Biphasic Peptide Amphiphile Nanomatrix Scaffold for Enhanced Osteogenic Response

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Statement of Purpose: The goal of this study was to develop a biphasic scaffold to enhance osteogenic response for bone tissue regeneration. The native bone microenvironment consists of an organic protein matrix reinforced with an inorganic calcium phosphate network. There is currently a need in bone tissue engineering for scaffolds that can recreate both components and facilitate the regrowth of bone. Peptide amphiphile (PA) based scaffolds offer an attractive solution to this problem. PAs are self-assembling molecules consisting of a hydrophobic alkyl tail and hydrophilic peptide head. The peptide head can be inscribed with specific cellular adhesive ligands and can mimic particular extracellular matrix proteins. The nanofibers will from hydrogels with the addition of calcium ions. It was hypothesized that a biphasic composite hydrogel consisting of PA-RGDS and PA-S nanofibers with hydroxyapatite nanoparticles (HANPs) could be created and would facilitate better bone regeneration in vivo when compared to controls.

Materials And Methods: Self-assembly of the hydrogels were induced by combing various PA/HANPs mixtures with 0.1M $CaCl_2$. TEM was taken to confirm scaffold formation. After inducing self-assembly with embedded HANPs, the viscoelastic properties of different hydrogels were measured using rheometry. Human mesenchymal stem cells (HMSCs) were encapsulated at a density of 50,000 cells per gel in four different conditions: PA-RGDS/PA-S (1:1) with 50% HA and 0% HA and PA-S with 50% HA and 0% HA. Gene expression for runt-related transcriptional factor-2 (Runx2), alkaline phosphatase (ALP), osteocalcin (OCN), and collagen type I were assessed was assessed at days 0, 7, 14, and 28. An *in vivo* study was performed on a critical sized femoral defect rat model. 6 mm defects were created in the rats and each defect was stabilized with an intramedullary threaded k-wire. After stabilization, three experimental groups were created and implanted into the defect sites. The conditions were: defect only, PA-RGDS/PA-S, and PA-RGDS/PA-S with 50% HANPs. Goldner's trichrome staining was performed at 4-weeks for each condition.

Results: TEM images confirmed that both PA-RDGS/PA-S, and PA-S hydrogels were self-assembled into nanofibers, and HANPs were uniformly dispersed (*Fig 1*).

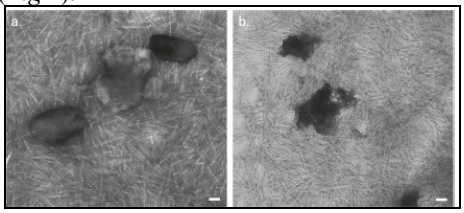

Figure 1. TEM images of biphasic PA hydrogels self-assembled with 50% HA concentration in (a) PA-RGDS/PA-S (1:1) and (b)PA-S.

Viscoelastic properties for both PA-RGDS/PA-S (1:1) and PA-S was the greatest with the addition of 50% HA. This concentration of HA was used in the subsequent experiments. HMSCs encapsulated in the biphasic PA-RGDS/PA-S (1:1) with 50% HA scaffolds showed the greatest expression of key osteogenic genes. PA-RGDS/PA-S (1:1) with 50% HA showed a dramatic stimulation of collagen type I expression at 14 days when compared to other conditions (*Fig 2)*.

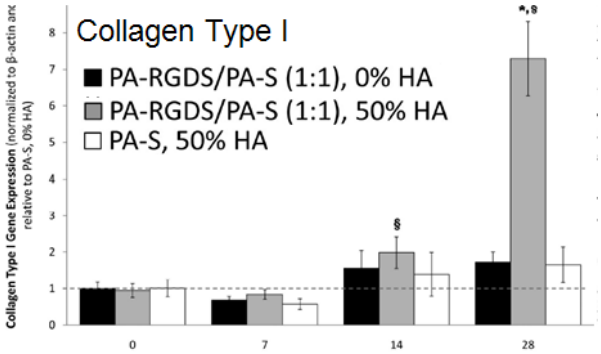

Figure 2. Collagen type I expression of hMSCs.

Additionally, histological assessments of the femoral defects at 4 weeks showed the biphasic PA-RGDS/PA-S (1:1) with 50% HA showed bone formation across the defect and calcified mineralization across the entire void.

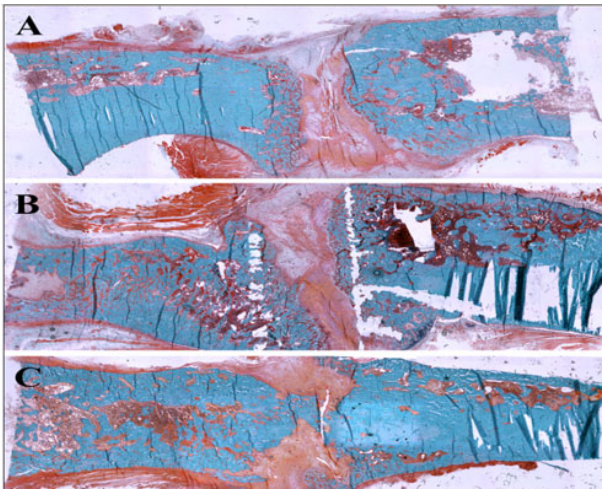

Figure 3. Goldner's trichrome staining (a) defect only, (b) PA-RGDS/PA-S (1:1) hydrogel, 0% HA and (c) biphasic PA-RGDS/PA-S (1:1), 50% HA. Osteoid stains dark pink, and calcified bone tissue appears green.

Conclusion: The biphasic PA nanomatrix demonstrated the most effective osteoinduction and comparative bone healing response. These results provide innovative insights into a promising biomimetic solution for bone tissue engineering.

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