Original Article

Efficacy of Chitra granules (porous hydroxyapatite crystals) as an alloplastic bone graft

Karthikeyan Maruthmuthu, Shajee Thomas[2], Sivakumar Muniapillai[1]

Departments of Oral Surgery, and [1]Oral Pathology, Madha Dental College and Hospital, Chennai, Tamil Nadu,
[2]Department of Oral Surgery, People's College of Dental Sciences and Research Centre, Bhopal, Madhya Pradesh, India

ABSTRACT

Introduction: Bone grafting is a gold standard technique in restoring the bone defects created due to the surgical removal of cysts and tumors as well as surgical corrections of skeletal deformities. The aim of the case series was to evaluate clinically, radiographically, and ultrastructurally the efficacy of Chitra granules, a porous hydroxyapatite synthetic bone graft, substituting as a restorative material in the maxillary bone defects. **Materials and Methods:** In this study, six patients were divided into two groups according to the size of the bone defect after the removal of periapical intraosseous lesions. Then, the defect was filled with Chitra granules mixed with sterile saline and the surgical site was closed as per regular surgical protocols. The surgical region was evaluated clinically and radiographically at regular intervals to assess healing and bone formation. Also, a bone biopsy was taken 3-6 months after implantation of bone graft for scanning electron microscopic study. **Results:** New bone formation with indistinct graft-surgical margin was evident in the postoperative radiographic evaluation after 6 months. Scanning electron microscopy revealed trabeculae of bone formation at the periphery and fibrous connective tissue growth in the pores of the granules along with ossification of fibrous connective tissue in and around the granules. **Conclusion:** In conclusion, with the encouraging results in the literature on the role of hydroxyapatite as an osseous filling material, Chitra granules as a biomaterial can be considered as a suitable alternative to autogenous grafts, allogenous grafts, and xenografts.

Key words: Allogenous grafts, chitra granules, osseous defect, porous hydroxyapatite crystals

INTRODUCTION

Bone grafting is generally required to restore the bone defects created by cyst or tumor removal, pseudoarthrosis, and surgical correction of skeletal deformities. Restoration of bone defects not only improves the function but also the esthetics, especially in the maxillofacial region where surgical treatment may lead to major facial disfigurement and unaesthetic appearance.[1] Grafting can be done with live bone, incised from the same individual (autogenous), from a donor of the same species (allogenous) and from the bones of other species (xenograft).[2]

The complications associated with the autogenous bone grafting like morbidity at the donor site, potential iatrogenic nerve damage, and rapid resorption of the graft material after being loaded with dentures, and graft rejection, immunogenic response, and infections associated with allograft and xenograft prompted the search for synthetic bone grafts, leading the discovery of synthetic bone grafts that are biocompatible and osteoconductive.[3]

The aim of this study is to evaluate the role of one such biocompatible and osteoconductive synthetic bone graft

Address for correspondence:
Dr. M. Karthikeyan, Department of Oral Surgery,
Madha Dental College and Hospital,
Kundrathur, Chennai - 600 069, Tamil Nadu, India.
E-mail: karthikeyanomfs@gmail.com

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material, Chitra granules, in maxillofacial defects as an effective bone-filling material.

MATERIALS AND METHODS

The study included a total number of six cases (two males and four females) of periapical osseous defects, including two cases of inflammatory granuloma of maxilla, two cases of periapical granuloma of maxilla, a case of adenomatoid odontogenic tumor (AOT) of maxilla, and a case of residual defect due to dentoalveolar fracture of maxilla. The inclusion criteria for the study were patients with periapical osseous defects of age range between 17-40 with no systemic complications, no history of allergy, and no local factors compromising wound healing. The patients were divided into two groups according to the size of the osseous defect. Group I and group II consisted of three cases each with periapical osseous defects less than 4 cm in diameter and more than 4 cm in diameter, respectively. The mean period of evaluation was 8 months. As per surgical principles, all the patients underwent thorough preoperative evaluation on the following aspects: Case history, clinical evaluation, radiological examination, hematological evaluation, and systemic evaluation.

