Injectable Calcium Phosphate Cement as a Bone-Graft Material Around Peri-implant Dehiscence Defects: A Dog Study

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Purpose: *Peri-implant dehiscence defects occur frequently after dental implant placement. Various graft materials and techniques are proposed for treatment. In this study, an injectable calcium phosphate cement (Augmentech, Wetzlar, Germany) applied to a peri-implant defect was investigated.* ***Materials and Methods:*** *Standardized buccal dehiscence defects (5.8 × 3.8 mm) were surgically created after implant site preparation in the right proximal tibiae of 5 beagle dogs. Fifteen stepped cylindrical implants (13 × 3.8 mm diameter) were inserted (3 per dog), and Augmentech injectable calcium phosphate cement was injected into the dehiscences. The bone at the distal side of the implant was left intact to serve as a control. Postsurgically, each dog received double staining of 2 fluorescent labels for estimation of bone cell activity at baseline and after 11 weeks of healing. The animals were sacrificed after 12 weeks. Dissected blocks were processed for histologic, histomorphometric, and fluorescence microscopic analysis, ie, percentage of bone-to-implant contact (BIC) and percentage linear bone height (LBH) were measured. Student* t *and Mann Whitney* U *tests were used for statistical analysis (*P < *.05).* ***Results:*** *Healing was uneventful in all dogs. Augmentech injectable calcium phosphate cement showed good space maintenance and osteoconductive properties with no foreign body reaction. BIC was 34.42 (± 19.88) and 37.00 (± 21.33) (*P = *.375), while LBH was 84.23 (± 19.73) and 96.10 (± 6.66) (*P = *.125) for test and control sites, respectively.* ***Conclusion:*** *Within the limits of the present study, it was concluded that Augmentech injectable calcium phosphate cement may be a suitable material for the treatment of buccal dehiscence defects around dental implants.* Int J Oral Maxillofac Implants 2008;23:1053–1062.

Keywords: *calcium phosphate, dehiscence, dental implant, dog study, grafting*

Sufficient bone volume is among the prerequisites for optimal implant placement and primary stability. However, localized bone defects may occur as a result of periodontal disease, traumatic injury, or tooth extraction. These bone defects may present serious esthetic deformities and insufficient bone volume for implant placement. Many attempts and methods have been presented for the restoration and preservation of alveolar bone morphology.[1,2] Autogenous bone grafts are considered the gold standard but experience limited use due to complex harvesting procedures and additional costs.[3,4] Further, awareness of disease transmission via contaminated blood and tissue has raised concerns about the use of allograft materials.[5,6]

Also, there is an increasing tendency to accelerate rehabilitation and shorten the period that patients are asked to live without functional teeth. There are immediate implantation trials employing a variety of techniques and protocols that have been performed after tooth extraction.[7–9] Immediate implantation cases are frequently associated with peri-implant bone defects due to incongruity of extraction socket and implant body, which requires a bone graft to fill the defect gap.[10]

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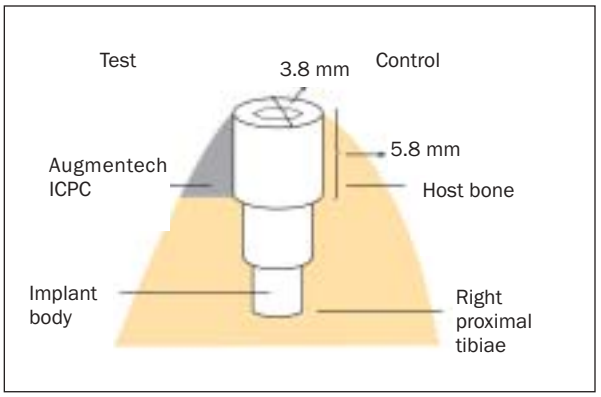


Fig 1 Schematic presentation of the experimental design. The stepped cylindrical implant was used as reference to create the dehiscence defect.

