Electronic Supplementary Material (ESI) for Biomaterials Science.

Angiopoeitin-1 derived peptide QHREDGS promotes osteoblast differentiation, bone matrix deposition and mineralization on biomedical materials

Nicole Feric,[a] Calvin Cheng,[b] M. Cynthia Goh,[b,c] Vyacheslav Dudnyk,[d] Val Di Tizio[e] and Milica Radisic* [a,e]

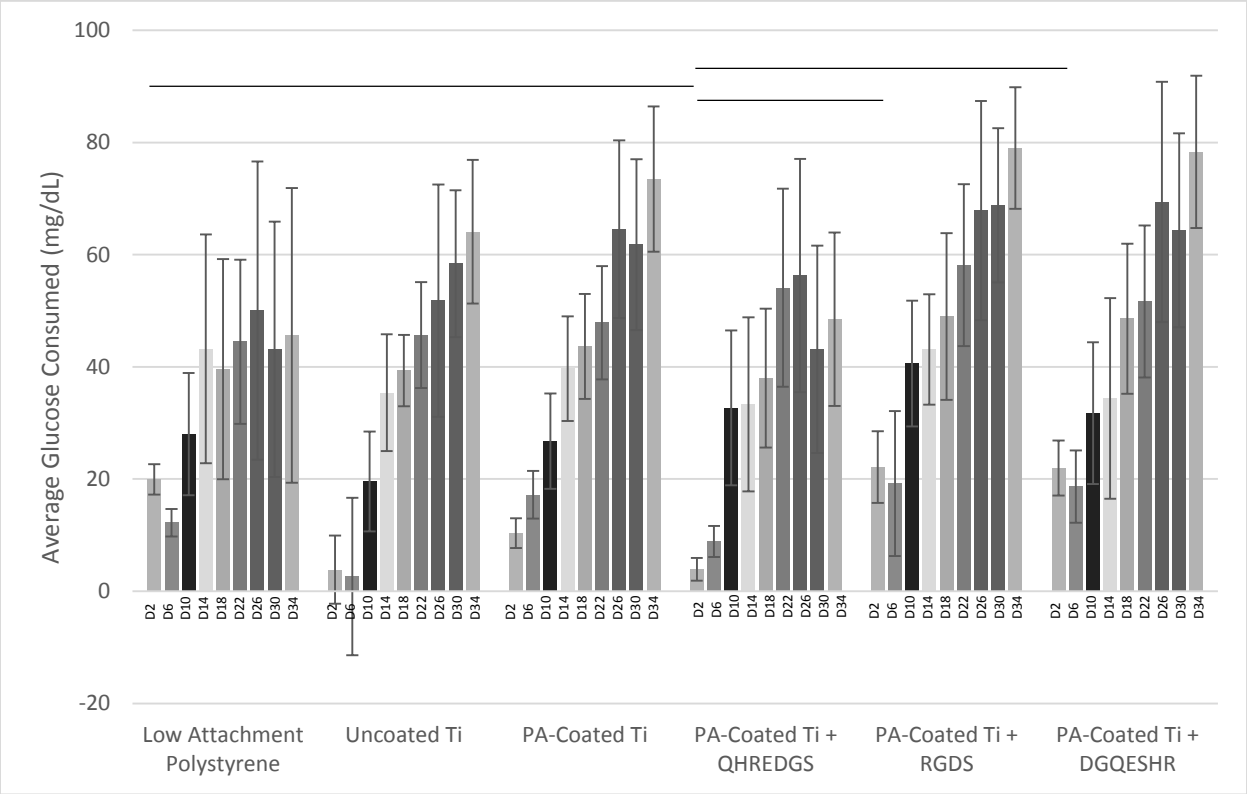
[a] Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario, M5S 3G9 Canada

[b] Department of Chemistry, University of Toronto, Toronto, Ontario M5S 3E5, Canada

[c] Institute for Optical Sciences, University of Toronto, Toronto, Ontario M5S 1A7, Canada

