REGENERATED KERATIN FIBERS FROM CHICKEN FEATHERS FOR TEXTILE AND BIOMEDICAL APPLICATIONS

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This dissertation focuses on dissolution of keratin from chicken feathers and subsequent development of normal and ultrafine fibers for textile and biomedical applications. In the last few decades, efforts have been made to transform the largely-available waste material, chicken feathers into fibers but they have yielded no success. In addition, keratin is preferred in biomedical applications due to the existence of cell-binding motifs in its molecular structures. However, 100% keratin ultrafine fibers have not been developed also due to lack of proper dissolution methods. Regarding the structures of scaffolds, three-dimensional (3D) fibrous structures possess advantages over two-dimensional (2D) structures as tissue engineering scaffolds since they show higher structural similarity to the natural extracellular matrices.

In this research, dissolution conditions are studied in order to obtain keratin solution with good spinnability. First, keratin is extracted from chicken feathers with backbones preserved after cleavage of inter- and intramolecular disulfide bonds using cysteine. Sodium dodecyl sulfate (SDS) is applied to dissolve keratin for spinning and mechanism of dissolution of keratin with SDS is investigated. Normal keratin fibers are wet spun and 3D ultrafine keratin fibrous scaffolds are produced via electrospinning. Increasing SDS concentration intensifies ordered conformation of keratin and firstly increases and then decreases viscosity of solution, suggesting continuous disentanglement of keratin

molecules and enhancement in inter- and intramolecular electrical repulsion. The diameters of obtained fibers as small as 20 microns also prove good drawability of keratin solution. The change of crystallinity is found to be consistent with that of tensile properties. In addition, structures composed of three-dimensionally oriented ultrafine pure keratin fibers are electrospun. The 3D scaffolds are water-stable. The adipose-derived mesenchymal stem cells penetrate more deeply and distribute more evenly in the 3D keratin fibrous structures comparing to commercial 3D scaffolds and electrospun 2D polylactic acid (PLA) scaffolds. The dissolution and 3D electrospinning methods are applied to wheat glutenin, another highly-crosslinked plant protein for adipose tissue engineering.

DEDICATION

I dedicate this dissertation to my family. My hearty gratitude goes to my loving parents, Zhongxing Xu and Zhenxiu Tu, whose complete trust and unconditional love accompanied me during this long and tough journey. My younger brother Donglei Xu and sister-in-law Xiaofeng Lin, have never left my side and always provided me with support whenever I needed. My uncle Dehua Tu and aunt Liying Ji are very special people in my life. They have been showing me love and care from my early childhood, and have been very supportive since I made my decision to pursue academic goals. In loving memory of my dear grandmas and grandpa, Qiaoda Gao, Yumei Miao and Quanda Xu.

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CHAPTER 1

INTRODUCTION

1.1. Regenerated fibers for industrial applications

Global fiber production in 2012 approached 85.8 million tons, of which approximate 50.6 million tons were synthetic fibers and about 30 million tons were cotton fibers (Rauschendorfer, 2013). Synthetic fibers are not sustainable because of limited petroleum reserves and rising oil prices; while production of cotton, the major natural fiber, has been decreasing. Therefore, to satisfy the increasing global consumption of fibers and to resolve the problem of limited resources, it is necessary to develop fibers from alternative resources with large availability at a low price.

Chicken feathers could be prospective resources to produce regenerated protein fibers. The US poultry industry produces more than 4 billion pounds of chicken feathers each year (Xia et al., 2012). Some of the feathers are autoclaved or hydrolyzed and then used as animal feed with low nutritional value (Coward-Kelly et al., 2006), and the rest are disposed through landfill, which occupy land and have potential to transmit viruses and pathogens (Yamamoto et al., 2010).

Efforts have been made to explore wide industrial applications of chicken feathers. Chicken feathers have been used as reinforcements to develop light-weight composites (Huda and Yang, 2009; Reddy and Yang, 2010), exploded via high-density steam into powders (Zhao W., 2012), and hydrolyzed, grafted or acetylated and then compression molded into thermoplastic films (Jin et al., 2011; Hu et al., 2011). In 2000s, U.S. Department of Agriculture (USDA) launched projects to transform chicken feathers into

feather-based industrial products, such as filter membranes, disposable utensils and containers have been developed (Durham, 2009). In biomedical fields, structures such as sponge scaffolds, coatings and conduit filling made from keratin have been widely investigated (Tachibana et al., 2002; Reichl, 2009; Sierpinski et al., 2008).

