Original Article

Pulp-dentin Complex Response to RU-HBM1, a Novel Resin Modified Glass Ionomer Cement Prototype, in Deep Cavity Preparation of Porcine Teeth

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Abstract

The objective of this study was to evaluate the response of the pulp-dentin complex to Research Unit-Herbal Medicine, Biomaterial and Material 1 (RU-HBM1), a resin-modified glass ionomer (RMGI) cement prototype, in deep cavity of porcine teeth compared with commercial RMGIs. Six 14-week-old pigs were used in this study. Deep class V cavity was created on the buccal surface of canine, premolar, and molar teeth. The teeth were randomly lined with the design cement bases: RU-HBM1, Vitrebond™ (VB), or GC-Gold Label Light-Cured Universal Restorative (GC). After that, the cavity walls were treated with 30% phosphoric acid and washed. Then, a bonding agent was applied and the cavities were restored using light-cured resin-based material (Filtek™ Z250XT, 3M ESPE). The teeth were extracted at 7, 30, and 70 days post-operation, sectioned and stained for histopathological evaluation. Data were collected and analyzed using SPSS program. It was found that the RU-HBM1, VB, and GC groups had no inflammatory reaction at all time points of observation except one sample in the VB group had mild reaction at day 7. Reactionary dentin formation was detected in all groups at 30 and 70 days post-treatment. Normal pulp tissue pattern and cellular organization were observed in all groups at all time points of observation. There was no significant difference in the overall histopathological scores of the RU-HBM1, VB, and GC groups at all time points of evaluation (p>0.05). In conclusion, RU-HBM1, a novel resin-modified glass ionomer cement prototype, is biocompatible for deep cavity prepared in intact teeth.

Keywords: animal study, biocompatibility, histopathology, reactionary dentin, RMGI

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Introduction

In 2011, Thailand imported dental materials and related instruments valued at approximately 4.1 billion baht, or 11% of the total imported medical and dental materials and equipment (Kruthkul, 2012). This expense increased by 16% compared to that of 2010, and was 1.54% of GDP. Therefore, the development of low-cost basic dental materials is an important strategy to solve this problem.

Light-cured resin modified glass ionomer (RMGI) cement is generally used as a liner, base or restoration (Croll and Nicholson, 2002; Mitra and Kedrowski, 1994). RMGI is composed of two parts: powder, which is mainly fluoro-aluminosilicate glass, and liquid, which is a mixture of light- and chemical-sensitive polyalkenoic acid and methacrylate monomer. The dual curing reaction involving both acid-base reaction and light-activated polymerization provides RMGI more advantageous clinical properties including a longer working time and a shorter setting time compared to conventional glass ionomer cement (Darwell, 2009; Primus, 2013).

Our research group has recently reported the physical properties and cytotoxicity of a light-cured RMGI prototype, RU-HBM1, as a liner or base (Thunyakitpisal et al., 2015). RU-HBM1 has met the requirements of ISO 9917-2:2010 and ISO 9917-2:1998 for working time, depth of cure, and flexural strength. RU-HBM1 was also biocompatible with dental pulp cells after 48 hours of culture. The cost of this material is much lower than that of similar imported products. For clinical safety, however, *in vivo* biocompatibility test of RU-HBM1 on pulp tissue is still required. The purpose of this study was to evaluate the response of pulp-dentin complex after the application of RU-HBM1 as base in deep class V cavity of sound porcine teeth. The results for RU-HBM1 were compared to the available commercial RMGIs.

Materials and Methods

The commercial light-cured RMGIs, Vitrebond™ (VB; A3 color, 3M ESPE, St. Paul, MN, USA) and GC-Gold Label Light-Cured Universal Restorative (GC; GC Company, Tokyo, JAPAN) were used as reference materials in this study. The expiration date of VB and GC were more than 6 months after the completion of the experiments. The powder and liquid components of VB, GC and RU-HBM1 are shown in Table 1. A light activator (Halogen Curing Light, Elipar™ 2500, 3M ESPE, USA) was used to cure the materials at an intensity of 700 mW/cm^2.

