CHAPTER 3

Materials and Methods

3.1 Patient selection and abutment conditions

The experiments were conducted on 43 teeth in 18 subjects (11 males and seven females). The patients were aged 18-30 years (average 22.8 years). All the subjects were healthy, had no systemic disease and did not take any medication. All of them had lost the mandibular first molar on one or both sides. Those teeth were to be replaced with a fixed partial denture because the subjects rejected a removable partial denture and could not afford dental implant restorations.

Of the 43 teeth, 20 were second premolars and 20 were second molars, which needed to be prepared for abutments of 20 full-coverage, three-unit bridges. The other three teeth were the first premolars adjacent to abutment teeth; they served as un-operated controls. All of the teeth in the experiment were vital and intact, had completely formed roots, and had normal position and alignment. No teeth had shifting, drifting, rotation, supra-occlusion or infra-occlusion. There was no periodontal pocket formation, bleeding on probing, pus exudation or gingival recession. Scaling and polishing were performed one to two weeks before starting the experiment to remove calculus deposition and to eliminate gingival inflammation. If there was caries or filling restoration, the depth of the cavity or the restoration was less than 0.5 mm into the dentine with absence of any hypersensitivity. The edentulous areas were free from retained roots or incomplete healing of the soft tissue. There were no impacted or erupted teeth distal to the second molar abutments. On periapical radiographic examination, the periodontal ligaments along the roots of all teeth were intact, and there were no radiolucent lesions around the roots. The ratio of the crown height to the root length of all teeth was at least 1:1.5.

The study was approved by the Human Experimentation Committee, Faculty of Dentistry, Chiang Mai University (Certificate no. 39/2015), and complied with the principles of the Declaration of Helsinki. The experimental procedures were clearly explained to each subject and written informed consent was granted by all subjects. The privacy rights of the subjects were respected at all times.

3.2 Abutment preparation and final impression

All the subjects were allowed to relax in the dental chair unit for ten minutes before starting the experiments. All the abutment teeth were prepared for full-coverage, three-unit bridges by the same operator. The teeth were anesthetized with 1.8 ml. of 4% articaine with epinephrine 1:100,000 (Ubistesin™ Forte, 3M ESPE, Seefeld, Germany) by an inferior alveolar nerve block, lingual nerve block and buccal nerve block technique. The tooth preparation was started five minutes after administration of the local anesthetic. The tooth reduction was approximately 1.0-1.5 mm from the enamel surface under light pressure and water spray coolant. A depth cut of each surface was made before total preparation to control the amount of tooth reduction of each tooth. The occlusal and buccal surfaces were reduced 1.5 mm while the mesial, distal and lingual surfaces were reduced by 1 mm. The cervical finished line of the preparation was a chamfer and was located slightly above the gingival margin to prevent creating gingival inflammation which might have interfered with the recording of blood flow from the teeth. The preparation was performed using new, medium-grit, diamond burs of the same size and same shape to control the sharpness of the burs and to reduce the pressure and the heat which might be generated from dull burs. All experiments were conducted with the same airotor handpiece on the same dental unit to control the water coolant and the speed of the cutting instrument.

Before taking the final impression, the gingival tissues were retracted using the double cord technique with plain retraction cords without chemical agents of two sizes, No. 0 and No. 1 (Ultrapack® UltradentInc, South Jordan, UT, USA). Cord No. 0 was packed first followed by cord No. 1 with light pressure (Figure 3.1). The retraction cords were left in the gingival sulcus for five minutes and then the final impression was made using the double-mixed, single impression technique. The retraction cords on the top level of both abutments were removed slowly and then the impression material,

light-body polyvinylsiloxane (Express™ XT Light body 3M, Deutschland GmbH, Neuss, Germany), was injected around the abutments. Putty-type polyvinylsiloxane (Express™ XT Putty Soft 3M, Deutschland GmbH) in a perforated tray was then inserted into the mouth. The impression was inspected after setting and removed. The die and master cast were made to prepare the final restoration, which was porcelain-fused-to base metal alloy bridge. The study cast of the antagonist was made from an irreversible hydrocolloid impression material (Kromopan® LASCOD SpA, Florence, Italy). The jaw relation record was made using polyvinylsiloxane bite registration material (Blu Mousse®Parkell Inc., Edgewood, NY, USA).

