Comparative Evaluation of Shear Bond Strength and Microleakage of Biodentine in Primary and Permanent Dentin: An *In Vitro* study

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Abstract

Introduction: This study is undertaken to evaluate and compare the shear bond strength of Biodentine to primary and permanent dentin after 2 days, 7 days, and 14 days and microleakage in primary and permanent dentin. **Materials and Methods:** A total of 60 primary and 60 permanent teeth were selected for study out of which 30 primary and 30 permanent teeth were evaluated for microleakage and rest teeth were divided into 3 subgroup of primary containing 10 sample in each and same for permanent teeth, which were evaluated for shear bond strength at 2 days, 7 days, and 14 days. Coronal dentin was exposed, and teeth were embedded into self-cure acrylic. For shear bond testing, a standardized PVC mold was placed over exposed dentin and restored with Biodentine and evaluated after 2 days, 7 days, and 14 days using a universal testing machine. For microleakage testing, a standardized class I cavity was prepared with 2 mm depth and filled with Biodentine and subject to thermocycling than submerged in 2% methylene blue dye for 24 h. After removing from the dye, teeth were cleaned and sectioned longitudinally and studied under a stereomicroscope. **Results:** Data were collected, tabulated, and analyzed using analysis of variance test and unpaired *t*-test. A significant difference ($P < 0.0001$) in shear bond strength existed at 2 days and 7 days in Biodentine-Primary dentin samples and same for Biodentine-Permanent dentin samples while no significant difference ($P > 0.05$) in shear bond strength existed at 7 days and 14 days in Biodentine-Primary dentin samples and same for Biodentine-Permanent dentin samples. Statistically insignificant difference existed between shear bond strength of Biodentine to permanent dentin and Biodentine to primary dentin (at 2 days [$P = 0.1095$, 7 days ($P = 0.0895$), and 14 days ($P = 0.0716$]). Biodentine exhibits less microleakage in both permanent (0.833 + 1.085) and primary teeth (0.666 + 0.922), which was statistically insignificant ($P = 0.524$). **Conclusion:** Biodentine exhibits the same level of shear bonding and microleakage in both primary and permanent dentin.

Keywords: Biodentine, Microleakage, Permanent dentin, Primary dentin, Shear bond strength.

Introduction

The most important objective of dental treatment in in pediatric dentistry is the preservation of functional primary teeth until natural exfoliation. To maintain normal form and function of a tooth, enamel and dentin need to be retained; if lost due to caries etc., they must be replaced through the preservation and treatment of the pulp-dentine complex by early and well-indicated pulp therapies.[1-3]

Dentine plays an important role in preserving the vitality of the tooth.[4] Loss of dentin is perhaps one of the major losses that hamper the integrity of the tooth structure to a significant extent. Whether in the coronal portion or the radicular one, the dentin loss must be substituted with an artificial material, which can restore the physiological integrity of the tooth structure.[5] A dentin substitute or a base, when used, reduces post-operative sensitivity caused by materials used to restore the tooth and also protects the pulp.[6]

From time immemorial, many materials have been studied for this purpose. Many medications, antibiotics, and anti-inflammatories have been used as pulp capping agents. If successful, this procedure precludes the need for more invasive,

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more extensive, and more expensive treatment. There has been constant research on the factors that may promote the healing of pulpal wounds and maintain vitality.[7-13]

Calcium hydroxide (CH) has enjoyed dominance since its introduction by Hermann in 1920 for root canal fillings and has been the gold standard and generally accepted agent of choice for pulpcapping procedures. Since then, CH has been recommended by several authors for direct pulp capping; however, it took until the middle of 20th century until it was regarded as the standard of care.[13-24]

Biodentine is a tricalcium silicate-based material designed as a permanent dentin substitute, is bioactive and biocompatible such as mineral trioxide aggregate (MTA)[25,26] and possesses the same good sealing abilities as other calcium silicate cements (CSCs). The microhardness, flexural, and compressive strength of Biodentine are higher than those of other CSCs and more comparable to dentine. Besides having a fast setting time and a high compressive strength any material used for posterior fillings must also have the ability to create a bond between the material and the dentine such as glass ionomer cements (GICs) or dentine adhesives. Thus, Biodentine can also be used as an alternative to GIC in restorative dentistry.

