博 士 論 文

Histological evaluation of a novel phosphorylated pullulan-based pulp capping material: An in-vivo study

新規リン酸化プルランベース覆髄材料の組織学的評価：in-vivo研究

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by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGHURES

LIST OF ABBREVIATIONS

PPL	Phosphorylated Pullulan
MTAPPL	Mineral Trioxide Aggregate Containing Phosphorylated Pullulan
NCMTA	Nex-Cem MTA
SB	Super Bond
EDTA	Ethylenediamine Tetraacetic Acid
DMP1	Dentin Matrix Protein
MTA	Mineral Trioxide Aggregate
PL	Pullulan
TBB	Tributylborane
MMA	Methyl Methacrylate
4-META	4-Methacryloyloxyethy Trimellitate Anhydride
PMMA	polymethyl methacrylate
BSA	Bovine Serum Antibody
ICI	Inflammatory Cell Infiltration
MTBF	Mineralized Tissue Barrier Formation

ABSTRACT

The properties of a pulp capping material play a major role in the prognosis of pulp capping treatment. A pulp capping material is considered ideal when it exhibits favourable pulpal response, good sealing ability, excellent biocompatibility, effective antibacterial property, and ease of manipulation. In these regards, performance of several materials has already been evaluated. A new biomaterial, Phosphorylated Pullulan (PPL) has recently gained attention in this field because of its bioadhesive behavior, high biocompatibility, and dentin regeneration ability. Based on PPL's above-mentioned advantageous properties, an innovative Mineral Trioxide Aggregate based material was introduced by adding phosphorylated pullulan (MTAPPL).

This study aimed to evaluate the dental pulp responses to novel mineral trioxide aggregate containing Phosphorylated Pullulan (MTAPPL) pulp capping material after direct pulp capping.

Ninety-six cavities were prepared from fifty-six male Wister rat's maxillary first molar teeth. The pulps were intentionally exposed and randomly divided into four groups according to pulp capping materials: an experimental pulp capping material containing Mineral Trioxide Aggregate with Phosphorylated Pullulan (MTAPPL, GC, Tokyo, Japan), an another experimental material containing Phosphorylated Pullulan (PPL, GC, Tokyo, Japan), conventional Nex-Cem MTA (NCMTA, GC, Tokyo, Japan) and Super Bond (SB, Sun Medical, Shiga, Japan). The rats were observed postoperatively after direct pulp capping at four observational time intervals of 1, 3, 7, and 28 days. Six teeth were allocated for each time interval. The maxillary sections, together with molar teeth were dissected and fixed in 4% paraformaldehyde solution for 24 hours at 4ºC. The tissues were demineralized in 10% EDTA solution and embedded in paraffin. The 5 µm-thick serial sections were cut in the mesiodistal

direction and prepared according to routine histological techniques to observe pulpal reactions with a scoring system. DMP1 and CD34 antigen were used to check odontoblast differentiation and pulpal vascularization, respectively. Statistical analysis was performed using the Kruskal-Wallis test and the Bonferroni corrected Mann–Whitney U test, for evaluating differences between the groups during each observation period. The level of significance was set at .05.

On day 1, mild inflammatory cells were present in MTAPPL and NCMTA groups, few inflammatory cells were present in PPL group, whereas SB showed mild to a moderate inflammatory response. Significant difference was observed between PPL and SB groups ($p < 0.05$). On day 3, moderate to severe inflammatory cells were present in PPL and SB group, whereas MTAPPL and NCMTA groups showed a mild inflammatory response. No significant difference was observed between all the groups ($p > 0.05$). On day 7, a thin layer of mineralized tissue formation was observed in all tested groups with no or mild inflammatory response. No significant difference was observed between the groups ($p > 0.05$). On day 28, no inflammatory responses were observed in the group MTAPPL and NCMTA, whereas PPL and SB groups showed a mild inflammatory response. significant difference was observed between MTAPPL and SB groups ($p < 0.05$). Complete mineralized tissue barrier formation was observed in the group MTAPPL, NCMTA and PPL with no significant difference ($p > 0.05$). the SB group exhibited incomplete mineralized tissue barrier formation, which was significantly different from NCMTA, MTAPPL, and PPL ($p < 0.05$). Positive staining of CD34 and DMP1 was observed in all the group.

This study demonstrated that MTAPPL material showed similar favourable cellular and inflammatory pulp responses and mineralized tissue formation as pulp capping material. Therefore, MTAPPL can be considered as promising direct pulp capping material.

CHAPTER 1

INTRODUCTION

The management of dental pulp exposures mainly caused by caries, traumatic injuries and during operative procedures, has brought interest for many researchers and clinicians [1]. Direct pulp capping is a procedure in which a biocompatible pulp capping material is placed directly over the exposure site, followed by a coronal seal to prevent disease progression, facilitate healing and repair [2,3]. An ideal pulp capping material should demonstrate favourable biological properties, good sealing ability, potent antimicrobial properties, and desirable handling properties.