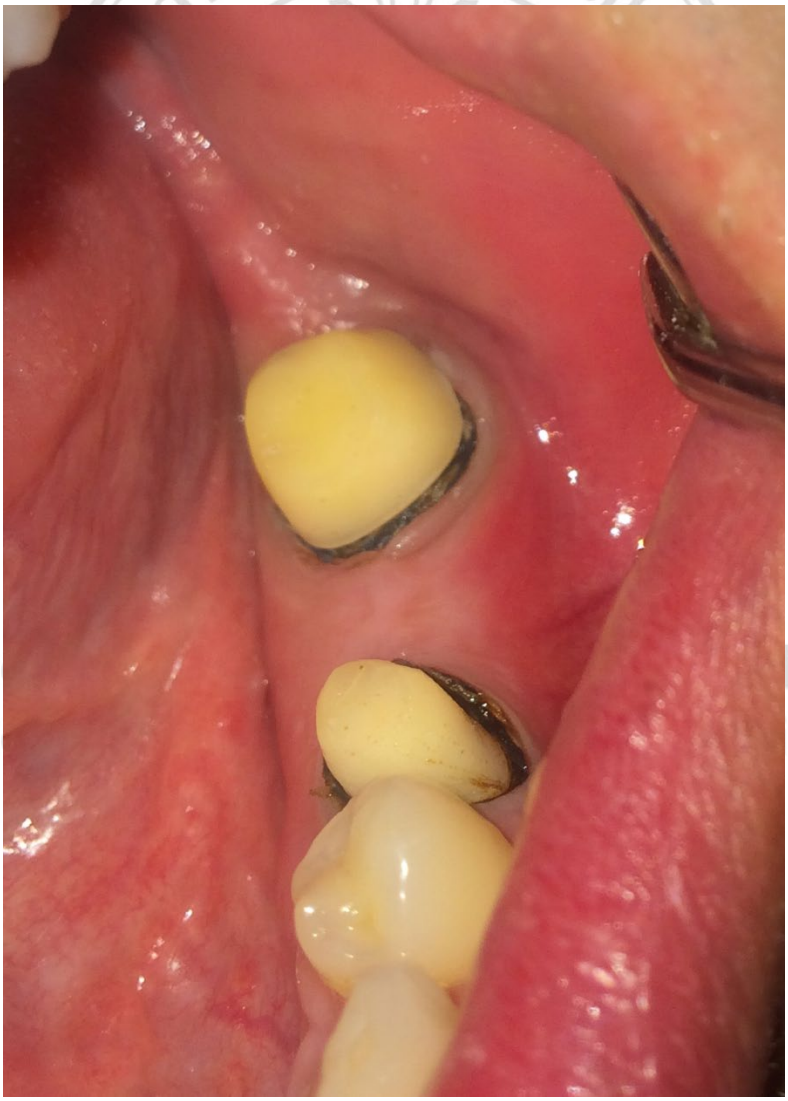


Figure 3.1 Abutment preparation and gingival retraction.

3.3 Temporary bridge and temporary cementation

After the preparation of the abutment teeth had been completed, a temporary bridge made of auto-polymerizing acrylic resin (UnifastTrad GC Corporation, Tokyo, Japan) from the study cast was relined and fixed in place with two types of temporary cement (Figure 3.2). In Group I, 10 temporary bridges were cemented on 10 premolars and 10 molars with eugenol-containing temporary cement (Temp-Bond, Kerr Corporation, Romulus, MI, USA.). In Group II, the other 10 temporary bridges were cemented on 10 premolars and 10 molars with non-eugenol temporary cement (Temp-Bond NE, Kerr Corporation).

