Original Article

Subcutaneous implantation of the light-activated pit and fissure resin-based sealant prototypes LAS-clear and LAS-opaque resulted in a mild transient tissue reaction

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Abstract

The objective of this study was to evaluate the biocompatibility of the light-activated pit and fissure resin-based dental sealant prototypes, LAS-clear and LAS-opaque, after subcutaneous implantation in rats for 7, 30, and 60 days. The commercial light-activated resin-based sealants, Delton®-clear (DC), Helioseal® clear (HC), Helioseal® opaque (HO), and Clinpro™ Sealant (CL) served as control. Twenty-four 8-week-old male Sprague-Dawley rats were used in this study. Two rows of three 5 mm subcutaneous incisions were created on the back of each rat parallel to the midline. The materials were randomly implanted and the incisions were closed using sutures. The implants were left *in situ* for 7, 30, and 60 days. The implanted samples and the surrounding skin were removed, sectioned, and stained for histopathological evaluation. Results revealed that the scores of inflammatory cell infiltration of the DC, HC, LAS-clear, HO, CL, and LAS-opaque groups were not significantly different at 7, 30, and 60 days post-operation (p>0.05). The average total inflammatory cell infiltration score was highest at day 7 and lowest at day 30. Even though all the material revealed a persistent inflammatory response mediated by lymphocytes and macrophages at all time point evaluation, the tissue reaction of all groups to material implantation was that of typical wound healing. The tissue response scores of the DC, HC, LAS-clear, HO, CL, and LAS-opaque were not significantly different at 7, 30, and 60 days post-operation. The tissue response observed was material encapsulation without marked pathology. The capsule thickness was gradually increased in a time-dependent manner. In conclusion, the LAS-clear and LAS-opaque materials were biocompatible with the subcutaneous layer.

Keywords: animal study, biocompatibility, histopathology, light-activated pit and fissure dental sealant, local tissue reaction

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Introduction

Light-activated pit and fissure resin-based dental sealants are used as a physical barrier to cover caries-susceptible pits and fissures in teeth, preventing caries development (Beauchamp et al., 2009 and Tinanoff et al., 2015). This type of sealant is composed of a liquid which is a mixture of light- and chemical-sensitive dimethacrylate monomers with optional pigmentation. Light-activated polymerization gives these sealants advantageous clinical properties such as a longer working time and a shorter setting time compared with autopolymerized resin-based sealants and higher retention rates compared with UV-light-polymerizing materials, compomers, and glass-ionomer-cement-based sealants (Kühnisch et al., 2012 and Tinanoff et al., 2015).

Our research group has recently described the properties of the light-activated resin-based sealant prototype I (LAS-clear) and light-activated resin-based sealant prototype II (LAS-opaque), which are clear and opaque sealants, respectively (Thunyakitpisal et al., 2016). LAS-clear and LAS-opaque met the requirements of ISO 6874:2005 and ISO 9917-2:1998 for depth of cure and flexural strength, respectively. Moreover, in the previous research, it was found that LAS-clear and LAS-opaque were biocompatible with gingival fibroblasts after 48 h of exposure. Importantly, the cost of these materials is much lower than that of similar imported products. To investigate the biocompatibility of LAS-clear and LAS-opaque prior to their clinical use, local tissue reaction in an *in vivo* animal model was assessed after implanting these materials subcutaneously in rats. The results of LAS-clear and LAS-opaque were compared with those of four available commercial resin-based pit and fissure sealants.

Materials and Methods

Sealants and sample preparation: The commercial light-activated resin-based dental pit and fissure sealants, Delton®-clear (DC; Dentsply, USA), Helioseal® clear (HC; Ivoclar Vivadent, Liechtenstein), Helioseal® opaque (HO; Ivoclar Vivadent, Liechtenstein), and Clinpro™ Sealant (CL; 3M™ ESPE™, USA), were selected as reference materials in this study. The expiration date of the commercial sealants was more than 6 months after the completion of the study. The major components of DC, HC, HO, CL, LAS-clear, and LAS-opaque are shown in Table 1. A light activator (Halogen Curing Light, Elipar™ 2500, 3M™ ESPE™, USA) was used to activate the materials at an intensity of 700 mW/cm^2. Each dental sealant (n=24) was placed into a customized stainless steel mold (2x2x2 mm^3). A polyester film and a glass slide were placed on both sides of the mold. The dental sealant was cured using activating-light exposures of 20 sec per the manufacturer's instructions. The specimen was light-cured on the opposite side of the mold in the same fashion. After removing the specimens from the mold, any flash was removed using scissors, and the samples were kept at 37^{o}C for 24 hrs.

