HISTOLOGIC STRUCTURE AND MINERAL COMPONENTS OF SECONDARY DENTIN FORMED BY ENDOCHONDRAL BONE MATRIX GELATIN IMPLANTATION IN RABBIT PULP CAVITY

A. G. Sobhani[*1], A. Shoa-Kazemi[1], B. Niknafs[1], A. Hedaiatpour[1] and A. Sobhani[2]

1) Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

2) Department of Animal Sciences, Faculty of Agriculture, University of Moghadas Ardebili, Ardebil, Iran

Abstract- Many investigators use bone matrix gelatin for bone induction but it is used rarely for repair of teeth defects. This study was designed to evaluate secondary dentin formation by endochondral bone matrix gelatin (E-BMG) in rabbit. E-BMG was prepared from tibia and femur of 4 Deutsche-Poland rabbits with average ages of 4-6 months. The prepared E-BMG was implanted in right incisor teeth pulps of 8 rabbits as experimental groups and left incisor teeth pulps selected as control groups. The light and scanning electron microscopic studies were performed on days 28 and 60 after operation. Also, new secreted matrix was analyzed on experimental, control and normal groups. The histological results showed secondary dentin and osteodentin formation in experimental group on day 28 after operation. The scanning electron microscopic observation on day 60 after operation in experimental group showed mineralized mass on site of E-BMG implantation. In contrast, in control group no mineralized mass was shown. Analyzing of new secreted matrix in experimental group showed the high deposition of calcium and phosphate on E-BMG implantation site. Results of present investigation indicate that implantation of E-BMG in pulp cavity could induce secondary dentin and osteodentin formation in rabbit. E-BMG could be a suitable biomaterial for secondary dentin formation in pulp cavity.

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Key words: Secondary dentin formation, osteodentin, bone matrix gelatin

INTRODUCTION

In recent decades, progress in repair of tooth and bone defects has been considerable. Dental pulp and its contents (vessels and nerves) are injured in many dental defects. Routine treatment of dental pulp injuries are excision of these contents and filling of pulp by specific materials. Dental pulp must be kept healthy, because it is active and live tissue. Furthermore many investigators try to invite new methods for repair of dental pulp without impairment of pulp tissue.

Senn in 1889 was the first researcher who substituted exogenic (bovine) muriatic acid demineralized bone matrix for healing of skull defects in 14 normal dogs (1). One year later, in 1890, Miller used decalcified bone chips as grafts for bone repair (2). In 1957 Ray and Holloway and in 1963 Hejna and Ray reported an enhanced repair of rat skull defects filled with demineralized bone matrix and showed bone growth from the bony rim (3, 4). Recently, some researchers have used demineralized bone matrix (DBM) or bone matrix gelatin (BMG) for induction of osteogenesis within

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*** Corresponding Author:**
A. G. Sobhani, Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran, P. O. Box: 14155-7313
Tel: +98 21 64432348
Fax: +98 21 6419072
E-mail: sobhania@sina.tums.ac.ir

the muscle or subcutaneous regions (5, 6). Urist in 1965 was the first to conduct a series of important experiments for preparation of BMG. Reddi (1972, 1976) used a simple method for preparation of DBM and reported early stages of cell proliferation induced by prepared material (6, 7).

Many investigators have worked hard and demonstrated some methods for dentin induction (8, 9). For stimulation of ectomesenchymal pulp cells and induction of them to odontoblast cells and finally dentin formation, Alliot *et al.* used calcium hydroxide and hydroxyapatite (10). Li *et al.* used growth hormone and insulin-like growth factor for repair of bone and tooth (11). Tziafas *et al.* used DBM in dog's dental pulp and reported that this material could induce ectomesenchymal pulp cells to odontoblast and form secondary dentin in pulp cavity (7, 12). Nakashima *et al.* used recombinant human bone morphogenic protein (rh-BMP) and other biomaterial agents for induction of dentin formation on canine amputated pulp and reported a positive result in dentin formation (13-16). Rutherford *et al.* used osteogenic protein-1 in pulp cavity of monkeys for new dentin formation (9). A literature review revealed only a few studies about the use of endochondral bone matrix gelatin (E-BMG) in pulp cavity for dentin formation (15, 17). Unfortunately, these papers had not explained details of histologic changes of pulp tissue and mineral component of new secreted dentin induced by BMG. In this study we decided to determine the above-mentioned parameters, formed by E-BMG in rabbit's pulp cavity.

