ความเป็นพิษของพอร์ตแลนด์ซีเมนต์สองชนิดที่ผลิตในประเทศไทยที่ผสมกับบิสมัทออกไซด์ต่อเซลล์จากกระดูกเบ้าฟันของมนุษย์

นางสาว ถนอมศุก เจียรนัยไพศาล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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CYTOTOXICITY OF TWO THAI WHITE PORTLAND CEMENTS MIXED WITH BISMUTH OXIDE IN HUMAN ALVEOLAR OSTEOBLASTS

Miss Thanomsuk Jearanaiphaisarn

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Endodontology
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Faculty of Dentistry
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Thesis Title	**CYTOTOXICITY OF TWO THAI WHITE PORTLAND CEMENTS MIXED WITH BISMUTH OXIDE IN HUMAN ALVEOLAR OSTEOBLASTS**
By	Miss Thanomsuk Jearanaiphaisarn
Field of Study	Endodontology
Advisor	Assistant Professor Chootima Ratisoontorn, D.D.S., Ph.D.

Accepted by the Faculty of Dentistry, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

W. Tasachan ..... Dean of the Faculty of Dentistry

(Associate Professor Wacharaporn Tasachan, D.D.S.)

THESIS COMMITTEE

Piyanee Panitvisai ..... Chairman

(Associate Professor Piyanee Panitvisai, D.D.S.)

..... Advisor

(Assistant Professor Chootima Ratisoontorn, D.D.S., Ph.D.)

Anchana Panichuttra ..... Examiner

(Assistant Professor Anchana Panichuttra, D.D.S., Ph.D.)

Mettachit Nawachinda ..... External Examiner

(Associate Professor Mettachit Nawachinda, D.D.S.)

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การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบความเป็นพิษของพอร์ตแลนด์ซีเมนต์สีขาวสองชนิดที่ผลิตในประเทศไทยตราช้างและตรากิเลนที่ผสมกับบิสมัทออกไซด์ กับไวท์โปรรูทเอ็มทีเอ ต่อเซลล์สร้างกระดูกเบ้าฟันมนุษย์ โดยประเมินความมีชีวิตของเซลล์ ลักษณะรูปร่างและการยึดเกาะของเซลล์บนผิววัสดุ ผสมวัสดุแต่ละชนิดกับน้ำกลั่นแล้วนำไปแช่ในอาหารเลี้ยงเซลล์ จากนั้นนำอาหารเลี้ยงเซลล์ที่ 1 3 7 และ 14 วัน ไปเลี้ยงเซลล์สร้างกระดูกเบ้าฟันมนุษย์ วัดความมีชีวิตของเซลล์ด้วยวิธีเมธิวเตตระโซเลียม และคำนวณเป็นค่าร้อยละความสัมพันธ์ของเซลล์ที่มีชีวิตในกลุ่มทดลองต่อกลุ่มควบคุม วิเคราะห์ความแตกต่างของค่าเฉลี่ยร้อยละความสัมพันธ์ของเซลล์ที่มีชีวิตเปรียบเทียบระหว่างวัสดุและระหว่างช่วงเวลา ด้วยสถิติความแปรปรวนแบบทางเดียวที่ระดับ นัยสำคัญ 0.05 และตรวจสอบลักษณะรูปร่างและการยึดเกาะของเซลล์ที่เลี้ยงบนวัสดุที่เวลา 24 และ 72 ชั่วโมงด้วยกล้องจุลทรรศน์อิเล็กตรอนชนิดส่องกราด จากการศึกษาพบว่าสารสกัดที่ 1 วันของทุกวัสดุมีความเป็นพิษมากกว่าช่วงเวลาอื่นๆโดยเฉพาะกิเลน ร้อยละความสัมพันธ์ของเซลล์ที่มีชีวิตของสารสกัดที่ 1 3 และ 7 วัน มีความแตกต่างกันระหว่างวัสดุ อย่างไรก็ดีสารสกัดที่ 14 วัน ไม่มีความแตกต่างกันอย่างมีนัยสำคัญ เซลล์สามารถยึดเกาะและแผ่ตัวได้ดีบนวัสดุทั้ง 3 ชนิดที่เวลา 24 และ 72 ชั่วโมง การศึกษานี้แสดงให้เห็นว่า ณ วันที่ 3 7 และ 14 พอร์ตแลนด์ซีเมนต์สีขาวที่ผสมกับบิสมัทออกไซด์ทั้งสองชนิดมีความเป็นพิษต่อเซลล์สร้างกระดูกเบ้าฟันมนุษย์ไม่มากไปกว่าไวท์โปรรูทเอ็มทีเอ เซลล์สามารถเกาะบนวัสดุทั้งสองชนิดได้คล้ายคลึงกับเมื่อเกาะบนไวท์โปรรูทเอ็มทีเอ

ภาควิชา ทันตกรรมหัตถการ ลายมือชื่อนิสิต .....

สาขาวิชา วิทยาเอ็นโดดอนต์ ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

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THANOMSUK JEARANAIPHAISARN: CYTOTOXICITY OF TWO THAI WHITE PORTLAND CEMENTS MIXED WITH BISMUTH OXIDE IN HUMAN ALVEOLAR OSTEOBLASTS. ADVISOR: ASST.PROF. CHOOTIMA RATISOONTORN, 118 pp.

The purpose of this study was to compare the cytotoxicity of white ProRoot® MTA and two Thai White Portland cement mixed with bismuth oxide; Chang and Kilan to primary human alveolar bone osteoblasts by assessing cell viability and investigating cell morphology and attachment. Chang, Kilan and white ProRoot® MTA were mixed with distilled water. Human alveolar bone osteoblasts were exposed to material extracts from different time: 1, 3, 7, 14 days. Cell viability was assessed using Methyltetrazolium assay and showed as relative percent of the survival rate of experimental group to control group. Differences in mean cell viability between materials and extraction times were analyzed by One-way ANOVA ($p < 0.05$). In addition, cells were seeded on the material disc and incubated for 24 and 72 hours. Cell morphology and attachment were observed by scanning electron microscope. All day 1 material extracts were more toxic than other time points especially Kilan extracts. There were differences of percents cell viability of material extract at day 1, 3 and 7. However at day 14 those of all material extracts were not different. Cells were able to attach and well spread on all material within both 24 and 72 hours. This study showed that Chang and Kilan were not more toxic than white ProRoot® MTA to primary human alveolar bone osteoblasts at days 3, 7 and 14. Cells could attach to Chang and Kilan in a similar fashion to white ProRoot® MTA.

Department : Operative Dentistry Student's Signature : Thanomsuk Jearanaiphaisarn

Field of Study : Endodontology Advisor's Signature : [signature]

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LIST OF ABBREVIATIONS

AA	ascorbic acid
ALP	alkaline phosphatase
BSP 2	bone sialoprotein 2
Chang	White Portland cement (Chang brand) mixed with bismuth oxide
COLIA2	type I collagen alpha 2
DMEM	Dulbecco's Modified Eagle's Medium
DMEM/F12	Dulbecco Minimal Essential Medium with F12 nutrient mixture (1:1)
DMSO	dimethyl sulfoxide
FBS	fetal bovine serum
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
HOB	human alveolar bone osteoblast
IRM	Intermediate Restorative Material
Kilan	White Portland cement (Kilan brand) mixed with bismuth oxide
MTA	Mineral Trioxide Aggregate
MTT	methyltetrazolium
OCN	osteocalcin
OPN	osteopontin
PBS	phosphate buffer solution
PCR	polymerase chain reaction
SEM	scanning electron microscope

CHAPTER I

INTRODUCTION

Background of the present study

Pulpal and periradicular diseases develop when the pulpal and periapical tissues are exposed to bacterial contamination [1]. Complete cleaning and shaping of the root canal system and sealing them in three dimensions with aseptic technique can result in healing of the periradicular lesions. However, the success rate of non-surgical root canal treatment is between 45-98.7% [2-4]. The causes of failure are inadequate cleaning, loose-fitting root canal filling or leakage of coronal restoration. Although the preferred treatment of failing endodontic cases is non-surgical retreatment, this may not be achieved because of the complexity of root canal systems or physical barriers such as post and core restoration, separated instruments. Surgical endodontic therapy becomes indicated when non-surgical retreatment is impractical or unlikely to improve the previous result. Its success is reported to range between 58-96% [5, 6]

The objectives of surgical endodontic therapy are eliminating infected periradicular tissue and preparing favorable environment for healing of the surgical wound. The surgical procedures include identification of the apex, osteotomy, apical root resection, retro-preparation and placement of retro-filling. The apical seal is the most important factor affecting success in surgical endodontics [7]. Therefore, the retro-filling material must provide an apical seal that inhibits the leakage of residual irritants from the root canal into the periradicular tissues. There are many retro-filling materials such as amalgam, intermediate restorative material (IRM) or superEBA. However, these are not the ideal retro-filling material [8].

Mineral trioxide aggregate (MTA), a well-known retro-filling material, develops from Portland cement [8, 9]. There are two types; grey MTA and white MTA. Nowadays, mineral trioxide aggregate is popular in endodontics (retrograde filling, repairing

perforation, direct pulp capping) because it has many favorable properties; biocompatibility both *in vitro*[10-13] and *in vivo* [14-16], good sealing ability [17-19], induce periradicular tissue regeneration [15, 16, 20], antimicrobial effect [21], radiopaque and dimensional stability [22]. Although MTA has several favorable retro-filling material properties, it is very expensive, not easy to handle and has long setting time [9].

Portland cement is a material which the major chemical components are similar to MTA [23]. A number of previous studies had compared MTA with Portland cement indicated that they are similar in chemical composition except for the inclusion of bismuth oxide in MTA [24-26]. In addition, *in vitro* [23, 27, 28] and *in vivo* [29] studies have also shown that Portland cement has properties similar to MTA.

Although mineral trioxide aggregate is a preferred retro-filling material, it is very expensive. This may not be affordable for the majority of Thai people. Portland cement which is much cheaper than MTA may be used as an alternative retro-filling material. Recently, a study showed that two Thai Portland cements mixed with bismuth oxide have almost similar chemical constituents and physical properties to white MTA (White ProRoot® MTA) [30]. However, there is no report of cytotoxicity between MTA and these Thai Portland cements. Therefore this study focuses on the cytotoxicity of two Thai Portland cements mixed with bismuth oxide comparing to white ProRoot® MTA

Research Question

Do the two Thai White Portland cements with bismuth oxide affect cell viability and cell morphology of human alveolar bone osteoblasts similar to white ProRoot® MTA?

Research Objectives

1. To compare cytotoxic effect of two Thai White Portland cements with bismuth oxide and white ProRoot® MTA in human alveolar bone osteoblasts using Extract test and MTT assay.

2. To investigate and compare cell morphology and attachment of human alveolar bone osteoblasts in contact with two Thai White Portland cements with bismuth oxide and white ProRoot® MTA by scanning electron microscope.

Hypothesis

1. Null hypothesis H_O: Cytotoxicity of two Thai White Portland cements with bismuth oxide in human alveolar bone osteoblasts is not different from that of white ProRoot® MTA.

   Alternative hypothesis H_A: Cytotoxicity of two Thai White Portland cements with bismuth oxide in human alveolar bone osteoblasts is different from that of white ProRoot® MTA.
2. Null hypothesis H_O: Cell morphology and attachment of human alveolar bone osteoblasts in contact with two White Portland cements with bismuth oxide are not different from those of white ProRoot® MTA.

   Alternative hypothesis H_A: Cell morphology and attachment of human alveolar bone osteoblasts in contact with two White Portland cements with bismuth oxide are different from those of white ProRoot® MTA.

Field of Research

To compare the cytotoxicity of two new material and white ProRoot® MTA in human alveolar bone osteoblasts by MTT assay and Scanning electron microscope.

Keywords

Bismuth oxide, Cytotoxicity, Human alveolar osteoblast, Mineral Trioxide Aggregate, White Portland cement

Research design

Laboratory experimental study

Limitations of Research

1. The number of bone samples investigated in this study is restricted due to the limitation of time and grant support.
2. This is an *in vitro* study which may not represent clinical situation.
3. The number of White ProRoot® MTA samples is limited due to the cost of the material.

Obstacles

1. Bacterial contamination
2. Slow cell proliferating rate, Not enough cells for testing
3. Material samples are broken when removed from plastic molds

Benefits of Research

1. To obtain basic cytotoxic background of the materials for further animal and clinical studies.
2. If Thai White Portland cements with bismuth oxide is biocompatible to human osteoblast equally or better than MTA, these white Portland cements may be used as a substitution of white ProRoot® MTA.
3. Because Portland cement is inexpensive, it will reduce the cost of endodontic surgery or other endodontic procedures.

Ethical consideration

In this study, informed consents were obtained prior to bone sample collection from patient undergone third molar extraction. The protocol was approved by the Ethics Committee of Faculty of Dentistry, Chulalongkorn University.

CHAPTER II

LITERATURE REVIEW

Surgical endodontic therapy is a procedure to eliminate infected periradicular tissue and prepare favorable environment for healing of the surgical wound. Among several steps of surgical endodontic therapy, retro-preparation and placement of retro-filling are the two of critical steps because they influence apical seal which is an important factor to support the success of surgical endodontic therapy. Consequently, choosing an appropriate retro-filling material will bring successful therapeutic result.

The requirements of an ideal retro-filling material are that the material should: [8, 31]

1. Easy to manipulate
2. Radiopaque
3. Non absorbable
4. Well tolerated by periradicular tissue
5. Promote healing
6. Adhere and seal the root canal system in three dimensions
7. Nontoxic
8. Adhere to dentinal wall of root end preparation
9. Biocompatibility
10. Dimensional stability
11. Moisture insensitivity

Many materials have been suggested as retro-filling materials including gutta percha, amalgam, polycarboxylate cement, zinc phosphate cement, IRM, EBA, Cavit, glass ionomer, resin composite, MTA, gold foil, cyanoacrylate, Diaket, Titanium screw, Teflon [32]. However, only some materials have been commonly used in clinical practice. These materials are zinc oxide-eugenol cements (IRM and superEBA), glass ionomer cement, resin composite, resin glass ionomer hybrids and mineral trioxide aggregate (MTA) but none of these can fulfill all ideal retro-filling properties.

Mineral Trioxide Aggregate (MTA)

Mineral Trioxide Aggregate (MTA) was developed at Loma Linda University in the 1990s as a retro-filling material. In 1998, it received acceptance by the US Federal Drug Administration and became commercially available as ProRoot® MTA (Tulsa Dental Products, Tulsa, OK, USA). The principal components of the grey-colored formula are tricalcium silicate, bismuth oxide, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, and calcium sulfate dehydrate. Up to 2002, only grey MTA was available, and in that year white MTA was introduced as ProRoot® MTA. Both formulae are 75% Portland cement, 20% bismuth oxide, and 5% gypsum by weight [33]. The differences between grey MTA and white MTA are the concentrations of carborundum (Al_2O_3), periclase (MgO) and especially FeO which are lower in the white MTA than in the grey MTA [34]. When mixed with sterile water, hydration of the MTA powder results in a colloidal gel that solidifies into a hard structure consisting of discrete crystals in an amorphous matrix.

MTA was introduced to use in many endodontic procedures including retro-filling [32], apexification [35], pulp capping material [36, 37] , pulpotomy [38] and repair root perforation [39, 40]. MTA has several good retro-filling properties; biocompatibilities *in vitro* and *in vivo*, good sealing ability, promoting regeneration of the periradicular tissue, antimicrobial effect, radiopacity, dimension stability and moisture insensitivity. It is also capable of inducing hard-tissue deposition [11, 41]. Because of superior properties of MTA, it is more popular than others. However, it is very expensive, not easily handling and has long setting time.

Electron probe microanalysis of MTA powder showed that calcium and phosphorous are the principal ions in this material. These ions are also the main components of dental hard tissue [9]. However, energy dispersive analysis with X-ray (EDAX) could not detect the presence of phosphorus in MTA. They concluded that the previous study by Torabinejad may be contaminated by prior immersion in phosphate solution [34, 42].

Portland Cement

Portland cement is a hydraulic material made by heating a limestone and clay mixture and pulverizing. It is a basic ingredient of concrete, mortar or stucco. White Portland cement has main chemical constituents (as shown in Table 1) like MTA. When Portland cement was mixed with water, its chemical compound constituents undergo chemical reactions that cause it to set.

Chemical Name	Chemical Formula	Shorthand Notation	Percent by Weight
Tricalcium silicate	$3CaO \times SiO_2$	C_3S	60
Dicalcium silicate	$2CaO \times SiO_2$	C_2S	19
Tricalcium aluminate	$3CaO \times Al_2O_3$	C_3A	11
Tetracalcium aluminoferrite	$4CaO \times Al_2O_3 \times Fe_2O_3$	C_4AF	1
Others			9

Table 1. Chemical constituents of White Portland cement

(From: American Society for Testing and Materials. Portland cement. ASTM C150)

From several studies, it was proved that Portland cement and MTA have similar major constituents except bismuth oxide which was found in MTA but not in Portland cement [24-26, 28]. When MTA, Portland cement and white Portland cement were compared physical properties; pH, radiopacity, setting time, solubility, dimensional change and compressive strength, it was found that they are similar [22]. From previous study, white ProRoot® MTA and two Thai white Portland cement mixed with bismuth oxide have also shown comparable chemical constituents and physical properties [30].

Bismuth oxide

Bismuth oxide is yellow powder, no odor and insoluble in water. Industrially, it is considered one of the less toxic of the heavy metals [43]. Bismuth oxide is incorporated into specialty polymers and materials for bone implants, dental prosthetic devices, catheters, sutures and surgical instruments to make them detectable by x-rays without

the toxicity or carcinogenicity associated with other heavy metals. Bismuth oxide is added to Portland cement to improve radiopacity of material [30].

Biocompatibility of MTA and Portland cement

The biocompatibility assessment of MTA encompassed *in vitro* cell culture techniques using either established cell lines or primary cell cultures. These studies were performed in several cell types including osteoblast, fibroblast, periodontal ligament cell, cementoblast, and others. Torabinejad who did the first MTA cytotoxicity test reported that MTA is less cytotoxicity to mouse fibroblast (L-929) than super-EBA and IRM but more than amalgam when tested by agar overlay method [13]. However, from radiochromium-labeled assay, MTA is less cytotoxicity than amalgam at 24 hours [13]. This result is in agreement with Keiser's study which tested in primary periodontal ligament cells. At 24 hours, MTA is less toxicity than amalgam and super-EBA [44]. Moreover, MTA is less toxic than Retroplast [45], Ketac molar [46] and Glass ionomer cement [27].

The attachment and morphology of human periodontal ligament fibroblasts to MTA in human teeth was evaluated using a scanning electron microscope. In freshly prepare-MTA, cells were round, low density and lacked attachment to MTA but in 24 hour set-MTA, cells were round and flattened and appeared to be tightly attached to MTA. As incubation period increased, fibroblast cells increased in number and became tightly attached to the material [47]. Koh et al. investigated the morphology of osteoblast-like cells (MG-63) in the presence of MTA and IRM by scanning electron microscopy. They reported that at 1 and 3 days, there are flatted and adhered cells on MTA but rounded and sparse cells on IRM [10]. In addition, human alveolar bone cells were attached and spread out onto MTA within 24 hours and proliferated to form a matrix-like layer within 7 days [41].

From *in vivo* tests, MTA not only present less periradicular inflammation than amalgam Super-EBA and IRM in tibia and mandible of Guinea pigs but also was the

material most often observed with hard tissue deposition when implanted MTA in the tibia of Guinea pigs [14]. Moreover, a layer of cementum can be formed over MTA but cannot be formed on amalgam as root-end filling in monkeys [16].

Biocompatibility of Portland cement was compared to MTA *in vitro* and *in vivo*. In cytotoxicity testing, Portland cement has the level of cell viability similar to MTA in mouse fibroblast (L-929) [29], human osteoblast-like cell (Saos-2) [27, 48], human endothelial cell (ECV 304) [49], mouse lymphoma cell (L5178Y) [50] and Chinese hamster ovary cell (CHO K-1) [51]. Moreover, from scanning electron microscopy, characteristic of cells in presence of MTA and Portland cement are similar [27, 48].

The genotoxicity of MTA and Portland cements in mouse lymphoma cells and Chinese hamster ovary cells demonstrated that the single cell gel (comet) assay failed to detect DNA damage after a treatment of cells by MTA and Portland cements for concentrations up to 1000 microgram/ml. These results implied that MTA and Portland cements are not genotoxins [51, 52].

Cell and tissue reactions to MTA and Portland cement by implanting material into bone cavities of the guinea pig mandible were tested by Saidon et al. Bone healing and minimal inflammatory response were observed adjacent to ProRoot® MTA and Portland cement implants. Both materials were well tolerated and confirmed the similarity between Portland cement and MTA at 2 and 12 weeks [29]. The pulpal response of dogs' teeth after pulpotomy and direct pulp protection with MTA Angelus, ProRoot® MTA, Portland cement and white Portland cement, it was found that all materials demonstrated similar results when used as pulp-capping materials. Pulp vitality was maintained in all specimens with hard tissue bridge. All materials performed equally well as pulp protection materials following pulpotomy [38].

Bismuth oxide which is not in Portland cement is added to Portland cement to improve radiopacity of material. Human osteosarcoma cell line (SaOS-2) cannot grow on

bismuth oxide. This growth inhibition may be due to the surface roughness or chemical releasing solutions of the material [53]. However, Portland cement mixed with 4:1 proportion bismuth oxide was not toxic to human osteosarcoma cell line [23]. Pure Portland cement and MTA demonstrated less cytotoxicity to immortalized human periodontal ligament cells (IPDL) at 12 and 24 hours when compared to Portland cement mixed with bismuth oxide. However, at 48 and 72 hours the number of cell viability of Portland cement mixed with bismuth oxide at the ratio of 4:1, 6:1 and 8:1 increased to the levels of those of MTA and pure Portland cement groups [54]. Moreover, bismuth oxide did not induce DNA damage in human lymphocytes in single cell gel (comet) assay [55].

Cytotoxicity testing

In vitro cytotoxicity screening methods have been widely used to evaluate biocompatibility of material. From International Standard Organization (ISO) 10993-5, there are three categories for cytotoxicity test:

1) Extract test which allows both qualitative and quantitiative assessment of cytotoxicity The advantages of extract test are 1) can examine the effects of materials on cells that are both distant to and in contact with them, 2) can easily clean by filtration, 3) simulates the immediate postsurgical root end environment in which toxic elements of the retro-filling material leach into the surrounding fluids in the periradicular tissue and 4) can dilute extraction fluid to observe a possible dose-response relationship for determine the most ideal concentration for the sensitivity of the cells tested [44]
2) Direct-contact test which allows both qualitative and quantitative assessment of cytotoxicity
3) Indirect-contact test
   - *Agar diffusion test* – qualitative assessment of cytotoxicity. This assay is not appropriate for leachables that cannot diffuse through the agar layer, or that should react with agar.

- *Filter diffusion test* – qualitative assessment of cytotoxicity

The most frequently used method for evaluation MTA effect to cell proliferation and viability was scanning electron microscopy (SEM) followed by enzyme assay [56]. Scanning electron microscope was widely used for morphological assay in order to observe the changes in cell morphology, adhesion and spreading on dental material. Adhesion and spreading of the cells on a materials surface are the initial phase of cellular function. Rajaraman et al. examined the adhesion and spreading of fibroblasts cells in cultured and found that prior to adhesion, cells were spherical or ovoid in shape and were covered with surface blebs and/or short microvilli. Adhesion was initiated by contact of the microvilli with the substratum. This was followed by formation of long filopodia at the point of contact and a decreased number of surface blebs and/or microvilli on cell surfaces that were not in contact with substratum. Cell spreading then occurred, with peripheral expansion of cytoplasmatic webs (lamellipodia) composed of many filopodia. After that, flattening of the cells into a polygonal shape appeared with decreasing in number of microvilli and filopodia [57]. The persistence of rounded cells with little or no spreading suggested that the surface material may be toxic [58].

