# Growth factors for treating diabetic foot ulcers (Review)

**Martí-Carvajal AJ, Gluud C, Nicola S, Simancas-Racines D, Reveiz L, Oliva P, Cedeño-Taborda J**


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[Intervention Review]

# Growth factors for treating diabetic foot ulcers

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## ABSTRACT

### Background

Foot ulcers are a major complication of diabetes mellitus, often leading to amputation. Growth factors derived from blood platelets, endothelium, or macrophages could potentially be an important treatment for these wounds but they may also confer risks.

### Objectives

To assess the benefits and harms of growth factors for foot ulcers in patients with type 1 or type 2 diabetes mellitus.

### Search methods

In March 2015 we searched the Cochrane Wounds Group Specialised Register, The Cochrane Central Register of Controlled Trials (CENTRAL) (*The Cochrane Library)*, Ovid MEDLINE, Ovid MEDLINE (In-Process & Other Non-Indexed Citations, Ovid EMBASE and EBSCO CINAHL. There were no restrictions with respect to language, date of publication or study setting.

### Selection criteria

Randomised clinical trials in any setting, recruiting people with type 1 or type 2 diabetes mellitus diagnosed with a foot ulcer. Trials were eligible for inclusion if they compared a growth factor plus standard care (e.g., antibiotic therapy, debridement, wound dressings) versus placebo or no growth factor plus standard care, or compared different growth factors against each other. We considered lower limb amputation (minimum of one toe), complete healing of the foot ulcer, and time to complete healing of the diabetic foot ulcer as the primary outcomes.

### Data collection and analysis

Independently, we selected randomised clinical trials, assessed risk of bias, and extracted data in duplicate. We estimated risk ratios (RR) for dichotomous outcomes. We measured statistical heterogeneity using the $I^2$ statistic. We subjected our analyses to both fixed-effect and random-effects model analyses.

**Main results**

We identified 28 randomised clinical trials involving 2365 participants. The cause of foot ulcer (neurologic, vascular, or combined) was poorly defined in all trials. The trials were conducted in ten countries. The trials assessed 11 growth factors in 30 comparisons: platelet-derived wound healing formula, autologous growth factor, allogeneic platelet-derived growth factor, transforming growth factor $\beta 2$, arginine-glycine-aspartic acid peptide matrix, recombinant human platelet-derived growth factor (becaplermin), recombinant human epidermal growth factor, recombinant human basic fibroblast growth factor, recombinant human vascular endothelial growth factor, recombinant human lactoferrin, and recombinant human acidic fibroblast growth factor. Topical intervention was the most frequent route of administration. All the trials were underpowered and had a high risk of bias. Pharmaceutical industry sponsored 50% of the trials.

Any growth factor compared with placebo or no growth factor increased the number of participants with complete wound healing (345/657 (52.51%) versus 167/482 (34.64%); RR 1.51, 95% CI 1.31 to 1.73; $I^2$ = 51%, 12 trials; *low quality evidence*). The result is mainly based on platelet-derived wound healing formula (36/56 (64.28%) versus 7/27 (25.92%); RR 2.45, 95% 1.27 to 4.74; $I^2$ = 0%, two trials), and recombinant human platelet-derived growth factor (becaplermin) (205/428 (47.89%) versus 109/335 (32.53%); RR 1.47, 95% CI 1.23 to 1.76, $I^2$= 74%, five trials).

In terms of lower limb amputation (minimum of one toe), there was no clear evidence of a difference between any growth factor and placebo or no growth factor (19/150 (12.66%) versus 12/69 (17.39%); RR 0.74, 95% CI 0.39 to 1.39; $I^2$ = 0%, two trials; very *low quality evidence*). One trial involving 55 participants showed no clear evidence of a difference between recombinant human vascular endothelial growth factor and placebo in terms of ulcer-free days following treatment for diabetic foot ulcers (RR 0.64, 95% CI 0.14 to 2.94; P value 0.56, *low quality of evidence*)

Although 11 trials reported time to complete healing of the foot ulcers in people with diabetes , meta-analysis was not possible for this outcome due to the unique comparisons within each trial, failure to report data, and high number of withdrawals. Data on quality of life were not reported. Growth factors showed an increasing risk of overall adverse event rate compared with compared with placebo or no growth factor (255/498 (51.20%) versus 169/332 (50.90%); RR 0.83; 95% CI 0.72 to 0.96; $I^2$ = 48%; eight trials; *low quality evidence*). Overall, safety data were poorly reported and adverse events may have been underestimated.

**Authors' conclusions**

This Cochrane systematic review analysed a heterogeneous group of trials that assessed 11 different growth factors for diabetic foot ulcers. We found evidence suggesting that growth factors may increase the likelihood that people will have complete healing of foot ulcers in people with diabetes. However, this conclusion is based on randomised clinical trials with high risk of systematic errors (bias). Assessment of the quality of the available evidence (GRADE) showed that further trials investigating the effect of growth factors are needed before firm conclusions can be drawn. The safety profiles of the growth factors are unclear. Future trials should be conducted according to SPIRIT statement and reported according to the CONSORT statement by independent investigators and using the Foundation of Patient-Centered Outcomes Research recommendations.

## PLAIN LANGUAGE SUMMARY

**Growth factors for treating diabetic foot ulcers**

**What are diabetic foot ulcers?**

People who suffer from diabetes mellitus (usually referred to as 'diabetes') can develop wounds (ulcers) on their feet and ankles. These diabetic foot ulcers can take a long time to heal, and affect quality of life for people with diabetes. In some people, failure of these ulcers to heal can contribute to the need for some level of amputation on the foot. Any treatments that encourage diabetic foot ulcers to heal will be valuable.

**What are growth factors?**

Growth factors are substances that occur naturally in the body. They promote growth of new cells and healing of wounds. Treatment of diabetic foot ulcers with growth factors may improve the healing of ulcers.

**The purpose of this review**

This Cochrane review tried to identify the benefits and harms of treating diabetic foot ulcers with growth factors in addition to providing standard care (i.e. pressure relief, removal of dead tissue from the wound, infection control and application of dressings).

**Findings of this review**

The review authors searched the medical literature up to 3 March 2015, and identified 28 relevant medical trials, with a total of 2365 participants. The trials were performed in ten different countries, generally in out-patient settings. All the trials had low numbers of participants, which makes potential overestimation of benefits and underestimation of harms more likely. Half of the trials were sponsored by the pharmaceutical industry that produces these growth factors.

The trials tested 11 different types of growth factor, usually by applying them to the ulcer surface. Growth factors had no effect on the risk of having one toe or more amputated when compared with either another growth factor, or placebo (inactive fake medicine), or standard care alone (evidence from four trials). However, when compared with placebo or no growth factor, growth factors seemed to make complete healing of ulcers (wound closure) more likely to occur (evidence from 12 trials).

**Shortcomings of the trials included in this review**

None of the trials reported data on participants' quality of life. Harms caused by treatments were poorly reported, so the safety profile of growth factors remains unclear.

It is clear that more trials are required to assess the benefits and harms of growth factors in the treatment of diabetic foot ulcers. These trials should be well-designed, conducted by independent researchers (not industry-sponsored), and have large numbers of participants. They should report outcomes that are of interest to patients, such as: how many of the participants' ulcers healed, and how long the healing took; any level of amputation in the foot; quality of life; ulcer-free days following treatment; and harms caused by treatment, including whether there are any potential cancer risks.

## SUMMARY OF FINDINGS FOR THE MAIN COMPARISON *[Explanation]*

**Any growth factor compared with placebo or no growth factor for diabetic foot ulcer**

**Patient or population:** foot ulcers in people with diabetes
**Settings:** outpatient
**Intervention:** any growth factor
**Comparison:** placebo or no growth factor

| Outcomes | Illustrative comparative risks* (95% CI) | | Relative effect (95% CI) | No of Participants (studies) | Quality of the evidence (GRADE) | Comments |
|---|---|---|---|---|---|---|
| | Assumed risk | Corresponding risk | | | | |
| | Placebo or no intervention | Any growth factor | | | | |
| **Complete wound closure**<br>Follow-up: 4 to 24 weeks | **346 per 1000**[1] | **523 per 1000**<br>(454 to 599) | **RR 1.51**<br>(1.31 to 1.73) | 1316<br>(12 studies) | ⊕⊕◯◯<br>**low**[2,3] | 1.- Growth factors investigated included autologous growth factor (1 trial); platelet-derived wound healing formula (2 trials); recombinant human platelet-derived growth factor (becaplermin) (5 trials), recombinant human basic fibroblast growth factor (2 trials), recombinant human epidermal growth factor (1 trial), and transforming growth factor (1 trial)<br>2.- Trials differed in quality. |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Lower limb amputation (minimum of one toe)**<br>Follow-up: 8 to 20 weeks | **174 per 1000**[1] | **123 per 1000**<br>(64 to 235) | **RR 0.74**<br>(0.39 to 1.39) | 219<br>(2 studies) | ⊕○○○<br>**very low**[4,5] | Platelet-derived wound healing formula (1 trial), and recombinant human epidermal growth factor (1 trial) |
| **Ulcer-free days following treatment for diabetic foot ulcers (free from any recurrence)**<br>Follow-up: 12 weeks | See comment | See comment | Not estimable | 55<br>(1 study) | See comment | Trial authors reported recurrence of ulcer in 27% (4/15) of participants receiving growth factor (recombinant human vascular endothelial growth factor) versus 33% (3/9) in placebo group. Hazard ratio was calculated using data transformation |
| **Time to complete healing of the diabetic foot ulcer** | See comment | See comment | Not estimable | 0<br>(0) | See comment | Meta-analysis was not possible due to the unique comparisons within each trial, failure to report data, with or without a high rate of withdrawals |
| **Quality of life** | See comment | See comment | Not estimable | 0<br>(0) | See comment | None of the trials assessed this outcome. |
| **Adverse events (non-serious and serious)**<br>Follow-up: 5 to 20 weeks | **412 per 1000**[1] | **404 per 1000**<br>(325 to 502) | **RR 0.98**<br>(0.79 to 1.22) | 385<br>(4 studies) | ⊕⊕○○<br>**low**[4,6] | Recombinant human epidermal growth factor (1 trial), recombinant human platelet-derived growth factor (1 trial), recombinant human vascular endothelial growth factor (1 trial), thrombin-induced, platelet-released platelet- |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | derived wound healing formula (1 trial) |

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
**CI:** Confidence interval; **RR:** Risk ratio; **HR:** Hazard ratio;

GRADE Working Group grades of evidence
**High quality:** Further research is very unlikely to change our confidence in the estimate of effect.
**Moderate quality:** Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
**Low quality:** Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
**Very low quality:** We are very uncertain about the estimate.

[1] Assumed risk is based on the risks for the control group in the meta-analysis.
[2] Downgraded one level for limitations in design and execution: Eleven out thirteen trials assessing this outcome have high risk for selection bias. And outcome assessment was performed in unclear fashion.
[3] Downgraded one level for inconsistency ($I^2$: 51%).
[4] Downgraded one level for limitations in design and execution.
[5] Downgraded two levels for imprecision: small sample size and very low rate of events conducting to wide confidence intervals.
[6] Downgraded one level for imprecision: Low rate of adverse events resulting in wide confidence intervals.

# BACKGROUND

See Appendix 1 for medical and epidemiological terms.

## Description of the condition

It is estimated that in 2011, approximately 366 million people had diabetes, that is 7.0% of the world's population (Bakker 2012a). Around 80% of these people live in low- or middle-income countries. By 2030, the global estimate is expected to rise to 552 million - that is 8.3% of the adult population (Bakker 2012a). The development of foot ulcers is a major complication of diabetes mellitus (Boulton 2005; Lipsky 2004; Rathur 2007; Richard 2008; Sibbald 2008). The International Working Group on the Diabetic Foot defines a foot ulcer as a full thickness wound involving the foot or ankle (Lavery 2008), that is, "a wound penetrating through the dermis" (Schaper 2004). A wound is a break in the epithelial integrity of the skin and may be accompanied by disruption of the structure and function of underlying normal tissue (Enoch 2008).

### Epidemiology of the foot ulcer in people with diabetes

The proportion of diabetic foot ulcers among people with diabetes mellitus varies across studies, ranging from 5% to 43% (Appendix 2). There are four classification systems for diabetic foot ulcers that are summarised in Appendix 3 (Ince 2008; Lavery 1996; Schaper 2004; Wagner 1981). Outcomes for diabetic foot ulcers are predicted by ulcer area, presence of peripheral arterial disease, duration of diabetes, and presence of osteomyelitis (infection of bone) (Ince 2007; Lavery 2009; Oyibo 2001).

There is a close relationship between the presence of a diabetic foot ulcer and the amputation of a toe or a lower limb (Boulton 2008; Bakker 2012b; Younes 2004). Indeed, Boulton 2008 and Bakker 2012a reported that more than 85% of such amputations were preceded by an active foot ulcer. Amputation is a major complication for people with a diabetic foot ulcer (Bartus 2004; Schaper 2012a), and is a risk factor for increased mortality (Izumi 2009). The incidence of amputations is higher in people with diabetes (range 0.64 to 5.25 per 1000 person-years) than in people without diabetes (0.03 to 0.24 per 1000 person-years) (Schaper 2012a). The reported annual incidence of major amputation in industrialised countries ranges from 0.06 to 3.83 per 1000 diabetic people (Jeffcoate 2005). The incidence varies between countries, races, and communities (Jeffcoate 2005), however, there is concern about the methods used to calculate incidence and prevalence of amputation in people with diabetes (Van Houtum 2008). The incidence of reamputation in diabetic people with history of amputation within two years is almost 50% (Kanade 2007). Reamputation could be due to poor selection of the original amputation level through efforts to save as much of the lower extremity as possible (Skoutas 2009).

### Diabetic foot ulcer pathways

The commonest causes of foot ulcers in people with diabetes are peripheral neuropathy (nerve damage), foot deformity, external trauma, peripheral vascular disease, and peripheral oedema (Boulton 2008; Figueroa-Romero 2008; Quattrini 2008; Schaper 2012b; Szabo 2009). Other significant risk factors include being over 75 years of age, use of insulin, poor psychosocial status, hyperkeratosis (thickening of the outermost layer of skin), macrovascular and microvascular complications, and duration of diabetes (Chao 2009; Iversen 2008; Leymarie 2005).

## Description of the intervention

Many studies have experimented with biological agents, aiming to modify the pathophysiology of diabetic foot ulcers. Growth factors are examples of these biological agents, and are considered to be a potentially important technological advance in the area of wound healing (Papanas 2007).

Growth factors are platelet-derived, endothelium-derived, or macrophage-derived, and include granulocyte colony-stimulating factor, platelet-derived growth factor, epidermal growth factor, transforming growth factor, fibroblast growth factor, vascular endothelial growth factor, insulin-like growth factor, and keratinocyte growth factor (Amery 2005; Barrientos 2008; Bennet 2003; Blair 2009; Cruciani 2009; Foster 2009; Galkoswka 2006; Grazul-Bilska 2003; Rozman 2007; Smyth 2009). Growth factors are administered topically (on the surface) (Afshari 2005; Agrawal 2009; Bhansali 2009; Chen 2004; d'Hemecourt 1998; Driver 2006; Hanft 2008; Hardikar 2005; Holloway 1993; Jaiswal 2010; Kakagia 2007; Landsman 2010; Lyons 2007; Niezgoda 2005; Richard 1995; Robson 2002; Saldalamacchia 2004; Steed 1992; Steed 1995a; Steed 1995b; Steed 1996; Tan 2008; Tsang 2003; Uchi 2009; Viswanathan 2006; Wieman 1998a), or intra lesionally (within the wound) (Fernández-Montequin 2007; Fernández-Montequin 2009).

## How the intervention might work

Normal wound healing has four phases: coagulation, inflammation, migration/proliferation, and remodelling (Papanas 2008). Sheehan 2006 observed that a 53% or greater reduction in the area of a foot ulcer area after four weeks of observation was a robust predictor of healing at 12 weeks. Since chronic wound healing may be limited by a lack of the necessary growth factors, healing may be speeded up by replacing or stimulating these growth factors, so enhancing the formation of granulation tissue, that precedes healing, within the wounds (Amery 2005; Barrientos 2008; Bennet 2003; Galkoswka 2006; Grazul-Bilska 2003; Köveker 2000; Pradhan 2009; Viswanathan 2006). See Appendix 4 for wound-healing and tissue-forming ability of growth factors.

## Why it is important to do this review

Diabetic foot ulcers represent a pervasive and important problem for people suffering from diabetes mellitus. Foot-related problems are responsible for up to 50% of diabetes-related hospital admissions (Albert 2002; Boulton 2001; Boulton 2005). Foot ulcers cause a low quality of life and often lead to lower extremity amputation (Armstrong 2008; Boutoille 2008; Goodridge 2006; Herber 2007; Kinmond 2003; Meatherall 2005; Price 2004; Ribu 2008; Schaper 2012a; Valensi 2007). Amputation causes prolonged hospitalisation, rehabilitation, and an increased need for home care and social services (Ali 2008; Ashry 1998; Girod 2003; Habib 2010; Lantis 2009; Redekop 2004; Siriwardana 2007; Van Acker 2000; Viswanathan 2005; Willrich 2005). Management of the diabetic foot has major economic consequences for patients, their families and society (Jeffcoate 2003; Jeffcoate 2004; Milman 2001; Rathur 2007; Smith 2004), and quality of life for caregivers is also unsatisfactory (Nabuurs-Frassen 2005).

Several randomised clinical trials (RCTs) have assessed the benefits and harms of growth factors for treating diabetic foot ulcers, and they need a critical appraisal for risk of systematic errors that can cause bias (that is, could cause overestimation of benefits and underestimation of harms) and risk of random errors (that is, play of chance). Several narrative reviews and meta-analyses have assessed the use of growth factors for treating diabetic foot ulcers, but these have been prone to errors (that is, lack of rigorous assessment of bias risks; no or insufficient evaluation of the risks of random errors; no evaluation of statistical heterogeneity; poor reporting of search methods; and potential conflicts of interest as authors of the reviews were also trialists of the included trials) (Hinchliffe 2008; Papanas 2008).

A systematic review of the most up to date evidence, including a rigorous assessment of the quality of that evidence, may help clinicians and clinical researchers make informed decisions about the use of growth factors for treating diabetic foot ulcers.

# OBJECTIVES

To assess the benefits and harms of growth factors for diabetic foot ulcers in patients with type 1 or type 2 diabetes mellitus.

# METHODS

## Criteria for considering studies for this review

### Types of studies

Randomised controlled trials (RCTs) in any setting.

### Types of participants

Adults (>18 years of age) with a diabetic foot ulcer of any aetiology.

### Types of interventions

See Appendix 1.

#### Experimental interventions

1. Platelet-derived wound healing formula
2. Autologous growth factor
3. Allogeneic platelet-derived growth factor
4. Transforming growth factor $\beta 2$
5. Arginine-glycine-aspartic acid (RGD) peptide matrix
6. Recombinant human platelet-derived growth factor (becaplermin)
7. Recombinant human epidermal growth factor
8. Recombinant human basic fibroblast growth factor
9. Recombinant human vascular endothelial growth factor (telbermin)
10. Recombinant human lactoferrin
11. Recombinant human acidic fibroblast growth factor

In addition to receiving the experimental intervention (growth factors) participants also received standard care (see below).

Trials of granulocyte-colony stimulating factors were excluded as they are the focus of another Cochrane review (Cruciani 2009).

#### Control interventions

1. Standard care (for example, antibiotic therapy, debridement, wound dressings) alone or plus placebo.

We noted whether the standard care was delivered similarly to intervention groups and noted any differences between intervention groups.

### Types of outcome measures

#### Primary outcomes

1. Complete wound healing (defined as 100% epithelialisation or skin closure without drainage).
2. Lower limb amputation (minimum of one toe).
3. Time to complete healing of the diabetic foot ulcer.

#### Secondary outcomes

1. Ulcer-free days following treatment for diabetic foot ulcers (free from any recurrence).
2. Quality of life (as measured by a validated scale).
3. Adverse events: number and type of adverse events defined as any untoward medical occurrence - not necessarily having a causal relationship with the treatment. We reported separately on adverse events that led, and did not lead, to treatment

discontinuation. We defined serious adverse events according to the International Conference on Harmonisation (ICH) Guidelines as any event that at any dose results in death, is life-threatening, requires in-patient hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability, or is a congenital anomaly/birth defect, and any important medical event that may have jeopardised the patient or requires intervention to prevent it (ICH-GCP 1997). All other adverse events were considered non-serious.

## Search methods for identification of studies

### Electronic searches

The following electronic databases were searched to identify reports of relevant randomised clinical trials:

1. The Cochrane Wounds Group Specialised Register (searched 03 March 2015);
2. The Cochrane Central Register of Controlled Trials (CENTRAL) (*The Cochrane Library* 2015, Issue 2);
3. Ovid MEDLINE (1946 to March 2, 2015);
4. Ovid MEDLINE (In-Process & Other Non-Indexed Citations) (March 2, 2015);
5. Ovid EMBASE (1974 to March 2, 2015);
6. EBSCO CINAHL (1982 to March 3, 2015).

We used the following search strategy in The Cochrane Central Register of Controlled Trials (CENTRAL):

1. MeSH descriptor Foot Ulcer explode all trees
2. MeSH descriptor Diabetic Foot explode all trees
3. diabet* NEAR/3 ulcer*:ti,ab,kw
4. diabet* NEAR/3 (foot or feet):ti,ab,kw
5. diabet* NEAR/3 wound*:ti,ab,kw
6. (#1 OR #2 OR #3 OR #4 OR #5)
7. MeSH descriptor Intercellular Signaling Peptides and Proteins explode all trees
8. MeSH descriptor Insulin-Like Growth Factor Binding Proteins explode all trees
9. growth NEXT factor*:ti,ab,kw
10. EGF or FGF or PDGF:ti,ab,kw
11. plermin or regranex or becaplermin:ti,ab,kw
12. (#7 OR #8 OR #9 OR #10 OR #11)
13. (#6 AND #12)

This strategy was adapted to search Ovid MEDLINE, Ovid EMBASE and EBSCO CINAHL (please see Appendix 5). The MEDLINE search was combined with the Cochrane Highly Sensitive Search Strategy for identifying randomised trials in MEDLINE: sensitivity- and precision-maximizing version (2008 revision) (Lefevbre 2011). The EMBASE and CINAHL searches were combined with the trial filters developed by the Scottish Intercollegiate Guidelines Network (SIGN) (SIGN 2010). There were no restrictions with respect to language, date of publication or study setting.

### Searching other resources

The following web sites were also searched:

1. Food and Drug Administration (http://www.fda.gov/);
2. European Medicines Agency (http://www.emea.europa.eu);
3. International Working Group on the Diabetic Foot (http://iwgdf.org/);
4. MedWatch The FDA Safety Information and Adverse Event Reporting Program (http://www.fda.gov/Safety/MedWatch/default.htm);
5. Medicines and Healthcare products Regulatory Agency ( http://www.mhra.gov.uk/index.htm);
6. Scirus (www.scirus.com);
7. CenterWatch (http://www.centerwatch.com);
8. Evidence in Health and Social Care (http://www.evidence.nhs.uk/);
9. Dailymed (http://dailymed.nlm.nih.gov/dailymed/about.cfm).
10. WHO International Clinical Trials Registry Platform Search Portal (http://apps.who.int/trialsearch/).

We also checked the reference lists of all the potentially relevant trials identified by the above methods.

## Data collection and analysis

We summarised data using standard Cochrane Collaboration methodologies (Higgins 2011).

### Selection of studies

Two review authors (AJM-C, SN) independently assessed each reference identified by the search against the inclusion criteria. We resolved disagreements that arose through discussion. Those references that appeared to meet the inclusion criteria were retrieved in full for further independent assessment by two review authors.

### Data extraction and management

One review author independently extracted data (SN) from the included trials using a spreadsheet data extraction form and two review authors (AJM-C, DSR) checked the data entered. We extracted the following data: eligibility criteria, demographics (age, sex, country), characteristic of the ulcers (anatomic site, size, number of ulcers, presence of infection, duration of ulceration), type of diabetes mellitus, duration of diabetes mellitus, ulcer treatments, and outcomes assessed. We discussed any discrepancies between review authors in order to achieve a final consensus.

### Assessment of risk of bias in included studies

Independently, three review authors (AJM-C, SN, DSR) assessed the risk of bias of each included trial using the domain-based evaluation as described in the *Cochrane Handbook for Systematic Reviews of Interventions* 5.1.0 (Higgins 2011). See, Appendix 6 for details.

Three review authors (LR, PO, JCT) checked these assessments. The review authors discussed discrepancies and achieved consensus.

#### Overall risk of bias

We made explicit judgements about whether the RCTs were at high risk of bias, according to the criteria given in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). We assessed the risk of bias as being high if any of the above domains was assessed as being at unclear or high risk of bias.

Trials that had adequate generation of allocation sequence, allocation concealment, blinding, handling of incomplete outcome data, and no selective outcome reporting, and that were without other risks of bias were considered to be trials with a low risk of bias. We explored the impact of the risk of bias through undertaking subgroup analyses.

### Measures of treatment effect

For binary outcomes, such as incidence of complete wound healing, amputation, and adverse events, we calculated the risk ratio (RR) with 95% confidence intervals (CI) for each. For ulcer-free days following treatment, a time-to-event outcome, we calculated the hazard ratio (HR) with 95% CI (Zavala 2007).

### Dealing with missing data

We assessed the percentage of dropouts for each included trial, and for each intervention group, and evaluated whether an intention-to-treat (ITT) analysis had been performed or could have been performed from the available published information. We contacted authors to resolve some queries on this issue.

In order to undertake an ITT analysis, we sought data from the trial authors on the number of participants in treatment groups, irrespective of compliance and whether or not participants were later thought to be ineligible, or otherwise excluded from treatment or lost to follow-up. If this information was not forthcoming, we undertook a complete patient analysis, knowing that it might be biased.

We included patients with incomplete or missing data in sensitivity analyses by imputing them according to the following scenarios (Hollis 1999).

- Extreme case analysis favouring the experimental intervention ('best-worse' case scenario): none of the drop-outs/participants lost from the experimental arm, but all of the drop-outs/participants lost from the control arm experienced the outcome, including all randomised participants in the denominator.
- Extreme case analysis favouring the control ('worst-best' case scenario): all drop-outs/participants lost from the experimental arm, but none from the control arm experienced the outcome, including all randomised participants in the denominator.

### Assessment of heterogeneity

We quantified statistical heterogeneity using the $I^2$ statistic, which describes the percentage of total variation across trials that is due to heterogeneity rather than sampling error (Higgins 2003). We considered statistical heterogeneity to be present if $I^2$ was greater than 50% (Higgins 2011). When significant heterogeneity was detected (i.e. when $I^2$ exceeded 50%), we attempted to identify the possible causes of heterogeneity.

### Assessment of reporting biases

We assessed publication bias and other bias by a funnel-plot (Sterne 2011). We calculated Egger's test with Comprehensive Meta-analysis software (CMA 2011).

### Data synthesis

We calculated pooled estimates and 95% confidence intervals (CI) using fixed-effect model.

### Subgroup analysis and investigation of heterogeneity

We had anticipated clinical heterogeneity in the effect of the intervention and we planned to conduct the following sub-group analyses had the data had been available. Furthermore, subgroup analysis would be performed only for complete wound healing (primary outcome).

We could not perform preplanned analyses for clinical subgroups (insulin-using compared to non insulin-using participants, severity and depth of wound, and use or not of antibiotics (Appendix 7; Appendix 8)) due to a lack of available data.

We conducted the following preplanned subgroup analyses.

1. Duration of follow-up: trials with less 20 weeks of follow-up compared to trials with 20 weeks or more of follow-up.
2. Type of growth factor.

The subgroup analyses were only performed for the outcome of complete wound closure.

### Sensitivity analysis

We performed the following sensitivity analysis in order to explore the influence of these factors on the intervention effect size.

1. Repeating the analysis taking attrition bias into consideration: 'Best-worst case' scenario versus 'Worst-best case' scenario

#### 'Summary of findings' tables

We used the principles of the GRADE system to assess the quality of the body of evidence associated with specific outcomes where possible (complete wound closure, lower limb amputation, ulcer-free day following treatment for diabetic foot ulcers, time to complete healing of the diabetic foot ulcer, quality of life, and adverse events) (Guyatt 2011f). We constructed 'Summary of findings' tables using the GRADE software. The GRADE approach appraises the quality of a body of evidence on the basis of the extent to which one can be confident that an estimate of effect or association reflects the item being assessed. The quality of a body of evidence considers within-study risk of bias (methodologic quality), the directness of the evidence, heterogeneity of the data, precision of effect estimates, and risk of publication bias (Balshem 2011; Guyatt 2011a; Guyatt 2011b; Guyatt 2011c; Guyatt 2011d; Guyatt 2011e; Guyatt 2011f; Guyatt 2011g; Guyatt 2011h; Guyatt 2011i; Guyatt 2012).

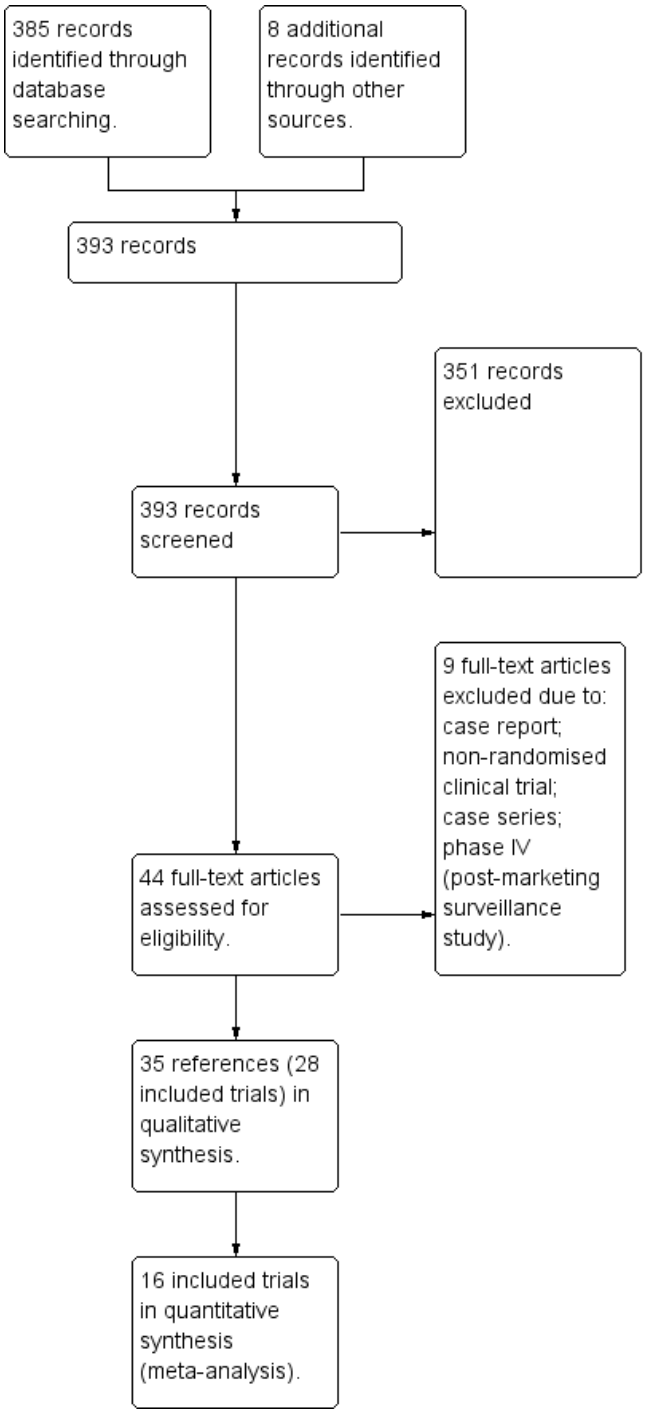
# RESULTS

## Description of studies

### Results of the search

We identified 424 references using our search strategies. Twenty-eight trials (35 references) involving 2365 participants met our inclusion criteria (Afshari 2005; Agrawal 2009; Bhansali 2009; Chen 2004; d'Hemecourt 1998; Driver 2006; Fernández-Montequin 2007; Fernández-Montequin 2009; Hanft 2008; Hardikar 2005; Holloway 1993; Jaiswal 2010; Kakagia 2007; Landsman 2010; Lyons 2007; Niezgoda 2005; Richard 1995; Robson 2002; Saldalamacchia 2004; Steed 1992; Steed 1995a; Steed 1995b; Steed 1996; Tan 2008; Tsang 2003; Uchi 2009; Viswanathan 2006; Wieman 1998a). See Figure 1 for details of the flow of studies.

**Figure 1. Study flow diagram.**



## Included studies

Tables of Characteristics of included studies show a detailed description of the studies.

### Growth factors and populations assessed in the trials

The 28 RCTs reported 11 different growth factors compared with several different control interventions.

The experimental interventions included both non-recombinant and recombinant growth factors. The non-recombinant growth factors investigated were: platelet-derived wound healing formula (Holloway 1993; Steed 1992); autologous growth factors (Driver 2006; Kakagia 2007; Saldalamacchia 2004); allogeneic platelet-derived growth factor (Steed 1996); transforming growth factor $\beta 2$ (Robson 2002); arginine-glycine-aspartic acid (RGD) peptide matrix (Steed 1995b). The recombinant growth factors were recombinant human platelet-derived growth factor (Agrawal 2009; Bhansali 2009; d'Hemecourt 1998; Hardikar 2005; Jaiswal 2010; Landsman 2010; Niezgoda 2005; Steed 1995a; Wieman 1998a); recombinant human epidermal growth factors (Afshari 2005; Chen 2004; Fernández-Montequin 2007; Fernández-Montequin 2009; Tsang 2003; Viswanathan 2006); recombinant human basic fibroblast growth factors (Richard 1995; Tan 2008; Uchi 2009); recombinant human vascular endothelial growth factor (Hanft 2008); recombinant human lactoferrin (Lyons 2007); and recombinant human acidic fibroblast growth factor (Tan 2008).

Twenty trials compared growth factors against no growth factor or against placebo (without or with co-interventions). The comparisons were: no growth factor (d'Hemecourt 1998; Fernández-Montequin 2009; Jaiswal 2010; Saldalamacchia 2004); saline solution (Bhansali 2009; Driver 2006; Holloway 1993; Richard 1995; Steed 1992; Steed 1995b; Steed 1996); or placebo (Agrawal 2009; Hanft 2008; Hardikar 2005; Lyons 2007; Robson 2002; Steed 1995a; Uchi 2009; Viswanathan 2006; Wieman 1998a). The characteristics of the placebo were not sufficiently described in Agrawal 2009, Hardikar 2005, Steed 1995a, Uchi 2009, or Wieman 1998a. Accordingly, only four trials used an appropriate placebo (Hanft 2008; Lyons 2007; Robson 2002; Viswanathan 2006).

Two trials compared one growth factor versus another growth factor, or different doses of the same growth factor (with or without co-interventions). Tan 2008 compared recombinant human acidic fibroblast growth factor versus recombinant human basic fibroblast growth factor. Fernández-Montequin 2007 compared two doses of recombinant human epidermal growth factor, 75 µg and 25 µg.

Six trials compared growth factors versus other interventions (with or without co-interventions): silver sulphadiazine (Afshari 2005); insulin (Chen 2004); oxidized regenerated cellulose/collagen biomaterial (Kakagia 2007); moisture-regulating dressing (Landsman 2010); oasis wound matrix (Niezgoda 2005); and actovegin (Tsang 2003).

The co-interventions used most frequently in both the experimental and the control groups were: wound debridement (Afshari 2005; Agrawal 2009; Bhansali 2009; d'Hemecourt 1998; Driver 2006; Fernández-Montequin 2007; Fernández-Montequin 2009; Hanft 2008; Hardikar 2005; Jaiswal 2010; Kakagia 2007; Lyons 2007; Robson 2002; Steed 1992; Steed 1995a; Steed 1995b; Tsang 2003; Uchi 2009; Wieman 1998a); wound dressing (Afshari 2005; Agrawal 2009; d'Hemecourt 1998; Hardikar 2005, Kakagia 2007; Landsman 2010; Lyons 2007; Robson 2002; Steed 1992; Steed 1995a; Tan 2008); antibiotics - topical (Chen 2004), and systemic (Afshari 2005; d'Hemecourt 1998; Fernández-Montequin 2007; Hardikar 2005; Lyons 2007; Viswanathan 2006); glycaemic control (Agrawal 2009; Chen 2004; Fernández-Montequin 2007; Hardikar 2005; Richard 1995; Viswanathan 2006); and offloading of local pressure on the foot ulcer (Bhansali 2009; d'Hemecourt 1998; Driver 2006; Hanft 2008; Hardikar 2005; Jaiswal 2010; Landsman 2010; Lyons 2007; Niezgoda 2005; Richard 1995; Robson 2002; Steed 1992; Steed 1995a; Steed 1995b; Steed 1996). Two trials did not report the use of any co-intervention (Holloway 1993; Saldalamacchia 2004).

