氏名		岸本　祐輝
学位の種類		博士（　工学　）
学位記番号		甲　第　６６５　号
学位授与の日付		平成２９年３月２０日
学位授与の要件		信州大学学位規程第５条第１項該当
学位論文題目		Studies on water based processing of electrospun silk fibroin nanofiber non-woven fabric for cell scaffold
論文審査委員	主査	教授　森川英明
		教授　玉田靖
		准教授　寺本彰
		准教授　田中稔久
		主任研究員　寺本英敏　（農業・食品産業技術総合研究機構）

論　文　内　容　の　要　旨

Silk fibroin (SF) has been extensively studied as one of the promising material for biomedical applications, because it exhibit several biological properties including good biocompatibility, biodegradability and excellent mechanical properties. Recently, electrospun SF nanofiber non-woven fabric consisting of fine fibers from tens of nanometers to a few micrometers in diameter have been specifically examined for the development of cell scaffolds in tissue engineering due to its biocompatibility, porous structure and large surface area to volume.

The electrospun SF nanofiber non-woven fabric is expected to be a candidate for the manufacturing new organic/inorganic materials with advanced structure and properties. Montmorillonite (MMT) clay is known to have biocompatibility, bio-activity, osteoinductivity and large surface area to volume. Therefore, the electrospun SF nanofiber non-woven fabric/MMT nanocomposite material is expected to be candidate for the producing new composite materials for tissue engineering like bone regeneration due to its biocompatibility, osteoinductivity and large surface area to volume.

Though, SF has been widely reported as safe and biocompatible, almost studies are used harmful solvent as the electrospinning SF solution such as hexafluoro-2-propanol, formic acid, hexafluoroacetone and trifluoroacetic acid. These solvents should be avoided to use for fabrication of silk nanofiber aim to apply for biomedical applications. To solve this problem, several studied to produce electrospun SF nanofiber non-woven fabric from aqueous solution with high concentration (17 wt%~) have been reported. However, such high concentrated SF aqueous solution are disadvantageous for industrial processing because high concentrated SF aqueous solution transform into gel easily than low concentration by interactions including hydrophobic interactions and hydrobonds. Therefore, electrospinning of SF aqueous solution at low concentration is ideal processing.

On the other hand, many studies have demonstrated that the seeded cells on the electrospun SF nanofiber non-woven fabrics were well adhered and growth on their surface. However, seeded cells cannot proliferate into inner space of the SF nanofibers due to its smaller pore size than cells. Because three dimensional construction with porous structure mimicking a tissue extracellular matrix (ECM) produces a cell scaffold that facilitates tissue regeneration, three dimensional high porous nanofiber non-woven fabric is necessary for regeneration of tissues.

Therefore, the focus on this study are development of electrospun SF nanofiber non-woven fabric/MMT nanocomposite materials for bone tissue engineering, development of electrospun SF nanofiber non-woven fabric using SF aqueous solution at low concentration, and development of three dimensional SF nanofiber non-woven fabric with larger pore size than cells for three dimensional tissue regeneration.

In chapter 2, the nanoSF/MMT composite were successfully prepared using electrospun SF nanofiber fabric, which had been immersed in MMT aqueous suspension. The nanostructured SF/MMT composite material were characterized by SEM, FTIR and XRD. And their ultrastructures were successfully visualized by high resolution TEM and EDS mapping analysis. High resolution TEM analysis and EDS mapping results indicated that O, Al, Si and Mg atoms, which are constituent elements of MMT were distributed on the surface of SF nanofibers. This creation of a nanocomposite in which SF nanofibers are surrounded by thin layers of MMT, each with a thickness of approximately 1.2 nm. It is suggested that this nanocomposite is a candidate for new biocompatible application as scaffold for tissue engineering like bone regeneration.

In chapter 3, I report a water based processing for the preparation of electrospun SF nanofiber non-woven fabric. Firstly, the effect of degumming conditions of *Bombyx mori* silk cocoon on the molecular weight distribution of SF was investigated. And then, the effect of the molecular weight distribution and pH of SF aqueous solution on electrospinnability were investigated. It was found that the degumming of *Bombyx mori* silk cocoons without alkaline reagents is effective to keep the molecular weight and narrower distribution of SF in the higher range. The spinning solution pH was important for electrospinning of a SF aqueous solution at low concentration. As a result of electrospinning, I could obtain the electrospun SF nanofiber non-woven fabric from 5 wt% of SF aqueous solution at pH 10.5. Tensile stress, strain and toughness of electrospun SF nanofiber non-woven fabrics depended on the molecular weight distribution of SF.

In chapter 4, I report the fabrication method for the preparation of three dimensional SF nanofiber non-woven fabric by wet electrospinning. To control the pore size in SF nanofiber non-woven fabric, citric acid buffered solution at pH 3.8 supplemented with tert-butyl alcohol (t-BuOH) was used as the liquid bath instead of dry flat plate or rotating drum. The t-BuOH concentration influenced the apparent pore size and porosity of the SF nanofiber non-woven fabric. The maximum pore size was formed at 30 Vol% of t-BuOH. The isoelectronic solution of citric acid buffer at pH 3.8 and t-BuOH can make the structure of SF nanofiber non-woven fabric crystallized without the following treatment such as alcohol and water vapor treatment. The initial cell adhesion on wet electrospun SF nanofiber non-woven fabric was significantly lower than on electrospun SF nanofiber non-woven fabric, but no difference of proliferation was observed by cell growth curves. Cells adhered and proliferated on both the surface and the inner spaces of wet electrospun SF nanofiber non-woven fabric, while cells adhered only on the surface of electrospun SF nanofiber non-woven fabric consisting of high fiber density and small pore size. The wet electrospun three dimensional SF nanofiber non-woven fabric is anticipated for use as a cell scaffold to simulate an ECM frame structure.