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The Use of Light/Chemically Hardened Polymethylmethacrylate, Polyhydroxylethylmethacrylate, and Calcium Hydroxide Graft Material in Combination With Polyanhydride Around Implants and Extraction Sockets in Minipigs: Part II: Histologic and Micro-CT Evaluations

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Abstract

Background—This report is the second part of the previously published study on the impact of light/chemical hardening technology and a newly formulated composite graft material for crestal augmentation during immediate implant placement.

Methods—A total of 48 implants were placed into the sockets of the mesial roots of freshly extracted mandibular premolar teeth in three minipigs. Crestal areas and intrabony spaces were randomly augmented with light-hardened graft materials including a composite graft consisting of polymethylmethacrylate, polyhydroxylethylmethacrylate, and calcium hydroxide (PPCH) plus polyanhydride (PA); PPCH graft; and PA graft, or left untreated. Distal sockets not receiving implants and the sockets of first molars (n = 60) were randomly treated with one of the graft materials or left empty. In addition, two molar sockets were treated with the original PPCH graft material. Quantitative microcomputed tomography (micro-CT) was used to assess alveolar bone structure and tissue compositions. Histologic evaluations included descriptive histology to assess

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the peri-implant wound healing, as well as histomorphometric measurements to determine bone-to-implant contact (BIC).

Results—Both trabecular and cortical bone measurements by micro-CT did not reveal any significant differences among the groups. Sites augmented with PPCH+PA resulted in significantly greater BIC surface than PPCH alone and no-graft-treated implants ($P < 0.05$) histologically. Stained ground sections showed complete bone formation between bone and implant surface in the PPCH+PA group, whereas sites without augmentation showed large gaps between bone and implant surfaces, indicating a slower bone apposition and less BIC surface compared to all other groups. Similar to implant sections, all materials showed positive outcome on trabecular and cortical bone formation in extraction sockets with an intact crestal cortical bone.

Conclusion—Histologic evaluations supported the previous findings on implant stability and function and confirmed that PPCH+PA provides a greater BIC with a well-organized implant–bone interface and is useful in crestal augmentation during immediate implant placement.

Keywords

Alveolar ridge augmentation; dental implants; immediate dental implant loading

Immediate implant placement involves the insertion of a dental implant directly into the fresh socket after tooth extraction.¹ Compared to delayed implantation, immediate implants have several advantages, including reduced number of surgeries, shortened treatment time, ideal positioning in extraction sockets, preservation of bony walls and soft-tissue contours, and a rapid prosthetic phase.^{2,3} Primary stability is essential for a predictable and successful osseointegration⁴ and can be achieved by a passive mechanical fixation within the bone. Subsequent wound healing, bone resorption, and new bone formation facilitate secondary implant stability. Therefore, a successful osseointegration is determined not only by the primary stability but also by the extent of the secondary implant stability.⁵ Bone-to-implant contact (BIC) is critical for the osseointegration and needs to be established by both the primary and secondary stability.

The loading can also be initiated immediately when occlusion can be restored within the first 72 hours after the surgery.⁶ However, immediate loading may increase the risk for failure attributable to micromovement caused by the occlusal forces. Micromovement of the implant fixture leads to soft-tissue encapsulation around the implant and results in failure of the osseointegration.^{7–9} Therefore, although immediate implantation is becoming more widely accepted, conventional loading initiated after 3 to 8 months is still considered critical for a successful osseointegration.¹⁰ However, the quest for reducing micromovement and shortening the loading time continues, and several technologic advances have been adopted.