Twenty-six trials administered the intervention topically; two trials involving recombinant human epidermal growth factor used intralesional administration (Fernández-Montequin 2007; Fernández-Montequin 2009).

The intervention was administered: once daily in 12 trials (Afshari 2005; Agrawal 2009; Bhansali 2009; d'Hemecourt 1998; Hardikar 2005; Jaiswal 2010; Landsman 2010; Steed 1992; Steed 1995a; Steed 1996; Tan 2008; Uchi 2009); daily during the six weeks for which participants were inpatients, then twice a week for 12 weeks in one trial (Richard 1995); twice daily in three trials (Lyons 2007; Viswanathan 2006; Wieman 1998a); once a week in one trial (Niezgoda 2005); twice a week in two trials (Robson 2002; Steed 1995b); or three times a week on alternate days in three trials (Fernández-Montequin 2007; Fernández-Montequin 2009; Hanft 2008). Six trials did not report on the frequency of administration (Chen 2004; Driver 2006; Holloway 1993; Kakagia 2007; Saldalamacchia 2004; Tsang 2003).

The mean age of participants was 59.1 years (standard deviation (SD) ± 4.16), and most were male (66.6% (SD ± 16.1%)). Ten trials included participants with type 1 and type 2 diabetes mellitus ( Driver 2006; Fernández-Montequin 2007; Fernández-Montequin 2009; Hanft 2008; Hardikar 2005; Jaiswal 2010; Landsman 2010; Niezgoda 2005; Viswanathan 2006; Wieman 1998a). Eighteen trials did not report the type of diabetes mellitus explicitly (Afshari 2005; Agrawal 2009; Bhansali 2009; Chen 2004; d'Hemecourt

1998; Hardikar 2005; Kakagia 2007; Lyons 2007; Richard 1995; Robson 2002; Saldalamacchia 2004; Steed 1992; Steed 1995a; Steed 1995b; Steed 1996; Tan 2008; Tsang 2003; Uchi 2009). Trials included participants with target foot ulcers at nine different sites (fore-foot, mid-foot, hind-foot, internal and external edge, sole, plantar surface, ankle). Six trials included participants with neuropathic ulcers (Hardikar 2005; Lyons 2007; Richard 1995; Robson 2002; Steed 1992; Steed 1996). The remaining 22 trials did not report the cause of the foot ulcers. Generally, the trials were conducted in the out-patient (ambulatory) setting.

#### Location of trials

The trials were conducted in ten countries: three in China (Chen 2004; Tan 2008; Tsang 2003); two in Cuba (Fernández-Montequin 2007; Fernández-Montequin 2009); one in Greece (Kakagia 2007); five in India (Agrawal 2009; Bhansali 2009; Hardikar 2005; Jaiswal 2010; Viswanathan 2006); one in Iran (Afshari 2005); one in Italy (Saldalamacchia 2004); one in Japan (Uchi 2009); and eleven in the USA (d'Hemecourt 1998; Driver 2006; Hanft 2008; Holloway 1993; Landsman 2010; Robson 2002; Steed 1992; Steed 1995a; Steed 1995b; Steed 1996; Wieman 1998a). One trial was conducted in both Canada and the USA (Niezgoda 2005), and another in both France and Italy (Richard 1995).

#### Trial methods

All trials were conducted using the parallel group trial design. Eighty-two per cent of the trials (23/28) were conducted without reporting an *a priori* estimation of sample size. Trials were small with sample sizes ranging from 13 to 382 participants, with a median sample size of 60 and a mean of 87 (± SD 76). Fourteen trials had follow-up periods of less than 20 weeks (range five to 18 weeks) (Afshari 2005; Agrawal 2009; Driver 2006; Fernández-Montequin 2007; Fernández-Montequin 2009; Hanft 2008; Jaiswal 2010; Kakagia 2007; Richard 1995; Saldalamacchia 2004; Steed 1995b; Tan 2008; Uchi 2009; Viswanathan 2006). Thirteen trials had a follow-up of 20 weeks or more (range 20 weeks to 26 weeks) (Bhansali 2009; d'Hemecourt 1998; Hardikar 2005; Holloway 1993; Landsman 2010; Lyons 2007; Niezgoda 2005; Robson 2002; Steed 1992; Steed 1995a; Steed 1996; Tsang 2003; Wieman 1998a). One trial did not report length of follow-up (Chen 2004). In the trials, the units of randomisation and analysis were the participants. In terms of assessing the stage of the ulcer - trials variously used Wagner's classification, the University of Texas Diabetic classification, or the International Association Enterostomal Therapy classification for (Appendix 3). Appendix 9 shows the methods for assessing ulcer dimension.

### Excluded studies

We excluded nine studies for the following reasons: case reports (Acosta 2006; Miller 1999; Tuyet 2009); non-RCTs (Aminian 2000; Saad Setta 2011); case series (Embil 2000; Hong 2006); and phase IV study (post-marketing surveillance study) (Mohan 2007; Yera-Alos 2013). See the Characteristics of excluded studies table.

#### Ongoing trials

We identified six ongoing trials (NCT00521937; NCT00709514; NCT00915486; NCT00926068; NCT01060670; NCT01098357). Full details are shown in the table of Characteristics of ongoing studies.

#### Studies awaiting classification

Four citations are 'Awaiting classification' (Gomez-Villa 2014; Morimoto 2013; Singla 2014; Young 1992; see Characteristics of studies awaiting classification for details).

## Risk of bias in included studies

The risk of bias in the included trials is summarised in Figure 2 and Figure 3, and detailed in the Characteristics of included studies table.

**Figure 2. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.**

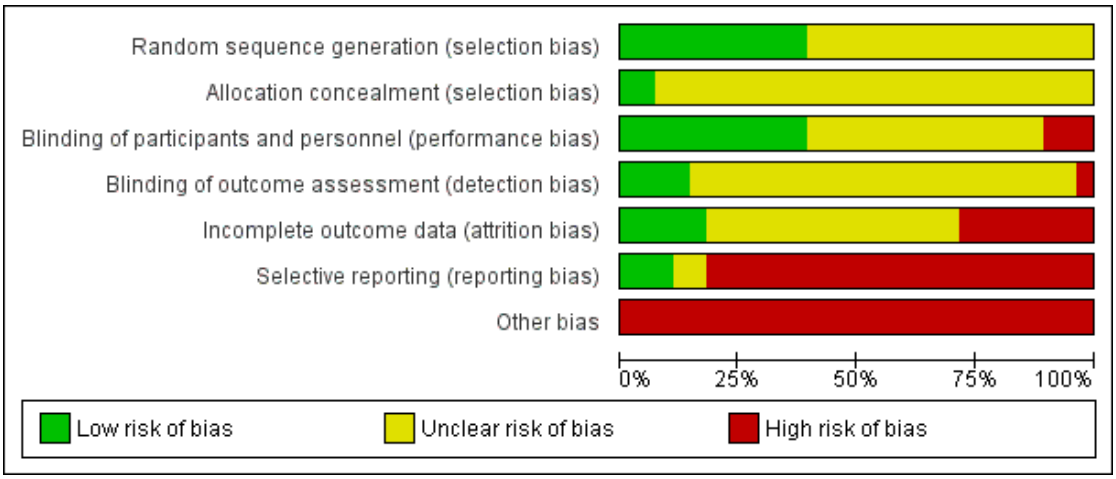
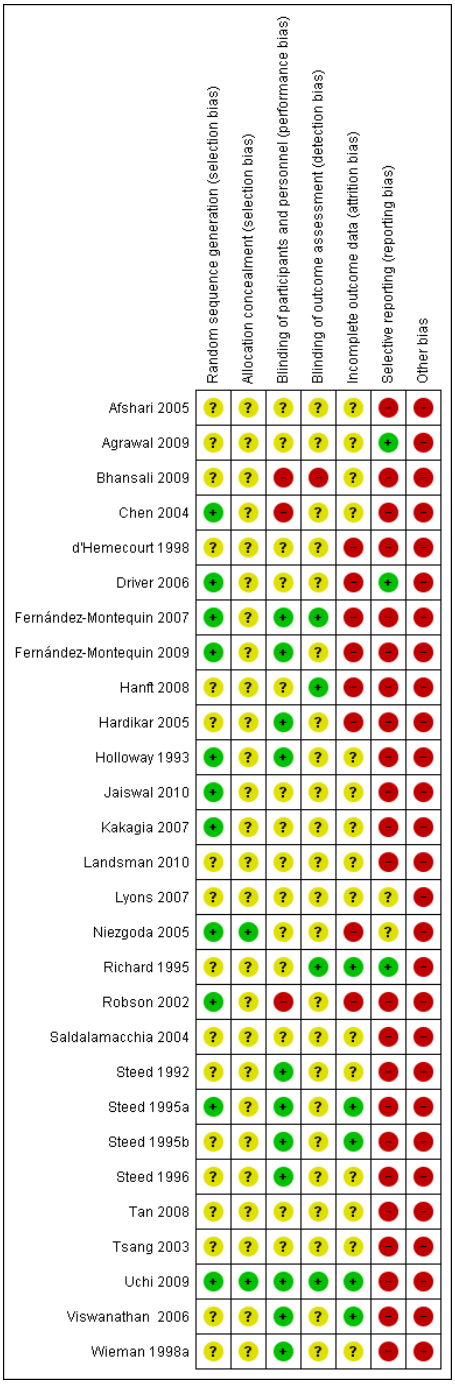


**Figure 3. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.**



### Allocation

#### Random sequence generation

The risk of bias arising from the method of generation of the allocation sequence was considered to be low in eleven trials (Chen 2004; Driver 2006; Fernández-Montequin 2007; Fernández-Montequin 2009; Holloway 1993; Jaiswal 2010; Kakagia 2007; Niezgoda 2005; Robson 2002; Steed 1995a; Uchi 2009). The remaining 17 trials had unclear risk of bias for this domain.

#### Allocation concealment

The risk of bias arising from the method of allocation concealment was considered to be low in two trials (Niezgoda 2005; Uchi 2009). The remaining 26 trials had an unclear risk for this domain.

### Blinding

The risk of bias due to lack of blinding of participants and personnel was rated as low in 11 trials (Fernández-Montequin 2007; Fernández-Montequin 2009; Hardikar 2005; Holloway 1993; Steed 1992; Steed 1995a; Steed 1995b; Steed 1996; Uchi 2009; Viswanathan 2006; Wieman 1998a). The risk of bias of performance bias was high in the remaining 17 trials.
In four trials outcome assessment was clearly reported as blinded, and detection bias was considered to be low (Fernández-Montequin 2007; Hanft 2008; Richard 1995; Uchi 2009). Blinding of outcome assessors was unclear or not performed in the remaining 24 trials, so the risk of detection bias was considered to be high.

### Incomplete outcome data

Risk of attrition bias was rated as low in six trials (Richard 1995; Robson 2002; Steed 1995a; Steed 1995b; Uchi 2009; Viswanathan 2006), but high in the remaining 22 trials.

### Selective reporting

Risk of selective outcome reporting bias was rated as low in three trials (Agrawal 2009; Driver 2006; Richard 1995), two trials was rated as having unclear risk (Lyons 2007; Niezgoda 2005), and rated as high in the remaining 23 trials. It was mainly due to these trials neither measured nor reported complete wound closure or safety data.

### Other potential sources of bias

Risk of other bias was rated as high in all 28 trials due to bias in the presentation of data or design bias.

Accordingly, all trials were considered to have an overall high risk of bias.

## Effects of interventions

See: **Summary of findings for the main comparison** Any growth factor compared with placebo or no growth factor for diabetic foot ulcer

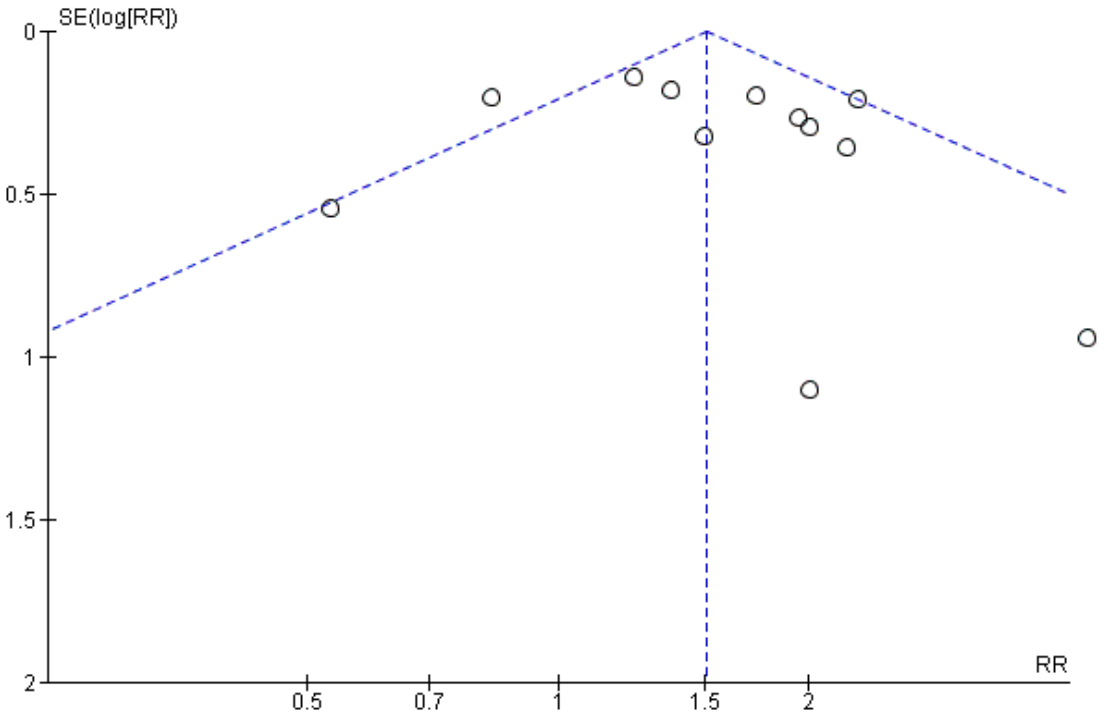
### Primary outcomes

#### Complete wound closure (defined as 100% epithelialisation or skin closure without drainage)

#### Any growth factor versus placebo or no growth factor

Meta-analysis of 12 trials showed that growth factors, when considered as a group, increased the incidence of complete wound healing compared with placebo or no growth factor (345/657 (52.51%) versus 167/482 (34.64%); RR fixed-effect model 1.51 95% CI 1.31 to 1.73; $I^2$ = 51%, *low quality evidence due to limitation in design, execution or both, and inconsistency*) (d'Hemecourt 1998; Hanft 2008; Hardikar 2005; Holloway 1993; Jaiswal 2010; Richard 1995; Robson 2002; Saldalamacchia 2004; Steed 1992; Steed 1995a; Uchi 2009; Viswanathan 2006; Wieman 1998a). See Analysis 1.1. Figure 4 shows a funnel-plot of this meta-analysis.

**Figure 4. Funnel plot for comparison effect of any growth factor versus placebo or no growth factor on 100% complete wound closure.P-value (two tailed) for Egger's test = 0.43**




***Subgroup analysis of trials with follow-up of less than 20 weeks compared to trials with follow-up of 20 weeks or longer***

Meta-analysis of five trials with follow-up of less than 20 weeks shows uncertainty due to imprecision (small sample size and low rate of event) in the proportion of complete wound healing comparing any growth factor versus placebo or no growth factor (102/167 (61.07%) versus 60/119 (50.42%); RR 1.24, 95% CI 1.00 to 1.55; $I^2$ = 57%; P value 0.05) (Jaiswal 2010; Richard 1995; Saldalamacchia 2004; Uchi 2009; Viswanathan 2006). Meta-analysis of seven trials with a follow-up of 20 weeks or longer showed an increase in the incidence of complete wound healing comparing any growth factor versus placebo or no growth factor (243/490 (49.59%) versus 107/363 (29.47%); RR 1.65, 95% CI 1.38 to 1.98; $I^2$ = 34%) (d'Hemecourt 1998; Hanft 2008; Hardikar 2005; Holloway 1993; Steed 1992; Steed 1995a; Wieman 1998a). The subgroup test showed high inconsistency between the two groups ($I^2$ = 73.5%, P value 0.05). See Analysis 2.1.

*Subgroup analysis by type of growth factor*

One trial comparing autologous growth factor versus placebo or no growth factor showed inconclusive results regarding complete wound closure due to high imprecision (2/7 (28.57%) versus 1/7 (14.28%); RR 2.0, 95% CI 0.23 to 17.34; P value 0.53) (Saldalamacchia 2004). Meta-analysis of two trials comparing platelet-derived wound healing formula versus placebo showed a significant increase in the likelihood of participants with complete wound healing receiving growth factor (36/56 (64.28%) versus 7/27 (25.92%); RR 2.45, 95% CI 1.27 to 4.74, $I^2$ = 0%) (Holloway 1993; Steed 1992). Meta-analysis of five trials showed that recombinant human platelet-derived growth factor (becaplermin) increased the proportion of the participants with complete wound healing compared with placebo (205/428 (47.89%) versus 109/335 (32.53%); RR 1.47, 95% CI 1.23 to 1.76, $I^2$ = 74%) (d'Hemecourt 1998; Hardikar 2005; Jaiswal 2010; Steed 1995a; Wieman 1998a). Meta-analysis of two trials comparing recombinant human basic fibroblast growth factor versus placebo or no growth factor showed inconclusive results regarding proportion of participants with complete wound healing (60/106 (56.60%) versus 27/59 (45.76%); RR 1.23, 95% CI 0.88 to 1.72, $I^2$ = 62% P value 0.22) (Richard 1995; Uchi 2009). One trial comparing recombinant human epidermal growth factor versus placebo showed an increase in the incidence of complete wound healing using growth factor (25/29 (86.28%) versus 14/28 (50%); RR 1.72, 95% CI 1.16 to 2.57) (Viswanathan 2006). One trial compar-

ing recombinant human vascular endothelial growth factor versus placebo showed no clear evidence of a difference regarding complete wound closure (15/29 (51.72%) versus 9/26 (34.61%); RR 1.49, 95% CI 0.79 to 2.82; P value 0.21) (Hanft 2008). The subgroup test showed no significant difference between the two groups ($I^2$ = 0%, P value 0.55). However, the quality of the evidence showed in this subgroup analysis should be considered either low or very low. It is due to severe imprecision (wide confidence intervals) based on small sample size and low number of event (complete wound closure), inconsistency and limitations of design and execution of these trials. See Analysis 3.1.

#### *Sensitivity analysis taking attrition into consideration*

Eight of the 12 trials combined for this outcome reported the exact number of participants with missing data in the intervention and the control groups. These trials involved 1043 participants ( d'Hemecourt 1998; Hanft 2008; Hardikar 2005; Holloway 1993; Steed 1995a; Uchi 2009; Viswanathan 2006; Wieman 1998a).

#### *'Best-worst case' scenario*

In a best-worst case scenario analysis where none of the drop-outs/participants were lost from the experimental arm, but all of the drop-outs/participants lost from the control arm experienced the outcome, including all randomised participants in the denominator, meta-analysis of eight trials showed a higher likelihood of complete wound healing in the participants receiving any growth factor compared with those exposed to placebo or no growth factor (417/607 (68.69%) versus 142/436 (32.56%); RR 2.09, 95% CI 1.81 to 2.41; $I^2$ = 57%; P value 0.00001).

#### *'Worst-best case' scenario*

In a worst-best case scenario analysis (all drop-outs/participants lost from the experimental arm, but none from the control arm experienced the outcome, including all randomised participants in the denominator) we did not find clear evidence of a difference in the proportion of participants assigned to any growth factor with complete wound healing compared with placebo or no growth factor (318/607 (52.38%) versus 218/436 (50%); RR 1.05, 95% CI 0.93 to 1.19; $I^2$ = 60%; P value 0.43).

A test for subgroup differences showed a significant difference ($I^2$ = 98.2%; P value 0.0001). See Analysis 4.1.

### Individual growth factor versus active control

There is inconclusive evidence of a difference between autologous growth factor and oxidized regenerate cellulose/collagen biomaterial regarding complete wound healing (4/34 (11.76%) versus 2/17 (11.76%); RR 1.00, 95% CI 0.20 to 14.93; P value 1.00) (Kakagia 2007). One trial reported inconclusive effects when recombinant human platelet-derived growth factor (becaplermin) was compared with OASIS Wound Matrix for achieving complete wound healing (10/36 (27.77%) versus 18/37 (48.64%); RR 0.57, 95% CI 0.31 to 1.06; P value 0.08) (Niezgoda 2005). There is not conclusive results when recombinant human epidermal growth factor was compared with silver sulphadiazine for reaching complete wound healing (7/30 (23.33%) versus 2/20 (10%); RR 2.33, 95% CI 0.54 to 10.11; P value 0.26) (Afshari 2005). There was a higher proportion of complete wound healing in participants allocated to recombinant human epidermal growth factor than those receiving actovegin (32/42 (76.19%) versus 8/19 (42.10%); RR 1.81, 95% CI 1.04 to 3.15; P value 0.04) (Tsang 2003).

### Lower limb amputation (minimum of one toe)

No trials described the extent of the amputation (Fernández-Montequin 2007; Fernández-Montequin 2009; Holloway 1993; Tsang 2003).

**Any growth factor versus placebo or no growth factor**

Meta-analysis of two trials showed no clear difference in number of lower limb amputations for growth factors, considered as a group, compared with placebo or no growth factor (19/150 (12.66%) versus 12/69 (17.39%); RR fixed-effects model 0.74, 95% CI 0.39 to 1.39; $I^2$ = 0%; P value 0.34, *low quality evidence due to limitation in design, execution or both, and imprecision*) (Fernández-Montequin 2009; Holloway 1993). See Analysis 1.2.

**Individual growth factor versus active control**

One trial comparing recombinant human epidermal growth factor versus actovegin showed no clear evidence of a difference regarding the incidence of lower limb amputation (2/42 (4.76%) versus 2/19 (10.52%); RR 0.45, 95% CI 0.07 to 2.98; P value 0.41) (Tsang 2003). Meta-analysis of two trials comparing two doses of recombinant human epidermal growth factor, 75 $\mu$g and 25 $\mu$g, showed no clear difference regarding lower limb amputation (15/76 (19.73%) versus 16/66 (24.24%); RR 0.82, 95% CI 0.44 to 1.52; $I^2$ = 0%; P value 0.52) (Fernández-Montequin 2007; Fernández-Montequin 2009).

### Time to complete healing of the diabetic foot ulcer

Fifteen trials assessed time to complete healing of the diabetic foot ulcer. However, no trial reported hazard ratios or information that would allow us to calculate it. Most trials did not state explicitly that all participants achieved complete healing (Bhansali 2009; Chen 2004; d'Hemecourt 1998; Driver 2006; Fernández-Montequin 2007; Fernández-Montequin 2009; Hanft 2008; Hardikar 2005; Holloway 1993; Niezgoda 2005; Robson 2002; Steed 1995a; Steed 1995b; Viswanathan 2006; Wieman 1998a), see Appendix 10 for details.

### Secondary outcomes

#### Ulcer-free days following treatment for diabetic foot ulcers (free from any recurrence)

One trial comparing recombinant human vascular endothelial growth factor (29 participants) versus placebo (26 participants) showed inconclusive difference in terms of ulcers-free days following treatment (HR 0.64, 95% CI 0.14 to 2.94 P value 0.56) (Hanft 2008).

#### Quality of life

None of the included trials addressed quality of life.

#### Adverse events

**Any growth factor versus placebo or no growth factor**

Meta-analysis of four trials reporting number of participants with events showed no clear evidence of a difference between all growth factors when considered as a group compared with placebo or no growth factor in terms of adverse events (non-serious and serious) (109/232 (46.98%) versus 63/153 (41.17%); RR 0.98, 95% CI 0.79 to 1.22; $I^2$ = 0%; P value 0.85, *low quality evidence*) (Fernández-Montequin 2009; Hanft 2008; Hardikar 2005; Holloway 1993). See Analysis 1.4.

**Individual growth factor versus placebo or no growth factor**

One trial comparing arginine-glycine-aspartic acid peptide matrix with placebo reported adverse events as follows: "0.65 events per patient (N = 26) in arginine-glycine-aspartic acid peptide matrix compared with 1.16 (N = 29) in the placebo group" (Steed 1995b). One trial showed no clear difference in overall adverse events when recombinant human platelet-derived growth factor (becaplermin) was compared with placebo (31/61 (50.81%) versus 34/57 (59.64%); RR 0.85, 95% CI 0.61 to 1.18; P value 0.34) (Steed 1995a).

One trial comparing recombinant human platelet-derived growth factor versus placebo reported an incidence of serious adverse events similar across comparison groups (25%, 30% and 24% either recombinant human platelet-derived growth factor 30 $\mu$g/g or 100 $\mu$g/g, and placebo, respectively (Wieman 1998a).

Meta-analysis of two trials comparing recombinant human platelet-derived growth factor (becaplermin) with placebo showed no clear difference between treatment groups in terms of: infection (35/95 (36.84%) versus 28/127 (22.04%); RR 1.57, 95% CI 0.37 to 6.71, $I^2$ = 88%; P value 0.54); cellulitis (11/165 (6.66%) versus 17/127 (13.38%); RR 0.49, 95% CI 0.24 to 1.02, $I^2$ = 0%; P value 0.06); peripheral oedema (9/165 (5.45%) versus 16/127 (12.59%); RR 0.44, 95% CI 0.20 to 0.96, $I^2$ = 0%; P value 0.04); pain (17/165 (10.30%) versus 16/125 (12.8%); RR 0.78, 95% CI 0.41 to 1.49, $I^2$ = 0%; P value 0.45); or skin ulceration (14/165 (8.48%) versus 10/127 (7.87%); RR 1.08, 95% CI 0.49 to 2.37, $I^2$ = 0%; P value 0.85) (d'Hemecourt 1998; Steed 1995a). See Analysis 6.2 to Analysis 6.6.

Meta-analysis of two trials comparing recombinant human basic fibroblast growth factor with placebo did not find a difference in terms of infection (3/106 (2.83%) versus 3/59 (5.08%); RR 0.77, 95% CI 0.18 to 3.29; $I^2$ = 0%; P value 0.72) (Richard 1995; Uchi 2009). Analysis 7.2.

One trial showed no clear evidence of a difference between recombinant human basic fibroblast growth factor versus placebo in terms of adverse events (4/97 (4.12%) versus 3/51 (5.88%); RR 0.26, 95% CI 0.02 to 2.83; P value 0.27) (Uchi 2009). One trial showed no clear difference between recombinant human epidermal growth factor group and placebo in terms of any adverse event (65/101 (64.35%) versus 31/48 (64.58%); RR 1.00, 95% CI 0.77 to 1.29; P value 0.98), or any severe adverse event (8/101 (7.92%) versus 2/48 (4.16%); RR 1.90, 95% CI 0.42 to 8.61; P value 0.40) (Fernández-Montequin 2009). One trial comparing recombinant human vascular endothelial growth factor with placebo showed inconclusive results in the incidence of adverse events during the six-week treatment period (14/29 (48.27%) versus 13/26 (50%); RR 0.97, 95% CI 0.56 to 1.65; P value 0.90) or the 12-week observation period (5/26 (19.23%) versus (6/23 (26.08%); RR 0.74, 95% CI 0.26 to 2.10; P value 0.57). This trial also did not show a conclusive difference in terms of serious adverse events during the six-week treatment period (2/29 (6.89%) versus 3/26 (11.53%); RR 0.60, 95% CI 0.11 to 3.30; P value 0.56) or 12-week observation period (3/26 (11.53%) versus 3/26 (11.53%); RR 1.00, 95% CI 0.22 to 4.50; P value 1.00) (Hanft 2008).

**Individual growth factor versus active control**

One trial comparing recombinant human platelet-derived growth factor (becaplermin) with OASIS Wound Matrix did not find clear evidence of a difference in terms of treatment related events (10/36 (27.77%) versus 17/37 (45.94%); RR 0.60, 95% CI 0.32 to 1.14; P value 0.12) (Niezgoda 2005). One trial comparing different doses of recombinant human epidermal growth factor, 75 $\mu$g versus 25 $\mu$g, showed no difference in terms of burning sensation (5/23 (21.73%) versus 2/18 (11.11%); RR 1.96, 95% CI 0.43 to 8.94 P value 0.39; 41 participants), or local pain (4/23 (17.39%) versus 3/18 (16.66%); RR 1.04, 95% CI 0.27 to 4.08; P value 0.95; 41 participants) (Fernández-Montequin 2007). One trial showed evidence of a difference in reduction of local wound pain in participants who received recombinant human acidic fibroblast growth factor compared with those who received recombinant human basic fibroblast growth factor (2/104 (1.92%) versus 6/35 (17.14%); RR 0.11, 95% CI 0.02 to 0.53; 9 P value 0.006) (Tan 2008).

## DISCUSSION

## Summary of main results

This Cochrane systematic review of growth factors for treating foot ulcers in people with diabetes included 28 randomised clinical trials that incorporated 2365 participants. Trials evaluated 11 different experimental growth factors compared with several different control interventions. Overall, the trials had a high risk of bias and were underpowered. Most of the trials, 82% (23/28), did not report an *a priori* sample size estimation. Drug companies sponsored at least 14 of the trials. The trials were conducted in 10 countries (Canada, China, Cuba, France, Greece, India, Iran, Italy, Japan, and the USA). In general the trials were conducted in the out-patient (ambulatory) setting. The reporting of trial participants characteristics was ill-defined with regard to their type of diabetes mellitus and etiology of their diabetic foot ulcer.

Meta-analysis was possible only on six types of experimental growth factors: platelet-derived growth factor, autologous growth factor; platelet-derived wound healing formula, recombinant human platelet-derived growth factor, recombinant human basic fibroblast growth factor, and human epidermal growth factor.

We were able to meta-analyse data on trial participants with complete wound healing. Meta-analysis of 12 trials showed that all growth factors, when considered as a group, seemed to increase the proportion of participants with complete wound healing significantly compared with placebo or no growth factor. The quality of the estimate was qualified as low due to limitations in design and execution of included trials, and inconsistency (Summary of findings for the main comparison).

We were able to meta-analyse data on lower limb amputation (minimum of one toe). One meta-analysis of two trials showed no clear evidence that growth factors, when as considered as a group, reduced the risk of lower limb amputation compared with placebo or no growth factor. Evidence was downgraded to very low due to pitfalls in design and execution of included trials, and a very small sample size and very low number of events (Summary of findings for the main comparison). Another meta-analysis of two trials that compared two doses of recombinant human epidermal growth factor, 75 $\mu$g and 25 $\mu$g, did not show a significant difference between the two doses.

Eleven trials reported time to complete healing of the diabetic foot ulcer, however, meta-analysis was not possible due to the unique comparisons within each trial, failure to report data, with or without a high rate of withdrawals. One trial comparing recombinant human vascular endothelial growth factor versus placebo showed inconclusive result on ulcer-free days following treatment. Trials did not report data on quality of life. Growth factors compared against placebo or no growth factor showed no difference in terms of any adverse event. However, overall, safety data were poorly reported and adverse events may have been underestimated. Evidence was considered as low due to limitations in design and execution, and low number of event (Summary of findings for the main comparison).

Trials with a 20-week or longer follow-up seemed to be more effective in increasing the number of participants with complete wound closure than trials with a follow-up of less than 20 weeks. However, there was no an conclusive difference between these groups.

We conducted a subgroup analysis of trials by type of growth factor. There was an inconclusive difference between growth factor versus placebo or no growth factor in terms of the number of participants with complete wound closure. This is could be due to small sample size and low number of events.

In terms of complete wound closure we found a clear difference between the 'best-worse case' scenario and the 'worst-best case' scenario in sensitivity analyses that took attrition into consideration. It should be interpreted as inconsistency due to missing data.

## Overall completeness and applicability of evidence

This Cochrane review found evidence suggesting that growth factors might be useful for increasing complete wound closure of foot ulcers in people with diabetes , though this conclusion is based on randomised clinical trials with a high risk of bias due to pitfalls in design and execution of the included trials. Therefore, and based on GRADE findings, future research are a need to know with a better certainty the clinical benefits of growth factors for treating diabetic foot ulcers. Furthermore, the safety profile of all the growth factors is unclear.

The results in this review are based on data from trials that included a broad range of participants with different co-morbidities, who received different treatment approaches. That heterogeneity downgraded the quality of evidence. We cannot rule out that the calculations of the potential effects have been overestimated due to poor methodological quality (bias risks, design, analysis and the small information size. Therefore, these three variables, i.e., high heterogeneity, pitfalls in methodology, and small sample size and low number of events, even after meta-analysis, depleted the quality of evidence. Futhermore, we cannot exclude an underestimation of harms. A caveat concerning the safety of recombinant human platelet-derived growth factor (becaplermin) has been outlined recently; this relates to the risk of cancer in people who use three tubes or more compared to that in non-users (FDA 2008; Papanas 2010). However, an observational study reported that this growth factor does not appear to increase the risk of cancer or cancer mortality (Ziyadeh 2011), though further high-quality data are clearly needed.

## Quality of the evidence

GRADE assessments were conducted on outcomes of both meta-analysis and non-pooled trials. None of the trials was graded as providing strong evidence, primarily because of small sample sizes (even after meta-analysis) which generate wide confidence intervals

with low precision of estimate of the intervention effects, and the high risk of bias due to a lack of adequate randomisation methods, lack of blinding, high attrition, and unclear reporting of outcomes. Quality of evidence also had to be downgraded due to inconsistency. We can't reject a potential detection bias -wound healing is a fairly subjective outcome- where is there is no blinded outcome assessment or unclear for this, even though due to clear definition of complete wound closure.

See Summary of findings for the main comparison for complete assessment and rationale for ratings.

This review assessed the impact of missing data on the effect of intervention in increasing the proportion of participants with complete wound closure (Analysis 4.1) using best/worst and /best case scenarios. If the amount of missing data is large, the conclusion on the difference between the comparison groups is not valid (Hollis 1999). This Cochrane review found a significant subgroup difference comparing all trials, best/worst and /best case scenarios (Analysis 4.1).

This Cochrane review has identified the following issues that should be considered when planning future trials: inconsistent information concerning the healing percentage of wound closure definition provided by trial reports, differences in definitions of outcomes, and inconsistency of reported outcomes need to be avoided. Trials should adopt an agreed set of core outcomes for each medical condition (Clarke 2007). This approach may reduce the impact of outcome reporting bias (Kirkham 2010).

The impact of outcome reporting bias may be reduced by adopting the recommendations of *The Patient-Centered Outcomes Research Institute* (PCORI) (PCORI 2012). This organisation was established by United States Congress as an independent, non-profit organisation, created to conduct research to provide information about the best available evidence to help patients and their healthcare providers make more informed decisions. PCORI's research is intended to give patients a better understanding of the prevention, treatment and care options available, and the science that supports those options (Gabriel 2012; Basch 2012; PCORI 2012; Selby 2012).

### Potential biases in the review process

There is a group of biases called significance-chasing biases (Ioannidis 2010), which includes publication bias, selective outcome reporting bias, selective analysis reporting bias, and fabrication bias. Publication bias represents a major threat to the validity of systematic reviews, particularly in reviews that include small trials. However, this Cochrane review has a low risk of publication bias due to the meticulous trial search that was performed, and the fact that we emailed the main authors of a number of the trials identified. Selective outcome reporting bias operates through suppression of information about specific outcomes and has similarities to publication bias of whole studies or trials, in that 'negative' results remain unpublished (Ioannidis 2010). We were surprised to find how few times amputations or mortality were reported in the trials. This Cochrane review found that 75% of the included randomised clinical trials had high risks of selective outcome reporting. For example, adverse events were not reported (Afshari 2005; Bhansali 2009; Chen 2004, Jaiswal 2010; Kakagia 2007; Landsman 2010; Saldalamacchia 2004; Steed 1992; Steed 1996; Tan 2008; Tsang 2003), or were poorly reported in a total of 16 trials (Hardikar 2005; Holloway 1993; Lyons 2007; Viswanathan 2006; Wieman 1998a). These 16 trials incorporated 55% of the randomised participants (1289/2365) included in this review. This review found no evidence of asymmetry of the funnel plot for complete wound closure (Figure 4).

### Agreements and disagreements with other studies or reviews

The results of our review are similar to the findings from other systematic reviews (Buchberger 2010; O'Meara 2000) and a narrative review of complete closure of diabetic foot ulcer using growth factors (Wieman 1998b).