Several reports have compared restorative material bond strength to permanent tooth dentin with varying results. Primary dentin has been assumed to be different from permanent dentin due to the variable amounts of mineral components, as well as morphological and structural differences. There might be substantial differences in the properties of primary dentin compared to permanent dentin.

This study is undertaken to evaluate and compare shear bond strength of Biodentine to primary and permanent dentin after 2 days, 7 days, and 14 days and microleakage in primary and permanent dentine.

Materials and Methods

The purpose of this *in vitro* study is to evaluate and compare the shear bond strength of Biodentine to primary and permanent dentin after 2 days, 7 days, and 14 days and microleakage in primary and permanent dentin. This study was carried out in the Department of Paediatric and Preventive Dentistry in association with the Department of Oral Pathology and Microbiology in Daswani Dental College and Research Centre, Ranpur Kota.

Inclusion criteria

Intact, freshly extracted primary and permanent teeth were included in this study.

Exclusion criteria

Grossly carious tooth, teeth which are attrited, abraded, restored, and with surface cracks/defects.

In the present study, 60 primary teeth and 60 permanent extracted human teeth were collected. The extracted teeth were cleaned of soft tissue and debris. Surface debridement of all teeth was done using a hand scaling instrument. The extracted teeth were stored in deionized water at room temperature till the commencement of the study, for not more than 1 week. 30 primary and 30 permanent teeth - used for microleakage testing; 30 Primary and 30 permanent teeth - for testing shear bond strength.

Samples were divided into the following groups and subgroups:

For shear bond testing

- Group S.1-Biodentine placed over primary dentin:
  - Subgroup S.1-2: Testing after 2 days
  - Subgroup S.1-7: Testing after 7 days
  - Subgroup S.1-14: Testing after 14 days.
- Group S.2-Biodentine placed over permanent dentin:
  - Subgroup S.2-2: Testing after 2 days
  - Subgroup S.2-7: Testing after 7 days
  - Subgroup S.2-14: Testing after 14 days.

For microleakage testing

Groups	Restorative material	Properties to be evaluated	Time of testing	No. of samples
M.1	Biodentine	Microleakage in primary dentine	After 24 h	30
M.2	Biodentine	Microleakage in permanent dentine	After 24 h	30

Methodology for preparation of dentin surface

Horizontal indentations were placed on the radicular portion. The root portion of each tooth was embedded into an acrylic mold with the occlusal surface of the tooth parallel to the base. Molds were then filled with cold-cure acrylic resin, leaving the crown portion of the tooth exposed. A high-speed fine diamond disk with copious water spray was used to expose coronal dentin of the occlusal surface, cut perpendicular to the long axis of the tooth.

Shear bond testing

After exposure of coronal dentin, abrasive paper was used subsequently to obtain flat dentin surface. Specimens were then stored in distilled water at room temperature. All samples were rinsed with distilled water and air dried directly before the application of the test material. A PVC mold of 3.5 mm inner diameter and 3 mm was placed on the dentine samples and was completely filled with test material. Biodentine capsule was manipulated with the help of an amalgamator and the mix was condensed into the mold with the help of a cement carrier and filling instrument, avoiding any air entrapments, voids, or gaps. Molds were removed after (15 min) confirming the initial set of the cement. Specimens produced were stored in distilled water at 37°C for 24 h before subjected to shear bond testing using universal testing machine. Shear bond strength for 10 specimen out of each group were tested after 2 days, 7 days, and 14 days. For Shear bond strength testing, the universal testing machine was utilized. The acrylic cylinders were secured to the test base clamp and placed on the static lower table of the universal testing machine while the crosshead assembly was secured to the mobile upper member. The crosshead was positioned so that the leading edge would contact the bonded Biodentine- dentin interface. The crosshead speed was set to 0.5 mm/min extension. For each sample, the maximum force achieved before breaking the bond was recorded. Shear bond strength was then calculated by dividing the maximum force by the surface area of the bonded composite resin interface (Surface Area = 9.625 mm^2) (1 N/mm^2 = 1 MPa). Notes were also taken regarding the date and time of Biodentine specimen preparation, as well as the associated lot number for each Biodentine capsule used. The mean, standard deviation, at 95% confidence intervals were calculated for each group using Statistical Package for the Social Sciences 20.0 software. Statistical analysis was done, and results were tabulated.

