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論文題目	Preparation of Microporous Cell-engineering Scaffolds from Liquid Crystalline Polysaccharide		
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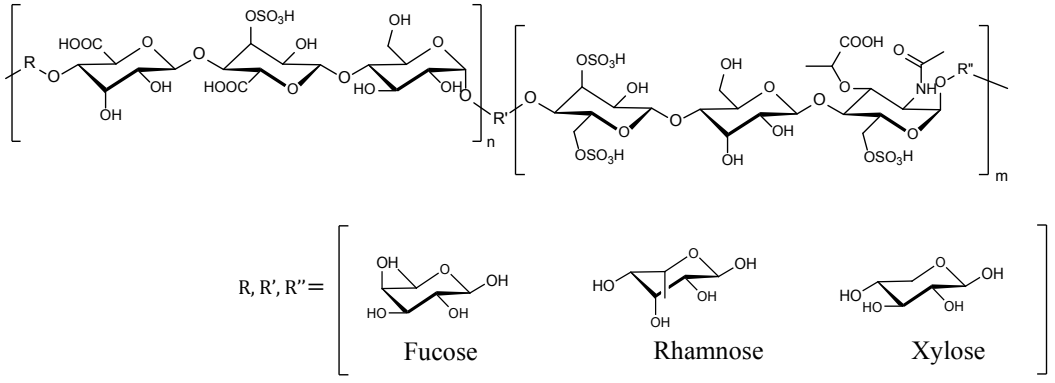
論文の内容の要旨

Background

Scaffold is a significant material of cell-engineering treatment. It possesses important functions of cells supporting materials that allowed for cells growth and new tissue formation. In order to become a cells supporting material, the scaffold need basic requirements such as biocompatibility, biodegradability, high porosity, and shape orientation. The microporous materials are the general formation of scaffolds. It has high water adsorption capacity and abundant interconnecting pore. The high water content that resembles the native tissue allowed for cells attachment and penetration. Sacran (Figure 1), polysaccharide, is extracted from Aphanothece sacrum cyanobacteria. The polymer contains various kinds of sugar residues such as Glc, Gal, Man, Xyl, Rha, Fuc, Ara, GalN, and Mur. It also consists of many functional groups such as hydroxyl, carboxylic, sulfate and amide. The amide sugar, acting like glycosaminoglycan, is the main content found in the extra cellular matrix. By this reason, sacran was selected for scaffold preparation. Moreover, liquid crystal behavior (LC) was observed in sacran solution. In the field of polymer orientation study, experiment conducted on LC has been considered to be a challenging practice. Thus, sacran is one of the most suitable materials for making scaffolds with



property (b) and one direction of fibroblast L929 cells orientation (c).**Figure 1.**

orientation controllability. Here a new microporous scaffold using LC polysaccharide with controlled orientation is presented. This scaffold was prepared by simple methods of solvent casting and freeze-drying. The characteristic in pore size, porosity, water adsorption capacity and mechanical properties were clarified. Moreover, the cell orientation capacity was confirmed.

Aim:

- **(i)** To prepare microporous materials scaffolds using sacran polymer.
- **(ii)** To study the biocompatibility of the scaffolds.
- **(iii)** To prepare sacran hydrogels with micro-patterned on the surface.
- **(iv)** To study the orientation property of sacran anisotropic porous and micro-patterned hydrogels.
- **(v)** To evaluate the orientation of cell on sacran materials.

Results and Discussions:

Chapter II, the surface selective microporous hydrogels with porous structure on side surface and flat on the top (Figure 2) were prepared by a combination of solvent casting and freeze drying techniques. Sacran water solution was casted at 60 °C to produce in-plane orientation thin films. The thin films were physical cross-linked at temperature 60, 80, 100, 120 and 140 °C without cross-linking agent. Then swollen hydrogels with in-plane orientation were created by water immersion of that cross-linked films. Finally, the swollen hydrogels were subjected to freeze dry process. The final products revealed an in-plane porous structure like a tunnel with pore size and porosity of 10-35 μm and 42-80 %, respectively. This is due to the sublimation of water on side surface parallel to the in-plane orientation of sacran polymer chains. In addition, they showed proper mechanical properties in a broad application. At high

