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Fibrous cellulose nanocomposite scaffolds prepared by partial dissolution for potential use as ligament or tendon substitutes

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ABSTRACT

Fibrous cellulose nanocomposites scaffolds were developed and evaluated for their potential as ligament or tendon substitute. The nanocomposites were prepared by partial dissolution of cellulose nanofiber networks using ionic liquid at 80 °C for different time intervals. Scanning electron microscopy study indicated that partial dissolution resulted in fibrous cellulose nanocomposites where the dissolved cellulose nanofibers formed the matrix phase and the undissolved or partially dissolved nanofibers formed the reinforcing phase. Mechanical properties of the composites in simulated body conditions (37 °C and 95% RH) after sterilization using gamma rays was comparable to those of natural ligaments and tendons. Stress relaxation studies showed stable performance towards cyclic loading and unloading, further confirming the possibility for using these composites as ligament/tendon substitute. *In vitro* biocompatibility showed a positive response concerning adhesion/proliferation and differentiation for both human ligament and endothelial cells. Prototypes based on the cellulose composite were developed in the form of tubules to be used for further studies.

1. Introduction

Cellulose being a natural polymer, insoluble in water and degradable in nature by microbial and fungal enzymes and having limited degradation in animal and human tissues, is a potential raw material for medical implants (Miyamoto, Takahashi, Ito, Inagaki, & Noishiki, 1989). Cellulose based materials like cellulose sponge and cellulose acetate, etc. are found to induce only negligible foreign body and inflammatory responses and are therefore considered as biocompatible (Märtson, Viljanto, Hurme, & Saukko, 1998; Miyamoto et al., 1989). In the recent years bacterial cellulose comprising of nanofibers is being the most widely used form of cellulose in biomedical application owing to its properties like high mechanical properties, biocompatibility, biodegradability, etc. (Bodin, Bäckdahl, Gustafsson, Risberg, & Gatenholm, 2006; Kim, Cai, & Chen, 2010; Märtson et al., 1998; Miyamoto et al., 1989; Ping et al., 2009; Svensson et al., 2005; Zaborowska et al., 2010). Cellulose has also been successfully used as scaffold for tissue engineered meniscus and blood vessels (Bodin et al., 2006; Svensson et al., 2005).

In the human body the main function of the tendons is to transfer the force due to muscle contraction to the bones whereas ligaments stabilize the joints preventing abnormal movements (O'Connor & Zavatsky, 1963). Though natural tendons and ligaments are capable of withstanding high stresses, injuries are common in tendons and ligaments, e.g. rupture of the anterior cruciate ligament (ACL), the primary and most important stabilizer for knee (Fu, Bennet, Latterman, & Ma, 1999). In order to facilitate rapid recovery and rehabilitation, artificial prosthesis to replace or repair natural ligaments and tendons have been explored (Bolton & Bruchman, 1983; Chazal et al., 1985; De Santis et al., 2004; Gissefalt, Edberg, & Flodin, 2002; Jenkins, Forster, Mckibbin, & Ralis, 1977; Legnani, Ventura, Terzaghi, Borgo, & Albisetti, 2010; McCartny, Tolin, Schwenderman, Freidmen, & Woo, 1993; Meyers, Chen, Lin, & Seki, 2008; Migliaresi & Nicolais, 1980; Nachemson & Evans, 1968). However, most of these materials did not possess the same biomechanical properties like the native structure and were known to show irreversible elongation, rupture and formation of wear debris. Experimental and clinical ligament reconstruction studies have generally demonstrated poor long-term results due to persistent pain, sterile effusions, arthritis, and mechanical breakdown of the synthetic polymers (McCartny et al., 1993).

During the 1970s and 80s, various synthetic materials were designed to act as a permanent ligament/tendon replacement device (Bolton & Bruchman, 1983; Jenkins et al., 1977; McCartny et al., 1993). Synthetic polymers clinically evaluated for ACL reconstruction include polytetrafluoroethylene (Gore-Tex), polyethylene terephthalate (Dacron; Stryker-Meadox and

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Leeds-Keio ligaments), carbon fibers (Integraft), and braided polypropylene (Kennedy Ligament Augmentation Device) (Bolton & Bruchman, 1983; Jenkins et al., 1977; McCartny et al., 1993). In the recent years several composite materials have been developed and studied with the aim to obtain suitable materials to replace natural ligaments and tendons (Chazal et al., 1985; De Santis et al., 2004; Hukins, Leahy, & Mathias, 1999; Iannace, Sabatini, Ambrosio, & Nicolais, 1995; Meyers et al., 2008; Migliaresi & Nicolais, 1980; Nachemson & Evans, 1968). Artificial tendon made of composites consisting of poly (HEMA) reinforced with polyethylene terephthalate (PET) fibers as well as PET reinforced polyurethane composites for use as ligament prostheses were reported earlier (De Santis et al., 2004; Migliaresi & Nicolais, 1980).

De Santis et al. (2004) studied ligament and tendon substitution with carbon fiber composites and reported a measured maximum strength of 28 MPa and strain of 30% for natural ligament and a maximum strength of 38 MPa and strain of 18%, for tendons. Nachemson and Evans (1968) reported a lower strength value for the human ligament, being only 4.4 ± 3.6 MPa and Chazal et al. (1985) reported for human ligament a strength of 15 ± 5 MPa with a strain of about 20%. This wide variation in mechanical properties indicate that the mechanical behaviour of biological tissues like tendons/ligaments is complex and is influenced by extraction location and method, age of the person, testing conditions of temperature and humidity used, loading rate, shape of the test piece, etc.

