

The Use of Light/Chemically-Hardened Poly-Methyl-Methacrylate, Poly-Hydroxyl-Ethyl-Methacrylate and Calcium Hydroxide Graft Material in Combination with Polyanhydride around Implants and Extraction Sockets in Minipigs: Part II: Histological and μ CT Evaluations

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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: Light-hardened poly-methyl-methacrylate, poly-hydroxyl-ethyl-methacrylate, and calcium hydroxide plus polyanhydride (PPCH+PA) composite graft), PPCH graft alone, PA graft alone, were compared to each other and to a no graft control group. All mandibular premolar teeth were extracted; a total of 48 implants were placed into sockets of the mesial roots in three minipigs. Crestal areas and intrabony spaces were randomly augmented with graft materials or left untreated. Distal sockets not receiving implants and the sockets of first molars (n=60) were randomly treated with one of graft materials or left empty. In addition, two molar sockets were treated with original PMMA/PHEMA and calcium hydroxide (PPCH) graft material. Quantitative microcomputed tomography (μ CT) was used to assess alveolar bone structure and tissue compositions following crestal augmentations. Histological evaluations included descriptive histology to assess the peri-implant wound healing as well as histomorphometric measurements to determine bone-to-implant contact (BIC).

Results: Although PPCH alone showed the least impact on trabecular bone formation, both trabecular and cortical bone measurements by μ CT did not reveal any significant differences between 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. Villanueva-stained sections showed complete bone formation between bone and implant surface in the PPCH+PA group, while 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: Histological evaluations supported the earlier findings on implant stability and function and confirmed that PPCH+PA provides a greater BIC contact with a well-organized implant-bone interface and is useful in crestal augmentation during immediate implant placement.

KEY WORDS:

Ridge augmentation, implants, stability, immediate loading, post-extraction sockets, synthetic graft materials

Immediate implant placement involves the insertion of a dental implant directly into the fresh socket after tooth extraction¹. 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 during placement of the implant. Subsequent wound healing, bone resorption and new bone formation facilitate secondary implant stability. A successful osseointegration is therefore determined not only by the primary stability, but also by the extent of the secondary implant stability⁵. Bone-to-implant contact (BIC) is critical for the osseointegration and needs to be established by both the primary and secondary stability.

In addition to the implant placement, the loading can also be initiated immediately where occlusion can be restored within the first 72 hours following the surgery (Consensus Conference of the EAO)⁶. On the other hand, immediate loading may increase the risk for failure due to the micro-movement caused by the occlusal forces. The micro-movement of the implant fixture leads to soft tissue encapsulation around the implant and results in failure of the osseointegration⁷⁻⁹. Therefore, while immediate implantation is getting more widely accepted, conventional loading initiated after 3-8 months is still considered critical for a successful osseointegration¹⁰. Yet, the quest for reducing the micro-movement and shortening the loading time continues and several technological advances have been adopted.

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 implant¹¹. 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, 13, 14}. Vertical crestal augmentation¹⁵ and split crest technique^{16, 17} have been successfully used for decreasing the voids. Many grafting materials have also been tested for reducing spaces around implants, increasing the BIC, and enhancing the stability in order to increase the success of the immediate implantation and loading. These include freeze-dried bone allografts, hydroxyapatite, and synthetic grafts¹⁸⁻²² all of which have advantages and short-comings. In our previous paper, we showed that a newly formulated chemically hardened graft material, poly-methyl-methacrylate, poly-hydroxyl-ethyl-methacrylate, and calcium hydroxide polymer graft composed of polymethylmethacrylate (PPCH-PA) 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 those areas where cancellous bone was present. In the present report (Part II), we aimed to study the impact of this application on the qualitative

parameters of the tissues. We demonstrate further evidence in the impact of this composite polymer graft material on crestal augmentation which primarily assists the secondary implant stability following immediate implant placement and immediate loading using quantitative microcomputed tomography (μ CT), histomorphometric analysis of BIC and qualitative radiological and histological characteristics of newly formed bone around implants. The impact of the composite polymer on new bone formation in extraction sockets was also evaluated.

MATERIALS AND METHODS

Technology and Products

An FDA cleared, biocompatible micro-porous composite of ***poly-methyl-methacrylate, poly-hydroxy-ethyl-methacrylate and calcium hydroxide*** (PPCH) bone replacement composite graft material containing polyanhydride (PA)** was obtained from the Sponsor^{††}. **PA** is a rapidly resorbable, synthetic bone substitute material, which 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 3-phase system** is a device to deliver individual doses of synthetic bone graft materials, PPCH or PA or their combination, PPCH+PA, to the surgical site. In this study, PPCH+PA composite graft material and the individual formulations of PPCH and PA were applied manually using the 3-phase delivery system and hardened *in situ* with standard dental curing light to support immediate implants placed in fresh extraction sockets in mini-pigs as adjunct to primary apical fixation as well as at the crestal areas to provide immediate bridging between implant and the bone at the coronal sites. In addition, the materials were also used as crestal augmentation material after tooth extraction for socket healing without implants. For this purpose, one molar tooth sockets and sockets of the premolar teeth not received implants were augmented with either PPCH+PA, PPCH or PA using 3-phase delivery system where all the graft materials were hardened by light cure. One group of sockets was treated with the original PMMA+PHEMA+CH without light/chemical hardening potential as the positive control.

