The receptor activator of nuclear factor κ-B ligand (RANKL) is a cytokine that helps remodel bone by forming osteoclasts. During tooth movement phases, osteoclasts resorb the hyalinization areas of alveolar bone, triggering tooth movement. We determined the differences in RANKL concentrations in early orthodontic treatment between preadjusted edgewise appliances (PEA) and self-ligating (SL) bracket systems. Gingival crevicular fluid was retrieved before treatment and at 1, 24, and 168 hours after treatment from five maxillary anterior proximal sites on the mesiolabial side of the upper right-to-left canines. An enzyme-linked immunosorbent assay was used to determine RANKL concentrations. The results showed differences in RANKL concentrations between the SL and PEA groups at 1, 24, and 168 hours after bracket insertion. The RANKL concentration at 168 hours in the SL group was higher compared to that in the PEA group, whereas that in the PEA group decreased to the baseline value after 24 hours. RANKL concentration in the passive SL bracket system was higher compared to that in the PEA system due to differences in the force and mechanotherapy of the SL brackets.

1. Introduction
The prevalence of malocclusion cases in Indonesia is considered to be relatively high, reaching up to 80% of the population. Malocclusion is ranked as the third most common dental condition after caries and periodontal diseases. This high incidence, along with the increase in the knowledge of the general population regarding malocclusion, has led to a higher demand for orthodontic treatment. In general, orthodontic treatment includes the use of edgewise and preadjusted bracket systems. The emergence of the preadjusted edgewise appliance (PEA) and self-ligating (SL) bracket systems contributes to innovations in orthodontic treatment.
Compared to SL brackets, the use of PEA brackets in orthodontic patients is known to cause more friction [1]. Since the SL system does not force the muscles or periodontal tissues to move beyond their capacity, it can produce harmonious dental movement [1–3]. However, the differences in friction between the two bracket systems can make it difficult to determine the amount of force that must be applied by orthodontic tools on the alveolar bone. The application of orthodontic force results in inflammation, which triggers formation of osteoclasts in the supporting tissue of the teeth [3,4]. Osteoclasts dissolve the mineral components of the bone, which results in resorption of alveolar bone. This, in turn, allows the tooth to shift to its intended position [3]. The receptor activator of nuclear factor κ-B ligand (RANKL) is a cytokine known to induce formation of osteoclasts that resorb alveolar bone [4].

Orthodontic tooth movement is the result of alveolar bone resorption that moves in the same direction as that of the applied force. Alveolar bone resorption can happen as early as the leveling and aligning stages, and it increases with more significant tooth movement. Radiographic images can be used to evaluate alveolar bone resorption; however, this can be done only after bone demineralization reaches 55%–60% [6]. An alternative method would be use of cone-beam computed tomography (CBCT), but this is limited to observing bone density proximal to the teeth. Another method to evaluate alveolar bone resorption is the use of a bone resorption biomarker. RANKL acts as an indicator of alveolar bone resorption [8–10]. Studies examining RANKL concentration within the gingival crevicular fluid (GCF) using edgewise brackets state that an increase in RANKL concentration occurs along with the amount of force applied. For example, the larger the force applied, the higher the RANKL concentration produced [9–10].

Nishijima et al. [10] conducted an in vitro research study on RANKL concentration in periodontal ligament (PDL) cells, which created higher compression compared to that in the RANKL control group (219.3± 26.9 vs.7.7± 5.8 pg/µL, respectively). RANKL concentration was evaluated using an enzyme-linked immunosorbent assay (ELISA), and it increased over 24 hours but decreased after 168 hours application of orthodontic forces [10]. Immunohistochemical test results are consistent with the aforementioned findings, since they showed that RANKL protein expression increased after application of orthodontic forces [12]. Therefore, we used PEA and SL brackets in the GCF to determine the differences in RANKL concentrations between orthodontic treatments.

2. Methods
The Dental Research Ethics Committee, Faculty of Dentistry Universitas Indonesia reviewed and approved this research protocol (12/Ethical Approval/FKGUI/III/2017). The protocol of this prospective clinical trial study was explained to the patients, and each patient signed an informed consent form before inclusion in this research. The inclusion criteria were orthodontic patients aged 15–35 years, use of SL and PEA brackets, light-to-medium crowding, no signs of active caries, healthy periodontal tissue, no systemic diseases, and patient not undergoing any treatments. The test subjects were split into three groups (two experimental and one control). A subject was excluded if they consumed nonsteroidal anti-inflammatory drugs during the course of the experiment.

