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Insoluble elastin reduces collagen scaffold stiffness, improves viscoelastic properties, and induces a contractile phenotype in smooth muscle cells

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ABSTRACT

Biomaterials with the capacity to innately guide cell behaviour while also displaying suitable mechanical properties remain a challenge in tissue engineering. Our approach to this has been to utilise insoluble elastin in combination with collagen as the basis of a biomimetic scaffold for cardiovascular tissue engineering. Elastin was found to markedly alter the mechanical and biological response of these collagen-based scaffolds. Specifically, during extensive mechanical assessment elastin was found to reduce the specific tensile and compressive moduli of the scaffolds in a concentration dependant manner while having minimal effect on scaffold microarchitecture with both scaffold porosity and pore size still within the ideal ranges for tissue engineering applications. However, the viscoelastic properties were significantly improved with elastin addition with a 3.5-fold decrease in induced creep strain, a 6-fold increase in cyclical strain recovery, and with a four-parameter viscoelastic model confirming the ability of elastin to confer resistance to long term deformation/creep. Furthermore, elastin was found to result in the modulation of SMC phenotype towards a contractile state which was determined via reduced proliferation and significantly enhanced expression of early (α-SMA), mid (calponin), and late stage (SM-MHC) contractile proteins. This allows the ability to utilise extracellular matrix proteins alone to modulate SMC phenotype without any exogenous factors added. Taken together, the ability of elastin to alter the mechanical and biological response of collagen scaffolds has led to the development of a biomimetic biomaterial highly suitable for cardiovascular tissue engineering.

1. Introduction

A major challenge in cardiovascular tissue engineering is the design and fabrication of biomaterials with suitable biological instructive cues to guide cell behaviour, while additionally supporting the challenging haemodynamic mechanical environment once implanted *in vivo* [1,2]. These instructive cues are chemical, physical, and mechanical in nature and play a major role in governing cellular adhesion, migration, proliferation, and differentiation, while encouraging synthesis of appropriate proteins/glycosaminoglycans [3,4]. Cells natively receive a large quantity of these cues through interaction with the extracellular matrix (ECM) and so a biomimetic approach to biomaterial design has a number of innate advantages. Natural-based materials, such as collagen and fibrin, are thus ideal candidates to construct biomaterial scaffolds from as they can form part of an adaptive tissue which is mechanically and biologically responsive to the haemodynamic environment [5,6]. The ubiquitous nature of collagen in the body and comparatively good mechanical properties in comparison to other natural polymers has thus led to its widespread use for cardiovascular tissue engineering as a cardiac cell delivery patch [7] and vascular graft among others [8,9].

Despite numerous advances in biofabrication methods for natural polymers, these collagen-based scaffolds often do not meet the mechanical requirements for the dynamic cardiovascular

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environment. Often this stems from the propensity of collagen-alone to creep over time, a process which would lead to aneurysm formation *in vivo* if utilised as a vascular graft [10–12]. In order to address some of the mechanical limitations of natural polymers such as collagen, composites have been developed in order to improve the strength of the scaffolds while also retaining biological activity [13–16]. Natural polymers, such as collagen and fibrin, have been combined with synthetic polymers, such as polylactic acid and polyethylene terephthalate, in order to further enhance the mechanical properties of the vessels [17,18]. While the strength of vascular grafts can be improved via the incorporation of synthetic polymers, this also generally reduces the compliance of the scaffolds as the synthetic polymers are relatively stiff. Consequently, despite some advances in the area, many of these grafts suffer from a compliance mismatch in comparison to a native vessel [19]. Additionally, biodegradable synthetic polymers, such as PGA, have been shown to dedifferentiate SMCs due to their hydrolysis products [20,21] which may also lead to a lowering of local pH levels and a resulting inflammatory response [22]. Consequently, recent research has begun to focus on creating composites of purely natural polymers to engineer a regenerative niche which can guide cell behaviour and ultimately promote tissue regeneration.

In native vasculature, elastin serves to dampen the pulsatile flow of blood by its efficient storage of elastic-strain energy. From a tissue engineering perspective, the high extensibility and resilience of this protein, coupled with its low stiffness, may allow it closer match the mechanical properties of native cardiovascular tissue. In particular, the addition of elastin to vascular scaffolds has been shown to alter the mechanical response of scaffolds through increasing compliance and reducing thrombogenicity [16]. In addition, elastin has been attributed with activating pathways which govern proliferation and differentiation of vascular cells. Specifically, elastin has been found to stimulate gene expression of the SMC markers α-SMA and calponin *in vitro* for MSCs seeded on elastin coated substrates [21,23]. Elastin can bind to the 67 kDa elastin/laminin binding protein which has been shown to be involved in mechanotransduction [24], ECM assembly [25], cell chemotaxis and proliferation [26]. It is clear that emulation of native tissue composition by elastin incorporation would therefore address many of the biological and mechanical issues seen in this field. However, there is a dearth of research on native elastin in tissue engineering due to the proteins large size and insolubility which makes it incompatible with many biofabrication techniques. Previous studies utilizing elastin have primarily focused on initial incorporation methods and characterisation [27,28] or the effects of elastin *in vivo* [29,30]. Thus, the effects of native elastin on biomaterial properties have yet to be fully elucidated for cardiovascular tissue engineering.