Animal Model

Study protocol and experimental design were reviewed and approved by the Boston University Medical Center Institutional Animal Care and Use Committee (BUMC IACUC) prior to study initiation. Three minipigs (adult male; 18-24 months old; average weight 35 kg) were purchased from the breeder^{‡‡} for this particular experiment and acclimatized for 7 days at the BUMC Laboratory Animal Science Center (LASC) prior to any study procedures. The minipigs were housed in metal pens separately throughout the study maintained at a temperature of 24 ± 2 °C, a relative humidity of 55%, and fed with regular minipig diet according to manufacturer's guidelines and tap water *ad libitum*. Following the implant surgeries, the animals were placed on a semi-liquid diet for two weeks.

Surgical and post-operative procedures were described elsewhere²³ and summarized in Figure 1. Briefly, parallel wall, screw-type, 3.25 mm in diameter and 11.5 mm or 13

mm in length titanium implants^{ss} were placed after atraumatic extractions of four premolar and first molar teeth in each quadrant using the surgical drill system^{***} under general anesthesia. Extractions were performed by elevation of a mucoperiosteal flap, separation of the roots using multiblade burs and separation disks, and incision of periodontal ligament with an Orban scalpel^{†††}, as needed. The implants were inserted subcrestal with primary stability at the apical area. A total of 48 implants, 16 implants in each pig, were inserted. The healing abutments (4 mm collar height)^{†††} with internal connection were screwed in occlusion for immediate loading and left unsplinted.

The distribution of implant and socket augmentation per treatment groups is shown in Table 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 3-phase delivery system and dental curing light was used to harden the graft material for implant augmentation according to manufacturer's guidelines. The placement of the graft material around the implants and on the crestal areas constituted a modified ridge augmentation procedure insuring tight closure without any exposure of the surface and flaps were primarily closed using 4-0 resorbable sutures. Primary closure of flaps was obtained by splitting the flap, where possible, and using matrix suture techniques.

Out of a total of 12 quadrants in three minipigs, four quadrants (two maxillary, two mandibular, selected randomly) were either treated with PPCH+PA or PA graft materials whereas two quadrants (one maxillary and one mandibular) received either PPCH alone or no graft (Table 1). Crestal augmentation was performed in 36 sockets using the same materials assigned for each quadrant. Sockets with crestal augmentation were randomly assigned to treatment groups; PPCH+PA, PPCH, PA and original PPCH (first three materials were light cured).

The animals were anesthetized using general anesthesia for a clinical follow-up and oral hygiene procedures including brushing and irrigation with chlorhexidine digluconate at 2 and 6 weeks post-operatively. At 12 weeks, all animals were euthanized by an overdose of pentobarbital (120 mg/kg, iv). Maxillae and mandibles were removed en bloc using an oscillating bone saw and high speed cutting discs under continuous saline irrigation. Then, each implant was dissected with the surrounding bone using a diamond saw. Specimens were fixed in 10% formalin for 5 days. Half of the bone blocks (n=23; one implant was lost at 6 weeks postoperatively) containing implants were used for clinical and mechanical analyses and for undecalcified histology. The remaining 24 implant-bone blocks were demineralized by immersion in EDTA (10% EDTA solution in distilled water, pH 7.4 at 4°C) with continuous agitation for about 6 weeks for histologic and histomorphometric analyses. Four mandibles representing each treatment group was used for μ CT evaluations of bone. Since 48 distal sockets treated with crestal augmentation were too close to the bone surrounding implants, they were evaluated together with the histological analysis of implant-bone interface. The remaining individual molar sockets (n=12) were evaluated by μ CT for bone volume, bone density and bone mass and later processed for decalcification/sectioning and histologic evaluation.

Clinical, Macroscopic and Radiographic Evaluations

First clinical outcome, stability and function were tested. These were reported in the Part I of this study²³. Briefly, visual inspection, periodontal probing of the peri-implant sulcus, application of force (pullout force load) and mobility^{§§§} measurements were used to determine implant stability (mobility) and radiographic evaluations were made to examine radiographic bone levels. Soft tissue evaluations showed no adverse reaction, abscess, or impaired wound healing at all sites including implants and sockets²³. Assessments described below were made at 12 weeks on harvested bone sections both around implants and in the sockets. Immediately after sacrificing the animals at 12 weeks, the maxillae and mandibles were harvested en bloc and split in half and fixed in 10% formalin. Before block sectioning, the radiographs were taken with a digital X-ray^{****} from all implant and socket augmentation sites. Radiographs were evaluated for implant threads covered by the crestal bone (reported previously in ref #²³) and for sockets in all groups for bone formation. The radiographic evaluations in sockets were only observational; qualitative assessments were made for organization of trabecular bone, cortical bone formation and continuation, residual graft materials, if any, were assessed.

Quantitative Microcomputed Tomography (μ CT)

Alveolar bone structure and tissue compositions were examined by quantitative microcomputed tomography (μ CT) following crestal augmentations around implants and in socket preservation with all materials tested in the study. Four specimens obtained from the mandible of the animals representing each of the treatment groups (PPCH-PA, PPCH, PA and original PPCH) were evaluated for structural aspects of the bone by μ CT at 12 weeks.

Immediately before scanning, individual quadrants stored in 10% formalin at 4°C were allowed to calibrate to room temperature. Scans were performed using a Scanco μ CT 40 system^{††††} located in the Orthopaedic and Development Biomechanics Laboratory. Specimens were kept in 10% formalin solution during scanning in order to minimize decomposition of the tissue throughout the duration of the scan (~20 minutes). During the scanning they were maintained in a uniform position using a custom foam jig to orient the specimen in the scanner. Samples were binarized using the same parameters for filter and threshold. μ CT scans were carried out at a resolution of 36 μ m/voxel.

