



Synthesis of Decellularized Cartilage Extracellular Matrix Microparticles



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Introduction

Microparticles, spherical particles with varying size in the range of 1 and 1000 μm in diameter, has been getting attention for their ability to encapsulate both water-insoluble and water soluble agent and larger surface-to-volume ratio in drug delivery.

Decellularization of harvested extracellular matrix can provide cell signaling cues to synthetic materials, which will promote cell growth in specific desired area, and has been considered as a promising material in tissue engineering.

Cartilage ECM (cdECM) is a collagenous network mainly composed of Type II collagen and Aggrecan, and it is highly avascular, which gives very low regeneration capabilities. In this study, Cartilage Decellularized Extracellular Matrix (cdECM) has been investigated for its synthesis, since there is no existing protocol for this specific material.

Materials / Methods

1. Digest cdECM with pepsin 1%
2. olive oil + Span 80 into 3 neck flask
3. Add digested cdECM solution into flask and let it emulsify
4. Add room temperature acetone and stir for 1.5 hour
5. Vacuum filter cdECM solution with acetone to wash and collect residue
6. Add 0.1% Tween 80 solution and 3.76mL of glutaraldehyde (25%) into new 3 neck flask
7. Add residue collected from previous steps and stir at 500 rpm for 20 hours
8. Add glycine into flask to quench the crosslinking and let it stir
9. Gradually wash the formed particles from water to acetone using vacuum filtration
10. Freeze-dry residue and sieve

Site Information:

Rice University

6500 Main St, Houston, TX 77030

Dr. Antonios Mikos



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Result

Initial Trial

-Particles formed (Range: 11-40 μm - Mean diameter: 22.047 μm)

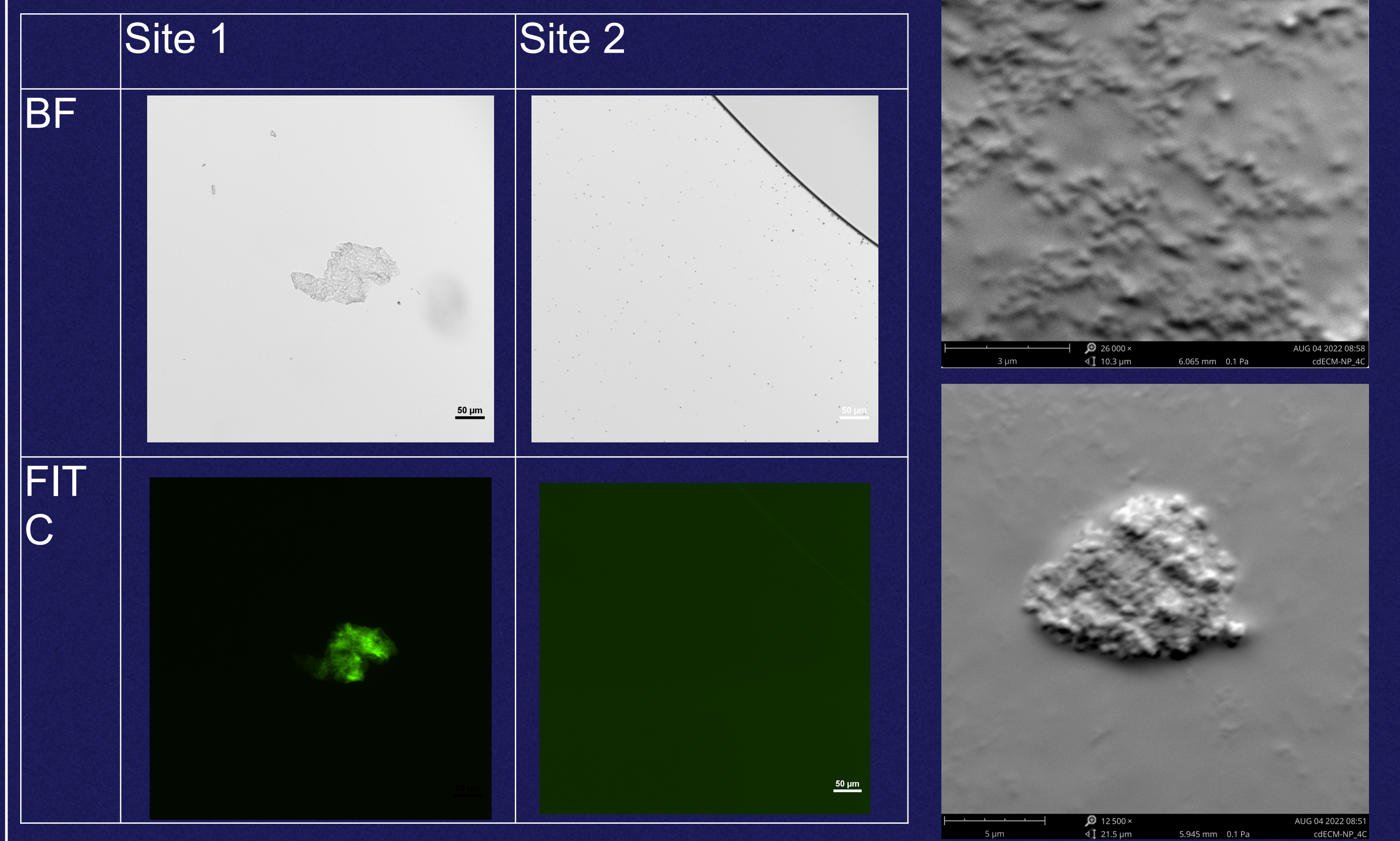
Observed microparticles during the synthesis, but after acetone wash, we no longer saw particles.

Potentially microparticles weren't crosslinked enough, where there was potentially change required in amount of glutaraldehyde and volume of acetone washes

Second Trial

Aggregates formed (Confirmed it was collagen through its natural fluorescence)

Yield by Mass: 13.3%



Personal Experience

Coming up with a protocol for materials that has not been established in the current field was very challenging. By looking at similar materials and their fabrication method, I not only understood the process but also obtained skills in literature review.

I feel extremely lucky to be given with this opportunity to work in the field I want to pursue in the future, meeting great people that I still keep in touch with. I will continue to learn more about this field in the future, and I'm very excited!

Discussion:

Due to the limited time, I was only able to troubleshoot 1 time after the initial attempt, but for next troubleshooting, there should be decreased amount of surfactant, decreasing glutaraldehyde.

ECM is a lot complex than general mono-composed polymers since it has more diverse compositions.