Table 1 Resin Modified Glass Ionomer Cements (RMGIs) used in this study

Material	Composition	Recommend light curing duration	Powder/Liquid Ratio (g/g)	Manufacturer
GC Gold Label Light-cured Universal Restoration (Lot no. 1211081)	Powder Fluoroaluminosilicate glass Liquid Polyacrylic acid; HEMA; TEGDMA; Photoinitiator	20 seconds	3.2/1	GC, Japan
Vitrebond™ (Lot no. N516621)	Powder Fluoroaluminosilicate glass Liquid Polyacrylic acid; HEMA; TEGDMA; Water; Photoinitiator	30 seconds	1.4/1	3M™ ESPE™, USA
RU-HBM1	Powder Fluoroaluminosilicate glass Liquid Polyacrylic acid; HEMA; TEGDMA; Photoinitiator	20 seconds	1.6/1	Research Unit of Herbal Medicine, Biomaterial and Material for Dental Treatment Chulalongkorn University

Animal study: Six 14-week-old Landrace-Large white-Duroc bred pigs (*Sus scrofa domesticus*) were obtained from the research-purposed farm in Nakhon Pathom Province. The protocol was approved by the Chulalongkorn University Animal Care and Use Committee (IACUC) of the Faculty of Veterinary Science. Two weeks before the operation, all subjects received scaling and root planning. The animals were omitted from food and water 6 hours before the operation. Sedation and anesthesia were accomplished using a mixture of xylazine hydrochloride 1 mg/kg, tiletamine hydrochloride, zolazepam hydrochloride (Zoletil®, Virbac laboratories, France) 3 mg/kg, and ketamine hydrochloride 2 mg/kg intramuscularly, followed by intravenous propofol 4 mg/kg. The animals were maintained using an anesthetic vaporizer containing isoflurane (2% in 100% oxygen).

After local anesthesia (2% mepivacaine with 1:20,000 levonordefrin, SEPTODONT Inc., New Castle, Delaware, USA), the operation area was isolated using rubber dam. The sound teeth were polished with a rubber cub and prophylactic paste, and 3% hydrogen peroxide and 0.2% chlorhexidine solution were applied, respectively. Class V cavity, 2 mm in depth, was created in the cervical part of labial/buccal surface using a round, high speed diamond bur with a

diameter of 2 mm under water spray coolant. The bur was changed after every four cavity preparations. The final cavity area was 1.5 mm in width x 2 mm in depth x 3 mm in length. The axial wall was smoothed using an inverted bur at low speed under water irrigation. Three cavities on canine, second premolar, and first molar of each quadrant were prepared to make a total of twelve cavities in one animal. After that, the teeth were irrigated with 0.9% normal saline. To prevent the chance of the same treatment on the same tooth of animal, cluster random sampling was created. Four experimental groups were assigned as shown in Table 2. The groups were randomly divided into the 4 quadrants in each animal.

Table 2 Treatment assignment per group in this study

	Canine	Second premolar	First molar
Group 1	VB	GC	RU-HBM1
Group 2	GC	RU-HBM1	VB
Group 3	RU-HBM1	VB	GC
Group 4	random	random	random

The powder and the liquid of each material were prepared and mixed following the manufacturer's instructions. The cement was loaded into a cavity with a thickness of 1 mm. After light cured activation, enamel and the cavity walls (dentin) were etched with 30% phosphoric acid (Scotchbond™ Universal Etchant, 3M ESPE, Neuss, Germany) for 15 seconds, rinsed with copious water, and briefly dried with air stream. A bonding agent (Adpter™ Single Bond2, 3M ESPE, USA) was immediately applied and light-cured for 10 seconds. A resin-based material (Filtek™ Z250XT, 3M ESPE, St. Paul, MN, USA) was used to restore the cavity and photo-irradiated for 40 seconds. The filling was polished with the flamed shape white stone, if necessary, to smooth the surface.

Two animals were sacrificed at 7, 30 and 70 days post-operation. The teeth were then extracted and fixed with 10% neutralized formalin buffer for 48 hr. After that the teeth were decalcified with 10% formic acid-sodium citrate method for 30 days. Dehydration of the teeth was carried out by ethanol-xylene, followed by paraffin-embedding. A serial section of 5 µm thickness was created parallel to the vertical axis of the teeth. Pulp-dentin complex response was evaluated using hematoxylin and eosin staining. Histopathological evaluation was scored according to the grading criteria of Tarim et al. (1998), Shimada et al. (2004), and Faraco and Holland (2004), which, with some modifications, consists of inflammatory cell response, reactionary dentin formation, and soft tissue organization (Table 3).

