Review Article

Vascular endothelial growth factor for in vivo bone formation: A systematic review

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ABSTRACT

Background: To achieve optimal bone formation one of the most influential parameters has been mentioned to be adequate blood supply. Vascular endothelial growth factor (VEGF) is hereby of particular interest in bone regeneration, because of its primary ability to induce neovascularization and chemokine affection for endothelial cells (EC), and is considered to be the main regulator of vascular formation. However, the growth factor has yet to be implemented in a clinical setting in orthopaedic intervention surgery. We hypothesised that the development of VEGF in vivo for bone formation in the last decade had progressed towards clinical application since the latest systematic review from 2008.

Objective: This systematic review recapped the last 13 years of in vivo bone regeneration using vascular endothelial growth factor (VEGF).

Method: A total of 1374 articles were identified using the PubMed search string (vegf or “vascular endothelial growth factor”) and (osteogen* or “bone formation” or “bone regeneration”). By 3 selection phases 24 published articles were included by the criteria of being in vivo, using only VEGF for bone formation, published after 2007 and written in English. Articles in vitro, written in different languages than English and older than 2007 was excluded. The most recent systematic review on this subject was published in 2008, with the latest included study from 01 to 11-2007. All included studies were classified based on animal, type of defect, scaffold, control group, type of VEGF, release rate, dosage of VEGF, time of evaluation and results. Each study was evaluated for risk of bias by modified CAMARADES quality assessment for the use in experimental animal studies. The score was calculated by peer review journal publication, use of control group, randomisation of groups, justified VEGF dosage, blinding of results, details on animal model, sample size calculation, comply with ethics and no conflict of interest.

Results: No clinical trials or human application studies were obtained from our search. Experimentally, 11 articles using solely VEGF for bone formation had a group or a timepoint significantly better than the corresponding control group. 18 articles revealed no significant difference of VEGF compared to the control group and 1 article reported a significant decreased bone growth using VEGF compared to control.

Conclusion: Based on these results no clinical studies have yet been performed. However, indications in the best use of VEGF from experimental studies could be made towards that the optimal release is within the first three weeks, in defect models, with the best effect before eight weeks. Future designs should incorporate this with standardised and reproducible models for verification towards clinical practice.

The translational potential of this article: This systematic review aims to assess the existing literature to focus on methodologies and outcomes that can provide future knowledge regarding the solitary use of VEGF for bone regeneration in a clinical setting.
**Introduction**

Bone loss and defect due to trauma or infection is a very specialised area in the field of orthopaedics, and knowledge of the physiological parameters in bone formation is subject to an ongoing investigation. Research on bone formation has attracted increasing interest due to an increasing elderly population and fracture rate globally, where a significant percentage of fractures has inadequate defect healing due to infections, surgical procedures and more fragile bone structure [1,2]. One well-known factor in achieving sufficient bone healing is to secure sufficient blood vessel contribution to the defect, with some research on growth factors focusing on the use of the angiogenic protein vascular endothelial growth factor (VEGF).

**VEGF in bone research**

VEGF is of particular interest in bone regeneration due to its primary ability to induce neovascularisation [10,15,22]. The VEGF protein also carries an indirect ability to differentiate MSCs into the osteogenic lineage [3,7]. Furthermore, it has a chemokine effect on surrounding endothelial cells (ECs) that can increase the number of vessels in a localised area [8]. These effects contribute to achieving a more controlled use of both the stimulating effect of VEGF and regenerative effects of the MSCs. Notably, the inhibition of VEGF has led to non-union models [9], making the growth factor essential in the bone healing process.

The VEGF family consists of different members, including VEGFA, -B, -C and -D, and placenta growth factor-1/2 (PIGF1/2) [8,10] (Fig. 1). The

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**Fig. 1.** VEGF family and receptors for the signal pathway to vessel distribution. Primary ossification center (POC), hypertrophic chondrocyte (HC) [12].

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**Fig. 2.** Illustration of the search strategy for the systematic review. A total of twenty-four articles were included.

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most commonly used isomer of VEGF in the field of bone research is the human VEGF165 (rVEGF165)—a member of VEGFA—due to its elevated potency and effect [24].

