Can Losartan controlled release improve the bio-hybrid cardiac patch in preventing left ventricle pathological remodeling?

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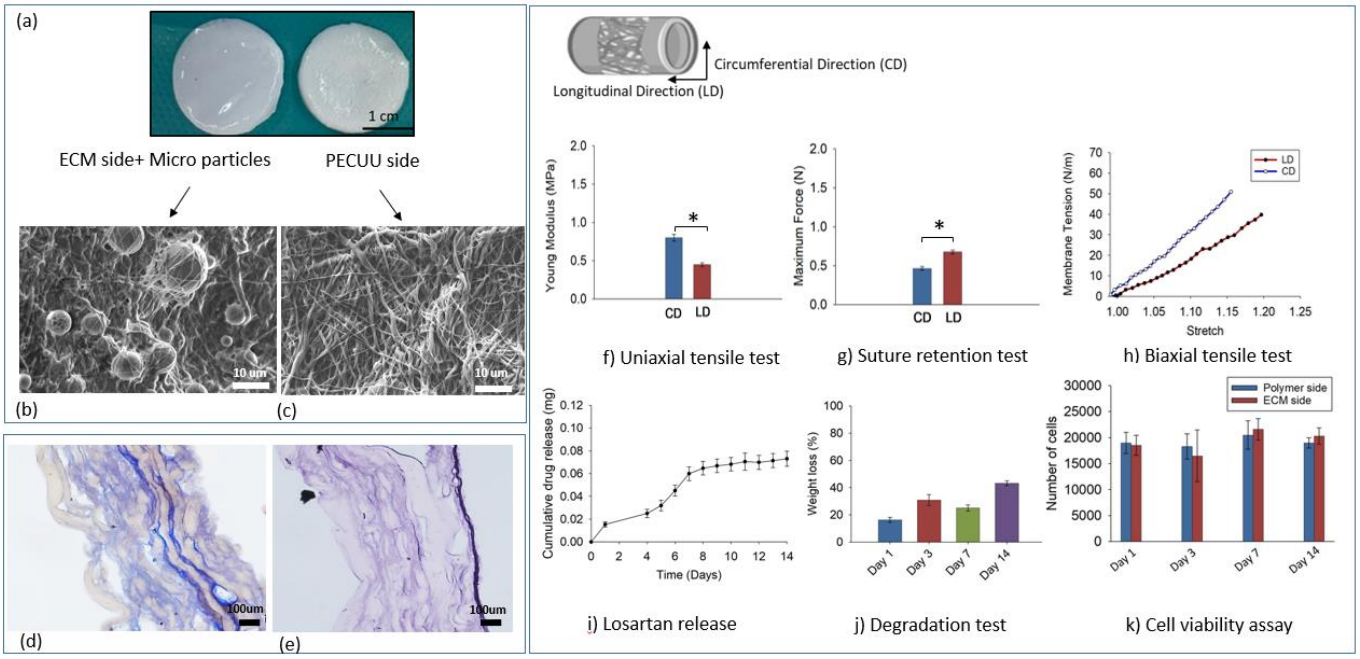

Figure 1. Patch in-vitro characterization: a) Gross examination of the bio-hybrid cardiac patch with the ECM rich side shown on the left and the polymer side shown on the right; b) SEM images of the ECM layer augmented with drug-loaded microparticles; and c) of the polymer layer. Histology of the patch d) Masson trichrome (MT) and e) Hematoxylin and Eosin (H&E). Biomechanical tests were performed in both circumferential and longitudinal directions including:(f) uniaxial tensile test, (g) suture retention, and (h) biaxial tensile test under equi-strain conditions. (i) Losartan release, (j) Accelerated hydrolytic degradation test, and (k) cell (murine myoblasts C2C12,) viability assays were conducted on both sides of the scaffold

Keywords: Biohybrid cardiac patch, cardiac tissue derived extracellular matrix, drug loaded microparticle,

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Objective

Cardiac patches (CP) are a promising alternative to prevent pathological remodeling initiated by a heart attack. In previous studies we demonstrated the efficacy of the biohybrid approach on rat chronic infarction model at 8 and 16 weeks from the injury. This study aims to further enhance the efficacy of the bio-hybrid approach by the controlled release of Losartan. The abstract presents the full in-vitro characterization of the scaffold that will be evaluated on a rat chronic infarction model.

