**Bone Extracellular Matrix-Mimicking Self-Assembled Biphasic Nanomatrix for Bone Tissue Regeneration**

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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] To recreate the basic assembly, peptide amphiphiles (PAs) functionalized with isolated cellular ligands from bone ECM molecules have been combined with inorganic hydroxyapatite (HA) nanocrystals, creating a biphasic hydrogel to interface with human mesenchymal stem cells (hMSCs). We hypothesize the biphasic hydrogel will promote enhanced osteogenic differentiation of hMSCs driven by inscribed ligands and HA, allowing for faster bone healing in vivo. The PAs developed contain MMP-2 enzyme degradable sites and either isolated ECM ligands from fibronectin (PA-RGDS) or no ligand (PA-S) as a negative control. The biphasic hydrogels were first optimized with different amounts of HA. Then efficacy of biphasic nanomatrix gel to promote osteogenic differentiation of hMSCs and better bone healing were tested in vitro and in vivo.

**Methods:** PAs self-assembled with HA as 3D biphasic hydrogels by $Ca^{2+}$ charge modulation. Different HA (%) amounts (0, 33.3, 50, 66.7%) tested by viscoelastic rheometry using dynamic oscillatory shear (0.1-10 Hz). After optimization, biphasic PA hydrogel with 50% HA transplanted into critical size femoral defect (6 mm) of athymic rats with k-wire intermedullary stabilization and evaluated by x-rays over 4 weeks.

**Results:** Creating biphasic PA hydrogels by including HA is the necessary next step for developing bone ECM mimetic scaffolds. Previously shown, PA hydrogels can be controlled by merging bioactive PAs (e.g. PA-RGDS) with the stronger gelating PA-S, as the molar ratio (Mr=PA-RGDS/PA-S) of 1:1 was found most stable.[2] Thus, PA-RGDS/PA-S (Mr=1:1) and PA-S hydrogels have been used for all biphasic gel studies. To first create the biphasic hydrogels, different HA (%) amounts were rheologically tested. It was found that increasing HA greatly improved viscoelasticity before oversaturation caused a drop-off. Specifically, the ratio of G'/G" peaked at 50% HA for both biphasic gels (*Table 1*). Thus, viscoelasticity and stability can be directly controlled within the biphasic hydrogels by incorporating different HA amounts, and HA (%) has been optimally fixed at 50% for all biological studies.

| HA % | G'/G" [a] | |
|---|---|---|
| Concentration | PA-RGDS / PA-S (1:1) | PA-S |
| 0% | 5.024 | 6.130 |
| 33.3% | 3.048 | 9.285 |
| 50% | 7.993 | 9.665 |
| 66.7% | 3.963 | 6.959 |

[a] ratio of storage modulus (G') to loss modulus (G") at 10 Hz

Table 1. Ratio of storage modulus to loss modulus for biphasic PA hydrogels with different HA (%) amounts.

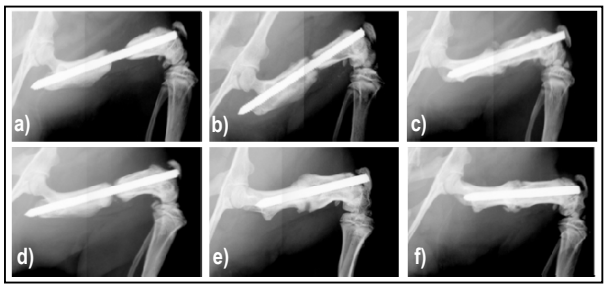


Fig. 1. X-rays after 2 (a-c) and 4 (d-f) weeks of 6 mm critical size femoral rat defects. Three groups – *Defect only* (a,d), *Gel only* (b,e), biphasic *Gel+50% HA* (c,f). All gels are PA-RGDS/PA-S (Mr=1:1).

Next, a pilot animal study was conducted using PA-RGDS/PA-S (Mr=1:1) hydrogels, along with biphasic gels containing 50% HA. The hydrogels with and without HA were then implanted into a rat femoral defect model of 6 mm (*Fig. 1*) and qualitatively evaluated by x-rays. After 2 weeks, it was progressively found that as the nanomatrix gel (*Gel only*) was incorporated and then the biphasic nanomatrix gel (*Gel+HA*), the callus formation across the injury defect increased, indicating a promoted healing response. For *Defect only* condition, the defect size remained relatively unaffected. However, in the presence of *Gel only*, there was increased callus formation based on new bone growth after 2 weeks, resulting in a smaller defect size. The bone healing response is further increased by the *Gel + 50% HA*, as transplantation with the biphasic gel led to new callus formation that bridged the gap across the original critical defect size. After four weeks, the same general trend was observed. While smaller in size, the segmental defect still remained visibly evident for *Defect only*. However, *Gel only* displayed new callus formation that connected the gap across the defect area, indicating comparatively better new bone growth. For *Gel + 50% HA*, a more complete bone healing response was found, as the defect void continued to be filled by new bone formation after initially bridging the segmental gap after 2 weeks. Thus, the x-rays support increased bone formation as promoted by the biphasic hydrogel.

**Conclusion:** This research project uses a bottom-up tissue engineering approach to recreate the natural building blocks of bone ECM, employing PAs and HA to create a biphasic nanomatrix hydrogel for bone healing. The inclusion of HA stabilizes the biphasic hydrogel and adds the last biomimetic component needed. Further in vitro and in vivo studies still remain, especially with encapsulated hMSCs; however, promising levels of bone regeneration have been observed, evidenced by the critical size femoral defect animal study. Thus, new insights into enhanced bone regenerative biomaterials are being developed by following the principles of nature tissue formation.

**References:**

1. Allori *et al*. Tiss Eng Part B Rev 2008; 14(3):275-83.
2. Anderson *et al*. ACS Nano 2009; 3(11):3447-54.