Acta Biomaterialia 43 (2016) 160–169

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Full length article

Multifunctional biomaterials from the sea: Assessing the effects of chitosan incorporation into collagen scaffolds on mechanical and biological functionality

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ARTICLE INFO

Article history:
Received 21 January 2016
Received in revised form 6 May 2016
Accepted 7 July 2016
Available online 8 July 2016

Keywords:
Marine biomaterials
Chitosan
Collagen
Osteogenesis
Chondrogenesis

ABSTRACT

Natural biomaterials such as collagen show promise in tissue engineering applications due to their inherent bioactivity. The main limitation of collagen is its low mechanical strength and somewhat unpredictable and rapid degradation rate; however, combining collagen with another material, such as chitosan, can reinforce the scaffold mechanically and may improve the rate of degradation. Additionally, the high cost and the risk of prion transmission associated with mammal-derived collagen has prompted research into alternative sources such as marine-origin collagen. In this context, the overall goal of this study was to determine if the incorporation of chitosan into collagen scaffolds could improve the mechanical and biological properties of the scaffold. In addition the study assessed if collagen, derived from salmon skin (marine), can provide an alternative to collagen derived from bovine tendon (mammal) for tissue engineering applications. Scaffold architecture and mechanical properties were assessed as well as their ability to support mesenchymal stem cell growth and differentiation. Overall, the addition of chitosan to bovine and salmon skin-derived collagen scaffolds improved the mechanical properties, increasing the compressive strength, swelling ratio and prolonged the degradation rate. Mesenchymal stem cell (MSC) attachment and proliferation was most improved on the bovine-derived collagen scaffold containing a 75:25 ratio of collagen:chitosan, and when MSC osteogenic and chondrogenic potential on the scaffold was assessed, a significant increase in calcium production ($p < 0.001$) and sulfated glycosaminoglycan (sGAG) production ($p < 0.001$) was observed respectively. Regardless of chitosan content, the bovine-derived collagen scaffolds out-performed the salmon skin-derived collagen scaffolds, displaying a larger pore size and higher percentage porosity, more regular architecture, higher compressive modulus, a greater capacity for water uptake and allowed for more MSC proliferation and differentiation. This versatile scaffold incorporating the marine biomaterial chitosan show great potential as appropriate platforms for promoting orthopaedic tissue repair while the use of salmon skin-derived collagen may be more suitable in the repair of soft tissues such as skin.

Statement of Significance

Collagen is commonly used in tissue engineering due to its biocompatibility; however, it has low mechanical strength and an unpredictable degradation rate. In addition, high cost and risk of prion transmission associated with mammalian-derived collagen has prompted research into alternative collagen sources, namely, marine-derived collagen. In this study, scaffolds made from salmon-skin collagen were compared to the more commonly used bovine-derived collagen with a focus on orthopaedic applications.

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http://dx.doi.org/10.1016/j.actbio.2016.07.009
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To improve the mechanical properties of these scaffolds, another marine biomaterial, chitosan, was added to produce scaffolds with increased mechanical stability. The collagen-chitosan composites were also shown to support mesenchymal stem cell differentiation towards both bone and cartilage tissue. This multi-functional scaffold therefore has potential in both bone and cartilage regeneration applications.
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1. Introduction

Biomaterial scaffolds for orthopaedic tissue engineering applications should be capable of integrating with native tissue, allowing for cell infiltration and proliferation, and to degrade at a rate proportional to new tissue formation, all without causing an immunological response. The material composition of the scaffold plays an important role in directing cell behaviour; natural biomaterials such as collagen show promise due to their inherent bioactivity [1–4]. The mechanical properties of biomaterials for orthopaedic applications must also be considered; in seminal work by Engler and colleagues, it was shown that mesenchymal stem cells (MSCs) differentiate toward different phenotypes depending on the stiffness of the substrate upon which they are seeded [5]. As well as influencing cell behaviour, scaffolds must be strong enough to withstand physical manipulation subjected during implantation and be straight-forward to fabricate, sterilize and shape to the size required [6]. The main limitation of natural materials, such as collagen, in orthopaedic tissue engineering applications, is their low mechanical strength and somewhat unpredictable degradation rate; however combining two or more materials can reinforce the scaffold mechanically and may slow the rate of degradation.

