

# Effect of Bisphosphonate Risedronate Hydrogels towards Number of Osteoclast during Relapse Tooth Movement

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## Introduction

Relapse (tooth movement in its original position) has made undesirable result of the orthodontic treatment. It became a complex problem in orthodontic which caused by many potential factors. Eric *et al.* in 2003 reported that relapse is commonly caused by intrinsic factor related to periodontal ligament and alveolar bone, but to some extents, extrinsic factors such as face structure growth, soft tissue pressure and interdilatation are also believed to influence the process. Relapse happen immediately after orthodontic appliance removed and decrease its speed after three days, this movement usually followed by alteration the amount and distribution of osteoclast. Osteoclast is the multinuclear cell origin of differentiation granulocyte-macrophage progenitor cell in bone marrow. It has a function for bone remodeling specifically resorption, when it completed osteoclast will be apoptotic.

One of the strategies to manipulate bone remodeling known in orthodontics area is by the application of an analogous phosphonate drug to inhibit bone resorption, e.g. bisphosphonate. Bisphosphonate risedronate is Nitrogen-containing bisphosphonate that has an ability to adhere calcium and inhibit differentiation of osteoclast. In this study, we proposed hydrogel system as described by Saito and Tabata. Bp-risedronate hydrogel is type of Drug Delivery System that has local effect on inhibiting resorption alveolar bone.

## Objective

Determine the effect of Bp-risedronate hydrogels toward number of osteoclast during tooth movement after orthodontic treatment.

## Result

Table 1. Number of osteoclast in the groups with and without bisphosphonate risedronate on day 3, 7, 14 and 21 upon completing stabilization period

Duration After Stabilization Period (day)	Number of osteoclast		
	Control	Bp-risedronate 250 µmol/L	Bp-risedronate 500 µmol/L
0	6.620±2.235	2.240±0.879	1.880±0.408
3	7.280±1.502	1.860±0.924	2.240±0.699
7	4.540±1.585	2.540±0.691	1.600±1.000
14	3.700±1.343	1.040±0.114	1.300±0.925
21	1.560±0.230	0.640±0.151	0.780±0.455

Table 2. One way Anova test summary on number of osteoclast (p<0.05)

Duration After Stabilization Period (day)	F	df	p-value
0	16.950	2	0.000
3	38.162	2	0.000
7	8.474	2	0.005
14	12.068	2	0.001
21	13.025	2	0.001

Table 1 showing that number of osteoclast were significantly different between groups at day 0, 3, 7, 14, and 21 (p<0.05). Group B and group C has lower osteoclast rather than group A. Statistical analysis also shows that there was significant different among groups (p<0.05), as shown in Table 2.