Table 1 Resin-based pit and fissure dental sealants used in this study

Material	Composition	Recommended light activating duration (sec)	Manufacturer
Delton®-clear (Lot no. 140218)	Aromatic and aliphatic dimethacrylate monomers; Light activators	20	Dentsply Professional, USA
Helioseal® clear (Lot no. S03682)	Bis-GMA and TEGDMA; Photoinitiator	20	Ivoclar Vivadent, Liechtenstein
Helioseal® opaque (Lot no. S39123)	Bis-GMA and TEGDMA; Titanium dioxide Photoinitiator	20	Ivoclar Vivadent, Liechtenstein
Clinpro™Sealant (Lot no. N478759)	Bis-GMA and TEGDMA Titanium dioxide; Coloring agent Photoinitiator	20	3M ESPE, USA
LAS-clear	Bis-GMA and TEGDMA; Photoinitiator	20	Research Unit of Herbal Medicine, Biomaterial, and Material for Dental Treatment, Faculty of Dentistry, Chulalongkorn University
LAS-opaque	Bis-GMA and TEGDMA; Titanium dioxide Photoinitiator	20	Research Unit of Herbal Medicine, Biomaterial, and Material for Dental Treatment, Faculty of Dentistry, Chulalongkorn University

Animal study: Twenty-four 8-week-old male Sprague-Dawley rats were obtained from the National Laboratory Animal Center, Mahidol University, Nakhon Pathom, Thailand. The rats were given access to food and water *ad libitum*, and maintained at 25±1°C with a 12 h light/12 h dark cycle during the course of experiment. The protocol was approved by Chulalongkorn University Animal Care and Use Committee (IACUC No. 1232005). All procedures were carried out by the same investigator. Anesthesia was performed using a mixture of 10 mg/kg xylazine hydrochloride (L.B.S. Laboratory Ltd., Thailand) and 75 mg/kg ketamine hydrochloride (Calypsol®, Gedeon Richter Ltd., Hungary) administered intramuscularly. The hair on the back of each rat was shaved and swabbed with 70% alcohol. Two rows of three 5 mm incisions were created on the back of each rat through the skin and underlying subcutaneous tissue parallel to the midline. Subcutaneous pockets were gently created using tissue forceps. Each animal received DC, HC, LAS-clear, HO, CL, and LAS-opaque samples, which were randomly inserted into the pockets and the locations were recorded. The incision was closed with non-absorbable polyamide monofilament sutures (Dafilon®, Braun, Germany), then wiped with povidone iodine solution.

Eight animals were sacrificed at 7, 30, and 60 days post-implantation. The skin samples were excised and fixed with 10% neutral buffered formalin for 5 days. The sealant specimens were removed and the trimmed skin samples were histologically processed, followed by embedding in paraffin wax. Serial sections of 4 μm thickness were cut and stained with hematoxylin and eosin (HE), and masson trichrome (MT) stains. For histopathological evaluation, randomly selected area was assessed. Based on ISO10993-6:2007(E) and the FDI method with some modifications and editions, the histopathological evaluation was scored according to inflammatory cell infiltration (I) and tissue response (TR) to the implanted material (Table 2). To score the presence of inflammatory cell type and number, five high-power (x40) magnification fields were randomly scanned from all slides. The number of each cell type was then counted per high-power fields (Morse et al., 1981). Fibrous capsule thickness was assessed for each specimen using a light microscope (Olympus CX-21, Tokyo, Japan) connected to a video camera (Touptek LCMOS series C-mount USB2.0 CMOS camera, Zhejiang, P.R. China) and to a computer and software (Touptek ToupView, Zhejiang, P.R. China).

Table 2 Histopathological criteria and grading

Number of inflammatory cell infiltration/ level of inflammation
0 Absent
1 Little inflammation, few inflammatory cells presented around the material (<5 cells/high-power field [HPF])
2 Mild inflammation around the material (5-25 cells/HPF)
3 Moderate inflammation with infiltration of cells around the material (25-125 cells)
4 Severe inflammation with heavy infiltration of cells covering the material (>125 cells)
Note: Cells were counted under a 400 x field (HPF).
Tissue response
Neovascularization:
0 None
1 Minimal capillary proliferation, focal, 1-3 buds
2 Groups of 4-7 capillaries with slightly supporting fibroblastic structures
3 Broad band of capillaries with supporting fibroblastic structures
4 Extensive band of capillaries with supporting structures
Fibroplasia
0 None
1 Narrow band, the fibrous capsule formation thinner than 50 μm
2 Moderately thick band, the fibrous capsule formation thickness between 50 and 150 μm
3 Thick band, the fibrous capsule formation thickness more than 150 μm, but less than 300 μm
4 Extensive band, the fibrous capsule formation thickness equal or more than 300 μm
Hemorrhage
0 Absent, only beneath the incision area
1 A foci around the material
2 Two foci around the material
3 Extensive hemorrhage around the material
4 Hematoma around the material

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

Results

None of the animals died during or after the surgery. No skin rubor was detected in any groups. Representative histopathological images of each group at days 7, 30, and 60 post-implantation are shown in Figures 1, 2, and 3, respectively. A summary of the inflammatory cell infiltration and tissue response scores of each group at days 7, 30, and 60 post-implantation is presented in Table 3.

