# Ideal Bone Defects Distance on Orthodontic Tooth Movement for Preparation of hADMSC-Scaffold Chitosan Intervention

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#### ABSTRACT

**Introduction:** Malocclusion can cause the risk of caries and periodontitis requires orthodontic treatment. Mechanical force during Orthodontic Tooth Movement (OTM) resulting in bone apposition and resorption is widely considered in both periodontal and orthodontic research. Bone defects occur because OTM is needed to accelerate bone regeneration to prevent relapses with the intervention of hADMSC - Scaffold chitosan for that required the ideal distance of bone defects between M1-M2.

**Objective:** This research aimed to calcify ideal bone defects distance on Orthodontic Tooth Movement for Preparation of hADMSC - Scaffold Chitosan Intervention by performing X-ray CR and Micro-CT.

**Methods:** This research using 3 Wistars at the time of treatment 4.6, 8, and 10 days. The NiTi 0.010-inch close coil spring length 8 mm drawn with 10 gf to 10.5 mm and X-ray CR and Micro-CT was examined. X-ray CR provides oualitative data and Micro-CT provides ouanitative and oualitative data.

qualitative data and Micro-CT provides quantitative and qualitative data. **Results:** The average width of the gap with a value of 0.404743 mm can be an ideal interdental distance for the preparation of interventions hADMSC - chitosan scaffold to be able to provide bone regeneration effect on the gap of M1 - M2.

**Conclusion:** The treatment on the 10th day can provide an ideal gap to be able to intervene hADMSC - chitosan scaffold to be able to provide closure to bone defects as bone regeneration.

Keywords: bone defect, malocclusion, micro-CT, orthodontic tooth movement, X-ray CR

#### **INTRODUCTION**

Malocclusion is one of the risk factors for caries, as the inadequate alignment of the teeth causes bacterial plaque and hinder its removal.<sup>1,2</sup> The correction of deviating tooth and jaw positions is central tenets in orthodontic therapy. Based on this assumption, an ideal occlusion will be achieved through orthodontic treatment and is considered as one of the basic requirements for perseverance and endurance of good oral health.<sup>3,4</sup>

Orthodontic treatment is an integral treatment for improving chewing, speech, aesthetic functions, and also for preventing caries and periodontal disease. Fixed appliances have become an essential part of comprehensive orthodontic treatment as that enables three-dimensional control of tooth movement.<sup>5</sup> Orthodontic treatments are based on tooth movement caused by the periodontal ligament, that connects tooth roots and alveolar bone. The forces given to a tooth produce stress on the ligament where the bone formation will be triggered on the traction area; and bone resorption and tooth movement will be promoted on the compression area.<sup>6,7</sup>

Orthodontic treatment will improve hygiene because it facilitates access to teeth and reduces occlusal trauma. It also aligns teeth and balance occlusion. However, there **Keywords:** bone defect, malocclusion, micro-CT, orthodontic tooth movement, X-ray CR

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are some chances that fixed orthodontic appliances will create a high supragingival biofilm accumulation and degrade periodontal health.<sup>8,9</sup> Periodontitis is an infective disease mainly caused by the dental biofilm, and its pathological manifestation is associated with how the host response against the microbial infection at the tooth/gingival interface. Moderate or advanced loss of bone and connective tissue attachment can get worse due to the migration of the teeth, especially if there are unfavorable occlusal forces. In these conditions, interdisciplinary studies that involve periodontal and orthodontic therapies to restore periodontal health, esthetics, as well as reestablish its function are necessary.<sup>10</sup> Mechanical force during Orthodontic Tooth Movement (OTM) resulting in bone apposition and resorption is widely considered in both periodontal and orthodontic research. During OTM, all components such as the osseous structure, periodontal ligament, and the soft tissue components, move along with the tooth. The same condition also applies to patients with reduced yet healthy periodontal tissues.11,12

Tissue engineering consists of three components which are scaffolds, cells, and growth factors. Those three components are called the triad of network engineering. Mesenchymal stem cells (MSC) play an active role in

and maintaining tissue repairing homeostasis Osteoblasts have the potential to regenerate bone toward effector cells. Human Adipose-Derived their Mesenchymal Stem Cells (hADMSC) is a multipotent cell characterized into osteogenic, chondrogenic, and adipogenic.<sup>13,14</sup> Chitosan nanoparticles are an effective material in nanotechnology especially for biomedical applications purposes.<sup>15,16</sup> Bone defects occur because OTM is needed to accelerate bone regeneration to prevent relapses with the intervention of hADMSC -Scaffold chitosan for that required the ideal distance of bone defects between M1-M2. This research aimed to calcify ideal bone defects distance on the Orthodontic Tooth Movement for Preparation of hADMSC - Scaffold Chitosan Intervention by performing X-ray CR and Micro-CT.