Surgical techniques

Surgical treatments for all six patients were performed under local anesthesia with adrenaline. All periapical osseous defects were approached intraorally with a buccal approach [Figure 1]. Through crevicular incision, trapezoidal mucoperiosteal flaps were raised. Care was taken not to create any button holes on the mucoperiosteum and a perfect hemostasis was achieved. In group I patients, the lesions were enucleated and thorough curettage was done. The cavity was irrigated with normal saline; apicectomy and retrograde filling with glass ionomer cement were done as required. In group II patients, bur cuts were made on the margins of the cyst and bone in order to give good clearance from the lesion. The lesion was enucleated and thorough curettage was done [Figure 2]. Care was taken to prevent any perforation of the nasal mucosa. Chitra granules (Topnotch Health Care Products Pvt. Ltd, Kerala, India) were mixed with sterile saline before its application, for easy manipulation. The cavities were inspected for residual lining and packed with Chitra granules, filling the entire cavity [Figure 3] and in some cases cavity were overfilled with graft material. Stents were not used. The closure was carried out with 3.0 catgut and with 4.0 vicryl for group I and group II patients, respectively.

All the patients were treated as outpatient and were prescribed a postoperative regimen of capsule amoxicillin (250-500 mg), tablet metronidazole (400 mg), tablet ibuprofen (400 mg), and chlorhexidine mouth wash for a period of 5 days.

All the patients were reviewed at regular intervals at first week, third week, sixth week, eight week, 12th week, and 18th week postoperatively for the possible postoperative events associated with surgical site like infection, dehiscence, and edema, and regular radiographs were taken at first month, second month, third month, and sixth month for the study of resorption and migration of the particles and recorded individually.

Preparation of the bone samples for scanning electron microscope (SEM)

The secondary surgery was carried out at 3-6 months after implantation of bone grafts. The mucoperiosteal flap was raised from the grafted site. With the help of a bone scoop, bone sample were taken from the center as well as from the periphery of the grafted site.

Initially the bone samples were fixed in formalin and later treated with glutaraldehyde for 24 h followed by treatment with alcohol for 20 days. Finally, the bone samples were fixed in 100% acetone in a sterile plastic tube. The samples were taken to Indian Institute of Technology, Chennai, for scanning electron microscopic study. The specimens were dried on a filter paper, completely dehydrated, and coated with gold particles. The specimens were viewed under SEM at various magnifications (350-2000 ×). Photomicrographs were taken and analyzed.

RESULTS

Postoperative clinical evaluation revealed no major postoperative events or complications except mild infection in one patient during the first week, mild dehiscence of 2 mm and 1 mm in four patients during first and third postoperative week, and all the patients showed mild to moderate edema during the first week [Table 1]. Also there was extrusion of excess graft material found in the surgical margins in three patients. On subsequent inspection at sixth week, all the initial postoperative events subsided and the healing was satisfactory. Radiographic examination revealed distribution of granules in the first month onwards. Resorption was seen after 2 months at the margin of grafted site, which showed dense area of granules interfacing with normal bone [Figure 4]. Early postoperative radiographs showed less distinct margins in the interface regions. Over time, the margins were seen to become less prominent and in all cases after 6 months the margins were indistinct. The graft in the early radiographs showed a high radiodensity. In later radiographs, the density was less and at the sixth month the radiodensity of the graft was similar to that of the surrounding bone [Figure 5]. Throughout the follow-up period, migration of particles from the implanted site was not seen. A clear plane of cleavage between mucoperiosteum and graft material was evident during the second surgery and no graft was adherent to the flap. SEM revealed that there was fibrous connective tissue formation in the pores of the Chitra granules. SEM photomicrographs showed trabeculae of bone formation in the periphery of the grafted site. Fibrous bands at different areas in the granules and ossification of

