CALCIUM-BASED NEUTRAL AND BIORESORBABLE SELF-SETTING INJECTABLE BONE PUTTY

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ABSTRACT

With recent developments in minimally invasive techniques for orthopedic surgery, a critical need has evolved for an injectable, bioresorbable bone substitute that can immediately harden isothermally *in situ*. To meet this demand, a buffered viscous bone putty with adjustable hardening time was developed. The implant is composed of a neutral-pH mixture of calcium-based bioceramics including bioresorbable nanocrystalline hydroxyapatite. Numerous *in vitro* studies demonstrate the material to be highly biocompatible. Canine bilateral humerus defect model studies demonstrate excellent remodeling of implanted material with full resorbtion at 12 to 18 weeks. Radiographic observations of healing fractures in clinical evaluation studies have confirmed that implant resorbtion and bone regeneration occur at a clinically acceptable rate.

Key words: Nanocrystalline hydroxyapatite, bone graft, bone substitute, bone void filler, injectable bone putty, synthetic bone, bioresorbable, bone cement

1. INTRODUCTION

The use of synthetic biocompatible, bioresorbable materials is increasing in orthopedic, plastic and dental surgery.[4] Such materials are typically needed to augment autologous bone grafts or to fill bone voids or augment bone loss (e.g. bone loss caused by periodontal disease, bone defect or cavity due to trauma, cancer/disease or surgery, and spinal fusion). The quality of a bone graft substitute is determined by its osteoconductive, osteoinductive and osteogenic properties. The remodeling process for osteoconductive materials is well characterized. Differences amongst osteoconductive

materials leads to differential rates of remodeling with varied levels of secondary inflammation and fibrosis at the remodeling sites. Osteoconductive materials when placed in regions of good vascularity with exposure to medullary elements have abundant access to growth factors and proteins important to bone production and remodeling as well as osteoprogenitor cells.

In orthopedic surgery, autologous bone grafts still represent the gold standard for bone grafting procedures.[3] The grafts contain osteoconductive, osteoinductive and osteogenic elements. Unfortunately, iliac crest grafts are associated with high rates of perioperative morbidity.[1,13] Limited supply is frequently an issue for larger-demand cases, revision cases or when local autograft sites are used.

Allograft bone materials offer osteoconductive elements with varied osteoinductive properties depending on the type of tissue processing. While allograft use is widespread, risk of disease transmission continues to raise concerns.[8] Recent reports of allografts contaminated by bacteria have revived these concerns and driven the demand for synthetic materials. Optimal processing of allografts has yet to be determined.[5] Multiple types of processing destroy the osteoinductive elements within the grafts and potentially weaken mechanical properties.[2]

Hydroxyapatite (HA), calcium phosphates, calcium salts (e.g. calcium carbonate and calcium sulfate) and bioactive glasses are good candidates for human bone hard tissue replacement.[7,14] For comparable crystal size, HA is in general more stable than other calcium phosphates. In native bone, approximately 95% of mineral phases are composed of a specific crystalline HA impregnated with impurities. Many apatitic bone cements have demonstrated excellent biocompatibility properties and usefulness for

clinical applications.[6]

A number of biocompatible bone graft substitutes, bone cements and bone pastes reported in the literature describe compositions that can harden after mixing. In the most common formulation, the powder is a calcium-based material and the liquid is a polymer or sometimes an aqueous solution. Setting and curing times have been reported to range from minutes to hours and sometimes days. For example, tetracalcium phosphate based cements set after 15 to 30 minutes (a fairly long time by surgical room standard) before fully converting to a solid mass of HA *in vivo* within 4 to 6 hours. In some cases, unpredictable resorbtion times may result when the injected putty does not set reproducibly or cures over an extended period of time. Highly alkaline or acidic cements (e.g. calcium sulfate) can potentially be harmful to surrounding soft tissues and lead to fibrous encapsulation.[9,10,12]

Although such products have been used in orthopedic and orthodontal surgery, the first generation of cements does not provide solutions that are easily injectable, neutral-pH, isothermic, quickly and reproducibly self-setting, and fully bioresorbable.

This article reviews the development and properties of an injectable calcium-based neutral-pH bioresorbable bone graft substitute. The first section describes the physical characteristics of the putty. Later sections summarize the *in vitro* and *in vivo* animal studies and the preliminary results of on-going clinical cases.