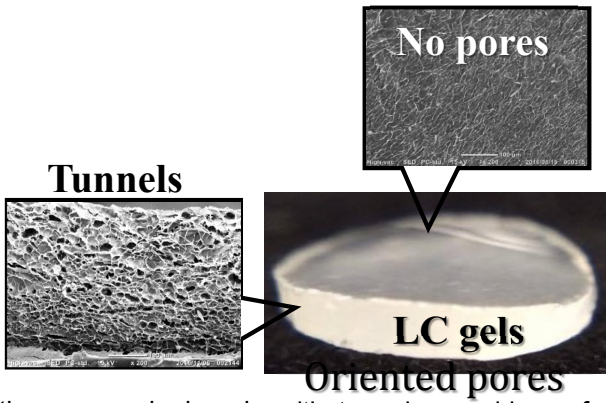


Figure 2. Surface selective porous hydrogels with tunnels on side surface while did not showed porous morphology on the top surface.

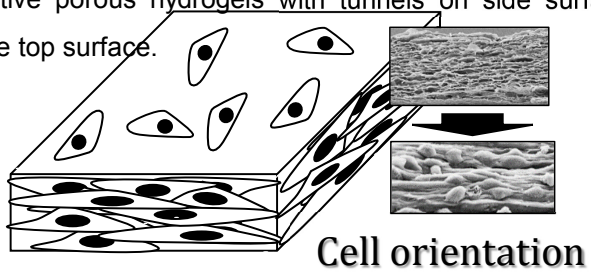


Figure 3. Fibroblast L929 cells attached on surface selective porous scaffold. Randomly orientation is presented on the top surface whereas perfectly orientation is revealed side surface. Additionally, the cell density on the top surface was lower than that of side surface.

cross-linking temperature, the anisotropic porous materials showed low porosity, fine-size pores, and minimal water adsorption. Conversely, the mechanical properties value such as moduli, cross-linking degree and toughness were very high. For low temperature cross-linking, the opposite set of values were observed. The water adsorption was between 9 to 186 times to that of dry material, and the elastic modulus was 3 to 585 kPa. The results reveals that the properties of the materials depends on temperature cross-linking. The surface selective microporous hydrogels were successfully prepared and precisely controlled for their properties.

There are various applications of porous materials, and the tissue engineering scaffold is considered to be one of the most significant. **In chapter III,** the biocompatibility and cell orientation capacity were studied

using mouse fibroblast cell L929 as a model in the cell culture experiment. The surface selective microporous hydrogels showed favorable cell compatibility property. The morphology of cells attachment was analyzed. The cells orientation on side surfaces is parallel to the in-plane orientation of polymer chains. The scaffolds can be altered to mimic the native tissue that represents uni-direction of the muscle orientation (Figure 3). Moreover, the water contact angle and protein adsorption were studied on the materials which were annealed at 100, 120 and 140 °C. The water contact angle was revealed to be 95 to 37°, and the protein adsorption were 36 to 96 μg per 1 mg. In the results, water contact angle, protein adsorption and cell orientation are related to cross-linking temperature, similar to the above-mentioned properties. However, the cell attached on top of the surface were randomly oriented. Another method was employed to control the cell orientation on the top surface of the scaffolds.

In **chapter IV** the scaffolds were casted on polystyrene, with micro-patterned on its surface. The pattern was set in a bar-shape mold with a diameter of 400 μm. The bars were arranged in parallel. The space between bars was fixed at 200, 250 and 300 μm. Sacran scaffolds with surface orientation were prepared with the same procedure to that of surface selective porous scaffolds except for the mentioned patterned substrate surface. The pattern of the scaffold revealed orientation perpendicular to that of bar molds. During the drying process, LC domains were slightly arranged to form an in-plane orientation like a layer. Looking at the side of bar molds, the top point of each bar has the sacran layer accumulated. The point is called nucleation point of orientation. Then the ends of polymer chains are aligned between bars. Polarization optical microscope technique was used to confirm the orientation of LC domains, and the results showed a clear and complete visible orientation. After that, the mouse fibroblast cell L929 was used in cell culture experiment. The distribution of cell orientation degree mimics the polymer orientation on the top surface. Finally, the orientation of cell was efficiently controlled on sacran LC polymer (Figure 4).