Calcium hydroxide has been considered as the 'gold standard' of direct pulp capping [4-6]. It has potent antimicrobial properties and can induce hard tissue formation [6,7]. However, it has some drawbacks such as low mechanical properties and poor adhesion to dentine resulting in microleakage and formation of tunnel defects inside the mineralized tissue barrier [2,8]. Nowadays with current advancements in endodontic biomaterials, mineral trioxide aggregate (MTA) is considered the material of choice for vital pulp therapy procedures including direct pulp capping because of its favourable biological profile [9], induction of hard tissue formation [10] and good adhesion to the dentine [11]. Studies showed that direct pulp capping using MTA demonstrated a higher clinical success rate than calcium hydroxide [1,12,13]. However, MTA has some drawbacks such as long setting time, difficult handling properties, discolouration potential and high cost [6,14]. Several bioactive glass and bioceramic based materials have been introduced to overcome these shortcomings [2,6,15].

Phosphorylated Pullulan (PPL) is a biomaterial that has recently gained attention because of its bioadhesive behaviour, high biocompatibility, and tissue regeneration ability [16,17]. Pullulan

(PL) is an uncharged, linear polysaccharide, aerobically formed by multiple strains of polymorphic yeast-like fungus, *Aureobasidium pullulans* in starch and sugar cultures [18]. Because of its favourable biological profile, PL is being tested for various biomedical applications such as gene delivery, selective drug therapy, tissue engineering and wound healing. It is also used in diagnostic applications such as perfusion, receptor and lymph node target-specific imaging and vascular compartment imaging [19]. PL's has low viscosity sustains even after exposure to heat and/or changes in pH [20]. PL has substantial mechanical stability and other functional properties such as adhesiveness, ability to form a film, and enzymatically mediated degradability [19]. Phosphorylated functional monomers incorporated in PPL have been used effectively as dental adhesives for bonding resin composites to dental structures [21].

Based on PPL's above-mentioned advantageous properties, an innovative MTA-based material was introduced by adding phosphorylated pullulan (MTAPPL) [17]. Results showed that the phosphorylation of biodegradable polysaccharide pullulan combined dentine's adhesion capacity with the gradual dissolution of internalized bioactive components [16]. However, information on the pulp tissue responses to MTAPPL as a pulp-capping material is scarce. This study aimed to evaluate dental pulps' responses in rat molars after direct pulp capping with MTAPPL and compared with PPL and conventional MTA materials. The null hypothesis was that there is no difference between the experimental materials with regards to pulpal inflammation and mineralized tissue formation.

CHAPTER 2

MATERIALS AND METHODS

This study was approved by the Committee for Laboratory Animals and Breeding Faculty (#16-0086).

2.1 Preparation of materials:

The pharmacopoeial polysaccharide PL was chemically functionalized with dihydrogen phosphate groups to chemically bridge the biomaterial with the hard tissue via ionic binding of the phosphate functional group to calcium of apatite as mentioned in a previous study [16].

This study was composed of four experimental groups as follows:

Group 1: An experimental MTA containing Phosphorylated Pullulan (MTAPPL, GC, Tokyo, Japan). The experimental MTAPPL was prepared by adding 5% PPL to the original combination of MTA. The MTAPPL powder was mixed according to the manufacturer instructions at a water to powder ratio of 1:3 on a sterile glass slab for 1 min. The mixture was applied directly onto the exposed pulp, followed by coronal restoration using Super Bond (SB, Sun Medical, Shiga, Japan).

Group 2: An experimental Phosphorylated Pullulan (PPL, GC, Tokyo, Japan); the powder was mixed according to the manufacturer instructions with the same technique used in Group 1, followed by coronal restoration using SB.

Group 3 (positive control): Conventional Nex-Cem MTA (NCMTA, GC, Tokyo, Japan) was used. The powder was mixed according to the manufacturer instructions with the same technique used in Group 1, followed by coronal restoration using SB.

Group 4 (negative control): No capping material was used. SB was mixed using the brush-dip technique according to manufacturer instructions and placed over the exposed pulp.

Table 1 shows the composition of each of the materials used in this study.

Table 1 Tested materials and their compositions according to the manufacturers

Materials	Manufacturer	Components
Mineral trioxide aggregate containing phosphorylated pullulan (MTAPPL)	GC, Tokyo, Japan	Portland cement (60%), Bismuth oxide (20%), Calcium sulfate dehydrate (5%), PPL (5%), Other (10%)
Phosphorylated Pullulan (PPL)	GC, Tokyo, Japan	Pure Phosphorylated Pullulan
Nex-Cem MTA (NCMTA)	GC, Tokyo, Japan	Portland cement (60-80%), Bismuth oxide (20-40%)
Super Bond (SB)	Sun Medical, Shiga, Japan	Initiator – TBB, Monomer – MMA, 4-META, Powder – PMMA powder

2.2 Study samples:

A total of 56 male Wister rats (Hokudoh, Hokkaido, Japan), aged 8-9 weeks and weighed between 220-300 g were included in this study. A total of 96 non-carious maxillary first molars were treated with the direct pulp capping procedure, as discussed in the following section.

2.3 Direct pulp capping procedures:

2.3.1 Sedation:

The rats were sedated with inhalation anesthesia (Wako pure chemical, Osaka, Japan), and intraperitoneally injected with a combination of medetomidine (ZENOAQ, Koriyama, Japan), midazolam (Maruishi Pharmaceutical, Osaka, Japan), and butorphanol (Meiji Seika Pharma co, Tokyo, Japan) at a dose of 0.15mg/kg+2mg/kg+2.5mg/kg body weight/rat, respectively.