After preparation and temporary cementation for one day, the temporary bridge was removed, the prepared teeth were cleaned with pumice, the pulpal blood flow was measured, as described below, and the temporary bridge was recemented with the same type of temporary cement. Seven days after tooth preparation, the temporary bridge was removed, the prepared teeth were cleaned with pumice and the permanent bridge was tried in, adjusted and permanently fixed with resin cement (RelyX^{TM} U200, 3M ESPE). At each follow-up visit, each subject was asked whether there was any pain or other symptom associated with the fitting of the bridge.

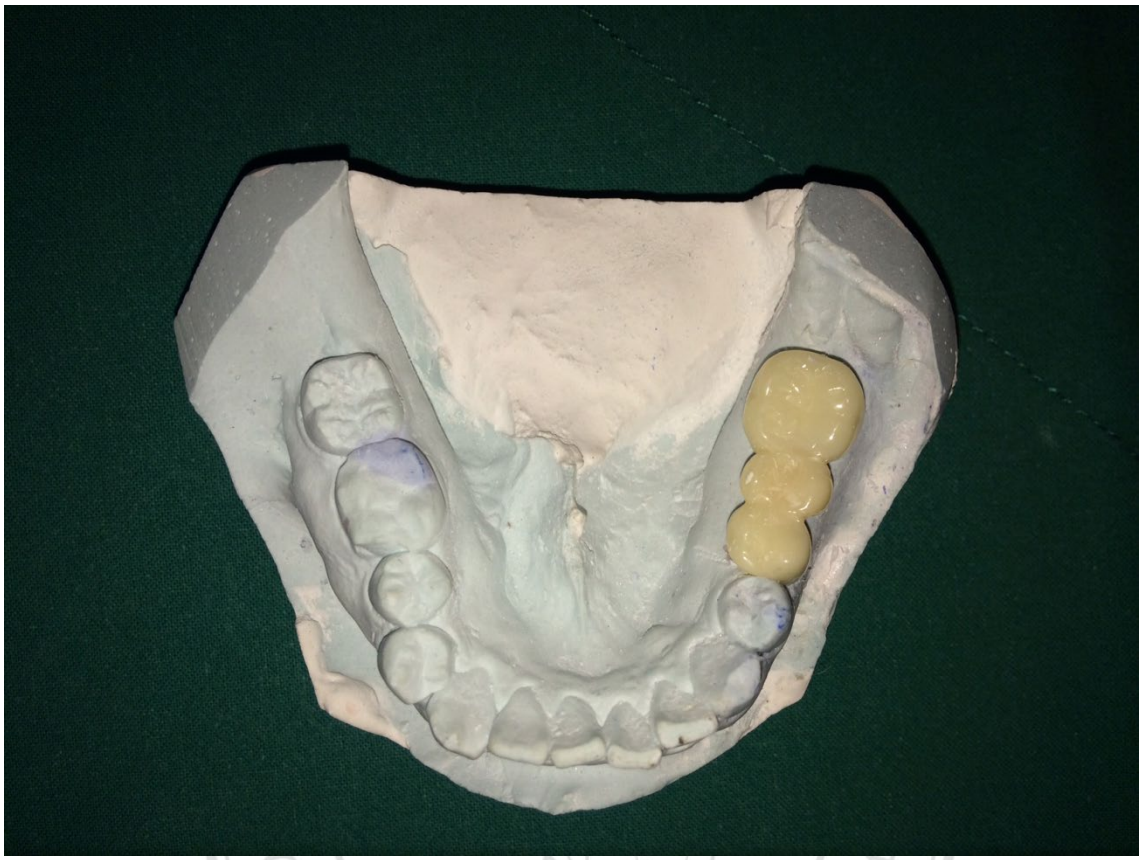


Figure 3.2 Temporary bridge prepared from the study cast.

