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Biodegradation of Injectable Calcium Phosphate Bone Cements: A Dental Perspective

Volkan Arısan

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Abstract

The prevalence of tooth loss and edentulism is among the most ubiquitous diseases of human history. Dental implants have been widely used for the treatment of any type of edentulism. However, the absence of sufficient bone volume usually limits the placement of implants. Many techniques and materials have been developed to restore an adequate volume of bone for future implant placement, but the process of biodegradation and replacement with new bone tissue is still under debate for all grafting materials. Among the available biomaterials, calcium phosphates (CaP) have been under the spot light for their advantages such as the ease of production and lack of disease transfer. The preparation of the material in two phases allowed self-hardening and subsequent space maintenance where it applied. This was of critical importance in load-bearing implants and joint prosthesis where rapid and strong healing is required. The injectability also allowed a better handling and manipulation in compromised areas such as the oral cavity. A novel form of injectable calcium phosphate cement (iCaP) with two distinct formulations was tested on dog tibiae. Healing and ossification at 4 and 12 weeks were assessed by histologic and histomorphometric analysis. No adverse reactions or negative consequences were noted. Mean new bone formation was 22.12 (SD, 15.68), 18.62 (SD, 13.11), and 9.56 (SD, 11.11)% in the groups 1, 2, and the control, respectively. Statistically, significant higher new bone formation was evident in the groups 1 and 2 as compared to the control group ($p < 0.01$). However, these differences were no more discernable after 12 weeks of healing. The results of the present investigation showed excellent *in vivo* biocompatibility but insufficient biodegradation of the iCaP in the center of the defect area. Further attempts are required to expedite the biodegradation of the iCaP.

Keywords: Calcium phosphate, dental implant, dentistry, bone graft, bone defect, bone regeneration, histology, biomaterial

1. Introduction

Calcium phosphate is the group name of minerals consisting of diverse ions. The fundamental ion group calcium (Ca_2^+) is coupled by the orthophosphates—a basic ion of distinctive atoms and molecules within varying organisms to form many different compounds essential to vitality [1]. Metaphosphates and pyrophosphates and occasionally hydroxide or hydrogen ions also contribute this family forming various combinations of crystalline configurations [2]. Mineralization of these ions and molecules constitutes the main frame of multicellular residents (the bone) including the human [3]. Some of the important subcategories of the calcium phosphates are as follows:

1.1. Mono calcium phosphate

Monocalcium phosphate is an inorganic compound mainly found as monohydrate salts. Monocalcium phosphates are widely used in the food industry to enhance baking, rising, and longevity issues. One of the main ingredients in baking powder is monocalcium phosphate. It is also used as a leavening agent in various processes. Phosphoric acid is used to treat the monocalcium phosphates to sustain their purity. Together with the fluorapatite, it can be converted to superphosphates, which are used in fertilizers [3].

1.2. Di-calcium phosphates

This form of the calcium phosphates mainly consists of the "brushite"-type calculus and could be formulated as monohydrate dihydrate and anhydrous crystalline structures. They are used as a dietary supplement in many of the low-calorie foods [4].

1.3. Tri-calcium phosphates

The calcium phosphate of phosphoric acid is named as tri-calcium phosphate and mainly derived from inorganic substances such as whitlockite mineral rocks. Tri-calcium phosphates are one of the main metabolic products of bone tissue and—the beta tri-calcium phosphates—especially have been under serious investigation by many researchers. With the evolution of sophisticated techniques and controlled sinterization methods, a biphasic form was supplied and termed as "biphasic calcium phosphate." This final form provided significant benefit, especially in bone surgery. A powder and liquid form which could be provided in a small physical carrier could be mixed and injected to the desired area like a tool paste. The material can be shaped and contoured *in situ,* and a hardening process takes place within 10 min. This is of utmost importance in bone defects requiring space maintenance. A semi-permeable membrane could be played over the hardened biphasic calcium phosphate body for further osteogenesis [5].

1.4. The calcium apatite

It forms the hydroxyapatite by a hexagonal crystalline system which 70% of the body bone mass is made of. Accordingly, hydroxyapatite can be considered as a principal component of

the human skeleton, including the teeth dentin and enamel. The carbonated calcium-deficient hydroxylapatite which is a modified form of the hydroxyapatite is the foremost mineral in teeth tissues. Regarding the importance of the material, many synthesis methods were proposed including wet chemical deposition, sol–gel route (wet-chemical precipitation), electrodeposition, and biomimetic deposition. Hence, many studies yielded an enhanced response of the bone tissues when the hydroxyapatite is implanted. Consequently, many modern joints or hip prostheses are coated by hydroxyapatite to facilitate healing and os-seointegration (direct bone deposition on the titanium surface). This approach was also used in the dental implants, but higher production costs and relatively lower bone-contact surface of dental implants did not justify its feasible use. Blocks of porous hydroxyapatite are also used for the restoration of the bone defects and local drug delivery [4].

Besides the mentioned types of calcium phosphates, there are other crystalline configurations such as octacalcium phosphate, tetracalcium phosphate, and amorphous calcium phosphate [6].

