THE VARIATION OF MECHANICAL CHARACTERISTIC OF COLLAGEN SCAFFOLD WITH MESENCHYMAL STEM CELL

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1 Introduction

In the tissue engineering, scaffolds influence the space of cells by functioning as an extracellular matrix (ECM), supplying the surface contact for cells and surrounding parts, mechanical stimulation for cells [1]. The ideal scaffolds are biodegradable with adjustable degradation rate that fits in the rate of tissue regeneration [2]. Moreover, the scaffolds should have high mechanical strength to protect the defect part from surrounding tissues and also highly porous structure to provide enough space for cell proliferation and ECM formation [3].

Porous structures of natural polymer such as collagen have widely been used as scaffolds in tissue engineering. Collagen guarantees excellent biological conditions, for example, it stimulates generation and differentiation of cells as extracellular matrix [4]. Collagen scaffolds usually have high porosity [5,6] and interconnected porous structures for cell proliferation [7]. On the other hand, bioactive ceramics such as β-tricalcium phosphate (β-TCP) and hydroxyapatite has also widely been used in bone tissue engineering because of excellent oseteoconductivity, cellular adhesion, accelerated differentiation and mechanical property. It is also noted that β-TCP has faster degradation rate than crystalline hydroxyapatite [8].

Recently, in the field of bone tissue engineering, regenerated bone graft has been one of the primary concerns instead of autografts and allografts. A regenerated graft may be developed by culturing and differentiating mesenchymal stem cells in porous scaffold [9]. In this case, the biochemical and biomechanical culture conditions and the structures and compositions of the scaffold are important factors controlling the quality of the regenerated bone graft.

In this study, porous collagen and collagen/β-TCP composite scaffolds were fabricated by the freeze-drying method. Rat bone marrow mesenchymal stem cells (rMSC) were then cultured in these scaffolds up to 28 days in order to assess the effect of cell growth on the mechanical behavior of the scaffolds.

2 Experimental

2.1 Preparation of collagen/β-TCP scaffold

Type 1 collagen solution (Nippon Meat Packers Inc.) was used to fabricate pure collagen and collagen/β-TCP scaffolds by the freeze drying method. The collagen solution and β-TCP powder (weight ratio 90:10) were mixed by using a magnetic stirrer. The mixed solution was poured into silicon rubber molds, and then frozen at -80˚C in a freezer and freeze-dried using a vacuum pump. The freeze-dried scaffolds were cross linked by glutaraldehyde vapor at 37˚C for 4 hours. After cross-linking, the scaffolds were treated with 0.1M glycine water solution to block unreacted aldehyde, afterwards, they were washed by deionized water and lyophilized [10].The porous microstructures were observed by a field emission electron microscope (FE-SEM).

2.2 Cell culture

rMSC (DS Pharma Biomedical Co.) were cultured in cell growth medium consisting of alpha-minimal essential medium (α-MEM) supplemented

with 10% fetal bovine serum and 1% penicillin-streptomycin. 100,000 cells suspended in 10μl of α-MEM were seeded in each of the scaffolds and then they were incubated for 1 hour to make cells adhered. After 1 hour incubation, these scaffolds were transferred to a 12-well plate containing 2 ml of differentiation medium per well. The differentiation medium was composed of cell growth medium and the supplement of osteoblast differentiation (KE-200, DS Pharma Biomedical Co.) including MEM, β-glycerophosphate, L-ascorbic acid and dexamethasone. The plate was then incubated at 37 ˚C in a humidified atmosphere of 5% CO_2. The α-MEM was changed twice per week.

2.2 Experimental methods

Compression tests of the scaffolds with proliferated cells were conducted periodically by using a conventional testing machine at a loading-rate of 1 mm/min to evaluate compressive mechanical properties such as the stress-strain relation and the elastic modulus. Three specimens were tested after they were removed from the media periodically. As the control group, scaffolds without cells seeded were tested as well as the scaffolds with cells.