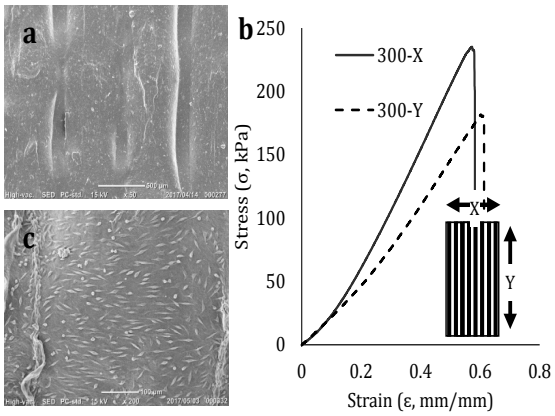


Figure 4. Sacran film with micro-patterned on the top surface (a) showed anisotropic mechanical

Conclusions

The microporous scaffolds with cell-orientation capacity was successfully prepared using sacran LC polymer. They revealed favorable results of pore properties, water adsorption capacity and mechanical properties. Furthermore biocompatibility and cell alignment were also confirmed. The angle of cell attached on materials was highly oriented, mimicking the native tissue behavior. According to the development of technology for human's bioengineering, the field of tissue engineering scaffolds is growing and progressing continuously. Today, the scaffolds are mainly the work of laboratory and research. However, it has the potential to be utilized, especially to save many lives on this planet, in the future.

Keywords: sacran, scaffold, liquid crystalline gels, cell-orientation, cell-engineering

論文審査の結果の要旨

ハイドロゲルは分子レベルの孔を持つ三次元ネットワークからなり、水分を含ませることが可能であるため細胞工学用基板として広く研究されてきた。一方、その構造制御は難しく、配向構造と細胞制御との関係はいまだ不明な点が多い。本論文では、ラン藻由来液晶性多糖類であるサクランのハイドロゲルの配向構造を制御するために先に見出されてきたキャスト法を応用し、かつゲルにマイクロ孔を形成させるために凍結乾燥法を活用した。これにより種々のサイズのマイクロ孔を持つ配向ゲルを各種作成しその構造と細胞接着・進展挙動との相関を明らかにすることを目的として研究を進め、以下のように纏めた。

第一章では、サクラン、ハイドロゲル、多孔性材料に関する研究背景を述べ、従来報告されてきた論文をレビューすることで、本論文の位置づけを行い、目的、意義を述べた。

第二章では、ハイドロゲルの前駆体であるサクランフィルムの加熱架橋条件を振り、一連の面内配向サクランハイドロゲルを作成し、それを凍結乾燥することでフィルム側面のみが多孔質となったハイドロゲルを作成できることを見出した。また、孔のサイズを加熱温度により制御できることを明らかにした。

第三章では、前章で合成した多孔質サクランゲルにL929マウス線維芽細胞を播種したところ、ゲルの側面には上面よりもはるかに多くの細胞が接着していることを確認した。しかも、側面に於いては細胞が一方向に配向して伸展することが見出された。

第四章では、前章で上面の広い面積の部分で接着細胞の伸展性を制御できなかったことを受け、サクランフィルムのキャスト時に前章までの平らな基板上へのキャストではなくマイクロサイズのポリスチレンの棒を一定間隔で並べた基板の上にキャストすることで、サクランが棒の短軸方向に配向することを見出した。かつ、その配向性は棒の間隔を変えることで制御できることも確認した。その配向性はハイドロゲル化後も維持され、その上

にL929マウス線維芽細胞を播種したところ、ゲルの上面でも細胞がサクランの配向に沿って並ぶことが見いだされた。

第五章では、全ての章を総括し、当該多孔性分子配向ゲルの異方的構造と膨潤性および細胞接着・伸展性との相関を纏めて説明した。以上、本論文は各種サクランより多孔性ハイドロゲルを初めて設計・合成し、かつ構造機能相関を明確にするなど学術的に貢献するところが大きい。よって博士（マテリアルサイエンス）の学位論文として十分価値あるものと認めた。