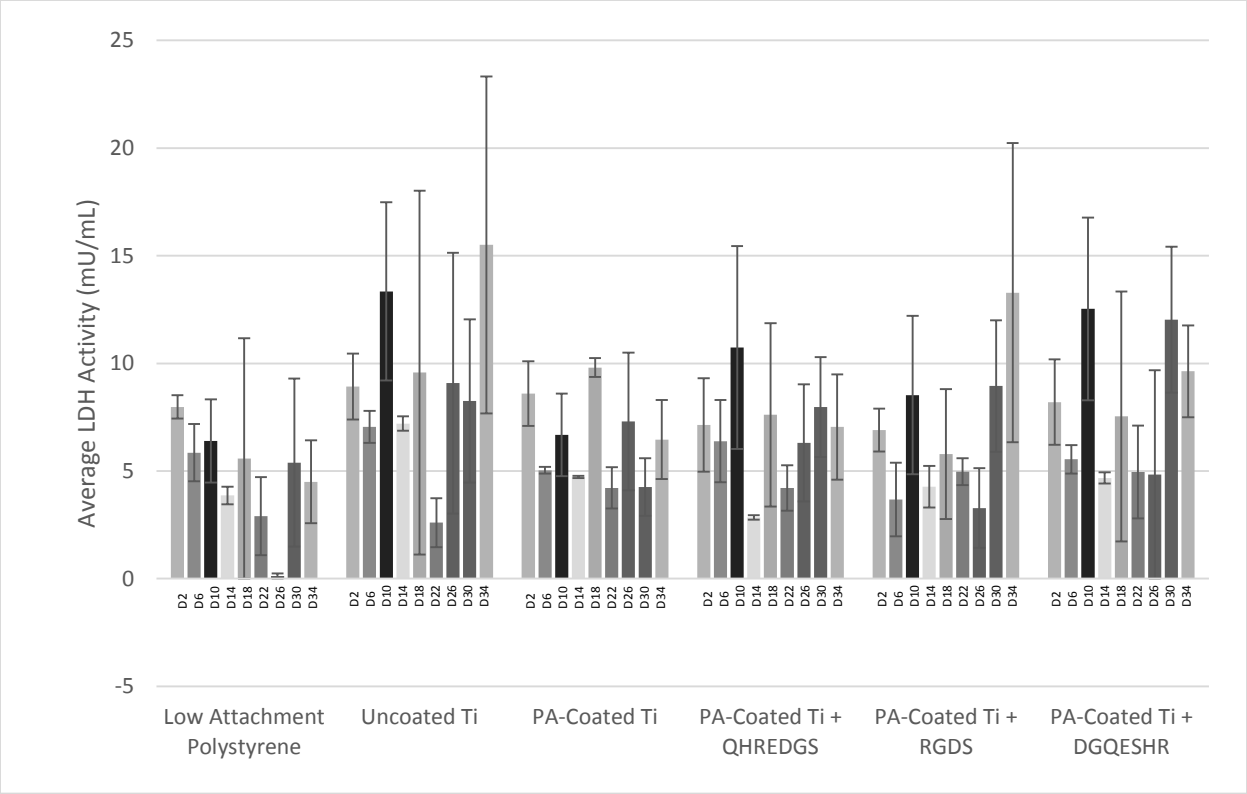
[d] Covalon Technologies Ltd., Mississauga, Ontario L4Z 3E6, Canada

[e] Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario M5S 3H6, Canada. E-mail: m.radisic@utoronto.ca; Tel.: +1 416 946 5295; Fax: +1 416 978 4317



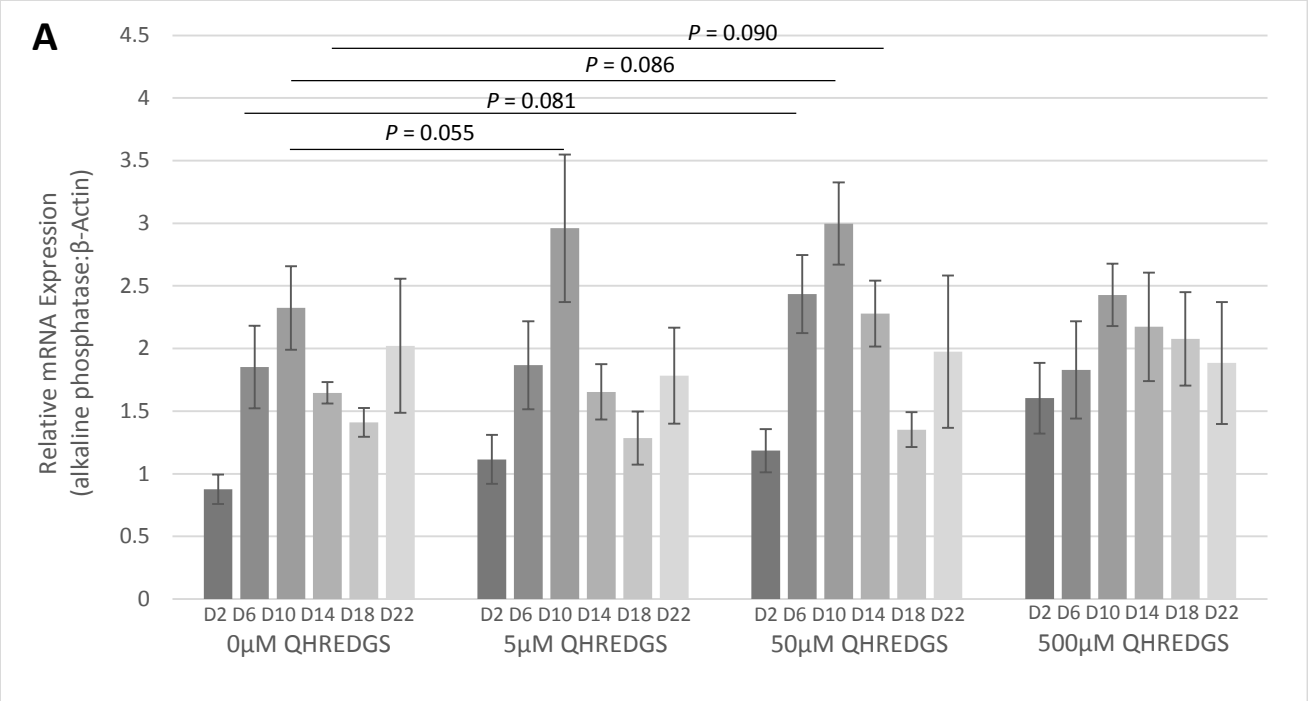
SUPPLEMENTARY FIGURE 1. Glucose consumption of osteoblasts seeded onto titanium (Ti) plates