Subsequent analysis of the scans was carried out using the software of Scanco Medical. Measurements of the bone (total volume), and density (volume fraction and mineral density) were made directly from the μ CT image data of each specimen. A global threshold algorithm was used which applied a fixed, constant threshold to all specimens using grey level. A constrained 3D Gaussian filter (filter width = 0.8, filter support = 1 voxel) was used to partially suppress image noise. The total volume, mineralized volume fraction, and mineral density were then calculated using standard routines provided by the system manufacturer. The mineral density was calculated in the same manner described above. Trabecular and cortical data were then extracted and processed to obtain morphometric parameters, bone mineral density (BMD: mm

HA/cm³) and bone mineral content (BMC), which were calculated for both area of interests (ROI).

For these measurements, an area of interest, ROI (outlined using the standard square measuring tool) surrounding each implant was defined as: distal of first implant, mesial of second implant and distal of first implant, distal of second implant, mesial of third implant and distal of second implant, distal of third implant, mesial of fourth implant and distal of third implant and distal of fourth implant. In addition the molar site treated with socket preservation was defined as augmentation site. Measurements were done for each sample.

The following measurements were obtained from the μ CT scanning for both trabecular and cortical bone: TV: The total volume of tissue enclosed by the contours and has been measured; BV: The volume of the voxels that are above the threshold (8192), i.e. the volume of bone (or mineralized) tissue; BV/TV: Bone volume fraction is the ratio of bone volume to total volume. This parameter is described as a percentage of the total volume that is bone; BMD: The average mineral density of only those voxels above the threshold (i.e., voxels which are considered to be bone tissue.); BMC: The average mass of bone mineral over those voxels above the threshold (i.e., those considered to be bone).

Decalcified Sections and Histological Analysis

Maxilla and mandible were dissected free and block sections of each of the implants and sockets with surrounding bone were prepared and placed in individual vials containing 10% formalin for fixation for at least 72 hours. Following 6 weeks of demineralization in 0.5M ethylene diamine tetra-acetic acid (EDTA); implants were gently removed en route for embedding in paraffin. Five (5) μ m serial sections were made in a buccolingual longitudinal orientation using a microtome. The sections were stained alternatively with either hematoxylin and eosin stain (H&E) or Masson's trichrome stain for qualitative histological evaluations. H&E stained sections were evaluated for qualitative observations including epithelium/connective tissue organizations, cell infiltrations, bone structures around implants as well as histomorphometric measurements of BIC. For BIC measurements, the distance from implant surface to the connective tissue or to the bone tissue was measured at the crestal areas. BIC was calculated using linear measurements performed on each sample. Measurements were made at the coronal aspects for the distance between the sub-epithelial connective tissue and the implant surface and the distance between the bone crest and the implant surface. Masson's trichrome stained sections were evaluated for qualitative assessments of new bone-collagen formation and mature bone at the implant interface.

Ground Sections and Analysis

Samples were dehydrated in a graduated series of ethanol and infiltrated in Exakt Technovit 7200 VLC^{****} according to the manufacturer's instructions. Samples were cut using an Exakt 300 parallel band saw system and an Exakt 400CS grinding and polishing device. Samples were then mounted on plastic slide holders. Surfaces were ground and polished using a graded series of Exact polishing papers with a smallest size of P2500. Thickness of ground sections was between 15 and 25 mm in average. Qualitative

histological assessments were made for bone-implant interface i.e., mature bone formation versus connective tissue formation, the features of the bone formed around implants specifically at the crestal levels augmented by the tested materials. Implant to bone interface at the coronal, mid and apical portions of the implant for each slide was evaluated.

Statistical Analysis

The unit of measurements 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 for original PPCH). Significant differences between test groups and controls for μ CT and histomorphometric measurements were assessed by analysis of variance (ANOVA). Pairwise multiple comparison analysis was performed by 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^{ssss}.

RESULTS

Clinical and Radiological Evaluations

Results of the clinical and radiological evaluations were reported in Part I of this study²³. All sites treated with implants with or without augmentation materials healed without major complications. A few sites exhibited some minor gingival inflammation due to heavy calculus deposition around the implants at each time point (2, 6 and 12 weeks). Some abutments were found lost and replaced, loose abutments were tightened. At 6 weeks, one implant was lost most probably due to a trauma in animal that had an anesthesia complication and had to be intubated by mouth. No further health problem in other animals was observed; none of the implants became loose or lost during this period. Occlusal surfaces of the abutments exhibited contact points (tattered and shining), which indicated function. At sacrifice, all implants showed good healing results and clinical stability except the one lost at 6 weeks. All sockets with augmentations showed complete clinical healing without complications (Figure 1).

The radiographic measurements around implants using the thread count were previously published²³. In all sockets, bone formation was uninterrupted and complete at 12 weeks (Figure 2A, depicted by blue arrows). While it was difficult to differentiate the residual graft particles from the newly formed bone in other groups, the sites treated with original PPCH showed micro particles 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 newly formed bone at the crestal areas revealed the reformation of cortical bone.