At 7 days post-implantation, there was no significant difference in the average total inflammatory cell infiltration score and tissue response score among the DC, HC, LAS-clear, HO, CL, and LAS-opaque groups ($p<0.05$, Table 3). The average total

inflammatory cell infiltration scores of the DC, HC, LAS-clear, HO, CL, and LAS-opaque groups were 3.5±1.41, 3.63±0.74, 3.13±0.83, 3.25±1.04, 3.13±0.99, and 3.13±1.25, respectively. Most of the inflammatory cells were lymphocytes and macrophages. Little neutrophils were found in one, two, and one samples of the HC, LAS-clear, and LAS-opaque groups, respectively. The average total tissue response scores of the DC, HC, LAS-clear, HO, CL, and LAS-opaque groups, which mainly obtained from fibroplasia, were 1.38±0.52, 1.38±0.51, 1.38±0.52, 1.25±0.46, 1.38±0.52, and 1.25±0.46, respectively. A few fibroblasts and *de novo* extracellular matrix production were found around the sample site (Fig. 1). A loose, poorly organized, discontinuous fibrous capsule was observed using MT staining.

Table 3 Histopathological criteria and evaluation of the sealants at day 7, day 30, and day 60 post-implantation (n=8)

Time	Inflammatory cell infiltration (I)	Materials					
		DC	HC	LAS-C	HO	CL	LAS-O
Day 7	Neutrophil	0	0.13±0.35	0.25±0.46	0	0	0.13±0.35
	Eosinophil	0	0	0	0	0	0
	Mast Cells	0	0	0	0	0	0
	Lymphocytes	1.75±0.71	1.75±0.46	1.50±0.53	1.63±0.52	1.50±0.53	1.50±0.53
	Plasma cells	0	0	0	0	0	0
	Macrophages	1.75±0.71	1.75±0.46	1.38±0.52	1.63±0.52	1.63±0.52	1.50±0.53
	Giant Cells	0	0	0	0	0	0
	Average total score	3.5±1.41	3.63±0.74	3.13±0.83	3.25±1.04	3.13±0.99	3.13±1.25
Day 30	Neutrophil	0	0	0	0	0	0
	Eosinophil	0	0	0	0	0	0
	Mast Cells	0	0	0	0	0	0
	Lymphocytes	1.0±0.58	0.86±0.69	0.71±0.76	0.81±0.63	0.64±0.51	0.6±0.52
	Plasma cells	0	0	0	0	0	0
	Macrophages	0.86±0.38	0.86±0.69	0.71±0.76	0.81±0.63	0.64±0.51	0.6±0.52
	Giant Cells	0	0	0	0	0	0
	Average total score	1.86±0.9	1.71±1.38	1.43±1.51	1.6±1.26	1.27±1.09	1.2±1.03
Day 60	Neutrophil	0	0	0	0	0	0
	Eosinophil	0	0	0	0	0	0
	Mast Cells	0	0	0	0	0	0
	Lymphocytes	1.17±0.58	1.17±0.58	1.42±0.51	1.23±0.43	1.15±0.35	1.25±0.45
	Plasma cells	0	0	0	0	0	0
	Macrophages	1.17±0.58	1.17±0.58	1.42±0.51	1.15±0.58	1.08±0.53	1.17±0.39
	Giant Cells	0	0	0	0	0	0
	Average total score	2.33±1.15	2.33±1.15	2.83±1.03	2.38±0.76	2.23±0.83	2.41±0.79

The same superscript letter means a significant difference between the groups (p<0.05).
DC=Delton clear; HC=Helioseal clear; LAS-C=LAS-clear; HO=Helioseal opaque; CL=Clinpro; LAS-O=LAS-opaque