Bone formation can be divided into intramembranous and endochondral ossification. The primary stimulator of VEGF expression in the osteoblast-like cell line is hypoxia-induced factor 1α (HIF-1α), while the release of HIF-1α is related to the initiation of fracture repair in the endochondral ossification [13,14]. The initial stage of endochondral bone formation is the cartilage base structure created by the chondrocytic progenitor cells within regions of low blood perfusion. Then, the chondrocytes proliferate and enlarge. This initiates the attraction and incorporation of endothelial cells, osteoblastic precursor cells, haematopoietic cells and osteoclasts that result in cartilage becoming degraded and replaced by trabecular bone and bone marrow [15,16].

**The challenges for using VEGF for bone regeneration**

Generally, abundance of blood vessels around the healing site will enable more nutrients and regenerative cells to be transported from the bloodstream to the area of bone formation, with more waste products from the healing site being moved away from the desired location [17]. However, the VEGF protein appears to have a narrow therapeutic window [4], which implies that if the dosage of VEGF is too high, this can cause malformed and non-functional vessels as well as a potentially toxic effect [4,18].

Furthermore, VEGF has a half-life between 4 and 24 h [19,20]. This short period makes it difficult to use in the surgical setting if a long-term effect on the defect is desired without reaching the toxicity threshold. This issue has caused multiple delivery methods in experimental designs to prolong the release of VEGF [21].

Theoretically, the VEGF protein should have the capability to enhance the regeneration of bone defects, especially endochondral ossification. The growth factor is already applied in human clinical trials for other purposes [22] and would have the natural capability to transition to the field of bone research. However, to our knowledge, clinical trials using VEGF in this field have not yet been published.

**Objective of this study**

This systematic review aspires to collect all existing in vivo results on the solitary use of vascular endothelial growth factor for bone growth compared to control, as evaluated by new bone formation. Additionally, we evaluate whether these results indicate any promising progress towards release methods and dosages that could be applied in a focused experimental design and translated into human clinical use. Our hypothesis is that solitary VEGF stimulation in the current in vivo literature...
| Ref          | Animal               | Type                        | Scaffold                          | Control                           | Type of VEGF       | Release                        | Dosage                  | TOE       | Results                                           | BV/TV | Quality score |
|--------------|----------------------|-----------------------------|-----------------------------------|-----------------------------------|--------------------|--------------------------------|--------------------------|-----------|---------------------------------------------------|-------|--------------|
| Amirian 2015 | Rat                  | Cranial defect model        | Pectin-biphasic calcium phosphate | Empty defect                     | rhVEGF             | Gelatin hydrogel scaffolds    | Total concentration VEGF: Unknown 75% released in 15 days | 2 weeks/4 weeks | MicroCT: VEGF significantly better than control |       | 4            |
| Keeney 2010  | Mice                 | Intra-femoral defect        | Collagen/calcium phosphate        | Only scaffold                     | Therapeutic plasmid | VEGF165 Plasmid DNA           | 0.35ug/mm^3              | 30 days   | Significant more bone in scaffold + VEGF165      |       | 3            |
| Lohse 2015   | Rat                  | Mandible                   | calcium carbonate granules        | Scaffold and empty defect         | rhVEGF165          | Polycaprolactone (PDLLA)      | 0.24ug/1.5ug/6ug, ~50% release first 3 days, low constant release till 5 weeks. | 4 weeks/13 weeks | Only significantly better with 1.5ug after 4 weeks. NS other timepoints and dosages |       | 3            |
| Çakır-Özkan 2017 | Rabbit              | Mandible                   | PLA-PEG                           | Only scaffold                     | rhVEGF165          | Gelatin                        | 750ng/scaffold Release 60% first 2 days, constant release for more than 14 days. | 4 weeks/8 weeks | Significantly better than control.                |       | 4            |
| L Zhang 2014  | Beagle               | Femoral neck fracture model | Cannulated (titanium) screws      | Cannulated screw fibrin glue      | VEGF               | PLGA/Fibrin glue               | Unknown total VEGF in fibrin glue, 21.5% released after 3 days. Steady with 1.71% to 42 days with 88% cumulative release. | 4 weeks/12 weeks | VEGF had significant better results in week 8 and week 12 (p < 0.01) |       | 6            |
| Lv 2015      | Rabbit               | Femoral condyle defect model| Titanium scaffold or empty defect | Empty titanium scaffold           | rhVEGF165          | Fibrin glue                    | 0.5ug VEGF, Steady 100% release in 96 h 0.6%/hour steadily from 12 to 96 h | 4 weeks    | Significantly better than control.               |       | 4            |
| Khojasteh 2017 | Dog (mongrel)        | Mandible defect             | B-TCP                             | Scaffold only                    | VEGF               | PLGA microspheres             | Release: Burst 60 ng/ml 8h Steady 14 days release total 200 ng/ml. | 8 weeks | No significant difference to control              |       | 4            |
|              | Rat                  | Ectopic                     | Scaffold + granules               | rhVEGF165                        | PDLLA/CaCO3        |                                |                          |           | Histomorphometry:                               |       | 4            |