Table 3 Histopathological criteria and grading

Degree of Inflammation: Cells were counted under a 40x objective lens (1 high-power field [HPF])	
Score	
1	Little or no inflammatory cell present in the pulp beneath the axial wall (1-3 cells=high-power field [HPF])
2	Mild inflammation beneath the axial wall (4-10 cells=HPF)
3	Moderate inflammation (11-50 cells) beneath the axial wall and involved in coronal pulp
4	Severe inflammation or abscess formation beneath the axial wall
Reactionary Dentin Formation	
Score	
1	No reactionary dentin deposition beneath the axial wall
2	Small thin rim of reactionary dentin deposition beneath the axial wall (the thickness of reactionary dentine was less than twice the thickness of predentin)
3	Partial reactionary dentin deposition beneath the axial wall (the thickness of reactionary dentin was between twice and four times the thickness of predentin)
4	Complete reactionary dentin deposition beneath the axial wall (the thickness of reactionary dentin was more than four times of thickness of predentin)
Soft Tissue Organization	
Score	
1	Normal or almost normal soft tissue organization beneath the axial wall Continued odontoblastic layer were found and well organized.
2	Partial loss of soft tissue organization Discontinued or absence of odontoblastic layer beneath the axial wall but central part of pulp normal Few cells and some collagen fibers appearing in the pulp tissue that is distant from axial wall
3	Total loss of general pulp morphology and cellular organization Some free spaces were found.

Statistical analysis: Statistical analysis was performed using the SPSS program for Windows, version 22.0 (SPSS, Chicago, IL). The score of histopathological data were analyzed by Kruskal-Wallis and Bonferroni multiple comparison. Value of $p<0.05$ was considered to be statistically significant.

Results

None of the animals died during and after the experiment. One sample from the VB group at 7 days post-experiment and one sample from the GC group at 70 days post-experiment were damaged during the histological processing. Therefore, there were 7 teeth in

the VB group and 7 teeth in the GC group at 7 and 70 days post-treatment, respectively, for further histopathological evaluation. Histopathological images representing each group are demonstrated in Figure 1. A summary of the dental pulp response scores of all the groups is presented in Table 4.

No inflammatory reaction was observed in all the RU-HBM1, VB, and GC groups except one mild reaction in the VB group at day 7. There was no significant difference in the degree of inflammation scores between the RU-HBM1, VB, and GC groups at 7, 30, and 70 days post-experiment (p>0.05).

Table 4 Histopathological evaluation of dental pulp responses at 7 (A), 30 (B), and 70 (C) days post-operation

A)

Group	Day 7												
	Inflammation					Reactionary dentin formation					Tissue organization			
	1	2	3	4	mean score	1	2	3	4	mean score	1	2	3	mean score
CG (n=8)	8	-	-	-	1.0	6	2	-	-	1.25	8	-	-	1.0
VB (n=7)	6	1	-	-	1.14	6	1	-	-	1.14	7	-	-	1.0
RU-HBM1 (n=8)	8	-	-	-	1.0	6	2	-	-	1.25	8	-	-	1.0

B)

Group	Day 30												
	Inflammation					Reactionary dentin formation					Tissue organization			
	1	2	3	4	mean score	1	2	3	4	mean score	1	2	3	mean score
CG (n=8)	8	-	-	-	1.0	-	1	4	3	3.25	8	-	-	1.0
VB (n=8)	8	-	-	-	1.0	-	2	4	2	3.0	8	-	-	1.0
RU-HBM1 (n=8)	8	-	-	-	1.0	-	2	4	2	3.0	8	-	-	1.0

C)

Group	Day 70												
	Inflammation					Reactionary dentin formation					Tissue organization			
	1	2	3	4	mean score	1	2	3	4	mean score	1	2	3	mean score
CG (n=7)	7	-	-	-	1.0	-	1	3	3	3.29	7	-	-	1.0
VB (n=8)	8	-	-	-	1.0	-	3	3	2	2.88	8	-	-	1.0
RU-HBM1 (n=8)	8	-	-	-	1.0	-	2	4	2	3.0	8	-	-	1.0

At day 7, slight reactionary dentin formation was observed in all the groups. The average scores of reactionary dentin formation of the RU-HBM1, VB, and GC groups were 1.25, 1.14, and 1.25, respectively (Figure 1, Table 4). The reactionary dentin was clearly detected at day 30 and day 70 post-operation in all the groups (Figures 2 and 3). The average scores of reactionary dentin formation of the RU-HBM1, VB, and GC groups at day 30 were 3.00, 3.00, and 3.25, respectively. At 70 days post-experiment, the average scores of reactionary dentin formation of the RU-HBM1, VB, and GC groups were 3.25, 2.9, and 3.3, respectively. There was no significant difference in the reactionary dentin formation scores between the RU-HBM1, VB, and GC groups at 7, 30, and 70 days post-experiment (p>0.05).

