Biomimetic Engineering of a Fully Bio-based System in Nanomedicine*

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Abstract— Design of materials and devices by mimicking the fascinating systems found in nature, have enabled scientists to discover new techniques to treat diseases from diagnosis to therapeutic care. With the recent advances in nanomedicine, the length-scale of this design has further extended down into a nano-sized array. Inspired by the two natural assemblies found in nature; cellulose and collagen; we have designed a new class of green functional material with nano-sized arrangement. The fabricated material composed of collagen hydrogel reinforced by cellulose nanowhiskers in order to effectively enhance the rigidity of collagen and to better mimic the morphology and profile features existed in biological tissues. The biocompatibility of the hydrogel nanocomposite was also investigated by the invasion and proliferation of human bone marrow derived mesenchymal stem cells around the materials at 8 day of culture. We believe that our biomimetically engineered platform in this study could increase the biomedical applications of fully bio-based systems such as scaffolding in tissue engineering.

I. INTRODUCTION

Today, biomimetic strategies have enabled scientists to mastermind the structure and functions of biological systems and apply the relevant principals in order to design materials and devices that transform medical treatment from diagnosis to therapeutic care. In tissue engineering for example, a biomimetic scaffolding material as an artificial extracellular matrix (ECM) plays an important role in regulating and controlling cellular interactions with the material at a molecular level and subsequently in directing the tissue regeneration [1]. The receptor binding to the ligand determines the strength of the cell adhesion to the surface of the scaffold material; the rate of cell migration on or within the scaffold material; and the formation of cytoskeleton organization in cultured cells [2]. Thereby, the biomimetic scaffolding material should address key parameters of biological responses such as the receptor-ligand affinity, the ligand density, and the ligand spatial distribution [3]. Perhaps, the surface morphology and the bulk topology of the biomimetic scaffolding material has a leading effect on its successful functioning as an attractive substrate for the protein adsorption and the subsequent cells adhesion [4]. The traditional design of an ECM scaffold has mainly focused on the properties of the biomimetic materials at the macroscopic and microscopic levels, as shown in Fig. 1A, 1B. New developments in nanomedicine however 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 [5, 6]. Nano-fibrous assemblies, such as shown in Fig. 2C, have a beneficial impact on the cellular response and the system biocompatibility via the increase in particle surface area, porosity, and available binding sites of the bio-scaffold material [7-9].

Additionally, natural ECM is typically a porous hydrogel comprised of protein and polysaccharide nanofibers which offer mechanical support as well as biochemical signals to cells [10]. In this study, to biomimetically engineer such an entity; we have selected two fascinating bio-based materials: collagen and cellulose. Collagen, as the most abundant protein in body, has long been investigated for extensive use in biomedical applications due to its excellent biocompatibility, safe biodegradability, and very low antigenecity [11]. However, the material made of pure collagen typically presents a poor water resistance with low mechanical stability and fast biodegradation without some form of matrix modification [12]. Cellulose, on the other hand, as the most abundant biopolymer on earth, has offered unique characteristics to design materials with superior structural diversity and functional versatility. Down to its hierarchy, the extracted cellulose nanowhiskers (CNWs)
II. EXPERIMENTAL METHODS

A. Materials

Cellulose nanowhiskers (CNWs) were extracted from microcrystalline cellulose precursor (Sigma-Aldrich) by using the technique described in [15]. The CNW suspension was then gently freeze-dried and delicately dispersed in an acetone suspension to reduce the interaction between the hydroxyl group on the nanocrystal surfaces while to improve the level of nanocrystal dispersion within the biopolymer collagen matrix. This technique notably reduced the agglomeration of the CNWs while preserved the nature of the nanocrystals without using toxic chemicals. Microfibrillar, type I collagen isolated from bovine Achilles tendon (Sigma-Aldrich) was initially dissolved in diluted acetic acid and homogenized upon magnetic stirring prior to nanocomposite fabrication. The pre-dispersed CNWs was then added into the collagen suspension and allowed to form a homogenous hydrogel by using the method described in [16]. The collagen-cellulose hydrogel nanocomposite was then stored at 4°C to maintain the gel stability and to control further hydration of the material prior to study its behavior. The human bone marrow derived mesenchymal stem cells (H-MSCs) were plated and prepared in form of aggregates following the instructions in [17]. The H-MSC aggregates were then embedded in the pre-sterilized hydrogel nanocomposite while supported by a woven nylon mesh ring in order to form a three-dimensional sandwich assay before incubating at 37°C and 5% CO₂ [17-19].