## AUTHORS' CONCLUSIONS

### Implications for practice

There is insufficient evidence from RCTs to recommend or refute the use of growth factors in treating diabetic foot ulcers. The results are based on the results of 28 RCTs with a high risk of bias. There is a paucity of information for other main clinical outcomes such as lower limb amputation, time to complete healing of the diabetic foot ulcer and ulcer-free days following treatment for diabetic foot ulcers (free from any recurrence). There is an absence of information on mortality, and quality of life. In addition, adverse events data remain unclear. Therefore, prescription of growth factors for treating people with diabetic foot ulcers can not be supported or rejected until new evidence from a large, high-quality trial becomes available and alters this conclusion.

### Implications for research

This systematic review has identified the need for well-designed, adequately powered RCTs to assess the benefits and harms of growth factors with complete wound closure, lower limb amputation, and adverse events as the primary outcomes. Since epidemiological studies have connected to the recombinant human platelet-derived growth factor (becaplermin) to a five-fold increase in cancer mortality in people who used more than three tubes of it compared to non-users, potential risk needs to be ruled out in large randomised clinical trials before wider use can be recommended. The trials should be designed according to the SPIRIT statement

(Chan 2013), and reported according to the CONSORT statement for improving the quality of reporting of efficacy; the trials should also provide better reports of harms/adverse events encountered during their conduct (Ioannidis 2004; Moher 2010; Schulz 2010). Future trials should be planned following the Foundation of Patient-Centered Outcomes Research recommendations (Basch 2012; Gabriel 2012; McKinney 2012). Potential trials should also include clinical outcomes such as, incidence of lower limb amputation (minimum of one toe, with the extent of amputation being specified, and data the incidence of different extents of amputation also reported separately), time to complete healing of the diabetic foot ulcer, quality of life, ulcer-free days following treatment for diabetic foot ulcers (free from any recurrence), and adverse events.

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* *Indicates the major publication for the study*

## CHARACTERISTICS OF STUDIES

### Characteristics of included studies *[ordered by study ID]*

**Afshari 2005**

| | |
|---|---|
| Methods | 1. Parallel-design (2 arms)<br>2. Study period: 4 weeks<br>3. Country: Iran<br>4. Unit of randomisation: participant<br>5. Unit of analysis: participant |
| Participants | 1. Randomised: 50 (50 ulcers)<br>i) Intervention group: 60% (30/50) (30 ulcers)<br>ii) Control group: 40% (20/50) (20 ulcers)<br>2. Age (range)<br>i) Intervention group (27-77 years)<br>ii) Control group (32-75 years)<br>3. Gender (male)<br>i) Intervention group: 72.7%<br>ii) Control group: 53.3%<br>4. Inclusion criteria<br>i) Grade 1 or 2 ulcer (grade 1: superficial ulcer; grade 2 deep ulcer to tendon, capsule, or bone)<br>ii) Ulcer with adequate perfusion, as indicated by an ABPI and ultrasound<br>5. Exclusion criteria: not reported |
| Interventions | 1. Intervention group: epidermal growth factor formulation (Hebermin®: Herber Biotec®) containing 1 mg recombinant human epidermal growth factor/1000 mg silver sulphadiazine in a hydrophilic base<br>2. Control: placebo consisting of 1% silver sulphadiazine in the same hydrophilic base<br>Co-interventions: both groups received wound debridement and irrigation with normal saline solution, systemic antibiotic therapy and daily wound dressing |
| Outcomes | Primary: complete wound closure |
| Notes | 1. A priori sample size estimation: not reported<br>2. Sponsor: not reported |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Quote: "patients were entered to the study randomly until a total of 50 patients" (p 760)<br>Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |

**Afshari 2005** (*Continued*)

| | | |
|---|---|---|
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Unclear risk | Quote: " . . . The placebo formulation contained . . . in the same hydrophilic base . . . " (p 760) |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Incomplete outcome data (attrition bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit judgment of 'low risk' or 'high risk'<br>Withdrawals were not reported |
| Selective reporting (reporting bias) | High risk | This trial did not report safety data |
| Other bias | High risk | 1. There was imbalance between the groups with regard to: number of participants and at least three major variables: sex (% male), age and wound size<br>2. Bias of the presentation data, see Appendix 1 |

**Agrawal 2009**

| | |
|---|---|
| Methods | 1. Parallel-design (2 arms)<br>2. Study period: 12 weeks<br>3. Country: India<br>4. Unit of randomisation: participant<br>5. Unit of analysis: participant<br>6. Follow-up: 12 weeks |
| Participants | 1. Randomised: 28<br>i) Intervention group: 50% (14/28) (number of ulcers not reported)<br>ii) Control group: 50% (14/28) (number of ulcers not reported)<br>2. Age (years, mean ± standard deviation)<br>i) Intervention group (54.38 ± 8.7)<br>ii) Control group (56.24 ± 8.75)<br>3. Gender (male)<br>i) Intervention group: 64.3% (9/14)<br>ii) Control group: 71.4% (10/14)<br>4. Inclusion criteria<br>i) ≥ 30 years of age<br>ii) A glycaemic target of HbA1c ≤ 7.0%, but HbA1c > 7.0% was not an exclusion criterion |

| | |
|---|---|
| | iii) Ulcer stage I, II, III or IV according to the Wagner (1981) classification<br>iv) Foot ulcer duration of > 3 months, free of infection, with an adequate lower-limb blood supply demonstrated by a TcPO2 ≥ 30 mmHg<br>v) Free of, or a moderate degree of, peripheral vascular disease<br>5. Exclusion criteria<br>i) Active neoplastic disease<br>ii) Diagnosis of active infection<br>iii) Those who had received immunosuppressive therapy during the preceding 3 months<br>iv) Those with liver disease, pulmonary tuberculosis, thyroid disorder, uraemia, alcoholism, or renal insufficiency, and those undergoing vascular reconstruction or receiving steroid or anticoagulant therapy |
| Interventions | 1. Intervention group: recombinant human platelet-derived growth factor (becaplermin) (0.01%) gel/day.<br>2. Control: placebo gel, no further details provided<br>Co-interventions: both groups received debridement, dressing, pressure relief and glycaemic control |
| Outcomes | Healing: participants categorised as complete responders, partial responders, non-complete responders (p 84) |
| Notes | 1. A priori sample size estimation: not provided<br>2. Sponsor: not reported |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Quote: "participants were randomised to receive either . . . " (p 81)<br>Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Incomplete outcome data (attrition bias)<br>All outcomes | Unclear risk | Imbalance between groups for participants lost after randomisation (18% (5/28) in total)<br>Control group: 36% (5/14) during the final |

**Agrawal 2009** (*Continued*)

| | | |
|---|---|---|
| | | week (week 12), reasons not reported<br>Experimental group: none |
| Selective reporting (reporting bias) | Low risk | The study protocol is not available but it is clear that the published report included all expected outcomes, including those that were pre-specified |
| Other bias | High risk | 1. Quote " . . . matched for all variables except sex and ulcer area . . . " (p 83)<br>2. Design bias, see Appendix 1 |

**Bhansali 2009**

| | |
|---|---|
| Methods | 1. Parallel-design (2 arms)<br>2. Country: India<br>3. Follow-up period: 20 weeks<br>4. Unit of randomisation: participant<br>5. Analysis unit: participant |
| Participants | 1. Randomised: 20 (24 ulcers)<br>i) Intervention group: 50% (10/20) (13 ulcers)<br>ii) Control group: 50% (10/20) (11 ulcers)<br>2. Age (years, mean ± SD)<br>i) Intervention group: 51.7 ± 13.6<br>ii) Control group: 49.5 ± 8.83<br>3. Gender (male)<br>i) Intervention group: 70% (7/10)<br>ii) Control group: 50% (5/10)<br>4. Inclusion criteria<br>i) > 20 years of age with type 1 or 2 diabetes<br>ii) At least 1 neuropathic plantar ulcer of Wagner grade ≥ 2 without X-ray evidence of osteomyelitis<br>iii) ABPI > 0.9<br>5. Exclusion criteria: not reported |
| Interventions | 1. Intervention group: recombinant human platelet-derived growth factor (becaplermin) 0.01% gel (once daily)<br>2. Control: placebo consisting of moist saline (once daily)<br>Co-interventions: debridement at baseline, offloading |
| Outcomes | Incidence of complete wound closure, duration of complete healing, rate of healing and safety |
| Notes | 1. A priori sample size estimation: not reported<br>2. Sponsor: not reported |
| ***Risk of bias*** | |

**Bhansali 2009** (*Continued*)

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Quote "Patients were randomized . . " (p e14)<br>Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | High risk | Open label (p e14) |
| Blinding of outcome assessment (detection bias)<br>All outcomes | High risk | Open label (p e14) |
| Incomplete outcome data (attrition bias)<br>All outcomes | Unclear risk | Insufficient reporting of attrition/exclusions to permit a judgement of 'low risk' or 'high risk' (e.g. number randomized not stated, no reasons for missing data provided) |
| Selective reporting (reporting bias) | High risk | Safety data were not reported |
| Other bias | High risk | Bias in the presentation of data and design bias, see Appendix 1 |

**Chen 2004**

| | |
|---|---|
| Methods | 1. Parallel-design (2 arms)<br>2. Country: China (Zhenjiang Centre People's Hospital, Guangdong Province)<br>3. Phase: unclear<br>4. Follow-up period: not reported<br>5. Unit of randomisation: participant<br>6. Analysis unit: participant |
| Participants | 1. Randomised: 59<br>i) Intervention group: 30 (59.84%)<br>ii) Control group: 29 (49.15%)<br>2. Age (years)<br>i) Intervention group: 70 (mean, SD not reported)<br>ii) Control group: 68 (mean, SD not reported)<br>iii) Overall: 44-92<br>3. Gender (male)<br>i) Intervention group: 18%<br>ii) Control group: 17% |

| | |
|---|---|
| | 4. Inclusion criteria: not reported<br>5. Exclusion criteria: not reported |
| Interventions | 1. Intervention group: recombinant human epidermal growth factor spray<br>2. Control: regular insulin 8 U<br>Co-interventions: 3% hydrogen peroxide, normal saline, ethacridine lactate (rivanol), gentamicin, diabetic diet, blood sugar control |
| Outcomes | Unclear<br>This trial reported data for time-to complete healing of diabetic foot ulcers |
| Notes | 1. A priori sample size estimation: not reported<br>2. Sponsor: not reported<br>3. Dates during which trial conducted: not reported<br>4. Financial disclosures: not reported<br>5. Funding/support: not reported<br>6. Disclosure statement: not reported |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Random number table |
| Allocation concealment (selection bias) | Unclear risk | No information about allocation concealment<br>Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | High risk | Difference between intervention and control treatments easy to identify |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Incomplete outcome data (attrition bias)<br>All outcomes | Unclear risk | No participants lost to follow-up |
| Selective reporting (reporting bias) | High risk | Only one outcome reported; it was not prespecified<br>Author reported 'time-to complete healing of the diabetic foot ulcer' as a continuous measure |

**Chen 2004** (*Continued*)

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|---|---|---|
| Other bias | High risk | Failure to report inclusion/exclusion criteria<br>Bias of presentation of the data, see Appendix 1<br>Design bias, see Appendix 1 |

**d'Hemecourt 1998**

| | |
|---|---|
| Methods | 1. Parallel-design (2 arms)<br>2. Country: USA<br>3. Study period: 20 weeks<br>4. Unit of randomisation: participant<br>5. Analysis unit: participant |
| Participants | 1. Randomised: 172<br>i) Intervention group 1 (becaplermin): 20% (34/172)<br>ii) Intervention group 2 (sodium carboxymethylcellulose (NaCMC)): 41% (70/172)<br>iii) Control group: 39% (68/172)<br>2. Age (years; mean ± standard deviation): overall: 58.3 ± 12.13<br>i) Intervention group 1 (becaplermin): 58.5 ± 11.90<br>ii) Intervention group 2 (NaCMC): 56.9 ±13.02<br>iii) Control group: 59.6 ±11.2<br>3. Gender (male)<br>i) Intervention group 1 (becaplermin): 70.6% (24/34)<br>ii) Intervention group 2 (NaCMC): 70.0% (49/70)<br>iii) Control group: 79.4% (54/68)<br>4. Inclusion criteria<br>i) Presence of chronic diabetic ulcer on lower extremity for at least 8 weeks prior to the study<br>5. Exclusion criteria<br>i) Osteomyelitis affecting the area of the target ulcer<br>ii) Target ulcer area after debridement (measured by multiplying length by width) < 1 $cm^2$ or > 10 $cm^2$<br>iii) > 3 chronic ulcers present at baseline<br>iv) Ulcers resulting from any cause other than diabetes<br>v) Use of concomitant medications known to affect wound healing (corticosteroids, chemotherapy, or immunosuppressive agents)<br>vi) Women who were pregnant, nursing, or of childbearing potential who were not using an acceptable method of birth control |
| Interventions | 1. Intervention groups<br>i) Intervention group 1: recombinant human platelet-derived growth factor (becaplermin) gel 100 $\mu$g/g (Regranex® Gel 0.01%)<br>ii) Intervention group 2: sodium carboxymethylcellulose (NaCMC) aqueous-based gel<br>2. Control group: good wound care<br>Co-interventions: all groups received good wound care consisting of initial sharp de- |

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| | bridement of ulcers to remove nonviable tissue, daily moist saline dressing changes, offloading of pressure, and systemic control of infection |
| Outcomes | 1. Primary: percentage of participants achieving complete wound closure without drainage or need for dressing<br>2. Secondary:<br>i) time to achieve complete wound closure<br>ii) relative ulcer area from baseline to endpoint<br>iii) wound evaluation score (6 parameters: erythema, oedema, purulence, necrotic tissue, fibrin, and drainage) |
| Notes | 1. A priori sample size estimation: not reported<br>2. Sponsor: not reported |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Quote "patients were randomly assigned in a 2:2:1 ratio to one of three treatment groups" (p 71)<br>Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Unclear risk | Quote "were conducted in double-blind fashion" (p 71)<br>Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Incomplete outcome data (attrition bias)<br>All outcomes | High risk | Overall lost: 24% (41/172 participants)<br>1. Intervention group 1 (becaplermin): 26% (9/34)<br>2. Intervention group 2 (NaCMC): 16% (11/70)<br>3. Control group: 31% (21/68)<br>Reasons: main reasons for withdrawals were<br>1. Adverse event**:**<br>i) Intervention group 1 (becaplemerin): 15%<br>ii) Intervention group 2 (NaCMC): 11% |

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|---|---|---|
| | | iii) Control group: 24%<br>2. Lost to follow-up:<br>i) Intervention group 1 (becaplemerin): 6%<br>ii) Intervention group 2 (NaCMC): 3%<br>iii) Control group: 1%<br>3. Participants' choice:<br>i) Intervention group 1 (becaplemerin): 3%<br>ii) Intervention group 2 (NaCMC): 0%<br>iii) Control group: 4%<br>4. Other:<br>i) Intervention group 1 (becaplemerin): 3%<br>ii) Intervention group 2 (NaCMC): 1%<br>iii) Control group: 1% |
| Selective reporting (reporting bias) | High risk | One or more outcomes of interest in the review were reported incompletely so that they could not be entered in a meta-analysis This trial did not report time to complete wound closure |
| Other bias | High risk | Bias in the presentation of data and design bias, see Appendix 1 |

**Driver 2006**

| | |
|---|---|
| Methods | 1. Parallel-design (2 arms)<br>2. Country: USA (14 sites)<br>3. Follow-up: 12 weeks<br>4. Unit of randomisation: participant<br>5. Analysis unit: participant |
| Participants | 1. Randomised: 72<br>i) Intervention group: 56% (40/72)<br>ii) Control group: 44% (32/72)<br>2. Age (years ±(SD))<br>i) Intervention group: 56.4 ± 10.2<br>ii) Control group: 57.5 ± 9.1<br>3. Gender (male %)<br>i) Intervention group: 80% (32/40)<br>ii) Control group: 84% (27/32)<br>4. Inclusion criteria<br>i) People with type 1 or type 2 diabetes aged 18-95 years, with an ulcer of at least 4 weeks' duration |

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| | ii) HbA1c < 12<br>iii) Index foot ulcer located on the plantar, medial, or lateral aspect of the foot (including all toe surfaces)<br>iv) Wound area (length x width) measurement between 0.5 $cm^2$ and 20 $cm^2$<br>v) Wounds located under a Charcot deformity had to be free of acute changes and must have undergone appropriate structural consolidation<br>vi) Index ulcer had to be clinically noninfected (cultures taken, but infection diagnosed through clinical signs and symptoms rather than culture results) and full-thickness without exposure of bone, muscle, ligaments, or tendons (University of Texas Treatment-Based Diabetic Foot Classification System: Grade 1A)<br>vii) Ulcer free of necrotic debris, foreign bodies, sinus tracts, tunnelling, and undermining after debridement<br>viii) Comprised of healthy vascularized tissue, and at least 4 cm from any additional wound<br>ix) Adequate limb perfusion assessed by examination and non-invasive vascular testing, ABPI and toe brachial index<br>x) Pregnant or lactating women; both men and women had to be willing to use a medically-accepted form of birth control throughout the trial and for 6 months following<br>5. Exclusion criteria<br>i) People currently enrolled in another investigational device or drug trial or previously enrolled (within last 30 days) in investigative research of a device or pharmaceutical agent<br>ii) ≥ 50% decrease in ulcer area during the 7-day screening period<br>iii) Ulcer due to non-diabetic aetiology<br>iv) Blood vessels non-compressible for ABPI testing<br>v) Evidence of gangrene in ulcer or on any part of the foot<br>vi) Radiographic evidence consistent with diagnosis of acute Charcot foot<br>vii) Currently receiving or had received radiation or chemotherapy within 3 months of randomisation<br>viii) Use of topical, oral or IV antibiotic/antimicrobial agents or medications within 2 days (48 hours) of randomisation<br>ix) Growth factor therapy administered within 7 days of randomisation (e.g. autologous platelet-rich plasma gel, becaplermin, bilayered cell therapy, dermal substitute, extracellular matrix)<br>x) Screening serum albumin level < 2.5 g/dL<br>xi) Screening haemoglobin < 10.5 mg/dL<br>xii) Screening platelet count < 100 $x10^9$/L<br>xiii) Undergoing renal dialysis, known immune insufficiency, known abnormal platelet activation disorders i.e. gray platelet syndrome, liver disease, active cancer (except remote basal cell of the skin), eating/nutritional, haematologic, collagen vascular disease, rheumatic disease, or bleeding disorders<br>xiv) History of peripheral vascular repair within 30 days of randomisation<br>xv) Known or suspected osteomyelitis<br>xvi) Surgical correction (other than debridement) required for ulcer to heal<br>xvii) Exposed tendons, ligaments, muscle, or bone in Index ulcer<br>xviii) Known psychological, developmental, physical, emotional, or social disorder, or any other situation that might interfere with compliance with study requirements or |

**Driver 2006** (*Continued*)

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| | healing of the ulcer, or both<br>xix) History of alcohol or drug abuse within the year prior to randomisation<br>xx) Inadequate venous access for blood draw<br>xxi) Religious or cultural conflict with the use of platelet gel treatment |
| Interventions | 1. Intervention group: platelet-rich plasma gel (AutoloGel™, Cytomedix, Inc, Rockville, Md), frequency of administration not reported<br>2. Control group: saline gel (Normlgel®, Mölnlycke Health Care, Norcross, Ga), frequency of administration not reported<br>Co-interventions: cleaning and interim wound assessment of vital signs, offloading orthosis walker |
| Outcomes | Primary: proportion of participants with a healed wound |
| Notes | 1. A priori sample size estimation: yes (p 72)<br>2. Sponsor: not reported |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote "was electronically generated, blocked per investigational center" (p 70) |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Unclear risk | Quote " . . . to maintain blinding of the investigators, investigative sites staff, patients, sponsor, and CRO staff, and monitor" (p 70)<br>This trial did not report the approach used to guarantee adequate blinding |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Quote ". . . to maintain blinding of the investigators, investigative sites staff, patients, sponsor, and CRO staff, and monitor" (p 70)<br>This trial did not report the approach used to guarantee adequate blinding |
| Incomplete outcome data (attrition bias)<br>All outcomes | High risk | Overall lost: 44% (32/72 participants)<br>Reasons: failure to complete treatment (25%; 8/32) and protocol violations (75%; 24/32) |
| Selective reporting (reporting bias) | Low risk | The study protocol was not available, but it is clear that the published reports included all expected outcomes, including those that |

**Driver 2006** (*Continued*)

| | | |
|---|---|---|
| | | were pre-specified |
| Other bias | High risk | Bias in the presentation of data and design bias, see Appendix 1 |

**Fernández-Montequin 2007**

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|---|---|
| Methods | 1. Parallel-design (2 arms)<br>2. Country: Cuba (5 sites)<br>3. Follow-up: 5-8 weeks<br>4. Unit of randomisation: participant<br>5. Analysis unit: participant |
| Participants | 1. Randomised: 41<br>i) Intervention group 1 (rhEGF 75 µg): 56% (23/41)<br>ii) Intervention group 2 (rhEGF 25 µg): 44% (18/41)<br>2. Age (years, mean ± SD)<br>i) Intervention group 1 (rhEGF 75 µg): 63 ± 12<br>ii) Intervention group 2 (rhEGF 25 µg): 67.5 ± 19.5<br>3. Gender (male)<br>i) Intervention group 1 (rhEGF 75 µg): 52.2%<br>ii) Intervention group 2 (rhEGF 25 µg): 56%<br>4. Inclusion criteria<br>i) Diabetic participants (type 1 or 2) of both sexes<br>ii) > 18 years<br>iii) Grade 3 or 4 foot ulcer according to Wagner's classification, with high risk of amputation<br>5. Exclusion criteria<br>i) Foot ulcer area $\leq$ 1 $cm^2$<br>ii) Hb < 100 g/l<br>iii) Uncontrolled chronic diseases (coronary or heart disease, diabetic coma or ketoacidosis, renal failure defined as a creatinine > 200 µmol/L and oligoanuria)<br>iv) Malignancies, psychiatric or neurological diseases that could impair proper reasoning for consent<br>v) Pregnancy and nursing |
| Interventions | 1. Intervention group 1: intralesional injections of rhEGF 75 µg (Citoprot-P®; Herber Biotec, Havana, Cuba), in hospital, 3 times/week (alternate days)<br>2. Intervention group 2: intralesional injections rhEGF 25 µg (Citoprot-P®; Herber Biotec, Havana, Cuba), in hospital, 3 times/week (alternate days)<br>Co-interventions: standardised wound care regimen, ulcers were sharply debrided, gangrenous and necrotic tissue removed whenever necessary, and broad-spectrum antibiotics and metabolic control as required |
| Outcomes | Primary: response at 5 to 8 weeks (percentage of ulcer area covered by granulation tissue)<br>1. Complete (75-100%)<br>2. Partial (50-75%)<br>3. Minimal (25-50%) |

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|---|---|
| | 4. No response (<25%)<br>Secondary efficacy endpoints<br>1. Time to obtain complete response<br>2. Complete healing (no exudates or need of dressing)<br>3. Prevention of limb amputation<br>4. Recurrence during 1-year-follow-up |
| Notes | 1. Sample size a priori calculation: yes (pp 335-6)<br>2. Sponsor: Curative technologies Inc, Heber Biotec SA, and The Ministry of Public Health of Cuba<br>3. Role of sponsor: financed the study, "the authors received free drug (rhEGF) . . . " (p 341)<br>4. Conflict of interest: some authors were employees of the Centre for Biological Research, which is part of the Centre for Genetic Engineering and Biotechnology, Havana network, where rhEGF is produced and the new formulation was developed<br>5. E-mail was sent to main author |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote "computer-generated random list" (p 335) |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | Quote "both vials were indistinguishable . . . " (p 335) |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | Quote "both vials were indistinguishable . . . " (p 335) |
| Incomplete outcome data (attrition bias)<br>All outcomes | High risk | Loss of participants: 36.5% (15/41)<br>1. Adverse event: Intervention group 1 = 66% (4/6); Intervention group 2 = 33.3% (3/9)<br>2. Mortality: Intervention group 1 = 4% (1/23)<br>3. Voluntary withdrawal: Intervention group 2 = 22.2% (2/9)<br>4. Lesion progression: Intervention group 1 = 33.3% (2/6);Intervention group 2 = 44.4% (4/9) |

**Fernández-Montequin 2007** (*Continued*)

| | | |
|---|---|---|
| Selective reporting (reporting bias) | High risk | One or more outcomes of interest in the review were reported incompletely so that they could not be entered into a meta-analysis |
| Other bias | High risk | Bias in the presentation of data and design bias, see Appendix 1 |

**Fernández-Montequin 2009**

| | |
|---|---|
| Methods | 1. Parallel-design (3 arms)<br>2. Country: Cuba (20 sites)<br>3. Follow-up: 8 weeks<br>4. Unit of randomisation: participant<br>5. Analysis unit: participant |
| Participants | 1. Randomised: 149<br>i) Intervention group 1: 53 (35.6%)<br>ii) Intervention group 2: 48 (32.2%)<br>iii) Control group: 48 (32.2%)<br>2. Loss prior to randomisation: 54%<br>i) Uncompensated chronic diseases (54)<br>ii) Hb < 10 g/dL (25)<br>iii) HbA1c >10% (18)<br>iv) Antecedents/suspected of malignancies (17)<br>v) Refused consent (13)<br>vi) Other exclusion criteria (48)<br>3. Age in years: median (25th-75th percentiles)<br>i) Intervention group 1: 63 (55-69)<br>ii) Intervention group 2: 65.5 (56-72)<br>iii) Control group: 64.0 (51-70)<br>4. Gender (male)<br>i) Intervention group 1: 28 (52.8%)<br>ii) Intervention group 2: 21 (43.8%)<br>iii) Control group: 27 (56.3%)<br>5. Inclusion criteria<br>i) People with type 1 or 2 diabetes<br>ii) Aged ≥18 years<br>iii) Presence of Wagner's grade 3 or 4 diabetic foot ulcers: > 1 $cm^2$<br>6. Exclusion criteria<br>i) Revascularisation surgery possible (for ischaemic ulcers)<br>ii) Hb < 100 g/L<br>iii) Uncompensated chronic diseases such as signs of heart failure, diabetic coma or ketoacidosis<br>iv) Renal failure (creatinine > 200 mg/dL)<br>v) Malignancies<br>vi) Psychiatric or neurological diseases (use of immunosuppressing drugs or |

| | |
|---|---|
| | corticosteroids)<br>vii) Pregnancy and nursing |
| Interventions | 1. Intervention group 1: 75 $\mu$g rhEGF (Herberprot-P®, Herber Biotec, Havana)<br>2. Intervention group 2: 25 $\mu$g rhEGF (Herberprot-P®, Herber Biotec, Havana). In both Intervention groups the product was dissolved in 5 ml of water for injection, 3 times/week, on alternate days<br>3. Control group: placebo, characteristics and administration schedules not reported<br>Co-intervention: standard wound care |
| Outcomes | 1. Primary: proportion of participants with partial or complete response after 2 weeks of treatment<br>2. Secondary:<br>i) Complete response rate at 8 weeks (Quote " . . . and > 75% (complete response) (p 434)<br>ii) Time-to complete response<br>iii) Complete wound closure<br>iv) Need for amputation<br>v) Recurrences within 1-year follow-up |
| Notes | 1. A priori sample size estimation: yes (p 434)<br>2. Sponsor: Heber Biotec SA and The Ministry of Public Health of Cuba<br>3. Role of sponsor: supplied the investigational product, supported the work<br>4. Conflict of interest: some authors were employees of the Centre for Biological Research, which is part of the Centre for Genetic Engineering and Biotechnology (CIGB), Havana network, where rhEGF is produced and the new formulation was developed and produced (p 440)<br>5. E-mail was sent to main author |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote " Randomisation was simple, central and stratified by investigation sites" (p 434) |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | Quote "both vials were indistinguishable . . . " (p 434) |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Placebo, characteristics of nature and schedules were not given |

| | | |
|---|---|---|
| Incomplete outcome data (attrition bias) All outcomes | High risk | Loss of participants: 27% (40/149)<br>1. Adverse event: Intervention group 1 = 22% (2/9); Intervention group 2 = (26% (4/15); Control group = 19% (3/16)<br>2. Lost to follow-up: Intervention group 1 = 22% (2/9); Intervention group 2 = 13% (2/15); Control group = 13% (2/16)<br>3. Mortality: Intervention group 1 = 22% (3/9); Intervention group 2 = 13% (2/15); Control group = 13% (2/16)<br>4. Lesion progression: Intervention group 1 = 44% (4/9); Intervention group 2 = 27% (4/15); Control group = 19% (3/16)<br>5. No response at week 2: Intervention group 1 = 11% (1/9); Intervention group 2 = 33% (5/15); Control group = 50% (8/16)<br>Quote "Nine patients in the lower EGF dose and placebo groups switched treatment at week 2 and are defined as non healers in further analysis. This design could have some impact on outcome regarding granulation rates at the week 8 visit and closure rates at 1 year follow-up" (p 437)<br>It is likely that the principle of ITT analysis was violated |
| Selective reporting (reporting bias) | High risk | One or more outcomes of interest in the review were reported incompletely so that they could not be entered in a meta-analysis<br>Time-to-complete response was reported using odds ratio (p 435) |
| Other bias | High risk | 1. This trial did not report hazard ratios for time to complete wound closure<br>2. Bias in the presentation of data and design bias, see Appendix 1 |

**Hanft 2008**

| | |
|---|---|
| Methods | 1. Parallel-design (2 arms)<br>2. Country: USA (9 sites)<br>3. Follow-up period: 12 weeks<br>4. Unit of randomisation: participant<br>5. Analysis unit: participant |
| Participants | 1. Randomised: 55<br>i) Intervention group (telbermin): 53% (29/55)<br>ii) Control group (placebo): 47% (26/55)<br>2. Age (years; mean and range)<br>i) Intervention group (telbermin): 59.5 (42-74)<br>ii) Control group (placebo): 59.3 (38-81)<br>iii) All participants: 59.4 (38-81)<br>3. Gender (male)<br>i) Intervention group (telbermin): 66% (19/29)<br>ii) Control group (placebo): 69% (18/26)<br>4. Inclusion criteria<br>i) People aged 18-80 years with type 1 or 2 diabetes mellitus<br>ii) HbA1c $\leq$ 12%<br>iii) Grade 1A ulcer, as defined by the University of Texas Diabetic Wound Classification<br>iv) Chronic ulcer with a duration of $\geq$ 4 weeks but < 6 months<br>v) Ulcer area, following sharp debridement, of 1-4 $cm^2$<br>vi) ABPI of 0.6-1.2 on the study foot<br>vii) For females of childbearing potential, use of an effective method of contraception<br>viii) Subjects with Charcot or another deformity of the study foot were included provided the deformity did not involve the study ulcer<br>5. Exclusion criteria<br>i) Active ulcer infection or cellulitis of any ulcer<br>ii) Ulcers with an aetiology unrelated to diabetes<br>iii) Active osteomyelitis in the study foot<br>iv) Subjects with ulcers related to an incompletely healed amputation wound<br>v) Use of any investigational drug/therapy on the study foot within the past month<br>vi) Previous use of platelet-derived or other growth factors on the study ulcer within the past 3 months<br>vii) Immunosuppressive treatment<br>viii) History of neoplasia or current neoplasia (with the exception of non-melanoma skin cancer)<br>ix) Proliferatuve diabetic retinopathy or wet age-related macular degeneration<br>x) Connective tissue disease<br>xi) Pregnancy or lactation<br>xii) Multiple ulcers on the study foot<br>xiii) Renal failure (serum creatinine of > 3.0 mg/dL)<br>xiv) Poor nutritional status (albumin of < 3.0 g/dL)<br>xv) Known hypersensitivity to any ingredients of telbermin, placebo or vehicle including usually inactive substances in the formulation of telbermin or placebo gel<br>xvi) Known prior instability likely to affect completion of required study visits during the treatment period |

| | |
|---|---|
| Interventions | 1. Intervention group: recombinant human vascular endothelial growth factor (telbermin), 72 μg/cm$^2$ over the entire ulcer surface and margins, 3 times/week, for 6 weeks<br>2. Control: placebo (formulated bulk solution without telbermin) in methylcellulose gel for 6 weeks<br>Co-interventions: standard good wound care including periodic sharp debridement at the clinician's discretion, also offloading |
| Outcomes | 1. Primary:<br>i) Safety, measured as incidence of clinically significant hypotension (a drop of ≥ 35 mmHg in systolic blood pressure relative to pre-dose level) 60 minutes after application of the study drug during the first week of treatment.<br>ii) Efficacy, assessed as percentage reduction in total ulcer surface area at day 43 (up to day 49 as the week 7 study visit could occur any time between days 43 and 49 for scheduling flexibility) from baseline (day 1), as determined by quantitative analysis of the planimetric tracings<br>2. Secondary<br>i) Safety:<br>a) incidence of clinical significant ulcer infection, defined as increased discharge and malodorous exudates from the ulcer, fever (≥ 38.6 °C) and white blood cell count > 10000/μl<br>b) development of anti-telbermin antibodies<br>c) incidence of adverse events<br>ii) Efficacy:<br>a) percentage reduction in total ulcer surface area at days 29 and 84 from baseline (day 1)<br>b) incidence of ulcer healing at days 29, 43 and 84<br>c) time (days) to complete ulcer healing<br>d) time (days) to recurrence for subjects whose ulcers had healed completely before day 84<br>e) incidence of increased total ulcer surface area (more than 15%), compared with baseline (day 1)<br>f) incidence of a progression in ulcer stage (University of Texas Classification Diabetic Wound Classification) |
| Notes | 1. A priori sample size estimation: yes (p 32)<br>2. Sponsor: Genentech, South San Francisco, CA<br>3. Role of sponsor: assistance with writing |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |

**Hanft 2008** (*Continued*)

| | | |
|---|---|---|
| Blinding of participants and personnel (performance bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | Quote " . . . a masked, third-party, reading centre . . performed the quantitative measurements" (p 31) |
| Incomplete outcome data (attrition bias)<br>All outcomes | High risk | Lost participants: total loss = 20% (11/55) : Intervention group 24% (7/29); Control group 15% (4/26)<br>Reasons:<br>1. infections: Intervention group 14% (1/7); Control group 25% (1/4)<br>2. mortality: Intervention group 14% (1/7); Control group 0% (0/4)<br>3. lost to follow-up: Intervention group 0% (0/7); Control group 25% (1/4)<br>4. participant choice: Intervention group 42% (3/7); Control group 50% (2/4) |
| Selective reporting (reporting bias) | High risk | One or more outcomes of interest in the review were reported incompletely so they could not be entered in a meta-analysis<br>Quote "Median time to complete healing was 58 days for telbermin-treated subjects. This could not be estimated for placebo-treated subjects because fewer than 50% healed completely during the 12-week study period." (p 35) |
| Other bias | High risk | Bias in the presentation of data and design bias, see Appendix 1<br>Funding bias<br>Many trial authors are or have been employed by Genentech or own Genentech stock |

**Hardikar 2005**

| | |
|---|---|
| Methods | 1. Parallel-design (2 arms)<br>2. Country: India (8 sites)<br>3. Follow-up: 20 weeks<br>4. Unit of randomisation: participant<br>5. Analysis unit: participant |

**Hardikar 2005** (*Continued*)

| | |
|---|---|
| Participants | 1. Randomised: 113<br>i) Treatment group: 55 (48.67%)<br>ii) Control group: 58 (51.33%)<br>2. Age (years mean ± standard deviation (SD))<br>i) Treatment group: 54.5 ± 9.9<br>ii) Control group: 54.7 ± 9.0<br>3. Gender (male)<br>i) Treatment group: 73% (40/55)<br>ii) Control group: 69% (40/58)<br>4. Inclusion criteria<br>i) Type 1 or 2 diabetes mellitus<br>ii) Age $\geq$ 18 years but $\leq$ 80 years<br>iii) At least 1, but < 3 full-thickness chronic neuropathic ulcers of at least 4 weeks duration on the lower extremity<br>iv) Only ulcers categorized as stage III or stage IV (according to the Wound, Ostomy, and Continence Nurses Society)<br>v) Ulcers with infection control as determined by a wound evaluation score could be included<br>vi) Evidence of adequate perfusion of the foot, assessed by colour arterial Doppler ultrasonography<br>5. Exclusion criteria<br>i) Arterial venous ulcers<br>ii) Ulcers caused by osteomyelitis or burns<br>iii) Poor nutritional status (serum total proteins < 6.5 g/dL)<br>iv) Persistent infection<br>v) Life-threatening concomitant diseases<br>vi) Deformities like Charcot foot<br>vii) Chronic renal insufficiency (serum creatinine > 3 mg/dL)<br>viii) Uncontrolled hyperglycaemia (HbA1c > 12%)<br>ix) History of use of corticosteroids or immunosuppressants<br>x) Hypersensitivity to the gel components of the intervention<br>xi) Women who were pregnant or nursing<br>xii) Women of childbearing age who were not taking contraceptives or were not willing to use them |
| Interventions | 1. Intervention group: recombinant human platelet-derived growth factor (becaplermin), 0.01% gel containing 100 $\mu$g of experimental drug, daily application<br>2. Control: placebo, details not reported<br>Co-interventions: standard wound care regimen consisting of appropriate sharp surgical debridement; daily ulcer cleaning and dressing; and offloading (e.g. crutches or wheelchair), or, where possible, complete bed rest. During this 1-week period, before the baseline visit (visit 2), a regimen of daily wound cleaning and dressing with appropriate non-weight bearing was followed. The use of antidiabetic medication and appropriate use of systemic antibiotics was advised during the treatment period |
| Outcomes | 1. Primary: percentage of participants achieving complete wound closure<br>2. Secondary<br>i) Time to achieve complete wound closure<br>ii) Percentage reduction in the ulcer surface area at each visit |