MATERIALS AND METHODS

Preparation of E-BMG

Allogenic E-BMG was prepared according to the method of Urist (17-20) as briefly described below.

Four male Deutsche-Poland rabbits with average ages of 4-6 months (1.5-2 kg body weight) were killed by lethal dose of chloroform. Diaphyseal shafts of long bones (femur and tibia) were collected and dissected free of muscles. Bone marrow exactly removed and bones were cut into chips. Liquid nitrogen was used to freeze the bone shafts in this procedure to avoid possible denaturation of proteins. The bones lipid was removed by chloroform/methanol (1/1), demineralized in 0.6 NHCL and extracted successively with solution of 2.0 M $CaCl_2$, 0.5 M ethylenediamine tetra acetic acid (EDTA), 0.8 M LiCl and water (55 degrees centigrade) to remove soluble proteins. Then, the bone chips using liquid nitrogen were pulverized in sample chamber and sieved. All the process was performed exactly in sterile condition.

Implantation of E-BMG

Eight male Deutsche-Poland rabbits were randomly divided to 2 equal groups (4 experimental and 4 controls) and anesthetized with an intramuscular injection of diazepam (2 ml/kg body weight) and ketamine (1 ml/kg body weight). The gingival covering of incisive tooth was dissected and cleaned. The bleeding was controlled by sterile gas compression. From buccal surface a duct with 5 mm diameter was made. The operation site was washed by sterile normal saline. Right inferior incisive teeth were selected as experimental groups and left inferior incisive teeth were chosen as control groups. In decreasing operation temperature during dental hand piece usage, we used normal saline droplet. After bleeding control, in experimental groups 2 mg of E-BMG was implanted in pulp cavity and then the canal filled with oogenol and ZnO mixture. In control groups no E-BMG particles were used and the canal filled only with oogenol and ZnO mixture. The day of BMG implantation was designated as day 0. The animals were held by standard condition.

Microscopic examination and chemical analysis

For harvesting of specimens and evaluation of histologic changes and mineral component of new secreted dentin half of animals were killed by overdose of chloroform on day 28 and other half were killed on day 60 after operation. The incisive teeth were extracted from mandible by bone scissor. The harvested teeth were fixed in 10 percent formaldehyde for 7 days and decalcified by 5 percent three chloride acetic acid for 20 days, embedded in hard paraffin and sectioned at 6 micron thickness. Sections as 18 micron intervals were stained by Hematoxylin and Eosin (H&E). The slides were

viewed microscopically and photos obtained. Some of the samples were selected for scanning electron microscopy (SEM) and chemical analyzing. These samples were not decalcified but dried in 40 degree centigrade temperature. The samples were held on special pedicles and coated by gold for SEM photography. In preparing SEM photograph and chemical analyzing of new secondary dentin, which was induced by E-BMG we used Philips XL-3D Poland Machine. Finally, the data were analyzed statistically using analysis of variance (ANOVA) tests for multiple comparisons among the means at a confidence level of 95% (INSTAT software program).

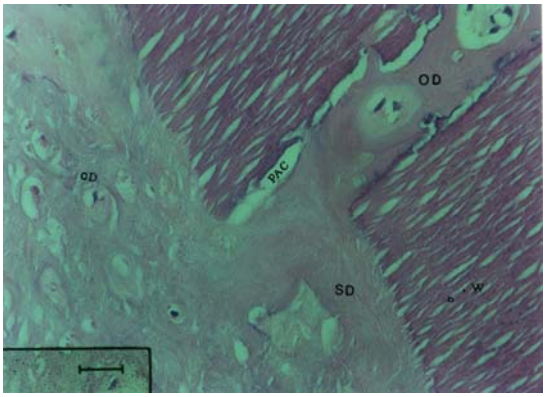

Fig. 2. Photomicrograph of secondary dentin (SD) in marginal zone attached to normal dentin. Pulp amputated cavity (PAC) is filled with produced osteodentin (OD) in experimental group at day 28. H and E staining: Original magnification: × 400.

RESULTS

Histologic findings

At day 28 after operation in experimental group the BMG particles were absorbed almost completely and osteodentin like tissue was visible adjacent to BMG particles (Fig 1). In this site the cells and osteodentin lacunae were detected (Fig. 1). Also, secondary new dentin induced by BMG particles spread from the host dentin to pulp cavity space (Fig. 2). New secreted matrix in pulp cavity was of 2 types: 1) Attached to host dentin a narrow space was filled by secondary dentin, which did not resemble primary normal dentin. In this region the dentinal tubules were visible but they were not having regular arrangement (Fig. 1), and 2) Central zone of pulp cavity was filled with new osteodentin matrix accompanied by some amount of lacunae. On this day, canal defects of some of the samples near pulp cavity were filled with osteodentin tissue (Fig. 1).