Methyltetrazolium assay is one of the functional assays that use tetrazolium salt MTT ((3-(4, 5,dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to measure mitochondrial dehydrogenase activity. It is a pale yellow substrate that produces a dark blue formazan product when cleaved by active mitochondria, and so the reaction only occurs in living, metabolically active cells [59]. The numbers of viable cells was directly proportional to the amount of formazan product. The amount of MTT-formazan produced can be determined spectophotometrically. MTT assay is simple, rapid, reliable, no radioisotope [46]. It is an inexpensive screening that purposes of a large number of samples in a short time [60]. So MTT is popular to use in many cytotoxicity tests [23, 44, 46, 61]

Periradicular wound healing

The ideal healing response after periradicular surgery is the re-establishment of an apical attachment apparatus including cementum overlying the resected root-end surface, periodontal ligament (PDL) and osseous repair [62, 63]. However, histological examination of biopsy specimens revealed three types of tissue response: healing with reformation of periodontal ligament; healing with fibrous tissue (scar); and moderate to severe inflammation without scar tissue [64]. The favorable healing is regeneration of bone followed by reformation of periodontal ligament and cementum. Biocompatibility of retro-filling material to osteoblasts is one of important factors to periradicular healing after endodontic surgery.

Studies that used established cell line have the advantage of enhanced reproducibility of results and are recommended by the ISO for preliminary cytotoxicity screening. For specific sensitivity testing to stimulate the *in vivo* situation, primary cell strains derived from living tissue are necessary and are also recommended by the ISO-10993 [44]. Primary osteoblasts have a diploid chromosome pattern, are characterized by growing slowly and have a finite lifespan. Established cell lines, on the other hand, have an aneuploid chromosome pattern, tend to multiply rapidly and have unlimited lifespan [65]. The summary of characteristics of permanent and primary cell lines is shown in Table 2.

Permanent cell line (Established cell line)	Primary cell line
Standardized	Donor dependent (less standardized)
More available	Derived from viable tissue
More repeatability and reproducibility	Less repeatability, reproducibility
High growth rate	Limited life span, limited number
Different biological properties from human cell	Close to human
Suitable for screening test or initial test	Suitable in metabolic activity, cell function studies

Table 2. Characteristics of permanent cell line and primary cell line [66]

Additionally, primary osteoblasts form mineralized nodules when exposed to differentiation medium while MG-63 cells which are permanent cell lines do not form nodules [65]. According to different characteristics, primary osteoblasts are more appropriate than permanent cells for testing material cytotoxicity in cell culture. Cells derived from human alveolar bone demonstrated osteoblast characteristics including the expression of bone maker genes, collagen type I, alkaline phosphatase, bone sialoprotein, osteopontin, osteocalcin and form mineralized nodules. These cells showed potential as an *in vitro* model of human osteoblasts from alveolar bone for further alveolar bone related studies [67]. Therefore, this study chose to perform cytotoxicity test in primary human alveolar bone osteoblasts.

CHAPTER III

MATERIALS AND METHODS

Materials

1. White Portland cement (Kilan brand, Universal White cement Co., LTD)
2. White Portland cement (Chang brand, The Siam White cement Co., LTD)
3. White ProRoot® MTA (Dentsply, USA)
4. Bismuth oxide (Fluka, Spain)
5. Intermediate Restorative Material (IRM®: Caulk, Dentsply, USA)
6. Sterile Distilled water
7. Glass slab
8. Metal spatula
9. Plastic ring mold
10. Cover slip
11. Scapel blade
12. Blade No.15
13. 35-mm. tissue culture dish (Corning, USA)
14. 60-mm. tissue culture dish (Corning, USA)
15. 24-well-flat-bottom plate (Nunc, Denmark)
16. 96-well-flat-bottom plate (Costar, USA)
17. 75 cm^2 cell culture flask (Corning, USA)
18. 25 cm^2 cell culture flask (Corning, USA)
19. CO_2 incubator
20. Laminar flow hood
21. Pasteur pipette
22. Pipette tip 20, 200, 1000 µl
23. Pipette 10, 25 ml
24. Hemocytometer
25. Phase contrast light microscope
26. Microplate reader

27. Dulbecco Minimal Essential Medium with F12 nutrient mixture (1:1) (DMEM/F12) (Gibco BRL, USA)
28. Heat-inactivated Fetal Bovine Serum (FBS) (Gibco BRL, USA)
29. 0.25% trypsin-EDTA (Gibco BRL, USA)
30. DMEM without phenol red (Gibco BRL, USA)
31. MTT (3-(4,5,dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Sigma, USA)
32. Dimethyl sulfoxide (DMSO)
33. Penicillin G (Gibco BRL, USA)
34. Streptomycin (Gibco BRL, USA)
35. Amphotericin B (Gibco BRL, USA)
36. L-glutamine (Gibco BRL, USA)
37. Phosphate Buffer Saline (PBS)
38. 2.5% glutaraldehyde in 0.1 M Cacodylate Buffer
39. Hexamethyldisilazane (HMDS) (Sigma-Aldrich, USA)
40. Ethanol
41. Scanning electron microscope (JSM-5410LV, JOEL, Japan)
42. 5-bromo-4chloro-3-indolylphosphate/nitroblue-tetrazolium salt, BCIP/NBT (Sigma *Fast*)

Methods

The protocol for this study is based on ISO 10993: Biological evaluation of medical devices.

Part 1: Evaluation and testing

Part 5: Tests for in vitro cytotoxicity

Part 12: Sample preparation and reference materials

1. Primary human alveolar bone osteoblast culture

Bone samples were collected from patients who undergone surgery of alveolar bone. Informed consent was obtained prior to inclusion in the study. The protocol was approved by the Ethics Committee of Faculty of Dentistry, Chulalongkorn University. The method to obtain human osteoblasts from the alveolar bone was described by Wongyaofa [67]. The bone samples were collected from patients only when there were alveolar bone pieces attached to the tooth roots or tissues that were removed during the routine extraction or surgical removal of the impacted third molars. The size of bone samples was about 2-5 mm. The bone samples immediatedly placed in a sterile tube containing Dulbecco's modified Eagle's medium supplemented with 2 mM L-glutamine, penicillin G (50 U/ml), streptomycin (50 µg /ml), amphotericin B (2.5 µg/ml) and 10% heat-inactivated fetal bovine serum (FBS). The samples were washed twice with phosphate buffer solution (PBS) to remove blood clots and adherent erythrocytes. The surrounding soft tissues were removed by scraping with a sterile scalpel. The samples were cut into small pieces and then 3-4 bone pieces were transferred to a 35-mm tissue culture dish containing 2 ml of Dulbecco Minimal Essential Medium with F12 nutrient mixture (1:1) (DMEM/F12) supplemented with 2 mM L-glutamine, penicillin G (50 U/ml), streptomycin (50 µg /ml), amphotericin B (2.5 µg/ml) and 10% heat-inactivated fetal bovine serum (FBS) (cell culture medium), incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Culture medium was changed twice weekly. When the cells migrating from the explants were confluent, they would be subcultured into a culture flask. To determine alkaline phosphatase (ALP) expression, cells in passage 1 were plated in a 35-mm tissue culture dish and cultured in inducing agents [Dulbecco Minimal Essential Medium with F12 nutrient mixture (1:1) (DMEM/F12) supplemented with 2 mM L-glutamine, penicillin G (50 U/ml), streptomycin (50 µg /ml), amphotericin B (2.5 µg/ml), 10% heat-inactivated fetal bovine serum (FBS), ascorbic acid (50 µg/ml) and 10 mM of Beta-glycerophosphate for 14 days. Then, cells were washed three times with PBS and fixed with 70% alcohol for 10 minutes. Alkaline phosphatase activity was tested using 500 µl of the substrate for ALP [5-bromo-4chloro-3-indolylphosphate/ nitroblue-tetrazolium salt, BCIP/NBT 228.7 mg in 10 ml of distilled water]. Positive

stained cells were included in this study. Cells in passage 2 were either frozen in fetal bovine serum/ dimethyl sulfoxide (DMSO) (9:1) at -80°C for further use, or subculture for experimental purposes. Cells in the 3rd or 4th passage were investigated the alkaline phosphatase activity, mineralized nodule formation and the expression of bone marker genes by methods described in previous study [67] to prove that these cells were osteoblasts. Cells in the 3rd to 5th passage from 3 different patients were used in this experimental study.

2. Sample preparation

The materials used in this study were White ProRoot® MTA and two commercially Thai White Portland cements approved by TISI 133 2518 (1975) shown in Table 3.

Materials	Manufacturing company
1.Thai White Portland cement a) Chang b) KILAN	 a) The Siam White cement Co., LTD b) Universal White cement Co., LTD
2. White ProRoot® MTA	Tulsa Dental Products, Tulsa, OK, USA

Table 3. Materials for cytotoxicity testing in this study.

Thai White Portland cements were mixed with bismuth oxide (Fluka, Spain) in ratio 4:1 by weight, using Grinding machine (Retsch S1000 F, F. Kurt Retach GmbH &Co., KG, Germany) for homogeneous powder. One gram of each white Portland cement mixed with bismuth oxide was mixed with 0.3 ml distilled water. White ProRoot® MTA was mixed with distilled water according to the manufacturer's instruction.

Sixteen standard cylinder discs of 6 mm in diameter and 1 mm in height for each tested material were prepared under aseptic conditions and then incubated for 3 hours in 95% humidity at 37°C. After removing specimens from plastic molds, all samples of each material were placed into a 60-mm tissue culture dish.

3. Viability test of cells cultured in material extracts by Methyltetrazolium (MTT) assay

- *Preparation of Extracts*

220 µl/sample of DMEM/F12 supplemented with 2 mM L-glutamine, penicillin G (50 U/ml), streptomycin (50 µg /ml), amphotericin B (2.5 µg/ml) was added into the culture dishes which had the material discs in the bottom and then incubated at the condition of 5% CO_2, 37 ºC for 1, 3, 7 or 14 days (16 samples/ material/ day group) (figure 1). Culture medium was replaced with new medium everyday until the period of extraction was due. Twenty four hours before extracting time, culture medium was replaced with cell culture medium with 10% FBS.

- *Cell cuture*

Primary human osteoblasts were seeded at 10,000 cells/well/200 µl of culture medium in a 96-well- tissue culture plate and incubated for 24 hours at 37°C 5% CO_2. After incubation, the medium in cell culture was replaced by 180 µl of extracted medium that was drawn from each 60-mm culture dish (16 wells/ material / day group) and cells were incubated for 24 or 72 hours (8 wells/ incubate time) at 37°C 5% CO_2. Fresh cell culture medium with serum was used as negative control and the day 1 extract of IRM® was used as a positive control.

Figure 1. Samples in 60-mm culture dishes filled with culture medium

- *MTT assay*

After the cells reached their incubation period of 24 or 72 hours, the extracted medium was removed from wells and then the wells were washed with phosphate buffer solution two times. The wells were added with 100 µl of media without phenol red containing 0.5 mg/ml of MTT powder (3-(4,5,dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Sigma Chemical Co., St. Louis, MO, USA). After 3-hour incubation, MTT solution was removed and then 200 µl of dimethyl sulfoxide (DMSO) was added into each well to dissolve the formazan crystal. The number of viable cells was calculated from spectrophotometer measurement at 570 nm wavelength. The percents cell viability relative to the control group (cell grew in the regular culture medium without extract) were calculated.

4. Cell morphology and attachment on material by Scanning Electron Microscope (SEM)

Two prepared samples from each material which were incubated with culture medium for 1 day were placed into the bottom of 96-well tissue culture plate and then cultured primary human osteoblasts were seeded on material at 10,000 cells/well/200 µl of cultured medium and incubated for 24 or 72 hours (2 samples/ incubate time). Cells cultured on glass slide were used as control. After incubation, the medium was removed and the disc of tested material along with the cells grown on their surface were washed three times with cacodylate-buffered solution, fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer 200 µl for 120 minutes at room temperature. After that the material discs were washed again in cacodylate-buffered solution. The material discs were dehydrated in ascending grades of ethanol, dried with Hexamethyldisilazane (HMDS) 5 minutes and mounted on copper stubs and sputter-coated with 15 nm gold palladium. Material surface, cell morphology and attachment on material discs were examined at center and peripheral area of material by using a scanning electron microscope at 500X and 1000X (JSM-5410LV, JOEL, Japan) compared with the cells of control sample and other materials.

5. Statistical analysis

Results of the data obtained from MTT assay of extracted medium from materials were reported as mean of percent cell viability ± standard deviation (SD).

For statistical analysis, the One-sample Kolmogorov-Smirnov test was used to test the normal distribution of the data. If the data was normal distribution and equal variances, the One-way Analysis of Variance (ANOVA) followed by Bonferroni test for post hoc multiple comparisons at the 95% confidence interval will be used for testing differences in cell viability by MTT assay. If the data was normal distribution but variances were not equal, Brown-Forsythe was used and followed by Tamhane's T2 test for post hoc multiple comparisons.

6.Budgets

Budget for tissue culture	20,000 BHT
Budget for RT-PCR	60,000 BHT
Budget for Reagent:	10,000 BHT
(ascorbic acid, beta-glycerophosphate, BCIP/NBT, Alizarin red S, MTT solution, Cacodylate buffer, HMDS)	
Budget for Material	85,000 BHT
Budget for SEM	10,000 BHT
Budget for document and copying	2,000 BHT
Total	187,000 BHT

CHAPTER IV

RESULTS

Primary cells derived from human alveolar bone

Cells at the 3rd to 5th passage were used in this study. Three primary human alveolar bone osteoblast cell lines, HOB1, HOB2 and HOB3, that positive stained to alkaline phosphatase substrate were used in our experiments. HOB1 was collected from alveolar trabecular bone of lower right third molar surgery of 29-year-old female. HOB2 was collected from alveolar trabecular bone of lower right third molar surgery of 20-year-old female. HOB3 was collected from alveolar cortical bone of upper left third molar surgery of 26-year-old male. HOB1 and HOB2 were more spindle-shaped and grew faster than HOB3 cells.

Alkaline phosphatase activity

Alkaline phosphatase activity could be detected from day 14 to day 28. In all three primary cell lines, the highest activity was observed at day 28.

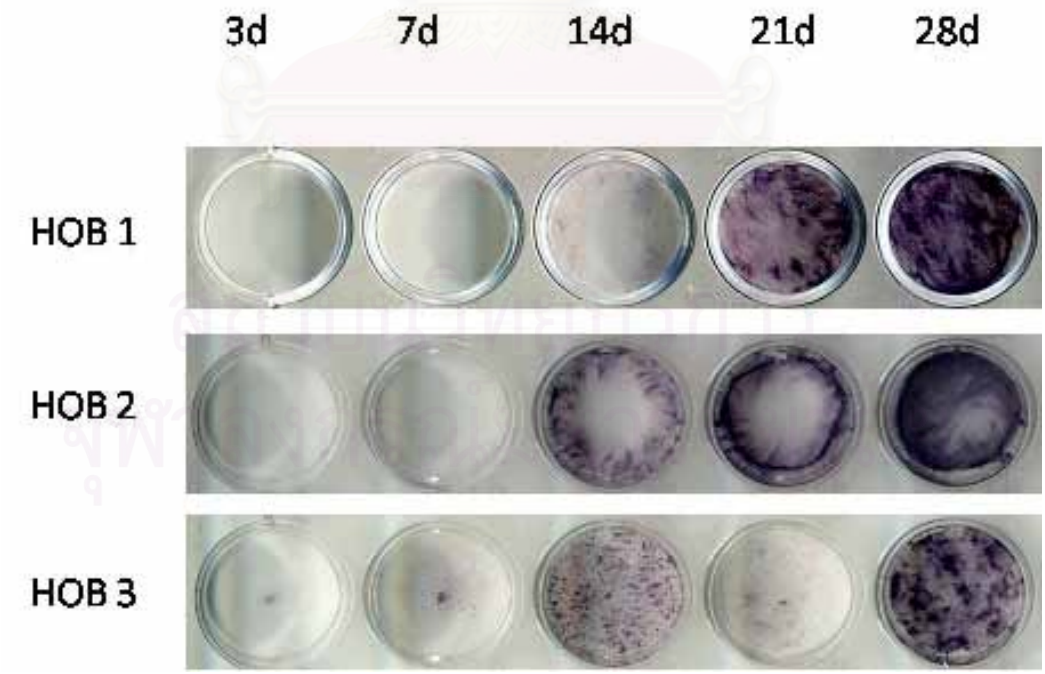

Figure 2. Alkaline phosphatase activity. Cells were cultured with 50 µg/ml of ascorbic acid and 10 mM of β-glycerophosphate for 3, 7, 14, 21 and 28 days.

Alizarin red S staining of mineralized nodules

The mineralized nodules were first detected in day 14 in all primary cell lines and the nodules increased gradually from day 14 to day 28 (Figure 3).

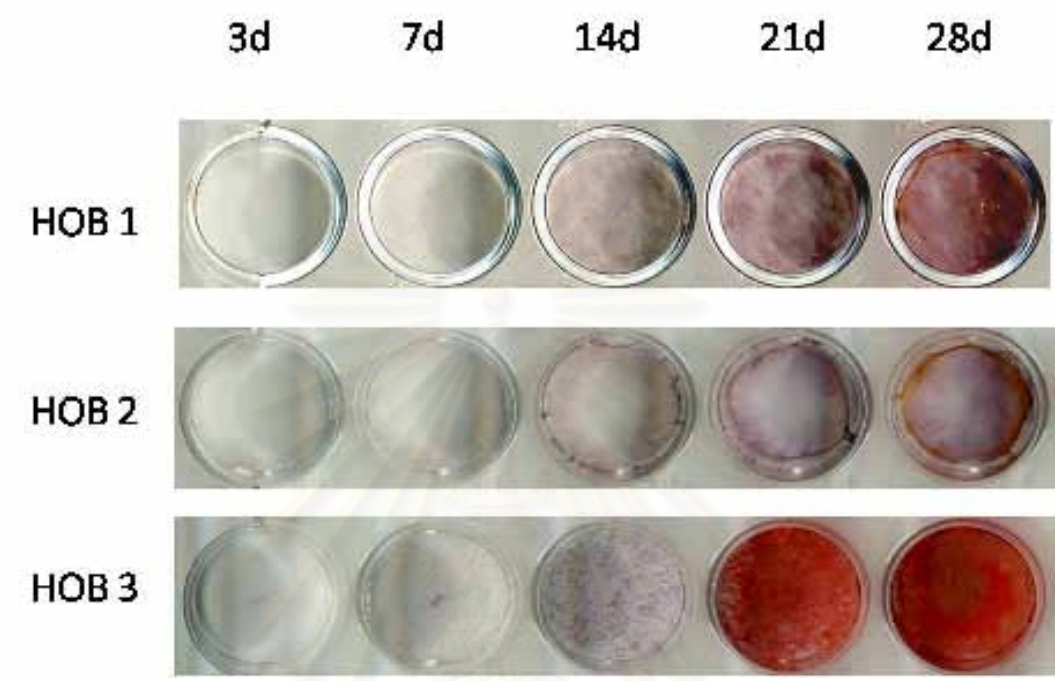

Figure 3. Alizarin red S staining. Cells were cultured with 50 µg/ml of ascorbic acid and 10 mM of β-glycerophosphate for 3, 7, 14, 21 and 28 days.

Osteogenic maker gene expression by RT-PCR analysis

RT-PCR analysis of RNA extracts from human alveolar bone osteoblasts at day 3 to day 28 show that these primary human alveolar bone osteoblasts expressed osteogenic makers; COLIA2, ALP, BSP2, OPN and OCN (figure 4-6).

HOB1 and HOB3 expressed COLIA2 at all time points. HOB2 also expressed COLIA2 at all times but the expression was low at days 3 and 14 and was high at days 7 and 28. For HOB1 and HOB3, ALP expression was up-regulated gradually until day 28. For HOB2, ALP was first detected at day 3 and then down-regulated at days 7 and 14. It was up-regulated at day 21 and down-regulated again at day 28.

For BSP2, HOB1 was first detected at day 3 and increased gradually until day 28. BSP2 expression of HOB2 was detected at day 3 and then decreased at day 7 and then up-regulated at day 14 to day 28. For HOB3, BSP2 expression was first detected at day 3 and then declined at days 7, 14 and increased at day 21 and declined again at day 28. All primary lines showed similar OPN expression. OPN was detected at day 3 and then down-regulated at days 7 and 14 and up-regulated again at day 21 to day 28.

For OCN expression, all primary lines first expressed OCN at day 3. HOB1, OCN expression was up-regulated gradually until day 28. HOB2 and HOB3, OCN expression could be detected at day 3. It was then down-regulated at day 7 to day 14 and then it was up-regulated at day 21 to the highest level of expression at day 28.

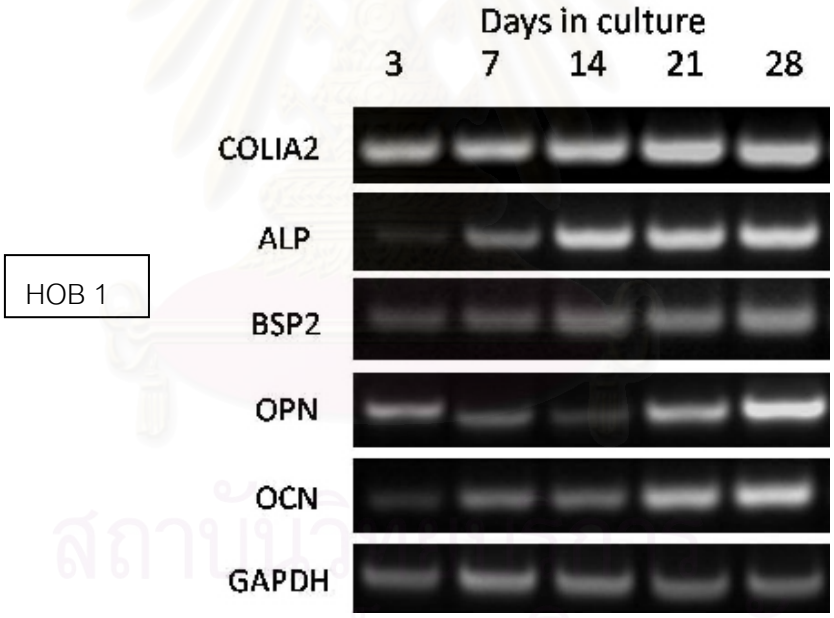

Figure 4. COLIA2, ALP, BSP2, OPN, OCN and GAPDH mRNA expression of HOB1. Cells were cultured with 50 µg/ml of ascorbic acid and 10 mM of β-glycerophosphate for 3, 7, 14, 21 and 28 days.