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|---|---|
| | iii) Total wound evaluation score at endpoint<br>iv) Safety |
| Notes | 1. A priori sample size estimation: not reported<br>2. Sponsor: Research and Development Department, Virchow Biotech Pvt Ltd, Hyderabad, India<br>3. Role of sponsor: supported the study |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | Quote " the authors are not privy to the differences in the formulation available commercially" (p 2/11)<br>Comment: both comparisons probably had similar appearances |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Incomplete outcome data (attrition bias)<br>All outcomes | High risk | Lost to follow-up: total = 19% (21/113)<br>Intervention group: 13% (7/55) and placebo group 24% (14/58)<br>Imbalance between comparison groups: 11%.<br>Reasons:<br>1. lost to follow-up: Intervention group 1/7; Control group 6/14<br>2. no compliance: Intervention group 2/7; Control group 1/14<br>3. Intervention group 4/7; Control group 6/14<br>4. other reasons: Intervention group 0/7; Control group 1/14<br>Comment: reason for missing outcome data likely to be related to true outcome, with either imbalance in numbers or reasons for missing data across intervention groups |

**Hardikar 2005** (*Continued*)

| | | |
|---|---|---|
| Selective reporting (reporting bias) | High risk | Safety data were poorly reported, and, therefore, could not be entered in a meta-analysis<br>This trial did not report hazard ratios for assessing time to complete wound closure |
| Other bias | High risk | Design bias, bias of presentation bias, see Appendix 1<br>Funding bias |

**Holloway 1993**

| | |
|---|---|
| Methods | 1. Parallel-design (4 arms), 2-phase study<br>2. Country: USA<br>3. Follow-up: 20 weeks<br>4. Unit of randomisation: participant<br>5. Analysis unit: participant |
| Participants | 1. Total randomised participants: unclear<br>i) First phase<br>a) Randomised: 14<br>b) Intervention group: number not specified<br>c) Control group: number not specified<br>ii) Second phase<br>a) Randomised: 97 (number of wounds: not specified)<br>b) Randomised and treated: 81 (91 wounds)<br>c) Randomised and analysed: 70 (77 wounds)<br>platelet-derived wound healing formula 0.01 dilution group: 15<br>platelet-derived wound healing formula 0.033 dilution group: 13<br>platelet-derived wound healing formula 0.1 dilution group: 21<br>Control (placebo) group: 21<br>2. Age of randomised and analysed participants: (mean years): 59.4 to 62.6 years<br>3. Gender (male):<br>i) platelet-derived wound healing formula 0.01: 73% (11/15)<br>ii) platelet-derived wound healing formula 0.033: 77% (10/13)<br>iii) platelet-derived wound healing formula 0.1: 81% (17/21)<br>iv) Placebo: 67% (14/21)<br>4. Inclusion criteria<br>i) People with diabetes mellitus with at least 1 chronic, nonhealing diabetic ulcer of 8 weeks duration<br>ii) Wounds 500 $mm^3$-50,000 $mm^3$<br>5. Exclusion criteria<br>i) Wounds possibly containing malignant cells<br>ii) Pre-existing diseases or terminal disease<br>iii) Pregnant, nursing or of childbearing potential |

**Holloway 1993** (*Continued*)

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|---|---|
| Interventions | 1. Intervention group<br>i) First phase: thrombin-induced, platelet-released platelet-derived wound healing formula 0.01 dilution<br>ii) Second phase: thrombin-induced, platelet-released platelet-derived wound healing formula (0.01, 0.1, and 0.033 dilutions)<br>2. Control group:<br>i) First phase: physiologic saline solution<br>ii) Second phase: isotonic platelet buffer containing 2-[4-(2-hydroxyethyl) piperazin-1-yl]ethanesulfonic acid (HEPES), glucose sodium chloride and potassium chloride (pH 6.6) |
| Outcomes | Primary: healed (functional assessment points 3 & 4 in original paper):<br>"3: 100% epithelized; maturing skin with a small amount of drainage; requires protective dressing only<br>4: 100% epithelized; maturing skin with a small amount of drainage; no dressing required" (p 200) |
| Notes | 1. This trial had 2 phases using 2 random sequence generations:<br>i) first phase included the first 14 participants, platelet-derived wound healing formula 0.01 dilution (experimental group) and physiologic saline solution as placebo<br>ii) second phase (second randomisation) was conducted to include 2 additional dilutions of platelet-derived wound healing formula (0.1 and 0.033) and a new placebo (isotonic platelet buffer containing 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid, glucose sodium chloride and potassium chloride).<br>2. A priori sample size estimation: not reported<br>3. Sponsor: Curative Technologies, Inc<br>4. Role of sponsor: financial |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote " . . . computer generated list of random numbers . . . " (p 200) |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | Quote "the appearance of the double-blind medication and the packaging were identical for drug and placebo to prevent unblinding" (p 200) |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |

| | | |
|---|---|---|
| Incomplete outcome data (attrition bias) All outcomes | Unclear risk | Lost 16% (16/97) post randomisation because failed to meet inclusion criteria - no details provided regarding loss according to group<br>Loss of randomised and treated participants (total = 81) by comparison group<br>1. Intervention group 1 = 1<br>2. Intervention group 2 = 3<br>3. Intervention group 3 = 6<br>4. Control group = 1<br>5. All intervention groups = 10<br>Quote "eleven patients with 14 wounds were excluded from the efficacy analysis due to noncompliance with protocol" (p 201)<br>Reasons:<br>1. Lost to follow up:<br>i) Intervention group 2: 33% (1/3)<br>2. Non compliance:<br>i) Intervention group 1: 100% (1/1)<br>ii) Intervention group 2: 66% (2/3)<br>iii) Intervention group 3: 50% (3/6)<br>iv) Control group: 100% (1/1)<br>3. Early amputation:<br>i) Intervention group 3: 33% (2/6)<br>4. Occluded vascular graft:<br>i) Intervention group 3: 16% (1/6)<br>Imbalance between comparison groups: unclear<br>Quote "four patients were lost to follow up after 12 weeks and 5 patients missed the last or second-to-last visit" (p 201)<br>Authors did not report whether these losses were in addition to those specified above and did not supply information about the timing of the losses |
| Selective reporting (reporting bias) | High risk | One outcome (complete wound closure) was reported incompletely<br>This trial did not report safety data |
| Other bias | High risk | Bias of the presentation data and information bias, see Appendix 1<br>Quote " . . . The first 14 patients were given |

**Holloway 1993** (*Continued*)

| | | |
|---|---|---|
| | | either 0.01 dilution of CT-102 or a placebo consisting of physiologic saline solution. The study was later revised to include two additional dilutions of CT-102 at 0.1 and 0.033. At that time a new randomization scheme was generated. A limited analysis conducted on the first 14 patients to ensure that there were no problems with the protocol. The placebo solution was at that time changed to an isotonic platelet buffer containing $N^1$-2-hydroxiethiyl piperazine-N-2-ethanesulfonic acid (HEPES), glucose sodium chloride and potassium chloride (pH=6.6) . . . " (p 200)<br>Funding bias |

**Jaiswal 2010**

| | |
|---|---|
| Methods | 1. Parallel-design (2 arms)<br>2. Country: India<br>3. Follow-up: 10 weeks<br>4. Unit of randomisation: participant<br>5. Analysis unit: participant |
| Participants | 1. Randomised: 50<br>i) Intervention group: 25 (50%)<br>ii) Control group: 25 (50%)<br>2. Age (mean years)<br>i) Intervention group: 56.2<br>ii) Control group: 49.9<br>3. Gender (male)<br>i) Intervention group: 76% (19/25)<br>ii) Control group: 92% (23/25)<br>4. Inclusion criteria<br>i) Type 1 or type 2 diabetes mellitus and chronic ulcers of at least 4 weeks duration of IAET stage III and IV<br>5. Exclusion criteria:<br>i) ABPI < 0.9 |
| Interventions | 1. Intervention group: recombinant human platelet-derived growth factor (Plermin, Dr Reddy's Laboratories Ltd) 0.01% gel topical application<br>2. Control group: topically applied placebo (KY Jelly (Ethnor) - a lubricating jelly containing glycerin 11.2%, methylparaben 0.1% and propylparaben 0.04%)<br>Co-interventions: standardised regimen of good wound care; pressure offloading for participants with plantar ulcers |
| Outcomes | Primary outcome: healing or percent reduction in size of the wound |

**Jaiswal 2010** (*Continued*)

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|---|---|
| Notes | 1. Sample size a priori calculation: not reported<br>2. Sponsor: not reported |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote "on the basis of computer generated numbers" (p 32) |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Incomplete outcome data (attrition bias)<br>All outcomes | Unclear risk | Insufficient reporting of attrition/exclusions to permit a judgement of 'low risk' or 'high risk' (i.e. no reasons provided for missing data) |
| Selective reporting (reporting bias) | High risk | Information on time to healed and safety was not provided |
| Other bias | High risk | Bias of presentation data, see Appendix 1 |

**Kakagia 2007**

| | |
|---|---|
| Methods | 1. Parallel-design (3 arms)<br>2. Country: Greece<br>3. Study period: 8 weeks<br>4. Unit of randomisation: participant<br>5. Unit analysis: participant |
| Participants | 1. Enrolled: 72<br>2. Randomised: 51<br>i) Intervention group 1 (oxidized regenerated cellulose/collagen biomaterial): 33.33% (17/51)<br>ii) Intervention group 2 (autologous growth factor delivered by gravitational platelet separation system): 33.33% (17/51)<br>iii) Intervention group 3 (both above interventions): 33.33% (17/51)<br>3. Age (years, mean ± standard deviation (SD)) |

| | |
|---|---|
| | i) Intervention group 1 (oxidized regenerated cellulose/collagen biomaterial): 58 ± 10<br>ii) Intervention group 2 (Autologous growth factor delivered by gravitational platelet separation system): 57 ± 12<br>iii) Intervention group 3 (both above interventions): 61 ± 9<br>4. Gender (male)<br>i) Global: 43.13% (22/51).<br>5. Inclusion criteria<br>i) Diabetic participants with significant soft tissue defects of the foot that had been present for at least 3 months<br>ii) Target ulcers ≥ 2.5 cm in any dimension after debridement<br>6. Exclusion criteria<br>i) Previous treatment with vacuum, hyperbaric oxygen, corticosteroid, immunosuppressive agents, radiation, or growth factors<br>ii) Anaemia<br>iii) Presence of cellulitis or venous stasis<br>iv) Inadequate perfusion determined by toe pulses of < 40<br>v) Osteomyelitis<br>vi) Malignancy in the wound<br>vii) Inability to attend clinics for follow-up |
| Interventions | 1. Intervention group 1: oxidized regenerated cellulose/collagen biomaterial (Promogran, Johnson & Johnson, New Brunswick, NJ)<br>2. Intervention group 2: autologous growth factor delivered by gravitational platelet separation system (GPS, Biomet)<br>3. Intervention group 3: combination of both treatments above<br>Co-interventions: all wounds were sharply debrided prior to the first application of dressings and were assessed weekly for 8 weeks |
| Outcomes | Change in ulcer dimensions within the 8-week follow-up |
| Notes | 1. A priori sample size estimation: not reported<br>2. Sponsor: not reported |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote " . . . random number generator" (p 388) |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |

**Kakagia 2007** (*Continued*)

| | | |
|---|---|---|
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Incomplete outcome data (attrition bias)<br>All outcomes | Unclear risk | Insufficient reporting of attrition/exclusions to permit a judgement of 'low risk' or 'high risk' (i.e. no reasons provided for missing data) |
| Selective reporting (reporting bias) | High risk | This trial did not report safety data |
| Other bias | High risk | Bias of presentation data, see Appendix 1<br>Author did not supply information on wound area at the end of the trial |

**Landsman 2010**

| | |
|---|---|
| Methods | Randomized, controlled trial, parallel design (2 arms)<br>Country study: USA<br>Intention to treat: unclear<br>Follow up period: 20 weeks<br>Unit of randomization: unclear<br>Unit of analysis: ulcers |
| Participants | Randomised: 32, no further details provided<br>Age (years)<br>Intervention group (becaplermin plus TheraGauze): 58.1<br>Control group (TheraGauze): 56.2<br>Gender (male): not reported<br>Inclusion criteria<br>1. Forefoot or midfoot ulcer<br>2. Wagner grade 1 or 2<br>3. Tolerate offloading with healing shoe, fixed ankle walker, or non-weight-bearing<br>4. Age 18-70 years<br>5. Insulin dependent diabetes mellitus or non insulin dependent diabetes mellitus (IDDM or NIDDM)<br>6. HbA1c $\leq$ 10% of total Hb<br>7. Palpable dorsalis pedis and posterior tibial pulses<br>8. 1-8 $cm^2$ wound surface area<br>Exclusion criteria<br>i) Active infection, including purulent discharge, cellulitis, or both<br>ii) Exposed bone<br>iii) Osteomyelitis associated with ulcer<br>iv) Dorsal ulcer<br>v) Ischaemic ulcers<br>vi) Evidence of gangrene<br>Withdrawal: not reported |

| | |
|---|---|
| Interventions | 1. Intervention group: recombinant human platelet-derived growth factor (becaplermin, Regranex 0.01%) daily in accordance with the manufacturer's recommendations, plus moisture-regulating dressing (TheraGauze®, Soluble systems LLC, Newport News, Virginia)<br>2. Control group: moisture-regulating dressing (TheraGauze®, Soluble systems LLC, Newport News, Virginia) applied directly to the wound surface every other day<br>Co-interventions: standard wound debridement as needed, offloading with a fixed ankle walker (Royce Medical Diabetic Boot; Ossur Medical, Camarillo, California), and dressing changes every other day |
| Outcomes | 1. Time to closure (full epithelialisation)<br>2. Rate of change in wound surface area |
| Notes | 1. A priori sample size estimation: not reported<br>2. Sponsor: Soluble systems LLC, Newport News, Virginia<br>3. Role of the sponsor: sponsor funded the study<br>4. Conflict of interest: the main author is a paid consultant for Soluble systems LLC<br>5. Email was sent to the main author requesting data on main outcome |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Quote "Professional, independent monitoring and centralized randomization" (p 156) |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Incomplete outcome data (attrition bias)<br>All outcomes | Unclear risk | Insufficient reporting of attrition/exclusions to permit a judgement of 'low risk' or 'high risk' (i.e. no reasons provided for missing data) |
| Selective reporting (reporting bias) | High risk | One outcome (Time to closure (full epithelialization) was reported incompletely so that it could not be included in the meta-analysis. This trial did not report safety data |

| | | |
|---|---|---|
| Other bias | High risk | Design bias and bias of presentation data, see Appendix 1<br>Funding bias |

**Lyons 2007**

| | |
|---|---|
| Methods | 1. Parallel-design (3 arms), 2 phases<br>2. Country: USA (7 sites)<br>3. Follow-up period: 6 months<br>4. Unit of randomisation: participant<br>5. Analysis unit: participant<br>6. The study was conducted in 2 phases: phase 1 was an open-label, sequential, dose-escalation design. Phase 2 was a single-blind, randomized, stratified, placebo-controlled pilot study to evaluate the efficacy of the 2 highest dose levels below the maximum tolerated dose (if any, up to 8.5% talactoferrin gel) of topically applied talactoferrin compared with placebo. |
| Participants | 1. Randomised: 46<br>i) Intervention group 1 (talactoferrin (recombinant human lactoferrin) 2.5% gel): 32.6% (15/46)<br>ii) Intervention group 2 (talactoferrin (recombinant human lactoferrin) 8.5% gel): 32.6% (15/46)<br>iii) Control group (placebo gel): 34.8% (16/46)<br>2. Age (years, mean ± standard deviation (SD))<br>i) Intervention group 1 (talactoferrin (recombinant human lactoferrin) 2.5% gel): 58 ± 10<br>ii) Intervention group 2 (talactoferrin (recombinant human lactoferrin) 8.5% gel): 53 ± 15<br>iii) Control group (placebo gel): 56 ± 14<br>3. Gender (male)<br>i) Intervention group 1 (talactoferrin (recombinant human lactoferrin) 2.5% gel): 93% (14/15)<br>ii) Intervention group 2 (talactoferrin (recombinant human lactoferrin) 8.5% gel): 80% (12/15)<br>iii) Control group (placebo gel): 56% (9/16)<br>4. Inclusion criteria<br>i) Age:≥ 18 years<br>ii) Diabetes mellitus with HbA1c between 6%-13%<br>iii) Presence of 1 or more diabetic neuropathic foot ulcers at, or below, the ankle that had not healed or decreased in size (≥ 30%) within the prior 4 weeks despite appropriate standard treatment<br>iv) Index ulcer required to be full thickness, extending through the dermis, but without tendon, muscle, joint capsule, or bone exposure, and without sinus tract, with post debridement size of 0.5 to 10 $cm^2$<br>v) Transcutaneous oxygen tension of ≥ 30 mmHg or ABPI ≥ 0.7<br>5. Exclusion criteria<br>i) Target ulcer from any cause other than diabetes |

| | |
|---|---|
| | ii) Signs of clinical wound related infection, including - but not limited to - cellulitis, osteomyelitis, gangrene, or deep tissue infection in the study extremity<br>iii) Active Charcot's foot on the limb under study<br>iv) Prior treatment of the target ulcer with Regranex (Ortho-McNeil Pharmaceutical, Inc, New Brunswick, NJ) within the previous 14 days, or autologous or allogeneic graft, or Dermagraft to the target ulcer within the preceding 4 weeks |
| Interventions | 1. Intervention group 1: talactoferrin (recombinant human lactoferrin) 2.5% gel applied topically twice daily for 12 weeks<br>2. Intervention group 2: talactoferrin (recombinant human lactoferrin) 8.5% gel applied topically twice daily for 12 weeks<br>3. Control group: placebo gel applied topically twice daily for 12 weeks, no details provided about the nature of placebo<br>Co-interventions: standard care consisted of:<br>1. initial and periodic (as needed) sharp debridement;<br>2. twice daily saline dressing change, including cleansing with saline;<br>3. offloading using standardised devices (DH Pressure Relief Walkers; Royce Medical, Camarillo, CA);<br>4. systemic control of any infection |
| Outcomes | Primary endpoint of phase 2: percentage of participants achieving ≥ 75% closure of the target ulcer |
| Notes | 1. A priori sample size estimation: no<br>2. Sponsor: Agennix Inc, Houston, TX, and the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institute of Health<br>3. Role of the sponsor: supplied the drugs and placebo for study (p 50) and support in part (p 49) |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Quote " . . . randomization was central . . . " (p 50) |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Unclear risk | Quote " . . . and none of the personnel of any of the site were informed of the blinding code before completion of the study" (p 50) |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |

**Lyons 2007** (*Continued*)

| | | |
|---|---|---|
| Incomplete outcome data (attrition bias) All outcomes | Unclear risk | Insufficient reporting of attrition/exclusions to permit a judgement of 'low risk' or 'high risk' (i.e. no reasons provided for missing data) |
| Selective reporting (reporting bias) | Unclear risk | The study protocol is not available, but it is clear that the published reports included all expected outcomes, including those that were pre-specified<br>However, this trial failed to provide separate data for each study arm |
| Other bias | High risk | Bias of presentation data, see Appendix 1 |

**Niezgoda 2005**

| | |
|---|---|
| Methods | 1. Parallel-design (2 arms)<br>2. Study period: 12 weeks<br>3. Follow-up: 6 months<br>4. Country: USA and Canada (9 sites)<br>5. Unit of randomisation: participant<br>6. Analysis unit: participant |
| Participants | 1. Enrolled: 98<br>2. Randomised: 98<br>i) Intervention group 1 (OASIS Wound Matrix): 51% (50/98); 37 completed assigned treatment<br>ii) Intervention group 2 (Regranex gel): 49% (48/98); 36 completed assigned treatment<br>iii) Cross-over: "Patients whose wounds were not healing by 12th week were given the option to cross over to the other treatment arm" (p 260). 12 participants in the Regranex Gel group crossed over to receive OASIS, 1 participant healed; 9 participants in the OASIS arm crossed over to receive Regranex Gel, 2 healed<br>3. Age (years, mean ± standard deviation (SD))<br>i) Intervention group 1 (OASIS Wound Matrix): 58 ± 2.3<br>ii) Intervention group 2 (becaplermin (Regranex gel)): 57 ± 1.9<br>4. Gender (male)<br>i) Intervention group 1 (OASIS Wound Matrix): 62% (23/37)<br>ii) Intervention group 2 (becaplermin (Regranex gel)): 58% (21/36)<br>5. Inclusion criteria<br>i) ≥18 years<br>ii) Type 1 or type 2 diabetes mellitus<br>iii) Ulcer size 1-49 $cm^2$<br>iv) Ulcer depth required to extend through both the epidermis and dermis<br>v) Grade I, Stage A (University of Texas classification)<br>vi) Present for > 1 month and nonhealing<br>vii) Viable wound bed with granulation tissue |

| | |
|---|---|
| | 6. Exclusion criteria<br>i) Exposed bone, tendon, or fascia<br>ii) Clinically defined and documented severe arterial disease<br>iii) History of radiation therapy to the ulcer site<br>iv) Ulcer of nondiabetic pathophysiology<br>v) Receiving corticosteroids or suppression of the immune system<br>vi) History of collagen vascular disease malnutrition (albumin < 2.5 g/dL)<br>vii) Known allergy to porcine-derived products<br>viii) Known hypersensitivity to any component of Regranex Gel (e.g. parabens)<br>ix) Religious or cultural objection to the use of porcine products<br>x) Uncontrolled diabetes (HbA1c > 12%)<br>xi) Previous organ transplant<br>xii) Clinically infected ulcer<br>xiii) Signs of cellulitis, osteomyelitis, necrotic or avascular ulcer bed<br>xiv) Undergoing haemodialysis<br>xv) Insufficient blood supply to the ulcer (TcPO2 < 30 mmHg or toe-brachial index < 0.70)<br>xvi) Active Charcot or sickle cell disease<br>xvii) Received treatment with any other investigational drug or device within the last 30 days<br>xviii) Unable to comply with the procedures described in the protocol<br>xix) Enrolled in a clinical evaluation for another investigational wound care device or drug |
| Interventions | 1. Intervention group 1: recombinant human platelet-derived growth factor (becaplermin (Regranex gel)), applied topically daily and removed after 12 hours<br>2. Control group: OASIS Wound Matrix (HEALTHPOINT, Ltd, Fort Worth, TX) applied topically weekly<br>Co-interventions: standard wound care, pressure-relief shoes (DH Pressure Relief Shoe; Royce Medical Co, Camarillo, CA) |
| Outcomes | Primary outcome: incidence of complete wound healing by 12 weeks |
| Notes | 1. A priori sample size estimation: not reported<br>2. Sponsor: Cook Biotech Incorporated, West Lafayette, IN, and DH Pressure RElief Shoe; Royce Medical Co, Camarillo, CA<br>3. Role of sponsors: provided the study supplies, including treatment products, dressing supplies, and pressure-relief shoes<br>4. Conflict of interest: one trial author is a research scientist with Cook Biotech Incorporated |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote " were assigned to a treatment group using a centralized computer system" (p 259) |

**Niezgoda 2005** (*Continued*)

| | | |
|---|---|---|
| Allocation concealment (selection bias) | Low risk | Quote " individual investigators were blinded to the size of the block" (p 259) |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Incomplete outcome data (attrition bias)<br>All outcomes | High risk | Lost post randomisation (treated): total = 26% (25/98)<br>1. Experimental group: 26% (13/50)<br>2. Control group: 25% (12/48)<br>3. Imbance between groups: 1%<br>Reasons for loss:<br>1. infection: Intervention group = 23% (3/13); Control group = 17% (2/12)<br>2. mortality: Intervention group = 8% (1/13); Control group = (0/12)<br>3. non-adherence to follow up visits: Intervention group = 31% (4/13); Control group = 58% (7/12)<br>4. hospitalisation and related to the study or target wound: Intervention group = 23% (3/13); Control group = 0% (0/12)<br>5. tendon/ bone exposure: Intervention group = 8% (1/13); Control group = 0% (0/12)<br>6. withdrew consent: Intervention group = 0% (0/13); Control group = 17% (2/12)<br>7. received other wound care therapy: Intervention group = 8% (1/13); Control group = 8% (1/12) |
| Selective reporting (reporting bias) | Unclear risk | One or more outcomes of interest in the review were reported incompletely so that they could not be included in a meta-analysis |
| Other bias | High risk | Bias of presentation data, see Appendix 1<br>Funding bias |

**Richard 1995**

| | |
|---|---|
| Methods | 1. Parallel-design (2 arms)<br>2. Country: France and Italy<br>3. Study period: 18 weeks<br>4. Unit of randomisation: participant<br>5. Analysis unit : participant |
| Participants | 1. Randomised: 17<br>i) Intervention group (basic fibroblastic growth factor): 53% (9/17)<br>ii) Control group (physiologic saline solution): 47% (8/17)<br>2. Age (years, mean ± SD)<br>i) Intervention group (basic fibroblastic growth factor): 61.9 ± 10.0<br>ii) Control group (physiologic saline solution): 63.6 ± 7.9<br>3. Gender (male)<br>i) Intervention group (basic fibroblastic growth factor): 100% (9/9)<br>ii) Control group (physiologic saline solution): 87.5% (7/8)<br>4. Inclusion criteria<br>i) Typical, chronic, nonhealing neuropathic ulcer on the plantar surface of the foot<br>ii) Grade 1-3, according to Wagner′ s classification<br>iii) After mechanical excision, the largest part of the wound had to measure more than 0.5 cm<br>iv) Vibration perception threshold higher than 30 V either at the big toe or at the medial malleolus<br>5. Exclusion criteria: not reported |
| Interventions | 1. Intervention group: basic fibroblastic growth factor (Farmitalia Carlo Erba, Milano, Italy) 50 $\mu$g (5 $\mu$g/ml), applied once a day on an in-patient basis during the first 6 weeks. During the last 12 weeks, it was applied twice a week, and participants were allowed to return home if ulcer progression was satisfactory<br>2. Control group: placebo (normal saline), applied once a day on an in-patient basis during the first 6 weeks. During the last 12 weeks, it was applied twice a week, and participants were allowed to return home if ulcer progression was satisfactory<br>Co-interventions:<br>1. Intensive insulin therapy using 3 subcutaneous injections a day or continuous subcutaneous insulin infusion maintained during the entire experimental period<br>2. Participants were totally non weight-bearing |
| Outcomes | 1. Healing<br>2. Improvement<br>3. No progression<br>4. Worsening<br>5. Ulcer perimeter reduction (% of initial perimeter)<br>6. Time for 50% healing (weeks) |
| Notes | 1. A priori sample size estimation: not reported<br>2. Sponsor: Farmitalia Carlo Erba Laboratory, Milano, Italy; P Dang<br>3. Role of sponsor: supported the study; reviewed the manuscript |
| ***Risk of bias*** | |

**Richard 1995** (*Continued*)

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'Yes'or 'No |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | Quote " . . . ulcer perimeter and area were measured by one of us (J.P.D), unaware of the patient's identity, data of photographs, and nature of the treatment" (p 65)<br>Comment: J.P.D is Jean-Pierre Daures |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All participants were analysed |
| Selective reporting (reporting bias) | Low risk | The study protocol is not available but it is clear that the published reports included all expected outcomes, including those that were pre-specified |
| Other bias | High risk | Design bias, see Appendix 1<br>Funding bias |

**Robson 2002**

| | |
|---|---|
| Methods | 1. Parallel-design (5 arms)<br>2. Country: USA (15 sites)<br>3. Follow-up: 20 weeks<br>4. Unit of randomisation: participant<br>5. Analysis unit: participant |
| Participants | 1. Randomised: 177<br>i) Intervention group 1 (transforming growth factor ß2 0.05%): 24.3% (43/177)<br>ii) Intervention group 2 (transforming growth factor ß2 0.5%): 24.85% (44/177)<br>iii) Intervention group 3 (transforming growth factor ß2 5%): 24.85% (44/177)<br>iv) Control group 1 (standard wound care): 14% (24/177)<br>v) Control group 2 (placebo): 12% (22/177)<br>2. Age (years, mean ± standard deviation):<br>i) Intervention group 1 (transforming growth factor ß2 0.05%): 56 ± 11<br>ii) Intervention group 2 (transforming growth factor ß2 0.5%): 56 ± 12 |

| | |
|---|---|
| | iii) Intervention group 3 (transforming growth factor ß2 5%): 56 ± 8<br>iv) Control group 1 (standard wound care): 55 ± 9<br>v) Control group 2 (placebo): 60 ± 10<br>3. Gender (male)<br>i) Intervention group 1 (transforming growth factor ß2 0.05%): 77%<br>ii) Intervention group 2 (transforming growth factor ß2 0.5%): 77%<br>iii) Intervention group 3 (transforming growth factor ß2 5%): 77%<br>iv) Control group 1 (standard wound care): 92%<br>v) Control group 2 (placebo): 82%<br>4. Inclusion criteria<br>i) ≥ 18 years of age<br>ii) People with diabetes mellitus and a neuropathic ulcer present for at least 8 weeks on the plantar surface of the forefoot, toes, metatarsals, or dorsum of the foot<br>iii) Ulcer 1-20 $cm^2$ in area after debridement, and of full thickness without exposed bone or tendon<br>iv) Adequate peripheral arterial circulation determined by an ABPI 0.7-1.3, or TcPO2 measurement on the foot ≥ 30 mmHg<br>5. Exclusion criteria:<br>i) Radiographically documented osteomyelitis<br>ii) Clinical infection of the ulcer<br>iii) Use of systemic steroids within the previous 30 days<br>iv) HbA1c greater than 13%<br>v) Serum creatinine > 2.5 mg/dL<br>vi) Serum albumin < 2 mg/dL |
| Interventions | 1. Intervention group 1 (transforming growth factor ß2 0.05%): topical collagen sponges containing recombinant human transforming growth factor-ß2 at 0.05 µg/$cm^2$ applied twice weekly<br>2. Intervention group 2 (transforming growth factor ß2 0.5%): topical collagen sponges containing recombinant human transforming growth factor-ß2 at 0.5 µg/$cm^2$ applied twice weekly<br>3. Intervention group 3 (transforming growth factor ß2 5%): topical collagen sponges containing recombinant human transforming growth factor-ß2 at 5.0 µg/$cm^2$ applied twice weekly<br>4. Control group 1 (standard wound care): sharp debridement, coverage with non-adherent dressing, and weight offloading for the affected foot<br>5. Control group 2 (placebo): placebo |
| Outcomes | Primary<br>1. Proportion of participants with complete ulcer closure at or before week 21<br>2. Percentage of ulcer area reduction by week 21<br>Secondary<br>1. Proportion of participants with complete closure of the ulcer at each weekly visit<br>2. Percentage of ulcer area reduction at each weekly visit<br>3. Time to wound closure<br>4. 3-month follow-up assessment of durability of wound closure<br>5. Safety |

| | |
|---|---|
| Notes | 1. A priori sample size estimation: not reported<br>2. Sponsor: Genzyme Corporation<br>3. Role of sponsor: created computer-generated treatment randomisation lists, generated the coded labelling key, provided editorial comment and review, program management, management of the clinical trial |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote " . . . computer-generation patients numbers" (p 3) |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | High risk | Quote "Double blinding for all 4 groups receiving collagen sponge was maintaining by code labelling for all collagen-sponge packaging materials . . . the standardized care group could not be blinded since these patients did not receive a collagen sponge like the other four treatment groups" (p 2/10) |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Incomplete outcome data (attrition bias)<br>All outcomes | High risk | Lost post randomisation: 15% (27/177)<br>Lost after treatment: 7% (11/150)<br>Total lost: 21% (38/177)<br>Reasons: not reported<br>Losses by intervention group: not reported<br>Potentially inappropriate application of simple imputation |
| Selective reporting (reporting bias) | High risk | Safety was reported incompletely so the data could not be entered in a meta-analysis |
| Other bias | High risk | Bias of presentation data, see Appendix 1 |

**Saldalamacchia 2004**

| | |
|---|---|
| Methods | 1. Parallel-design (2 arms)<br>2. Country: Italy<br>3. Follow-up period: 5 weeks<br>4. Unit of randomisation: participant<br>5. Analysis unit: participant |
| Participants | 1. Randomised: 14<br>i) Intervention group: 50% (7/7)<br>ii) Control group: 50% (7/7)<br>2. Age (years, mean ± SD)<br>i) Intervention group: 61.1 ± 9.4<br>ii) Control group: 58.1 ± 7.8<br>3. Gender (male)<br>i) Intervention group: 57.14% (4/7)<br>ii) Control group: 28.57% (2/7)<br>4. Inclusion criteria<br>i) Diabetic participant<br>ii) Grade 2 or 3 ulcer according to Wagner, lasting for at least 8 weeks, and without signs of infection<br>5. Exclusion criteria: not reported |
| Interventions | 1. Intervention: topic application of autologous platelet gel plus standard care plus, frequency not reported<br>2. Control: standard care |
| Outcomes | Reduction rate (%)<br>Complete healing or reduction of at least 50% |
| Notes | 1. This trial was reported as a letter to editor<br>2. A priori sample size estimation: not reported<br>3. Sponsor: not reported |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Quote ". . . were randomly . . . " (p 395) |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Unclear risk | Quote "observers were blind with respect to treatment assignments" (p 395) |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Quote "observers were blind with respect to treatment assignments" (p 395) |

**Saldalamacchia 2004** (*Continued*)

| | | |
|---|---|---|
| Incomplete outcome data (attrition bias) All outcomes | Unclear risk | Insufficient reporting of attrition/exclusions to permit a judgement of 'low risk' or 'high risk' (i.e. no reasons provided for missing data) |
| Selective reporting (reporting bias) | High risk | This trial did not report safety data |
| Other bias | High risk | Bias of presentation data, see Appendix 1 |

**Steed 1992**

| | |
|---|---|
| Methods | 1. Parallel-design (2 arms)<br>2. Follow-up: 20 weeks<br>3. Country: USA<br>4. Unit of randomisation: participant<br>5. Analysis unit: participant |
| Participants | 1. Randomised: 13<br>i) Intervention group (platelet-derived wound healing formula, homologous): 54% (7/13)<br>ii) Control group (placebo):46% (6/13)<br>2. Age: (years, ± SD)<br>i) Intervention group (platelet-derived wound healing formula, homologous): 58.7 ± 12.4<br>ii) Control group (placebo): 54.2 ± 12.9<br>3. Gender (male)<br>i) Intervention group (platelet-derived wound healing formula, homologous): 71.4% (5/7)<br>ii) Control group (placebo): 67% (4/6)<br>4. Inclusion criteria<br>i) A neurotrophic ulcer of the lower extremity that had not healed after at least 8 weeks of standard treatment<br>ii) Diabetes mellitus<br>iii) Platelet count $\geq$ 100,000/$mm^3$<br>iv) $TcPO_2$ > 30 mmHg<br>5. Exclusion criteria<br>i) Clinical signs of infection such as erythema, induration, tenderness, fever, or chills |
| Interventions | 1. Intervention group: platelet-derived wound healing formula was applied to a cotton gauze sponge and placed on the ulcer in the evening. The intervention was prepared from blood donors.<br>2. Control group: placebo (normal saline) was applied to a cotton gauze sponge and placed on the ulcer in the evening<br>Co-interventions: normal cotton gauze applied to the wound for the next 12 hours; participants were supplied with a half-shoe (IPOS North American Niagara Fakks, NY) that transferred the weight to their heel and could be used for balance, they also had access to wheel-chairs, crutches, or walkers to avoid weight-bearing; debridements |