Chitosan is a biocompatible, biodegradable polysaccharide derived from chitin, which can be isolated from many marine species [7]. The most common source of chitin is crab and shrimp exoskeletons, of which at least 2.3 million metric tons are produced each year as food waste [8]. Chitosan is a very interesting material as it can be processed into hydrogels [9,10], nanofibers [11], beads [12], microparticles [13], nanoparticles [14–16] and porous scaffolds [17–19] and has been used in a wide range of tissue engineering applications such as in wound healing [20], and in drug and gene delivery [16,21–24]. Of particular interest in orthopaedic tissue engineering, chitosan has been shown to promote MSC osteogenesis [25] and, being a linear polysaccharide, has an analogous molecular structure to hyaluronic acid, a non-sulfated glycosaminoglycan which is a major component of articular cartilage, and has been shown to support MSC chondrogenesis.

Collagen is the most abundant protein found in the human body, serving as the major component of the extracellular matrix [4,26,27]. It is biocompatible and biodegradable and readily allows for cell adhesion, proliferation and differentiation [28]. For this reason collagen is in widespread use in tissue engineering research, however, it has low mechanical strength and rapid degradation rate, which are limiting its use commercially. Cross-linking methods can increase the mechanical properties of collagen however the incorporation of another material, forming a composite has shown the most promise in improving the scaffolds characteristics [29–31]. Due to the interesting properties of chitosan outlined above, the main aim of this study was to investigate the potential of collagen-chitosan composite scaffolds for orthopaedic tissue engineering applications.

A further caveat in choosing biomaterials for tissue engineering is the source; as collagen is such a ubiquitous material, it can be extracted from numerous sources, most commonly bovine and porcine connective tissue such as skin and tendon. However, while mammalian-derived tissues are subject to extensive processing to reduce risk of disease transmission and immunogenicity, there remain concerns about transmission of prions such as Creutzfeldt-Jakob Disease (CJD), although there have been no reports of this to date [32–35]. Religious restriction is also an important issue as three major religions; Judaism, Islam and Hinduism prohibit the use of products derived from either bovine or porcine origin [36]. For these reasons, there has been interest in alternative sources of collagen. While recombinant technology can be used to make collagen, it is extremely expensive and yields are low and inefficient for most tissue engineering applications [37]. Alternatively, marine-derived collagen is an easily accessible source of collagen and can be obtained from many different sources including fish skin, jellyfish and marine sponges [38–40]. As up to 75% of fish weight is discarded as a food waste, it is possible to obtain large amounts of collagen cheaply [41]. One drawback to marine-derived collagen is that it has lower hydroxyproline content than the more commonly used bovine-derived collagen. Hydroxyproline functions to stabilise the collagen triple helix conformation and a high hydroxyproline content indicates greater thermal stability [42]. The denaturation temperature of marine-derived collagen is thus reported to be approximately 40 °C [41] whereas bovine-derived collagen has a reported denaturation temperature of 95 °C [43].

The objectives of this study was thus to investigate the effect of the incorporation of chitosan on the morphological and mechanical properties of collagen scaffolds and also to determine if marine-derived collagen, isolated from salmon skin, might serve as a viable alternative to mammal-derived collagen, isolated from bovine tendon, in tissue engineering applications. In addition, the potential of the resultant collagen-chitosan composite scaffold to enhance both MSC-mediated osteogenesis and chondrogenesis was assessed.

2. Materials and methods

All materials were provided by Sigma Aldrich, Ireland unless otherwise stated.

2.1. Isolation of collagen from salmon skins

Salmon skins were obtained from a local market and kept frozen until use. Scales and muscle were removed and skins were further washed with water and cut into pieces of about 2 × 2 cm. Fats were removed from salmon skins by immersion in 10% ethanol for 48 h, under stirring (with change of solution at least twice a day). Salmon skins were then treated with 0.1 M NaOH (1:10 w/v), during 3 × 2 h, to remove non-collagenous proteins as described previously [41,44–46]. After thorough washing with water, salmon skins were dissolved in 0.5 M acetic acid (HOAc) (1:10 w/v), during 72 h, under stirring. The resulting mixture was centrifuged and the supernatant, containing the acid soluble collagen, was further vacuum filtered to remove non-soluble impurities. Salmon-skin collagen was recovered by salting out and after centrifugation; collagen was resuspended in 0.5 M HOAc, dialysed against 0.1 M HOAc and freeze-dried until further use. All the extraction procedure was conducted at 4 °C and the resulting

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