Methods

The bio-hybrid CP was fabricated using double-stream electrospinning of Poly (ester-carbonate urethane) urea (PECUU) and cardiac extracellular-matrix (ECM) gel obtained from decellularized porcine hearts. Poly (D, L-lactide-co-glycolide) (PLGA) microparticles (MPs) loaded with drugs were embedded in the ECM-gel. Scanning electron

Microscopy and digital image analysis was adopted to characterize the patch microstructure (e.g. fiber orientation, diameter and intersection density). Cell vitality and proliferation were evaluated with Alamar Blue for 14 days and histological staining (Masson's Trichrome, Hematoxylin & Eosin) performed at pre-set intervals. Patch topology, Young's Modulus, and suture retention were measured via a dial indicator gauge - biquintic interpolation and uniaxial tensile testing (UTT), respectively. Biomaterial anisotropy was evaluated via biaxial tensile testing (BTT). Accelerated hydrolytic degradation tests were performed for 14 days. Losartan release was assessed over a 14-day period using a Franz Cell. C2C12 cell culture was adopted to assess cell infiltration.

Results

The custom-algorithm identified fiber orientation index of 0.65 and 0.77 (0.5 pure isotropy, 1 pure anisotropy) for the ECM-side and polymer-side, respectively, indicating a predominant fiber alignment of the polymer side towards a preferential direction. As rat native LV anisotropy dictates an OI of 0.8, the patch processing allowed to achieve physiologically relevant values of structural anisotropy. This was also confirmed by BTT and by UTT which demonstrated that the patch circumferential direction was stiffer (1.34MPa) than the longitudinal (0.89MPa). Process stability in terms of thickness homogeneity with average value of 390 μm was confirmed by the limited STD of 7.39 μm. Cell viability assays and H&E staining demonstrated that the patch is non-toxic and supports cell-growth and infiltration.

Conclusions

Results demonstrate our capacity to couple the biohybrid approach (polymer rich/control on mechanics, ECM rich/bioactivity) with physiologically relevant anisotropy and with the controlled release of Losartan. Future in-vivo evaluation on rat coronary ligation model will assess if and how controlled release can synergistically enhance cardiac patch efficacy in preventing pathological remodeling.

Cardiac patches (CP) are a promising alternative to prevent pathological remodeling following heart attacks. This study builds on previous research demonstrating the biohybrid approach's effectiveness in a rat chronic infarction model at 8 and 16-weeks post-injury, aiming to enhance this efficacy through the controlled release of Losartan.

Methods:
The bio-hybrid CP was created using double-stream-electrospinning, combining Poly(ester-carbonate-urethane) urea (PECUU) with cardiac extracellular matrix (ECM) gel derived from decellularized porcine hearts. Drug-loaded Poly (D, L-lactide-co-glycolide) (PLGA) microparticles were incorporated into the ECM-gel. The patch's microstructure was characterized using scanning-electron-microscopy and digital image analysis, focusing on fiber orientation, diameter, and intersection density. Cell viability and proliferation were assessed using Alamar-Blue over 14 days, along with histological staining at predetermined intervals. Patch topology, Young's Modulus, and suture retention were measured via a dial indicator gauge-biquintic interpolation and uniaxial-tensile-testing (UTT), respectively. Biomaterial anisotropy was evaluated via biaxial-tensile-testing (BTT). hydrolytic degradation and Losartan release were evaluated over 14 days. C2C12 cell culture was adopted to assess cell infiltration.

Results:
The study utilized a custom algorithm to identify fiber orientation index of 0.65 for the ECM-side and 0.77 for the polymer-side, indicating a significant alignment in the polymer. The patch's anisotropy was relevant to physiological conditions, with mechanical testing showing the circumferential direction was stiffer (1.34 MPa) compared to the longitudinal (0.89 MPa). The patch's average thickness was 390±7.39 μm. Cell viability assays and histological evaluations confirmed the patch's biocompatibility, promoting cell growth and infiltration.

conclusions:
This research successfully integrates a biohybrid approach that combines mechanical control with bioactivity, achieving physiologically relevant anisotropy and enabling the controlled release of Losartan. Future studies will investigate the in-vivo performance of this cardiac patch in a rat coronary ligation model to determine the synergistic effects of Losartan on preventing pathological remodeling post-infarction.