Microleakage testing

Cavity preparation

A standardized Class I cavity preparation was done on occlusally exposed dentinal surfaces using a carbide bur in a high-speed airotor handpiece with water sprays for placing 2 mm base layer of Biodentine. All internal line angles were rounded. The teeth were randomly divided into 2 groups of 30 teeth.

Group M.1	Group M.2
Primary dentin (30)	Permanent dentin (30)

Restorative procedure

Prepared cavities were restored with 2-mm-thick layer of Biodentine. One capsule of Biodentine was opened and liquid was dispensed into the capsule and the capsule was placed inside the amalgamator and mixed for 30 s. Then the mix was carried using cement carrier and condensed against the cavity wall. Excess material was removed within 12 min after which the cement sets hard. All specimens were subjected to 500 rounds of thermocycling at 5/55° with a dwell time of 60s. Two coats of nail varnish were applied on the entire tooth except 1 mm from the restoration margin. Teeth were then placed in 2% methylene blue dye for 24 h at room temperature. After removal of the specimens from the dye solution, the superficial dye was removed with a pumice slurry and rubber cup. The specimens were then sectioned longitudinally with double-sided diamond discs, and two sections were obtained. The extent of dye penetration was studied under a stereomicroscope. The part of the sectioned tooth that showed a greater amount of microleakage was scored. Microleakage was scored using following criteria:

Scoring	Dye penetration	Extent of leakage
0	0	No dye penetration
1	0–0.7 mm	Dye penetration up to the first third of the prepared cavity wall
2	0.7–14 mm	Dye penetration up to the second-third of prepared cavity wall
3	Up to 2 mm	Dye penetration into the entire prepared cavity wall, but not including the pulp floor of the cavity
4	>2 mm	Dye penetration, including the pulpal floor of the cavity

Results

In the present study, 60 primary teeth and 60 permanent extracted human teeth were collected. 30 primary and 30 permanent teeth - used for microleakage testing; 30 Primary and 30 permanent teeth - for testing shear bond strength. Statistical analysis was done by using SPSS software version 26.0. Unpaired student 't' test and Repeated Measure of ANOVA was applied. Signiifcance level is fixed at $P < 0.05$.

Table1reveals Comparative evaluation of mean shear bond strength of biodentine on primary dentine& Permanent dentine at 2 days, 7 days & 14 days. It shows that mean shear bond strength was signficantly increased from 2 days to 14 days among both primary & permanent dentine.($P<0.001$). it also shows that there was statistically no signiifcant difference between primary and parmament dentine at 2, 7, & 14 days.($P>0.05$) (Table1).

Table 2 reveals Comparative evaluation of mean Microleakage of biodentine on primary dentine& Permanent dentine. Mean microleakage was higher among permanent dentin (0.833 + 1.085) as compare to primary dentin(0.666 + 0.922). There was statistically no signiifcant difference found in mean Microleakage of biodentine on primary dentine& Permanent dentine.($P=0.523$)(Table 2).

Table 3 reveals Microleakage scores of biodentine obtained in primary dentine and permanent dentine samples . Microleakage were assessed from score 0 to score 4. Score 1 were found among maximum samples follwed by score 2. (Table 3).

Table 1: Comparative evaluation of mean shear bond strength of Biodentine on primary dentine and Permanent dentine at 2 days, 7 days, and 14 days

Groups	Shear bond strength			Repeated measure of ANOVA
	After 2 days n=10	After 7 days n=10	After 14 days n=10	
	Mean±SD	Mean±SD	Mean±SD	Significance "*P*" Value
Primary dentine	3.450±0.552	8.570±0.618	8.910±0.521	<0.001 (HS)
Permanent dentin	3.840±0.481	9.010±0.467	9.360±0.529	<0.001 (HS)
Student "*t*" test value	1.684	1.796	1.916	
P-value	0.109 (NS)	0.089 (NS)	0.071 (NS)	

ANOVA: Analysis of variance, SD: Standard deviation

Table 2: Comparative evaluation of mean microleakage of Biodentine on primary dentine and permanent dentine at 2 days, 7 days and 14 days

Groups	Microleakage
n=30	Mean±SD
Primary dentine	0.666±0.922
Permanent dentin	0.833±1.085
Student '*t*' test value	0.6424
P-value	0.523 (NS)

