Qatar Foundation Annual Research Conference 2016 (ARC'16) 22nd-23rd March 2016,
Qatar National Convention Centre, Doha, Qatar

Valve tissue engineering with living absorbable threads

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Abstract: *One of the fundamental aspects of tissue engineering (TE) is the population of three-dimensional (3D) scaffolds with appropriate cells that recapitulate the physiological situation. This is particularly valid for heart valve engineering as this tissue contains different cell types arranged in distinct regions. Therefore, a technique was developed that utilises a 44 filament PCL yarn to create "living threads" based on thin biodegradable PCL fibers of different diameters (23 -243 um), as a first step to provide specific spatial organisation of cells in a tissue engineered valve. These versatile fibers can be used to produce scaffolds of 3D shapes identical to the cup-like structure of a normal human valve while preserving the particular orientation of both the cells and the fibers (see **Figure 1. a-d**). We aimed to assess their ability to bind to stem cells and to measure the mechanical strength of the fibres.*

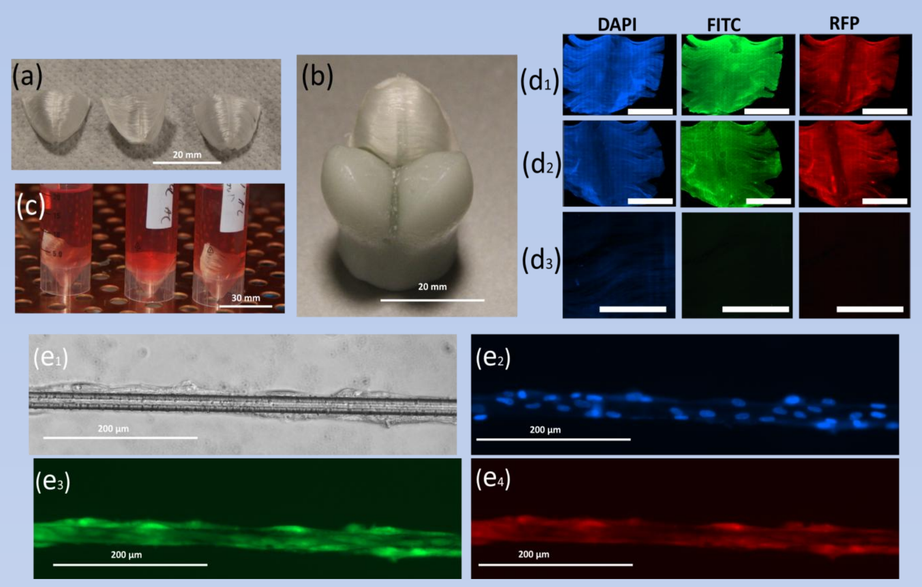

Figure 1. Preparation of leaflet shaped scaffold. Scaffolds made out of circumferentially aligned PCL thread (220Dtex, 44f), free standing (a), and on the mold (b) after culturing the cells (c). MSCs on leaflets-shaped scaffolds were visualized using DAPI, FITC staining and in RFP channel (d1 and d2). Control - aligned PCL yarns with no cells (d3). Scale bars are1 cm. MSCs on a single filament of PCL yarn (e). Cells were visualized as described in (d).

Methods

The 44 filaments (f), 220 dtex, 177 µm in diameter thread was obtained from EMS-GRILTECH (Domat, Switzerland). The PCL yarns were produced by a melt-spinning process. The polymer resins were melted in an extruder at specific temperatures and pumped to a spin pack. The exact melt throughput was controlled by a gear pump with an accurately defined volume. In the spin pack, the melt was distributed to the spinneret, where every capillary gets the same amount of melt to assure a proper diameter uniformity of each filament. After extruding from the spinneret, the liquid filaments solidify by quenching with cold air. Then, the threads were stretched on godet rolls to their final size/diameter and wound up on a winder to bobbins for further processing. The other threads; consisting 1, 3, and 22 filaments were obtained by manually splitting the yarn into the desired count of filaments. Prior to use, the threads were washed in isopropanol for 20 min and dried.

Human mesenchymal stem cells (MSCs) /PCL yarn interaction was assessed by culturing cells on different types of yarns over 4 days (see **Figure 1.e**). The viability of cells on the threads and leaflets was confirmed by staining them using CellTracker™ Green CMFDA fluorescent dye (see **Figure 1.e$_3$**).
To determine the stiffness of the materials, the single thread of PCL yarn was tested in a Bose Electroforce uniaxial setting. The thread was cut into 4 cm pieces and was tested in dry and wet conditions (phosphate buffer saline – PBS). For wet conditions, the samples were prepared as described above (washed in isopropanol for 10 min, then washed twice in PBS for 10 min each and then kept overnight in PBS before the test was carried out). The tests were conducted in a basin of PBS. All tensile tests were done at a rate of 0.1 mm/s. Mechanical properties of yarns cultured with MSCs for 8 days were as well tested in wet conditions.

