Jaw bone regeneration in relation to position of dental implant

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Abstract

Objective: To evaluate the correlation of molecular parameters of bone cells such as BMP2, FGF23, RUNX2 from osteotomies sites and bone type according to Cone Beam Computer Tomography (CBCT), surgeon tactile sensation.

Materials and methods: Study designs: Experimental cross sectional study. The bone specimens were collected during operation. The specimens were placed in refrigerator at -80°C and then extracted for RNA. The cDNA product was used for TaqMan RT-PCR reaction analysis.

Results: Among total 34 implant sites, 21 (62%) were from male with mean age 51 years old. The majority of bone type was type 3+4 with 28 (82.4%) from CBCT and 22 (64.7%) from surgeon tactile, respectively. Gene expression profile demonstrated that *BMP2, FGF23,* and *RUNX2* were expressed; 52.9%, 23.5% and 41.2%.The gingival thickness and smoking was found significantly difference with wound healing but not significant difference with *BMP2, FGF23* and *RUNX2* gene expression ($p < 0.05$).

Conclusion: Molecular parameters such as BMP2 and FGF23 gene expression were correlated with bone quantity and quality evaluation in term of wound healing including the thickness of soft tissue and amount of hard tissue. This study suggested the possibility of osteogenic gene profile matching the human jawbone

Keyword: osteointegration, bone quality, bone quantity, bone density ,gene expression, dental implant

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Introduction

Dental implant is world wild use with high success rate and long term stability. Many factors are still considered to achieve osteointegration. The term bone quality is immeasurable there are four difference type of cell present bone modeling and remodeling. Osteoblasts, bone lining cell ,osteoclast and osteocyte. There are four categories with different degrees of density[1]. One of the methods used to evaluate is based on the perception of the clinician at the time of preparation at the surgical bed[2]. Regarding to the differences in bone quality, the lower density found the significant lower dental implant survival rate[3,4,5]. Furthermore there are some study reported that more failure in type III than in type II bone[6] However, there was no statistically significant difference for implant failure, bone density and quality, tooth loss, implant type area, or the surgical protocol. There are many factors related osteointegration such as implant material, implant design, surface condition, surgical technique, implant loading and status of the bone[7]. Osteointegration is influence for implant loading and long-term clinical success. During the initial remodeling, the cell initially remove the necrotic debris and lead to expression of cell surface proteins and production of cytokines. This cytokine-regulated cellular recruitment and express growth factors such as fibroblast growth factor, transforming growth factor, epithelial growth factor as well as bone morphogenic protein. The objective of this study is to evaluate the correlation activity of bone cell at the osteotomies site by gene expression with CBCT and surgeon tactile sensation.

Materials and methods

As for description of mean and standard deviation regarding 34 samples were shown in table 1. Age was in range 20-79 years mean was 51 years old. Samples were collected from 34 osteotomies dental implant sites.

After doing history taking and clinical examination, the participants will be selected based on inclusion and exclusion criteria. General information (age, sex, smoking history, concomitant systemic diseases, and drug allergy), position of implant at maxillary or mandible, duration of edentulous, periodontal disease, length and width of space (mm) then radiographic images: periapical and panoramic radiographs and CBCT (Cone Beam Computed Tomography) will be performed to evaluate the classification of bone types will be done by radiologist according to the original classification system proposed by ratio of crestal thickness per total bone thickness and crestal thickness at osteotomy site below 5 mm. from the crest. Then surgeon's tactile perception was measure during first drilling. These classification methods categorize bone types into two groups: 1+2, 3+4 according to the distribution of cortical and trabecular bone. Then measurement gingival thickness was by the periodontal probe A drill 1.8 mm will be used for drilling at implant site and measure surgeon tactile sensations. To categories bone types into two groups: 1+2 and 3+4 then use autologous bone harvest from (MegaGen Co.,LTD). Then implant (Intralock Implant. Co.,Ltd, Dentapex,Thailand) will be placed according to the surgical stent at that time apply healing abutment. Suture the surgical site and provide medication. Amoxycylin 500 mg Paracetamol 500 mg, Ibuprofen 400 mg for 3 days. The score of wound healing was measure and the plaque score was recorded modified by classifying healing of skin following trauma in patients with diabetes mellitus 15(IDS A guidelines) and plaque score by reference Mombelli (Mpi) will be measured at day 7 in the same day stitch off. in case wound healing were not very good will stich off after 14 days This study was a descriptive cross sectional

study. Ethics approved by MU-DT/PY-IRB 2013/033.0807, Mahidol University, Bangkok, Thailand.