Time	Tissue response (TR)	Materials					
		DC	HC	LAS-C	HO	CL	LAS-O
Day 7	Fibroplasia	1.38±0.52	1.25±0.46	1.38±0.52	1.25±0.46	1.0±0.0	1.13±0.35
	Neovascularization	0	0	0	0	0.25±0.46	0
	Hemorrhage	0	0.13±0.35	0	0	0.13±0.35	0.13±0.35
	Average total score	1.38±0.52	1.38±0.51	1.38±0.52	1.25±0.46	1.38±0.52	1.25±0.46
Day 30	Fibroplasia	1.57±0.53	1.86±0.38	1.57±0.53	1.54±0.52	1.36±0.5	1.6±0.52
	Neovascularization	0	0	0	0	0	0
	Hemorrhage	0	0	0	0	0	0
	Average total score	1.57±0.53	1.86±0.38	1.57±0.53	1.54±0.52	1.36±0.5	1.6±0.52
Day 60	Fibroplasia	2.0±0.0	1.91±0.29	2.0±0.0	1.62±0.51	1.62±0.51	1.83±0.39
	Neovascularization	0	0	0	0	0	0
	Hemorrhage	0	0	0	0	0	0
	Average total score	2.0±0.0	1.91±0.29	2.0±0.0	1.62±0.51	1.62±0.51	1.83±0.39

The same superscript letter means a significant difference between the groups (p<0.05).
DC=Delton clear; HC=Helioseal clear; LAS-C=LAS-clear; HO=Helioseal opaque; CL=Clinpro; LAS-O=LAS-opaque

At 30 days post-implantation, there was no significant difference in the average total inflammatory cell infiltration score and tissue response score among the DC, HC, LAS-clear, HO, CL, and LAS-opaque groups (p>0.05, Table 3). The average total inflammatory cell infiltration scores of the DC, HC, LAS-clear, HO, CL, and LAS-opaque groups were 1.86±0.9, 1.71±1.38, 1.43±1.51, 1.6±1.26, 1.27±1.09, and 1.2±1.03, respectively. All groups were characterized by macrophages and lymphocytes localized adjacent to the implant sites. The average total tissue response scores of the DC, HC, LAS-clear, HO, CL, and LAS-opaque groups were 1.57±0.53, 1.86±0.38, 1.57±0.53, 1.54±0.52, 1.36±0.5, and 1.6±0.52, respectively. A thin, moderately organized, and continuous fibrous capsule was present around the sample sites (Fig. 2).

At 60 days post-implantation, there was no significant difference in the average total inflammatory cell infiltration score and tissue response score among the DC, HC, LAS-clear, HO, CL, and LAS-opaque

groups (p>0.05, Table 3). The average total inflammatory cell infiltration scores of the DC, HC, LAS-clear, HO, CL, and LAS-opaque groups were 2.33±1.15, 2.33±1.15, 2.83±1.03, 2.38±0.76, 2.23±0.83, and 2.41±0.79, respectively. The average total tissue response scores of the DC, HC, LAS-clear, HO, CL, and LAS-opaque groups were 2.0, 1.91±0.29, 2.0, 1.62±0.51, 1.62±0.51, and 1.83±0.39, respectively. Macrophages and lymphocytes were still the major defensive cells surrounding the samples. A thick, dense, and well-organized fibrous capsule around the samples was observed (Fig. 3).

Discussion

According to ISO 10993-6, subcutaneous implantation is an established method for evaluating the biocompatibility of dental materials and biomaterials. Subcutaneous implantation is a relatively high-throughput, low-cost screening method, thus, it is commonly used during the development of a new material prior to testing it on a target organ (Diller et al., 2014 and Kidd et al., 2002). Similar to other dental restorative composite materials, pit and fissure dental sealants cover only the outer surface of the tooth; therefore, subcutaneous implantation is a popular method to evaluate the biocompatibility of these materials (Pătroi et al., 2013).

Wound healing is typically composed of three overlapping phases: inflammation, proliferation, and remodeling (Singer and Clark, 1999). During the acute inflammation phase, polymorphonuclear cells are the first defensive cells to infiltrate the wound site to eliminate foreign agent and dead cells prior to proliferative phase initiation. In the present study, a few neutrophils were detected only during the first 7 days post-operation. This finding suggested acute inflammation during the first week post-operation. Scattered resident lymphocytes and macrophages around the implanted material were also observed in all groups, indicating the shift from acute to chronic phase. At 30 and 60 days post-implantation, all specimens showed mild to moderate inflammatory reactions with lymphocytes and macrophages. This suggests that the materials may release particulates that may, in turn, induce a continuance local inflammatory reaction. At day 7, one focal hemorrhage was detected in one of the eight samples of the HC, LAS-clear, CL, and LAS-opaque groups. This tissue reaction could be associated with trauma during surgical procedure rather than properties of the materials because no hemorrhage was found in any samples at days 30 and 60 post-implantation.