1.5. CaP cement

It is defined as a powder or a mixture of powders which following the mixing into water or an aqueous medium to a paste, reacting in room or normal body temperature by the formation of a precipitate containing of one or more calcium phosphate crystals and sets by the entan-glement of the crystals of that precipitate. In the hardening phase, the material can be injected into any desired area. This greatly improves the manipulation [7].

1.6. Self-hardening injectable iCaP

The self-setting forms of CaP were introduced by Brown and Chow [8]. The material gained high popularity and interest due to its mechanical and biologic properties [8]. When used as a grafting material, its application promoted a higher level of bone repairing with some noted problematic issues. The difference of the crystalline composition and final amorphous form was found to be not favorable in terms of biocompatibility [9]. This was overcome by different sintering and preparation techniques leaving a higher percentage of organic form in the latest setting.

In almost all clinical and experimental studies, the material was accepted and healed rapidly [3]. However, histologic sections revealed limited biodegradation of the material. This was especially critical when a functional implant is to be placed on the area. Any load-bearing functional implant is expected to be surrounded by living bone tissue, which is called as osseointegration [10]. Upon the use of alloplastic materials around load-bearing implants, the replacement of the grafting material was not of a concern. The inert behavior of the materials was favored rather than the bone replacement (creeping substitution). The widespread use of dental implants then yielded the need of bone replacement of any graft material applied for the treatment of the lost bone volume. Up until then, the term "resorption" was being used to define the dissolution of the grafting materials. However, this was rather associated with a pathologic response so "biodegradation" was used instead of resorption [11].

1.7. Biodegradation

Occasionally defined as "resorption" can be defined as the dissolution of the graft material by the body biology [1]. Specifically, when used in bone defects, the CaP are expected to be removed by polymorph nucleated cells and simultaneously replaced by bone deposition by osteoblasts and other bone-inducing cells. In the description of this process, the "resorption" term was being used to define a group of inflammatory cells and subsequent tissue repair. In contrast to the repair process, biodegradation was then found appropriate for defining the dissolution of organic materials for a regeneration result [12]. Biodegradation is especially used in product packing when environmental issues are considered [7]. The biodegradation potential is distinct in different graft materials (**Table 1**).

Type of graft transfer	Bone-inducing effect (osteoinductive)		Space maintenance (osteoconductivity)	Vital cell proliferation (osteogenesis)
Within the same species (autografts)	Potency	✔✔	✔	✔✔✔
	Biodegradation characteristic	Surface resorption		
Between the different species (allografts)	Potency	✔	✔✔	✔
	Biodegradation characteristic	Inconsistent and/or incomplete		
Transfer from non-vital/ synthetic sources (alloplasts)	Potency	✔	✔✔✔	Not applicable
	Biodegradation characteristics	Incomplete		

✔: Poor; ✔✔: moderate; ✔✔✔: strong.

Table 1. Biodegradation and osteogenesis potential in various types of bone grafts.

1.8. The relevance of the biodegradation in oral reconstruction

The skeleton constitutes the principal internal framework that allows the structural integrity of the organs. It also provides many biomechanics aspects of mobility [13]. More than 200 different types of bones create a critical support for many of the organs and elements. Among these, the face and jawbones that are embriogenetically derived from the pharyngeal arch to have unique features from rest of the body bone that is constructed via the endochondral ossification. The neural crest that the skull is originating attains its particular characteristics for specific purposes such as the protection of the brain tissue and formation of the first digestion component: the jaws and teeth. Any loss or damage in the jaws or teeth was one of the major health problems that the humanity faced upon the early ages. Due to its accessibility, the calcium phosphates-based materials were the first choice in the repair and restoration of jawbones [14]. Initial application of the raw material was not practical due to the powder form. Following particulate forms, improved the outcomes. However, they yielded difficulty in the

contention of the defect area in the presence of bleeding [15]. Especially in the oral cavity, the conceded are may be subjected to forces and irritating factors resulting with the geometric violation of the graft area and a poor outcome (**Table 2**).
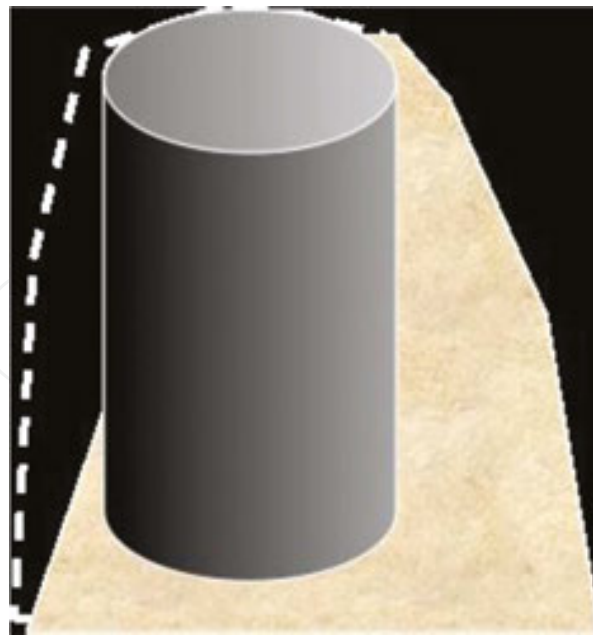
Name	Characteristics and biodegradation
Calcium phosphates (CaP)	Constitute the inorganic matrix of the bone
Resorbable hydroxyapatite (HA)	Rapid biodegradation. Small particles may lead foreign body reaction
Tri-calcium phosphate (TCP)	High biocompatibility and moderate biodegradation
Dense HA	Resistant to biodegradation. Long-term space maintainer
Porous HA	Faster biodegradation than dense HA
Bioglass	Non-degradable. High biocompatibility in bone
Calcium carbonate	Inconsistent biodegradation
Calcium sulfates (the Paris plaster)	Rapid biodegradation

