課題番号　:F-19-HK-0064
利用形態　:機器利用
利用課題名（日本語）　:繊維芽細胞の初期配向が創傷癒合に与える影響
Program Title (English)　:Effect of pre-alignment of fibroblasts on wound healing
利用者名（日本語）　:ランアナン [1)], 大橋俊朗 [2)]
Username (English)　:Lan Yanan [1)], Toshiro Ohashi [2)]
所属名（日本語）　:1）北海道大学工学院、2）北海道大学大学院工学研究科
Affiliation (English)　: 1) Graduate School of Engineering, Hokkaido University, 2) Faculty of Engineering, Hokkaido University。
キーワード／Keyword　: Stamping, Stretching, Migration velocity, リソグラフィ・露光・描画装置

1. 概要（Summary）

Wound healing proceeds through a complex collaborative process, which includes early stage of inflammation, intermediate stage of cell proliferation and migration, and mature stage of barrier remodeling. Previous studies have shown that fibroblast alignment was observed on the mature stage of wound scar. It can therefore be assumed that it would be more efficient to accelerate the wound repair rate if cells are aligned to a particular direction prior to wound closure. In this study, fibroblasts are pre-aligned to evaluate the effect of cell alignment on wound closure rate. For cell alignment, cells were cultured on a polystyrene Petri dish treated with fibronectin by using a PDMS stamp that has micropatterned gratings with 15 μm line and 15 μm spacing. The result would provide improved understanding of alignment of fibroblasts on wound healing.

2. 実験（Experimental）

【利用した主な装置】

電子ビーム描画装置（ELS 3700）; 両面マスクアライナ（MA-6)

【実験方法】

To micropattern cells in the present study, a device fabricated by a series of techniques: photolithography and soft lithography in a cleanroom of Open Facility, Hokkaido University. To get a line and space of 15 μm aligned stamp in this study, a double casting was used. First, The thickness of 50 μm of SU-8 coated on the silicon wafer followed by the revolution rate of spin coating. After the spin coating, it was soft-baked sequentially on 65ºC hotplate for 5 minutes and 95ºC hotplate for 20 minutes. To increase the physical stability of SU-8 and adhesion to the wafer, it was subjected to hard baking at 190ºC for 20 hours. After a positive mold was firstly created on a silicon wafer to produce a silicon mold, then multiple microgroove substrates can be made from negative molds. To minimize adhesion effect, the surface of the silicon mold was salinized.

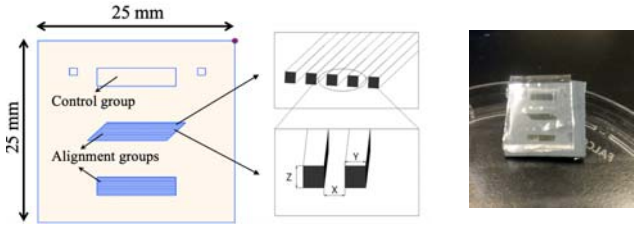

Figure 1. The design of the device used in this experiment. A 50μm of thickness Z, and 15 μm of line Y and space X device was designed on an area of 25 mm^2 silicon wafer.

3. 結果と考察（Results and Discussion）

In this study, we have developed a new experimental methods to observe wound healing where aligned fibroblasts were prepared before wound closure by micropatterned techniques (Figure 1). For the device fabrication, we first patterned a photomask based on the different curvature design using Electron Beam Lithography (EBL). Then, the fabrication process followed by photolithography and soft-lithography process. By using the fabricated device, migration rate of pre-aligned fibroblasts (aligned) and control groups (non-aligned) can be observed.

4. その他・特記事項（Others）

I would like to thank Dr. Agus Subagyo, and DC student Mr. Mazlee Bin Mazalan, for their guidance and technical assistant during the fabrication process of the tortuous microchannel.

5. 論文・学会発表（Publication/Presentation）

1) Effect of Alignment of Fibroblasts on Wound Healing by Using Micropatterning Technique, Lan Yanan, 日本機械学会第 32 回バイオエンジニアリング講演会, 2019

6. 関連特許（Patent）

-none-