Dehiscences are the most common type of bone defects clinicians face during implant surgery, especially after tooth extraction.[11] This type of defect does not heal without treatment[12] and may possess a biomechanical risk at implant loading.[13] Several treatment modalities and materials are described with satisfactory results for dehiscences resulting from extraction or peri-implant pathology.[14]

Guided bone regeneration with a barrier membrane has become an accepted method of treating peri-implant bone defects adjacent to dental implants.[15] Despite the success of this method, membrane exposure and subsequent potential bacterial colonization can result in inflammation and/or reduced bone fill.[16]

Synthetic materials made of calcium phosphate ceramics have shown their osteocompatibility and usefulness as an implant material.[17–19] These materials offer great potential for bone regeneration because they have a chemical composition similar to the biological apatite of bone tissue.[20] Animal and human studies with block, granulated, and powder forms of calcium phosphate ceramics have proven their efficacy as bone-substitute materials.[21–23] In addition, a powder-and-liquid form, which hardens after mixing and is designed for injection into the bone defect, also provided favorable results.[24–26]

The purpose of this study was to determine the efficacy of an injectable calcium phosphate cement (ICPC) for the treatment of experimental peri-implant bone dehiscence defects. Therefore, the injectable calcium phosphate cement material was evaluated for its capacity to create and maintain space for the regeneration of bone. In addition, the biodegradation/substitution of the injectable calcium phosphate cement and the nature of the newly formed bone were examined.

MATERIALS AND METHODS

The injectable calcium phosphate cement used in this study (Augmentech, Wetzlar, Germany) comes in a ready-to-mix tube with powder and liquid components. The powder consists of tricalcium phosphate, magnesium phosphate, magnesium hydrogen phosphate, and strontium carbonate, while the liquid is a diammonium hydrogen phosphate solution. The powder component was sterilized with 27 kGy gamma radiation.

Prior to application, the tube was inserted into a mixing apparatus (Silamat, Vivadent, Schaan, Liechtenstein), shaken for 15 seconds, and placed into a hand instrument for manual injection in retrograde fashion. Physical setting was achieved after approximately 10 minutes. Elasticity of the hardened cement was reported to be 230 N/cm^2, 2.3 MPa in vivo.

Implants

Fifteen titanium stepped cylindrical implants (Frialit-II, Friadent-Dentsply, Mannheim, Germany) 13 × 3.8 mm in diameter were used. A stepped implant design was preferred to facilitate the standardization of the surgical model and histological process (Fig 1).

Animals

Five male beagle dogs about 3 years of age weighing between 34 to 42 kg were used in the study. The Ethics Commission of Istanbul University approved the study protocol. Animals were monitored for 15 days prior to surgery to reveal any possible pathology. A standard diet was used during this observation period.

Surgical Procedure

The proximal tibia was chosen as the experimental site for its ease of access and low risk of wound complications. The right proximal tibiae were shaved and washed and disinfected with betadine. Xylazin (Rompun, Bayer, Germany) 1.5 mg/kg IM was administered as a premedication. After 10 minutes, ketamin (Ketanest, Alfasan, The Netherlands) 10 mg/kg IM was injected as anesthesia. Animals were cardiologically monitorized during the surgery. After ensuring the status of anesthesia, a full thickness flap was raised and the cortical bone was exposed in the proximal tibiae. Implant beds were prepared according to the written protocol of the Frialit-II (Friadent-Dentsply, Mannheim, Germany) implant system for the 13 × 3.8 mm diameter stepped cylindrical implant. Three implant beds, 1.5 cm apart, were prepared. After the preparation of the implant beds, bone at the mesial side of the implants was removed to the level of the first coronal step level (3.8 mm × 5.8 mm) using round and fissure burs and a rotation

speed of 850 rpm under saline irrigation. Standardization was verified using periodontal probes. After the bone preparation, the surgical site was rinsed thoroughly with saline and cleaned of debris. The bone at the distal side of the implants was left intact and served as a control. The implants were then placed into the prepared sockets. Each animal received 3 implants. All implants achieved good primary stability following insertion. Defects were checked again 3-dimensionally for the standardization of the created dehiscence defects. Healing caps were screwed on the implants, and hemostasis was established by moisture gauze application. The Augmentech injectable calcium phosphate cement was prepared according to the manufacturer's instruction and injected into the defects. Excess cement was removed and the remaining cement was shaped to the defect's geometry (Fig 2). After setting of the cement, wound was closed in layers using 3.0 vicryl and silk sutures. Radiographs were taken to check the surgical area and implants. A profilactic antibiotic regimen (amoxicillin and clavuonic acid, Sysnulox, Pfizer, Belgium, 140 mg + 35 mg) 20 mg/kg IM was administered for 7 days. To follow the pattern of osteogenesis, tetracyclin hydroclorur (Tetra, Mustafa Nevzat Ilaç Sn, Istanbul, Turkey, 10 mg/kg, IV) was injected at 4 weeks after implantation and alizarin complexone (Sigma Aldrich, Cologne, Germany, 25 mg/kg IV) was injected at 11 weeks after implantation.