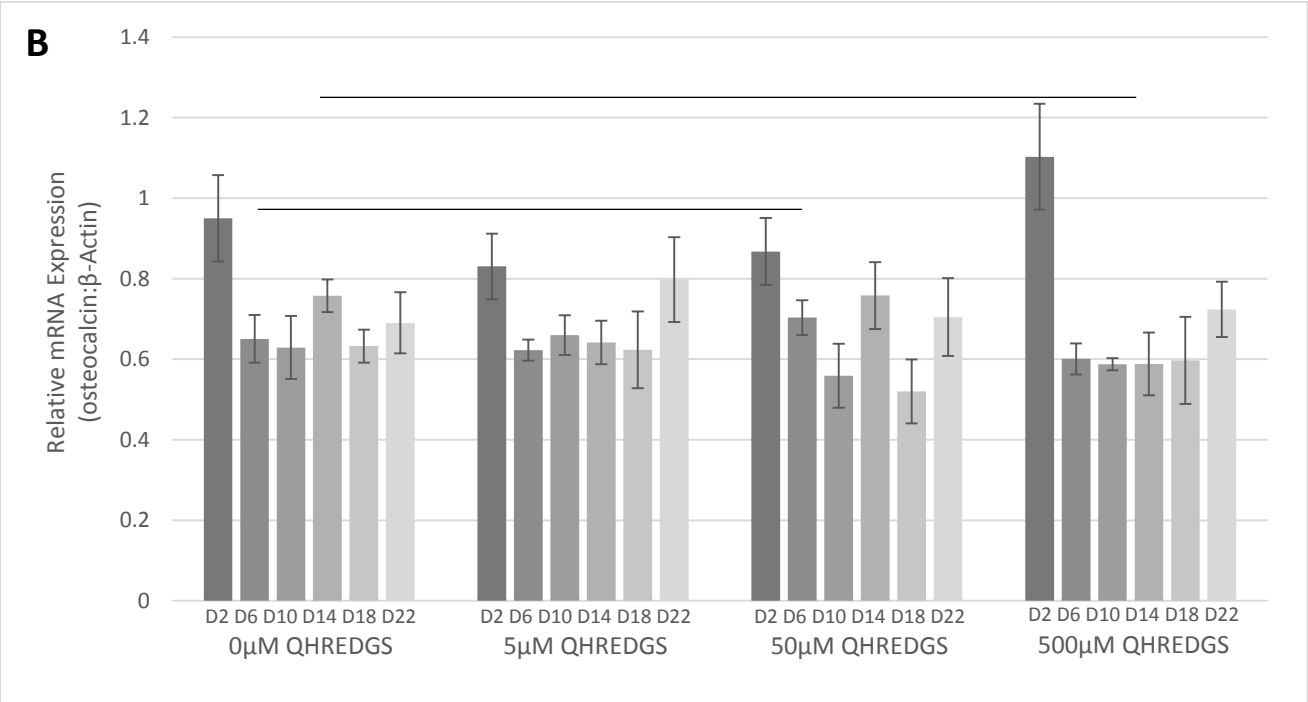
MG-63 osteoblast-like cells were grown to confluence on low attachment polystyrene tissue culture plastic or Ti plates without polyacrylate (PA)-coating (*Uncoated Ti*), with PA-coating but no peptide conjugated (*PA-Coated Ti*), or with PA coating and with either QHREDGS, RGDS or DGQESHR (scrambled) peptide conjugated. Cells were cultured in osteogenic medium beginning on day 12. Conditioned media was collected on the indicated days (D) and the glucose concentration therein was measured by ELISA. Data presented are the mean ± SEM and the lines indicate statistical significance ($P < 0.05$; one-way ANOVA and Student-Newman-Keuls post-hoc analysis; n=3).

