Biomaterials 32 (2011) 8990–8998

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

The development of collagen-GAG scaffold-membrane composites for tendon tissue engineering

Steven R. Caliari [a], Manuel A. Ramirez [b], Brendan A.C. Harley [a,c,*]

[a] Dept. Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
[b] Dept. Bioengineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
[c] Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

ARTICLE INFO

Article history:
Received 6 June 2011
Accepted 14 August 2011
Available online 30 August 2011

Keywords:
Collagen
Scaffold
Membrane
Composite
Tendon
Mechanical properties

ABSTRACT

Current tissue engineering approaches for tendon defects require improved biomaterials to balance microstructural and mechanical design criteria. Collagen-glycosaminoglycan (CG) scaffolds have shown considerable success as *in vivo* regenerative templates and *in vitro* constructs to study cell behavior. While these scaffolds possess many advantageous qualities, their mechanical properties are typically orders of magnitude lower than orthopedic tissues such as tendon. Taking inspiration from mechanically efficient core–shell composites in nature such as plant stems and porcupine quills, we have created core–shell CG composites that display high bioactivity and improved mechanical integrity. These composites feature integration of a low density, anisotropic CG scaffold core with a high density, CG membrane shell. CG membranes were fabricated via an evaporative process that allowed separate tuning of membrane thickness and elastic moduli and were found to be isotropic in-plane. The membranes were then integrated with an anisotropic CG scaffold core via freeze-drying and subsequent crosslinking. Increasing the relative thickness of the CG membrane shell was shown to increase composite tensile elastic modulus by as much as a factor of 36 in a manner consistent with predictions from layered composites theory. CG scaffold-membrane composites were found to support tendon cell viability, proliferation, and metabolic activity *in vitro*, suggesting they maintain sufficient permeability while demonstrating improved mechanical strength. This work suggests an effective, biomimetic approach for balancing strength and bioactivity requirements of porous scaffolds for tissue engineering.

1. Introduction

Tendons are specialized connective tissues that transmit tensile loads between bone and muscle. Their functional capacity derives from a unique extracellular matrix (ECM) composed primarily of type I collagen arranged in a highly organized hierarchy of parallel, crosslinked fibrils [1,2]. Tendon and ligament injuries are common among both recreational and elite athletes as well as the elderly. Of the near 35 million musculoskeletal injuries in the US every year, approximately 50% involve tendons and ligaments with a cost to the US health care industry in the tens of billions of dollars per year [3]. The most serious injuries require surgical intervention; such tendon and ligament injuries are responsible for hundreds of thousands of surgical procedures each year in the US [2,3].

One of the key challenges of orthopedic tissue engineering is to create biomaterials that can support tissue regeneration while remaining mechanically competent. Due to the need for mechanical competence, the most common biomaterial designs for tendon and ligament tissue engineering are electrospun polymer mats [4–6] and woven fibrous materials [7,8]. While these constructs can promote cell alignment and be designed with tensile moduli approaching the level of tendon, they are dense substrates that permit limited cell penetration compared to the traditional tissue engineering target for a fully three-dimensional biomaterial structure. As an alternative, porous scaffold biomaterials typically have highly tunable 3D microstructural features, show significantly heightened levels of permeability, and can be fabricated from a range of natural, biodegradable materials. However, the relative density (ρ^*/ρ_s; 1% porosity where ρ^* is the density of the porous foam and ρ_s is the density of the solid it is constructed from) of these porous scaffolds differentially affects a number of critical scaffold properties. Notably, increasing scaffold ρ^*/ρ_s increases both its specific surface area, impacting cell attachment, and its elastic

* Corresponding author. Dept. of Chemical and Biomolecular Engineering, Institute of Genomic Biology, University of Illinois at Urbana-Champaign, 110 Roger Adams Laboratory, 600 S. Mathews Ave., Urbana, IL 61801, USA. Tel.: +217 244 7112; fax: +217 333 5052.
E-mail address: bharley@illinois.edu (B.A.C. Harley).

0142-9612/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2011.08.035

modulus, which varies with $(\rho^*/\rho_s)^2$ [9–12]. However, increasing scaffold ρ^*/ρ_s also increases steric hindrance to cell penetration and, critically, reduces scaffold permeability [13], negatively impacting cell penetration into the porous structure and long-term survival. Due to the high porosity (>90%) typically required for most tissue engineering scaffolds to adequately support cell bioactivity [14], these materials are often orders of magnitude too soft for orthopedic applications such as for tendon. Mechanical stimulation of cell-seeded scaffold constructs has been used to marginally improve construct mechanical properties, however not to the level of native tendon or ligament [15–18]. Coupled with the known anisotropy of native tendon tissue, strategies to produce mechanically-reinforced, anisotropic biomaterial scaffolds may present a pathway toward developing bioactive tendon regeneration templates.