SD: Standard deviation

Table 3: Microleakage scores of Biodentine obtained in primary dentine and permanent dentine samples

Group	Microleakage score									
	0		1		2		3		4	
	No	Percentage	No	Percentage	No	Percentage	No	Percentage	No	Percentage
Primary dentine (*n*=30)	17	56.66	8	26.66	3	10	2	6.66	0	0
Permanent dentine (*n*=30)	15	50	9	30	3	10	2	6.66	1	3.33

Table 4: Biodentine components and properties

Component	Function
Tricalcium silicate	Main component of the powder regulates the setting reaction
Dicalcium silicate	Additional core material
Calcium carbonate and oxide	Acts similar to filler
Iron oxide	Contributes to shade/color
Zirconium oxide	Radio pacifier
Calcium chloride	Accelerator
Hydro soluble polymer	Reduces the viscosity to increase workability Water reducing agent

Discussion

One of the main goals of restorative procedures in primary and permanent teeth is the removal of carious tissue and its replacement with suitable material. Adaptation of the restorative material to cavity margins and internal cavity surface is of great importance for the long-term performance of restoration to control the marginal leakage, which may occur due to dimensional changes in restorative material and which leads to a lack of adaptation of material to cavity preparation, leading to recurrent caries and pulpal disease.

In 2011, the French manufacturer Septodont introduced the material Biodentine (Septodont, Saint Maur Des Fosses, France). According to the manufacturer, Biodentine has many potential uses. Biodentine has been advocated for use as an endodontic restorative material for procedures including apexification, root perforation repair, and repair of internal or external root resorption. With regard to vital pulp therapy, it has been advocated as a direct and indirect pulp-capping medicament, as well as for pulpal coverage in pulpotomy procedures and apexogenesis. As a direct restorative material, Biodentine can be used as a temporary restoration or as a permanent dentin replacement under definitive composite restorations (Bachoo *et al*. 2013).

(Bachoo *et al*. 2013, Septodont, Saint Maur Des Fosses, France).

The extracted teeth were stored in deionized water at room temperature till the commencement of the study, for not more than 1 week. ISO (1991) reported that no other chemical agent than deionized water should be used since other agents may be absorbed by, and alter tooth substance. After exposure of mid-coronal dentin, abrasive paper was used subsequently to obtain a flat dentin surface. Teeth were embedded in self-cure acrylic resin. Specimens were then stored in distilled water at room temperature. All samples were rinsed with distilled water and air dried directly before the application of the test material.

For standardization of samples, a PVC mold of 3.5 mm inner diameter and 3 mm was placed on the dentine samples and was completely filled with test material. Biodentine is distributed in single application units of the liquid and powder forms to be mixed immediately before placement (Koubi *et al*. 2013). According to the manufacturer, the powder is composed of tricalcium silicate, dicalcium silicate, calcium carbonate, iron oxide, and zirconium oxide, while the liquid component is made up of water, calcium chloride, and a partially modified polycarboxylate to serve as a superplasticizing and water reducing agent (Han *et al*. 2013, Raskin *et al*. 2012). First, the liquid portion is added to the powder capsule, and then the capsule is triturated. Once mixed, Biodentine has a consistency that is dense and can be carried with a spatula and then gently condensed within the cavity preparation with amalgam condensers (Raskin *et al*. 2012).

Biodentine does not require dentin or enamel to be etched before application, nor does it require a bonding agent (Koubi *et al*. 2013). Biodentine is applied directly into the cavity preparation and, when used as a direct pulp-capping medicament, placed directly over the exposed pulpal tissues as well. For posterior teeth, Biodentine has been used for both class I (occlusal) and class II (interproximal) cavity preparations.

For filling the mold with Biodentine, a capsule was gently tapped on a hard surface to loosen the powder. Once a capsule was opened, it was placed on the white capsule holder and then a single-dose container of liquid was detached and gently tap on the sealed cap to force all the liquid down container, cap was twisted to open it and care was taken so that no drop of liquid falls out of the single dose container. 5 drops from the single-dose container was poured into the capsule and slightly mixed with mixing sticks and closed and placed on amalgamator for mixing, at a speed of 4000–4200 rotations/min for 30 s. After 30 s capsule was opened, and thick consistency Biodentine was collected with the instrument supplied in the box. Then, the mix was condensed into the mold with the help of a cement carrier and filling instrument, avoiding any air entrapments, voids, or gaps.