RU-HBM1, VB, and GC did not alter the pulp tissue organization. Normal appearance of the odontoblast layer underneath the axial wall of cavity was observed in all the groups. There was no significant difference in the soft tissue organization scores between the RU-HBM1, VB, and GC groups at the periods of assessment (p>0.05).

For the overall histopathological scores (inflammation, reactionary dentin formation, soft tissue organization), there was no statistical difference among the RU-HBM1, VB, and GC groups at the periods of assessment (p>0.05) (Table 4).

Discussion

Because of their enhanced physical and mechanical properties, and biocompatibility, RMGI has been widely accepted as the material of choice for dental lining and cement to support overlying resin and amalgam restoration (de Souza Costa et al., 2006 and 2007; Uzzaman et al., 2005; Mickenautsch et al., 2010). Composite resin and amalgam restoration material has been used to fill in tooth cavity after removing infected dentin and tooth preparation. However, this filling is very rigid causing fracture of the remaining tooth structure especially the underlying dentin. In addition, the temperature of food and drink could transfer from the filling material to the underlying dentin and pulp causing tooth sensitivity. To limit these disadvantages, lining material and cement base is recommended to thermally insulate the pulp and mechanically support the teeth.

GC and VB are worldwide RMGIs that have been clinically proven for their biocompatibility and efficiency for more than a decade (Mount, 1994; Mousavinasab et al., 2008; Nicholson and Czarnecka, 2008). Therefore, both GC and VB are suitable as reference to evaluate the biocompatibility of RU-HBM1 as lining and base material. The pulpal response of RU-HBM1 was assessed using porcine teeth containing the histological and biological pulp responses similar to human teeth (Shayegan et al., 2009; Leites et al., 2011). In this study, the tooth preparation for filling was created, based with selective materials, and restored with filling material to simulate the clinical procedure, which is more clinically relevant than *in vitro* tests.

Inflammation is the first sign of body reaction against foreign body. From our data, except one sample

of VB at day 7 post-experiment, little or no inflammatory reaction was detected in the RU-HBM1, VB, and GC groups at days 7, 30, and 70 post-experiment. The normal pulp tissue organization was also observed. These finding agreed with the studies of de Souza Costa et al. (2007) and Uzzaman et al. (2005).

