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Synthetic and Natural Fibrous Scaffolds for Soft Tissue Engineering Applications

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ABSTRACT

Fibrous scaffolds have been extensively studied as grafts for damaged tissue, owing to their physical architecture mimicking the native tissues like articular cartilage and skin. Developing mechanical robust fibrous scaffolds is therefore a critical issue to prevent scaffold failure that limits their applications in tissue engineering. This paper demonstrates our latest development of synthetic and natural fibrous scaffolds having physical architectures and mechanical properties comparable to that of native biological soft tissues. Synthetic fibrous scaffold was produced from gelatin solution using electrospinning technique while natural fibrous scaffold was extracted from small intestinal submucosa (SIS) of cattle. The SIS membrane was first decellurized and further reinforced with alginate hydrogel to form 3D composite scaffold. The physical architectures of both synthetic and natural fibrous scaffolds including thickness and microstructure morphology were characterized. SIS fibrous membrane reinforced with alginate hydrogel demonstrated more than 10 times of increment in scaffold thickness. Through scanning electron microscope (SEM) visualization, the synthetic fibrous scaffold demonstrated microstructures that mimic nanometer fiber and porous structure of soft collagenous tissues. Uniaxial tensile and fracture tests were performed to determine the tensile properties and fracture toughness of fibrous scaffolds. Both types of scaffolds showed tensile strength (0.81 – 38.30 MPa) and fracture toughness (0.86 – 32.52 kJ/m^2) comparable to natural soft collagenous tissues. The developed tissue engineered scaffolds not only exhibit physical architectures mimicking native tissue structures but also demonstrate mechanical properties comparable to the native soft tissues.

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Introduction

Tissue engineering has great potential in offering solution and transcending the limitation of current treatment of damaged tissue. It aims to develop a biological substitute that regenerates and restores the function of damaged tissue. A typical approach of tissue engineering involves seeding cells on a tissue engineered 3D scaffold, which acts as supportive matrix for regeneration and proliferation of cells. One of the important criterions in scaffold design is the physical architecture which including thickness and microstructure of scaffold. Scaffold thickness affects the cell growth rate [1] and sometimes sufficient scaffold thickness is required to total replace entire tissues. For instance, scaffold with thickness 1 – 5 mm is needed for resurface articular cartilage of entire joint [2]. Besides scaffold thickness, the microstructure of scaffold was found greatly influences mechanical properties [3 – 5], protein permeation and absorption [6 – 7], biological responses and cell behaviors including morphology, adhesion, proliferation and differentiation [1, 7 – 9].

Researches on mimicking the architecture of scaffold to native biological tissues have been extensively studied in order to ensure the functionality of scaffold both in vitro and in vivo. Scaffolds with fibrous design have been considered as grafts for damaged tissues, owing to their physical architecture mimicking the native tissues like articular cartilage and skin. Moreover, such scaffold design was found to promote biological activities and cell behavior and offers nutrient transport [10, 11]. Therefore, various scaffold fabrication techniques including electrospinning [10, 12], freeze drying [8, 13], phase separation and self-assembly have been conducted to develop the 3D fibrous scaffolds. Electrospinning is one of the commonly used techniques due to its flexibility and simplicity in producing the fibrous scaffolds with different microstructure morphology by direct adjusting the electrospinning parameters [14].

Besides mimicking the physical architecture of native biological tissues, the mechanical properties of scaffold are one of the important features that need to be taken account as well. Recent researches showed that there is an improvement and better control in tissue regeneration by applying chemical and mechanical stimuli on the seeded scaffolds using bioreactor [1, 15 – 16]. However, such external loading can induce failure and consequently cause loss of scaffold function. In this regard, the mechanical properties of tissue engineered scaffold become critical. Therefore, developing mechanical robust scaffolds with physical architecture similar to that native tissue is crucial to

prevent failure in bioreactor and thus suits their potential tissue engineering applications.

In the present study, synthetic and natural fibrous scaffolds were prepared to mimic the physical architecture and mechanical properties of soft tissues. Synthetic fibrous scaffold was prepared using electrospinning technique while natural fibrous scaffold was extracted from SIS of cattle and further reinforced with alginate hydrogel. They were mechanically tested in uniaxial tensile and fracture tests and it was found that their tensile strength and fracture toughness were comparable to soft collagenous tissue. Hence, these tough fibrous membranes can be treated as potential scaffolds candidate for soft tissue engineering applications.

