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Compressed collagen constructs with optimized mechanical properties and cell interactions for tissue engineering applications

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ABSTRACT

In this study, we are introducing a simple, fast and reliable add-in to the technique of plastic compression to obtain collagen sheets with decreased fibrillar densities, representing improved cell-interactions and mechanical properties. Collagen hydrogels with different initial concentrations (1.64 mg/mL–0.41 mg/mL) were compressed around an electrospun sheet of PLGA. The scaffolds were then studied as non-seeded, or seeded with 3T3 fibroblast cells and cultured for 7 days. Confocal microscopy and TEM imaging of non-seeded scaffolds showed that by decreasing the share of collagen in the hydrogel formula, collagen sheets with similar thickness but lower fibrous densities were achieved. Nanomechanical characterization of compressed collagen sheets by AFM showed that Young's modulus was inversely proportional to the final concentration of collagen. Similarly, according to SEM, MTS, and cell nuclei counting, all the scaffolds supported cell adhesion and proliferation, whilst the highest metabolic activities and proliferation were seen in the scaffolds with lowest collagen content in hydrogel formula. We conclude that by decreasing the collagen content in the formula of collagen hydrogel for plastic compression, not only a better cell environment and optimum mechanical properties are achieved, but also the application costs of this biopolymer is reduced.

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1. Introduction

Biocompatible scaffold systems as cell delivery vehicles play an important role in tissue repair and regeneration. Among various forms of scaffold materials, naturally derived polymers possess outstanding characteristics (i.e. biocompatibility, low immunogenicity, capability of being naturally remodeled by cells) for tissue regeneration applications [1]. Collagen is a natural polymer with extensive utilization in regenerative medicine. Collagen type I is the most commonly used collagen due to its abundance, ubiquity, and biocompatibility [2,3]. Hydrogel scaffolds of collagen, although very conventional and capable of rapid introduction of cells, suffer from low mechanical strength. It (the low mechanical strength) originates from low protein to water ratio [4], leading to smaller collagen fibril density in collagen hydrogels compared to their natural equivalent [5]. Moreover, collagen hydrogels (or hyper-hydrated gels) are unstable materials, as they undergo self-compression under their own weight as soon as transferring a freshly cast hydrogel to another surface (glass surface or a blotting set) causing the liquid to expel out of the hydrogel [6]. To increase fibrillar density of collagen-based scaffolds and/or improve their mechanical properties, researchers have performed different studies including 3-D microfabrication methods in collagen matrices [7,8], crosslinking [9], or making high concentration collagen scaffolds of around 10–45 mg/mL via ultracentrifugation [10] or evaporation methods [11]. While these methods are either complicated (3-D microfabrication methods) or can be toxic to cells (crosslinking procedures), the technique of plastic compression of collagen hydrogels [6] has attracted a great deal of attention for tissue engineering studies [4,5,12–18]. In this procedure, which was introduced by Brown et al. in 2005 [6], excess water of the collagen hydrogel is removed via mechanical compression, leading to a denser and stronger construct compared to conventional gels. This technique has been applied in different fields of tissue engineering and regenerative

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Table 1
Formulations to prepare different collagen hydrogels for plastic compression.

Collagen hydrogel formula	Volumetric percentage (v/v%)		
	Collagen	MEM 10X	Alfa-MEM
C80 (1.64 mg/mL collagen in final hydrogel)	80	10	10
C60 (1.23 mg/mL collagen in final hydrogel)	60	7.5	32.5
C40 (0.82 mg/mL collagen in final hydrogel)	40	5	55
C20 (0.41 mg/mL collagen in final hydrogel)	20	2.5	77.5

medicine such as in the cornea [12,14], tendon [19], bone [4], and urinary bladder [16,20] regeneration. The collagen sheet obtained from compression method, although stronger than conventional collagen hydrogels, still suffers from handling difficulties [17] and is not strong enough for some specific tissue engineering applications in need of high mechanical strength and/or exposed to frequent cycles of loading and unloading [16,17]. To improve the mechanical properties of plastic compressed (PC) sheets, multilayer fabrication through stacking, rolling, or serial compression of PC sheets have been offered by different research groups [3,4,6]. Another solution is to perform the plastic compression over a supporting substrate so that a hybrid construct including collagen can be achieved [16–18]. In this way, the supporting substrate (usually a synthetic polymeric sheet) is responsible for fulfilling the mechanical requirements and the PC collagen provides the cells with a fibrous protein construct similar to natural connective tissue. Such hybrid constructs have shown satisfactory outcomes from both cell behavior and mechanical aspects [16–18], and have the potential to be implemented for tissue engineering purposes with specific requirements of layered cell seeding and high mechanical strength.