(continued on next page)
| Ref       | Animal Type | Scaffold | Control | Type of VEGF | Release                  | Dosage | TOE       | Results                                                                 | BV/TV | Quality score |
|-----------|-------------|----------|---------|--------------|--------------------------|--------|-----------|--------------------------|-------|---------------|
| Moser 2018 [27] |             | PDLLA/CaCO3 composite granules |         |              | 25ug VEGF/g polymer      |        |           | 4 weeks/13 weeks          | No significant difference to control | Histomorphometry: 4 weeks: Control: 0.1% VEGF: 0.1% 13 weeks: Control: 0.1% VEGF: 0.1% |
| W Zhang 2014 [38] | Rabbit Skull defect | Silk scaffold | Silk scaffold with water | VEGF | Silk scaffold + water absorption | 6ug/scaffold | 12 weeks | No significant difference to control | uCT BV/TV control: 4.12 VEGF: 10.14 Histomorphometry: Control: ~28% VEGF: ~44% | 3 |
| W Zhang 2011 [39] | Rabbit Sinus floor elevation surgery | Silk hydrogel | Silk gel alone | rhVEGF165 | Silk hydrogel        | 1000ug/ml * 0.200 ml = 4 ug per scaffold No burst release. At least 24 days release | 4 weeks/12 weeks | No significant difference between VEGF and control | 4 |
| Quinlan 2017 [28] | Rat Calvarial defect model | Collagen-Hydroxyapatite scaffold | Empty defect and only scaffold | rhVEGF165 | Alginate microparticles | 1ug/mg (1.6 ug/scaffold) Burst release till day 7. Steady release till 8 weeks. | 8 weeks | Significantly better than empty defect. NS against scaffold alone. Significant more new bone in VEGF group | uCT: Empty defect: 0.4% Empty scaffold: 1.8% VEGF: 3.2% Histomorphometry: (um2, 105) Empty defect: 0.9 um2 x 105 Empty scaffold: 1.9 um2 x 105 VEGF: 5.4 um2 x 105 | 7 |
| Schilephake 2015 [29] | Rat Tibia head placement | Titanium implant | Empty implants with empty DNA nucleotide surface | rhVEGF165 | DNA oligonucleotide | 750ng/screw 53% released within week 1. | 1 week/4 weeks/13 weeks | Significant lower bone formation in week 4. NS in week 1 and 13. | uCT: Empty control: 4.0% Surface control: 4.3% VEGF: 5.9% 4 weeks Empty control: 17.6% Surface control: 20.3% VEGF: 5.9% 13 weeks No values mentioned | 7 |
| Behr 2011 [35] | Mouse Calvarial model | Collagen sponge | PBS soaked collagen sponges | VEGFA | Collagen sponge | 200ng/mouse | 2 weeks/4 weeks/8 weeks/12 weeks | VEGF significantly better than control. | uCT 2 weeks: Control:1.2% VEGF:81.0% 4 weeks: | 6 |

