Acta Biomaterialia 29 (2016) 188–197

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Production of ascorbic acid releasing biomaterials for pelvic floor repair

Naşide Mangır [a,b], Anthony J. Bullock [a], Sabiniano Roman [a], Nadir Osman [a,b], Christopher Chapple [b], Sheila MacNeil [a,*]

[a] Department of Materials Science Engineering, Kroto Research Institute, University of Sheffield, United Kingdom
[b] Royal Hallamshire Hospital, Urology Clinic, Sheffield, United Kingdom

ARTICLE INFO

Article history:
Received 27 March 2015
Received in revised form 10 September 2015
Accepted 14 October 2015
Available online 19 October 2015

Keywords:
Extracellular matrix
Collagen
Polylactic acid
Pelvic floor tissue engineering

ABSTRACT

Objective: An underlying abnormality in collagen turnover is implied in the occurrence of complications and recurrences after mesh augmented pelvic floor repair surgeries. Ascorbic acid is a potent stimulant of collagen synthesis. The aim of this study is to produce ascorbic acid releasing poly-lactic acid (PLA) scaffolds and evaluate them for their effects on extracellular matrix production and the strength of the materials.
Materials and methods: Scaffolds which contained either L-ascorbic acid (AA) and Ascorbate-2-Phosphate (A2P) were produced with emulsion electrospinning. The release of both drugs was measured by UV spectrophotometry. Human dermal fibroblasts were seeded on scaffolds and cultured for 2 weeks. Cell attachment, viability and total collagen production were evaluated as well as mechanical properties.
Results: No significant differences were observed between AA, A2P, Vehicle and PLA scaffolds in terms of fibre diameter and pore size. The encapsulation efficiency and successful release of both AA and A2P were demonstrated. Both AA and A2P containing scaffolds were significantly more hydrophilic and stronger in both dry and wet states compared to PLA scaffolds. Fibroblasts produced more collagen on scaffolds containing either AA or A2P compared to cells grown on control scaffolds.
Conclusion: This study is the first to directly compare the two ascorbic acid derivatives in a tissue engineered scaffold and shows that both AA and A2P releasing electrospun PLA scaffolds increased collagen production of fibroblasts to similar extents but AA scaffolds seemed to be more hydrophilic and stronger compared to A2P scaffolds.

Statement of significance

Mesh augmented surgical repair of the pelvic floor currently relies on non-degradable materials which results in severe complications in some patients. There is an unmet and urgent need for better pelvic floor repair materials. Our current understanding suggests that the ideal material should be able to better integrate into sites of implantation both biologically and mechanically.
The impact of vitamin C on extracellular matrix production is well established but we in this study have undertaken a critical comparison of two derivatives of vitamin C as they are released from a biodegradable scaffold. This strategy proved to be equally useful with both derivatives in terms of new tissue production yet we observed significant differences in mechanical properties of these biomaterials.

1. Introduction

Stress urinary incontinence (SUI) and pelvic organ prolapse (POP) are two major public health issues that can affect the physical, social and psychological wellbeing of affected women, usually aged >40 years [1,2]. Surgical treatment of both conditions often involves implantation of biomaterials to provide support. Although there is a reasonable initial success rate, 20–30% of women will subsequently require re-operation due to complications or recurrence [3,4]. The complications related to synthetic non-absorbable mesh implants are particularly concerning for both patients and physicians [5,6]. Tissue engineering has potential to overcome such complications through the use of degradable synthetic matrices with appropriate strength and elasticity that

* Corresponding author.
E-mail address: s.macneil@sheffield.ac.uk (S. MacNeil).

http://dx.doi.org/10.1016/j.actbio.2015.10.019
1742-7061/

release bioactive factors conducive to integration of the material and tissue remodelling.

Healthy pelvic floor connective tissues are thought to be in a state of continuous remodelling involving the synthesis and degradation of collagen and other ECM components [7]. Abnormalities in this degradation-synthesis equilibrium are not only implicated in the pathophysiology of pelvic floor defects but may also put any repair at risk of failure as the underlying primary abnormality in collagen turnover persists. Degradation of collagen is mediated by a family of matrix metalloproteinases (MMP), whose activity is regulated at multiple levels including synthesis, activation, activity inhibition (tissue MMP inhibitors) and synthesis of collagen involves a cascade of post-translational modification reactions leading to a mature, high tensile strength support structure. Elevated MMP and reduced MMP inhibitor levels were found in paravaginal tissues of women with urinary incontinence [8] and also reduced collagen content was evident in endopelvic fascia of women with SUI and POP compared to healthy women [9,10]. Thus defective/decreased collagen synthesis is a part of the disease process in women with SUI and/or POP and incorporation of stimulants of collagen synthesis into biomaterials could be a reasonable strategy in pelvic floor tissue engineering.