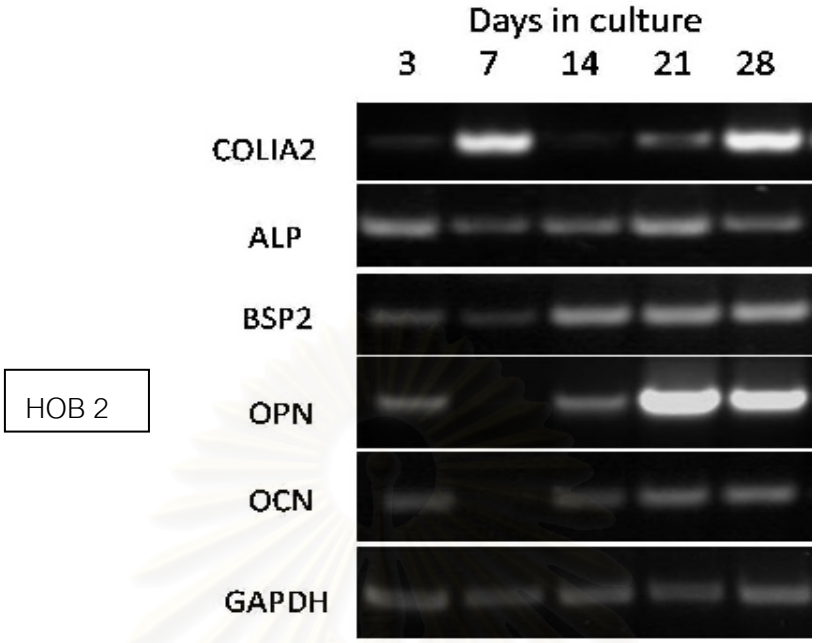

Figure 5. COLIA2, ALP, BSP2, OPN, OCN and GAPDH mRNA expression of HOB2. Cells were cultured with 50 µg/ml of ascorbic acid and 10 mM of β-glycerophosphate for 3, 7, 14, 21 and 28 days.

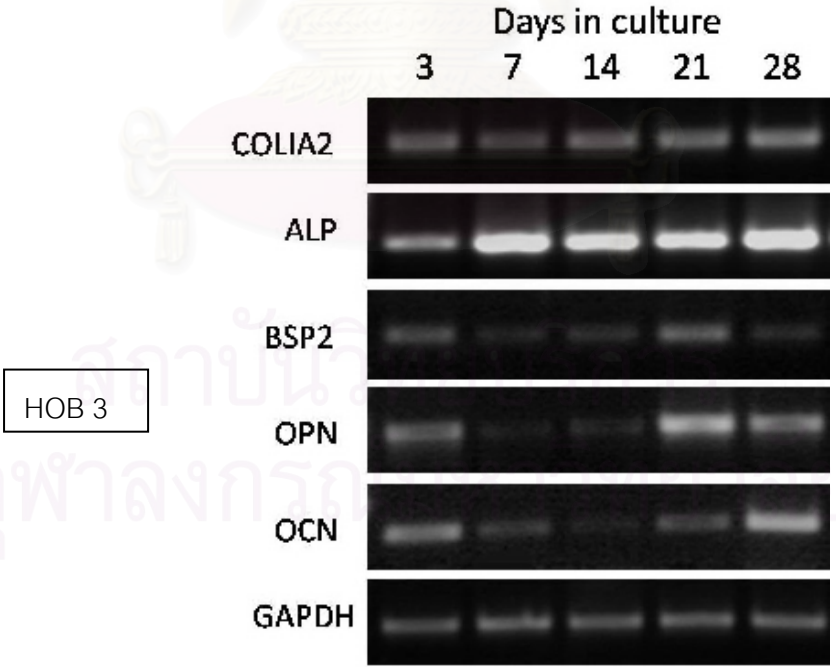

Figure 6. COLIA2, ALP, BSP2, OPN, OCN and GAPDH mRNA expression of HOB3. Cells were cultured with 50 µg/ml of ascorbic acid and 10 mM of β-glycerophosphate for 3, 7, 14, 21 and 28 days

Viability of cells cultured in material extracts by MTT assay

Relative percents cell viability of the primary human osteoblasts treated with material extracts for 24 and 72 hours were shown in Figure 7-12. IRM® was confirmed to be highly toxic, showing cell viability at 0.23 % and 14.44% for 24- and 72-hour incubation, respectively.

- HOB1

At 24-hour incubation in extracts, relative percents cell viability of all material extracts at all time points were more than 90%. At 72-hour incubation, relative percent cell viability tended to be more than that at 24-hour incubation for all material extracts especially at days 3 and 7. Their relative percents of cell viability were more than 100. This is with only exception for the Kilan extract at day 1 for both 24- and 72-hour exposure time of which percent cell viability is 12.81% and 7.38%, respectively (Figure 7).

The cytotoxicity of day 1 extract from Kilan was statistically significantly more than those from white ProRoot® MTA and Chang ($p < 0.001$) in both 24- and 72-hour incubation groups. In contrast, percents cell viability of day 1 Chang extract was not statistically significantly different to day 1 white ProRoot® MTA extract when incubate for 24 hours. However, at 72-hour incubation, day 1 Chang extract showed significantly less percent cell viability comparing to white ProRoot® MTA ($p < 0.001$). Nonetheless, the percents cell viability of day 1 Chang extract was nearly 100% showing that day 1 Chang extract was not cytotoxic.

For day 3 extracts, Chang and Kilan showed more percents cell viability comparing to white ProRoot® MTA with statistically significant difference for Chang at both 24- and 72-hour incubation and at 72-hour incubation for Kilan ($p < 0.05$). There were no significant differences of percents cell viability between all tested materials at days 7 and 14, except for the day 7 Chang extract at 72-hour incubation had significantly more percent cell viability than white ProRoot® MTA extract.

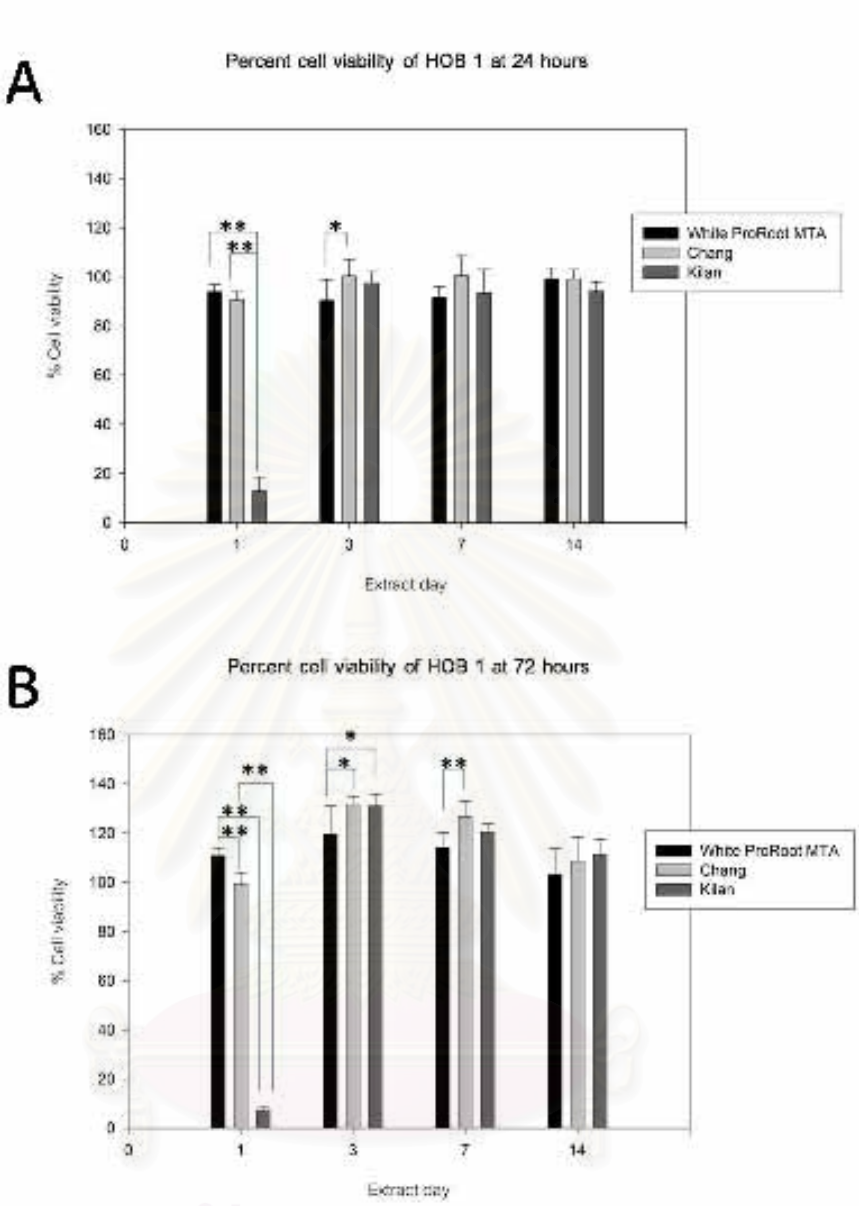

Figure 7. The percent cell viability of HOB1 treated with material extracts for 24 hours (A) and 72 hours (B) relative to untreated control (medium) by MTT assay (* = statistically significantly different at $p < 0.05$, ** = statistically significantly different at $p < 0.001$)

Percents cell viability of the primary human osteoblasts treated with extracts from different time points for 24 and 72 hours were shown in Figure 8.

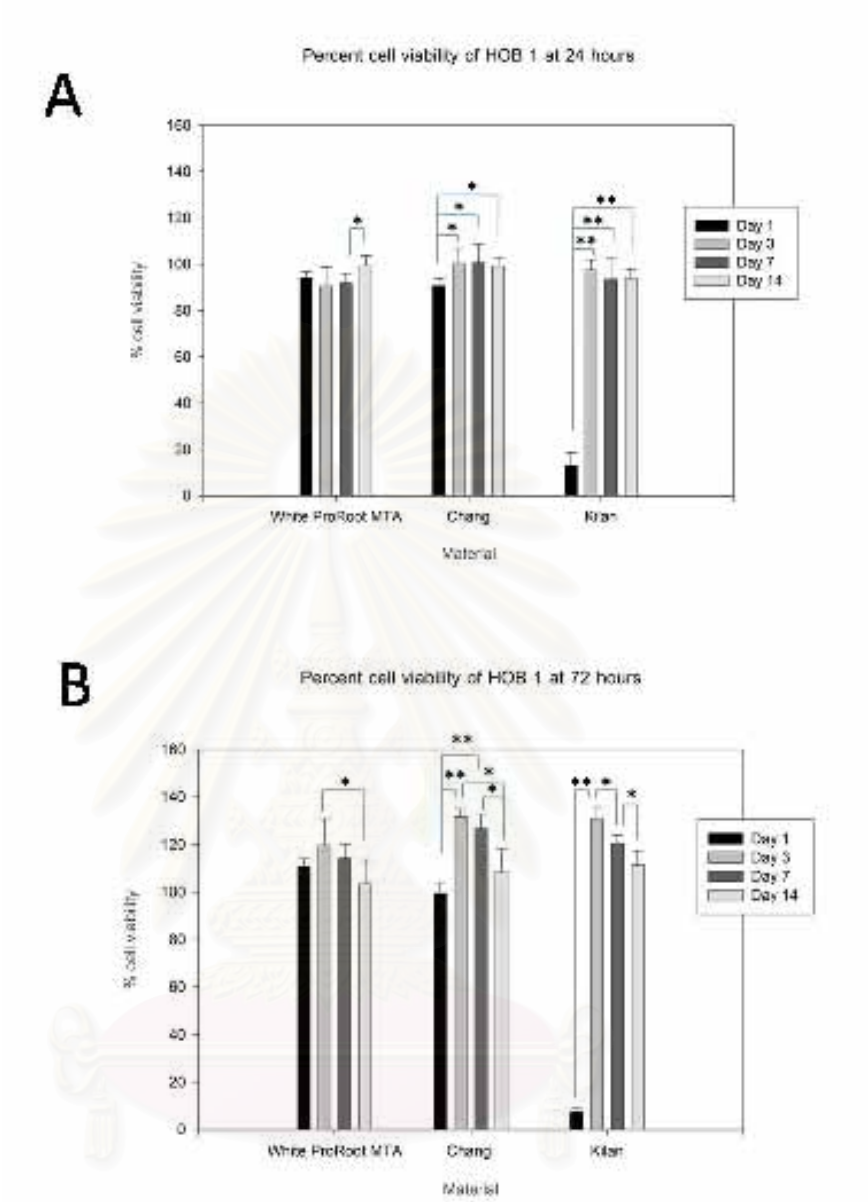

Figure 8. The percent cell viability of HOB1 treated with material extracts at different time points for 24 hours (A) and 72 hours (B) relative to untreated control (regular medium) by MTT assay (* = statistically significantly different at $p < 0.05$, ** = statistically significantly different at $p < 0.001$)

For white ProRoot® MTA, at 24-hour incubation in extracts, percent cell viability of day 14 white ProRoot® MTA extract was statistically significantly more than that of day 7 ($p < 0.05$). In 72-hour incubation groups, percent cell viability of day 3 was more than

that of day 14 ($p < 0.05$). Day 1 extracts of Kilan and Chang showed less percent cell viability than extracts of other time points in 24-hour incubation groups ($p < 0.001$ and $p < 0.05$, respectively). However, at 72-hour incubation, percents cell viability of Kilan extracts at day 3, day 7, day 14 and day 1 were in descending order. For Chang extracts, the percents cell viability of days 3 and 7 were more than those of days 1 and 14.

- HOB2

Relative percents cell viability of all material extracts at all time points were more than 80 with only exception for the Kilan extract at day 1 for 72-hour exposure time. At 72-hour incubation, at days 3 and 7 relative percent cell viability tended to be more than 24-hour incubation for all material extracts especially Kilan and Chang at day 3 which showed relative percents cell viability more than 100 (Figure 9).

The cytotoxicity of day 1 extract from Kilan was statistically significantly more than those from white ProRoot® MTA ($p < 0.001$) and Chang ($p < 0.05$) in 72-hour incubation groups. In contrast, percent cell viability of day 1 Kilan extract was statistically significantly more than that of day 1 white ProRoot® MTA extract when incubate for 24 hours ($p < 0.05$). At 72-hour incubation, day 1 Chang extract showed significantly less percent cell viability comparing to white ProRoot® MTA ($p < 0.001$).

For day 3 extracts, Chang and Kilan showed more percents cell viability comparing to white ProRoot® MTA with statistically significant difference for both 24- ($p < 0.05$) and 72-hour incubation ($p < 0.001$). In 72-hour incubation groups, day 3 extract of Chang showed more percents cell viability than that of Kilan ($p < 0.05$). For day 7 extracts, Kilan showed statistically significantly more percents cell viability than white ProRoot® MTA and Chang for both 24- and 72-hour incubation. However, percent cell viability of day 7 extracts of Chang and white ProRoot® MTA were not different. There were no significant differences of percents cell viability between all tested materials at day 14.

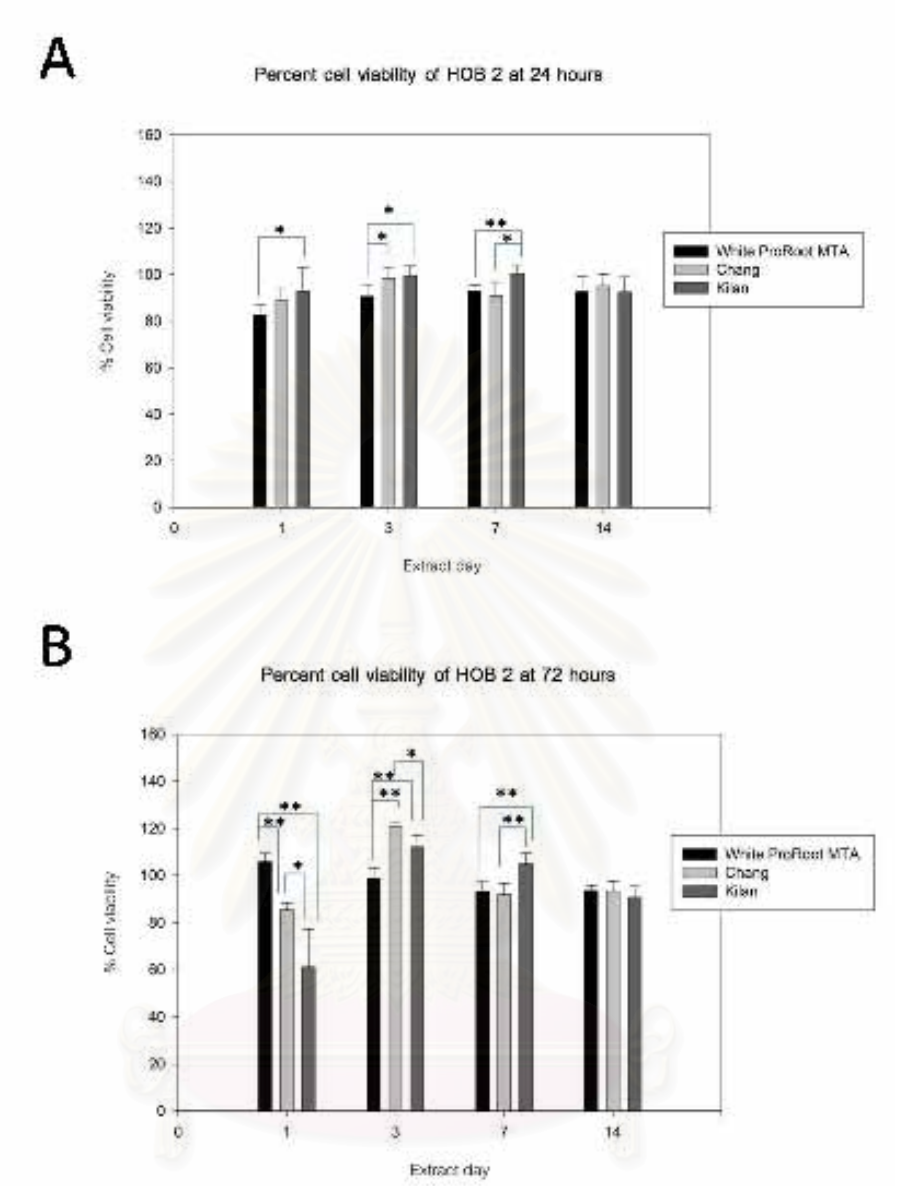

Figure 9. The percent cell viability of HOB2 treated with material extracts for 24 hours (A) and 72 hours (B) relative to untreated control (medium) by MTT assay (* = statistically significantly different at $p < 0.05$, ** = statistically significantly different at $p < 0.001$)

Percents cell viability of the primary human osteoblasts treated with extracts from different time points for 24 and 72 hours were shown in Figure 10. For white ProRoot® MTA extracts, the percent cell viability of day 1 extracts was less than extracts of other days in 24-hour incubation groups ($p < 0.05$). In contrast, for 72 hours, the

percent cell viability of day 1 extract was the highest comparing to other days extract. For Kilan extracts, there were no significant differences of percent cell viability between extract time points at 24-hour incubation but at 72-hour incubation, percents cell viability of days 3 and 7 were more than those of days 1 and 14 ($p < 0.001$). For Chang extracts, percent cell viability of day 3 extract was significantly higher than those of days 1 and 7 ($p < 0.05$) for 24-hour incubation. Correspondingly, for 72-hour incubation, the percent cell viability of day 3 extract was more than those of others ($p < 0.001$).

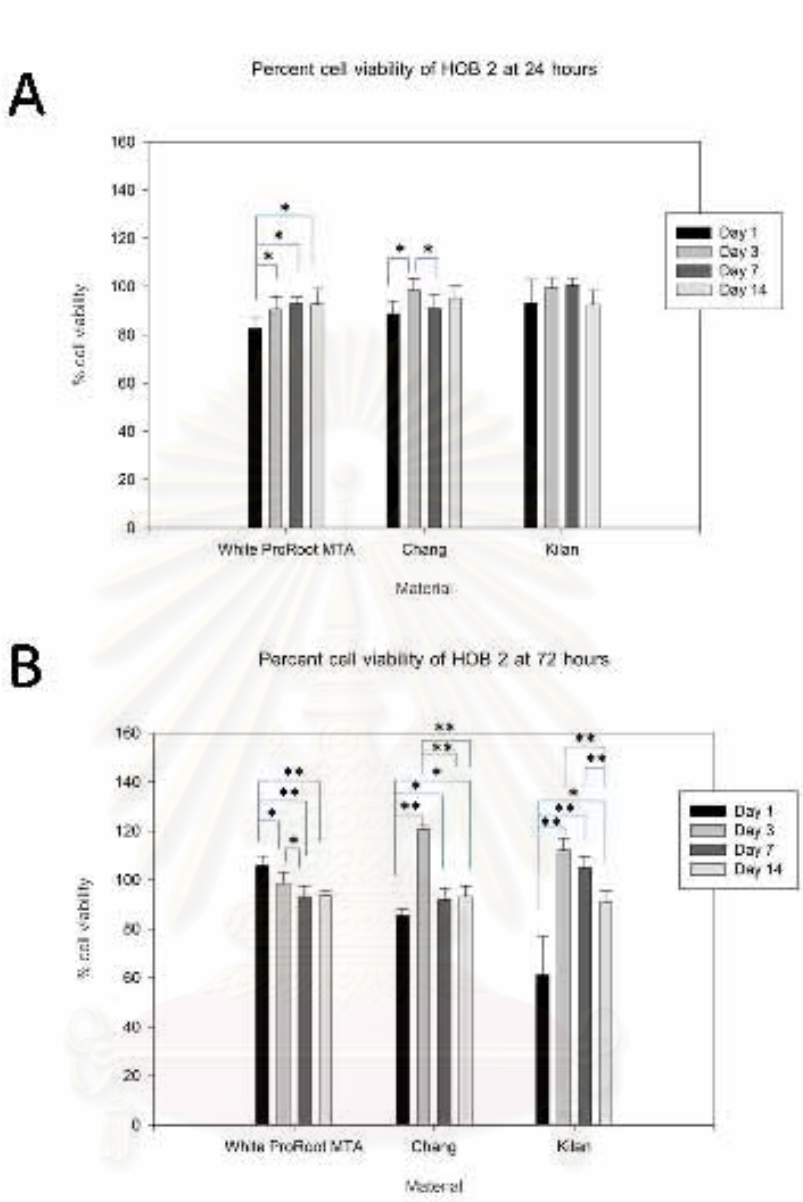

Figure 10. The percent cell viability of HOB2 treated with material extracts at different time points for 24 hours (A) and 72 hours (B) relative to untreated control (regular medium) by MTT assay (* = statistically significantly different at $p < 0.05$, ** = statistically significantly different at $p < 0.001$)

- HOB3

At 24- and 72-hour incubation in extracts, relative percents cell viability of all day 1 material extracts were the least comparing to days 3, 7 and 14 extracts. Relative percent cell viability tended to increase gradually at time. This is with exception for day

14 Kilan extract at both 24- and 72-hour incubation and day 7 white ProRoot® MTA extract at 24-hour incubation (Figure 11).

