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Review article

Silk fibroin as biomaterial for bone tissue engineering

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ABSTRACT

Silk fibroin (SF) is a fibrous protein which is produced mainly by silkworms and spiders. Its unique mechanical properties, tunable biodegradation rate and the ability to support the differentiation of mesenchymal stem cells along the osteogenic lineage, have made SF a favorable scaffold material for bone tissue engineering. SF can be processed into various scaffold forms, combined synergistically with other biomaterials to form composites and chemically modified, which provides an impressive toolbox and allows SF scaffolds to be tailored to specific applications. This review discusses and summarizes recent advancements in processing SF, focusing on different fabrication and functionalization methods and their application to grow bone tissue *in vitro* and *in vivo*. Potential areas for future research, current challenges, uncertainties and gaps in knowledge are highlighted.

Statement of significance

Silk fibroin is a natural biomaterial with remarkable biomedical and mechanical properties which make it favorable for a broad range of bone tissue engineering applications. It can be processed into different scaffold forms, combined synergistically with other biomaterials to form composites and chemically modified which provides a unique toolbox and allows silk fibroin scaffolds to be tailored to specific applications. This review discusses and summarizes recent advancements in processing silk fibroin, focusing on different fabrication and functionalization methods and their application to grow bone tissue *in vitro* and *in vivo*. Potential areas for future research, current challenges, uncertainties and gaps in knowledge are highlighted.

Contents

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1. Introduction

Bone tissue engineering (TE) is a promising strategy to regenerate bone and is regarded as a future alternative to current clinical treatments. Not only can tissue engineered constructs be transplanted as a graft, but also pave the way for three-dimensional (3D) tissue models which help to investigate tissue abnormalities and to analyze them both at the cellular and molecular level [1,2]. In this whole process, the patient's own cells could be used which would be a key tool for personalized medicine. The goal is to create 3D bone tissues by combining cells, scaffolds and to some extent also growth factors or mechanical stimuli. One of the main challenges is the choice of an appropriate biomaterial which can mimic the natural bone tissue matrix with its mechanical and biological characteristics to support tissue development. Various materials have been tested for bone TE purposes [3,4]. Silk fibroin (SF) has been proven to be a promising biomaterial for scaffold fabrication in general and its remarkable mechanical properties predestine it for bone TE applications [5]. There is a steady increase in number of publications and citations on the use of SF as scaffold material for bone TE applications over the last 10 years which supports its significance and potential as a biomedical material for bone TE. This review seeks to highlight its characteristics and the various modification options of SF as a biomaterial in bone TE.

2. Bone biology

Bone TE relies on our knowledge of bone structure, composition, mechanics and tissue formation which makes it crucial to have a fundamental understanding of bone biology. Approximately 35% of the bone tissue is made of an organic part while the remaining 65% is inorganic matrix [6]. The organic extracellular matrix (ECM) of bone consists of complex self-assembled macromolecules such as collagens, which make up 90–95% of the organic ECM, osteocalcin, osteopontin, osteonectin, bone sialoprotein, hyaluronan and proteoglycans [7]. The inorganic mineral phase of bone consists of hydroxyapatite (HA) as well as carbonate and inorganic salts [8]. Guiding stem cells along the osteogenic lineage is a critical step in bone regeneration and it is well known that the ECM plays a main role in regulating the stem cell fate [9–11]. Not only does this network serve as a scaffold for cells; it also helps in immobilizing growth factors and cytokines [12]. The water content in cortical bone can vary between 18% and 30% and shows a direct relationship to bone porosity, which increases through aging and osteoporosis [13]. Nano-composite structures made of collagen and HA contribute to the strength and hierarchical architecture of bone [14]. The overall bone structure is divided into cortical bone, which is more compact, and cancellous bone, which appears more sponge-like and whose pores are filled with bone marrow or fat. Bone is a highly dynamic tissue which is continually renewed and remodeled through formation and resorption processes of bone-forming osteoblasts and bone-resorbing osteoclasts as an adaption to mechanical loads, regulatory factors such as hormones and cytokines, and other environmental parameters [15]. Osteoclasts are multinucleated cells derived from hematopoietic stem cells [16], which dissolve crystalline HA through release of hydrochloric acid and a mixture of proteases which degrades the organic bone matrix rich in collagen fibers [17]. Their role in bone remodeling involves the removal of cracks after fracture or micro damage occurs but they also function as immune cells and can secrete cytokines that can affect surrounding cells [18,19]. Their differentiation and function is controlled by osteoprotegerin, receptor activator of nuclear factor (NF)-kappaB (RANK) and RANK ligand (RANKL) [20]. Osteoblasts are derived from mesenchymal stem cells (MSCs) located in the bone marrow and differentiate towards the osteogenic lineage [21]. Once they become encapsulated within their own matrix, they acquire a stellate morphology and are referred to as osteocytes [22,23]. Osteocytes comprise more than 90% of all bone cells in adult animals and are considered to be the major cell type responsible for sensing mechanical strain and translating it into biochemical responses which affect bone remodeling [24]. *In vivo*, osteocytes communicate to adjacent osteocytes via a dense and interconnected canalicular network containing cell processes for rapid signal transduction [25]. When mechanically activated, they produce bone morphogenetic proteins, Wnts, prostaglandin E_2 and nitric oxide, which can modulate the recruitment, differentiation, and activity of osteoblasts and osteoclasts [26–30]. This ability makes osteocytes the essential mediator in orchestrating bone remodeling in response to mechanical stimulation [31]. MSCs are a valuable cell source for tissue regeneration because of their ability to self-renew and to differentiate along the osteogenic lineage [32]. Their recruitment, homing and subsequent differentiation also plays an important role in the repair of bone fractures [33]. Readily accessible sources of adult MSCs are bone marrow, adipose tissue or peripheral blood, of which bone marrow mesenchymal stem cells (BMSCs) have been most studied [34]. BMSCs can be expanded *in vitro* to 50 population doublings [35] which allows to increase their number such that a sufficient amount can be prepared for terminal differentiation and tissue regeneration.

3. Silk fibroin as a biomaterial

The behavior of cells which adhere to a surface is strongly influenced by the biomaterial characteristics. Molecular structure, mechanical properties and surface topography on micrometer and nanometer scale will influence adhesion, migration, proliferation, differentiation, and cell signaling [10,36–42]. In addition to soluble factors, sensing a biomaterial's physical properties plays a decisive role in cellular function and fate. The following paragraph will address the biomaterial characteristics of SF.

3.1. Structure and mechanical properties

Silk is composed of two major proteins: SF (fibrous protein) and sericin (globular protein). SF is a protein isolated from different animals in the form of an aqueous protein solution. The ability to produce silk has evolved multiple times among insects such as *Bombyx mori*, spiders, mites and beetles with diverse functions [43]. With such a variety of silk, it is not surprising that its