μ CT Evaluations

Figure 2B demonstrates both the implant-bone interface and socket preservation sites treated with PPCH+PA. New bone was densely deposited around the implant while the socket showed a normal bone reformation with well-organized trabeculation and cortical bone formation at the extraction site. The new bone formation was characterized by well-organized trabecular and cortical structure around the implant and within the augmented

sites. All materials showed positive results on trabecular and cortical bone formation in extraction sockets (Figure 3).

Tables 2 and 3 present the μ CT results for trabecular bone volume, bone density (BMD) and bone mineral content (BMC) for all groups. Both trabecular and cortical bone measurements did not reveal any significant difference between the groups. Light/chemically hardened PPCH showed the least effect on trabecular bone formation; however, the difference was not significant.

Histological 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 (Figure 4). The surrounding bone of the PA, PPCH and no graft groups had a loose structure with high number of spaces filled with fat and connective tissue. Some areas of implants were covered by thin trabecular bone (Figures 4B-D). With most of the implants in PPCH+PA group, however, a mineralized tissue and newly formed lamellar bone, with increased density around cervical and middle parts of the implants was observed. The newly formed bone was detectable with no signs of inflammation or connective tissue formation. A healthy Haversian system in close contact with the implant surface, especially within the thread of the implant was observed. In addition, osteocytes within numerous lacunae were seen localized around the threads especially in the coronal portion of the implants (Figure 4A).

The distance between the sub-epithelial connective tissue and BIC was significantly less in groups PPCH+PA and PA compared to both PPCH ($p=0.02$ for both) and no 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 (Figure 4E). Likewise, groups treated with PPCH+PA and PA resulted in significantly greater BIC surface at the crest compared to implants without graft ($p=0.001$, and $p=0.003$, respectively). The difference between PPCH alone group however, was not statistically significant for any group ($p=0.13$ and 0.19 , respectively).

Evaluation of Masson's trichrome-stained sections was done on a descriptive basis only. No statistical procedures were performed. These sections allowed clear visualization of the presence of new mature bone formation and collagen deposition within the newly formed bone and bone matrix. Masson's Trichrome stained sections showed new bone formation at the coronal and mid portions of the implant where a direct bone-implant contact was observed with regard to PPCH+PA composite graft material (Figure 5). The new bone was incorporated by a newly formed bone matrix with numerous osteoblasts. The connective tissue was consisted 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 to newly formed connective tissue at the implant bone interface. The bone matrix between the old and new bone was loosely organized and contained fewer osteoblast compared to PPCH+PA sections.

Microscopic Analysis of Undecalcified Sections

Formalin-fixed, undecalcified sections stained with Villanueva staining were analyzed for qualitative structures of bone implant interface specifically at the crestal level for all groups. No gap or connective tissue formation was found between bone and implant surface in the PPCH+LH treated samples (Figure 6, panel A). Higher magnification (100x) images indicated the newly formed bone in contact with implant surface. The PA treated sections showed connective tissue formation at the coronal part of the implant in close contact to implant surface. The newly formed bone was also seen in PA treated sections, but the major structure connecting implant was immature bone/connective tissue. (Figure 6B).

Implant sites treated with PPCH has also shown a newly formed bone in conjunction with connective tissue at the coronal portion of the implant surface (Figure 6C). The coronal threads of the implant were in contact to connective tissue but also with newly formed bone with collagen fibers. Sites without augmentation showed less mature bone formation and less bone-implant contact surface compared to all other groups (Figure 6D). In these sections, the BIC was loosely organized and mainly formed by immature bone/connective tissue formation.

Alveolar Ridge Augmentation and Socket Preservation at the Extraction Sockets

Figure 7 demonstrates the presence of compact bone with many osteocyte lacunae and regularly distributed vascular structures located in marrow spaces and in Haversian canals were detectable in all sections. 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 connective tissue as a foreign body reaction. PPCH+PA-treated specimens demonstrated a densely organized connective tissue and new bone formation compared to all other groups. Mature bone formation was less in the PA, PPCH and no graft groups and mainly formed by immature bone/connective tissue. Sections from non-grafted specimens showed loosely organized bone matrix and less new bone formation between host bone particles (Figure 7).

DISCUSSION

The present study demonstrates that a newly formulated poly-methyl-methacrylate, poly-hydroxyl-ethyl-methacrylate and calcium hydroxide graft material in combination with polyanhydride (PPCH+PA) provides immediate crestal support by bridging the gaps between the implant and the socket walls and maintains the contact the implant-bone contact until the bone-implant-contact 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, due to occlusal forces that potentially cause micromovement of the implants during healing period leading to soft tissue encapsulation around the implant and resulting in failure of the osseointegration process. Recently however, the results of

a multicenter study with Straumann implants showed that early loaded (after 6 weeks of healing) implants were successfully restored and loaded with implant success rates of greater than 99% two years after restoration²⁴. More recently, another multicenter study with immediately or early loaded Straumann SLActive implants reported comparable implant survival rates of 98% and 97%, respectively, to conventional loading²⁵.

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 mandible while 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 also indicated 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 role. However, due to lack of sufficient randomized controlled clinical trials and sufficient statistical power, the results should be carefully interpreted especially in 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 the optimal bone-to-contact on implant surfaces. In these cases, augmentation of the space at the crestal areas is equally important as the primary stability. The present study tested 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 following tooth extraction. We have previously published the stability and functional properties of the augmented crestal bone surrounding implants with light/chemically hardened PPCH + PA graft material after immediate implant placement. 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, we further demonstrate that PPCH+PA has capabilities of restoring bone at the crestal areas during implant placement into fresh extraction sockets by histological and μ CT 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 challenges²⁹. 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 rate³⁰⁻³². A retrospective 10- year clinical study testing TCP-beta 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 quality³³.

