

Recellularizing Engineered Cartilage for Pediatric Airways

Leah Levin¹, Paul Gehret², Riccardo Gottardi^{2,3}

¹COL 2023, College of Arts and Sciences, ²Department of Bioengineering, School of Engineering and Applied Sciences

³Department of Pediatrics, Perelman School of Medicine



Abstract

Decellularization shows promise as a scaffold fabrication technique for tissue engineering, but cartilage poses recellularization challenges with its dense matrix and sparse cell population. We evaluated cell reinvasion using image analysis with ImageJ in a new scaffold derived from cartilage decellularized using a novel technique. For different seeding protocols we compared cell density, invasion distance, and nuclei to nuclei separation. Scaffolds seeded with 200k cells and incubated for 1 week in a Transwell plate and 1 week in a Bioreactor proved to be the best condition. Next, we will assess differentiation in these scaffolds.

Decellularization Shows Promise for Tissue Regeneration

Commercialized acellular scaffolds

Prima™ Plus CorMatrix ECM™

Cartilage Limitations

- Dense matrix
- Sparsely populated cells
- Important mechanical properties

Hyaline Cartilage 100 μm

Classic Fabrication Approach

Remove cells → Reinvade cells

Novel Approach for Cartilage using Porcine Menisci

Enzymatically remove blood vessels and elastin to create channels for cell migration

Scaffold for tracheal cartilage

Recellularization Approach

1. Punching scaffolds from the digested, decellularized porcine menisci
2. Seeding stem cells at various cell densities
3. Placing scaffolds in Transwell plate with serum gradient
4. Placing scaffolds in a spinner flask bioreactor

Recellularized Scaffolds Match Density of Native Tissue

1 Week of Invasion

2 Weeks of Invasion

T = Transwell
B = Bioreactor

1 Week Invasion

Density (cells/mm²)

2 Week Invasion

Density (cells/mm²)

Best condition: no significant difference from native

Invasion Distance Shows a Uniform Distribution after 2 Weeks

Measuring distance from surface

Native

1w 200k Transwell

1w 400k Transwell

2w 200k Transwell & Bioreactor (Best condition)

2w 200k Transwell

2w 400k Transwell

Nearest Nuclei to Nuclei Distances Increase with Time

Native Meniscus

1w 200k Transwell

1w 400k Transwell

2W 200k Transwell & Bioreactor

2k 200k Transwell

2W 400k Transwell

Average: 33.8125

Average: 29.4763

Average: 27.1878

Average: 32.2108

Average: 32.7221

Average: 37.350

Conclusions and Further Directions

Best condition:

- 200k cells
- 1 week Transwell
- 1 week Bioreactor
- Repeat with 400k cells, 1 week Transwell, 1 week Bioreactor
- Study differentiation of stem cells in the scaffold

References

Rana et. al. *Tissue, Engineering and Regenerative M*

Alta et. al. *Science Translational Medicine* 2012

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