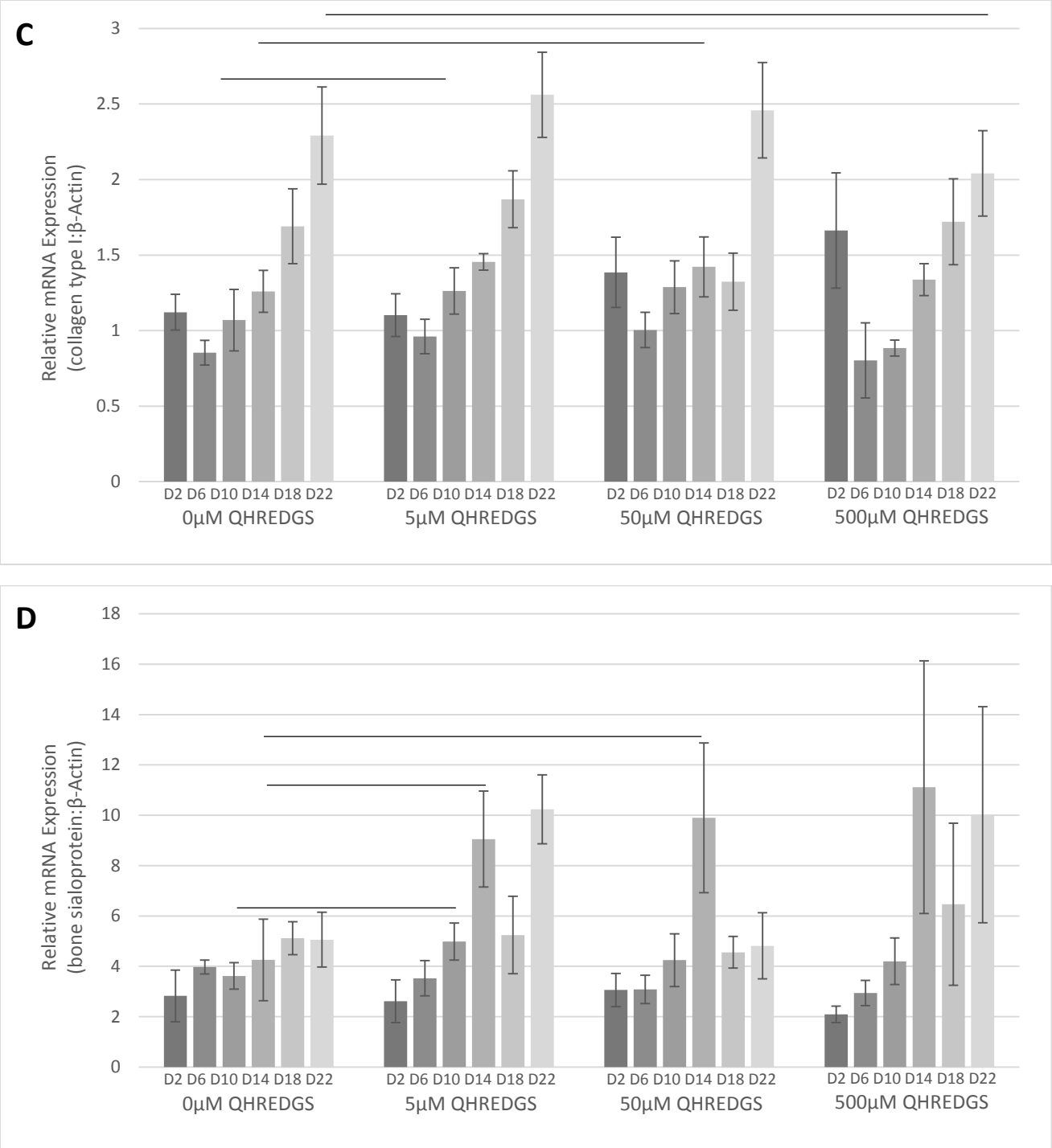


SUPPLEMENTARY FIGURE 2. Lactate dehydrogenase (LDH) activity of osteoblasts seeded onto Ti plates

MG-63 osteoblast-like cells were grown to confluence on low attachment polystyrene tissue culture plastic or Ti plates without PA-coating (*Uncoated Ti*), with PA-coating but no peptide conjugated (*PA-Coated Ti*), or with PA coating and with either QHREDGS, RGDS or DGQESHR (scrambled) peptide conjugated. Cells were cultured in osteogenic medium beginning on day 12. Conditioned media was collected on the indicated days (D) and the LDH activity therein was measured by ELISA. Data presented are the mean ± SEM (n=3).


A
P = 0.090
P = 0.086
P = 0.081
P = 0.055
Relative mRNA Expression (alkaline phosphatase:β-Actin)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
D2 D6 D10 D14 D18 D22
0μM QHREDGS
5μM QHREDGS
50μM QHREDGS
500μM QHREDGS

