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Preparation and Characterization of the Silk Fibroin 3D Scaffolds with Porous and Interconnected Structure

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Abstract

Silk fibroin (SF) is wildly used in the field of biomedical science. Considering the contradiction between suitable pore size and outstanding mechanical properties, this research focused on the topic of preparing the 3D scaffolds with large pore sizes and high interconnectivity. For preparing the silk fibroin 3D scaffold samples, three kinds of porogens were applied in two methods which paraffin spheres were used in freeze-drying. Sodium chloride (NaCl) and sodium carbonate (Na_2CO_3) were used in leaching respectively. Different pore size of porogens were used to control the pore size and porosities of prepared scaffold samples. Some basic properties of scaffold samples were characterized including surface morphology, chemical structure, porosity and mechanical performance. The best porosity of sample reached to 93% in the research.

Keywords: Silk fibroin; Paraffin spheres; Tissue engineering; Porous scaffold

1 Introduction

The silk fashions can be traced back to thousands of years ago. However, the silk did not be considered as biomaterial or other kinds of advanced materials until 1990s. One of the most emerging subjects in this century, tissue engineering, contents varieties of knowledge not only limit to molecular biology but also material engineering [1]. Tissue engineering tries to heal or replace human organs and tissues, if injured, by means of certain scaffolds. The main function of silk as biomaterial, regenerated silk fibroin scaffold, is the enhancement and support to the organs and assistance of tissue reconstruction [2].

Biomaterials for tissue engineering applications could be itemized as following classifications: (1) non-biodegradable materials, such as nylon, dacron and expanded polytetrafluoroethylene (ePTFE). This type of biomaterial is the initially developed one, and is widely applied in the clinical environment now. (2) Artificial synthetic biodegradable polymers: Diversified polymers have already been exploited as tissue engineering, including poly Glycolide (PGA), poly L-lactic

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acid (PLA), poly Caprolactone (PCL), poly L-lactide-co-glycolide (PLGA), polyethylene oxide (PEO), *et al.* The major advantages of synthetic materials are the wealth of sources, convince in processing and adjustable biochemistry or physical properties [3]. The disadvantage of polymers cannot be ignored. The hydrophobic polymers lead to the difficulty of cells adhesion and proliferation. Also, the degradation products of artificial synthetic material are acids, which bring about inflammatory reaction [4]. (3) Natural materials: Apart from silk fibroin, collagen, fibrous protein gel and chitosan are taken into consideration for tissue engineering. This kind of biomaterial outperforms the synthetic polymers in cells adhered growth [5]. Easy preparation and low toxicity are the other superiority for natural material whilst its mechanical properties usually under instability when the degradation starts [6]. (4) Combined extracellular matrix materials: It is the unity of artificial synthetic polymer and natural material. It is believed of combined extracellular matrix could hold the good characters from its both raw materials. However, the preparation materials and methods are still need to explore in the future studies.

Silk fibroin, derived from *Bombyx mori*, is a widely used natural protein polymer [7, 8]. The raw silk is consisted of two insoluble proteins, sericin and fibroin [9]. The sericin is smooth and glossy, which coats two independent filaments of fibroin. To obtain pure fibroin, the norming stage is boiling the silk to degum the sericin. The dissolve of fibroin is a huge topic for many researchers. Matsumoto (1996) was one of the first to dissolve the fibroin by using neutral salt ($LiBr \cdot H_2O$—EtOH—H_2O) [10]. A similar study by Park reached different method, finding $CaCl_2$—EtOH—H_2O ternary solution could dissolve fibroin in room temperature [11]. The two methods stated above are the most favorable of dissolve fibroin.

In recent years, silk fibroin has been applied to 3D tissue engineering scaffolds, but there is a contradiction between its aperture size and mechanical properties [12]. In terms of bone scaffolds, the porous size of scaffolds has a significant effect on the long-term implant efficiency of bone filler and bone tissue [13], while the cellular metabolic efficiency is also related to the scaffold pore size [14].

The research of our predecessors shows that efficacious preparation method of the 3D scaffold needs to take into account the two points, intrinsic advantages of the mechanical properties and biocompatibility, for the material. At present, the focus of 3D tissue engineering scaffold research is to enable cells could grow inside scaffolds [1], a three-dimensional and porous space, to make sure these cells have the desired morphology and function of the human bodies. Large pore size three-dimensional silk fibroin scaffolds can give cells enough space to grow in, whereas the scaffolds have good connectivity pores is also the effect of this experiment hope to achieve.

Further, the chosen processing method also needs to be able to control the key parameters, pore size and porosity, in 3D scaffolds. On the one hand, higher porosity scaffolds facilitate cell growth and migration [15]. On the other hand, the larger pore size scaffolds will improve newly grown cells to adhere to the inner surface because of the higher specific surface area to satisfy the replacement and recovery of the function of the human organ [16]. In particular, the mechanical properties of the scaffolds are of particular importance for the hard tissue parts of the body, such as bone and cartilage tissue. Although the mechanical properties depend mainly on the nature of the material, processing also has some impact on it [17, 18]. In general, scaffold morphology is very important for its function. Therefore, the preparation methods of tissue engineering scaffold capable of producing irregular three-dimensional shape are better [19]. The current situation is that scientists have found a variety of techniques for the preparation of tissue engineering scaffolds, each of which has obvious advantages, but there is no single method efficacious enough