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Facile preparation of a novel mulberry silk fibroin scaffold for three-dimensional tumor cell culture

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ABSTRACT

Three-dimensional (3D) culture methods, building a bridge between the traditional two-dimensional (2D) monolayer cell culture and the *in-vivo* conditions, launch a new era in cell culture. A reliable and stable method for 3D cell culture is required for further cytology research or chemosensitivity tests, considering its high similarity with conditions *in vivo*. Here we present a novel 3D tumor cell culture model based on mulberry silk fibroin derived from *Bombyx mori*, which is easy to access in China. This is a biocompatible 3D silk fibroin scaffold, without adding any toxic organic solvents, surfactants or other toxic agents during the preparation process. We investigated its microstructure and confirmed that the gastric cancer cell lines were successfully seeded and grown on the scaffold. In conclusion, the silk fibroin scaffold, as a promising 3D cell culture model, has the potential for more accurate cytology research and clinical chemosensitivity tests in the future.

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1. Introduction

By using cell culture methods, cells isolated from the original tissue source could be grown under controlled conditions. Based on the cell lines in the traditional 2D cell culture flask, modern cytology research could be done and methods including MTT could be used to predict the effectiveness of chemotherapeutic drugs before treatment [1]. Nevertheless, the accuracy and reliability of the cytology research and chemosensitivity test based on monolayer cell culture are still controversial.

Cells in 3D models were aggregated as multi-cellular spheroids. The characteristics of cells in 3D models, including differentiation and proliferation, are more like that *in vivo*. Therefore, the 3D culture models have shown great improvement over the traditional 2D cell culture model, building a bridge across the *in-vitro* and *in-vivo* conditions [2].

Recently, there are various methods for 3D cell culture: forced-floating methods, hanging drop methods, agitation-based approaches, matrices, scaffolds, micro-fluidic cell culture platforms and the 3D printing technology [3,4]. However, there are still many defects in these methods, such as labor intensive, expensive specialized equipment, immature technology, low stability and biocompatibility [5].

In this study, we present a novel material, the mulberry silk fibroin (SF), to build a scaffold for 3D cell culture model. As a biologic protein, silk fibroin is the major content of silk that has been used to produce surgical suture. With good biocompatibility, *in-vivo* biodegradability [6–8] and low immunogenicity [9], the silk fibroin, as a natural biomacromolecule, has been fabricated into nanoparticles, and gels [10], with good cell affinity [11]. Thus, it is a material with potential biomedical application. In this study, we developed a facile method to prepare mulberry silk fibroin scaffold, and applied it in 3D tumor cell culture. As previous studies have been done on the *Antheraea mylitta*-based scaffold for seeding of liver and breast cancer cell lines [12,13], the novelty of this study lies in the finding that *Bombyx mori* could be also used to build a silk fibroin scaffold, as a 3D model for the gastric cancer cell line.

2. Experimental

Preparation of the SF aqueous solution: The SF aqueous solution was prepared as previously reported [14]. Briefly, after cut into small pieces, cocoons were degummed in boiled 0.5% sodium