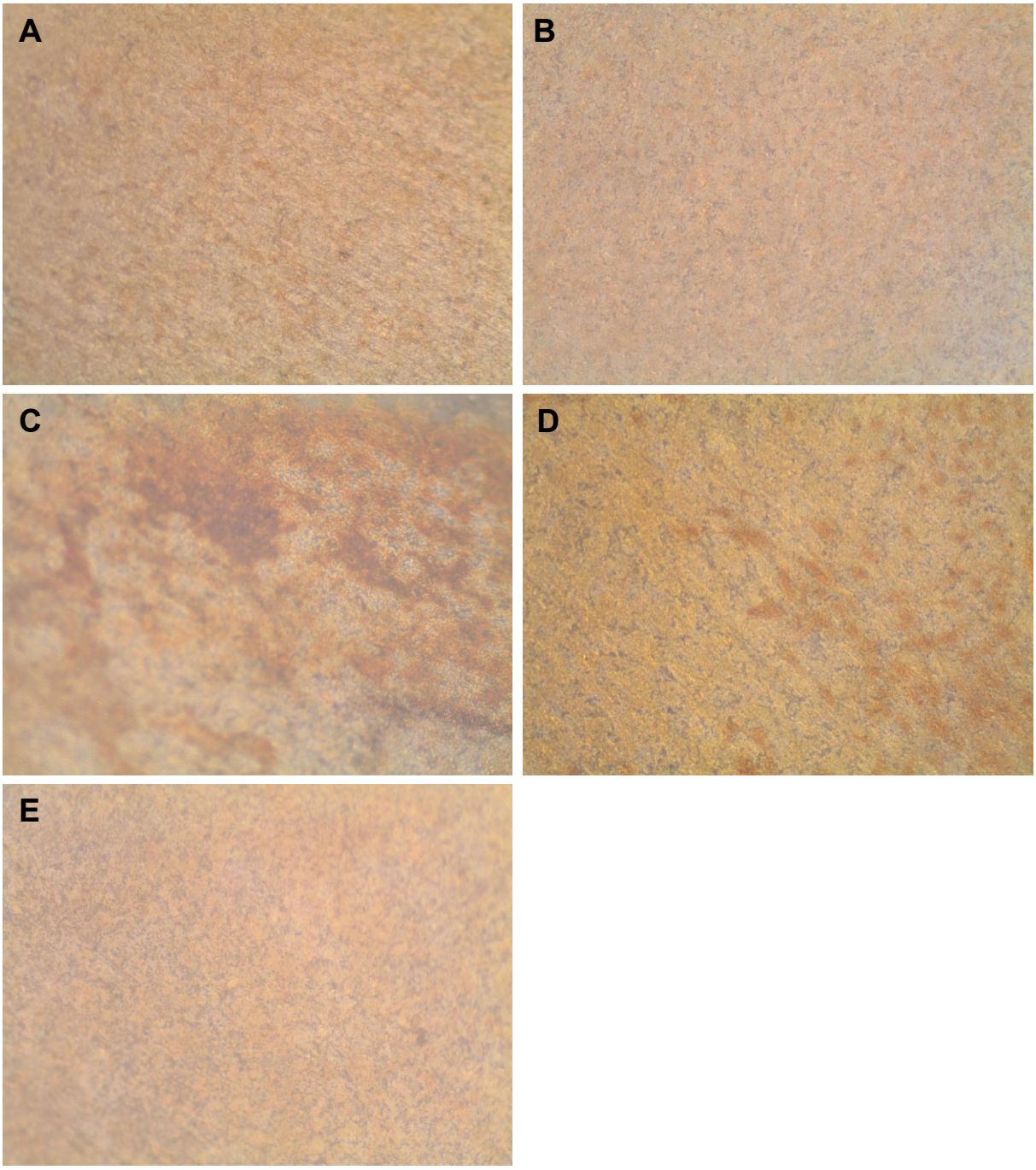

B
Relative mRNA Expression (osteocalcin:β-Actin)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
D2 D6 D10 D14 D18 D22
0μM QHREDGS
5μM QHREDGS
50μM QHREDGS
500μM QHREDGS



SUPPLEMENTARY FIGURE 3. Relative expression of osteogenic genes in cell monolayers grown on tissue culture plastic in the presence of 0, 5, 50 or 500μM soluble QHREDGS

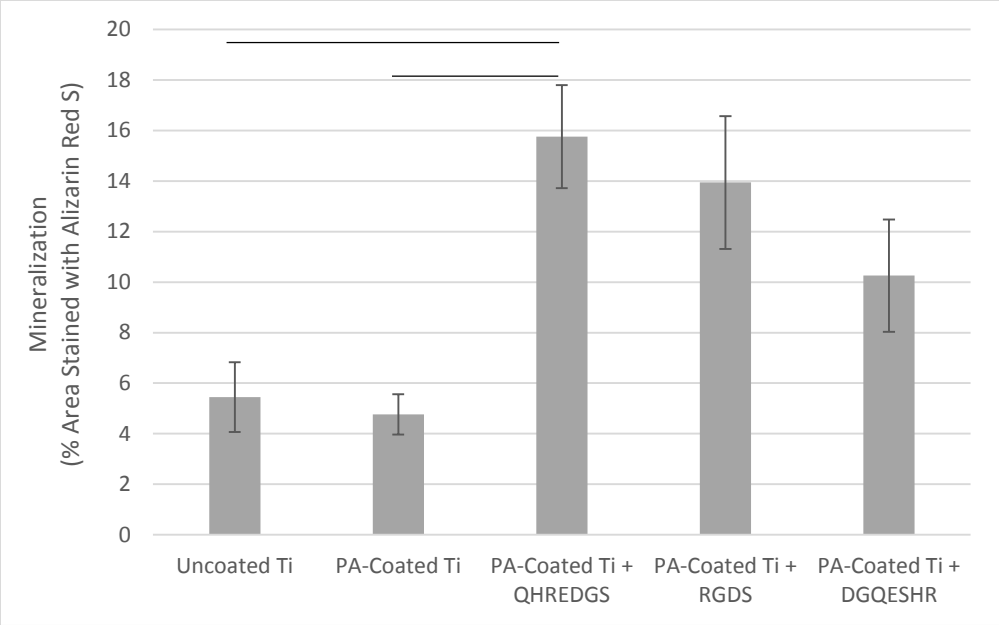
MG-63 osteoblast-like cell monolayers were grown on tissue culture plastic in the presence or absence of soluble QHREDGS. The cells were cultured for up to 22 days in medium supplemented with 50μg/mL ascorbic acid, 100nM dexamethasone and 10mM β-glycerophosphate (osteogenic media) with 5, 50 or 500μM soluble QHREDGS or equivolume PBS added to the medium with each change. Cells were lysed

and RNA was extracted on the indicated days (D) and qPCR was performed on the resultant cDNA using primers for the indicated genes: (A) alkaline phosphatase, (B) osteocalcin, (C) type I collagen and (D) bone sialoprotein. Gene expression was normalized by dividing by the expression of the housekeeping gene β-actin. The data is graphed to indicate the changes in gene expression with time in the difference treatment groups. Data presented are the mean ± SEM (n=3) and the lines indicate statistical significance ($P < 0.05$, unless noted, n=3).