2.3.2 Cavity preparations:

After achieving analgesia, the teeth were cleaned with a physiological saline solution, followed by cotton soaked with 75% ethanol for surface disinfection (Wako pure chemical, Japan). Cavities were prepared on the maxillary first molars' mesial surface using a #1/2 sterile stainless-steel round bur (Shofu, Kyoto, Japan). The burs were changed after the preparation of every two cavities in the same rat. The cavities and cutting instruments were irrigated with sterile distilled water to prevent pulp damage from heat. The pulp was exposed through each cavity remaining thin dentine using a sterile stainless-steel K file size 20 (Mani, Tochigi, Japan). Bleeding was controlled by pressing sterile paper points over the exposure sites for 1-2 minutes. After the control of bleeding, the exposure site was dried by paper points and then directly capped with each test material, except for the negative control. All cavities were subsequently restored with SB, as instructed by the manufacturer. The opposite teeth cusp tip was slightly trimmed to be out of occlusion to minimize occlusal forces. The rats were observed postoperatively after direct pulp capping at four observational time intervals of 1, 3, 7, and 28 days. Six teeth were allocated for each time interval. Teeth with traumatic pulpal exposures with the bur, suspected fracture and dislodged coronal restoration were excluded.

2.4 Sample preparation and histological staining:

At each time interval, the rats were sacrificed using intraperitoneal injections with an overdose of anaesthetic solution. The maxillary sections were dissected and fixed in 4% paraformaldehyde solution along with the molar teeth (Nacalai Tesque Inc, Kyoto, Japan) for 24 hours at 4ºC and then rinsed with running water for 6 h. The tissues were demineralized in 10% EDTA (pH-7.4) (Wako pure chemical, Osaka, Japan) at room temperature for 21 days. SB material was carefully removed from the cavities after demineralization and rinsed for 24 h with running water. After that, the samples were dehydrated in ascending ethanol grades,

dealcoholized by xylene, and embedded in paraffin. The 5 µm-thick serial sections were cut in the mesiodistal direction using a sliding microtome (Yamato, Saitama, Japan). The slides were numbered, and median sections were selected for hematoxylin-eosin (H&E) staining to determine the pulp tissue's response and mineralized tissue formation.

2.5 Immunohistochemistry examination:

Immunohistochemical staining was performed using a rabbit anti-CD34 monoclonal antibody (dilution 1:200), and a rabbit anti-DMP1 (dentine matrix protein 1) polyclonal antibody (dilution 1:500) as primary antibodies. Sections from each time interval were selected for CD34 and 28-days sections for DMP1 antibody staining. All the sections were deparaffinized in xylene, rehydrated in a graded alcohol series (ethanol 70%, followed by 80%, 90%, and 100%), and then blocking endogenous peroxide activity with 3% hydrogen peroxide for 10 min. Sections were blocked for 15 min with 1% BSA (Bovine serum antibody) solution and then incubated at room temperature for 60 min with the primary antibodies. The sections were subsequently incubated at room temperature for 30 min with the following peroxide-linked secondary antibodies with 3,3'-diaminobenzidine as a chromogenic substrate and countered with a hematoxylin stain solution.

2.6 Observation items and evaluation criteria:

The stained sections were observed under a light microscope for inflammatory cell infiltration (ICI), and mineralized tissue barrier formation (MTBF). Modified versions [22] of the ISO 10993 and 7405 standards were used to evaluate pulp tissue changes after direct pulp capping, as shown in Table 2.

Table 2 Evaluation criteria used for histological analysis of the pulps after direct pulp capping.

	Inflammatory cell infiltration
0	Absence or presence of a few scattered inflammatory cells in the pulp (none)
1	Mild acute/chronic cell lesions (mild)
2	Moderate inflammatory cell lesions seen as abscesses or densely stained infiltrates of polymorphonuclear leucocytes, histiocytes, and lymphocytes in one-third or more of the coronal pulp and/or the mid-pulp (moderate)
3	Pulp necrosis due to severe degree of infection or lack of tissue in one-half or more of the pulp (severe)
	Mineralized tissue barrier formation
0	No mineralized tissue barrier formation (none)
1	A layer of scattered and foggy mineralized tissue deposition (initial)
2	Partial/incomplete mineralized tissue barrier formation extending to more than one-half of the exposure site but not completely closing the exposure site (partial)
3	Complete mineralized tissue barrier formation (complete)

2.7 Statistical analysis

Kruskal-Wallis test and the Bonferroni corrected Mann–Whitney U test for each time interval were used for data analysis. The level of significance was set at 0.05.

CHAPTER 3

RESULTS

Statistical result of the histological evaluation is given in Fig. 1.

