Geometry of artificial ECM
Three-Dimensional Structure of Titanium-Web (TW) Promotes Differentiation of Human Bone Marrow Mesenchymal Cells into Osteoblasts

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Abstract: We demonstrate that a new culture system using a titanium web (TW) consisted of titanium fibers (50 mm diameter) can promote differentiation of mesenchymal cells into osteoblasts without conventional application of dexamethasone. Human mesenchymal stem cells (hMSC, Cambrex) and MC3T3-E1 were cultured on titanium web (87% of porosity). ALP/DNA in hMSC at 2weeks was 2 times higher and MC3T3-E1 at 4 weeks was 4 times higher on TW than those on the conventional plastic dishes. Effect of 3D structure of TW is self-evident and the results will open a new age of 3D bone cell biology using TW.

Keywords: three-dimensional culture, scaffold, titanium fibers, bone marrow mesenchymal cells, stem cells

Introduction

There are increasing needs for efficient three-dimensional (3D) scaffold for cell culture systems, since it is becoming clear that there are remarkable differences in cellular behavior between the cells cultured on three-dimensional environment and cells cultured on the conventional flat dishes.[1)] But until recently only a few solid 3D biomaterials with sufficient porosity for cell growth have been provided. We were the first to show that titanium web (TW) consisted of fibers with diameter of 50 μm could provide a suitable environment for bone ingrowth *in vivo*.[2-8)] One of the advantages of TW is its mechanical strength, maintaining a very stable 3D geometry as an artificial extracellular matrix. In this paper, we demonstrate that human bone-derived mesenchymal stem cells (hMSC) differentiate into osteoblasts more rapidly in TW culture than those cultured on plastic dishes.

Materials and Methods

Cell Culture on Titanium Web

Human mesenchymal stem cells (Cambrex Co., USA) were plated at 3.1 x 10^3 cells/ml, grown in the mesenchymal stem cell growth medium (MSCGM, Cambrex Co., USA) supplemented by 10% fetal calf serum, 50 units/ml of Penicillin and 50 ng/ml of Streptomycin. Cultures were maintained until 90% confluent at 37℃ in 5% CO^2 in air. To increase cell numbers, subculture was carried out every 3 days by dissociation with 0.05% trypsin and 0.02% EDTA in physiological buffered saline (PBS).

For experiments, cells in the logarithmic growth phase were dissociated, and inoculated at 1 x 10^5 cells/15 mm plastic dish (Falcon). On the bottom of each well, TW (1.5 x 13 mm) was placed. TW is an unwoven sheet consisted of titanium fibers with a diameter of 50 μm, equipped with 87% porosity and average pore size of 200 μm.[2-8)]

For one group of cultures, MSCGM, which was augmented by 0.1 μM dexamethasone, 0.05 mM ascorbic acid-2-phosphate and 10 mM β-glycerophosphate was used as DEX (+) group. For another group, the same medium except dexamethasone was used as DEX (-) group. Cultures were maintained up to 28 days by feeding every 3 days.

MC3T3-E1 cells, provided by Dr, H. Kodama were grown and cultured in α-modified Eagle's minimal essential medium (α - MEM) supplemented with 10% fetal calf serum as described above. But the medium for MC3T3-E1 cells was not augmented with DEX, β-glycerophosphate, or ascorbic acid-2-phosphate.[9)] in a group of experiment used MC3T3-E1, TW were coated with hydroxyapatite by a modified method reported previously.[6)]

Biochemical Analysis

At the end of culture, insoluble contents of well including TW was washed three times with PBS (-) and extracted in 500 μl of 2M NaCl/0.02M Tris-HCl (pH7.4) for DNA or 500 μl of 0.2% IGEPAL CA-630 (Sigma-Aldrich Co., USA)/10mM/Tris-HCl/1 mM MgCl2 pH 7.4. Alkaline phosphatase activity (ALP) was quantified by the method of Kind and King 3), and DNA content by the method using fluorescent dye (Hoechst 33258). [10)]

Morphological Analysis

At the end of culture, TW with cells and matrix were fixed in 10% neutral formaldehyde, embedded in polyester resin (Rigolac, Oken, Japan), cut and polished into 80 μm section by Maruto Systems (Crystal Cutter Nova MC-415Y and Speed Rap ML-521-d). Sections were stained with Cole's hematoxylin and eosin. In some cases, edge parts of the TW were directly observed by microscopy during culture.