Methodology

Synthetic fibrous membrane preparation

Synthetic fibrous membrane was produced using electrospinning technique. Gelatin powder was first dissolved in a mixture of glacial acetic acid and water to form a gelatin solution. The solution was then loaded into a syringe and was pumped through a blunt tip needle at constant feed rate by a syringe pump (KD Scientific, USA). A voltage was supplied between the needle and a metal collector using a unit of high voltage power supply.

Natural fibrous membrane preparation

The preparation of natural fibrous membrane was reported in previous study [17]. Fresh SIS from cattle was kept frozen before being processed. The SIS was defrosted for about 10 hours before decellularization (Figure 1a). Layer of mucosa, serosa and muscular were removed by scraping and flushing water to the SIS repeatedly until a white layer of submucosa could be seen (Figure 1b). The submucosa layer was then cut into sheet form and left to dry (Figure 1c).

The SIS membrane was prepared in three different conditions which were dehydrated, hydrated and reinforced with alginate. The dried submucosa layer was referred to dehydrated SIS. For hydrated SIS, the submucosa layer was immersed in PBS solution prior to testing (Figure 1d). For SIS-alginate preparation, the dehydrated SIS was soaked in 3 wt. % of sodium alginate solution for 20 minutes (Figure 1e) and crosslinked with 200 mM of calcium chloride solution overnight (Figure 1f). The SIS-alginate composite was rinsed with distilled water two to three times before and after crosslinking process. The composite scaffold was kept in distilled water until used.

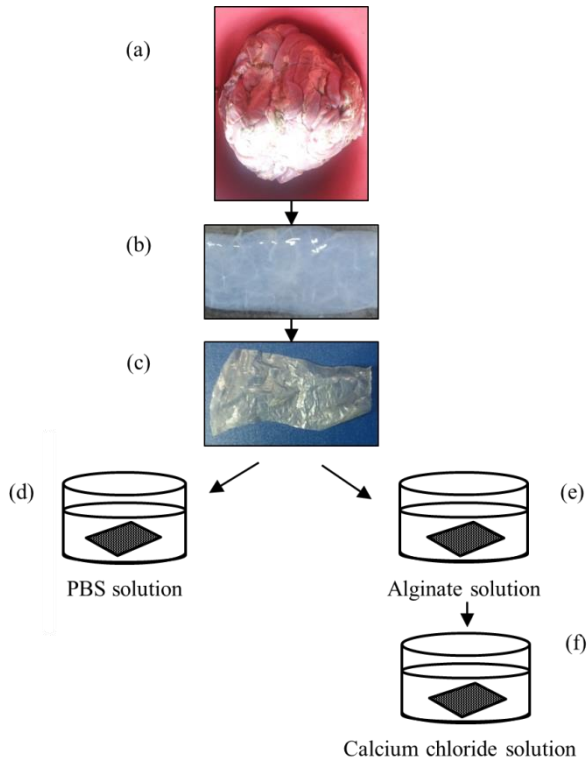

Figure 1: Natural fibrous membrane preparation.

Membrane thickness measurement

Thickness of the synthetic and natural fibrous membranes was determined using a digital caliper with 0.01 mm precision. The membrane thickness was taken at three different points of each type of membrane and averaged as mean thickness.

Microstructure imaging

The morphology of synthetic fibrous membrane was characterized by Scanning Electron Microscope (SEM, Hitachi, USA). Prior to SEM visualization, a thin layer of gold was coated on membrane surface.

Mechanical testing

Uniaxial tensile and fracture test were conducted on all fibrous scaffolds with a universal testing machine (Lloyd Instruments Ltd, UK). For both mechanical tests, all the membranes were cut into rectangular form with dimension of 3 mm x 24 mm. A notch with 8 mm length was introduced to the edge of

scaffolds for fracture test purpose. Load cell of 500 N and 10 N were used for natural and electrospun fibrous membranes, respectively. All the membranes were separated at constant test speed of 3 mm/min until failure.

