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Multi-layered collagen-based scaffolds for osteochondral defect repair in rabbits

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ABSTRACT

Introduction: Identification of a suitable treatment for osteochondral repair presents a major challenge due to existing limitations and an urgent clinical need remains for an off-the-shelf, low cost, one-step approach. A biomimetic approach, where the biomaterial itself encourages cellular infiltration from the underlying bone marrow and provides physical and chemical cues to direct these cells to regenerate the damaged tissue, provides a potential solution. To meet this need, a multi-layer collagen-based osteochondral defect repair scaffold has been developed in our group.

Aim: The objective of this study was to assess the *in vivo* response to this scaffold and determine its ability to direct regenerative responses in each layer in order to repair osteochondral tissue in a critical-sized defect in a rabbit knee.

Methods: Multi-layer scaffolds, consisting of a bone layer composed of type I collagen (bovine source) and hydroxyapatite (HA), an intermediate layer composed of type I and type II collagen and HA; and a superficial layer composed of type I and type II collagen (porcine source) and hyaluronic acid (HA), were implanted into critical size (3 × 5 mm) osteochondral defects created in the medial femoral condyle of the knee joint of New Zealand white rabbits and compared to an empty control group. Repair was assessed macroscopically, histologically and using micro-CT analysis at 12 weeks post implantation.

Results: Analysis of repair tissue demonstrated an enhanced macroscopic appearance in the multi-layer scaffold group compared to the empty group. In addition, diffuse host cellular infiltration in the scaffold group resulted in tissue regeneration with a zonal organisation, with repair of the subchondral bone, formation of an overlying cartilaginous layer and evidence of an intermediate tidemark.

Conclusion: These results demonstrate the potential of this biomimetic multi-layered scaffold to support and guide the host reparative response in the treatment of osteochondral defects.

Statement of Significance

Osteochondral defects, involving cartilage and the underlying subchondral bone, frequently occur in young active patients due to disease or injury. While some treatment options are available, success is limited and patients often eventually require joint replacement. To address this clinical need, a multi-layer collagen-based osteochondral defect repair scaffold designed to direct host-stem cell mediated tissue formation within each region, has been developed in our group. The present study investigates the *in vivo* response to this scaffold in a critical-sized defect in a rabbit knee. Results shows the scaffolds ability to guide the host reparative response leading to tissue regeneration with a zonal organisation, repair of the subchondral bone, formation of an overlying cartilaginous layer and evidence of an intermediate tidemark.

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1. Introduction

Cartilage has a limited capacity to regenerate following injury and thus poses a significant clinical problem. Osteochondral defects involving the smooth cartilage lining of the articulating surface and the underlying subchondral bone, are particularly problematic as they frequently occur in young active patients due to diseases such as osteochondritis dissecans, or as a result of traumatic injury to the joint. While some treatment options, such as microfracture, mosaicplasty and autologous chondrocyte implantation (ACI), are available to orthopaedic surgeons, further developments are required in order to improve success rates and achieve long lasting defect repair. Failure of these treatment options can eventually lead to the requirement for joint replacement. Cartilage is avascular and chondrocytes, the cellular components of cartilage, are present in low numbers and have poor mitotic ability. These inherent properties of cartilage tissue contribute significantly to its limited regenerative capacity. Regenerative medicine offers some promise in the area but the identification of a suitable cell source poses a significant challenge in the development of strategies for cartilage repair. Autologous chondrocytes, harvested and expanded prior to implantation into the site of damage, is one commonly used cell source. This approach has a number of associated limitations, most notably the requirement for a two-step procedure, and high costs associated with the technique. Other potential cell sources are under investigation, including chondrocytes from non-articular sources and MSCs harvested from various locations including the bone marrow, adipose tissue and peripheral blood; however, their use clinically is still limited. An urgent clinical need thus remains for an off-the-shelf, low cost, one-step approach to chondral and osteochondral defect repair. In order to meet this need and overcome the requirement for an external cell source, there is now growing focus on the use of a biomaterials-based approach, where rather than supply cells to the defect site, the ideal biomaterial might encourage the infiltration of cells from the underlying bone marrow and provide physical and biochemical cues to direct these cells to regenerate the damaged tissue [1–3].