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Table 2 (continued)

| Ref       | Animal | Type              | Scaffold                        | Control                                    | Type of VEGF | Release               | Dosage | TOE          | Results                                      | BV/TV | Quality score |
|-----------|--------|-------------------|---------------------------------|--------------------------------------------|--------------|-----------------------|--------|--------------|----------------------------------------------|-------|---------------|
| Geuze 2012 [46] | Beagle | Ectopic           | BCP scaffold                    | Calcium phosphate BCP scaffold mixed with microparticles or hydrogel without growth factors | rhVEGF165    | Sustained release: PLGA microparticles. Fast release: Hydrogel (gelatin) | 0.4ug per ectopic implant | 9 weeks | No significant difference to control.       |                   |               | 8               |
| Hernandez 2012 [40] | Rabbit | Bone defect       | PLGA porous scaffold            | Empty defect and empty scaffold            | rhVEGF165    | PLGA microspheres     | 4 mg (0.35ug)/20 mg (1.75ug) | 2 weeks | 2 weeks: VEGF (1.75) significantly better than all groups. | 4 weeks | Control: 10% | 4 | 4               |
| Kempen 2009 [30] | Rat    | Critical sized femur shaft model, subcutaneous model | Empty defects (only orthotopic) and empty scaffold | VEGF | Gelatine Hydrogel     | 2.0ug/scaffold 58% release during first 3.5 days. Total release after 2 weeks | 8 weeks | Subcutaneous: No significant difference between control and VEGF. Orthotopic: No significant difference between VEGF and empty scaffold or empty defect. | Subcutaneous: Empty scaffold: 0% VEGF: 0% Orthotopic: uCT new bone: Empty defect: 28mm3 Empty scaffold: 28mm3 VEGF: 30mm3 | 4               |
| Casap 2008 [41] | Rabbit | Mandible distraction | Injections                      | No injection                               | rVEGF165     | —                     | After 14 days Sug/ul. for 4 days. 60 days | No significant difference to control (p = 0.057) | MicroCT BV/TV: Control: 2.5% VEGF: 1.3% Histomorphometry scoring: Control 4 weeks: 0.5 12 weeks: 1 VEGF | 6               |
| Patel 2008 [55] | Rat    | Cranial defect    | Gelatin microspheres in porous PPF scaffold | Blank Gelatin microspheres or empty defect | VEGF | Gelatin | 0.24ug/mm³ | 4 weeks/12 weeks | No significant difference to control |                   |               | 6               |

(continued on next page)
| Ref       | Animal | Type                        | Scaffold                          | Control                           | Type of VEGF | Release                        | Dosage | TOE | Results                                      | BV/TV | Quality score |
|-----------|--------|-----------------------------|-----------------------------------|-----------------------------------|--------------|--------------------------------|--------|-----|----------------------------------------------|------|--------------|
| Yang 2010 | Rabbit | Radial diaphysis            | BTCP coated with fibrin sealant    | Scaffold and untreated rhVEGF165 | Absorption fibrinogen | 2.6ug VEGF/scaffold 90% release after 7 days. | 4 weeks, 8 weeks, 12 weeks | 4 weeks: 0.5 12 weeks: 1 MicroCT Empty defect: 4w: 7% 12w: 16% Control 4 weeks: 4% 12 weeks: 8% VEGF 4 weeks: 2% 12 weeks: 6% microCT new bone/TV | 5    |
| Yonamine 2010 | Rat    | calvaria                    | PLGA microspheres                  | Empty defect/sham surgery VEGF165 | PLGA microspheres | 1ug per 500ul No release from day 0-7. Full release till day 21, 2eg | 12 weeks | 12 weeks | No significant difference to control | 3    |
| Wu 2012   | rabbit | Mandibular distraction      | Plasmid pIRES injection            | pIRES and normal saline hVEGF165 | Plasmid | 2 weeks/4 weeks/8 weeks | Significant difference to control in both bone types. | 7    |
| pigs      | Bio-oss| rhVEGF165                   | Fibrin glue                        | 8ug/ml, 3 ml total               |               |                                |                    | (continued on next page) | 5    |
will demonstrate a pattern and method for human application in bone formation for a potential solution in the use in orthopaedic surgery.

Materials and methods

Design

Inclusion criteria include in vivo studies in both animals and humans using VEGF compared to empty defect or empty control, where the outcome measure is bone growth within a region assessed either by micro-CT or histomorphometry. All human studies were included. If a study used VEGF in combination with other hormones or growth factors, only the solely VEGF groups were included. Articles were restricted to English articles with full text available that were published during the last thirteen years. Exclusion criteria were all ex vivo studies, reviews, explanatory articles, conference abstracts, lectures and newspaper articles (Fig. 2).

