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Original Research

Histological evaluation of cell behavior in soft tissue around a 4-META/MMA-TBB resin cement applied to the bone tissue in rats

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ABSTRACT

Objectives: The aim of this study was to evaluate the behavior of the soft tissue in rats around a 4-META/MMA-TBB resin and other types of bone cement.
Methods: 4-META/MMA-TBB resin (SBV) and PMMA bone cement (SIMP) were applied to wounds made in the left femurs of 24 male Sprague-Dawley rats. Rats were sacrificed at 3 or 7 days after the operation; then, histological evaluation and immunohistochemical evaluation using a CD68 antibody were performed.
Results: Histologically, at 3 days after the operation, inflammation against operative stress was observed in all groups. At 7 days, marked formation of granulation tissue was recognized in the SIMP group compared with the SBV and control groups. Immunohistochemically, at 7 days, a number of CD68-positive cells were recognized in the SIMP group compared with the SBV and control groups.
Conclusion: These results suggest that SBV has a superior biocompatibility to SIMP in the soft tissue around the rat femur.

1. Introduction

Polymethylmethacrylate (PMMA) has been employed as a bone cement since the 1960s [1] in orthopedics. Originally used for the fixation of prostheses, these cements have more recently been employed to stabilize compression fractures of vertebrae [2–4] and to treat vertebral tumors [5]. Although clinical successes have been reported, PMMA is beset with inherent drawbacks, including the risk of thermal and chemical necrosis to healthy bone tissue [6,7], impaired functioning of the immune system [8,9] and a variety of systemic and cardiovascular reactions [10]. Therefore, orthopedic treatment using dental cement instead of PMMA bone cement has been considered.

4-META/MMA-TBB [4-(2-methacryloxyethyl) trimellitic, a hydride/methyl methacrylate-tributylborane] resin (Super Bond C&B®), is widely utilized as a dental adhesive in Japan. Previous studies have reported its excellent adhesive properties with tooth hard tissues, including enamel [11], dentin [12] and cementum [13], and a moderate biocompatibility with dental pulp [14] and periodontal tissue [15,16]. Furthermore, several studies in recent years have reported its bond strength to bone [17,18]. Consequently, we considered 4-META/MMA-TBB resin as a candidate agent alternative to PMMA bone cement. However, little information comparing the biocompatibility of 4-META/MMA-TBB resin and other bone cements is available.

The aim of this study was to evaluate the biocompatibility of 4-META/MMA-TBB resin and several bone cements around the soft tissue of rats.

2. Materials and methods

2.1. Materials

4-META/-MMA-TBB resin cement (Super Bond C&B® and Catalyst V; SBV) was purchased from Sun Medical, Moriyama, Japan. PMMA bone cement (Surgical Simplex® P; SIMP) was purchased from Stryker Japan, Osaka, Japan.

☆ Asian AOMS: Asian Association of Oral and Maxillofacial Surgeons; ASOMP: Asian Society of Oral and Maxillofacial Pathology; JSOP: Japanese Society of Oral Pathology; JSOMS: Japanese Society of Oral and Maxillofacial Surgeons; JSOM: Japanese Society of Oral Medicine; JAMI: Japanese Academy of Maxillofacial Implants.

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2.2. Surgical procedure

Twenty-four male Sprague-Dawley rats (250–300 g each) were used in this study. This study protocol was approved by the Committee for the Guidelines for Treatment of Experimental Animals at the Tokyo Dental College. All animals were deeply anesthetized by inhalation of diethyl ether and an intraperitoneal injection of sodium thiopental (Ravonal, Tanabe Seiyaku, Osaka, Japan).

Surgery was performed on the left femur of each rat. An incision was made in the skin and dissection was continued through the subcutaneous tissue and muscle. The periosteum was carefully resected and the lateral aspect of the femur was exposed. To prevent the loss of cement materials, a wound (major axis; 3 mm, minor axis; 1 mm, depth; to the bone marrow) was formed with a diamond burr (diameter; 1 mm) with chilled sterile saline. After astriction with a swab soaked with hemostatic agent (TD Zett Jelly, Bee Brand Medico Dental, Osaka, Japan), the area was washed with sterile saline, and was then dried with air. Super Bond with Catalyst V® and Surgical Simplex® P were then put in the wounds according to the manufacturer's instructions. Four rats were used for each material series. As a control, coagulated blood was placed in the wound instead of the resin. The soft tissue was sutured with 4–0 suture material (PERMAHAND, Ethicon Inc., Johnson & Johnson, Belgium). The rats were fed powdered food (Oriental Yeast, Tokyo, Japan) during the experimental period, and were sacrificed with an overdose of the same anesthesia immediately, or at 3 or 7 days after the operation.

2.3. Histological and immunohistochemical analysis

Femurs from each animal were resected en bloc with the surrounding soft tissue including muscle and fascia and were fixed with 10% neutral buffered formalin for 48 h. Specimens were infiltrated with acetone to solubilize the resin and were then decalcified with 10% acetic acid for 1 week. After dehydration in a graded ethanol series, the specimens were paraffin embedded, serially sectioned at 3 μm and stained with hematoxylin and eosin (H–E) or used for immunohistochemistry.

