Silk Hydrogels for Tissue Engineering

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Abstract Silk hydrogels have been highlighted in the past decade as potential matrices for tissue engineering and regenerative medicine applications. In this mini review, the biological attributes of silk proteins, as well as methods reported in literature to fabricate silk hydrogels will be discussed.

1 Introduction

The field of tissue engineering (TE) which aims to repair, regenerate or replace damaged tissue has been extensively researched in the past decade as an alternative to organ transplantation. The general approach has been centred on combining cells, growth factors and tissue engineering matrices, with the goal of engineering functional tissues in vitro, which can then be implanted into the body. Hydrogels, which are highly hydrated polymeric network have been highlighted as potential tissue engineering matrices, due to their structural similarity to the native extracellular matrix [1, 2]. Several materials ranging from synthetic to natural polymers, have been fabricated into hydrogels, and shown to support cellular growth and differentiation [2]. In particular, silk proteins, which have been traditionally used in biomedical applications as sutures and drug delivery systems, have also been translated into tissue engineering matrices in the form of hydrogels. This review will focus on the main attributes of silk proteins as biomaterials, as well as methods to fabricate silk hydrogels for tissue engineering applications.

2 Hydrogels as tissue engineering matrices

Hydrogels are defined as hydrophilic polymeric networks which are capable of absorbing water ranging from ten to a thousand times their dry weight [3]. They are structurally similar to the native extracellular matrix (ECM) in their hydrated state, which enables facilitation of nutrient diffusion and waste removal through the TE matrix. In an ideal scenario, when cells are encapsulated within a hydrogel, the hydrogel should be able to support cell remodelling and ECM secretion, which results in a functional engineered tissue that can be transplanted (Figure 1). Hydrogels can be classified based on the forces between the crosslinked networks (physical or covalent), or the nature of the polymer used for fabrication (synthetic or natural) [2]. Physical hydrogels have either molecular entanglements, ionic bonding, or hydrogen bonding holding the network together that can be reversible, while covalent gels are hydrogels with covalently crosslinked networks that are permanent [3]. These gels can be fabricated from a range of synthetic or natural polymers.

In general, synthetic hydrogels have less batch-to-batch variability with good chemical and mechanical stability. Current synthetic polymeric hydrogel systems reported in literature include poly(vinyl alcohol) (PVA), poly(ethylene oxide) (PEO), poly(ethylene glycol) (PEG), poly(N-isoproplyacrylamide) (NIPAAm), and poly(propylene furmarate) (PPF) [1, 2, 4-8]. However, although the reported synthetic hydrogels have good physico-mechanical properties, their application as scaffolds for TE remain limited due to the lack of biological sequences available to facilitate cellular functions. Moreover, most of these polymers are hydrophilic and resist cell attachment [9, 10].

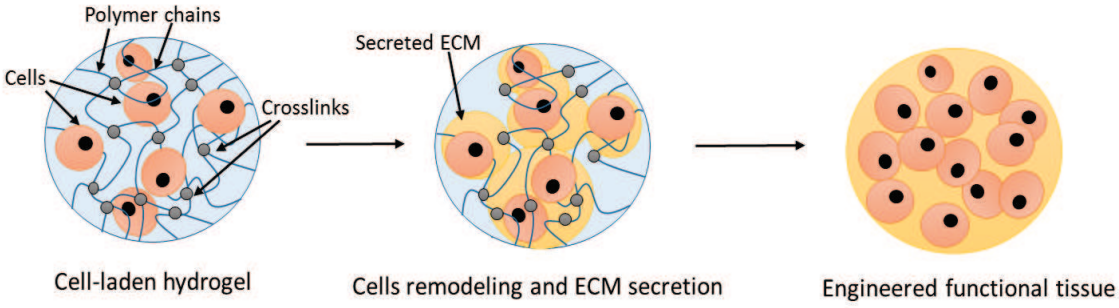

Figure 1: Schematic of tissue formation in cell-laden hydrogels.

On the other hand, hydrogels fabricated from naturally derived polymers, such as chitosan, collagen, gelatin, hyaluronic acid, fibrinogen and fibrin, are known to have good bio-functionality to support cellular growth, proliferation and differentiation [11-16]. However, these natural hydrogels are generally weak with limited mechanical stability as TE matrices. Therefore, there exists a need to engineer TE scaffolds that have the advantages of both synthetic and natural polymers, with good physico-mechanical properties and also with the ability to support cellular function. In recent years, hydrogels fabricated from silk proteins have been identified as potential candidates to meet these criteria.

