Safety and efficacy of Chitra-CPC calcium phosphate cement as bone substitute

A. C. Fernandez*, Mira Mohanty, H. K. Varma and Manoj Komath

Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram 695 012, India

Calcium phosphate cements (CPCs) have gained importance in orthopaedics and dentistry as repair materials for bony/dentinal defects. They are aqueous-based, mouldable and osteoconductive materials which set into hydroxyapatite, the basic mineral of bone and teeth. A CPC product 'Chitra-CPC' has been developed. This communication compiles the safety and efficacy evaluation of Chitra-CPC. The evaluation plan consisted of acute systemic toxicity test (in mice for systemic response), intracutaneous reactivity test (in rabbits for skin response), pyrogen test (in rabbits for presence of pyrogens) and maximization sensitization test (in guinea pigs for allergic skin response). Soft tissue response was tested by implantation in rabbit paravertebral muscle, with histological evaluation at 1, 4 and 12 weeks post-implantation. The efficacy of the product to heal bone defects was investigated by implanting in rabbit femur with hydroxyapatite ceramic granules as the control. Local effects at macroscopic and microscopic levels were assessed at time periods of 4, 12, 26 and 52 weeks post implantation.

The cement did not show any adverse effects in the acute systemic toxicity. Nor did it elicit any erythemic or edematous reactivity in the intracutaneous reactivity test. The maximization sensitization study did not show any adverse skin response and the pyrogen test did not evoke undue temperature rise. In the muscle implantation test, there was no haemorrhage, infection or necrosis. Localized vascularization was present near the implanted region. Chronic inflammation was observed in 1 week, which became mild by 12 weeks with the evidence of repair. Bone implantation studies showed that efficacy of Chitra-CPC and hydroxyapatite granules in bone healing is comparable. Both materials were found to be osteoconductive, but with the difference that Chitra-CPC resorbed progressively allowing simultaneous new bone formation. This proves the osteotransductivity of Chitra-CPC, which is the ideal property for a bone substitute.

Keywords: Biocompatibility, bone substitute, bone implantation, calcium phosphate cements, osteoconductivity.

CALCIUM phosphate cements (CPCs) are new generation bone substitutes, with potential clinical applications in orthopaedics and dentistry. These are aqueous-based two-component cements, having a powder part containing calcium and phosphorus ingredients and a liquid part consisting of a solution of phosphates. The mixing of the components leads to the formation of a putty or paste, which undergoes isothermal setting retaining the shape with sufficient mechanical strength. The attractive feature of CPCs is that the set mass is hydroxyapatite, the basic inorganic component of bone and teeth. Synthetically prepared hydroxyapatite, which has a generic chemical formula $Ca_{10}(PO_4)_6(OH)_2$, is already in use as a bone substitute in the form of ceramic granules and blocks. Hydroxyapatite ceramics are biocompatible (accepted by the body without any adverse reaction) and osteoconductive (gets integrated with host bone). The mouldability of CPCs gives them an edge over hydroxyapatite ceramics in skeletal repair. The material has excellent biocompatibility and is reported to have the property of osteotransductivity (i.e. active resorption at bony sites, facilitating bone remodelling)[1,2].

Intensive research activities occurred during the past two decades to develop CPC formulations for various clinical applications[1–3]. Significant success has been reported for CPCs of putty consistency in bone grafting, especially in the reconstruction of non-load bearing areas[3,4].

In an indigenous venture, scientists at the Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Thiruvananthapuram, had developed a novel formulation of CPC[5]. This patented (Indian IPR) formulation, named 'Chitra-CPC', has enhanced viscous and cohesive properties than conventional CPC. Chitra-CPC could be mixed in varying consistencies, from mouldable putty to injectable paste. This flexibility provides immense advantage in clinical application as a bone and dentine substitute[5,6].

In putty form, Chitra-CPC has excellent workability enabling the shaping according to requirements so as to fill in intricate cavities, which is difficult to achieve with ceramic granules or blocks. The paste form of Chitra-CPC is fully injectable through a narrow cannula or a delivery needle of 18/19 gauge size[6]. Easy delivery could be achieved even to the sites of limited accessibility. The full injectability opens up the possibility of minimally invasive fracture management of cancellous bone.

Chitra CPC has been subjected to extensive materials characterization which proved it to be a clinically viable material[5,6]. This communication deals with safety and efficacy evaluation of Chitra CPC, to validate it for human clinical use.

The essential details about the components and ingredients of Chitra-CPC have been described elsewhere[5]. The cement consists of powder and liquid parts, which are to be mixed in appropriate wetting ratio to obtain a self-setting putty/paste. The powder part is composed of an equimolar mix of 100 μm particles of tetracalcium phosphate (TTCP–$Ca_4(PO_4)_2O$) and dicalcium phosphate dihydrate (DCPD–$CaHPO_4.2H_2O$) added with an optimum quantity of gelling agent in dry powder form. The liquid part is distilled

*For correspondence. (e-mail: acfernandez04@yahoo.com)

water containing disodium hydrogen phosphate (Na_2HPO_4, in 0.2M concentration) as the setting accelerator. The optimum wetting ratio is 0.8 ml of liquid per gram powder. The cement was prepared and allowed to set for various tests.

Evaluation of the safety and efficacy of a biomedical product is essential not only for its scientific validation, but also to satisfy the requirement for regulatory approvals. The rationale is to ensure that the product meets the intended clinical needs and does not invoke any adverse or toxic response when used in patients. Appropriate test plans are to be made for each product according to national and international standards and guidelines[7–9].