**Steed 1992** (*Continued*)

| | |
|---|---|
| Outcomes | 1. Funtional assessment (healing)<br>2. Reduction in wound volume |
| Notes | 1. A priori sample size estimation: not reported<br>2. Sponsor: Curative technologies, Inc<br>3. Role of sponsor: supported part of this research<br>4. Half shoes IPOS North American, Niagara Falls, NY |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | Quote " . . . were identical in appearance. Neither the investigators nor the patients were able to distinguish between the two products" (p 1599) |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Incomplete outcome data (attrition bias)<br>All outcomes | Unclear risk | Insufficient reporting of attrition/exclusions to permit a judgement of 'low risk' or 'high risk' (i.e. no reasons provided for missing data) |
| Selective reporting (reporting bias) | High risk | This trial did not report safety data |
| Other bias | High risk | Design bias, see Appendix 1<br>Funding bias<br>Imbalance in wound area ($mm^2$) and initial volume ($mm^3$) at baseline |

**Steed 1995a**

| | |
|---|---|
| Methods | 1. Parallel-design (2 arms)<br>2. Country: USA (10 sites)<br>3. Follow-up period: 20 weeks<br>4. Unit of randomisation: participant<br>5. Analysis unit: participant |
| Participants | 1. Randomised: 118 (118 ulcers)<br>i) Intervention group (recombinant human platelet-derived growth factor): 51.7% (61/118) (61 ulcers)<br>ii) Control group: 48.30% (57/118) (57 ulcers)<br>2. Age (years, mean)<br>i) Intervention group (recombinant human platelet-derived growth factor): 63.2<br>ii) Control group: 58.3<br>3. Gender (male)<br>i) Intervention group (recombinant human platelet-derived growth factor): 86.8% (53/61)<br>ii) Control group: 80.7% (46/57)<br>4. Inclusion criteria<br>i) At least 19 years of age<br>ii) At least one ulcer between 1-100 $cm^2$ in area<br>iii) Free of infection according to clinical examination and radiographs<br>iv) Adequate arterial blood supply assessed by measuring TcPO2 of $\geq$ 30 mmHg on the dorsum of the foot, or at the margin of the ulcer if the ulcer was on the plantar surface<br>5. Exclusion criteria<br>i) Women of childbearing potential<br>ii) Nursing mothers<br>iii) Hypersensitivity to any component of the study gel<br>iv) > 3 ulcers<br>v) Ulcers caused by large-vessel arterial ischaemia, venous insufficiency, pressure, or necrobiosis lipoidica diabeticorum<br>vi) Osteomyelitis, malignant or terminal disease<br>vii) Alcohol or substance abuse<br>viii) People who had participated in a clinical trial of an investigational drug or device within the previous 30 days<br>ix) Thermal, electrical, or radiation burn wounds at the site of the target ulcer<br>x) People receiving corticosteroids, immunosuppressive agents, radiation therapy or chemotherapy<br>xi) Vascular reconstruction during the previous 8 weeks |
| Interventions | 1. Intervention group: recombinant human platelet-derived growth factor (becaplermin) (rhPDGF-BB (Chiron Corp, Emeryville, CA; Johnson and Johnson, New Brunswick, NJ)) applied to the target ulcer once a day at a dose calculated to approximate 2.2 µg of rhPDGF-BB/$cm^2$ ulcer area; 12 hours later the residual gel was removed by mild irrigation with saline<br>2. Control group: placebo gel administered in the same way as the intervention gel. Details of the nature of the placebo were not reported<br>Co-interventions: a saline-moistened gauze dressing was placed over the target ulcer and gel, and the foot wrapped with a roll of gauze. Pressure relief for the target ulcer achieved |

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|---|---|
| | by means of crutches, wheelchairs, orthotic shoes, or other methods. Sharp debridement could be performed at each office visit if the investigator thought it was necessary |
| Outcomes | 1. Primary: complete healing (100% wound closure with no drainage present and no dressing required)<br>2. Secondary:<br>i) Time to complete wound closure<br>ii) Percentage reductions in area of target ulcers |
| Notes | 1. A priori sample size estimation: not reported<br>2. Sponsor: RW Johnson Pharmaceutical Research Institute (Raritan, NJ)<br>3. Role of sponsor: the sponsor prepared the computer-generated randomizations schedule for each centre before study initiation, and was responsible for the conduct of the trial and all analyses |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote " . . . computer generation . . . " (p 73) |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | Quote " . . . were no differences of colour, consistency, or odour between the placebo and rhPDGF-BB gel" (p 72) |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | Lost post randomisation: total = 27% (32/118)<br>Intervention group = 23% (14/61); Control group = 32% (18/57)<br>Imbalance between group: 9%<br>Reasons<br>1. Death: Intervention group = 0/14; Control group = 2/18<br>2. Adverse experiences: Intervention group = 6/14; Control group = 6/18<br>3. Treatment failure: Intervention group = 3/14; Control group = 5/18<br>4. Noncompliance: Intervention group = 1/14; Control group = 1/18<br>5. Intercurrent medical problem: |

**Steed 1995a** (*Continued*)

| | | |
|---|---|---|
| | | Intervention group = 2/14; Control group = 2/18<br>6. Lost to follow up: Intervention group = 2/14; Control group = 1/18<br>7. Other: Intervention group = 0/14; Control group = 1/18 |
| Selective reporting (reporting bias) | High risk | One outcome of interest (time to complete wound closure) in the review was reported incompletely so the data could not be entered in a meta-analysis |
| Other bias | High risk | Bias of presentation data and design bias, see Appendix 1<br>This trial did not report hazard ratio for time to complete wound closure<br>Funding bias |

**Steed 1995b**

| | |
|---|---|
| Methods | 1. Parallel-design (2 arms)<br>2. Country: USA (10 sites)<br>3. Follow-up period: 10 weeks<br>4. Unit of randomisation: participant<br>5. Analysis unit: participant |
| Participants | 1. Randomised: 65 participants<br>i) Intervention group (arginine-glycine-aspartic acid peptide matrix): 61.5% (40/65)<br>ii) Control group: 38.5% (25/65)<br>2. Age (years, means ± SD)<br>i) Intervention group (arginine-glycine-aspartic acid peptide matrix): 61.8 ± 1.9 years<br>ii) Control group: 61.0 ± 2.2 years<br>3. Gender (male)<br>i) Intervention group (arginine-glycine-aspartic acid peptide matrix): 72.5% (29/40)<br>ii) Control group: 80% (20/25)<br>4. Inclusion criteria<br>i) People with foot ulcers of at least 1 month's duration that penetrated through the epidermis and into the dermis without exposure of bone or tendon<br>ii) $\geq$ 18 years of age<br>iii) Ulcer surface area of 1-15 $cm^2$<br>iv) Free of infection according to clinical examination and radiographs<br>v) Adequate arterial blood supply i.e. TcPO2 $\geq$ 30 mmHg on the dorsum of the foot or at the margin of the ulcer if the ulcer was on the plantar surface<br>5. Exclusion criteria<br>i) People receiving medications that might adversely affect healing (systemic |

| | |
|---|---|
| | corticosteroids or antineoplastic agents)<br>ii) Medical conditions that might retard healing (immune system diseases, systemic lupus erythematosus, scleroderma, rheumatoid arthritis, osteomyelitis, bleeding disorders, Raynaud's, or cancer requiring chemotherapy) |
| Interventions | 1. Intervention group: arginine-glycine-aspartic acid peptide matrix (Argidence Gel, formerly Telio Derm Gel, Telios Pharmaceutics, San Diego, CA) applied with a change of dressing twice/week<br>2. Control group: normal saline placebo plus standard wound care<br>Co-interventions: debridements as required; shoes designed to relieve pressure on the study ulcer |
| Outcomes | 1. Completely healed<br>2. Safety |
| Notes | 1. A priori sample size estimation: not reported<br>2. Sponsor: Telios Pharmaceuticals, San Diego, CA<br>3. Role of sponsor: supported this research |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | Quote "A member of the study support staff other than the investigators applied the treatment . . . identical syringes were used to administer RGD peptide matrix and saline placebo" (p 3) |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | Lost post randomisation: total = 22% (14/65)<br>Intervention group = 20% (8/40); Control group = 24% (6/25)<br>Imbalance between group: 4%<br>Reasons<br>1. Adverse events: Intervention group = 4/8; Control group = 2/6<br>2. Other: Intervention group = 4/8; |

**Steed 1995b** (*Continued*)

| | | |
|---|---|---|
| | | Control group = 4/6 |
| Selective reporting (reporting bias) | High risk | The safety profile was incompletely reported |
| Other bias | High risk | Design bias and bias of presentation of data, see Appendix 1<br>Funding bias |

**Steed 1996**

| | |
|---|---|
| Methods | 1. Parallel-design (2 arms)<br>2. Country study: USA<br>3. Follow-up period: 20 weeks<br>4. Unit of randomisation: participant<br>5. Analysis unit: participant |
| Participants | 1. Randomised: 36 (number of participants in each arm and number of wounds: not reported)<br>2. Age: not reported<br>3. Gender: not reported<br>4. Inclusion criteria<br>i) Diabetic<br>ii) Neurotrophic foot ulcers<br>iii) TcPO2 on the dorsum of the foot ≥ 30 mmHg<br>iv) Clinically significant diabetic neuropathy<br>5. Exclusion criteria<br>i) ≥ 3 ulcers<br>ii) Infected bone |
| Interventions | 1. Intervention group: growth factors released from platelets (Curative Technologies, Inc., Setauket, N.Y.) were applied to the wound and covered with cotton gauze each evening. The vehicle for the platelet releasate was buffered saline solution, identical in appearance to the placebo.<br>2. Control group: buffered saline solution was applied to the wound and covered with cotton gauze each evening.<br>Co-interventions: half-shoe to redistribute weight, also used crutches, wheelchairs, or walkers for offloading |
| Outcomes | Recurrence rate of the diabetic neurotrophic ulcer |
| Notes | 1. This trial did not report the number of participants according to randomisation group or the characteristics of the groups. Trial reported the participants that healed and ulcer recurrence<br>2. A priori sample size estimation: not reported<br>3. Sponsor: not reported<br>4. Email sent to the main author requesting information on data |

**Steed 1996** (*Continued*)

| *Risk of bias* | | |
|---|---|---|
| **Bias** | **Authors' judgement** | **Support for judgement** |
| Random sequence generation (selection bias) | Unclear risk | Quote "patients were randomized to receive . . ." (p 231) |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | Quote "identical in appearance to the placebo" (p 231) |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Incomplete outcome data (attrition bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Selective reporting (reporting bias) | High risk | Safety data were not reported |
| Other bias | High risk | Design bias and Bias of presentation data, see Appendix 1 |

**Tan 2008**

| | |
|---|---|
| Methods | 1. Parallel-design (2 arms)<br>2. Country: China<br>3. Follow-up period: 6 weeks<br>4. Unit of randomisation: participant<br>5. Analysis unit: participant |
| Participants | 1. Number enrolled: not reported<br>2. Randomised:139<br>i) Intervention group 1 (rhaFGF):104 (75%)<br>ii) Intervention group 2 (rhbFGF): 35 (25%)<br>3. Age (mean years (range)): 52.2 ± 17.31 (18-75), information not reported by comparison group<br>4. Gender: not reported<br>5. Inclusion criteria<br>i) Chronic skin wound without other growth factor treatments (definition of "chronic state" was not reported)<br>ii) Skin wounds of ≥ 2 cm diameter through the full skin thickness<br>iii) Wound unhealed after at least 8 weeks of routine treatments<br>6. Exclusion criteria: not reported |

**Tan 2008** (*Continued*)

| | |
|---|---|
| Interventions | 1. Intervention group 1: rhaFGF 100 U/0.1 ml/$cm^2$ dissolved in normal saline solution and applied topically<br>2. Intervention group 2: rhbFGF 100 U/0.1 ml/ $cm^2$ dissolved in normal saline solution and applied topically<br>Co-interventions: sterile cotton dressing without antibiotics |
| Outcomes | Outcomes were not described explicitly as primary end points<br>Quote "after 6-week treatment, the wounds were divided into four categories: complete healing, significant healing if more than 50% of the wound are had healed; effective healing, if 20-50% of the wound area had healed; ineffective healing, if less than 20% of the wound had healed" (p 434) |
| Notes | 1. A priori sample size estimation: not reported<br>2. Sponsor: National Natural Science Foundation, Ministry of Education Incubation Foundation Technology Innovative Project, Program of New Century Excellent Tallents in University, Zhejian Provincial Program for the Cultivation of high-level Innovative Health Talents, Juvenile Diabetes Research Foundation, and Wenzhou Medical College for the Chinese-American Research Institute for Diabetic Complications<br>3. Role of sponsors: to provide grants |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Quote " . . . prospective study by randomly . . ." (p 433) |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Incomplete outcome data (attrition bias)<br>All outcomes | Unclear risk | Insufficient reporting of attrition/exclusions to permit a judgement of 'low risk' or 'high risk' (i.e. no reasons provided for missing data) |
| Selective reporting (reporting bias) | High risk | One or more outcomes of interest in the review were reported incompletely so that they could not be entered in a meta-analysis. This trial did not report safety data |

**Tan 2008** (*Continued*)

| | | |
|---|---|---|
| Other bias | High risk | 1. This study did not report baseline characteristics<br>2. Sampling bias, bias of presentation bias, design bias, see Appendix 1 |

**Tsang 2003**

| | |
|---|---|
| Methods | 1. Parallel-design (3 arms)<br>2. Country: China (Diabetes Ambulatory Care Centre)<br>3. Follow-up period: 24 weeks<br>4. Unit of randomisation: participant<br>5. Analysis unit: participant |
| Participants | 1. Randomised:48.03% (61/127 enrolled)<br>i) Intervention group 1 (Actovegin plus recombinant human epidermal growth factor 0.02%): 34.4% (21/61)<br>ii) Intervention group 2 (Actovegin plus recombinant human epidermal growth factor 0.04%): 34.4% (21/61)<br>iii) Control group (Actovegin 5%): 31.2% (19/61)<br>2. Age (years)<br>i) Intervention group 1: 68.76 ± 10.45<br>ii) Intervention group 2: 62.24 ± 13.68<br>iii) Control group: 64.37 ± 11.67<br>3. Gender (male)<br>i) Intervention group 1: 61.9% (13/21)<br>ii) Intervention group 2: 28.57% (6/21)<br>iii) Control group: 52.63% (10/19)<br>4. Lost before randomisation: total = 51.96% (66/127). Losses due to:<br>i) wound healing<br>ii) ulcer above malleoli<br>iii) ulcer grade ≥ 3<br>iv) ABPI < 0.7<br>v) refused consent<br>5. Inclusion criteria<br>i) Ulcer, Wagner grade 1 or 2<br>ii) Ulcer located below the ankle<br>iii) Ulcer with adequate perfusion, as indicated by an ABPI ≥ 0.7<br>6. Exclusion criteria<br>i) Very poor sugar control<br>ii) Ulcers with severity ≥ grade 3 |
| Interventions | 1. Intervention group 1: daily local application of Actovegin plus 0.02% recombinant human epidermal growth factor, covered with sterile gauze<br>2. Intervention group 2: daily local application of Actovegin plus 0.04% recombinant human epidermal growth factor, covered with sterile gauze<br>3. Control group: daily local application of Actovegin 5% cream only, covered with sterile gauze<br>Co-interventions: standard wound care consisted of debridement of necrotic tissue and |

**Tsang 2003** (*Continued*)

| | |
|---|---|
| | reduction of callus |
| Outcomes | Complete healing defined as full epithelialisation of the wound with an absence of discharge |
| Notes | 1. A priori sample size estimation: not reported<br>2. Sponsor: Bio-Click Technologies Ltd, Hong Kong<br>3. Role of sponsor: provided recombinant human epidermal growth factor<br>4. Actovegin is a protein free calf blood extract (NYCOMED Austria) (p 1857) |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Quote "randomization was performed by drawing envelopes" (p 1857) |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Unclear risk | Quote " . . . patients and physicians were blind to the hEGF concentrations" (p 1858)<br>This trial did not reported how blinding was performed |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Incomplete outcome data (attrition bias)<br>All outcomes | Unclear risk | Insufficient reporting of attrition/exclusions to permit a judgement of 'low risk' or 'high risk' (i.e. no reasons provided for missing data) |
| Selective reporting (reporting bias) | High risk | This trial did not report safety data |
| Other bias | High risk | Design bias, see Appendix 1<br>Funding bias<br>This was the first trial by this clinical group that assessed recombinant human epidermal growth factor, however, it did not investigate safety |

**Uchi 2009**

| | |
|---|---|
| Methods | 1. Parallel-design (3 arms)<br>2. Country: Japan<br>3. Follow-up period: 8 weeks<br>4. Unit of randomisation: participant<br>5. Analysis unit: participant |
| Participants | 1. Randomised: 150<br>i) Intervention 1 group (basic fibroblast growth factor 0.001%): 32.7% (49/150), 1 participant withdrew consent before starting treatment<br>ii) Intervention 2 group (basic fibroblast growth factor 0.01%): 33.3% (50/150), 1 participant did not receive growth factor as ulcer healed before starting treatment<br>iii) Control group (placebo): 34.0% (51/150)<br>2. Age (years, mean ± SD)<br>i) Intervention 1 group (basic fibroblast growth factor 0.001%): 61.0 ± 13.0<br>ii) Intervention 2 group (basic fibroblast growth factor 0.01%): 59.8 ± 13.8<br>iii) Control group (placebo): 60.2 ± 11.7<br>3. Gender (male)<br>i) Intervention 1 group (basic fibroblast growth factor 0.001%): 66.6% (32/48)<br>ii) Intervention 2 group (basic fibroblast growth factor 0.01%): 71.4% (35/49)<br>iii) Control group (placebo): 72.5% (37/51)<br>4. Inclusion criteria<br>i) ≥ 20 years of age<br>ii) Attending physicians selected a targeted ulcer in presence of multiple ulcers<br>iii) Ulcers measuring < 900 $mm^2$, not reaching the periosteum (Wagner grade 2)<br>iv) ABPI at rest ≥ 0.9 in participants with no palpable pulsation in either artery<br>5. Exclusion criteria<br>i) Severe artery calcification due to maintenance haemodialysis or diabetes<br>ii) Malignant tumour or history of malignant tumour<br>iii) History of hypersensitivity to basic fibroblast growth factor<br>iv) Women with confirmed or suspected pregnancy; nursing women; women who desired to become pregnant during the trial<br>v) Patients receiving oral administration or injection of adrenocortical steroid (equivalent to > 20 mg/day of prednisolone) |
| Interventions | 1. Intervention 1: 5 puffs of basic fibroblastic growth factor at 0.001% (equivalent to 3 µg of basic fibroblastic growth factor) sprayed 5 cm from the target ulcer once a day, for 8 weeks<br>2. Intervention 2: 5 puffs of basic fibroblastic growth factor at 0.01% (equivalent to 30 µg of basic fibroblastic growth factor) sprayed 5 cm from the target ulcer once a day, for 8 weeks<br>3. Control: 5 puffs placebo (characteristics not reported) sprayed 5 cm from the target ulcer once a day for 8 weeks<br>Co-interventions: appropriate treatments to control blood glucose levels |
| Outcomes | Primary outcomes:<br>1. Ulcer shrinkage<br>2. Safety |

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|---|---|
| Notes | 1. A priori sample size estimation: yes (p 463)<br>2. Sponsor: Kaken Pharmaceutical Co, Ltd<br>3. Role of sponsor: data collection and pre-specified statistical analysis |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Quote " . . . computer-generated randomisation program" (p 462) |
| Allocation concealment (selection bias) | Low risk | Quote " . . . assigned to groups by telephone or fax at the KCB-1 Registration Center (ADJUST Co., LTD., Kokkaido, Japan)" (p 462) |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | Quote " . . . participants in the blinded trial included physicians, evaluators, patients, and monitor." (p 462) |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Low risk | Quote " . . . participants in the blinded trial included physicians, evaluators, patients, and monitors." (p 462) |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | Lost post randomisation (withdrew within 4 weeks): total = 6% (9/150)<br>Reasons<br>1. Basic fibroblast growth factor 0.001% group: 1 participant withdrew within 4 weeks because of an adverse reaction<br>2. Basic fibroblast growth factor 0.01% group: 4 withdrawals; 1 participant withdrew within 4 weeks because of an adverse reaction, and 3 were excluded within 4 weeks because of protocol violations<br>3. Placebo group: 4 withdrawals; 1 adverse reaction; 1 died because of renal failure; 1 withdrew because ulcer healed; and there were no photographs for 1 |
| Selective reporting (reporting bias) | High risk | This trial did not report safety data |
| Other bias | High risk | Bias in presentation of data. see Appendix 1<br>Funding bias |

**Viswanathan 2006**

| | |
|---|---|
| Methods | 1. Parallel-design (2 arms)<br>2. Phase III trial<br>3. Country: India<br>4. Follow-up period: 15 weeks<br>5. Unit of randomisation: participant<br>6. Analysis unit: participant |
| Participants | 1. Randomised: 60<br>i) Intervention group (recombinant human epidermal growth factor): 50% (30/60)<br>ii) Control group: 50% (30/60)<br>2. Age (years): not reported<br>3. Gender (male):not reported<br>4. Inclusion criteria<br>i) Target ulcers 2-50 $cm^2$ in area<br>ii) People available for the 15-week study period who could adhere to the treatment regimen<br>iii) Healthy men or women aged 18-65 years at the time of consent<br>iv) Women of non-child bearing potential (e.g. surgically sterilised), or, if of child bearing potential, must have a negative pregnancy test, or have used adequate contraceptive precautions (as confirmed by the investigator) 30 days prior to screening and the baseline visit, and agreed to continue such precautions up to week 15<br>v) Controlled diabetes mellitus (types 1 and 2) and foot ulcers<br>vi) Ulcers that remained open without healing for more than 2-3 weeks (irrespective of the ambulatory treatment administered)<br>vii) ABPI reading $\geq$ 0.75<br>5. Exclusion criteria<br>i) Ulcer $\geq$ Wagner grade 3<br>ii) Those with life-threatening gastrointestinal, hepatic, renal, endocrine, hematological, or immunologic disorder, or serious cardiac failure (New York Heart Association Grades 3 and 4)<br>iii) Any of the following factors: hypertension grade 3; known case of hypersensitivity to the ingredient(s); uncontrolled diabetes mellitus (type 1 or 2), diabetic ketoacidosis or coma<br>iv) Pregnant women and nursing mothers<br>v) Past history of, or current, acute or chronic autoimmune disease<br>vi) Chronic alcohol abuse (40 mL/day for at least 6 months)<br>vii) Treatment known to impair wound healing, including but not limited to: corticosteroids, immunosuppressive drugs, cytotoxic agents, radiation therapy, and chemotherapy, within 1 month prior to the initial visit<br>viii) Use of any marketed, investigational, or herbal medicine or non-registered drug for wounds or burns in the past 6 months<br>ix) Clinically relevant abnormal haematology or biochemistry values (in the opinion of the investigator)<br>x) Any criteria that, in the opinion of the investigator, suggest non-compliance with the study<br>xi) Evidence of systemic or local infection, such as purulent drainage, osteomyelitis, or nonviable tissue that cannot be removed by debridement<br>xii) Treatment with a dressing containing any other growth factors or other |

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|---|---|
| | biological dressings within the 30 days preceding the screening visit<br>xiii) Participation in another clinical study within the 30 days preceding the screening visit or during the study |
| Interventions | 1. Intervention group: topical application of recombinant human epidermal growth factor gel twice daily until the wound healed or until the end of week 15, whichever was earlier<br>2. Control group: topical application of placebo (water based) gel twice daily until the wound healed or until the end of week 15, whichever was earlier<br>Co-interventions: normal dose of insulin prescribed; oral and intravenous antibiotics for prevention of infection |
| Outcomes | 1. Percentage of healing<br>2. Duration of healing<br>3. Quality of healing and epithelisation |
| Notes | 1. Trial did not report the baseline characteristics of the groups<br>2. A priori sample size estimation: not reported<br>3. Sponsor: Bharat Biotech International Limited, Hyderabad, India<br>4. Role of sponsor: financial support<br>5. Conflict of interest: main author was paid an investigator's fee for conducting the study, another author is a consultant for the company that provided the study supplies and that supported study financially |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | Quote "the tubes containing either rhEGF or placebo were similar" |
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | Lost: 5% (3/60): Intervention group = 1; Control group = 2 |
| Selective reporting (reporting bias) | High risk | One or more outcomes of interest in the review were reported incompletely so that they could not be entered in a meta-analysis<br>Quote "The recorded adverse events were |

**Viswanathan 2006** (*Continued*)

| | | |
|---|---|---|
| | | 1 case of rash, 3 cases of pain, and 2 cases of skin irritation" (p 4/14)<br>This trial did not report adverse event data according to comparison group |
| Other bias | High risk | Design bias and bias of presentation bias, see Appendix 1<br>Funding bias: main author was paid an investigator's fee for conducting the study, another author is a consultant for the company that provided the study supplies and that supported study financially |

**Wieman 1998a**

| | |
|---|---|
| Methods | 1. Parallel-design (3 arms)<br>2. Country: USA (23 sites)<br>3. Follow-up period: 20 weeks<br>4. Unit of randomisation: participant<br>5. Analysis unit: participant |
| Participants | 1. Randomised: 382<br>i) Intervention 1 (recombinant human platelet-derived growth factor 30 µg/g): 34.6% (132/382)<br>ii) Intervention 2 (recombinant human platelet-derived growth factor 100 µg/g): 32.2% (123/382)<br>iii) Control group (placebo): 33.2% (127/382)<br>2. Age (years, means ± SD)<br>i) Intervention 1 (recombinant human platelet-derived growth factor 30 µg/g): 58 ± 11.3<br>ii) Intervention 2 (recombinant human platelet-derived growth factor 100 µg/g): 57 ± 11.5<br>iii) Control group (placebo): 58 ± 11.8<br>3. Gender (male)<br>i) Intervention 1 (recombinant human platelet-derived growth factor 30 µg/g): 62% (82/132)<br>ii) Intervention 2 (recombinant human platelet-derived growth factor 100 µg/g): 67% (82/123)<br>iii) Control group (placebo): 72% (91/127)<br>4. Inclusion criteria<br>i) Aged ≥19 years with type 1 or type 2 diabetes<br>ii) At least 1 full thickness (stage III or IV, as defined in the International Association of Enterostomal Therapy guide to chronic wound staging chronic ulcer of the lower extremities. If > 1 lower-extremity ulcer present, the target ulcer was the one that, in the opinion of the investigator, would take the longest time to heal with good wound care practice<br>iii) Target ulcer present for at least 8 weeks despite previous treatment<br>iv) TcPO2 on the limb with the target ulcer = 30 mmHg |

**Wieman 1998a** (*Continued*)

| | |
|---|---|
| | 5. Exclusion criteria<br>i) Osteomyelitis present in the area of the target ulcer<br>ii) Target ulcer area < 1 $cm^2$ or > 40 $cm^2$ after debridement<br>iii) Sum of the areas of all ulcers present > 100 $cm^2$<br>iv) Ulcers resulting from any cause other than diabetes (e.g. electrical, chemical, or radiation insult)<br>v) Concomitant diseases (e.g. connective tissue disease), treatment (e.g. radiation therapy), or medication (e.g. corticosteroids, chemotherapy, or immunosuppressive agents) that would present safety hazards or interfere with evaluation of the study medication<br>vi) Women who were pregnant, nursing, or of childbearing potential and not using either an intrauterine device or oral contraception<br>vii) People with cancer |
| Interventions | 1. Intervention group 1: topically applied recombinant human platelet-derived growth factor gel (becaplermin, 30 µg/g), twice daily, morning and evening<br>2. Intervention group 2: topically applied recombinant human platelet-derived growth factor gel (becaplermin, Regranex Gel 0.01%, 100 µg/g), twice daily, morning and evening<br>3. Control: topically applied placebo gel, twice daily, morning and evening<br>Co-intervention: standardised regimen of good wound care |
| Outcomes | Primary<br>1. Percentage of participants that achieved complete healing within the 20-week study period<br>2. Safety<br>Secondary: time required to achieve complete healing |
| Notes | 1. A priori sample size estimation: not reported<br>2. Sponsor: not reported |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Quote "patients were randomized to one of three . . . " (p 823)<br>Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Blinding of participants and personnel (performance bias)<br>All outcomes | Low risk | Quote "the placebo gel was identical to the vehicle component of the gel formulation containing the active drug" (p 823) |

| | | |
|---|---|---|
| Blinding of outcome assessment (detection bias)<br>All outcomes | Unclear risk | Insufficient information provided to permit a judgment of 'low risk' or 'high risk' |
| Incomplete outcome data (attrition bias)<br>All outcomes | Unclear risk | Lost post randomisation: total = 19% (73/382)<br>1. Intervention group 1 = 21% (28/132)<br>2. Intervention group 2 = 17% (21/123)<br>3. Control group = 19% (24/127)<br>Reasons<br>1. Lost to follow up: Intervention group 1 = 1/28; Intervention group 2 = 1/21; Control group = 2/24<br>2. Adverse events: Intervention group 1 = 17/28; Intervention group 2 = 13/21; Control group = 13/24<br>3. Non compliance: Intervention group 1 = 4/28; Intervention group 2 = 3/21; Control group = 3/24<br>4. Protocol violation: Intervention group 1 = 2/28; Intervention group 2 = 2/21; Control group = 3/24<br>5. Other: Intervention group 1 = 4/28; Intervention group 2 = 2/21; Control group = 3/24 |
| Selective reporting (reporting bias) | High risk | One or more outcomes of interest in the review were reported incompletely so they could not be entered in a meta-analysis |
| Other bias | High risk | Quote "the time to complete healing, defined as the number of days until the patients achieved a functional assessment score of 1, was analyzed using Cox's proportional hazards model" (p 824)<br>Design bias and bias of data presentation, see Appendix 1 |

**Abbreviations**
ABPI = ankle-brachial pressure index
CT-102 = thrombin-induced platelet-released platelet-derived wound healing formula
Hb = haemoglobin
HbA1c = glycated haemoglobin
IAET = The International Association of Enterostomal Therapists (now known as the Wound, Ostomy, Continence Nurses' Society (WOCN))
ITT = intention to treat (analysis)

IV = intravascular
rhaFGF = recombinant human acidic fibroblast growth factor
rhbFGF = recombinant human basic fibroblast growth factor
rhEGF = recombinant human epidermal growth factor
TcPO2 = transcutaneous oxygen tension

## Characteristics of excluded studies *[ordered by study ID]*

| Study | Reason for exclusion |
|---|---|
| Acosta 2006 | Case report |
| Aminian 2000 | Not an randomised clinical trial |
| Embil 2000 | Case series |
| Hong 2006 | Case series |
| Miller 1999 | Case report |
| Mohan 2007 | Phase IV (post-marketing surveillance study) |
| Saad Setta 2011 | Not an randomised clinical trial |
| Tuyet 2009 | Case report |
| Yera-Alos 2013 | Phase IV (post-marketing surveillance study) |

## Characteristics of studies awaiting assessment *[ordered by study ID]*

**Gomez-Villa 2014**

| | |
|---|---|
| Methods | |
| Participants | |
| Interventions | |
| Outcomes | |
| Notes | awaiting full text retrieval |

**Morimoto 2013**

| | |
|---|---|
| Methods | |
| Participants | |
| Interventions | |
| Outcomes | |
| Notes | awaiting full text retrieval |

**Singla 2014**

| | |
|---|---|
| Methods | |
| Participants | |
| Interventions | |
| Outcomes | |
| Notes | awaiting full text retrieval |

**Young 1992**

| | |
|---|---|
| Methods | 1. Phase I/II, double-blind, placebo-controlled, parallel design (3 arms)<br>2. Country: United Kingdom<br>3. Intention to treat: unclear<br>4. Follow-up period: not reported<br>5. Unit of randomisation: not reported**<br>6. Analysis unit: participant<br>**Neither the title nor abstract of this study indicated whether it was randomised. We have not been able to find the address of the authors, enquiries are ongoing |
| Participants | 1. Participants: 25<br>i) Intervention 1 (PDGF high dose): 14 (56%)<br>ii) Intervention 2 (low dose and placebo): 11 (44%)<br>2. Gender (male): not reported<br>3. Age (years, means ± SD): not reported<br>4. Inclusion criteria: not given, so it reported<br>i) Diabetic participants<br>ii) Neuropathic foot ulceration<br>5. Exclusion criteria: not reported |
| Interventions | 1. Intervention 1: daily application of PDGF gel 0.2 ml/$cm^2$<br>2. Intervention 2: participants applied placebo, 10 mg/ml, 100 mg/ml or 1000 mg/ml<br>Co-interventions: appropriate pressure relief with orthoses or casts and weekly chiropody |
| Outcomes | Not stated, so it reported complete healing, safety |

**Young 1992** (*Continued*)

| | |
|---|---|
| Notes | 1. Data extracted from abstract<br>2. A priori sample size estimation: no<br>3. Sponsor: not reported<br>4. This trial reported these results:<br>i) high dose group: complete healing (5/14)<br>ii) low dose group and placebo: complete healing: 1/11<br>iii) Quote: "no significant differences in median healing rates were found"<br>5. This trial did not report safety data, however, the authors stated " topical PDGF is well tolerated"<br>6. It is unclear what the trial authors meant by 'high dose' and 'low dose |

**Abbreviation**
PDGF = platelet-derived growth factor

## Characteristics of ongoing studies *[ordered by study ID]*

**NCT00521937**

| | |
|---|---|
| Trial name or title | A prospective, randomised, multi-centre, blind-observer, controlled, parallel-group study comparing the efficacy and safety of DERMAGEN® versus conventional treatment in the treatment of diabetic neuropathic foot ulcer |
| Methods | 1. Allocation: randomised<br>2. Endpoint classification: efficacy study<br>3. Intervention model: parallel assignment<br>4. Masking: single blind (outcomes assessor)<br>5. Primary purpose: treatment<br>6. Country: France |
| Participants | 1. Enrolled: 388<br>2. Inclusion criteria<br>i) Age $\geq$ 18 years<br>ii) to here Documented, stable type 1 or 2 diabetes mellitus (confirmed by HbA1c at least every 4 months)<br>iii) Neuropathic foot ulcer located on the plantar surface of the forefoot<br>iv) Ulcer surface area 1-15 $cm^2$ (after mechanical debridement of the ulcer)<br>v) Palpable pulse evidenced on both feet (presence of dorsalis pedis pulse and posterior tibial pulse) or in absence of one pulse, an SPI by Doppler > 0.9 on the target limb<br>vi) Presence of diabetic foot ulcer for at least 4 weeks prior to enrolment<br>vii) Ulcer extending through the dermis without exposure of muscle, tendon, bone, or joint capsule<br>3. Exclusion criteria<br>i) Typical Charcot's foot<br>ii) Decrease or increase in the size of the ulcer by $\geq$ 50% during the run-in period<br>iii) Presence of osteitis (eq Br osteomyelitis) at the inclusion visit (evidenced with a radiological lesion facing the wound (bone erosion or disappearance of the cortical bone))<br>iv) Clinical evidence of PEDIS* grade 3, or 4 infection at the inclusion visit<br>v) People who cannot have/use an offloading method |

**NCT00521937** (*Continued*)

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|---|---|
| | vi) Working people who could not be on sick-leave during the study period<br>vii) Known allergy to collagen, streptomycin, penicillin and/or products of bovine origin<br>viii) People requiring dialysis<br>ix) Untreated psychiatric disorder<br>x) Clinical evidence of gangrene on any part of the affected foot<br>xi) People receiving corticosteroids, NSAIDs, immunosuppressive or cytotoxic agents, or systemic agents that could affect wound repair or any treatment that might interfere with the assessment of the study treatment |
| Interventions | Intervention: Dermagen®<br>Control: Conventional treatment |
| Outcomes | 1. Primary outcome measure: complete wound closure at week 12: time-frame = 12 weeks; not designated as a safety issue<br>2. Secondary outcome measure: time to complete wound healing: time-frame = 24 weeks; not designated as a safety issue |
| Starting date | January 2009 (date of first enrolment) |
| Contact information | Name: Olivier Chosidow, MD, PhD<br>Address: not reported<br>Telephone: not reported<br>Email: not reported<br>Affiliation: Hôpital Tenon, Paris |
| Notes | * Pedis is a classification system for diabetic foot ulcers in people with diabetes mellitus (Schaper 2004). See Appendix 3 for details.<br>Target sample size: 388<br>Register: ClinicalTrials.gov<br>Last refreshed on: 21 December 2010<br>Main ID: NCT00521937<br>Date of registration: 27 August 2007<br>Primary sponsor: Laboratoires Genévrier<br>Recruitment status: active, not recruiting<br>URL: NCT00521937<br>Source(s) of monetary support: Laboratoires Genévrier |