Sample preparation and surgical procedure

Bone Specimens from patients were collected by autologous bone harvesting (MegaGen Co.,LTD) and transferred into 2ml Eppendorf tube and place on ice and then centrifuged and cleaned by PBS (Phosphate buffer solution). After that specimens were placed in refrigerator at -80°C. Primary closure was obtained for all the surgical sites and the patient was instructed to maintain hygiene around the surgical site using a soft-bristle toothbrush. Recall appointment and measure wound healing according to One week following the dental procedure, the patients were recalled for evaluation of the wound healing and the sutures were removed and the site irrigated with normal saline solution. The healing of the soft tissues around the surgical area was visually evaluated and classified into "good" or "not good" according to a modified soft tissue healing index used for classifying healing of skin following trauma in patients with diabetes mellitus 15(IDS A guidelines) and measure plaque score by modified Mombelli (Mpi) score.

RNA extraction

Approximately 50 - 100 mg of bone tissue was grinded in liquid nitrogen by using a baked mortar and pestle, and then removed the powder to 1 ml of TRIzol and centrifuged at 12,000 g for 10 min. The upper part was supernatant will be transferred to a new tube and add 200 µl of chloroform followed by 15 min of centrifugation at 12,000 g. Isopropanol 500 µ ;l per 1 ml of TRIzol was added for RNA sedimentation (incubate 10 min at RT), then, centrifuged at 12,000 g for 10 min. Supernatant was discarded and 1 ml of 75% ethanol per 1 ml of TRIzol was added, then, centrifuged at 7,500 g for 5 min (2 times). Eventually, RNA pellet was dried at room temperature for 10-15 min before dissolve in 0.1 % DEPC–treated water. RNA concentrations were determined using spectrophotometry (A260) and the purity was assessed from the A260:A280 ratio. The RNA sample was stored at -80°C until used. The quality of RNA was checked on a 1% agarose gel containing 0.5 µg of ethidium bromide (EtBr)/ml. The modified RNA extraction from Seriwatanachai et al. 2008[8] was used in this procedure.

Reverse transcription

Total RNA from bone was subjected to cDNA synthesis. To remove genomic DNA, RNA samples were incubated with 1 unit of deoxyribonuclease I (DNase I) per µg RNA at 25°C for 15 min. The reverse transcription was conducted using iScriptTM Select cDNA Synthesis kit according to manufacturer's protocol (Bio-Red, USA). Each 20 µl reaction contained: 1 µg of total RNA sample, 2 µl Oligo(dT) primer, nuclease-free water (variable volume), 4 µl 5x iScript select reaction mix and 1 µl iScript reverse transcriptase. The reverse transcription reaction was performed at 42°C for 60-90 min, and then at 85°C for 5 min to heat-inactivate the reverse transcriptase. Finally, the cDNA product was stored at -20°C to 4°C until used

Realtime-PCR

The reagent used in realtime PCR was TaqMan Universal PCR Master Mix, Applied Biosystems, USA). Expression of target genes was normalized with GAPDH expression. Applied Biosystems (ABI), and the real-time PCR reaction was performed on the ABI 7500 Sequence Detection System (Applied Biosystems, Foster, CA, USA).

All the analyses were calculated using a Pearson's chi-square or Fisher's Exact Test,

Exact 2-tailed, p-value < 0.05 as statistically significant cut off point. Descriptive analyses performed for molecular parameters will be expressed along with the mean and standard deviation. SSPS 18.0 for Windows (Chicago, IL, USA) was used for data analysis.

Results

General information of participants and site of implants were shown in table 1, the mean and standard deviation of crestal bone thickness at left mandible, right mandible, left maxillar, and right maxillar were 1.20 ± 0.18 respectively. The average bucco-lingual width and crestal thickness were 8.03 ± 1.35 and 1.31 ± 0.83, respectively. Regarding gingival thickness was 2.95±1.08 respectively.

Among 34 implant sites, 13 (38%) were from female and 21 (62%) from male. Regarding associated systemic diseases, 27 (79.4%) samples were from normal persons, 1 (2.9 %) from diabetics, 3 (8.8%) from hypertension, and 3 (8.8%) from hypertension with hypothyroidism patients and 11.8% have periodontal disease. Nine out of 34 samples (26.5%) were collected from smoking patients.