Chitra-CPC falls under 'resorbable calcium salt bone void filler device' according to the US FDA classification[9]. The selection of tests has been done following the related guidelines. Chitra-CPC has already passed the biological screening tests of cytotoxicity and haemolysis according to the requirement of the standard, thereby qualifying for the *in vivo* toxicological tests[5]. The plan of toxicological tests of Chitra-CPC has been prepared on the basis of ISO 10993 – 'Biological evaluation of medical devices'[8]. The toxicological tests selected were: acute systemic toxicity, intracutaneous (intradermal) reactivity test, pyrogen test, maximization sensitization test and muscle implantation. (Supplementary toxicological tests like carcinogenicity, genotoxicity, reproductive/developmental toxicity and biodegradation are excluded because the end-product is hydroxyapatite, the safety of which had already been established[10]). Long-term implantation in animal bone according to ISO 10993 Part 6 ('Tests for local effects after implantation') was selected to assess the efficacy of Chitra-CPC as a bone substitute.

The evaluation plan has been discussed in the Biological Evaluation Committee of the Biomedical Technology Wing, SCTIMST, and was duly approved. Permission to use animals was obtained from the Institutional Animals Ethics Committee, SCTIMST. Inbred strains of animals were used in all the experiments and the animal husbandry was maintained in accordance with ISO 10993-2 ('Animal welfare requirements'). In the case of mice, polypropylene cages having steel top and paddy husk bedding were provided for housing, with five numbers per cage. Rabbits and guinea pigs were housed individually in anodized aluminium/stainless steel cages. Commercially available standard feed and potable water were given *ad libitum*. All the animal experiments were done in an animal surgical facility at aseptic conditions.

The acute systemic toxicity test assesses the systemic response of the extracts (in normal saline and cotton seed oil) in mice.

The extracts were prepared by incubating 4 g of the set cement (in pellet form) with normal saline and cottonseed oil, for 1 h at 121°C. Aliquots from each extract were taken and pH values checked. The extraction medium alone was used as control in each case.

Healthy and active Swiss Albino mice of either sex, in the weight range 17–23 g, were randomly selected for the experiment. Ten mice each (5 males and 5 females) were used for test as well as control. The saline and cotton seed oil test extracts and their controls were administered intravenously and intraperitoneally, at a dose of 50 ml/kg body wt. Observations were made immediately after injection and at 24, 48 and 72 h.

The intracutaneous (intradermal) reactivity test evaluates the local responses in rabbits to the extracts of the material (in normal saline and cotton seed oil) following intracutaneous injection.

The extraction process was the same as that in the previous test. Healthy, smooth-skinned, adult New Zealand white rabbits (three animals of about 2 kg weight) were selected and prepared by closely clipping the fur on the back, taking care that the skin is free of mechanical trauma or signs of irritation. Eight sites were selected on each side for the aseptic subcutaneous injection of the extracts at a dose of 0.2 ml per site. The test extracts were injected at the five upper sites (normal saline extract on the left and cotton seed oil extract on the right) and their controls at the three lower sites.

Observations were made for erythema and oedema, immediately after injection and at 24, 48 and 72 h, and graded. The primary irritation scores were obtained by subtracting the average scores of the controls from that of the test (separately for erythema and oedema). The primary irritation index for each test extract was found by taking the average scores for all the animals. For comparison, the mean scores were converted into response categories as: 0 to 0.4 – negligible, 0.5 to 1.9 – slight, 2 to 4.9 – moderate, and 5 to 8 – severe.

The pyrogen test is to check the presence of any pyrogenic substances of both endotoxin and non-endotoxin origin, by measuring the rise in body temperature of rabbits following intravenous injection of the extract.

The test extract was prepared by exposing a surface area of 480 cm^2 of the cement (by casting in sheet form) to a quantity of 160 ml of sodium chloride injection (containing 0.9% NaCl). Extraction was done for 1 h at 37°C and the medium was then filtered with sterilized Whatman filter paper, into a pyrogen-free beaker.

The animals used were healthy, adult New Zealand white rabbits with an average weight of 2 kg, acclimatized for 7 days at a controlled temperature of 22 ± 3°C. Arrangements were made to measure the rectal temperatures of the animals in a rabbit restrainer. Three rabbits whose body temperatures did not exceed 39.8°C with daily variations not more than 1°C, were selected for the experiment. Rectal temperatures recorded 30 min prior to the test were taken as reference value.

In the experiment, the extract was injected intravenously into the marginal ear vein of each rabbit at a dose of 10 ml per kg body wt and rectal temperatures were recorded at 30 min interval, starting from the instant of in-

jection. If the body temperature raises ≥ 0.5°C in any animal, presence of pyrogenic substance in the sample is confirmed.

The maximization sensitization test is used to determine the potential of the material to produce skin sensitization in guinea pig using extracts.

The material extract was made by treating 2 g cement pellet with 10 ml of physiological saline, for 24 h at 70°C, followed by filtering. Physiological saline alone was used as control. Mixtures of these in equal volumes with Freund's complete adjuvant were also prepared for the experiment.

Healthy, adult Hartley strain guinea pigs (of either sex, in the weight range 300–500 g) were used for the experiment, with ten animals in the test group and five in the control group. For the experiment, the dorsal intra-scapular region (for intradermal or topical application) and flank region (for challenge dose) of each animal were clipped and prepared aseptically. The course of experiment was distributed as intradermal induction phase, topical induction phase and challenge phase.