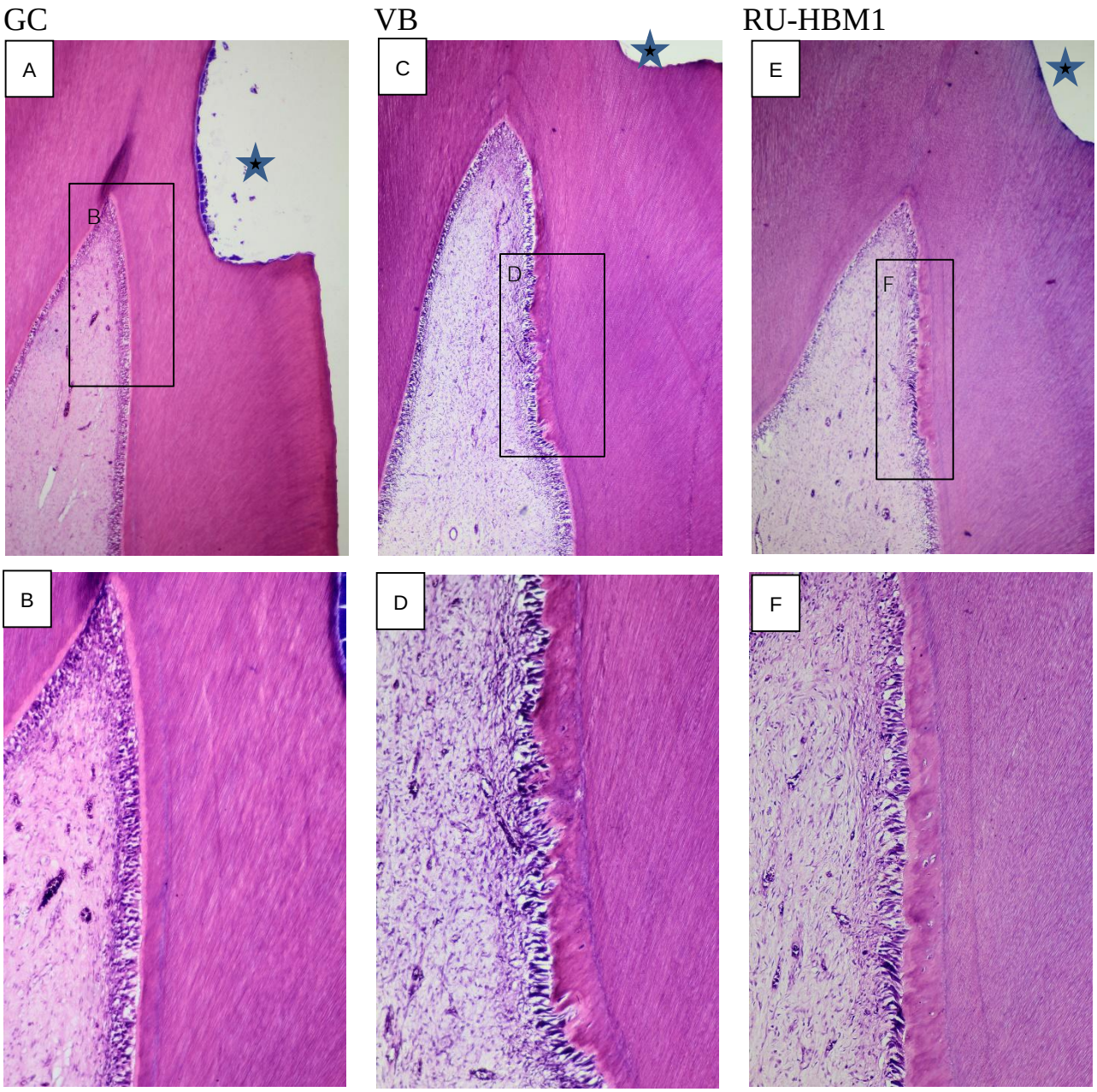

Figure 1 Histopathology of dental pulp at day 7 post-experiment: GC-treated group (A, B), VB-treated group (C, D), and RU-HBM1-treated group (E, F). The GC group revealed thickening of predentin layer without reactionary dentine formation, score=0. The VB and RU-HBM1 groups showed irregular thin rim of reactionary dentin beneath the axial wall of cavity, score=2. ★ = experimental tooth cavity, rectangular line = higher magnification of dentin and pulp beneath the axial wall of cavity in B, D, and F

Reactionary dentin, a tertiary dentin, is synthesized by activated odontoblast and deposited beneath the stimulated area (Bleicher, 2014; Couve et al., 2014). Following mild irritant and injury to the pulp, the survived odontoblasts secrete extracellular matrix and deposit mineralization. We observed the initial phase of reactionary dentin formation in all groups since day 7 post-experiment, and 100% reactionary dentin formation of all groups at day 30 post-operation. The effect of VB and GC on reactionary dentin of primate teeth was also reported (Duque et al., 2006).

From our observation, only the average score of reactionary dentin of each material group was clearly different at each time point compared with those of the inflammation score and tissue organization score. GC had the highest average score of reactionary dentin formation at days 30 and 70, while VB had the lowest average score at days 30 and 70. The patented limitations restricted our knowledge of the exact amounts of the components in the commercial RMGIs. Thus, this study could not clearly explain why each RMGI material had different average scores of reactionary dentin. A practical explanation is that each

RMGI has different composition and percentage of each component. The copolymeric of polycarboxylic acids between acrylic acid and itaconic acid was used in VB, while polycarboxylic acids were used in GC and RU-HBM1 (Pameijer et al., 2015; Khoroushi et al., 2012; Darvell, 2009). With the extra carboxylic acid side chain, itaconic acid has more acidity (pK_a 3.69) than acrylic acid (pK_a 4.08) (Ibarra-Montaño et al., 2015). The stronger acidity of VB could irritate the underneath dental pulp and subsequently induce an inflammatory response higher than those of GC and RU-HBM1. The high amount of water (30-40%) in the liquid part of VB makes it porous after polymerization and causes release of incomplete polymerized resin-based material, and leaching of non-polymerized monomers to the pulp tissue underneath through the transdentinal diffusion and open dentinal tubule (Pameijer et al., 2015; Primus, 2013). The non-polymerized camphoroquinone and Bis-GMA induce oxidative stress, DNA damage, apoptosis, and cytotoxicity interference in odontoblast activity and eventually reactionary dentin formation (Volk et al., 2009; Yano et al., 2011). However, taken together with the data of inflammation reaction, normal pulp tissue organization, and reactionary dentin formation, our finding suggests the biocompatibility of RU-HBM1, GC and VB.