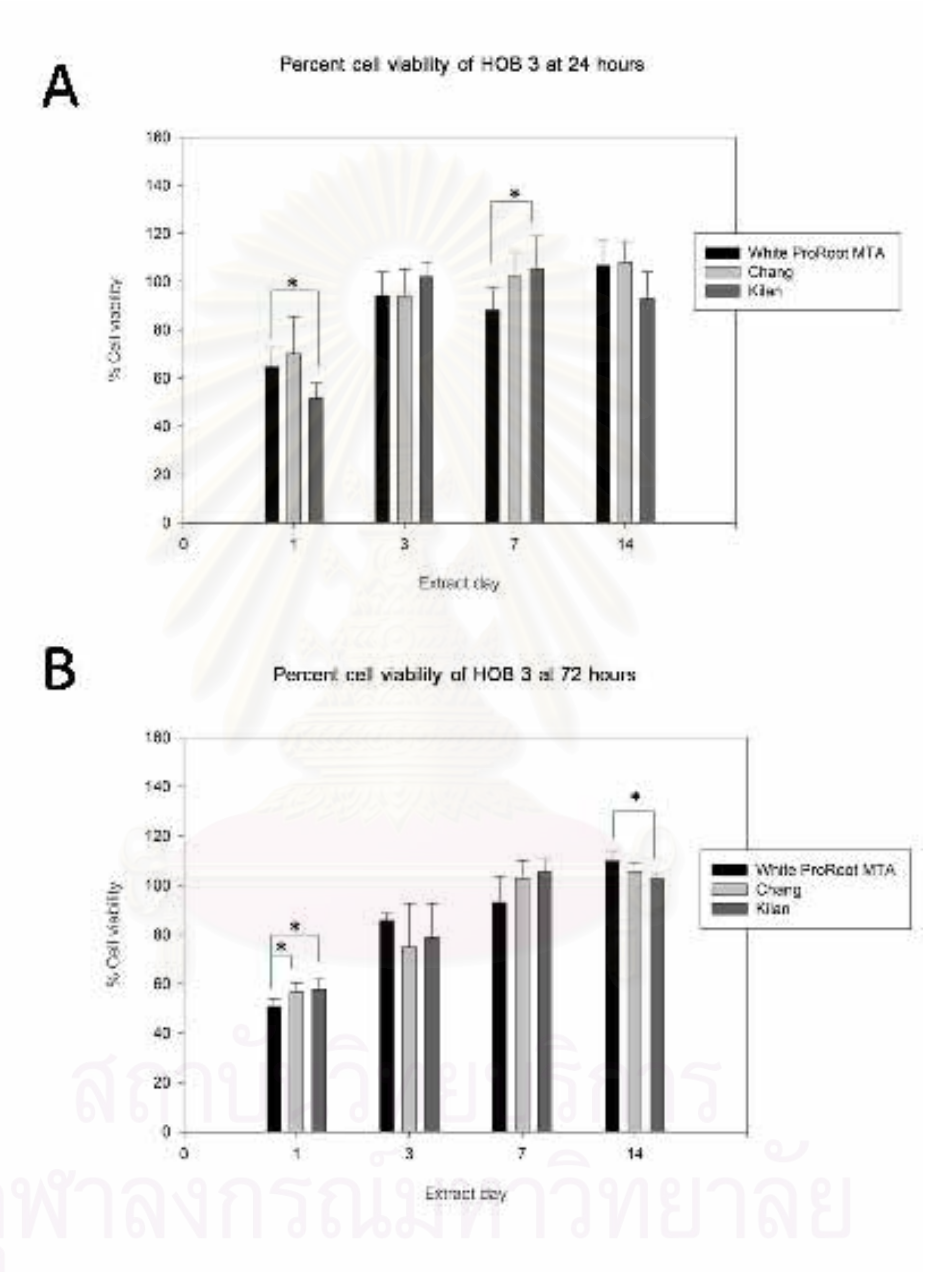

Figure 11. The percent cell viability of HOB3 treated with material extracts for 24 hours (A) and 72 hours (B) relative to untreated control (medium) by MTT assay (* = statistically significantly different at $p < 0.05$)

The cytotoxicity of day 1 extract from Kilan was statistically significantly more than those from white ProRoot® MTA ($p < 0.05$) in 24-hour incubation. In contrast, percents cell viability of day 1 Kilan and Chang extract were statistically significantly more than that of day 1 white ProRoot® MTA extract when incubated for 72 hours ($p < 0.05$). There were no significant differences of percents cell viability between all tested materials at day 3.

For day 7 extracts, Kilan showed more percents cell viability comparing to white ProRoot® MTA with statistically significant difference at 24-hour incubation ($p < 0.05$). However, for 72-hour incubation, there were no significant differences of percents cell viability between all tested materials. For day 14 extract, in 24-hour incubation group, there were no significant differences of percents cell viability between all tested materials. However, Kilan extract had significantly less percent cell viability than white ProRoot® MTA extract at 72-hour incubation.

Percents cell viability of the primary human osteoblasts treated with extracts from different time points for 24 and 72 hours were shown in Figure 12. For white ProRoot® MTA extracts, percent cell viability of day 14 was the highest and that of day 1 was the lowest significantly in both 24- and 72-hour incubation groups. For Kilan extracts, at 24-hour incubation, percent cell viability of day 1 was less than extracts of other days ($p < 0.001$) but at 72-hour incubation, percents cell viability of days 7 and 14 extracts were more than those of days 1 ($p < 0.001$) and 3 ($p < 0.05$) extracts. Like Kilan, at 24-hour incubation percent cell viability of day 1 Chang extract was less than extracts of other days and at 72-hour incubation percent cell viability of day 14 Chang extracts was significantly more than those of days 1 ($p < 0.001$) and 3 ($p < 0.05$) extracts.

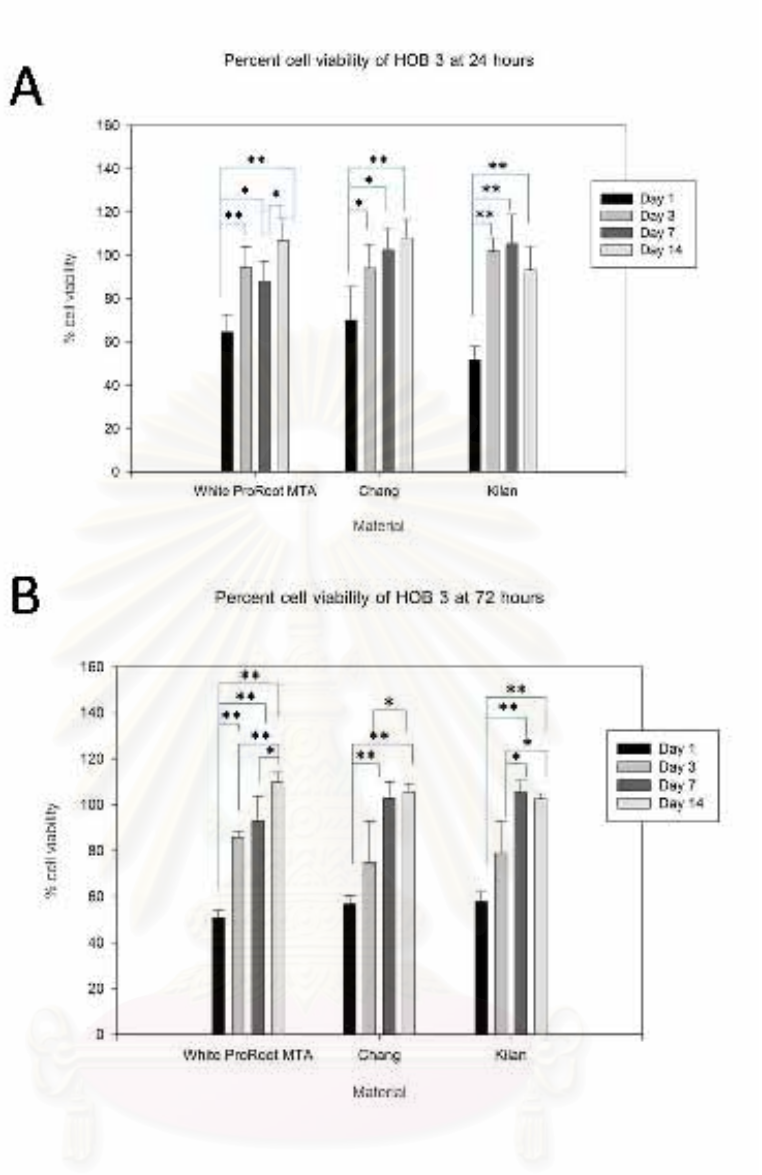

Figure 12. The percent cell viability of HOB3 treated with material extracts at different time points for 24 hours (A) and 72 hours (B) relative to untreated control (regular medium) by MTT assay (* = statistically significantly different at $p < 0.05$, ** = statistically significantly different at $p < 0.001$)

Cell morphology and attachment on materials by Scanning Electron Microscope (SEM)

Characteristics of material surface

The characteristics of material surface were examined by scanning electron microscope at 1000X magnification. White ProRoot® MTA surface had different sizes and

several crystal shapes; round, clubbing, pyramid (Figure 14). Kilan surface was similar to White ProRoot® MTA surface but does not have clubbing crystal (Figure 15). On the other hand, Chang surface had small round crystals which were uniform in size (Figure 16).

Cell morphology and attachment on material

Human alveolar bone osteoblasts grew on glass cover slip demonstrated a large number of cells in similar shape. They dispersed normally on the surface at 24 and 72 hours. They appeared to be well spread and attached to the glass within 24 hours. At 72 hours, there are more cellular extensions and processes which well attached to the glass slide (Figure 13).

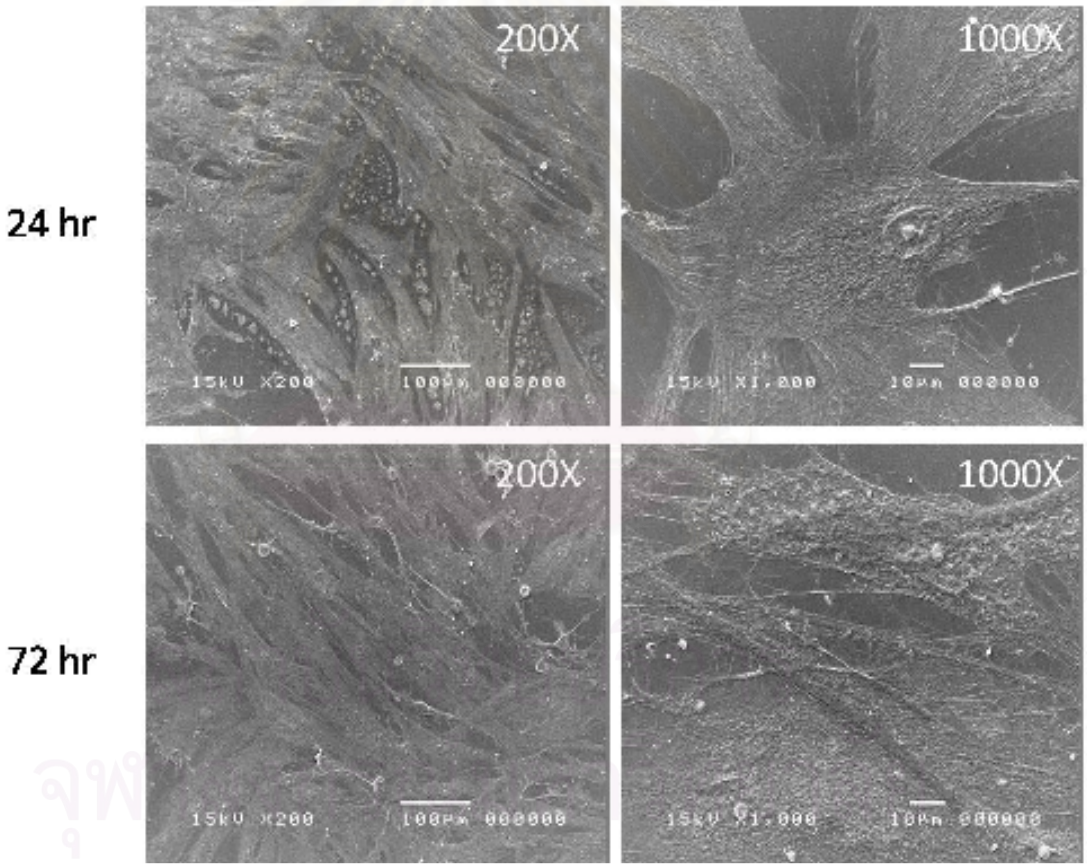

Figure 13. Scanning electron micrographs of HOB1 attached to glass slide at 24- and 72-hour incubation (magnification 200X, 1000X)

On white ProRoot® MTA, human alveolar bone osteoblasts dispersed throughout the surface at 24- and 72-hour incubation. The cells were polygonal shape which was similar to that seen in glass cover slip but fewer cells were observed. Their processes were interacting with crystals of MTA. At 72 hours, the cells showed more lamellipodial extension and interacted with adjacent cells. Parts of lamellipodia were inserted in between material crystals (Figure 14).

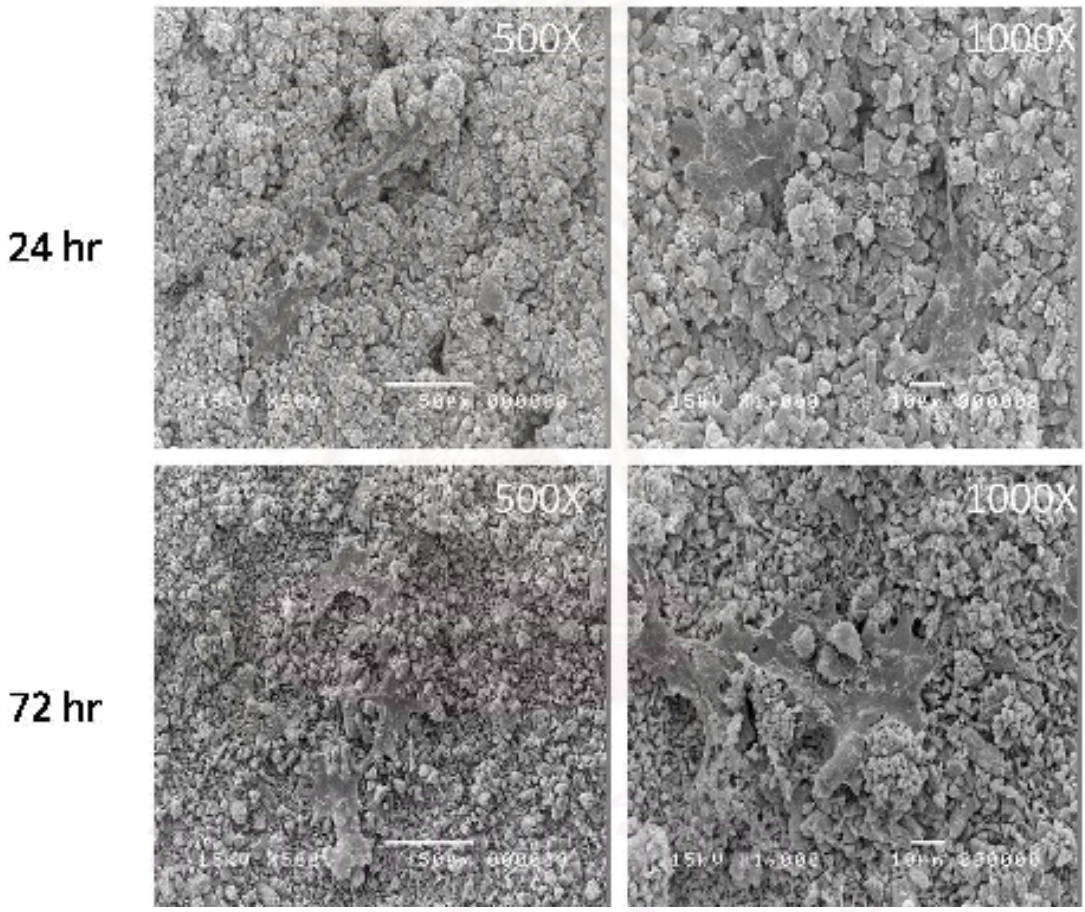

Figure 14. Scanning electron micrographs of HOB1 attached to White ProRoot® MTA at 24- and 72-hour incubation (magnification 500X, 1000X)

Human alveolar bone osteoblasts dispersed and attached on Kilan similar to cells on white ProRoot® MTA. Cell morphology on Kilan was similar to that seen on the white ProRoot® MTA. The cells on Kilan attached and spread well on the surface in 24 and 72 hours (Figure 15).

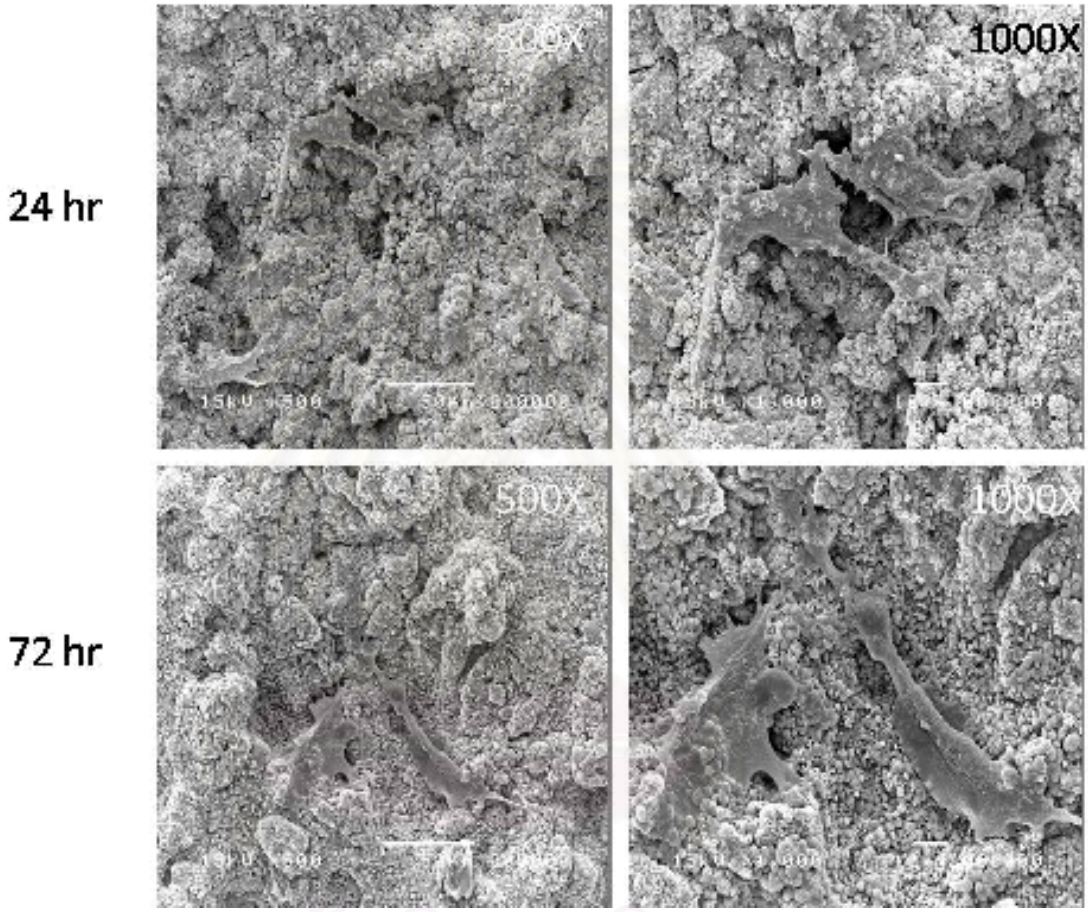

Figure 15. Scanning electron micrographs of HOB1 attached to Kilan at 24- and 72-hour incubation (magnification 500X, 1000X)

The dispersion and attachment of the human alveolar bone osteoblasts observed on Chang were similar to that on white ProRoot®MTA or Kilan. The morphology of cells on Chang was similar to white ProRoot®MTA or Kilan but they were more extended on Chang than those on white ProRoot®MTA or Kilan at 24 hours. They attached well on Chang surface with lamellipodia and filopodia (Figure 16). Moreover, at 72 hours, we observed that the surface of Chang had more cell number than the surface of White ProRoot®MTA or Kilan.

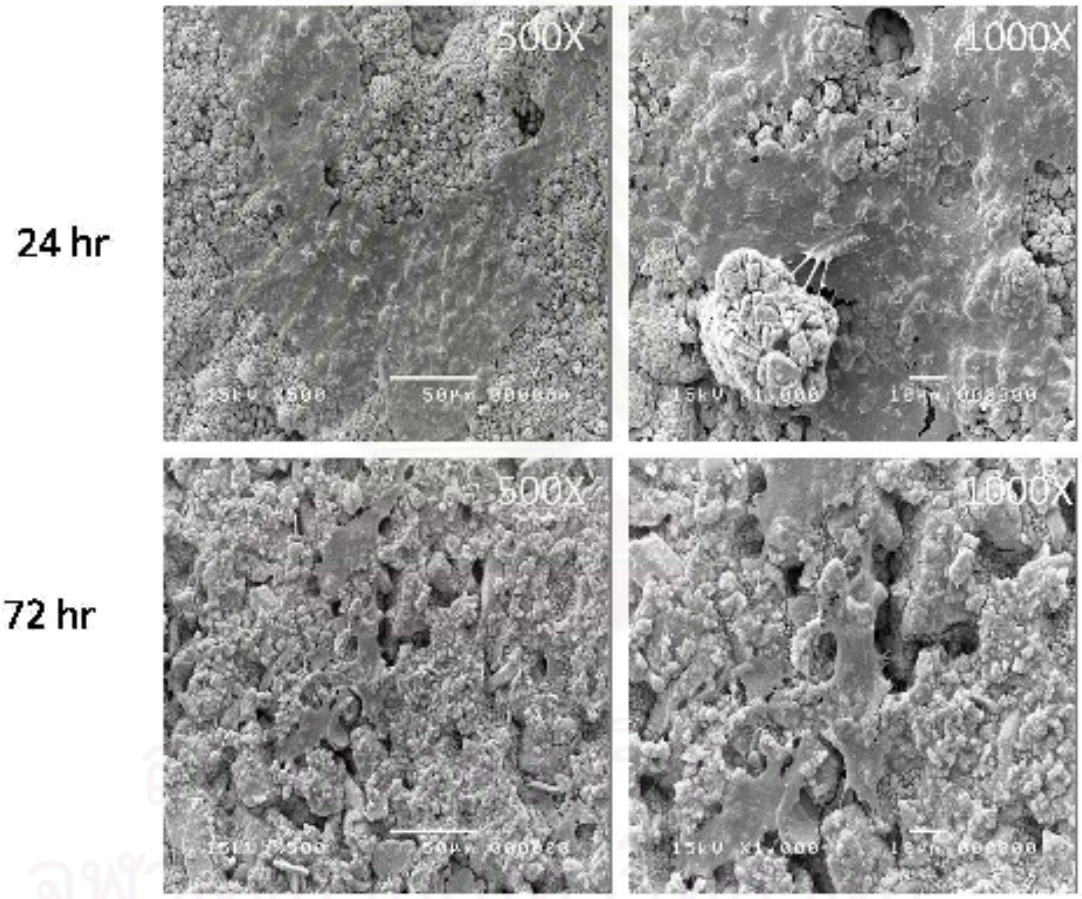

Figure 16. Scanning electron micrographs of HOB1 attached to Chang at 24- and 72-hour incubation (magnification 500X, 1000X)

The morphology and attachment of human osteoblasts observed on IRM® was different from that seen on others. The cells were round and poorly attached to IRM surface within both 24 and 72 hours (Figure 17). This morphology showed that IRM® was toxic to primary human alveolar bone osteoblasts.