In the intradermal induction phase, the test solutions (extract in physiological saline and its mixture with Freund's complete adjuvant) were intradermally injected to the clipped intrascapular regions to the test group, at a dose of 0.1 ml. The same doses of the control solutions (and its mixture with Freund's complete adjuvant) were given to the control animals. The topical induction phase was conducted after seven days in the injected animals. The test and control extracts were topically applied to the respective groups, onto the intrascapular region of each guinea pig (after pre-treating with 10% sodium lauryl sulphate) using a 2×4 cm filter paper patch. Occlusive dressings were given to cover the patches and both were removed after 48 h. The challenge phase was conducted 14 days after the topical application, in which the test material extract was applied in both the test and control animals using filter paper patches and covered with an occlusive dressing. The dressings were removed after 24 h for observation.

The appearance of the challenge skin sites of test and control animals was observed at 24, 48 and 72 h after removal of dressings and patches. The skin reactions were scored as follows – for erythema: no signs – 0, slight erythema – 1, well defined erythema – 2, moderate erythema – 3 and severe erythema to slight eschar formation – 4; and for oedema, no signes – 0, slight oedema – 1, well-defined oedema – 2, moderate oedema (1 mm) – 3 and severe oedema (> 1 mm) – 4.

Muscle implantation investigates the biological response of muscle tissues (of rabbits) to the material for different time periods up to 12 weeks, according to ISO 10993.

The implantation was done in healthy, adult New Zealand white rabbits (of either sex with body weight about 2.5 kg) having well-developed paravertebral muscles. Three animals were used per period with three periods (1, 4 and 12 weeks post implantation) of observation. The test samples were prepared by filling the cement into sterile Teflon tubes (2 mm dia and 5 mm length) and bare Teflon tubes were used as controls. Four test samples and three controls were implanted per animal in the paravertebral muscles.

Each animal was anaesthetized and fur on either side of the spine was clipped followed by swabbing with 70% alcohol. Incisions were made into the paravertebral muscle (four sites 25 mm apart) to insert the test material intramuscularly along one side of the spine. The control materials (three sites) were implanted intramuscularly in the muscle on the contra lateral side. The skin incisions were then closed using sterile sutures.

At the end of each implantation period, the animals were euthanized with an overdose of thiopentone sodium, and the implanted materials were excised along with the surrounding tissues. The explants were macroscopically examined for haemorrhage, necrosis and infection, and then fixed in 10% buffered formalin for histopathological evaluation.

The excised tissue blocks were dehydrated initially in ethyl alcohol in ascending order of concentration and subsequently subjected to clearing in 100% chloroform. They were then impregnated in molten paraffin twice and embedded in wax. Next, 5 μm thick sections were cut using a Rotary Microtom (Leica) and stained with Haematoxylin and Eosin after de-paraffinization. Observations were made using a Leica Diaplan stereo microscope.

The sections were evaluated for biological response. Parameters studied included fibrosis/fibrous capsule formation, inflammation, degeneration, necrosis, cellular response (of polymorphonuclear leukocytes, lymphocytes, plasma cells, eosinophils, macrophages and multinucleated cells), presence of material debris, fatty infiltration, granuloma formation and tissue in-growth. The histological grading system adopted to analyse the biological response was as follows: Severity of degeneration (determined by the presence of nuclear debris and capillary wall breaking), 0 – not present, 0.5 – minimally present, 1 – mild, 2 – moderate and 3 – severe; Severity of necrosis (determined by the presence of nuclear debris and capillary wall breaking), 0 – not present, 0.5 – minimally present, 1 – mild, 2 – moderate, 3 – severe; Cellular response (based on the number and distribution of cells average of five fields at magnification 400X), 0 – 0 cells, 0.5 – 1 to 5 cells, 1 – 6 to 15 cells, 2 – 16 to 25 cells, 3 – >26 cells; and other parameters (like the presence of material debris, fatty infiltration, granuloma and tissue in growth), 0 – not present, 0.5 – minimally present, 1 – mild, 2 – moderate, 3 – severe.

Chitra-CPC is a bone substitute material intended for the healing of defects of the hard tissue. The efficacy of the product to heal bone defects had been investigated following the guidelines[8] in the standard ISO 10993. The method is to fill the material in critical size defects in

rabbit femur and histologically assess the local effects at both macroscopic and microscopic levels. This is a long-term implantation test, in which the healing pattern is evaluated at time periods of 4, 12, 26 and 52 weeks post implantation. For comparison of efficacy, commercially available hydroxyapatite ceramic granules ('Periobone-G') were used as the control material.

For long-term bone implantation, healthy, adult New Zealand white rabbits (of either sex in the weight range 2.0–2.5 kg) were selected, with four animals per period. All the surgical procedures were carried out in the animal procedure room by a toxicologist with a veterinary surgeon for the anesthesia. Pre-medication (0.25 mg Atropine and 1–5 mg Diazepam per kg body wt, as intra-muscular injections) was given to the animals, followed by anesthesia (5 mg Xylazine and 80 mg Ketamine per kg body wt, as intra-muscular injections) after 20 min. Both the sides of the hind legs were shaved and wiped with antiseptic solution. After placing the animal on lateral recumbency, the skin along the craniolateral border of the shaft of the femur bone was incised to open the subcutaneous fat and superficial fascia. The skin margins were undermined and retracted and the *fascia lata* was incised along the cranial border of the biceps and *vastus lateralis* muscle to reveal the shaft of the femur.