After 12 weeks of complication-free healing, the animals were sacrificed by an overdose of pentobarbital (Abbot Laboratories, Chicago, Illinois, 65 mg/kg, IV). Bone segments containing the implants were harvested en bloc, immersed in a 3% phosphate buffered formalin solution, and stored at 4°C for 2 weeks.

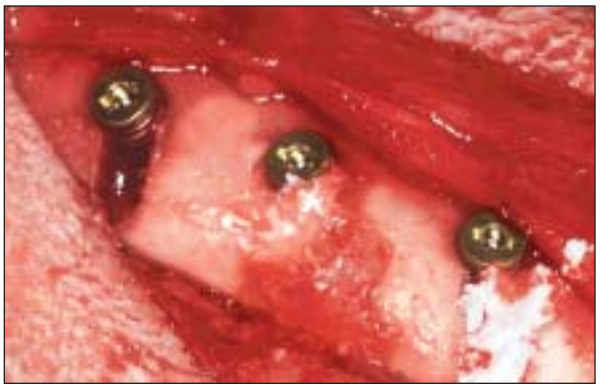


Fig 2 Augmentech injectable calcium phosphate cement was injected into dehiscence defects.

Histologic Procedures

After fixation, each block containing 3 implants was dehydrated in ethanol and embedded in methylmethacrylate. Following polymerization, the blocks were reduced in size using a saw and grinding machine. Nondecalcified sections (195 µm) were prepared with a modified diamond blade saw microtome technique as described by Klein et al.[27] At least 4 sections per implant were obtained in a longitudinal direction parallel to the axis of the implant. Three sections were stained with methylene blue and basic fuchsin and one section was used without any staining for fluorescence microscopy. Finally, sections were evaluated with a light and fluorescence microscope (Leica DMRBE, Leica Microsystems, Wetzlar, Germany). Three sections from each implant were included for histologic and histomorphometric analysis. Biocompatibility, signs of inflammation, the role of Augmentech injectable calcium phosphate cement in bone ingrowth, bone-to-implant contact (BIC), and vertical linear bone height (LBH) in the dehiscence zone were evaluated. The dehiscence defect region (test) and the native bone region in the opposite wall of implant body's first coronal step (control) were investigated and compared. The resorption of the cement, bone-to-cement contact, and the substitution with new bone tissue were also evaluated and compared to the native bone in the other side of the implant wall.

Histomorphometric Analysis

To objectively measure the bone-regenerative efficacy of the Augmentech injectable calcium phosphate cement in the dehiscence area, the following measurements were performed on digital images (resolution 2,600 × 1,640 dots per inch) obtained by a digital camera (Sony DXC151P, Nagasaki, Japan) using image-analysis software (Leica Quin, Leica Microsystems Imaging Solutions, London, UK).

- BIC was defined as the percentage of bone in direct contact with the implant surface without any tissue in between (Fig 3).
- LBH, the distance between the coronal top level of the bone and the first step, was measured. This value was calculated in relation to the distance between the coronal top and first step level of the implant surface (Fig 4).

All measurements were repeated for test and control sites, and the presented data were based on the average of the 3 measured sections.

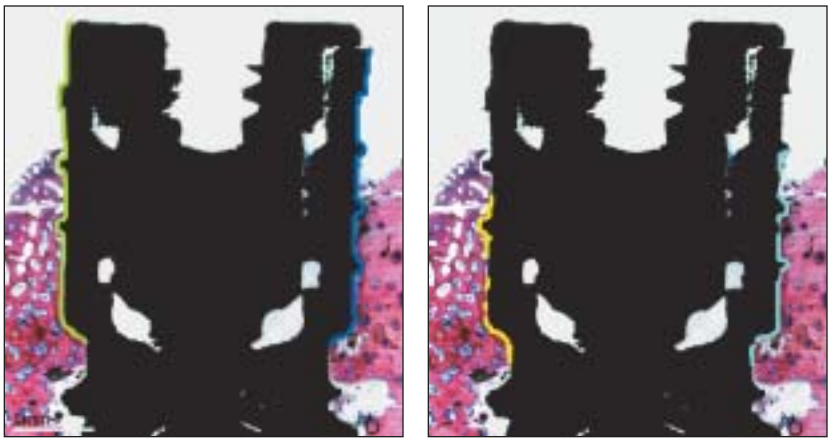


Fig 3 Schematic presentation of bone-to-implant contact (BIC) measurement. Green = Implant surface length in dehiscence side (test). Dark blue = Implant surface length along the first step of the implant body (control). Yellow = New bone tissue in contact with the implant surface (test). Light blue = Native bone tiisue in contact with the implant surface (control).

