Collagen Scaffolds Reinforcement Through Cells remodeling For Vascular Tissue Engineering

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Introduction:

The replacement of small-caliber blood vessels (diameter < 6 mm) remains a major challenge for cardiovascular surgeons when there is a lack of functional autologous secondary blood vessel for replacing the diseased one. Today, synthetic polymers are often used as small blood vessels substitutes but still more than 50% of them fail due to plaque deposition and internal hyperplasia within 5 years of implantation (1). Inadequate mechanical and biological properties are mainly responsible for this clinical complication. In this context, vascular tissue engineering (VTE) holds a promise to create functional blood vessels *in vitro* with high clinical performances. Type I collagen offers an appropriate environment for the growth of cells in terms of biological concerns as it is the main component of the extracellular matrix. However, the low mechanical properties of collagen scaffolds hinder the VTE strategy and impede further culture under mechanical stimulation in a bioreactor (2). Hence, a prior step aiming to improve the mechanical properties of the scaffold is mandatory before culturing in a bioreactor. This is generally achieved using physical or chemical agents that can affect the integrity of the material. In this context, letting vascular cells naturally remodel the scaffold into a reorganized material prior to dynamic culture in bioreactor might be a suitable step.

This work aims to investigate how the mechanical properties of collagen gel scaffolds can be naturally enhanced through remodeling induced by cells during static culture.

Materials and methods

Collagen tubes preparation. Type I collagen was extracted from rat-tail tendons and solubilized in acetic acid solution (0.02 N) at a concentration of 4 g/L according to a protocol previously described (3). Smooth muscle cells (SMC) were extracted from the aorta of a young pig. The collagen solution (2 g/L) was mixed with NaOH (15 mM), and Hepes (20 mM) in deionized water. DMEM (1.1X), which eventually contained SMC (10^6 cells/mL), was added to the last solution. This mixture was then poured in a tubular mould and let jelify for 30 min at room temperature. Static culture was achieved by immersing the collagen tube with its mandrel into culture medium contained 50 mL centrifuge tube specifically modified with gas exchange filter.

Collagen thickness measurements. The thickness of the constructs was obtained through laser-guided measurements using a scanning laser interferometer (Series 183B, LaserMike 136). For the first 12 hours and then every day of static culture, the collagen tubes were removed from the 50 mL centrifuge tube in sterile conditions and placed in front of the laser beam so as to measure the constructs external diameter.

Mechanical properties measurements. Stepwise stress relaxation tests were performed on the arterial constructs after cell remodeling. Collagen tubes were cut in rings and attached to the grips of an Instron 5848 Microtester (Instron Corporation, Norwood, MA, USA). Samples were stretched to

10% at a rate of 5%/s. The relaxation of the stress was then monitored for 300s. Afterward, the stress relaxation was measured every strain of 10% till failure.

Results:

Laser-guided thickness measurements showed a very fast contraction of the gel by SMC (Figure 1). This collagen remodeling resulted in great differences in the mechanical and viscoelastic behaviour of the gels as shown in Figure 2.

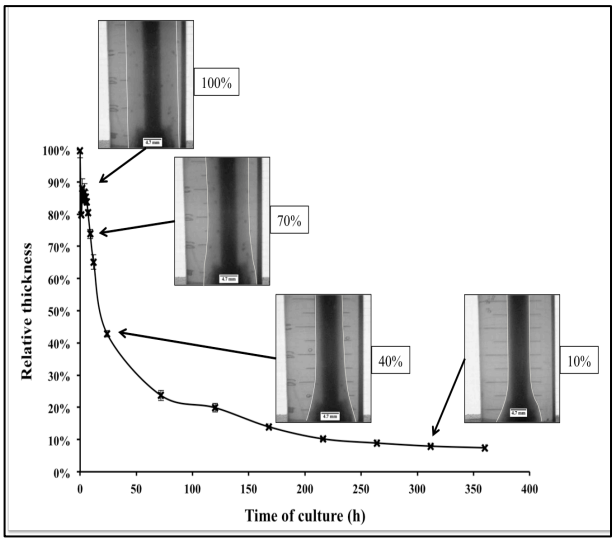

Figure 1: Contraction of collagen tubes seeded with SMC as a function of the time of culture (n=3). Pictures show the contraction of an arterial construct, which is around a mandrel and immersed in a 50 mL centrifuge tube containing medium culture.

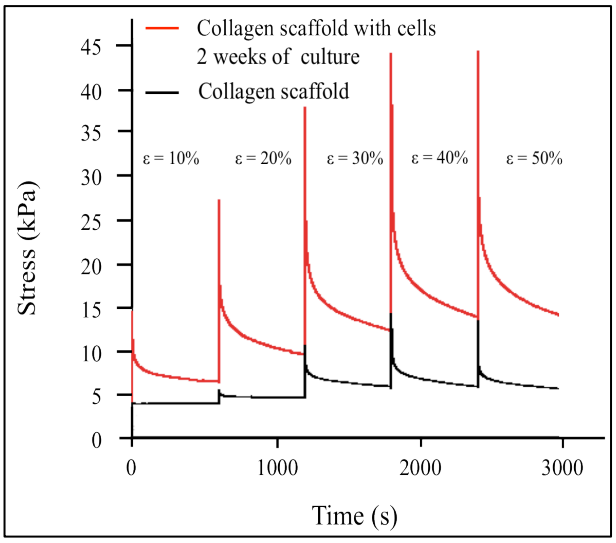

Figure 2: Stepwise stress relaxation tests on collagen gel scaffolds and collagen gels seeded with SMC after 2 weeks of culture. ε represents the strain at which the stress relaxation test has been performed.

Discussion:

The initial thickness of the samples was approximately 5 mm. The constructs contracted for more than 60% in less than 24h (Figure 1). After 2 weeks of static culture, the thickness of the samples drastically decreased and reached around 400 µm, which attests to the important remodeling of the collagen scaffold by the SMCs. The remodeling of the scaffold by the SMC strongly affected the mechanical properties of the arterial construct (Figure 2). For each strain, the maximum load supported by the arterial construct cultured for 2 weeks is many times higher than the one of the scaffold, especially at low strains. The remodeling of the scaffold by the SMC has a great influence on the viscoelastic response of the material since the time for reaching the stress at equilibrium is higher for the sample containing SMC.

Culturing statically SMC on tubular collagen gel scaffolds leads to a material with enhanced mechanical properties that allow further maturation with appropriate mechanical stimulation in a bioreactor. This presentation will shed light on the close link existing between the mechanical improvement and the change in the organization of the cells and the collagen fibrils occurring during remodeling.

References:

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3. Rajan N et al. Nature Protocols. 2006;1(6):2753–8.