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 crosslinkable prepolymer. When the crosslinkable 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 was used as a uniform threshold for microCT bone measurements. The threshold for bone and graft material was determined using visual image, intensity (grey level) and histogram analysis. Segmentation/thresholding is very important for morphometric analysis and we realize that using a global thresholding could have an impact on the information obtained by this image analysis³⁴, however, we also believe that this impact in this study is very small as we have used a larger voxel size that is suitable for cortical bone comparing the histological images and microCT imaging³⁵⁻³⁷.

In conclusion, the results of this present study confirmed the previous report²³ in showing that the newly formulated light/chemically hardened graft material PPCH+PA was beneficial in immediate implant placement following tooth extraction and resulted in greater BIC and histological bone formation in quality and quantity over three months. In addition, the PPCH+PA resulted in crestal bone augmentation over extractions 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, poly-methyl-methacrylate, poly-hydroxy-ethyl-methacrylate and calcium hydroxide composite bone replacement graft material containing polyanhydride 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. Further studies in animals studying different healing times and longer durations and well-controlled human clinical studies need to be carried out for further evaluation of this newly formulated bone substitute material.

ACKNOWLEDGEMENT

This study was supported in part by Biopiant, R&D/LLC, Westport, CT and USPHS grant DE019938 (T.E.V.D). The authors thank Prof. Devon A. Shipp, Department of Chemistry and Biomolecular Science, Clarkson University Potsdam, New York; the veterinary and animal care personnel, Mimi Crowley, Sharron Kirchain, Michelle Callihan, Rosa Yip, and Sergio Maura at the BUMC LASC; Zachary Mason, Boston University, Department of Biomedical Engineering, Laboratory of Orthopaedic and Developmental Biomechanics, for his technical assistance during μ CT imaging and Emilie Goguet-Surmenian, Boston University Department of Periodontology, for her assistance in histology. Implants and the implant equipment were kindly donated by Biomet 3i, Inc., Palm Beach Gardens, FL. Specialty surgical instruments were a gift from Hu-Friedy, Inc., Chicago, IL. Drs. Hasturk, Kantarci, Ghattas, Dangaria, Abdallah, Diekwisch and Van Dyke declare no financial interest. Dr. Ashman, CEO of the Biopiant, R/D, is the inventor and has issued patents on the materials tested.

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Submitted June 06, 2012; accepted for publication December 13, 2013.

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)

Figure 2.

Qualitative radiographic and μ CT evaluations. A. Digital radiographs obtained ex-vivo were used to qualitatively evaluate the bone formation in extractions sockets. All materials showed bone formation at 12 weeks (blue arrows). The original PPCH was still detectable at the surface of the socket at the crestal level (red arrow). B. μ CT scans were taken ex-vivo using only 12 mandibular blocks containing implants and sockets. Micrographs depict the areas of both implants and sockets treated with PPCH+PA. The newly formed cortical bone at the crestal area of implant and socket augmentation site are detectable.

Figure 3.

μ CT evaluations and quantitative analysis. A. Although all materials tested resulted in new bone formation and complete bone healing, μ 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 where all grafts resulted in trabecular and cortical bone formations successfully without statistically significant difference. $p>0.05$; ANOVA with LSD post hoc test.

Figure 4.

Histological evaluations around implants. A. PPCH+PA group showed a mineralized tissue and newly formed bone, with increased density around cervical and middle parts of the implants. The newly formed bone was detectable with no signs of inflammation or connective tissue formation. B, C and D. PA, PPCH and no graft groups had a loose structure with high number of spaces filled with fat and connective tissue. E. Histometrical analysis. Sites augmented with PPCH+PA and PA treatment resulted in statistically significantly greater BIC surface than PPCH and no graft treated implants (Original magnification at 25x and 100x). *= Significant compared to PPCH and no graft groups; #=significant compared to no graft. $p<0.05$; ANOVA with LSD post hoc test.

Figure 5.

Masson's Trichrome staining around implants. New bone formation at the coronal and mid portions of the implant with a direct bone-implant contact 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 to newly formed connective tissue at the implant bone interface (Original magnifications at 25x and 100x).

Figure 6.

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

Figure 7.

Histological 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. Histological analysis revealed that PPCH+PA-treated specimens demonstrated

densely organized connective tissue and a complete new bone formation compared to all other groups. B, C. and D. Bone formation was delayed in the PA, PPCH and non-grafted groups characterized by large medullary spaces and connective tissue presence. In the non-grafted sites, loosely organized bone matrix was the major finding observed around the host bone particles (E: Epithelium, B: Bone, CT: Connective Tissue; original magnifications 25x and 100x).