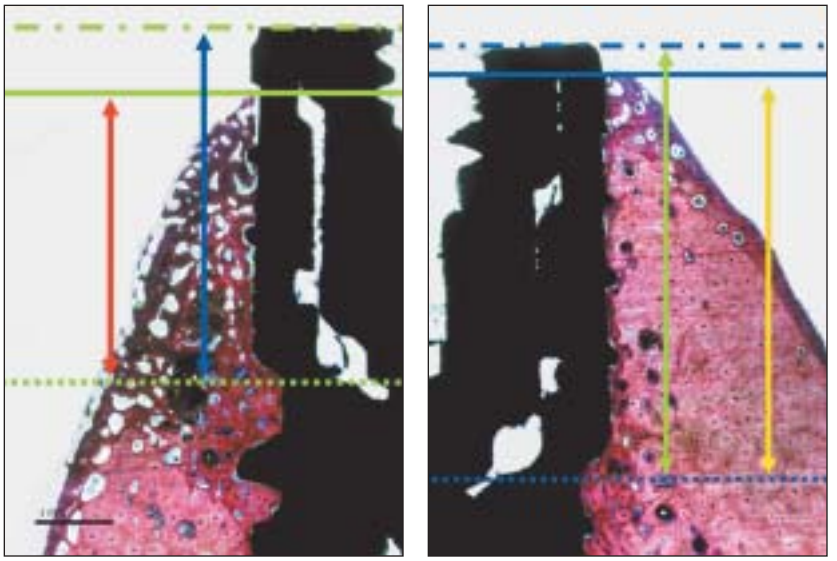


Fig 4 Schematic presentation of the linear bone height (LBH) measurement.

Test (left)
- Defect lining
- Top of the implant
- Measured length of first implant step
- Coronal bone contact
- Vertical bone height

Control (right)
- Defect lining
- Top of the implant
- Measured length of first implant step
- Coronal bone contact
- Vertical bone height

STATISTICAL ANALYSIS

Results were compared using SPSS 10.0.1 (SPSS, Chicago, Illinois). A 2-tailed Student *t* and Mann Whitney *U* tests were used for the comparision of the BIC and LBH values of test and control groups. Results were represented by ± 0.5 error range with a statistical significance of $P < .05$.

RESULTS

Healing was uneventful in all animals, except in dog 3 in which the sutures were partly removed 4 days after surgery. However, this site healed properly after daily cleaning and dressing.

Radiologic examination revealed no pathology around implants or defect sites.

Light Microscopic Observations

All implants were osseointegrated, and there was no sign of an inflammatory response. Augmentech injectable calcium phosphate cement was in direct contact with the host bone in all sections. Augmentech injectable calcium phosphate cement was replaced with new bone, sparsely woven bone tissue characterized by large marrow spaces. This new bone tissue was in direct contact with the implant surface. Evidently, Augmentech injectable calcium phosphate cement served as a good scaffold for new bone regeneration and maintained its given shape during the 12-week healing period. The Augmentech injectable calcium phosphate cement in the defect sites (test) was replaced by new bone, but a circular core of nonresorbed cement in the center of the dehiscence defect was seen in most of the sections (Fig 5). At higher magnification, resorption lacunae,

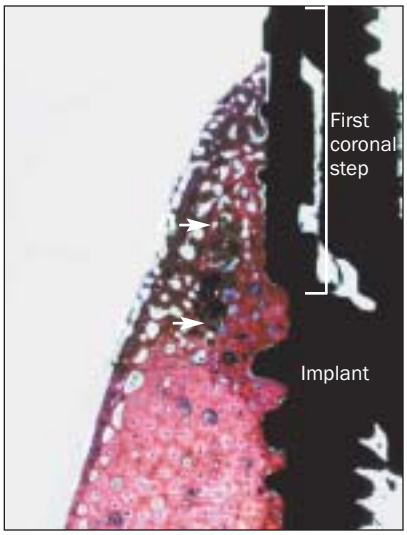


Fig 5 Implant no. 1 in dog 4. Test, methylene blue, basic fuchsin staining. Original magnification × 16. (Dark pink areas indicate newly formed bone and light pink areas are the host bone.) Augmentech injectable calcium phosphate cement core is largely being replaced by new bone with large marrows. Remnants of cement are present, especially in the center of the graft core *(arrows)*.