The initial gap between the bone and the implant is one of the most important factors that affect the BIC during the immediate implant placement and loading in addition to primary stability and surface roughness of the implant.¹¹ Osseointegration is not predictable when implants are placed without a close fit to the bone. Immediate implant placement in fresh extraction sockets commonly have voids adjacent to the surfaces.^{12–14} Vertical crestal augmentation¹⁵ and split-crest technique^{16,17} have been used successfully for decreasing the

voids. Many grafting materials have been tested for reducing spaces around implants, increasing the BIC, and enhancing the stability to increase the success of the immediate implantation and loading. These include freeze-dried bone allografts, hydroxyapatite, and synthetic grafts,^{18–22} all of which have advantages and shortcomings. In a previous paper, the present authors showed that a newly formulated light/chemically hardened graft material, polymethylmethacrylate, polyhydroxyethylmethacrylate, and calcium hydroxide (PPCH) plus polyanhydride (PA) polymer graft, was useful in immediate implant placement after tooth extraction in minipigs and resulted in greater stability and a well-organized implant-bone interface with immediate loading, especially in the areas with cancellous bone. In the present report, the impact of this application on the qualitative parameters of the tissues is investigated. Additional evidence is provided for the impact of this composite polymer graft material on crestal augmentation, which primarily assists the secondary implant stability after immediate implant placement and immediate loading using quantitative microcomputed tomography (micro-CT), histomorphometric analysis of BIC, and qualitative radiologic and histologic characteristics of newly formed bone around implants and on bone fill in extraction sockets.

MATERIALS AND METHODS

Technology and Products

A Food and Drug Administration–cleared, biocompatible, microporous composite bone replacement graft material containing PPCH and PA^{**} was obtained from the sponsor of this study.^{††} PA is a rapidly resorbable, synthetic bone substitute material that can be light and/or chemically hardened, allowing for immediate bridging and stability. Light/chemical hardening technology is a proprietary combination of light- and chemical-based processes that harden bone substitute materials in situ. The three-phase system is a device to deliver individual doses of synthetic bone graft materials, PPCH, PA, or their combination, to the surgical site. In this study, PPCH+PA composite graft material and the individual formulations of PPCH and PA are applied manually using the three-phase delivery system and hardened in situ with standard dental curing light to provide immediate bridging between the implant and bone at the coronal sites. In addition, the materials were also used as crestal augmentation material after tooth extraction for socket healing without implants.

Animal Model

The study protocol was reviewed and approved by the Boston University Medical Center (BUMC) Institutional Animal Care and Use Committee before study initiation. Three adult male minipigs (18 to 24 months old; average weight: 35 kg) were purchased from the breeder^{††} and acclimatized for 7 days at the BUMC Laboratory Animal Science Center before any experimentation. The minipigs, housed in metal pens separately throughout the study, were maintained at a temperature of 24°C ± 2°C and a relative humidity of 55%, and the animals were fed a regular diet according to the guidelines of the manufacturer and tap

^{**}Kerr, a division of Sybron Dental Specialties, Orange, CA.

^{††}Göttingen Minipigs, Marshall BioResources, North Rose, NY.

water *ad libitum*. The animals were placed on a semiliquid diet for 2 weeks after the implant surgeries.

Surgical and postoperative procedures were described previously²³ and are summarized in Figure 1. Briefly, parallel-wall, screw-type, 3.25-mm-diameter, 11.5-or 13-mm-long titanium implants^{§§} were placed after atraumatic extractions of four premolar and first molar teeth in each quadrant using the surgical drill system^{|||} under general anesthesia. The implants (n = 48; 16 implants per pig) were inserted subcrestally with primary stability at the apical area. The healing abutments (4-mm collar height)^{¶¶} were screwed in occlusion for immediate loading and left unsplinted.

The distribution of implant and socket augmentation per treatment group is shown in Figure 1. The crestal areas and all bony sites at the boundaries of implants were filled with either PPCH+PA, PPCH, or PA graft material using a three-phase delivery system, and dental curing light was used to harden the graft materials using a modified ridge augmentation procedure ensuring tight closure around implants and over the sockets. Flaps were closed using 4-0 resorbable sutures. Primary closure was obtained by splitting the flap when possible and using matrix suture techniques.

There were a total of 12 quadrants in three minipigs (PPCH+PA = four quadrants: two maxillary and two mandibular; PA = four quadrants: two maxillary and two mandibular; PPCH = two quadrants: one maxillary and one mandibular; no graft = two quadrants: one maxillary and one mandibular). Crestal augmentation was performed in 36 sockets randomly assigned to four treatment groups: 1) PPCH+PA; 2) PPCH; 3) PA; and 4) PPCH without light/chemical hardening (original PPCH) as the positive control.