In the current study the attempt have been to use wood based cellulose in nanoscale to develop artificial ligaments and tendons with mechanical properties similar or better than natural ligaments or tendons. While developing artificial tendons or ligaments biocompatibility, mechanical properties as well as resistance to *in vivo* moisture, temperature conditions and cyclic loading conditions need to be considered. Since natural tissues function at 37 °C it is advantageous to measure the properties at this temperature, even though elevated temperatures may dehydrate the specimen and affect its properties (Hukins et al., 1999).

Cellulose in nanoscale has been successfully isolated from various plant and animal resources and is of great interest due to its renewable nature, good mechanical properties and large specific surface area (Hubbe, Rojas, Lucia, & Sain, 2008; Oksman, Mathew, & Sain, 2009). The current report is unique because of the use of biobased materials for the development of medical application and is expected to provide superior mechanical properties as well as biocompatibility and non-toxicity as required for biomaterials. The fibrous nanocomposite preparation by partial dissolution has been reported earlier by researchers where micro-sized fibers were used as starting materials (Duchemin, Mathew, & Oksman, 2009; Nishino, Matsuda, & Hirao, 2004). Nanofibers were used in this study considering the possibility to get more homogeneous and uniform product as well as improved fiber matrix interaction and mechanical properties owing to high surface area available for nanocelluloses. The processing and characterisation of the nanocomposites, especially in simulated body conditions of 37 °C and high relative humidity conditions (98%) are included in this work. This study is expected to provide valuable insights on the use of fibrous nanosized cellulose in the development of artificial ligaments and tendons. The developed fibrous cellulose composite is finally fabricated into a prototype.

2. Experimental

2.1. Materials

Special cellulose (Domsjö Fabriker AB, Sweden) from Norway spruce with a cellulose content of >94% was used as starting material for the fibrillation process. Nanofibers were isolated from the special cellulose by mechanical fibrillation method, which is reported elsewhere (Mathew, Stelte, & Oksman, 2009). Fig. 1A shows the fibrous nanocellulose with diameters in the range of 10–40 nm obtained after the mechanical isolation process.

1-Butyl-3-methylimidazolium chloride ($[C_4mim]Cl$) with a purity >95% from BASF (category #38899) was used for the partial dissolution, without further purification.

2.2. Methods

2.2.1. Preparation of fibrous nanocomposites

The fibrous nanocomposites were prepared in two steps, first a low concentration aqueous nanofiber suspension (0.5 wt%) was used to prepare nanofiber networks. This suspension was filtered using a filter press under vacuum until all the water was drained off. The final drying was done by heating at 100 °C between two heated plates for 1 h and then for 6 h at 60 °C at 35 MPa pressure. These nanofiber networks are referred to as NF_0.

The second step was to prepare fibrous nanocomposites. Nanofiber networks were dried thoroughly in a vacuum oven to remove any residual moisture and treated with 15 mL ionic liquid at 80 °C for 90 and 120 min, to prepare fibrous nanocomposites with two degrees of dissolution. The partially dissolved networks were then plunged into water to stop dissolution and to precipitate the dissolved cellulose. After that, the networks were thoroughly washed in excess of water to remove all residual ionic liquid. The excess of water was then wiped off using a paper towel, and dried in a hot press at 60 °C. The prepared fibrous nanocomposites were referred to as NF_{90} and NF_{120} based on time of dissolution.

2.2.2. Prototype development

The cellulose nanofiber networks were prepared and coated with ionic liquid. These coated films were rolled into tubules and kept at 80 °C for 90 min to carry out partial dissolution. After that, partially dissolved tubules were kept immersed in water for 12 h to stop the dissolution and precipitate the dissolved cellulose. The tubes were rinsed in a water bath for 48 h to remove all residual ionic liquids.

2.3. Characterisation

2.3.1. Scanning electron microscopy (SEM)

The morphology of the composites as well as the prototypes was studied using a JEOL JSM 6460 LV, scanning electron microscope. The samples were cryogenically fractured using liquid nitrogen and sputter coated with gold to avoid charging.

2.3.2. X-ray diffraction (XRD)

Crystallinity studies of the composites were done using a Philips X'pert MRD X-ray diffractometer, Philips Electronics N.V. (USA) operated at 40 kV and 45 mA. The samples were exposed for a period of 1.5 s for each angle of incidence (θ) using a Cu Kα X-ray source with a wavelength (λ) of 1.541 Å. The angle of incidence was varied from 5° to 40° at increments of 0.05°. The percentage crystallinity index (C_{Ir}) was measured using the Segal empirical method (Segal, Creely, Martin, & Conrad, 1959):

$$C_{Ir} \quad (\%) = \frac{(I_{200} - I_{am})}{I_{200}} \times 100 \tag{1}$$

where I_{200} is the intensity value for the crystalline cellulose, and I_{am} is the intensity value for the amorphous cellulose.

2.3.3. Water uptake

The samples used for water sorption studies were circular discs, 20 mm in diameter, cut from films conditioned at 5% relative humidity. The cut samples initial weight and dimensions were