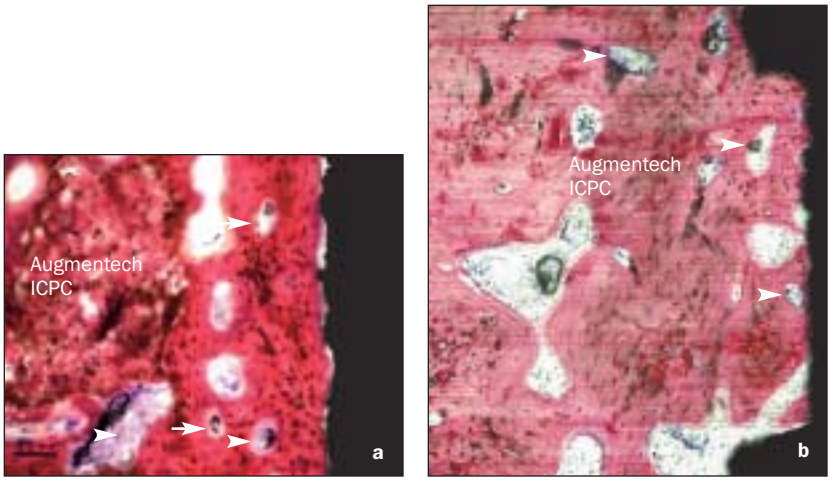
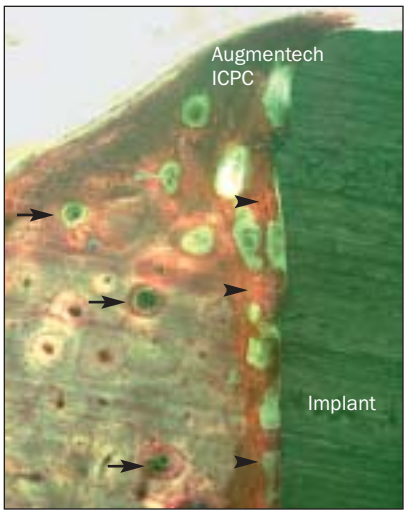


Fig 6 *(a)* Implant no. 1 in dog 4 (test). Methylene blue, basic fuchsin staining. Original magnification × 250. Randomly ordered blood vessels and primary osteons show the ongoing bone formation *(arrows)*. Resorption lacunae near to Augmentech injectable calcium phosphate cement body *(arrowheads)*. *(b)* Implant no. 3 in dog 1 (test). Methylene blue, basic fuchsin staining. Original magnification × 250. Residues of Augmentech injectable calcium phosphate cement can be observed appearing as brown-black areas. Resorption lacunae are present within the Augmentech injectable calcium phosphate cement area *(arrowheads)*.

Fig 7 Implant no. 2 in dog 3 (test). Methylene blue, basic fuchsin staining. Original magnification × 100. Dehiscence area is almost completely replaced with new bone characterized by the presence of secondary osteons and remodeling activity. Resorption lacunae are also visible *(arrows)*. Woven bone is still present at the bone-implant interface *(arrowheads)*.



including osteoclast-like cells, were seen, which implied that the material was still actively being replaced by new bone (Figs 6 and 7). Typical osseointegration process and remodeling activity were observed in control sections. There were no signs of inflammation or foreign body reaction. Secondary osteons could be discerned. At higher magnification, native bone could be seen in direct contact with the implant surface.

Fluorescence Microscopy

New bone formation began before the fourth week after surgery. The Augmentech injectable calcium phosphate cement was replaced by new bone tissue characterized by the presence of woven bone and primary osteon formation. Osteogenic activity was dense at the defect bottom as the cement body was mostly replaced by new bone (light green color indicates the fourth week of tetracycline labeling).

