Spatiotemporal delivery of dual angiogenic growth factors for the development of prevascularized collagen gel

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Objectives

Serious injuries can affect bone function significantly, and treatment costs for bone defect repairs account for $2.5 billion in the U.S annually. Osteogenic growth factor (GF) delivery has provided an alternative to auto- and allografts in the treatment of critical bone defects.

Delivery of multiple GFs to synergize vascular and osteogenic responses in bone healing have shown promise in animal models and could eventually provide an alternative to bone grafting in the treatment of critical-sized bone defects.

Given the critical dependence of bone regeneration on adequate vascularization, temporally controlled delivery of vascular endothelial growth factor (VEGF) could augment the regeneration of vascularized bone tissue for challenging clinical scenarios at risk for non-union.

However, VEGF alone can form vessels that are leaky and unstable, and thus co-delivery of platelets derived growth factor (PDGF) with VEGF may enhance the establishment of matured vascular networks.

Therefore, the goal of this study is to evaluate the effects of dual angiogenic GFs on vascularization and to regenerate the bone in a critical composite tissue injury model by delivering both osteogenic and dual angiogenic GFs (BMP-2, VEGF and PDGF).

Methods

The effects of various single and multiple GFs on the promotion of vascularization of microvascular fragments (MVFs) in the collagen gel were determined using collagen gel-based in situ delivery of GFs. Collagen gels and alginate were prepared using our previously reported method.

The gels were assayed to characterize the binding and release properties of BMP-2, VEGF, and PDGF. 100 ng of the GFs in 0.1% BSA (1ml) were added to the gels and placed in a rotary shaker overnight to determine the depletion of GF from solution. The solution was centrifuged and fresh BSA-PBS was added to monitor the release of bound GFs from gels at 1, 2, 3, 5, 7, 10, 14, 21 and 28 days. GF release was then measured by ELISA (BMP-2 Quantikine ELISA Kit: R&D Systems).

MVFs were obtained from adipose tissue of epididymal fat pads of Lewis rats, suspended at 20,000 fragments/mL of collagen gel, and cultured in serum-free media. Quantification of vessel growth in the collagen gel using various GFs combination was done on day 9 of culture. The gels were fixed, and rhodamine labeled with Griffonia simplicifolia (GS-1) lectin (Vector Laboratories, Burlingame, CA) was used to stain vessel structures and imaged using a Zeiss LSM 700 confocal microscope. ImageJ was used for the quantification of branch number and total length.

Three different groups were tested for the treatment of composite non-union femur segmental defect model – resulting from adjacent soft tissue loss. Surgeries were performed on 13-week-old female SASCO Sprague–Dawley rats (*250 g) using procedures approved by the Georgia Institute of Technology Institutional Animal Care and Use Committee. In vivo radiographs (Faxitron MX-20; Faxitron Bioptics, LLC, AZ) and micro-computed tomography (μCT; Viva CT; Scanco, Switzerland) were obtained at 4 weeks post-surgery for qualitative and quantitative analysis of bone regeneration.

Results and Discussion

The bound fraction was found to be above ~90% for BMP-2, VEGF, and PDGF, verifying increased loading efficiency of the GFs in the gels. Both the groups exhibited sustained release of GFs over 28 days, further supporting strong electrostatic binding and retention of GFs.

In vitro study evaluated the effects of dual angiogenic GF on vascularization of MVFs in collagen gel. Collagen gel treated with angiogenic GFs (VEGF and PDGF) proved to facilitate the stimulation of MVFs growth in vitro, as demonstrated by the total network length and number of branches.

In vivo study show increased defect bone volume for co-delivery of BMP-2, VEGF and PDGF irrespective of dose ratio when compared to 2.5 μg BMP-2 dose (G1 vs G2 or G3). Results indicating that dual angiogenic GFs are required to achieve stable and matured blood vessel formation that augment the regeneration of vascularized bone tissue in critical tissue injury.