Based on previous literature

A thirteen-year duration was selected due to an existing systematic literature review produced in 2008 on the effect of VEGF on bone formation [23]. The latest included article from this study was from 01 to 11/2007.

Studies were identified through PubMed using the search string (vegf or “vascular endothelial growth factor”) and (osteogen* or “bone formation” or “bone regeneration”). Studies were then filtered for full text only, human or animal studies, and publication date from 1 January 2007 to 01 July 2019. Studies were then screened by title and abstract, and the full texts of eligible studies were analysed for final inclusion (Fig. 2).

Phases for inclusion

Study selection was divided into different phases. In phase one, articles were evaluated by their title and abstract. In phase two, articles were assessed based on their full text by primary inclusion and exclusion criteria without any evaluation of results. In the final phase, relevant studies were subject to quality assessment using a scoring system.

The extracted data from each included article

The following data were extracted from the included studies: journal type (peer-review or not), animal model details, tissue type (e.g. cranial or ectopic bone formation), type of scaffold, type of control group, type of randomisation, type of VEGF and its release system, justification for VEGF dose and/or release rate, experiment duration, type and magnitude of outcome, type of blinding, sample size considerations, and ethics and disclosure statements. The corresponding author of the study was contacted if any of the aforementioned information was missing from the articles. Data extraction was performed by CHD and verified by KK. At any disagreements, the third or fourth authors were included, and a discussion was initiated until agreement by all authors.

Risk of bias assessment

Assessing the risk of bias among articles was inspired by Macleod et al., while the pooling of animal experimental data was provided by CAMARADES for experimental animals [24] (Table 1). This assessment consisted of 10 items, with each providing 1 point. Three items were removed, and two items were added to highlight the objective of this systematic review. This meant that questions regarding the control of temperature, the blinded induction of ischaemia, and the use of anaesthetic without significant intrinsic neuroprotective activity were removed. Instead, the justification of a control group and VEGF dosage were added.
The total assessment of the risk of bias was based on scientific quality according to peer-reviewed articles, control groups, random allocations to intervention groups, blinded evaluations and sample size calculation. Furthermore, the reproducibility of dosage and animal models, any conflict of interest and animal regulations by approval mentioned in the article.

Statistics

Statistics were calculated by one-way ANOVA for the comparison of different parameters, when applicable. A p-value of less than 0.05 was considered significant.

Results

A total of 1374 articles were identified using the PubMed search. During the title/abstract screening, 1304 articles were excluded, leaving 70 articles for the full-text analysis. The full-text analysis excluded 46 articles: 1 used a modified adenovirus, 5 were ex vivo studies, 21 were missing bone volume/total volume evaluation by either histomorphometry or microCT scan and 19 articles did not have a group with the solitary use of VEGF for bone formation (Fig. 2).

Data extracted from each article

The content of each article is illustrated in Table 2. No studies used VEGF in human studies.

Experimental models

The experimental models were as follows: rats [25-33], mice [34,35], rabbits [36-43], beagles [44-47] and pigs [48]. The types of defect were as follows: cranial/calvaria/skull defects [25,28,31,32,35,44,48], intra-femoral implantation [34], mandible [26,33,36,41,43,45,47], femoral neck fracture model [44], femoral condyle defect [37,40], femoral shaft defect [30], ectopic [27,46], sinus floor elevation surgery [39], tibia head implant [29] and radial diaphysis [42].

Release methods for VEGF

VEGF was released by different delivery systems as follows: gelatine [30,31,34,36,46], plasmid DNA [34,43], poly-d,L-lactic acid (PDLLA) [26], fibrin glue [37,42,44], PLGA microspheres [32,33,40,45], PDLLA/COS [27], silk scaffold [38], hydrogel [39], alginate microparticles [28], PLGA microparticles [46], DNA oligonucleotide [29], Bio-Oss [48], hydroxyapatite [47], collagen sponge [35] and injection [41].

VEGF dosages

The dosage of VEGF was mentioned as either a total dosage, a dosage per area, or an amount in fluid. Doses were as follows: 0.24ug [26], 0.35ug [40], 0.4ug [46], 0.5ug [37], 1.5ug [26,27], 1.75ug [40], 2ug [30,43], 2.6ug [42], 3ug [47], 6ug [26,27,38], 20ug [39], 24ug [48], 200 ng [33,35], 750 ng [36], 200ng/100ul [45], 1ug/500ul [32], 5ug/ul [41], 75 ng/mm² [29], 2.1ug/mm² [34], 0.24ug/mm² [31], 1ug/mg [28] and two studies did not mention dosage [25,44].