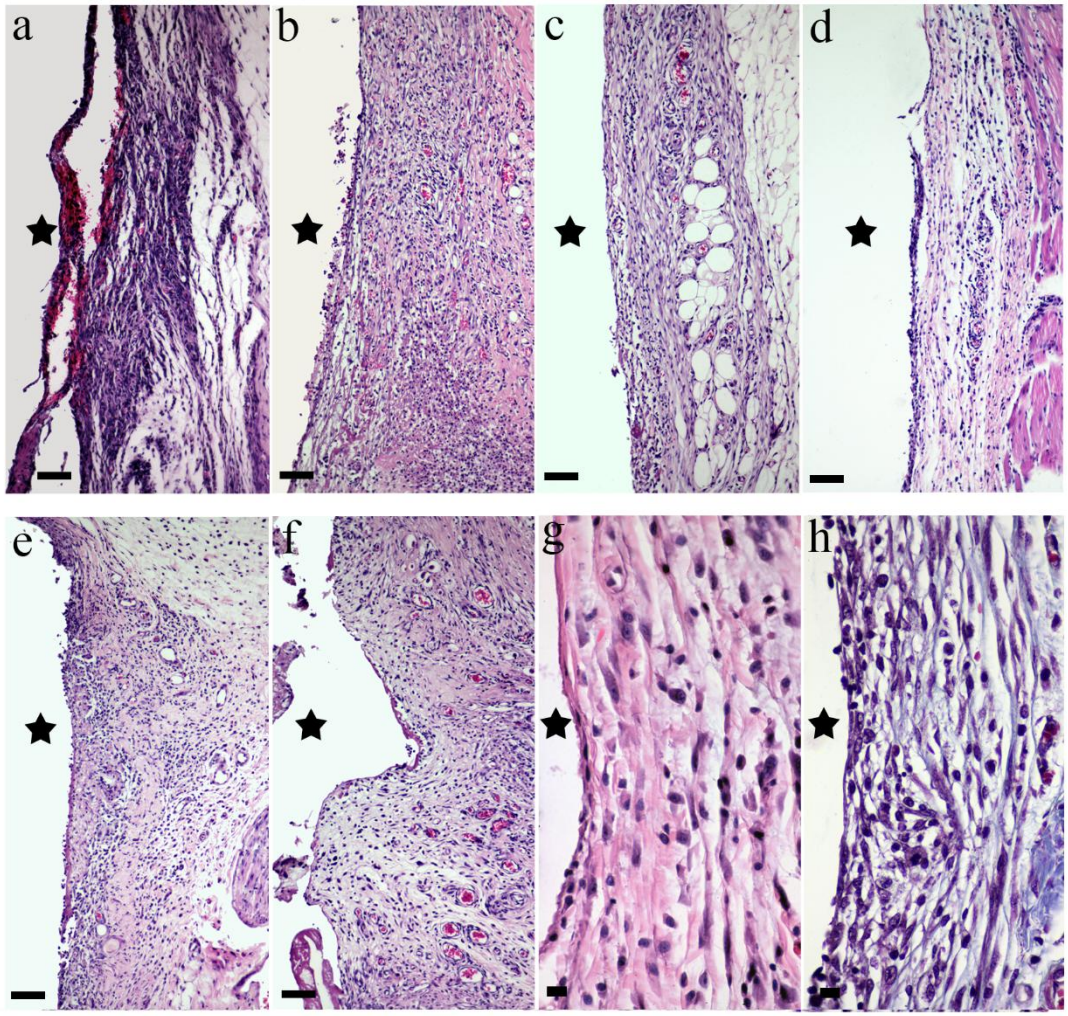

Figure 1 Histopathology of the subcutaneous tissue at day 7 post-implantation: DC (a), HC (b), LAS-clear (c), HO (d), CL (e), and LAS-opaque (f) groups. A loose, poorly organized, discontinuous capsule was observed in all groups. Mild inflammatory

cell infiltration in the LAS-opaque group seen at higher magnification (g). Loose fibrous tissue in the LAS-clear group (h) as shown by MT stain, ★ = experimental material, a-g HE stain, bar = 100 µm.