**NCT00709514**

| | |
|---|---|
| Trial name or title | A phase II, double-blind, placebo-controlled clinical evaluation of DCB-WH1 in healing of chronic diabetic foot ulcers |
| Methods | 1. Allocation: randomised<br>2. Endpoint classification: safety/efficacy study<br>3. Intervention model: parallel assignment<br>4. Masking: double blind (subject, caregiver, investigator, outcomes assessor)<br>5. Primary purpose: treatment<br>6. Country Taiwan |

**NCT00709514** (*Continued*)

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|---|---|
| Participants | 1. Enrolled: 50<br>2. Inclusion criteria<br>i) ≥ 20 years with diabetes mellitus (type 1 or 2) and a cutaneous ulcer on the foot that has been present for at least 2 weeks<br>ii) Grade 1 target ulcer according to a modified Wagner system, which includes wounds involving the epidermis, the dermis, the hypodermis or the subcutaneous fat but not the tendon or joint capsule. The thickness of these layers should be approximately 0.2-8 mm and wounds should have an area of 3-15 $cm^2$ post debridement<br>iii) ABPI ≥ 0.80<br>iv) Study ulcer should show "infection control" as judged by the investigator<br>v) Free of any necrotic or infected soft and bony tissue<br>vi) Signed informed consent form<br>3. Exclusion criteria<br>i) Ulcers caused by venous or arterial insufficiency, osteomyelitis<br>ii) Poor nutritional status (albumin < 3 g/dl), poor diabetic control (HbA1c > 10%), anaemia (Hb < 10 g/dL), leukocyte count < 1000/$mm^3$<br>iii) Requiring prostaglandin treatment<br>iv) Requiring treatment with corticosteroids, immunosuppressive or chemotherapeutic agents, radiotherapy<br>v) Presence of necrosis, purulence or sinus tracts that cannot be removed by debridement<br>vi) Presence of connective tissue disease, renal failure (eGFR ≦ 30 ml/min/1.73 $m^2$), abnormal liver function (AST, ALT > 2.5 x upper limit of normal range), malignancy<br>vii) Vascularisation surgery performed < 8 weeks before entry into the study<br>viii) History of cerebrovascular events, coronary intervention (stent or CABG) or myocardial infarction, within 6 months prior to study<br>ix) Female patient with a positive pregnancy test, or breastfeeding, or unwilling to use appropriate contraceptive methods during study |
| Interventions | 1. Experimental intervention: 1.25% DCB-WH1 ointment topically applied twice daily<br>2. Placebo intervention: no details reported |
| Outcomes | Primary outcome measure: incidence of complete ulcer closure: time-frame = 12 weeks; designated as a safety issue |
| Starting date | May 2008 (date of first enrolment) |
| Contact information | Name: David Yeh, Director<br>Address: not reported<br>Telephone: +886 2 26558098<br>Email: davidyeh@microbio.com.tw<br>Affiliation: not reported |
| Notes | PEDIS is<br>Target sample size: 50<br>Register: ClinicalTrials.gov<br>Last refreshed on: 8 March 2011<br>Main ID: NCT00709514<br>Date of registration: 27 June 2008<br>Primary sponsor: Oneness Biotech Co, Ltd |

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| | Recruitment status: completed<br>URL: http://clinicaltrials.gov/show/NCT00709514<br>Source(s) of monetary support: Oneness Biotech Co, Ltd |

**NCT00915486**

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|---|---|
| Trial name or title | A randomized, multi-center, controlled, parallel group, dose finding study of the efficacy and safety of topically applied I-020201 as an adjunct to good standard-of-care versus good standard-of-care alone in patients with chronic diabetic foot ulcers |
| Methods | 1. Allocation: randomised<br>2. Endpoint classification: safety/efficacy study<br>3. Intervention model: parallel assignment<br>4. Masking: double blind (subject, caregiver, investigator, outcomes assessor)<br>5. Primary purpose: treatment<br>6. Country:Czech Republic, Germany, Hungary, Romania, Russian Federation,Serbia |
| Participants | Enrolled: 210<br>1. Inclusion criteria<br>i) Age $\geq$ 18 years<br>ii) Provided written informed consent<br>iii) Women of childbearing potential with a negative result from pregnancy test at screening who agree to use an acceptable birth control method (hormonal or IUD), or abstinence, throughout the trial<br>iv) Type 1 or type 2 diabetes mellitus with HbA1c $\leqq$ 12%<br>v) Only 1 diabetic foot ulcer on the foot to be treated, on or below the ankle<br>2. Exclusion criteria<br>i) Pregnant or breast-feeding<br>ii) Known or suspected allergies to any of the components of the I-020201<br>iii) Uncontrolled anaemia (Hb < 9 g/dL in women and < 10 g/dL in men)<br>iv) Hypoalbuminaemia (albumin < 3 g/dL)<br>v) Overtly infected target ulcer (as judged by investigator)<br>vi) Highly exuding wounds (wounds that require a daily dressing change)<br>vii) Osteomyelitis<br>viii) Systemic infections<br>ix) Acute Charcot foot and severe chronic Charcot deformity<br>x) ABPI < 0.7 or ankle systolic pressure < 70 mmHg<br>xi) One of the following:<br>a) monophasic or biphasic flow (with loss of reverse flow) in either foot artery, or a toe on Doppler waveform analysis on the dorsalis pedis and posterior tibial arteries<br>b) brachial index < 0.7<br>c) TcPO2 < 40 mmHg<br>xii) Suspicion, presence or history of systemic or local cancer or tumour of any kind |
| Interventions | 1. Group 1: Good Standard of Care: Experimental (GSoC): twice per week<br>2. Group 2: GSoC + vehicle (topical fibrin as an adjunct to GSoC twice per week)<br>3. Group 3: GSoC + I-020201 (33 $\mu$g) topical as an adjunct to GSoC twice per week<br>4. Group 4: GSoC + I-020201 (100 $\mu$g) topical as an adjunct to GSoC twice per week<br>5. Group 5: GSoC + I-020201 (300 $\mu$g) topical as an adjunct to GSoC twice per week |

**NCT00915486** (*Continued*)

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|---|---|
| Outcomes | 1. Primary outcome: percentage reduction in ulcer surface area: time-frame = 4 weeks after start of treatment<br>2. Secondary outcomes<br>i) Changes in systemic PDGF-AB and antibody levels against TG-PDGF.AB and aprotinin: time-frame: 1, 4, 12, 16 and 20 weeks after start of treatment<br>ii) Changes in vital signs, body weight, physical examination and laboratory parameters: time-frame: throughout the study and 28 weeks after start of treatment<br>iii) Incidence of complete wound closure (full re-epithelialisation with confirmation 4 weeks afterwards): time-frame = 12 and 16 weeks after start of treatment<br>iv) Incidence of complete wound closure (full re-epithelialisation with confirmation 4 weeks afterwards): time-frame = whole study period (28 weeks after start of treatment)<br>v) Incidence of participants with ulcer recurrence: time-frame = up to 16 and 28 weeks after start of treatment<br>vi) Incidence of treatment failure defined as < 30% decrease in ulcer size: time-frame = after 8 weeks of treatment<br>vii) Incidence of treatment-related AEs (systemic and at the target ulcer) and all AEs/SAEs: time-frame = whole study period<br>viii) Time to complete wound closure (full re-epithelialisation with confirmation 4 weeks afterwards): time-frame = at any time during the study |
| Starting date | May 2009 (date of first enrolment) |
| Contact information | Name: Mitra Safari<br>Address: not reported<br>Telephone: +41 44 200 5600<br>Email: mitra.safari@kuros.ch<br>Affiliation: not reported |
| Notes | 1. Target sample size: 210<br>2. Register: ClinicalTrials.gov<br>3. Last refreshed on: 25 January 2011<br>4. Main ID: NCT00915486<br>5. Date of registration: 5 June 2009<br>6. Primary sponsor: Kuros Biosurgery AG<br>7. Recruitment status: completed<br>8. URL: http://clinicaltrials.gov/show/NCT00915486<br>9. Source(s) of monetary support: Kuros Biosurgery AG |

**NCT00926068**

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|---|---|
| Trial name or title | Safety and efficacy of HO/03/03 10 µg in the treatment of plantar neuropathic diabetic foot ulcers (Truheal) |
| Methods | 1. Allocation: randomised<br>2. Endpoint classification: safety/efficacy study<br>3. Intervention model: parallel assignment<br>4. Masking: double blind (subject, investigator)<br>5. Primary purpose: treatment<br>6. Country: USA |

**NCT00926068** (*Continued*)

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|---|---|
| Participants | 1. Enrolled: 196<br>2. Inclusion criteria<br>i) Age 18-80 years, extremes included<br>ii) Diagnosed with diabetes mellitus type 1 or 2<br>iii) A documented single, target, non-healing, plantar neuropathic diabetic foot ulcer with a minimal duration of 4 weeks prior to giving informed consent<br>iv) Ulcer size at randomisation: Wagner grade 1, 2.0 -10 $cm^2$ extremes included, or Wagner grade 2, 1.0-10 $cm^2$ extremes included<br>v) Single target ulcer on the study foot: Wagner grade 1 or 2 (does not involve abscess or osteomyelitis)<br>vi) Target ulcer area decreased by $\leq$ 30%, or $\leq$ 0.1 cm/week edge healing rate measured, between post-debridement values at screening and at randomisation, if debridement clinically indicated<br>vii) HbA1c $\leq$ 12%<br>viii) Ankle to Brachial Index (ABI) on study foot: 0.7 $\leq$ ABI $\leq$ 1.2 or ABI > 1.2 and toe pressure > 50 mm Hg (ABI measured by Doppler; toe blood pressure measured by toe cuff)<br>ix) Diabetic neuropathy confirmed by neurological testing<br>x) Participants available for entire study period, and able and willing to adhere to protocol requirements<br>xi) Signed informed consent form prior to any study protocol-related procedure<br>3. Exclusion criteria<br>i) Medical history, current or within the last 2 years, of abuse of alcohol, barbiturates, benzodiazepines, amphetamines, narcotics, cocaine, psychoactive drugs or other substances that interfere with treatment compliance<br>ii) Use of growth factors, skin graft or participation in an investigational study within 30 days prior to the start of the screening period<br>iii) Women who are pregnant, lactating, of childbearing potential, or post-menopausal for < 2 years and not using a medically approved method of contraception, or who test positive on a blood-based pregnancy test<br>iv) A documented medical history of HIV, HBV or HCV<br>v) A documented significant cardiac, pulmonary, gastrointestinal, endocrine (other than diabetes mellitus type 1 or 2), metabolic, neurological, hepatic or nephrologic disease and/or receiving dialysis<br>vi) Anaemia (Hb < 9 g/dL for women, or < 10 g/dL for men) or white blood cell count > 11,000/$\mu$L or platelet count < 100,000/$\mu$L or impaired renal function (creatinine > 3 mg/dL) or liver function tests > 3 times upper normal laboratory values or any indication of malnourishment (albumin < 2.8 g/dL) or any other clinically significant biochemistry, haematology and urinalysis tests<br>vii) Any clinically significant illness during the 4 weeks preceding the screening period<br>viii) Current or previous (within last 5 years) malignancy, other than basal cell carcinoma, or is treated by radio/chemotherapy<br>ix) Any signs of clinical infection in the wound (which could be linked to raised body temperature, abscess, osteomyelitis, necrosis or erythema)<br>x) Received any antibiotic treatment during the screening period<br>xi) Evidence of infection or osteomyelitis on a plain foot X-ray at screening<br>xii) Bed-ridden or unable to come to the clinic<br>xiii) > 1 target non-healing diabetic foot ulcer per subject<br>xiv) Plantar neuropathic diabetic foot ulcer is located on an active Charcot foot<br>xv) Hind foot ulcer or foot deformity/condition that prevents the use of offloading footwear<br>xvi) Revascularisation leg surgery within the last 6 months, or a candidate for revascularisation surgery |

**NCT00926068** (*Continued*)

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| | during the course of the study<br>xvii) Glucocorticosteroid treatment (prednisone > 10 mg/day or equivalent)<br>xviii) Inability to stop alternative wound healing treatment (e.g. becaplermin or other topical products) following debridement |
| Interventions | 1. Intervention: HO/03/03 10 µg<br>2. Control: placebo comparator |
| Outcomes | 1. Primary outcome measures<br>i) Complete ulcer closure: time-frame = up to 14 weeks inclusive; not designated as a safety issue<br>ii) Time to event analysis to determine time for incidence of 100% study wound closure per unit of time (days) and the incidence of 100% wound closure per unit of time using the log rank test<br>2. Secondary outcome measures<br>i) Percentage change in wound area at 4 weeks: time-frame = 4 weeks; not designated as a safety issue<br>ii) 75% wound closure by or on study week 14: time-frame = up to 14 weeks inclusive: not designated as a safety issue<br>iii) Incidence of AEs, changes in vital signs, physical examination, electrocardiogram and laboratory tests from baseline to termination: time-frame = 14 weeks; designated as a safety issue<br>iv) Incidence of 100% closure according to the Fisher exact 2-tailed test: time-frame = up to 14 weeks inclusive; not designated as a safety issue<br>v) Percentage change in granulation tissue at 4 weeks: time-frame = 4 weeks; not designated as a safety issue<br>vi) Incidence of improved ulcers; not designated as a safety issue |
| Starting date | February 2010 (date of first enrolment) |
| Contact information | Name: Talma Gotteiner, MPharm<br>Address: not reported<br>Telephone: +97289407188<br>Email: talma@healor.com<br>Affiliation: not reported |
| Notes | 1. Target sample size: 146<br>2. Register: ClinicalTrials.gov<br>3. Last refreshed on: 22 March 2011<br>4. Main ID: NCT00926068<br>5. Date of registration: 22 June 2009<br>6. Primary sponsor: HealOr<br>7. Recruitment status: recruiting<br>8. URL: http://clinicaltrials.gov/show/NCT00926068<br>9. Source(s) of monetary support: HealOr |

**NCT01060670**

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|---|---|
| Trial name or title | A multi-center, randomized, controlled clinical trial to evaluate the safety and effectiveness of Integra® Dermal Regeneration Template for the treatment of neuropathic diabetic foot ulcers |
| Methods | 1. Allocation: randomised<br>2. Endpoint classification: safety/efficacy study<br>3. Intervention model: parallel assignment<br>4. Masking: open label<br>5. Primary purpose: treatment<br>6. Country: USA |
| Participants | 1. Enrolled: 350<br>2. Inclusion criteria<br>i) Type 1 or 2 diabetes mellitus<br>ii) HbA1c $\leq$ 12%<br>iii) Diabetic foot ulcer located below the ankle and/or on the bottom of the foot that has been present for 30 days and is of sufficient size to qualify for the study<br>iv) Good vascular perfusion of the affected limb<br>3. Exclusion criteria<br>i) Gangrene, infection, or osteomyelitis<br>ii) Sensitivity to bovine collagen or chondroitin, or both<br>iii) Ulcers resulting from other health conditions besides diabetes<br>iv) Conditions or laboratory values that are not within the specified ranges |
| Interventions | 1. Intervention: dermal replacement device. Application in diabetic foot ulcer<br>2. Control: moist wound therapy<br>3. Co-intervention: saline plus secondary dressing and conventional wound therapy |
| Outcomes | 1. Primary outcome measure: incidence of complete wound closure: time-frame = 16 weeks; not designated as a safety issue<br>2. Secondary outcome measures<br>i) Time to complete wound closure: time-frame = 28 weeks; not designated as a safety issue<br>ii) Incidence of recurrence: time-frame = 28 weeks; not designated as a safety issue<br>iii) Incidence of adverse events: time-frame = 28 weeks; designated as a safety issue |
| Starting date | April 2010 (date of first enrolment) |
| Contact information | Name: Nicola Fenty-Stewart, PhD<br>Address: not reported<br>Telephone: 1-609-275-0500 (http://www.integralife.com/index.aspx?redir=contact) Accessed on 3 September 2014<br>Email: nicolafs@amarexcro.com<br>Affiliation: not reported |
| Notes | 1. Target sample size: 350<br>2. Register: ClinicalTrials.gov<br>3. Last refreshed on: 3 June 2010<br>4. Main ID: NCT01060670<br>5. Date of registration: 31 January 2010<br>6. Primary sponsor: Integra LifeSciences Corporation |

**NCT01060670** (*Continued*)

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| | 7. Recruitment status: completed<br>8. URL: http://clinicaltrials.gov/show/NCT01060670<br>9. Source(s) of monetary support: Integra LifeSciences Corporation |

**NCT01098357**

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|---|---|
| Trial name or title | A phase I/II, multicentre, randomised, controlled, and open-label trial comparing the efficacy and safety of three dose regimens of BioChaperone PDGF-BB to becaplermin gel for the treatment of diabetic foot ulcer |
| Methods | 1. Allocation: randomised<br>2. Endpoint classification: safety/efficacy study<br>3. Intervention model: parallel assignment<br>4. Masking: open label<br>5. Primary purpose: treatment<br>6. Country: India |
| Participants | 1. Enrolled 192<br>2. Inclusion criteria<br>i) Aged ≥ 18 years, with type 1 or 2 diabetes mellitus<br>ii) Single full-thickness plantar ulcer of the extremity (below the malleolus) extending through the epidermis and dermis, but not involving bone, tendons, ligaments or muscles (grade IA as defined by University of Texas Diabetic Wound Classification)<br>iii) Chronic ulcer of at least 6 weeks duration despite appropriate wound care<br>iv) Ulcer area (greatest length by greatest width), following sharp debridement, of 1-10 $cm^2$<br>v) Well-controlled infection or cellulitis (systemic antibiotic therapy)<br>vi) Peripheral neuropathy assessed by Semmes-Weinstein monofilament test or by bio esthesimeter (vibration perception threshold)<br>vii) Adequate arterial blood supply measured by (colour) doppler ultrasonography, ABPI > 0.60, or ankle systolic pressure > 70 mmHg, or toe pressure > 30 mmHg. ABPI should be < 1.3 (which is frequently related to medial artery calcification<br>viii) Women required to be surgically sterile, post-menopausal, or be non-nursing and agree to practice adequate contraception and have a negative pregnancy test at screening<br>ix) Provide signed informed consent before any study procedure<br>3. Exclusion criteria<br>i) Ulcer of non-diabetic cause or origin, e.g. electrical, chemical or radiation insult, bedsores, vascular ulcer or Charcot deformity ulcers<br>ii) Active ulcer infection assessed by clinical examination and radiographically, if necessary. Presence of necrosis, purulence or sinus tracts that cannot be removed by debridement<br>iii) Active osteomyelitis affecting the area of the target ulcer<br>iv) Poorly-controlled diabetes (uncontrolled glycaemia: HbA1c ≥ 12%), renal failure (serum creatinine > 3.0 mg/dL), poor nutritional status (albumin < 3.0 g/dL or total protein < 6.5 g/dL)<br>v) Known connective tissue or malignant disease<br>vi) Concomitant treatment with corticosteroids, immunosuppressive agents, radiation therapy, or anticancer chemotherapy<br>vii) Use of investigational drug/device within 30 days<br>viii) Topical application of any advance wound care on this wound (growth factor, antiseptics, antibiotics or debriders) within 7 days<br>ix) Vascular reconstruction within 8 weeks. |

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| | x) Expected noncompliance with the protocol (i.e. not available for the duration of the trial, or noncompliant with treatment or wound care), or felt to be unsuitable by the Investigator for any other reason |
| Interventions | After the screening visit, the eligible participant population randomly receive 1 of the following:<br>1. Intervention 1: BioChaperone™ PDGF-BB 25 µg/cm² applied as a spray every 2 days for 20 weeks<br>2. Intervention 2: BioChaperone™ PDGF-BB 12.5 µg/cm² applied as a spray every 2 days for 20 weeks<br>3. Intervention 3: BioChaperone™ PDGF-BB 4 µg/cm² applied as a spray every 2 days for 20 weeks<br>4. Intervention 4: beclapermin (Regranex) gel 6.25 µg/cm² applied daily for 20 weeks<br>Experimental BioChaperone PDGF-BB is a new formulation of the B isoform dimer of recombinant human platelet-derived growth factor (rhPDGF-BB) containing the new excipient Biochaperone, a dextran modified polymer. The finished product is administered as a sterile spray<br>Regranex: active comparator becaplermin gel (Regranex® Gel 0.01%, Systagenix, formerly and Johnson & Johnson) is a topical gel of rhPDGF-BB contained in a gel tube<br>All 4 groups to be assessed weekly till the 8th week (visit 10); then once every 2 weeks (14 day duration) thereafter till the end of study. The maximum number of visits expected is 16. The study data will be presented at the end of 20 weeks |
| Outcomes | 1. Primary outcome measures:<br>i) Incidence of complete wound closure: time-frame = 20 weeks; not designated as a safety issue<br>ii) Incidence of complete wound closure<br>2. Secondary outcome measures<br>i) Time to achieve complete wound closure: time-frame = 20 weeks (duration of study); not designated as a safety issue<br>ii) Time to achieve complete wound closure<br>iii) Percentage reduction in total ulcer surface area at each visit: time-frame = 20 weeks (study duration); not designated as a safety issue<br>iv) Incidence of complete wound healing at week 10: time-frame = 10 weeks; not designated as a safety issue<br>v) Safety measures: time-frame = 20 weeks (duration of study); designated as a safety issue<br>a) Treatment-related adverse events with investigator's assessment of seriousness, severity, duration and relationship to study medication<br>b) Wound-related infections<br>c) Changes in standard laboratory tests (haematology, biochemistry and detection of antibodies) |
| Starting date | June 2010 (date of first enrolment) |
| Contact information | Not reported |
| Notes | Target sample size: 192<br>Register: ClinicalTrials.gov<br>Last refreshed on: 2 November 2010<br>Main ID: NCT01098357<br>Date of registration: 1 April 2010<br>Primary sponsor: Virchow Group<br>Recruitment status: completed<br>URL: http://clinicaltrials.gov/show/NCT01098357<br>Source(s) of monetary support: Virchow Group, and Adocia |

| | |
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| | Secondary sponsor(s): Adocia |

**Abbreviations**
ABPI = ankle-brachial pressure index
AE = adverse event
ALT = alanine transaminase
AST = aspartate transaminase
CABG = coronary artery bypass graft
eGFR = epidermal growth factor receptor
Hb = haemoglobin
HbA1c = glycated haemoglobin
HBV = Hepatitis B virus
HCV = Hepatitis C virus
HIV = human immunodeficiency virus
PDGF = platelet-derived growth factor
PDGF-AB = platelet-derived growth factor (specific form)
rhPEGF-BB = recombinant human platelet-derived growth factor
min = minute(s)
NSAID = non-steroidal anti-inflammatory
SAE = serious adverse event
SPI = systolic pressure index
TcPO2 = transcutaneous oxygen tension
TG-PDGF.AB = transglutaminase- platelet-derived growth factor AB

## DATA AND ANALYSES

### Comparison 1. Any growth factor versus placebo or no growth factor

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1 Complete wound closure | 12 | 1139 | Risk Ratio (M-H, Fixed, 95% CI) | 1.51 [1.31, 1.73] |
| 2 Lower limb amputation (minimum of one toe) | 2 | 219 | Risk Ratio (M-H, Fixed, 95% CI) | 0.74 [0.39, 1.39] |
| 3 Ulcer-free days following treatment for diabetic foot ulcers (free from any recurrence) | 1 | | Hazard Ratio (Fixed, 95% CI) | 0.64 [0.14, 2.94] |
| 4 Adverse events (non-serious and serious) | 4 | 385 | Risk Ratio (M-H, Fixed, 95% CI) | 0.98 [0.79, 1.22] |

### Comparison 2. Any growth factor versus placebo or no growth factor (subgroup analysis of trials with follow-up < 20 weeks versus follow-up ≥ 20 weeks)

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1 Participants with complete wound closure | 12 | 1139 | Risk Ratio (M-H, Fixed, 95% CI) | 1.51 [1.31, 1.73] |
| 1.1 Trials with length of follow-up < 20 weeks | 5 | 286 | Risk Ratio (M-H, Fixed, 95% CI) | 1.24 [1.00, 1.55] |
| 1.2 Trials with length of follow up ≥ 20 weeks | 7 | 853 | Risk Ratio (M-H, Fixed, 95% CI) | 1.65 [1.38, 1.98] |

### Comparison 3. Any growth factor versus placebo or no growth factor (subgroup analysis by type of growth factor)

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1 Complete wound closure | 12 | 1137 | Risk Ratio (M-H, Fixed, 95% CI) | 1.50 [1.30, 1.73] |
| 1.1 Autologous growth factor (AGF) | 1 | 14 | Risk Ratio (M-H, Fixed, 95% CI) | 2.0 [0.23, 17.34] |
| 1.2 Platelet-derived wound-healing formula (PDWHF) | 2 | 83 | Risk Ratio (M-H, Fixed, 95% CI) | 2.45 [1.27, 4.74] |
| 1.3 Recombinant human platelet-derived growth factor (rHuPDGF) | 5 | 763 | Risk Ratio (M-H, Fixed, 95% CI) | 1.47 [1.23, 1.76] |

| | | | | |
|---|---|---|---|---|
| 1.4 Recombinant human basic fibroblast growth factor (rHubFBGF) | 2 | 165 | Risk Ratio (M-H, Fixed, 95% CI) | 1.23 [0.88, 1.72] |
| 1.5 Recombinant human epidermal growth factor (rHuEGF) | 1 | 57 | Risk Ratio (M-H, Fixed, 95% CI) | 1.72 [1.16, 2.57] |
| 1.6 Recombinant human vascular endothelial growth factor (rHuVEGF) | 1 | 55 | Risk Ratio (M-H, Fixed, 95% CI) | 1.49 [0.79, 2.82] |

**Comparison 4. Any growth factor versus placebo or no growth factor (sensitivity analyses considering attrition)**

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1 Complete wound closure | 12 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 1.1 All trials | 12 | 1139 | Risk Ratio (M-H, Fixed, 95% CI) | 1.51 [1.31, 1.73] |
| 1.2 Best-worst case scenario | 8 | 1049 | Risk Ratio (M-H, Fixed, 95% CI) | 2.06 [1.79, 2.38] |
| 1.3 Worst-best case scenario | 8 | 1043 | Risk Ratio (M-H, Fixed, 95% CI) | 1.05 [0.93, 1.19] |

**Comparison 5. Platelet derived wound healing formula (PDWHF) versus control**

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1 Complete wound closure | 2 | 83 | Risk Ratio (M-H, Fixed, 95% CI) | 2.45 [1.27, 4.74] |
| 2 Lower limb amputation (minimum of one toe) | 1 | 70 | Risk Ratio (M-H, Fixed, 95% CI) | 2.2 [0.11, 43.95] |

**Comparison 6. Recombinant human platelet-derived growth factor (rHuPDGF) versus placebo**

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1 Complete wound closure | 5 | 753 | Risk Ratio (M-H, Fixed, 95% CI) | 1.45 [1.21, 1.73] |
| 2 Adverse event: infection | 2 | 222 | Risk Ratio (M-H, Fixed, 95% CI) | 2.05 [1.41, 2.97] |
| 3 Adverse event: cellulitis | 2 | 292 | Risk Ratio (M-H, Fixed, 95% CI) | 0.49 [0.23, 1.01] |
| 4 Adverse event: peripheral oedema | 2 | 292 | Risk Ratio (M-H, Fixed, 95% CI) | 0.44 [0.20, 0.96] |
| 5 Adverse event: pain | 2 | 290 | Risk Ratio (M-H, Fixed, 95% CI) | 0.77 [0.41, 1.48] |
| 6 Adverse event: skin ulceration | 2 | 292 | Risk Ratio (M-H, Fixed, 95% CI) | 1.08 [0.49, 2.37] |

**Comparison 7. Recombinant human basic fibroblast growth factor (rHubFBGF) versus placebo**

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1 Complete wound closure | 2 | 165 | Risk Ratio (M-H, Fixed, 95% CI) | 0.81 [0.59, 1.11] |
| 2 Adverse event: infection | 2 | 165 | Risk Ratio (M-H, Fixed, 95% CI) | 0.75 [0.18, 3.20] |

**Comparison 8. Recombinant human epidermal growth factor versus active control**

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1 Lower limb amputation (minimum of one toe) | 3 | | Risk Ratio (M-H, Fixed, 95% CI) | Subtotals only |
| 1.1 Recombinant human epidermal growth factor versus actovegin | 1 | 61 | Risk Ratio (M-H, Fixed, 95% CI) | 0.45 [0.07, 2.98] |
| 1.2 Recombinant human epidermal growth factor 75 $\mu$g dose versus 25 $\mu$g dose | 2 | 142 | Risk Ratio (M-H, Fixed, 95% CI) | 0.79 [0.43, 1.47] |

## Analysis 1.1. Comparison 1 Any growth factor versus placebo or no growth factor, Outcome 1 Complete wound closure.

Review: Growth factors for treating diabetic foot ulcers

Comparison: 1 Any growth factor versus placebo or no growth factor

Outcome: 1 Complete wound closure

| Study or subgroup | Any growth factor n/N | Control n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| d'Hemecourt 1998 | 15/34 | 15/68 | | 5.3 % | 2.00 [ 1.11, 3.59 ] |
| Hanft 2008 | 15/29 | 9/26 | | 5.1 % | 1.49 [ 0.79, 2.82 ] |
| Hardikar 2005 | 39/55 | 18/58 | | 9.4 % | 2.28 [ 1.50, 3.48 ] |
| Holloway 1993 | 31/49 | 6/21 | | 4.5 % | 2.21 [ 1.09, 4.50 ] |
| Jaiswal 2010 | 15/25 | 18/25 | | 9.6 % | 0.83 [ 0.56, 1.25 ] |
| Richard 1995 | 3/9 | 5/8 | | 2.8 % | 0.53 [ 0.18, 1.55 ] |
| Saldalamacchia 2004 | 2/7 | 1/7 | | 0.5 % | 2.00 [ 0.23, 17.34 ] |
| Steed 1992 | 5/7 | 1/6 | | 0.6 % | 4.29 [ 0.67, 27.24 ] |
| Steed 1995a | 29/61 | 14/57 | | 7.7 % | 1.94 [ 1.14, 3.27 ] |
| Uchi 2009 | 57/97 | 22/51 | | 15.4 % | 1.36 [ 0.95, 1.95 ] |
| Viswanathan 2006 | 25/29 | 14/28 | | 7.6 % | 1.72 [ 1.16, 2.57 ] |
| Wieman 1998a | 109/255 | 44/127 | | 31.4 % | 1.23 [ 0.93, 1.63 ] |
| **Total (95% CI)** | **657** | **482** | | **100.0 %** | **1.51 [ 1.31, 1.73 ]** |

Total events: 345 (Any growth factor), 167 (Control)

Heterogeneity: $Chi^2$ = 22.64, df = 11 (P = 0.02); $I^2$ =51%

Test for overall effect: Z = 5.67 (P < 0.00001)

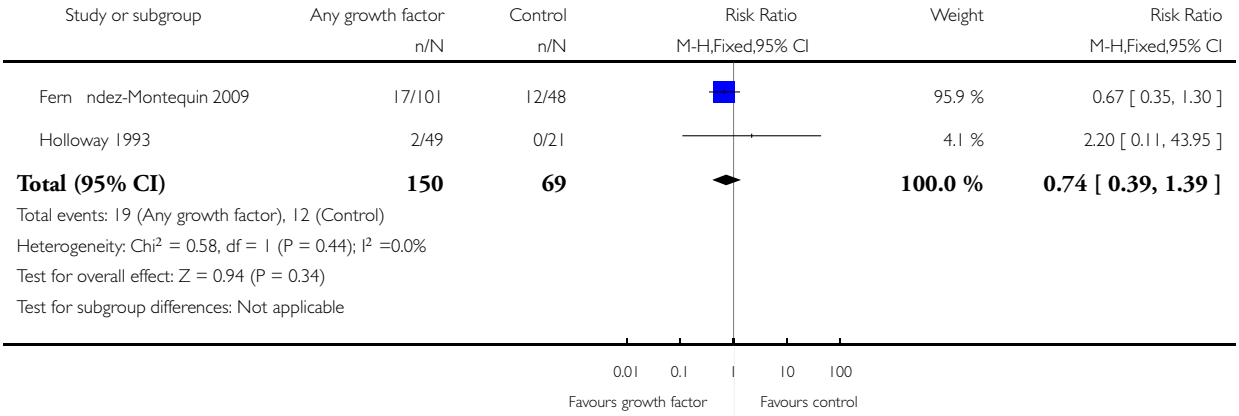
Test for subgroup differences: Not applicable

### Analysis 1.2. Comparison 1 Any growth factor versus placebo or no growth factor, Outcome 2 Lower limb amputation (minimum of one toe).

Review: Growth factors for treating diabetic foot ulcers

Comparison: 1 Any growth factor versus placebo or no growth factor

Outcome: 2 Lower limb amputation (minimum of one toe)

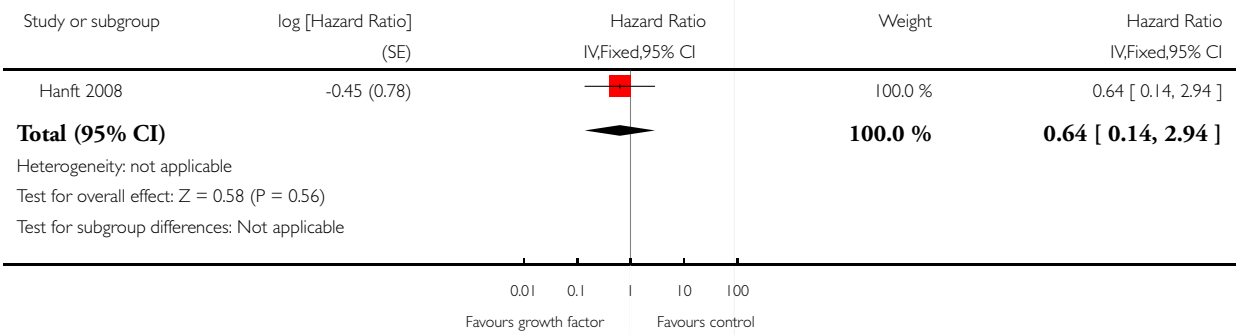



### Analysis 1.3. Comparison 1 Any growth factor versus placebo or no growth factor, Outcome 3 Ulcer-free days following treatment for diabetic foot ulcers (free from any recurrence).

Review: Growth factors for treating diabetic foot ulcers

Comparison: 1 Any growth factor versus placebo or no growth factor

Outcome: 3 Ulcer-free days following treatment for diabetic foot ulcers (free from any recurrence)



### Analysis 1.4. Comparison 1 Any growth factor versus placebo or no growth factor, Outcome 4 Adverse events (non-serious and serious).

