Available online at www.sciencedirect.com
ScienceDirect
Acta Biomaterialia 5 (2009) 1006–1018
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Density–property relationships in mineralized collagen–glycosaminoglycan scaffolds

Biraja P. Kanungo, Lorna J. Gibson *

Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

Received 26 August 2008; received in revised form 18 November 2008; accepted 25 November 2008
Available online 11 December 2008

Abstract

Mineralized collagen–glycosminoglycan scaffolds have previously been fabricated by freeze-drying a slurry containing a co-precipitate of calcium phosphate, collagen and glycosaminoglycan. The mechanical properties of the scaffold are low (e.g. the dry Young's modulus for a 50 wt.% mineralized scaffold is roughly 780 kPa). Our previous attempt to increase the mechanical properties of the scaffold by increasing the mineralization (from 50 to 75 wt.%) was unsuccessful due to defects in the more mineralized scaffold. In this paper, we describe a new technique to improve the mechanical properties by increasing the relative density of the scaffolds. The volume fraction of solids in the slurry was increased by vacuum-filtration. The slurry was then freeze-dried in the conventional manner to produce scaffolds with relative densities between 0.045 and 0.187 and pore sizes of about 100–350 μm, values appropriate for bone growth. The uniaxial compressive stress–strain curves of the scaffolds indicated that the Young's modulus in the dry state increased from 780 to 6500 kPa and that the crushing strength increased from 39 to 275 kPa with increasing relative density. In the hydrated state, the Young's modulus increased from 6.44 to 34.8 kPa and the crushing strength increased from 0.55 to 2.12 kPa; the properties were further increased by cross-linking. The modulus and strength were well described by models for cellular solids.

Keywords: Mineralized scaffolds; Collagen; Mechanical characterization; Microstructural characterization; Relative density

1. Introduction

Scaffolds for tissue regeneration are defined as: "three-dimensional open-cell porous structures synthesized from either natural or synthetic polymers which have the potential to support attachment, migration and multiplication of living cells" [1]. Although unproven, a widely believed design paradigm for scaffolds is that mimicking the composition of the natural tissue as closely as possible improves the capacity for regeneration [2]. The ability of a scaffold to regenerate tissue depends on its pore size, pore shape, porosity, biodegradability and mechanical properties. The average pore diameter must be large enough for cells to migrate through the pores yet small enough to retain an appropriate specific surface area for sufficient cell binding. For example, pore sizes in excess of 100 μm are optimal for bone growth [2–4]. Equiaxed pore shape and homogeneity are optimum for uniform cell adhesion and distribution of extracellular matrix proteins. Scaffolds must have large enough porosity (generally greater than 90%) and interconnectivity for effective transfer of cells and metabolites [5]. The degradation rate of the scaffold has to be roughly equal to the regeneration rate of the tissue. Furthermore, cells have been observed to be sensitive to the mechanical properties of the scaffold, which in turn affects the overall construct bioactivity [6].

Ideally, scaffolds should be similar to their natural counterparts in terms of chemical composition and physical structure. For this reason, natural polymers such as collagen are of major interest. To this end, collagen–glycosaminoglycan (CG) scaffolds have been developed and used clinically for skin regeneration and experimentally for nerve regeneration over the past three decades [7–19].

* Corresponding author. Tel.: +1 617 253 7107; fax: +1 617 258 6275.
E-mail address: ljgibson@mit.edu (L.J. Gibson).

1742-7061/$ - see front matter © 2008 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2008.11.029

Composite scaffolds of collagen or gelatin with ceramics (e.g. hydroxyapatite and tricalcium phosphate), i.e. mineralized CG (MCG) scaffolds, have been developed to regenerate hard tissues such as bone [20–23]. The most recent fabrication technique improves upon this mineralization process by forming a triple co-precipitate of mineral, collagen and glycosaminoglycan, without using a titrant, by controlling the molarity of the reactant acid and molar ratios of the different calcium sources [24–30]. Due to the in situ co-precipitation of the mineral phase, calcium phosphate crystals form within the collagen fibers, resulting in a more uniformly mineralized scaffold. Freeze-drying is then used to fabricate porous scaffolds from the triple co-precipitated slurry. These MCG scaffolds have regenerated subchondral bone at 16 weeks in a 4 mm diameter and 6 mm deep defect site at the knee joint in a goat model [31].