Table 2. Classification of CaP materials and their biodegradation characteristics.

1.9. Injectable CaP in clinical dentistry

Amorph and hydrous forms opened a new horizon with the cement-like setting feature. In this form, a powder (amorph component) and liquid (hydrous form) component is mixed to create a reaction of hardening just like a cement. While in its hardening stage, the material is in a "flowable" state allowing easy transfer and shaping according to the defect-site-specific conditions. This feature is then used as "inject ability" for enhancing the practical aspect of the calcium phosphate-based bone-grafting sequence. The primary results were favorable except one phenomenon present in almost all histologies. The core of the material was found intact *in situ* despite the abundant bone formation in the graft-to-host bone border. Initial observations revealed that the material was highly biocompatible but lacked porosity yielding a bulky end product. As a result, the body fluids could penetrate the material only from the surface but did not reach to the core of the material. This aspect may of course be advantageous in some specialties of dentistry such as endodonty. The material applied to the root of the natural teeth after the removal of the vital structures known as the root canal therapy. The material successfully sealed the canals (even the side canals known as the *isthmus*) hermetically and caused no foreign material reaction the tooth-apex. The resistance of the material to resorption and biodegradation allowed long-term stability and success of the treatment known as the root canal therapy. However, in the restoration of the lost bone volume, the lack of complete translation of the injectable calcium-phosphate-bone cement (iCaP) into the bone tissue was a challenge [16]. Especially in oral implantology where a titanium fixture is expected being surrounded by living bone tissue (osseointegration) and any intact form of graft material was undesirable (**Figure 1**).

Figure 1. A dental implant placed into the alveolar crest should be surrounded by living bone tissue. Any lack of bone that is described as the dehiscence defects (intermittent lines) should be covered by a graft material with an osteogenesis potential.

1.10. Biodegradation of injectable CaP

Many attempts have been undertaken to overcome the issues related to biodegradation of the iCaP. A series of powder and mixture settings was formulated and experimented on animal models to reveal the best configuration. The hydrous component allowed fine-tuning of the setting time and flowability characteristics, while the powder component was mainly related with the final hardness of the cement. Concomitantly, a porous character was obtained for gaining better flow and penetration by bodily cells and fluids. A delicate balance of the setting time, ease of injectability, and the final setting hardness was a constant challenge in the development stage in many previous studies [17].

2. Experimental study

In order to optimize the biodegradation of the injectable CaP, three different formulations were deduced be tested experimentally to achieve an objective comparison. In this respect, a dog model was chosen due to the available bone volume. The tibia was the choice of defect creating since the oral cavity may pose significant infection and mastication forces risks. After the retrieval of an ethical approval, six beagle dogs were housed for the experiment, and the tibias were exposed after the general anesthesia. Three standard bone defects were created in the proximal tibia of animals, and the mentioned iCaP formulations were mixed and injected into the defects. One defect was left empty to serve as control. All animals were injected with fluorochrome labels in the first and last week of healing to discriminate the pattern of healing

on the histology. The animals were sacrificed after 4 weeks, and bone biopsies were obtained from the defect sites. The samples were processed according to the non-decalcified histology protocol. The histologic slices were obtained and subjected to histomorphometric analysis for objective comparison.

2.1. The injectable CaP cements

The cements were founded as powder and liquid components in a capsule carrier and are readily available for mixing (**Figure 2**).

Figure 2. The injectable CaP was provided in a ready-to-mix tube. The powder and liquid form is separated by a plastic barrier. Prior to the placement to the shaker (i.e., amalgamator), the barrier plastic is removed thereby allowing the mixing of the components. The material is shaken for 30 s for proper viscosity.

After mixing, the iCaP cement can be injected to the defect and cured in a similar manner to others cement materials.

