A Cardiac Patch for Delivering Therapeutic Stem Cells to the Heart Following Myocardial Infarction

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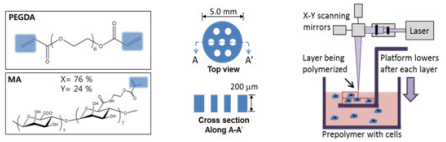
Introduction

Cardiovascular disease is the leading cause of death for both men and women worldwide. Despite advances in post-infarction treatment, heart failure that develops after the heart attack still remains a major cause of death. Current treatments focus on supporting the healthy tissue following a heart attack, but do not actively prevent the degradation of the myocardial tissue that is characteristic of a typical infarction. Cardiovascular myocytes are unable to reenter the cell cycle and replicate, meaning that any myocardial tissue death will be replaced with non-contractile scar tissue. The decrease in overall function of the heart tissues leads to overcompensation of the remaining heart muscles. We hypothesize that a patch encapsulating pro-survival factor producing stem cells can be placed directly on the heart surface and can generate enough beneficial growth factors to help prevent the degradation of the cardiac tissue and allow the heart to maintain its pre-infarction function and efficiency.

Background

Hydrogel Patches

Patches were created by polymerizing a PEGDA/MA solution with cells suspended within the mixture. Size, shape, thickness and internal structures can be controlled through X-Y scanning mirrors and a movable platform (Figure 1)



a. Material Design b. Microvascular stamp c. 3D Fabrication by SLA

Figure 1: PEDGA and MA were the base materials used for the hydrogen patches (A). Internal structures can be created within the patches (B) through control of a movable platform and scanning mirrors (C)

Cell Encapsulation

Cells encapsulated using the SLA technique are viable and have been shown to divide. (Figure 2).

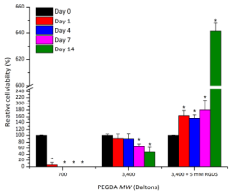


Figure 2: NIH/3T3 fibroblasts were encapsulated in the hydrogels and quantitatively evaluated in 20% PEGDA-700 and -3400 hydrogels with or without RGD peptides

Aims

- Provide an in vivo model to test post-infarction treatments
- Develop methods to transfer hydrogel patches onto living, beating cardiac tissue
- Increase adhesion of hydrogel patches to the heart surface to ensure patches are maintained on the target tissues after chest cavity closure and evacuation

Methods

Surgical Method

- 9-10 wk old female C57BL mice
- Mice are intubated using a 20G angiocath tube
- The skin layer and muscle layers are cut to expose the ribcage
- A thoracotomy is performed to gain access to the heart
- A single suture is placed in the LAD to occlude blood flow to the apex of the heart and create an infarct (Figure 3)
- Treatment patches are transferred to the surface of the heart and monitored briefly for correct placement
- Each layer is sutured closed individually to insure a suitable seal so pressure gradients can be properly reached
- PE-10 tubing is placed just prior to closing in order to remove air from the chest cavity before final close

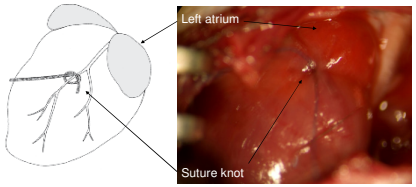
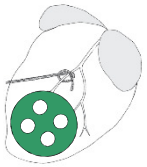


Figure 3: Suture placement in the LAD, just below and the the right of the left atrium

Patch Preparation

- Patches are prepared by the SLA method, as previously described
- An additional adhesion layer is created just prior to surgery by:
  1) Adsorption of chitosan (5.0 mg/ml) for 2h
  2) Dipping in 200 mM FeCL3 for 5s
  3) Dipping in 10 wt % HA-DA or PVA-DA for 5s
- Patches are then transferred to the target area of the heart