Extensive microstructural and mechanical characterization of CG and of MCG scaffolds of varying mineral content has been reported by Harley et al. [32] and by Kanungo et al. [30], respectively. Critical mechanical properties of scaffolds include elastic modulus, E^*, compressive crushing strength, σ^*, and compressive crushing strain, ε^*. The mechanical properties of different MCG scaffolds (along with the triple co-precipitated scaffolds) have been compared in the literature [30]. The triple co-precipitated mineralized scaffolds have relative densities (the density of the cellular solid, ρ^*, divided by that of the solid from which it is made, ρ_s) of roughly 0.03–0.04; that of trabecular bone varies from 0.05 to 0.60 [33,34]. The mechanical properties of human compact and trabecular bone, along with 50 wt.% MCG scaffold (with a relative density, ρ/ρ_s, of 0.04), are listed in Table 1.

It is critical that the scaffold should have sufficient stiffness and strength to maintain its shape and size during surgical procedures such as implantation and to enhance bone in-growth while preventing encroachment of non-osseous tissue and competing cell types after implantation [35]. The optimal requirements for the above properties vary depending on the defect site and there are no established optimal magnitudes of the mechanical properties for bone scaffolds [36]. The current triple co-precipitated MCG scaffold (with a ρ/ρ_s of 0.04) can be crushed by hard thumb pressure. Hence, it is critical to improve the mechanical properties of MCG scaffolds such that they can be functionally suitable for bone regeneration. The mechanical properties (E^* and σ^*) of the scaffold depend on those of the solid (E_s and σ_{fs}) they are made from as well as the relative density of the scaffold, (ρ^*/ρ_s) [5,30,32,37]. The overall properties of the scaffolds can be improved by either improving the properties of the solid it is made from or by increasing the relative density of the scaffold. Previous attempts to increase the mechanical properties of the scaffold by increasing the mineral content led to scaffolds with poorer mechanical properties due to the introduction of defects [30]. Our previous attempts to improve the mechanical properties by increasing the volume fraction of the components of the slurry have not been successful due to the difficulty in mixing the viscous slurry at higher volume fractions of the mineral, collagen and GAG [32]. In this paper we describe a new technique to improve the mechanical properties by increasing the relative density of the scaffold by a vacuum filtration technique.

Table 1
Mechanical properties of human compact bone, trabecular bone and 50 wt.% MCG scaffold with $\rho^*/\rho_s = 0.04$ [30,63–65].

	Condition	E^* (MPa)	σ^* (MPa)
Human compact bone	Wet	10,000–20,000	110–254
	Dry	16,500–33,000	170–390
Human trabecular bone	Wet	90–1000	1–30
	Dry	150–1650	1.5–45
50 wt.% MCG scaffold ($\rho^*/\rho_s = 0.04$)	Wet	0.004–0.015	0.0003–0.002
	Dry	0.7–1	0.03–0.1

2. Materials and methods

2.1. Fabrication of mineralized collagen–glycosaminoglycan suspension

A mineralized CG suspension (50 wt.% mineral) was fabricated using microfibrillar, type I collagen isolated from bovine achilles tendon (Sigma–Aldrich Chemical Co, St. Louis, MO), chondroitin-6-sulfate (GAG) isolated from shark cartilage (Sigma–Aldrich), phosphoric acid (H_3PO_4) (EMD Chemicals Inc., Gibbstown, NJ), calcium nitrate ($Ca(NO_3)_2{\cdot}4H_2O$) and calcium hydroxide ($Ca(OH)_2$) (Sigma–Aldrich). The suspension was prepared by combining collagen (0.019 wt.%), GAG (0.002 wt.%), calcium nitrate (0.004 wt.%) and calcium hydroxide (0.009 wt.%) in a solution of 0.14 M phosphoric acid (pH 1.47) through a triple co-precipitation method described elsewhere [5,24–30]. Briefly, the collagen was mixed with phosphoric acid solution at 15,000 rpm and 4 °C in an overhead blender (IKA works Inc., Wilmington, NC) for 60 min. Glycosaminoglycan was added to the collagen–phosphoric acid mix using a peristaltic pump (Manostat, New York, NY) and mixed for another 60 min at 15,000 rpm. Dry-mixed calcium nitrate and calcium hydroxide were added to the collagen–GAG—phosphoric acid solution and mixed for additional 15 min at 15,000 rpm. The suspension was mixed for an additional 24 h on a magnetic stirrer at room temperature and was stored at 4 °C. The co-precipitate within the slurry had a mass of 0.042 g per 1 ml of slurry; we refer to the density of the co-precipitate within the slurry as 0.042 g ml^{-1}.

2.2. Densification of the slurry

The above slurry (denoted as 1×, where $1\times \equiv 0.042$ g ml^{-1}) was densified using the set-up shown in Fig. 1. A cylindrical polysulfone mold (McMaster Carr Supplies, Dayton, NJ), with an inside diameter of 5.7 cm, was perforated at the base with holes 1 mm in diameter for filtering the solvent to the aluminum base. Two filter papers with