Table 1. Case details and periodic review

Patient name and age	Diagnosis	Treatment	Postoperative events				
			Follow-up	Infection	Dehiscence	Edema	Migration
Ms. Nazia Begum 17 years	Adenomatoid odontogenic tumor in relation to 11, 12, 13	Resection and reconstruction with porous hydroxyapatite	First week	Absent	Absent	Mild	Absent
			Third week	Absent	Absent	Absent	Absent
			Sixth week	Absent	Absent	Absent	Absent
			Eighth week	Absent	Absent	Absent	Absent
			12th week	Absent	Absent	Absent	Absent
			18th week	Absent	Absent	Absent	Absent
Mr. Kannan 25 years	Residual defect due to dentoalveolar fracture in relation to 11, 12, 13	Curettage and reconstruction with porous hydroxyapatite	First week	Absent	Mild (2 mm)	Absent	Absent
			Third week	Absent	Mild (1 mm)	Moderate	Absent
			Sixth week	Absent	Absent	Absent	Absent
			Eighth week	Absent	Absent	Absent	Absent
			12th week	Absent	Absent	Absent	Absent
			18th week	Absent	Absent	Absent	Absent
Ms. Ananthi 17 years	Periapical granuloma in relation to 12, 13	Curettage and reconstruction with porous hydroxyapatite and extraction 12, 13	First week	Mild	Absent	Moderate	Absent
			Third week	Absent	Absent	Absent	Absent
			Sixth week	Absent	Absent	Absent	Absent
			Eighth week	Absent	Absent	Absent	Absent
			12th week	Absent	Absent	Absent	Absent
			18th week	Absent	Absent	Absent	Absent
Ms. Kanchana 17 years	Inflammatory granuloma in relation to 22, 23	Curettage and reconstruction with porous hydroxyapatite and extraction 22	First week	Absent	Mild (2 mm)	Moderate	Absent
			Third week	Absent	Mild (1 mm)	Absent	Absent
			Sixth week	Absent	Absent	Absent	Absent
			Eighth week	Absent	Absent	Absent	Absent
			12th week	Absent	Absent	Absent	Absent
			18th week	Absent	Absent	Absent	Absent
Ms. Vilvarani 30 years	Chronic granuloma in relation to 22, 23	Curettage and reconstruction with porous hydroxyapatite and extraction 22	First week	Absent	Mild (1 mm)	Mild	Absent
			Third week	Absent	Absent	Absent	Absent
			Sixth week	Absent	Absent	Absent	Absent
			Eighth week	Absent	Absent	Absent	Absent
			12th week	Absent	Absent	Absent	Absent
			18th week	Absent	Absent	Absent	Absent
Mr. Soundrarajan 40 years	Inflammatory granuloma in relation to 21, 22	Curettage and reconstruction with porous hydroxyapatite	First week	Absent	Mild (1mm)	Mild	Absent
			Third week	Absent	Absent	Absent	Absent
			Sixth week	Absent	Absent	Absent	Absent
			Eighth week	Absent	Absent	Absent	Absent
			12th week	Absent	Absent	Absent	Absent
			18th week	Absent	Absent	Absent	Absent

fibrous connective tissue in and around the granules were also evident [Figures 6 and 7].

DISCUSSION

The idea of bone grafting began with a description of bone structure in 1674, by the Dutch surgeon scientist Antony Van Leeuwenhoek, in his letter dated June 1 1974, addressed to the publisher of the Philosophical Transaction, after the Dutch surgeon Job Van Meekren in 1668, described the first bone grafting procedure using the graft derived from a dog's skull to restore the traumatic defects in a soldier's cranium.[4] The first clinical autograft was performed in Germany, by Philips Von Walter in 1820. Barth and Curtis published their work on bone transplantation. William MacEwen in 1879 performed first allografting procedure.[5]

Grafting can be done with live bone, incised from the same individual (autogenous), from a donor of the same species (allogenous) and from the bones of other species (xenograft).[3] Any bone graft used to bridge osseous defects has two functions: Biologically, it supplies bone cells to induce the formation of osteogenic tissues and mechanically it displaces the soft tissues and acts as a porous matrix within which osteoconduction can occur.[6]

In oral and maxillofacial surgery, bone grafting is indicated to encourage healing of ununited fractures, to enhance the union at osteotomy site and for reducing the time required to reach sufficient strength and to resist movement at the bone ends and early relapse,[7] to restore continuity of bone following an osteotomy, to restore excised segment of either maxilla or mandible, as an onlay to restore bone contour or to increase the thickness of the bone where there has been severe atrophy[8] and to fill residual cavities after removal of benign cyst or tumors to hasten healing and to restore bone contour.[9]

As the allogenic and xenografts pose the risk of immunogenic response and infections,[10] in the past there has been tremendous use of autogenous bone graft in

restoring bony defects in maxillofacial region. Extensive use of autogenous bone grafts has been restricted by the limitations inherent in their procurements such as use of general anesthesia, post-grafting complication like donor site morbidity, chances of iatrogenic nerve damages, and lastly patient has to stay in the hospital for a longer time. Moreover, these grafts have a tendency for resorption under stress.[3]

Due to their own disadvantages of the autogenous bone grafts, newer and simple methods were sought after. Many authors have substantiated the use of alloplastic bone substitutes in the maxillofacial surgery[11-14] and pointed out that sintered biomaterials have several qualities when used in restoring alveolar and osseus defects.[15]

In the present study, the material used is porous hydroxyapatite crystals (Chitra granules), which is easily available in Indian market named as an "orthogran." Chitra granules are prepared by a precipitation method starting from calcium solution and ammonium dihydrogen phosphate solutions. Both solutions are maintained at pH above 10 by adding ammonium solution. The phosphate solution is then added drop by drop to calcium solution under continuous stirring. After the completion of