Before insertion of the brackets, a study model was molded to calculate the degree of crowding using Little’s Irregularity Index and electronic digital calipers toward the mesial contact of the 6th anterior tooth in the upper jaw. A0.01-inchnickel-titanium (NiTi) wire was used to install the PEA (MBT; Ormco, Glendora, CA, USA) and SL (Damon Q; Ormco) brackets in the upper jaw of the test subject.

The GCF samples were retrieved from the test subjects via an intra crevicular technique using paper points at five spots in the gingival sulcus proximal to the anterior teeth, while using the Offenbacher method. GCF retrieval was conducted at four different timeframes: before bracket insertion (T0), and 1 (T1), 24 (T2), and 168 (T3) hours after insertion. Paper points (ISO no. 30) were applied to the mesiolabial and distolabial sides of the anterior maxilla for 30 seconds. Then, the sample collected on the
paper point was inserted into an Eppendorf tube containing 200 μL phosphate-buffered saline and stored at −80 °C.

RANKL concentration analyses of the GCF samples were conducted using a sandwich ELISA (Human RANKL; Elabscience, Wuhan, China). All the subjects and controls were tested twice using the ELISA, and the resulting data showed the cytokine concentrations of the GCF (pg/μL).

The analysis was done using SPSS Statistics for Windows (IBM Corp., Armonk, NY, USA) was used to analyze the RANKL concentration results between the experimental and control groups. The differences in the RANKL concentrations were evaluated with a one-way and repeated measures analysis of variance (ANOVA).

3. Results

The RANKL concentrations in the experimental group yielded statistically significant results ($p < 0.01$; Fig. 1), while those in the control group showed no statistically significant difference ($p > 0.01$); therefore, the control group reflected a good standard.

![Figure 1](image.png)

**Figure 1.** RANKL concentrations (ng/mL) for the PEA, SL bracket, and control groups using a pair wise comparison test (Bonferroni). *$P < 0.05$.

The difference in the RANKL concentrations in the PEA group between T0 and T2 was statistically significant ($p < 0.05$). However, the differences for the same group at T0, T1, and T3 hours were not statistically significant ($p > 0.05$; Table 1).

**Table 1.** Differences in the RANKL concentrations of the PEA, SL bracket, and control groups.

| System   | Mean (SD) | p value | T0 vs. T1 | T1 vs. T2 | T2 vs. T3 |
|----------|-----------|---------|-----------|-----------|-----------|
| PEA      | 0.027 (0.007) | 0.040 (0.005) | 0.071 (0.005) | 0.022 (0.006) | 0.062 | 0.000* | 0.000* |
Table 1. Continue

|          | T0    | T1    | T2    | T3    | SL     | PEA vs. Control | SL vs. Control | PEA vs. SL |
|----------|-------|-------|-------|-------|--------|----------------|----------------|-----------|
| SL       | 0.027 | 0.069 | 0.100 | 0.150 | 0.000* | 0.048*         | 0.198          |           |
|          | (0.006) | (0.008) | (0.012) | (0.014) | (0.004) | (0.034) | (0.030) | (0.029) |
| Control  | 0.031 | 0.034 | 0.030 | 0.029 | 1.00   | 1.00           | 1.00           |           |

Pairwise comparison test (Bonferroni), *P < 0.05

The RANKL concentrations in the SL group showed statistically significant differences between T0, T1, T2, and T3, and between T1 and T2 and T3 after insertion of the SL bracket (p < 0.05). However, the differences between T2 and T3 were not statistically significant (p > 0.05; Table 1).

Subsequently, we conducted a test to check for differences in RANKL concentrations among the groups at the same time points. Figure 2 shows statistically significant differences between average RANKL concentrations at T1, T2, and T3 (p < 0.05). However, no statistically significant difference was observed in RANKL concentrations among the PEA, SL, and control groups at T0 (p > 0.05). At T1, T2, and T3, we found statistically significant differences between the SL, PEA, and control groups (p < 0.05; Table 2).