In control group, within the pulp cavity no indication of pulp cells differentiation was seen and sectioned pulp canal did not show any repaired tissue (Fig. 3).

At day 60 after operation in experimental group SEM examination showed new secondary heterogeneous matrix formation in pulp cavity. This matrix accompanied some spherical mineral

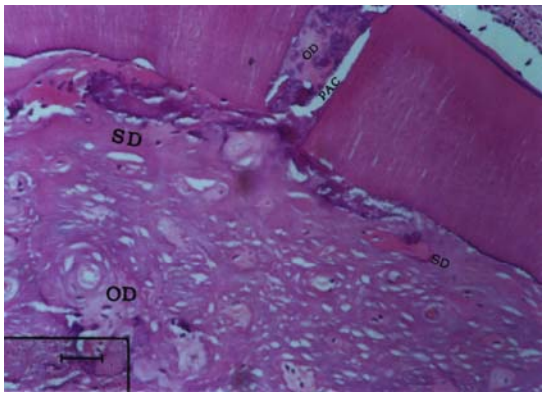

Fig. 1. Photomicrograph of secondary dentin (SD) in marginal zone and osteodentin (OD) in central zone of pulp cavity in experimental group at day 28. Some unabsorbed E-BMG particles are visible in pulp amputated cavity (PAC). H and E staining: Original magnification: × 400.

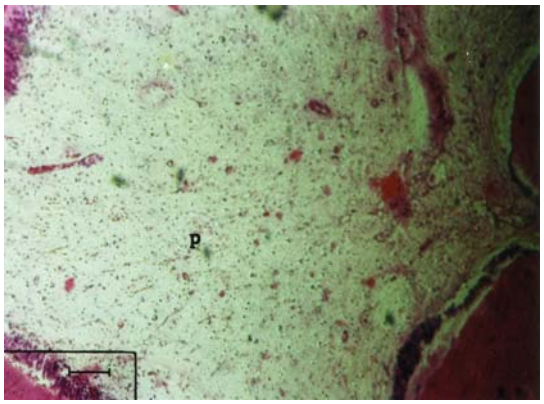

Fig. 3. Photomicrograph from amputated dental cap from control group, showing no indication of pulp cell differentiation and repair. No dentin or osteodentin was found at day 28 in pulp cavity (P). H and E staining: Original magnification: × 400.

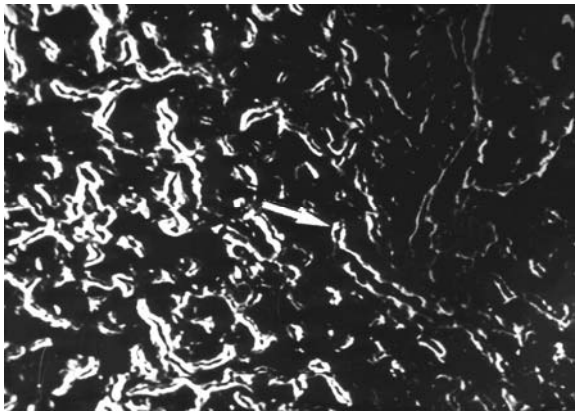

Fig. 4. Scanning electron micrograph from pulp cavity in experimental group, showing crystal trabeculae (CT), irregular dentinal tubule (arrow) and spherical calcium and phosphate mass at day 60. Original magnification: × 2000.

mass and a number of narrow tubules which crossed through the new secreted matrix and opened to the outside (Fig. 4). These changes were not visible in control group (Fig. 5).

Statically, chemical analysis of new secreted matrix within the pulp cavity in experimental group, control group and normal dentin showed significant accumulation of calcium (P<0.0001) and moderate increase in phosphates level in experimental group. The accumulation of calcium in experimental group resembled normal dentin. But in control group levels of calcium and phosphate were much lower compared to other groups (Table 1).