Fracture toughness determination

The fracture energy, Gc was determined from both notched and unnotched samples. Followed the Eq. (1) described by Rivlin and Thomas [18],

$$\text{Fracture energy, } Gc = W_o l_o \quad (1)$$

where W_o is elastically stored energy per unit volume required to initiate fracture and l_o is the initial length of sample.

Result

Membrane thickness

For the case of electrospun fibrous scaffold, the thickness can be varied from few hundred microns to millimeters, depending on the electrospinning duration. Thicker electrospun scaffold can be collected at longer electrospinning duration. From the previous study [17], the dehydrated and hydrated SIS membranes had similar thickness, which is 0.03 ± 0.00 mm and 0.04 ± 0.01 mm, respectively. When the SIS membrane was reinforced in alginate hydrogel, a significant increment in thickness by one order of magnitude was achieved. The thickness of SIS-alginate was found 0.41 ± 0.01 mm.

Microstructure morphology

The microstructure morphology of gelatin electrospun scaffold was demonstrated in fibrous form (Figure 2). The gelatin fibers were randomly oriented and overlapped on each other. No formation of bead defect was observed in the SEM image.

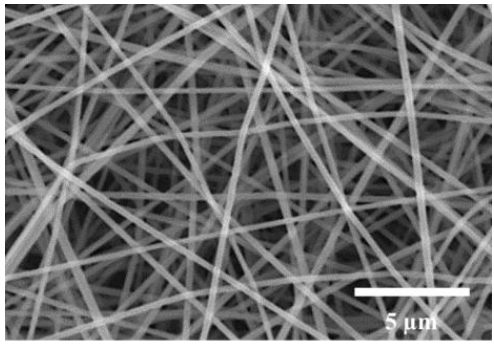

Figure 2: SEM image of gelatin electrospun scaffold.

Mechanical properties of fibrous membranes

Figure 3a shows the comparison of tensile strength, σ for both synthetic and natural fibrous membranes. Dehydrated SIS membrane demonstrated the highest tensile strength, followed by hydrated SIS, gelatin electrospun fibrous membrane and SIS-alginate composite scaffold. The tensile strength of electrospun membrane was found three times greater than SIS-alginate composite membrane.

Gelatin electrospun membrane and hydrated SIS membrane exhibited similar fracture strain (Figure 3b). The fracture strain was increased when the SIS membrane was in hydrated and in composite form. Reinforcement of SIS-alginate composite resulted in highest fracture strain of 0.93 ± 0.14 which was four times larger than electrospun and hydrated SIS membranes.

Fracture toughness of all fibrous membranes was determined using the Equation (1). The hydrated SIS membrane showed the greatest fracture toughness, followed by dehydrated SIS membrane, gelatin electrospun scaffold and SIS-alginate membrane (Figure 3c). No much significant difference in fracture toughness for both gelatin electrospun and SIS-alginate composite membranes.

