Human BMSC Specific Attachment on Apatite Surfaces Using Phage-Derived Peptide Sequences

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Statement of Purpose: Cell based tissue regeneration strategies to treat critical sized defects are a promising alternative to current standards of care. A biomaterial carrier is often required to support and guide progenitor cell driven tissue regeneration at the defect site. Improving attachment of specific regenerative cell populations to biomaterials can improve tissue regeneration outcomes[1]. In order to encourage human bone marrow stromal cell (hBMSC) specific attachment on apatite surfaces, we identified peptide sequences with high affinity towards apatite (VTKHLNQISQSY,VTK) and clonally derived hBMSCs(DPIYALSWSGMA, DPI) using phage display[2,3]. The primary aims of this study were to measure apatite binding affinity, hBMSC adhesion strength, specificity and morphology when apatite and cell specific peptides are combined into a dual functioning peptide.

Methods: Mineral binding sequences were identified using a 12 mer Ph.D.12 Phage Display Library, computational modeling, the bioinformatics tool RELIC and *in vitro* binding assays[2]. Cell specific peptide sequences were identified by screening a12 mer Ph.D.12 Phage Display Library against clonally derived hBMSC, and analyzing sequences using RELIC and immunohistochemistry. Known cell binding (RGD) and mineral binding EEEEEEE (E7) acidic peptide motifs were also tested as controls [4]. Peptides were incubated with HA powder at physiologic conditions. Langmuir isotherms of bulk versus bound peptide were constructed to determine dissociation constants (K_D). Each peptide laden apatite film and no peptide controls were incubated with 75k cells for 3hrs at 37^{o}C and 5% CO_2 in serum-free media. Cells were subsequently subjected to detachment forces ranging from 0-10^{-5} dynes using a centrifuge. Adherent cell fractions were determined using the WST-1 assay and half-cell detachment forces (τ_{50}) were determined by fitting sigmoidal curves to the data points. Samples were fixed and stained with Rho-Phalloidin/DAPI, imaged using a confocal microscope and histomorphometric analysis was conducted using Image J (NIH). Four samples from each peptide group and 10 fields per sample were imaged and analyzed for histomorphometric analysis.

Results: DPI-VTK had the lowest K_D 2.83 ± 0.57 μM, compared to 7.36 ± 0.35 μM for RGD-VTK, 74.1 ± 3.47 μM for RGD-E7, and 74.6 ± 2.89 μM for VTK..DPI-VTK and cell binding control RGD-VTK demonstrated higher binding affinity than previously reported RGD-E7 peptide ($p < 0.01$). Dual peptides RGD-VTK and RGD-E7 showed the highest initial cell attachment when no force was applied ($p < 0.01$). However as detachment forces were applied, larger cell fractions were adherent to DPI-VTK and, as a result, the τ50 value of 0.48 μdynes for DPI-VTK was significantly higher than VTK, RGD-VTK, and RGD-E7 values of 0.05, 0.01 and 0.05μdynes, respectively ($p<0.01$). DPI-VTK bound strongly to hBMSCs, compared to pre-osteoblasts and fibroblasts (Fig. 1 $p<0.01$). Although, RGD-VTK bound weakly to hBMSCs, it promoted stronger attachments to MC3T3s and MDFs ($p <0.01$). Total hBMSC spread area was greater on RGD-VTK and RGD-E7 than DPI-VTK (Fig. 2A). However, normalizing to the number of cells indicated more spread area/cell on DPI-VTK compared to RGD-VTK and RGD-E7 (Fig. 2B).

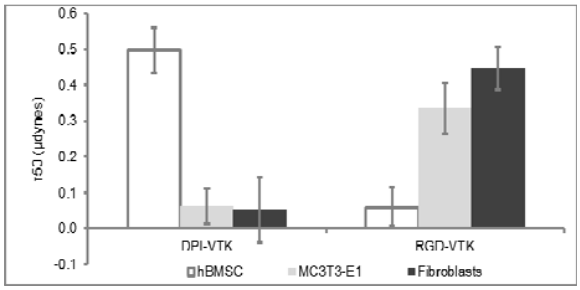

Figure 1. Half-cell detachment forces on dual-peptide coated apatite films (*$p<0.01$, n=6)

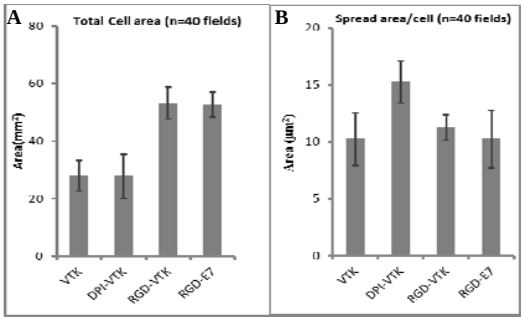

Figure 2. Histomorphometric analysis of hBMSCs on peptide laden biomimetic apatite surfaces (n=40 fields)

Conclusions: Dual-functioning phage derived peptides have a high apatite binding affinity and facilitate hBMSC specific attachment on apatite surfaces. Both cell and mineral binding sequences contribute to apatite binding affinity and cell specificity. Centrifugation assay and histomorphometry indicate that DPI-VTK improved hBMSC initial attachment on apatite surfaces. This could result from a greater number of contacts or more specific contacts with cell surface receptors. Taken together, the results indicate phage display is a promising strategy to identify peptide sequences that promote the attachment of specific cell populations on a biomaterial.

References

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