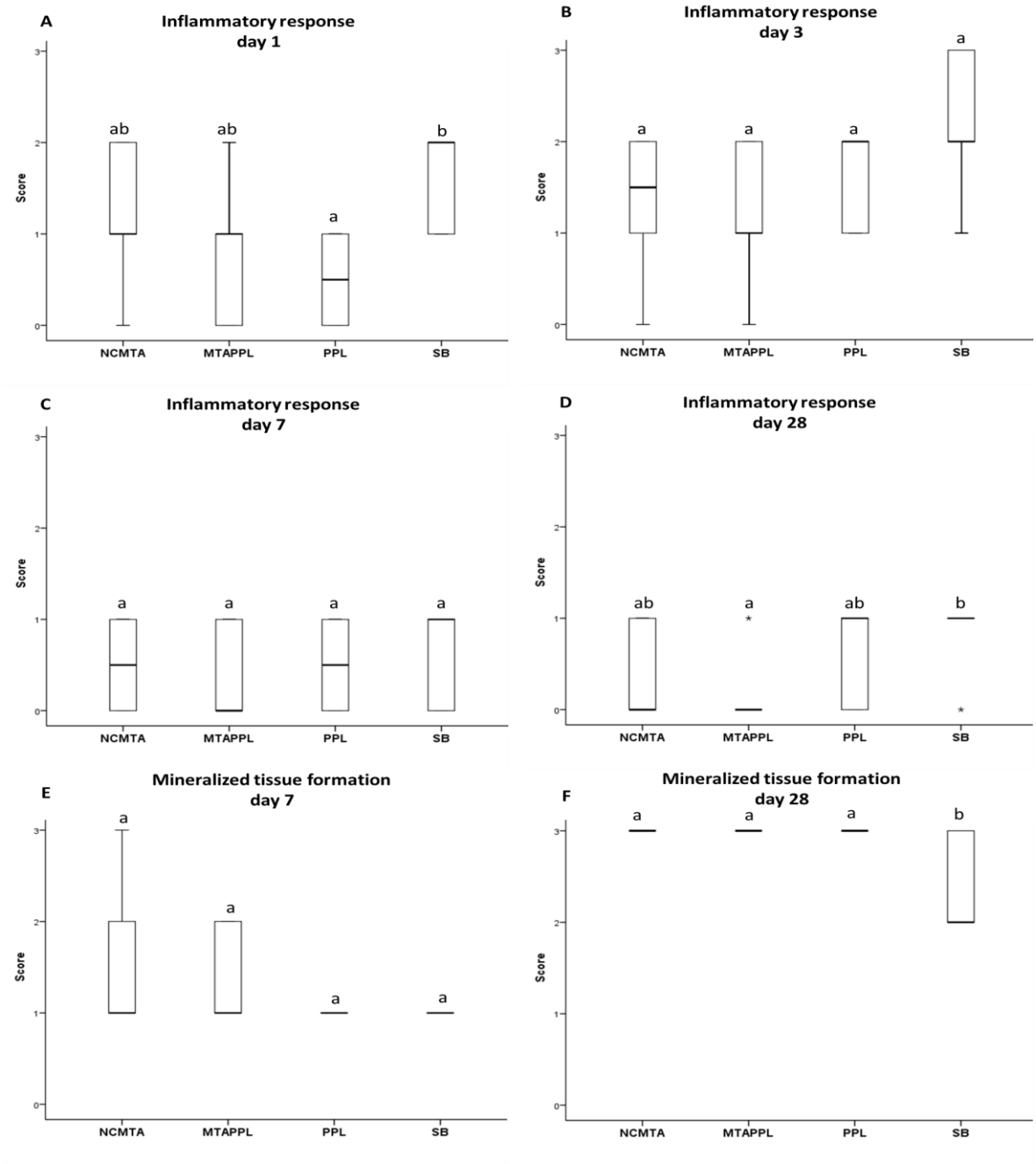

Figure 1: Box plot of data. (A) Inflammatory response at day1 (B) Inflammatory response at day3 (C) Inflammatory response at day 7 (D) Inflammatory response at day 28 (E) Mineralized tissue formation at day 7 (F) Mineralized tissue response at day 28. The letters a and b indicate a statistically significant difference between groups ($p < .05$).

3.1 Inflammatory cell infiltration (ICI)

On day 1, mild inflammatory responses including hyperaemia and inflammatory cell infiltration (mainly polymorphonuclear leucocytes), and areas of necrosis were observed at the exposure site in MTAPPL and NCMTA groups. A few scattered inflammatory cells were present in the PPL group, whereas SB showed a mild to moderate inflammatory response (Fig. 2). A significant difference was observed between PPL and SB groups ($p < 0.05$).