Tetracalcium phosphate (TTCP: $Ca_4(PO_4)_2O$) and an hydrous di-calcium phosphate $CaHPO_4$ are mixed 2:5 molar and obtained the $Ca(H_2PO_4)_2 . H_2O + Ca_2NaK(PO_4)_2$. The mixture is diluted with 0.5 ml distilled water. The powder was sterilized via 27 kGy gamma radiation. The hydrous part of cements was divided into two groups. In the group 1, the hemi-hydrate part excluded. In group 2, the hemi-hydrate part was included but the concentrations were 5.
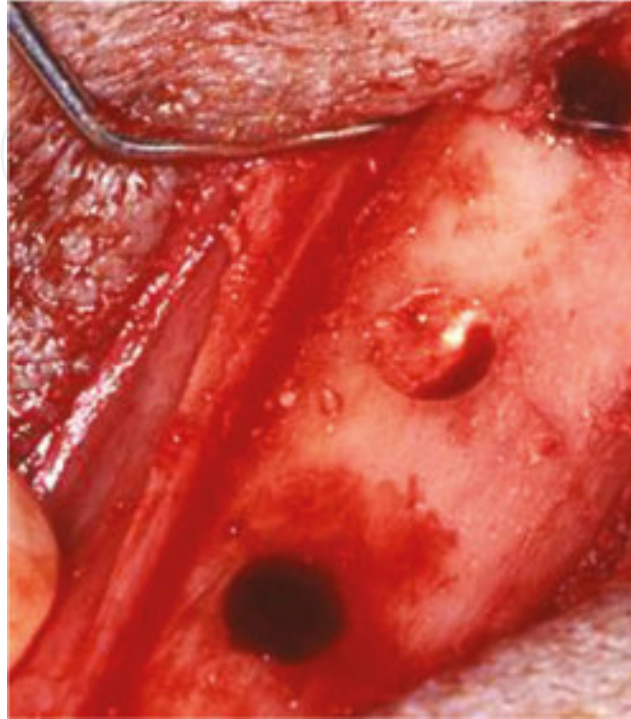
The powder and liquid form was provided in an application tube ready to be inserted in to a mixer. The separator is removed, and the power and liquid forms are mixed for 20 s.

2.2. Surgical procedures

All surgical procedures were performed under general anesthesia. The left tibia of the dog was shaved and disinfected with povidone-iodine. During all surgical procedures, the animals were pre-anesthetized with Xylazine (Rompun/ Bayer, Germany) 1.5 mg/kg intramuscularly [i.m.] and anesthetized by Ketamine (Ketanest/ Alfasan, Holland) 10 mg/kg i.m. and maintained by isoflurane 3.5% (volume/volume) (Forane, Abbott Laboratories, Rungis Cedex, France). They were administered through an endotracheal tube. Ten, the bone surface in the proximal tibia region was exposed by an incision followed by skin and periosteal elevation.

Three recipient sites were prepared, using a drill of 3.8 mm in diameter to a depth of 13 mm to obtain standard defect sites. A minimum of 1.5 mm was sustained between defects to provide adequate healing conditions for the defects.

Figure 3. Two standard bone defects (a diameter and depth of 3.8 and 13 mm respectively) were prepared for the injection of groups 1 and 2 (middle and the right defect). One defect (on left side) was left empty to serve as control.

After achieving bleeding control, iCaP cement capsule placed in amalgamator for 20 s, after that iCaP cement injected in all defect except one defect for each animal left empty (**Figure 3**).

After the removal of the residual cement, 10 min time was exceeded for the setting of the material. Then, the periost and dermis sutured with vicryl 3–0 suture material (Ethicon, Polyglactin 910, Chicago, A.B.D). In order to monitor patterns of bone formation, hydrochloric tetracycline (at the 4th week (Tetra, Mustafa Nevzat İlaç Sanayı, Istanbul, Turkey) and Alizarin complexone (Sigma-Aldrich, Bonn, Germany); (at the 11th week) were injected intravenously (I.V.). The animals were fed with standard diet throughout the 12-week recovery period. At the end of the 12 weeks, the animals were sacrificed by an overdose of sodium pentobarbital (Abbot Lab. Chicago A.B.D).

2.3. Histologic preparation and analysis

The block biopsies taken from the proximal tibias, stored in phosphate-buffered solution and maintained at 4°C. The blocks were dehydrated with a series of alcohols and put into a transparent flask filled with methyl methacrylate. This was essential for the non-decalcified histologic sections. Following the polymerization, the blocks were removed from the flask and using the Donath technique, 395 μm non-decalcified sections were obtained. A total of three

sections were taken from each defect. The first two sections stained with methylene blue and basic fuchsin, and the third section with the thickness of 390 µm was taken without staining for fluorescence analysis. For the analysis, the sections were magnified under ×100 light magnification of a stereo microscope. The new bone and residual area was calculated manually using dedicated software (Olympus Image Analyzer, Tanaka, Japan). The percentage of new bone and the residual graft area was calculated.

2.4. Statistical analysis

The normality of the results was controlled by the Shapiro–Wilk Normality test. Student t-test was used for the statistical analysis. All analyses were performed by the GraphPad Prism software (San Diego, California, USA).

