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Research Article

Diameter of a Hollow Center in Cylindrical Hydroxyapatite Scaffold for Hard Tissue Formation

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Abstract The purpose of this study was to obtain an appropriate geometry of porous hydroxyapatite scaffold for hard tissue formation in most of the pores. Porous cylindrical hydroxyapatite structures with a hollow center as a scaffold were manufactured. The diameter of a hollow center was 2 or 3 mm. Bone marrow cells were obtained from bone shafts of femora of 6-week-old male Fischer 344 rats. The cell/hydroxyapatite composite scaffolds were implanted in dorsal subcutis of 7-week-old male Fischer 344 rats. They were removed 8 weeks postoperatively. Serially sectioned paraffin specimens were made and observed histologically. The ratio of pores including hard tissue to the number of total pores per a square centimeter of the cross-sectional area in the scaffolds was calculated. In the scaffold with a hollow center measuring 2 mm in diameter, a new hard tissue formation was observed near the superficies and the number of pores including hard tissue was $260.6 \pm 60.8/\text{cm}^2$. In the scaffold with a hollow center measuring 3 mm in diameter, new hard tissue was observed between superficies and the wall of the hollow center. The number of pores including hard tissue was $589.6 \pm 73.3/\text{cm}^2$.

Keywords hydroxyapatite; scaffold; bone marrow cells; hollow center; in vivo

1 Introduction

Some previous reports proved hard tissue formation by bone marrow stem cells in porous hydroxyapatite block used as a scaffold [1,3]. However, it may be difficult to induce hard tissue in all pores of the scaffold. All pores must be filled with hard tissue to enhance physical strength of the scaffold. The tooth consists of cylindrical hard tissue including dentin and pulp in the central portion.

We reported that the scaffold with a hollow center showed new hard tissue formation in many pores between the external superficies and the wall of the hollow. On the other hand, in the scaffolds without a hollow center, hard tissue formation was observed in only a few pores in the area near the external superficies [2].

In this study, two types of scaffolds with a hollow center of which diameter was different were designed and manufactured. The purpose of this study was to confirm the possibility of hard tissue formation in pores of the scaffold with the different diameter of the hollow center.

2 Materials and methods

The Animal Welfare Committee of Osaka Dental University approved the experimental procedures on use and care of animals. This study was performed under the Guidelines for Animal Experimentation at Osaka Dental University.

Porous cylindrical HA structures with a hollow center as a scaffold for bone and tooth regeneration were manufactured. The sizes of the scaffolds with 55% total porosity were 8 mm in diameter and 10 mm in height. The diameter of a hollow center was 2 or 3 mm. They were manufactured by Hoya Corporation.

Bone marrow cells were obtained from bone shafts of femora of 6-week-old male Fischer 344 rats. A primary culture for 1 week was enforced for the cells. Then, subculture for 1 week was performed with MEM containing 15% fetal bovine serum, 1 mM β-glycerophosphate, 10 nM dexamethasone, 82 μg/mL ascorbic acid and antibiotics. The bone marrow cells were released from substratum. The hydroxyapatite scaffolds were immersed in bone marrow cell suspension at 1×10^7 cells/mL concentration. The cell/ hydroxyapatite composite scaffolds were implanted in dorsal subcutis of 7-week-old Fischer male 344 rats. They were removed 8 weeks postoperatively, embedded in paraffin and 5 μm of serial sections were made. The sections were stained with hematoxylin-eosin.

The calculated cross-sectional area of the hydroxyapatite scaffold with a hollow center measuring 2 mm in diameter was $0.471\,\text{cm}^2$ and one with that measuring 3 mm in diameter was $0.455\,\text{cm}^2$. The ratio of pores including hard

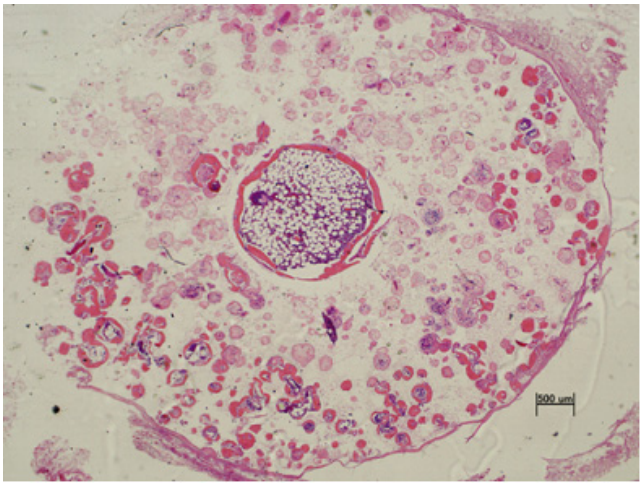

Figure 1: Micrograph of scaffold with a hollow center measuring 2 mm in diameter.