3.4 Pulpal blood flow recording

Pulpal blood flow was recorded with a Moor Type MBF3D/42 blood flow monitor (Moor Instruments, Axminster, UK) (Fig. 3.3). During recording, opaque black rubber dam (Four D Rubber Co. Ltd., Heanor, UK) was applied around the crowns of the teeth, extended from the mandibular canine to the second molar on the same side, in order to reduce the blood flow signals picked up from tissues outside the tooth (97) (Fig. 3.4).

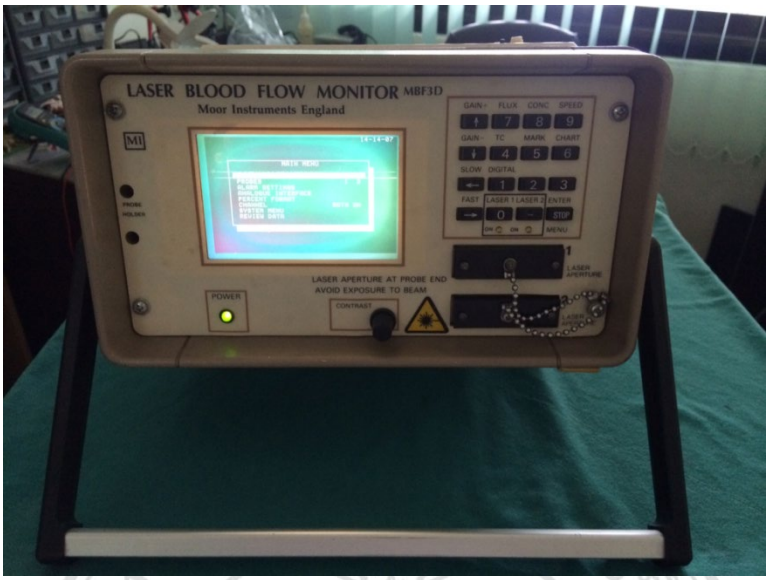


Figure 3.3 Moor Type MBF3D/42 laser Doppler flow meter.

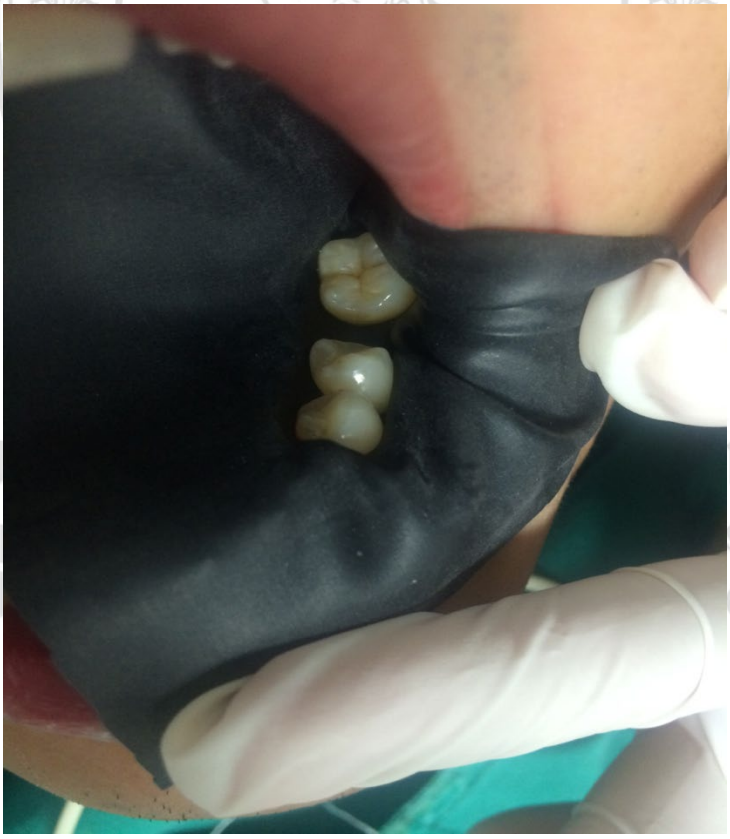


Figure 3.4 Opaque rubber dam was applied around the crowns of the teeth to reduce the blood flow signals from other tissues.