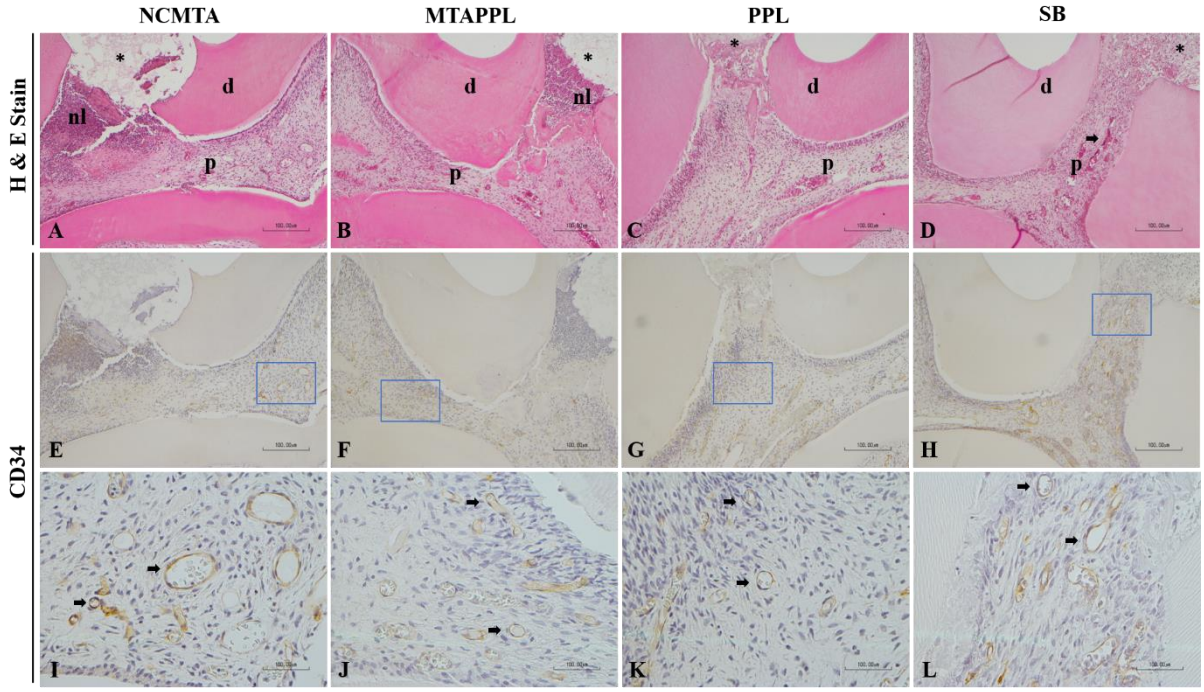

Figure 2: Histology of rat pulps at day 1 stained with hematoxylin-eosin (A-D) and immunohistochemistry with CD34 antigen (E-L). H&E stained sections showed partial pulp tissue necrosis at the exposure site in the NCMTA and MTAPPL groups (A and B, respectively). Only a few scattered inflammatory cells are present in the PPL group (C), and mild to moderate inflammation with a limited accumulation of polymorphonuclear leukocytes and congested blood vessels (arrow) were observed in the SB group (D). No mineralized tissue deposition was observed in all experimental groups. I–L present a high-magnification view of the areas demarcated by the blue rectangular in E–H. In all groups, positive staining of CD34 antigen (I-L) showed less differentiated$CD34^+$ blood vessels (arrows).

p, pulp: d, dentin, nl, necrotic layer, Asterisk (*), indicates pulp exposure sites.

On day 3, moderate to severe inflammatory cells were present in PPL and SB group, whereas MTAPPL and NCMTA groups showed mild inflammatory responses with local infiltration of polymorphonuclear leucocytes and vascular proliferation indicating acute inflammatory responses to the exposure site (Fig. 3). No significant difference was observed between all the groups ($p > 0.05$).

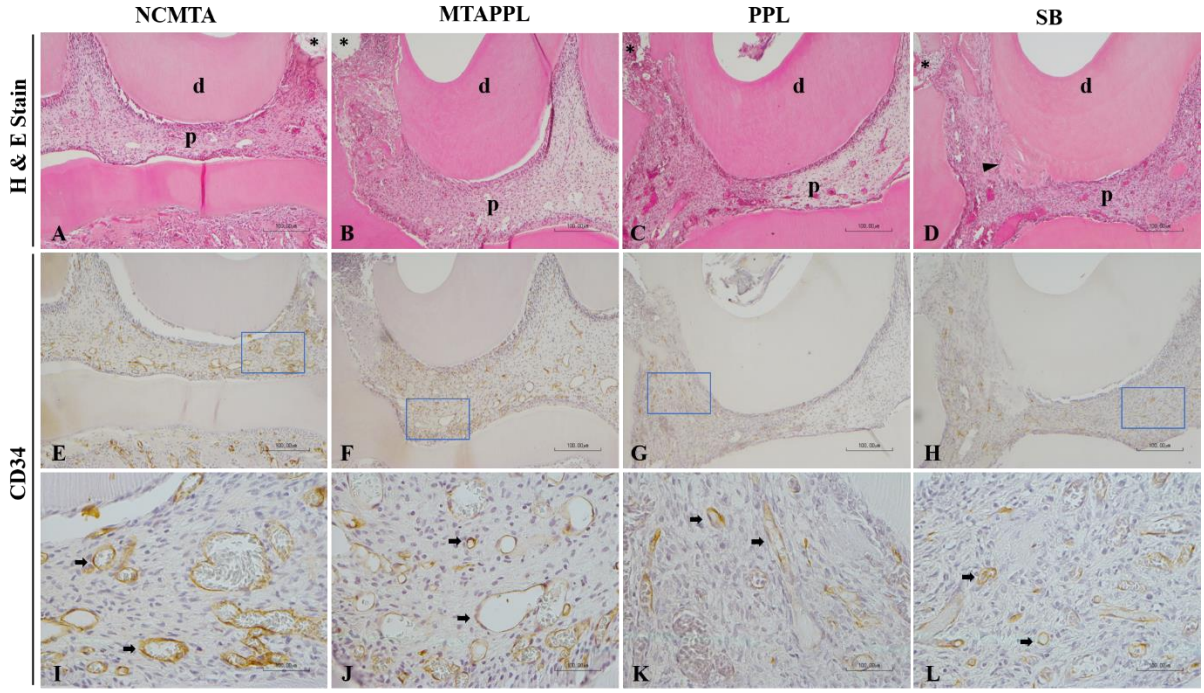

Figure 3: Histology of rat pulps at day 3 stained with hematoxylin-eosin (A-D) and immunohistochemistry with CD34 antigen (E-L). H&E stained sections showed mild inflammatory responses in the NCPPL and MTAPPL groups (A and B, respectively). Moderate inflammation with infiltration of inflammatory cells in the group PPL (C) and severe inflammation with extensive inflammatory cell infiltration was observed in the group SB (D), respectively. No mineralized tissue deposition was observed in the NCMTA, MTAPPL, and PPL groups, whereas SB group (D) showed a thin layer of mineralized tissue deposition (arrowhead). I–L present a high-magnification view of the areas demarcated by the blue rectangular in E–H. In all groups, positive staining of CD34 antigen (I-L) showed more differentiated$CD34^+$ blood vessels (arrows).

p, pulp: d, dentin, Asterisk (*), indicates pulp exposure sites.