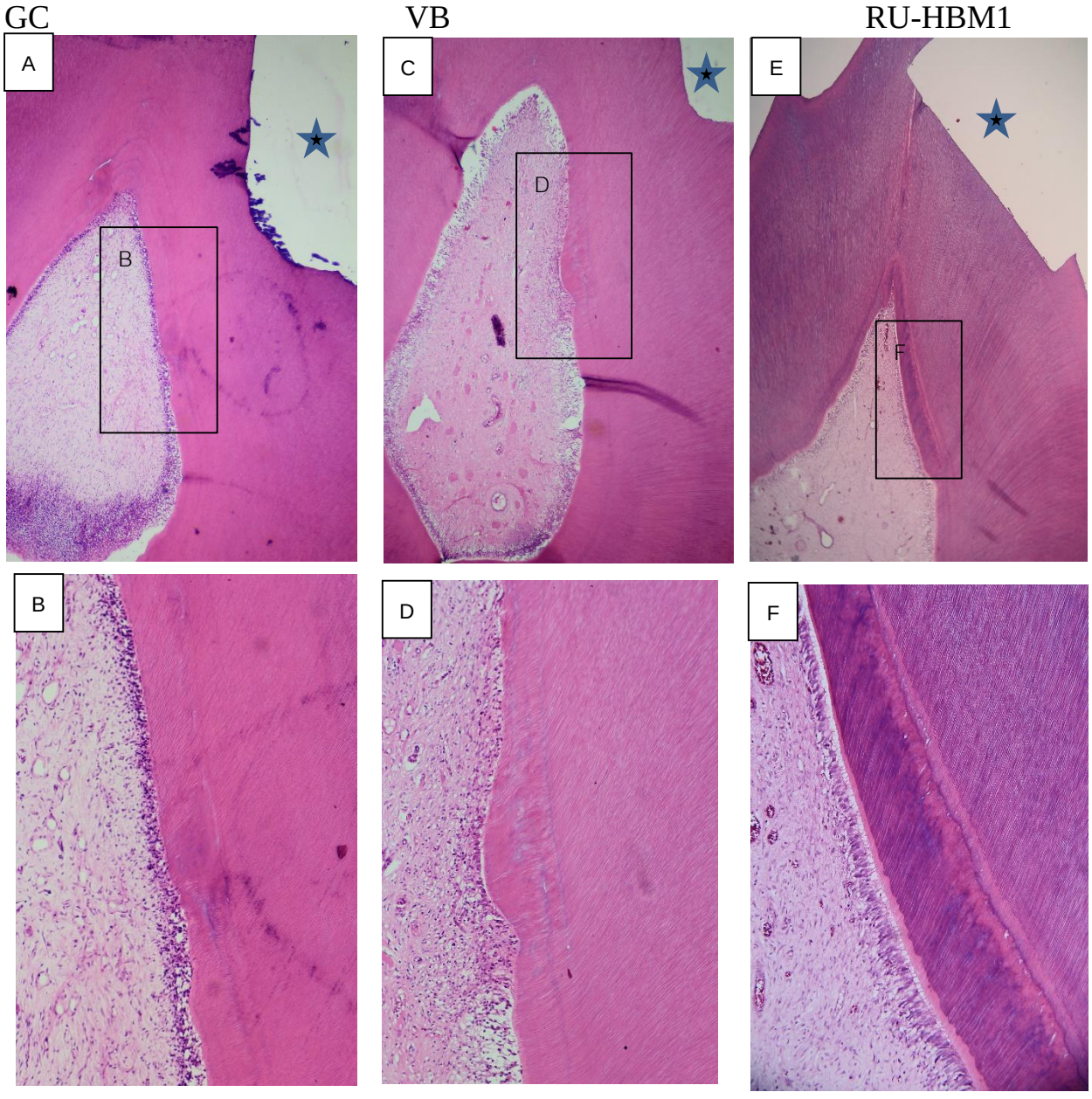

Figure 2 Histopathology of dental pulp at day 30 post-experiment: GC-treated group (A, B), VB-treated group (C, D), and RU-HBM1-treated group (E, F). The smooth thick layer of reactionary dentin was observed in all groups: GC score=2, VB and RU-HBM1 score=3. ★ = experimental tooth cavity, rectangular line = higher magnification of reactionary dentin and pulp beneath the axial wall of cavity in B, D, and F