Review: Growth factors for treating diabetic foot ulcers

Comparison: 1 Any growth factor versus placebo or no growth factor

Outcome: 4 Adverse events (non-serious and serious)

| Study or subgroup | Any growth factor n/N | Control n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| Fern ndez-Montequin 2009 | 65/101 | 31/48 | | 54.0 % | 1.00 [ 0.77, 1.29 ] |
| Hanft 2008 | 14/29 | 13/26 | | 17.6 % | 0.97 [ 0.56, 1.65 ] |
| Hardikar 2005 | 7/53 | 10/58 | | 12.3 % | 0.77 [ 0.31, 1.87 ] |
| Holloway 1993 | 23/49 | 9/21 | | 16.2 % | 1.10 [ 0.62, 1.95 ] |
| **Total (95% CI)** | **232** | **153** | | **100.0 %** | **0.98 [ 0.79, 1.22 ]** |

Total events: 109 (Any growth factor), 63 (Control)

Heterogeneity: $Chi^2$ = 0.46, df = 3 (P = 0.93); $I^2$ =0.0%

Test for overall effect: Z = 0.19 (P = 0.85)

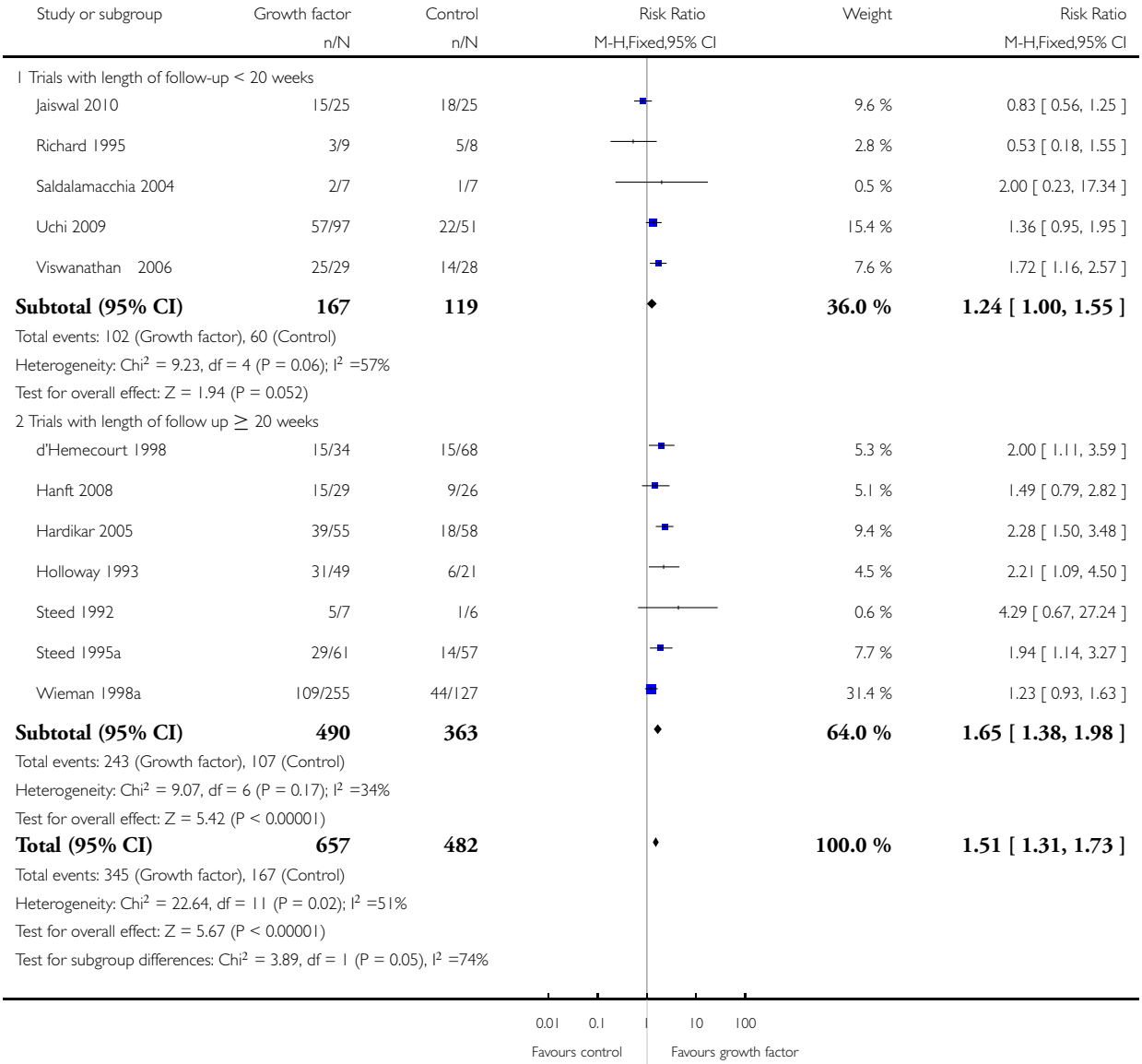
Test for subgroup differences: Not applicable

**Analysis 2.1. Comparison 2 Any growth factor versus placebo or no growth factor (subgroup analysis of trials with follow-up < 20 weeks versus follow-up ≥ 20 weeks), Outcome 1 Participants with complete wound closure.**

Review: Growth factors for treating diabetic foot ulcers

Comparison: 2 Any growth factor versus placebo or no growth factor (subgroup analysis of trials with follow-up < 20 weeks versus follow-up ≥ 20 weeks)

Outcome: 1 Participants with complete wound closure



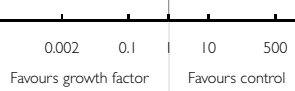
## Analysis 3.1. Comparison 3 Any growth factor versus placebo or no growth factor (subgroup analysis by type of growth factor), Outcome 1 Complete wound closure.

Review: Growth factors for treating diabetic foot ulcers

Comparison: 3 Any growth factor versus placebo or no growth factor (subgroup analysis by type of growth factor)

Outcome: 1 Complete wound closure

| Study or subgroup | Any growth factor n/N | Control n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| 1 Autologous growth factor (AGF) | | | | | |
| Saldalamacchia 2004 | 2/7 | 1/7 | | 0.5 % | 2.00 [ 0.23, 17.34 ] |
| **Subtotal (95% CI)** | **7** | **7** | | **0.5 %** | **2.00 [ 0.23, 17.34 ]** |
| Total events: 2 (Any growth factor), 1 (Control) | | | | | |
| Heterogeneity: not applicable | | | | | |
| Test for overall effect: Z = 0.63 (P = 0.53) | | | | | |
| 2 Platelet-derived wound-healing formula (PDWHF) | | | | | |
| Holloway 1993 | 31/49 | 6/21 | | 4.5 % | 2.21 [ 1.09, 4.50 ] |
| Steed 1992 | 5/7 | 1/6 | | 0.6 % | 4.29 [ 0.67, 27.24 ] |
| **Subtotal (95% CI)** | **56** | **27** | | **5.1 %** | **2.45 [ 1.27, 4.74 ]** |
| Total events: 36 (Any growth factor), 7 (Control) | | | | | |
| Heterogeneity: $Chi^2$ = 0.43, df = 1 (P = 0.51); $I^2$ =0.0% | | | | | |
| Test for overall effect: Z = 2.66 (P = 0.0077) | | | | | |
| 3 Recombinant human platelet-derived growth factor (rHuPDGF) | | | | | |
| d'Hemecourt 1998 | 15/34 | 15/68 | | 5.4 % | 2.00 [ 1.11, 3.59 ] |
| Hardikar 2005 | 37/53 | 18/58 | | 9.2 % | 2.25 [ 1.47, 3.43 ] |
| Jaiswal 2010 | 15/25 | 18/25 | | 9.6 % | 0.83 [ 0.56, 1.25 ] |
| Steed 1995a | 29/61 | 14/57 | | 7.8 % | 1.94 [ 1.14, 3.27 ] |
| Wieman 1998a | 109/255 | 44/127 | | 31.5 % | 1.23 [ 0.93, 1.63 ] |
| **Subtotal (95% CI)** | **428** | **335** | | **63.4 %** | **1.47 [ 1.23, 1.76 ]** |
| Total events: 205 (Any growth factor), 109 (Control) | | | | | |
| Heterogeneity: $Chi^2$ = 15.17, df = 4 (P = 0.004); $I^2$ =74% | | | | | |
| Test for overall effect: Z = 4.19 (P = 0.000028) | | | | | |
| 4 Recombinant human basic fibroblast growth factor (rHubFBGF) | | | | | |
| Richard 1995 | 3/9 | 5/8 | | 2.8 % | 0.53 [ 0.18, 1.55 ] |
| Uchi 2009 | 57/97 | 22/51 | | 15.4 % | 1.36 [ 0.95, 1.95 ] |
| **Subtotal (95% CI)** | **106** | **59** | | **18.3 %** | **1.23 [ 0.88, 1.72 ]** |
| Total events: 60 (Any growth factor), 27 (Control) | | | | | |
| Heterogeneity: $Chi^2$ = 2.66, df = 1 (P = 0.10); $I^2$ =62% | | | | | |
| Test for overall effect: Z = 1.23 (P = 0.22) | | | | | |
| 5 Recombinant human epidermal growth factor (rHuEGF) | | | | | |

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| Study or subgroup | Any growth factor n/N | Control n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| Viswanathan 2006 | 25/29 | 14/28 | | 7.6 % | 1.72 [ 1.16, 2.57 ] |
| **Subtotal (95% CI)** | **29** | **28** | | **7.6 %** | **1.72 [ 1.16, 2.57 ]** |
| Total events: 25 (Any growth factor), 14 (Control) | | | | | |
| Heterogeneity: not applicable | | | | | |
| Test for overall effect: Z = 2.68 (P = 0.0073) | | | | | |
| 6 Recombinant human vascular endothelial growth factor (rHuVEGF) | | | | | |
| Hanft 2008 | 15/29 | 9/26 | | 5.1 % | 1.49 [ 0.79, 2.82 ] |
| **Subtotal (95% CI)** | **29** | **26** | | **5.1 %** | **1.49 [ 0.79, 2.82 ]** |
| Total events: 15 (Any growth factor), 9 (Control) | | | | | |
| Heterogeneity: not applicable | | | | | |
| Test for overall effect: Z = 1.24 (P = 0.21) | | | | | |
| **Total (95% CI)** | **655** | **482** | | **100.0 %** | **1.50 [ 1.30, 1.73 ]** |
| Total events: 343 (Any growth factor), 167 (Control) | | | | | |
| Heterogeneity: $Chi^2$ = 22.26, df = 11 (P = 0.02); $I^2$ =51% | | | | | |
| Test for overall effect: Z = 5.62 (P < 0.00001) | | | | | |
| Test for subgroup differences: $Chi^2$ = 4.02, df = 5 (P = 0.55), $I^2$ =0.0% | | | | | |

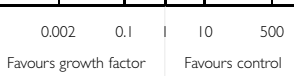
## Analysis 4.1. Comparison 4 Any growth factor versus placebo or no growth factor (sensitivity analyses considering attrition), Outcome 1 Complete wound closure.

Review: Growth factors for treating diabetic foot ulcers

Comparison: 4 Any growth factor versus placebo or no growth factor (sensitivity analyses considering attrition)

Outcome: 1 Complete wound closure

| Study or subgroup | Any growth factor n/N | Control n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| 1 All trials | | | | | |
| d'Hemecourt 1998 | 15/34 | 15/68 | | 5.3 % | 2.00 [ 1.11, 3.59 ] |
| Hanft 2008 | 15/29 | 9/26 | | 5.1 % | 1.49 [ 0.79, 2.82 ] |
| Hardikar 2005 | 39/55 | 18/58 | | 9.4 % | 2.28 [ 1.50, 3.48 ] |
| Holloway 1993 | 31/49 | 6/21 | | 4.5 % | 2.21 [ 1.09, 4.50 ] |
| Jaiswal 2010 | 15/25 | 18/25 | | 9.6 % | 0.83 [ 0.56, 1.25 ] |
| Richard 1995 | 3/9 | 5/8 | | 2.8 % | 0.53 [ 0.18, 1.55 ] |
| Saldalamacchia 2004 | 2/7 | 1/7 | | 0.5 % | 2.00 [ 0.23, 17.34 ] |
| Steed 1992 | 5/7 | 1/6 | | 0.6 % | 4.29 [ 0.67, 27.24 ] |
| Steed 1995a | 29/61 | 14/57 | | 7.7 % | 1.94 [ 1.14, 3.27 ] |
| Uchi 2009 | 57/97 | 22/51 | | 15.4 % | 1.36 [ 0.95, 1.95 ] |
| Viswanathan 2006 | 25/29 | 14/28 | | 7.6 % | 1.72 [ 1.16, 2.57 ] |
| Wieman 1998a | 109/255 | 44/127 | | 31.4 % | 1.23 [ 0.93, 1.63 ] |
| **Subtotal (95% CI)** | **657** | **482** | | **100.0 %** | **1.51 [ 1.31, 1.73 ]** |
| Total events: 345 (Any growth factor), 167 (Control) | | | | | |
| Heterogeneity: Chi² = 22.64, df = 11 (P = 0.02); I² =51% | | | | | |
| Test for overall effect: Z = 5.67 (P < 0.00001) | | | | | |
| 2 Best-worst case scenario | | | | | |
| d'Hemecourt 1998 | 28/34 | 15/68 | | 6.2 % | 3.73 [ 2.33, 5.99 ] |
| Hanft 2008 | 16/29 | 9/26 | | 5.8 % | 1.59 [ 0.86, 2.97 ] |
| Hardikar 2005 | 44/59 | 18/58 | | 11.2 % | 2.40 [ 1.59, 3.63 ] |
| Holloway 1993 | 40/49 | 6/21 | | 5.2 % | 2.86 [ 1.43, 5.69 ] |
| Steed 1995a | 43/61 | 14/57 | | 8.9 % | 2.87 [ 1.77, 4.65 ] |
| Uchi 2009 | 62/97 | 22/51 | | 17.8 % | 1.48 [ 1.05, 2.10 ] |
| Viswanathan 2006 | 26/29 | 14/28 | | 8.8 % | 1.79 [ 1.21, 2.65 ] |
| Wieman 1998a | 158/255 | 44/127 | | 36.2 % | 1.79 [ 1.38, 2.31 ] |
| **Subtotal (95% CI)** | **613** | **436** | | **100.0 %** | **2.06 [ 1.79, 2.38 ]** |

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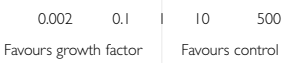
| Study or subgroup | Any growth factor n/N | Control n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| Total events: 417 (Any growth factor), 142 (Control) | | | | | |
| Heterogeneity: $Chi^2$ = 15.02, df = 7 (P = 0.04); $I^2$ =53% | | | | | |
| Test for overall effect: Z = 9.87 (P < 0.00001) | | | | | |
| 3 Worst-best case scenario | | | | | |
| d'Hemecourt 1998 | 15/34 | 26/68 | | 7.1 % | 1.15 [ 0.71, 1.87 ] |
| Hanft 2008 | 15/29 | 11/26 | | 4.8 % | 1.22 [ 0.69, 2.16 ] |
| Hardikar 2005 | 37/53 | 32/58 | | 12.5 % | 1.27 [ 0.95, 1.69 ] |
| Holloway 1993 | 31/49 | 7/21 | | 4.0 % | 1.90 [ 1.00, 3.60 ] |
| Steed 1995a | 29/61 | 32/57 | | 13.6 % | 0.85 [ 0.60, 1.20 ] |
| Uchi 2009 | 57/97 | 26/51 | | 14.0 % | 1.15 [ 0.84, 1.58 ] |
| Viswanathan 2006 | 25/29 | 16/28 | | 6.7 % | 1.51 [ 1.06, 2.15 ] |
| Wieman 1998a | 109/255 | 68/127 | | 37.3 % | 0.80 [ 0.64, 0.99 ] |
| **Subtotal (95% CI)** | **607** | **436** | | **100.0 %** | **1.05 [ 0.93, 1.19 ]** |
| Total events: 318 (Any growth factor), 218 (Control) | | | | | |
| Heterogeneity: $Chi^2$ = 17.33, df = 7 (P = 0.02); $I^2$ =60% | | | | | |
| Test for overall effect: Z = 0.80 (P = 0.43) | | | | | |
| Test for subgroup differences: $Chi^2$ = 50.43, df = 2 (P = 0.00), $I^2$ =96% | | | | | |



### Analysis 5.1. Comparison 5 Platelet derived wound healing formula (PDWHF) versus control, Outcome 1 Complete wound closure.

Review: Growth factors for treating diabetic foot ulcers

Comparison: 5 Platelet derived wound healing formula (PDWHF) versus control

Outcome: 1 Complete wound closure

| Study or subgroup | PDWHF n/N | Control n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| Holloway 1993 | 31/49 | 6/21 | | 88.6 % | 2.21 [ 1.09, 4.50 ] |
| Steed 1992 | 5/7 | 1/6 | | 11.4 % | 4.29 [ 0.67, 27.24 ] |
| **Total (95% CI)** | **56** | **27** | | **100.0 %** | **2.45 [ 1.27, 4.74 ]** |

Total events: 36 (PDWHF), 7 (Control)

Heterogeneity: $Chi^2 = 0.43$, df = 1 (P = 0.51); $I^2 = 0.0\%$

Test for overall effect: Z = 2.66 (P = 0.0077)

Test for subgroup differences: Not applicable


### Analysis 5.2. Comparison 5 Platelet derived wound healing formula (PDWHF) versus control, Outcome 2 Lower limb amputation (minimum of one toe).

Review: Growth factors for treating diabetic foot ulcers

Comparison: 5 Platelet derived wound healing formula (PDWHF) versus control

Outcome: 2 Lower limb amputation (minimum of one toe)

| Study or subgroup | PDWHF n/N | Control n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| Holloway 1993 | 2/49 | 0/21 | | 100.0 % | 2.20 [ 0.11, 43.95 ] |
| **Total (95% CI)** | **49** | **21** | | **100.0 %** | **2.20 [ 0.11, 43.95 ]** |

Total events: 2 (PDWHF), 0 (Control)

Heterogeneity: not applicable

Test for overall effect: Z = 0.52 (P = 0.61)

Test for subgroup differences: Not applicable

## Analysis 6.1. Comparison 6 Recombinant human platelet-derived growth factor (rHuPDGF) versus placebo, Outcome 1 Complete wound closure.

Review: Growth factors for treating diabetic foot ulcers

Comparison: 6 Recombinant human platelet-derived growth factor (rHuPDGF) versus placebo

Outcome: 1 Complete wound closure

| Study or subgroup | rHuPDGF n/N | Placebo n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| d'Hemecourt 1998 | 15/34 | 15/58 | | 9.3 % | 1.71 [ 0.96, 3.04 ] |
| Hardikar 2005 | 37/53 | 18/58 | | 14.4 % | 2.25 [ 1.47, 3.43 ] |
| Jaiswal 2010 | 15/25 | 18/25 | | 15.1 % | 0.83 [ 0.56, 1.25 ] |
| Steed 1995a | 29/61 | 14/57 | | 12.1 % | 1.94 [ 1.14, 3.27 ] |
| Wieman 1998a | 109/255 | 44/127 | | 49.2 % | 1.23 [ 0.93, 1.63 ] |
| **Total (95% CI)** | **428** | **325** | | **100.0 %** | **1.45 [ 1.21, 1.73 ]** |

Total events: 205 (rHuPDGF), 109 (Placebo)

Heterogeneity: $Chi^2$ = 14.16, df = 4 (P = 0.01); $I^2$ =72%

Test for overall effect: Z = 4.02 (P = 0.000057)

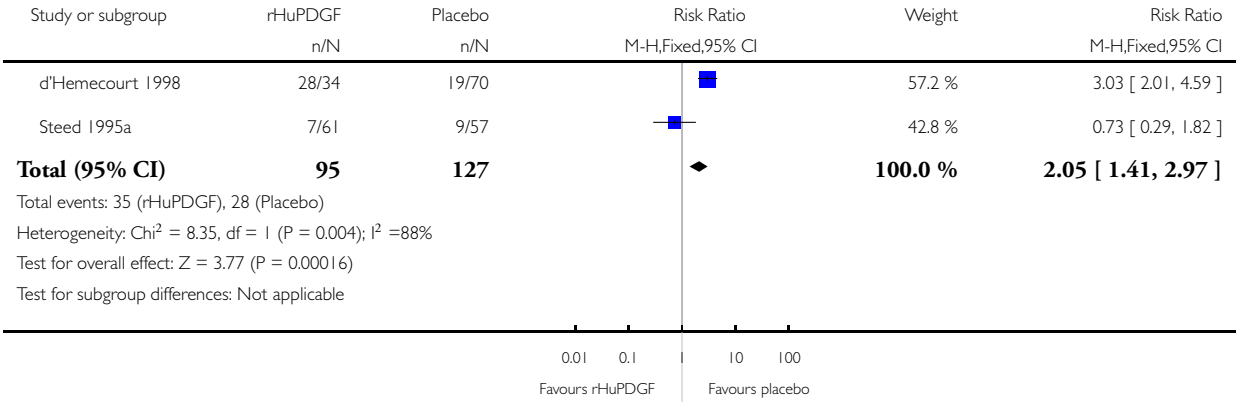
Test for subgroup differences: Not applicable

### Analysis 6.2. Comparison 6 Recombinant human platelet-derived growth factor (rHuPDGF) versus placebo, Outcome 2 Adverse event: infection.

Review: Growth factors for treating diabetic foot ulcers

Comparison: 6 Recombinant human platelet-derived growth factor (rHuPDGF) versus placebo

Outcome: 2 Adverse event: infection

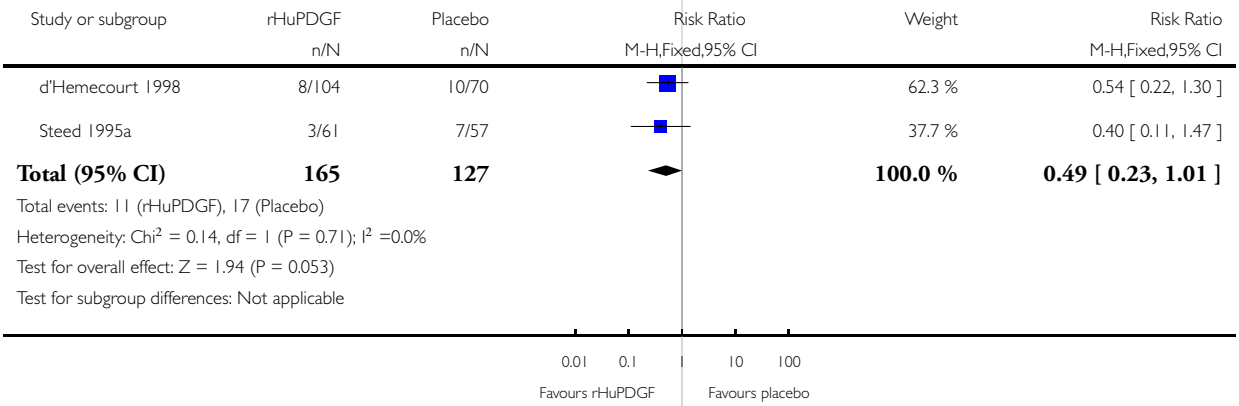



### Analysis 6.3. Comparison 6 Recombinant human platelet-derived growth factor (rHuPDGF) versus placebo, Outcome 3 Adverse event: cellulitis.

Review: Growth factors for treating diabetic foot ulcers

Comparison: 6 Recombinant human platelet-derived growth factor (rHuPDGF) versus placebo

Outcome: 3 Adverse event: cellulitis



### Analysis 6.4. Comparison 6 Recombinant human platelet-derived growth factor (rHuPDGF) versus placebo, Outcome 4 Adverse event: peripheral oedema.

Review: Growth factors for treating diabetic foot ulcers

Comparison: 6 Recombinant human platelet-derived growth factor (rHuPDGF) versus placebo

Outcome: 4 Adverse event: peripheral oedema

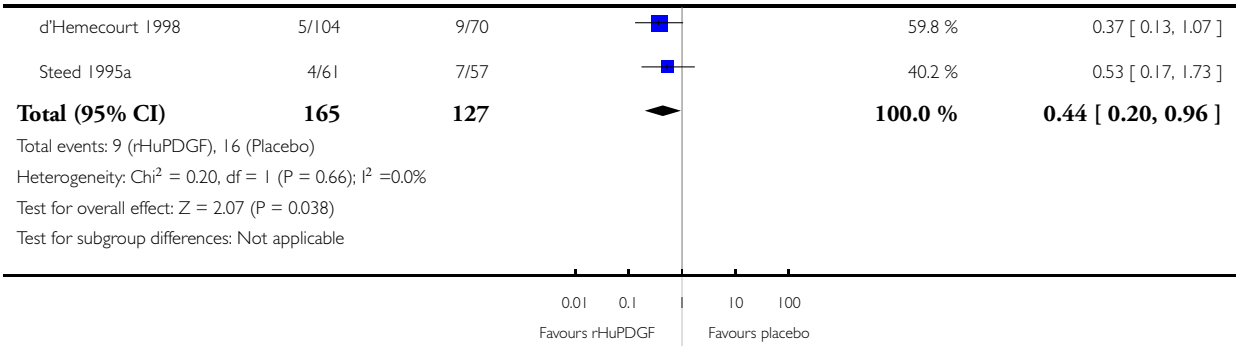
| Study or subgroup | rHuPDGF n/N | Placebo n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| d'Hemecourt 1998 | 5/104 | 9/70 | | 59.8 % | 0.37 [ 0.13, 1.07 ] |
| Steed 1995a | 4/61 | 7/57 | | 40.2 % | 0.53 [ 0.17, 1.73 ] |
| **Total (95% CI)** | **165** | **127** | | **100.0 %** | **0.44 [ 0.20, 0.96 ]** |

Total events: 9 (rHuPDGF), 16 (Placebo)

Heterogeneity: $Chi^2$ = 0.20, df = 1 (P = 0.66); $I^2$ =0.0%

Test for overall effect: Z = 2.07 (P = 0.038)

Test for subgroup differences: Not applicable

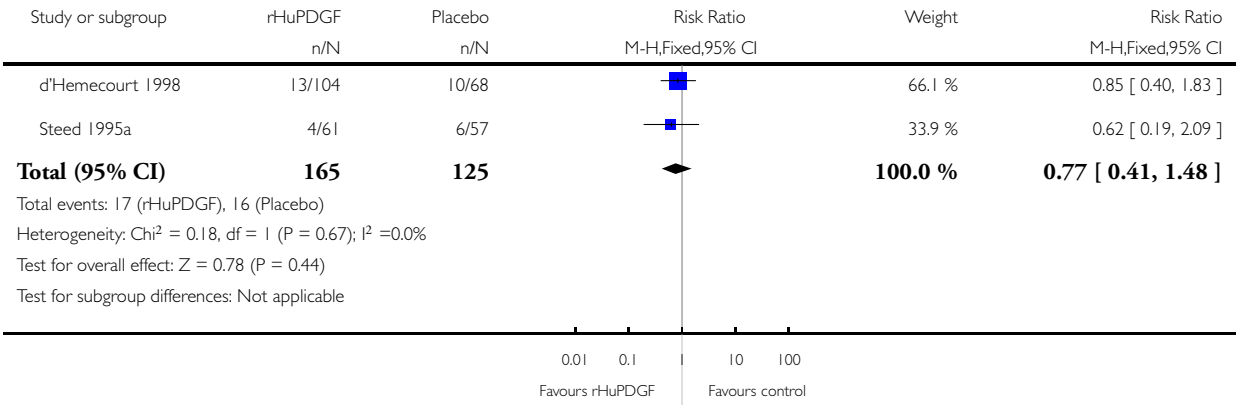


### Analysis 6.5. Comparison 6 Recombinant human platelet-derived growth factor (rHuPDGF) versus placebo, Outcome 5 Adverse event: pain.

Review: Growth factors for treating diabetic foot ulcers

Comparison: 6 Recombinant human platelet-derived growth factor (rHuPDGF) versus placebo

Outcome: 5 Adverse event: pain

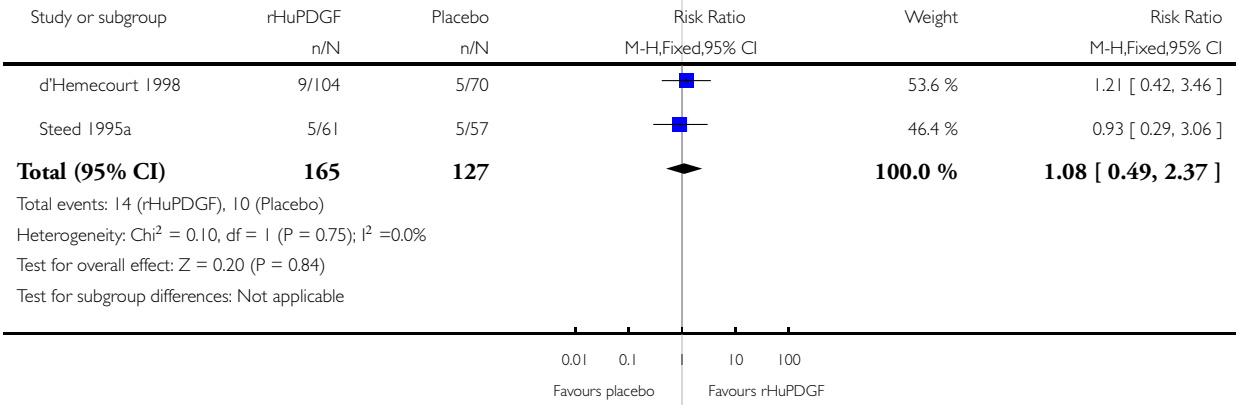



### Analysis 6.6. Comparison 6 Recombinant human platelet-derived growth factor (rHuPDGF) versus placebo, Outcome 6 Adverse event: skin ulceration.

Review: Growth factors for treating diabetic foot ulcers

Comparison: 6 Recombinant human platelet-derived growth factor (rHuPDGF) versus placebo

Outcome: 6 Adverse event: skin ulceration



### Analysis 7.1. Comparison 7 Recombinant human basic fibroblast growth factor (rHubFBGF) versus placebo, Outcome 1 Complete wound closure.

Review: Growth factors for treating diabetic foot ulcers

Comparison: 7 Recombinant human basic fibroblast growth factor (rHubFBGF) versus placebo

Outcome: 1 Complete wound closure

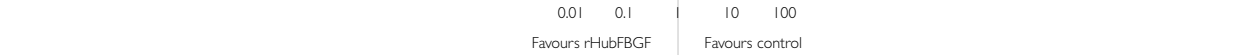
| Study or subgroup | rHubFBGF n/N | Control n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| Richard 1995 | 6/9 | 3/8 | | 7.7 % | 1.78 [ 0.65, 4.87 ] |
| Uchi 2009 | 40/97 | 29/51 | | 92.3 % | 0.73 [ 0.52, 1.02 ] |
| **Total (95% CI)** | **106** | **59** | | **100.0 %** | **0.81 [ 0.59, 1.11 ]** |

Total events: 46 (rHubFBGF), 32 (Control)

Heterogeneity: $Chi^2$ = 2.75, df = 1 (P = 0.10); $I^2$ =64%

Test for overall effect: Z = 1.33 (P = 0.18)

Test for subgroup differences: Not applicable



### Analysis 7.2. Comparison 7 Recombinant human basic fibroblast growth factor (rHubFBGF) versus placebo, Outcome 2 Adverse event: infection.

Review: Growth factors for treating diabetic foot ulcers

Comparison: 7 Recombinant human basic fibroblast growth factor (rHubFBGF) versus placebo

Outcome: 2 Adverse event: infection

| Study or subgroup | rHubFBGF n/N | Control n/N | Risk Ratio M-H,Fixed,95% CI | Weight | Risk Ratio M-H,Fixed,95% CI |
|---|---|---|---|---|---|
| Richard 1995 | 2/9 | 2/8 | | 61.8 % | 0.89 [ 0.16, 4.93 ] |
| Uchi 2009 | 1/97 | 1/51 | | 38.2 % | 0.53 [ 0.03, 8.23 ] |
| **Total (95% CI)** | **106** | **59** | | **100.0 %** | **0.75 [ 0.18, 3.20 ]** |

Total events: 3 (rHubFBGF), 3 (Control)

Heterogeneity: $Chi^2$ = 0.10, df = 1 (P = 0.75); $I^2$ =0.0%

Test for overall effect: Z = 0.39 (P = 0.70)

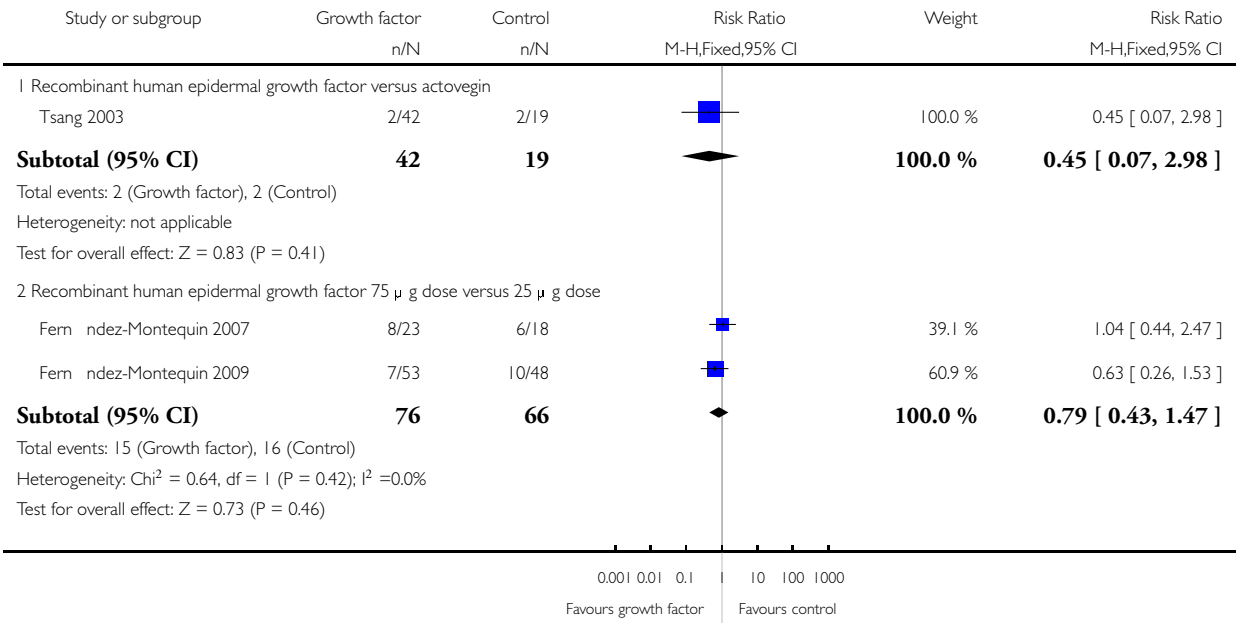
Test for subgroup differences: Not applicable

**Analysis 8.1. Comparison 8 Recombinant human epidermal growth factor versus active control, Outcome 1 Lower limb amputation (minimum of one toe).**

Review: Growth factors for treating diabetic foot ulcers

Comparison: 8 Recombinant human epidermal growth factor versus active control

Outcome: 1 Lower limb amputation (minimum of one toe)




# APPENDICES

## Appendix 1. Glossary of medical and epidemiological terms

| Terms | Definition | Reference |
|---|---|---|
| Ankle Brachial Index | Comparison of the blood pressure between the brachial artery and the posterior tibial artery. It is a predictor of peripheral arterial disease | http://www.ncbi.nlm.nih.gov/mesh |

(*Continued*)

| | | |
|---|---|---|
| Actovegin | A biological drug - a calf blood haemodialysate - manufactured from a natural source | Buchmayer 2011 |
| Amputation | The removal of a limb or other appendage or outgrowth of the body | http://www.ncbi.nlm.nih.gov/mesh |
| Arginine-glycine-aspartic acid (RGD) peptide matrix (Argidene Gel®, formerly Telio-Derm Gel®, Telios Pharmaceuticals, San Diego, CA, USA) | This peptide matrix contains the arginine-glycine-aspartic acid amino acid sequence, through which cells in vivo become attached to macromolecules of extracellular matrix via surface integrin receptors. The matrix (intervention) is a sterile non-preserved clear viscous gel, formulated in phosphate-buffered saline and dispensed from a single-use syringe. The functional ingredient of RGD peptide matrix is a complex formed by the combination of a synthetic 18-amino acid peptide and sodium hyaluronate. It also contains added unconjugated sodium hyaluronate as a viscosity-increasing agent, and, therefore, does not need to be prepared from patient's samples | O'Meara 2000 |
| Attrition bias | A type of selection bias due to systematic differences between the study groups in the quantitative and qualitative characteristics of the process of loss of their members during study conduct, i.e., due to attrition among subjects in the study | Porta 2008 |
| Autologous platelet gel | See 'platelet-rich plasma' | Lacci 2010 |
| Basic fibroblast growth factor (bFGF) (Farmitalia Carlo Erba, Milan, Italy) | A heparin-building, single-chain peptide of 146 amino acids, ubiquitously distributed in mesoderm- and neuroectoderm-derived tissues. This is a potent mitogen for all cell types involved in the healing process. It is highly angiogenic and chemotactic for fibroblasts and endothelial cells. bFGF is produced by recombinant DNA technology using *Escherichia coli* type b | O'Meara 2000 |
| Bias in the presentation of data | Error due to irregularities produced by digit preference, incomplete data, poor techniques of measurement, technically poor laboratory procedures, or an intentional attempt to mislead | Porta 2008 |

(*Continued*)

| | | |
|---|---|---|
| Burning sensation | An abnormal feeling of burning in the absence of heat | http://www.healthline.com/hlc/burning-sensation |
| Callus | A hard, thickened area of skin occurring in parts of the body that are subjected to pressure or friction, particularly the soles of the feet and the palms of the hands | O'Meara 2000 |
| Cellulitis | An acute, diffuse, and suppurative inflammation of loose connective tissue, particularly the deep subcutaneous tissues, and sometimes muscle, most commonly seen as a result of infection of a wound, ulcer, or other skin lesion | http://www.ncbi.nlm.nih.gov/mesh |
| Co-intervention | In a randomised controlled trial, the application of additional diagnostic or therapeutic procedures to members of either, some or all of the experimental and control groups | Porta 2008 |
| Connective tissue disease | A heterogeneous group of disorders, some hereditary, others acquired, characterised by abnormal structure or function of one or more of the elements of connective tissue, i.e. collagen, elastin, or the mucopolysaccharides | http://www.ncbi.nlm.nih.gov/mesh |
| CT-102 activated platelet supernatant (APST) (Curative Technologies, Setauket, NY, USA) (synonym: platelet-derived wound-healing formula (PDWHF)) | A combination of growth factors released from $\rho$-granules of human platelets by thrombin | O'Meara 2000 |
| Debridement | The removal of foreign material and dead or contaminated tissue from, or adjacent to, a wound until the surrounding healthy tissue is exposed | O'Meara 2000 |
| Design bias | The difference between a true value and that obtained through the faulty design of a study. Examples include uncontrolled studies where the effects of two or more processes cannot be separated because of lack of measurement of key causes of the exposure or outcome (confounding); also studies performed on poorly-defined populations or with unsuitable control groups | Porta 2008 |

