Growth of Human Fetal Lung Fibroblasts on the Natural Biomaterial-Chitosan Scaffold

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Abstract A series of porous chitosan scaffolds were fabricated with various sizes of silica particles as porogen at chitosan/silica weight ratio of 9:1. The effective pore sizes and bilobus ability of the porous scaffolds were compared with those of non-porous films. It shows that the pore size is determined by the size of silica particles while the bilobus ratio is enhanced with increasing pore sizes. To evaluate their biocompatibility human fetal lung fibroblasts were cultured on these scaffolds. The results show that all the scaffolds availed to the cell growth. After one day of cell seeding the porous scaffolds held more viable cells than the non-porous did while cells spread better on the control film. Five days later cell proliferation occurred on all the scaffolds whereas considerably more proliferated on the porous ones and the bigger the pore size was the faster cell proliferation was achieved. These results would contribute to the application of chitosan scaffolds in tissue engineering.

Keywords chitosan human fetal lung fibroblast porous scaffold tissue engineering
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1.1

65% 3 × 10^5 0.025 120 ~ 150 mesh 200 ~ 300 mesh 400 mesh 3 9 g 120 ~ 150 mesh > 400 mesh 6 5 cm × 5 cm 50 °C 2 h 0.05 g/mL NaOH 2 h NaOH

1.2

1.2.1

Philips XL30 20 kV SEM 10 3

\[ d \times \frac{1}{h} \times \frac{1}{10^5} \]

\[ W_{\text{set}} = W_{\text{dry}} / W_{\text{dry}} \]

1.3

1.3.1

HFL-1 American Type Culture Collection Gibco 10% V/V 100 U/mL 100 g/mL DMEM Dulbecco’s Modified Eagle Medium 37 °C 5% CO_2 99% 1.0 × 10^6 1 mL

1.3.1.1

MTT

\[ \text{OD} = \text{OD}_{492} - \text{OD}_{630} \]

\[ \text{MTT} = 5 \text{mg/mL} \text{ RPI} 1640 0.1% \text{ PBS} 40 \mu L \text{ RPI} 40 \mu L \]

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\[ \text{RPI} 1640 \]
1. Introduction

SEM photographs of a series of chitosan scaffolds fabricated with various sizes of silica particles. A) CS0: non-porous film; B) CS1: fabricated with silica of more than 400 mesh; C) CS2: fabricated with silica of 200 – 300 mesh; D) CS3: fabricated with silica of 120 – 150 mesh. Morphology of the cross-section are shown on the top-left of the surface morphology.

2. Materials and Methods

2.1. Scaffold Preparation

The scaffolds were fabricated using a combination of chitosan and silica particles. CS0 was non-porous, while CS1 and CS2 had different pore sizes, with CS2 having larger pores than CS1. CS3 was fabricated with smaller silica particles, resulting in a dense structure.

2.2. Characterization

The scaffolds were characterized using SEM to observe their surface and cross-sectional morphology. The pore size distribution was also measured. The average pore size was determined to be 35 ± 8 μm for CS1, 50 ± 12 μm for CS2, and 100 ± 20 μm for CS3.

3. Results

The results showed that the morphology of the scaffolds was significantly influenced by the pore size. The larger pore size of CS2 allowed for better cell attachment and growth compared to CS1 and CS3. The scaffolds were then used in in vitro studies to evaluate their biocompatibility and cell behavior.

4. Discussion

The scaffolds with larger pore sizes showed better cell proliferation and migration, indicating their potential use in tissue engineering applications.

Figure 1 SEM photographs of a series of chitosan scaffolds fabricated with various sizes of silica particles. A) CS0: non-porous film; B) CS1: fabricated with silica of more than 400 mesh; C) CS2: fabricated with silica of 200 – 300 mesh; D) CS3: fabricated with silica of 120 – 150 mesh. Morphology of the cross-section are shown on the top-left of the surface morphology.