Regarding bone types according to CBCT, Panoramic X-ray and Surgeon tactile, commonest the bone type was bone type 2, 6 (17.6%), 8 (23.5%), and 12 (35.3%), respectively. Regarding gingival thickness, thick biotype is 31 (91.2%) and thin biotype is 3 (9.8%). (Table 2)

There are from maxilla were 12 (35.3%) and mandible were 22 (64.7%) respectively. After operation per oral amoxacillin 500 mg tid plus paracetamol 500 mg qid for 3 days were given. Regarding wound healing, good status wound healing was 73 % and not good status of wound healing was 27%.There are significant relationship between type of bone from CBCT and OPG (panoramic) with p-value 0.018 but we could not found the significant relationship between CBCT and surgeon tactile sensation (p > 0.05). But we could not find significant relationship between all 3 gene expressions with wound healing (p > 0.05). For 3 gene expression were not related with wound healing with p-value 0.14,0.06, 0.46 respectively. In case of smoking and wound healing we found the significant relationship with p-value 0.048 from Fisher's-exact test.We found the significant relationship between gingival thickness and wound healing with p-value 0.01 but we could not found the significant relationship betwwe crestal thickness and wound healing.(p>0.05)

Regarding gene expression, undetermined and determined gene expression of *BMP2, RUNX2* and *FGF23*, were; 16 (47.1%) and 18 (52.9%), 19 (55.9%) and 15 (44.1%), 26 (76.5%) and 8(23.5%), respectively. (Table 3)

Table 1 General information of the participants and site of implants (34 sample)

Thickness at site of implants (34 samples)	Range(mm)	Mean±SD
Bucco-lingual width (mm)	6-10	8.78±0.962
Crestal bone at left mandible (mm)	1.2-2	1.61±0.19
Crestal bone at right mandible (mm)	1.2-2	1.65±0.21
Crestal bone at left maxillar (mm)	0.3-1.1	0.73±0.38
Crestal bone at right maxillar (mm)	0.2-1.2	0.73±0.32
Bucco lingual width	5.3-10.7	8.03±1.36
Gingival thickness (mm)	1.0-5.23	2.95±1.09
Crestal thickness (mm)	0-2	1.14±0.61

Table 2 Information regarding site of implants (34 samples)

Types	Type of bone	n (%)
CBCT result	Bone type 1+2	6 (17.6)
	Bone type 3+4	28 (82.4)
Panoramic X-ray result	Bone type 1+2	8 (23.5)
	Bone type 3+4	26 (76.5)
Type of bone (Surgeon tactile)	Bone type 1+2	12 (35.3)
	Bone type 3+4	22 (64.7)
Gingiva biotype	Thick	31 (91.2)
	Thin	3 (8.8)

Table 3 Gene expression of *BMP2, FGF23*, and *RUNX2*

Type	Type of gene expression	n (%)
BMP2	Undetermined gene expresion	16 (47.1)
	Determined Gene expression	14 (52.9)
FGF23	Undetermined gene expresion	26 (76.5)
	Determined Gene expression	8 (23.5)
RUNX2	Undetermined gene expresion	19 (55.9)
	Determined Gene expression	14 (41.2)

Relation of gene expression and wound healing were found that the expression of 3 genes do not relate with wound healing but in case of good wound healing we found more *FGF23* ($p > 0.05$).However we found significant relationship between *BMP2* and mandibular bone. ($p > 0.05$). (**Figure 1.**).

Relation of gene expression with gingival thickness was mentioned in table 4. Mean of gingival thickness in determined gene expression of *BMP2* and *FGF23* were higher than undermined gene expression, but there were no statistical significance. ($P > 0.05$) On the other hand, mean of gingival thickness in determined gene expression of *RUNX2* was statistically lower than undermined gene expression. ($p < 0.05$)

Relation of gene expression with gingival thickness at ossteotomy site was mentioned in table 4. Mean of gingival thickness at ossteotomy site in determined gene expression of *BMP2* and *FGF23* were statistically significant higher than undermined gene expression, p-value 0.005 and 0.04, respectively.