2. METHODS AND MATERIALS

2.1 Physical Characteristics

The bone putty described in this article has been designed to meet a number of criteria for use as an injectable bone graft substitute. First, it must have adequate hydraulic properties to flow easily during injection. It must have sufficiently high viscosity to be moldable or stay in place during and after injection. The implant must harden *in situ* as quickly as possible even in wet or bloody environments once injected so that the potential for migration is mitigated. The hardening process must be as close to isothermic as possible to prevent thermal damage to surrounding tissues and also to prevent the destruction of osteoinductive elements. The implant must be biocompatible and bioresorbable. The pH of the implant must be as neutral as possible and its composition as close to bone. Finally, the phases and microstructure of the device must be such that resorbtion can occur at a clinically acceptable rate.

To meet all these criteria, a mixture of calcium-based bioceramics was designed. For this bone putty, the regeneration of the hydroxyapatite in the bone tissue is mostly provided by the mixture of calcium phosphate and bioresorbable nanocrystalline HA (shown in Fig.1). The nanocrystals of HA were synthesized using a process described in detail elsewhere.[11]

Once in contact with water, the powder (in its commercial form cleared by the FDA) is workable and injectable for 3 minutes. It then hardens progressively for an additional 2 minutes. The total preparation procedure is complete in 5 minutes. The hardening and setting times are adjustable. The microstructure of the implant is revealed in Fig. 2. During the hardening phase, the temperature increases by less than 10°C and

never exceeds body temperature. The pH of test samples shaped into small beads and placed in porcine liquid blood was measured as a function of time (at 72, 96, 264 and 504 hours). The measurements showed that during the entire time, the pH remained neutral.

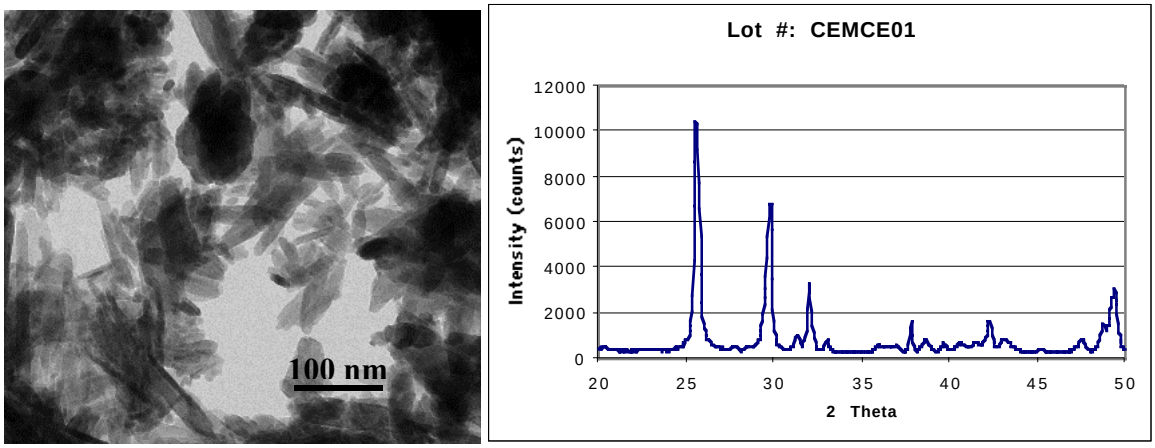

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Figure 1: a) Transmission electron micrograph and b) x-ray spectrum of the bioresorbable nanocrystalline HA. The small size of the crystals makes it easier for bone cells to transfer calcium phosphate through their membrane.

