Background: Following tooth extraction, remodeling and resorption of the alveolar bone at the extraction site characterize wound healing. This produces a reduction in ridge volume and difficulties in delayed placement of implants in an ideal position. Medical grade calcium sulfate hemihydrate (MGCSH) has been proposed as a graft material in extraction sockets to minimize the reduction in ridge volume. The aim of the present study was to investigate the influence of MGCSH on the histopathologic pattern of intrasocket regenerated bone and to evaluate histologically the healed MGCSH grafted extraction socket site 3 months postextraction.

Methods: MGCSH was grafted in 10 fresh human extraction sockets in 10 patients. Five post-extraction sockets were used as controls. At 3 months a cylindrical tissue specimen, 2.5 mm in diameter, was trephined from the previously grafted site followed by implant placement. Non-decalcified specimens were sectioned at a cross-horizontal plane and stained with fast green, toluidine blue, and Van Kossa stains for histological and histomorphometrical examination.

Results: Histologically, MGCSH was not observed in most of the specimens. Newly formed bone with lamellar arrangements was identified in all the horizontal sections with no difference between apical, medium, and coronal areas. The mean trabecular area in the coronal sections was 58.6% ± 9.2%; in the medium sections, 58.1% ± 6.2%; and in the apical sections, 58.3% ± 7.8%. The differences were not statistically significant.

Conclusion: MGCSH seems to be an ideal graft material in extraction socket bone regeneration because it is almost completely resorbable, and it allows a new trabecular bone arrangement at 3 months.

Regeneration of alveolar bone lost or injured as a result of disease or trauma may pose therapeutic problems in implant dentistry because bone defects often fail to heal, or heal with a type of tissue different from the original tissue with respect to morphology and function. This represents a particular problem following tooth extraction. Following tooth extraction, the first 24 hours are characterized by formation of a blood clot. Within 2 to 3 days the blood clot contracts and is replaced by formation of granulation tissue with blood vessels and collagen fibers. After 4 days, an increased density of fibroblasts is visible in the clot and the proliferation of fibroblasts from wound margins is apparent. Remodeling of the sockets begins with the presence of osteoclasts inducing bone resorption. One week after extraction, the socket is characterized by granulation tissue consisting of a vascular network, young connective tissue, osteoid formation in the apical portion, and epithelial coverage over the wound. Three weeks following extraction, there is dense connective tissue overlying the residual sockets, which are now filled with granulation tissue. A trabecular pattern of bone starts to emerge. Two months after extraction, bone formation in the socket is not yet complete, with the presence of not fully mineralized osteoid, the bony height of the original socket has not been reached, and the trabecular pattern is still undergoing remodeling.

Controlled clinical studies have documented an average of 4.0 to 4.5 mm horizontal bone resorption following extraction procedures. Other studies have documented significant dimensional changes in the surrounding alveolar bone following extraction procedures. The resorption and remodeling process represents a particular problem in implant placement especially in the anterior maxilla where the dimension and morphology of the alveolar ridge cannot easily accommodate implants.

Guided bone regeneration procedures are used to preserve the height and width of the alveolar bone for future implant placement. Other techniques, such as grafting of autogenous bone and bone substitute materials; i.e., allogenic, alloplastic, and xenogenic substances have also been used for this purpose. Two histological studies reported positive socket healing responses with...
allografts\textsuperscript{24} and xenografts\textsuperscript{23} while others have shown poor results with demineralized freeze-dried bone allograft (DFDBA), bovine bone, and even autogenous bone when implanted into sockets following extraction.\textsuperscript{18-25}

Calcium sulfate was one of the first bone substitutes used in orthopedics and dentistry because it is readily available, easily sterilized, inexpensive, completely and rapidly resorbable, and biocompatible.\textsuperscript{26-29} In addition, calcium sulfate is osteoconductive; it is not osteoinductive in itself, but in the presence of bone and/or periosteum it almost always becomes osteogenic.\textsuperscript{30,31} In a previous study,\textsuperscript{32} MGCSH was histologically and histomorphometrically examined at augmented sinus floor sites. The material was well tolerated by the host and was completely resorbed. This current pilot study was designed to histologically and clinically evaluate the healing of extraction sockets at 3 months where MGCSH was used as filler material in ridge preservation procedures, compared to untreated sites.