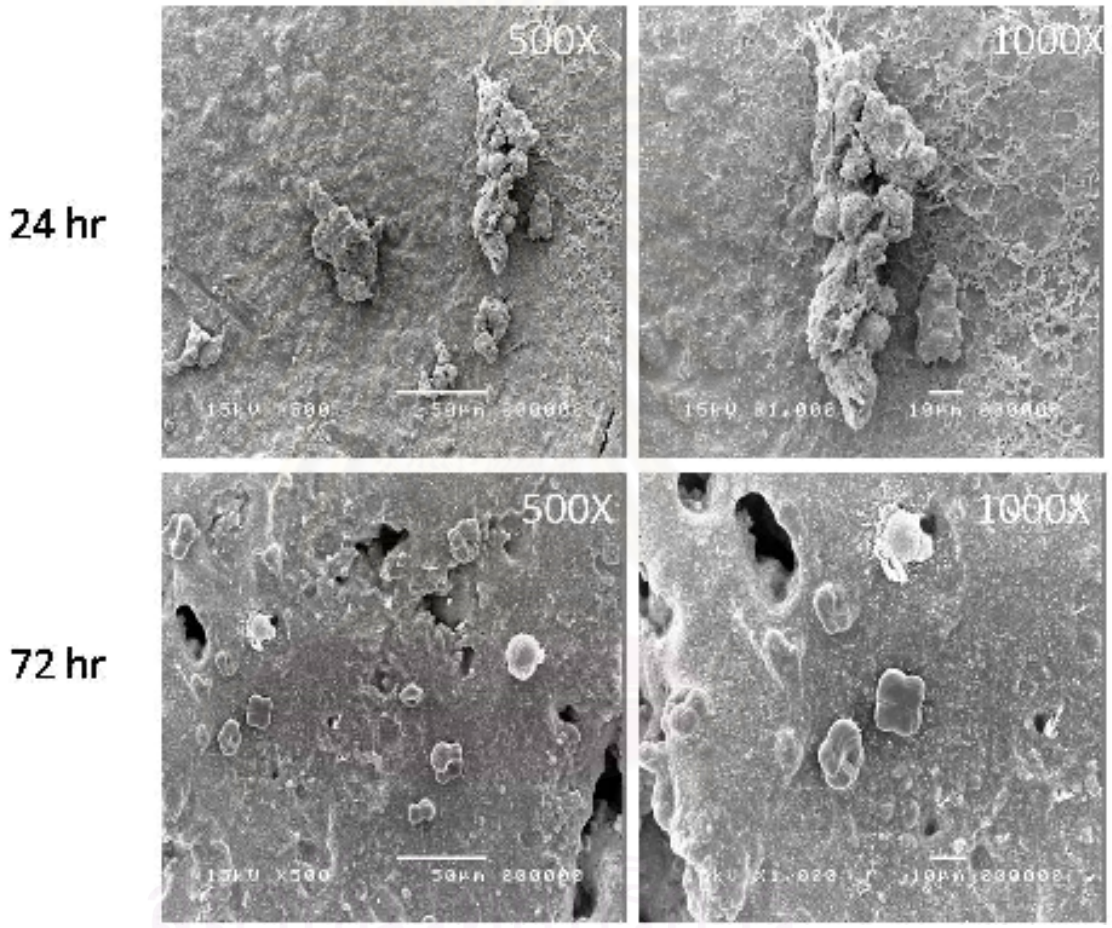

Figure 17. Scanning electron micrographs of HOB1 attached to IRM® at 24- and 72-hour incubation (magnification 500X, 1000X)

CHAPTER V

DISCUSSION AND CONCLUSION

There are several *in vitro* studies about biocompatibility of MTA [13, 41, 47, 53, 68]. Most studies used established cell lines because they are highly proliferative, easily available, and more reproducible than primary cell lines. However, established cell line and primary cell lines have some different biological properties. For example rat calvaria primary osteoblasts cultured in medium with β-glycerophosphate and dexamethasone could produce mineralized nodules but MG-63 which is an osteosarcoma cell line could not [65]. In addition, ISO recommended using primary cell strains derived from living tissues for specific sensitivity testing to simulate the *in vivo* situation. Osteoblast is one of the important cells for periradicular tissue healing after endodontic surgery. Therefore, primary human alveolar bone osteoblasts were used in this study to test the cytotoxicity effect of retrofilling materials. The results of RT-PCR, alkaline phosphatase activity assay and Alizarin red S staining showed that all primary cell strains of this study expressed osteoblastic markers and can produce mineralized nodules when cultured in medium with ascorbic acid and β-glycerophosphate. This means that all primary human osteoblast strains were osteoblasts. However, each strain showed different pattern of mRNA expression. This demonstrated that there are some biological differences among three strains which were used in this study.

In this study, we used primary human alveolar bone osteoblasts from three people. The cell viability results of each cell line were different. One possible reason was each cell line had different genetic backgrounds which influence cell response to material extracts. The difference of osteoblastic marker expression between primary cell lines showed that each cell line was at different stage of differentiation which affects cell proliferation and cell response to material extracts. RT-PCR showed that HOB3 expressed high ALP earlier than HOB1 and HOB2 corresponding to the results of ALP activity and mineralized nodule formation which showed that HOB3 started to produce ALP and nodules at day 3 while HOB1 and HOB2 started at day 14. These results

implied that HOB3 was later differentiation stage than HOB1 and HOB2. The differentiation stage of osteoblast may due to type of bone. Magnusson et al. reported that osteocalcin content of cortical bone from diaphysis was more than 3-fold greater than that of trabecular bone from diaphysis and greater trochanter [69]. Osteocalcin was osteoblastic marker of late differentiation [70] therefore, cortical bone may have late differentiated osteoblasts more than trabecular bone. HOB3 was obtained from cortical bone and expressed osteocalcin at day 3 more than HOB1 and HOB2 which were obtained from trabecular bone. However, one study showed that the osteoblasts from maxilla and mandible were not different in cell proliferation and expression of cell differentiation markers such as collagen I, ALP, and osteocalcin [71]. Gender may be one factor that affects cell viability results. The previous studies showed that the number of cell from iliac crest and long bone increased significantly with time in culture and were significantly higher in women than in men [72] as our observation that HOB1 and HOB2, collected from females, grew faster than HOB 3 which collected from male. In this study, most of the results from MTT assay of HOB1 and HOB2 were more similar comparing to HOB3 which could be due to their differentiation stage, gender of the donor, and type of the bone sample.

The cytotoxicity of material extracts was compared at different time points (day 1, 3, 7 and 14) by MTT assay. For day 1 extracts, at 24-hour incubation all materials were the highest toxic comparing to other days especially for Kilan in HOB1 and HOB3. This result was in agreement with Kim et al.'s study which tested in virus transfected human periodontal ligament cells and Guven et al.'s studies which tested in primary human gingival fibroblasts. These studies showed that ProRoot® MTA and Portland cement mixed with bismuth oxide was toxic at day 1 [54, 73].

Cell viability at 24-hour incubation was used to assess toxicity of material extracts while cell viability at 72-hour incubation was used to assess recovery of cell activity after exposing to material extract. In all primary cell lines, percent cell viability of day 1 Chang extract and white ProRoot® MTA extract were not significantly different in

24-hour incubation. This showed that the toxicity of day 1 Chang extract and white ProRoot® MTA extract were not different. For Kilan, day 1 extract was significantly more toxic than day 1 white ProRoot® MTA extract at 24-hour incubation. Although percent cell viability of HOB2 was high at 24-hour incubation, it obviously decreased at 72-hour incubation. These results showed that at day 1 Kilan extract was more toxic than white ProRoot® MTA extract. The significantly more toxicity at 24-hour incubation of day 1 Kilan when compared to white ProRoot® MTA was similar to the result of Kim et al.'s study in PDL cells which showed that Portland cement mixed with bismuth oxide was more toxic than ProRoot® MTA at 24-hour incubation. [54]. The reasons why Kilan has much more toxic than Chang at day 1 may be the differences of pH and final setting time. From the previous study, the pH of Kilan at the first 20 min was higher than that of Chang [30] thus, high alkaline from day 1 Kilan extract may affect cell viability. In addition, Kilan had about 30 min longer final setting time than Chang [30] as a result Kilan may release toxic substance for a longer period of time than Chang.

At 72-hour incubation, percents cell viability of day 1 Chang and Kilan extracts were significantly less than that of white ProRoot® MTA with only exception in HOB3 that percents cell viability of Chang and Kilan extracts were significantly more than that of white ProRoot® MTA. This means that HOB3 responded to day 1 material extracts different from other cell lines as a result, the cell activity of HOB3 at 72-hour incubation was different from those of HOB1 and HOB2.

In every cell lines, percents cell viability of day 3 Chang and Kilan extracts were higher than those of day 1 at both 24- and 72-hour incubations. Percents cell viability of all day 3 material extracts were nearly that of culture medium at 24-hour incubation and more than 100% at 72-hour incubation only exception in HOB3. The results showed that Chang and Kilan at day 3 were not more toxic than white ProRoot® MTA and may also encourage cell proliferation. This is in agreement with the Camilleri et al.'s result which showed that day 3 white Portland cement without gypsum mixed with bismuth oxide extract enhanced cell activity compared with the control medium [23]. For white

ProRoot® MTA, percent cell viability of day 3 extract was more than 85% which is in agreement with Vajrabhaya et al.'s study that test in primary PDL cell (89.10%) [46].

For day 7 extract, Chang and Kilan were not more toxic than white ProRoot® MTA. Percents cell viability of Chang and Kilan extracts were nearly that of culture medium at 24-hour incubation. In addition, most percents cell viability of Chang and Kilan extracts were more than 100% at 72-hour incubation. This showed that at day 7 Chang and Kilan were not toxic and may also encourage cell proliferation. The only previous study that tested cytotoxicity of MTA extract more than 3 days is Camilleri et al.'s. They reported that day 7 white Portland cement without gypsum mixed with bismuth oxide extract was toxic and had less viable cells than white ProRoot® MTA extract at 24-hour incubation. However, at 72-hour incubation, viable cells increased to nearly those of control medium [23]. The difference of their results and the present study may due to gypsum which was in our white Portland cements but not in white Portland cement of Camilleri et al's. Calcium sulfate (gypsum), one of ingredients in white Portland cement, can retard setting time of cement [74]. Thus, our white Portland cements set slower than that of Camilleri et al. [30, 74]. The slower setting of our materials may lead to more releasing of calcium ions which increase cell proliferation [75]. In addition, gypsum also releases calcium ions. The effect of calcium ions from gypsum was proved by Lazary et al. [76]. Their study showed that mouse pre-osteoblastic cell (MC3T3-E1) proliferation on gypsum was increased by almost 2 folds compared to cells grew on culture plate. Moreover, when cultured on gypsum, they exhibited an increased ALP activity [76].

There were no differences of percent cell viability of all material extracts at day 14 only exception in HOB3 that white ProRoot® MTA extract had more percent cell viability than Kilan extract at 72-hour incubation. However, percents HOB3 viability of day 14 extracts of all materials were more than 100%. All material extracts were not toxic and may promote proliferation at day 14.

All materials had the highest toxicity in the first day. However, the percents cell viability of all material extracts were more than 90 and the most differences between percents cell viability were not exceed than 10%. These results demonstrated that only exception for day 1 extract, Chang, Kilan and white ProRoot® MTA was not toxic. This showed that our two Thai white Portland cement mixed with bismuth oxide were not more toxic than white ProRoot® MTA at days 3, 7 and 14. The cause of difference of cell viability between our white Portland cements mixed with bismuth oxide and white ProRoot® MTA may be due to their hydration reaction. Perhaps the hydration reaction of Chang and Kilan was different from that of white ProRoot® MTA at different time points.

In this study, we found that material extracts promote cell proliferation. At 72-hour incubation, viable cells of days 3, 7 and 14 extracts of all materials in HOB1 and those of day 3 extracts of Chang and Kilan in HOB2 were higher than that of culture medium. The reason for this may be due to the hydration product of MTA and Portland cement, calcium hydroxide, which chemically decomposed into calcium and hydroxyl ions [77]. The enhancement of cell proliferation by continuous release of calcium ions from MTA was confirmed by Takita et al. They reported that the increasing of human dental pulp cell proliferation when increased concentration of calcium ions by releasing from MTA or adding calcium chloride as a source of calcium ions [75]. Park et al. showed that calcium ions accelerate osteoblast proliferation and increase ALP, OPN and OCN mRNA levels when cells grown on calcium ion incorporated titanium surface than on untreated titanium surface [78]. The change of osteoblast metabolism after contact with MTA or MTA product was confirmed by previous studies. Huang et al. reported that in MTA group, extracellular regulated kinase (ERK)-1 and -2 which were involved in the osteoblast proliferation and differentiation were more than those in control group [79]. In agreement with Tani-Ishii et al.'s study, it showed that in the presence of MTA, osteoblasts grew faster and produced more OCN and COLI which involved in mineralization [68]. These studies showed that MTA and Portland cements allow cell growth and the expression of matrix proteins involved in mineralization.

Besides the extract tests, scanning electron microscope was used to investigate cell morphology and attachment of human alveolar bone osteoblasts in contact with two Thai White Portland cements with bismuth oxide and white ProRoot® MTA. Scanning electron microscope is the most frequently used method to evaluate the material effect on cell proliferation and viability. Moreover, adhesion and spreading of the cells on a materials surface are the initial phase of cellular function which shows that material is biocompatible. The morphology of cell and the attachment to materials will exhibit the biocompatibility or toxicity [56, 58].

We used SEM to compare cell morphology and attachment of primary human osteoblast on material surface between three experimental materials in qualitative aspect. HOB1, HOB2 and HOB3 exhibited the osteoblastic marker and the ability to produce mineralized nodules. This showed that all strains were active osteoblasts. However, HOB1 had more cell number than other strains which adequate for SEM procedure. Thus, we used only HOB1 for the sample to test cell morphology and attachment on materials.

Material evaluation by SEM revealed that surfaces of three materials were different. The crystals of white ProRoot® MTA were various shapes such as bead, oval, clubbing or round. The crystal character of Kilan was similar to white ProRoot® MTA but it had no clubbing shaped crystals. Chang was different from others. It had only small round crystals. Although the surfaces of these materials were different, the previous study reported that these materials had similar chemical elements [30].

When contact to white ProRoot® MTA, human alveolar bone osteoblasts were polygonal in shape and could attach on material surface within 24 hours and more spreading in 72 hours. Likewise, the earlier studies with SaOS-2 showed that cells could attach MTA surface within 24 hours [27, 58]. In addition, Al-Rabeah et al. reported that human alveolar bone osteoblasts well attached and spread out over grey and white MTA surface within 24 hours and their processes also interacted with adjacent cells [41].

Human alveolar bone osteoblast morphology on two Thai white Portland cement mixed with bismuth oxide were polygonal shape which was similar to that on white ProRoot® MTA. However, we observed that the cells on Chang spread better than those on Kilan and white ProRoot® MTA at 24 hours. The cell attachment on Kilan and Chang was similar to those on white ProRoot® MTA. The result was in agreement with Abdullah et al.'s study which showed that SaOS-2 could attach and spread on MTA and Portland cement within 12, 24, 48 and 72 hours [27]. Furthermore, Gandolfi et al. showed that SaOS-2 was polygonal and attached well on tetrasilicate cement which made from white Portland cement and bismuth oxide in 72 hours [48]. The cell morphology and well attachment of human alveolar bone osteoblasts on Chang, Kilan and white ProRoot® MTA both at 24- and 72-hour incubations showed that these materials were biocompatible. Human alveolar bone osteoblasts could attach and dispersed thoughout the three experimental material surfaces.

SEM also showed that at 72 hours Chang had more cell density than Kilan and white ProRoot® MTA which was in the same result of cell viability by MTT assay of day 3 Chang extract. It is possible that Chang may release more calcium ions than others at day 3. Chang, Kilan and white ProRoot® MTA were less toxic than IRM® which showed marked rounding of the cells and depletion of cell numbers. The cell morphology and attachment on IRM® were similar to those of other studies [10, 58, 80]. The toxic component of IRM® is eugenol [81].

There were some limitations in the present study. First, this was an *in vitro* experiment using primary human alveolar bone osteoblasts, the results from this study can only assess the cytotoxicity of two Thai white Portland cements mixed with bismuth oxide and white ProRoot® MTA on human alveolar bone osteoblasts. Postsurgical healing is complex and involved both cellular and extracellular events. Second, Due to the limitation of time, the number of bone samples investigated in this study was limited, thus the results of this study cannot be completely represent the total population. Further *in vitro* experiment using other periradicular cells such as cementoblast, PDL cells was

recommended. *In vivo* and clinical investigations are needed before these Thai white Portland cements mixed with bismuth oxide can be used in clinical practice.

In conclusion, the results of this study demonstrate that two Thai white Portland cements mixed with bismuth oxide were not more toxic than white ProRoot® MTA to primary human alveolar bone osteoblasts at days 3, 7 and 14. However, at day 1, Kilan was more toxic than white ProRoot® MTA and Chang. The primary human alveolar bone osteoblasts could attach to Chang and Kilan in a similar fashion to white ProRoot® MTA.

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APPENDICES

Table 1. MTT assay results for cytotoxicity of extracted medium from materials to human alveolar bone osteoblasts line 1 (HOB 1) for 24 hours

	% Cell viability (mean ± SD)			
Extract day / Material	1 day	3 day	7 day	14 day
MTA	93.98 ± 2.83	90.43 ± 8.36	91.72 ± 4.34	99.26 ± 4.46
Chang	90.62 ± 3.26	100.31 ± 6.81	100.60 ± 7.90	99.24 ± 3.67
Kilan	12.81 ± 5.74	97.46 ± 4.54	93.57 ± 9.41	94.05 ± 3.86

Table 2. MTT assay results for cytotoxicity of extracted medium from materials to human alveolar bone osteoblasts line 2 (HOB 2) for 24 hours

	% Cell viability (mean ± SD)			
Extract day / Material	1 day	3 day	7 day	14 day
MTA	82.58 ± 4.26	90.67 ± 5.09	92.89 ± 2.68	92.67 ± 6.42
Chang	88.76 ± 5.35	98.42 ± 4.83	90.85 ± 5.93	95.17 ± 5.12
Kilan	93.01 ± 9.80	99.66 ± 4.00	100.58 ± 2.93	92.52 ± 6.43

Table 3. MTT assay results for cytotoxicity of extracted medium from materials to human alveolar bone osteoblasts line 3 (HOB 3) for 24 hours

	% Cell viability (mean ± SD)			
Extract day / Material	1 day	3 day	7 day	14 day
MTA	64.57 ± 8.09	94.31 ± 9.73	88.11 ± 9.22	106.75 ± 10.15
Chang	70.08 ± 15.40	94.11 ± 11.05	102.59 ± 9.54	108.11 ± 8.71
Kilan	51.6 ± 6.30	102.09 ± 6.08	105.47 ± 13.40	93.18 ± 11.11

Table 4. MTT assay results for cytotoxicity of extracted medium from materials to human alveolar bone osteoblasts line 1 (HOB 1) for 72 hours

	% Cell viability (mean ± SD)			
Extract day / Material	1 day	3 day	7 day	14 day
MTA	110.82 ± 3.18	119.64 ± 11.39	114.11 ± 6.15	103.32 ± 10.45
Chang	99.39 ± 4.62	131.66 ± 3.29	126.63 ± 6.42	108.75 ± 9.53
Kilan	7.38 ± 1.61	131.03 ± 4.87	120.54 ± 3.34	111.38 ± 6.06

Table 5. MTT assay results for cytotoxicity of extracted medium from materials to human alveolar bone osteoblasts line 2 (HOB 2) for 72 hours

	% Cell viability (mean ± SD)			
Extract day / Material	1 day	3 day	7 day	14 day
MTA	105.79 ± 4.01	98.69 ± 4.48	93.22 ± 4.05	93.53 ± 2.06
Chang	85.43 ± 2.75	120.58 ± 2.32	92.03 ± 4.58	93.3 ± 4.18
Kilan	61.16 ± 16.12	112.16 ± 4.95	104.95 ± 4.53	90.73 ± 4.76

Table 6. MTT assay results for cytotoxicity of extracted medium from materials to human alveolar bone osteoblasts line 3 (HOB 3) for 72 hours

	% Cell viability (mean ± SD)			
Extract day / Material	1 day	3 day	7 day	14 day
MTA	50.77 ± 3.23	85.74 ± 2.77	93.10 ± 10.66	109.87 ± 4.17
Chang	56.85 ± 3.58	74.86 ± 17.82	102.89 ± 7.06	105.63 ± 3.62
Kilan	57.83 ± 4.73	78.93 ± 13.71	105.35 ± 5.13	102.56 ± 2.32

Table 7. Normal distribution of MTT assay results for cytotoxicity of extracted medium from materials to human alveolar bone osteoblasts line 1 (HOB 1)

NPar Tests White ProRoot MTA & 24-hour incubation

One-Sample Kolmogorov-Smirnov Test

Material	Extract day	Incubate time			% cell viability
White ProRoot MTA	1 day	24 hrs	N		8
			Normal Parameters[a,b]	Mean	93.9811
				Std. Deviation	2.82702
			Most Extreme Differences	Absolute	.188
				Positive	.140
				Negative	-.188
			Kolmogorov-Smirnov Z		.532
			Asymp. Sig. (2-tailed)		.940
	3 day	24 hrs	N		8
			Normal Parameters[a,b]	Mean	90.4292
				Std. Deviation	8.35959
			Most Extreme Differences	Absolute	.238
				Positive	.180
				Negative	-.238
			Kolmogorov-Smirnov Z		.674
			Asymp. Sig. (2-tailed)		.754
	7 day	24 hrs	N		8
			Normal Parameters[a,b]	Mean	91.7186
				Std. Deviation	4.33926
			Most Extreme Differences	Absolute	.185
				Positive	.155
				Negative	-.185
			Kolmogorov-Smirnov Z		.524
			Asymp. Sig. (2-tailed)		.947
	14 day	24 hrs	N		8
			Normal Parameters[a,b]	Mean	99.2604
				Std. Deviation	4.46010
			Most Extreme Differences	Absolute	.215
				Positive	.215
				Negative	-.200
			Kolmogorov-Smirnov Z		.607
			Asymp. Sig. (2-tailed)		.854

a. Test distribution is Normal.

b. Calculated from data.

NPar Tests Chang & 24-hour incubation

One-Sample Kolmogorov-Smirnov Test

Material	Extract day	Incubate time			% cell viability
Chang	1 day	24 hrs	N		8
			Normal Parameters [a,b]	Mean	90.6238
				Std. Deviation	3.25831
			Most Extreme Differences	Absolute	.199
				Positive	.123
				Negative	-.199
			Kolmogorov-Smirnov Z		.564
			Asymp. Sig. (2-tailed)		.908
	3 day	24 hrs	N		8
			Normal Parameters [a,b]	Mean	100.3065
				Std. Deviation	6.80677
			Most Extreme Differences	Absolute	.266
				Positive	.153
				Negative	-.266
			Kolmogorov-Smirnov Z		.752
			Asymp. Sig. (2-tailed)		.623
	7 day	24 hrs	N		8
			Normal Parameters [a,b]	Mean	100.5985
				Std. Deviation	7.89518
			Most Extreme Differences	Absolute	.269
				Positive	.167
				Negative	-.269
			Kolmogorov-Smirnov Z		.762
			Asymp. Sig. (2-tailed)		.607
	14 day	24 hrs	N		8
			Normal Parameters [a,b]	Mean	99.2361
				Std. Deviation	3.67141
			Most Extreme Differences	Absolute	.205
				Positive	.205
				Negative	-.140
			Kolmogorov-Smirnov Z		.580
			Asymp. Sig. (2-tailed)		.889

a. Test distribution is Normal.

b. Calculated from data.

NPar Tests Kilan & 24-hour incubation

One-Sample Kolmogorov-Smirnov Test

Material	Extract day	Incubate time			% cell viability
Kilan	1 day	24 hrs	N		7
			Normal Parameters [a,b]	Mean	12.8121
				Std. Deviation	5.73901
			Most Extreme Differences	Absolute	.232
				Positive	.232
				Negative	-.156
			Kolmogorov-Smirnov Z		.614
			Asymp. Sig. (2-tailed)		.845
	3 day	24 hrs	N		8
			Normal Parameters [a,b]	Mean	97.4601
				Std. Deviation	4.54275
			Most Extreme Differences	Absolute	.295
				Positive	.170
				Negative	-.295
			Kolmogorov-Smirnov Z		.834
			Asymp. Sig. (2-tailed)		.491
	7 day	24 hrs	N		8
			Normal Parameters [a,b]	Mean	93.5675
				Std. Deviation	9.40897
			Most Extreme Differences	Absolute	.233
				Positive	.187
				Negative	-.233
			Kolmogorov-Smirnov Z		.659
			Asymp. Sig. (2-tailed)		.778
	14 day	24 hrs	N		8
			Normal Parameters [a,b]	Mean	94.0541
				Std. Deviation	3.85730
			Most Extreme Differences	Absolute	.220
				Positive	.149
				Negative	-.220
			Kolmogorov-Smirnov Z		.621
			Asymp. Sig. (2-tailed)		.835

a. Test distribution is Normal.

b. Calculated from data.