A number of biomaterials-based approaches to osteochondral defect repair have emerged in recent years. Simplistic non-layered approaches have yielded little success [4,5] and there has thus been a move towards optimised layered materials for osteochondral defect repair. However, to date such materials tend to consist of separate cartilage and bone repair scaffolds that are fused together using sutures or biological glues rather than truly addressing the integrated layered structure of osteochondral tissues. In addition, the research focus has been mainly on the chondral region of the defect site and has thus neglected the subchondral bone and calcified cartilage regions. It is now recognised that subchondral bone injury can play an important role in the development and progression of degenerative joint disease [6,7]. Thus regeneration of healthy subchondral bone within the defect site is essential in order to achieve completed repair within an osteochondral defect [8]. The calcified cartilage and tidemark regions of the tissue also play a significant role by providing a stable interface between bone and cartilage and are critical in preventing vascular invasion from the subchondral bone into the chondral region which can lead to undesirable bony ingrowth. While some devices aimed at osteochondral defect repair have recently emerged onto the market place, including TruFit (Smith and Nephew, MA, USA), MaioRegen (Fincernamica, Provincia di Ravenna, Italy) and Agili-C (Cartiheal, Kfar Saba, Israel), until now, no solution exists that addresses the complex challenge of repairing the cartilage, calcified cartilage and the underlying subchondral bone in osteochondral lesions. To meet this need, a biomimetic multi-layered collagen-based scaffold, has recently been

designed and developed within our research group for the repair of osteochondral defects [9,10]. This biomimetic scaffold mimics the stratified composition of native osteochondral tissue by seamlessly integrating three distinct collagen-based layers using a novel iterative layering freeze-drying technique. The result is a multi-layered scaffold consisting of a base layer, previously optimised alone for bone repair [11,12], composed of type I collagen and hydroxyapatite (HA); an intermediate layer composed of type I and type II collagen and HA; and a superficial layer, previously optimised for cartilage repair, composed of type I and type II collagen in addition to the glycosaminoglycan hyaluronic acid (HyA) (Fig. 1). The specific composition of each layer has been previously optimised within our group, with the inclusion of HA shown to increase the osteogenic properties of collagen scaffolds [11–13] and the inclusion of HyA and type II collagen shown to result in improved chondrogenic properties [14–16]. This multi-layered scaffold is characterised by seamless integration between layers, a high porosity and a highly interconnected pore structure [9]. The extracellular matrix macromolecules and biomechanical properties of this scaffold are designed to direct the differentiation of cells to produce cartilage, calcified cartilage and bone within each region of the scaffold. This scaffold has shown promising results *in vitro* and thus in order to assess its potential for translation into clinical use, assessment *in vivo* in an animal model is required.

The objective of this study was to assess the *in vivo* response of this biomimetic collagen-based multi-layered scaffold and determine its ability to facilitate the repair of osteochondral tissue in a critical-sized, weight bearing defect in a rabbit knee. The specific aims were to evaluate the scaffold's ability to support host cell infiltration and matrix deposition *in vivo*, and to investigate if the composition and micro-structure will direct regenerative responses within the distinct layers of the scaffold leading to tissue regeneration with a zonal organisation similar to that of native osteochondral tissue i.e. superficial articular cartilage, intermediate calcified cartilage and deep subchondral bone.

2. Materials and methods

2.1. Multi-layered scaffold fabrication

Multi-layered collagen-based osteochondral scaffolds were fabricated as previously described [9]. Briefly, this involved fabrication of individual collagen-based suspensions for the bone layer, intermediate layer and cartilage layer of the scaffold. The bone layer contains 0.5% (w/v) microfibrillar bovine tendon type I collagen (Col1) (Collagen Matrix Inc., NJ, USA) and 1% (w/v) Hydroxyapatite (HA) powder (Captal 'R' Reactor Powder, Plasma Biotol, UK). The intermediate layer contains type I collagen (Col1), type II collagen (Col2) (Porcine type 2 collagen, Biom'up, Lyon, France) in 0.5 M acetic acid and adding HA to give final concentrations of 0.5% (w/v) Col1, 0.5% (w/v) Col2 and 0.2% (w/v) HA. The cartilage layer consists of 0.125% (w/v) Col1, 0.375% (w/v) Col2 and 0.05% (w/v) HyA. A novel 'iterative layering freeze-drying' process was used to fabricate multi-layered scaffolds [9,10]. Briefly, 11.7 ml of the base layer suspension was pipetted into a stainless-steel tray (internal dimensions, 60 mm × 60 mm) before being freeze-dried (Virtis Genesis 25EL, Biopharma, Winchester, UK) at a constant cooling rate of 1 °C min⁻¹ to a final freezing temperature of –40 °C [17,18]. Following freeze-drying, the base layer was cross-linked using 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDAC)/N-hydroxysuccinimide (NHS) (Sigma–Aldrich, Arklow, Ireland) crosslinking at a ratio of 5:2 M and a 6 mM concentration of EDAC g⁻¹ of collagen. The intermediate layer was formed by pipetting 3.9 ml of the intermediate layer suspension on top of the hydrated base layer scaffold and freeze-drying as before. Following

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