(*Continued*)

| | | |
|---|---|---|
| Diabetes Mellitus, type 1 | A subtype of diabetes mellitus that is characterized by insulin deficiency. It is manifested by the sudden onset of severe hyperglycemia, rapid progression to diabetic ketoacidosis, and death unless treated with insulin. The disease may occur at any age, but is most common in childhood or adolescence | http://www.ncbi.nlm.nih.gov/mesh |
| Diabetes Mellitus, type 2 | A subclass of diabetes mellitus that is not insulin-responsive or dependent (NIDDM) . It is characterized initially by insulin resistance and hyperinsulinemia; and eventually by glucose intolerance; hyperglycemia; and overt diabetes. Type II diabetes mellitus is no longer considered a disease exclusively found in adults. Patients seldom develop ketosis but often exhibit obesity | http://www.ncbi.nlm.nih.gov/mesh |
| Diabetic coma | A state of unconsciousness that is a complication of diabetes mellitus. It occurs in cases of extreme hyperglycaemia or hypoglycaemia that may occur as a complication of insulin therapy | http://www.ncbi.nlm.nih.gov/mesh |
| Diabetic ketoacidosis | A life-threatening complication of diabetes mellitus (primarily of type 1) exacerbated by severe insulin deficiency and extreme hyperglycaemia. It is characterised by the metabolism of fatty acids (ketosis); dehydration; and depressed consciousness leading to coma | http://www.ncbi.nlm.nih.gov/mesh |
| Epidermal growth factor (EGF) | This growth factor stimulates keratinocyte proliferation and locomotion, and inhibits fibroblast proliferation. It is a chemoattractant for mesodermal and epidermal cells | O'Meara 2000 |
| Gangrene | The death and decay of part of the body due to a deficiency in, or the cessation of, the blood supply. Causes can include disease, injury, atheroma in major blood vessels, frostbite and severe burns. Dry gangrene is the death and withering of tissues caused by the cessation of the local blood circulation; moist gangrene is the death and putrefactive decay of tissue due to bacterial infection | O'Meara 2000 |

(*Continued*)

| | | |
|---|---|---|
| Growth factors | A group of multifunctional peptides thought to promote cellular proliferation, migration and protein synthesis. They may be derived from platelets, endothelial cells, monocytes, tissue macrophages, fibroblasts or epidermal cells | O'Meara 2000 |
| Hypersensitivity | Heightened reactivity to an antigen, which can result in pathologic reactions to subsequent exposure to that particular antigen | http://www.ncbi.nlm.nih.gov/mesh |
| Hypotension | Abnormally low blood pressure that can cause inadequate blood flow to the brain and other vital organs. A common symptom is dizziness, but greater negative impacts occur when there is prolonged deprivation of oxygen and nutrients | http://www.ncbi.nlm.nih.gov/mesh |
| Information bias | A flaw in measuring exposure, covariate, or outcome variables that results in different quality (accuracy) of information between comparison groups. The information bias may not be independent of selection biases. Bias in an estimate arising from measurement errors | Porta 2008 |
| International Association Enterostomal Therapy classification for assessing the stage of ulcers | **Stage I**<br>An observable pressure-related alteration of intact skin, which, when compared to adjacent skin or an opposite area on the body, may include changes in one or more of the following: skin temperature (warmth or coolness), tissue consistency (firm or boggy feel), and/or sensation (pain, itching). In lightly-pigmented skin, an ulcer appears as a defined area of persistent redness, while in darker skin, an ulcer may appear with persistent red, blue or purple hues<br>**Stage II**<br>Partial-thickness skin loss involving epidermis or dermis, or both. The ulcer is superficial and presents as an abrasion, blister, or shallow crater<br>**Stage III**<br>Full-thickness skin loss involving damage to or necrosis of subcutaneous tissue that may extend down to, but not through, underlying fascia. The ulcer presents clinically | http://www.wocn.org/pdfs/WOCN_Library/Position_Statements/staging.pdf |

(*Continued*)

| | | |
|---|---|---|
| | as a deep crater with, or without, undermining of adjacent tissue<br>**Stage IV**<br>Full-thickness skin loss with extensive destruction, tissue necrosis, or damage to muscle, bone or supporting structures (e.g. tendon, joint capsule). Undermining and sinus tracts also may be associated with stage IV pressure ulcers | |
| Ischaemia | A deficiency of blood in a body part due to functional constriction, or actual obstruction, of a blood vessel | O'Meara 2000 |
| Impaired glucose tolerance | A pathological state in which the blood glucose level is less than approximately 140 mg/100 ml of plasma at fasting, and above approximately 200 mg/100 ml plasma at 30-, 60-, or 90-minutes during a glucose tolerance test. This condition is seen frequently in diabetes mellitus, but also occurs with other diseases and malnutrition | http://www.ncbi.nlm.nih.gov/mesh |
| Neuropathic ulcer | An ulcer that usually occurs on the plantar surface of the foot. These ulcers are often associated with sensory neuropathy and, therefore, are often painless. They are typically surrounded by callus tissue, as they occur at sites of high mechanical pressure | O'Meara 2000 |
| Oedematous (oedema) | Abnormal fluid accumulation in tissues or body cavities. Most cases of oedema are present under the skin in subcutaneous tissue | http://www.ncbi.nlm.nih.gov/mesh |
| Peripheral vascular disease | A general or unspecified disease of the blood vessels outside the heart | O'Meara 2000 |
| Platelet-derived growth factors | Mitogenic peptide growth hormone carried in the alpha-granules of platelets released when platelets adhere to traumatised tissues. Connective tissue cells near the traumatised region respond by initiating the process of replication | http://www.ncbi.nlm.nih.gov/mesh |
| Platelet-enriched plasma | See platelet-rich plasma | Lacci 2010 |
| Platelet releasate | See platelet-rich plasma | Lacci 2010 |

(*Continued*)

| | | |
|---|---|---|
| Platelet-rich concentrate | See platelet-rich plasma | Lacci 2010 |
| Platelet-rich plasma | Portion of the plasma fraction of autologous blood having a platelet concentration above baseline | Lacci 2010 |
| Recombinant human form of platelet-derived growth factor (rhPDGF-BB), homodimer (Chiron Corp, Emeryville, CA, USA) | Growth factor produced from genetically engineered yeast cells into which the gene for the $\beta$-chain of PDGF has been inserted | O'Meara 2000 |
| Skin ulceration (ulcer) | A lesion on the surface of the skin, or a mucous surface, produced by the sloughing of inflammatory necrotic tissue | http://www.ncbi.nlm.nih.gov/mesh |
| Toe-brachial index (TBI) | Ratio of the brachial and big toe systolic pressures: the brachial pressure is obtained by Doppler and the toe pressure by photo plethysmography | http://www.deh-inc.com/index.cfm?fuseaction=productdetail&productid=56 |
| Transforming growth factor | Hormonally-active polypeptides that can induce the transformed phenotype when added to normal, non-transformed cells | http://www.ncbi.nlm.nih.gov/mesh |
| University of Texas diabetic classification of ulcers | Stages<br>A: No infection or ischaemia<br>B: Infection present<br>C: Ischaemia present<br>D: Infection and ischaemia present<br>Grading<br>0: Epithelialised wound<br>1: Superficial wound<br>2: Wound penetrates to tendon or capsule<br>3: Wound penetrates to bone or joint | http://www.fpnotebook.com/Surgery/Exam/UnvrstyOfTxsDbtcWndClsfctn.htm |
| Wagner's classification of foot ulcers | Grade 1: Superficial diabetic ulcer<br>Grade 2: Ulcer extension<br>a) Involves ligament, tendon, joint capsule or fascia<br>b) No abscess or osteomyelitis<br>Grade 3: Deep ulcer with abscess or osteomyelitis<br>Grade 4: Gangrene to portion of forefoot<br>Grade 5: Extensive gangrene of foot | http://www.fpnotebook.com/surgery/Exam/WgnrUlcrClsfctn.htm |

## Appendix 2. Frequency of foot ulcers in people with diabetes

| Study/year/country | Frequency of foot ulcers | Frequency of amputation | Study design and follow-up time | Participants (setting and sample size) | Notes |
|---|---|---|---|---|---|
| Rith-Najarian 1992 **USA** | 41/358: 11.4% | 14/358: 3.9%<br>14/41: 34.1% | Prospective cohort, 32 months | N = 406 (results came from 358) diabetic American-Indian people.<br>Community setting | |
| Veves 1992 **UK** | 35% | Not available | Prospective observational study, 30 months | N = 86 (mean age: 53.3 years; range: 17.7 to 77 years) | |
| Young 1994 **UK** | Cumulative incidence:<br>VPT < 15 V: 2.9%<br>VPT > 25 V: 19.8% | Not available | Prospective observational study | N not available | |
| Humphrey 1996 **Australia** | Not available | Study cohort: 8.1 per 1000 person-years<br>Nationally: 7.6 per 1000 person-years | Population-based survey, 12 years | Nauruans (N = 1564; age: ≥ 20 years) | NIDDM |
| Lee 1993 **USA** | Not available | 18.0/1000 person-years | Prospective observational study, 9.9 ± 4.3 years | Oklahoma Indians with NIDDM. (N = 1012)<br>Data based on: 875 participants | NIDDM |
| Lehto 1996 **Finland** | Not available | Male: 5.6%<br>Female: 5.3% | Prospective observational study, 7 years | N = 1044 NIDDM<br>Gender: 571 male, 473 female<br>Age: 45 to 64 years | NIDDM |
| Moss 1996 **USA** | Not available | Cumulative incidence:<br>younger-onset: 5.4%;<br>older-onset: 7.3% | Prospective observational study, 10 years | Primary care setting | |
| Nelson 1988 **USA** | Not available | Amputation performed in 84 participants<br>NIDDM participants: 95% (80/84) | Prospective observational study, 12 years | Pima Indians of the Gila River Indian Community in Arizona<br>N = 4399 | NIDDM |

(*Continued*)

| | | | | | |
|---|---|---|---|---|---|
| Abbott 2002 **UK** | 2% per year | Not available | Prospective observational study, 2 years | Community healthcare setting<br>N = 9710 (diabetic patients) | |
| Winkley 2007 **UK** | 43.2% (recurrences) | 15.5% | Prospective population-based cohort study, 18 months | N = 253 | |
| Ramsey 1999 **USA** | 5.8% | 15.6% | Retrospective cohort, 3 years | N = 8905 | |

**Abbreviations**
NIDDM = non insulin-dependent diabetes mellitus
UK = United Kingdom
USA = United States of America
VPT = vibration perception threshold

## Appendix 3. Clasification systems for diabetic foot ulcers in people with diabetes mellitus

| **Wagner classification** (Wagner 1981)<br>**Components**:<br>1. ulcer depth<br>2. presence of osteomyelitis or gangrene | **University of Texas Wound Classification System** (Lavery 1996)<br>**Components**:<br>1. ulcer depth<br>2. wound infection<br>3. lower-extremity ischaemia | **The SINBAD system** (Ince 2008)<br>**Components:**<br>1. site (of ulcer)<br>2. ischaemia<br>3. neuropathy<br>4. bacterial infection<br>5. area<br>6. depth | **PEDIS system** Schaper 2004<br>**Components:**<br>1. perfusion<br>2. extent/size<br>3. depth/tissue loss<br>4. infection<br>5. sensation |
|---|---|---|---|
| **Grade 0:** pre- or post-ulcerative lesion<br>**Grade 1:** partial/full thickness ulcer<br>**Grade 2:** probing to tendon or capsule<br>**Grade 3:** deep with osteitis<br>**Grade 4:** partial foot gangrene<br>**Grade 5:** whole foot gangrene | **Grade 0:** pre- or post-ulcerative site that has healed<br>**Grade 1:** superficial wound not involving tendon, capsule, or bone<br>**Grade 2:** wound penetrating to tendon or capsule<br>**Grade 3:** wound penetrating bone or joint<br>Each wound grade has 4 stages:<br>**Stage A:** clean wounds<br>**Stage B:** non ischaemic infected wounds<br>**Stage C:** ischaemic non-infected wounds | **Ulcer site:** forefoot (distal to tarso-metatarsal joint = 0; mid-foot/hindfoot = 1)<br>**Ischaemia:** blood flow relatively intact (at least one pulse palpable on the affected foot = 0; evidence of ischaemia i.e. neither pulse palpable with signs of reduced tissue perfusion with, or without, gangrene = 1)<br>**Neuropathy**:<br>absent = 0; present = 1 (based on a routine examination using either Neurotips or 10 g nylon monofilaments) | **GRADE 1**<br>No symptoms or signs of PAD in the affected<br>foot, in combination with palpable dorsal pedal and posterior tibial artery, or<br>1. ankle-brachial index 0.9-1.10, or<br>2. toe-brachial index > 0.6, or<br>3. TcPO2 > 60 mmHg<br>**GRADE 2**<br>Symptoms or signs of PAD, but not of CLI: |

(*Continued*)

| | | | |
|---|---|---|---|
| | **Stage D:** ischaemic infected wounds | **Bacterial infection:** absent = 0; present = 1<br>**Area**: <1 $cm^2$ = 0; ≥1 $cm^2$ = 1<br>**Depth**: superficial = 0; deep (tendon, periosteum, joint capsule, or bone) = 1 | 1. presence of intermittent claudication (in case of claudication, additional non-invasive assessment should be performed), as defined in the document of the International Consensus on the Diabetic Foot, or<br>2. ankle-brachial index < 0.9, but with ankle pressure > 50 mmHg, or<br>3. toe-brachial index < 0.6, but systolic toe blood pressure > 30 mmHg, or<br>4. TcPO2 30-60 mmHg, or<br>5. other abnormalities on non-invasive testing, compatible with PAD (but not with CLI)<br>**GRADE 3**<br>CLI, as defined by:<br>1. systolic ankle blood pressure < 50 mmHg, or<br>2. systolic toe blood pressure < 30 mmHg, or<br>3. TcPO2 < 30mmHg |

**Abbreviations**
CLI = critical limb ischaemia
PAD = peripheral arterial disease
TcPO2 = transcutaneous oxygen pressure

## Appendix 4. Sources of growth factors

| Growth factor | Sources | Wound-healing and tissue-forming ability | Biologic activities | Source |
|---|---|---|---|---|
| **Epidermal growth factor (EGF)** | Blood vessel cells, outer skin cells<br>Fibroblasts, and many other cell types | 1. Stimulates the proliferation of epidermal and epithelial cells, fibroblasts, and embryonic cells<br>2. Chemoattractant for fibroblasts and epithelial cells<br>3. Stimulates re- | Cell proliferation | Foster 2009; Rozman 2007 |

(*Continued*)

| | | | | |
|---|---|---|---|---|
| | | epithelialisation, augments angiogenesis<br>4. Influences the synthesis and turn-over of extracellular matrix | | |
| **Platelet-derived growth factor (PDGF)** | Fibroblasts, smooth muscle cells, chondrocytes, osteoblasts, mesenchymal stem cells | 1. A and B isoforms are potent mitogens for fibroblasts, arterial smooth muscle cells, chondrocytes, and epithelial and endothelial cells<br>2. Potent chemoattractant for haematopoietic and mesenchymal cells, fibroblasts, and muscle cells, stimulates chemotaxis toward a gradient of PDGF<br>3. Activates transforming growth factor alpha, stimulates neutrophils and macrophages, mitogenesis of fibroblasts and smooth muscle cells, collagen synthesis, collagenase activity, and angiogenesis | Chemoattraction, cell proliferation | Foster 2009; Rozman 2007 |
| **Transforming growth factor alpha (TGF-$\alpha$)** | Blood vessel cells, outer skin cells | 1. Resembles epidermal growth factor, binds to the same receptor<br>2. Stimulates mesenchymal, epithelial, and endothelial cell growth, endothelial chemotaxis, controls the epidermal development<br>3. Stimulates the proliferation of endothelial cells, more potent than epidermal growth factor | Cell proliferation | Rozman 2007 |

(*Continued*)

| | | | | |
|---|---|---|---|---|
| | | 4. Promotes the generation of osteoblasts, influencing them to lay down bone matrix during osteogenesis<br>5. Affects bone formation and remodeling by inhibition of the synthesis of collagen and release of calcium | | |
| **Transforming growth factor beta (TGF-ß1)** | Blood vessel tissue, outer skin cells<br>Fibroblasts, monocytes<br>Osteoblasts-highest levels of TGF-$\beta$r | 1. Stimulates fibroblast chemotaxis and proliferation and stimulates collagen synthesis<br>2. Decreases dermal scarring<br>3. Growth inhibitor for epithelial and endothelial cells, fibroblasts, neuronal cells, haematopoietic cell types, and keratinocytes<br>4. Antagonizes the biological activities of epidermal growth factor, platelet derived growth factor, acidic fibroblast growth factor and basic fibroblast growth factor | Promotes matrix synthesis | Foster 2009; Rozman 2007 |
| **Fibroblast growth factor; acidic. (aFGF or FGF-1)** | Blood vessels<br>Fibroblasts, other cell types | 1. Participates in proliferation, differentiation, angiogenesis, and cell migration<br>2. A mitogen for skin-derived keratinocytes, dermal fibroblasts, and vascular endothelial cells | Angiogenesis, fibroblast proliferation | Foster 2009; Rozman 2007 |
| **Fibroblast growth factor; basic (bFGF or FGF-2)** | Blood vessels, smooth muscle, skin fibroblasts, other cell types | 1. Stimulates the growth of fibroblasts, myoblasts, osteoblasts, neuronal cells, | Angiogenesis, fibroblast proliferation | Foster 2009; Rozman 2007 |

(*Continued*)

| | | | | |
|---|---|---|---|---|
| | | endothelial cells, keratinocytes, and chondrocytes<br>2. Stimulates angiogenesis, endothelial cell proliferation, collagen synthesis, wound contraction, matrix synthesis, epithelialisation, and keratinocyte growth factor production | | |
| **Vascular endothelial growth factor (VEGF/ VEP)** | Blood vessel cells | 1. Stimulates the proliferation of macrovascular endothelial cells<br>2. A strong angiogenic protein, induces neovascularisation<br>3. Induces the synthesis of metalloproteinase, which degrades interstitial collagen types 1, 2, and 3 | Angiogenesis | Foster 2009; Rozman 2007 |
| **Endothelial cell growth factor (ECGF)** | Blood vessel cells | 1. Cell growth, migration, new blood vessel growth<br>2. Anti-apoptosis (anti-cell death) | Endothelial cell proliferation, angiogenesis | Foster 2009; Rozman 2007 |
| **Lactoferrin** | Polymorphonuclear leukocytes | 1. Suppresses free radical-mediated damage and decreases accessibility of the metal to invading bacterial, fungal and neoplastic cells<br>2. An iron-binding protein that was originally characterized as a milk protein. | A glycoprotein with antibiotic, anti-inflammatory and immunomodulatory activity | Weinberg 2003; Andersen 2004 http://www.ncbi.nlm.nih.gov/mesh |

## Appendix 5. Search strategies

MEDLINE
1 exp Foot Ulcer/
2 exp Diabetic Foot/
3 (diabet* adj3 ulcer*).tw.
4 (diabet* adj3 (foot or feet)).tw.
5 (diabet* adj3 wound*).tw.
6 or/1-5
7 exp "Intercellular Signaling Peptides and Proteins"/
8 exp Insulin-Like Growth Factor Binding Proteins/
9 growth factor*.tw.
10 (EGF or FGF or PDGF).tw.
11 (plermin or regranex or becaplermin).tw.
12 or/7-11
13 6 and 12
14 randomized controlled trial.pt.
15 controlled clinical trial.pt.
16 randomized.ab.
17 placebo.ab.
18 clinical trials as topic.sh.
19 randomly.ab.
20 trial.ti.
21 or/14-20
22 (animals not (humans and animals)).sh.
23 21 not 22
24 13 and 23
EMBASE
1 exp Foot Ulcer/
2 exp Diabetic Foot/
3 (diabet* adj3 ulcer*).tw.
4 (diabet* adj3 (foot or feet)).tw.
5 (diabet* adj3 wound*).tw.
6 or/1-5
7 exp Growth Factor/
8 growth factor*.tw.
9 (EGF or FGF or PDGF).tw.
10 (plermin or regranex or becaplermin).tw.
11 or/7-10
12 6 and 11
13 Clinical trial/
14 Randomized controlled trials/
15 Random Allocation/
16 Single-Blind Method/
17 Double-Blind Method/
18 Cross-Over Studies/
19 Placebos/
20 Randomi?ed controlled trial$.tw.
21 RCT.tw.
22 Random allocation.tw.
23 Randomly allocated.tw.
24 Allocated randomly.tw.
25 (allocated adj2 random).tw.

26 Single blind$.tw.
27 Double blind$.tw.
28 ((treble or triple) adj blind$).tw.
29 Placebo$.tw.
30 Prospective Studies/
31 or/13-30
32 Case study/
33 Case report.tw.
34 Abstract report/ or letter/
35 or/32-34
36 31 not 35
37 animal/
38 human/
39 37 not 38
40 36 not 39
41 12 and 40
CINAHL
S20 S15 and S19
S19 S16 or S17 or S18
S18 lower extremity N3 ulcer* or AB lower extremity N3 ulcer*
S17 TI (varicose ulcer* or venous ulcer* or leg ulcer* or foot ulcer* or (feet N1 ulcer*) or stasis ulcer* or crural ulcer*) or AB (varicose ulcer* or venous ulcer* or leg ulcer* or foot ulcer* or (feet N1 ulcer*) or stasis ulcer* or crural ulcer*)
S16 (MH "Leg Ulcer+")
S15 S1 or S2 or S3 or S4 or S5 or S6 or S7 or S8 or S9 or S10 or S11 or S12 or S13 or S14
S14 TI ( diatherm* or microwave* ) or AB ( diatherm* or microwave* )
S13 (MH "Microwaves")
S12 (MH "Diathermy+")
S11 TI ( monophasic or galvanic ) or AB ( monophasic or galvanic )
S10 TI interferential therap* or AB interferential therap*
S9 TI ( TENS or NMES ) or AB ( TENS or NMES )
S8 TI high voltage or AB high voltage
S7 TI ( low intensity or low frequency ) or AB ( low intensity or low frequency )
S6 TI ( direct current or pulsed current or alternating current ) or AB ( direct current or pulsed current or alternating current )
S5 TI electric* current or AB electric* current
S4 TI electric* stimulation or AB electric* stimulation
S3 TI ( electromagnetic* or electrotherap* ) or AB ( electromagnetic* or electrotherap* )
S2 (MH "Electric Stimulation+")
S1 (MH "Electromagnetics+")

## Appendix 6. Assessment of risk of bias in included studies

**Generation of allocation sequence (checking for possible selection bias)**

1. Low risk (any truly random process, e.g. random number table, computer random number generator, that is likely to produce comparable groups).
2. High risk (any non-random process, e.g. odd or even date of birth, hospital or clinic record number, that is unlikely to produce comparable groups).
3. Unclear risk, if the trial was described as randomised, but the method used for the allocation sequence generation was not described.

**Allocation concealment (checking for possible selection bias)**

1. Low risk (e.g. telephone or central randomisation or consecutively-numbered, sealed, opaque envelopes; allocation is unlikely to be foreseen in advance or become known later).
2. High risk (open random allocation or unsealed or non-opaque envelopes, alternation, date of birth; allocation could be foreseen in advance or become known later).
3. Unclear risk, if the trial was described as randomised, but the method used to conceal the allocation was not described.

**Blinding or masking (checking for possible performance and detection bias)**

We assessed the adequacy of blinding separately for participants, carers/personnel and outcome assessors, and also for different outcomes or classes of outcomes.

1. Low risk: participants, carers/personnel and/or outcome assessors blinded regarding the intervention participants received, or lack of blinding could not have affected the results.
2. High risk: participants, carers/personnel and/or outcome assessors were not blinded regarding the intervention participants received and this could have affected the results.
3. Unclear risk: blinding of participants, carers/personnel and outcome assessors was not reported.

**Incomplete outcome data (checking for possible attrition bias through withdrawals, dropouts, protocol deviations)**

1. Low risk (any one of the following):
   i) no missing outcome data;
   ii) reasons for missing outcome data were unlikely to be related to true outcome (for survival data, censoring unlikely to be introducing bias);
   iii) missing outcome data were balanced in numbers across intervention groups, with similar reasons for missing data across groups;
   iv) for dichotomous outcome data, the proportion of missing outcomes compared with observed event risk was not enough to have a clinically relevant impact on the intervention effect estimate;
   v) for continuous outcome data, plausible effect size (difference in means or standardised difference in means) among missing outcomes was not enough to have a clinically relevant impact on observed effect size;
   vi) missing data have been imputed using appropriate methods.
2. High risk (any one of the following):
   i) reason for missing outcome data likely to be related to true outcome, with either imbalance in numbers or reasons for missing data across intervention groups;
   ii) for dichotomous outcome data, the proportion of missing outcomes compared with observed event risk enough to induce clinically relevant bias in intervention effect estimate;
   iii) for continuous outcome data, plausible effect size (difference in means or standardised difference in means) among missing outcomes enough to induce clinically relevant bias in observed effect size;
   iv) 'as-treated' analysis done with substantial departure of the intervention received from that assigned at randomisation; potentially inappropriate application of simple imputation.
3. Unclear risk (either of the following):
   i) insufficient reporting of attrition/exclusions to permit a judgement of 'low risk' or 'high risk' to be made (e.g. number randomised not stated, no reasons for missing data provided);
   ii) the study did not address this outcome.

**Selective outcome reporting bias**

1. Low risk (either of the following):
   i) the study protocol is available and all the pre-specified (primary and secondary) outcomes were reported in the final report;
   ii) the study protocol was not available but it was clear that the published reports included all expected outcomes.
2. High risk (any one of the following):
   i) not all of the study's pre-specified primary outcomes have been reported;
   ii) one or more primary outcomes is reported using measurements, analysis methods or subsets of the data (e.g. sub scales) that were not pre-specified;

iii) one or more reported primary outcomes were not pre-specified (unless clear justification for their reporting is provided, such as an unexpected adverse effect);

iv) one or more outcomes of interest in the review are reported incompletely so that they cannot be entered in a meta-analysis;

v) the study report fails to include results for a key outcome that would be expected to have been reported for such a study.

3. Unclear risk: insufficient information available to permit a judgement of 'low risk' or 'high risk' to be made.

#### Other biases

We described for each included study any important concerns we had about other possible sources of bias (academic bias, bias in presentation data, etc.)

1. Low risk of bias, the trial appears to be free of other components that could put it at risk of bias.
2. Unclear risk, the trial may or may not be free of other components that could put it at risk of bias.
3. High risk of bias, there are other factors in the trial that could put it at risk of bias.

## Appendix 7. Wound-based severity grade for diabetic foot ulcers, according to the damaged anatomic sites

| **Grade** (Beckert 2006) | **Anatomic sites** |
|---|---|
| 1 | Dermis |
| 2 | Subcutaneous |
| 3 | Fascia |
| 4 | Muscle |
| 5 | Bone |

## Appendix 8. Diabetic ulcer severity score for foot ulcers in people with diabetes

| **Severity** (Beckert 2006) | **Score** |
|---|---|
| Absent pedal pulses | Yes = 1<br>No = 0 |
| Bone involvement | Yes = 1<br>No = 0 |
| Site of ulceration | Toe = 0<br>Foot = 1 |

(*Continued*)

| | |
|---|---|
| Number of ulcers | Multiple = 1<br>Single = 0 |

## Appendix 9. Methods used in included trials for evaluating skin wound evolution

| Study | METHOD |
|---|---|
| d'Hemecourt 1998 | " . . . The area of the target ulcer was also measured (length by width)." (p 71) |
| Driver 2006 | "Wounds were assessed and measured (length, width, and depth using a metric tape measure at each visit. The measurements and other wound variables including undermining or tunnelling, characteristics of wound exudates (i.e., presence, colour, amount, and odour), necrotic tissue, and granulation tissue were documented." (p 71) |
| Fernández-Montequin 2007 | "…a standardized photograph was taken to permit further audit of result." (p 335) |
| Fernández-Montequin 2009 | "Ulcer areas and percent granulation were measured by planimetry from a manual tracing on a transparent grid sheet."(p 434) |
| Hanft 2008 | "Weekly 35 mm photographs documented the physical features of the ulcers, while the surface area was measured using quantitative planimetric tracing of the ulcer margin." (p 31) |
| Hardikar 2005 | " . . . greatest length by greatest width . . ." (p 142) |
| Holloway 1993 | ". . . each scheduled visit the wounds were evaluated for length, width, depth and granulation tissue. " (p 200) |
| Jaiswal 2010 | "The ulcer area was calculated, by obtaining the impression of the ulcer floor on a sheet of cellophane paper and transferring on to a graph paper." (p 32) |
| Kakagia 2007 | "All wounds were photographed digitally at initial debridement and then once weekly with a reference marker of scale in three dimensions. Computarized planimetry was used . . . to compare the progression of wound healing in the three groups." (p 389) |
| Lyons 2007 | "Ulcer area was determined by tracing the debridement target ulcer onto an acetate medium at screening, . . . An image of the acetate was obtained by scanning a photocopy of the acetate containing the metering device affixed to the photocopy. For the analysis of healing, the area of the target ulcer was determined by planimetry of the image of the acetate . . . " (p 51) |
| Niezgoda 2005 | ". . . photo planimetry . . . " (p 260) |
| Richard 1995 | " . . . the length and width of the ulcer were measured and photograph was taken . . . " (p 67) |

(*Continued*)

| | |
|---|---|
| Robson 2002 | "Specific ulcer evaluation included photography, size and depth measurements . . . The areas of unclosed ulcers were obtained using digitised image analysis . . . of ulcer outline tracings made on double thickness plastic sheets . . . " (p 134) |
| Saldalamacchia 2004 | " . . . the wounds area was estimated by considering the wound like an ellipses whose diameters were the largest and shortest dimensions of the wound." (p 395) |
| Steed 1992 | " . . . length, width, depth were measured and the ulcers were photographed at each visit." (p 1599) |
| Steed 1995a | "([area at baseline-current area]/area at baseline)*100." (p 73) |
| Steed 1995b | "Ulcer area was determined by manually tracing the ulcer outline on the acetate and calculating the area of the tracing using computerized planimetry." (p 40) |
| Tan 2008 | " . . . were photographed weekly with a . . . camera with MD 50 nm lens and range flash" (p 434) |
| Tsang 2003 | "Throughout the study, ulcerate areas were overlaid with grid paper for size reference in photography . . . " (p 1858) |
| Uchi 2009 | " . . . photographed target ulcers during each visit . . . digital camera . . . " (p 462) |
| Viswanathan 2006 | " . . . Wound measurements were divided into 3 major groups: ruler based assessment schemes, transparent tracings, and optical methods . . . " (p 188) |
| Wieman 1998a | "At each visit, the area of the target ulcer was measured (length multiplied by width)." (p 824) |

## Appendix 10. Time to complete healing: RCTs not included in the meta-analysis

| Study | Results | Reasons |
|---|---|---|
| Bhansali 2009 | Intervention group: 90 days<br>Control group: 120 days | Reported incomplete information for calculating hazard ratio |
| d'Hemecourt 1998 | Intervention group I: 98 days<br>Intervention group II: 85 days<br>Control group: 141 days | Reported incomplete information for calculating hazard ratio |
| Driver 2006 | Intervention group: 45 days<br>Control group: 85 days | Reported incomplete information for calculating hazard ratio |
| Fernández-Montequin 2007 | Intervention group: 144.2 days<br>Control group: 136.5 days | Reported incomplete information for calculating hazard ratio |

(*Continued*)

| | | |
|---|---|---|
| Fernández-Montequin 2009 | Intervention group I: 98 days<br>Intervention group II: 84 days<br>Control group: 140 days | Reported incomplete information for calculating hazard ratio |
| Hanft 2008 | Treatment group: 58 days<br>Control group: could not be estimated | Reported incomplete information for calculating hazard ratio |
| Hardikar 2005 | Intervention group: 57 days (at week 20)<br>Control group: 96 days (at week 20) | Reported incomplete information for calculating hazard ratio |
| Holloway 1993 | Intervention group: 140 days<br>Control group: could not be determined | Reported incomplete information for calculating hazard ratio |
| Niezgoda 2005 | Intervention group: 73 days<br>Control group: 60 days | Reported incomplete information for calculating hazard ratio |
| Robson 2002 | Intervention group I: 16 weeks<br>Intervention group: 12 weeks<br>Intervention group: 13 weeks<br>Control group I: 9 weeks<br>Control group II: could not be determined | Reported incomplete information for calculating hazard ratio |
| Steed 1995a | Intervention group: 30 days<br>Control group: 40 days | Reported incomplete information for calculating hazard ratio. |
| Steed 1995b | Intervention group: 4 weeks<br>Control group: 8 weeks | Reported incomplete information for calculating hazard ratio. |
| Wieman 1998a | Intervention group: 86 days<br>Control group: 127 days | Reported incomplete information for calculating hazard ratio. |
| Viswanathan 2006 | Intervention group: 8.5 weeks<br>Control group: 9.8 weeks | Reported incomplete information for calculating hazard ratio. |

## CONTRIBUTIONS OF AUTHORS

Arturo J Martì-Carvajal: conceived, designed and coordinated the review; extracted data; checked the quality of data extraction; undertook quality assessment; analysed and interpreted data; checked quality assessment; performed part of data analysis and interpretation; performed statistical analysis; checked the quality of the statistical analysis; completed the first draft of the review; performed part of writing and editing the review; made an intellectual contribution to the review; approved the final review prior to submission; advised on the review; secured funding; wrote to study author/experts/companies; provided data and is a guarantor of the review.

Christian Gluud: checked the quality of data extraction; undertook and checked quality assessment; analysed or interpreted data; performed part of data analysis and interpretation; checked the quality of the statistical analysis; performed part of writing and editing the review; made an intellectual contribution to the review; approved the final review prior to submission and advised on the review..

Susana Nicola: extracted data; checked the quality of data extraction; checked quality assessment; made an intellectual contribution to the review; approved the final review prior to submission and wrote to study author/experts/companies.

Daniel Simancas-Racines: checked the quality of data extraction; undertook and checked quality assessment; analysed or interpreted data; made an intellectual contribution to the review; and approved the final review prior to submission.

Ludovic Reveiz: checked the quality of data extraction; undertook and checked quality assessment; analysed or interpreted data; made an intellectual contribution to the review; approved the final review prior to submission and advised on the review.

Patricio Oliva: checked the quality of data extraction; undertook and checked quality assessment; analysed or interpreted data; made an intellectual contribution to the review and approved the final review prior to submission.

Jorge Cedeño-Taborda: checked the quality of data extraction; undertook and checked quality assessment; analysed or interpreted data; made an intellectual contribution to the review; approved the final review prior to submission and advised on the review.

### Contributions of editorial base

Nicky Cullum: edited the protocol; advised on methodology, interpretation and protocol content. Approved the final protocol prior to submission.
Jo Dumville: edited the review, advised on methodology, interpretation and review content. Approved the final review prior to submission.
Sally Bell-Syer: co-ordinated the editorial process. Advised on methodology, interpretation and content. Edited and copy-edited the protocol and review.
Rachel Richardson: edited the review.
Ruth Foxlee: designed the search strategy and edited the search methods section.

## DECLARATIONS OF INTEREST

Arturo Martí-Carvajal: was employed in 2004 by Eli Lilly to run a four-hour workshop on 'How to critically appraise clinical trials on osteoporosis and how to teach this'. He was employed in 2007 by Merck to run a four-hour workshop 'How to critically appraise clinical trials and how to teach this'. These activities are not related to his work with The Cochrane Collaboration or any Cochrane review.

Jorge A Cedeño-Taborda: was employed as Medical Director for Organon Venezolana SA from 2002 to 2007. In 2007 he was employed by Novartis de Venezuela as an outsourced medical adviser for the development of scientific material to support the marketing of Vildagliptine. None of these activities is related to the subject of this Cochrane review.

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- Copenhagen Trial Unit, Denmark.

Academic.

- Cochrane Hepato-Biliary Group, Denmark.

Academic.

- Iberoamerican Cochrane Network, Spain.

Academic.

## DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Based on Consolidated Standards of Reporting Trials (CONSORT) statement, we changed the term 'safety' into 'adverse events'. The term 'safety' may be considered to imply substantive evidence of an absence of harm. The term is often misused when there is simply absence of evidence of harm (Ioannidis 2004).