FACTORIATION AND CHARACTERIZATION OF PARTICLE-BASED INVERTED COLLOIDAL CRYSTALS

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INTRODUCTION
Each year, there are about 3 million musculoskeletal surgeries performed in the United States. Two primary therapeutic methods used to repair musculoskeletal damage, specifically concerning bone damage, are autografts and allografts. Autografting, involves removing bone material from a secondary site on the patient for implantation at the damaged site, while allografting encompasses inserting bone pieces from a donor into the patient.\textsuperscript{1} However, there are a few major problems with both of these techniques. One overall issue is they both require removal of bone, which can be a painful procedure. Autografting is specifically problematic because it requires more surgery/recovery time since bone is extracted from one area of the body for insertion at another site. Although allografting combats the problem of multiple surgeries on the patient, there is a lack of donors as well as a high potential for disease transmission and for the patients’ body to reject the bone donation.\textsuperscript{2, 3} In order to combat these grafting issues, scientists have developed biocompatible scaffolds to promote tissue regeneration. One regenerative technology still in development is an inverted colloidal crystal (ICC). An ICC structure, as seen in Figure 1, is characterized by having highly ordered, interconnected, porous networks, a solid backbone made of biocompatible materials and the ability to enhance cellular growth and nutrient diffusion due to the spherical cavities and porosity. ICC’s are typically created by forming colloidal crystals, embedding them within a solid material, such as a polymer, and subsequently removing the particles from the solid matrix, forming a porous material.\textsuperscript{4, 5}

Porosity is a key component of tissue scaffold design for cellular infiltration, nutrient diffusion and waste removal across the scaffold which are why ICC’s are proving effective.\textsuperscript{6} However, typical ICCs only offer homogeneous porosity with a lack of hierarchy in the design. By adding hierarchal features one could provide topological cues to cells to enhance bone tissue regeneration because hierarchal surface morphology more closely resembles bone vs the smooth backbone of ICCs.

One way to adjust hierarchy is through backbone customization and using particles as the scaffold backbone. By expanding current ICC technology, we are the first to synthesize a uniquely designed particle-based inverted crystal, with added porosity arising from the backbone structure. The structural differences between ICC’s and particle based inverted crystals are that the former has a solid material filling the interstitial spaces while the latter has small particles fused together in the spaces between pores. Figure 2 shows comparative schematics to demonstrate these structural differences. To produce these crystals, we organize two differently sized particle populations into a hexagonally packed array. The smaller particles act as the backbone and the larger ones as a template (or temporary placeholders) to be removed forming the pores. The particle-based inverted crystal is ultimately a unique structure with potential to further enhance bone tissue regeneration and improve upon current ICC regenerative therapeutics stemming from the structural design.

OBJECTIVE
We aim to increase the hierarchical complexity and porosity of ICCs by fabricating particle-based inverted crystals. Specifically, focus will be placed on observing the effects of varied heating conditions and chemical washing time periods on the small particle fusion and large particle removal. However, in order to fabricate this particle-based inverted crystal, a workable mold must first be designed that allows for easy and effective crystal fabrication.

SUCCESS CRITERIA
Three areas I have focused on include: 1) Designing a mold to allow for easy creation and removal of particle-based crystals. 2) Conducting repeatable tests to determine small particle fusion times and temperatures to anneal particles. 3) Altering the chemical washing steps for particle-based ICC pore formation.
METHOD

Figure 2B illustrates the particle-based inverted colloidal crystals fabricated from a highly ordered precursor structure (binary colloidal crystal). To create the binary crystal, two differently sized particle populations are organized by applying ultrasonic energy from a sonication bath to the particles in the mold. This energy allows the crystals to self-assemble into a crystalline formation on a 3D level. Two categories of crystals are created: 1) 1µm Polystyrene (PS) + 100µm Soda Lime (SL) particles and 2) 10µm PS + 100µm SL particles. The crystals are then placed in the oven where the small particles are annealed at their points of contact (120°C for 1µm backbone and 220°C for 10µm backbone) to form robust binary colloidal crystals in the mold. To create the particle-based inverted colloidal crystal, the large particles must be removed by washing the crystals in hydrofluoric acid (HF) for 6 days and for 1 day in deionized water.

RESULTS

Through collaborations with the machine shop, a final crystal fabrication mold was developed. This mold, made of stainless steel, promotes easy synthesis and extraction of crystals. Figure 3 shows images of the custom made mold and an example crystal extracted from this mold. Successful fusion of the small particle backbone was achieved in both the 1µm PS + 100µm SL and 10µm PS + 100µm SL crystal categories, as seen below in Figure 4A. Removal of the large particles (100µm SL) is not entirely effective, which is shown in Figure 4B, but the procedure is still being modified.

DISCUSSION

From both SEM images seen in Figure 4, along with many others (data not shown), qualitative analyses were done to modify crystal fabrication protocol. Alterations were made to achieve gentle small particle fusion, to enhance porosity, and complete large particle removal, to create smooth cavities as a substrate for cellular proliferation. Examples of these changes include varying the fusion times and temperatures to have small particles anneal at their points of contact to enhance porosity and diffusion potential across the scaffold. Other changes comprise varying the amount of time crystals spent washing in HF and deionized water. The HF specifically etches away at the large soda lime particles, so more focus was placed on changing the number of days in HF solution. Figure 4B shows that the large particle removal process still needs some work based on the lack of a clean, smooth surface, which is the desired result. Protocol alterations will continue to be made, such as increasing washing time in HF or use of certain lab machinery to invoke agitation to the crystal, to enhance HF infiltration into the sample for more thorough particle removal.

In future work, mechanical testing will be done to study the strength and elastic properties of the crystal to comprehend the type of load it can bear. Porosity determination and diffusivity testing will also be performed to compare if this was enhanced in comparison to ICC structures. These three characterization tests are important to perform because this particle-based inverted colloidal crystal technology is being designed as a potential translational therapy to encourage bone tissue regeneration.

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REFERENCES