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Design of an elasticized collagen scaffold: A method to induce elasticity in a rigid protein

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ABSTRACT

Type I collagen is widely applied as a biomaterial for tissue regeneration. In the extracellular matrix, collagen provides strength but not elasticity under large deformations, a characteristic crucial for dynamic organs and generally imparted by elastic fibers. In this study, a methodology is described to induce elastic-like characteristics in a scaffold consisting of solely type I collagen.

Tubular scaffolds are prepared from collagen fibrils by a casting, molding, freezing and lyophilization process. The lyophilized constructs are compressed, corrugated and subsequently chemically crosslinked with carbodiimide in the corrugated position. This procedure induces elastic-like properties in the scaffolds that could be repeatedly stretched five times their original length for at least 1000 cycles. The induced elasticity is entropy driven and can be explained by the introduction of hydrophobic patches that are disrupted upon stretching thus increasing the hydrophobic-hydrophilic interface. The scaffolds are cytocompatible as demonstrated by fibroblast cell culture.

In conclusion, a new straightforward technique is described to endow unique elastic characteristics to scaffolds prepared from type I collagen alone. Scaffolds may be useful for engineering of dynamic tissues such as blood vessels, ligaments, and lung.

Statement of Significance

In this research report, a methodology is presented to introduce elasticity to biomaterials consisting of only type I collagen fibrils. The method comprises physical compression and corrugation in combination with chemical crosslinking. By introducing elasticity to collagen biomaterials, their application in regenerative medicine may be expanded to dynamic organs such as blood vessels, ligaments and lung. The combination of strength and elasticity in one single natural biomaterial may also "simplify" the design of new scaffolds.

1. Introduction

Insoluble type I collagen is a biomaterial that has been extensively used in the field of tissue engineering and regenerative medicine as scaffolding material. In the native extracellular matrix, collagen provides structural support to tissues. Individual triple helical collagen monomers are densely packed into collagen fibrils that are subsequently organized in bundles called fibers which add strength and prevent the tissue from rupturing upon exposure to mechanical stress. Ideally, in tissue engineering the scaffold should

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temporarily take over the role of the native extracellular matrix and in this respect type I collagen is often applied. Collagen is widely available from animal sources, easy to purify, biodegradable and well-recognized for its excellent cross-species biocompatibility [1]. In addition, collagen is easy to modify with functional groups, such as glycosaminoglycans or growth factors, and moreover, its biodegradability can be regulated by crosslinking techniques [2]. Numerous type I collagen based scaffolds in the field of tissue engineering are already used in the clinical setting showing the value of collagen as a biomaterial [3].

Collagen-based scaffolds generally do not exhibit elastic properties at high deformations, which is of importance for the mechanical compliance of dynamic tissues. Many of these organs, such as lungs, heart valves, ligaments, blood vessels, skin, and bladder, need to be both strong and elastic to prevent ruptures and to reversibly deform [4,5]. The extracellular matrix of such organs contains elastic fibers, which give the tissue the required resilience and ensure that it regains its original shape after every deformation. Elastic fibers are mainly composed of the protein elastin which is responsible for the elasticity of the tissue [6]. The elastic fibers together with the collagen fibers largely determine the overall mechanical properties of a specific tissue.

In several studies, insoluble elastin-collagen scaffolds have been designed in order to mimic the natural matrix of elastic tissues [7,8]. However the use of insoluble elastin fibers comes with several disadvantages. A major problem is that elastin usually induces calcification *in vivo*, which may lead to life threatening side-effects [2,9–11]. Calcification in porcine heart valves for example, is a major clinical problem and has especially been observed in children [12], possibly in line with calcification of elastic fibrils in young animals [13]. Many attempts have been performed to improve clinical applicability including recellularization, surface treatment, enzyme treatment, but the clinical efficacy remains debatable [14]. In addition, the extreme insolubility of elastic fibers complicates the scaffold production process and will further complicate clinical approval since it is an additional animal derived component. Hydrolyzed or solubilized elastin are easier to handle and have been shown to stimulate synthesis of native elastin fibers [15], but they do not endow the initial scaffolds with major elastic properties. Conferring elasticity to collagen-only scaffolds would therefore be a major advantage in the design of functional scaffolds for tissue engineering of organs/tissues which are subjected to considerable deformations.