Clinical follow-up and oral hygiene procedures included brushing and irrigation with chlorhexidine digluconate at 2 and 6 weeks postoperatively. At 12 weeks, all animals were euthanized by an overdose of pentobarbital (120 mg/kg, intravenously). Maxillae and mandibles were removed en bloc using oscillating bone saws and cutting disks under saline irrigation. Then, each implant was dissected with the surrounding bone. Specimens were fixed in 10% formalin for 5 days. Half of the implant–bone blocks (n = 23; one implant was lost at 6 weeks postoperatively) were used for clinical and mechanical analyses and for subsequent undecalcified histology. The remaining 24 bone–implant blocks were further demineralized by immersion in EDTA for histologic and histomorphometric analyses.

Clinical, Macroscopic, and Radiographic Evaluations

Clinical outcome, stability, and function by visual inspection, periodontal probing of peri-implant sulcus, radiology, pull-out force test, and mobility were evaluated and reported in Part I of this study.²³ Soft-tissue evaluations showed no adverse reaction, abscess, or impaired wound healing at all sites, including implants and sockets.²³ Assessments described below were made at 12 weeks on bone sections both around implants and from the sockets. The radiographs were taken with a digital x-ray^{###} before histology and evaluated by

§§Osseotite Certain Microminiplant, Biomet 3i, Palm Beach Gardens, FL.

|||Osseocision, Biomet 3i.

¶¶Biomet 3i.

counting the implant threads covered by the crestal bone (reported previously.²³) The radiographic evaluations in the sockets were only observational; qualitative assessments were made for organization of trabecular bone, cortical bone formation and continuation, and for residual graft materials, if any.

Quantitative Micro-CT

Four mandibles representing each of the treatment groups (PPCH-PA, PPCH, PA, and original PPCH) were evaluated for structural aspects of the bone and tissue compositions at 12 weeks by micro-CT.

Immediately before scanning, specimens stored in 10% formalin at 4°C were allowed to acclimate to room temperature. Scans were performed using a cabinet micro-CT system^{***} located at the Boston University Orthopaedic and Development Biomechanics Laboratory. Specimens were kept in 10% formalin to minimize decomposition of the tissue and maintained in a uniform position using a custom foam jig to orient the specimen in the scanner during the scanning (≈ 20 minutes). Samples were binarized using the same parameters for filter and threshold. Scans were performed at a resolution of 36 $\mu\text{m}/\text{voxel}$.

Subsequent analysis of the scans was performed using software.^{†††} Measurements of the bone (total volume [TV]) and density (volume fraction and bone mineral density [BMD]) were made directly from the micro-CT image data. A global threshold algorithm was used that applied a fixed, constant threshold to all specimens using gray level. A constrained three-dimensional Gaussian filter (filter width: 0.8; filter support: 1 voxel) was used to partially suppress image noise. The TV, mineralized volume fraction, and BMD were then calculated using standard routines provided by the system manufacturer. The BMD was calculated in the same manner described above. Trabecular and cortical data were extracted and processed to obtain the morphometric parameters BMD (millimeters of hydroxyapatite per cubic centimeters) and bone mineral content (BMC). For these measurements, a region of interest (ROI; outlined using the standard square measuring tool), surrounding each implant and molar socket preservation sites was defined.

The following measurements were obtained from micro-CT scanning for both trabecular and cortical bone: 1) TV: the total volume of tissue enclosed by the contours; 2) bone volume (BV): the volume of the voxels that are above the threshold (8,192), e.g., the volume of bone (or mineralized) tissue; 3) BV/TV: bone volume fraction is the ratio of BV to TV (this parameter is described as a percentage of the TV that is bone); 4) BMD: the average mineral density of only those voxels above the threshold (e.g., voxels that are considered to be bone tissue); and 5) BMC: the average mass of bone mineral above the threshold (e.g., those considered to be bone).

##Schick Technologies, Long Island City, NY.