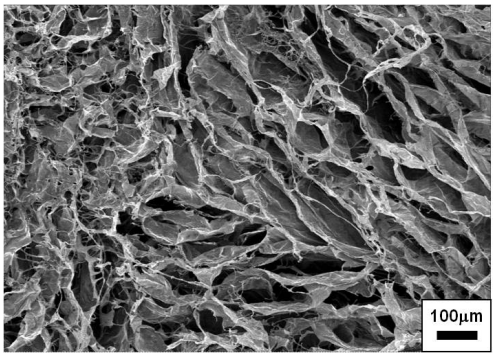
The surface of the specimen was observed using FE-SEM in order to characterize the proliferation behavior of the cells.

The cell number and alkaline phosphatase (ALP) activity were also evaluated using a spectrophotometric plate reader. Cell Counting Kit (DOJINDO) was used by following the instruction provided by the supplier. After the specimen soaked in PBS was placed into the centrifuging tube, the test reagent was added and reacted for 2 hours at 37˚C in a humidified atmosphere of 5% CO_2. The light absorption of the reaction solution was then measured by a plate reader at a wavelength of 450 nm. ALP was measured to evaluate the differentiation behavior quantitatively. Specimens for the assay of ALP were prepared by means of Fujita's protocol [11]. Briefly, the scaffolds with cultured cells were washed by PBS, and then frozen at -30˚C. After repeating the freezing and thawing process three times, the specimens were used for the assay. The LabassayTM ALP kit was used by following the instruction. After the specimen was placed in the centrifuging tube, the buffered substrate (p-nitrophenylphosphate disodium 6.7 mmol/l, pH 9.8) was added and reacted for 15 minutes at 37˚C in a humidified atmosphere of 5% CO_2. The reaction solution was stopped with aqueous sodium hydroxide (0.2 mol/l) and the production of p-nitrophenol was measured by a plate reader at a wavelength of 405 nm.

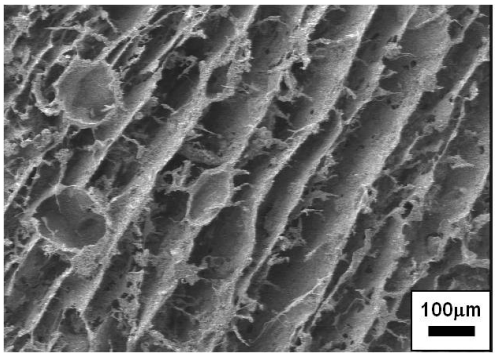
3 Results and discussion

FE-SEM micrographs of the cross-sections of the scaffolds are shown in Fig.1. It is seen that continuous porous structure was well constructed. The ranges of pore diameter in the collagen scaffold and the collagen/β-TCP scaffold are from 50 to 200μm and from 50 to 150μm, respectively. It has been reported that the minimal pore size is from 100 to 150μm required for tissue ingrowth and cell adhesion [12].

FE-SEM micrographs of the surface regions of the scaffolds with the proliferated cells after 7 and 14 days are shown in Figs.2 and 3. By comparing Figs.2 and 3 with Fig.1, it is clearly understood that the surface was totally covered by the proliferated cells and ECM such as collagen and mineralized

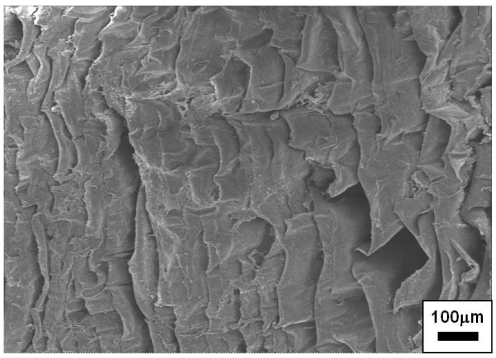


(a) Collagen scaffold.