In this manuscript, we describe a new method to induce elasticity in scaffolds consisting of only type I collagen fibrils, thereby circumventing the issues that are associated with the use of elastin. By compression and corrugation of lyophilized porous collagen constructs followed by carbodiimide crosslinking under physical restraint, we were able to alter the morphology, and induce elastic-like, entropy-driven, characteristics. The combination of both strength and elastic-like properties in a one-component scaffold offers new opportunities for tissue engineering of dynamic tissues.

2. Materials and methods

2.1. Type I collagen fibrils

Highly-purified type I collagen fibrils were obtained as previously described [16]. Briefly, bovine achilles tendons were pulverized under liquid nitrogen-cooled conditions using a universal cutting mill (Pulverisette19, Fritsch GmbH, Idar-Oberstein, Germany) with a sieve-opening of 0.5 mm. The purification process included washings with aqueous solutions of NaCl (1.0 M), urea (6.0 M), and acetic acid (0.25 M), acetone and demineralized water.

2.2. Scaffold construction

2.2.1. Preparation of a porous collagen scaffold

A 0.8% (w/v) collagen suspension was prepared by mixing purified type I collagen fibrils with 0.25 M acetic acid. This suspension was swollen overnight and homogenized using a Silverson L5 M-A laboratory mixer (Silverson, Chesham, UK) by mixing 3 min at 2500 rpm using a general purpose disintegrating workhead, followed by 3 min at 2500 rpm with a slotted workhead. The suspension was deaerated using centrifugation at 100*g*. All steps were performed at 4 °C to prevent denaturation of the collagen. The collagen suspension was poured into a 10 mL polypropylene mold with a 6 mm stainless steel (grade 304) mandrel inside and frozen for at least 4 h at −20 °C in aluminum freezing blocks. After removal of the mandrel the frozen constructs were lyophilized (Zirbus sublimator 500II, Bad Grund, Germany).

2.2.2. Preparation of compressed and corrugated collagen scaffolds

After freeze-drying, the porous constructs (P-tube), with the mandrels again put in place, were uniformly compressed around the mandrel by gently squeezing them between two plane surfaces under a rolling motion to create porous compressed scaffolds (PC-tube, Fig. 1A). Next, in order to create porous compressed and corrugated scaffolds (PCC-tube), two discs fitting exactly over the mandrels were positioned around each side of the scaffold and subsequently pushed together until 10 mm space was left in between (Fig. 1A). The discs were replaced by 6 mm rubber O-rings to hold the scaffold in its corrugated state. Next, the scaffolds were crosslinked using a zero-length crosslinking method applying EDC (Merck Schuchardt OHG, Hohenbrunn, Germany) and NHS (Fluka Chemie AG, Buchs, Switserland) [17]. In brief, scaffolds were crosslinked for 3 h at room temperature in 50 mM 2-morpholinoethane sulfonic acid (MES buffer, pH 5.0) (USB, Ohio, USA) containing 40% (v/v) ethanol, 33 mM EDC and 6 mM NHS and subsequently washed in 0.1 M Na_2HPO_4, (2x), 1 M NaCl (2x), 2 M NaCl (4x), and demineralized water (6x). Finally, scaffolds were placed in 70% (v/v) ethanol and stored at −20 °C until use.

2.3. Scaffold characterization

2.3.1. Macroscopic evaluation and video recording

Macroscopic images were taken and videos were recorded using a Canon 1d X with a EF macro lens camera (Canon, Melville, NY, USA). High-speed videos (1000 frames per second) were recorded using a Casio EX-ZR100 (Casio, Tokyo, Japan) with camera speed settings at HS1000 and resolution at 224 × 64 pixels.

2.3.2. Scanning electron microscopy

Scanning electron microscopy (SEM) was used to analyze the morphology and structure of the tubular scaffolds. Samples were lyophilized, fixed on a stub with double-sided carbon tape and sputtered with an ultrathin gold layer in a Polaron E5100 Coating System. Examination was performed in a JEOL SEM 6310 apparatus (JEOL Ltd, Tokyo, Japan) with an accelerating voltage of 10 kV.

2.3.3. Effect of fluids with different polarities on elastic-like characteristics

PCC-tubes were restrained in a stretched position and air-dried. Next, the stretched scaffolds were placed in fluids of different polarities (i.e. water, ethylene glycol, methanol, ethanol, 1-propanol, 1-butanol, acetone and chloroform (all from Sigma-Aldrich) [18]. Before use, non-polar fluids were dehydrated by adding anhydrous Cu(II)SO_4 crystals (Sigma-Aldrich). The ability of the stretched scaffold to return to its original corrugated state was