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Reduced hydraulic permeability of three-dimensional collagen scaffolds attenuates gel contraction and promotes the growth and differentiation of mesenchymal stem cells

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ABSTRACT

Optimal scaffold characteristics are essential for the therapeutic application of engineered tissues. Hydraulic permeability (k) affects many properties of collagen gels, such as mechanical properties, cell–scaffold interactions within three dimensions (3D), oxygen flow and nutrient diffusion. However, the cellular response to 3D gel scaffolds of defined k values has not been investigated. In this study, unconfined plastic compression under increasing load was used to produce collagen gels with increasing solid volume fractions. The Happel model was used to calculate the resulting permeability values in order to study the interaction of k with gel mechanical properties and mesenchymal stem cell (MSC)-induced gel contraction, metabolism and differentiation in both non-osteogenic (basal medium) and osteogenic medium for up to 3 weeks. Collagen gels of fibrillar densities ranging from 0.3 to >4.1 wt.% gave corresponding k values that ranged from 1.00 to 0.03 μm^2. Mechanical testing under compression showed that the collagen scaffold modulus increased with collagen fibrillar density and a decrease in k value. MSC-induced gel contraction decreased as a direct function of decreasing k value. Relative to osteogenic conditions, non-osteogenic MSC cultures exhibited a more than 2-fold increase in gel contraction. MSC metabolic activity increased similarly under both osteogenic and non-osteogenic culture conditions for all levels of plastic compression. Under osteogenic conditions MSC differentiation and mineralization, as indicated by alkaline phosphatase activity and von Kossa staining, respectively, increased in response to an elevation in collagen fibrillar density and decreased gel permeability. In this study, gel scaffolds with higher collagen fibrillar densities and corresponding lower k values provided a greater potential for MSC differentiation and appear most promising for bone grafting purposes. Thus, cell–scaffold interactions can be optimized by defining the 3D properties of collagen scaffolds through k adjustment.

1. Introduction

Collagens (types I–IV) are the dominant protein constituents of the connective tissue extracellular matrix (ECM), contributing to the biological and structural integrity of skin, tendons, blood vessels, cartilage and bone [1–3]. Due to its fibrillar structure, tensile properties, biocompatibility and biodegradability, collagen type I is a favorable protein substrate for diverse biomedical applications [4–8]. In particular, tissue engineering using in vitro reconstituted three-dimensional (3D) collagen type I gel scaffolds is increasingly applied to produce a biomimetic environment for cell growth [3,9].

Engineered tissue scaffolds should provide: (1) mechanical support for tissue replacement or regeneration, (2) an environment that favors cell integration, proliferation and cell differentiation for the desired therapeutic effect and (3) a permeable matrix that allows efficient nutrient, gas and waste diffusion [10–12]. The chemical composition, physico-mechanical properties (e.g. stiffness) and 3D architecture of the hydrated gels have been shown to affect proliferation, morphology, gene expression and the fate of stem cells [13–17]. Conversely, cell–scaffold interactions through cell integrin receptor heterodimers contribute to the physico-mechanical properties of scaffolds by transducing myosin activity along actin filaments, thereby producing contractile forces that remodel the matrix (e.g. pore size, permeability and density) [18,19]. Collagen gel contraction is further regulated, in part, by cell type, cell density and collagen concentration [19,20]. The relative difference between cell and matrix stiffness values has been

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identified as a key factor in matrix remodeling and contraction. Closely matched values of 3D collagen matrices and cells have been shown to not only change cell shape, but also result in matrix remodeling and contraction [21–23].

Permeability (k) is defined as the ability of a porous structure to transfer fluid through its interstices under an applied pressure which drives fluid flow [24,25]. Thus, the poroviscoelastic characteristic of scaffolds – stiffness, mass transport and, consequently, cell–cell and cell–scaffold interactions, cell signaling, morphogenesis and differentiation within 3D scaffolds – are each potentially influenced by k [23,26–30]. There are several important parameters affecting scaffold permeability, including porosity, pore size and distribution, interconnectivity and pore orientation [30].

Mesenchymal stem cells (MSCs) isolated from bone marrow are self-renewing cells that are capable of differentiating into osteoblasts, adipocytes and chondrocytes [31–33]. Since there is a balance between mechanical properties and permeability in scaffolds, it is hypothesized that the control of k may influence MSC-induced gel contraction, as well as growth and osteoblastic differentiation. However, measurement of the hydraulic permeability of hydrated gel scaffolds is difficult due to their structural fragility. Consequently, few data exist describing the permeability of naturally derived polymer scaffolds. In the case of collagen gel systems, a number of studies conducted under different conditions have been reported, with varied results for hydraulic permeability [29,30,33]. To tackle these experimental limitations a number of theoretical models have been developed to predict the permeability of various materials and biomaterials [29,33–35]. In the case of fibrillar materials the Happel model [35] is a useful method to describe the relationship between hydraulic permeability of a random array of long cylindrical fibers and the geometry. In this study, hydraulic permeability of different collagen gel scaffolds was calculated by applying the Happel model to govern geometry in these scaffolds.