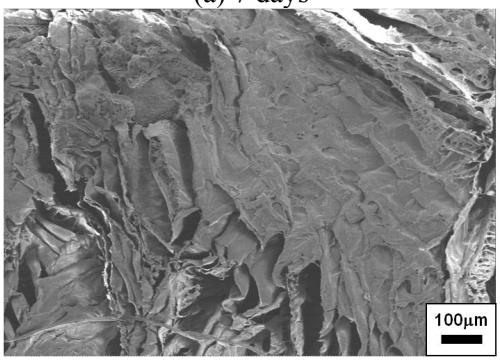


(b) Collagen/β-TCP scaffold.

Fig.1. Micro structure of the scaffolds.

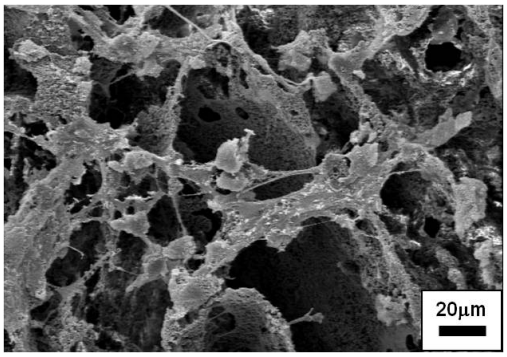


(a) 7 days

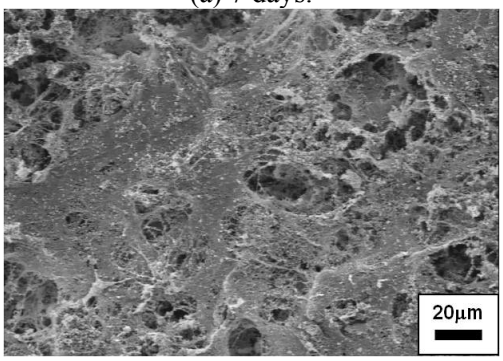


(b) 14 days

Fig.2 Cell growth behavior in collagen scaffold.



(a) 7 days.



(b) 14 days.

Fig.3 Cell growth behavior in β-TCP/collagen scaffold.

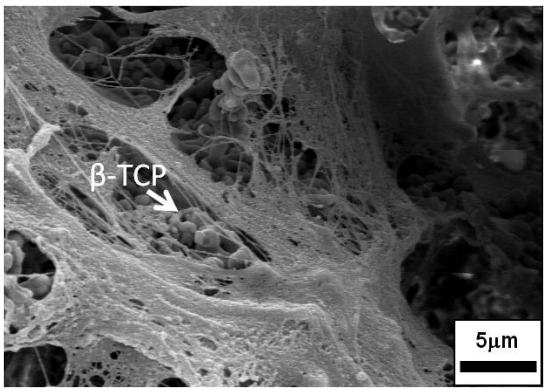


Fig.4 Cell growth behavior of β-TCP/collagen scaffold.

nodules produced by the cells after 7 and 14 days. It was also observed that the morphology has been changed such that the tissue-like structure became thick as culture period increased. A magnified view of the surface of the collagen/β-TCP scaffold is shown in Fig.4. It is noted that the cells grew proliferously and attached to the inside of the scaffold.

The variation of cell number is shown in Fig.5. In the case of the pure collagen scaffold, the cell number tended to increase up to 14 days and then, slightly decreased or almost kept constant up to 28 days. On the other hand, in the collagen/β-TCP scaffold, the cell number tended to increase gradually up to 28 days.

The variation of ALP activity is shown in Fig.6. The ALP of the pure collagen scaffold reached 2.5 times larger than the initial value in 14 days of culture. It is also seen that the ALP increased up to 14 days and afterwards, decreased up to 28 days. Such increase of ALP activity up to 14 days is likely due to the differentiation of rMSC to osteoblasts with increase of the cell number. The decreasing behaviors of ALP activity and cell number from 14 to 28 days are thought to be strongly related to the differentiation and the mineralization. In the case of collagen/β-TCP scaffolds, the ALP activity dramatically increased from 14 to 21 days and became twice of the initial value in 21 days of culture. It is also found that the maximum value of ALP activity of the collagen/β-TCP scaffold was about twice as large as that of the pure collagen scaffold, indicating that the distributed β-TCP particles are very effective for differentiation of the rMSCs into osteoblasts.