NPar Tests White ProRoot MTA & 72-hour incubation

One-Sample Kolmogorov-Smirnov Test

Material	Extract day	Incubate time			% cell viability
White ProRoot MTA	1 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	110.8223
				Std. Deviation	3.18442
			Most Extreme Differences	Absolute	.208
				Positive	.144
				Negative	-.208
			Kolmogorov-Smirnov Z		.589
			Asymp. Sig. (2-tailed)		.878
	3 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	119.6443
				Std. Deviation	11.39113
			Most Extreme Differences	Absolute	.303
				Positive	.155
				Negative	-.303
			Kolmogorov-Smirnov Z		.856
			Asymp. Sig. (2-tailed)		.456
	7 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	114.1054
				Std. Deviation	6.14724
			Most Extreme Differences	Absolute	.162
				Positive	.138
				Negative	-.162
			Kolmogorov-Smirnov Z		.458
			Asymp. Sig. (2-tailed)		.985
	14 day	72 hrs	N		7
			Normal Parameters[a,b]	Mean	103.3243
				Std. Deviation	10.44576
			Most Extreme Differences	Absolute	.224
				Positive	.224
				Negative	-.166
			Kolmogorov-Smirnov Z		.593
			Asymp. Sig. (2-tailed)		.873

a. Test distribution is Normal.

b. Calculated from data.

NPar Tests Chang & 72-hour incubation

One-Sample Kolmogorov-Smirnov Test

Material	Extract day	Incubate time			% cell viability
Chang	1 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	99.3871
				Std. Deviation	4.62460
			Most Extreme Differences	Absolute	.190
				Positive	.190
				Negative	-.175
			Kolmogorov-Smirnov Z		.538
			Asymp. Sig. (2-tailed)		.934
	3 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	131.6601
				Std. Deviation	3.29241
			Most Extreme Differences	Absolute	.240
				Positive	.240
				Negative	-.199
			Kolmogorov-Smirnov Z		.680
			Asymp. Sig. (2-tailed)		.745
	7 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	126.6349
				Std. Deviation	6.42498
			Most Extreme Differences	Absolute	.179
				Positive	.179
				Negative	-.134
			Kolmogorov-Smirnov Z		.507
			Asymp. Sig. (2-tailed)		.959
	14 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	108.7452
				Std. Deviation	9.52657
			Most Extreme Differences	Absolute	.276
				Positive	.276
				Negative	-.150
			Kolmogorov-Smirnov Z		.779
			Asymp. Sig. (2-tailed)		.578

a. Test distribution is Normal.

b. Calculated from data.

NPar Tests Kilan & 72-hour incubation

One-Sample Kolmogorov-Smirnov Test

Material	Extract day	Incubate time			% cell viability
Kilan	1 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	7.3792
				Std. Deviation	1.61163
			Most Extreme Differences	Absolute	.342
				Positive	.342
				Negative	-.173
			Kolmogorov-Smirnov Z		.967
			Asymp. Sig. (2-tailed)		.307
	3 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	131.0347
				Std. Deviation	4.86789
			Most Extreme Differences	Absolute	.217
				Positive	.201
				Negative	-.217
			Kolmogorov-Smirnov Z		.613
			Asymp. Sig. (2-tailed)		.846
	7 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	120.5376
				Std. Deviation	3.33986
			Most Extreme Differences	Absolute	.202
				Positive	.202
				Negative	-.113
			Kolmogorov-Smirnov Z		.572
			Asymp. Sig. (2-tailed)		.900
	14 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	111.3806
				Std. Deviation	6.05756
			Most Extreme Differences	Absolute	.198
				Positive	.109
				Negative	-.198
			Kolmogorov-Smirnov Z		.561
			Asymp. Sig. (2-tailed)		.911

a. Test distribution is Normal.

b. Calculated from data.

Table 8. Normal distribution of MTT assay results for cytotoxicity of extracted medium from materials to human alveolar bone osteoblasts line 2 (HOB 2)

NPar Tests White ProRoot MTA & 24-hour incubation

One-Sample Kolmogorov-Smirnov Test

Material	Extract day	Incubate time			% cell viability
White ProRoot MTA	1 day	24 hrs	N		8
			Normal Parameters[a,b]	Mean	82.5845
				Std. Deviation	4.26178
			Most Extreme Differences	Absolute	.157
				Positive	.132
				Negative	-.157
			Kolmogorov-Smirnov Z		.445
			Asymp. Sig. (2-tailed)		.989
	3 day	24 hrs	N		8
			Normal Parameters[a,b]	Mean	90.6722
				Std. Deviation	5.09070
			Most Extreme Differences	Absolute	.158
				Positive	.118
				Negative	-.158
			Kolmogorov-Smirnov Z		.446
			Asymp. Sig. (2-tailed)		.989
	7 day	24 hrs	N		8
			Normal Parameters[a,b]	Mean	92.8883
				Std. Deviation	2.68041
			Most Extreme Differences	Absolute	.268
				Positive	.180
				Negative	-.268
			Kolmogorov-Smirnov Z		.758
			Asymp. Sig. (2-tailed)		.613
	14 day	24 hrs	N		8
			Normal Parameters[a,b]	Mean	92.6699
				Std. Deviation	6.41638
			Most Extreme Differences	Absolute	.212
				Positive	.212
				Negative	-.150
			Kolmogorov-Smirnov Z		.599
			Asymp. Sig. (2-tailed)		.865

a. Test distribution is Normal.

b. Calculated from data.

NPar Tests Chang & 24-hour incubation

One-Sample Kolmogorov-Smirnov Test

Material	Extract day	Incubate time			% cell viability
Chang	1 day	24 hrs	N		8
			Normal Parameters[a,b]	Mean	88.7626
				Std. Deviation	5.34749
			Most Extreme Differences	Absolute	.208
				Positive	.117
				Negative	-.208
			Kolmogorov-Smirnov Z		.587
			Asymp. Sig. (2-tailed)		.881
	3 day	24 hrs	N		8
			Normal Parameters[a,b]	Mean	98.4229
				Std. Deviation	4.82825
			Most Extreme Differences	Absolute	.195
				Positive	.195
				Negative	-.135
			Kolmogorov-Smirnov Z		.552
			Asymp. Sig. (2-tailed)		.920
	7 day	24 hrs	N		8
			Normal Parameters[a,b]	Mean	90.8495
				Std. Deviation	5.92920
			Most Extreme Differences	Absolute	.194
				Positive	.194
				Negative	-.166
			Kolmogorov-Smirnov Z		.550
			Asymp. Sig. (2-tailed)		.923
	14 day	24 hrs	N		8
			Normal Parameters[a,b]	Mean	95.1699
				Std. Deviation	5.11788
			Most Extreme Differences	Absolute	.153
				Positive	.136
				Negative	-.153
			Kolmogorov-Smirnov Z		.434
			Asymp. Sig. (2-tailed)		.992

a. Test distribution is Normal.

b. Calculated from data.

NPar Tests Kilan & 24-hour incubation

One-Sample Kolmogorov-Smirnov Test

Material	Extract day	Incubate time			% cell viability
Kilan	1 day	24 hrs	N		8
			Normal Parameters[a,b]	Mean	93.0086
				Std. Deviation	9.80412
			Most Extreme Differences	Absolute	.136
				Positive	.126
				Negative	-.136
			Kolmogorov-Smirnov Z		.385
			Asymp. Sig. (2-tailed)		.998
	3 day	24 hrs	N		8
			Normal Parameters[a,b]	Mean	99.6585
				Std. Deviation	4.00386
			Most Extreme Differences	Absolute	.223
				Positive	.223
				Negative	-.172
			Kolmogorov-Smirnov Z		.629
			Asymp. Sig. (2-tailed)		.823
	7 day	24 hrs	N		8
			Normal Parameters[a,b]	Mean	100.5825
				Std. Deviation	2.93381
			Most Extreme Differences	Absolute	.173
				Positive	.145
				Negative	-.173
			Kolmogorov-Smirnov Z		.490
			Asymp. Sig. (2-tailed)		.970
	14 day	24 hrs	N		8
			Normal Parameters[a,b]	Mean	92.5243
				Std. Deviation	6.42665
			Most Extreme Differences	Absolute	.305
				Positive	.161
				Negative	-.305
			Kolmogorov-Smirnov Z		.863
			Asymp. Sig. (2-tailed)		.445

a. Test distribution is Normal.

b. Calculated from data.

NPar Tests White ProRoot MTA & 72-hour incubation

One-Sample Kolmogorov-Smirnov Test

Material	Extract day	Incubate time			% cell viability
White ProRoot MTA	1 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	105.7934
				Std. Deviation	4.00640
			Most Extreme Differences	Absolute	.181
				Positive	.172
				Negative	-.181
			Kolmogorov-Smirnov Z		.511
			Asymp. Sig. (2-tailed)		.956
	3 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	98.6930
				Std. Deviation	4.47734
			Most Extreme Differences	Absolute	.184
				Positive	.173
				Negative	-.184
			Kolmogorov-Smirnov Z		.522
			Asymp. Sig. (2-tailed)		.948
	7 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	93.2179
				Std. Deviation	4.04715
			Most Extreme Differences	Absolute	.123
				Positive	.102
				Negative	-.123
			Kolmogorov-Smirnov Z		.349
			Asymp. Sig. (2-tailed)		1.000
	14 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	93.5309
				Std. Deviation	2.06355
			Most Extreme Differences	Absolute	.179
				Positive	.123
				Negative	-.179
			Kolmogorov-Smirnov Z		.507
			Asymp. Sig. (2-tailed)		.960

a. Test distribution is Normal.

b. Calculated from data.

NPar Tests Chang & 72-hour incubation

One-Sample Kolmogorov-Smirnov Test

Material	Extract day	Incubate time			% cell viability
Chang	1 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	85.4348
				Std. Deviation	2.74810
			Most Extreme Differences	Absolute	.204
				Positive	.164
				Negative	-.204
			Kolmogorov-Smirnov Z		.578
			Asymp. Sig. (2-tailed)		.892
	3 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	120.5806
				Std. Deviation	2.31995
			Most Extreme Differences	Absolute	.151
				Positive	.102
				Negative	-.151
			Kolmogorov-Smirnov Z		.427
			Asymp. Sig. (2-tailed)		.993
	7 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	92.0284
				Std. Deviation	4.58480
			Most Extreme Differences	Absolute	.204
				Positive	.148
				Negative	-.204
			Kolmogorov-Smirnov Z		.578
			Asymp. Sig. (2-tailed)		.892
	14 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	93.3013
				Std. Deviation	4.18404
			Most Extreme Differences	Absolute	.240
				Positive	.194
				Negative	-.240
			Kolmogorov-Smirnov Z		.679
			Asymp. Sig. (2-tailed)		.746

a. Test distribution is Normal.

b. Calculated from data.

NPar Tests Kilan & 72-hour incubation

One-Sample Kolmogorov-Smirnov Test

Material	Extract day	Incubate time			% cell viability
Kilan	1 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	61.1595
				Std. Deviation	16.01783
			Most Extreme Differences	Absolute	.160
				Positive	.160
				Negative	-.146
			Kolmogorov-Smirnov Z		.452
			Asymp. Sig. (2-tailed)		.987
	3 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	112.1607
				Std. Deviation	4.94795
			Most Extreme Differences	Absolute	.178
				Positive	.170
				Negative	-.178
			Kolmogorov-Smirnov Z		.503
			Asymp. Sig. (2-tailed)		.962
	7 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	104.9457
				Std. Deviation	4.52729
			Most Extreme Differences	Absolute	.182
				Positive	.162
				Negative	-.182
			Kolmogorov-Smirnov Z		.516
			Asymp. Sig. (2-tailed)		.953
	14 day	72 hrs	N		8
			Normal Parameters[a,b]	Mean	90.7346
				Std. Deviation	4.75887
			Most Extreme Differences	Absolute	.170
				Positive	.170
				Negative	-.104
			Kolmogorov-Smirnov Z		.481
			Asymp. Sig. (2-tailed)		.975

a. Test distribution is Normal.

b. Calculated from data.

Table 9. Normal distribution of MTT assay results for cytotoxicity of extracted medium from materials to human alveolar bone osteoblasts line 3 (HOB 3)

NPar Tests White ProRoot MTA & 24-hour incubation

One-Sample Kolmogorov-Smirnov Test

Material	Extract day	Incubate time			% cell viability
White ProRoot MTA	1 day	24 hrs	N		6
			Normal Parameters [a,b]	Mean	64.5661
				Std. Deviation	8.08972
			Most Extreme Differences	Absolute	.241
				Positive	.139
				Negative	-.241
			Kolmogorov-Smirnov Z		.590
			Asymp. Sig. (2-tailed)		.878
	3 day	24 hrs	N		6
			Normal Parameters [a,b]	Mean	94.3096
				Std. Deviation	9.73470
			Most Extreme Differences	Absolute	.197
				Positive	.197
				Negative	-.147
			Kolmogorov-Smirnov Z		.482
			Asymp. Sig. (2-tailed)		.974
	7 day	24 hrs	N		6
			Normal Parameters [a,b]	Mean	88.1112
				Std. Deviation	9.22064
			Most Extreme Differences	Absolute	.158
				Positive	.158
				Negative	-.110
			Kolmogorov-Smirnov Z		.386
			Asymp. Sig. (2-tailed)		.998
	14 day	24 hrs	N		6
			Normal Parameters [a,b]	Mean	106.7493
				Std. Deviation	10.14964
			Most Extreme Differences	Absolute	.313
				Positive	.224
				Negative	-.313
			Kolmogorov-Smirnov Z		.768
			Asymp. Sig. (2-tailed)		.597

a. Test distribution is Normal.

b. Calculated from data.

NPar Tests Chang & 24-hour incubation

One-Sample Kolmogorov-Smirnov Test

Material	Extract day	Incubate time			% cell viability
Chang	1 day	24 hrs	N		6
			Normal Parameters[a,b]	Mean	70.0758
				Std. Deviation	15.40261
			Most Extreme Differences	Absolute	.327
				Positive	.213
				Negative	-.327
			Kolmogorov-Smirnov Z		.801
			Asymp. Sig. (2-tailed)		.542
	3 day	24 hrs	N		6
			Normal Parameters[a,b]	Mean	94.1087
				Std. Deviation	11.05225
			Most Extreme Differences	Absolute	.286
				Positive	.189
				Negative	-.286
			Kolmogorov-Smirnov Z		.701
			Asymp. Sig. (2-tailed)		.710
	7 day	24 hrs	N		6
			Normal Parameters[a,b]	Mean	102.5884
				Std. Deviation	9.54246
			Most Extreme Differences	Absolute	.191
				Positive	.191
				Negative	-.187
			Kolmogorov-Smirnov Z		.467
			Asymp. Sig. (2-tailed)		.981
	14 day	24 hrs	N		6
			Normal Parameters[a,b]	Mean	108.1124
				Std. Deviation	8.70903
			Most Extreme Differences	Absolute	.201
				Positive	.201
				Negative	-.119
			Kolmogorov-Smirnov Z		.493
			Asymp. Sig. (2-tailed)		.968

a. Test distribution is Normal.

b. Calculated from data.

NPar Tests Kilan & 24-hour incubation

One-Sample Kolmogorov-Smirnov Test

Material	Extract day	Incubate time			% cell viability
Kilan	1 day	24 hrs	N		6
			Normal Parameters[a,b]	Mean	51.5955
				Std. Deviation	6.29861
			Most Extreme Differences	Absolute	.235
				Positive	.206
				Negative	-.235
			Kolmogorov-Smirnov Z		.577
			Asymp. Sig. (2-tailed)		.894
	3 day	24 hrs	N		6
			Normal Parameters[a,b]	Mean	102.0862
				Std. Deviation	6.07771
			Most Extreme Differences	Absolute	.194
				Positive	.194
				Negative	-.176
			Kolmogorov-Smirnov Z		.475
			Asymp. Sig. (2-tailed)		.977
	7 day	24 hrs	N		6
			Normal Parameters[a,b]	Mean	105.4723
				Std. Deviation	13.40052
			Most Extreme Differences	Absolute	.179
				Positive	.141
				Negative	-.179
			Kolmogorov-Smirnov Z		.439
			Asymp. Sig. (2-tailed)		.991
	14 day	24 hrs	N		6
			Normal Parameters[a,b]	Mean	93.1761
				Std. Deviation	11.11060
			Most Extreme Differences	Absolute	.295
				Positive	.295
				Negative	-.223
			Kolmogorov-Smirnov Z		.723
			Asymp. Sig. (2-tailed)		.672

a. Test distribution is Normal.

b. Calculated from data.

NPar Tests White ProRoot MTA & 72-hour incubation

One-Sample Kolmogorov-Smirnov Test

Material	Extract day	Incubate time			% cell viability
White ProRoot MTA	1 day	72 hrs	N		6
			Normal Parameters[a,b]	Mean	50.7716
				Std. Deviation	3.22572
			Most Extreme Differences	Absolute	.222
				Positive	.167
				Negative	-.222
			Kolmogorov-Smirnov Z		.543
			Asymp. Sig. (2-tailed)		.929
	3 day	72 hrs	N		6
			Normal Parameters[a,b]	Mean	85.7377
				Std. Deviation	2.76581
			Most Extreme Differences	Absolute	.153
				Positive	.153
				Negative	-.119
			Kolmogorov-Smirnov Z		.375
			Asymp. Sig. (2-tailed)		.999
	7 day	72 hrs	N		6
			Normal Parameters[a,b]	Mean	93.1000
				Std. Deviation	10.65781
			Most Extreme Differences	Absolute	.396
				Positive	.223
				Negative	-.396
			Kolmogorov-Smirnov Z		.970
			Asymp. Sig. (2-tailed)		.304
	14 day	72 hrs	N		6
			Normal Parameters[a,b]	Mean	109.8747
				Std. Deviation	4.16564
			Most Extreme Differences	Absolute	.307
				Positive	.280
				Negative	-.307
			Kolmogorov-Smirnov Z		.751
			Asymp. Sig. (2-tailed)		.626

a. Test distribution is Normal.

b. Calculated from data.

NPar Tests Chang & 72-hour incubation

One-Sample Kolmogorov-Smirnov Test

Material	Extract day	Incubate time			% cell viability
Chang	1 day	72 hrs	N		6
			Normal Parameters [a,b]	Mean	56.8478
				Std. Deviation	3.57891
			Most Extreme Differences	Absolute	.201
				Positive	.181
				Negative	-.201
			Kolmogorov-Smirnov Z		.492
			Asymp. Sig. (2-tailed)		.969
	3 day	72 hrs	N		6
			Normal Parameters [a,b]	Mean	74.8621
				Std. Deviation	17.81658
			Most Extreme Differences	Absolute	.241
				Positive	.185
				Negative	-.241
			Kolmogorov-Smirnov Z		.591
			Asymp. Sig. (2-tailed)		.876
	7 day	72 hrs	N		6
			Normal Parameters [a,b]	Mean	102.8853
				Std. Deviation	7.05738
			Most Extreme Differences	Absolute	.250
				Positive	.250
				Negative	-.208
			Kolmogorov-Smirnov Z		.612
			Asymp. Sig. (2-tailed)		.849
	14 day	72 hrs	N		6
			Normal Parameters [a,b]	Mean	105.6345
				Std. Deviation	3.61693
			Most Extreme Differences	Absolute	.253
				Positive	.253
				Negative	-.213
			Kolmogorov-Smirnov Z		.621
			Asymp. Sig. (2-tailed)		.836

a. Test distribution is Normal.

b. Calculated from data.

NPar Tests Kilan & 72-hour incubation

One-Sample Kolmogorov-Smirnov Test

Material	Extract day	Incubate time			% cell viability
Kilan	1 day	72 hrs	N		6
			Normal Parameters [a,b]	Mean	57.8264
				Std. Deviation	4.72513
			Most Extreme Differences	Absolute	.224
				Positive	.224
				Negative	-.217
			Kolmogorov-Smirnov Z		.548
			Asymp. Sig. (2-tailed)		.925
	3 day	72 hrs	N		6
			Normal Parameters [a,b]	Mean	78.9346
				Std. Deviation	13.70643
			Most Extreme Differences	Absolute	.261
				Positive	.208
				Negative	-.261
			Kolmogorov-Smirnov Z		.639
			Asymp. Sig. (2-tailed)		.809
	7 day	72 hrs	N		6
			Normal Parameters [a,b]	Mean	105.3549
				Std. Deviation	5.13214
			Most Extreme Differences	Absolute	.223
				Positive	.223
				Negative	-.140
			Kolmogorov-Smirnov Z		.545
			Asymp. Sig. (2-tailed)		.928
	14 day	72 hrs	N		6
			Normal Parameters [a,b]	Mean	102.5591
				Std. Deviation	2.31506
			Most Extreme Differences	Absolute	.325
				Positive	.325
				Negative	-.155
			Kolmogorov-Smirnov Z		.797
			Asymp. Sig. (2-tailed)		.549

a. Test distribution is Normal.

b. Calculated from data.

