PHYSICO-CHEMICAL CHARACTERIZATION AND BIOLOGICAL TESTS OF SILK FIBROIN/COLLAGEN/CHITOSAN MATERIALS, CROSS-LINKED BY GLYOXAL SOLUTION

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Introduction

The large molecular compounds, derived from natural sources, are characterized by properties desired in tissue engineering: biocompatibility, biodegradability, lack of immune responses after introduction into the human body. Currently, polymer implants are most commonly manufactured with chitosan and collagen [1,2]. However, this kind of materials exhibit poor stability in water conditions, and poor mechanical properties. Therefore, it is necessary to search for new materials that can potentially be used in tissue engineering. For the production of biomaterials with better properties, mixtures of two or more biopolymers should be used [3]. Significantly better mechanical properties have been demonstrated for silk fibroin [4]. Silk fibroin is a protein component of raw silk, where it performs structural functions [4]. It was decided to create ternary mixtures based on the three polymers (silk fibroin, collagen and chitosan). In addition, such mixtures can be subjected to cross-linking process, to improve parameters such as stability in water conditions and regularity of pores. This type of modification is expected to improve the mechanical properties of materials, stability in water conditions and degradation resistance [5].

Glyoxal is a very simple organic compound with two aldehyde groups. It is the smallest dialdehyde. Glyoxal is a really common cross-linking agent of polysaccharides and proteins [6,7].

Materials and Methods

Chitosan (CTS) was supplied by Sigma-Aldrich (Poznań, Poland). Silk fibroin and collagen were obtained in house. Collagen was obtained from young rat tail tendons. Silk fibroin was prepared from Bombyx mori cocoons (Jedwab Polski Sp. z o.o., Milanówek, Poland), as a 5% concentrated solution. Then the solution was filtered. Chitosan and collagen were prepared as 1% solution in 0.1 M acetic acid.

Three types of mixtures were prepared. The first type included chitosan and collagen (50/50 weight ratio) mixtures with 10, 20 and 30% silk fibroin addition. The second type corresponded to silk fibroin and collagen (50/50 weight ratio) mixtures with 10, 20 and 30% chitosan addition, and the third type concerned 50/50 weight ratio silk fibroin/collagen mixtures with 10, 20 and 30% of chitosan addition. The mixtures were poured into 24-well polystyrene culture plates, frozen, and lyophilized (ALPHA 1–2 LDplus, CHRIST, -55 °C, 5 Pa, 48 h).

The following properties of the materials were measured: density and porosity, moisture content and swelling degree. Mechanical properties of the 3D materials under compression were studied. Additionally, metabolic activity of MG-63 osteoblast-like cells on materials was examined.

Results and Discussion

It was found that the materials were characterized by a high swelling degree and good porosity, which can be suitable for tissue engineering applications. None of the materials showed to be cytotoxic to MG-63 cells.

Conclusions

Glyoxal solution was a good cross-linking agent for three dimensional materials based on the blends of silk fibroin, collagen and chitosan. It was found that cross-linked materials were characterized by high swelling rate and adequate porosity, which can be suitable for tissue engineering applications. Mechanical properties vary depending on the blends composition. The highest Young's modulus among the studied scaffolds was observed for SF/CTS/10Coll scaffold. None of the studied materials was cytotoxic to MG-63 cells. The cross-linking of ternary biopolymer blends with glyoxal may be a new way of materials' modification which offers a cheaper alternative to the existing methods of chemical cross-linking.

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