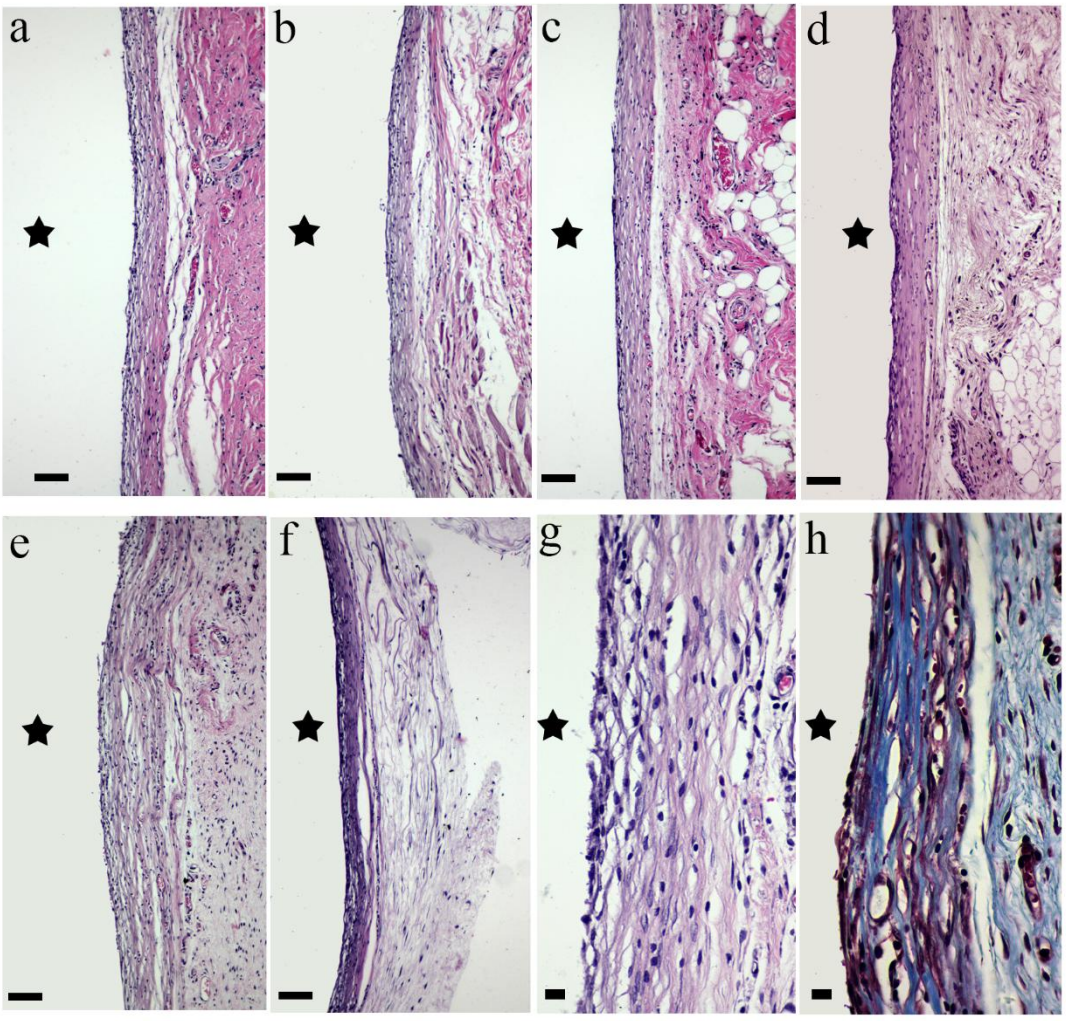

Figure 2 Histopathology of the subcutaneous tissue at day 30 post-implantation: DC (a), HC (b), LAS-clear (c), HO (d), CL (e), and LAS-opaque (f) groups. A compact, moderately organized, thin capsule was observed in all groups. Mild inflammatory cell infiltration in the LAS-opaque group seen at higher magnification (g). Compact, moderately organized, thin capsule in the LAS-clear group (h) as shown by MT stain, ★ = experimental material, a-g HE stain, bar = 100 µm.

Interestingly, two samples of CL were found to exhibit new capillary formation at day 7 post-operation. The coloring agent in this sealant was an extra component compared with the other sealants. Thus, the angiogenesis activity of CL likely resulted from this specific component formulation leaching into the surroundings. The effect of coloring agent, curcumin, on new blood vessel formation has been reported (Ajaikumar et al., 2008)

A fibrous capsule is an organized granulation tissue consisting of fibroblasts, fibrocytes, collagen fibers, and new capillaries found surrounding an implanted material. Regarding biocompatibility of material, generally, the chronic phase could not be longer than two weeks after implantation. However, in this study, it was observed that the thickness and organization of the fibrous capsule increased following implantation. The same response of body after subcutaneous implantation of dental adhesive resin has been reported (de Souza Costa et al., 2000 and Costa et al., 1997). The possible explanation is that the unpolymerized resin monomers are released from the specimen into the surrounding connective tissue and activate the inflammation and tissue responses (de Souza Costa et al., 2000; Munksgaard et al., 1990; Geurtsen et al., 1998; Kaga et al., 2001).