Studies in favour of using VEGF

The results were based on new bone regenerated in the bone defect region and compared to the control as were follows. Eleven articles had a group, time point or dosage with significantly better results in the VEGF group compared to the control (Fig. 2). Of which seven articles showed better bone growth at all time points and dosages for the VEGF group [25,34–37,42,43]. One article only showed significantly better results when compared to empty control and not an empty carrier [28]. One article favoured VEGF at 8 and 12 weeks [44]. One article favoured VEGF at week two, four, and eight [40], and another article favoured VEGF at a dosage of 1.5ug VEGF at week four [26]. Of these 11 articles, 6 had empty scaffold as the control group [25,26,35,36,42,43], while four articles had an empty defect as the control group [28,34,40,43] and four compared the combined scaffold and release method without VEGF [37,40,42,44]. Evaluations were conducted after an average of 5.8 weeks.

Studies not in favour of using VEGF

In total, 18 articles had a group, time point or dosage with no significant (NS) difference in bone formation in the VEGF groups compared to the control group (Fig. 2). Twelve articles showed NS compared to control for all groups included in the study [27,28,30–33,38,39,41,45–48]. One article showed NS compared to the scaffold [28]. One article with the dosage of 0.24ug and 6ug in weeks 4 and 13, and 1.5ug at week 4 [26], while 1 article was NS in the 4-week group [44]. One article in weeks 1 and 13 [29] and one in week 12 [40]. Of these results, eight had a control of only scaffold [26,27,32,33,38,39,45–47], two had an empty defect [36,41], two had the scaffold and release method combined [44,46], three had both the empty defect and scaffold group [28,36,40,48] and one had an empty defect and release material [31]. The average evaluation was conducted after eight weeks.

One article had a group in week four with significantly lower bone formation in the VEGF group compared to both empty defect and scaffold [29] (Fig. 2).

The time of evaluation was significantly lower in the 11 articles that showed better results with the use of VEGF compared to the 18 articles showing NS compared to the control (p < 0.05).

Risk of bias

The characteristics of included studies are illustrated in Table 2. The quality scores of the 24 articles range from 3 to 8 with an average of 5.08 points out of 10. The average quality score of the 11 studies that had a group, time point or dosage with a significantly better bone formation than the control was 4.81, whereas the average of the 18 articles with no significant difference was 5.3. No statistical differences were observed between the groups (p > 0.05). The only article with a lower quantity of bone formation with the use of VEGF had a score of 7.

Discussion

This article conducted a systematic review that collated and assessed recent progress in the solitary use of VEGF for bone formation over the past 13 years alongside any progress made towards clinical application. A total of 1374 articles were found by the search criteria. Phase one included 70 articles by title and abstract, while phase two excluded 46 articles based on the full-text analysis. Ultimately, a total of 24 articles met the criteria for inclusion. These were quality scored using eight validated questions and one modified for the purpose of this review. Notably, the various models and methodologies used in these studies made statistical comparisons difficult. However, some very exciting indications could be extracted from the articles, such as the most efficient use of VEGF appearing to occur in defect models with a release of VEGF within the first three weeks, and evaluation studies with an early focus of eight weeks or less exhibiting the improved use of VEGF. For future study designs, this review serves as an inspiration for the modification and improvement of VEGF use for bone formation.

In 2008, a systematic review of the use of VEGF for bone formation was produced for future applications in bone research [23].
The study concluded that the existing evidence on the use of VEGF for bone formation is positive, but difficult to direct due the use of a lot of different models. The study recommended the potential use of VEGF in fracture healing and suggested a focus on future implementation. The most recent study included in this review was from January 2007. To our knowledge, no systematic review has been performed on this matter since. This point highlights the relevance of a follow-up study to evaluate further progress regarding the clinical use of this growth factor in bone surgery to promote more efficient healing.

However, when comparing the conclusion from these two reviews, similar indications and conclusions of the beneficial use of VEGF for bone formation are present. Both studies have not been able to make statistical heterogeneity analysis due to both lack of descriptive methodology and different fracture and animal models. The conclusion from both studies is that VEGF has been seen in several studies to have a beneficial effect which could indicate potential use.

