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Fabrication of cardiac patch by using electrospun collagen fibers

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ABSTRACT

Synergy between micro-nanotechnology and regenerative medicine can lead to new tools for health improvement. In this study, we investigate the efficacy of electrospun scaffolds – fabricated using clinically approved collagen – as supports for cardiomyoblast culture. The scaffolds were prepared using non-toxic solvents and crosslinking agents and characterized by scanning electron microscopy and contact angle measurements. Among different types of collagen samples, we found that atelocollagen can produce better quality of electrospun fibers than acid and basic fibrous collagen. Our results also show that the cell culture performance can be improved by adjusting the crosslinking conditions. Typically, increasing the concentration of citric acid of the cross-link agents from 5% to 10% w/w and the post-crosslink baking time from 1.5 to 2.5 h led to significant increases of the cellular colonization of the scaffold, showing three-dimensional growth of cardiac cells due to the specific morphology of the fibrous scaffolds. Finally, in vivo tests of the biocompatibility of the fabricated scaffolds have been done using a mouse model of dilated cardiomyopathy. As expected, the biocompatibility of the scaffold was found excellent and no visible inflammation was observed after the implantation up to two weeks. However, 5% citric acid electrospun collagen scaffolds was less resistant in vivo, proving again the importance of the processing parameter optimization of the electrospun scaffolds.

1. Introduction

Engineered scaffolds have been widely used as structural and functional supports on which cells are seeded for the generation of cell therapy products. In the field of cardiac therapy, this approach is challenging but holds a real promise for improving function of the chronically failing myocardium [1]. Previously, a large number of investigations have been devoted to the culture of cardiac cells using scaffolds made of synthetic polymers. In particular, nanofibrous scaffolds obtained by electrospinning of poly-L-lactic acid (PLLA) and poly-(caprolactone) (PCL) could be produced for the formation of functional cardiac cell layers [2]. However, because of the lack of cell affinity these synthetic polymers are inherent less attractive than natural polymers – such as collagen, fibrinogen, elastin – for in vivo applications [3]. Among them, collagen constitutes one of the main proteins of the extracellular matrix, and this allows for a close simulation of the natural fibrillar structure of cardiac tissue [4]. Accordingly, it is interesting to consider electrospun collagen scaffolds as supports and carriers of cardiac cells that can be implanted for repair of the failing myocardium [5].

Different types of clinically approved collagen are now available but the ability of making fibrous scaffolds out of them has yet to be demonstrated. One of the issues is the crosslinking process, which uses chemical reagents to render the fibrous structures stable in the culture medium and after implantation. The most common crosslinkers for collagen fibers are glutaraldehyde – well known for its efficiency but somehow toxic – and combinations of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) – a system which is less

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toxic but often causes a film-like morphology after the immersion of the fibers in water [6] [7]. Alternatively, a citric acid (CA) based system has been recently applied and shown the requested biocompatibility [8].

In this work, we study the electrospinning parameters of clinically approved collagen in order to produce optimal nanofiber scaffolds for three-dimensional culture of cardiac cells. In particular, we were interested in creating a patch form of scaffold that can be used for therapy purposes. Our results show that the colonization of cardiac cells is critically dependent on the crosslink parameters and that our collagen scaffolds are fully compatible with the implantation requirements, thereby providing a way towards a scaffold based cardiac cell therapy.

2. Materials and methods

2.1. Collagen solutions preparation and electrospinning

Different types of clinically approved collagen, including atelocollagen, acid fibrous, basic fibrous were provided by Biom'Up (Saint-Priest, France) in form of dry material after lyophilization. For electrospinning, the raw collagen materials were dissolved using a solvent system containing ethanol, water and a variety of salts [7]. Specifically, the buffer solution is composed of the following salts dissolved in deionized water: potassium chloride, sodium chloride, potassium phosphate monobasic, sodium phosphate dibasic heptahydrate. The salts concentration is 20× in the collagen buffer solution. The final solvent system consists of buffer solution and ethanol in a ratio 1:1 v/v with a collagen concentration of 16% w/v.