The biocompatibility of RU-HBM1 and GC to dental pulp tissue corresponded with our previous *in vitro* study, which demonstrated that RU-HMB1 and GC were biocompatibile with pulpal cells (Thunyakitpisal et al., 2015). However, it should be noted that VB was biocompatibile with dental pulp tissue, while it was cytotoxic to dental pulp cell. One possible explanation is the remaining dentin effectively

functions as a barrier for the penetration/diffusion of by-product and non-polymerized monomer. Another explanation is that the dimension of VB base of this study was about 1 mm high x 1.5 mm wide x 3 mm long, which is less than the 2 mm high x 2 mm wide x 25 mm long sample in the previous cytotoxicity test. Therefore, the amount of by-product and non-polymerized monomer was less than its toxicity level.

It should be noted that this experiment was performed on sound tooth, not on infective teeth. Therefore, clinical trial evaluation of this RU-HBM1 material should be performed. In conclusion, the resin-based dental cement RU-HBM1 has acceptable biocompatibility for deep cavity prepared in intact tooth.

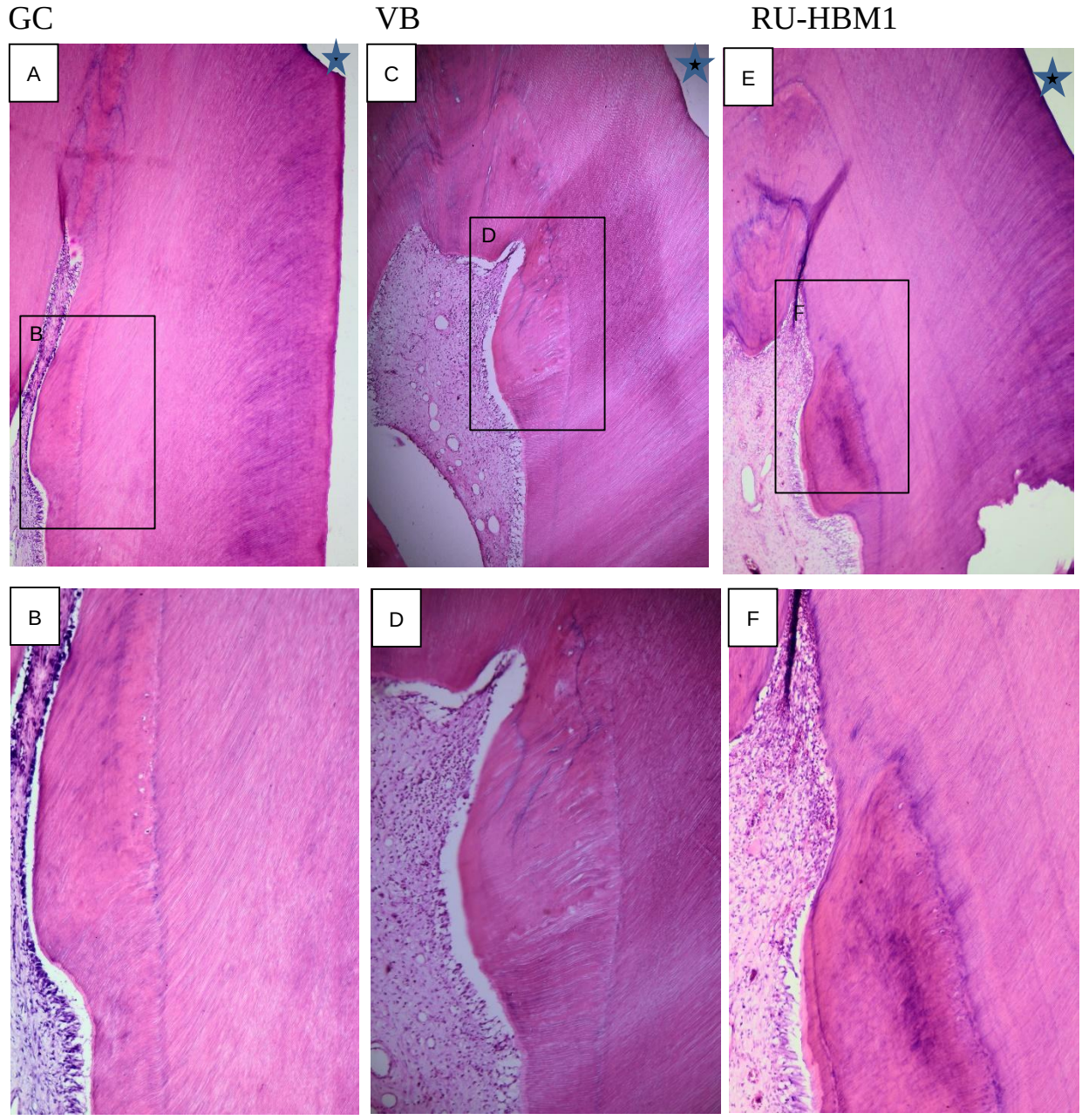

Figure 3 Histopathology of dental pulp at day 70 post-experiment: GC-treated group (A, B), VB-treated group (C, D), and RU-HBM1-treated group (E, F). The thick layer of reactionary dentin was detected in all groups: GC, VB, and RU-HBM1 score=4. ★ = experimental tooth cavity, rectangular line = higher magnification of reactionary dentin and pulp beneath the axial wall of cavity in B, D, and F

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บทคัดย่อ

