Cellulose nanofiber based composites for use as ligament or tendon substitute

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ABSTRACT
Cellulose nanofibers were isolated from softwood pulp by mechanical fibrillation process and were utilized to develop biobased nanocomposites for biomedical application. Different processing techniques were attempted to develop nanocomposites for use as artificial ligament or tendon and the composites mechanical properties at room conditions as well as at simulated body condition (37 °C and 98 % RH) were studied. These initial studies indicated that the inherent properties like low toxicity, biocompatibility and biodegradability together with excellent mechanical properties of nanocelluloses makes cellulose based nanocomposites an excellent candidate for load bearing components in biomedical applications.

INTRODUCTION
Although significant progress has been made toward understanding the anatomy, composition, biomechanics and healing of ligaments and tendons there are still no graft or prosthesis ideally suited to substitute natural ligaments/tendons so far. Natural tendons and ligaments are capable of withstanding high stresses and during the 1970s and 80s, various synthetic materials were designed to act as a permanent ligament/tendon replacement devices. Synthetic polymers clinically evaluated for ACL reconstruction include polytetrafluoroethylene (Gore-Tex), polyethylene terephthalate (Dacron; Stryker-Meadox and Leeds-Keio ligaments), carbon fibres (Integraft), and braided polypropylene (Kennedy Ligament Augmentation Device). In fact, these materials did not possess the same biomechanical properties of the native structure and were known to fatigue, stretch, and/or particulate over time. Though there are reports on development of composite materials for biomedical applications, especially for tendon and ligament replacement, most of them are based on synthetic polymers.

Research in the field of artificial ligaments and tendons indicates that mechanical performance comparable to that of natural ligaments/tendons and biocompatibility are the primary requirements for materials considered for artificial ligament/tendon application. The current report is unique because of the use of biobased raw materials with remarkable mechanical properties for developing the ligament or tendon substitute. The nanocellulose fibres were isolated from wood using chemical and mechanical treatments and were utilized to develop composite materials having mechanical properties similar or better than natural ligaments or tendons. Nanosized cellulose fibers used is expected to result in a homogeneous and uniform product as well as improved fibre-matrix interaction owing to high surface area available for nanocelluloses. The processing and properties of the nanocomposites, especially in the body conditions of 37° C and high relative humidity conditions (98 %) are reported here. This study is expected to provide valuable insights on the use of nanosized cellulose fibres in the development of artificial ligaments and tendons. The in vitro biocompatibility studies on these nanofiber based composites materials were looked into and were finally fabricated into a prototypes and is being evaluated for in vivo bio mechanical performance.

EXPERIMENTAL
Materials
Collagen fibres obtained from horse tendons by pH induced regeneration (Resorba, Germany) and cellulose nanofibres were produced out of never dried softwood pulp by mechanical fibrillation. The ionic liquid used for cellulose as well as the crosslinking agents for collagen was procured from Sigma Aldrich GmH, Germany.

Processing of nanocomposites
Composite 1. The nanofiber networks were prepared from cellulose nanofiber suspensions and treated with ionic liquid to prepare partially dissolved networks which is then precipitated and washed with water to remove the residual reagents and dried to obtain flat films
Composite 2. Cellulose nanofiber suspensions were thoroughly mixed with collagen suspensions and casted on Petri dishes to obtain flat films. The films were neutralised and subsequently treated with cross linking agents to obtain uncross linked and cross slinked composites respectively. These films were washed thoroughly and dried in a vacuum oven.
Characterisation
Microscopy studies were carried out on the developed using a JOEL JSM 6460 LV, scanning electron microscope. The samples were cryo-fractured using liquid nitrogen and the fracture surfaces were sputter coated with gold to avoid charging.

Mechanical properties of the composites after sterilization were studied using a Shimadzu Autograph AG-X Universal Tester. The strain rate used was 2mm/min and the load cell was of 1kN. The testing was conducted at 2 different conditions a) at room temp and b) at 37°C and 95% RH.

The samples used for moisture sorption studies were circular discs 12 mm in diameter, cut from films conditioned at 5 % relative humidity. Phosphate buffer solution (PBS) with pH of 7.3 was used as the sorption medium and uptake was determined by gravimetric method.

RESULTS AND DISCUSSION

Composite 1

All-cellulose nanocomposites prepared by partially dissolution using ionic liquids were studied for tendon and ligament applications. Figure 1 compares the micro structural morphology of undissolved nanofiber networks (Figure 1a) with those after dissolution (Figure 1b). The undissolved network shows layers of nanofibers with homogeneous morphology. The partially dissolved composites showed core-shell morphology with more dissolution on the surface layers compared to the inner layers. The dissolved cellulose nanofibers formed the matrix phase with the undissolved/ partially dissolved nanofibers formed the reinforcing phase.

The undissolved and dissolved nanofiber networks were tested for tensile performance in room conditions as well as before and after sterilization (using gamma rays) in simulated body conditions. The results showed that simulated body conditions and sterilization decreased the tensile strength but increased the strain for the studied materials and were comparable to that of natural ligament or tendon, in simulated body conditions even after sterilization. The partially dissolved mats exhibited a strength of 30MPa and a strain of 30 % at 37 °C and 95% RH, after sterilization.

Composite 2

The morphology studies on the nanocomposites based on collagen and cellulose nanofibers developed by pH induced fibrillation of collagen indicated partial fibrillation of collagen resulting in a composite with collagen fibres and cellulose nanofibers embedded in the collagen matrix. The tensile strength of the composites increased from 56 MPa to 96 MPa with addition of nanofibers which increased further to 190 MPa when the collagen phase was cross linked. After sterilization these materials showed mechanical performance similar to that of natural tendons and ligaments, in simulated body conditions.

The moisture uptake in PBS medium showed that both cellulose nanofibers and the cross linking decreases moisture uptake. Table 1 shows the moisture update data for collagen and the composites with and without cross linking. The addition of cellulose nanofibres as well as the matrix crosslinking was found to stabilise the composites towards different moisture and pH conditions as well as increase the mechanical properties.

CONCLUSION

Cellulose nanofiber based composite materials were developed as the load bearing component of ligament/tendon substitutes. The initial studies indicated that nanocelluloses having inherent properties like low toxicity, biocompatibility and biodegradability together with excellent mechanical properties are excellent candidates to develop cellulose based nanocomposites for biomedical applications. Prototypes based on these composites are being evaluated using in vitro studies for biocompatibility and in vivo studies for biomechanical performance in order to develop these materials to a commercial level.

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References