CHAPTER III

MATERIALS AND METHODS

MATERIALS

1. Elastomeric ligatures

The elastomeric ligatures, Power "O" modules (Size .120, No. 640-0078, Ormco, U.S.A.) were selected as samples in this investigation. Their general configurations were doughnut-shaped elastomers with 0.120 inch of outer diameter. Not only are they popularly used in clinics but also the Ormco Corporation claims that they provide a tighter fit and greater control over a six to eight week period. They are gray injection molded ligatures which are reshaped to decrease force decay and improve fit (Ormco® catalogue, 1999). They are available in a package of 20 sticks with 50 modules attached per stick. The elastomeric ligatures were bought from a local vendor in Thailand. They were in their plastic package and preserved in a refrigerator before being randomly selected for testing.

2. Two percent glutaraldehyde solution

The Pose-Dex® Low foam, long life liquid activated glutaraldehyde (Pose Health Care, Thailand) was used as disinfectant in this investigation. It is widely used in hospitals and dental clinics. It consists of 2% glutaraldehyde and 98% inert ingredients. Two percent glutaraldehyde solution was prepared strictly following the company directions by adding 150 milliliters of activator into 5,000 milliliters of Pose-Dex® solution and mixing well. The activated solution changed color from colorless to light blue.

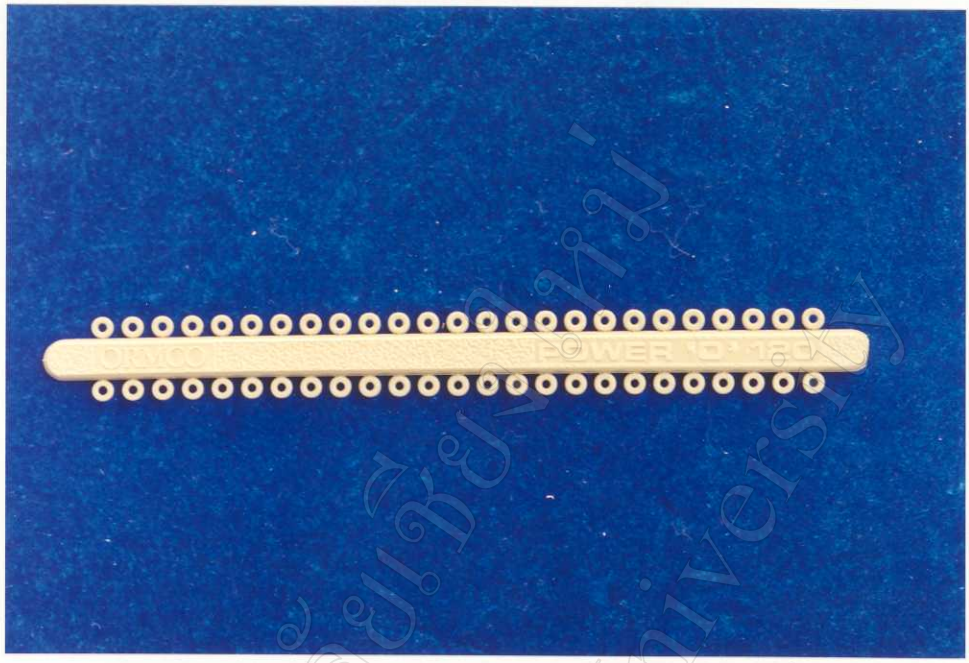

Figure 3.1 A stick of elastomeric ligatures, Ormco® Power "O" modules

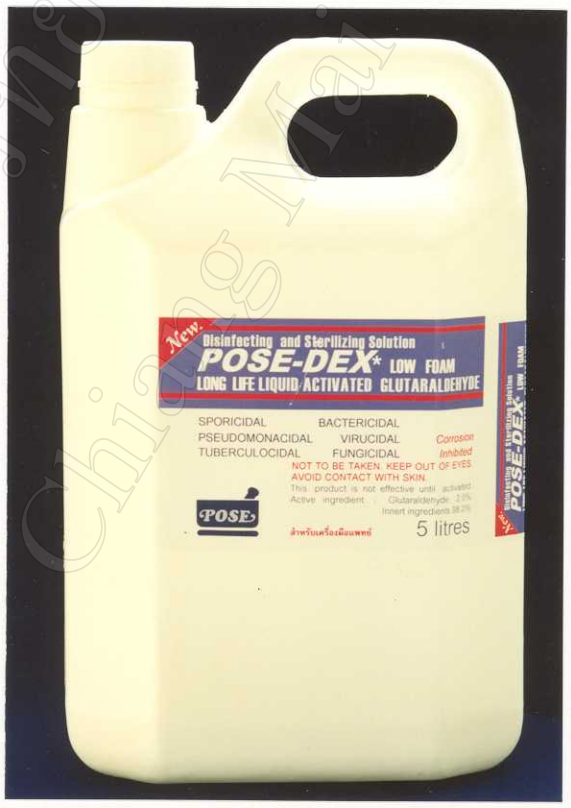

Figure 3.2 Two percent glutaraldehyde solution, Pose-Dex® Low foam, long life liquid activated glutaraldehyde

3. Instruments

1) A ten-times magnifying glass with scale (SKS10XSD, Japan) was used to measure the dimensions of elastomeric ligatures to the nearest of 0.1 millimeter.

Figure 3.3 A ten-times magnifying glass

2) A universal testing machine, LLOYD instruments LRX with 100 newtons load cell (Intro Enterprise Co, Ltd.), was connected to the computer to establish the generated force in grams. Each upper and lower crosshead had a designed vertical stainless steel hook attached, 0.46 millimeter in diameter, to hold an elastomeric ligature.

3) Twenty four rectangular alumimium bars (4 x 3 x 100 millimeters) with rounded angles were constructed by hand. The perimeters of the bars were approximated to that of the bracket base of the upper central incisor. Both ends of each bar were slightly tapered to facilitate the application and the removal of the ligatures.

