Adherence and Growth of Endothelial Cells on Silk Fibroin Dense Membranes

Andrea C. D. Rodas[1], Grínia M. Nogueira[2], Marisa M. Beppu[2], Bronislaw Polakiewick[3], Marina J.S. Maizato[4], Adolfo A. Leirner[4], Ronaldo N.M. Pitombo[3], Olga Z. Higa[1]
[1]Instituto de Pesquisas Energéticas e Nucleares (IPEN), Centro de Biotecnologia, São Paulo, Brazil.
[2]Faculdade de Engenharia Química – UNICAMP, Campinas, Brazil.
[3]Faculdade de Ciências Farmacêuticas (FCF-USP), Dep. de Tecnologia Bioquímico-Farmacêutica, São Paulo, Brazil.
[4]Divisão de Bioengenharia do Instituto do Coração (InCor) HC-FMUSP, São Paulo, Brazil.

Introduction

Natural silk is composed by two main components: sericin, which is a glue-like protein that surrounds fibroin, the silk filament. Silk fibroin (SF) can be processed into several shapes, such as membranes, gels or powder. Dense SF membranes present good mechanical and thermal resistance, vapor and oxygen permeability and biocompatibility, which make them suitable materials to cell adhesion and growth, enzymatic immobilization and contact lenses [1]. Other researches verified SF interaction with fibroblast [2], keratinocytes [3] and entholelial cells [4]. The evaluation of the ability of dense SF membranes to adhere and grow endothelial cells was the scope of the present study. This characteristic is desired to materials to be implanted such as cardiovascular devices, since the growth of a cell layer on the surface of the device may improve its characteristics such as mechanical resistance [5].

Materials and Methods

Preparation of silk fibroin dense membranes: Silk fibers from *Bombyx mori* silkworm were washed three times, for 30 min, in 0.5% (m/v) Na_2CO_3 solution at 85°C, rinsed with water, and dried at room temperature to remove sericin. Purified fibroin was dispersed in ternary solvent $CaCl_2/CH_3CH_2OH/H_2O$ (1:2:8 mole ratio) to a concentration of 10% (w/v), at 85°C until total dissolution. Silk fibroin dense membranes were obtained by casting the dialyzed SF solution on polystyrene plates. SF dense membranes were immersed in ethanol 70% (v/v) to induce crystallization and water stability.

Cell culturing: Human umbilical vein endothelial cells (HUVEC) from ATCC (CRL 1730) were maintained in F12 medium supplemented with antibiotic and antimicotic solution (final concentration: 100 units/mL penicillin, 100 µg/mL streptomycin and 0.025 µg/mL amphotericin), 2mM glutamine, 20µg/mL endothelial cell growth supplement, 90µg/mL heparin and 10% bovine fetal serum, at 37° C in a humidified 5% CO_2 atmosphere until they reached confluence. For subculturing and for experiments, cells were harvested using 0.05% trypsin and 0.02% EDTA in phosphate-buffered saline, pH 7.4.

Cell seeding onto SF membranes: Silk fibroin membranes were sterilized by humid heating and placed on the bottom of a 12 multiwell plate. Three wells without silk fibroin membrane were used as control. Cell suspension were seeded at a concentration of $10x10^3$ cells per well. The growing cells were accompanied daily in light inverted microscopy with phase filter and the culture medium was changed each three days. Digital photographic captures were performed in the 3rd and 14th day of culture.

Results and Discussion

Sterilization did not alter SF membranes stability. Figure 1 presents micrographs where HUVECs are adhered and grown onto SF membranes surface in a period of two weeks. The cells density increased in the same growth rate as well as onto membranes as onto control wells of the cell culture plate.

Conclusion

Silk fibroin dense membranes can be sterilized and are suitable materials for adherence and growth of endothelial cells. Due to its characteristics, this material can be used as cardiovascular material coating or manufacturing.

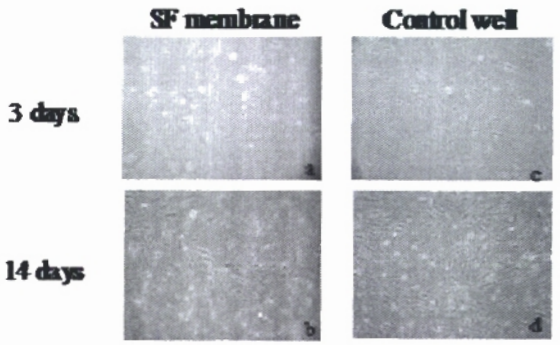

Figure 1. HUVEC growing onto SF membrane (a, b) and control well (c, d).

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