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Designing a tubular matrix of oriented collagen fibrils for tissue engineering

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ABSTRACT

A scaffold composed entirely of an extracellular matrix component, such as collagen, with cellular level control would be highly desirable for applications in tissue engineering. In this article we introduce a novel, straightforward flow processing technique that fabricates a small diameter tubular matrix constructed of anisotropic collagen fibrils. Scanning electron microscopy confirmed the uniform alignment of the collagen fibrils and subsequent matrix-induced alignment of human fibroblasts. The uniform alignment of the fibroblasts along the collagen fibrils demonstrated the ability of the aligned fibrils to successfully dictate the directional growth of human fibroblasts through contact guidance. Various non-cytotoxic cross-linking techniques were also applied to the collagen conduit to enhance the mechanical properties. Tensile testing and burst pressure were the two measurements performed to characterize the mechanical integrity of the conduit. Mechanical characterization of the cross-linked collagen conduits identified 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride cross-linking as the most promising technique to reinforce the mechanical properties of native collagen. An oriented conduit of biocompatible material has been fabricated with decent mechanical strength and at a small diameter scale, which is especially applicable in engineering cardiovascular tissues and nerve grafts.

1. Introduction

There is a considerable demand in the field of tissue engineering for biocompatible materials that demonstrate similar characteristics to the extracellular matrix (ECM). It is essential that the scaffolding material promotes normal cell growth and differentiation with good interaction between the individual cells and the surroundings [1]. Collagen, the most prevalent protein found in the human body, has long been established as a prime candidate for producing biomimetic tissue engineering scaffolds or matrices. Due to its high tensile strength, biodegradability, lack of immunogenicity issues, and its natural ability to favor cellular attachment and growth, collagen is a promising biomaterial for applications in wound healing and regeneration [2].

An individual collagen molecule is a semi-flexible rod approximately 300 nm in length and 1.5 nm in diameter. The rod itself consists of three polypeptide strands wound together in a left-handed triple helix. In nature collagen is commonly found as a structure of fibrous bundles, such as in the connective tissue of tendons and ligaments. The rod-shaped molecules of collagen can self-assemble to form remarkable hierarchical fibril structures of varying diameter. The process of forming fibrils, or fibrillogenesis, can be induced in vitro by exposing a solution of molecular collagen, which remains in its molecular state at acidic pH levels, to a neutral pH environment at physiological temperature. Fibrillogenesis produces a viscoelastic collagen gel capable of flowing under stress.

A desirable characteristic in biomaterial scaffolds is the ability to direct cellular growth. A correlation between the orientation of the substrate and the directional growth of cells was initially identified by Weiss as contact guidance [3]. Since the discovery of contact guidance techniques have been developed to fabricate anisotropic substrates of collagen due to the unique ability of aligned collagen to dictate the orientation of cells. The majority of native extracellular matrices found in tissues consist of an ordered structure with a defined orientation, a characteristic which has been shown to significantly contribute to normal tissue function [1]. Some of the existing methods that can produce oriented collagen gels use high strength magnetic fields, micro-fluidic channels, or electrospinning processes [4–6]. These techniques require complex equipment and may not be the most convenient processes suitable for high throughput commercial production.

In addition to the lack of oriented collagen substrates for tissue engineering, there is also a pressing need for small diameter, biocompatible conduit grafts that demonstrate mechanical integrity comparable with native tissues. The majority of tubular construct grafts are made up of synthetic materials. Dacron and expanded poly(tetrafluoroethylene) (e-PTFE) are the most widely used and have demonstrated great success in grafts that experience high blood flow, where thrombosis is unlikely to occur. However, these synthetic materials are not as suitable for smaller diameter

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conduits. Lower flow rates occur when the inner diameter is less than 6 mm, which can lead to thrombus formation and closure of the graft if the material collapses [7]. An alternative technique is to use natural materials, such as collagen, that contain similar components to the ECM. A key challenge in using natural, biodegradable components as the key material is the ability to demonstrate significant mechanical integrity and attributes that are characteristic of the original tissue.

In this study we address the demands for an anisotropic, small diameter, biocompatible conduit with respectable mechanical integrity with the development of a novel flow processing technique. The extrusion technique highlighted in this research is more convenient and straightforward than previously used methods. A gel-like collagen tube is produced by aligning the collagen molecules in their monomeric state using hydrodynamic flow and then initiating fibrillogenesis using a neutral pH buffer. Using fibrillogenesis to preserve the supramolecular order of collagen molecules was first established by Besseau and Giraud-Guille [8]. Acid-solubilized collagen solution is pushed through an annulus, creating a coating of collagen monomers aligned along the longitudinal axis of flow. Immediately afterwards the collagen is soaked in a neutral pH buffer to initiate the formation of fibrils and freeze the alignment of the collagen molecules. This manual extrusion is a flow processing technique that creates a hollow conduit consisting of oriented collagen fibrils, a structure similar to the native ECM and therefore favorable for cellular growth. The simplicity of the technique allows the process to be easily automated for the continuous production of small diameter conduits of arbitrary lengths, making them applicable to a variety of tissue engineering applications.