On day 7, no or mild inflammatory responses (few scattered inflammatory cells) were observed in all the groups (Fig. 3). The inflammatory cells were lymphocytes and plasma cells, with a few polymorphonuclear leucocytes and macrophages representing the chronic inflammation phase. No significant difference was observed between the groups ($p > 0.05$).

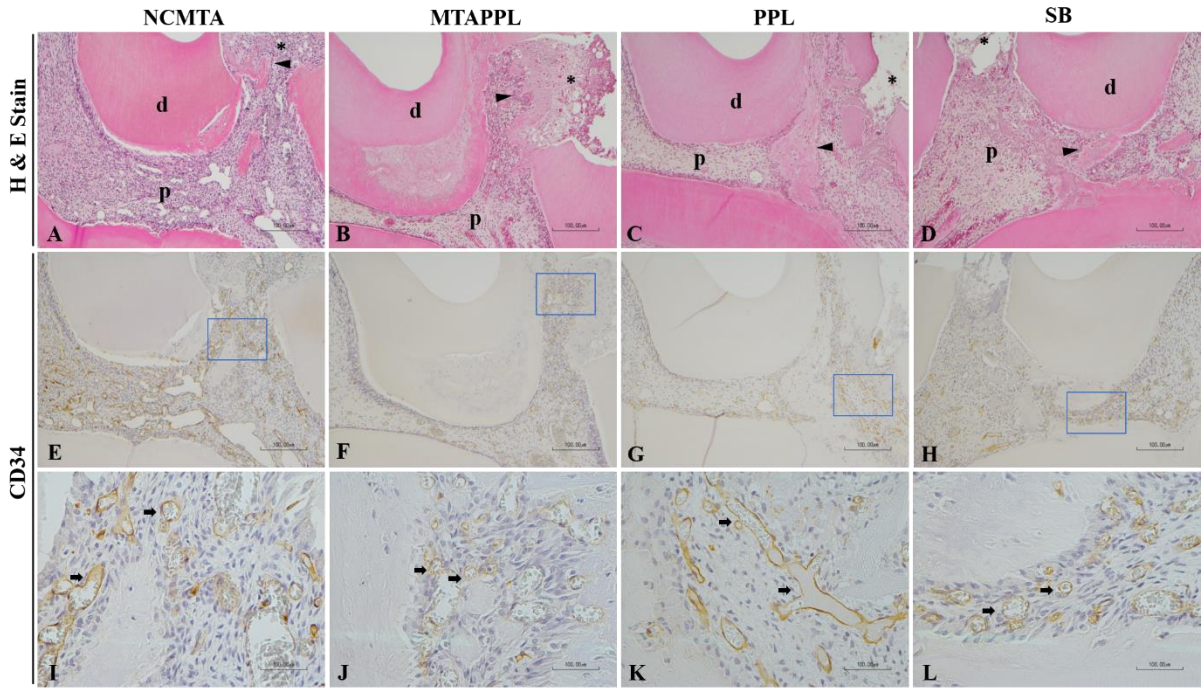

Figure 4: Histology of rat pulps at day 7 stained with hematoxylin-eosin (A-D) and immunohistochemistry with CD34 antigen (E-L). H&E stained sections showed no or presence of few scattered inflammatory cells with a thin layer of mineralized tissue deposition (arrowheads) in all groups (A-D). I–L present a high-magnification view of the areas demarcated by the blue rectangular in E–H. In all groups, positive staining of CD34 antigen showed differentiated$CD34^+$ blood vessels (arrows) just beneath the mineralized tissue deposition (I-L).

p, pulp: d, dentin, Asterisk (*), indicates pulp exposure sites.

At day 28, MTAPPL and NCMTA groups showed no inflammatory responses, whereas PPL and SB groups showed a mild inflammatory response (Fig. 5). A significant difference was observed between MTAPPL and SB groups ($p < 0.05$).