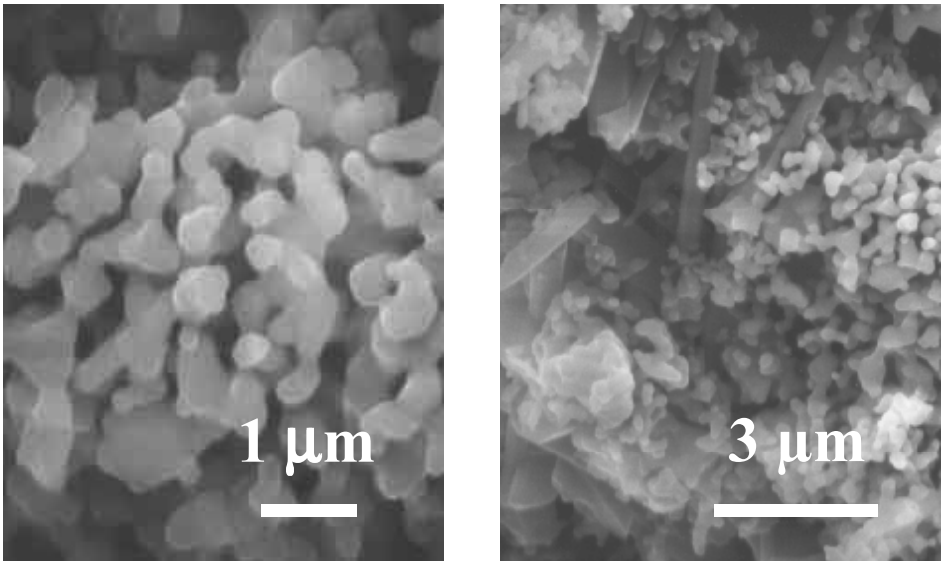


Figure 2: SEM photograph of the fracture surface of a hardened implant showing some of the details of the implant microstructure.

2.2 Implant Preparation

The implants characterized in this study including *in vitro* and *in vivo* testing were prepared using pre-measured volumes of powder and sterile water (see Fig. 3). The kit to prepare these injectable implants with the characteristics described below for a hardening time of about 5 minutes was cleared by the FDA under the trade name Cem-Ostetic. To prepare the putty, the water was poured into the jar containing the powder. The jar was used as a mixing container. Adding 0.4 to 0.6 ml (or cc) of water for each milliliter of powder produces a viscous paste with usable consistency. The putty was either molded into desired shapes and allowed to harden *ex situ* or inserted into a 10-guage syringe from the plunger side (pumping the putty from the needle orifice is more difficult) before being injected immediately *in situ* into a void. The molding or injection process could be completed within 2 minutes after adding water to the powder. Although the viscosity and hardening time of the putty are adjustable, for the chemical formulation reported here, the putty quickly begins to harden after 3 minutes. Once hardening occurs, the implant is no longer workable and must be allowed to solidify before stress can be applied. To eliminate the potential for cracking the implant while the implant begins to solidify, the molding or injection process must be completed within 3 minutes after the water is first in contact with the powder. Repairing or smoothing tiny cracks is possible by applying a few drops of sterile water onto the surface. After 3 minutes (mixing and molding/injection are now complete), the putty must be left to harden completely for an additional 2 minutes. Although the implant continues to get stronger if kept alone for another few minutes, it is strong enough to be used at that time.

Figure 3: Schematic summary of the putty preparation steps showing the containers for the powder and water. After pouring the content of the water vial, it is mixed for 1 minutes to produce a homogeneous paste. For the next two minutes, the putty can be molded and left to harden *ex situ* to make an implant or injected *in situ* to fill a void. The implant is ready for use 5 minutes after first contact between the water and the powder.

The implant can dry *ex situ* or be secured mechanically after injection *in situ*. The injected putty still hardens properly when injected into liquid or bloody environments. As seen in Figure 4, the putty retains its integrity after injection into blood.

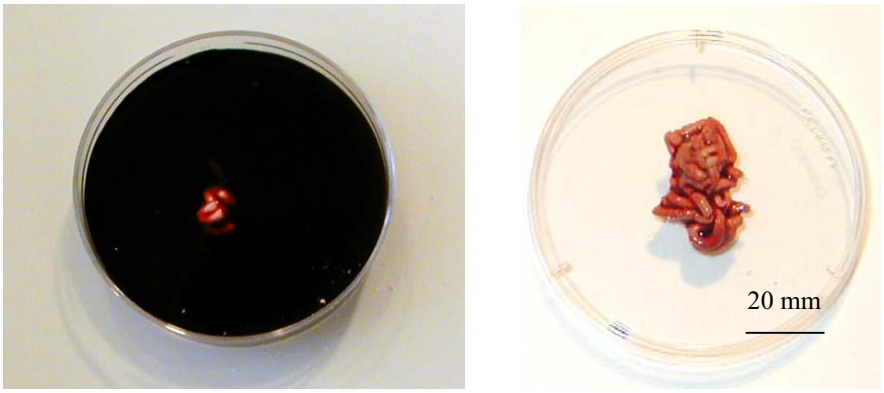


Figure 4: Putty in blood (left) immediately after injection and (right) taken out in one piece after 1 week. The device is still hard after 1 hour, 1 day, and 1 week in blood.