3 Silk proteins

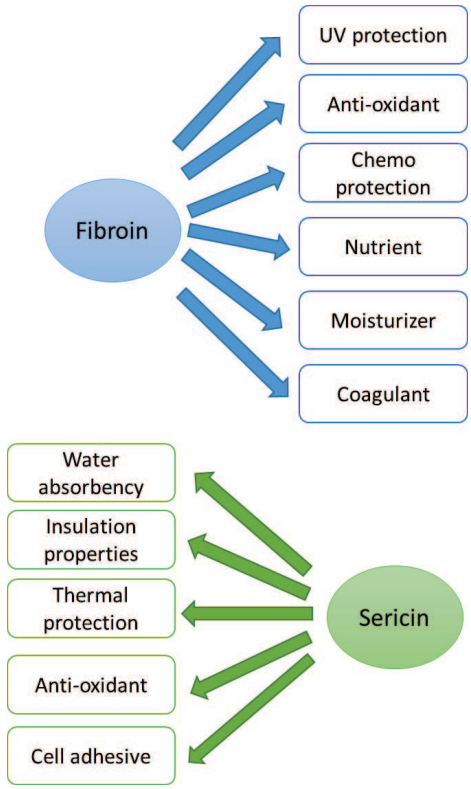

Figure 2: Biological attributes of silk fibroin and sericin [18].

Silk is a combination of fibrous proteins synthesised in specialised epithelial cells that line glands in silkworms, and has been successfully used as a suture material for centuries with great potential in biomaterial applications [17]. Before converted to silk fibers, silk proteins are synthesized by silk gland cells and stored in the lumen of the silk glands [18]. The usage of silk fibers is advantageous in the biomaterial aspect as it has properties that can rival synthetic polymers but requires less harsh processing conditions [18]. Silks have impressive mechanical properties, environmental stability, biocompatibility, controlled proteolytic biodegradability, morphologic flexibility and the ability for amino acid side change modification to immobilize growth factor [17].

The two major components in silk is fibroin and sericin, where fibroin is normally coated with sericin in the cocoons [18]. The biological attributes of these two silk proteins are listed in Figure 2. Sericin is secreted from the middle silk gland of a mature silkworm larva and acts as the glue that keeps fibroin together [19, 20]. Sericin produced by the most commonly researched domesticated type, Bombyx mori,(B. mori) consists of peptides with 3 major fractions of 150, 250, and 400 kDa [21]. This protein also exists in two kinds of conformation, random coils or β-sheets. [18].

On the other hand, fibroin is the major structural protein of silk which is secreted from the posterior silk gland [19]. Vepari et al reported that B. mori fibroin fibers are about 10-25 μm in diameter and contain a light protein chain (L-chain) with molecular weight of approximately 26 kDa, and heavy protein chain (H-chain) of approximately 390 kDa, where both L- and H-chain are linked by a disulphide bond [17]. Similarly to sericin, fibroin also exists in random coils and β-sheets conformation. By heating, stretching or immersing fibroin in a polar solvent, the protein conformation undergoes transition from random coil to β-sheet (Figure 3). This β-sheet transformation also corresponds to higher mechanical durability, where higher amount of β-sheet formation corresponds to higher mechanical strength [22].

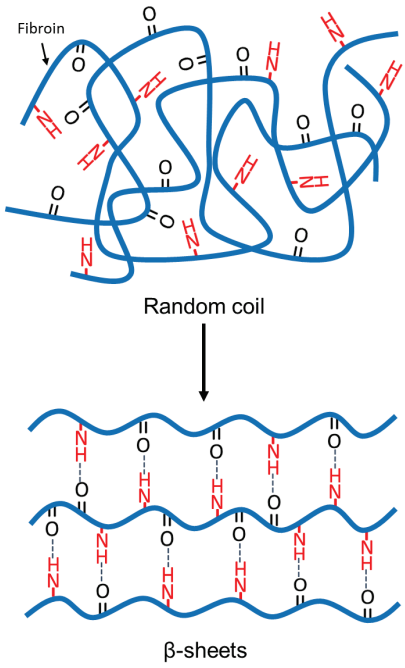

Figure 3: Random coil to β-sheet transformation of silk fibroin.

