LNP-HA Scaffold Architecture and Osteogenic Properties

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WeARE Research Area

The research focuses on the incorporation of Laponite in HA scaffolds to study the osteogenic properties of Laponite (charges) on human bone marrow mesenchymal cells. The histological portion focuses on utilizing histomorphometry to analyze the cell layer on the LNP-HA scaffolds.

Background

In this study, Laponite Nano Particles (LNPs) are modified with a different type of emulsifier. This agent is well known as a basic compound with three amine groups used frequently as an emulsifier for o/w emulsions. Because the emulsions are not stable solutions under various conditions, the LNPs were modified with this emulsifier permanently [1]. Eventually, the modified LNPs will be mixed with the HA slurry for further LNP-HA scaffold optimization.

LNPs and HA powder (TAL Materials, Ann Arbor, MI) have been used as the main components of the scaffolds, Trimethylamine (TEA) (Sigma Aldrich, St. Louis, MO) as the modification agent, Polyurethane sponges (EN Murray, Denver, CO) for coating, N,N-dimethylformamide (Sigma Aldrich, St. Louis, MO), Carboxymethylcellulose (Sigma Aldrich, St. Louis, MO), Ammonium Polyacrylate dispersant, and Polyvinyl Alcohol (M_w: 89,000- 98,000, Sigma Aldrich, St. Louis, MO) were all used in HA slurry preparation.

Objectives

- To investigate the effects of Laponite on HA scaffold architecture for bone tissue engineering applications.
- To evaluate the osteogenic properties of the LNP-HA scaffold.
- To evaluate the ability of cells to adhere to the LNP-HA scaffold.

Methodology

Scaffold Preparation:
1g of LNPs were completely mixed with 5 ml deionized water until it turned into a semi-transparent solution. Then, 1ml TEA was added to the stock suspension and the mixture was stirred vigorously for 48 hours. The mixture was then centrifuged with maximum speed for 20 minutes. After centrifugation and being washed with DI water three times, NPLs were kept in a vacuum chamber for two days to remove the residual water. HA slurry was prepared similarly to previous studies [2]. Different slurries were prepared based on various ratios of HA and LNPs. A control sample was made of only HA and 2 other groups with 10 and 25% w: w of LNPs were prepared.

Cell culture:
Human bone marrow mesenchymal stem cells (HBMSCs) were purchased and subcultured in growth media (79% DMEM, 20% FBS, 1% Antibiotics) until the cells reached 80% confluency. Then, sterilized samples were soaked in media for 2 hours before cell seeding. The cells were then seeded on each scaffold at 400,000 cells per sample. Seeded cells were fed every two days and samples were fixed in 4% paraformaldehyde solution on day 14.

Scaffold Histology:
The first step in preparing the samples for histomorphometry was the dehydration process of the samples. Then, the samples were embedded in Technovit and sectioned and grinded down using EXAKT's cutting and grinding equipment. Samples were then polished to 1200 grit with a final thickness of 190 µm, 170 µm, and 180 µm, respectively for samples 1, 2, and 3. The samples were sectioned in the manner shown in figure 1. Finally, the samples were stained for 5 minutes with Alizarin red, then 5 minutes with Aniline blue, then 3 minutes with Biebrich scarlet.

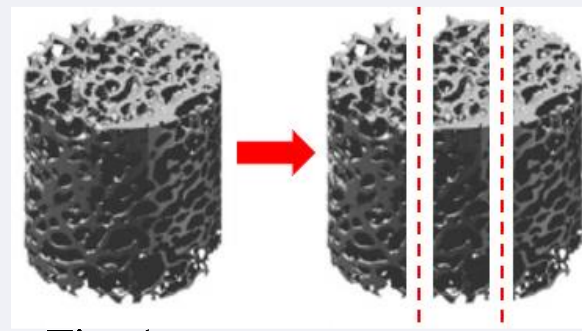

Fig. 1
Cutting schematic of the scaffold samples

Scaffold histomorphometry was performed with respect to the following factors: Trabecular thickness [Tb.th. = (4 / 1.199) x (SS / SV)], Trabecular number [Tb.n. = $((4 / \pi) \times (SV / TV))^{-0.5}$ / (Tb.th.)], and trabecular separation [Tb.sp. = ((4 / π) x (TV / BV) – 1) x (Tb.th.)]. Scaffold volumetric and surface to volume ratios were calculated from 2D area and perimeter data.

Results

Scaffold Architecture:
Table 1 shows the bone histomorphometry analysis parameters. ImageJ and the BoneJ plugin along with photoshop aided with obtaining this data.