The probe of the instrument (o.d. 1.5 mm) (Fig. 3.5) was supported on the tooth with an opaque stent. The stent was prepared from self-curing acrylic resin on a study model of the mandibular teeth. It extended from the mandibular canine to the second molar and covered the buccal and occlusal surfaces of these teeth (Fig. 3.6 A& B). Holes to fit the probe were drilled through the stent 2 mm from the gingival margin and perpendicular to the enamel surface. These holes ensured that the probe was replaced in the same position throughout the experiment, in contact with either the enamel or dentin surface(Figs. 3.7 A& B, 3.8).

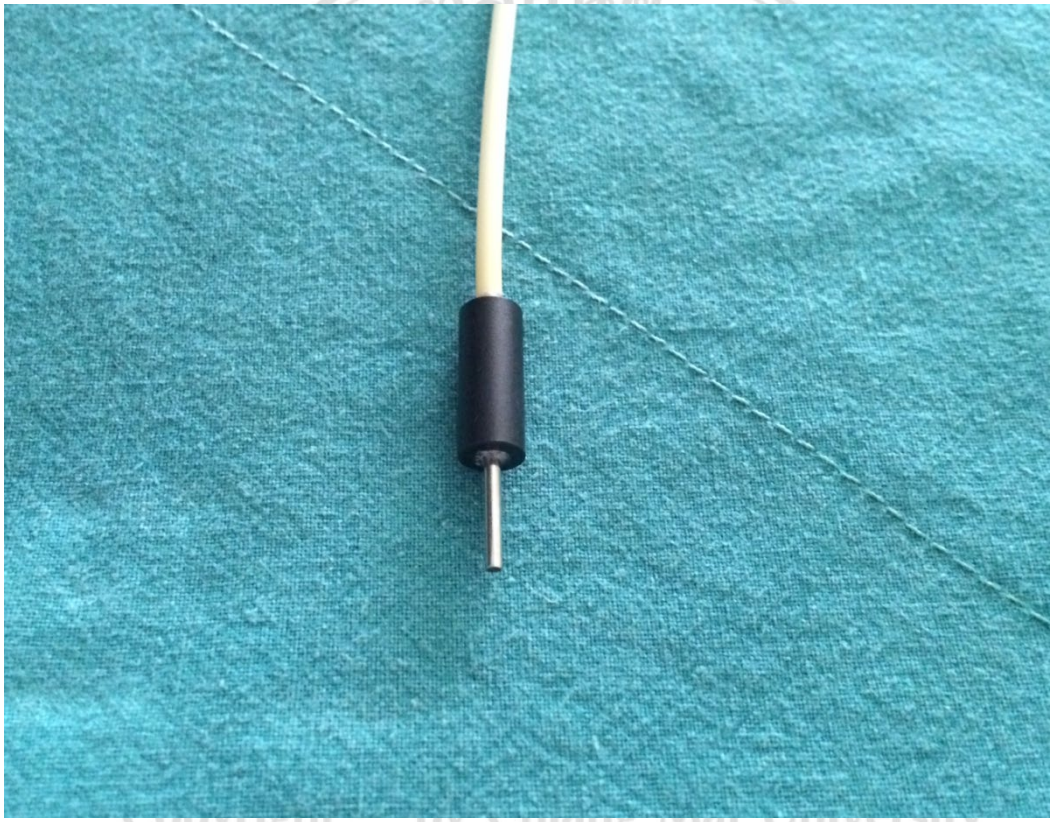
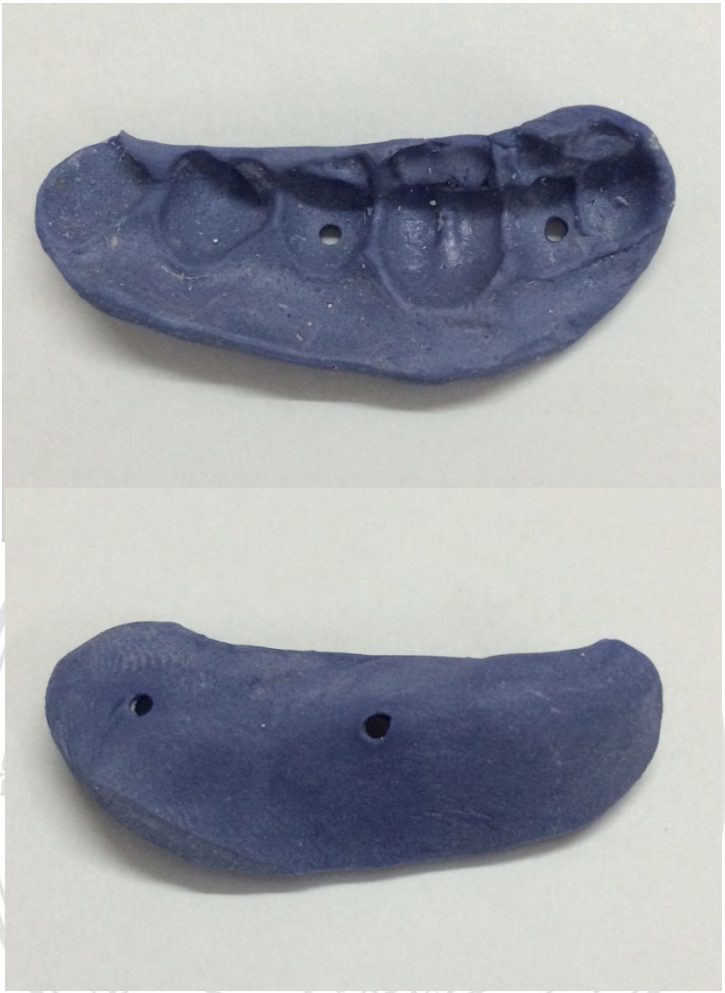