3. BIOCOMPATIBILITY AND SAFETY STUDIES

The biocompatibility and safety of the Cem-Ostetic™ putty were demonstrated before receiving clearance by the FDA in the US and CE marking in Europe. The *in vitro* and *in vitro* tests described in this section demonstrated the biocompatibility, effectiveness, and safety of the putty. The tests include long-term bone implantation, acute and chronic cytotoxicity, sensitization, mutagenicity, genotoxicity, and pyrogenicity. The long-term implantation studies concluded that the putty remodels well and were consistent with anticipated results on an osteoconductive material. Surgically created defects were rapidly repopulated with bone cells and the graft material was replaced by new bone. The putty was found to be nontoxic in prolonged contact with tissue and was determined to be osteoconductive, biocompatible, safe and effective in bone repair. These studies are summarized below.

3.1 *In vitro* Biocompatibility and Safety

In vitro studies for cytotoxicity, sensitization, systemic toxicity, genotoxicity and pyrogenicity were first performed on the putty.

3.1.1 Cytotoxicity

The ISO elution test (MEM extract) was used for this cytotoxicity study. Multiple cultures of L-929 (ATCC cell line CCL 1, NCTC clone 929) mammalian fibroblast cells were prepared. The cell cultures were plated 24 to 48 hours prior to applying extract medium. The test article was considered non-toxic with a grade of 0 at 24 hours and slightly reactive with a grade of 1 at 48 hours. Both negative and reagent controls were considered non-toxic with a grade of 0. The positive control was considered toxic with a grade of 4 (severe reactivity). Thus, the putty met the requirements of USP and ISO and

is not cytotoxic.

3.1.2 Kligman Sensitization

The Kligman (guinea pig) maximization method was used to perform the protocol. The putty was extracted in 0.9% USP sodium chloride and cottonseed oil. Each extract was intradermally injected and occlusively patched to ten test guinea pigs (per extract) in an attempt to induce sensitization. The vehicle was similarly injected and occlusively patched to five control guinea pigs (per vehicle). Following a recovery period, the test and control animals received a challenge patch of the appropriate putty extract and the reagent control. All sites were scored at 24, 48, and 72 hours after patch removal. Under the conditions of this study, the putty extracts showed no evidence of causing delayed dermal contact sensitization in the guinea pigs.

3.1.3 Acute Systemic Toxicity

Twenty Albino Swiss mice were used for the test. The 0.9% USP sodium chloride for injection and cottonseed oil extracts of the putty did not induce a significantly greater biological reaction than the control articles, when tested in 20 Albino Swiss mice. None of the test or control animals exhibited overt signs of toxicity at any of the observation points.

3.1.4 Genotoxicity

The Ames (Salmonella typhimurium reverse mutation) test was performed. The plate incorporation and spot test methods were used for evaluation. The tests were performed both with and without metabolic activation using the S-9 activation system. None of the five tester strains produced two-fold increases in the number of spontaneous revertants. The spot tests showed no zone of increased reversion or of inhibition and the

extract tested against the five strains did not meet the criteria for a potential mutagen.

3.1.5 Pyrogenicity Tests on Rabbits

The 0.9% USP sodium chloride for injection extract of the putty. Four New Zealand White rabbits were used for this test. The temperature of injected rabbits was monitored and was found to increase by less than 0.3°C. The temperature increase of the control rabbit was 0.2°C. The increase in temperature did not exceed the test limit for the maximum individual temperature rise. Based upon the criteria of the protocol, the putty was determined to be non-pyrogenic.