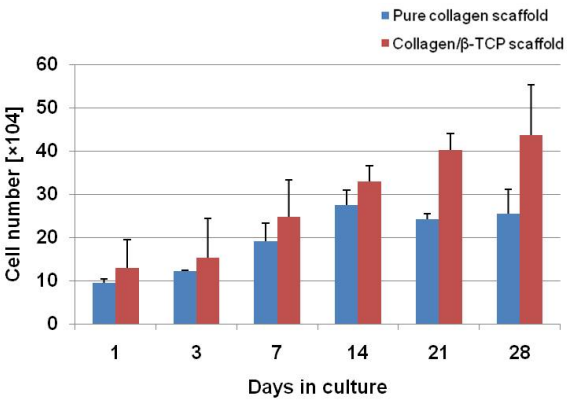


Fig.5 Variation of the number of stem cells.

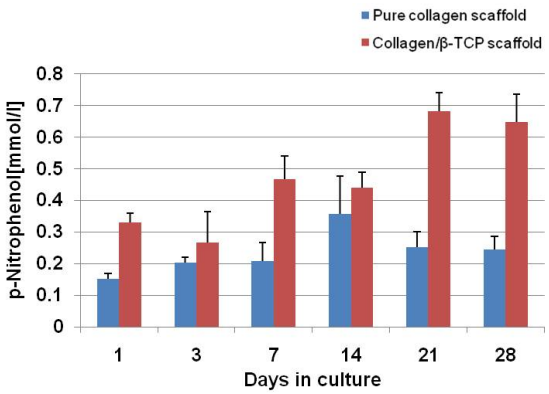


Fig.6 Variation of ALP activity.

The variation of the compressive modulus is shown in Fig.7. In the collagen/β-TCP scaffolds, the modulus tended to increase with increase of culture period, while the modulus of the pure collagen scaffold did not show such change. Actually, the modulus of the pure collagen scaffold tended to decrease up to 14 days and then recovered to the almost same value with the initial modulus at 28 days. These changes of the macroscopic modulus of the scaffolds with cells are thought to be strongly related to the degradation of collagen, the differentiation of rMSC to osteoblasts and therefore, formation of collagen and calcification.

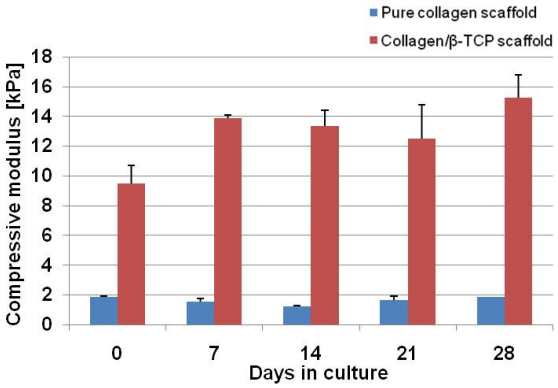


Fig.7 Variation of compressive modulus.

4 Conclusions

Effects of rMSC culture in two different types of collagen scaffolds were examined in this study. The macroscopic mechanical property such as the compressive modulus was correlated with the microscopic cell growth behavior within the porous structures of the scaffolds. The conclusions are summarized as follows:

(1) The compressive elastic moduli of the collagen/β-TCP scaffold tended to increase with increase of culture period and this is mainly due to the cell proliferation, differentiation and subsequent ECM formation and calcification. The modulus of the pure collagen scaffold tended to decrease up to 14 days due to the degradation of the scaffold and then increase up to 28 days because of the proliferation .

(2) FE-SEM studies showed that the cells were well penetrated into the porous structure of the collagen/β-TCP scaffold, on the other hand, for the pure collagen scaffold, the cell proliferation was observed mainly on the seeding surface.

(3) The cell number and ALP activity of the collagen/β-TCP scaffold increased gradually. On the contrary, in the case of pure collagen scaffold, cell number and ALP activity increased up to 14 days, afterwards, decreased up to 28 days.

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