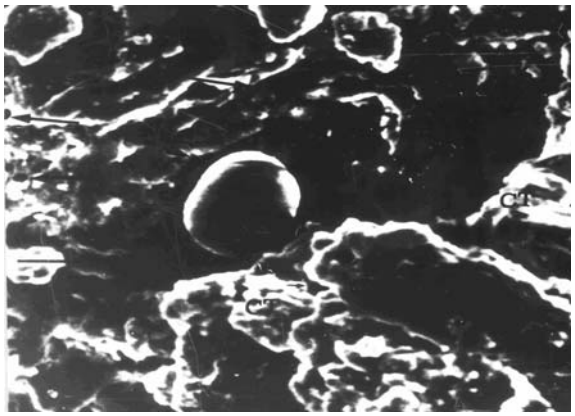

Fig. 5. Scanning electron micrograph from pulp cavity in control group at day 60. Pulp cavity has restricted mineralization and have low level of calcium and phosphate deposition. Original magnification: × 1000.

Table 1. Comparison of calcium and phosphate levels of produced matrix mass 60 days after operation in experimental and control groups with normal dentin*

Mineral elements	Experimental group	Control group	Normal dentin
P	22.19±10.37	17.73±5.31	19.20±5.05
Ca	63.99±5.15†	28.80±3.63	67.70±6.41

*Data are given as mean±SD.

† Shows significant difference with control group which is similar to normal dentin.

DISCUSSION

Repair of dentin defects in human represents a major challenge for dentists. Traditionally, derivatives of calcium hydroxide are used as the best material for pulp capping or repair of some tooth defects (21, 22) but use of these materials is often compromised by non-union, stress fracture and absorption.

Recently some of researchers used demineralized dentin, bone matrix, recombinant human bone morphogenic protein (BMP)-2 and -4, osteogenic protein-1, growth hormone and insulin-like growth factor 1 and so on for induction of pulp mesenchymal cells and dentin formation (8,9,11). In present research we used E-BMG in pulp cavity and studied histologic changes and mineral components of samples by light microscopy and scanning electron microscopy at days 28 and 60 after E-BMG implantation. The results showed that E-BMG could induce heterotopic osteodentin and dentin formation in pulp cavity. Nakashima used allogenic dentin for dentin induction and observed osteodentin and dentin. He also reported that during this period, there was a decrease in secreted osteodentin matrix, disappearance of osteodentin cells and change in lacunae and their size in experimental groups. He believed that this condition had resulted from the mineralization of osteodentin (15). In present study we observed that implanted particles of E-BMG in pulp cavity had been replaced by osteodentin matrix on 28th day after operation but in the zone next to normal dentin, new secreted matrix resembled dentin. Our results were different from Nakashima's study which showed that osteodentin has been formed before secondary dentin. SEM study by Nakashima

and Tziafas have shown new secreted mineralized matrix in experimental groups after 28 days. This mineralized matrix had homogenous structure with a few holes on matrix surface (16). In present study SEM photographs from new mineralized matrix in experimental groups after 60 days of surgery showed lots of holes with irregular shapes. These differences might be due to studying of samples after a long time (60 days). This situation causes increasing mineralization in new secreted matrix.

According to Alliot *et al.*, hydroxyapatite crystals in mineralized matrix were as separated masses, which had spherical or bobble shape with smooth or cavitated surfaces (10). In present study, we observed these kinds of masses in most of the SEM micrographs. But these spherical crystals had smooth appearance without any cavities, which were suspended within mineralized matrix. Alliot *et al.* used hydroxyapatite and BMP to repair dentin (10). They studied SEM micrographs after 28 days in experimental groups and observed tubular structure like secondary dentin. These tubules were opened to matrix surface irregularly. They also reported that secondary dentin had been produced around the dentinal tubule. In present study we observed tubular structures which had pass crystal layer and opened to matrix surface. This condition could be a sign of secondary dentin formation. Nakashima analyzed quantitatively dentin formation around inductive substance and compared it with control groups on 60th day after operation and reported that the rate of secondary dentin formation was 80-82 percent in experimental groups and 42 percent in control group (8).

X-ray and electron probe examination of mineral substance in muscular tissue induced by E-BMG has been performed by Yamashita. He analyzed spherical calcified mass surrounding the BMG particles and found the amount of calcium, phosphate, silicon, sulphur and potassium deposition (23, 24). In present study, chemical analysis of new secreted matrix has been performed and we found lots of calcium and phosphate within exposed pulp cavity in experimental group. Results of our study showed that amount of calcium and phosphate in experimental group was similar to normal dentin and had great difference with control group.

In conclusion, results of this study indicate that E-BMG can induce differentiation of pulp's ectomesenchymal cells to odontoblast like cells and finally osteodentin and secondary dentin production. It seems that E-BMG can be used as a clinical material to repair dentin defects.

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