Developing regenerated keratin fibers could not only provide new sources for fiber industry to alleviate the fiber shortage, but also add value to poultry industry and address related environmental concerns. Chicken feathers contain about 90 wt% of keratin. As small linear proteins with only a few bulky side groups and molecular weight higher than 10 kDa, feather keratin meets the molecular requirements for fiber spinning (Poole et al., 2008). Keratin has about 7% cysteine, which could serve as crosslinking sites to form water-stable fibers (Arai et al., 1983).

To the best of our knowledge, no efficacious method has been developed to produce regenerated keratin fibers, though relevant research could date back to more than seventy years ago. In 1943, regenerated keratin fibers were fabricated via wet spinning of protein-surfactant complexes in the laboratory (Harris and Brown, 1947; Lundgren, 1941; Lundgren and O'Connell, 1944). Another patent issued in 1948 described a two-step process to produce regenerated keratin fibers (Evans and Shore, 1948). A short report published in *Nature* in 1949 also indicated successful regeneration of keratin fibers from wool (Wormell and Happey, 1949). However, the mechanical properties of the fibers were not reported. Nevertheless, we tried the methods and found that the results could not be repeated, and we did not find any other reports regarding successful repetition of the methods as well. The latest report regarding spinning of pure regenerated keratin fibers was in 2008. Fan dissolved extracted feather keratin in ionic liquid for wet spinning (Fan,

2008). However, the obtained fibers showed tensile strength as low as 23 MPa. Composite fibers using keratin as one component had also been developed. Keratin and polyvinyl alcohol (PVA) composite fibers have been produced via wet spinning (Bin, 2011). Nevertheless, incorporation of high amounts of unsustainable petroleum-based PVA and toxic crosslinker glutaraldehyde prevented wide applications of the composite fibers.

One prerequisite of producing keratin fibers is to obtain linear keratin molecules with preserved backbones. The keratin in natural feathers is a network crosslinked via disulfide bonds. Alkaline treatment randomly destroys backbones and disulfide bonds in feather keratin, and resulted in short molecules that could not be spun without addition of synthetic polymers (Bin, 2011). Extraction of feather keratin with highly reductive thiol could keep the molecular backbones intact but dissociate the disulfide crosslinks. However, fibers could not be developed if the extracted linear keratin molecules remained entangled in solution (Jia et al., 2012). In addition, most widely used thiols, such as mercaptoethanol and dithiothreitol cannot be used in large scale, because they are either environmentally hazardous or high in price. Keratin also has been reduced and extracted using sodium sulfites with a low yield, due to their relatively low reducibility. Moreover, ionic liquids could dissolve keratin mainly by interrupting hydrogen bonds instead of disulfide bonds (Xie et al., 2005; Idris et al., 2013). The resultant fibers with diameters ranging from about 75 to 110 μm inferred poor keratin spinnability (Fan, 2008), which could be due to the non-linearity of obtained molecules and remained molecular entanglement (Ghosh and Banerjee, 2001). Furthermore, disentanglement and alignment of linear polymers in solution are the other key factors for successful development of

satisfactory fibers. If the spinning dope contained randomly folded polymers, the drawability of keratin could be insufficient to generate fine fibers.

Using surfactant is a feasible approach to disentangle and align keratin in solution. The use of SDS to disentangle and align proteins, carbohydrates and synthetic polymers has been reported widely (Thuresson et al., 1996; Stenstam et al., 2001). Expansion of polymers was ascribed to increased electrical repulsion among molecules, as well as unraveling of polymer chains from assemblies (Fan, 2008). Water-insoluble proteins were assembled into random coils in water via strong hydrophobic interaction, which has a potential to be interrupted by surfactants. However, limited study has been done on the effect of surfactant on conformational change of water-insoluble proteins.

1.2. Ultrafine fibrous structures for tissue engineering

Tissue engineering scaffolds are designed as temporary artificial extracellular matrices (ECMs) to support attachment, proliferation and development of cells (Shastri, 2009). Ideal scaffolds should be capable of closely mimicking the topographies and spatial structures of native ECMs, in order to facilitate cells to grow and differentiate following the patterns similar to that found in native tissues and organs (Bhattarai et al., 2006; Dvir et al., 2011).

