Osteogenic Differentiation of Human Mesenchymal Stem Cells Synergistically Enhanced by Biomimetic Peptide Amphiphiles Combined with Conditioned Media

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Statement of Purpose: An attractive strategy for bone tissue engineering is to use extracellular matrix (ECM) analogous biomaterials to govern biological response via synthetic cell-ECM interactions. Thus, peptide amphiphiles (PAs) were studied as ECM-mimicking materials to guide osteogenic differentiation of human mesenchymal stem cells (hMSCs). PAs were functionalized with ECM isolated ligands (i.e. PA-RGDS, PA-DGEA), and along with negative controls (PA-S, tissue culture plastic (TCP)), osteoinductive potential was studied with or without conditioned media (dexamethasone, β-glycerol phosphate, ascorbic acid). It was hypothesized that ligand-functionalized PAs would synergistically enhance osteogenic differentiation in combination with conditioned media. Comparative evaluations independent of condition media also evaluated ability of PAs to promote osteoinduction by inscribed ligands alone, allowing for therapeutic effectiveness under physiological conditions.
Methods: PAs self-assembled as 2D coatings by solvent evaporation. hMSC attachment and proliferation studied on 2D scaffolds by PicoGreen assay. Osteoinductivity assessed by histochemical staining for alkaline phosphatase (ALP) and RT-PCR analysis of osteogenic markers (i.e. Runx2, ALP, osteocalcin (OCN)) using the $2^{-\Delta\Delta Ct}$ method normalized to GAPDH and PA-S control group. ANOVA analysis used for statistical significance ($p<0.05$).
Results: We previously showed that PAs can guide osteogenic differentiation based only on synthetic cell-ligand interactions.[1] For a more comprehensive evaluation, this study investigated osteoinductivity of PA-RGDS and PA-DGEA by including gene expression and adding conditioned media for comparison, looking into potential synergistic effects on osteoinduction. hMSCs were grown in conditioned media for 28 days on four coating surfaces,

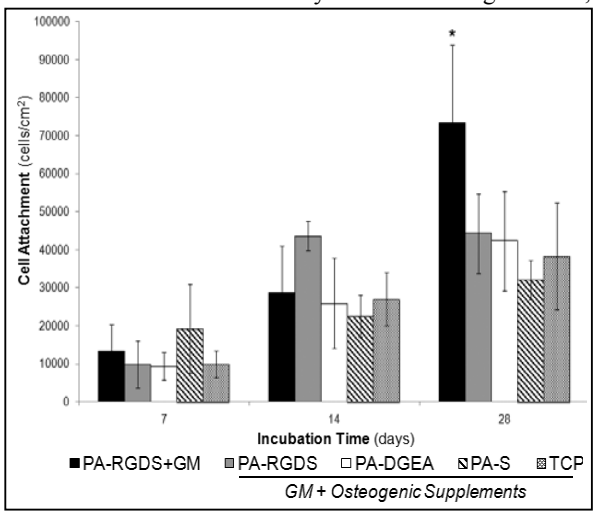

Fig. 1. Cell attachment of hMSCs. All samples cultured in conditioned media except for PA-RGDS+GM, which used growth media (GM) only. *PA-RGDS+GM significantly greater attachment than PA-DGEA, PA-S, and TCP.

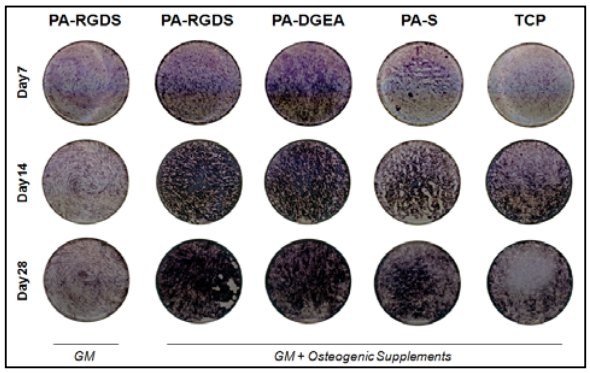

Fig. 2. ALP histochemical staining to qualitatively evaluate osteogenic differentiation over 28 days.

with the exception of PA-RGDS also cultured in normal GM. No significant differences in cell attachment (*Fig.1*) observed for all surfaces after 7 and 14 days. However, attachment for PA-RGDS+GM was significantly higher by day 28. Thus, conditioned media slowed proliferation, hinting that the cells had shifted to a differentiated osteogenic state. While hMSCs on PA-RGDS in GM only became over confluent, suggesting delayed osteoinduction by ligand signaling alone. Next, ALP activity was qualitatively evaluated using histochemical staining (*Fig.2*). It was found that greater ALP activity directly correlated to including conditioned media and the incorporated bioactive ligands (PA-RGDS, PA-DAGEA) greatly enhanced the level of activity, as depicted by bluish-purple precipitation. Also, PA-RGDS in GM induced a visible level of ALP activity comparable to negative controls after 7 days, but this was not maintained long-term. Finally, gene expressions of Runx2, ALP, and OCN were quantified over 28 days to assess osteoinduction, evaluating prominent markers spanning the differentiation process. PA-RGDS and PA-DGEA in conditioned media expressed greater gene expressions promoted much faster than control surfaces. However, evidence supporting osteoinductive potential of PA-RGDS without supplemental aid was also demonstrated. Besides visible levels of ALP activity, PA-RGDS cultured in GM only produced gene expression values very similar to control surfaces aided by conditioned media, or in the case of OCN, was even able to exceed the expressed amount.
Conclusion: Both of the ligand-functionalized PAs were found to synergistically enhance the level of visualized ALP activity and osteogenic gene expression compared to the control surfaces lacking biofunctionality. Guided osteoinduction was also observed without supplemental aid on the PA scaffolds, but at a delayed response and not to the same phenotypic levels. Thus, the biomimetic PAs foster a symbiotic enhancement of osteogenic differentiation, demonstrating the potential of ligand functionalized biomaterials for future bone tissue repair.
References:

1. Anderson *et al*. Biomacromolecules 2009; 10:2935-44.