Influence of surface properties of silk fibroin-based biomaterials on cell behaviors

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Introduction

Silk fibroin (SF) is a natural polymer produced by *Bombyx mori* silkworms which forms the core fiber in cocoons. SF-based biomaterials are expected to be useful for clinical applications due to their good biocompatibilities, suitable strength, and so on. In our previous studies using keratinocytes and fibroblasts, SF-based biomaterials induced the enhanced cell migrations and the up-regulated expressions of wound repair-related genes[1-3]. The unique properties of SF-based biomaterials are thought to be an important factor of interaction with cells. The heavy chain of SF has highly repetitive crystalline domains, such as Gly-Ala-Gly-Ala-Gly-Xaa, and amorphous domains, and these crystalline domains contribute to form the β-sheet structure (Fig. 1). The contents and the distribution of β-sheet structure in SF-based biomaterials are assumed to influence on the interaction with cells. In this study, SF films were prepared using a combination of heat drying and alcohol aqueous solution treatments to induce structural changes of SF molecules, and the relationship between secondary structure of SF molecules and cell behaviors on SF-based biomaterials was evaluated.

Fig.1 The heavy chain of silk fibroin

Materials and Methods

I. Film preparation

SF aqueous solutions were prepared from the degummed silk fibers of *Bombyx mori* silkworm cocoons.
Fibroin soln. (10 mg/ml) was cast on substrates.
Films were dried at r.t.

↓ overnight

Films were treated with 80% alcohol aqueous solution.
Treated films were dried at r.t..

↓ overnight

II and III

Table 1. Preparation condition and abbreviates of SF films.

Temp.	80% alcohol aqueous solution					
	-	MeOH	EtOH	PrOH	BtOH	HFIP
25	Rt/NON	Rt/Me	Rt/Et	Rt/Pr	Rt/Bt	Rt/HFIP

**MeOH: Methanol, EtOH: Ethanol, PrOH: 2-Propanol, BtOH: *t*-Butyl Alcohol, HFIP: 1,1,1,3,3,3-Hexafluoro-2-propanol

II. Structural analysis

ATR-FTIR

ATR-IR spectra were collected using a FT-IR spectrometer (JASCO FT/IR-4100) equipped with an ATR unit in the range 4000-500 cm^{-1} at 4 cm^{-1} of spectral resolution.

Raman imaging

Raman spectra were collected using a confocal microRaman spectrometer (JASCO NRS-5100) equipped with an 1800 line/mm grating monochromator, a CCD Detector, and objective lenses. The excitation source was a 532 solid-state laser. An objective lens of 20x magnification was used. Raman spectra were recorded in the range 4000-100 cm^{-1} at 2 cm^{-1} of spectral resolution.

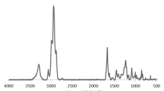

Fig. 2 Raman spectrum of SF film.

III. *In vitro* evaluation

Cell proliferation

Fibroblasts (NIH/3T3) were seeded on SF-coated 96 well plates at a density of 1.0 x 10^3 cells/well.

↓ Incubation for 4, 24, 48, and 72 hours, at 37 ºC

Reagents of WST-1 were added to wells.

↓ Incubation for 30 min and 1 hour, at 37 ºC

Absorbance (@450 and @650 nm) were measured.

Cell migration

NIH/3T3 were seeded on SF-coated glass bottom dishes at a density of 7.5 x 10^4 cells/dish.

↓ Incubation for 1 hour, at 37 ºC

Supernatant were removed.
Cells were washed with 2 mL of PBS.
Medium (3 mL) were added to cells.

↓ Time lapse imaging at 5 min intervals, for 4.5 hours at 37 ºC

Pathways of single NIH/3T3 were tracked using MtrackJ software for quantitative evaluation.

Results and discussion

II. Structural analysis of SF films

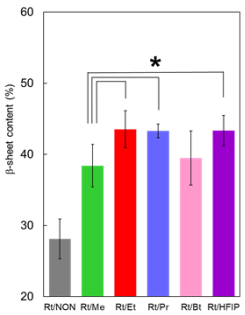

Fig. 3 Relative intensities of β-sheet in SF films (n=5).

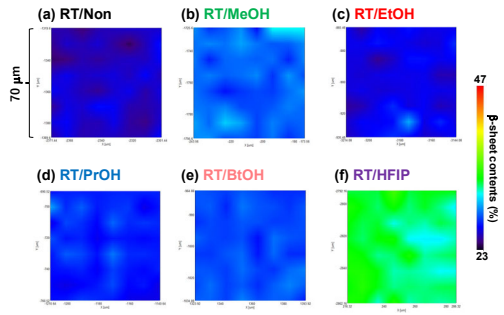

Fig. 4 Distributions of β-sheet in alcohol-treated SF films determined by raman imaging.

- Contents and distributions of β-sheet depended on the type of alcohols.
- Various properties of alcohol molecules in alcohol/water mixtures, such as diffusivity, excess enthalpy, surface tension and so on, were thought to be related to structural changes to β-sheet in films.

- Compared to PS, the enhanced proliferation and the faster migration of fibroblasts were observed on SF films.
- Fibroblasts on alcohol-treated films moved faster than cells on non-treated film.
- Secondary structures of SF films would influence on cell behaviors.

III. Cell behaviors of SF films

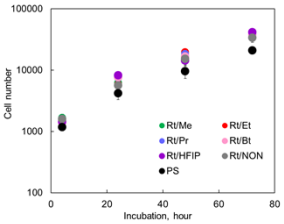

Fig. 5 Cell proliferation curves on SF films (n=5).

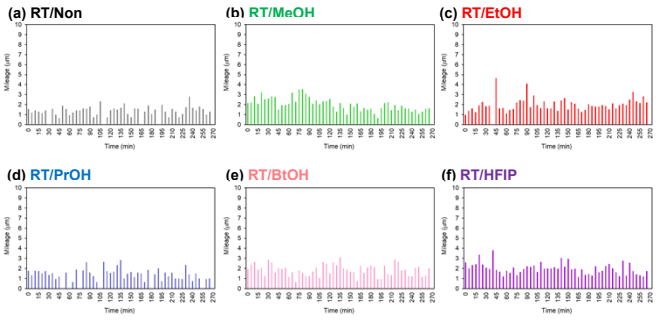

Fig. 6 Migration of a single fibroblast on SF films treated with 80% methanol aqueous solution was measured using time-lapse microscopy at 5 min for 4.5 hours.

Conclusion

Fibroblasts might recognize and interact with the surfaces of SF-based biomaterials. Although further studies are required to assess the specific interaction between SF molecules and cells, SF-based biomaterials are anticipated to be useful for applications such as wound dressings.

References

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