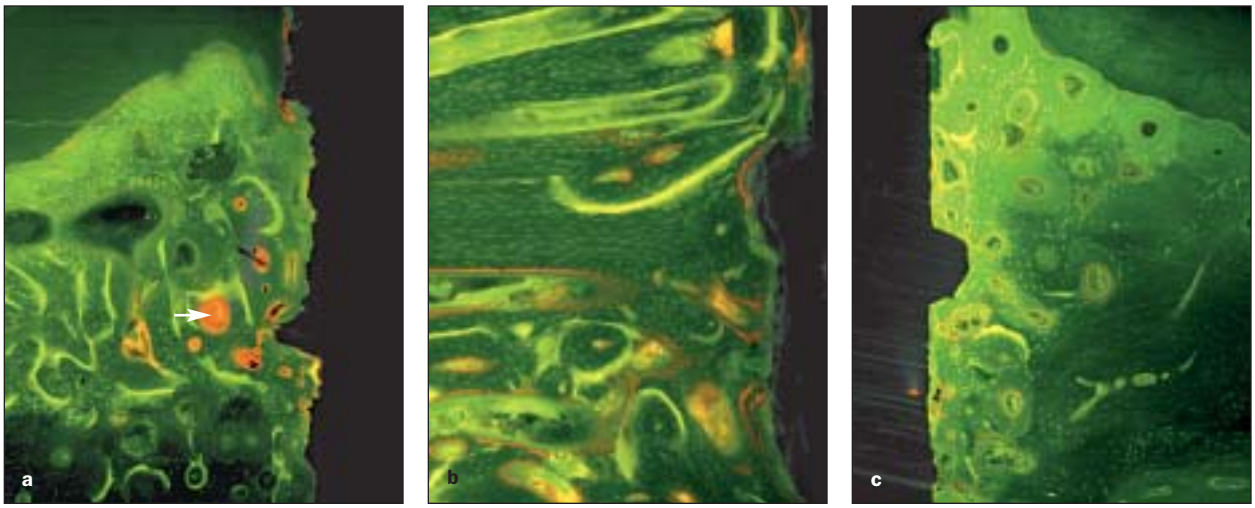


Fig 8 Fluorescence microscopy, tetracyclin hydrochlorur and alizarin complexon staining. *(a)* Implant no. 1 in dog 1 (test). Original magnification × 100. New bone formation is marked by light-green staining. Orange staining shows a resorption lacuna *(arrow)*. *(b)* Implant no. 3 in dog 2 (test). Original magnification × 250. Bone formation has already started in the fourth week. The bone-implant interface is still remodeling (orange staining). *(c)* Implant no. 1 in dog 1 (control). Original magnification × 100, active remodeling is characterized by orange staining.

At the end of the eleventh week, small fragments and particles of the Augmentech injectable calcium phosphate cement were still being replaced. Typical osseointegration and remodeling activity could be observed along the bone-implant interface in both the test and control sides. Despite ongoing osteogenic activity at the end of week 11, a circular, nonresorbed Augmentech injectable calcium phosphate cement body was present in the sections. Lamellar staining patterns of alizarin (red-orange) reveal bone apposition at the border of this Augmentech injectable calcium phosphate cement body. A typical osseointegration process, ie, resorption and remodeling of new and pre-existing bone structures, was visible in the control side.

The Augmentech injectable calcium phosphate cement material used in the study was dense and lacked macroporosity (< 1 mm). Nevertheless, the cement body was infiltrated with resorption lacuna-like structures; small lacunas stained green and larger lacunas stained orange-red. This reveals that bone apposition initiated near the small lacunas before the fourth week and near the large ones after the fourth week of implantation (Fig 8).

Bone Histomorphometry

Measured BIC values in test and control sites are listed in Table 1. Two-tailed Student *t* and Mann Whitney *U* tests showed no significant difference between the test and control sites ($P = .375$). Mean BIC values in control sites (37.00 ± 21.33) were slightly higher than test sites (34.42 ± 19.88). Due to very low BIC values detected in some of the histologic slices, a large data range was observed in the data series (Fig 9).

Measured LBH values in test and control sites are listed in Table 2. Two-tailed Student *t* test showed no significant difference between the test and control sites ($P = .125$) (Fig 10). The mean bone levels in control and test sites were 96.10 ± 6.66 and 84.23 ± 19.73, respectively.

DISCUSSION

Oral implants placed in dog tibiae were used to evaluate the behavior of an injectable calcium phosphate cement bone substitute in a peri-implant dehiscence model in this study. Densitometric and histometric similarity of dog bone to human bone was previously reported,[28] and calcium phosphate was tested in dogs in similar studies.[29,30] Also, bone grafts were already tested for their suitability to regenerate peri-implant bone defects in dog tibiae.[31,32] Therefore, dog tibiae seem to be suitable for testing a peri-implant dehiscence model as they have abundant bone volume working zones as compared to the alveolar crest.

In addition, alveolar ridge deformities occur after the removal of teeth in the dog mandible. These ridge deficits may interfere or hamper the standardization of the planned dehiscence model.[33] All this information formed the basis for the decision to choose the dog tibia as the experimental site in the current study. Further, a large number of studies report a lack of various degrees of bone fill and

Table 1 Mean and Standard Deviation (SD) Values of BIC in Augmentech ICPC-Filled Defects (test) and Control Regions (P = .375)