4 Silk hydrogels: Fabrication techniques and applications

For the past decades, it has been thought that the sericin fraction of silk causes unwanted inflammatory responses and hence has not been the major focus of biomaterial research. Sericin, which is the glue component of the silk cocoons are normally isolated using heat in basic conditions. These isolation conditions have been shown to cause denaturation and degradation of the protein, which subsequently affects the mechanical properties of the resultant hydrogels. In order to prevent heat denaturation during sericin isolation, Teramoto et al. researched genetically modified silkworms (Sericin-Hope) whose cocoons contain 99% sericin. As these Sericin-Hope cocoons contain almost no fibroin, the heat treatment was not required to separate the sericin from fibroin [23, 24]. However, although these hydrogels were shown to have significantly higher elastic modulus than the conventionally used domesticated silkworms, their mechanical properties were still not on par with fibroin hydrogels [23, 24].

Fibroin, the fibrous part of the silkworm cocoon, has been characterised to have abundance of hydrophobic amino acid groups such as glycine, sericin and alanine, which are capable to form physical crosslinks without addition of any chemical crosslinkers [25]. This approach is beneficial as concerns associated with toxicity of chemical crosslinkers are eliminated. However, fibroin hydrogels formed by this self-assembly approach require a long crosslinking time that can take up to days. Kim et al. showed that silk fibroin solution (2% w/v, pH 6.4 - 6.8, 37 °C) required 30 days for complete gelation [20]. These large hydrophobic domains of fibroin also allow its gelation time to be tailored by a number of factors such as temperature, ionic concentration, pH and salt concentration [26]. The different factors used to control fibroin gelation are:

pH changes pH closer to fibroin isoelectric point accelerate gelation [27-29]

Temperature changes Fibroin solution crosslinks faster at higher temperature. The resultant hydrogels are also mechanically stiffer [20, 27, 30]

Freeze-thawing Porosity of fabricated fibroin hydrogels depend on the number of freeze-thaw cycles [31-33]

For example, Ayub et al. showed that decreasing the pH of the fibroin solution to 3-4 successfully facilitated gel formation within two days [34]. It was hypothesised that the lower pH initiated protonation of the carboxyl groups, which subsequently reduce repulsion between the fibroin polymer chains and led to formation of crosslinks [29, 35]. It has also been showed that the fibroin protein structures are converted from random coils to β-sheets during the crosslinking process [34]. As the physico-mechanical properties of the fibroin gels are directly related to the amount of β-sheets formed, many researchers have focused on different methods to induce β-sheet transition [20, 27, 36, 37]. The compressive modulus of these fibroin gels fabricated using different methods can vary from 60 kPa to 7 GPa [26]. Chemical crosslinking of fibroin solution using different solvents and chemicals has also been studied to fabricate fibroin hydrogels. Although these gels are normally mechanically stronger and require shorter gelation time, the chemicals or conditions used are normally quite harsh for cells in terms of TE applications. For example, typical chemical crosslinkers include glycerol, sodium dodecyl sulfate and sodium N-lauroyl sarcosinate that are not cyto-compatible to cells at high concentrations [35, 38-40].

Fibroin hydrogels have gained huge popularity as TE matrices in the past decade for various applications such as bone engineering, cartilage engineering, nerve engineering, immunoisolation of cells, drug delivery and injectable void fillers. For example, mesenchymal stromal cells seeded into macroporous fibroin hydrogels were able to proliferate into osteoblasts and promote bone formation [41, 42]. Similarly. Fini et al. reported that the presence of fibroin gels in a cranial defect promoted bone healing by increasing the rate of osteoblasts proliferation and differentiation [43]. The rate of healing was significantly better than the FDA approved synthetic polymer, poly(lactide-co-glycolic acid) (PLGA) [41]. Fibroin gels have also been used to repair peripheral nerve injuries, where conduits coated with fibroin gels and immobilised with nerve growth factors successfully promoted nerve regeneration of a 14 mm rat sciatic nerve injury [44]. These hydrogels have good chemical and mechanical stability in vivo post implantation, which can be used as void fillers for surgical reconstruction and soft tissue augmentation [45].

All these examples highlighted the potential of fibroin hydrogels as tissue engineering matrices, mainly due to its inherent biocompatibility and biofunctionality properties, as well as tailorable physico-mechanical properties.

5 Conclusion and future outlooks

In conclusion, this review has outlined the main attributes of the two silk proteins, sericin and fibroin, mainly focusing on methods to fabricate them into hydrogels and their applications. Although these hydrogels have shown great potential as TE matrices, there is still lack of data on the in vivo performance of these materials to demonstrate that they are safe for clinical use. As the TE field is trending towards bridging the gap between in vitro and in vivo studies, future experiments should focus on evaluating the in vivo degradation, functionality and mechanical properties of the silk hydrogels, to confirm that these gels meet the clinical requirements.

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