Results

Patch Placement

Initial adhesion tests are shown in Figure 4. Hydrogel to heart surface transfer protocols were developed using mouse cadavers. Thoracotomy access was checked for accessibility of the apex of the heart and the feasibility of applying the patch within the space constraints of the chest cavity. Hydrostatic forces were enough to hold the patch to the heart surface, but still allowed movement along the surface for final placement (Fig. 4A). Next, the patch was placed on the beating heart of a live mouse and again hydrostatic forces were enough to maintain contact with the surface even with an average mouse heartbeat of 500-600 bpm (Fig. 4B). After chest closure and evacuation animals were kept on the ventilator for 10 minutes before euthanasia and reopening the chest. Hydrostatic forces were not sufficient to retain patch on the heart surface.

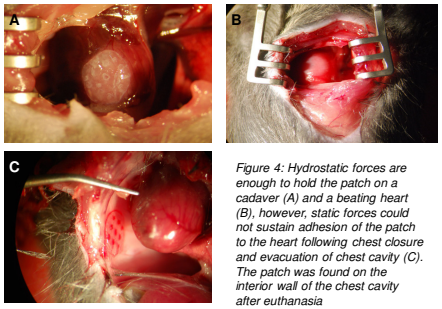


Figure 4: Hydrostatic forces are enough to hold the patch on a cadaver (A) and a beating heart (B), however, static forces could not sustain adhesion of the patch to the heart following chest closure and evacuation of chest cavity (C). The patch was found on the interior wall of the chest cavity after euthanasia

Increasing Patch Adhesion

An additional adhesive component was incorporated into the hydrogel patches to increase patch retention on the heart. Patches were prepared by soaking in a polysaccharide solution. Just prior to transferring into the animal, the patch was dipped serially into a solution of FeCl3 followed by a solution of anionic polysaccharides as described in the "Methods" section. As an additional measure, the thickness of the patch was decreased to allow for better conformation to the heart surface. The patch remained on the heart surface post suturing and evacuation of the chest cavity (Figure 5).

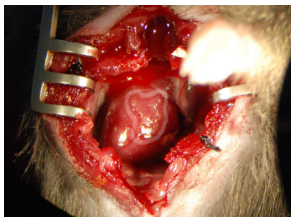


Figure 5: Thin patches prepared with an additional "adhesive" layer remains on the heart surface after chest closure and subsequent euthanasia.

Conclusions and Future Directions

Cell retention at the site of damaged or compromised myocardium remains a problem for stem cell therapies following myocardial infarction or for chronic ischemia. Engineered hydrogels show great promise as a delivery vehicle for stem cells due to the high level of control over mechanical properties and ease of chemical modification. This level of control allows for the optimization of hydrogels for maintaining stem cell viability in the harsh post-infarction, reperfusion environment. One difficulty has been the ability to apply and retain these patches on living tissues. Using a polysaccharide-based "adhesive" we have shown that hydrogels can be adhered to living tissues. This technique avoids suturing directly to the heart tissue which can cause scarring. Future constructs will incorporate adhesive elements into cardiac patches using SLA technology (Fig. 6).

Figure 6: Adhesive layers can be incorporated into hydrogel patches during fabrication as a think layer at one surface of the patch or incorporated as a regular pattern throughout the patch.

Future directions of this work will include the measurement of heart function via echocardiography and PET/SPECT with and without heart patches following myocardial infarction. Advanced cardiac patches will take advantage of the powerful SLA technique to incorporate multiple cell types in patch sections uniquely designed to either support extended survival in the hydrogel environment or designed to release cells at the heart surface using biodegradable hydrogels.

Acknowledgments

- This work was funded by US Army Grant (W81XWH-08-1-0701) and a Fellowship from Carle Foundation Hospital.
- Special thanks to Prof. Dave Geenen, Assistant Professor of Physiology in Medicine, Department of Medicine, Section of Cardiology, for guidance and training in establishing mouse infarct model

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