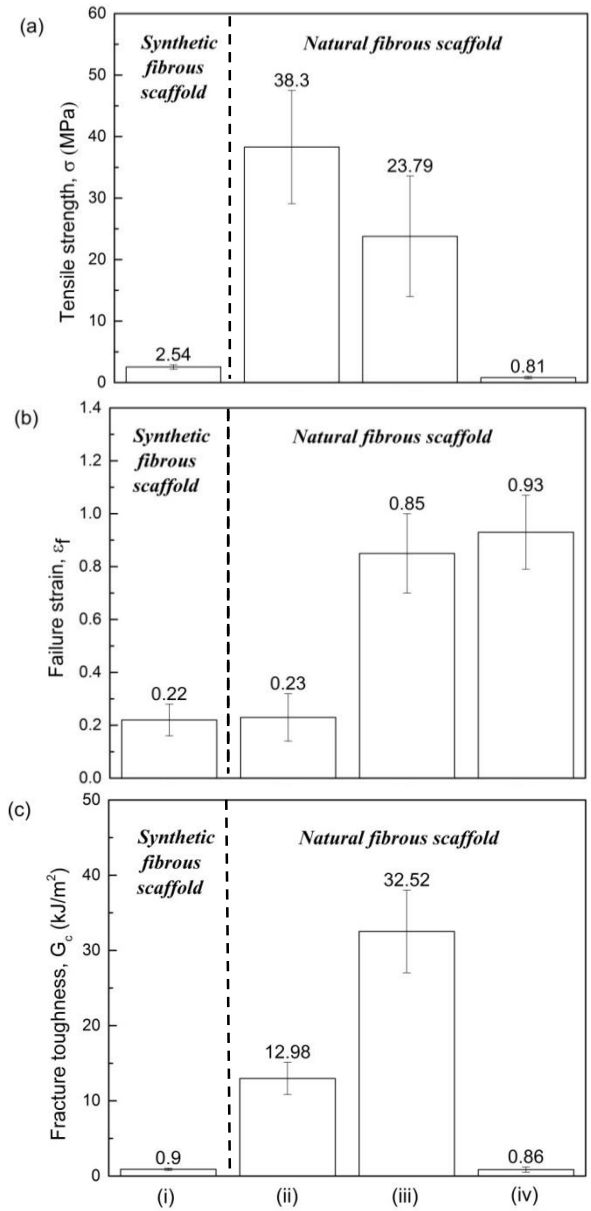

Figure 3: (a) Tensile strength σ, (b) failure strain ε_f and (c) fracture toughness G_c of (i) gelatin electrospun fibrous membrane, (ii) dehydrated SIS, (iii) hydrated SIS and (iv) SIS-alginate composite membrane.

Discussion

Both synthetic and natural fibrous membranes showed their features in mimicking the native biological soft collagenous tissues in term of physical architecture including thickness and microstructure morphology. In this study, the thickness of fibrous membranes was increased in two ways: modulate the electrospinning duration and reinforcement of hydrogel. Both of the ways provided alternatives for preparing 3D tissue engineered scaffolds with adequate thickness. Longer electrospinning duration yielded thicker electrospun fibrous membrane [19]. Reinforcement of alginate hydrogel had significantly increased the thickness of SIS membranes as compared to the dehydrated and hydrated SIS membranes. Similar thickness increment was formed in the case of PCL-alginate hydrogel composite, depending on concentration of alginate hydrogel [20]. The microstructure morphology of gelatin membrane mimicked the fibrous structure of extracellular matrix (ECM) of natural soft collagenous tissues like articular cartilage [21]. Such fibrous microstructure in gelatin membrane can be acted as natural ECM for cells when culturing on the membrane.

Besides the physical architecture, mechanical properties of fibrous membrane are another important feature in developing scaffold which mimics the functional properties of native tissues. Although the dehydrated SIS membrane exhibited greatest tensile strength, the fracture toughness was not the highest among others. This is attributed to the small resistance of membrane to initiate crack propagation during the fracture test. Meanwhile, the reinforcement of SIS fibrous membrane into alginate hydrogel enhanced the resistance of crack propagation during fracture test. Such reinforcement caused the membrane to become more robust to failure than dehydrated membrane and pure hydrogel [20, 22 – 23]. Formation of fiber bundles in front of notch tip resisted the crack propagation and aided energy dissipation prior to failure [24]. Such fiber bundle represented the toughening mechanism in the SIS-alginate membrane.

In our study, the tensile strength of electrospun gelatin membrane and SIS-alginate composite membrane was 2.54 ± 0.38 MPa and 0.81 ± 0.18 MPa, respectively. Meanwhile their fracture toughness was similar, around 0.8 – 0.9 kJ/m^2. Both types of scaffolds had substantially increased the tensile strength and toughness of single gelatin and alginate which was around 0.01 – 0.1 MPa and 0.01 – 0.1 kJ/m^2, respectively [25 – 26]. Such enhancement in mechanical properties was comparable to the natural soft collagenous tissues including cartilage, cornea and skin with tensile strength around 1 – 10 MPa and fracture toughness in the range of 1 – 10 kJ/m^2 [27 – 30].

Conclusion

Both synthetic and natural fibrous membranes exhibited physical architecture and mechanical properties comparable to native biological soft collagenous tissue. The synthetic fibrous membrane produced by electrospinning technique demonstrates microstructure morphology mimicking the fibrous structure that exists in native soft tissue like articular cartilage and skin. The physical architecture of natural SIS membrane was altered through reinforced the SIS membrane with alginate hydrogel to improve the scaffold thickness for more than 10 times. In term of mechanical properties, both types of membranes showed tensile strength and fracture toughness comparable to natural soft collagenous tissues. Hence, these membranes can be treated as potential scaffolds candidate for soft tissue engineering application.

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