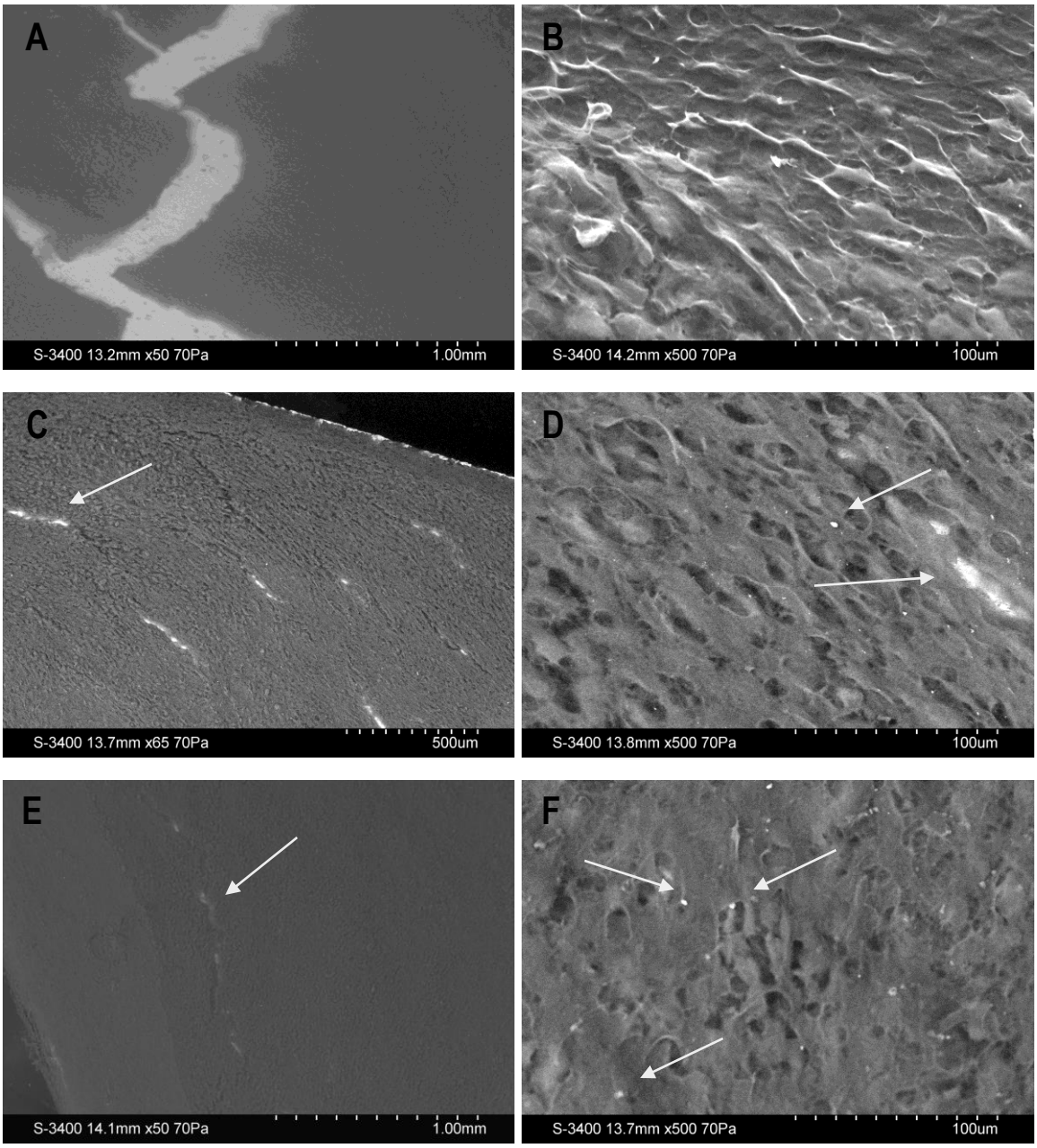
SUPPLEMENTARY FIGURE 4. Alizarin Red S and von Kossa staining of Ti plates seeded with osteoblasts and culture in non-osteogenic medium

Representative Alizarin Red S and von Kossa staining images of Ti plates with various coatings seeded with MG-63 osteoblast-like cells and cultured in non-osteogenic media for 22 days. **(A)** Ti plates without the PA-coating. **(B)** PA-coated Ti plates without any peptide immobilized. **(C)** PA-coated Ti plates immobilized with QHREDGS peptide. **(D)** PA-coated Ti plates immobilized with RGDS peptide. **(E)** PA-coated Ti plates immobilized with DGQESHR (scrambled) peptide.