3. Results

The injection and the manipulation of the CaP were easy, and the material was set in almost 15 min. However, this period of time can be considered long for the clinical work. Also, in the presence of bleeding, the setting of the material was compromised and particles washed away by the blood. After the self-hardening, the manipulation of the soft tissues for suturing was rather easy.
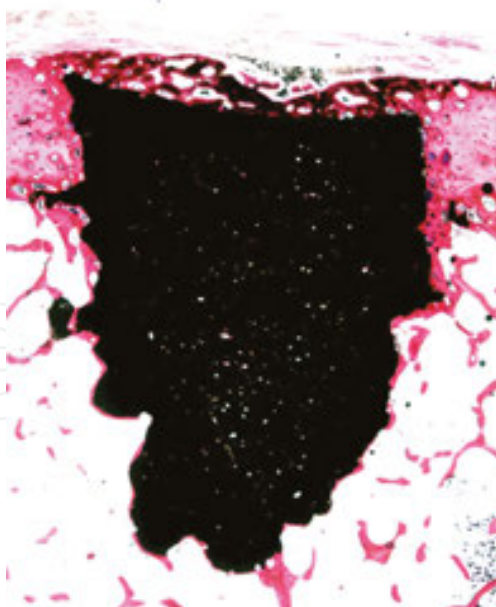
Throughout the healing period (up to12 weeks), no local or general problems or complications were observed in the animals. The surgical area healed quickly in all dogs without any signs of a developed pathology. Radiographic examination showed no pathological changes at the surgical zone.

3.1. Light microscopic examination

All the histological cross-section views of the CaP graft material have showed bone integration. A dense trabecular bone structure was also noticed around the grafted area. A thin cortical bone layer surrounded a trabecular woven bone. In this layer, the particles of the CaP cement were in the center of the defect and substituted by a thick layer of trabecular bone tissue. Almost in all the histological sections, the coronal aspect of the injectable CaP graft was surrounded by a layer of cortical bone. At high magnification, the Howship lacunae (osteoclast resorption lacunae) were seen at the zone adjacent to the graft material. A continuous layer of osteoid deposition followed biodegradation of the graft material. The outer part of the graft core was replaced with new bone, but the core part (inner part) of the cement was not biodegraded. In contrary to the outer part (the sections in contact with the native bone), the center section was still intact. The graft material was strictly placed into the defect which was in a complete contact with the surrounding bone tissue, and no fibrous tissue or inflammatory reaction was observed in any of the histological sections. At the early stage of healing active angiogenesis and osteogenesis have been shown, and no inflammatory symptoms were seen. (**Figures 4** and **5**)
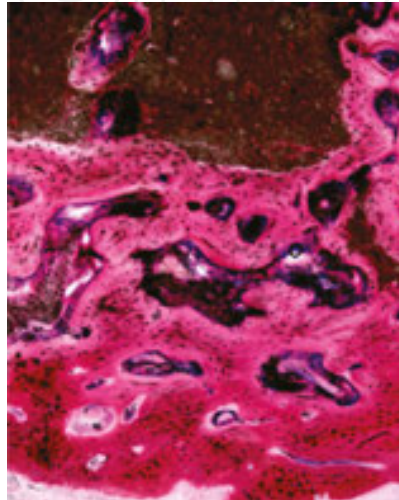
Figure 4. Group 1. Four weeks healing. Basic fuchsin and toluidine staining. Original magnification ×10. The un-resorbed bulk material can be seen in the middle of the defect. Osteoid deposition is evident in the borders of the material. No inflammatory evidence is visible.
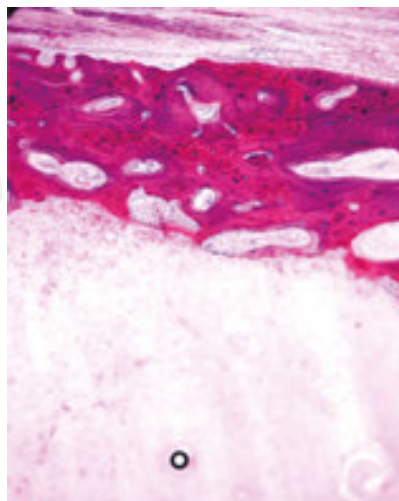

Figure 5. Group 2. Four weeks healing. Basic fuchsin and toluidine staining. Original magnification ×10. The apical portion of the material has been replaced by living bone tissue characterized by large trabecular zones. The material is not biodegraded completely as marked by the dark area. However, the iCaP shows excellent integration with the host bone.

At 12th week, the process of new bone apposition and mineralization was still visible. Moreover, the graft found at the base of the defect and next to the cortical bone has been degraded by osteoclast-like cells. At higher magnification, both dense trabecular area and unlimited osteoblast like cells, primary, and secondary osteons were found in the space between the vessels at the regenerated bone. In this time frame, it was evident that the formation of osteoid tissue began growing from the defect walls and continues toward the center (**Figures 6** and **7**).

