Development of three-dimensional myocardium as an alternative to heart transplant

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Purpose

Limited capacity of heart tissue to regenerate itself is one of the main reasons why myocardial infarction (MI) becomes a progressively debilitating disease that cannot be stopped by pharmacotherapy (1-3). The goal of myocardial tissue engineering (MTE) is to provide a microstructure mimicking the microenvironment of the myocardium by replacing the infarcted area (4). We designed 3D-printed scaffolds to mimic the architecture of the myocardium, providing a more desirable microenvironment for the cells to regenerate the myocardial tissue and induce vascularization for a better integration with the native myocardium.

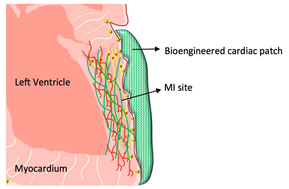

***Figure 1**: Bioengineered patches implanted on ischemic tissue after myocardial infarction: The implanted patches can regenerate myocardium after MI.*

Objectives

1. To optimize the concentration and ratio of two natural polymer combinations as the main matrix (scaffold) based on cell viability and printability studies.
2. To design the scaffolds mimicking the architecture of the myocardium (Figure 2).
3. To fabricate the designed scaffolds using three-dimensional (3D) printing technique.

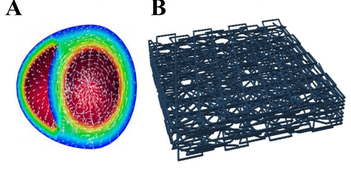

***Figure 2**: The orientation of fibers in native myocardium (A) vs the designed scaffolds based on the orientation of the fibers in native myocardium (B). In the native heart, the orientation of the fibers in epicardial and right ventricular surfaces is -70 degrees, and it gradually changes until it reaches +80 degrees on the endocardial surfaces (right). In our design, the first layer of the fibers are printed with -70 degrees orientation and every layer the orientation of the fibers increases with +10 degrees. As a result, after printing 15 layer the orientation of the last layer of fibers are +80 degrees.*

Methods

Two natural polymers, alginate and gelatin, were selected as components of 3D-printed scaffolds. Various concentrations of the polymer solutions (1-3% w/w) were prepared. The viability of cardiomyocytes and endothelial cells was studied through MTT assay (Figure 3) and Live/Dead assay (Figure 4). Two polymer combinations, (Alginate2%Gelatin 1%) and (Alginate 3%Gelatin1%), showing the highest cell viability for both cell lines, were selected.

3D-printing parameters were optimized for the selected polymer combinations. 3D printing was performed with nine different printing parameters including the distance between each two adjacent fibers and the diameter of each fiber (Figure 5) . Two different parameters were selected for each polymer combination. The scaffolds were printed with a specific layout, mimicking myocardium architecture (Experimental structure). A lattice structure (a simple structure with layers orientated with 0 and 90 degrees) was designed and printed as a control for structure. The scaffolds were imaged using scanning electron microscope (Figure 6).

Results

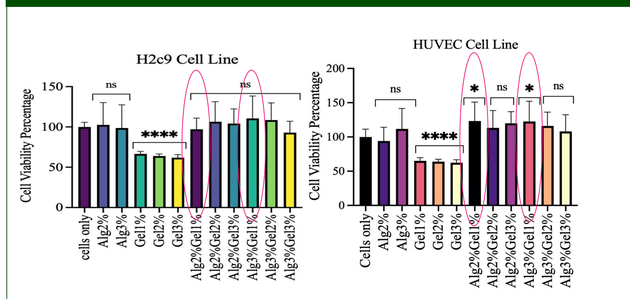

***Figure 3**:Cell viability of endothelial cell line (HUVEC: Human Umbilical Vein Endothelial Cells) and cardiac cell line (H9c2) after 48 hrs. exposure to different combinations of the two polymers using MTT assay.*. Al2%Gel1% (p=0.0193) and Alg3%Gel1% (p=0.0247) samples showed statistically higher cell viability compared to controls. The rest of the combinations of the two polymers did not show any statistical difference compared to controls (right). Alg2%Gel1% and Alg3%Gel1% were chosen for further studies.

