Porous Laponite Nanoclay-Hydroxyapatite Composite Scaffolds: Characterization and Understanding Cell-Material Interactions

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Statement of Purpose: Hydroxyapatite (HA), as a biocompatible ceramic, has been widely used in bone tissue engineering due to its high chemical and physical resemblance to native bone minerals. HA scaffolds have been modified with different materials to improve mechanical properties and osteoconductivity. Laponite is a synthetic nanoclay that has shown promise in promoting osteogenic differentiation. In this study, we developed highly porous HA-Laponite nanoclay scaffolds (0-50% by volume nanoclay) and investigated their potential for bone tissue engineering applications.

Materials and Methods: Laponite nanoclays were mixed with 5 ml deionized water and Trimethylamine [1]. Then, the pH was adjusted to 10.5 by adding NaOH and HCl solution. Then the mixture was stirred vigorously for 48 hours. Then the mixture was centrifuged with maximum speed for 20 minutes followed by centrifugation and washing with DI water three times. HA slurry was prepared similar to our previous studies [2]. Different slurries were prepared based on various ratios of HA and LNPs. The control sample was made of only HA and 4 other groups with 5, 10, 25, and 50% of LNPs were sintered. Scanning Electron Microscopy showing pore architecture and surface grain size, XRD analysis to characterize the crystallinity, EDX elemental analysis, Mechanical testing, Porosity, 3D pore structure characterization by MicroCT figures and architectural analysis, and 4-week degradation study were conducted. *In vitro* evaluation was conducted by seeding samples with bone marrow human mesenchymal cells and the study continued for 21 days (n=4). Immunohistochemistry imaging (Actin, DAPI, and Osteopontin), cell proliferation (Alamar Blue), and alkaline phosphatase assays were performed to evaluate the preliminary impact of the combination of Laponite and hydroxyapatite on cellular behavior.

Results: Results showed that nanoclay did not having a significant impact on porosity; however, resulted in changes in grain shape. In addition, while there is a significant decline in Young's modulus, strength at failure, and toughness in 25% nanoclay scaffolds compared to pure HA scaffolds; grain stabilization at higher volume fractions resulted in mechanical properties being comparable between 100% HA and 50% nanoclay scaffolds.

Initial in vitro results showed a significant increase (*$p<0.05$) in the cell proliferation in group 50%. ALP also improved when HA percentage increased. IHC imaging results indicated a complete cellular attachment to the 3D scaffolds over 21 days. Cells in all groups showed osteopontin expression; however, more osteopontin expression was observed in group HA 90%.

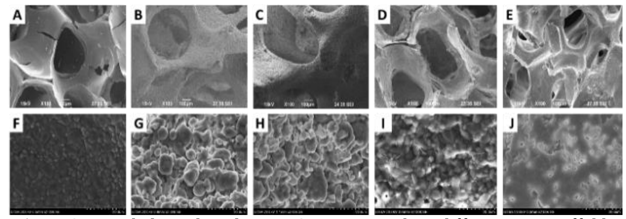

Figure 3: Morphological Evaluation. SEM images show differences in scaffold pore size and structure. 100% HA (A&F), 95% HA and 5% Laponite(B&G), 90% HA and 10% Laponite (C&H), 75% HA and 25% Laponite(D&I), 50% HA and 50% Laponite (E&J).

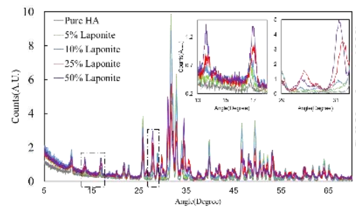

Figure 2: XRD Spectra of HA-Laponite nanoclay scaffolds

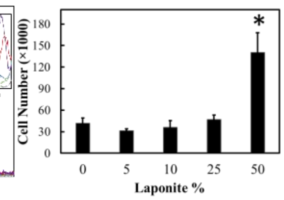

Figure 3: Cell Proliferation on tested groups during 24 hours.

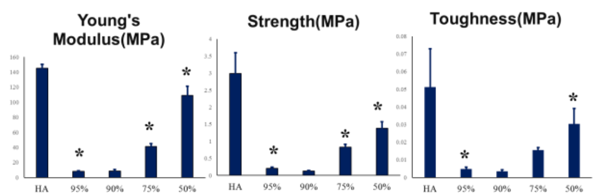

Figure 1: Figure 4. Young's Modulus, Strength, and Toughness of HA-Laponite nanoclay scaffolds

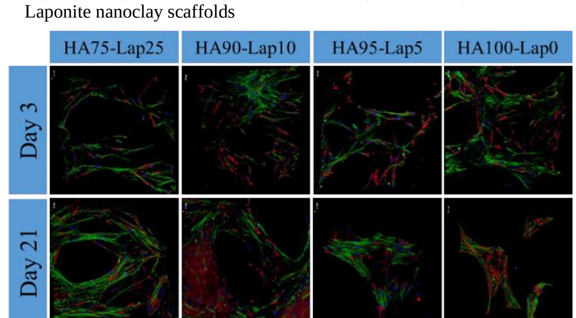

Figure 1: Immunohistochemistry evaluation of HA-Laponite nanoclay scaffolds. (Actin: green, DAPI: Blue, Osteopontin: Red) at 20X.

Conclusion: Our results showed that the highest percentage of Laponite ratio that can be used in HA scaffolds is 50%. Laponite caused the change in grain size. Grain stabilization at higher volume fractions resulted in mechanical properties being comparable between 100% HA and 50% nanoclay scaffolds.

References: 1. Li, W., et al. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2012. 400: p. 44-51.

2. Appleford, M.R., et al., Journal of biomedical materials research Part A, 2009. 89(4): p. 1019-1027.