Table 1.**Distribution of Treatments per Sites (n=3 minipigs)**

Group	Number Implants / sockets	Maxilla	Mandible
PPCH + PA	16/20	8/10	8/10
PA	16/20	8/10	8/10
PPCH	8/10	4/5	4/5
No graft	8/6	4/3	4/3
Original PPCH	0/4	0/2	0/2
Total	48/60	24/30	24/30

Table 2. **μ CT Assessments in Trabecular Bone at Treated Areas with Socket Augmentation for All Groups (Mean $\pm$ SD)**

Treatment (n=5/group)	BV/TV (%)	BMD	BMC
Original PPCH	31.5 $\pm$ 8.5	778.5 $\pm$ 43.4	24.8 $\pm$ 18.9
PPCH	27.7 $\pm$ 12.1	773.9 $\pm$ 22.0	28.5 $\pm$ 12.4
PA-LH	24.2 $\pm$ 5.7	761.2 $\pm$ 22.2	34.9 $\pm$ 23.0
PPCH-PA	24.2 $\pm$ 7.1	764.2 $\pm$ 33.5	22.7 $\pm$ 6.8

Table 3. **μ CT Assessments in Cortical Bone at Treated Areas with Socket Augmentation for All Groups (Mean $\pm$ SD)**

Treatment	BV/TV (%)	BMD	BMC
Original PPCH	75.7 $\pm$ 6.3	807.9 $\pm$ 33.3	60.1 $\pm$ 21.0
PPCH-LH	73.2 $\pm$ 2.1	798.7 $\pm$ 28.2	76.7 $\pm$ 14.0
PA	78.5 $\pm$ 3.9	783.0 $\pm$ 47.3	59.5 $\pm$ 20.7
PPCH-PA	76.1 $\pm$ 13.3	778.8 $\pm$ 27.7	72.4 $\pm$ 24.7

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†† Bioplant R&D, LLC, 1771 Post Rd East; Westport, CT 06880-5606

‡‡ Göttingen® Minipigs, Marshall BioResources, 5800 Lake Bluff Road, North Rose, NY

§§ Osseotite® Certain® Microminipiant™, Biomet 3i, Inc. Palm Beach Gardens, FL

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‡‡‡ Biomet 3i™, Implant Innovations, Inc., Palm Beach Gardens, FL

§§§ Periotest, Medizintechnik Gulden, Modautal, Germany

**** Schick Technologies Inc., Long Island City, NY

†††† Scanco Medical, Bassersdorf, Switzerland

‡‡‡‡ EXAKT Technologies, Oklahoma City, OK, USA

§§§§ Statistical Package for the Social Sciences® version 11.0 for Windows (SPSS Inc., Chicago, Chicago, IL USA)

Figure 1

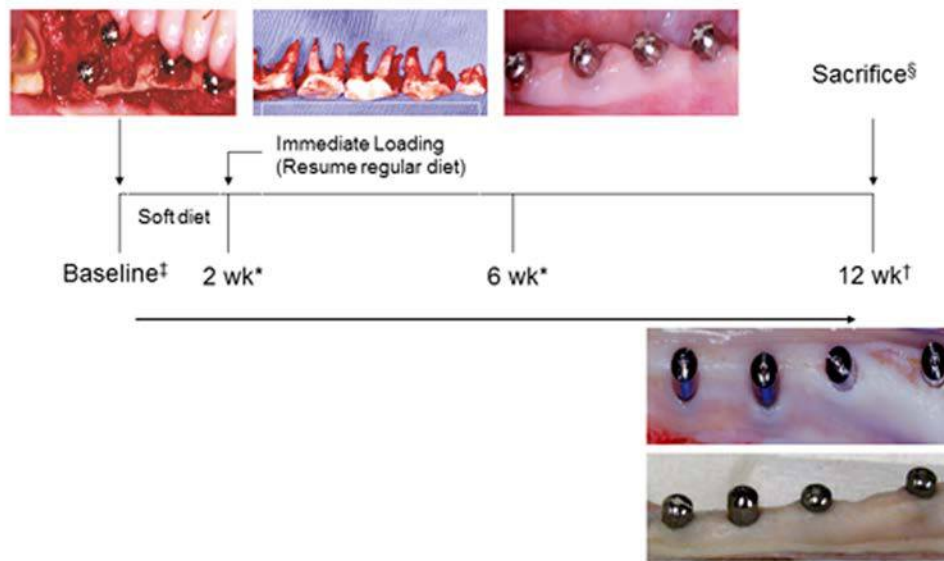


Figure 2A

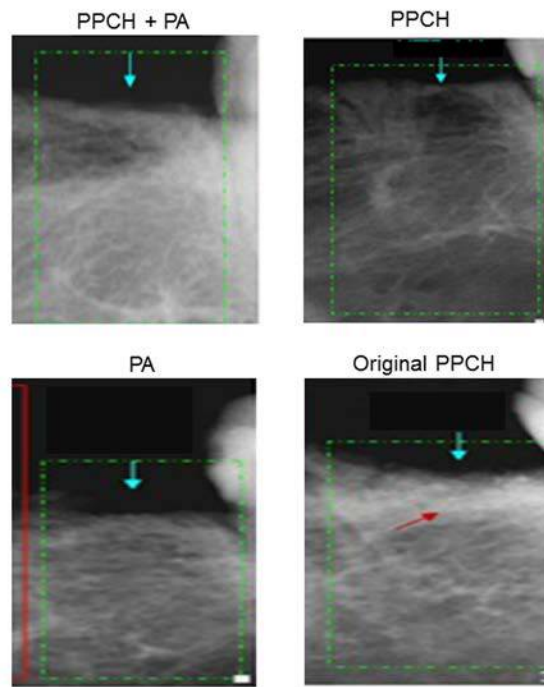
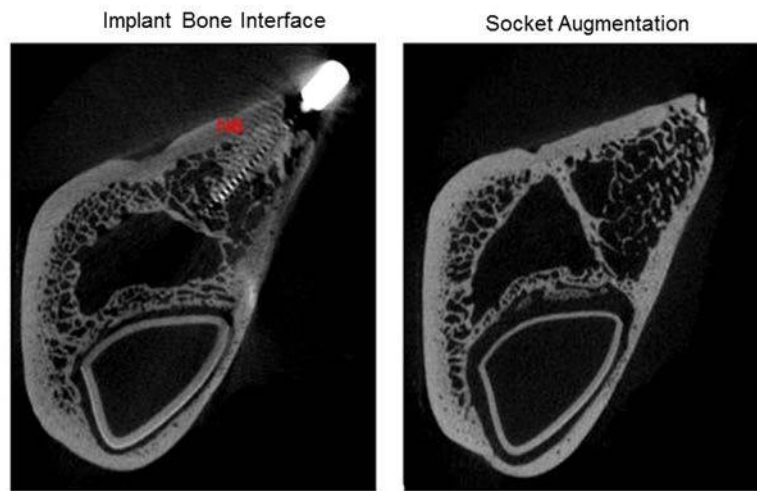


