課題番号　:F-18-UT-0102
利用形態　:機器利用
利用課題名（日本語）　:破骨細胞の制御に適した電子線リソグラフィによるマイクロパターンの作製
Program Title (English)　:Fabrication of micro pattern for the optimization of osteoclast control by Electron Beam Lithography
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1. 概要（Summary）

In bone remodeling, it is important to control the differentiation of osteoclast by the topographic surface and to elucidate its process in view of intracellular signal transduction. In this study, the podosome formation stimulated by micro-topographic substrate are investigated after confirming the effect of microtopography on osteoclast differentiation.

2. 実験（Experimental）

【利用した主な装置】

超高速大面積電子線描画装置, 汎用 ICP エッチング装置

【実験方法】

Silicon wafer mold with micropattern on the surface was made by electron-beam lithography. Polydimethylsiloxane (PDMS) solution mixed with curing agent was poured on the silicon mold and cured to prepare the micropatterned substrates.

Osteoclast precursor cells were cultured on the fabricated substrates and were induced into osteoclast. At day4, immunofluorescence staining was performed for identifying podosmes.

3. 結果と考察（Results and Discussion）

The fabricated substrates for cell culture were observed with a scanning electron microscope (SEM). Pattern were designed in line-shape with 2μm of width, 2 μm of height and 1, 5, and 10μm of width respectively (Figure 1).

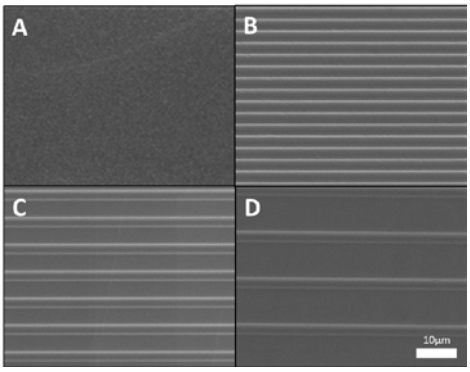

Figure 1 Micropatterned substrate (A; flat, Interval of line B; 1 μm, C; 5 μm, D; 10 μm)

The cytoskeleton and adhesion of differentiated osteoclasts on the pattern were observed by fluorescence staining (Figure 2). Cytoskeletal-adhesive structure known as podosome was formed. In addition, the observation that the adhesion sites were formed on the dent was confirmed by staining vinculin, actin, and DAPI.

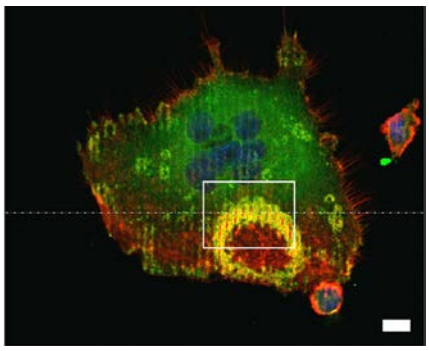
Figure 2 Immunofluorescence image of podosome in the differentiated osteoclasts at 1μm.

4. その他・特記事項（Others）

なし

5. 論文・学会発表（Publication/Presentation）

(1) Heonuk Jeong, 日本機械学会 第 31 回バイオエンジニアリング講演会, 平成 30 年 12 月 14 日.

6. 関連特許（Patent）

なし