This review presents an overview of all articles using VEGF and approaches could hereby inspire and be compared for future designs. It must be implied that studies for in vivo studies are based on previous literature and not local methods, experience, and regular descriptions. Based on these findings the lack of clinical trials can be correlated to a narrow window of the VEGF dosage and release, the insufficient focus of the research on specific type of bone healing processes i.e. POC, long bone, SOC etc, and specific location for the fracture in the case of mechanical strength and the cortical-trabecular ratio.

The consideration in using VEGF in vivo should in general be addressed to monitored effect on affected tissue to not induce irregularized tissue growth, as all stimulants in tissue engineering. Furthermore, isolated VEGF has been identified with malignant cell growth [49]. This is why general side effects and systemic evaluation must be addressed when using VEGF in designs with local approach [50].

When using VEGF locally, some dosages have shown to cause edema around the healing site and in later stages, non-union [4,18,29]. Before any clinical trials can be initiated reproducible results must be presented for a well-defined clinical treatment and side effect profile. In case of inspiration for this development, this review can be relevant in both developing and inventing future strategies.

A statistical comparison of all included studies proved difficult due to the inconsistencies in variables such as administration, model, defect, animal, release and dosage across studies. Moreover, an internal difference noted by Schliepakhe et al. had one group showing NS results in weeks 1 and 13, but a lower amount of bone in week 4. This illustrates that the use of the same design can yield different results depending on the time of observation. The time of observation was between 1 and 13 weeks across all included studies. Moreover, the majority of the included studies had an evaluation between 4 and 8 weeks (58%). Comparison according to outcome showed a significantly shorter evaluation period in the studies achieving a better effect of VEGF compared to the control. This could be an indication that the greatest effects of VEGF occur within the early stage of the bone regeneration process.

Although dosages were reported in different units, a general observation was that the lowest reported single dosage used in all included studies was 0.2ug [33,35], while the highest was 24ug [48]. The two dosages with 0.2ug were reported by Behr et al. (significantly better results with VEGF) and by Das et al. (no significant difference). Furthermore, Schmitt et al. used 24ug and noted no significant difference compared to control. In studies where VEGF showed significantly better results in one group, 2.1ug/mouse was used in an intra-femoral implementation [34], 0.5ug in a rabbit femoral condyle defect [37], 200 ng in a mouse skull defect [35], 2ug in a rabbit mandible distraction model [43], 750 ng in a rabbit mandible defect [36] and 2.6ug in a rabbit radial diafysis [42], while a rat cranial defect model used an unknown dosage [25]. The only group with a worse effect of VEGF than control used 750 ng/cm² in a rat tibial head implant [29]. Based on these markers, it is not possible to provide a dose–response curve in any of the models. However, the highest single dosage with consistent positive response of VEGF was 2.6ug. This interval serves as a marker for future studies working with the same animal and defect model with the use of VEGF—even in combination studies.

In translational medicine, the dosages and release rates are essential to provide sufficient evidence for the clinical applications of products [40]. Release rates are defined as burst or continuous release. The release pattern of the growth factors has already been previously studied with a focus on prolonging the effect of VEGF [51]. In the use of VEGF on bone formation, the general consensus is that VEGF has the greatest effect when released in the natural systemic peak of 2–3 weeks [50] which, in smaller rodent and humans, is calculated to be at approximately week 1–3 [52,53]. Theoretically, VEGF will be concentrated in the inflammatory phase during the first week after a fracture within a defect. However, in the cartilage phase, inhibition has been shown to stimulate the osteogenic lineage [54]. These statements would suggest that VEGF had an optimal effect in endochondral ossification and that the release should be stopped before the cartilage phase of the bone formation process. This would imply that the optimal release occurs within the first weeks of a defect. By this assumption, we grouped the included studies into burst release groups, with full release occurring within the first three weeks [25,30,32,33,37,41,42,45] and continuous release occurring for more than three weeks [26–28,39,44]. The remaining studies have no information regarding release after three weeks [29,36,40] or have an unknown release profile [31,34,35,38,43,46–48].