Plastic compression was used to rapidly generate tissue scaffolds with controlled collagen fibrillar densities (CFDs) approaching those of native tissues. Plastic compression significantly improves the biomechanical properties of collagen scaffolds without adverse effects on the viability and metabolism of resident cells [36–39]. In the current work collagen matrices of three different fibrillar densities and associated k values were produced by PC and seeded with MSC in order to assess the effect of induced microstructural changes on MSC-induced gel contraction, metabolism and differentiation over the course of 3 weeks. By defining cell–scaffold properties in long-term cultures, k can be applied to engineer optimal scaffolds for bone tissue engineering.

2. Materials and methods

2.1. Collagen gel preparation

Collagen scaffolds were prepared by adding 0.4 ml of 10× Dulbecco's modified Eagle's medium (DMEM) (Sigma, St. Louis, MO) to 1.6 ml of sterile rat tail type I collagen dissolved in 2.2 mg ml^{-1} acetic acid (First Link Ltd., Birmingham, UK) and neutralized with 5 M NaOH, resulting in a final volume of 2 ml. Collagen solution (0.9 ml) was distributed into 24-well plates (15.6 mm in diameter) and placed in a tissue culture incubator for 30 min at 37 °C for collagen gel polymerization.

2.2. Plastic compression and permeability calculations

In order to generate gels with different CFDs plastic compression using different static stresses of 0, 343, 690 and 1022 N m^{-2} (referred to as PC0, PC1, PC2 and PC3, respectively), was applied (Table 1). As made highly hydrated collagen gels were transferred to a porous support consisting of (bottom to top) absorbent paper blot layers, a stainless steel mesh and a polymer mesh (Fig. 1A) [37]. Gel specimens underwent unconfined compression by loading either one, two or three glass microscope slides (75 × 25 × 1 mm^3) on top of the hydrated gels for 2 min. Thereupon, Happel's model for flow through a random array of long cylindrical fibers [35] was used to theoretically predict the hydraulic permeability of partially compressed collagen gels as a function of solid volume fraction. This model may be summarized as:

Table 1
Effect of plastic compression for 2 min on collagen fibrillar density (CFD) and solid volume fraction (σ).

Sample	Compressive stress (Nm^{-2})	Mass loss (%)	CFD (wt.%)	σ
PC0	0		0.29 ± 0.03	0.002 ± 0.44 × 10^{-4}
PC1	343	87.89 ± 0.75	2.39 ± 0.10	0.017 ± 14.32 × 10^{-4}
PC2	690	91.19 ± 0.83	3.29 ± 0.03	0.024 ± 27.68 × 10^{-4}
PC3	1022	93.01 ± 0.52	4.14 ± 0.12	0.030 ± 28.97 × 10^{-4}

To measure their CFD, gel specimens were snap frozen in liquid nitrogen followed by freeze drying for 48 h to determine the dry weight. Values are presented as means ± standard deviations.

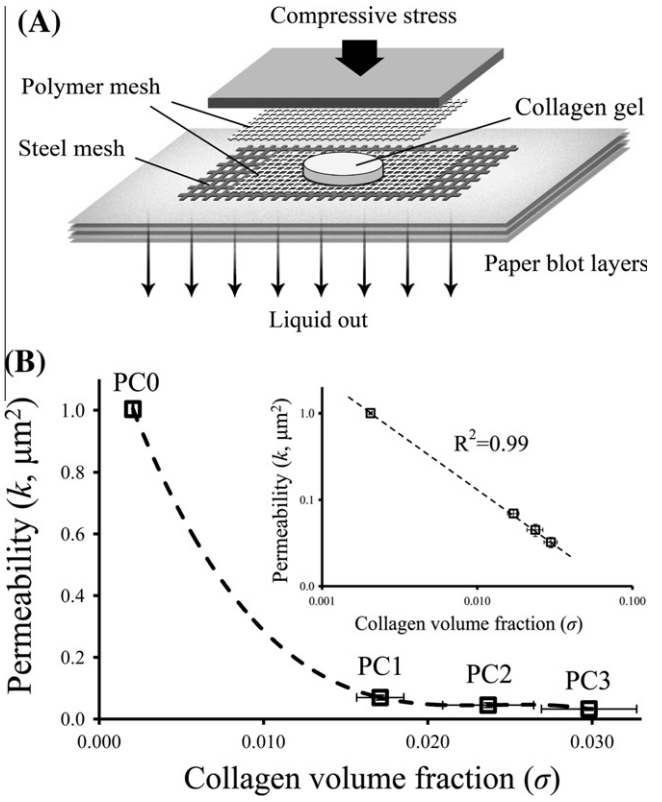

Fig. 1. Generation of collagen gel matrices of defined hydraulic permeability values (k). (A) Schematic representation of the experimental set-up used to apply increasing levels of plastic compression to highly hydrated collagen gel. Cast highly hydrated collagen gels were placed on a porous support consisting of (bottom to top) absorbent paper blot layers, a stainless steel mesh and a polymer mesh (reconstructed from [37]) and compressed using 343, 690 or 1022 Pa. (B) Hydraulic permeability of non-compressed and plastic compressed collagen gels as a function of collagen volume fraction (σ). Increasing the plastic compression level from PC0 to PC3 resulted in a decrease in k. When plotted on a logarithmic scale (inset), k–σ exhibited a linear relationship. Scaffolds with distinct initial k values were generated by modulating the compression stress. A minimum of four replicates were analyzed for each level of PC.

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