***Scanco μCT 40 system, Scanco Medical, Bassersdorf, Switzerland.

†††Scanco Medical

Decalcified Sections and Histologic Analyses

The implants and sockets with surrounding bone were dissected as block sections and fixed with 10% formalin for at least 5 days. After 6 weeks of demineralization in 0.5 M EDTA (10% EDTA solution in distilled water, pH 7.4 at 4°C), implants were gently removed for embedding in paraffin. Five micrometer serial sections were made in a buccolingual longitudinal orientation. The sections were stained alternatively with either hematoxylin and eosin (H&E) stain for the analyses of epithelium/connective tissue (CT) organization, cell infiltrations, or bone structures around implants or Masson's trichrome stain for qualitative evaluations including new bone-collagen formation and mature bone at the implant interface. For histomorphometric measurements of BIC, the distance from implant surface to the CT or to the bone tissue was measured at the crestal areas. Measurements were made at the coronal aspects for the distance between the subepithelial CT and the implant surface and the distance between the bone crest and the implant surface.

Ground Sections and Analyses

Samples were dehydrated in a graduated series of ethanol and infiltrated in embedding media^{†††} according to the instructions of the manufacturer. Samples were cut using a band saw system^{§§§} and a grinding and polishing device.^{||||} Samples were then mounted on plastic slide holders. Surfaces were ground and polished using a graded series of polishing papers^{¶¶¶} with the smallest size of P2500. Thickness of ground sections was 15 to 25 mm on average. Qualitative histologic assessments, e.g., mature bone formation versus CT formation, the features of the bone formed around implants specifically at the crestal levels augmented by the tested materials, were made at the coronal, mid, and apical portions of the bone-implant interface.

Statistical Analyses

The unit of measurement was the treated sites ($n = 16$ for each of the light-cured PPCH+PA and PA alone and $n = 8$ for light-cured PPCH and original PPCH). Significant differences among test groups and controls for micro-CT and histomorphometric measurements were assessed by analysis of variance (ANOVA). Pairwise multiple comparison analysis was performed by using a least significant difference (LSD) post hoc test. Two-tailed P values were calculated, and $P < 0.05$ was set as the level of statistical significance. All statistical evaluations were performed using a statistical package.^{###}

RESULTS

Clinical and Radiologic Evaluations

Results of the clinical and radiologic evaluations were reported in Part I of this study.²³ All sites treated with implants with or without augmentation materials healed without major complications. At sacrifice, all implants showed good healing results and clinical stability

^{†††}EXAKT Technovit 7200 VLC, EXAKT Technologies, Oklahoma City, OK.

^{§§§}EXAKT 300 parallel band saw system, EXAKT Technologies.

^{||||}EXAKT 400CS Grinding System, EXAKT Technologies.

^{¶¶¶}EXAKT polishing papers, EXAKT Technologies.

^{###}SPSS v.11.0 for Windows, IBM, Chicago, IL.

except the one lost at 6 weeks. All sockets with augmentations showed complete clinical healing without complications (Fig. 1).

In all sockets, bone formation was uninterrupted and complete at 12 weeks (see blue arrows in supplementary Fig. 1A in the online *Journal of Periodontology*). Although it was difficult to differentiate the residual graft particles from the newly formed bone in other groups, the sites treated with original PPCH showed microparticles with an outer surface containing calcium detectable at the surface of the socket at the crestal level (red arrow). The trabecular bone formation was without any fibrous tissue; the organization of newly formed bone at the crestal areas revealed the reformation of cortical bone.

Micro-CT Evaluations

Supplementary Figure 1B in the online *Journal of Periodontology* demonstrates both the implant–bone interface and socket preservation sites treated with PPCH+PA. New bone was densely deposited around the implant, whereas the socket showed a normal bone reformation with well-organized trabeculation and cortical bone formation at the extraction site.

Supplementary Tables 1 and 2 in the online *Journal of Periodontology* present the micro-CT results for BV, BMD, and BMC for all groups. Both trabecular and cortical bone measurements did not reveal any significant difference among the groups. Light/chemically hardened PPCH showed more dense bone formation in sockets; however, the difference was not significant (Fig. 2).