## **Materials and Methods**

### Animals

This research using 5-month-old Wistar rats weighing 200-300 grams with 3 rats at the time of treatment 4.6, 8, and 10 days. Previously a spring pull was made to prove 10 gf using a digital force gauge (Elecall type ELK-50, USA) and a digital caliper by pulling a NiTi 0.010 inch close coil spring length 8 mm (International GAC, Bohemia, NY) so that a spring length was obtained 10.5 mm. The animals were anesthetized by intramuscular injection of ketamine HCL at a dose of 95 mg/kg bw and xylazine premedication 5 mg/kg bw intraperitoneally for 30 minutes duration, then a NiTi 0.010 inch close coil spring length 8 mm (International GAC, Bohemia, NY) was placed between the upper left first molar (M1) and upper incisors (I1) by a 0.001-inch stainless steel bonding wire and bonded with orthodontic adhesive (Transbond TM XT, 3M Oral Care, St. Paul, MN). The springs are adjusted to produce 10 g of force (gf) at a spring length of 10.5 mm with a digital caliper. Given antibiotics gentamicin 2 mg/kg bw and ketoprofen 1 mg/kg bw every day for 3 days (Figure 1). This study has been evaluated and approved with ethical clearance number: 001/EC/LKS/RSMTH/VIII/2020.

#### X-Ray CR

In rats with an intramuscular injection of ketamine at a dose of 95 mg/kg bw and xylazine premedication 5 mg/kg bw intraperitoneally for x-ray CR examination carried out latero laterally and dorsal Vetro on day 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup>, and 10<sup>th</sup>. On the results of the x-Ray CR examination (CR 7 Vet Image Plate X-Ray Scanner, IM3, Australia), bone defect distance was measured at M1 and M2 interseptals.

#### Micro-CT

On day 8<sup>th</sup>, the rats were then terminated with an intramuscular injection of ketamine at a dose of 95 mg/kg bw and xylazine premedication 5 mg/kg bw intraperitoneally for the decapitation of the head was put immersed in a fixation solution that was buffered formaldehyde 10% to be examined Micro-Computed Tomography (Micro-CT). Samples were placed into containers arranged in a row on wax, then by scanning 360° with Micro-CT (Bruker SkyScan 1173 High Energy Micro-CT).<sup>17</sup>

#### Results

In the x-ray CR test to analyze the distance of interseptal bone defects in M1-M2 starting on day-4 and 6, descriptively did not obtain this distance by looking at the radiolucent image of the x-ray CR photo. On the 8<sup>th</sup> day and the 10th day, a radiolucent picture has been seen which will be proven by the next test, Micro-CT (Figure 2). In the Micro-CT examination, all samples were tested on the 10<sup>th</sup>-day treatment, so that the total gap volume, the gap volume fraction, and the average width gap for each treatment can be seen in Table 1. Micro-CT images in each treatment can be seen in Figure 3.

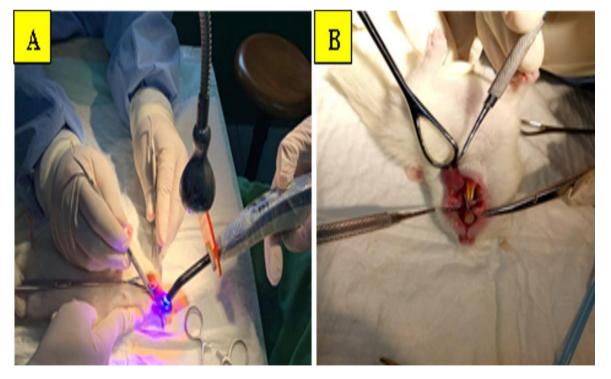
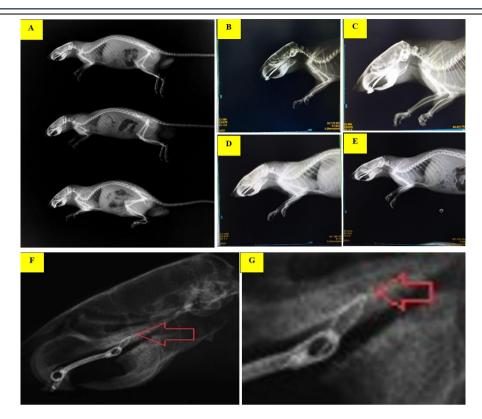


Figure 1: Animal; (A) M1 - I1 close coil spring is installed, and an adhesive is applied to I1 then UV irradiation with lightcuring; (B) close coil spring installation results.

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**Figure 2:** X-Ray CR; (A) Three rats were analyzed using x-Ray CR; (B) Observation on day 4; (C) Observation on day 6; (D) Observation on day 8; (E) Observation on day 10; (F, G) the radiolucent picture indicated by the direction of the arrow.