3.1.6 Summary

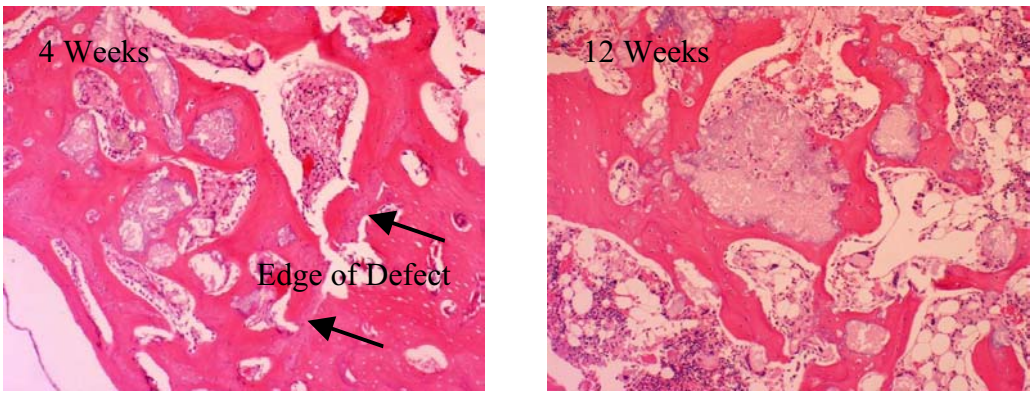
In summary, the putty described in this section is safe and exhibits excellent biocompatibility. No toxic or immune reactions were observed. The tests showed that putty is not a potential mutagen and that it is non-pyrogenic.

3.2. *In vivo* Biocompatibility and Safety Study

The bone putty was evaluated in an 18-weeks lupine-healing model. Three lateral mid-diaphyseal holes 2-mm in diameter were drilled bilaterally in sixteen New Zealand White rabbits. The bone putty was surgically implanted by injection into the two proximal holes in the femurs of four rabbits for each test interval. Control articles were implanted at the same time in the distal two defects. Following recovery, rabbits were monitored daily for general health and weighed prior to implantation and at termination. The femurs were excised and examined macroscopically. The bone tissue surrounding the implants and the soft tissue adjacent to the implant sites were evaluated microscopically for pathology. The results indicated that both the test and control articles were well tolerated following implantation for 4, 8, 12, and 18 weeks. All test implants were already remodeled into

cortical bone after 12 weeks. The defect sites appear the same as the surrounding bone radiographically. The observations revealed no evidence of local irritation from the test article following surgical implantation of the putty into the bone tissue of the rabbit. The microscopic evaluation of the bone tissue and the soft tissue adjacent to the implant sites indicated that both test and control articles were well tolerated following implantation for all study intervals. There was no macroscopic or microscopic evidence of a local adverse or toxic response to the insoluble or leachable components from the putty.

Results demonstrated that the bone putty did not interfere with bone regeneration in this animal model. Histologically, there was no evidence of osteolysis or excessive hyperostosis in any of the test implant sites at any time point. Histologically, bony ingrowth was observed from the margins of defects as expected for an osteoconductive material (see Fig. 5). No fibrotic response was noted.



a) b)

Figure 5: Histology after 4 and 12 weeks showing the resorbtion of the putty and the formation of new bone in the implanted site.

All cortical defects demonstrated bridging new callous formation. The amount of new bone within the cortical defect greatly increased as a function of time. Table 1 is a

summary of the results on hyperostosis in the defects and on the osteoconductivity grade as a function of implantation interval. The amount of hyperostosis and the osteoconductivity grade was determined using analyses performed on eight surgical defects. The osteoconductivity grade was measured both in the cortical defect and in the medullary cavity.

Bone growth and implant resorbtion occurred very quickly during the first 8 weeks. After 4 weeks, 53% (on average) bone growth was measured where the defect was created. Both bone remodeling and bone growth-rate began to decrease after 8 weeks since more than 80% of the bone was already reconstructed. The amount of remaining implant in the cortical defect observed at 18 weeks was negligible (i.e., less than 15%). Remaining material should eventually be completely removed during osteonal remodeling. Occasionally, defect sites in the cortical defect were completely healed and filled with trabecular bone after the 12 or 18-weeks intervals. After 12 weeks, the new bone within the cortical defect had numerous randomly arranged osteons and was moderately thick. At the edges of defects, this new bone merged with the surrounding cortical bone. It was remodeled to the point that the edge of the defect was difficult to identify and it was extremely difficult to differentiate it from surrounding healthy bone tissue. The new bone typically invaded and surrounded all remaining implant material in the defect. The interface between new bone and bone substitute was devoid of cells as normally observed for an osteoconductive material. There was no evidence of osteolysis in any of the cortical defects filled with the test article.