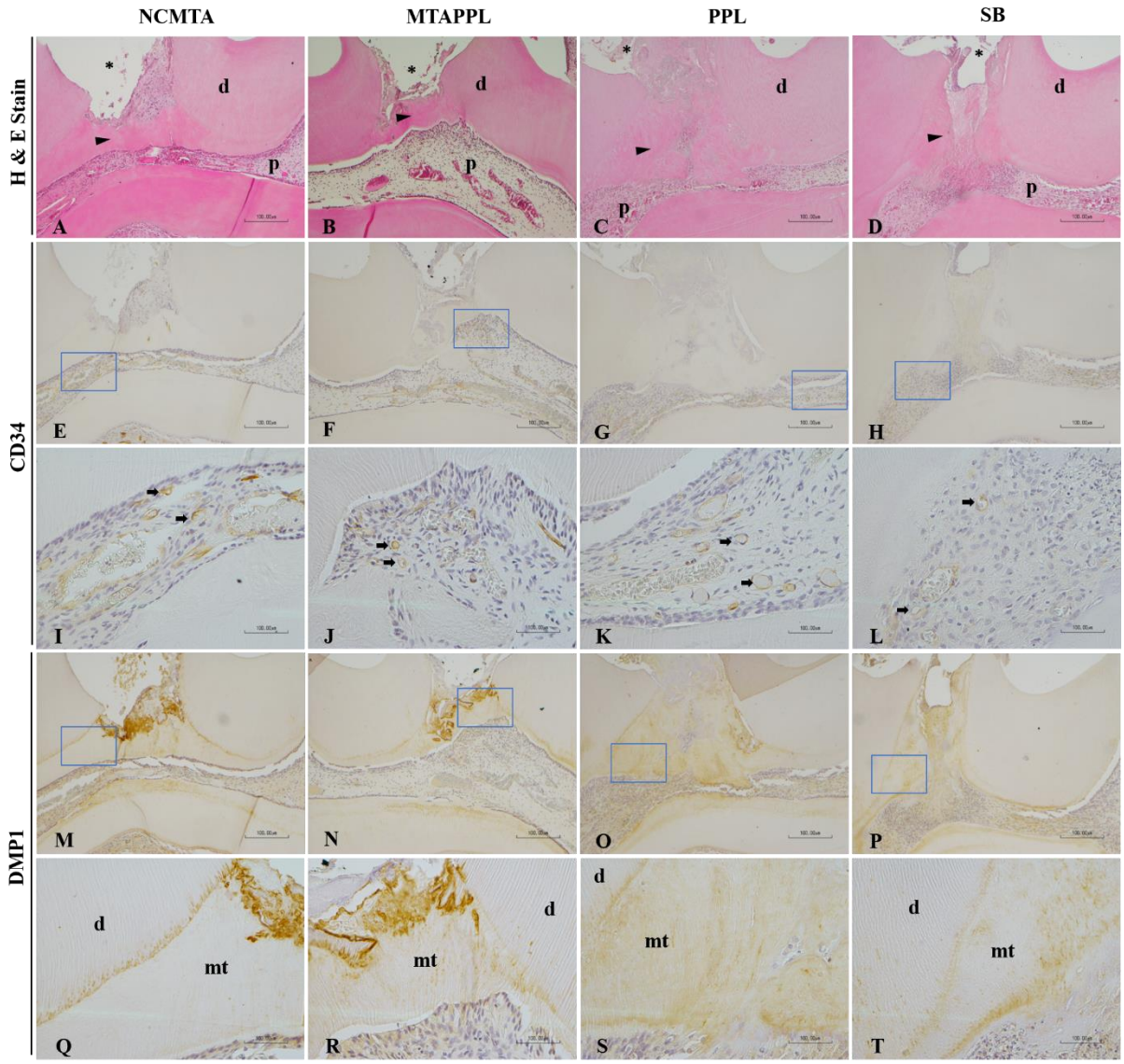

Figure 5: Histology of rat pulps at day 28 stained with hematoxylin-eosin (A-D) and immunohistochemistry with CD34 and DMP1 antigen (E-L and M-T, respectively). H&E stained sections showed no inflammation in the group NCMTA and MTAPPL; mild inflammation was observed in the group PPL and SB group (A-D). Complete mineralized tissue barrier formation (arrowheads) was observed in the group NCMTA, MTAPPL, and PPL. In contrast, incomplete mineralized tissue barrier formation (arrowhead) was observed in the group SB. .I–Land Q-T presents a high-magnification view of the areas demarcated by the blue rectangular in E–H and M-P, respectively. In all groups, positive staining of CD34 antigen showed differentiated$CD34^{+}$ blood vessels (arrows, I-L). Strong positive staining of DMP1 was observed in all the groups (M-T).

p, pulp: d, dentin, mt, mineralized tissue, Asterisk (*), indicates pulp exposure sites.

3.2 Mineralized tissue barrier formation (MTBF)

On day 1, no mineralized tissue formation was observed in all test groups. On day 3, only SB showed a thin layer of mineralized tissue. On day 7, thin mineralized tissue was observed in all the groups. Odontoblast-like cells were distributed beneath the newly formed mineralized tissue (Fig. 4). No significant difference was observed in mineralization between groups ($p > 0.05$).

On day 28, all specimens from all groups formed thicker mineralized tissue barriers. MTAPPL, NCMTA, and PPL groups exhibited complete mineralized tissue barrier formation and odontoblast like cells formation beneath the mineralized tissue barrier with no significant difference ($p > 0.05$). In contrast, the SB group exhibited incomplete mineralized tissue barrier formation. It did not exhibit odontoblast like cell formation beneath the mineralized tissue barrier (Fig. 5), which was significantly different from NCMTA, MTAPPL and PPL ($p < 0.05$).

3.3 Immunohistochemical staining

The CD34 antigen expression was positive in all experimental groups at all time intervals (Fig. 2-5). A down-regulation of random CD34+ cells and the development of well-defined micro-vessels identifiable on endothelial cell surfaces (arrows) from the intense expression of CD34 antigen were observed.