However we found significant relationship of classification of bone type according CBCT and panoramic radiograph. (table5)

Discussion

In the present study, The classification system most generally used is proposed by Lekholm & Zarb[1] that might be subjective but still no other classification or index that more reliable which uses conventional radiograph and surgeon's tactile sense. Misch 2008 will modify feeling of drill that correlation with the location of jaw and CT[9,10]. The anterior mandible region presents higher bone density than the posterior mandible, followed by anterior maxilla and posterior maxilla. Hence, the lower maxilla presents higher bone density than the upper maxilla[11]. The bone density correlate

with computer tomography. CT examination is approximately to be 0.06 mSv, comparable to 3 weeks equivalent natural exposure. Shapurian et al. 2006 correlated HU value with Lekhom and Zarb classification evaluated two observers. The agreement in assessment of bone quality was low (r=0.65, p<0.001)[12]. They concluded that this emphasized Lekhom and Zarb classification is subjective nature it impossible to use more than one observer. Several studies told sex and age effect to the cortical thickness of jaw bone[13]. Also did not correlate their measurements with marginal bone loss at a follow up. Trisi and coworkers reported a correlation between the surgeon's perception of 4 different classes of bone density, first based on hand-felt drilling resistance, however it's seem to be difficult to classification and compare with difference

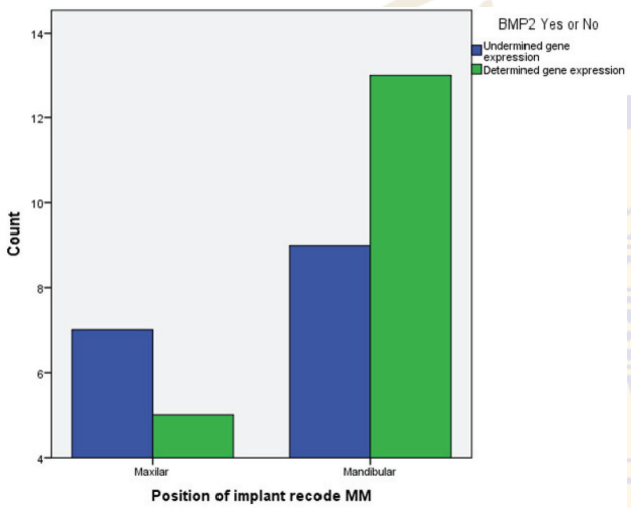
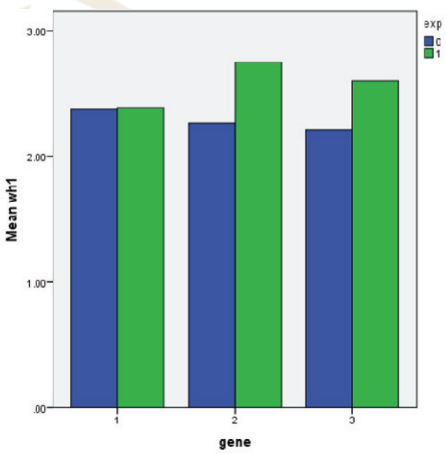


Figure 1 The relationship between position of implant and *BMP2, FGF23, RUNX2* gene expression



1=*BMP2* ,2=*FGF23*, 3=*RUNX2*

Figure 2 Gene expressions of *BMP2, FGF23,* and *RUNX2* and wound healing

Table 4 Relation of gingival thickness at ossteotomy site and *BMP2, FGF2, RUNX2* gene expression

Type	Gingival Thickness	N	Mean (mm)	SD	p
BMP2	Undetermined gene	16	0.86	0.53	**0.005***
	Determined gene	14	1.46	0.54	
FGF23	Undetermined gene	23	1.02	0.57	**0.041***
	Determined gene	7	1.55	0.59	
RUNX2	Undetermined gene	16	1.07	0.56	0.516
	Determined gene	14	1.22	0.67	

Independent t-test (Sig. 2-tailed)

Table 5 Relation of CBCT and rexbonet Crosstabulation

		panoramic		Total	sig
		2	3		
CBCT recode	2	4	2	6	0.018*
	3	4	24	28	
Total		8	26	34	

Fisher's Exact Test

surgeon for the ratings used most frequently it's difficult to compare type 2 and 3[14]. The gold standard is the amount of bone trabeculae assessed by morphometric. Considering the complexity and the multifactorial nature of bone-heating after implant osteotomy, it is possible to affirm that an appropriate surgical protocol should be adopted during drilling procedures, with emphasis on the control of biological and clinical factors, to promote the preservation of cell viability and consequent increase in the success rate of osseointegration. Bone usually varies in density from person to person, bone to bone in the skeleton, and from site to site in the same bone. Poor quality bone can be related to a lower success rate but all studies are always not differently in statistical term. The studies retrospective and couldn't control to use same implant surface and design and found type III was the lowest success rate[15]. The Tobacco use had an influence on the loss of dental implants[4].