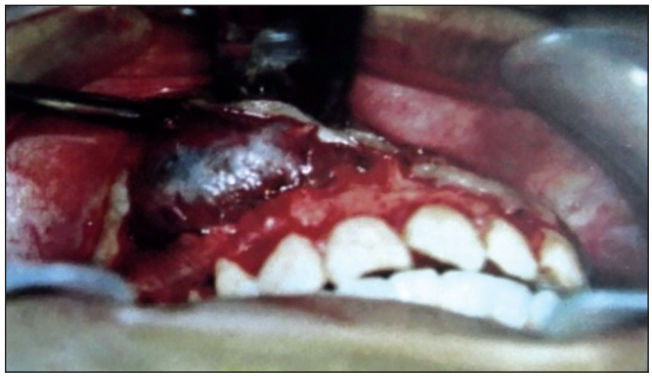

Figure 1. Surgical exposure of the tumor (AOT)

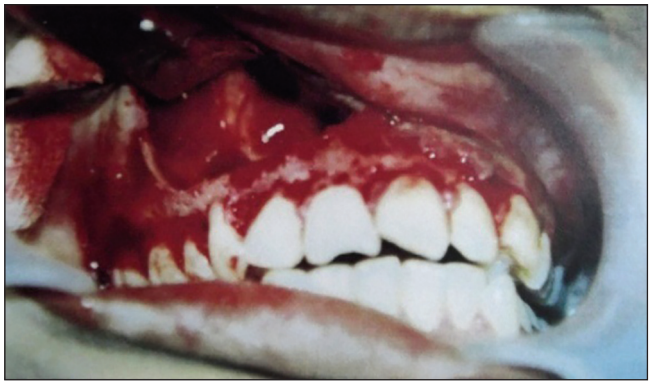

Figure 2. Bony defect after removal of the tumor

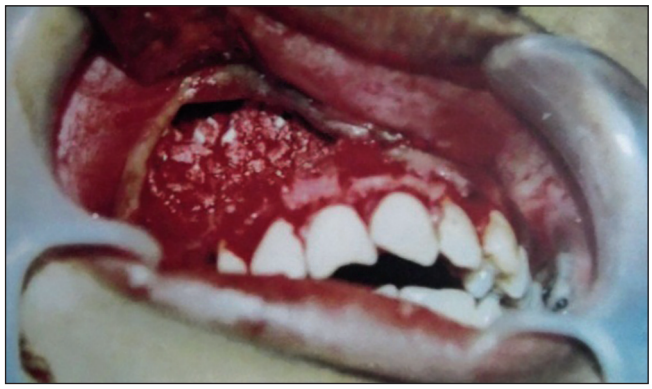

Figure 3. Defect filled with Chitra granules

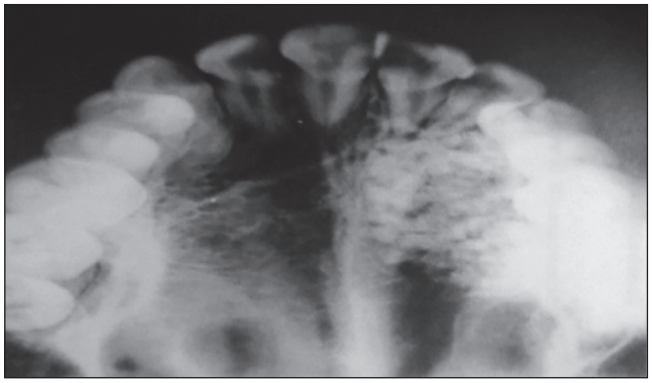

Figure 4. Postoperative radiograph after 3 months showing bone formation at the periphery

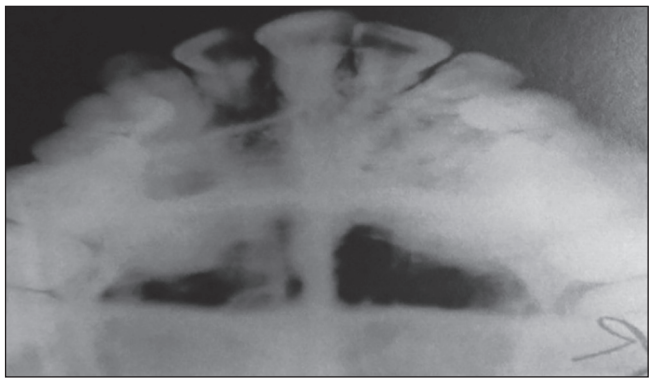

Figure 5. Postoperative radiograph after 6 months showing complete bone formation filling the defect