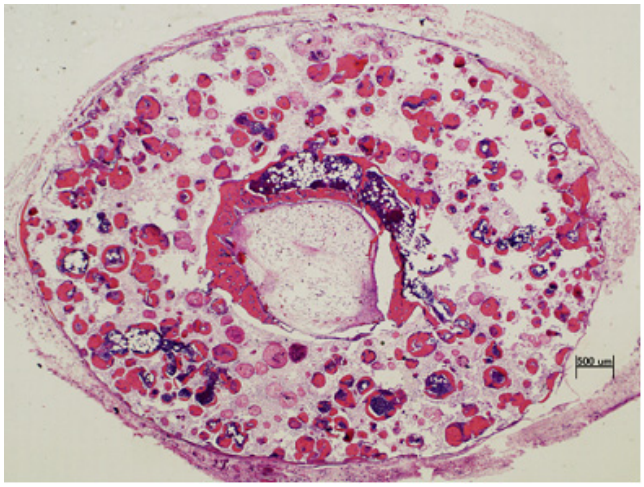

Figure 2: Micrograph of scaffold with a hollow center measuring 3 mm in diameter.

tissue to the number of total pores per a square centimeter of the cross-sectional area in the scaffolds was calculated.

Data are expressed as mean values ± standard deviations. The statistical significance of differences was determined by Student's t-test, with probability of less than 0.1% considered significant.

3 Results and discussion

In the scaffold with a hollow center measuring 2 mm in diameter, new hard tissue formation was observed near the superficies. A small quantity of hard tissue was formed in the pores in an area adjacent to a hollow center of the scaffold (Figure 1). The scaffold with a hollow center measuring 3 mm in diameter observed new hard tissue formation between superficies and the wall of the hollow center. Hard tissue was formed along the wall in a hollow center of the scaffolds (Figure 2). The number of pores including hard tissue was 260.6 ± 60.8/cm^2 in the scaffold with a hollow center measuring 2 mm in diameter. In the scaffold with that measuring 3 mm in diameter, the number of pores including hard tissue was 589.6 ± 73.3/cm^2 (Figure 3). The statistical analysis by Student's t-test revealed significantly a large number of pores including hard tissue in the scaffolds with a hollow center measuring 3 mm in diameter than those with one measuring 2 mm in diameter.

In the hollow center measuring 2 mm in diameter, most of connective tissue necrotized. On the other hand, granulation tissue was observed in the hollow center measuring 3 mm in diameter. Nutrition supply for hard tissue formation in the pores near the area of a hollow center would be enough in the scaffolds with a hollow center measuring 3 mm in diameter than that measuring 2 mm. The

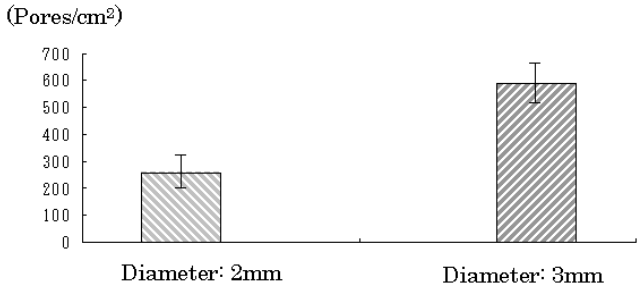

Figure 3: The ratio of pores including hard tissue to the number of total pores per a square centimeter of the cross-sectional area was calculated in the scaffolds.

results of this study shows that a hollow center with larger diameter may contribute to hard tissue formation in many pores of the scaffold. However, large diameter of a hollow center causes thinness of the scaffold. It should cause decrease of mechanical strength of the scaffold. Thickness of the scaffold is rather desirable. It is thought that the cylindrical porous hydroxyapatite scaffold should become a substitute of tooth in the future. The effect of a hollow center approved in this study may resemble a geometrical function of dental pulp cavity as a route for nutrition supply.

4 Summary

The ratio of pores including hard tissue in the scaffold with a hollow center measuring 3 mm in diameter was higher than that of scaffolds with the one measuring 2 mm in diameter. Hard tissue formation in most of the pores in the scaffold with a hollow center measuring 3 mm in diameter induced dense construction.

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