Three defects of 2 mm diameter were created 1 cm apart on the bone using a high-speed surgical micromotor drilling machine with burrs of appropriate size. The site was irrigated with saline and debris cleared-off using suction. Drilling depth was carefully adjusted to avoid entry of the burr into the bone marrow. The materials were transferred into the defect by an applicator. The test material (Chitra-CPC) was applied on the right femur and the control (Periobone-G granules in the size range 150–250 µm, mixed with saline) on the left. Utmost care was taken not to contaminate the surrounding areas by material remnants.

After completion of implantation, the retracted muscles were released and replaced. The cut subcutaneous fat and superficial fascia were sutured in the first tier and the skin incision closed by a second suture. Post-operatively the animals were kept under antibiotic cover for the first 3 days.

On completion of the experimental period, the animals were euthanized with an overdose of anesthetic. Femur was removed by autopsy and preserved in 10% buffered formalin. The implant sites were identified grossly after clearing the bone surface. The bone was cut perpendicular to the long axis using a high-precision saw into pieces, each including one implant site.

The excised blocks were dehydrated in ethyl alcohol in ascending order of concentration from 80 to 100%, the last step being repeated twice. The blocks were then embedded in PMMA resin. Multiple 100 µm thick sections were cut using a high-precision saw and stained with Stevenel's blue. The observations were done using a Leica Diaplan stereo microscope.

The biological response was evaluated as in the case of muscle implantation, with the same grading system. In addition, the status of the implanted material and new bone formation were examined in the sections. Microscopic images were recorded digitally using Leica DFC 320 camera and Leica QWin software (version v3.2.1).

In the acute systemic toxicity test, during the observation period, all the animals (both in test and control groups) were found normal. There were no adverse effects or loss in body weight or mortality in any of the animals. An increase in body weight (less than 10%) has been observed after 72 h.

No erythema or oedema was elicited by the saline extract of the test material in the intracutaneous (intradermal) reactivity test. When the cotton seed oil extract was applied, one animal showed erythema and oedema with a score of 0.5. This corresponds to a primary irritation index of 0.166, which is negligible.

The pyrogen test was uneventful; none of the animals showed any abnormality during the experimental period. The body temperatures were measured up to 180 min, at 30 min intervals. Changes in temperature recorded varied to –0.1°C in two animals and +0.2°C in one. These are less than the allowed limit (i.e. increase of +0.5°C after injection of the extract) and hence the material is concluded to be non-pyrogenic.

In the maximization sensitization test, the animals (both in test and control groups) did not show any abnormalities during the experimental period. The numerical grading for erythema and oedema was zero.

During the period of muscle implantation, the general condition of all the test animals was normal throughout the experimental period. Twelve test and 8 control materials were retrieved at the end of the one-week period, 11 test and 8 control materials at the end of 4 weeks and 11 test and 9 control materials at the end of 12 weeks. Localized vascularization observed around the test and control materials at 1 week post implantation was similar and comparable. Slight vascularisation was observed around the test samples at 4 and 12 weeks. There was no encapsulation, haemorrhage, infection or necrosis around the implanted material at any time period.

Results of the observations on histological sections are compiled in Table 1, taking one representative implant site for each period. The biological response of the tissue to the test material at 12 weeks post implantation was similar to that of the control material.

Sections of explants of the long-term bone implantation experiment were initially subjected to analysis of biological response. Biological response indicated that the material is safe for bone throughout the evaluation periods (4, 12, 26 and 52 weeks post implantation). No fibrosis/fibrous capsule formation or inflammation was seen in any of the samples. All the parameters of severity of degeneration, severity of necrosis and cellular response (leukocytes, lymphocytes, plasma cells, eosinophils, macro-

Table 1. Biological response in muscle implantation

	1 Week		4 Weeks		12 Weeks	
Period	T	C	T	C	T	C
Biological response						
Extent of fibrosis/fibrous capsule (μm)	0	0	16	14	13.5	3.5
Extent of inflammation (μm)	698	244	248	88	60	64
Severity of degeneration	3	2	1	0.5	0.5	0
Severity of necrosis	3	2	0.5	1	0.5	0.5
Cellular response						
Leukocytes	0	0	0	0	0	0
Lymphocytes	0.5	1	0.5	0	0	0
Plasma cells	0	0	0	0	0	0
Eosinophils	0	0	0	0	0	0
Macrophages	3	3	3	1	1	0.5
Multinucleated cells	0.5	0	0	0	0	0
Other parameters						
Presence of material debris	0	0	0.5	0	0.5	0
Fatty infiltration	0	0	2	0	1	0
Granuloma	0	0	0	0	0	0
Tissue in-growth	–	–	–	–	–	–

T, Test; C, Control.

phages and multinucleated cells) were recorded as zero in the grading. There was no fatty infiltration or granuloma formation. Material debris was present in all the samples, both in test and control groups. However, this was not graded because the distribution was highly non-uniform. The bone tissue in-growth seen in all the samples proves that both test and control materials are osteoconductive.

Detailed histological observations have been carried out to understand the nature of material resorption and bone remodelling at the implanted sites. At 4 weeks post implantation, only one test site could be recovered. The analysis could not be done properly as the material remnants got lost during sectioning. The control (hydroxyapatite granules) sites could be retrieved successfully, which showed the material remnant as dense grey areas with different shapes. Traces of new woven bone were found surrounding the granules. In either case, the cortical bone at the interface did not show any reaction to the implants. No inflammatory cells or fibrous tissue was observed at the interfaces. The periosteal surfaces were not healed.

