Fabrication of a Bioinspired Cellulose-based Composite with Biocompatible Surface as a Potential Scaffold in Vascular Tissue Engineering

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Statement of Purpose: As a temporary artificial extracellular matrix (ECM), scaffold plays a significant role in integrating the overall tissue structure while hosting the seeded cells to effectively accommodate their proliferation, migration and differentiation to form a new tissue. The traditional design of an ECM scaffold has mainly focused on the properties of the biomimetic materials at the macroscopic and microscopic levels, however the new developments in bio- and nano-technology in recent years revealed the role of nano-sized moieties in a human ECM and the crucial presence of a nano-structured bio-scaffold as a necessary component for the correct tissue development [1]. Nano-fibrous assemblies have beneficial impact on the cellular response and system biocompatibility via the increase in the particle surface area, porosity, and available binding sites of the scaffold [2-6]. In addition, the majority of naturally-occurring tissues exhibit a preferential alignment and a well-ordered structure [1, 5, 7], thereby the orientation of the nanofibrils in a designed scaffold could potentially tune a particular cell proliferation, migration, and differentiation. Among potential nano-structured biopolymers, cellulose could be a feasible candidate for a tissue-engineered scaffold, given its renewable and environmentally benign nature, and its abundance and excellent biocompatibility with the susceptibility to get aligned under an externally-applied field. Inspired by the fascinating properties of cellulose and its derivatives, we have designed a biomimetic nanocomposite with biocompatible surface and aligned nano-sized whiskers.

Methods: In our initial design, cellulose nanowhiskers (CNWs) were embedded in a matrix of cellulose acetate propionate (CAP) to fabricate a fully bio-based scaffold possessing a controlled biodegradability, 3D porosity, and non-acidic byproducts. To ensure uniform distribution, CNWs were delicately extracted from a multi-stage process including: a rigorous acid hydrolysis under controlled temperature, a repeated cycles of ultracentrifuge followed by a few minutes of ultrasonication, and a multiple dialysis exchange against distilled water to obtain a neutral aqueous suspension of CNWs with a pH near 6-7. Then, the suspension was gently freeze-dried and delicately dispersed in acetone prior to mixing with the matrices to reduce the hydroxyl group interactions and improve the quality of nanowhisker dispersion within the medium. This technique notably reduced the agglomeration of the CNWs while preserved the nature of the nanofibers without using toxic chemicals. In our current design, we have developed a collagen-reinforced scaffold by well-dispersed nano-sized cellulose whiskers to effectively enhance the mechanical/thermal stability of collagen and to better mimic the morphology and profile features existed in the ECM of a human vessel.

Results: Comparable to carbon nanotubes or Kevlar, CNWs impart a significant strength and a directional rigidity to the CAP matrix at only 0.2 wt.% yet double that at 3.0 wt.% and intensify to an almost twofold increase within a weak applied magnetic field of 0.3T. The aligned profile features not only improved the directionality and percolation of nanofibers within the medium, but also drastically lowered the optimum amount of CNWs required to obtain the best composite performance. In addition, the viscoelastic improvement of about twofold increase was also evidenced in the collagen-cellulose composite at only 3.0 wt.% of cellulose nanofillers. In order to verify the accuracy of our experimental data and to predict the unusual reinforcing effect of CNWs in cellulose-based nanocomposites at such low filler content, homogenization schemes such as the mean field approach and the percolation theory were also investigated in this study [8]. Based on these comparisons, the tendency of cellulose whiskers to interconnect with one another through strong hydrogen-bonding confirmed the formation of a three-dimensional rigid percolating network, fact which impart an excellent mechanical/thermal stability to the entire structure at such low filler concentration. In principle, the scaffold microstructure including porosity, pore size, pore shape, fibrous interconnectivity, and mechanical properties can significantly influence regular cellular activities and overall scaffold biofunctionality [9]. The successful grafting of our CNWs within the CAP or the collagenous matrix and the feasible microstructure of our fabricated nanocomposites could introduce a stable mechanical/ thermal system for further investigation on the protein adsorption and subsequent cells adhesion. In fact, the biocompatibility of our designed collagen-cellulose composite was confirmed by encapsulating human mesenchymal stem cells on the material surface, where the invasion and proliferation of the cultured cells were clearly evidenced at 8-day of culture.

Conclusions: We have designed a potential bioinspired scaffold material comprised of cellulose nanowhiskers embedded in a matrix of CAP or collagen. The well-dispersed CNW phase created a rigid percolating network within the host matrix, which imparted considerable mechanical/thermal stability to the entire nanocomposite at low filler content and substantially enhanced these properties upon the alignment of the nanofibers. We believe that our biocompatible nanohybrid platform not only could expand the biomedical applications of renewable cellulose-based materials but also could provide a potential scaffold candidate in tissue engineering of small diameter grafts.

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