Morphologies of ECMs vary according to functions of target tissues and cell types in the tissues (Knight et al., 2000; Roskelley et al., 1994; Yamada and Cukierman, 2007). For example, in skin tissue, the top layer is formed by compact packing of epithelial cells on a 2D fibrous ECM basement membrane. Three-dimensional spatial spreading of fibroblasts and immune cells occurs in the interior region of the skin tissue, and correspondingly the ECMs are constructed by stereoscopically and randomly oriented

ultrafine protein fibers (Smalley et al., 2006; Bosman and Stamenkovic, 2003). Fibrous structures with 3D orientation and random distribution can also be found in native ECMs in breast (Bissell et al., 2003), liver (Uygun et al., 2010), bladder (Zegers et al., 2003), lung (Petersen et al., 2010) and many other organs and tissues (Zhu et al., 2010). It has been reported that cells cultured on flat 2D substrates may differ considerably in morphology and differentiation pattern from those cultured in more physiological 3D environments (Cukierman et al., 2002; Griffith and Swartz, 2006). Therefore, it is reasonable to fabricate scaffolds with particular morphology and structure according to category and functions of original native tissues (Lu et al., 2010; Mikos et al., 2006; Spalazzi et al., 2008; Haycock, 2011).

Three-dimensional fibrous structures with spatially oriented fibers are preferred to the 3D non-fibrous structures due to their higher degree of similarity to native ECM structures in cartilage. The 3D architectures in natural cartilage ECMs are composed of collagen fibrils and proteoglycans, and play pivotal roles in imparting mechanical strength, acting as reservoirs for biomolecule delivery, providing biological and physical guidance and regulation to cell behaviors, such as proliferation, shaping, migration and differentiation in multiple aspects (Knight et al., 2000). Three-dimensional non-fibrous structures, such as hydrogels or sponges, were built up by randomly or regularly interconnected polymer slices. The slices could not function in the same manner as fibrils in natural ECMs in terms of guiding cell spreading and interaction, promptly transmitting mechanical and biological signals among cells. Besides, faster attachment of cells could also be resulted from more available adhering sites and more nutrients and bioactive molecules adsorbed onto the 3D fibrous scaffolds due to the larger surface areas.

Three-dimensional fibrous scaffolds are also advantageous in terms of mass transportation, which is critical for uniform distribution of cells throughout the scaffolds. Compared to 3D fibrous scaffolds, the 3D non-fibrous scaffolds could result in uneven distribution of nutrient and cells over large length scale. Inadequate transportation of nutrient and waste has been correlated with a decrease in tissue quality as a function of distance from the nutrient source, since the most distant regions could become metabolically inactive or even necrotic. Three-dimensional fibrous structures are more prone to maximize cellular viability by modulating nutrient and signaling gradients for the control of cell behavior and tissue formation. The fibrous scaffolds with larger surface area than the non-fibrous ones may also have higher loading of serum proteins, which played critical roles in cell attachments.

A few comparison studies based on synthetic and natural materials revealed the advantages of 3D fibrous scaffolds over non-fibrous ones in tissue engineering. In a study comparing 3D sponges and fibrous poly(ethylene glycol)-terephthalate/poly(butylene terephthalate) (PEGT/PBT) scaffolds for cartilage repair, the fibrous structures showed favorable mechanical properties and better *in vivo* production of GAGs in mice (Roskelley et al., 1994). 3D fibrous structure from another synthetic polymer poly(l-lactic acid) (PLLA) demonstrated better support of *in vitro* oriented differentiation of human embryonic stem cells than 3D solid walled structures (Yamada and Cukierman, 2007; Smalley et al., 2006). 3D fibrous structures from natural material chitosan also promoted chondrogenesis of stem cells better than 3D non-fibrous ones (Bosman and I. Stamenkovic, 2003). Attachment of osteoblasts on fibrous scaffolds were found much higher than on non-fibrous scaffolds (Woo et al., 2003). Mostly, cell attachment preceded

proliferation and differentiation, and thus the ultrafine fibrous scaffolds may provide a favorable environment for tissue growth. Fibrous structures for a broad applications of tissue engineering have been intensively investigated and summarized elsewhere (Bissell et al., 2003). Fabrication methods of micro- and nano-scale fibers have been briefly summarized in Table 1. There are good review papers for fabrications of fibers for biomedical applications (Uygun et al., 2010; Zegers et al., 2003).

1.3. Fabrication of 3D ultrafine fibrous scaffolds

There are mainly three technologies to fabricate 3D ultrafine fibrous scaffolds as shown in Table 1.1.

Molecular self-assembly is the spontaneous organization of individual molecules into structurally-defined stable arrangements through preprogrammed noncovalent interactions, such as hydrogen bonds, van der Waals forces, hydrophobic interactions and electrostatic interactions (Whitesides et al., 1991; Lehn, 1993; Ball, 1994; Zhang, 2003). Self-assembly is a bottom-up approach to create nanofibers from small building blocks, including small molecules, peptides and nucleic acids. In this research, keratin is an existing macromolecule and thus the self-assembly method will not be suitable.