Figure 3.4 A universal testing machine, LLOYD instruments LRX, with designed vertical stainless steel hooks

Figure 3.5 A rectangular aluminium bar with rounded angles and tapered ends

4) An incubator (Memmert, Memmert Corporation, Germany) was maintained at constant 37°C±1°C as normal body temperature.

Figure 3.6 An incubator

5) A sealed clear plastic box (15 x 15 x 21 centimeters) with 100% humidity. The plastic box was lined with 5-centimeter thickness of dense sponge soaked with a litre of distilled water 24 hours before testing and throughout the experiment. The dense sponge also supported 24 aluminium bars with ligatures stretched over them in upright position.

6) Elastomeric forceps were used to put on and take off the elastomeric ligatures from the aluminium bar.

Figure 3.7 A sealed clear plastic box

METHODS

1. Sample preparation

Eight hundred and forty modules of gray molded elastomeric ligatures were cut by sharp scissors from 17 sticks of 50 modules and then randomly separated into four groups, 210 modules in each group,

Group I (untreated elastomeric ligatures as a control group). They were left in room temperature at 27°C±2°C.

Group II (1-time glutaraldehyde treated elastomeric ligatures). They were totally immersed in a 2% glutaraldehyde solution for 10 minutes at room temperature at 27°C±2°C, then rinsed thoroughly with ultra pure water for 1 minute, and allowed to air dry for 60 minutes on a napkin.

Group III (2-time glutaraldehyde treated elastomeric ligatures). They were treated in the same way as group II, twice.

Group IV (3-time glutaraldehyde treated elastomeric ligatures). They were treated in the same way as group II, 3 times.

Each group was randomly divided into 7 subgroups, 30 modules each, according to time interval: initial, 1 day, 2 days, 7 days, 14 days, 21 days and 28 days. Subgroup 1 was the initial unstretched elastomeric ligatures, which were in passive state. Subgroup 2 to subgroup 7 were the elastomeric ligatures stretched over the rectangular aluminium bars to simulate conventional elastomeric ligation for 1 day, 2 days, 7 days, 14 days, 21 days and 28 days respectively. Therefore, each bar held 30 elastomeric ligatures of each subgroup.

All 24 rectangular aluminium bars with stretched ligatures were kept in order of group and subgroup in the sealed well-prepared plastic box with 100% humidity to facilitate the removal of ligatures for testing in sequence. The plastic box with ligatures stretched over the bars was placed in the incubator (Memmert, Memmert Corporation, Germany) at constant 37°C±1°C.