Molds were removed after (15 min) confirming the initial set of the cement. Specimens produced were stored in distilled water at 37°C for 24 h before being subjected to shear bond testing using a universal testing machine. For shear bond testing of Biodentine to primary dentin and for shear bond testing of Biodentine to permanent dentin, depending on the assigned grouping, samples were allowed to set for 2, 7, and 14 days.

After the allotted setting time, Shear bond strength for 10 specimen samples out of each group were analyzed after 2 days, 7 days, and 14 days. Specimens were fixed to a universal testing machine and loaded to failure. For shear bond strength testing, the universal testing machine (Shimadzu AG-X; Tokyo, Japan) with a crosshead speed of 0.5 mm/min was utilized. The acrylic cylinders were secured to the test base clamp and placed on the static lower table of the universal testing machine while the crosshead assembly was secured to the mobile upper member. The crosshead was positioned so that the leading edge would contact the bonded Biodentine-dentin interface. The crosshead speed was set to 0.5 mm/min extension. For each sample, the maximum force achieved before breaking the bond was recorded. Shear bond strength was then calculated by dividing the maximum force by the surface area of the bonded composite resin interface (Surface Area = 9.625 mm^2) (1 N/mm^2 = 1 MPa).

The result showed that the mean shear bond strength of Biodentine on permanent teeth at 2 days was 3.840 ± 0.4812 MPa while the mean shear bond strength of Biodentine on primary teeth at 2 days was 3.450 ± 0.552 MPa. Similar shear bond values were observed at Biodentine-permanent dentin interface in a study done by Kaup *et al*. at 2-day (3.14 ± 1.09) MPa. The result showed that the mean shear bond strength of Biodentine on permanent dentin 7 days was 9.010 ± 0.467 MPa while mean shear bond strength of Biodentine on primary dentin at 7 days was 8.570 ± 0.618 and similar values were observed in a study done by Kaup *et al*. at Biodentine-permanent dentin interface on 7 days (9.75 ± 2,19) MPa. The result showed that mean shear bond strength of Biodentine on permanent dentin after14 days was 9.360 ± 0.529 MPa, whereas the mean shear bond strength of Biodentine on primary teeth at 14 days was 8.910 ± 0.521 MPa. In a study done by Kaup *et al*. similar values were observed for shear bond strength of Biodentine-permanent dentin interface when evaluated at 14 days (9.34 ± 1.01 MPa. Pradeep *et al*. conducted study to evaluate the shear bond strength of Biodentine-dentin interface and MTA-dentin at different time interval. The result showed that the mean shear bond strength of Biodentine on permanent teeth after 2 days (7.97 ± 1.35), 7 days (9.69 ± 0.48), 1 month (11.21 ± 0.69), 6 months (11.61 ± 0.72) were in accordance with our study results.

According to the results of the present study, the lowest mean shear bond strength of Biodentine–primary dentine interface was measured at 2-day specimen while the highest mean shear bond strength of Biodentine–primary dentine interface was measured at 14 days. When analysis of variance (ANOVA) test was applied, there was a statistically significant difference between the shear bond strength of Biodentine-primary dentin interface at 2 days, 7 days, and 14 days. When unpaired *t*-test was applied between sample vales at 2 days and 7 days, there was statistically significant difference having $P < 0.0001$, revealing that a significant difference existed in values of mean shear bond strength of Biodentine–primary dentine interface measured at 2 days and values of mean shear bond strength of Biodentine–primary dentine interface measured at 7 days. When unpaired *t*-test was applied between sample vales at 7 days and 14 days, $P > 0.05$ was obtained, revealing that no significant difference in shear bond strength existed in values of mean shear bond strength of Biodentine–primary dentine interface measured at 7 days and values of mean shear bond strength of Biodentine–primary dentine interface measured at 14 days in Biodentine–primary dentin samples.