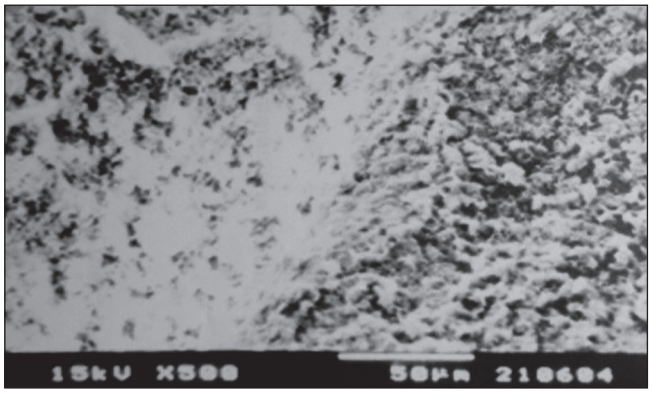

Figure 6. Electron micrograph reveals density of bone after surgical procedure

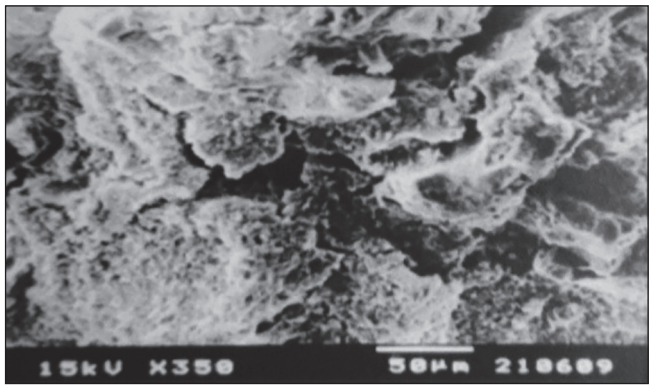

Figure 7. Electron micrograph shows trabeculae of bone formation

the precipitation, the stirring is continued for another 1 hr and aged for 24 hrs at room temperature. The precipitate is filtered and washed thoroughly to remove ammonia and other ions absorbed over the precipitate surface. After repeated washing with distilled water, the precipitate is then subjected to freeze drying in lyophilizer. The free flowing powder obtained is heat treated at various temperatures and later characterized in phase purity, composition, and morphology. The freeze-dried powder is first calcined and it is mixed with appropriate pore former and thoroughly mixed in a ball mill. The milled powder-pore former mixture is then compacted and heat treated at high temperature to get sintered porous blocks. These pellets are manually crushed using mortar and pestle into appropriate size fractions and separated using standard sieves. The granules are then subjected to ultrasound cleaning and dried in a hot air oven.[16]

The hydroxyapatite granules showed minimum hemolytic potential when subjected to hemolytic studies.[17] There was no change in physical and chemical properties of both powder and granules either by irradiation or by heat treatment. Non-immunogenecity has been tested by subcutaneous implantation in guinea pig. Mucous membrane irritation test has been done by applying the material on the vaginal epithelium of rabbit and no acute inflammation was observed.[18] Cytotoxicity test was done with L929 cell line and there was no obvious cytotoxic effect when using washed powders and granules. The desirable property of this material as described by Blijdrop 1988 is its biocompatibility of hydroxyapatite with bone at the interface by lesser toxicity to the mucosa and soft tissue.[19] Lamb, 1986 obtained polycrystalline forms of hydroxyapatite[20] and finally Herald V Cohen in 1980 obtained dense hydroxyapatite.[14] The alloplastic bone graft material used in this study is an indigenously manufactured material. They are available in blocks, which can be machined in required size and shapes according to the need.

Lamb (1986) suggested the use of palatally based buccal flaps for restoration of local defects, as it facilitated increased soft tissue cover and visibility to the implanted area and also facilitated accurate placement of the implant.[20] Based on this, all the cases in this study were approached through crevicular incision, and mucoperiosteal flaps were designed on the labial side, which fulfilled all the criteria for a basic requirement of a flap. The osseous defects were accessed through the labial approach; entire lesion was excised and curetted. As with the previous study of Lamb, normal saline was used for moistening the hydroxyapatite particles and easy manipulation was experienced. In few cases, moistening was not carried out and resulted in difficulty in manipulation.