Figure 6. Group 1. Twelve weeks healing. Basic fuchsin and toluidine staining. Original magnification, ×100. The floor of the defect is focused. High magnification in the apical portion reveals direct contact the living bone tissue with vital cells and ongoing ossification. Osteoid deposition is characterized by osteocytes (small dots) lines at the iCaP border. The iCaP material is being penetrated by small indentations (Howship lacuna) derived from the host bone tissue.
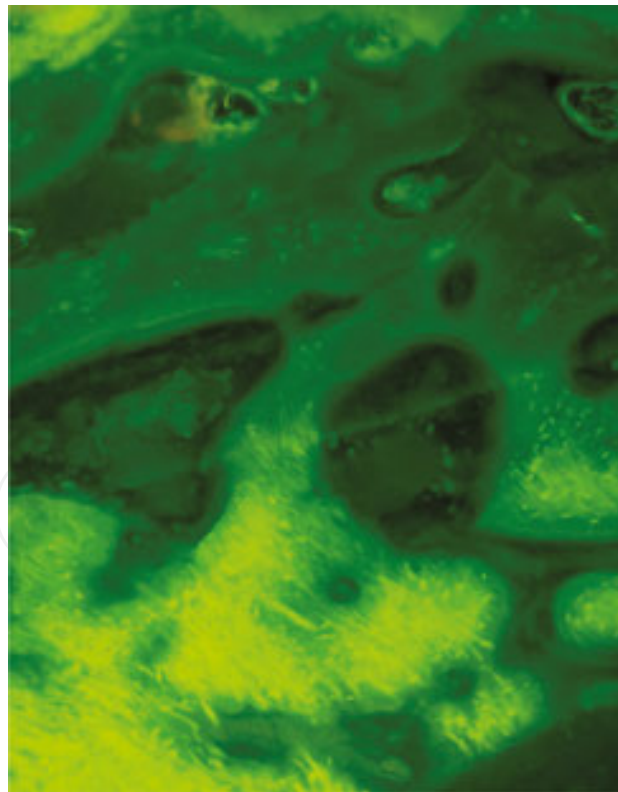

Figure 7. Group 2. Twelve weeks healing. Basic fuchsin and toluidine staining. Original magnification, ×100. Coronal part of the defect is focused. In the coronal aspect, the iCaP material is covered with new bone tissue. Despite the fragments of small ossification points, the iCaP material has not biodegraded (white bulk in the lower aspect of the image).
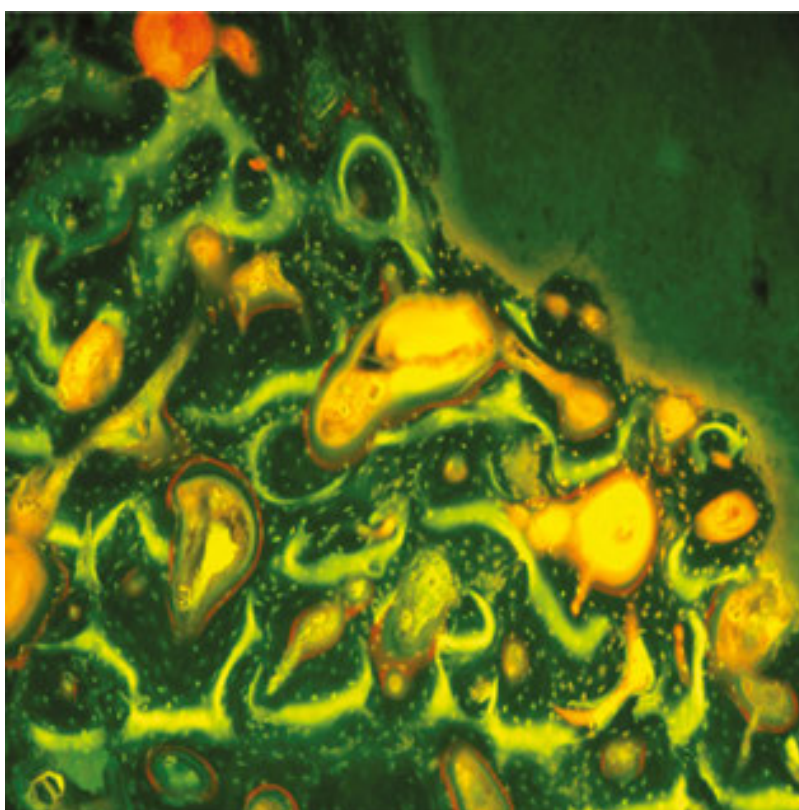
In the control defect, the cervical part of the defect was filled with soft tissue. On the contrary, the apical part was filled with mature bone. Low-mineralized primary osteoid was also seen especially at the borders of the defect in both the control and test groups.

3.2. Fluorescence microscopic examination

The use of fluorochrome labels allowed observation of the bone growth and the position of the new bone that occupied the grafted space in relation with the time frame. The tetracycline HCL applied in the 4th week stains the mineralization by green color (Native bone in dark green and new bone in light green). The alizarin complexone applied in the 11th week stains the mineralized tissues in orange color (Native bone dark orange/red/brown and new bone in light orange). Accordingly, tetracycline HCL stain was seen at borders of the defect at the 4th week sections. This indicates that the formation of this bone started shortly after CaP placement. The histological cross-section of the CaP-grafted area at the 12th week showed a continuous formation of bone and remodeling. It was evident that a bone turnover was ongoing throughout both the intervals. The rate of new bone formation was within normal physiology limits. The formation of the bone initiated from the borders of the defect towards the center (**Figures 8** and **9**).