Implant no.	Test (%)	Mean	Control (%)	Mean
Dog 1				
1	56.8		57.55	
2	65.75	57.80	63.75	52.61
3	50.85		36.54	
Dog 2				
1	11.81		1.34	
2	26.01	21.45	22.27	13.37
3	26.52		16.49	
Dog 3				
1	18.83		26.41	
2	50.49	33.17	54.87	37.89
3	30.2		32.38	
Dog 4				
1	42.69		73.58	
2	69.64	50.11	56.68	62.82
3	38.01		58.21	
Dog 5				
1	5.63		0.83	
2	15.2	9.55	14.27	18.31
3	7.82		39.82	
Mean		34.42		37.00
(SD)		(19.88)		(21.33)

Table 2 Mean and Standard Deviation (SD) Values of LBH in Augmentech ICPC-Filled Defects (Test) and Control regions (P = .125)

Implant no.	Test (%)	Mean	Control (%)	Mean
Dog 1				
1	69.4		98.21	
2	61.59	64.12	98.69	96.76
3	61.36		93.37	
Dog 2				
1	100.00		100.00	
2	100.00	100.00	100.00	100.00
3	100.00		100.00	
Dog 3				
1	67.47		96.81	
2	100.00	84.17	98.89	95.85
3	85.03		91.86	
Dog 4				
1	88.24		73.58	
2	97.97	74.74	100.00	90.70
3	38.01		98.51	
Dog 5				
1	97.25		96.86	
2	98.55	98.11	97.34	97.19
3	98.53		97.37	
Mean		84.23		96.10
(SD)		(19.73)		(6.66)

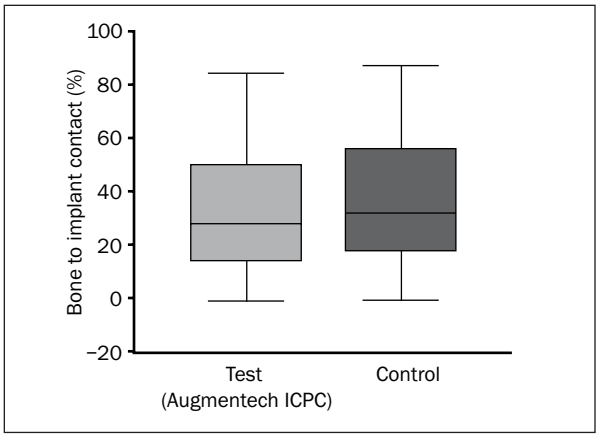


Fig 9 Box-whisker plot showing the distribution of the BIC values.

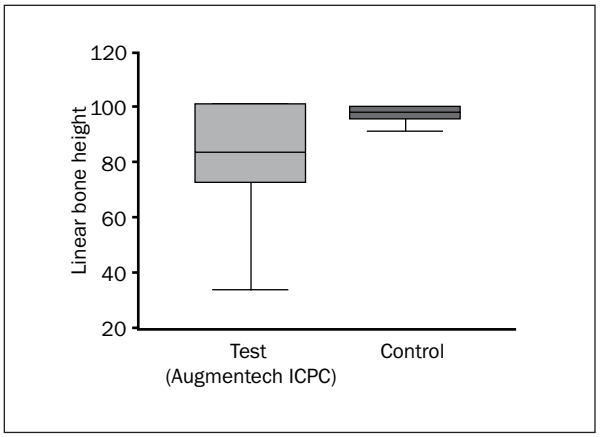


Fig 10 Box-whisker plot showing the distribution of LBH values.

osseointegration at defects which were left empty to serve as controls.[34] Consequently, it was decided to use the opposite side of each defect (intact bone) as a positive control and the use of an empty left defect was excluded. Twelve weeks of implantation was chosen as implant retrieval time because the wound healing process is completed within this time frame and it allows the comparison of implant integration, bone formation, and cement degradation. A bone graft should be biocompatible and nonsupportive of local pathogens, potential diseases, and cross-infection. Also, the graft body should maintain its mechanical stability and volume during the initial healing and then subsequently degrade completely to be replaced by new bone. Additionally, it should match the physical and chemical composition of natural bone trabeculae, provide calcium and phosphate ions, and serve as a scaffold for new bone ingrowth for establishing optimal osteogenic environment.[35] Microporosity and ease of handling and application is also desirable in a grafting material.

Membrane collapse and subsequent lack of bone volume are reported as a complication in guided bone regeneration surgery.[36] The Augmentech injectable calcium phosphate cement we used seems to be a reliable space-maintaining scaffold for bone ingrowth; this feature of similar injectable calcium phosphate cements was also reported in other studies.[37,38] Due to the material physical setting property, the geometric given shape of the defect was maintained clinically and histologically during the complete healing period. Test and control sites showed no statistically significant difference regarding the LBH values. The space maintaining property of the Augmentech injectable calcium phosphate cement was found to be satisfactory.