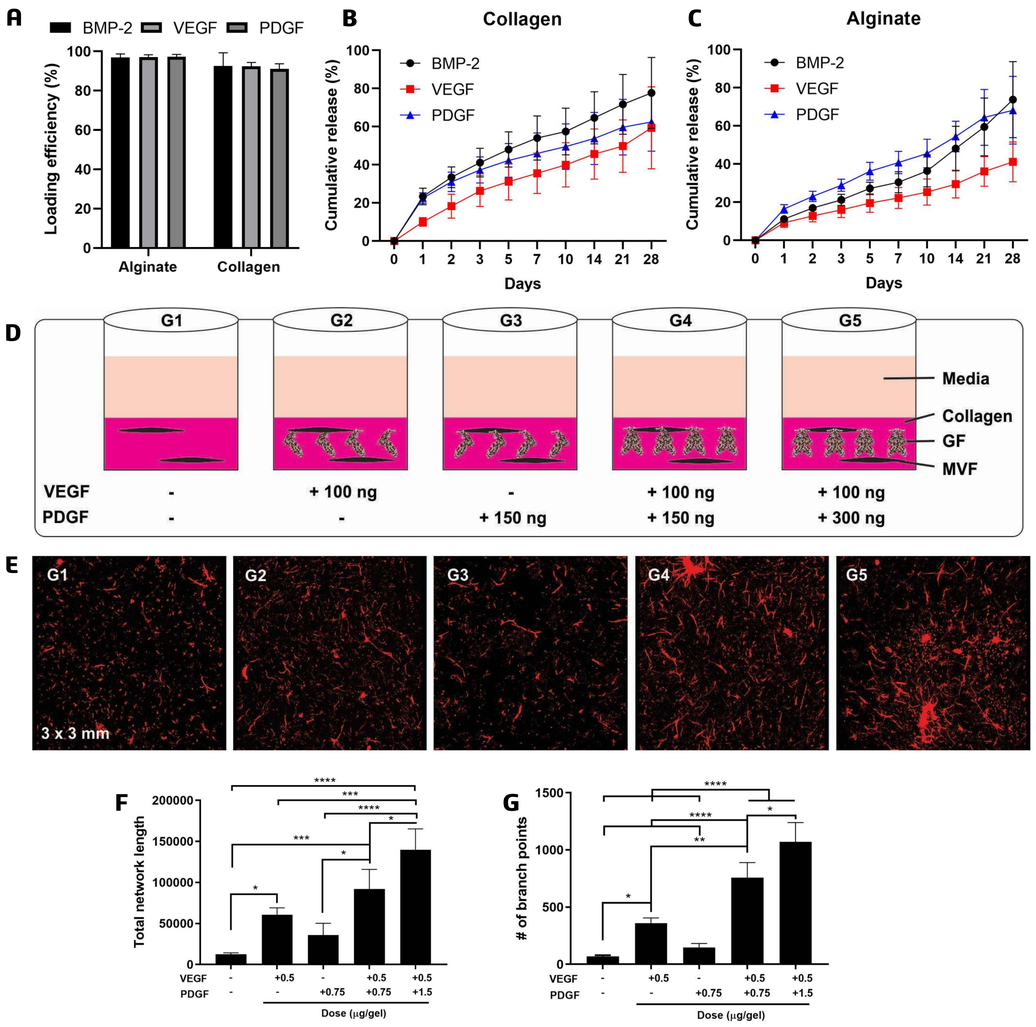

Figure 1. Collagen and hybrid alginate gel were used as GFs delivery vehicles. A, B, C) Loading efficiency and release kinetics show a sustained and steady delivery of VEGF and PDGF by the gels. D) Five different groups were examined (in vitro) by varying the GFs' dose, and combination (G1, G2, G3, G4 and G5), as schematically illustrated. E) Immunofluorescence staining images of MVF cultured collagen gel treated with various GFs, show dose and combination dependent MVF growth. F,G) The total network length, and number of branch-points quantified using ImageJ, respectively (significant differences **** p<0.0001, *** p<0.001, ** p<0.01, * p<0.05).