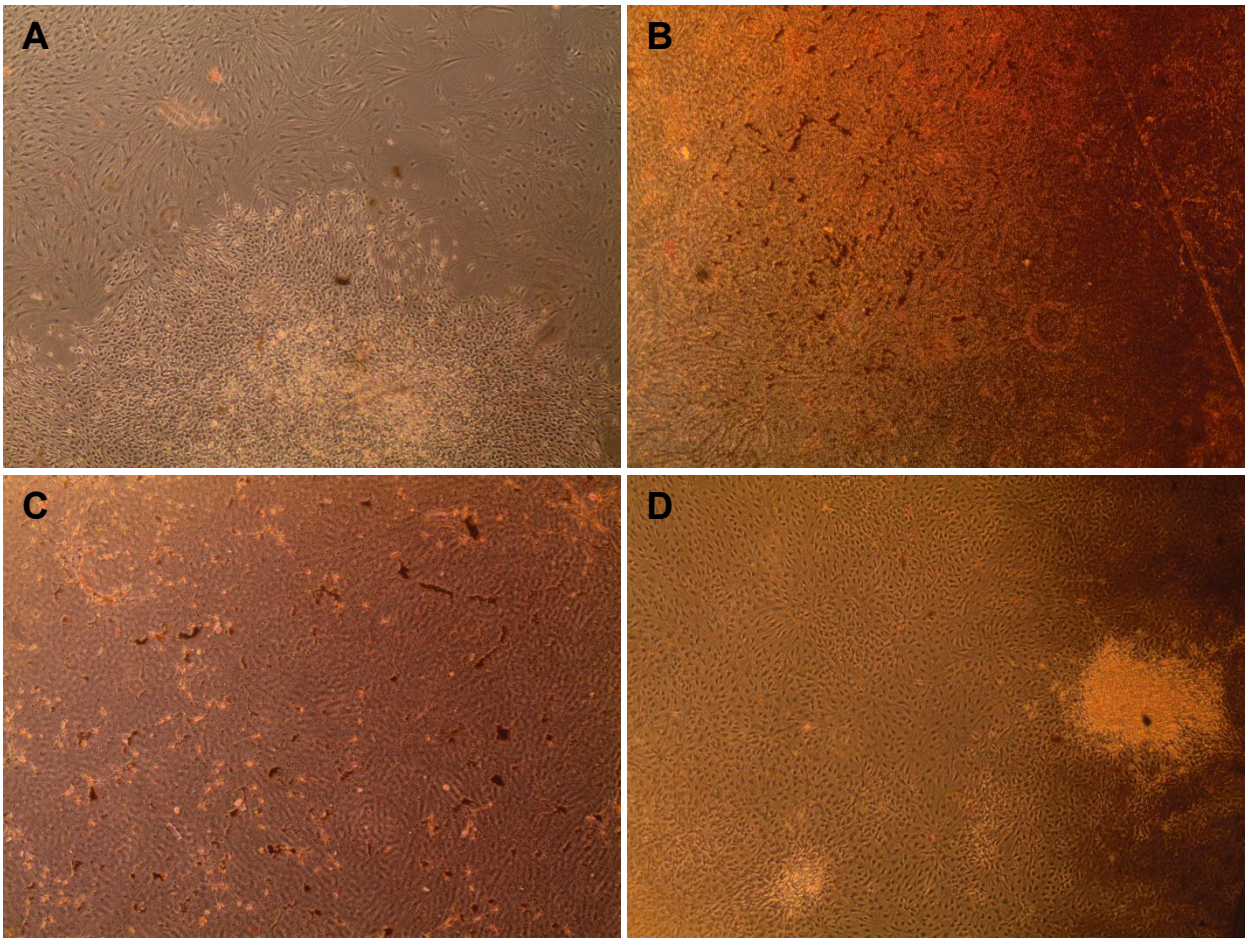
SUPPLEMENTARY FIGURE 5. Quantification of Alizarin Red S staining of Ti plates seeded with osteoblasts and culture in non-osteogenic medium

Ti plates with various coatings were seeded with MG-63 osteoblast-like cells and cultured in non-osteogenic media for 22 days then stained with Alizarin Red S. The percent of the surface area bound by Alizarin Red S was determined from red channel staining images. Data presented are the mean ± SEM (n=3) and the lines indicate statistical significance ($P < 0.05$; Kruskal-Wallis one-way ANOVA on ranks and Dunn's post-hoc analysis; n=3).



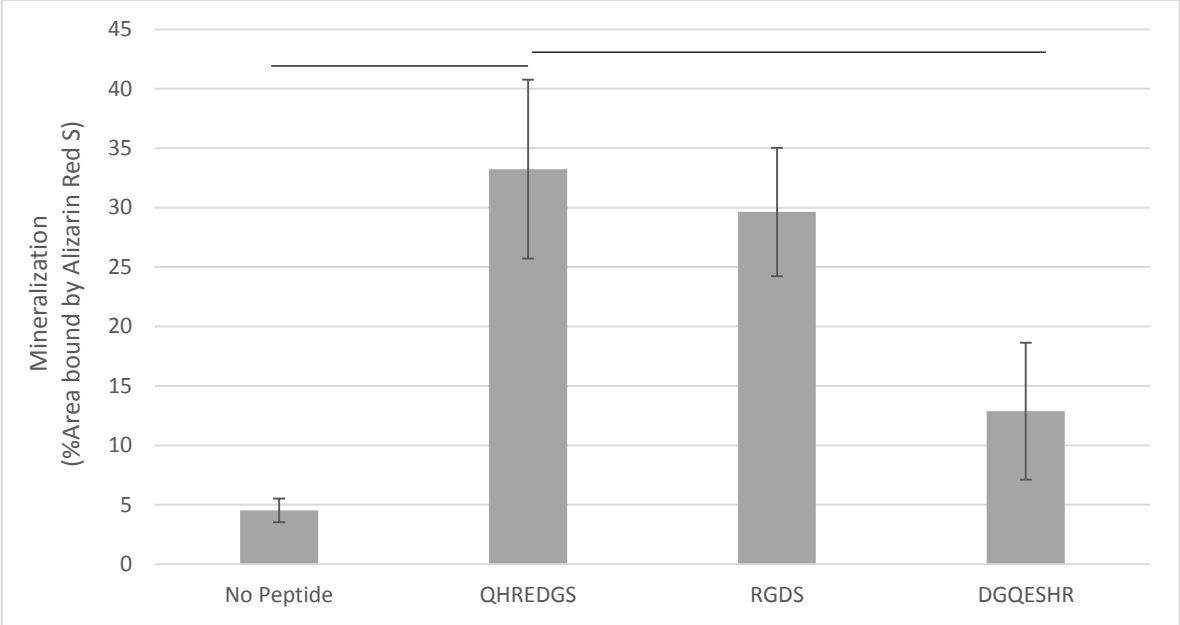
SUPPLEMENTARY FIGURE 6. Environmental scanning electron microscopy (ESEM) images of Ti plates seeded with osteoblasts and culture in non-osteogenic medium

