Compatibility of Porous chitosan scaffold with isolated mesenchymal stem cell *in vitro*

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Background

Tissue adhesive films developed from chitosan can be used for photochemical tissue bonding (PTB) when prepared in solution with rose bengal dyes. Work performed in the Lauto labs has shown that oligomeric chitosan can be freeze dried to create porous chitosan patches that can be used in PTB in conjunction with rose bengal dyes (see figure 1). These adhesive chitosan patches have similar mechanical properties to other non-porous adhesive films whilst providing a bio-favorable porous scaffold that mimics the natural ECM of many tissues. Human mesenchymal stem cells (hMSCs) have shown beneficial effects on ventricular function and infarct remodeling through paracrine effects when when immobilized in chitosan patches[1]. These patches have also been shown to improve retention and viability of hMSCs When compared to intramyocardial injections[2]. In this study, human mesenchymal stem cells (hMSCs) were tested to observe the culturing effects of these oligomeric chitosan patches on hMSC viability and stemness to evaluate there potential as cell carrying cardiac patches.

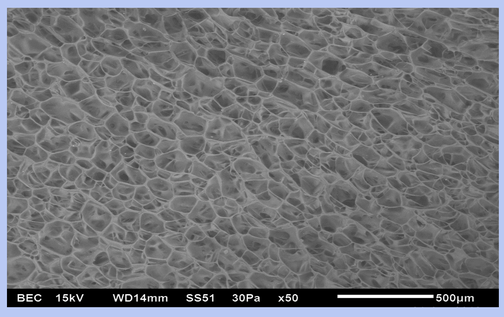
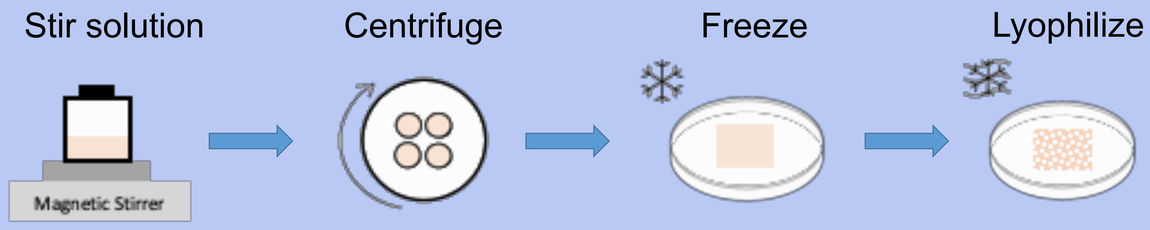


Figure 1: SEM image of 10% oligomeric chitosan patch pours.

Project Aims

1. Production of a porous biocompatible patch to retain hMSCs
2. Assessment of stemness of hMSCs cultured in chitosan patches.

Materials and methods



Chitosan patch development

Generate Mixture:
- 1.5% w/v medium weight chitosan
- 0.2% w/v oligomeric chitosan
- 2% v/v acetic acid in water
- In DI H_2O

↓ Stir Mixture for 1 week at room temperature

↓ Centrifuge Mixture to remove insoluble material

↓ Pipette 3mL of solution into a plastic petri dish

↓ Freeze for 24hours at -30ºC

↓ Freeze dry samples for 6hours at 0.1mbar and -50ºC

↓ Air dry samples at room temperature for 3 weeks or until stable in water

Culture of hMSCs

Place patch in a well of a 12 well plate

↓ Soak Patch in PBS for 30minutes

↓ Replace PBS with DMEM and incubate for 30minutes at 37ºC and 5% CO_2

↓ Aspirate DMEM and pipette $3x10^4$ Cells in 50uL of DMEM on to patch

↓ Incubate at 37ºC and 5% CO_2 for another 30minutes

↓ Gently add 1mL of culture media and culture cells at 37ºC and 5% CO_2

Stemness and viability

Cell viability was assessed by incubated for 1 h at 37 °C in a solution containing 2 µM calcein, for green staining of live cells, and 4 µM of ethidium homodimer-1, a red label for dead cells.

Chitosan patches washed with PBS and then fixed in 1% paraformaldehyde at room temperature for 30minutes

↓ Patches incubated in 0.1% Triton X-100 for 30mins at room temperature

Morphology: Cells incubated in tetra-rhodamine-phalloidin (1:100) in Bovine serum albumin

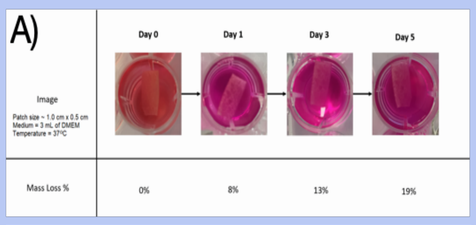
Stemness: Chitosan patches incubated in hStro-1 (1:200) and endoglin (1:100) in 2% goat serum.