According to the results of the present study, the lowest mean shear bond strength of Biodentine–permanent dentine interface was measured at 2 days specimen while the highest mean shear bond strength of Biodentine–permanent dentine interface was measured at 14 days. When the ANOVA test was applied, there were statistically significant differences between shear bond strength of Biodentine–permanent dentin interface at 2 days, 7 days and 14 days. When unpaired *t*-test was applied between sample vales at 2 days and 7 days, there was statistically significant difference having $P < 0.0001$, revealing that a significant difference existed in values of mean shear bond strength of Biodentine–permanent dentine interface measured at 2 days and values of mean shear bond strength of Biodentine–permanent dentine interface measured at 7 days. When unpaired *t*-test was applied between sample vales at 7 days and 14 days, $P > 0.05$ was obtained, revealing that no significant difference in shear bond strength existed in values of mean shear bond strength of Biodentine–permanent dentine interface measured at 7 days and values of mean shear bond strength of Biodentine–permanent dentine interface measured at 14 days in Biodentine-primary dentin samples.

In the present study, there were no statistically significant differences between shear bond strength values of Biodentine-permanent dentin interface at 2 days and shear bond strength values of Biodentine-primary dentin at 2 days ($P = 0.1095$ and there were no statistically significant differences between shear bond strength values of Biodentine-permanent dentin interface at 7 days and shear bond strength values of Biodentine-primary dentin at 7 days [$P = 0.0895$]. Similarly, there were no statistically significant differences between shear bond strength values of Biodentine-permanent dentin interface at 14 days and shear bond strength values of Biodentine-primary dentin at 14 days [$P = 0.0716$]). Similar to our study results, no significant differences were seen in the shear bond strength of Biodentine in primary versus the shear bond strength of Biodentine in permanent in a study conducted by Raju *et al*.[55]

In the present study, class 1 cavities were prepared in each sample with straight fissured burs using high speed, water-cooled handpiece. The cavity width represented approximately one-third the width of the occlusal table, with a depth of 2 mm below the DEJ without bevel as Biodentine can be used as base as a permanent dentin substitute under permanent restoration. Thermocycling is used to mimic intra-oral temperature variations and subject the restoration on the tooth to temperature extremes compatible with the oral cavity. The result of the present study revealed that Biodentine exhibits less microleakage in both permanent (0.833 + 1.085) and primary teeth (0.666 + 0.922), which was statistically insignificant

($P = 0.524$). This correlates with the findings of Koubi *et al*., Raju *et al*. (Permanent [0.76 + 0.83], primary [0.60 + 0.87], $P = 0.415$) studies.

The good marginal integrity of Biodentine is likely due to the outstanding ability of the calcium silicate materials to form hydroxyapatite crystals at the surface. When formed at the interface between the restorative material and the dentin walls, these crystals may contribute to the sealing efficiency of the material. Biodentine™ showed significantly less microleakage than GIC, because Biodentine™ is composed of a mixture of 5 µm round particles embedded in a calcium silicate hydrate matrix, which are very smaller than the size of MTA particle. Raskin *et al*. investigated the microleakage of Class II sandwich technique with a biocompatible Ca_3 SiO_5-based dentin substitute and found that Biodentine™ can be indicated in open-sandwich Class II restorations without any preliminary treatment.

Studies on the ultrastructure of the Biodentine-dentine interface following phosphate-buffered saline immersion have demonstrated the formation of a “mineral tag” studies by Han and Okiji also confirmed the formation of tag-like structures alongside an interfacial layer called as “mineral infiltration zone” by intertubular diffusion of carbonate from CSC into the dentin, following the denaturing effect of the strongly alkaline cement. The comparison of interfaces showed a dentin-mineral infiltration with the Biodentine. Thus far, researchers have concluded that there is low penetrance of silver nitrate solution as well as low glucose diffusion apparent between the tooth structure and Biodentine restoration (Pradelle-Plasse *et al*. 2009 and Koubi *et al*. 2012). Nevertheless, Biodentine has shown good adaptability toward dentine. In addition to the formation of apatite crystals, the nanostructure of calcium silicate hydrate may also explain the good sealing qualities of the CSC.

Conclusion

Hence, in the light of these data and the result obtained by our study, we conclude that Biodentine exhibits better shear bonding to dent in after 7 days in both primary and permanent teeth and less microleakage in primary dentin, so it can be used in both permanent and primary teeth. Since *in vitro* studies do not reflect all variables present in the mouth, the results of this *in vitro* study may not be extrapolated to clinical situations unless adequate clinical trials are conducted to test the *in vivo* efficacy of the material.

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