While plastic compression is considered as a technique leading to very dense fibrillar structures [3,4,21], we address a question in this study whether we could rather reduce the collagen fibrillar density in compressed sheets of collagen in the context of further optimizing the cellular interaction with the final scaffold. On the other hand, although there have been some studies so far about optimum collagen concentration in collagen hydrogels applied for tissue engineering [11,22,23], the effect of collagen concentration (lower concentrations compared to conventional collagen concentration applied for making PC sheets) on the overall mechanical and cell interaction behavior of compressed collagen sheets has yet to be defined. Hence, in this study we have offered some modifications to the conventional hydrogel formula applied for compression, aiming to achieve less dense but still strong collagen constructs. To this end, we have applied different formulae for collagen hydrogel preparation, compressed the gels onto supporting substrate (here PLGA electrospun sheet), and compared the final constructs from different aspects including: internal collagen micro-architecture, mechanical properties, and cell-scaffold behaviors including cell attachment, and proliferation. The reason we applied PLGA electrospun sheet as the supporting substrate was to enhance the mechanical properties of the whole scaffold, and to further develop the PC collagen-PLGA- PC collagen layered scaffolds applied in our previous studies [18]. Herein, we have focused on improving the functional properties of the collagen layer, and believe the modifications introduced in this study can be implemented in other hybrid scaffolds applying PC collagen.

2. Materials and methods

2.1. Preparation of the scaffold constructs

Four different collagen hydrogels were prepared according to the quantities mentioned in Table 1 and the protocols described earlier [6,17]. In brief, sterile rat-tail collagen type I solutions (2.06 mg/mL protein in 0.6% acetic acid; First Link Ltd, UK) were mixed with 10% Eagle's minimum essential medium (MEM; Invitrogen, Denmark), and neutralized with 2.5 M NaOH. Finally, alpha-MEM medium was added. Hydrogels were labeled as C80, C60, C40, and C20. For instance, C80 was the symbol for the conventional collagen hydrogel used for plastic compression in which 80% volumetric share of the hydrogel was made up of collagen with the original concentration of 2.06 mg/mL. In other words, C80 was equivalent to the hydrogel with collagen concentration of 1.64 mg/mL. It is worth noting that MEM 10X is the indicator for neutralization of collagen solution due to the change in color of phenol red dye in media. Therefore, the volumetric ratio of MEM 10X to collagen solution has been adjusted to be kept around 12.5% of collagen volume (similar to the ratio in conventional hydrogel formula).

1 mL of each of the prepared collagen hydrogels was cast into circular shaped molds (diameter of 34 mm) and were incubated at 37 °C for 20 min to undergo the gelation procedure. After that, PLGA electrospun sheet (with optimized structure as described earlier [18], average fiber diameter of 705 nm, and average thickness of 80 μm) was added onto the collagen gel, and was covered with another 1 mL of the same type of collagen hydrogel. After 20 min, the hybrid construct was transferred onto blotting elements, consisting of a layer of sterile 110 mm-thick nylon mesh (~40 mm mesh size) and a sterile 400-mm-thick stainless steel mesh (mesh size ~ 200 mm), which were placed on top of three sterile gauze pads (Fig. 1). The set gels were covered with a second nylon mesh and a loading plate (as a static weight) (120 g) for 5 min at room temperature, leading to the formation of hybrid constructs of PCX-PLGA-PCX; X was any of the values 80, 60, 40 or 20, representing the different collagen concentrations. PC80 (as an example) represents the C80 after experiencing the plastic compression procedure.

2.2. Scaffold characterization

2.2.1. Calculation of collagen concentration in compressed collagen sheets

If we assume that only water is expelled during compression of collagen hydrogel, we can calculate the theoretical collagen concentration in different hydrogels undergoing compression by measuring the initial volume of hydrogel and the volume of the final sheet based on the rule of no change in collagen mass before or after compression.

$$c_{pc} = \frac{c_0 \, . \, t_0}{t_{pc}} \quad (1)$$

where c_0 represent the concentration of collagen in the initial hydrogel, t_0 the thickness of initial gel and c_{pc} and t_{pc} the concentration and thickness of the compressed sheets, respectively.

2.2.2. Morphological characterization of hybrid constructs

2.2.2.1. Scanning electron microscopy. The morphology of electrospun nanofibers, compressed collagen sheets, and hybrid constructs of PCX-PLGA-PCX was studied with scanning electron microscopy (SEM). Specimens were fixed in 2.5% glutaraldehyde in cacodylate buffer overnight and then washed with cacodylate buffer. Subsequently, samples were dehydrated with increasing concentrations of ethanol (30%, 50%, 70%, 90%, and 100%) for 10 min each. Finally, the constructs were treated with hexamethyldisilazane (HMDS) for further water extraction. The dehydrated constructs were maintained in desiccators equipped with vacuum for overnight air-drying. After sputter-coating with platinum, Quanta FEG 3D scanning electron microscope (SEM) was used to observe surface of scaffolds. The average fiber diameter of the scaffolds was measured by applying ImageJ 1.46R (NH, Maryland, USA) to the SEM micrographs.

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