Porous collagen-glycosaminoglycan (CG) scaffolds have previously been used in a variety of tissue engineering applications, both *in vivo* as regenerative templates for skin, peripheral nerves, conjunctiva, and cartilage [19–21] as well as *in vitro* as 3D microenvironments to probe fundamental questions about cell behaviors and cell–matrix interactions [22–26]. These scaffolds have pore microstructures designed to simultaneously block cell-mediated contraction and scar tissue synthesis while supporting cell attachment, proliferation, efficient metabolite transport, and synthesis of a functional ECM [11,21]. Notably, their chemical composition and degradation kinetics have been specifically optimized to prevent platelet accumulation and organized wound contraction [21]. The CG scaffolds are traditionally fabricated via freeze-drying where the freezing rate [27] and final freezing temperature [22,28] can be manipulated to affect scaffold pore size and uniformity. Recently, a directional solidification approach has been introduced that enables fabrication of a series of highly anisotropic CG scaffolds characterized by aligned tracks of ellipsoidal pores for tendon tissue engineering applications [29]. A significant effect was observed for both pore shape (anisotropic vs. isotropic) and anisotropic scaffold pore size (50–250 μm) on the attachment, proliferation and metabolic activity of primary equine tendon cells [29]. These CG scaffolds possess the 3D structure, porosity (>95%), and bioactivity required of an orthopedic regeneration template, but mechanically are 2–3 orders of magnitude softer than native tendon ($E^* \sim 10^2$–10^3 MPa) [30,31]. Alternatively, a series of collagen membranes have recently been described for applications in skin [32], peripheral nerve [33], and bone [34] repair. These membranes provide an avenue to theoretically present the same combinations of bioactive ligands as CG scaffolds but with improved mechanical properties. However, these collagen membranes lack a 3D microstructure or the porosity required for cell integration.

While the multi-scale properties of tendon itself cannot be replicated by current biomaterials technologies, composite materials that mimic mechanically efficient core–shell structures in nature may hold promise for complex tissue engineering applications. Plant stems combine a porous core with a dense shell to aid osmotic transport (core) while maintaining sufficient tensile/bending stiffness (shell); bird beaks and porcupine quills can also combine a dense shell and porous core to enhance compressive strength and mechanical efficiency, respectively [10]. Core-shell composite designs have been used to engineer high strength structural materials [35], but have not seen extended application in the field of biomaterials. A composite biomaterial that combines, but does not functionally integrate, an agarose hydrogel and a nanofiber electrospun mat has recently been described for fibrous tissue (intervertebral disk, meniscus) regeneration applications [36]. A recently described liquid-phase co-synthesis methodology provides the ability to create composite CG scaffolds featuring multiple, distinct biomaterial compartments linked together by a continuous interface [37]. While originally developed to link two disparate scaffold compartments for osteochondral tissue engineering, this technology provides a pathway for creating a novel composite biomaterial that functionally integrates CG scaffold and membrane elements. We believe adding the CG membrane can significantly increase the tensile strength of the subsequent scaffold-membrane composite in a manner consistent with layered composite theory predictions [38].

This manuscript describes the development of a new class of core–shell CG biomaterial composites that integrate a high density (high tensile strength) isotropic CG membrane with a low density (highly porous) anisotropic CG scaffold (Fig. 1). We hypothesized that the CG membranes could be integrated with aligned CG scaffolds in a manner to maintain adequate permeability to support

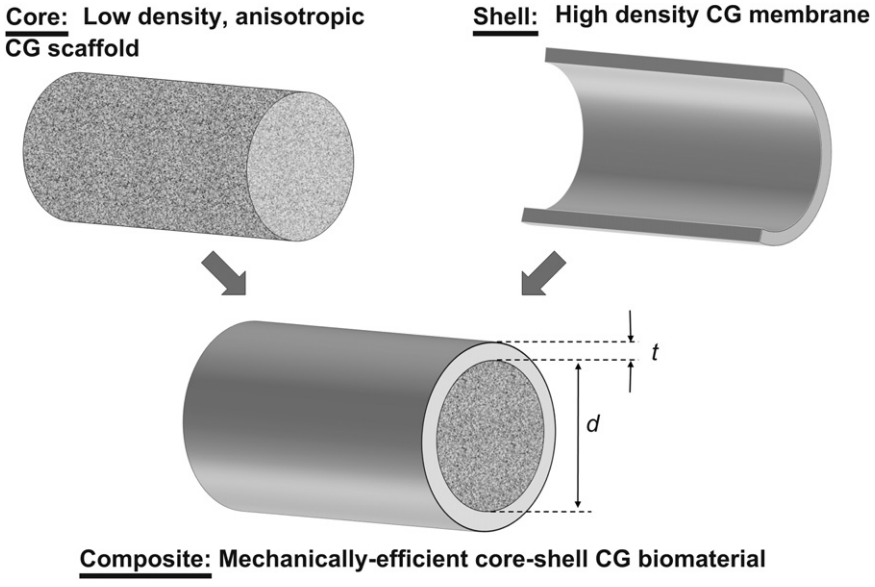

Fig. 1. Schematic of CG scaffold-membrane composite design.