In this study, we hypothesised that the presence of elastin in a porous collagen scaffolds would markedly alter the mechanical and biological response and, from a cardiovascular tissue engineering perspective, that its incorporation would produce a more natural viscoelastic response while inducing a more contractile SMC phenotype. The aim of the study was thus to elucidate the influence of elastin addition on the microstructural and mechanical properties of collagen scaffolds and to examine the biological response of smooth muscle cells when seeded on the composite scaffolds. Specifically, after elastin incorporation, we investigated the resultant scaffold microarchitecture via scanning electron microscopy and used histological techniques to quantify pore architecture and elastin distribution. Mechanical properties were investigated via compression and uniaxial tensile testing while viscoelastic response was assessed by examining creep and cyclical strain recovery analysis. Subsequently, we assessed the effect of elastin addition on SMC phenotypic modulation towards a synthetic or contractile phenotype by assessing cell proliferation and gene expression.

2. Materials and methods

2.1. Scaffold fabrication

2.1.1. Fabrication of collagen-elastin scaffolds

To fabricate Collagen-Elastin (CE) scaffolds, a freeze-drying process was used. Firstly, it was necessary to prepare a co-suspension of collagen and elastin in an aqueous acetic acid solution as per Fig. 1. This collagen-elastin suspension was produced by mixing 0.5% w/v of fibrillar Type I bovine collagen (Integra Life Sciences, Plainsboro, NJ) and elastin from bovine neck ligament (Sigma–Aldrich, Germany) in 0.05 M acetic acid. Elastin was blended into the collagen/acetic acid suspension in one of three different concentrations: 10 wt % (CE10), 35 wt % (CE35) and 100 wt % (CE100) elastin to collagen – which corresponds to the ratios found in dry native skin, lung and arterial tissues respectively. The suspension was then added to a mixing vessel, cooled to 4 °C, where it was blended using an overhead blender (Ultra Turrax T18, IKA Works Inc., Wilmington, NC) at a speed of 15,000 rpm to homogenise the suspension. A desiccator was used to degas the high viscosity suspension and it was then placed in a stainless steel pan and freeze-dried at a cooling rate of 0.9 °C/minute to a final freezing temperature of −40 °C (Advantage EL, Vir-Tis Co., Gardiner NY). This freeze-drying profile has been previously optimised by O'Brien et al. [31] to develop collagen-based scaffolds with a homogeneous pore structure.

Following freeze-drying the scaffolds were dehydrothermally (DHT) crosslinked as per Haugh et al. [32]. Briefly, the scaffolds were subjected to a DHT treatment of 105 °C for 24 h at 0.05 bar in a vacuum oven (Vacucell 22, MMM, Germany). This crosslinking method also sterilises the scaffolds for use in cell culture.

2.2. Scaffold microstructural analysis

2.2.1. Assessment of elastin distribution in the CE scaffolds

Histological analysis was performed to evaluate the spatial distribution of elastin within the freeze-dried scaffolds. Scaffold samples were placed in a solution of 10% formalin for 30 min followed by embedding in paraffin by an automatic tissue processor (ASP300, Leica, Germany). The samples were sectioned at 10 μm using a microtome (Leica RM 2255, Leica, Germany) and mounted on glass histology slides followed by deparaffinization in xylene. The slide were then stained with a modified Verhoeff-van Gieson staining procedure adapted from the manufacturer's instructions (Sigma–Aldrich, Dublin, Ireland) and coverslips were applied with DPX mountant. The mounted sections were imaged on a microscope (Nikon Eclipse 90i, Nikon, Japan) and digital images were recorded at 10× magnification using the attached control unit connected to a PC and imaging software (Nikon DS Camera control unit, Nikon, Japan with NIS Elements Basic Research V3.06, Nikon Instruments Europe, The Netherland).

2.2.2. Effect of elastin addition on scaffold mean pore size, porosity, and pore architecture

The scaffold mean pore size was determined using a polymer resin embedding technique as previously described [31]. Samples were embedded in a JB-4 glycomethacrylate (Polysciences, Germany) resin according to the manufacturers protocol and serially sectioned at 10 μm using a microtome (Leica RM 2255, Leica, Germany). Sections were stained with toluidine blue and digital images were acquired before quantification by a pore typography analyser script previously written for MATLAB® (MathWorks Inc, MA, USA) [33]. The software thresholds the images, identifies pore boundaries, and the mean pore size was calculated from best fit ellipses inside each pore. A minimum of 200 pores were analysed for each