Following extrusion the collagen conduits can undergo a cross-linking treatment to increase the structural integrity of the biomaterial. This study also uses various characterization methods to evaluate different cross-linking treatments in order to identify promising processes for reinforcing the collagen structure. Tensile testing and burst pressure measurements are convenient and reliable means of characterizing the mechanical properties of conduits. Uncross-linked samples were very susceptible to breaking when handled, thus they could not be tested for their mechanical properties. Only cross-linked samples were analyzed by mechanical testing.

2. Materials and methods

2.1. Dialysis of collagen

High concentration rat tail collagen type I was purchased from BD Biosciences at a stock concentration of 9–10 mg ml^{-1} in 0.02 N acetic acid, pH 3.5. The collagen was then further dialyzed against polyethylene glycol (Fluka) at 4 °C, using semi-permeable cellulose dialysis tubing of pore size 32 × 20.4 mm (Fisher). After 30–35 min the remaining collagen in the dialysis tubing reaches a final concentration of approximately 30 mg ml^{-1}.

2.2. Extrusion of collagen conduits

An aluminum syringe, consisting of two parts, is used to manually extrude the collagen tubes. The exterior part, which remains stationary, is shaped like a hollow cylinder with a tapering shaft. The collagen solution is injected into the hollow cylinder and extruded through the tapered end. The other part, which moves during extrusion, consists of a cylinder that fits flush with the cylindrical cavity of the outer piece and a thin rod, 5 cm in length. The diameter of the rod dictates the inner diameter of the collagen tubes, which is 1 mm. The annulus region through which the collagen is extruded has a thickness of 0.5 mm and the length of the cylindrical cavity is 7.5 cm. A schematic is shown in Fig. 1.

Fig. 1. Schematic of the syringe used to extrude collagen conduits. The diameter of the inner rod dictates the inner diameter of the collagen tubes, which is 1 mm. The annulus region, through which the collagen is extruded, has a thickness of 0.5 mm and the length of the cylindrical cavity is 7.5 cm. 0.1 ml of ~30 mg ml^{-1} collagen solution is injected into the hollow cavity of the syringe. The inner rod, which is 5 cm long, is then manually forced through the annulus, extruding the collagen at a volumetric flow rate of 0.124 cm^3 s^{-1}. The extrusion process coats the inner rod with a 0.5 mm layer of collagen, which is then immediately soaked in 10× PBS at 37 °C for 5 min to initiate fibril formation and stabilize the collagen fibril alignment.

A general purpose support stand, consisting of a weighted metal base plate and metal rod screwed in vertically, is used for the extrusion process. In preparation for extrusion the exterior cylinder is held stationary by a rubber-pronged clamp attached to the metal rod. The exterior cylinder is tilted to a near horizontal position in order to load the collagen solution and prevent collagen from spilling out due to gravity. Approximately 0.1 ml of the dialyzed collagen solution is injected into the hollow cavity of the syringe for the production of each collagen conduit. Once the collagen solution is loaded into the syringe the exterior cylinder can be quickly moved into a vertical orientation and the inner rod is pushed manually into the hollow cavity. The collagen solution is extruded at an approximate volumetric flow rate of 0.124 cm^3 s^{-1}, which corresponds to a wall shear rate of 221.11 s^{-1} in the thin annulus region. As collagen is extruded through the annulus the inner rod becomes coated with a thin layer (~0.5 mm) of collagen.

The collagen-coated rod is then immediately immersed in a buffer of 10× phosphate-buffered saline (PBS) (Gibco-Invitrogen) at 37 °C for 5 min in order to induce fibrillogenesis of the collagen molecules. The tube will become opaque, indicating the collagen fibrils have formed.

2.3. Cross-linking of collagen conduits

After fibrillogenesis has been induced in the collagen the 10× PBS is replaced with the solution for the preferred cross-linking treatment for the specified amount of time. The samples are then removed from the cross-linking solution and receive three washes in 1× PBS. The samples are stored in 1× PBS until they are needed for mechanical characterization or imaging. It is important that the tubes remain hydrated to retain their original structure.

2.3.1. Riboflavin–ultraviolet photo-treatment

The collagen conduit is immersed in 0.75 mM riboflavin (Sigma–Aldrich) dissolved in 1× PBS buffer. The riboflavin serves as a photo-initiator to cross-link the collagen fibrils when the sample is exposed to ultraviolet (UV) light at a wavelength of 265 nm for 1.5 h at room temperature [9].

2.3.2. Dimethyl suberimidate (DMS) chemical treatment

The sample is soaked in a solution of 1% dimethyl suberimidate (Sigma–Aldrich) in 0.2 M Tris, pH 8.5 for 2 h at room temperature [10].

2.3.3. UV–DMS photo and chemical combination

The sample is immersed in 0.75 mM riboflavin and 1% DMS dissolved in 0.2 M Tris buffer, pH 8.5, and exposed to UV light for 1.5 h. This provides a combination of photo-treatment and chemical cross-linking to reinforce the collagen material properties.