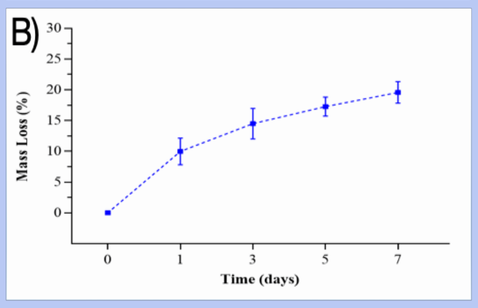
↓ Incubation in secondary antibody – anti-mouse 488 ((1:200) for hStro-1 and anti-rabbit 546 (1:200) for endoglin in 2% goat serum.

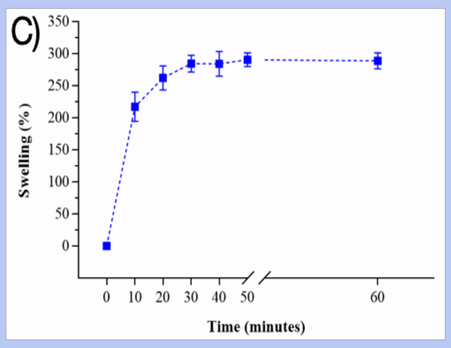
Results

Mechanical results

Property	Mean ± Standard deviation
E-modulus (Film was hydrated in PBS at ~ 25 °C)	1.30 ± 0.09 MPa (n = 10)
Tensile Strength (Film was hydrated in PBS at ~ 25 °C)	0.85 ± 0.08 MPa (n = 10)
Tensile Strain (Film was hydrated in PBS at ~ 25 °C)	90 ± 5 % (n = 10)
Mass Loss in PBS at 37 °C after 7 days	20 ± 2 % (n = 6)
Swelling ratio in PBS at 37 °C after 2 hours	288 ± 17 % (n = 6)
Pore Diameter	110 ± 24 µm (n = 300)







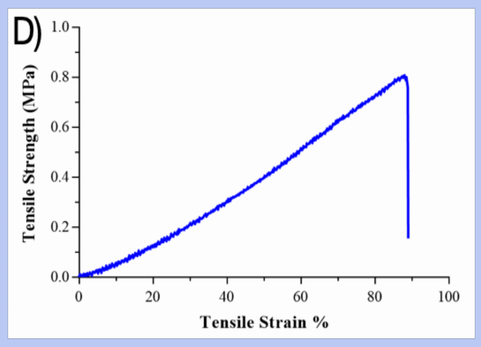


Figure 2: Summary of mechanical properties of a 10% oligomeric chitosan patch. The table summarizes the results A,B) solubility and mass loss over 7 days at 37C C) the percentage of swelling within the first two hours soaking in PBS at 37C D) the E-modulus curve as a relationship between tensile strength and tensile strain.