In the present study, foreign body giant cells, which are a marker of foreign body reaction, were not observed at any time points evaluated. These giant cells are originally derived from monocyte and macrophage, and usually found at the material interface (Sheikh et al., 2015). Our results suggest that these pit and fissure materials did not induce inflammatory cytokine or tissue reaction in the body.

The present study found that the average total inflammatory cell infiltration score of each material was highest at 7 days post-implantation, and lowest at 30 days post-implantation (Cintra et al., 2010 and Vosoughhosseini et al., 2012). The proprietary nature of the commercial pit and fissure dental sealants evaluated restricted our knowledge of their exact composition. Thus, the explanation why each sealant material had different inflammatory cell infiltration and tissue response could not be stated. A practical explanation is that each sealant has a different composition and percentage of each component.

During the first week the unpolymerized monomers are released from the surface to surrounding tissue and dramatically drop in the fourth week. After that the unpolymerized materials from the inner part slowly diffuse into an outer surface and are continuously released into surrounding tissue to stimulate persistent inflammation and fibrous encapsulation. Our inflammatory cell infiltration and tissue reaction results suggest the biocompatibility of LAS-clear and LAS-opaque to subcutaneous tissue after implantation for 60 days compared with the commercial materials. However, a long-term clinical study of LAS-clear and LAS-opaque should be performed.