Histologic and Histomorphometric Assessments

In all groups, no obvious bone resorption around implants was observed in H&E-stained sections. A direct BIC was seen in all sections. Osseous deposition revealed a lamellar and partially trabecular structure containing numerous osteoblasts at the surface of bone trabeculae (Fig. 3). The surrounding bone in the PA, PPCH, and no-graft groups had a loose structure with a high number of spaces filled with fat and CT. Some areas of implants were covered by thin trabecular bone (Figs. 3B through 3D). However, with most of the implants in the PPCH+PA group, a mineralized tissue and newly formed lamellar bone with increased density around cervical and middle parts of the implants were observed. The newly formed bone was characterized by a healthy Haversian system in close contact with the implant surface, especially within the thread of the implant. In addition, osteocytes within numerous lacunae were seen localized around the threads, especially in the coronal portion of the implants (Fig. 3A).

The distance between the subepithelial CT and BIC was significantly less in the PPCH+PA and PA groups compared to both the PPCH ($P = 0.02$ for both) and no-graft control groups ($P = 0.002$ and $P = 0.001$, respectively), indicating more BIC surface at the coronal sites in groups treated with PPCH+PA and PA (Fig. 3E). Likewise, these treatments resulted in significantly greater BIC surface at the crest compared to implants without graft ($P = 0.001$ and $P = 0.003$, respectively), while there was no statically significant difference compared to PPCH group ($P = 0.13$ and 0.19 , respectively).

Evaluation of Masson's trichrome-stained sections was done on a descriptive basis only. These sections allowed clear visualization of the presence of new mature bone formation and collagen deposition within the newly formed bone and bone matrix. New bone formation at the coronal and mid portions of the implant was observed in the sites treated with PPCH+PA composite graft material (Fig. 4). The new bone was incorporated by a newly formed bone matrix with numerous osteoblasts. The CT was composed of well-organized collagen fibers and fibroblasts. The normal appearance of bone with Howship lacunae and blood vessels was observed adjacent to the implant threads.

Sites augmented with PA, PPCH, and no graft demonstrated partial new bone formation in conjunction with newly formed CT at the implant–bone interface. The bone matrix between the old and new bone was loosely organized and contained fewer osteoblasts compared to PPCH+PA sections.

Microscopic Analyses of Undecalcified Sections

Formalin-fixed, undecalcified tetrachrome-stained^{****} sections were analyzed for qualitative structures of the implant–bone interface specifically at the crestal level. No gap or CT formation was found between the bone and implant surface in the PPCH+PA-treated samples (Fig. 5A). Higher-magnification ($\times 100$) images indicated the newly formed bone in contact with the implant surface. The PA-treated sites mainly consisted of CT in close contact with the implant surface at the coronal part with a lesser amount of new bone compared to PPCH+PA composite graft (Fig. 5B).

Implant sites treated with PPCH showed similar results to PA-treated sites; a newly formed bone in conjunction with CT was the major structure at the coronal portion (Fig. 5C). In general, the coronal implant threads were in contact with CT while newly formed bone with collagen fibers was also present in some areas. Sites without augmentation showed less bone formation and less BIC surface compared to all other groups (Fig. 5D). In these sections, the BIC was mainly formed by immature bone/CT formation.

Alveolar Ridge Augmentation and Socket Preservation at the Extraction Sockets

The presence of compact bone with many osteocyte lacunae and regularly distributed vascular structures located in marrow spaces and in Haversian canals was detectable in all sections (Fig. 6). No inflammatory cell infiltration was present at the augmented sites. Some sections (mostly PA and PPCH+PA treated) showed unresorbed graft particles encapsulated within the supracrestal CT as a foreign body reaction. PPCH+PA-treated specimens demonstrated a densely organized CT and more new bone formation compared to all other groups. In the PA, PPCH, and no-graft groups healing was mainly by immature bone/CT. Sections from non-grafted specimens showed loosely organized bone matrix and less new bone formation between host bone particles (Fig. 6).