Figure 3.5 The tip of the laser probe.



B

Figure 3.6 Opaque acrylic stent with holes to stabilize the laser probe. (A) Inner surface. (B) Outer surface

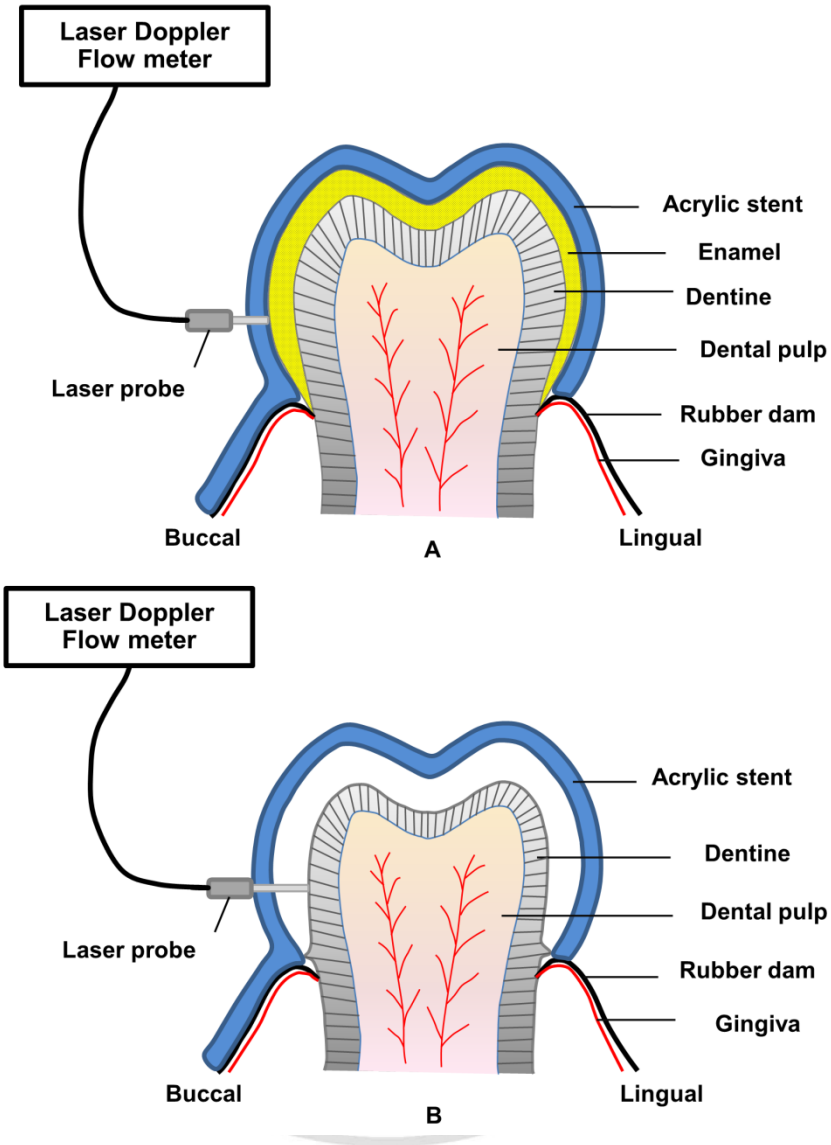


Figure 3.7 Diagram of the experimental set-up for pulpal blood flow recording. (A) Pulpal