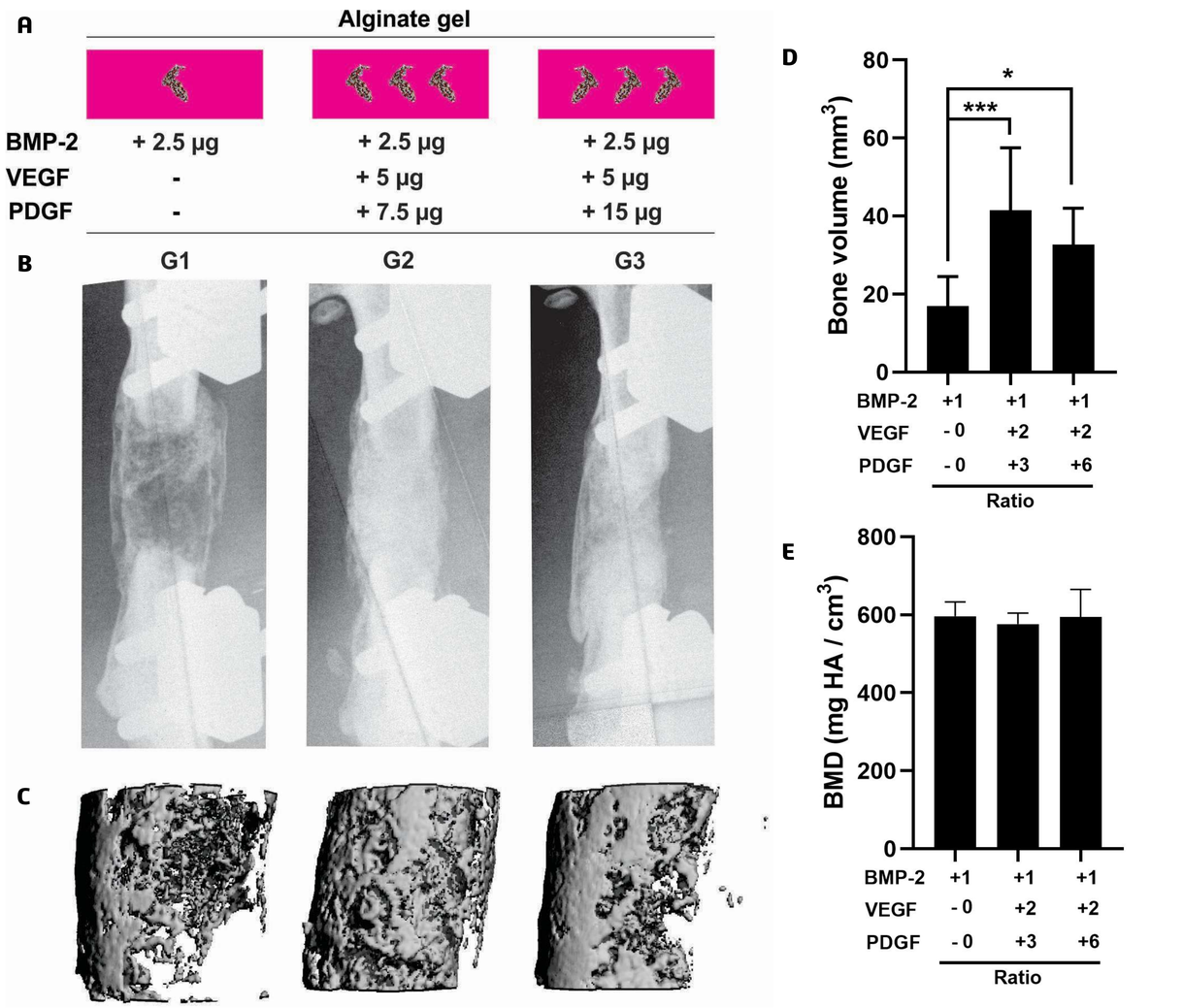

Figure 2. A) alginate hybrid system with PCL mesh used for spatiotemporal delivery of BMP-2, VEGF and PDGF to treat composite defect rat model which consists of a critically-sized 8 mm segmental femoral defect and volumetric muscle loss, analyzed at 4 weeks. B, C) X-ray images and MicroCT reconstructions at 4 weeks showed better bone formation within the segmental defect site treated with BMP-2, VEGF and PDGF than BMP-2 alone, irrespective of VEGF and PDGF dose ratio. Heterotopic ossification was not observed among all the groups. D) Regenerating bone volume in composite injuries was higher with G2 and G3 than G1. E) Bone mineral density in composite injuries showed no significant changes between groups. Plots are mean ± SD, n=8. (significant differences *** p<0.001, * p<0.05).

Summary. In situ delivery of VEGF in collagen gel shows enhanced MVF growth when compared to PDGF delivery and no GFs treatment. However, PDGF shows additive effect with the presence VEGF on the MVF growth irrespective VEGF and PDGF dose ratio. Results indicating that combination of VEGF and PDGF is necessary to achieve mature and stable blood vessels. In vivo results demonstrate that collagen gel achieves sustained release of triple GFs that facilitate early vascularization and synergistic bone healing. The findings of a combination of dose-dependent osteogenic and angiogenic GFs will aid the treatment of severe musculoskeletal injuries.

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