Preparation of an artificial skin by replication of its structure and function using fibrillogenesis

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Statement of Purpose: The most frequently used method for the development of biocompatible skin replacement is development of artificial skin using collagen, for the collagen composes major part of the native skin. For this, we focused on the structural aspect of native skin. The native skin possesses a distinguishable structure from other native tissues, where the density of the collagen decreases in the lower part of dermis. This induces different functionality where the epidermal layer is formed on the dense side and cell infiltration is occurred in the sparse side. A method of reconstruct an artificial skin which is effective for dermal reconstruction is essential.

Our group found that it is possible to obtain a collagen gel with stabilized collagen fibril structure by using fibrillogenesis and gelling contemporarily.[1)] Based on this, it is possible to prepare a collagen gel with gradient density by suppressing the diffusion of the collagen molecules and induce fibrillogenesis contemporarily. In this study, we are reporting on preparation of the collagen gel with gradient density and characterization of the physical and biological properties targeting the reconstruction of the artificial skin structure.

Methods: Two type of Gradient collagen gel was prepared by fibrillogenesis using cell culture insert. First, 3 collagen aqueous solution with different density (0.25wt%, 0.5 wt%, 2 wt%) were prepared. Then, each collagen aqueous solutions were inserted into a cell culture insert. The cell culture insert was put in the 6 well-plate filled with $NaCl/Na_2HPO_4$ aqueous solution for 24h at 4°C to obtain a fibrillized collagen gel (1LC). In order to construct a collagen gel with gradient density, 2 wt% collagen aqueous solution was put into the bottom of cell culture insert, then 0.5 wt% collagen aqueous solution was put on the 2 wt% collagen aqueous solution, before putting 0.25 wt% collagen aqueous solution on top. The cell culture insert was put in the 6 well-plate filled with $NaCl/Na_2HPO_4$ aqueous solution for 24h at 4°C to obtain a collagen gel with gradient collagen density (3LC). To compare the fibril density of the gel, the gradient structures of these gels were observed by atomic force microscope (AFM) and scanning electron microscope (SEM) and collagen staining kit to stain collagen fibril. To investigate strength of the gel, compressive test was executed using creep meter. Swelling test was executed by putting them into distilled water at room temperature. These samples were immersed until it reaches equilibrium. To study cell behavior on the 3LC, L929 was seeded on these matrices, and cell number was determined for 5days.

Results: Basically collagen molecules fibrillize in cell culture plate by the $NaCl/Na_2HPO_4$ diffusion. The gelling and fibrilligenesis process occurs contemporarily. The 1LC and 3LC are stable in water, and did not observe the hydrolysis and shrinkage at 37°C. The surface of the collagen matrices observed using AFM showed that the collagen fibrils were formed with 67nm of D-periodicity. The density gradient observed with collagen staining and SEM was clearly confirmed for 3LC. The dense collagen layer and the thin collagen layer existed without boundaries, implying that the layers were attached to each other by integration. This is thought to due to the fast diffusivity of the salts from outside inducing fibrillogenesis. Using this property, it is also possible to prepare a collagen and collagen-elastin separate layers attached to each other. The upper part of the 3LC showed partial aggregation of the collagen fibrils, while the on aggregation was not shown for below part, indicating that the gelling has occurred for the upper part of the 3LC. The swelling ratio and mechanical strength test result showed that collagen matrix possesses both physical properties gel and sponge.

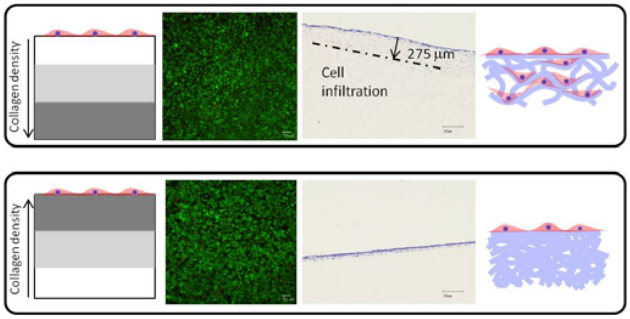

Figure 1. The schematic image of *in vitro* experiment and the images of cell proliferation. Cell proliferation on the sparse layer (top) and dense layer (below) on the 3LC. The cell intrusion into the collagen matrix is shown for 3LC.

Figure 1 shows the schematic images of cell adhesion and proliferation. The L929 cultured on the dense part and sparse part of the 3LC showed that the cell adhesivity was similar to each other. However, the cell starts to penetrate into the collagen matrix in the case of cell culture on the sparse part of collagen matrix. This is obvious for the density of the collagen fibril is much lower. Interestingly, the contraction of collagen matrix was not observed for all matrices.

Conclusions: We have successfully prepared a collagen gel with gradient structure. This is the first time that the collagen gel with gradient concentration within a single structure has been prepared. The collagen matrix is composed of collagen fibrils, which is expected to show different biological property *in vivo,* where epidermal layer and cell infiltration can be controlled by the different collagen fibril density.

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References 1) Nam K et al., J. Biomater Polym Edn. 2009; in press.