Except for SB, all groups showed positive staining for DMP1 in 28-day section, indicating odontoblasts and odontoblasts-like cells along the pulpal-dentine wall and newly formed mineralized tissue. Strong expression was observed in the MTAPPL and NCMTA groups (Fig. 5).

CHAPTER 4

DISCUSSION

Advances in biomaterials are at the forefront of endodontic research. Over the years, a number of endodontic biomaterials have been introduced for vital pulp therapy procedures, including direct pulp capping [23,24]. Well-controlled randomized clinical trials are crucial to gather strong evidence of clinical effectiveness for these materials. However, before any pulp capping agent can reach the stage of clinical testing in humans, it is important to be tested in animal models [25]. In this study, rat molars were chosen because of their anatomical and histological similarities to human molars [3]. The use of rat maxillary first molars is a convenient and appropriate model for assessing the biocompatibility of pulp capping materials [26-28].

MTA is considered the gold standard for endodontic biomaterials because of its favourable biological profile [29-32]. However, because of some drawbacks such as long setting time (which can reach up to 240 min), discolouration potential and high cost, a number of modified formulations have been introduced as potential alternatives [2,6,14].

According to the manufacturer, PPL can set in 5 min. It is generally accepted that PPL's improves the particles' surface contact with the mixing liquid, enabling rapid setting and ease of handling. The addition of PPL to MTA improves adhesion to hard tissue [25]. Because of this property, the MTAPPL combination could be used as a single visit direct pulp-capping material. Reported setting times have shown variations for other MTA based materials: 15 min [33], 12 min [6], 72 min [34].

The present investigation evaluated the pulp tissue responses after direct pulp capping with MTAPPL, PPL, and NCMTA. Our results showed that the cellular responses and hard tissue formation of MTAPPL are comparable to NCMTA, thus rejecting the null hypotheses. Inflammation has a controversial effect on pulp regeneration and repair [35-37]. While some authors in the past argued that inflammation had solely a negative effect due to the risk on pulp necrosis, others found that inflammation has a positive and necessary role in tissue repair [37]. This positive effect is thought to be promoted by the role of different growth factors and cytokines [20]. Therefore, any material meant to be placed directly over the exposed pulp tissue should retain the inflammatory reaction to the minimum, not passing beyond the stage that will lead to pulp necrosis [20].

This study also revealed that MTAPPL and NCMTA groups demonstrated mild inflammatory responses, whereas PPL group showed a moderate inflammatory response. Thus, our results confirm that the MTAPPL biomaterial is a safe option for pulp capping procedure. On day 7, all groups showed the formation of a thin mineralized tissue, which can increase the sealing ability, prevent bacterial leakage and stimulate the reparative capability of pulp cells through the deposition of calcium phosphate minerals along with the dentine-material interface [15]. These observations are similar to other studies that found little or no inflammatory cells and a thin layer of mineralized tissue formation when the pulp was capped with MTA [3,14,38]. On day 28, complete MTBF was associated with no pulpal inflammation in MTAPPL and NCMTA groups. These observations can be related to their favourable biocompatibility and bioactivity, as well as their excellent sealing ability and potent antibacterial properties [15,39]. On the other hand, the PPL group also showed complete MTBF with tunnel defects. This can lead to leakage and penetration of bacteria into the pulp tissue. The tunnels can result from the severity of pulp trauma and the number of vessels damaged during mechanical exposure. It was reported that

there are blood vessels within the tunnels, which maintain the calcium supply to the affected pulp tissue [5]. The calcium ions in the necrotic layer are responsible for the partial dystrophic calcification of the coagulation necrosis [5]. In the negative control group, SB exhibited partial MTBF with moderate pulpal inflammation after day 28. Except for SB, odontoblast like cells were observed in all groups after 28 days. Both MTAPPL and NCMTA showed similar histological responses in the dental pulp of rat molars.

DMP1 plays an important role in odontoblast differentiation and matrix mineralization [40]. As shown in Figure 5, the positive staining of DMP1 at the odontoblastic layer indicated the MTBF. This MTBF could be attributed to the favourable induction of MTAPPL and NCMTA on the proliferation of stem cells, their migration to the exposed pulp surface, and their differentiation into odontoblast-like cells as shown in other studies [41]. CD34 is an endothelial marker that is extensively used in IHC and has been recently used in dental pulp to identify pulpal vasculature [42]. CD34 antigen is a heavily glycosylated type I transmembrane protein [43]. In our study, the positive staining of the CD34 antigen revealed a homogenous and strong staining pattern of capillaries as well as large vessels (Fig. 2-5). The presence of well-defined microvessels in the dental pulp recognisable from the intense expression of CD34 antigen indicates the remodeling and vasculogenesis ability of dental pulp vessels [43,44]. The CD34 antigen expression represents neovessels with intense remodeling activity. The capillaries of the sub-odontoblastic layer, responsible for the pulpal reaction to any physical and chemical stimuli and pathological condition, are intensely stained with CD34 [45].

The setting accelerator of MTAPPL improves its handling properties [16], which was observed while manipulating the material in the course of this study. Superior handling properties may have an impact on the quality of bonding of the overlying final restoration.

CHAPTER 5

CONCLUSION

MTAPPL has favourable pulp responses and induce mineralized tissue formation. MTAPPL has the potential to be used in direct pulp capping.

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