Table 1: Amount of hyperostosis in defects and osteoconductivity grade for the putty (Cem-Ostetic™ lot # 00-CEM-90-1215, sterilization # 10021).

Test Intervals	**Hyperostosis (Average)**	**Osteoconductivity grade* (cortical defect)**
4 weeks	53%	4
8 weeks	84%	6
12 weeks	87%	6 or completely resorbed
18 weeks	89%	6 or completely resorbed

- "0" no evidence of bone growth,
- "1" thin discontinuous rim of new trabecular bone surrounding bone graft,
- "2" thin rim of new bone completely surrounding bone graft,
- "3" moderately thick discontinuous rim of new trabecular bone surrounding bone graft,
- "4" moderately thick rim of new bone completely surrounding bone graft,
- "5" thick discontinuous rim of new bone surrounding bone graft, and
- "6" thick rim of new trabecular bone surrounding bone graft.

The test sites containing the putty were radio-opaque. The implant as or slightly less dense than surrounding cortical bone. The visual appearance in radiographs of bone in implanted sites was the same as that of bone in adjacent areas of cortical bone for the 8, 12, and 18 weeks groups suggesting that the implant was resorbed and remodeled into cortical bone. These observations were obviously confirmed by post-mortem histology analysis of the implanted sites.

Both test and control articles were therefore very well tolerated during this study. Although well tolerated, implanting excess implant material should be avoided. The implant should be placed and constrained as closely as possible to the area to be filled. There was no microscopic evidence of adverse or toxic effects (including sections of periosteum, endosteum, cortical bone, bone marrow stroma, hematopoietic cells, and bone marrow stromal elements) in the bone tissue. There was also no evidence of

increased osteolytic activity in any of the bone sections examined. The cortical defects were typically filled by highly remodeled bone merging with the edges of the defect.

In conclusion, no evidence (macroscopic or microscopic) of local adverse or toxic response to the insoluble or leachable components of the putty was found. The implantation study in rabbits therefore determined that the bone putty was highly biocompatible and very safe.

4. BONE REMODELLING STUDY

4.1 Experimental Method

A bilateral canine humeral defect-healing model was used to assess the effectiveness of the putty. This study was designed to evaluate the effectiveness of the putty and that of a standard mixture of sintered HA (60 wt.%) and tri-calcium phosphate (40 wt.%) cleared by the FDA under the trade name of Bi-Ostetic.

Three cylindrical defects, measuring 6-mm by 12-mm, were surgically created in each proximal humerus of four bred mongrel/hound mix canines. The defects in each humerus were injected with the putty or filled with 1-mm porous granules of the sintered HA-TCP mixture. Bone wax was used to stop bleeding in the controls. The treatment location and order on each humerus were randomized prior to surgery.

Radiographs of all surgical sites were taken at 6, 12, 15 and 18 weeks. To better quantify the radiographic observations, animals were sacrificed after 13 weeks and 19 weeks. Each humerus was excised. The majority of soft tissue and the distal portion of the humerus were removed to allow penetration of formalin into the medullary cavity. The remaining proximal portion of each humerus was placed into 10% NBF and gently

shaken until adequately fixed. The samples were cut into 10-mm cross-sections using a diamond edged blade attached to a band saw in such a manner that each test or control article implant site was located at the cut edge or 1 to 2 mm from the cut edge. The sections of bone were decalcified using a commercially available solution until a cross-section of the bone distal to the implant site could be easily cut with a razor blade. All decalcified sections were infiltrated and embedded in paraffin, sectioned and stained with hematoxylin and eosin.

4.2 Radiographic Results

Defects filled with the putty healed faster than the sintered HA-TCP mixture and controls. By 19 weeks defects filled with putty were barely visible radiographically, regardless of their orientation (metaphysial vs. diaphysial) in the bone. As seen in Fig. 6, the HA-TCP mixture resorbed more slowly that the putty, regardless of defect location.

