Composite Structures 153 (2016) 392–400

Contents lists available at ScienceDirect

Composite Structures

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

Mechanical flexure behavior of bio-inspired collagen-reinforced thin composites

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ARTICLE INFO

Article history:
Received 8 February 2016
Revised 16 May 2016
Accepted 15 June 2016
Available online 17 June 2016

Keywords:
Bio-composite
Collagen
Fibers
Indentation
Finite element
Hyperelastic

ABSTRACT

Soft tissue can be viewed as a multi-layered composite structure reinforced with different collagen fiber systems used to control the overall mechanical properties. The objective of this study was to manufacture, test and model the mechanical behavior of a multidirectional collagen reinforced composite material system. To that end, novel bio-composite constructs were fabricated from long collagen fibers reinforced polyacrylamide–alginate (PAAm–Alg) matrix. The collagen fibers were aligned in multi-directions within thin circular matrix plates. The constructs were clamped and subjected to flexure using a rigid spherical indenter. Three dimensional finite element (FE) models were generated with continuum and beam elements representing the matrix and fibers, respectively. The hyperelastic behavior predicted by the calibrated FE models was validated based on the results of one tested configuration. The new bio-inspired composites can be tailored to generate similar mechanical behavior of native tissues. The FE model can be employed to design future complex constructs for soft tissue substitutes.

1. Introduction

Soft tissues are often considered as collagen fiber reinforced composite structures characterized by several hierarchical levels, from the nano-scale to the macro-scale. Similar basic materials (i.e. collagen and proteoglycans) with different structural organization can generate entirely different tissues such as corneas and tendons [1]. The structural level presents an additional complexity to the tissues by regional structural changes in fiber density and orientation [2]. These spatial heterogeneous structures differ in their oriented mechanical responses depending on their loading mode.

Biomimetics of soft tissues requires mechanical compatibility as well as biocompatibility [3]. Thus, a complete new construct should simultaneously mimic and perform mechanical behaviors similar to the physiological modes at the macro and micro-structural levels, in order to replace the native tissue. Development of fiber reinforced composite structures for soft tissue substitutes is a promising approach for tissue regeneration, for example, corneal laminates were fabricated of cross-plied gelatin nano-fibers embedded in a hydrogel matrix [4]. Multi-layered abdominal wall grafts made of collagen fibers were also suggested [5,6]. Vascular grafts [7] were produced from angle-plied collagen fibers embedded in elastin matrix. Furthermore, Angle-plied (±30°) laminates were fabricated based on PCL nano-fibers seeded with cells [8,9], arranged in an intervertebral discolor structure with agarose core as nucleus pulposus [10].

Direct mechanical tensile characterization methods of tissues are widely used. However, several studies have applied nano and macro-level indentations as viable techniques for mechanical testing of soft tissues and their substitutes [11–15]. A ball-indentation method applied at the center of a clamped hydrogel membrane was developed to derive the nonlinear mechanical behavior from the geometry and the membrane deflection [16,17]. This technique is axisymmetric and thus advantageous for anisotropic tissues that are subjected to flexure. Similar tests (macro and micro indentations) were conducted on several biomaterials (such as alginate and corneal cells seeded in a hydrogel matrix) and soft tissues (cornea and extracellular matrix membranes) to examine their nonlinear mechanical behavior [18–22].

Bio-mimetic based designs of complex material and geometrical constructs can be improved using computational models that allow to predict and tailor their mechanical behaviors. These *in silico* designs can be used to accelerate and improve bio-engineering functionalities [3,23]. Computational models have been used to

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http://dx.doi.org/10.1016/j.compstruct.2016.06.031
0263-8223/

simulate the mechanical behavior and failure of native tissues [24–30] in various numerical methods (e.g. homogenization method), to explore the tissue geometry in different scales [31,32] and to examine the influence of fiber orientation and anisotropic behavior [33–36]. Furthermore, numerical models have been used to compare bio-engineered constructs to native tissue structures under physiological loading modes [35,37], and to examine the influence of fiber fraction and orientation on reinforced composites with hyperelastic material behavior [33]. Thus, fabrication of new bio-composite structures integrated with computational simulations can lead to new classes of engineered soft-tissue substitutes [3].

This study demonstrates the fabrication of new multidirectional collagen-fiber reinforced composites and their mechanical behavior under flexural loading. We present a new hyperelastic material system of PAAm–Alg hydrogel [38] reinforced with long collagen fibers. The collagen fibers [33,39] were aligned in a plane of thin hydrogel sheets to create reinforcement every 30 degrees with a transparent optical center, bio-inspired by the cornea structure. These composite constructs were circumferentially clamped and mechanically tested by a rigid spherical indenter pushed onto their center. Three dimensional and hyperelastic FE models were generated to test the influence of different multi-directional fiber architectures on the mechanical behavior. Experimental indentations were performed to verify the predictions of the FE models.

2. Materials and methods

2.1. Bio-composite fabrication and material characterization

2.1.1. Isolation and purification of collagen fibers

Collagen fibers were harvested from soft coral sarcophyton sp. tissue stored in 70% ethanol as previously described [33,39]. The long collagen fibers were wrapped around a thin hexagonal stainless steel frame. To create organized fiber angle-plied arrays, the fibers were wrapped onto every parallel side (at 60° angles), avoiding the center of the hexagon. For the longitudinal samples, the fibers were wrapped onto a rectangular stainless steel frame to create a uniaxial arrangement of the fibers. The arranged collagen fibers were then washed with ethanol (70% v/v) and double-distilled water (DDW).