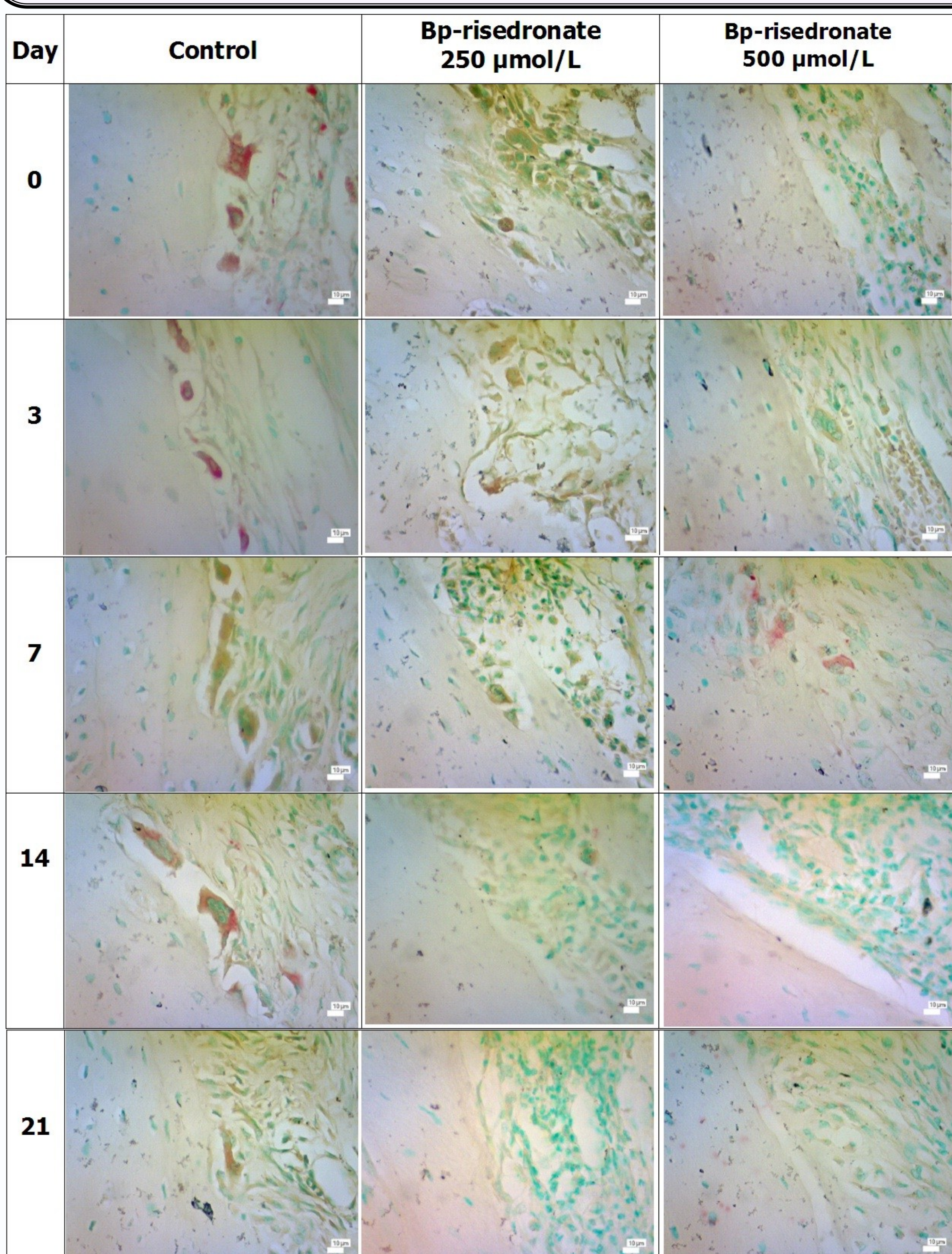
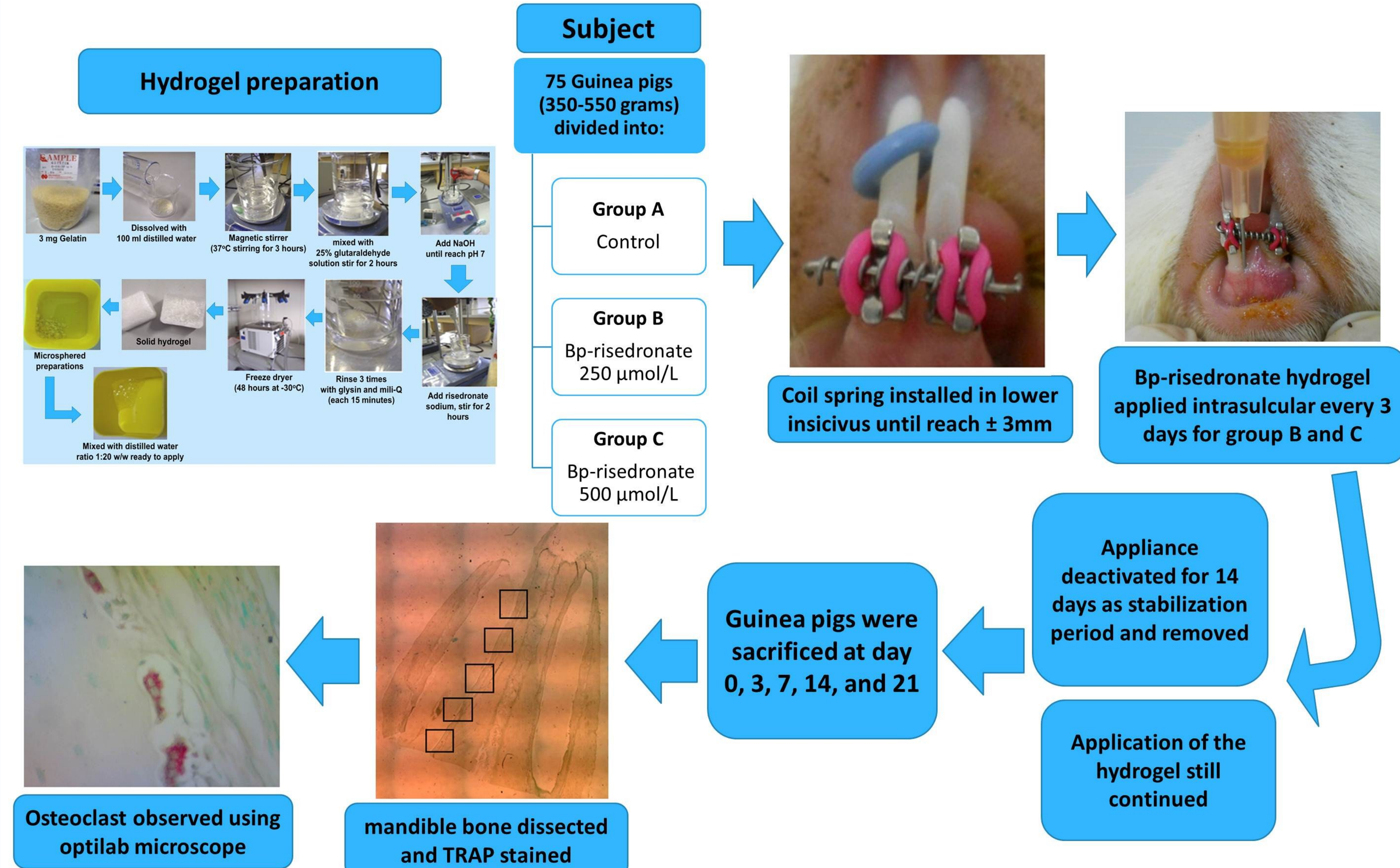


