

Supplementary Materials for

3D bioprinting spatiotemporally defined patterns of growth factors to tightly control tissue regeneration

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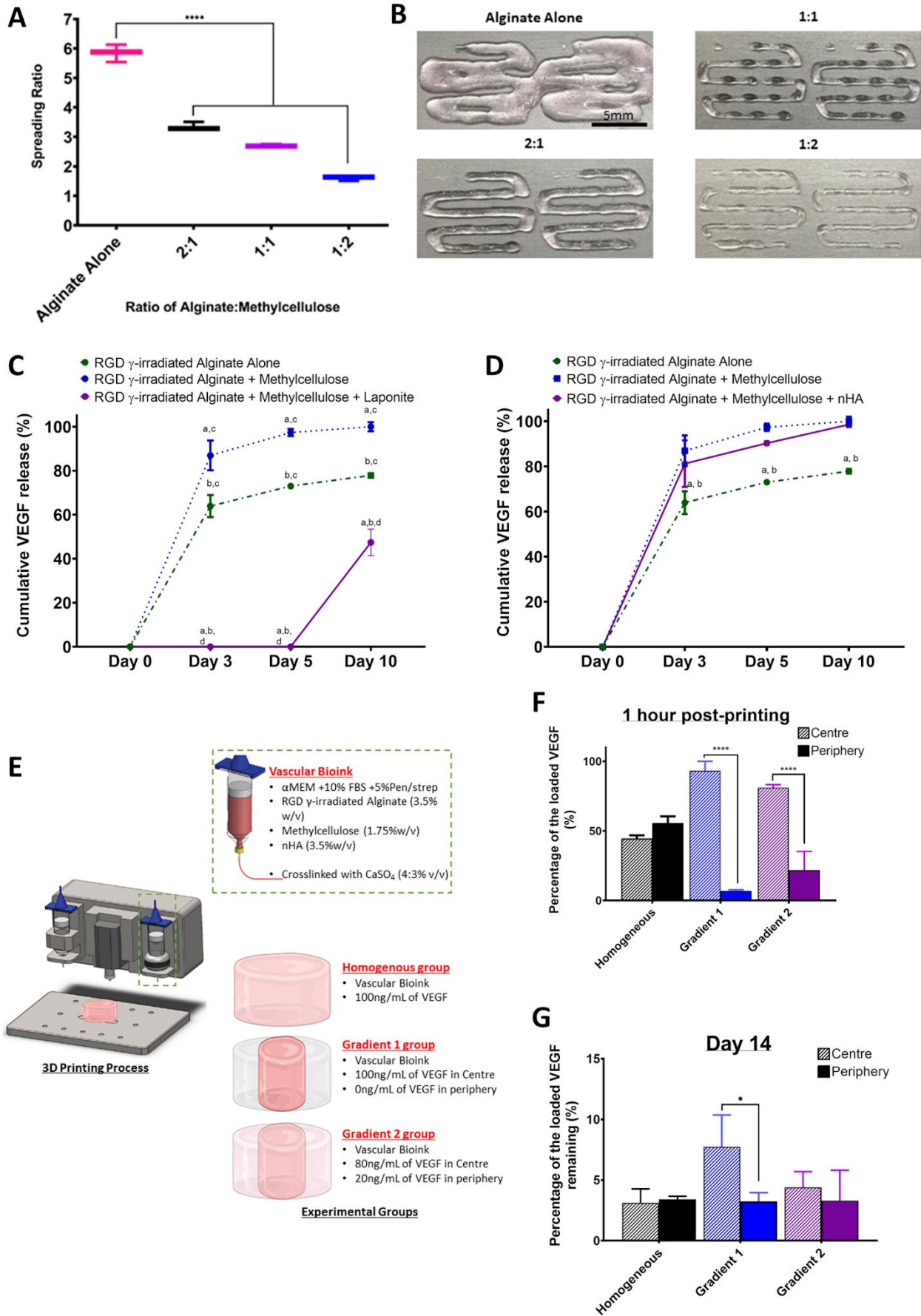
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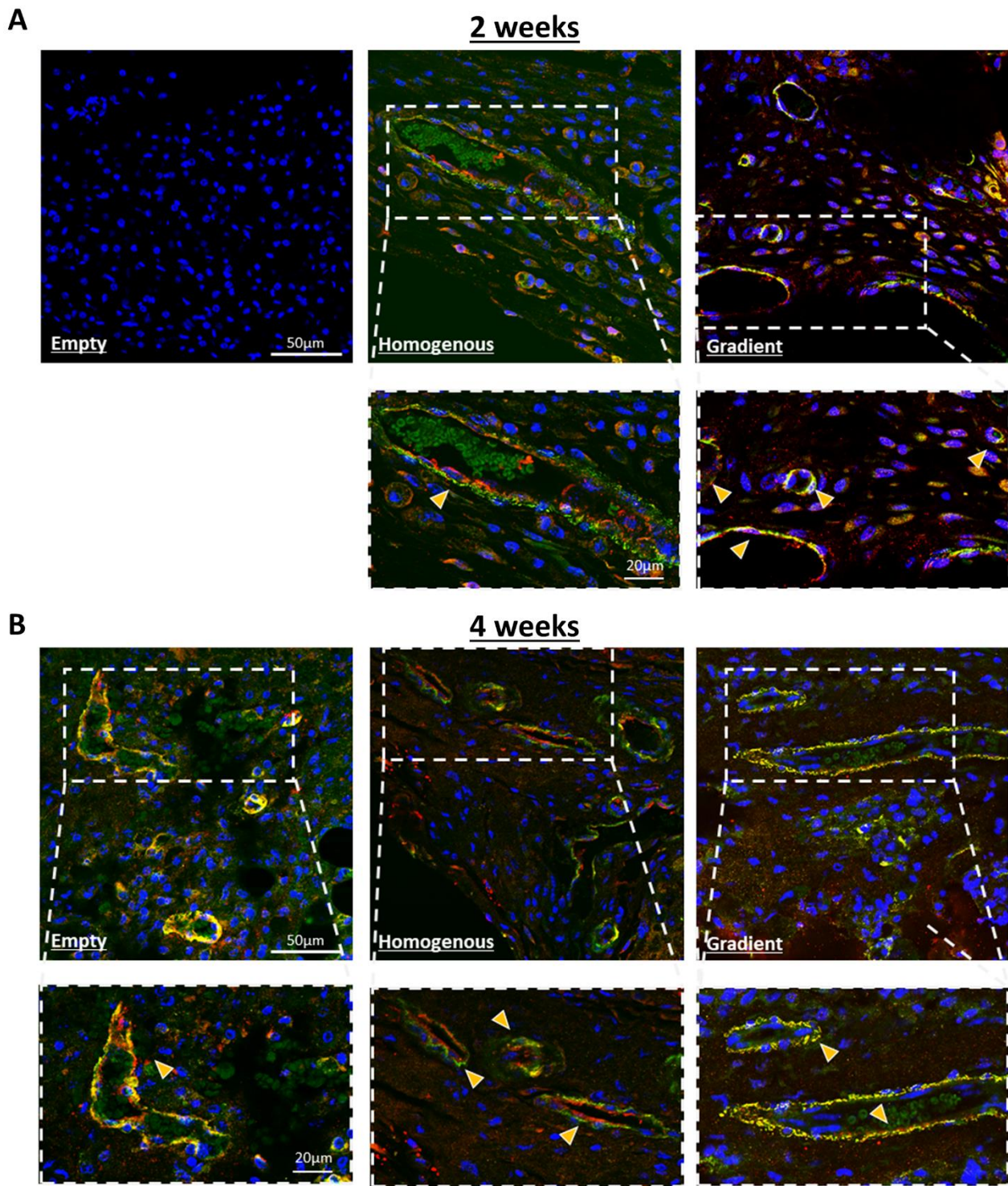
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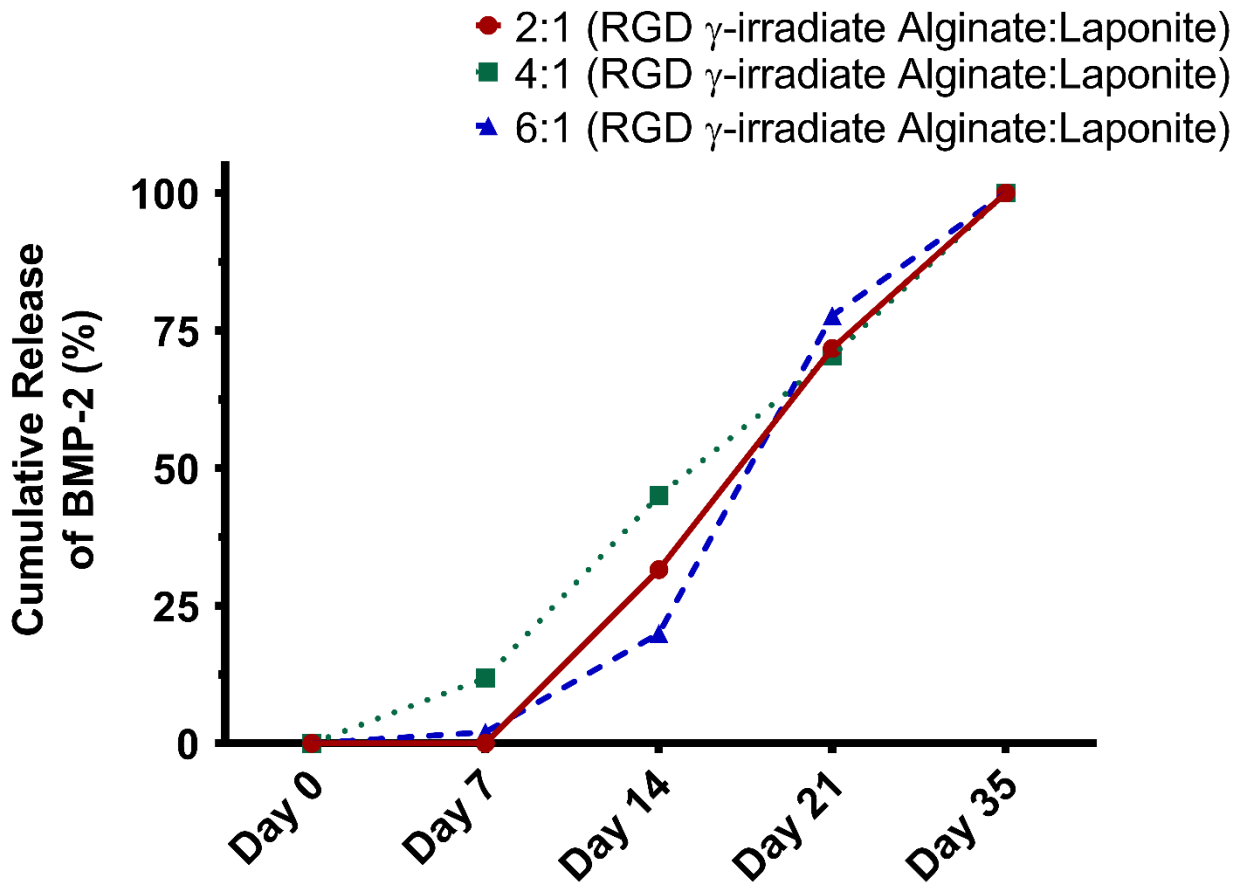
Supplementary Materials:



