Fabrication of Oriented Porous Scaffold for Engineering Myocardial Tissue

Zhuo Xiong[1,2], Ting Zhang[1,2], Le Jin[1,2], Renji Zhang[1,2], Yongnian Yan[1,2]
[1]Department of Mechanical Engineering, Tsinghua University, P.R.China
[2]Key Laboratory for Advanced Materials Processing Technology, Ministry of Education, P.R.China

Introduction: Various tissue engineering approaches have been established for engineering functional myocardial tissue *in vitro*. As cardiac myocytes must be highly organized in order to conduct signals for synchronize contractions[1], cell orientation becomes one of the key factors for cardiac tissue regeneration[2]. Several methods, such as mechanical strain and electrical coupling, have been used to improve cell alignment[2]. As scaffolds serve as a structural template for cell attachment and tissue formation[3], in this study, a kind of oriented porous scaffold were fabricated with self-developed "Oriented Thermally Induced Phase Separation (OTIPS)" technique. The morphology and mechanical properties of the scaffold were evaluated. Engineered myocardial tissues with guided cell alignment were generated and evaluated using this scaffold.

Methods: (1)Scaffold fabrication. 3%Chitosan(Sigma) and 0.4%collagen type I (Sigma) solution were mixed together, pre-cooled at 4□ for 24hr and then poured into a temperature controlled mold. The mixture were cooled and frozen under 1D gradient temperature field for 6hr, which were induced by controlling the top and bottom temperatures of the mold. After freeze-dried, Matrigel(BD) was coated on the obtained collagen-chitosan scaffold, and then sterilized with Co-60 radiation. (2)Scaffold characterization. Morphology of the scaffold was evaluated with Scanning Electron Microscope (SEM). Orientation Index (*OI*) was calculated using the method as previously reported[4]. Tensile properties were measured with mechanical tester(Sample size 5mm×2.5mm×15mm). (3)Cell seeding. Scaffolds were immersed in 100% ethanol and dried. Cardiac myocytes were isolated from 2-day-old neonatal rats and were seeded with a density of $2.5\times10^8/cm^3$. Constructs were statically cultured, and media was changed every 3days. (4)Cell morphology. After 7 days of cultivation, constructs were fixed with 4% paraformalclehyde, and cell morphology was evaluated by SEM and hematoxylin and eosin (H&E) staining.

Results: The fabricated scaffold had uniformly unidirectional pores with interconnected micro pores and fibers across the oriented direction (Fig. 1). This structure was obtained by controlling the crystallization of solvent with 1D gradient temperature field.

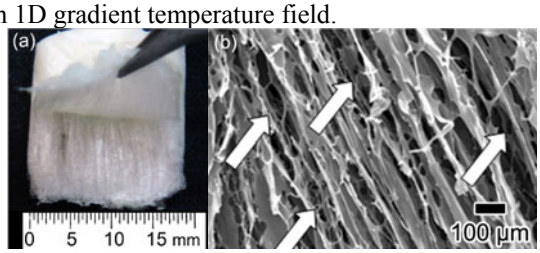

Figure 1. Image of oriented porous scaffold (a) and its high magnification with SEM (b).

The oriented structure can only be formed while temperature gradient *dT/dx* was higher than 4K/mm. *OI* value characterized the orientation degree of scaffold. And the results showed that *OI* value increased with *dT/dx* (Tab. 1), which means pores in the scaffold were more identically oriented while *dT/dx* increased.

Table 1. *OI* value with different temperature gradient.

dT/dx	4 K/mm	8 K/mm	20 K/mm
OI	0.901 ± 0.058	0.951 ± 0.029	0.984 ± 0.010

In this scaffold, chitosan served as the main mechanical support, and tensile strength increased with the chitosan concentration. Additionally, tensile strength can be obviously enhanced along the longitudinal direction, which was about 5 times higher than the transverse direction. (Fig. 2)

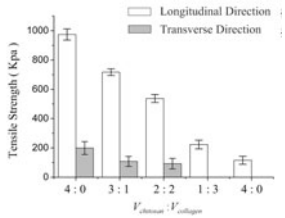

Figure 2. Tensile strength of the scaffolds in different directions and with different chitosan:collagen volume ratio.

After 7 days of culture *in vitro*, the seeded cardiac myocytes interconnected and attached to the scaffold (Fig. 3). Most of the cells aligned with orientation of the scaffold.

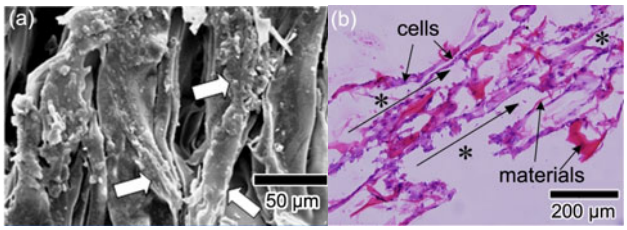

Figure 3. Cell morphology and histology assay. (a) SEM photo, (b) HE staining.

Conclusions: In this study, a kind of oriented porous scaffold was fabricated with OTIPS technique and firstly used for engineering myocardial tissue. The oriented morphology was controlled by 1D temperature gradient parameters and volume ratio of materials. Tensile strength was highly improved along the longitudinal direction. This scaffold mimicked the structure of native extracellular matrix and had similar mechanical properties. Cell culture results indicated that oriented structure guided cell growth direction and improved cell alignment, which offered potential advantages for further engineering of functional cardiac tissue.

References: [1] Cohen S, et al. Sci Am. 2004:291(5):44-51. [2] Eschenhagen T, et al. Circ Res. 2005:97(12):1220-1231. [3] Radisic M, et al. Philos Trans R Soc B-Biol Sci. 2007:362(1484):1357-1368. [4] Yang F, et al. Biomater. 2006:27(28):4923-4933.