Figure 1 shows a section from a typical test site with Chitra-CPC at 12 weeks post-implantation. The cement material could be identified as a greyish and homogenous mass composed of fine particles (Figure 1 *a*). Though osteointegration with host bone did not occur in most of the parts, new bone was observed to grow into the implanted area from the host bone boundary at some places (Figure 1 *b*). Implant material near the boundary was seen surrounded by lamellar bone (Figure 1 *c*). Evidence of deposition of new bone inside the cement mass was noted (Figure 1 *d*). The periosteal surface was seen healed (Figure 1 *a*) without any inflammatory cells or fibrous tissue at the interface. There was no evidence of bone resorption or remodelling. Bone marrow appeared normal.

In the control sites, new woven bone was observed around few areas of implant material. There was evidence of bone remodelling and Howship's lacuna on one edge of this material. However, osteoclasts were not noted. Bone marrow appeared normal.

Histological pictures of representative sites containing the test (Chitra-CPC) and the control (hydroxyapatite ceramic granules) are compared in Figure 2. Fine particulate mass of the test material was present surrounded by lamellar and woven bone (Figure 2 *a*, *c*, *e*). Haversian canals were also present. Periosteal surface was found healed and osteointegration with host bone was observed. The control sites were identified with dense grey, granular materials of different shapes (Figure 2 *b*, *d*, *f*). New woven bone was present throughout the implanted area, including the inter-granular regions. This was clearly demarcated from host bone on either side. The cortical bone at the interface did not show any reaction to the implants. Bone remodelling was evident with osteoblasts at the material–bone interface and osteoclast-like cells in resorption areas of the material. No inflammatory cells or fibrous tissue was observed at the interface. Periosteal surface was found healed. It is notable that the distinct boundaries appearing around the ceramic granules (Figure 2 *f*) are absent in the cement material (Figure 2 *e*). This is an indication of the phenomenon of material resorption and simultaneous new bone formation, i.e. osteotransduction[2].

Figure 3 is a comparison of the representative histological pictures of test and control sites at 52 weeks, post implantation. The cement material was still present at the test site, though in a lesser quantity. The healed part of

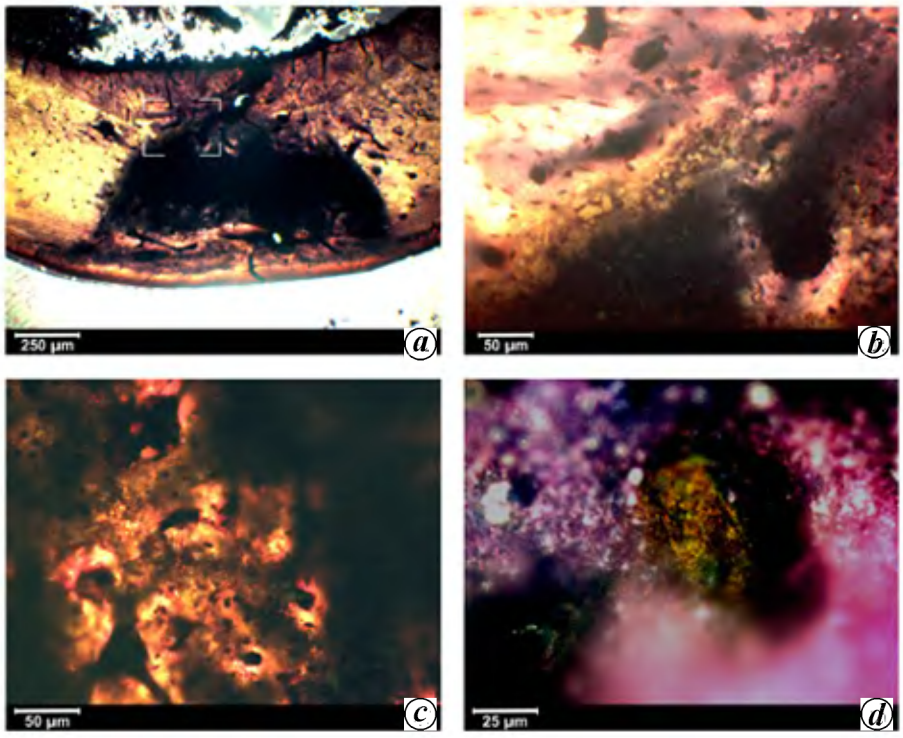

Figure 1. Chitra-CPC in rabbit femur 12 weeks post implantation (Stevenel's blue stained sections in resin). In the low magnification (***a***), the material (dark area) and the host bone are seen. In certain places at the interface (marked in the frame), new bone started growing into the material, which is shown magnified in (***b***). (***c***) Central area of the material with woven bone. (***d***) Evidence of new bone deposition inside the particulate material.

the implanted region was rich in lamellar bone, with well-formed Haversian canals. A remarkable degree of osteointegration to the host bone was observed (Figure 3 *a* and *c*). The hydroxyapatite ceramic granules were present at the control sites as dense grey areas. The boundaries indicated that they had undergone little resorption. Regions around the granules were inhabited by woven and lamellar bone, which integrated well with the host bone (Figure 3 *b* and *d*).

In both cases, the progress of bone remodelling was evident, in comparison with that observed at 26 weeks post implantation. Osteoblasts were present at the interstitial sites and osteoclast-like cells, in the resorption areas (Figure 3 *c* and *d*). No inflammatory cells or fibrous tissue was observed at the interfaces. Periosteal surfaces were completely healed.