Table 10. Two-way ANOVA Results of relation between variable of cell viability test of HOB 1

Two-way ANOVA 24-hour incubation

Between-Subjects Factors

		Value Label	N
Material	1	White ProRoot MTA	32
	2	Kilan	31
	3	Chang	32
Extract day	1	1 day	23
	2	3 day	24
	3	7 day	24
	4	14 day	24

Tests of Between-Subjects Effects

Dependent Variable: % cell viability

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	45629.477(a)	11	4148.134	122.462	.000
Intercept	745918.764	1	745918.764	22021.240	.000
Material	9686.322	2	4843.161	142.981	.000
Extract	16214.072	3	5404.691	159.559	.000
Material * Extract	23136.788	6	3856.131	113.842	.000
Error	2811.434	83	33.873		
Total	808890.607	95			
Corrected Total	48440.911	94			

a R Squared = .942 (Adjusted R Squared = .934)

Two-way ANOVA 72-hour incubation

Between-Subjects Factors

		Value Label	N
Material	1	White ProRoot MTA	31
	2	Kilan	32
	3	Chang	32
Extract day	1	1 day	24
	2	3 day	24
	3	7 day	24
	4	14 day	23

Tests of Between-Subjects Effects

Dependent Variable: % cell viability

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	95966.096[a]	11	8724.191	202.983	.000
Intercept	1087282.907	1	1087282.907	25297.504	.000
Material	10368.887	2	5184.444	120.625	.000
Extract	42891.601	3	14297.200	332.649	.000
Material * Extract	42597.527	6	7099.588	165.184	.000
Error	3567.327	83	42.980		
Total	1189098.315	95			
Corrected Total	99533.423	94			

a. R Squared = .964 (Adjusted R Squared = .959)

Table 11. Two-way ANOVA Results of relation between variable of cell viability test of HOB 2

Two-way ANOVA 24-hour incubation

Between-Subjects Factors

		Value Label	N
Material	1	White ProRoot MTA	32
	2	Kilan	32
	3	Chang	32
Extract day	1	1 day	24
	2	3 day	24
	3	7 day	24
	4	14 day	24

Tests of Between-Subjects Effects

Dependent Variable: % cell viability

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2180.071[a]	11	198.188	6.467	.000
Intercept	832975.230	1	832975.230	27180.766	.000
Material	727.895	2	363.948	11.876	.000
Extract	903.871	3	301.290	9.831	.000
Material * Extract	548.305	6	91.384	2.982	.011
Error	2574.244	84	30.646		
Total	837729.545	96			
Corrected Total	4754.315	95			

a. R Squared = .459 (Adjusted R Squared = .388)

Two-way ANOVA 72-hour incubation

Between-Subjects Factors

		Value Label	N
Material	1	White ProRoot MTA	32
	2	Kilan	32
	3	Chang	32
Extract day	1	1 day	24
	2	3 day	24
	3	7 day	24
	4	14 day	24

Tests of Between-Subjects Effects

Dependent Variable: % cell viability

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	19509.191[a]	11	1773.563	49.221	.000
Intercept	884092.250	1	884092.250	24535.829	.000
Material	662.450	2	331.225	9.192	.000
Extract	8715.697	3	2905.232	80.628	.000
Material * Extract	10131.045	6	1688.507	46.860	.000
Error	3026.747	84	36.033		
Total	906628.188	96			
Corrected Total	22535.938	95			

a. R Squared = .866 (Adjusted R Squared = .848)

Table 12. Two-way ANOVA Results of relation between variable of cell viability test of HOB 3

Two-way ANOVA 24-hour Incubation

Between-Subjects Factors

		Value Label	N
Material	1	White ProRoot MTA	24
	2	Kilan	24
	3	Chang	24
Extract day	1	1 day	18
	2	3 day	18
	3	7 day	18
	4	14 day	18

Tests of Between-Subjects Effects

Dependent Variable: % cell viability

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	22321.988[a]	11	2029.272	19.416	.000
Intercept	584228.139	1	584228.139	5589.933	.000
Material	478.995	2	239.497	2.292	.110
Extract	19136.454	3	6378.818	61.033	.000
Material * Extract	2706.539	6	451.090	4.316	.001
Error	6270.860	60	104.514		
Total	612820.987	72			
Corrected Total	28592.848	71			

a. R Squared = .781 (Adjusted R Squared = .740)

Two-way ANOVA 72-hour incubation

Between-Subjects Factors

		Value Label	N
Material	1	White ProRoot MTA	24
	2	Kilan	24
	3	Chang	24
Extract day	1	1 day	18
	2	3 day	18
	3	7 day	18
	4	14 day	18

Tests of Between-Subjects Effects

Dependent Variable: % cell viability

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	29962.236[a]	11	2723.840	41.691	.000
Intercept	524686.056	1	524686.056	8030.747	.000
Material	23.631	2	11.815	.181	.835
Extract	28758.667	3	9586.222	146.725	.000
Material * Extract	1179.938	6	196.656	3.010	.012
Error	3920.079	60	65.335		
Total	558568.372	72			
Corrected Total	33882.316	71			

a. R Squared = .884 (Adjusted R Squared = .863)

Table 13. One-way ANOVA and Post hoc results of mean differences of percents cell viability between material extracts in HOB 1

Oneway Day 1 extract & 24-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
4.053	2	20	.033

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	30814.487	2	15407.243	939.816	.000
Within Groups	327.878	20	16.394		
Total	31142.365	22			

Robust Tests of Equality of Means

% cell viability

	Statistic[a]	df1	df2	Sig.
Brown-Forsythe	879.202	2	12.506	.000

a. Asymptotically F distributed.

Multiple Comparisons

Dependent Variable: % cell viability
Tamhane

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	81.16902*	2.38834	.000	74.0956	88.2425
	Chang	3.35734	1.52515	.130	-.7854	7.5000
Kilan	White ProRoot MTA	-81.16902*	2.38834	.000	-88.2425	-74.0956
	Chang	-77.81168*	2.45606	.000	-84.9511	-70.6722
Chang	White ProRoot MTA	-3.35734	1.52515	.130	-7.5000	.7854
	Kilan	77.81168*	2.45606	.000	70.6722	84.9511

*. The mean difference is significant at the .05 level.

Oneway Day 3 extract & 24-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
2.020	2	21	.158

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	413.598	2	206.799	4.533	.023
Within Groups	957.961	21	45.617		
Total	1371.559	23			

Multiple Comparisons

Dependent Variable: % cell viability
Bonferroni

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	-7.03095	3.37702	.149	-15.8158	1.7539
	Chang	-9.87738*	3.37702	.024	-18.6622	-1.0926
Kilan	White ProRoot MTA	7.03095	3.37702	.149	-1.7539	15.8158
	Chang	-2.84644	3.37702	1.000	-11.6313	5.9384
Chang	White ProRoot MTA	9.87738*	3.37702	.024	1.0926	18.6622
	Kilan	2.84644	3.37702	1.000	-5.9384	11.6313

*. The mean difference is significant at the .05 level.

Oneway Day 7 extract & 24-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
2.346	2	21	.120

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	351.215	2	175.608	3.105	.066
Within Groups	1187.841	21	56.564		
Total	1539.057	23			

Multiple Comparisons

Dependent Variable: % cell viability

Bonferroni

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	-1.84897	3.76045	1.000	-11.6312	7.9333
	Chang	-8.87991	3.76045	.084	-18.6622	.9023
Kilan	White ProRoot MTA	1.84897	3.76045	1.000	-7.9333	11.6312
	Chang	-7.03095	3.76045	.227	-16.8132	2.7513
Chang	White ProRoot MTA	8.87991	3.76045	.084	-.9023	18.6622
	Kilan	7.03095	3.76045	.227	-2.7513	16.8132

Oneway Day 14 extract & 24-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
1.056	2	21	.366

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	143.891	2	71.945	4.473	.024
Within Groups	337.754	21	16.084		
Total	481.645	23			

Multiple Comparisons

Dependent Variable: % cell viability
Bonferroni

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	5.20631	2.00521	.051	-.0100	10.4226
	Chang	.02433	2.00521	1.000	-5.1919	5.2406
Kilan	White ProRoot MTA	-5.20631	2.00521	.051	-10.4226	.0100
	Chang	-5.18198	2.00521	.052	-10.3982	.0343
Chang	White ProRoot MTA	-.02433	2.00521	1.000	-5.2406	5.1919
	Kilan	5.18198	2.00521	.052	-.0343	10.3982

Oneway Day 1 extract & 72-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
4.147	2	21	.030

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	51457.823	2	25728.912	2261.895	.000
Within Groups	238.874	21	11.375		
Total	51696.697	23			

Robust Tests of Equality of Means

% cell viability

	Statistic[a]	df1	df2	Sig.
Brown-Forsythe	2261.895	2	14.377	.000

a. Asymptotically F distributed.

Multiple Comparisons

Dependent Variable: % cell viability
Tamhane

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	103.44304*	1.26184	.000	99.8585	107.0276
	Chang	11.43511*	1.98518	.000	5.9647	16.9055
Kilan	White ProRoot MTA	-103.44304*	1.26184	.000	-107.0276	-99.8585
	Chang	-92.00793*	1.73148	.000	-97.1105	-86.9054
Chang	White ProRoot MTA	-11.43511*	1.98518	.000	-16.9055	-5.9647
	Kilan	92.00793*	1.73148	.000	86.9054	97.1105

*. The mean difference is significant at the .05 level.

Oneway Day 3 extract & 72-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
1.313	2	21	.290

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	732.033	2	366.017	6.683	.006
Within Groups	1150.058	21	54.765		
Total	1882.092	23			

Multiple Comparisons

Dependent Variable: % cell viability
Bonferroni

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	-11.39044*	3.70016	.017	-21.0158	-1.7650
	Chang	-12.01579*	3.70016	.012	-21.6412	-2.3904
Kilan	White ProRoot MTA	11.39044*	3.70016	.017	1.7650	21.0158
	Chang	-.62536	3.70016	1.000	-10.2508	9.0000
Chang	White ProRoot MTA	12.01579*	3.70016	.012	2.3904	21.6412
	Kilan	.62536	3.70016	1.000	-9.0000	10.2508

*. The mean difference is significant at the .05 level.

Oneway Day 7 extract & 72-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
2.833	2	21	.081

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	628.101	2	314.051	10.442	.001
Within Groups	631.565	21	30.075		
Total	1259.666	23			

Multiple Comparisons

Dependent Variable: % cell viability

Bonferroni

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	-6.43225	2.74201	.087	-13.5652	.7007
	Chang	-12.52948*	2.74201	.000	-19.6624	-5.3965
Kilan	White ProRoot MTA	6.43225	2.74201	.087	-.7007	13.5652
	Chang	-6.09723	2.74201	.112	-13.2302	1.0357
Chang	White ProRoot MTA	12.52948*	2.74201	.000	5.3965	19.6624
	Kilan	6.09723	2.74201	.112	-1.0357	13.2302

*. The mean difference is significant at the .05 level.

Oneway Day 14 extract & 72-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
2.038	2	20	.156

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	248.899	2	124.450	1.609	.225
Within Groups	1546.830	20	77.342		
Total	1795.730	22			

Multiple Comparisons

Dependent Variable: % cell viability
Bonferroni

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	-8.05626	4.55154	.276	-19.9475	3.8350
	Chang	-5.42083	4.55154	.743	-17.3121	6.4704
Kilan	White ProRoot MTA	8.05626	4.55154	.276	-3.8350	19.9475
	Chang	2.63543	4.39720	1.000	-8.8526	14.1235
Chang	White ProRoot MTA	5.42083	4.55154	.743	-6.4704	17.3121
	Kilan	-2.63543	4.39720	1.000	-14.1235	8.8526

Table 14. One-way ANOVA and Post hoc results of mean differences of percents cell viability between material extracts in HOB 2

Oneway Day 1 extract & 24-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
2.693	2	21	.091

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	439.629	2	219.815	4.615	.022
Within Groups	1000.154	21	47.626		
Total	1439.784	23			

Multiple Comparisons

Dependent Variable: % cell viability
Bonferroni

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	-10.42416*	3.45059	.020	-19.4004	-1.4480
	Chang	-6.17811	3.45059	.263	-15.1543	2.7981
Kilan	White ProRoot MTA	10.42416*	3.45059	.020	1.4480	19.4004
	Chang	4.24605	3.45059	.696	-4.7302	13.2222
Chang	White ProRoot MTA	6.17811	3.45059	.263	-2.7981	15.1543
	Kilan	-4.24605	3.45059	.696	-13.2222	4.7302

*. The mean difference is significant at the .05 level.

Oneway Day 3 extract & 24-hour Incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
.330	2	21	.722

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	379.613	2	189.806	8.726	.002
Within Groups	456.807	21	21.753		
Total	836.420	23			

Multiple Comparisons

Dependent Variable: % cell viability
Bonferroni

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	-8.98634*	2.33199	.003	-15.0527	-2.9200
	Chang	-7.75072*	2.33199	.010	-13.8170	-1.6844
Kilan	White ProRoot MTA	8.98634*	2.33199	.003	2.9200	15.0527
	Chang	1.23562	2.33199	1.000	-4.8307	7.3019
Chang	White ProRoot MTA	7.75072*	2.33199	.010	1.6844	13.8170
	Kilan	-1.23562	2.33199	1.000	-7.3019	4.8307

*. The mean difference is significant at the .05 level.

Oneway Day 7 extract & 24-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
8.293	2	21	.002

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	421.570	2	210.785	12.412	.000
Within Groups	356.631	21	16.982		
Total	778.201	23			

Robust Tests of Equality of Means

% cell viability

	Statistic[a]	df1	df2	Sig.
Brown-Forsythe	12.412	2	13.344	.001

a. Asymptotically F distributed.

Multiple Comparisons

Dependent Variable: % cell viability

Tamhane

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	-7.69417*	1.40498	.000	-11.5045	-3.8838
	Chang	2.03883	2.30055	.781	-4.5744	8.6521
Kilan	White ProRoot MTA	7.69417*	1.40498	.000	3.8838	11.5045
	Chang	9.73301*	2.33888	.006	3.0730	16.3931
Chang	White ProRoot MTA	-2.03883	2.30055	.781	-8.6521	4.5744
	Kilan	-9.73301*	2.33888	.006	-16.3931	-3.0730

*. The mean difference is significant at the .05 level.

Oneway Day 14 extract & 24-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
.929	2	21	.411

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	35.388	2	17.694	.488	.620
Within Groups	760.651	21	36.221		
Total	796.040	23			

Multiple Comparisons

Dependent Variable: % cell viability

Bonferroni

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	.14563	3.00921	1.000	-7.6824	7.9737
	Chang	-2.50000	3.00921	1.000	-10.3280	5.3280
Kilan	White ProRoot MTA	-.14563	3.00921	1.000	-7.9737	7.6824
	Chang	-2.64563	3.00921	1.000	-10.4737	5.1824
Chang	White ProRoot MTA	2.50000	3.00921	1.000	-5.3280	10.3280
	Kilan	2.64563	3.00921	1.000	-5.1824	10.4737

Oneway Day 1 extract & 72-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
11.698	2	21	.000

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7989.188	2	3994.594	42.773	.000
Within Groups	1961.218	21	93.391		
Total	9950.406	23			

Robust Tests of Equality of Means

% cell viability

	Statistic[a]	df1	df2	Sig.
Brown-Forsythe	42.773	2	8.307	.000

a. Asymptotically F distributed.

Multiple Comparisons

Dependent Variable: % cell viability
Tamhane

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	44.63388*	5.83761	.000	27.0227	62.2450
	Chang	20.35858*	1.71768	.000	15.6238	25.0934
Kilan	White ProRoot MTA	-44.63388*	5.83761	.000	-62.2450	-27.0227
	Chang	-24.27530*	5.74590	.010	-41.8885	-6.6621
Chang	White ProRoot MTA	-20.35858*	1.71768	.000	-25.0934	-15.6238
	Kilan	24.27530*	5.74590	.010	6.6621	41.8885

*. The mean difference is significant at the .05 level.

Oneway Day 3 extract & 72-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
1.592	2	21	.227

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1950.235	2	975.118	58.611	.000
Within Groups	349.376	21	16.637		
Total	2299.612	23			

Multiple Comparisons

Dependent Variable: % cell viability
Bonferroni

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	-13.46766*	2.03942	.000	-18.7729	-8.1624
	Chang	-21.88757*	2.03942	.000	-27.1928	-16.5823
Kilan	White ProRoot MTA	13.46766*	2.03942	.000	8.1624	18.7729
	Chang	-8.41991*	2.03942	.001	-13.7252	-3.1147
Chang	White ProRoot MTA	21.88757*	2.03942	.000	16.5823	27.1928
	Kilan	8.41991*	2.03942	.001	3.1147	13.7252

*. The mean difference is significant at the .05 level.

Oneway Day 7 extract &72-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
.043	2	21	.958

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	815.510	2	407.755	21.129	.000
Within Groups	405.273	21	19.299		
Total	1220.783	23			

Multiple Comparisons

Dependent Variable: % cell viability

Bonferroni

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	-11.72788*	2.19652	.000	-17.4418	-6.0140
	Chang	1.18948	2.19652	1.000	-4.5244	6.9034
Kilan	White ProRoot MTA	11.72788*	2.19652	.000	6.0140	17.4418
	Chang	12.91736*	2.19652	.000	7.2035	18.6313
Chang	White ProRoot MTA	-1.18948	2.19652	1.000	-6.9034	4.5244
	Kilan	-12.91736*	2.19652	.000	-18.6313	-7.2035

*. The mean difference is significant at the .05 level.

Oneway Day 14 extract & 72-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
2.568	2	21	.101

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	38.561	2	19.281	1.302	.293
Within Groups	310.879	21	14.804		
Total	349.440	23			

Multiple Comparisons

Dependent Variable: % cell viability

Bonferroni

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	2.79633	1.92378	.483	-2.2081	7.8008
	Chang	.22955	1.92378	1.000	-4.7749	5.2340
Kilan	White ProRoot MTA	-2.79633	1.92378	.483	-7.8008	2.2081
	Chang	-2.56678	1.92378	.589	-7.5712	2.4377
Chang	White ProRoot MTA	-.22955	1.92378	1.000	-5.2340	4.7749
	Kilan	2.56678	1.92378	.589	-2.4377	7.5712

Table 15. One-way ANOVA and Post hoc results of mean differences of percents cell viability between material extracts in HOB 3

Oneway Day 1 extract & 24-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
5.263	2	15	.019

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1080.226	2	540.113	4.733	.025
Within Groups	1711.783	15	114.119		
Total	2792.009	17			

Robust Tests of Equality of Means

% cell viability

	Statistic[a]	df1	df2	Sig.
Brown-Forsythe	4.733	2	9.431	.038

a. Asymptotically F distributed.

Multiple Comparisons

Dependent Variable: % cell viability

Tamhane

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	12.97062*	4.18561	.036	.8583	25.0829
	Chang	-5.50964	7.10263	.844	-27.1625	16.1432
Kilan	White ProRoot MTA	-12.97062*	4.18561	.036	-25.0829	-.8583
	Chang	-18.48026	6.79354	.091	-39.9990	3.0385
Chang	White ProRoot MTA	5.50964	7.10263	.844	-16.1432	27.1625
	Kilan	18.48026	6.79354	.091	-3.0385	39.9990

*. The mean difference is significant at the .05 level.

Oneway Day 3 extract & 24-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
1.505	2	15	.254

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	248.314	2	124.157	1.467	.262
Within Groups	1269.275	15	84.618		
Total	1517.589	17			

Multiple Comparisons

Dependent Variable: % cell viability
Bonferroni

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	-7.77663	5.31094	.491	-22.0829	6.5297
	Chang	.20087	5.31094	1.000	-14.1054	14.5072
Kilan	White ProRoot MTA	7.77663	5.31094	.491	-6.5297	22.0829
	Chang	7.97750	5.31094	.461	-6.3288	22.2838
Chang	White ProRoot MTA	-.20087	5.31094	1.000	-14.5072	14.1054
	Kilan	-7.97750	5.31094	.461	-22.2838	6.3288

Oneway Day 7 extract & 24-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
.152	2	15	.861

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1038.627	2	519.313	4.381	.032
Within Groups	1778.263	15	118.551		
Total	2816.890	17			

Multiple Comparisons

Dependent Variable: % cell viability
Bonferroni

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	-17.36111*	6.28625	.044	-34.2946	-.4276
	Chang	-14.47716	6.28625	.108	-31.4107	2.4564
Kilan	White ProRoot MTA	17.36111*	6.28625	.044	.4276	34.2946
	Chang	2.88395	6.28625	1.000	-14.0496	19.8175
Chang	White ProRoot MTA	14.47716	6.28625	.108	-2.4564	31.4107
	Kilan	-2.88395	6.28625	1.000	-19.8175	14.0496

*. The mean difference is significant at the .05 level.

Oneway Day 14 extract & 24-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
.509	2	15	.611

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	818.367	2	409.183	4.061	.039
Within Groups	1511.539	15	100.769		
Total	2329.906	17			

Multiple Comparisons

Dependent Variable: % cell viability

Bonferroni

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	13.57323	5.79567	.100	-2.0388	29.1852
	Chang	-1.36306	5.79567	1.000	-16.9751	14.2490
Kilan	White ProRoot MTA	-13.57323	5.79567	.100	-29.1852	2.0388
	Chang	-14.93629	5.79567	.063	-30.5483	.6757
Chang	White ProRoot MTA	1.36306	5.79567	1.000	-14.2490	16.9751
	Kilan	14.93629	5.79567	.063	-.6757	30.5483

Oneway Day 1 extract & 72-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
.836	2	15	.452

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	175.294	2	87.647	5.774	.014
Within Groups	227.704	15	15.180		
Total	402.997	17			

Multiple Comparisons

Dependent Variable: % cell viability

Bonferroni

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	-7.05472*	2.24946	.020	-13.1142	-.9953
	Chang	-6.07619*	2.24946	.049	-12.1357	-.0167
Kilan	White ProRoot MTA	7.05472*	2.24946	.020	.9953	13.1142
	Chang	.97853	2.24946	1.000	-5.0809	7.0380
Chang	White ProRoot MTA	6.07619*	2.24946	.049	.0167	12.1357
	Kilan	-.97853	2.24946	1.000	-7.0380	5.0809

*. The mean difference is significant at the .05 level.

Oneway Day 3 extract & 72-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
15.004	2	15	.000

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	362.295	2	181.147	1.059	.371
Within Groups	2564.732	15	170.982		
Total	2927.027	17			

Robust Tests of Equality of Means

% cell viability

	Statistic[a]	df1	df2	Sig.
Brown-Forsythe	1.059	2	9.665	.384

a. Asymptotically F distributed.

Multiple Comparisons

Dependent Variable: % cell viability

Tamhane

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	6.80310	5.70841	.631	-12.6314	26.2376
	Chang	10.87564	7.36071	.482	-14.5042	36.2555
Kilan	White ProRoot MTA	-6.80310	5.70841	.631	-26.2376	12.6314
	Chang	4.07254	9.17693	.963	-22.5129	30.6580
Chang	White ProRoot MTA	-10.87564	7.36071	.482	-36.2555	14.5042
	Kilan	-4.07254	9.17693	.963	-30.6580	22.5129

Oneway Day 7 extract & 72-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
.744	2	15	.492

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	504.067	2	252.033	3.985	.041
Within Groups	948.672	15	63.245		
Total	1452.739	17			

Multiple Comparisons

Dependent Variable: % cell viability

Bonferroni

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	-12.25490	4.59147	.053	-24.6231	.1133
	Chang	-9.78528	4.59147	.150	-22.1535	2.5829
Kilan	White ProRoot MTA	12.25490	4.59147	.053	-.1133	24.6231
	Chang	2.46962	4.59147	1.000	-9.8986	14.8378
Chang	White ProRoot MTA	9.78528	4.59147	.150	-2.5829	22.1535
	Kilan	-2.46962	4.59147	1.000	-14.8378	9.8986

Oneway Day 14 extract & 72-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
.713	2	15	.506

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	161.914	2	80.957	6.785	.008
Within Groups	178.971	15	11.931		
Total	340.885	17			

Multiple Comparisons

Dependent Variable: % cell viability

Bonferroni

(I) Material	(J) Material	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
White ProRoot MTA	Kilan	7.31566*	1.99428	.007	1.9436	12.6877
	Chang	4.24029	1.99428	.151	-1.1318	9.6123
Kilan	White ProRoot MTA	-7.31566*	1.99428	.007	-12.6877	-1.9436
	Chang	-3.07537	1.99428	.432	-8.4474	2.2967
Chang	White ProRoot MTA	-4.24029	1.99428	.151	-9.6123	1.1318
	Kilan	3.07537	1.99428	.432	-2.2967	8.4474

*. The mean difference is significant at the .05 level.

Table 16. One-way ANOVA and Post hoc results of mean differences of percents cell viability between extract time points in HOB 1

Oneway White ProRoot® MTA & 24-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
6.091	3	28	.003

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	364.279	3	121.426	4.166	.015
Within Groups	816.175	28	29.149		
Total	1180.455	31			

Robust Tests of Equality of Means

% cell viability

	Statistic[a]	df1	df2	Sig.
Brown-Forsythe	4.166	3	16.702	.022

a. Asymptotically F distributed.

Multiple Comparisons

Dependent Variable: % cell viability

Tamhane

(I) Extract day	(J) Extract day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1 day	3 day	3.55197	3.11999	.867	-7.0389	14.1429
	7 day	2.26255	1.83102	.808	-3.4859	8.0110
	14 day	-5.27929	1.86697	.089	-11.1586	.6001
3 day	1 day	-3.55197	3.11999	.867	-14.1429	7.0389
	7 day	-1.28941	3.33001	.999	-12.0359	9.4571
	14 day	-8.83126	3.34991	.134	-19.6043	1.9417
7 day	1 day	-2.26255	1.83102	.808	-8.0110	3.4859
	3 day	1.28941	3.33001	.999	-9.4571	12.0359
	14 day	-7.54185*	2.20005	.024	-14.2705	-.8131
14 day	1 day	5.27929	1.86697	.089	-.6001	11.1586
	3 day	8.83126	3.34991	.134	-1.9417	19.6043
	7 day	7.54185*	2.20005	.024	.8131	14.2705

*. The mean difference is significant at the .05 level.