Additionally, we chose fifteen cases (five cases of each group) for immunohistochemical analysis. For immunohistochemical staining, a primary antibody against CD68 (Abcam, Cambridge, UK; diluted 1:100), which is expressed on the surface of macrophages, was used. Endogenous peroxidase activity was blocked by incubating the sections with 3% H_2O_2 in methanol for 30 min. To prevent non-specific reactions, sections were incubated with 3% bovine serum albumin for 30 min in a humidity chamber. After washing in PBS, sections were incubated with the primary antibody overnight at 4 °C. The sections were then incubated with a horseradish-peroxidase-conjugated secondary antibody [Histofine MAX-PO(MULTI), Nichirei, Tokyo, Japan] for 30 min in the humidity chamber. Finally, they were visualized using 0.01% 3,3′-diaminobenzidine, and were counterstained with hematoxylin. The labeling index was calculated from the analysis of about 200 cells in 1000 μm^2 area around those materials.

2.4. Statistical analysis

Data from labeling index of CD68 were analyzed for statistical significance using an analysis of variance (ANOVA) followed by the Fisher's test for multiple comparisons.

3. Results

3.1. Histological findings

At 3 days after the operation, a mild infiltration of lymphocyte in the quadriceps muscle was observed in the coagulated blood control (Fig. 1A and B), and moderate infiltrations of lymphocyte were observed in the SBV (Fig. 1C and D) and SIMP groups (Fig. 1E and F).

At 7 days after the operation, some granulation tissue had formed on the surface of the SBV group (Fig. 2C and D), and in contrast, marked formation of granulation tissue was recognized

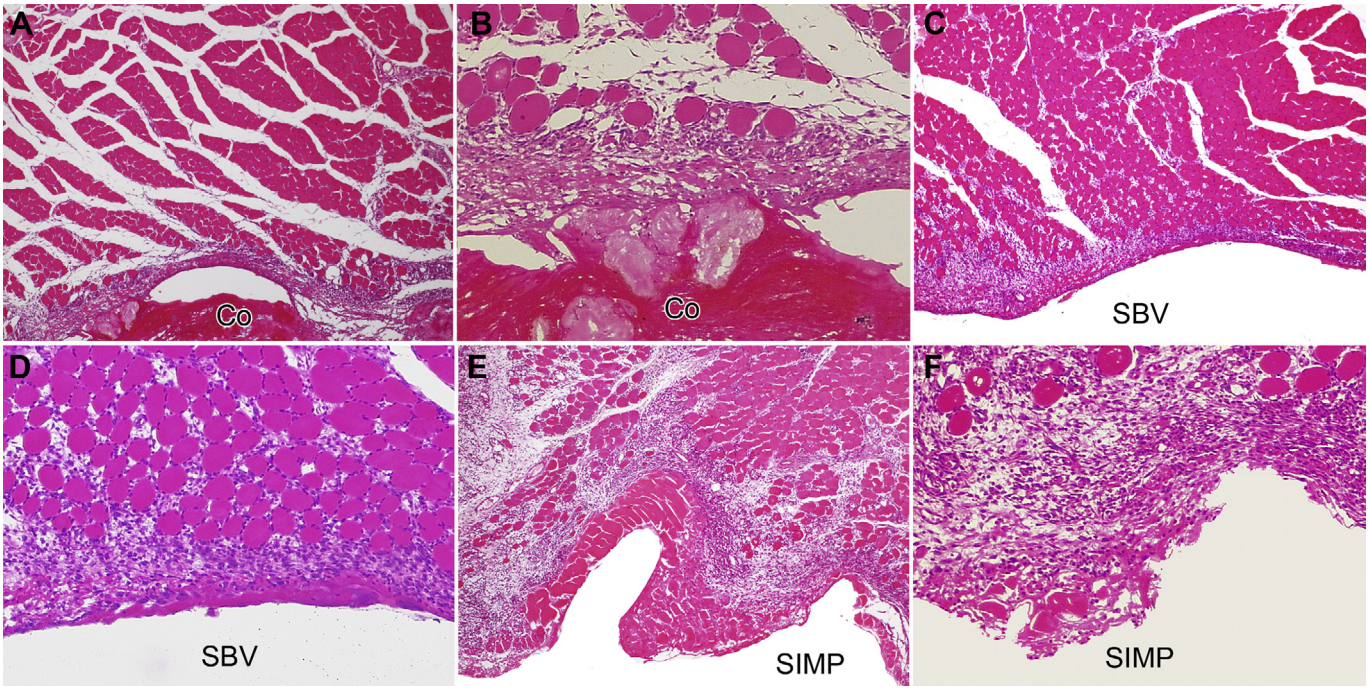

Fig. 1. Histological findings at 3 days after the operation. The lymphocytic infiltration was observed slightly in the control (A and B) group, however, a mild to moderate lymphocytic infiltration was recognized in the SBV (C and D) and SIMP (E and F) groups. "Co" represented coagulated blood (A, C, E: H–E stain, 40×; B, D, F: H–E stain, 200×).