2.1.2. Fabrication

Two arrayed collagen fiber hexagonal frames were stacked thus generating aligned fibers every 30° with a clear center which were then inserted into a round mold. The longitudinal samples were inserted into a rectangular mold. The samples were covered with 45 mM EDC/NHS crosslinker (N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride, Sigma–Aldrich, Israel)/(N-Hydroxysuccinimide, Sigma–Aldrich, Israel).

Fabrication of the PAAm–Alg hydrogel was performed as described by Sun et al. [38] using a mixture of 1:6 Acryl-Amide (Gibco-BRL) and alginate. The mixture was cross-linked with TEMED (Bio-Rad, USA), Bis-acryl amide (Bio-Rad) and ammonium persulphate (Bio-Rad). The hydrogel solution was inserted into the mold and covered the arranged collagen fibers. The sample was topped with a dialysis membrane (6000–8000 MWCO, Spectra Por, SpectrumLabs, USA) and covered with a 0.1 M $CaCl_2$ (Merck, USA) solution. The samples were photo-cross-linked under a UV light (12 W, Uvitec Cambridge) for an hour and then transferred to a 0.1 M $CaCl_2$ solution for 48 h at room temperature to continue the ionic gelation. The complete bio-composite construct was then removed from the mold and kept in the metal frame for the flexure test.

Rectangular samples (matrix and composite) were removed from the metal frame prior to the unidirectional loading. The matrix samples were fabricated as listed above, excluding the insertion of the collagen fibers.

Fiber fractions were determined using image analysis; images of the aligned fibers were taken with a digital microscope (AM311S, BigCatch, Taiwan) on a dark background. The images were processed into binary numerical arrays, followed by calculating the percentage of the white pixels (representing the fibers) from the dark background. The fiber fraction was normalized to the final bio-composite thickness.

2.1.3. Scanning electron microscopy

Collagen fibers and PAAm–Alg–collagen composites were examined by a scanning electron microscope (SEM) (Quanta 200 FEG Environmental Scanning Electron Microscope). The samples were fixed in a solution of 4% formaldehyde and dehydrated through a series of graded ethanol agents. The samples were sputtered with a gold–palladium alloy and then examined by a SEM under high-vacuum conditions.

2.2. Mechanical testing

Tensile testing of the longitudinal composites and PAAm–Alg matrix was performed using an Instron 5582 loading frame with a 100 N load cell at a rate of 0.05 mm·s^{-1}. Samples were preconditioned and then stretched to failure. Flexural loading was performed as shown in Fig. 1(a–d): a rigid spherical indenter with a diameter of 15.7 mm was connected to the Instron machine and pushed into the center of a clamped composite construct membrane. Displacements and loads were measured by the Instron and its Bluehill operating software. The construct was gripped in a cylindrical PMMA holder with a circumferential socket designed to hold the construct metallic frame. The rigid indenter was positioned to touch the composite membrane prior to the beginning of the test. The samples were preconditioned to 10 cycles of 0.1 mm and then pushed down to failure at a rate of 0.05 mm s^{-1}.

2.3. Finite element analysis

A FE model was generated to simulate the mechanical behavior of the fabricated composite laminates using Abaqus FE software (Dassault Systems, Simulia Corp., Providence, RI, USA). Longitudinal alginate–collagen calibrated composite model [33] was implemented with a new PAAm–Alg matrix, taken from the matrix-alone tensile measurements. It was subjected to unidirectional tension for the calibration of the new material system by comparing it to the experimental results. The stress–strain behavior of the materials was hyperelastic and described in Table 1. The collagen's effective hyperelastic mechanical behavior was fitted using an Ogden strain energy density function [40] (3rd order, Eq. (1)). A Marlow hyperelastic model Eq. (2) [41] was used for the PAAm–Alg matrix that was considered to be homogenous and isotropic. The volumetric part of the equations was neglected due to the nearly incompressible nature of the bio-composite.

$$U = \sum_{i=1}^{N=3} \frac{2\mu_i}{\alpha_i^2}\left(\bar{\lambda}_1^{\alpha_i} + \bar{\lambda}_2^{\alpha_i} + \bar{\lambda}_3^{\alpha_i} - 3\right) + \sum_{i=1}^{N=3} \frac{1}{D_i}(J_{el} - 1)^{2i} \tag{1}$$

$$U = U_{dev}(\bar{\lambda}_1 + \bar{\lambda}_2 + \bar{\lambda}_3) + U_{vol}(J_{el}) \tag{2}$$

Transverse and angle-plied (±30°) PAAm–Alg–collagen laminate models were generated with FVF (fiber volume fraction) of 15% and their unidirectional tensile behavior was simulated. Three constructs were built by stacking several oriented laminates into a rectangular-shaped matrix with a thickness of 1.5 mm and 40 mm of length and width. Every model consisted of 6 layers of embedded fibers with the following orientations: 0/90/0/90/0/90