Figure 2B



unedited

Figure 3

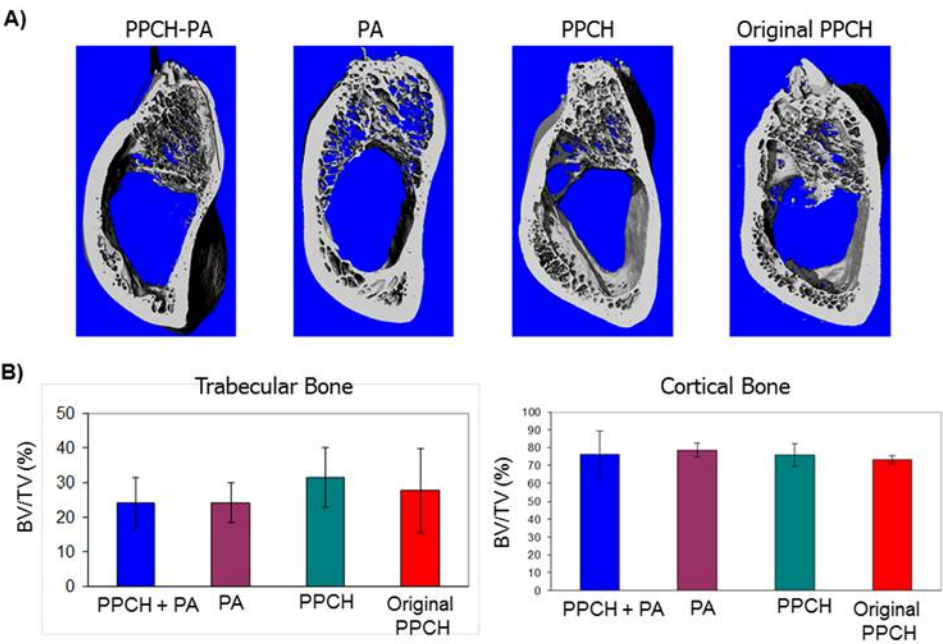


Figure 4

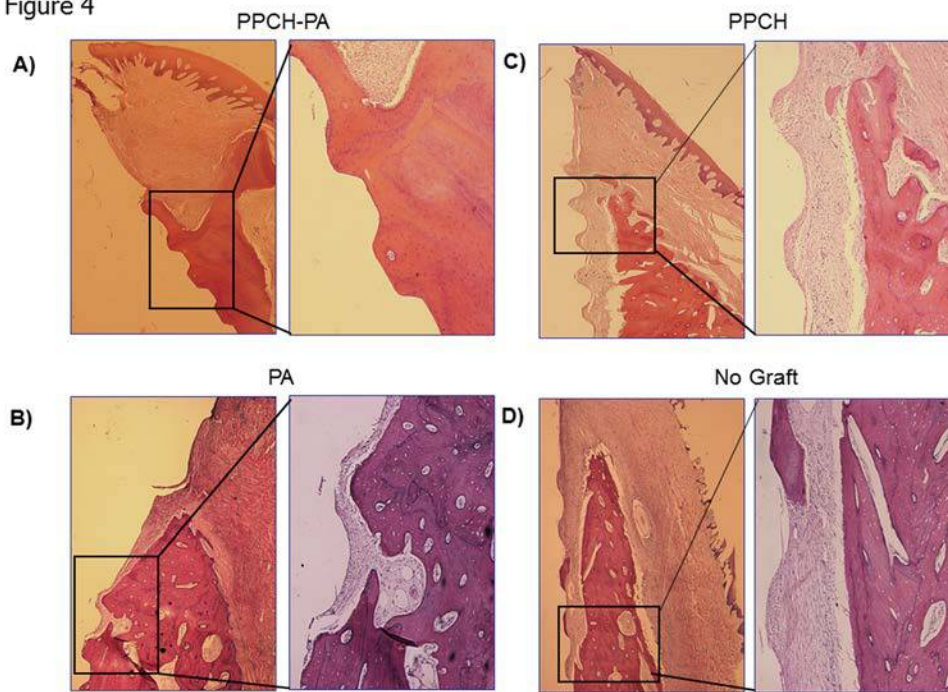


Figure 4