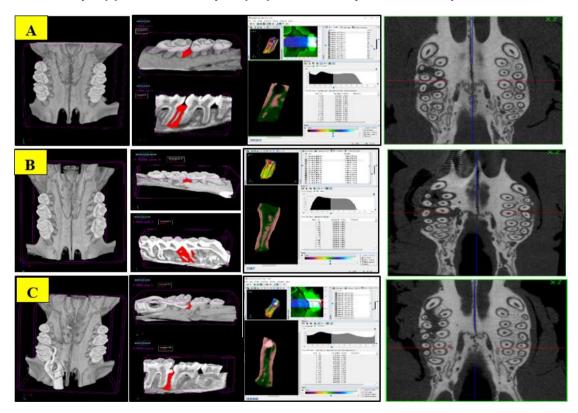


Figure 3: Micro-CT; (A) Sample 1; (B) Sample 2; (C) Sample 3.

Treatment	Total Volume Gap	Fraction Volume Gap	Average Width Gap
T1	1.870622 mm <sup>3</sup>	63.60517 %	0.418586 mm
T2	1.850875 mm <sup>3</sup>	62.96693 %	0.376769 mm
T3	1.527033 mm <sup>3</sup>	61.05372 %	0.418875 mm
	Total of Average Width Gap		0.404743 mm

Table 1. Width of Gap Distribution Table for Micro-CT.

#### Discussion

Remodeling changes in the area of compression and tension are characteristics that indicate tooth movement which was induced by orthodontic force. In this condition, osteoblasts and osteoclasts play role as the cellular effectors.<sup>18</sup> Orthodontic tooth movement had positive effects on preventing epithelial apical downgrowth and lower the depth of the pocket, although, bony defects boosted the bone healing. Chitosan has beneficial characteristics. as its biodegradability, such biocompatibility, bioactivity, nontoxicity, and polycationic nature.<sup>19</sup>

Bone reconstruction includes multiple physiological processes which exhibits encapsulated into nanospheres and loads into porous the nonlinear characteristics in the relative material and boundary biodegradable CaP scaffolds layer by layer after key condition was optimized. The interaction between the external environment parameters and validate the predictive power of the model by the and internal factors makes a dynamic balance between osteoclasts experimental training and testing data and osteoblasts to affect the bone formation.<sup>20</sup>

To analyze the width of the gap in the bone defect, x-Ray CR and Micro-CT were examined. In the x-Ray CR examination, there is a 2-dimensional visible gap with radiolucent between M1 - M2 which is not so clear in Figure 2 and Figure 3. The existing picture cannot be interpreted qualitatively only quantitatively. For this reason, a more accurate examination is needed namely Micro-CT examination by analyzing qualitatively and quantitatively. Micro-CT examination is done with Volume of Interest (VOI) in the area that appears in red. So that the total average width of the gap is 0.404743 mm and can be seen in Table 1.

The average width of the gap with a value of 0.404743 mm can be an ideal interdental distance for the preparation of interventions hADMSC - chitosan scaffold to be able to provide bone regeneration effect on the gap of M1 - M2. The ground method of orthodontic treatment is periodontal tissue remodeling, where pressure on alveolar bone starts building up and begins the movement of the tissues. Alveolar bone, periodontal ligament, and cementum reconstruction and regeneration will return to their normal structure when the teeth hit the target position.<sup>21</sup>

Tissue engineering is a study that focus on restoring damaged tissues function and structure. The combination of cells, scaffolds, and factors are commonly used to facilitate tissue regeneration. A scaffold is a three-dimensional structure which provide the environment for the cells to grow and do their differentiation. One of the most important aspects when designing a scaffold is choosing the right material. The material should have proper texture, such as elastic and stiff. Porous materials are necessary for waste diffusion, cellular infiltration, as well as for nutrient to enter. Biodegradable and bioresorbable scaffolds can create a stable structure until the body's own tissue can take over its function.<sup>22</sup>

Tissue engineering of bone and cartilage has progressed from simple to sophisticated materials with defined porosity, surface features, and the ability to deliver biological factors.<sup>23</sup> Bone defects caused by OTM from orthodontic treatment can cause gaps in the opposition in the distal region. The width of the gap is needed to prepare the chitosan scaffold to be properly placed in the gap. In this study, researchers searched for the ideal distance with a strength of 10 gf in what time of day so that the chitosan scaffold can be placed. The ideal distance on the 10th day can be an ideal distance to be able to provide sufficient space for intervention from the HADMSC - chitosan scaffold to be able to give bone regeneration effect on the gap of M1 - M2.

## Conclusion

We conclude that the treatment on the 10th day can provide an ideal gap to be able to intervene hADMSC chitosan scaffold to be able to provide closure to bone defects as bone regeneration.

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## **Conflict of Interest**

The authors declare no conflicts of interest.

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