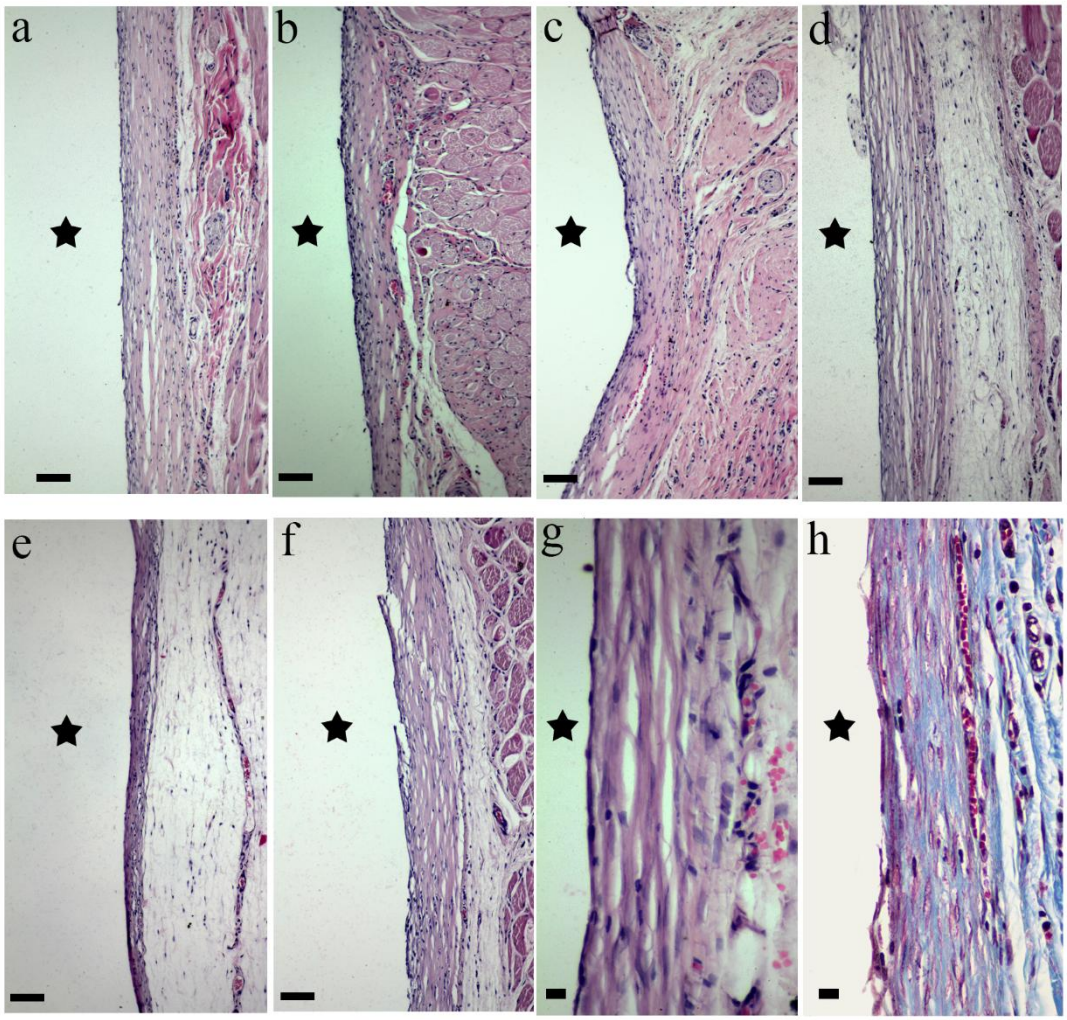

Figure 3 Histopathology of subcutaneous tissue at day 60 post-implantation: DC (a), HC (b), LAS-clear (c), HO (d), CL (e), and LAS-opaque (f) groups. A compact, dense, well-organized, thick capsule was observed in all groups. Slight inflammatory cell infiltration in the LAS-opaque group seen at higher magnification (g). Compact, dense, well-organized, thick capsule in the LAS-clear group (h) as shown by MT stain, ★ = experimental material, a-g HE stain, bar = 100 µm.

The biocompatibility of the DC, HC, HO, CL, LAS-clear, and LAS-opaque materials with subcutaneous tissue corresponds with our previous *in vitro* study that demonstrated that the DC, HC, HO, LAS-clear, and LAS-opaque materials were biocompatible with gingival fibroblasts (Thunyakitpisal et al., 2016). It should be noted that although CL was found to be biocompatible with subcutaneous tissue, it was cytotoxic to gingival fibroblasts. Therefore, the data obtained from *in vitro* conditions may not completely confirm the results obtained from *in vivo* environment. The complex orchestration of the effects of the vascular system, immune system, and inflammation is absent in the *in vitro* environment (Wataha, 2003). Without detoxification, buffering, and excretion systems present *in vivo*, the accumulation of toxic substances released from samples likely occurs.

In conclusion, the resin-based pit and fissure dental sealants LAS-clear and LAS-opaque have acceptable biocompatibility in the subcutaneous tissue.

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บทคัดย่อ

การฝังวัสดุเคลือบหลุมร่องฟันต้นแบบชนิดแข็งตัวด้วยการฉายแสง LAS-clear และ LAS-opaque ในชั้นใต้ผิวหนังทำให้เกิดการตอบสนองของเนื้อเยื่อชนิดอ่อนแบบชั่วคราว

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วัตถุประสงค์ของการศึกษา เพื่อประเมินความเข้ากันได้กับเนื้อเยื่อเมื่อฝังวัสดุเคลือบหลุมร่องฟันเรซิน แบบแข็งตัวด้วยการฉายแสงต้นแบบ LAS-clear และ LAS-opaque ใต้ชั้นผิวหนังของหนูแรท เปรียบเทียบกับวัสดุเคลือบหลุมร่องฟันเรซินแบบแข็งตัวด้วยการฉายแสงทางการค้า หนูแรทอายุ 8 สัปดาห์ได้รับการกรีดถึงชั้นใต้ผิวหนังจำนวน 2 แถว แถวละ 3 ช่อง ในแนวขนานกลางหลัง โดยมีความยาวช่องละ 5 มิลลิเมตร สุ่มใส่ด้วยวัสดุเคลือบหลุมร่องฟัน ได้แก่ Delton®clear (DC), Helioseal® clear (HC), Helioseal® opaque (HO), Clinpro™ Sealant (CL), LAS-clear และ LAS-opaque เย็บปิดผิวหนังด้วยไหมเย็บ เก็บตัวอย่างและเนื้อเยื่อโดยรอบในวันที่ 7 30 และ 60 หลังการทดลอง นำชิ้นเนื้อผ่านกระบวนการเตรียมเนื้อเยื่อ ตัดแผ่นเนื้อเยื่อบาง ย้อมสี และตรวจทางจุลพยาธิวิทยา และวิเคราะห์ผลด้วยโปรแกรมสำเร็จรูป SPSS การทดลองพบว่า กลุ่มทดลอง DC, HC, HO, CL, LAS-clear และ LAS-opaque มีการอักเสบแบบอ่อนที่ 7 วัน และไม่แสดงการอักเสบที่ 30 และ 60 วัน ทุกกลุ่มทดลองมีการตอบสนองของเนื้อเยื่อต่อวัสดุแบบการหายของแผลปกติ วัสดุถูกล้อมรอบด้วยเนื้อเยื่อเกี่ยวพันแบบไม่มีพยาธิสภาพ สรุปว่า LAS-clear และ LAS-opaque มีความเข้ากันได้กับชั้นเนื้อเยื่อใต้ชั้นผิวหนัง

คำสำคัญ: การศึกษาในสัตว์ ความเข้ากันได้ จุลพยาธิวิทยา วัสดุเคลือบหลุมร่องฟันแบบแข็งตัวด้วยการฉายแสง การตอบสนองของเนื้อเยื่อเฉพาะที่

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