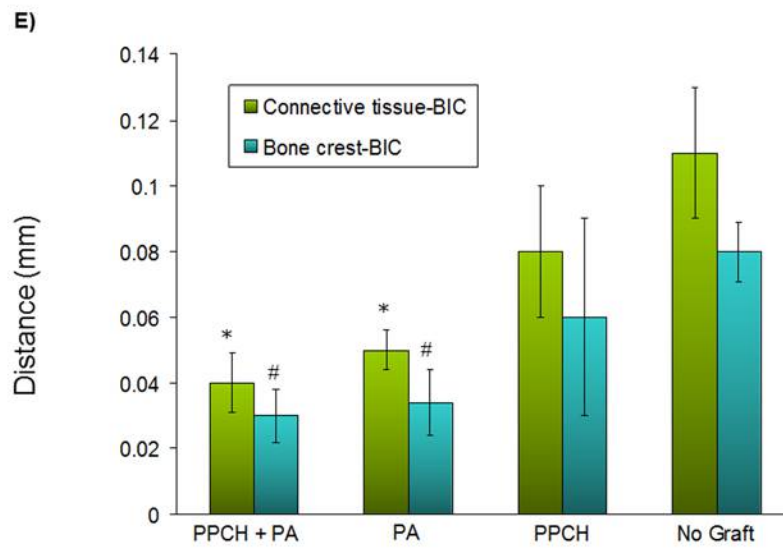


Figure 5

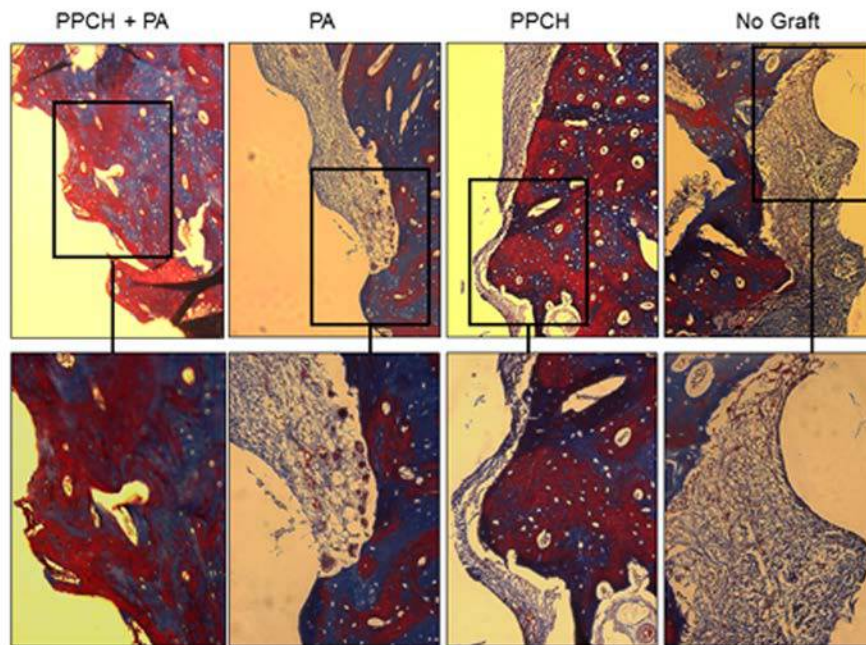


Figure 6

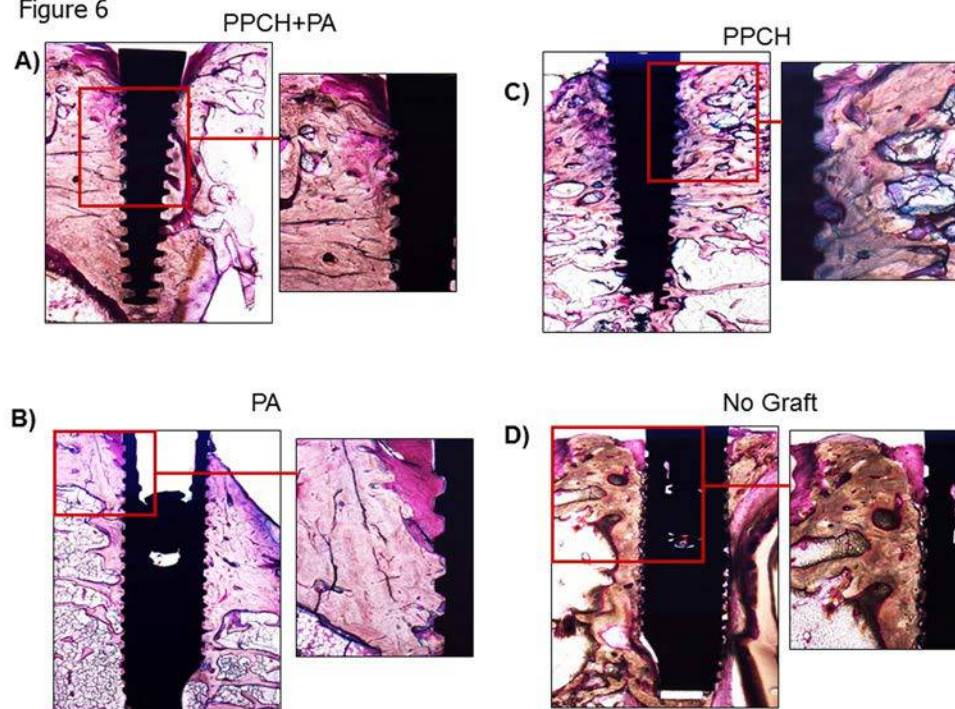


Figure 7