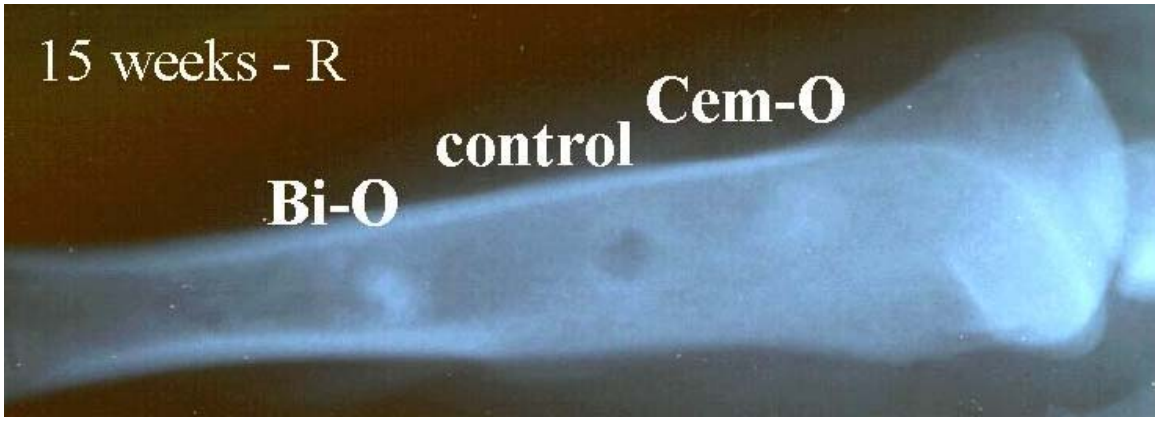


Figure 6: Radiograph at 15 weeks of the left humerus showing the three sites filled with porous granules of the sintered HA-TCP mixture (Bi-O on the left), the putty (Cem-O on the right) and the control in the center. The HA-TCP mixture resorbed more slowly with the material remaining clearly visible at all time regardless of defect location.

4.3 Histology Results

The histological analysis of the sections implanted with putty showed that amounts of bony trabeculae within the cortical and cancellous portions of the implant sites were similar to amounts of bone observed in the surrounding cancellous bone at both 13 and 19 weeks intervals. Control defects failed to fill with new bone at any time point. All results were consistent with observations in the lupine studies.

5 CLINICAL EVALUATION

The putty was first used as bone void filler in an array of clinical cases performed in several hospitals in northern California. As of this publication, follow-up has varied and clinical data continues to be collected. Initial clinical cases have targeted complex adult reconstruction of femoral and acetabular defects, metaphyseal fractures, iliac bone graft defects, and augmentation of spinal fixation. All cases have demonstrated appropriate radiographic remodeling of the grafted sites. The duration of radiographic remodeling has varied with anatomic location and patient demographics. The following shows some preliminary results of ongoing clinical cases to illustrate the performance of the putty.

5.1 Tibial plateau fracture

A split depression type tibial plateau fracture was treated in a 32-year old female. The putty was molded and placed in the metaphyseal defect augmenting the cannulated screw stabilization of the fracture. Radiographic evidence of extensive bone graft remodeling was evident at 6 and 8 weeks.