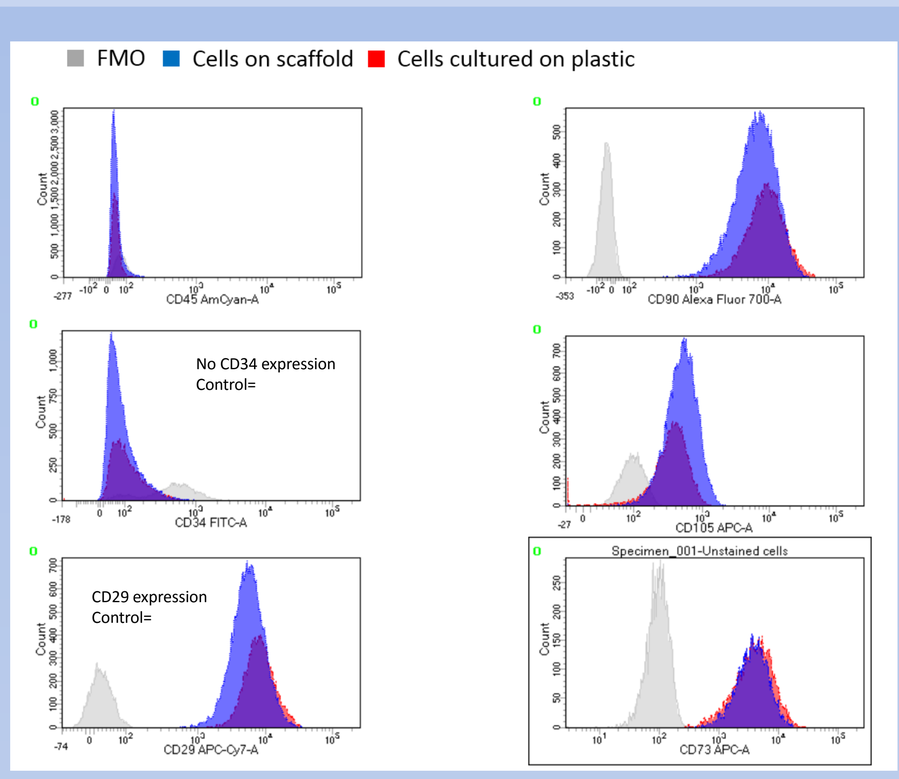

Figure 2. Comparison between cells cultured on the scaffold and plastic surface using the FMO (Fluorescence Minus One) control method.

Table 1. Results of FMO control method with comparison with standard sample.

antibody/cell	MSCs (standard)*	MSCs Scaffold	MSCs Plastic
CD105	✓	✓	✓
CD90	✓	✓	✓
CD73	✓	✓	✓
CD29	✓	✓	✓
CD45	X	X	X
CD34	X	X	X
Adherent on plastic	✓	✓**	✓

* According to Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy.
**After harvesting and seeding on TCP.

Results

The fibers readily attracted human MSCs which were firmly adhered (see **Figures 1, 2** and **Table 1**). It appeared that cell/yarn interaction was mediated through clumping of cells on the filaments. We confirmed that high concentration of FBS did not trigger unspecific cellular attachment as PCL threads were not colonized by cells in this condition. By evaluating nuclei alignment, we found that cells spontaneously oriented parallel to PCL fibers which is a favorable outcome, as it potentially enables a patterned deposition of ECM proteins in the cells and consequently might further improve the resulting leaflet mechanical anisotropy. Importantly, cells were able to bridge the space in-between fibers thus non-leaking constructs may be obtained.
Stiffness of 44-filament-PCL-yarn under dry conditions, wet (saline) conditions and wet conditions after 8-day cell seeding was of 359.5 ± 14.8, 372.6 ± 12.4, 348.2 ± 18.0 MPa, respectively.

Conclusion

PCL fibers shaped as yarns were used to produce dimensional shapes identical in shape to the cusp-like structure of a normal human valve.
Mechanical properties revealed that the yarns stiffness did not significantly change when cultured with cells over the period of 8 days. Yarns arranged in the shape of leaflets preserved the particular orientation of both the cells and the fibers. This has important functional implications as living absorbable fibers could be a valuable resource in tissue engineering of heart valves.

Acknowledgements

This research was funded by Sidra Medical and Research Center, Al Dafna, Doha, Qatar.