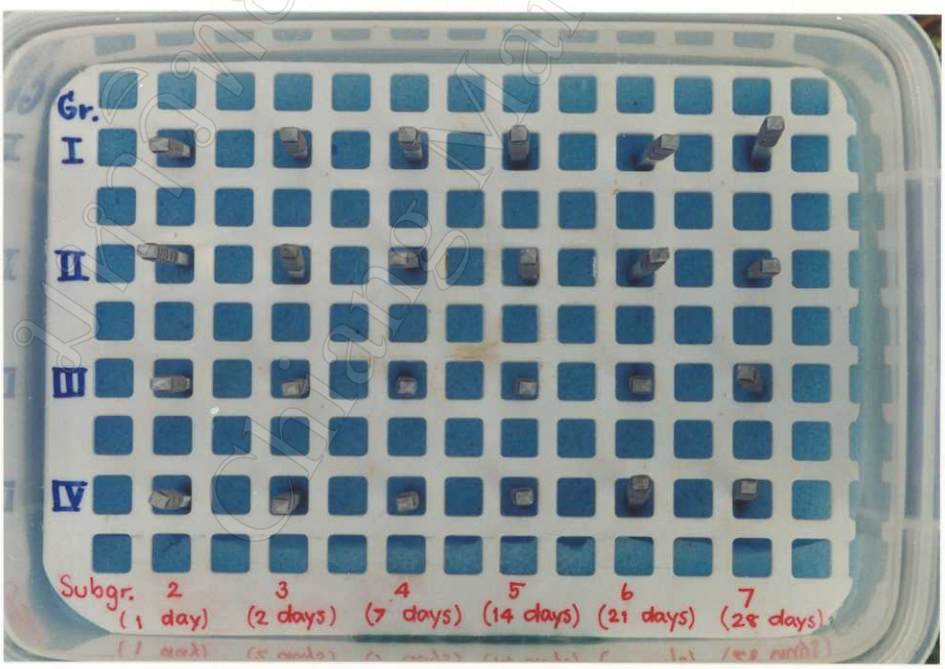

Figure 3.8 Twenty four aluminium bars with stretched elastomeric ligatures stored in a sealed plastic box.

2. Initial dimension measuring

The initial outside diameter (OD), the inside diameter (ID) and the wall thickness (WT) of all subgroup 1 samples of each group were measured by the ten-times magnifying glass with scale (SKS10XSD, Japan) to the nearest of 0.1 millimeter (Figure 3.9). All data of each group were separately recorded in millimeters.

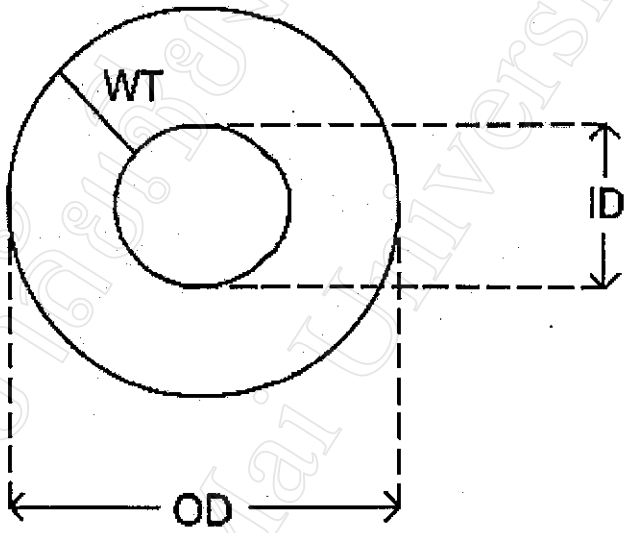

Figure 3.9 Dimensions of elastomeric ligature: the outside diameter (OD), the inside diameter (ID), and the wall thickness (WT).

3. Initial force testing

The initial force testing proceeded after the initial dimension measuring. All subgroup 1 samples of each group were tested for the initial force on the universal testing machine, LLOYD instruments LRX with 100 newtons load cell (Intro Enterprise Co Ltd.), which was connected to the computer to establish generated force in grams. The universal testing machine was set up to stretch the elastomeric ligatures to the displacement of 5.5 millimeters at the rate of 5.08 millimeters per minute as recommended by Kovatch *et al.* (1976). The 5.5 millimeters of displacement was calculated from the inter-bracket width of an upper central incisor twin bracket (Taloumis *et al.*, 1997).

Following the calibration procedure, each sample was suspended on the vertical hooks of upper and lower crossheads of the universal testing machine (Figure 3.10). The testing was initiated when the universal testing machine had been set at zero. The force-displacement curve was shown on the computer monitor and the force magnitude was read at the displacement of 5.5 millimeters and recorded in grams.