In the seven studies that had better bone formation than the control, three had a full release within the first three weeks [25,37,42] while the rest were unknown [34–36,43]. The only group worse than control had no information on the release [29]. The assumption made by these results supports the existing literature, which suggests that VEGF should be released within the first 3 weeks.

The amount of new bone varied from 0 to 1% in a mouse ectopic model [27] up to 92% in a mouse calvarial model [35], and up to 96% in a rat mandible distraction model [43]. Since the physiological effects of VEGF seem to be best in the endochondral state of healing, it follows that the ectopic designs exhibit low amounts of new bone. The other ectopic design in this review had only 4% of total bone formation [46]. These results suggest that VEGF is not very osteogenic outside of defects models and the effectiveness is location-dependent. By this assumption, VEGF has the potential to optimise existing chemokine effects, but not to establish new pathways for enhancement. This could theoretically indicate that a focus towards fracture models would be preferable if the optimal use of VEGF should be established.

In the calvarial mouse model by Behr et al., VEGF had the same effect on bone formation as BMP-2. As such, the authors focused on comparing the dosages of BMP-2 to the clinical dosages that are already used for BMP-2 and could be used for VEGF. This study design focused on the translational purpose and the release method of the collagen sponge that has already been used for clinical studies. This study could serve as a marker for future designs in larger animal models with a dosage of 200mg/mouse. This also seems to correlate to the dosage range for growth factors in a small animal model. Furthermore, BMP-2 was adjusted from a clinical dosage into an experimental dosage. Notably, being able to translate between different models and areas to use the design for multiple purposes is an important aspect of optimal VEGF use.

Overall, 15 of the studies included in this review have the primary purpose of combining the angiogenic stimulation of VEGF with bone morphogenic protein (BMP) or mesenchymal stem cells [25–27,30,33,36–40,43,45,46,48,55], whereas the group with only angiogenic stimulation serves as a secondary objective. The general outcome is that 11 of these studies with a combination treatment using BMP [25–27,30,33,36–38,46,48,55] showed superior results to treatment using VEGF alone, as measured by new bone formation. Khojasteh et al. showed superior results with a combination of mesenchymal stem cells. Moreover, Wu
et al. noted no difference between combination treatment and VEGF at week two, though the combination treatment was significantly better than VEGF alone at weeks four and eight. This illustrates that the time of treatment observation serves as an important factor in the measures of the outcome of bone formation with the use of these growth factors.

The majority of control groups in the included articles are empty defects, scaffolds only, or scaffold in combination with release material (Table 1). The clinical relevance for this approach must be considered limited since our intervention has the purpose of providing the same (or even better) results using existing procedures. However, while this can prove difficult in smaller animal models, it should be considered when translating into larger models to provide comparable results. An example of this is a study by Hernández et al., where the authors used the INFUSE model for the delivery of BMP-2—a method that is already established clinically and thus serves as a relevant control group.

In general, the use of VEGF in bone formation studies has shown strong potential over the last decade; however, no human trials have been performed to date. In order to evolve in the field, detailed description of methodologies would be particularly useful so that each study could be compared with existing results and developed to influence future designs. This would provide an opportunity to compare existing results using statistics, thereby creating the transparency necessary to build on existing literature. Notably, this field is currently delayed by the lack of reproducibility.

Conclusion

This review revealed that the development of VEGF is still progressing for the purpose of clinical application. The studies included in this systematic review provide valuable information on the use of VEGF for bone formation, such as:

- The releases of VEGF appearing to be optimal within the first three weeks following fracture.
- VEGF seems to have the best effect in fracture models, and in general seems to be better in defect models when compared to ectopic design. The single highest VEGF dosage for significant results was 2.6 ug per animal, regarding a variety of models and animals. Higher dosages did not have any improved effect compared to control.
- The studies with an observation shorter than eight weeks seem to have the best outcome.

Studies that combined VEGF with other osteogenic factors generally had a higher percentage of new bone formation compared to VEGF and/or control; however, this is also correlated to possible higher costs, more preparation efforts, and higher side effect profiles such as malignant growth.

Future research using solely VEGF should be inspired by the existing literature and focus on the development of published methodologies and results. This will ensure progression in the area while making it possible to translate the results of different models and animals for clinical trials.

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Conflict of Interest

The authors have no conflicts of interest to disclose in relation to this article.

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