****Villanueva's Osteochrome Bone Stain, PolySciences, Warrington, PA.

DISCUSSION

The present study demonstrates that a newly formulated PPCH+PA provides immediate crestal support by bridging the gaps between the implant and the socket walls and maintains the space until the BIC is achieved. Most importantly, this crestal “bridge” allows the soft tissue to localize and shape around the neck of the implant by providing guidance at the crestal level. The data also demonstrated that this structure was maintained during immediate functioning and was stable against chewing forces.

Several studies reported that the risk of implant failure increased when immediate loading was applied as a result of occlusal forces that potentially cause micromovement of the implants during the healing period, leading to soft-tissue encapsulation around the implant and resulting in failure of the osseointegration process. Conversely, recent reports indicated that early loaded (after 6 weeks of healing) implants can be successfully restored and loaded with implant success rates of >99% 2 years after restoration.²⁴ More recently, another multicenter study with immediately or early loaded dental implants^{†††} reported implant survival rates of 98% and 97%, respectively, comparable to conventional loading.²⁵ Moreover, a recent systematic review of studies evaluated immediate loading, and the implant and restoration prognosis has shown that overall implant success was 79% to 100% in the mandible, whereas the implant survival rates were 95.8% to 100% for both jaws. Another systemic review of studies with immediate loading concluded that immediate loading of implants placed into fresh extraction sockets displayed excellent implant prognosis with an implant survival rate of 95.6%.²⁶ Overall, these studies indicate that an appropriate patient selection, primary stability, splinting of implants during restoration, and the expertise of surgeons were the important criteria for the prognosis of immediately loaded implants and their restorations. In addition, good bone quality and use of long implants also played roles. However, because of a lack of sufficient randomized, controlled clinical trials and sufficient statistical power, the results should be carefully interpreted, especially in the maxilla and immediate loading of immediately placed implants.

A bone-wall defect, especially at the buccal aspect or crestal bone deficiency, is a major problem and often is the primary limiting factor for immediate implant placement. Although primary stability can still be achieved at the apical region, the gap between the bone walls and the implant becomes the determining factor for optimal BIC. In these cases, augmentation of the space at the crestal areas is equally important as the primary stability. The present study tests the potential of a newly formulated, chemically hardened PPCH+PA composite graft material in the stability and function of the immediately placed and loaded implants after tooth extraction. The stability and functional properties of the augmented crestal bone surrounding implants with light/chemically hardened PPCH+PA graft material after immediate implant placement has been published previously by the present authors. The results indicated that light-hardened PPCH+PA graft material is useful for promoting bone formation at crestal areas and supporting the stability of the implants. In this report, it is further demonstrated that PPCH+PA has the capabilities of restoring bone at the crestal areas during implant placement into fresh extraction sockets by histologic and micro-CT

^{†††}Straumann SLActive implants, Institute Straumann, Basel, Switzerland.

assessments. PPCH+PA was also useful in the socket preservation after tooth extraction, as indicated by well-organized trabecular and crestal bone formation.

Despite the development of new surgical approaches and techniques,^{27,28} several problems relating to immediate implant placement, including management of soft tissue and bone support at the coronal level still remain as important challenges.²⁹ Guided bone regeneration with barrier membranes and/or bone grafts were shown to improve osseointegration and stability of immediately placed implants without any change in healing and osseointegration rate.^{30–32} A retrospective 10-year clinical study testing β -tricalcium phosphate grafting in large extractions sockets after immediate implant placement showed no crestal bone loss in 72.1% of cases, and the result was similar to those placed in bone with favorable bone quality.³³

Although specific resorption time for this material is not known, PPCH and PA are completely degradable. The degradation time can be changed based on the indication. Depending on the specific application, the time required can be manipulated based on a number of factors, e.g., the ratio of the bone substitute and the cross-linkable prepolymer. When the cross-linkable prepolymer contains more than one type of monomer, the ratio of the monomers also plays a crucial role in the degradation/resorption time.