Oneway Chang & 24-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
1.591	3	28	.214

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	541.019	3	180.340	5.433	.005
Within Groups	929.333	28	33.190		
Total	1470.352	31			

Multiple Comparisons

Dependent Variable: % cell viability

Bonferroni

(I) Extract day	(J) Extract day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1 day	3 day	-9.68276*	2.88056	.014	-17.8605	-1.5050
	7 day	-9.97470*	2.88056	.010	-18.1524	-1.7970
	14 day	-8.61230*	2.88056	.035	-16.7900	-.4346
3 day	1 day	9.68276*	2.88056	.014	1.5050	17.8605
	7 day	-.29194	2.88056	1.000	-8.4697	7.8858
	14 day	1.07046	2.88056	1.000	-7.1073	9.2482
7 day	1 day	9.97470*	2.88056	.010	1.7970	18.1524
	3 day	.29194	2.88056	1.000	-7.8858	8.4697
	14 day	1.36240	2.88056	1.000	-6.8153	9.5401
14 day	1 day	8.61230*	2.88056	.035	.4346	16.7900
	3 day	-1.07046	2.88056	1.000	-9.2482	7.1073
	7 day	-1.36240	2.88056	1.000	-9.5401	6.8153

*. The mean difference is significant at the .05 level.

Oneway Kilan & 24-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
5.112	3	27	.006

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	36703.183	3	12234.394	309.898	.000
Within Groups	1065.926	27	39.479		
Total	37769.108	30			

Robust Tests of Equality of Means

% cell viability

	Statistic[a]	df1	df2	Sig.
Brown-Forsythe	312.283	3	17.811	.000

a. Asymptotically F distributed.

Multiple Comparisons

Dependent Variable: % cell viability

Tamhane

(I) Extract day	(J) Extract day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1 day	3 day	-84.64800*	2.69903	.000	-93.2062	-76.0898
	7 day	-80.75544*	3.97130	.000	-93.2824	-68.2284
	14 day	-81.24201*	2.56223	.000	-89.5490	-72.9350
3 day	1 day	84.64800*	2.69903	.000	76.0898	93.2062
	7 day	3.89257	3.69400	.898	-8.1391	15.9242
	14 day	3.40599	2.10699	.563	-3.0644	9.8764
7 day	1 day	80.75544*	3.97130	.000	68.2284	93.2824
	3 day	-3.89257	3.69400	.898	-15.9242	8.1391
	14 day	-.48657	3.59526	1.000	-12.4356	11.4625
14 day	1 day	81.24201*	2.56223	.000	72.9350	89.5490
	3 day	-3.40599	2.10699	.563	-9.8764	3.0644
	7 day	.48657	3.59526	1.000	-11.4625	12.4356

*. The mean difference is significant at the .05 level.

Oneway White ProRoot® MTA & 72-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
1.932	3	27	.148

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1038.918	3	346.306	4.925	.007
Within Groups	1898.492	27	70.315		
Total	2937.410	30			

Multiple Comparisons

Dependent Variable: % cell viability
Bonferroni

(I) Extract day	(J) Extract day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1 day	3 day	-8.82201	4.19269	.269	-20.7582	3.1141
	7 day	-3.28313	4.19269	1.000	-15.2193	8.6530
	14 day	7.49791	4.33984	.573	-4.8572	19.8530
3 day	1 day	8.82201	4.19269	.269	-3.1141	20.7582
	7 day	5.53888	4.19269	1.000	-6.3973	17.4750
	14 day	16.31991*	4.33984	.005	3.9648	28.6750
7 day	1 day	3.28313	4.19269	1.000	-8.6530	15.2193
	3 day	-5.53888	4.19269	1.000	-17.4750	6.3973
	14 day	10.78103	4.33984	.117	-1.5741	23.1361
14 day	1 day	-7.49791	4.33984	.573	-19.8530	4.8572
	3 day	-16.31991*	4.33984	.005	-28.6750	-3.9648
	7 day	-10.78103	4.33984	.117	-23.1361	1.5741

*. The mean difference is significant at the .05 level.

Oneway Chang & 72-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
5.272	3	28	.005

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5483.872	3	1827.957	44.513	.000
Within Groups	1149.839	28	41.066		
Total	6633.711	31			

Robust Tests of Equality of Means

% cell viability

	Statistic[a]	df1	df2	Sig.
Brown-Forsythe	44.513	3	17.962	.000

a. Asymptotically F distributed.

Multiple Comparisons

Dependent Variable: % cell viability

Tamhane

(I) Extract day	(J) Extract day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1 day	3 day	-32.27291*	2.00708	.000	-38.5171	-26.0287
	7 day	-27.24771*	2.79882	.000	-35.9462	-18.5493
	14 day	-9.35803	3.74404	.173	-21.5442	2.8282
3 day	1 day	32.27291*	2.00708	.000	26.0287	38.5171
	7 day	5.02519	2.55246	.378	-3.2255	13.2759
	14 day	22.91488*	3.56363	.001	10.8447	34.9851
7 day	1 day	27.24771*	2.79882	.000	18.5493	35.9462
	3 day	-5.02519	2.55246	.378	-13.2759	3.2255
	14 day	17.88969*	4.06257	.005	5.1825	30.5969
14 day	1 day	9.35803	3.74404	.173	-2.8282	21.5442
	3 day	-22.91488*	3.56363	.001	-34.9851	-10.8447
	7 day	-17.88969*	4.06257	.005	-30.5969	-5.1825

*. The mean difference is significant at the .05 level.

Oneway Kilan & 72-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
5.697	3	28	.004

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	78984.234	3	26328.078	1420.406	.000
Within Groups	518.997	28	18.536		
Total	79503.231	31			

Robust Tests of Equality of Means

% cell viability

	Statistic[a]	df1	df2	Sig.
Brown-Forsythe	1420.406	3	18.871	.000

a. Asymptotically F distributed.

Multiple Comparisons

Dependent Variable: % cell viability

Tamhane

(I) Extract day	(J) Extract day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1 day	3 day	-123.65548*	1.81293	.000	-129.8225	-117.4885
	7 day	-113.15841*	1.31111	.000	-117.4291	-108.8877
	14 day	-104.00139*	2.21617	.000	-111.6832	-96.3196
3 day	1 day	123.65548*	1.81293	.000	117.4885	129.8225
	7 day	10.49707*	2.08719	.002	3.9801	17.0140
	14 day	19.65409*	2.74751	.000	11.1896	28.1186
7 day	1 day	113.15841*	1.31111	.000	108.8877	117.4291
	3 day	-10.49707*	2.08719	.002	-17.0140	-3.9801
	14 day	9.15702*	2.44563	.020	1.3247	16.9893
14 day	1 day	104.00139*	2.21617	.000	96.3196	111.6832
	3 day	-19.65409*	2.74751	.000	-28.1186	-11.1896
	7 day	-9.15702*	2.44563	.020	-16.9893	-1.3247

*. The mean difference is significant at the .05 level.

Table 17. One-way ANOVA and Post hoc results of mean differences of percents cell viability between extract time points in HOB 2

Oneway White ProRoot® MTA & 24-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
2.532	3	28	.077

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	564.494	3	188.165	8.143	.000
Within Groups	647.028	28	23.108		
Total	1211.521	31			

Multiple Comparisons

Dependent Variable: % cell viability
Bonferroni

(I) Extract day	(J) Extract day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1 day	3 day	-8.08771*	2.40355	.013	-14.9112	-1.2642
	7 day	-10.30388*	2.40355	.001	-17.1274	-3.4804
	14 day	-10.08543*	2.40355	.001	-16.9089	-3.2619
3 day	1 day	8.08771*	2.40355	.013	1.2642	14.9112
	7 day	-2.21617	2.40355	1.000	-9.0397	4.6073
	14 day	-1.99772	2.40355	1.000	-8.8212	4.8258
7 day	1 day	10.30388*	2.40355	.001	3.4804	17.1274
	3 day	2.21617	2.40355	1.000	-4.6073	9.0397
	14 day	.21845	2.40355	1.000	-6.6051	7.0420
14 day	1 day	10.08543*	2.40355	.001	3.2619	16.9089
	3 day	1.99772	2.40355	1.000	-4.8258	8.8212
	7 day	-.21845	2.40355	1.000	-7.0420	6.6051

*. The mean difference is significant at the .05 level.

Oneway Chang & 24-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
.499	3	28	.686

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	450.669	3	150.223	5.306	.005
Within Groups	792.791	28	28.314		
Total	1243.461	31			

Multiple Comparisons

Dependent Variable: % cell viability
Bonferroni

(I) Extract day	(J) Extract day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1 day	3 day	-9.66032*	2.66054	.007	-17.2134	-2.1072
	7 day	-2.08693	2.66054	1.000	-9.6400	5.4662
	14 day	-6.40732	2.66054	.137	-13.9604	1.1458
3 day	1 day	9.66032*	2.66054	.007	2.1072	17.2134
	7 day	7.57338*	2.66054	.049	.0203	15.1265
	14 day	3.25299	2.66054	1.000	-4.3001	10.8061
7 day	1 day	2.08693	2.66054	1.000	-5.4662	9.6400
	3 day	-7.57338*	2.66054	.049	-15.1265	-.0203
	14 day	-4.32039	2.66054	.694	-11.8735	3.2327
14 day	1 day	6.40732	2.66054	.137	-1.1458	13.9604
	3 day	-3.25299	2.66054	1.000	-10.8061	4.3001
	7 day	4.32039	2.66054	.694	-3.2327	11.8735

*. The mean difference is significant at the .05 level.

Oneway Kilan & 24-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
4.227	3	28	.014

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	437.013	3	145.671	3.595	.026
Within Groups	1134.425	28	40.515		
Total	1571.438	31			

Robust Tests of Equality of Means

% cell viability

	Statistic[a]	df1	df2	Sig.
Brown-Forsythe	3.595	3	16.304	.036

a. Asymptotically F distributed.

Multiple Comparisons

Dependent Variable: % cell viability

Tamhane

(I) Extract day	(J) Extract day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1 day	3 day	-6.64989	3.74419	.498	-19.0996	5.7998
	7 day	-7.57390	3.61815	.347	-19.9971	4.8493
	14 day	.48436	4.14461	1.000	-12.5189	13.4876
3 day	1 day	6.64989	3.74419	.498	-5.7998	19.0996
	7 day	-.92401	1.75493	.996	-6.3694	4.5213
	14 day	7.13425	2.67705	.119	-1.3129	15.5814
7 day	1 day	7.57390	3.61815	.347	-4.8493	19.9971
	3 day	.92401	1.75493	.996	-4.5213	6.3694
	14 day	8.05825	2.49773	.055	-.1348	16.2513
14 day	1 day	-.48436	4.14461	1.000	-13.4876	12.5189
	3 day	-7.13425	2.67705	.119	-15.5814	1.3129
	7 day	-8.05825	2.49773	.055	-16.2513	.1348

Oneway White ProRoot® MTA & 72-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
1.327	3	28	.285

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	831.303	3	277.101	19.536	.000
Within Groups	397.148	28	14.184		
Total	1228.452	31			

Multiple Comparisons

Dependent Variable: % cell viability
Bonferroni

(I) Extract day	(J) Extract day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1 day	3 day	7.10037*	1.88307	.005	1.7544	12.4463
	7 day	12.57554*	1.88307	.000	7.2296	17.9215
	14 day	12.26251*	1.88307	.000	6.9166	17.6084
3 day	1 day	-7.10037*	1.88307	.005	-12.4463	-1.7544
	7 day	5.47517*	1.88307	.042	.1292	10.8211
	14 day	5.16214	1.88307	.063	-.1838	10.5081
7 day	1 day	-12.57554*	1.88307	.000	-17.9215	-7.2296
	3 day	-5.47517*	1.88307	.042	-10.8211	-.1292
	14 day	-.31302	1.88307	1.000	-5.6589	5.0329
14 day	1 day	-12.26251*	1.88307	.000	-17.6084	-6.9166
	3 day	-5.16214	1.88307	.063	-10.5081	.1838
	7 day	.31302	1.88307	1.000	-5.0329	5.6589

*. The mean difference is significant at the .05 level.

Oneway Chang & 72-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
2.371	3	28	.092

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5803.181	3	1934.394	150.358	.000
Within Groups	360.226	28	12.865		
Total	6163.407	31			

Multiple Comparisons

Dependent Variable: % cell viability
Bonferroni

(I) Extract day	(J) Extract day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1 day	3 day	-35.14578*	1.79341	.000	-40.2371	-30.0544
	7 day	-6.59356*	1.79341	.006	-11.6849	-1.5022
	14 day	-7.86652*	1.79341	.001	-12.9579	-2.7752
3 day	1 day	35.14578*	1.79341	.000	30.0544	40.2371
	7 day	28.55222*	1.79341	.000	23.4609	33.6436
	14 day	27.27926*	1.79341	.000	22.1879	32.3706
7 day	1 day	6.59356*	1.79341	.006	1.5022	11.6849
	3 day	-28.55222*	1.79341	.000	-33.6436	-23.4609
	14 day	-1.27295	1.79341	1.000	-6.3643	3.8184
14 day	1 day	7.86652*	1.79341	.001	2.7752	12.9579
	3 day	-27.27926*	1.79341	.000	-32.3706	-22.1879
	7 day	1.27295	1.79341	1.000	-3.8184	6.3643

*. The mean difference is significant at the .05 level.

Oneway Kilan & 72-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
7.718	3	28	.001

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12212.257	3	4070.752	50.226	.000
Within Groups	2269.373	28	81.049		
Total	14481.630	31			

Robust Tests of Equality of Means

% cell viability

	Statistic[a]	df1	df2	Sig.
Brown-Forsythe	50.226	3	10.922	.000

a. Asymptotically F distributed.

Multiple Comparisons

Dependent Variable: % cell viability
Tamhane

(I) Extract day	(J) Extract day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1 day	3 day	-51.00117*	5.92719	.000	-71.2955	-30.7069
	7 day	-43.78623*	5.88501	.000	-64.0898	-23.4827
	14 day	-29.57504*	5.90781	.006	-49.8726	-9.2775
3 day	1 day	51.00117*	5.92719	.000	30.7069	71.2955
	7 day	7.21495	2.37114	.052	-.0453	14.4751
	14 day	21.42613*	2.42716	.000	14.0019	28.8504
7 day	1 day	43.78623*	5.88501	.000	23.4827	64.0898
	3 day	-7.21495	2.37114	.052	-14.4751	.0453
	14 day	14.21119*	2.32226	.000	7.1067	21.3156
14 day	1 day	29.57504*	5.90781	.006	9.2775	49.8726
	3 day	-21.42613*	2.42716	.000	-28.8504	-14.0019
	7 day	-14.21119*	2.32226	.000	-21.3156	-7.1067

*. The mean difference is significant at the .05 level.

Table 18. One-way ANOVA and Post hoc results of mean differences of percents cell viability between extract time points in HOB 3

Oneway White ProRoot® MTA & 24-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
.529	3	20	.667

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5638.518	3	1879.506	21.588	.000
Within Groups	1741.217	20	87.061		
Total	7379.736	23			

Multiple Comparisons

Dependent Variable: % cell viability
Bonferroni

(I) Extract day	(J) Extract day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1 day	3 day	-29.74346*	5.38705	.000	-45.5120	-13.9749
	7 day	-23.54511*	5.38705	.002	-39.3136	-7.7766
	14 day	-42.18320*	5.38705	.000	-57.9517	-26.4147
3 day	1 day	29.74346*	5.38705	.000	13.9749	45.5120
	7 day	6.19835	5.38705	1.000	-9.5702	21.9669
	14 day	-12.43974	5.38705	.190	-28.2083	3.3288
7 day	1 day	23.54511*	5.38705	.002	7.7766	39.3136
	3 day	-6.19835	5.38705	1.000	-21.9669	9.5702
	14 day	-18.63809*	5.38705	.015	-34.4066	-2.8696
14 day	1 day	42.18320*	5.38705	.000	26.4147	57.9517
	3 day	12.43974	5.38705	.190	-3.3288	28.2083
	7 day	18.63809*	5.38705	.015	2.8696	34.4066

*. The mean difference is significant at the .05 level.

Oneway Chang & 24-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
2.115	3	20	.130

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5069.940	3	1689.980	12.844	.000
Within Groups	2631.492	20	131.575		
Total	7701.431	23			

Multiple Comparisons

Dependent Variable: % cell viability

Bonferroni

(I) Extract day	(J) Extract day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1 day	3 day	-24.03294*	6.62255	.010	-43.4179	-4.6479
	7 day	-32.51263*	6.62255	.001	-51.8976	-13.1276
	14 day	-38.03662*	6.62255	.000	-57.4216	-18.6516
3 day	1 day	24.03294*	6.62255	.010	4.6479	43.4179
	7 day	-8.47968	6.62255	1.000	-27.8647	10.9053
	14 day	-14.00367	6.62255	.283	-33.3887	5.3813
7 day	1 day	32.51263*	6.62255	.001	13.1276	51.8976
	3 day	8.47968	6.62255	1.000	-10.9053	27.8647
	14 day	-5.52399	6.62255	1.000	-24.9090	13.8610
14 day	1 day	38.03662*	6.62255	.000	18.6516	57.4216
	3 day	14.00367	6.62255	.283	-5.3813	33.3887
	7 day	5.52399	6.62255	1.000	-13.8610	24.9090

*. The mean difference is significant at the .05 level.

Oneway Kilan & 24-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
.860	3	20	.478

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11134.535	3	3711.512	39.107	.000
Within Groups	1898.151	20	94.908		
Total	13032.686	23			

Multiple Comparisons

Dependent Variable: % cell viability
Bonferroni

(I) Extract day	(J) Extract day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1 day	3 day	-50.49070*	5.62458	.000	-66.9545	-34.0269
	7 day	-53.87684*	5.62458	.000	-70.3406	-37.4130
	14 day	-41.58058*	5.62458	.000	-58.0444	-25.1168
3 day	1 day	50.49070*	5.62458	.000	34.0269	66.9545
	7 day	-3.38613	5.62458	1.000	-19.8499	13.0777
	14 day	8.91012	5.62458	.773	-7.5537	25.3739
7 day	1 day	53.87684*	5.62458	.000	37.4130	70.3406
	3 day	3.38613	5.62458	1.000	-13.0777	19.8499
	14 day	12.29626	5.62458	.245	-4.1675	28.7601
14 day	1 day	41.58058*	5.62458	.000	25.1168	58.0444
	3 day	-8.91012	5.62458	.773	-25.3739	7.5537
	7 day	-12.29626	5.62458	.245	-28.7601	4.1675

*. The mean difference is significant at the .05 level.

Oneway White ProRoot® MTA & 72-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
1.983	3	20	.149

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11138.527	3	3712.842	99.676	.000
Within Groups	744.983	20	37.249		
Total	11883.509	23			

Multiple Comparisons

Dependent Variable: % cell viability
Bonferroni

(I) Extract day	(J) Extract day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1 day	3 day	-34.96608*	3.52369	.000	-45.2803	-24.6518
	7 day	-42.32834*	3.52369	.000	-52.6426	-32.0141
	14 day	-59.10311*	3.52369	.000	-69.4174	-48.7889
3 day	1 day	34.96608*	3.52369	.000	24.6518	45.2803
	7 day	-7.36226	3.52369	.298	-17.6765	2.9520
	14 day	-24.13703*	3.52369	.000	-34.4513	-13.8228
7 day	1 day	42.32834*	3.52369	.000	32.0141	52.6426
	3 day	7.36226	3.52369	.298	-2.9520	17.6765
	14 day	-16.77477*	3.52369	.001	-27.0890	-6.4605
14 day	1 day	59.10311*	3.52369	.000	48.7889	69.4174
	3 day	24.13703*	3.52369	.000	13.8228	34.4513
	7 day	16.77477*	3.52369	.001	6.4605	27.0890

*. The mean difference is significant at the .05 level.

Oneway Chang & 72-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
22.310	3	20	.000

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9845.833	3	3281.944	33.393	.000
Within Groups	1965.639	20	98.282		
Total	11811.473	23			

Robust Tests of Equality of Means

% cell viability

	Statistic[a]	df1	df2	Sig.
Brown-Forsythe	33.393	3	7.461	.000

a. Asymptotically F distributed.

Multiple Comparisons

Dependent Variable: % cell viability

Tamhane

(I) Extract day	(J) Extract day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1 day	3 day	-18.01424	7.41888	.291	-47.9401	11.9116
	7 day	-46.03743*	3.23046	.000	-57.5034	-34.5715
	14 day	-48.78662*	2.07729	.000	-55.5676	-42.0056
3 day	1 day	18.01424	7.41888	.291	-11.9116	47.9401
	7 day	-28.02319	7.82344	.059	-57.0798	1.0334
	14 day	-30.77239*	7.42196	.044	-60.6874	-.8573
7 day	1 day	46.03743*	3.23046	.000	34.5715	57.5034
	3 day	28.02319	7.82344	.059	-1.0334	57.0798
	14 day	-2.74920	3.23751	.963	-14.2172	8.7188
14 day	1 day	48.78662*	2.07729	.000	42.0056	55.5676
	3 day	30.77239*	7.42196	.044	.8573	60.6874
	7 day	2.74920	3.23751	.963	-8.7188	14.2172

*. The mean difference is significant at the .05 level.

Oneway Kilan & 72-hour incubation

Test of Homogeneity of Variances

% cell viability

Levene Statistic	df1	df2	Sig.
9.414	3	20	.000

ANOVA

% cell viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8954.246	3	2984.749	49.357	.000
Within Groups	1209.458	20	60.473		
Total	10163.703	23			

Robust Tests of Equality of Means

% cell viability

	Statistic[a]	df1	df2	Sig.
Brown-Forsythe	49.357	3	8.012	.000

a. Asymptotically F distributed.

Multiple Comparisons

Dependent Variable: % cell viability

Tamhane

(I) Extract day	(J) Extract day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1 day	3 day	-21.10825	5.91880	.066	-43.5948	1.3783
	7 day	-47.52852*	2.84797	.000	-56.8398	-38.2172
	14 day	-44.73272*	2.14811	.000	-52.4070	-37.0585
3 day	1 day	21.10825	5.91880	.066	-1.3783	43.5948
	7 day	-26.42026*	5.97502	.023	-48.8238	-4.0167
	14 day	-23.62447*	5.67488	.046	-46.7666	-.4823
7 day	1 day	47.52852*	2.84797	.000	38.2172	56.8398
	3 day	26.42026*	5.97502	.023	4.0167	48.8238
	14 day	2.79580	2.29849	.840	-5.5439	11.1355
14 day	1 day	44.73272*	2.14811	.000	37.0585	52.4070
	3 day	23.62447*	5.67488	.046	.4823	46.7666
	7 day	-2.79580	2.29849	.840	-11.1355	5.5439

*. The mean difference is significant at the .05 level.

BIOGRAPHY

Miss Thanomsuk Jearanaiphaisarn was born on 5th April 1980 in Bangkok. She graduated with D.D.S. (Doctor of Dental Surgery) from the Faculty of Dentistry, Chulalongkorn University in 2004, and became a staff member at Faculty of Dentistry, Prince of Songkha University. She studied in a Master degree program in Endodontology at Graduate School, Chulalongkorn University in 2008.