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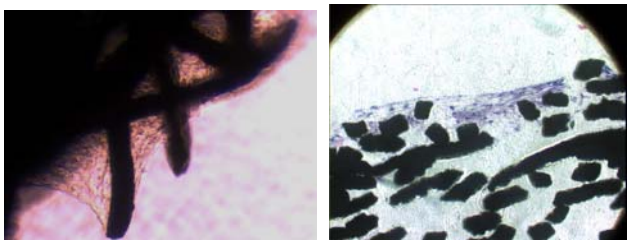

Fig. 1 Direct photomicrograph of cells, producing rich volume of collagen fibers on TW at 2 weeks (left), and perpendicular section of the cell ingrowth into TW after 4 weeks (right).

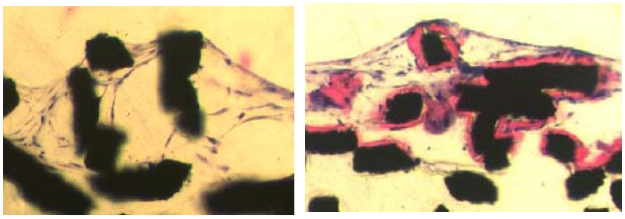

Fig.2 Photomicrographs of MC3T3-E1 cultured on TW for 6 weeks (left) and 8 weeks (right). The sections were made perpendicular to TW disk. Note the active matrix formation by the cells upon matrix-like layers. Black substances were 50 mm thick Ti fiber in TW.

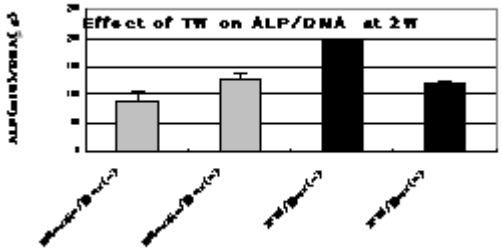

Fig. 3 Alkaline phosphatase activities of human mesenchymal stem cell cultured on titanium web (TW), compared with conventional plastic dishes at 2 week.

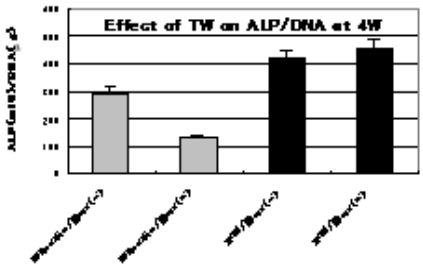

Fig. 4. Alkaline phosphatase activities of human mesenchymal stem cell cultured on titanium web (TW), compared with conventional plastic dishes at 4 week.

Results and Discussion

Figure 1 shows morphology of the MC3T3-E1 cells cultured on TW for 2 and 4 weeks. They were observed directly by light microscope at their edge of the scaffold (felt side), and by thin (80 μm) section perpendicular to TW disk (right side). From both patterns, it was revealed that the cells were actively developing in the rich networks of collagen fibers, supported three-dimensionally by titanium fibers within TW. The results indicate that TW is a feasible artificial extracellular matrix (ECM) for osteoblasts with its 3D geometry. Development of the osteoblasts within TW was more clearly shown at 6 weeks in Fig. 2 (left side). Interesting finding was that at 8 weeks matrix-like structures were formed on the surface of titanium fibers of TW, on and within which, cells grew. One possibility was that this structure related with hydroxyapatite, since TW used in this group of MC3T3-E1 culture was coated with hydroxyapatite. Precise identification of the matrix-like substances needs further analysis.

In Figs 3 and 4, the effects of TW upon alkaline phosphatase activity/DNA in the hMSC cells were shown. Cells were cultured with or without DEX. As a strong inducing reagent for osteoblasts differentiation, DEX is widely used in culture medium. Therefore we wanted to know whether the effect of DEX could be replaced by the 3D effect of TW. It was clearly shown that at 2 weeks, ALP of hMSC on TW without DEX was significantly higher than ALP of the cells cultured on plastic dishes, either with or without DEX. Also at 4 weeks, the same was true concerning ALP of hMSC on TW irrespective of presence of DEX. Reason is not clear why ALP in TW culture with DEX was lower than that without DEX at 2 weeks (Fig.3), and ALP in plastic culture without DEX was higher than that with DEX at 4 weeks (Fig.4).

It was clearly demonstrated that the effect of TW on hMSC could replace the effect of DEX both at 2 or 4 weeks. Again, ALP/DNA of MC3T3-E1cells on hydroxyapatite-coated TW was 4 times higher than those on plastic dishes (data not shown).

Mechanism behind the effect of 3D structure on cell differentiation is a central theme of geometry of artificial ECM. [1, 11] This mechanism inevitably involves adhesive proteins, cell surface receptors, and their intracellular signal transduction, for the most of which we need further studies. In this study TW was shown to be a strong tool with its advantages of mechanical stability and controllable pore sizes, compared with previously reported artificial ECM.

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