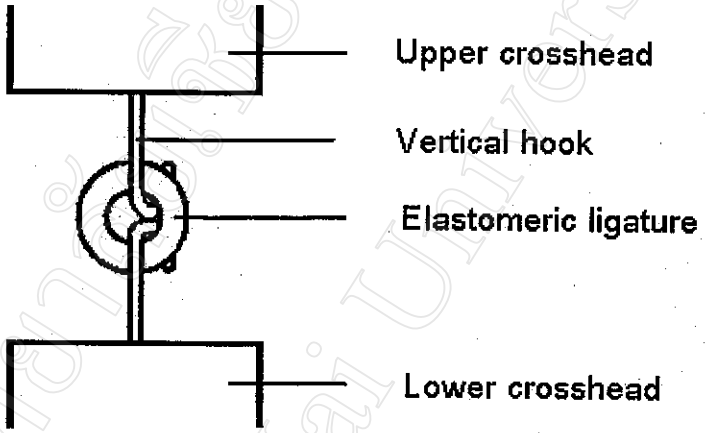

Figure 3.10 Loading an elastomeric ligature on the designed vertical hooks of upper and lower crossheads of the universal testing machine

4. Remaining force testing

At each specific time interval, all samples in each subgroup from four groups were removed from the alumimium bars for remaining force testing on the universal testing machine in the same manner as initial force testing. Since the inner diameter of an elastomeric ligature stretched from the bar was larger than initially, it was necessary to set the distance between the upper and lower vertical hooks according to the larger diameter of each subgroup. Therefore, the remaining force magnitude was read at the displacement corresponding to the change of hook distance. The distance between the upper and lower hooks was set at 2.5 millimeters; thus the force magnitude was read with the displacement of 3.0 millimeters.

Since the initial force of the four groups was different, it was necessary to standardize to 100% for comparison. Thus all recorded force magnitude was then converted to the percentage of remaining force by the formula:

$$\text{Percentage of remaining force (\%)} = \frac{\text{Remaining force (gram) in each time interval} \times 100}{\text{Initial force (gram)}}$$

STATISTICAL ANALYSIS

The data were proceeded by the SPSS for Window Release 9.0 program as following:

1. Descriptive analysis was used for determining means and standard deviations of:

1.1. the initial outside diameter, inside diameter and wall thickness of 1, 2, 3-time glutaraldehyde treated and untreated elastomeric ligatures,

1.2. the initial force of 1, 2, 3-time glutaraldehyde treated and untreated elastomeric ligatures,

1.3. the percentage of remaining force at seven time interval of 1, 2, 3-time glutaraldehyde treated and untreated elastomeric ligatures.

2. One way analysis of variance (ANOVA) was used for comparing the initial outside diameter, inside diameter and wall thickness among 1, 2, 3-time glutaraldehyde treated and untreated elastomeric ligatures. If there were significant differences, the multiple comparisons were tested. Therefore, null hypothesis (H_0) were:

2.1. H_0: there was no significant difference in the initial outside diameter among 1, 2, 3-time glutaraldehyde treated and untreated elastomeric ligatures,

2.2. H_0: there was no significant difference in the initial inside diameter among 1, 2, 3-time glutaraldehyde treated and untreated elastomeric ligatures,

2.3. H_0: there was no significant difference in the initial wall thickness among 1, 2, 3-time glutaraldehyde treated and untreated elastomeric ligatures.

3. One way analysis of variance (ANOVA) was used for comparing the initial force among 1, 2, 3-time glutaraldehyde treated and untreated elastomeric ligatures. If there

were significant differences, the multiple comparisons were tested. Therefore, null hypothesis (H_0) was:

H_0: there was no significant difference in the initial force among 1, 2, 3-time glutaraldehyde treated and untreated elastomeric ligatures.

4. One way analysis of variance (ANOVA) was used for comparing the percentage of remaining force:

4.1. at each time interval, one way analysis of variance (ANOVA) was used for comparing the percentage of remaining force among 1, 2, 3-time glutaraldehyde treated and untreated elastomeric ligatures. If there were significant differences, the multiple comparisons were tested. Therefore, null hypothesis (H_0) was:

H_0: there was no significant difference in the percentage of remaining force at each time interval among 1, 2, 3-time glutaraldehyde treated and untreated elastomeric ligatures.

4.2. for each group, one way analysis of variance (ANOVA) was used for comparing the percentage of remaining force among seven time intervals to 28 days. If there were significant differences, the multiple comparisons were tested. Therefore, null hypothesis (H_0) was:

H_0: there was no significant difference in the percentage of remaining force among seven time intervals to 28 days.