Ascorbic acid plays a crucial role in synthesis and post-translational modification of collagen. This is widely studied and thought to occur at multiple levels: [1] acting as a co-factor for the enzyme prolyl hydroxylase which is responsible for crosslinking of collagen fibrils thus forming the triple helix structure [11], [2] stimulating collagen gene expression via malondialdehyde [12] and [3] activation of collagen gene transcription and stabilization of procollagen mRNA [13,14]. Also ascorbic acid is a major antioxidant in human blood at concentrations of 40–80 μM. Safe intravenous administrations up to a final plasma concentration of 5 mM in cancer patients have been reported [15]. The toxic concentrations in culture conditions are consistently reported to be >0.5 mM [16]. The use of L-ascorbic acid (AA), the naturally occurring and metabolically active form of ascorbic acid in humans, to increase collagen production of fibroblasts *in vitro* dates back to 1970s [17]. Its use is limited by its low chemical stability in aerobic culture conditions [18]. Thus a variety of AA derivatives have been produced to increase its stability (e.g. Ascorbate-2-Phosphate [A2P], Na salt of ascorbate). A2P has been the most widely used synthetic form of AA in cell culture since Hata and Senoo proved the efficacy of the long acting and stable A2P [19,20]. There have been a few direct comparisons of AA analogues in cultured fibroblasts [21,22].

Electrospinning is a widely used technique in tissue engineering that produces scaffolds with micro/nano sized fibres while allowing purposeful modification of the 3D structure [23]. Also electrospun PLA scaffolds were shown to have mechanical properties close to those of healthy paravaginal tissue [24] while showing successful integration into native tissues in the short term [25]. Emulsion electrospinning is a modification of this technique to achieve successful incorporation of hydrophilic substances, such as AA, in the centre of hydrophobic polymer fibres resulting in a core–shell morphology that enables a sustained release of the hydrophilic content [26,27]. Thus the emulsion electrospun mats can serve as both vehicles to deliver bioactive factors and tissue scaffolds to provide structural support [28]. Previously successful encapsulation of vascular endothelial growth factors [29], Rhodamine B [30] and human nerve growth factor [31] into emulsion electrospun fibres has been demonstrated.

The aims of this study were to construct electrospun PLA scaffolds that are able to release the two most commonly used ascorbic acid derivatives (AA and A2P), to evaluate the comparative effectives of the two in terms of collagen production and finally to assess the impact of the AA and A2P on the mechanical properties of the electrospun scaffolds.

2. Materials and methods

2.1. Scaffold synthesis and characterisation

2.1.1. Preparation of emulsions

PLA polymer (Sigma–Aldrich) was dissolved 10% w/v in dichloromethane (DCM). Fifty microlitre of Span80 (Sigma–Aldrich) was added to the polymer solution and stirred at 250 rpm for 10 min. L-ascorbic acid (Sigma–Aldrich) and L-ascorbic acid 2-phosphate (SigmaLAldrich) were dissolved in distiled water and a total volume of 500 μl solution was added drop wise to the PLA-Span80 solution while stirring 1000 rpm with magnets for 15 min (Fig. 1), the final concentration being 0.0001, 0.001 and 0.01 g of either AA or A2P per gram of PLA. Unless stated otherwise, the medium concentration (0.001 g of AA and A2P per gram of PLA) was used in experiments. A control emulsion electrospun scaffold containing only 500 μl dH_2O (Vehicle scaffolds), without AA or A2P was also included together with a PLA only electrospun scaffold. All emulsions were freshly made and electro spun immediately.

2.1.2. Electrospinning conditions

The emulsions were loaded into 5 mL syringes with blunt tipped stainless steel needles. The emulsions were delivered at a constant feed rate of 40 μl/min using a programmable syringe pump (Aladdin 1000) and were electrospun horizontally with an accelerating voltage of 15 kV supplied by a high voltage power supply (Brandenburg, Alpha series III, UK). Fibrous mats were collected on aluminium foil sheets wrapped around an earthed aluminium rotating collector (rotating at 300 rpm) 15 cm from the tip of the needle. Scaffolds were produced and left to dry for 1 h in a fume hood.

2.1.3. AA and A2P release profile

All measurements of AA and A2P were performed using a UV-spectrophotometer (Thermo Scientific™ Evolution 220) at an absorbance wavelength of 252 nm. A calibration curve was initially constructed by measuring 8 concentrations of AA and A2P (lowest: 10 nM and highest: 100 μM) prepared in dH_2O and PBS, respectively. All solutions were freshly prepared and the absorbances were immediately measured. The calibration curve was linear with a correlation coefficient of $R^2 > 0.999$ for both AA and A2P and the lower limit of detection was 500 nM.

Initially the feasibility of using PBS to demonstrate AA release was investigated. For this three pieces of AA scaffolds (mean weight 0.022 ± 0.001 g) were placed into 4 mL of dH_2O or PBS. At 2, 4, 6, 24 and 72 h media was removed, absorbance read and the media was put back into the well. The concentration was determined using the calibration curve. The release of AA into dH_2O could be demonstrated with small error bars within the first 6 h of the experiment whereas we were unable to detect any amount of AA in PBS except at the first 2 h (Fig. 2). This suggested dH_2O as the sole media to study release of AA.

Release of AA and A2P from scaffolds were studied in dH_2O and PBS, respectively. Three pieces of AA and A2P scaffolds (mean weights: 0.0199 ± 0.002 and 0.0176 ± 0.002, respectively) were placed in 4 mL media and were kept in a dry incubator at 37 °C. A vehicle scaffold was taken as a control. At 2, 4, 6, 8, 10 h and 1, 2, 3, 7, 14, 21 and 28 days a sample was removed from the media, the absorbance measured and the concentration was determined with use of the calibration curve. All the media were then discarded and replaced with fresh media. Experiments were repeated three times.