Therefore in poor bone quality there are many surgical technique to modify to achieve the primary stability and secondary stability such as undersized preparation, osteotome, bone expander, the piezosurgery, the flapless procedure, and the bone stimulation by low-level laser therapy.

The gold standard of bone graft is autogenous bone. To understand the functional role of bone and soft tissue cell during healing we examined the effect of gene expression in bone tissue cells. To direct evaluate function and vitality of bone cell might be difficult. Relative to The RNA and cDNA will represent bone function. Bone morphogenetic proteins play an important role in the regulation in the bone induction and key cellular events[16]. Bone cell and growth factors are effect to cell proliferation and differentiation. Bone morphogenetic protein 2 *(BMP2)* is the most powerful cytokine that promotes differentiation of mesenchymal cells into osteoblasts in vitro and induce bone formation in vivo. *BMPs* are members of the transforming growth factor *(TGF)* β. The function of BMPs is to induce the maturation of osteoblasts in endochondral bone formation.

BMP2 is known to control the expression and functions of Runt-related gene 2 (*RUNX2*) through Smad signalling. It was noted that *BMP2* regulated Osterix expression independently through two distinct transcription factors, Runx2 and Msx2 were essential for osteoblast differentiation. *RUNX2*/Core-binding factor 1 (Cbfa1), is an essential transcription factor for osteoblast differentiation and bone.

RUNX2 protein was first detected in preosteoblasts during the early stages of osteoblast differentiation. RUNX2 is reduced during osteoblast maturation and bone development. Smad signaling is required for induction of Osterix, and that Osterix expression is regulated via both Runx2-dependent and –independent mechanisms by *BMP2* signaling. The Fibroblast Growth Factor 23 (*FGF23*) produced by osteocytes. Osteocytes are sensed mechanical strain and controlling bone formation and resorption. The Wnt/β-catenin signaling pathway plays a critical role in osteoblastic cell differentiation and bone formation. Wnt and B*MPs* have similar and overlapping effects.

Immunohistochemical analysis showed *FGF23* production of osteoblasts and granulation tissue in the fracture callus during bone healing. *FGF23* is involved in bone healing and is a promising candidate as an indicator for healing processes prone to reunion versus nonunion. In our study the *BMP2, RUNX2* and *FGF23* expression was not statistically significant difference between types of bones according to Cone-beam computer tomography panoramic radiography and surgeon tactile senses but we found the correlation between bone type from CBCT and panoramic radiograph significant difference ($p<0.05$). There are many factors

that can affect wound healing which interfere with one or more phases in this process, thus causing improper or impaired tissue repair. They are consisting of four highly integrated and overlapping phases: hemostasis, inflammation, proliferation, and tissue remodelling or resolution. Most chronic wounds are ulcers that are associated with ischemia, diabetes mellitus, venous stasis disease

However, we found relationship between better wound healing and *RUNX2*. Also history of periodontal disease, smoking, and metabolic disorders can affect gene expression same in our study we found significant difference in smoking patients. In clinical apply the genome microarray analyses at the osteotomy site to identify critical gene networks involved in osseointegration found that the circadian regularity system and cartilage extracellular matrix may be encourage the osseointegration by vitamin D[17]. In some study found dexamethasone can promote would healing[18]. Our limitations were small sample sizes. Therefore only 34 bone extracted samples. However, the results of the study showed that the method of gene extraction and observation of gene expression was valid and repeatable. Moreover, our finding indicated that *RUNX2* was expressed differently in statistical term difference gingival thickness. The thickness to protect bone resorption should be 1.8-3.9 mm[19]. Our results in jaws bone area according to animal research *BMP2* levels in mandibular were greater than maxillary sockets but we cannot find significant different between gene expression and maxilla or mandible. In oral cavity, *FGF23* presents a unique opportunity to simultaneously observe four different types of mineralized tissue such as bone, cementum, dentin, and enamel. *RUNX2* in osteoblasts reduces during bone development. Our results we found the relationship significant differences gene expression increase in periodontitis and smoking. Higher *FGF23* associated with and alcohol intake induce bone resorption[20] same with our study. Smoking has an adverse effect on fracture healing and bone regeneration. In smokers, *BMP2* gene expression of human periosteum was reduced[21]. The molecular evaluation at osteotomies sites were not found relationship significant difference with CBCT surgeon tactile sense and radiographic aspects[22]. But we found the correlations significant difference between *BMP2, RUNX2* and gingival thickness in term of wound healing then there have correlation in classification bone type from CBCT and OPG (Panoramic x-ray)($p<0.05$)