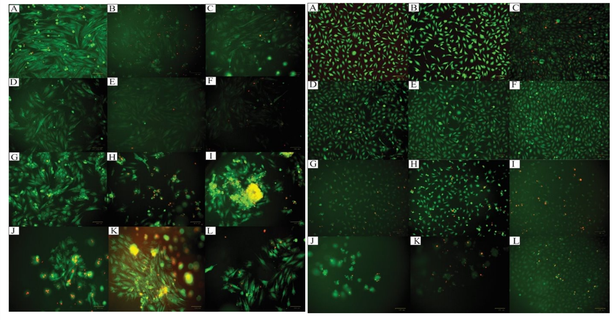

***Figure 4:** Cell viability of HUVEC cell line (right) and H9c2 cell line (left) after 48 hrs. exposure to different combinations of the two polymers using Live/Dead assay. A) Cells only, B) Alginate 2%, C) Alginate 3%, D) Gelatin 1%, E) Gelatin 2%, F) Gelatin 3%, G) Alg2%Gel1%, H) Alg2%Gel2%, I) Alg2%Gel3%, J) Alg3%Gel1%, K) Alg3%Gel2%, L) Alg3%Gel3%*

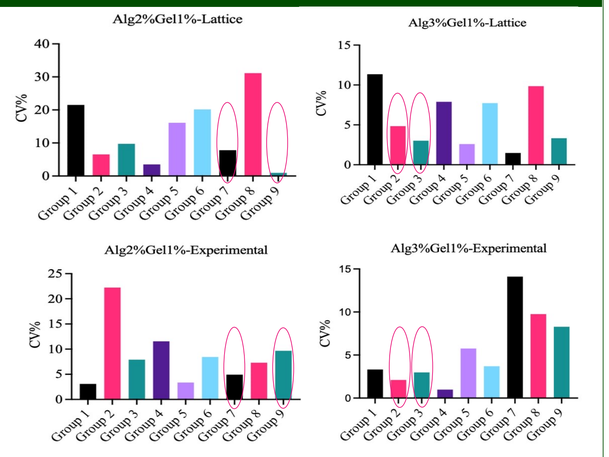

***Figure 5:** Coefficient of variations of the 3D-printed fibers for both polymer combinations and structures indicates printing parameters resulted in more fibers in the same range of the diameters. The samples with lower CV% were selected. The samples with CV<5% for Alg3%Gel1% (group2 and group3) and the samples with CV<10% for Alg2%Gel1% (group 7 and group 9) were chosen For further studies.*

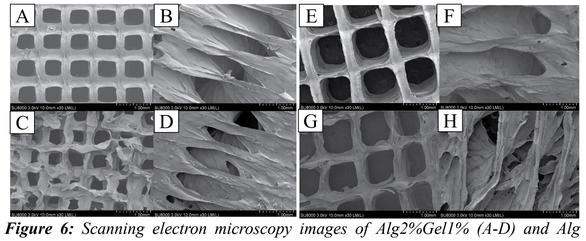

***Figure 6:** Scanning electron microscopy images of Alg2%Gel1% (A-D) and Alg 3%Gel1% (E-H). A) Alg2%Gel1%-group7, B) Alg2%Gel1%-group7, C) Alg2%Gel1%-group9, D) Alg2%Gel1%-group9, E) Alg3%Gel1%-group 2, F) Alg3%Gel1%-group 2, G) Alg3%Gel1%-group 3, H) Alg3%Gel1%-group 3. Scaffolds C and D (group 9) were eliminated for further studies due to lack of fidelity.*

Conclusion and future perspectives

Two combinations of the polymer with highest viability of both endothelial and cardiac cells were selected and following optimization of 3D-printing parameters, the scaffolds mimicking the microstructure of myocardium along with a lattice structure were 3D-printed.

Next step, the cell-laden polymer combinations will be printed to optimize the constructs for neo-vascularization.

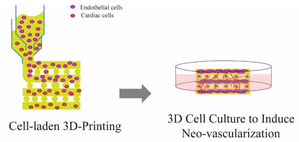

***Figure 7**: Promoting neo-vascularization*

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