blood flow recording on the enamel surface. (B) Pulpal blood flow recording on the exposed dentine surface.

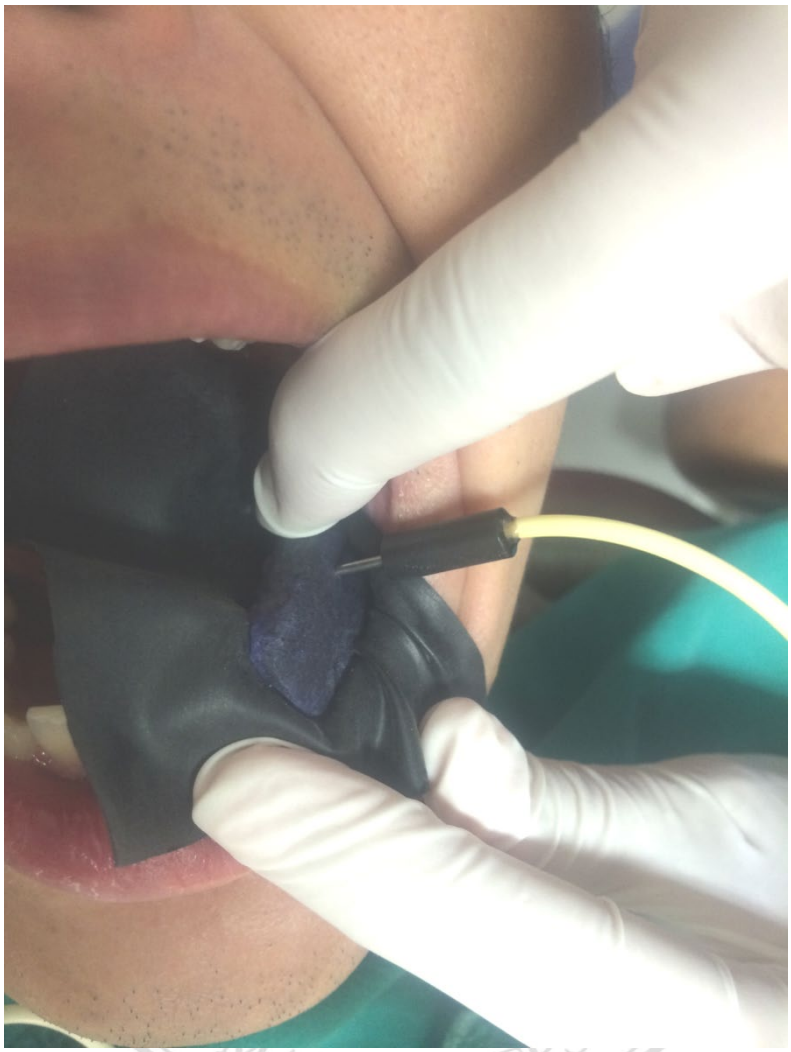


Figure 3.8 The rubber dam and the acrylic stent were applied on the teeth to stabilize the laser probe on the tooth surface.