Figure of histological slides stained with TRAP for Control, Bp-risedronate 250 µmol/L, and Bp-risedronate 500 µmol/L on day 0, 3, 7, 14 and 21 after stabilization period, showing abundant osteoclast along alveolar bone of the group treated without Bp-risedronate.

## Research Methods



## Statistical Analysis

The data were statistically analysed by Shapiro-Wilk test to determine whether the data were normally distributed and One Way Analysis of variance (ANOVA) was used to analyse whether there are significant differences between the groups.

## Histological analysis

Histological analysis was done by calculating the average number of osteoclasts. Data were obtained from 5 ROI (Region of Interest) on slides which were taken randomly using a light microscope completed with a digital camera (Olympus) attached. Active osteoclasts were seen to be in contact with bone, characterized by a multinucleate, irregular shape of nucleus with the bright red granular cytoplasm. The active osteoclasts were usually found either in contact with bone surface or within the lacunae.

## Discussion

This study suggested Bp-risedronate with hydrogel drug delivery effective to reduce osteoclast number. Applications of Bp-risedronate 250 µmol/L and Bp-risedronate 500 µmol/L to the subject were effective to inhibit osteoclast activity. Bp-risedronate is an analog statistic organic pirophosphonate (PPI). Pirophosphonate structure allow oxygen to be bond with phosphate (P-O-P), however Bp-risedronate structure oxygen were replaced with carbon atom (P-C-P). Binding of P-C-P give a resistance of enzyme degradation and strong bond with calcium hydroxyl apatite from bone structure (Rodan and Reszka, 2002). As the difference structure of Bp-risedronate, it can be distinguished into two types simple BPs and nitrogen contained BPs or N-BPs (Rogers, 2003). Nitrogen or group of amino will upgrade the potentation of anti resorbptive Bp-risedronate compared with Bp-risedronate without nitrogen (or amino). Non nitrogen Bp-risedronate is earliest generation of Bp-risedronate (such as etirodronate, klorodronate, and tiludronate) which has the closest characteristic of PPI. Otherwise second and third generation of Bp-risedronate (such as aledronate, srisedronate, ibandronate, pamidronate, and zoledronic acid) contained by nitrogen on the R2 chain (Drake *et al.*, 2008). N-Bp-risedronate (such as pamidronate, aledronate, and risedronate) will inhibit farnesyl pyrophosphate synthase (enzyme in mevalonate pathway). The inhibition will result unformed of isoprenoid geranylgeranyl pyrophosphate (GGPP). Unformation of GGPP may not lead into prenilation of several small protein GTPase (such as Ras, Rho, and etc) that responsible for cytoskeleton integrity and intra cell signaling on the osteoclast. Without integrity of cytoskeletal, osteoclast will unformed ruffled border that avoid resorption and apoptotic occur (Ghoneima *et al.*, 2010).

## Conclusion

This study indicates that Bp-risedronate hydrogels that applied intrasulcular affecting the reduction of osteoclast during relapse tooth movement. By using TRAP staining, it was shown that the number of osteoclasts decreased significantly in the group treated with bisphosphonate risedronate with gelatin hydrogel carrier compared to the group without bisphosphonate risedronate. Further studies related to other parameters to confirm the results have been continued in our laboratory to translate the results into clinical applications.

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