The histological observations establish both the materials to be osteoconductive. However, the performance of Chitra-CPC at the defect site differs significantly from that of the hydroxyapatite ceramic granules. Figures 1 *b*, 2 *e* and 3 *c* depict clearly the progressive resorption of the cement mass and simultaneous remodelling of the defect. This osteotransductive property is absent in the case of ceramic granules.

The results enable us to make an assessment of the safety and efficacy of Chitra-CPC. In the safety evaluation, the complete set of toxicological tests stipulated in the standard ISO 10993 for a bone implant has been done.

In acute systemic toxicity test, the material extracts (in normal saline and cotton seed oil) did not invoke any adverse systemic response in mice. Nor did it elicit any erythemic or oedematous reactivity when injected at the intracutaneous sites in rabbits. These results warrant the systemic and intracutaneous safety of Chitra-CPC in clinical application.

No signs of erythema and oedema, or any adverse skin response was seen in the maximization sensitization study in guinea pigs. Intravenous injection of the extract in rabbits in the pyrogen test did not cause any undue rise in the body temperature. The results cover the allergic and pyrogenic safety aspects of the material.

The cement was free from any adverse effects when implanted in rabbit paravertebral muscle. Histological analysis did not reveal any encapsulation, haemorrhage, infection or necrosis. Localized vascularization was present near the implanted region. The chronic inflammation observed at 1 week persisted around the implant site till 4 weeks post implantation. The inflammation was mild at 12 weeks post implantation, with evidence of repair. This ensures the tissue compatibility of Chitra-CPC.

The efficacy evaluation, in which Chitra-CPC was implanted in rabbit bone for 52 weeks with hydroxyapatite

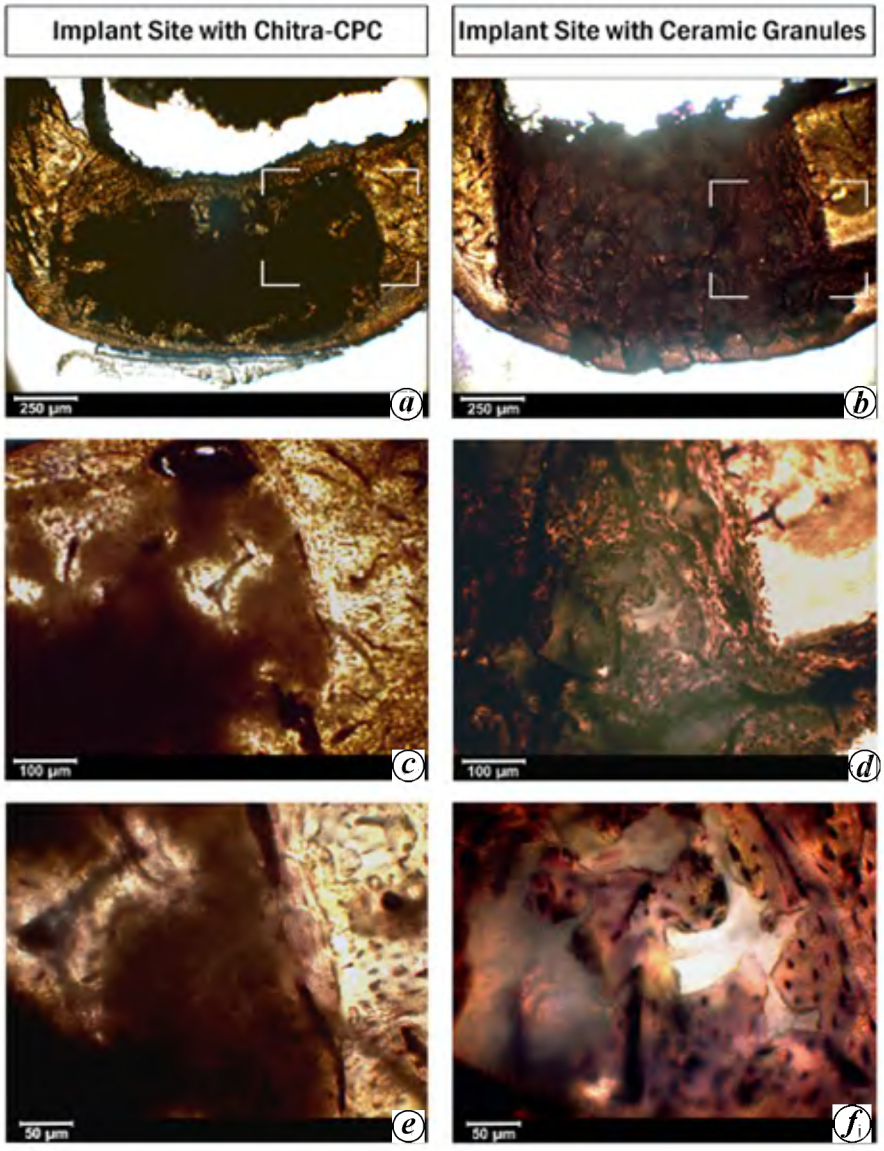

Figure 2. Chitra-CPC and hydroxyapatite ceramic granules in rabbit femur 26 weeks post implantation (Stevenel's blue stained sections in resin). ***a***, ***b***, Low magnification views of materials with adjoining host bone. The respective areas marked are shown magnified in (***c***) and (***d***). The cement appears (***c***) as dense mass with new bone around it. The granules in (***d***) have woven bone in the pores as well as in the interstitial areas. ***e***, ***f***, Further magnified views of the implanted site. Large number of osteoblasts and a few osteocytes are present in the cement, without any inflammatory cells or fibrous tissue (***e***). Distinct boundaries present around the ceramic granules (***f***) are absent in the cement material.

ceramic granules as control, demonstrates healing of the critical size defects without any adverse events. Biocompatibility of the materials is evident, as inflammatory cells or fibrous tissue was absent at any period of observation (Figures 1–3). New bone appeared at the material–bone interface along with the presence of osteoblasts by 12 weeks, in both cases. The implanted regions were engulfed with new bone by 26 weeks, where osteoblasts and few osteocytes were found. This was observed to integrate well with the host bone by 52 weeks post implantation. At this stage, lamellar bone with Haversian system was observed to form in the region where remodelling had occurred. The favourable healing events observed at the implant sites are expected because both the materials consist of calcium hydroxyapatite, the basic bone mineral.