ผลของเรซินมอดิฟายด์กลาสไอโอโนเมอร์ซีเมนต์ต้นแบบ RU-HBM1 ต่อการตอบสนองของเนื้อฟันและเนื้อเยื่อโพรงฟันของฟันสุกรที่เตรียมแควิตีลึก

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วัตถุประสงค์ของการศึกษา เพื่อประเมินผลการตอบสนองของเนื้อฟันและเนื้อเยื่อโพรงฟันต่อเรซินมอดิฟายด์กลาสไอโอโนเมอร์ซีเมนต์ต้นแบบ RU-HBM1 ในฟันสุกรที่เตรียมแควิตีระดับลึก เปรียบเทียบกับเรซินมอดิฟายด์กลาสไอโอโนเมอร์ซีเมนต์ทางการค้า สุกรทดลองอายุ 14 สัปดาห์ได้รับการกรอฟันเพื่อเตรียมแควิตีระดับลึกด้านแก้มของฟันเขี้ยว ฟันกรามน้อย และฟันกราม สุ่มปิดด้วยเรซิน มอดิฟายด์กลาสไอโอโนเมอร์ซีเมนต์ ได้แก่ RU-HBM1, Vitrebound (VB) และ GC-Gold Label Light-Cured Universal Restorative (GC) จากนั้นเตรียมผิวฟันด้วยกรดฟอสฟอริกความเข้มข้นร้อยละ 30 ทาแควิตีด้วย bonding agent และปิดทับด้วยวัสดุเรซินอุดฟันสีเหมือนฟันที่แข็งตัวด้วยการฉายแสง (Filtek™ Z250XT, 3M ESPE) ถอนฟันตัวอย่างในวันที่ 7 30 และ 70 หลังการทดลอง นำฟันดังกล่าวมาผ่านขบวนการเตรียมเนื้อเยื่อ ตัดแผ่นบาง ย้อมสี และตรวจทางจุลพยาธิวิทยา และวิเคราะห์ผลการทดลองโดยโปรแกรมสำเร็จรูป SPSS การทดลองพบว่ากลุ่มทดลอง RU-HBM1, VB และ GC ไม่แสดงการอักเสบของโพรงฟัน ยกเว้นหนึ่งตัวอย่างของกลุ่ม VB ที่พบการอักเสบระดับอ่อนในวันที่ 7 หลังการทดลอง พบการสร้างเนื้อฟันตอบสนองในทุกกลุ่มที่ 30 และ 70 วัน เซลล์เนื้อเยื่อโพรงฟันมีการจัดเรียงตัวปกติใน ทุกกลุ่มการทดลองของทุกช่วงเวลาการศึกษา ผลคะแนนประเมินทางจุลพยาธิวิทยาโดยรวมของทั้ง 3 กลุ่มทดลองในทุกช่วงเวลาไม่พบความแตกต่างทางสถิติที่ระดับความเชื่อมั่นร้อยละ 95 สรุปได้ว่า RU-HBM1 มีความเข้ากันได้กับเนื้อเยื่อโพรงฟันในฟันที่ถูกกรอเตรียมแควิตี ระดับลึก

คำสำคัญ: การศึกษาในสัตว์ ความเข้ากันได้ จุลพยาธิวิทยา เนื้อฟันตอบสนอง เรซินมอดิฟายด์กลาสไอโอโนเมอร์ซีเมนต์

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