Phage-derived peptides improve hBMSC adhesion contact distribution and proliferation on biomimetic apatite

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Statement of Purpose: Recruiting and promoting growth of specific regenerative cell populations on a biomaterial can improve the delivery of cell-based tissue regeneration therapies [1]. Extracellular matrix components have been used to functionalize biomaterials to encourage cell attachment. These cues lack cell specificity or can interfere with serum protein adsorption which negatively impact the regenerative processes [2]. We have identified peptides specific for human bone marrow stromal cells (hBMSC) (DPIYALSWSGMA, DPI) and apatite surfaces (VTKHLNQISQSY,VTK) using phage display [3], and demonstrated the conservation of mineral and mesenchymal stem cell specificity when the peptides are combined into a single bi-functional peptide (DPI-VTK) [4]. This study aims to characterize DPI-VTK's mediation of hBMSC spreading and cytoskeletal contact distribution in the presence and absence of serum proteins and to quantify cell proliferation on peptide coated substrates.

Methods: Commercially synthesized peptides (100µg/mL) were adsorbed to bone-like mineral (BLM) coated PLGA films. Experimental groups included no-peptide controls, mineral-binding peptide (VTK), dual peptide (DPI-VTK), and dual peptide with cell-binding control (RGD-VTK). Peptides were adsorbed for 3 hours and primary hBMSCs were added in the presence or absence of serum to assess cell spreading. After 18 hrs, cell adhesion contacts were immuno-fluorescence labeled with anti-Vinculin (Alexa 488) and nuclei were stained with DAPI. hBMSCs were next cultured on peptide-coated mineral films for a 10 day proliferation assay. Films were washed 3x to remove dead and weakly adherent cells and adherent cells were imaged using Rhodhamine-Pholloidin and DAPI. Image J was used to measure cell diameter, and identify the number and distribution of cell contacts, and number of adherent cells. Cell diameter was calculated using scale bars. Adhesion ROIs centered on each cell with counts for each sections from center (6) to periphery (1) (n=20 cells per group). Cell counts were attained using image J (n=40 fields at 40x per group). ANOVA with Tukey test for pairwise comparisons was done 2-way for culture conditions and peptide groups and 3-way for culture conditions, peptide groups and cell-substrate contact distribution.

Results: In serum-free media, cells interacting with DPI-VTK had a greater cell diameter than cells on RGD-VTK ($p<0.05$) or BLM ($p< 0.001$). In serum, cells attaching to DPI-VTK had a significantly greater cell diameter than cells attached to RGD-VTK ($p < 0.02$). This is consistent with studies showing a negative interaction between the RGD peptide and serum proteins [2]. RGD-VTK demonstrated more contacts than either BLM or DPI-VTK in the serum free condition ($p<0.001$). Both RGD-VTK ($p< 0.004$) and DPI-VTK ($p<0.014$) were able to recruit more cell contacts than BLM alone in serum. Cells on DPI-VTK demonstrated more contacts in serum compared to serum-free media ($p<0.002$), indicating a cooperative interaction between cells, serum proteins, and DPI-VTK. Contacts at periphery (sections 1-2) were significantly higher than at the center (1-2) ($p <0.01$) except in the serum-free and BLM condition. DPI-VTK and RGD-VTK demonstrate a greater number of cell contacts towards the periphery (sections 1-3) compared to BLM ($p<0.001$) in both culture conditions indicating a more active cell phenotype with favorable substrate interactions. Proliferation data indicates RGD-VTK had higher cell numbers at 18 hr compared to BLM($p <0.016$). However by day 7, there was an increase in cell number between DPI-VTK and BLM groups ($p<0.001$). Cell numbers on DPI-VTK were also higher than VTK and RGD-VTK on day 10 ($p < 0.05$).

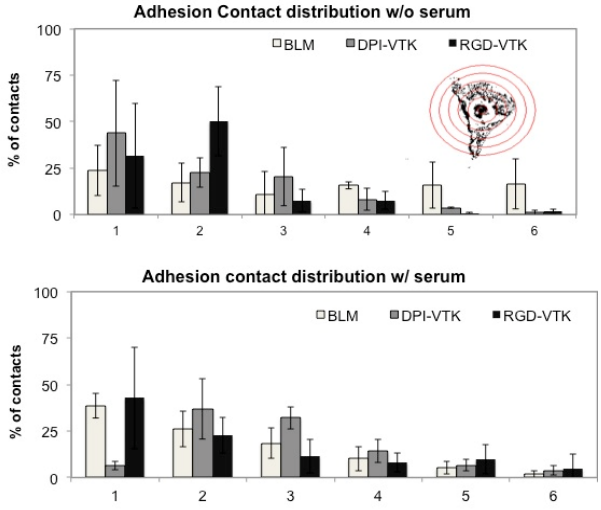

Figure 1 Adhesion contact distribution with the cell center at 6 and the periphery at 1

Conclusions: In addition to specifically tethering hMBSCs to mineralized biomaterials, DPI-VTK enhances cell spreading and proliferation on peptide-coated mineral substrates compared to controls. Cell spreading is greater on DPI-VTK surfaces compared to RGD-VTK in serum and greater than either RGD-VTK or BLM in the absence of serum. DPI-VTK's promotion of increased hBMSC adhesion contacts distributed at the cell periphery and increased spreading are both cell characteristics implicated in osteogenic mechano-transductive pathways. Taken together, the phage-derived peptide DPI-VTK can promote the specific recruitment of hMBSCs while encouraging favorable cell-substrate interactions and improving proliferation.

References

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