These results establish that both Chitra-CPC and hydroxyapatite ceramic granules are osteoconductive. However, they differ in resorption significantly, as evident from the histological pictures. Chitra-CPC gets resorbed as new bone grows in, while the granules undergo little resorption. This difference could be attributed to the bulk structure of the materials. The granules consist of hydro-

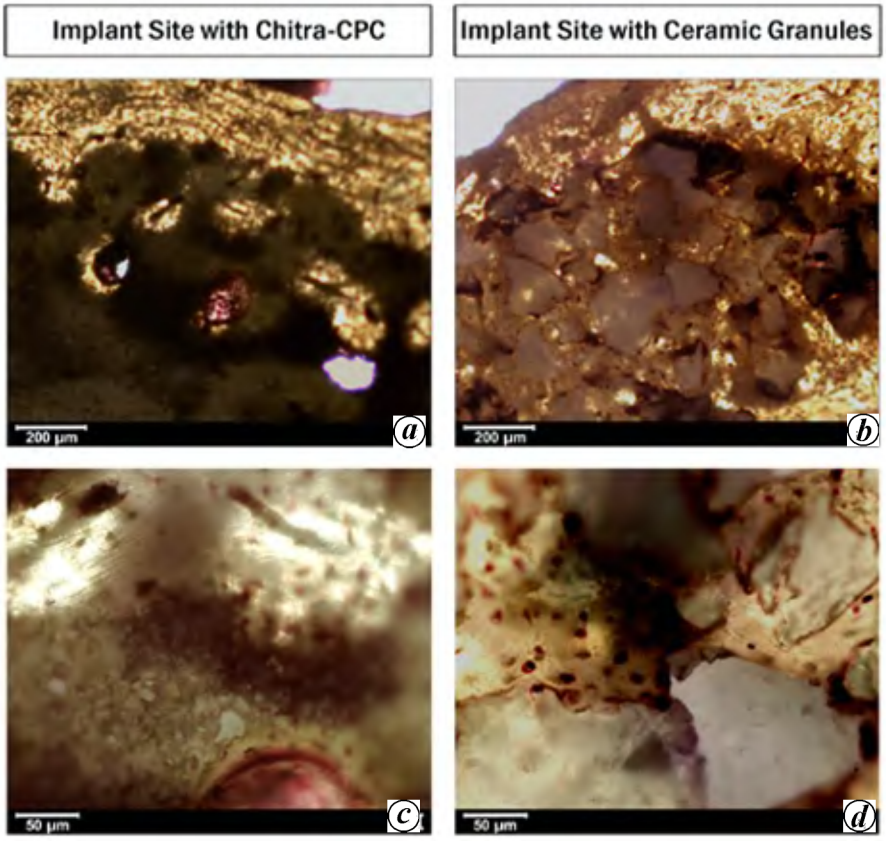

Figure 3. Chitra-CPC and hydroxyapatite ceramic granules in rabbit femur 52 weeks post implantation (Stevenel's blue stained sections in resin). Low magnification views of the respective sites are given in (*a*) and (*b*). Healing of the defect is evident on comparing these with Figure 2 (*a*) and (*b*). Unresorbed remnants of both the materials are present, with woven and lamellar bone deposition around. The central parts of (*a*) and (*b*) are shown magnified in (*c*) and (*d*) respectively. Figure 1 (*b*), Figure 2 (*e*) and (*c*) above describe clearly the osteotransductive property of Chitra-CPC.

xyapatite crystals sintered together in a size of 150 µm or above across, with internal pores. The rate of *in vivo* resorption is directly related to the osteoclastic activity at the surface[10]. Chitra-CPC has submicron-sized particles of hydroxyapatite, inter-grown to form a homogeneous mass during cement setting[6]. The inter-particle boundaries are weak enough to give way to newly growing bone and the particulate structure offers enormously large surface area for osteoblasts to act upon.

The most notable outcome of the histological analysis is the replacement of Chitra-CPC calcium phosphate cement by new bone, as the healing process progresses. This is the osteotransductive property, an ideal requirement for a bone substitute[2] which provides better strength to the healing site. Although the cement has low compressive strength compared to the ceramic granules[5], it has the clinical advantage of better defect repair.

This study projects CPC as an excellent option in skeletal repair instead of ceramic granules, in procedures involving non-load bearing sites. The osteotransductivity of the cement promotes bone healing and helps in regaining the strength of the defect site faster. Mouldability and injectability are additional advantages. Chitra-CPC could be used as bone-graft substitute, for augmentation and void-filling in orthopaedics. It finds use in dentistry as well, for infrabony defect management and in endodontic repair.