![Figure 2. RANKL concentrations between the experimental and control groups at T0, T1, T2, and T3 after insertion using a 1-way ANOVA. *p < 0.05.](image)

Table 2. Differences in RANKL concentrations at T0, T1, T2, and T3 after insertion.

| Time | Mean (SD) | PEA vs. Control | SL vs. Control | PEA vs. SL |
|------|-----------|----------------|----------------|------------|
| T0   | PEA       | SL             | Control        | PEA vs. SL |
|      | 0.027     | 0.027          | 0.031          | 0.004      | 0.005       | 0.000       |
|      | (0.007)   | (0.006)        | (0.004)        |            |             |             |
| T1   | PEA       | SL             | Control        | PEA vs. SL |
|      | 0.040     | 0.069          | 0.034          | 0.775      | 0.000*      | 0.000*      |
|      | (0.005)   | (0.008)        | (0.004)        |            |             |             |
| T2   | PEA       | SL             | Control        | PEA vs. SL |
|      | 0.071     | 0.100          | 0.030          | 0.000*     | 0.000*      | 0.000*      |
|      | (0.007)   | (0.006)        | (0.004)        |            |             |             |
Table 2. Continue

|     | (0.005) | (0.012) | (0.030) |
|-----|---------|---------|---------|
| T3  | 0.022   | 0.030   | 0.029   |
|     | (0.006) | (0.030) | (0.029) |

Pair wise comparison tests (Bonferroni and Tamhane). *P < 0.05.

4. Discussion

RANKL is a proinflammatory cytokine produced by osteoblasts, and it is activated by lymphocyte T as a regulating factor, fusion, and osteoclastogenesis [9,10]. The remodeling balance in the alveolar bone depends on the RANKL components, receptor activator of nuclear factor κ-B (RANK), and osteoprotegerin (OPG), which are in the tumor necrosis factor (TNF) superfamily. RANKL activates its specific receptor (RANK), which is located on the surface of the osteoclast and induces osteoclast maturity, resulting in resorption of the alveolar bone [11–13]. Tooth movement due to orthodontic forces can result in RANKL formation and osteoclastogenesis in the periodontal tissue [9]. Similar conditions were reported by Ogaswara [14], who found increases in RANKL concentrations in the osteoblasts and PDLs during tooth movement induced by orthodontic forces. Others have proved that orthodontic forces significantly increase RANKL production in the PDL, and an increase in orthodontic forces correlates with an increase in RANKL in the periodontal tissue [4-7].

The PEA group exhibited statistically significant differences in RANKL concentrations at T2 when compared to the other time periods. However, the RANKL concentrations at T0 and T1, and T3 hours showed no statistically significant differences when compared to each other. RANKL concentration at T3 decreased according to a study conducted by Taloumis et al. [15] in which force decay was apparent in the elastomer ligation after 24 hours in artificial saliva.

Statistically significant differences were noted in RANKL concentrations among the SL, PEA, and control groups at T1, T2, and T3. Our results showed that the average RANKL concentration was higher in the SL group compared to the PEA and control groups. According to Harradine [16], the superiority of the passive SL bracket system compared to conventional systems is due to the built-in slot that can open and close the archwire and keep it inside the bracket. This distributes the controlled force, causing frontal resorption. In addition, force decay from elastomeric ligation does not occur, unlike in conventional brackets.

Some studies mention that implementation of passive SL brackets showed significantly faster tooth movement during the leveling and aligning stages [17–19]. This is due to the reduced friction when using the passive SL compared to conventional brackets. In conventional brackets, the ligation system is conducted by conventional ligation, and an elastomeric or metal ligature may hinder sliding of the archwire through the bracket slots, which results in inefficient tooth movement. However, the passive SL bracket has a built-in mechanism ensuring that the archwire remains in the slot, and the force distribution occurs with frontal resorption [16].

The leveling and aligning effectiveness depends not only on the bracket system, but also on the archwire used. As shown by Pandis et al. the most important aspect of concern is not the archwire dimension, but the amount of force translated from the bracket to the tooth [18]. Another key factor that may influence tooth movement concerns the characteristics of the archwire surface itself. Currently, archwires that exhibit minimum friction already have been developed. For example, the passive SL bracket system uses a copper-NiTi (CuNiTi) arch wire in the initial phases of leveling, aligning, and rotation control. Moreover, a thermoelastic property is a mechanical property shape memory effect that is driven by temperature. The explanation for use of a CuNiTi arch wire shows that the memory shape still distributes the force, which means that after 24 hours of CuNiTi arch wire use, tooth movement still
occurs. This explains the average RANKL concentration increase between 24 and 168 hours after installation, which was not statistically significant.

5. Conclusion
This study demonstrated differences in RANKL concentrations between the SL and PEA bracket systems during the initial phases of orthodontic treatment at 1, 24, and 168 hours after bracket insertion. The RANKL concentration in the passive SL bracket system was higher at 168 hours compared to that of the PEA system due to differences in the force systems and mechanotherapy aspects of the SL bracket.

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