Osteogenic differentiation of human mesenchymal stem cells directed by extracellular matrix-mimicking ligands in a biomimetic self-assembled peptide amphiphile nanomatrix

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Statement of Purpose

Tissue engineering has great potential for clinical bone regeneration needs, such as bone tissue recovery and faster healing of fractures. Bone is a dense, connective tissue with an extracellular matrix (ECM) composed of organic collagen fibrils, inorganic minerals, and osteogenic cells. An ideal strategy is to emulate the bone hierarchical formation. It is hypothesized that a nanoscale, biomimetic scaffold composed of ECM-mimicking peptide amphiphiles (PAs) inscribed with cellular adhesive ligands can direct the osteogenic differentiation of human mesenchymal stem cells (hMSCs) isolated from bone marrow without osteogenic supplements (e.g. dexamethasone, β-glycerol phosphate). PAs are amphiphilic molecules able to self-assemble into higher order structures.[1] The designed PAs are functionalized with a peptide sequence composed of a matrix metalloproteinase-2 enzyme degradable site[2], coupled with adhesive ligands isolated from ECM proteins. Specifically, different PAs have been synthesized, inscribed with fibronectin/laminin-binding (PA-RGDS), collagen-binding (PA-DGEA), or heparin-binding (PA-KRSR) peptide signals, and the ability of each to influence cellular behaviors, specifically osteogenic different, was investigated.

Methods

All studies conducted on 2D PA coatings. PAs self-assembled onto glass coverslips by solvent evaporation and verified with TEM. Initial attachment (PicoGreen assay) and osteogenic differentiation of hMSCs on PA nanomatrices studied. Alkaline phosphatase (ALP) activity, cell morphology, and mineral deposition via von Kossa staining used as osteogenic differentiation markers. ANOVA analysis used for statistical significance.

Results

The novel PA nanomatrices were successfully synthesized and self-assembled by solvent evaporation into nanofibers with the adhesive ligands exposed to the outside. This self-assembly technique created 2D nanomatrix surfaces consisting of PA nanofibers with a typical diameter range of 6-10 nm (*Fig. 1*).

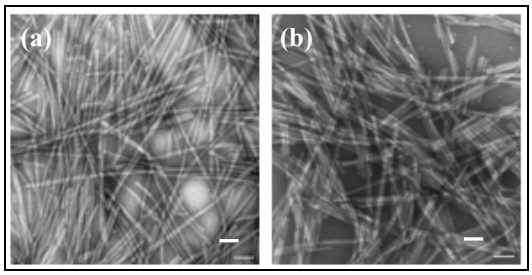

Fig. 1. TEM images of self-assembled (a) PA-RGDS and (b) PA-DGEA. Scale bar represents 50 nm.

Initial cell attachment found PA-RGDS to be significantly greater compared to PA-DGEA and PA-KRSR, demonstrating hMSCs can recognize the adhesive ligands. ALP activity results (*Fig. 2*), an early marker, showed the ability of the PA-RGDS nanomatrix to induce osteogenic differentiation without osteogenic media supplements compared to the other PA nanomatrix surfaces.

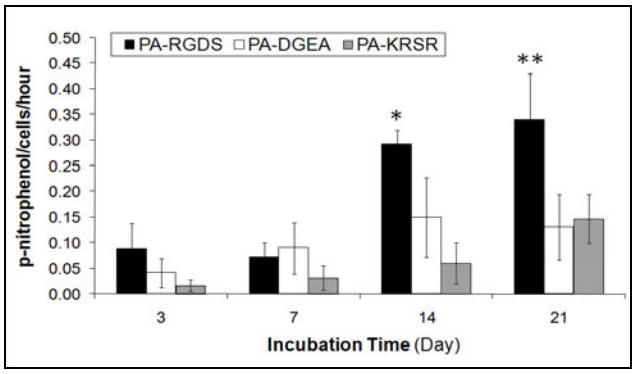

Fig. 2. ALP activity. *,**PA-RGDS significantly greater ($p<0.01$) than PA-DGEA and PA-KRSR ($n=4$).

Characteristic osteogenic morphology over 35 days was frequently observed on PA-RGDS and slightly on PA-DGEA, changing from spindle-shape to multilayered cuboidal clusters. PA-RGDS showed mineralized stains by days 28 and 35 (*Fig. 3*), validating mature osteogenic development. A few mineralized nodules were found on the PA-DGEA coating, and no osteogenic morphology or mineral deposits were observed on PA-KRSR or glass.

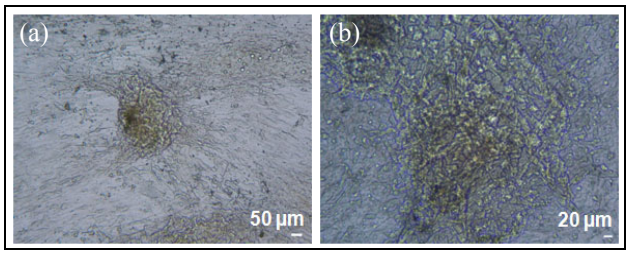

Fig. 3. Mineral deposition via von Kossa staining of PA-RGDS after (a) 28 and (b) 35 days.

Conclusion

The results show the ability of the ECM isolated signals to solely control cell behaviors. Most importantly, the PA-RGDS nanomatrix demonstrated the greatest potential to direct osteogenic differentiation without supplements. Thus, these results better define the importance of integrin-mediated binding for osteogenic differentiation in the absence of stimulatory factors. Overall, this research model demonstrates a new, versatile strategy for tissue regeneration by closely mimicking the principle of natural bone tissue formation.

References

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Acknowledgments

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