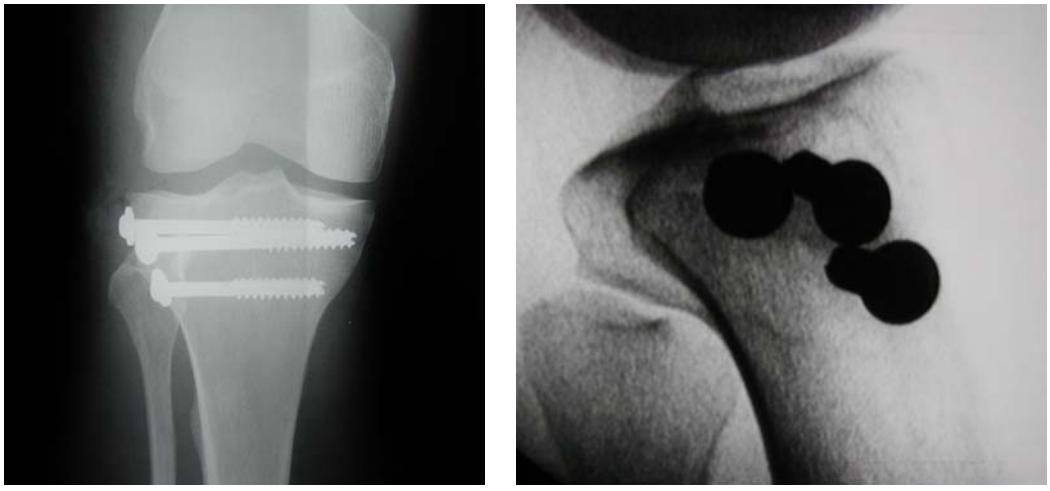


Figure 7: AP and lateral views of acute fixation of tibial plateau fracture treated with bone putty. Extensive bone graft remodeling was evident at 7 weeks.

5.2 Distal Radius fracture:

A closed complex distal radius fracture in a 70-year old female was treated with external fixation frame and percutaneous pin fixation. The putty was placed openly in the metaphyseal defect with a small incision. Complete remodeling of bone graft material was seen by 6 weeks, concurrent with clinical evidence of fracture healing.

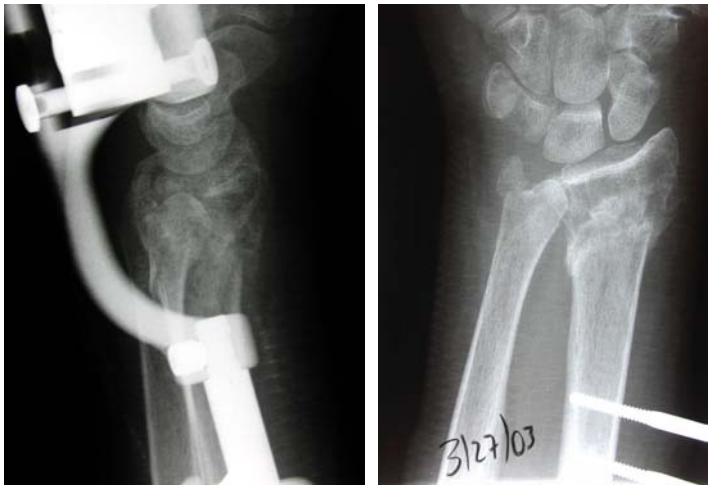


Figure 8: Distal radius fracture augmented with bone putty at the fracture site. The fracture healed uneventfully without collapse.

5.3 Acetabular Reconstruction:

A 42-year old female with a failed loose infected bi-lobed acetabular cup and loose femoral component underwent complex reconstruction of both components. At second stage of the revision with a cage construct, the acetabular bony defect was filled with allograft cancellous chips and augmented with Cem-Ostetic putty. X-rays at 6 weeks are consistent with incorporation of the graft material.

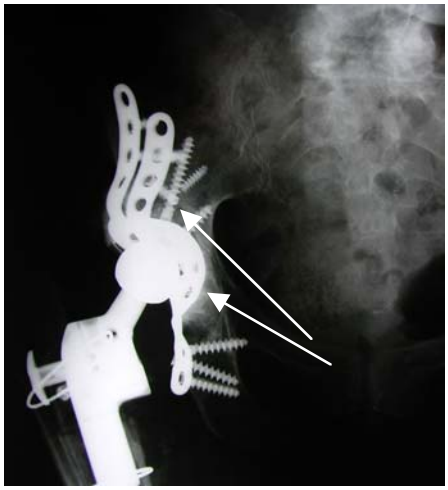


Figure 9: Bone putty augmentation of a reconstructive acetabular cage.

6. CONCLUSIONS

An injectable bioresorbable bone putty with adjustable hardening time is described. The performance characteristics of the putty meet the requirements for numerous bone augmentation procedures. The putty is self-setting, nearly isothermic, and can harden *in situ* even in wet or bloody environments. The components of the implant are designed to produce a neutral-pH putty that resembles bone hard tissue and do not contain polymers or plasticizers. Numerous *in vitro* studies demonstrate that the putty is highly biocompatible and non-pyrogenic.

Canine bilateral humerus defect model studies demonstrate excellent remodeling of implanted material with full resorbtion at 12 to 18 weeks. Radiographic observations of healing fractures in clinical cases confirm that implant resorbtion and bone regeneration occur at a clinically acceptable rate.

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