Ronald P. Desjardins (1985) enumerated the potential problems of hydroxyapatite particles like diffusion into adjacent areas, irregular distribution and extrusion, incorrect position, migration settling, and resorption.[21] In this study, there was extrusion of the excess graft material found in the surgical wound margins in three patients. In one case, there was insufficient material, but there was no diffusion in to the adjacent areas and stents were not used due to their minimal value. Closure was carried out with simple interrupted sutures with 3.0 catgut and watertight closure was achieved.

Badalian VA *et al*.,(2000) in a study suggested that regeneration of periapical tissue depends on the size of the periapical lesion.[9] In the current study, radiographic evaluation was done for all cases. Intraoral periapical X-rays, occlusal views, and orthopantomographs (OPGs) were taken to assess the periapical lesion. According to the lesion size, it was categorized into group 1 and group 2 for study convenience. Radiographically, the regeneration of periapical tissue and the behavior of the graft material were studied and found to be satisfactory postoperatively, which was in converse to Badalian VA's study.

Regular radiographs were taken at first month, second month, third month, and sixth month. It was found that distribution of the granules was seen in the first month onwards. Resorption was seen after second month at the margin of the grafted site, which shows dense area of granules interfacing with normal bone. Early postoperative radiographs showed less distinct margins in the interface regions. Over time, the margin was seen to become less prominent and in all cases after 6 months the margins were indistinct. The graft in early radiographs showed a high radiographic density. In later radiographs, the density was less at 6 months, showed density similar to that of surrounding bone. Throughout the follow-up for 3 months, there was no migration of particles from the implanted site. Resorption of some graft materials was encountered when insufficient graft material was placed; this was evident in one case after 2 months.

Che-Shoa Chang *et al*., (1991) studied histological findings from the specimens taken from implant site at

5 months and 1 year. In their study, there was new bone formation and investing fibrous tissue formation as early as 5 months.[22]

Chang *et al*., implanted particulate hydroxyapatite in experimentally created bone defects in monkeys and carried out histological studies at 1, 2, 3, 6, and 8 months. In the first month, only loose fibrous tissue was observed over the interparticulate space. Woven bone was seen in the second month, which progressed to new mature bone in the third month, and continuing maturation of the bone tissue was observed in the sixth and eighth months.[23] Ettel *et al*.,(1989) evaluated the histological response to porous hydroxyapatite in chronically inflamed and surgically created periodontal pockets in Rhesus monkeys. The results showed newly formed bone within the porous channels of the graft in direct apposition to the surface of graft particles.[13]

Bagambisa *et al*., studied about the interaction of osteogenic cells with hydroxyapatite implant materials *in vitro* and *in vivo* using scanning electron microscopy. They concluded that the implant bed cells showed an intimate contact with the osteoblasts depositing organic matrix and bone material in apposition to the graft material. No encapsulation or granulation tissue was observed.[24]

In this study, scanning electron microscopic study was done to analyze the bone formation. During secondary surgery, there was a clean plane of periosteal cleavage, and there was no adherence of fibrous tissues to the graft material. Osteointegration was seen at the periphery of the implant site and loose bone formation in the center. Examination with scanning electron microscopy revealed that there was fibrous connective tissue formation in the pores. SEM photomicrographs showed trabeculae of bone formation in the periphery of the grafted site. It also showed ossification of fibrous connective tissue in and around the granules and exhibited fibrous bands at different areas in the granules.

Based on the observation in this study, Chitra granules showed various advantages over the other conventional grafting techniques with respect to the following:

1. Ease of its availability in the commercial market and being indigenously manufactured, the cost of effectiveness is also well accepted.
2. Single stage outpatient procedure, not requiring admissions or general anesthesia.
3. It could be easily shaped according to the need of the surgeon.
4. The grafted site healed uneventfully without any significant complication.
5. The porous nature of the material acted as a good scaffold for osteosynthesis.
6. SEM study revealed new bone formation.

CONCLUSION

In the light of encouraging results in the literature on the role of hydroxyapatite as osseous-filling material, a clinical study was conducted on Chitra granules as a bioplastic bone substitute in the osseous defects in oral and maxillofacial region. Chitra granules are found to be a suitable alternative autogenous graft, allogenous graft, and xenografts for the repair of the osseous defects created by surgical procedures. Despite the limitation of the study where only six patients were treated, the study yielded good results. Hence, more number of randomized clinical trials with a larger sample size has to be evaluated further.

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