Figure 8. Fluorescence microscopy view of the 4 weeks healed defect in group 1. Light green staining reveals new bone formation. Original magnification, ×200. Coronal side of the defect is focused. Light green areas depicting ongoing osteogenesis around the iCaP area (right bottom corner). It is obvious that the osteogenic activity started soon after the placement of the iCaP.

Figure 9. Fluorescence microscopy view of the 12 weeks healed defect in group 2. Light green and orange staining reveals new bone formation. Original magnification, ×150. Apical side of the defect is focused. Orange circles and lines reveal bone active remodelation and formation in the 11th week. The iCaP is being replaced by trabeculas of new bone. The bone formation that is evident on the 4th week (light green staining) is being deposited by osteocyte lamellas (orange color) in the 11th week.

3.3. Histomorphometric analysis

Mean new bone formation was 22.12 (SD, 15.68), 18.62 (SD, 13.11), and 9.56 (SD, 11.11)% in the groups 1, 2, and the control, respectively. Statistically, significant higher new bone formation was evident in the groups 1 and 2 as compared to the control group ($p < 0.01$). However, these differences were no more discernable after 12 weeks of healing (**Figure 8**).

The rate of residual iCaP was similar in both time intervals. In the 4 weeks healing group, almost half of the iCaP was still present in both groups and there were no statistically significant differences between the groups 1 and 2. After 12 weeks, the biodegradation was evident. However, complete biodegradation of the iCaP was not evident in any groups 45.44 (SD, 22.16) and 41.20 (21.20)% for groups 1 and 2, respectively; (**Figure 9**).

Cellular evaluation of the histologic slices in the groups yielded no inflammatory response or foreign body reaction. Histomorphometry taken together with the fluorescence sections reveals that new bone formation initiated right after the surgery at the iCaP and host bone border. In both groups, the staining in the 4th and 11th weeks was similar. It is obvious that the cells involved in bone turnover infiltrate the iCaP body by small indentations and the

biodegradation was sparsely conducted in the roots of these indentations. The bulk and non-porous nature of iCaP, unfortunately, inhibited the infiltration of cells responsible in the biodegradation. This was characterized by orange stains in the 11th week, and there were not visible differences between the groups (**Table 3**).
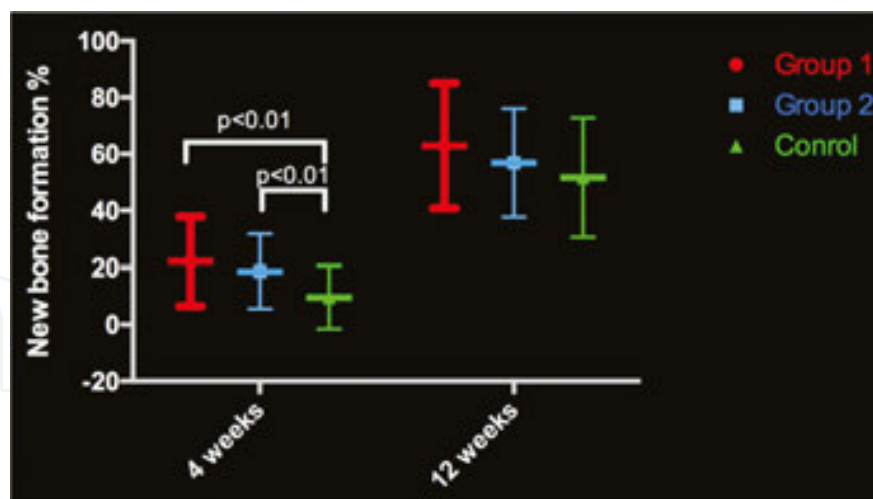
	Group 1	Group 2	Control
4th week (tetracycline staining)	Osteoid deposition and new bone formation visible in all histologic sections. Major mass of the iCaP is visible	Osteoid deposition and new bone formation visible in all histologic sections. No significant biodegradation is visible	Osteoid deposition and new bone formation visible at bottom side of the defect. The coronal aspect of the defect is lacking bridging
11th week	New bone formation is evident in all sections. A thin layer of new bone tissue is bridging over the iCaP. No sections reveal complete biodegradation of the iCaP	New bone formation and lamellar formation is evident in all sections. A thin layer of new bone is bridging the top of the defect. iCaP was not biodegraded and left intact, especially in the center of the defect	The bone defect is being filled with trabecular bone. The amount of new bone formation is less than the group 1 and 2