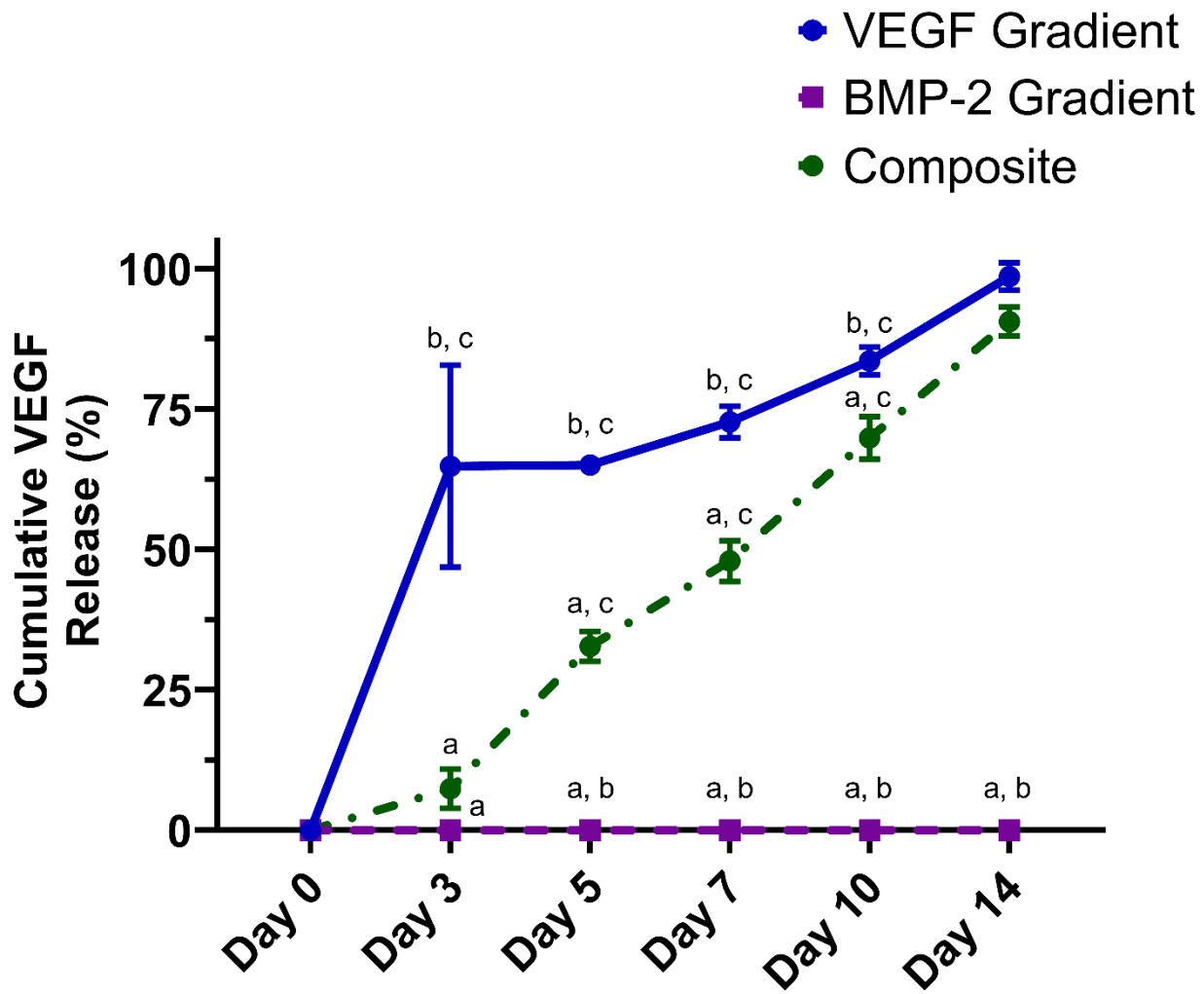
Supplementary Fig. 1. Development of growth factor releasing bioinks. (A) Spreading Ratio for RGD γ -irradiated alginate alone and various ratios of RGD γ -irradiated alginate to methylcellulose. Error bars denote standard deviation, *** $p < 0.001$, $n = 6$. (B) Representative images of the design pattern used to determine spreading ratios of the bioinks. (C) Cumulative release of VEGF into the media after the addition of Laponite. ^a $p < 0.05$ vs. RGD γ -irradiated alginate, ^b $p < 0.05$ vs. RGD γ -5 irradiated alginate + methylcellulose, ^c $p < 0.05$ vs. RGD γ -irradiated alginate + methylcellulose + Laponite. (D) Cumulative release of VEGF into the media after the addition of nHA. ^a $p < 0.05$ vs. RGD γ -irradiated alginate, ^b $p < 0.05$ vs. RGD γ -irradiated alginate + methylcellulose, ^c $p < 0.05$ vs. RGD γ -irradiated alginate + methylcellulose + nHA. All error bars denote standard deviation, $n = 6$. (E) Schematic of the 3D printed scaffold and experimental groups. Construct design (8mm in diameter, 4 mm in height). (F) VEGF ELISA on the centre 10 and periphery of the 3D printed scaffolds 1 hour post printing and (G) after 14 days of *in vitro* culture. All error bars denote standard deviation, * $p < 0.05$, **** $p < 0.0001$, $n = 6$.