In conclusion, molecular parameters such as *BMP2* and *FGF23* gene expression were correlated with bone quantity and quality evaluation. This study suggested the possibility of osteogenic gene profile matching the human jawbone microstructure.

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Competing interest :The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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International Abstract

Effect of double-layer application on dentin bond durability of one-step self-etch adhesives

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Taschner M, Kümmerling M, Lohbauer U, Breschi L, Petschelt A, Frankenberger R

Purpose: The aim of this in vitro study was 1) to analyze the influence of a double-layer application technique of four one-step self-etch adhesive systems on dentin and 2) to determine its effect on the stability of the adhesive interfaces stored under different conditions.

Materials and Methods: Four different one-step self-etch adhesives were selected for the study (iBondSE, Clearfil S^3 Bond, XenoV+, and Scotchbond Universal). Adhesives were applied according to manufacturers' instructions or with a double-layer application technique (without light curing of the first layer). After bonding, resin-dentin specimens were sectioned for microtensile bond strength testing in accordance with the nontrimming technique and divided into 3 subgroups of storage: a) 24 hours (immediate bond strength, T0), b) six months (T6) in artificial saliva at 37°C, or c) five hours in 10 % NaOCl at room temperature. After storage, specimens were stressed to failure. Fracture mode was assessed under a light microscope.

Results: At T0, iBond SE showed a significant increase in microtensile bond strength when the double-application technique was applied. All adhesive systems showed reduced bond strengths after six months of storage in artificial saliva and after storage in 10% NaOCl for five hours; however at T6, iBond SE, Clearfil S^3 Bond, and XenoV+ showed significantly higher microtensile bond strength results for the double-application technique compared with the single-application technique. Scotchbond Universal showed no difference between single- or double-application, irrespective of the storage conditions.

Conclusion: The results of this study show that improvements in bond strength of one-step self-etch adhesives by using the double-application technique are adhesive dependent

Effect of incorporating BisGMA resin on the bonding properties of silane and zirconia primers

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Statement of Problem: Some silane primers and some zirconia primers contain extra resins such as bisphenol A glycol dimethacrylate (BisGMA) in their formulations for better wetting. No studies exist on the bonding properties of zirconia and silane primers, which contain extra resins.

Purpose: The purpose of this study was to investigate the effect of incorporating BisGMA resin on the bonding properties of silane and zirconia primers.

Material and Methods: Silica-base lithium disilicate was etched and treated with BisGMA-incorporated Porcelain Primer, unmodified Porcelain Primer, or resin-containing Kerr Silane. Zirconia ceramic was airborne-particle abraded and treated with BisGMA-incorporated Monobond Plus, unmodified Monobond Plus, or BisGMA-containing ZPrime Plus. After primer treatment and cleaning with ethanol, the contact angles were measured to determine surface change (n=10). Shear bond strength tests were also performed to measure the adhesion strength between resin cements and ceramic surfaces (n=10). Data were statistically analyzed by 1-way ANOVA followed by the Tukey multiple comparison as a post hoc test (significance level .05).

Results: The incorporation of BisGMA resin did not significantly influence the bond strength or contact angle of the zirconia primer ($P>.05$), but it did significantly reduce those of the silane primer ($P<.05$). Resin-containing Kerr Silane (22 degrees, 23 MPa) had a similar contact angle and higher bond strength than the control (21 degrees, 18 MPa), but lower than Porcelain Primer (88 degrees, 34 MPa). Resin-containing ZPrime Plus (75 degrees, 29 MPa) had a similar contact angle and higher bond strength than both Monobond Plus (74 degrees, 18 MPa) and the control (15 degrees, 4 MPa).

Conclusions: The addition of BisGMA resin significantly inhibited the efficacy of silane-containing porcelain primers but did not affect that of phosphate-containing zirconia primers