In the present study, a threshold of 250 is used as a uniform threshold for micro-CT bone measurements. The threshold for bone and graft material was determined using visual image, intensity (gray level), and histogram analysis. Segmentation/thresholding is very important for morphometric analysis, and it is understood that using a global thresholding could have an impact on the information obtained by this image analysis,³⁴ but it is also believed that the impact in this study is very small because the present study uses a larger voxel size that is suitable for cortical bone when comparing the histologic images and micro-CT imaging.^{35–37}

CONCLUSIONS

The results of this present study confirm the previous report²³ by showing that the newly formulated light/chemically hardened graft material PPCH+PA was beneficial in immediate implant placement after tooth extraction and resulted in greater BIC and histologic bone formation in quality and quantity during 3 months. In addition, the PPCH+PA resulted in crestal bone augmentation over extraction sockets, preventing the soft-tissue migration, and acted within the same context as socket preservation. Within the limits of the small sample size, these findings show that the light/chemically hardened PPCH+PA can be safely and successfully used to perform crestal ridge augmentations around implants and in extraction sockets. In addition, PPCH+PA graft material has the potential to provide implant stability and more bone formation during immediate loading. Additional studies in animals using different healing times and longer durations and well-controlled human clinical studies should be performed for further evaluation of this newly formulated bone substitute material.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Study timeline. *Oral hygiene, implant check, and stability test measurements. †Stability test measurements, tissue harvesting. ‡All procedures were performed under general anesthesia. §Animals were euthanized by overdose pentobarbital injections (120 mg/kg). Groups included light-hardened materials PPCH+PA, PA, and PPCH; no graft and original PPCH.

Figure 2.

Micro-CT evaluations and quantitative analysis. **A)** Although all materials tested resulted in new bone formation and complete bone healing, micro-CT scans showed more densely organized new bone formation around implants and in the extraction sockets in specimens treated with PPCH+PA. **B)** Quantitative analysis yielded similar results in which all grafts resulted in trabecular and cortical bone formations successfully without statistically significant difference. $P > 0.05$; ANOVA with LSD post hoc test.

Figure 3.

Histologic evaluations around implants. **A)** The PPCH+PA group showed mineralized tissue and newly formed bone, with increased density around the cervical and middle parts of the implants. The newly formed bone was detectable with no signs of inflammation or CT formation. **B through D)** The PA, PPCH, and no-graft groups had a loose structure with a high number of spaces filled with fat and CT. **E)** Histometric analysis. Sites augmented with PPCH+PA and PA treatment resulted in statistically significantly greater BIC surface than the PPCH-and no-graft-treated implants (original magnification $\times 25$ and $\times 100$).

* $P < 0.05$, significant compared to the PPCH-and no-graft-treated groups; † $P < 0.05$, significant compared to no graft; ANOVA with LSD post hoc test.

Figure 4.

Masson's trichrome staining around implants. New bone formation at the coronal and midportions of the implant with a direct BIC was observed in sections treated with PPCH +PA composite graft material. Conversely, sites treated with PA, PPCH, and no graft demonstrated partial bone formation in conjunction with newly formed CT at the implant–bone interface (original magnifications $\times 25$ and $\times 100$).

Figure 5.

Ground sectioning of implant–bone blocks. Undecalcified sections were obtained and stained^{††††} for all groups. **A)** No gaps or CT formation was found between bone and implant surface in the PPCH+PA– treated samples. A successful BIC was detectable. **B)** In PA-treated sections, CT was the major structure at the coronal part of the implant in close contact to the implant surface. **C)** The implant sites treated with PPCH also showed newly formed bone in conjunction with CT at the coronal portion of the implant surface. **D)** Sites without augmentation were found to have less BIC surface compared to all other groups, indicating delayed bone apposition (original magnifications $\times 25$ and $\times 100$).

^{††††}Villanueva stain, PolySciences.

Figure 6.

Histologic evaluations in extraction sockets. H&E-stained sections were evaluated under light microscopy for new bone formation and tissue reaction. No inflammatory cell infiltration was seen at the augmented sites. **A)** Histologic analysis revealed that PPCH+PA –treated specimens demonstrated densely organized CT and a complete new bone formation compared to all other groups. **B through D)** Bone formation was delayed in the PA, PPCH, and no-graft groups characterized by large medullary spaces and CT presence. In the no-graft sites, loosely organized bone matrix was the major finding observed around the host bone particles (original magnifications $\times 25$ and $\times 100$). B = bone; E = epithelium.