

Bone Extracellular Matrix-Mimicking Self-Assembled Nanomatrix for Directing Osteogenic Differentiation of Human Mesenchymal Stem Cells without Soluble Factors

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Statement of Purpose: Tissue engineered biomaterials for bone regeneration are challenged to recapitulate the native osteoinductive environment at the basic nanostructure level of tissue formation. In simplest terms, bone extracellular matrix (ECM) consists of biologics, organic fibrous proteins, and inorganic minerals.¹ It is hypothesized that a biomimetic self-assembled nanomatrix of fibrous peptide amphiphiles (PAs) inscribed with ECM ligands and nano-hydroxyapatite (HA) can direct osteogenic differentiation of human mesenchymal stem cells (hMSCs) without osteogenic supplemented media (OSM). PA coating studies without HA were first conducted to assess nanostructure and ligand-directed cellular response, specifically osteogenic differentiation, before moving to stability and mechanical tuning of 3D hydrogels with embedded HA. Each PA evaluated expressed MMP-2 enzyme degradable sites and isolated ECM ligands from either fibronectin (PA-RGDS), collagen (PA-DGEA), or heparin (PA-KRSR). Negative (PA-RGES, PA-S) and positive (glass plus OSM) controls were included.

Methods: PAs self-assembled as 2D coatings by solvent evaporation or 3D hydrogels by Ca²⁺ charge modulation. TEM verified nanofiber structure. hMSC response studied on 2D scaffolds by initial attachment (PicoGreen assay) and osteogenic differentiation (alkaline phosphatase (ALP) activity, morphology, mineral deposition via von Kossa). 3D PA hydrogels modulated by a two-stage process using (1) composite method with PA-S and (2) adjusting HA ratios. ANOVA analysis used for statistical significance.

Results:

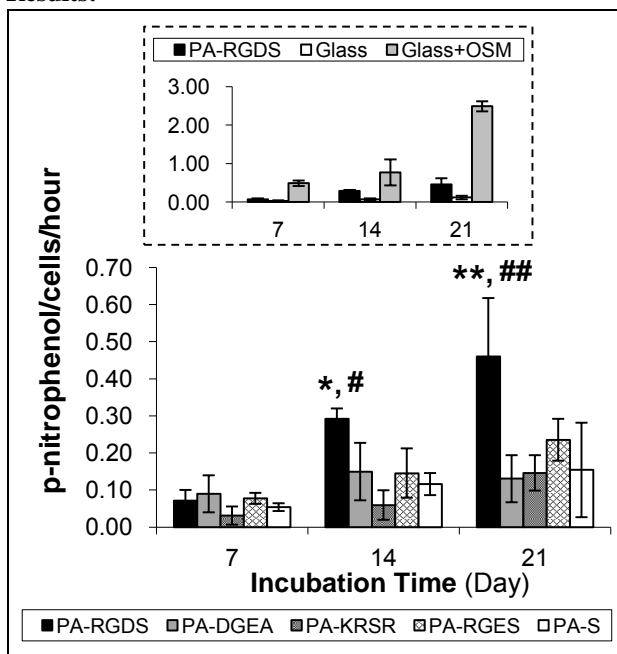


Fig. 1. ALP activity. *,**PA-RGDS significantly greater than other PAs. #,##PA-RGDS expressed significantly more than Day 7 ($p < 0.05$). PA-RGDS compared to glass and glass plus OSM depicted in top inset

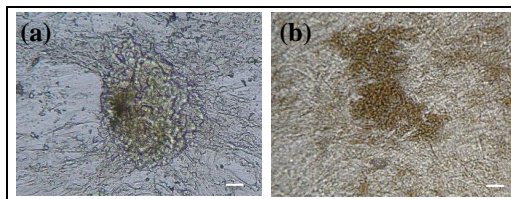


Fig. 2. Mineral deposition of (a) PA-RGDS and (b) glass plus OSM after 28 days. Scale bar equals 50 μ m.

PAs without HA were successfully synthesized and self-assembled into nanofibers with ligands exposed. Multilayered fibrillar networks (DIA=6-10 nm) were observed. Initial cell attachment found PA-RGDS to be greater than all other coatings, indicating hMSC can recognize the different ligands. ALP activity results (Fig. 1), an early marker, showed higher osteogenic potential without OSM on PA-RGDS compared to other PAs. OSM produced a 5-fold increase in ALP activity over PA-RGDS, indicating ligand-driven differentiation is in at an earlier stage. Characteristic osteogenic morphology over 35 days was frequently observed on PA-RGDS and slightly on PA-DGEA, changing from spindle-shape to cuboidal clusters. PA-RGDS showed mineral stains by day 28 that compared favorably to OSM control (Fig. 2), validating osteogenic maturation after the initial delay. A few mineral nodules were found on PA-DGEA, but no osteogenic morphology or mineral deposits observed on all other PA coatings without HA. With ligand-directed osteogenic potential established in 2D, biomimetic PA studies progressed to 3D hydrogels with HA. First, PAs were combined at various molar ratios (MR) with the stronger PA-S, improving stability by using a mechanically tunable composite hydrogel (Fig. 3). Next, HA was embedded in composite PAs for further stability and integration of the final inorganic component needed for a complete bone biomimetic. Different HA-PA weight ratios (%) were tested, and viscoelasticity improved with concentration.



Fig. 3. Composite PA nanomatrix hydrogel modulation.

Conclusion: PAs inscribed with isolated ligands were able to direct hMSC osteogenesis without OSM. Mechanically tunable hydrogels were developed, and HA integration efforts begun to create a true bone ECM mimetic. Further study of PA hydrogels with HA and corresponding cell response via osteogenic gene expression still remain for future experiments. Overall, these results establish the beginnings of a new, versatile approach to regenerate bone tissues by closely following natural tissue formation.

References:

1. Allori A *et al.* TISS Eng Part B Rev 2008; 14(3):275-83.