Cell culture results

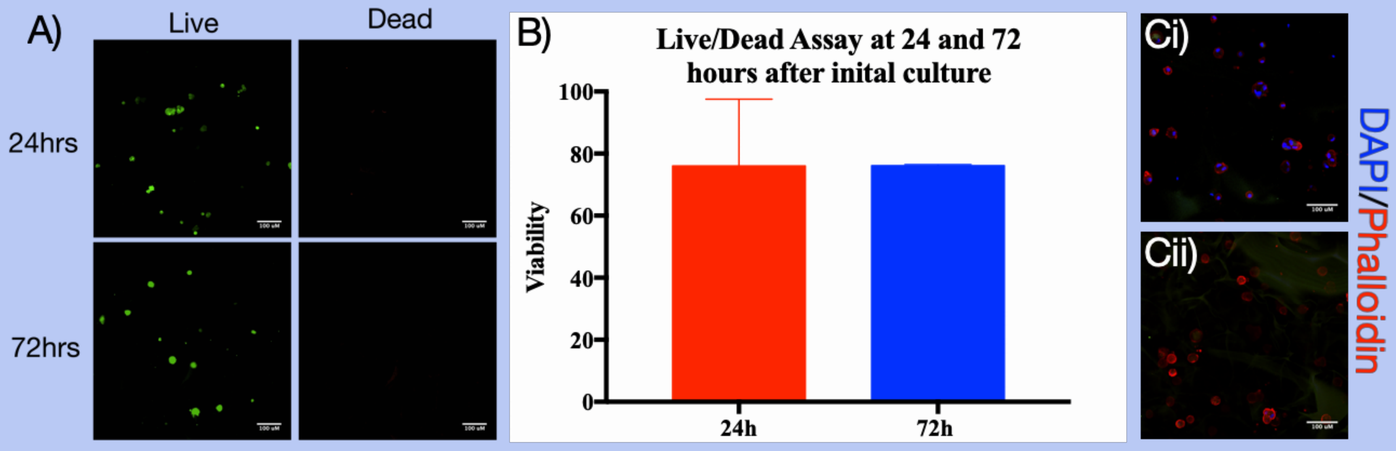


Figure 3: Viability and morphology of hMSCs cultured in a chitosan patch. Cell viability was assessed with calcein and ethidium bromide staining A) Green staining reveals live cells while red staining reveals dead cells B) A quantification of live and dead cells shows a maintained viability between days 1 and 3 with viability of 76.14 ± 10.78% and 76.03 ± 30.40% respectively. C) Cell morphology was assessed by rhodamine phalloidin (red) and DAPI (blue) staining's after 1day (Ci) and 5 days (Cii).

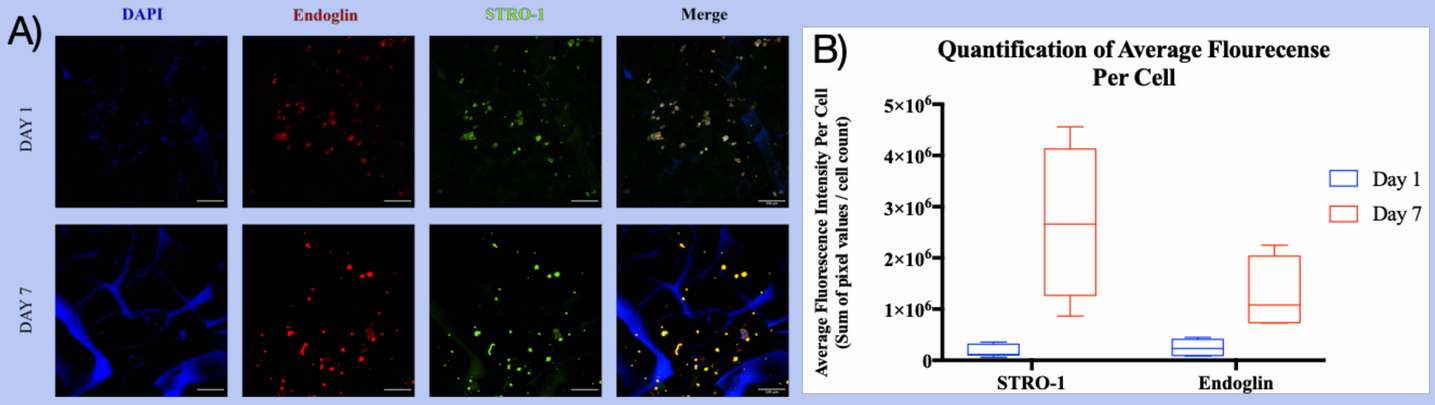


Figure 4: Immunohistochemistry staining of CD34 and CD105: Stemness markers expression analysis. (A) hMSC cultured on porous chitosan patches were analysed for the expression of STRO-1 (green) and endoglin (red). (B) Quantification of average fluorescence per cell. There was a significant increase in the expression of STRO-1 and Endoglin between days 1 and 7 (STRO-1: p = 0.0013, multiple T-tests, n = 7-4, Endoglin: p= 0.0039, multiple t-tests, n = 7-4). For images in (A) day 1, scale bar = 200 µm and for images in (A) day 7, scale bar = 100 µm.

Conclusion

In the present study, a novel biocompatible chitosan patch has been developed that can uphold tissue bonding strengths and degrade to allow tissue migration and prevent scar bound engraftment. These patches also support hMSCs in vitro while promotes stemness markers hStro-1 and Endoglin. This study suggests that these chitosan patches can promote functional survival of engrafted hMSCs to be used as a restraint to ventricular remodeling post-MI.

3D Rendering of cells within a chitosan patch

Observe a 3D reconstruction of hMSCs isolated within a porous chitosan patch containing 10% oligomeric chitosan. Cells are stained for stem cell markers CD34 and CD105.

Sources

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About the author

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