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Research Paper

A new class of bio-composite materials of unique collagen fibers

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ABSTRACT

A novel collagen-based bio-composite was constructed from micro-crimped long collagen fiber bundles extracted from a soft coral embedded in alginate hydrogel matrix. The mechanical features of this bio-composite were studied for different fiber fractions and in longitudinal and transverse loading modes. The tensile modulus of the alginate hydrogel was 0.60 ± 0.35 MPa and in longitudinal collagen-reinforced construct it increased up to 9.71 ± 2.80 for 50% fiber fraction. Ultimate tensile strength was elevated from 0.08 ± 0.04 MPa in matrix up to 1.21 ± 0.29 for fiber fraction of 30%. The bio-composite demonstrated hyperelastic behavior similar to human native tissues. Additionally, a dedicated constitutive material model was developed to enable the prediction of the longitudinal mechanical behavior of the bio-composite. These findings will allow tailor-designed mechanical properties with a quantitatively controlled amount of fibers and their designed spatial arrangement. This unique bio-composite has the potential to be used for a wide range of engineered soft tissues.

1. Introduction

Soft tissues can be viewed as an assembly of biomaterials that form a variety of heterogeneous material systems, each intended for distinct biological and mechanical environment.

Today, it is still a challenging task to develop scaffold for soft tissue repair that will provide appropriate mimic of native tissues. Tailoring the mechanical behavior of engineered tissues is essential, such as their resistance and response when subjected to tensile or compression loading forces, since mechanically unfitted implant may cause damage to the host tissue (Hollister, 2009). For instance, discrepancies in aortic tissue implant lead to differences in the local mechanical properties which ultimately can cause pseudo-aneurysm or intimal hyperplasia (Tremblay et al., 2009). These pathologies evolve due to local remodeling of the host tissue and are caused in part due to mechanical stress concentration.

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Collagen is a structural protein that functions as mechanical support and load bearing element in tissues. In addition, it holds a variety of biological functions as cell–matrix interactions and binder for other proteins (as fibronection, decorin, etc.) to promote essential cellular functions (Parenteau-Bareil et al., 2010). Thus, collagen-based materials serve as initiating basis in tissue engineering (Bowles et al., 2010; Caves et al., 2010a; Gentleman et al., 2003; Hahn et al., 2006; Parenteau-Bareil et al., 2010; Rafat et al., 2008; Zeugolis et al., 2008). Mammalian sourced collagens have been widely explored to produce scaffolds. In order to use them as biomaterials they demand processing and de-cellularization from tissues' associated cells, such processing may leave collagen with immunogenic residues. When they are not completely removed these residues can lead to inflammatory reactions (Fitzpatrick et al., 2010; Liao et al., 2008; Parenteau-Bareil et al., 2010). Additionally, processing procedures can reduce graft strength up to two orders of magnitude relative to the native collagen (Chen et al., 2008).

A variety of collagen strengthening methods, fiber formation and crosslinking techniques have been designed in order to construct the complex structure of native collagen or to develop suitable substitutes (Caves et al., 2010b; Kumbar et al., 2008; Tamayol et al., 2013; Telemeco et al., 2005; Zeugolis et al., 2008). Alternative collagen sources have been explored including marine (Benayahu et al., 2011; Jeong et al., 2007; Songa et al., 2006) and human recombinant collagen expressed in plants (Ruggiero et al., 2000). Achieving pure and strong collagen fibers as biomaterials is required for the various biomedical applications, combining strength and biocompatibility. The latter fibers can be embedded in matrix binders as layers or laminated in order to construct collagen-reinforced composites for tissue regeneration and repair. These matrices can be hydrogels as chitosan (Pok et al., 2013; Wright et al., 2012), gelatin (Pok et al., 2013) or gellan gum (Thorvaldsson et al., 2013). Such bio-composite might be considered advantageous since the hydrogel matrix provides soft and aqueous surroundings that benefit the resident cells, diffusion processes and three-dimensional structure, similar to the native proteoglycans and the stiff fibers allow mechanical robustness and structural strength (Pok et al., 2013; Thorvaldsson et al., 2013). Fiber reinforced bio-composites have recently been developed to provide desired mechanical and biological features for tissue-repair applications including cartilage (Wright et al., 2012), abdominal wall (Caves et al., 2011), blood vessels (Caves et al., 2010a; Kumar et al., 2013), cardiac tissue (Pok et al., 2013) and nucleus pulpous (Thorvaldsson et al., 2013).