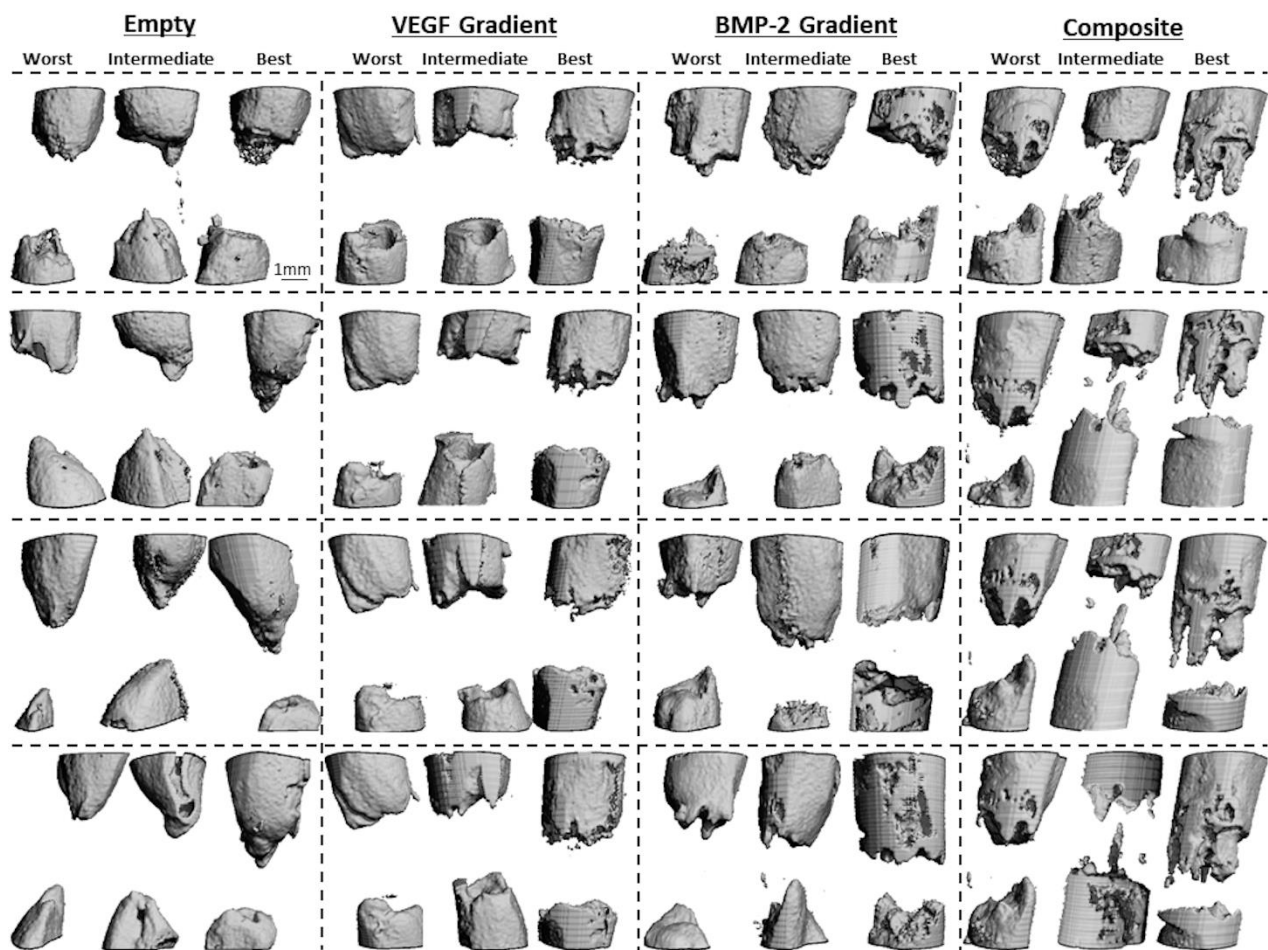
Supplementary Fig. 2. Mature vessels present at 2 and 4 weeks in both Homogenous and VEGF gradient groups. Immunohistochemical staining of nuclei (blue), vWF (red), and α -smooth actin (green) of the experimental groups at (A) 2 and (B) 4 weeks post-implantation. Images taken at 20X and 40X. Yellow arrows denote vessels with α -smooth actin and vWF dual staining.



Supplementary Fig. 3. Cumulative release of BMP-2 into the media of varying w/w ratio of Alginate to Laponite. Error bars denote standard deviation, $n=4$.



Supplementary Fig. 4. Cumulative release of VEGF into the media of the three different experimental groups. Error bars denote standard deviation, ^a $p < 0.05$ vs. VEGF Gradient, ^b $p < 0.05$ vs. Composite, ^c $p < 0.05$ vs. BMP-2 Gradient, $n = 6$



Supplementary Fig. 5. Bone regeneration over time, as determined by *in vivo* μ CT analysis. 3D reconstructions of the best, intermediate and worst healers from all four groups at all-time points.