1. Driessens, F. C. M., Planell, J. A. and Gil, F. J., Calcium phosphate bone cements. In *Encyclopedic Handbook of Biomaterials and Bioengineering*, Marcel Dekker, New York, 1995, vol. 2, pp. 855–877.
2. Driessens, F. C. M., Planell, J. A., Boltong, M. G., Khairoun, I. and Ginebra, M. P., Osteotransductive bone cements. *J. Eng. Med. (Proc. Inst. Mech. Eng., Part H)*, 1998, **212**, 427–435.
3. Weiss, D., Sachs, M. A. and Woodard, C. R., Calcium phosphate bone cements – a comprehensive review. *J. Long-Term Effects Med. Implants*, 2003, **13**, 41–47.
4. Bohner, M., Calcium orthophosphates in medicine: From ceramics to calcium phosphate cements. *Injury, Int. J. Care Injured (Suppl. 4)*, 2000, **31**, D37–D47.
5. Komath, M. and Varma, H. K., Development of a fully-injectable calcium phosphate cement for orthopaedic and dental applications. *Bull. Mater. Sci.*, 2003, **26**, 415–422.
6. Komath, M. and Varma, H. K., Fully-injectable calcium phosphate cement – A promise to dentistry. *Indian J. Dent. Res.*, 2004, **15**, 89–95.

7. von Recum, A. F., *Handbook of Biomaterials Evaluation, Scientific Techniques and Clinical Testing of Implant Materials*, Macmillan, New York, 1986.
8. International Standard ISO 10993, Biological evaluation of medical devices, International Organization for Standardization, Switzerland, 1997.
9. Class II special controls guidance document: Resorbable calcium salt bone void filler device: Guidance for Industry and FDA. US Department of Health and Human Services, Food and Drug Administration, Center for Devices and Radiological Health, Document issued on 2 June 2003.
10. Aoki, H., *Medical Applications of Hydroxyapatite*, Takayama Press, Tokyo, 1994.

ACKNOWLEDGEMENTS. We thank the staff of Toxicology Division and Implant Biology Division of our Institute for help during evaluation.

Received 3 April 2006; revised accepted 23 August 2006

Improvement in nearest neighbour weather forecast model performance while considering the previous day's forecast for drawing forecast for the following day

Dan Singh* and Ashwagosha Ganju

Research and Development Centre, Snow and Avalanche Study Establishment, Chandigarh 160 036, India

Nearest neighbour model for prediction of weather in terms of snow day/no snow day for consecutive three days in advance (lead time up to 72 h) was tested in two different modes of prediction for two different stations; Dhundi in Himachal Pradesh and Stage-II in Jammu and Kashmir (J&K), in the Pir Panjal range of NW Himalaya, with two different types of data. The data of station Stage-II are incomplete with less data of 12 winters (winter 1991–92 to winter 2003–04, missing data of 1994–95) and those of station Dhundi are complete with more data of 15 winters (winter 1989–90 to winter 2003–04). The model performance was tested with incomplete and complete data respectively, in two different modes. First, in mode I prediction of weather is made based on the probability of snowfall calculated from nearest days/nearest situations. Secondly, in mode II the prediction was made considering the previous day's probability of snowfall also, along with the probability of snowfall calculated from nearest days/situations, i.e. while forecasting for day-2 (lead time 48 h), probability of snowfall for day-1 (lead time 24 h) is also taken into account.

The model performance is found to be better for mode II compared to mode I for all three days except for day-1 forecast with incomplete data. The model performance is better for Stage-II compared to Dhundi in both the modes. Significant difference in the model performance for day-1 and day-2 forecasts is found between those with incomplete data compared to those with complete data. The model results are briefly discussed here.

Keywords: Nearest neighbour technique, snow and no snow day, weather prediction.

MANY case-based techniques have been proposed for prediction of weather and weather parameters[1–4]. These methods use different pattern-recognition techniques. Riordan and Bjarne[3] proposed a fuzzy case-based system for weather prediction. Following the approach of case-based reasoning, Singh *et al.*[5] proposed the nearest neighbour model for prediction of weather at a station in terms of snow day/no snow day and the expected snowfall amount under different well-established snowfall categories. The proposed model predicts weather for consecutive three days in advance based on the probability of snowfall calculated from nearest days/situations, where the previous day's probability of snowfall is not considered for drawing forecast for following day, i.e. while forecasting for day-2 (lead time 48 h), probability of snowfall for day-1 (lead time 24 h) is not taken into consideration.

In this communication, nearest neighbour model for weather prediction, where the previous day's probability of snowfall also has been taken into consideration along with the probability of snowfall calculated from nearest days/situations, for drawing forecast for the following day is proposed. The developed model has been tested for two different stations with two different types of data in NW Himalaya. The model performance improves for both the stations while considering the previous day's probability of snowfall along with the probability of snowfall calculated from nearest days/situations for drawing forecast for the following day, i.e. while forecasting for day-2, day-1 forecast has been taken into account. The model performance with incomplete data is found better for mode II compared to mode I for all three days, except for day-1 forecast. The model performance significantly differs for both the stations. A significant difference in the model performance is found for day-1 and day-2 forecasts for incomplete data compared to those with complete data.

The western Himalayan range comprises diverse climatic zones. The climatic conditions in these zones are briefly presented in Sharma and Ganju[6]. The present study area lies in Pir Panjal range of NW Himalaya. The model has been tested for two different stations, i.e. Dhundi in Himachal Pradesh and Stage-II in Jammu and Kashmir (J&K). The climatic conditions at Stage-II have been presented in Singh *et al.*[5].

Dhundi is one of the main research and field study stations of Snow and Avalanche Study Establishment (SASE),

*For correspondence. (e-mail: dan_@rediffmail.com)