Herein, we report the fabrication of a novel all-natural bio-composite material system based on unique long collagen fibers extracted from a soft coral (Benayahu et al., 2011) reinforcing an alginate hydrogel matrix (Haj-Ali et al., 2013). The fibers were isolated from the soft coral as folded bundles and when slightly stretched during extraction they reached up to 20 cm in length. The fiber bundles in their natural surroundings are heavily coiled in the coral mesenteries of the polyp (Benayahu et al., 2011). Vast morphological and biochemical analyses indicated that the fibers are resembled to type I collagen (Benayahu et al., 2011) by their amino acid composition as found by Nuclear Magnetic Resonance spectroscopy (NMR) and by mass using mass spectroscopy (MS). Transmission electron microscopy (TEM) and Masson trichrome staining also strengthen this determination. However, differences between the collagen from soft coral and mammalian sources do exist, such as the higher melting temperature of the coral collagen and its bundle form of nested helical packing. These collagen fibers present melting temperature of 68 °C that suggests high natural cross-linking degree. The fibers possess tensile strength of 39–59 MPa and stiffness of 0.34–0.54 GPa on isolated fibers (Benayahu et al., 2011). These unique collagen fibers consist of natural micro-crimping (coiling) which is essential for hyperelastic mechanical behavior of soft tissues (Caves et al., 2010c; Holzapfel, 2001). The collagen fibers are also biocompatible and shown to support cell growth both *in vivo* and *in vitro* studies (Benayahu et al., 2011).

In the current study, the soft coral fibers were aligned in specified controlled orientation to provide preferential mechanical properties, such as anisotropic stiffness and tensile strength. These properties can be tailored and controlled based on the volume of fibers (V_f) used in the reinforcement of the alginate matrix. The alginate hydrogel was derived from marine algae and has been widely used for biomedical applications (Ertesvig and Valla, 1998; Kuo and Ma, 2001; Lee and Mooney, 2012).

2. Materials and methods

2.1. Isolation and purification of collagen fibers

Soft coral *Sarcophyton* (Benayahu et al., 2011) was kept frozen pre-reaping and defrosted before fiber extraction. A piece of colony was reaped to expose the fibers and the exposed fibers were physically pooled out from the soft coral (Fig. 1a). They manually spun around thin U-shaped stainless steel wire to create unidirectional, straight and organized array of fiber bundles (Fig. 1b). The aligned fibers were carefully washed several times in water and then with 70% ethanol.

2.2. Bio-composite fabrication

The spun fiber bundles (Fig. 2a) were inserted to a dialysis membrane (6000–8000 MWCO, Spectra Por) together with 3 ml Sodium alginate solution (3% w/v in DDW, Protanal LF 10-60, FMC BioPolymer) (Fig. 2b). The alginate and collagen were cross-linked with a 45 mM EDC [*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride, Sigma-Aldrich] – NHS (*N*-Hydroxy-succinimide, Sigma-Aldrich) (Fig. 2d). The membrane was sealed, flattened and soaked in 0.1 M $CaCl_2$ (Merck) solution to enable ionic gelation of alginate hydrogel through diffusion for 48 h at room temperature (Fig. 2c). Then, the bio-composite was removed from membrane and frame (Fig. 2e). Matrix fabrication was conducted as for the bio-composite, excluding the fiber insertion and crosslinking.

2.3. Fibers quantification and orientation

The arranged fibers were photographed on a dark background (Samsung camera, 8 Megapixels) and images were processed