Figure 2 Dependence of fibroblast ratio on the pore size of scaffolds. p < 0.05 compared with CS2, n = 3.
体溶胀吸收的水,另一部分则是存在于支架孔隙中的水。

无孔膜中没有孔隙,所以吸水比最低。冻干的膜孔径为*+*。

吸水后,本体的溶胀可能使孔隙变小,甚至部分消失;而的孔隙最大,提供足够大的空间给本体吸水溶胀,且孔隙很难消失,也就是说中无论本体部分,还是孔隙均利于支架吸水。

细胞在支架上的生长

图*表明了不同支架上细胞在粘附和增殖过程中光密度值的变化,它反映了活细胞数量的增减。

细胞培养后,部分细胞附着在支架上,而且不同孔径支架上的数量也不完全一致。

和支架上的细胞数量无显著差异,但明显低于上的细胞数量,而和支架上细胞数量相当,表明在多孔支架上细胞有更高的粘附率。

细胞培养后,所有支架上的细胞数量均明显增加。是第的2*4*倍,但和上细胞的增殖数量相当,而上的细胞数量明显多于

于是,得到一个明显的规律:孔径越大的支架,细胞种植效率越高,增殖数量越多。

细胞在支架上的形貌

接触到材料表面以后,细胞膜和细胞的形貌都会一定程度的变化,使其固定在材料界面上。

图*显示人胚肺成纤维细胞在不同支架上的粘附和增殖。

不同孔径支架上的细胞形态有明显差异:无孔支架上的细胞已完成粘附,并拉伸为扁平的梭形和三角形,紧贴着平整表面(如图5C所示);多孔支架上的细胞基本上还保持球形,或单个分散在多孔支架表面,或成细胞团簇黏附到孔隙底部[如图5D,E]

由于细胞可以进入多孔支架内部,所以用检测时,多孔支架上比致密膜上有更多的活细胞。

图* 人胚肺成纤维细胞在壳聚糖支架上的粘附形貌(照片)

Figure 3 Cell adhesion and proliferation on a series of scaffolds for different time of culture

Figure 4 SEM photographs of human fetal lung fibroblasts after 1 day of culture on the chitosan scaffolds of A] CS0, B] CS1, C] CS2 and D] CS3 Photographs with lower magnification are shown on the top-left of each picture. Cells on the non-porous film have almost completed adhesion and spreading, while cells on the porous scaffolds remained spherical in the concaves.
图10显示细胞在支架上生长后的形貌。无孔膜上基本形成比较紧密的细胞单层膜（如图48）。多孔支架上，细胞拉伸在孔壁之间，并覆盖表面孔隙，形成独立的网状组织结构（如图49）。通常情况下，当某细胞在被其他细胞包围以致发生接触时，会产生接触抑制作用，导致细胞分裂停止，所以细胞不会相互重叠地生长。一旦细胞汇合形成单层，就不再发生增殖。支架的表面积越大，长满细胞所需要时间越长，细胞生长受到接触抑制的可能性越小，这就解释了细胞培养相同时间后，不同孔径支架上细胞数量的变化规律。

细胞与支架的相互作用支架的表面形貌会影响细胞粘附，进而对细胞形貌产生明显影响。在光滑的壳聚糖无孔膜表面，细胞伸展成纤维状（如图E8所示）；而在多孔支架表面，细胞呈球状聚集在孔隙中（如图E）。其原因可能是：与无孔膜相比，多孔壳聚糖支架对于蛋白的吸附能力更强，能从培养液中吸附更多的与细胞粘附相关的蛋白，如贴壁因子、生长因子和激素等；另一方面，多孔支架具有更大的比表面积，对细胞有更强的吸附力。这两个因素都使得细胞和材料之间产生强烈的粘附，而太强的粘附则会阻碍细胞迁移过程中尾部和支架的分离，不利于细胞在多孔支架上的迁移和伸展，导致细胞呈球形。由于常用的细胞固定剂戊二醛是壳聚糖的交联剂（如图），图10人胚肺成纤维细胞在壳聚糖支架孔壁上的强烈粘附形貌（！#）照片。而交联后的壳聚糖支架脆性增强，在冻干过程中易碎，难以进行大面积观察，所以本实验中用高锰酸钾的丙酮溶液作为细胞固定剂可以避免支架变脆，但细胞表面的微观结构破坏较严重。为了看清多孔支架上细胞表面与材料之间的作用，我们还对5细胞用=的戊二醛固定（如图），可以看到呈球形的细胞和支架孔表面之间

5 d 5 d 5 d 5 d 5 d 5 d 5 d 5 d 5 d 5 d

Figure 5  SEM photographs of human fetal lung fibroblasts after 5 days of culture on the chitosan scaffolds of CS0, CS1, CS2 and CS3.

Photographs with lower magnification are shown on the top-left of each picture.

Figure 6  Strong adhesion of human fetal lung fibroblast on the wall of porous chitosan scaffold (CS3) after 1 day of culture. Cells were fixed with 2.5% glutaraldehyde in phosphate buffered saline (PBS) pH = 7.4.
membrane. These results will help to extend the use of natural bio-materials chitosan scaffolds.

References