Representative ESEM images of Ti plates seeded with MG-63 osteoblast-like cells after 22 days of culture in non-osteogenic media. **(A-B)** Ti plates without the PA-coating. **(C-D)** PA-coated Ti plates immobilized with QHREDGS. **(E-F)** PA-coated Ti plates immobilized with RGDS. Images were taken at high (*right panels*) and low (*left panels*) magnification. Arrows indicate mineral deposits.



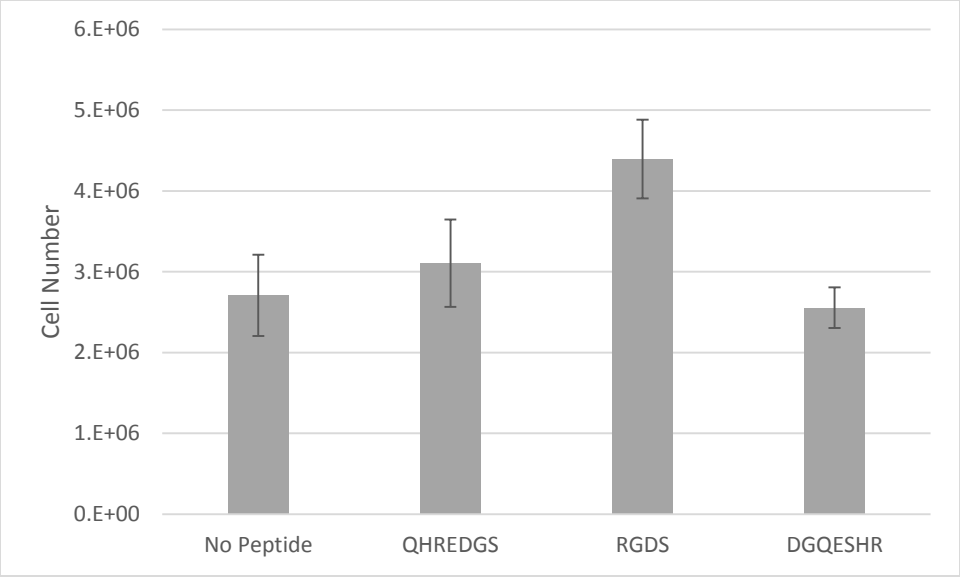
SUPPLEMENTARY FIGURE 7. Alizarin Red S and von Kossa staining of polyethylene glycol (PEG) hydrogels seeded with osteoblasts and culture in osteogenic medium

Representative Alizarin Red S and von Kossa staining images of PEG hydrogels seeded with MG-63 osteoblast-like cells and cultured for 41 days. Cells were cultured in osteogenic medium beginning on day 15. **(A)** PEG hydrogels without any peptide immobilized. **(B)** PEG hydrogels with QHREDGS peptide immobilized. **(C)** PEG hydrogels with RGDS peptide immobilized. **(D)** PEG hydrogels with DGQESHR (scrambled) peptide immobilized.



SUPPLEMENTARY FIGURE 8. Quantification of Alizarin Red S staining of polyethylene glycol (PEG) hydrogels seeded with osteoblasts and culture in osteogenic medium

PEG hydrogels with and without peptide immobilized were seeded with MG-63 osteoblast-like cells and cultured for 41 days. Cells were cultured in osteogenic medium beginning on day 15. The percent of the surface area bound by Alizarin Red S was determined from red channel staining images. Data presented are the mean ± SEM (n=3) and the lines indicate statistical significance ($P < 0.05$; one-way ANOVA and Student-Newman-Keuls post-hoc analysis; n=3).



SUPPLEMENTARY FIGURE 9. Cells attached to polyethylene glycol (PEG) hydrogels with or without peptide immobilized

The number of cells attached to PEG hydrogels with or without peptide immobilized after 41 days of culture. PEG hydrogels were seeded with MG-63 osteoblast-like cells and cultured for 41 days, wherein the medium was supplemented with 50µg/mL ascorbic acid, 100nM dexamethasone and 10mM β-glycerophosphate (osteogenic media) as of day 15. Data presented are the mean ± SEM (n=3).