The sensitivity of the laser Doppler flow meter was standardized as described by the manufacturers, and recordings were made with an upper bandwidth setting of 14.9 kHz and a time constant of 0.1 second. Blood flow was measured in arbitrary perfusion units (P.U.) (101). Both the flux and DC level (backscattered light intensity) signals from the blood flow monitor were digitized by a CED micro1401-3 data acquisition nit, with the Spike II Program (Cambridge Electronic Design Limited, Cambridge, UK) and stored on a laptop computer for further analysis.

The PBF signal was recorded seven times for each experiment. The first signal from the two abutment teeth and from the three ipsilateral un-operated, controls was recorded before administration of local anesthetic (LA). Recordings were made again five minutes after administration of local anesthetic. For these recordings, the tip of the laser Doppler probe was placed on the surface of the buccal enamel. Three recordings were made with the tip of the laser probe on the exposed dentin surface of the teeth after buccal side preparation, after complete preparation and after gingival retraction. The other two recordings were repeated one day and seven days later. PBF was also recorded from the buccal enamel of the control teeth at the same seven intervals as for the abutment teeth. Each subject was asked at each follow-up visit whether there were any pain or other symptoms associated with the fitting of the bridge.

After each experiment, records were made at different flow meter light intensities using a stationary reflector (white card) with the same level of illuminations as during recording from the tooth. These recordings were used to determine the output that has same values with zero blood flow in the recording. To record a signal from white card, the probe tip was inserted at the end of the black and opaque plastic syringe. The white card was attached to the piston of the syringe. The piston was pulled slowly and stopped when the signal was stable for at least five seconds. The recorded data were used to calculate the offset of the blood flow signal that would have been present while recording from the teeth due to noise in the detection system (102). For each set of blood flow values recorded form a tooth during the experiment, the mean and S.D. were calculated and the offset, determined as described above, was subtracted from the mean.

3.5 Statistical analysis

3.5.1 Effect of local anesthesia

The effect of local anesthesia on pulpal blood flow was evaluated by comparing the differences between the blood flow values recorded before and after local anesthesia in all abutment teeth and the control teeth (n=43), using Student's paired t-test.

3.5.2 Effect of tooth preparation

The effect of tooth preparation on pulpal blood flow was evaluated by comparing the differences between the blood flow values recorded after buccal preparation and

after complete preparation in the second premolar (n=20) and the second molar abutment teeth (n=20), using Student's paired t-test.

3.5.3 Effect of gingival retraction

The effect of gingival retraction on pulpal blood flow was evaluated by comparing the differences between the blood flow values recorded after complete preparation and after gingival retraction in the second premolar (n=20) and the second molar abutment teeth (n=20), using Student's paired t-test.

3.5.4 Effect of temporary cementation

The effect of temporary cementation on pulpal blood flow was analyzed by comparing the differences between the blood flow values recorded one day and seven days after complete preparation using two–way, repeated measures analysis of variance (RM ANOVA). The PBF values from premolars and molars were analyzed separately. Since the PBF values after complete preparation from Groups I and II were different, the PBF values after temporary cementation could not be compared. The PBF values from complete preparation were adjusted to 100% to produce the baseline values, and the values from one day and seven days were adjusted in comparison to the baseline values; as a result, the PBF values from each group could be compared. The statistical analyses were carried out with Sigmaplot® software (version 12, Systat Software Inc., San Jose, CA, USA). *P* values of less than 0.05 were considered significant.