Table 3. Evaluation of biodegradation in groups 1 and 2 as compared to the control group.
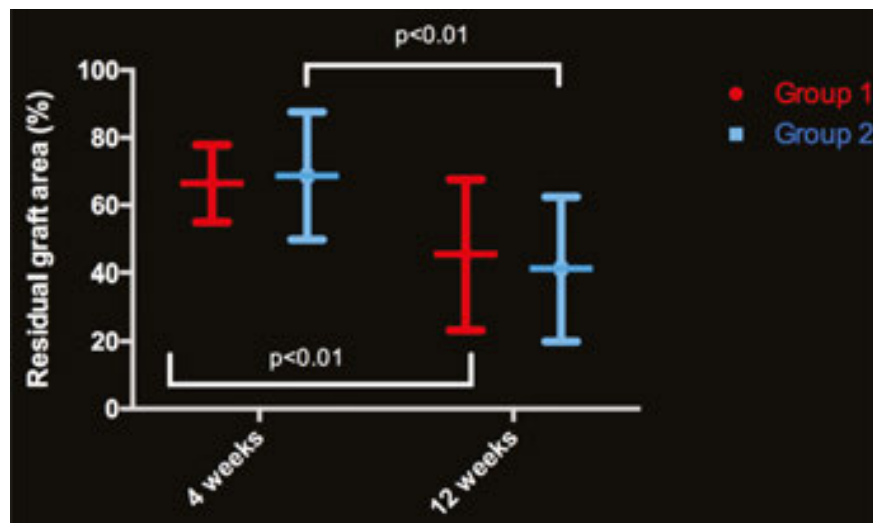
4. Discussion

The restoration of the lost bone volume would be one of the most concerned topics in the future of dentistry. Given the prevalence of edentulism and tooth loss, more people are likely to apply for a fixed prosthetic restoration approach. Dental implant is an excellent base for such purposes. Unfortunately, the bone volume rapidly decreases due to the atrophy in lack of the functional stimulus [16].

In the restoration of an alveolar bone defect, the autogenous bone transfers are regarded as the golden standard. However, they are difficult manage and prone to many complications in the short and long term. Hence, extensive surface resorption, especially in the iliac grafts, questions their effective use. Nevertheless, the contents of living bone cells are critical for rapid healing [3]. The iCaP lacks any living cells and does not incorporate relevant growth factors or bone-inducing elements. Therefore, its application and efficacy fundamentally depend on the vascularization of the native bone [18]. Moreover, the open nature of the iCaP allows integration of any desired elements, cells, or components that may be tailored according to the site-specific needs. The results of the present investigation are promising, and the iCaP seems suitable for further development for efficient and inexpensive bone regeneration purposes (**Figures 10** and **11**).

Figure 10. New bone formation measured in the time intervals.

Figure 11. Residual graft area measured in the time intervals.

After 4 weeks of healing, a considerable amount of new bone was evident in both groups as compared to the control defect. Nevertheless, there were no statistically significant differences in between these two groups. This may render that the inclusion of hemi-hydrate groups did not yield any positive influence in terms of new bone formation.

After 12 weeks of healing, the level of new bone was above 50% in all the defects and there were no statistically significant differences in the groups or the control defect. This may point the long healing period or the insufficient dimensions of the graft. Even so, there is no consensus on the critical size defects of the tibia in terms of a dental implant osteotomy.

The residual graft was also investigated to corroborate the results of new bone formation. In the 4 weeks, more than 60% of the injectable CaP material was still present in the defects. After 12 weeks, the rate of residual CaP decreased below 40% in group 2. The difference was

statistically significant in none of the time intervals. Histologic section yielded a core material was left non-resorbed right at the center of the defect. Ooms et al. [19] in a study on dog, placed an injectable porous form CaP graft material into the standardized defects in combination with an experimental titanium implant and found that this material was highly ossified in the whole area, especially in infection cases or in case of few walled bony defects, and also he found out that the material outcome might be high if vascularization is supported. It may be proposed that further porosity is required around the iCaP to allow vascularization and body-fluid penetration.

Many different attempts and experiments have been performed in a purpose to increase the biodegradation of CaP cement. It is thought that increasing the solubility of CaP inside the bone results in accelerating the period of biodegradation. Solubility increases in direct proportion to the surface area [20]. The body fluids and their contents such as phagocytic cells expedite the resorption period of the graft when the contact area between the fluids and the graft material increases. As a result, the hardened graft material porosity is expanded so to rise the infiltration of fluids and blood between the graft. Daculsi et al. [21] produced a porous CaP cement with interporous distance ranges between 100–500 micrometer to introduce a good environment for cell growth.

In the present experimental model, new bone regeneration was ascertained without any complications that might occur thereby of using a barrier membrane such as exposure of the soft tissue and infection. This may be attributed to site of the tibia as the area of the defect. Nonetheless, applying the graft into the oral bone might also have good prognosis with no complications as it was observed in the tibia. Some researchers reported that the use of membrane may be unnecessary as the material acts as a space maintainer. In this study, the four-walled bony defect was involved so that no barrier membrane was used. Hence, new bone formation was occurred in both groups.

5. Conclusions

The injectable CaP cement has an excellent biocompatibility and a good space maintaining ability. No pathologic findings encountered during the period of the present and similar other studies. Injectable from of the CaP greatly improves the applicability of the material. However, the biodegradation of the present iCaP was similar to those of previous observations. The application of the CaP yielded better new bone formation as compared to the empty control defects. The center of the graft seen intact at the end of 12 weeks. The inclusion of a hemi-hydrate component did not effect effected neither the new bone formation nor the residual graft. In both groups and time intervals, in the injectable CaP was not degraded completely at the end of 12 weeks of recovery time. Supplementary methods are required to fasten the biodegradation process.

Acknowledgements

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