B. Microscopy Imaging

The morphology of the aqueous suspension of CNWs was probed using an AFM NanoScope (Multimode Scanning Probe Microscope (SPM), Veeco 3000). A droplet of the suspension was initially dried on a glass slide prior to imaging and the scans were obtained in air with commercial Si Nanoprobe SPM tip of 1.6 μm in tapping mode. The microstructure of the hydrogel nanocomposite was imaged by a scanning electron microscope (JOEL SEM) at an accelerating voltage of 5 kV. Prior to imaging, several drops of the collagen-CNW hydrogel were deposited on silicon wafers that were pre-cleaned with piranha solution and ethanol, and allowed to dry at room temperature to remove the moisture from the surfaces. Then, the silicon wafers was sputter-coated with gold for less than a minute to increase the conductivity of the samples prior to SEM imaging. The growth and invasion of H-MSCs around the fabricated hydrogel were digitally imaged using an optical microscope from different regions of the three fabricated specimens of the same hydrogel to ensure the accuracy of the biocompatibility measurements and the homogeneity of the nontoxic samples.

III. RESULTS AND DISCUSSION

In principle, the architecture of a bio-scaffold has a promising role in promoting cells adhesion, migration, and differentiation throughout the material. Scaffolds with the nano-sized features provide a larger surface area and promote a higher affinity for the protein adsorption and cells adhesion by increasing the binding sites available to the membrane receptors [6, 9]. The morphology of our CNWs shown in Fig. 2 confirmed the successful fabrication of nano-sized fibers in an aqueous solution.

In general, it is well known that the mechanical behavior of a composite material greatly depends on its fabrication technique and the experimental conditions. In a CNW-based composite, these parameters strongly affect the topological dispersion of nanowhiskers and hence the final properties of the nanocomposite system [20]. A poor fiber-dispersion generally increases the probability of gas entrapment and air bubble formation while adversely introduces a gradient of nanowhisker concentration within the medium, which ultimately reduces the intended mechanical performance of the nanocomposite system [14]. Thus, our pre-dispersion technique described in the previous section introduced a homogeneous suspension and a uniform surface feature as the CNWs were percolated within the collagenous medium. This delicate pre-dispersion method notably reduced the formation of inhomogeneous regions such as air bubbles, while also inhibited the CNW flocculation during the hydrogel nanocomposite fabrication. The sample homogeneity was
investigated using the thermogravimetric analysis (TGA) of different regions of the hydrogel nanocomposite as described in our previous study [16]. The smooth TGA profiles of our fabricated nanocomposites with no indication of a separate degradation stage suggested the successful grafting of CNWs within the host matrix. Additionally, the well separation of nanofibers observed from the SEM images in Fig. 3 and Fig. 4 verified the non-agglomeration of fillers throughout the medium. We believe that the quality of the CNW dispersion and their favorable interactions have a significant role in the formation of a three-dimensional percolating network and in the mechanical behavior of the nanocomposite material as previously investigated [14].

Research studies have also confirmed the superior increase of cell adhesion and neo-tissue development in a nano-scaled environment and on a microstructure that is smaller than the diameter of the cultured cells [21]. Thereby, the nano-fibrous landscape of our fabricated material exhibited in the SEM image shown in Fig. 3 could constitute a practical scaffolding platform for cells to attach and render their normal activities until they secrete their own ECM and form a new tissue.

Furthermore, the leading requirements; such as mass-transport for cell nutrition, open channels for cell migration, and surface features for cell attachment, promote the formation of a porous landscape in the design of a bio-scaffold [22]. A controlled porous structure allows the diffusion of fluids and gases deep into the material, encouraging cell seeding throughout, in order to form and regenerate new tissue [23, 24]. Likewise, a porous landscape could effectively alter the biological functioning of the material by providing a balance between the temporary mechanical/chemical stability of the bio-scaffold and the effective mass transport of the cultured cells. The 3D aggregates of the small-sized pores in Fig. 4 presents the potential capability of our uniform porous assembly in creating a viable cell migration avenue for further tissue regeneration.

The biocompatibility and nontoxicity of the fabricated collagen-cellulose hydrogel nanocomposite were studied through encapsulating human bone marrow derived mesenchymal stem cells (H-MSCs) on the material surface. The radial invasion and proliferation of the cultured H-MSCs around our hydrogel material in Fig. 5 presented the formation of a three-dimensional assay consisting the hydrogel nanocomposite/H-MSCs/nylon ring as the structural support.

Likewise, the growth of cells from the aggregated bundles in day 2 into sprouting around the hydrogel nanocomposite material in day 8 confirmed the viable candidacy of our designed system as a biomimetic scaffolding platform for cells to render their normal activities and outgrow (Fig. 6).

Finally, the H-MSCs sprouting out of their proliferation sites shown in Fig. 7 once again confirmed the biocompatibility and nontoxicity of our designed biomimetic hydrogel system.
Figure 7. Sprout of H-MSCs around the hydrogel nanocomposite seen under phase contrast microscope at 10-x magnification.

IV. CONCLUSION

In this study, we have biomimetically engineered a fully bio-based hydrogel material comprised of collagen and cellulose nanowhiskers. This bio-functional assembly was intended to potentially resemble the structural features existed in natural extracellular matrix of human tissues while to provide a mechanically rigid platform. The radial invasion of cultured human bone marrow derived mesenchymal stem cells around the fabricated hydrogel nanocomposite confirmed the biocompatibility and nontoxicity of our system and its potential as a bio-scaffold in tissue engineering.

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