For the crosslinking of the collagen scaffolds a system containing CA, glycerol as extending agent and sodium hypophosphite (SHP) as catalyst was used [8]. The three crosslinking components were added to the collagen solution before the electrospinning. Different concentrations of CA and SHP were studied: 5% CA with 2.1% SHP and 10% CA with 4.2% SHP, keeping constant the glycerol amount to 3% (weight percentage based on the weight of collagen).

In electrospinning, the solution is fed through a thin needle opposite to a collecting plate and a high voltage is applied to form a jet of the solution that travels from the needle to the grounded collecting plate (Fig. 1). In our study, the collagen solution was loaded to a syringe with a 23 gauge blunt needle and the flow rate was controlled via a pump at 1 ml/h. By applying voltage of 13.5 kV and keeping a distance of 5 cm between the needle tip and the grounded collector, fibers were deposited to the latter at room temperature (20 ± 2 °C). After 8 min of continuous electrospinning, collagen scaffolds were obtained that could be easily peeled off from the collector (Fig. 2).

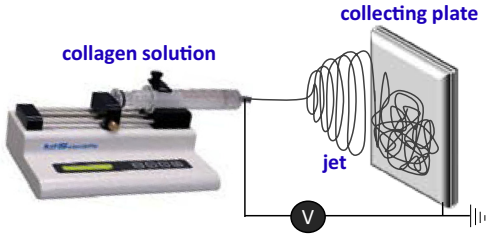

Fig. 1. Schematic representation of the electrospinning apparatus.

2.2. Post-electrospinning treatment

After electrospinning, a crosslinking post-treatment was applied in order to make the scaffolds insoluble in cell culture medium and facilitate their handling. The carboxyl groups of CA reacted with the additional hydroxyl groups introduced by glycerol in order to form covalent bridges between the collagen fibers. The scaffolds were baked at 150 °C for three different periods (1.5 h, 2 h, 2.5 h).

The crosslinked scaffolds were mounted onto handing devices for biologic assays. Cell crowns® holders were utilised for this purpose and then placed in 24-well plates for cell culture.

2.3. Characterization methods

The structure of the electrospun collagen scaffolds was studied after the steps of electrospinning and crosslinking treatment using scanning electron microscopy (SEM) and contact angle measurements.

The SEM measurements were performed using the e-LiNE (Raith). A thin gold layer of 5 nm was deposited prior to measurements and images at different magnifications were obtained on at least three different areas in the same sample. The measurements were performed under extra high tension of 10 kV.

The contact angle measuring instrument was the Drop Shape Analyzer – DSA 30 (Krüss). The sessile drop method was utilized and the measurements took place at room temperature (20 °C). The volume of the applied droplets of deionized water was 1.5 μl. The contact angle data were obtained by averaging over five measurements in different areas on the sample surface.

2.4. Cardiomyoblasts culture

The H9c2 cell line derived from embryonic rat heart tissue was used in this study. Cells were cultured in high glucose DMEM + Glutamax supplemented with 10% foetal bovine serum and 1% penicillin/streptomycin. They were seeded on the collagen scaffold after passage using trypsin-EDTA. After two days of culture, scaffolds were washed with PBS and fixed with 1% paraformaldehyde solution. After 2 washing steps, they were incubated with 5% bovine serum albumin (BSA) during 1 hour. Then Alexa Fluor® 488 Phalloidin (Life technologies) was used to stain

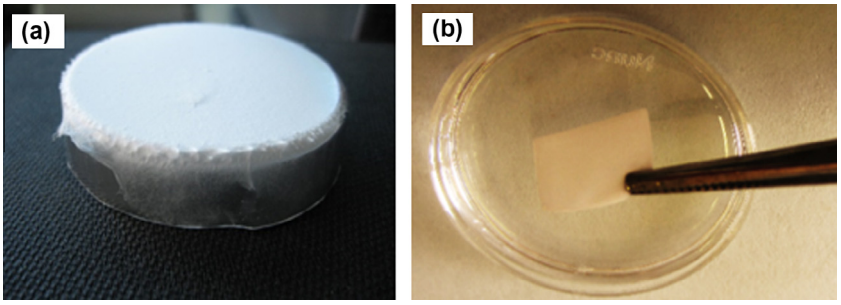

Fig. 2. Images of the atelocollagen fibrous scaffold (a) deposited onto aluminum collector, (b) after the peeling-off.