Hjörting-Hansen et al declared that the length of the calcium phosphate setting period enables enough working time, but the material is negatively affected by bleeding, which causes dissolution and erosion.[39] A similar observation was made in this study. When proper hemostasis was not established, Augmentech injectable calcium phosphate cement was washed away by bleeding during the flowable phase.

No foreign body reaction or inflammatory response was observed histologically around the Augmentech injectable calcium phosphate cement in this study. Further, despite the 12 weeks of healing, the Augmentech injectable calcium phosphate cement was not completely degraded and replaced by new bone. The center portion of the Augmentech injectable calcium phosphate cement remained as a circular zone surrounded by new bone tissue in direct contact with cement and also covering the facial layer of the dehiscence defect in almost all sections. This bone biocompatible behavior of calcium phosphate materials was also reported in previous studies.[40,41]

Biodegradation characteristics of graft materials vary depending on their chemical properties, physical form, and porosity. Crystal configuration, density, and the chemistry of the material is also important in the biodegradation process.[42–45] Low porosity of injectable calcium phosphate cement reduced the infiltration of body liquids and cells.[46,47] A relationship between bone ingrowth and porosity (pore size, pore morphology, and degree of pore interconnectivity) has also been reported in previous studies.[48–50] Although the material used in this study cannot be considered porous, it showed favorable biodegradation and substition performance. However, it has to be noted that subtle differences in the aforementioned parameters can have a serious effect on biodegradation and final outcome.[51,52] For optimal regeneration, the biodegradation rate of the bone graft substitute should equalize the rate of bone formation.[53] A slow biodegradation rate can result in the maintenance of a foreign material and incomplete bone fill for implant installation. For example, in a study by Ooms et al,[54] injectable calcium phosphate cement was placed in the defects created in the femoral condyles of goats. The presence of intact injectable calcium phosphate cement was observed even at the end of 24 weeks. In a study by Comuzzi et al,[55] custom-made stepped titanium implants were inserted into prepared bone beds in the goat femur; the space between bone and steps was filled with an injectable calcium phosphate cement. A polylactic acid membrane was also used in a group of implants to observe the effect on osteogenesis. The cement-filled biopsies showed abundant bone fill, and the use of a membrane did not result in additional benefits. After 12 weeks, a nonresorbed core of injectable calcium phosphate cement was seen in all specimens.

Fluoroscence microscopy revealed that the material had started being infiltrated by osteoclast-like cells and osteoblasts at the beginning of the first week. At the end of 12 weeks of healing, resorption lacunae with active cells depositing layers of osteoid were still visible. Evidently, degradation and bone apposition was still ongoing in the tweleth week. Healing of the peri-implant dehiscence started from the lateral and apical bone wall of the defect. Similar observations were also reported by Boticelli et al[56] in a dog study investigating marginal healing of implants inserted in the mandible.

Guided bone regeneration has shown good results in achieving bone augmentation around implants,[57] but it has the risk of exposure and subsequent inflammation.[36,58] The mean BIC in the defect zone was 34.42% in this study. The range of BIC values in other studies is large. Zablostky et al[59] reported a BIC of over 75% in the region of peri-implant dehiscences after 8 weeks of healing with e-PTFE (expanded polytetrafluoroethylene) membranes in dog mandibles. Boticelli et al[60] removed the buccal bone wall of overprepared implant beds and placed titanium implants in the dog mandible. After 4 months of healing, resolution of all defects was observed with a mean BIC of 35%. Oh et al[61] used collagen membranes in dehiscence-type defects created in the dog mandible. After 16 weeks of healing, BIC rate was between 30% and 57%, and the impact of membrane exposure on BIC was serious. In the current study, there was no statistically significant difference between test and control groups; therefore, we can conclude that the Augmentech injectable calcium phosphate cement regenerated bone supports implant-to-bone contact.

LBH values in test and control sides did not show statistical difference. The LBH value is reported to be 0 in some sections, and this was caused by separation of the Augmentech injectable calcium phosphate cement area during microtome sectioning, which resulted in a large standard deviation.

CONCLUSIONS

Within the limits of this experimental model, it can be concluded that:

- Augmentech injectable calcium phosphate cement is an osteoconductive material, but biodegradation is not completely finished in 12 weeks.
- Bone-implant contact was similar in the Augmentech injectable calcium phosphate cement–filled defect side compared to the untreated control side.
- Bone height at the Augmentech injectable calcium phosphate cement–filled defect was similar to the untreated control side.

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