Table 1. *Histomorphometry values of LNP-HA scaffolds*

Parameter	Abbreviation	Sample 1	Sample 2	Sample 3	Units
Scaffold Volume/Total Volume	SV/TV	0.28430	0.49368	0.35036	%
Scaffold Surface/Total Volume	SS/TV	0.02305	0.03930	0.02442	mm^{-1}
Scaffold Surface/Scaffold Volume	SS/SV	0.08107	0.07961	0.06971	mm^{-1}
Trabecular Thickness	Tb.Th	0.27046	0.26558	0.23255	mm
Trabecular Number	Tb.N	2.22449	2.98530	2.87209	mm^{-1}
Trabecular Separation	Tb.Sp	0.94082	0.41936	0.61256	mm

An inverted microscope was utilized to observe the samples and take pictures using the BIOQUANT software, then the pictures of the scaffold were stitched together using Microsoft ICE. Pictures of the samples were taken at 10x then stitched together to see the full sample. Then, pictures were taken at 40x for better visualization and analysis. These pictures are shown in figures 2, 3, and 4.

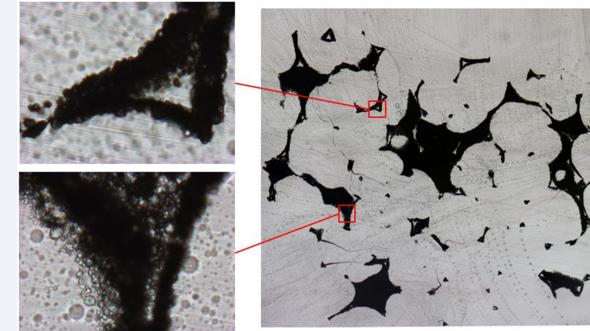

Fig. 2
Sample 1 microscope photos

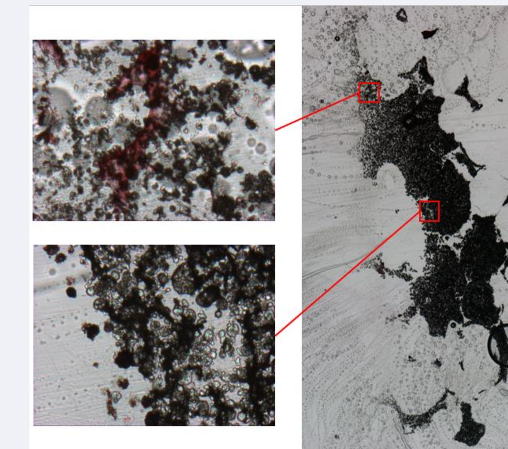

Fig. 3
Sample 2 microscope photos

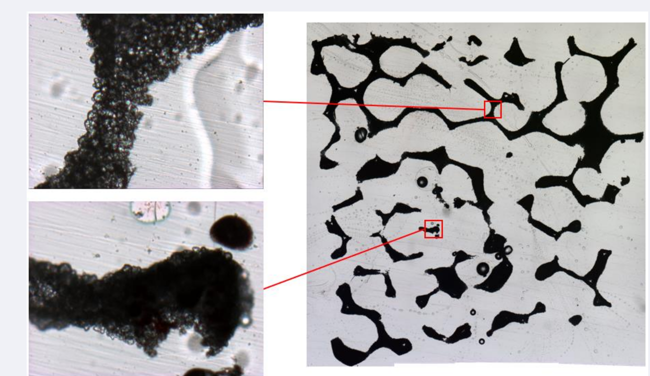

Fig. 4
Sample 3 microscope photos

Cell Attachment:
It was observed that there was little to no cell layer in all three samples. This is shown by the minimal amount of staining on the LNP-HA scaffolds, indicating that the cells were not thriving in this environment. Sample 2 has a small cell layer that could be seen at 40x, indicated by the light red staining while sample 3 was almost completely dead.

Skills and Experience

I gained experience in utilizing specialized cutting and grinding equipment during this project as well as the BIOQUANT, Microsoft ICE, ImageJ, and photoshop software programs. I also built on my time management and problem-solving skills, especially when COVID-19 happened. I had to adapt to and overcome the issues of not being able to discuss aspects of the project with my PI face-to-face and fully relying on communicating via email. I spent a lot more time obtaining data through calculations and digital analysis than I would've had to if I was able to utilize the resources in the lab, however, I did gain experience in two new software programs because of this.

What I Learned

I learned numerous new laboratory techniques and processes. I was able to improve and build on my engineering and lab knowledge. I also learned how altering certain properties of HA scaffolds affect the ability of cells to adhere to their surface and thrive in that environment. Additionally, I learned how to adapt to circumstances due to the COVID-19 outbreak. I learned how to utilize what I had available to me due to the lack of availability of the programs and equipment at the lab and make observations with the data I had.

Future Plans

The next steps in this research would be to determine what went wrong or what caused the cells to not successfully adhere to the LNP-HA scaffold. If a potential issue is found, then the study can be repeated with necessary changes to determine if there is a better outcome. Additionally, the calculations can be verified using BIOQUANT once we have lab access again.

Acknowledgments

This work is supported by the USDA National Institute of Food and Agriculture, Interdisciplinary Hands-on Research Traineeship and Extension Experiential Learning in Bioenergy/Natural Resources/Economics/Rural project, U-GREAT (UnderGraduate Research, Education And Training) program (2016-67032-24984).

References

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2. Appleford, M.R., et al., *In vivo study on hydroxyapatite scaffolds with trabecular architecture for bone repair*. Journal of biomedical materials research Part A, 2009. **89**(4): p. 1019-1027.