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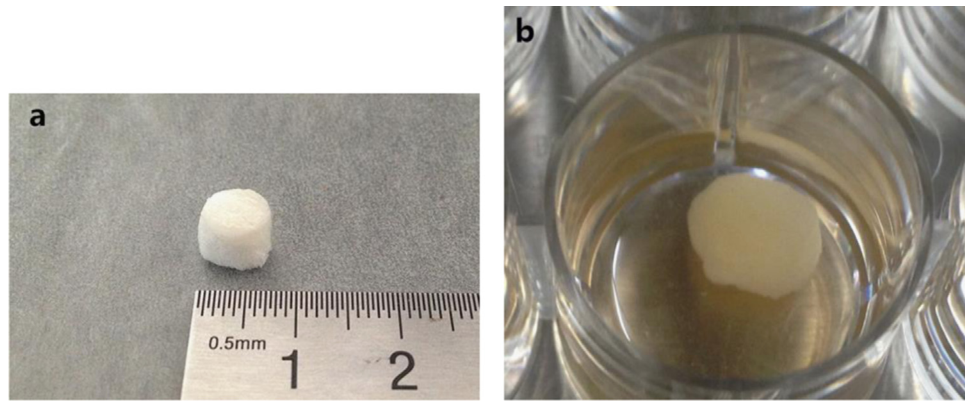
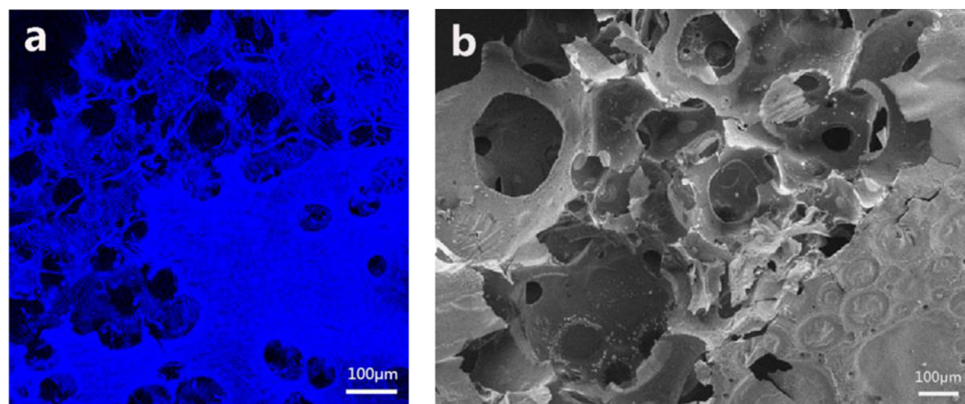


Fig. 1. (a) Appearance of the SF scaffold after treatment of absolute ethanol: cylindrical, loose and spongy; (b) The cell-laden scaffold was incubated in the 24-well cell culture plate, on day 7.



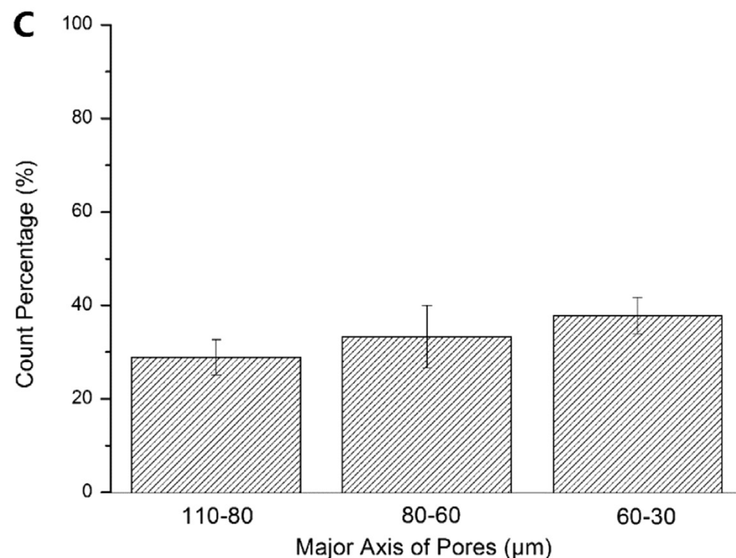


Fig. 2. (a) Microstructures of the SF scaffold under the laser scanning confocal microscope; (b) Microstructures of the SF scaffold under the scanning electron microscope: porous microstructures with unequal-sized pores; and (c) Measurement and analysis of the major axis of pores in the 3D scaffold.

carbonate for 30 min twice, washed with deionized water thoroughly, followed by air drying. The degummed dry SF was dissolved in a ternary solution (molar ratio $CaCl_2/CH_3CH_2OH/H_2O = 1:2:8$) at 70 °C for 4 h. The resulting solution was dialysed (MWCO 14000 Da) against deionized water for 4 days to remove salts and ethanol. After being centrifuged (10,000 rpm for 15 min) and filtered through 0.22 μm filter for sterilization, the SF aqueous solution (50 mg/mL) was stored at 4 °C before use. For the fabrication of the scaffolds, the concentration of SF solution was diluted to 2 wt% with sterilized Milli-Q water. The final fibroin concentration was determined by weighting the residual solid after drying a predetermined volume of solution at 60 °C for 24 h.

Fabrication of 3D fibroin scaffolds: The SF scaffold was prepared as previously reported with mild modification [13]. First, 500 μl of silk fibroin solution (2 wt%) was added into Freezing Tube. Then they were frozen at −20 °C for 24 h, followed by freeze-drying

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