**MATERIALS AND METHODS**

**Study Population**

Ten patients (three males, seven females; age range 35 to 58 years) with no systemic disorders participated in the study. All patients signed informed consent forms, and the study was approved by the Ethics Committee of the University of Chieti, Italy. Maxillary and mandibular single tooth/teeth extraction(s) (incisor, canines, and/or premolars) was scheduled, followed by restoration with implants at 3 months. Teeth with ongoing pathoses, i.e., periapical radiograph radiolucency and/or periodontal abscess, were included in the study. Alveoli with severe ridge resorption \( \geq 50\% \) of the socket were excluded.

Labial and palatal local infiltration with lidocaine 1:100,000 was performed. An intracrevicular incision was extended to the mesial and distal teeth and a conservative mucoperiosteal flap was raised. Careful tooth/teeth extraction was performed. The MGCSH was grafted (Figs. 1A, 1B, and 1C) in the extraction socket using different consistencies: in the apical portion, MGCSH prehardened particles (G 170) were grafted; in the middle portion, MGCSH was compacted with dry gauze against the first layer; and in the coronal portion a fast set solution was used to quickly obtain the hardest consistency possible. No osteopromotive regenerative barrier was used. In five patients with four teeth scheduled for extraction, one site served as a control and the other three were treated with MGCSH. In all patients primary closure of the flap was performed.

Postoperative systemic antibiotics (amoxicillin) were prescribed for 1 week. An antiseptic solution, 0.2\% chlorhexidine mouthwash, was used for 45 seconds twice daily for 2 weeks. Sutures were removed after 10 to 14 days. Radiographic examinations were performed every 15 days to continually monitor the MGCSH. At 3 months, extraction sites were re-entered for implant placement (Figs. 1D and 1E). Osteotomy for implant insertion was performed in an axial coronal-apical direction using a 3.00 mm external diameter trephine bur. Cylindrical sample cores, 7 mm in length, of newly formed intrasocket tissue were
obtained from all sites (Fig. 1E). Following removal of cores, osteotomies were completed and the implants inserted. Before histological processing, tissue samples were marked to identify the coronal and apical sites. Samples were fixed in 4% buffered formaldehyde, dehydrated in graded series of alcohol from 50% to 100%, and embedded in methylmethacrylate.

Cross-sections of 70 micron were obtained with a diamond saw microtome, stained with fast green, toluidine blue, and modified Van Kossa and observed with a microscope.

**Histomorphometry**

Histomorphometric measurements were performed in all the stained slides. Only preserved, rounded sections were examined. The core area of every section (0.1 mm²) was chosen for histomorphometric analysis and area fraction percentage of each component in each section was measured automatically using a software program. To evaluate bone quality, the trabecular bone volume was measured according to the nomenclature approved by the American Society of Bone and Mineral Research.

Statistical analysis was expressed using mean values and standard deviations of the measurements. The differences among the recorded values of the coronal, middle, and apical sections of the test sites were analyzed using a paired t test. A P value <0.05 was considered statistically significant.

**RESULTS**

Upon surgical reentry at 3 months, the volume of the augmented extraction socket sites was clinically well preserved (Fig. 1D). Bucco-lingual dimension of the augmented alveolar ridge enabled safe insertion of the titanium implant.

Histologic examination revealed new bone formation in all the treated specimens with an almost complete absence of MGCSH, absence of connective tissue, and inflammatory cells. In all the sections (Figs. 2, 3, and 4) bone presented a trabecular arrangement with no differences between the apical, medium, and coronal levels. In general, control sites showed less new bone formation than treated sites (Fig. 5).

**Histomorphometric Observations**

In the coronal sections (Fig. 2) of MGCSH treated sites, average trabecular bone area fraction was 58.6% ± 9.2%; in medium sections it was 58.1% ± 6.2% (Fig. 3); and in the apical sections it was 58.3% ± 7.8% (Fig. 4). These differences were not statistically significant. In all the apical sections stained with Von Kossa we found a high calcium ion content and the presence of an amorphous substance around bone trabeculae, close to the surface. Based on composition, as well as position, this was interpreted as a product or a residue of the grafted material. We did not find organized connective tissue and foreign material in any section.

The percentage of trabecular bone did not exceed 46% in the control sections and there were no differences between the coronal and the apical portions. Due to the small number of control specimens, statistically significant comparisons with the treated sites could not be made.

**DISCUSSION**

The final goal of any grafting procedure is to achieve formation of 100% living bone tissue surrounding the
implants. The presence of a reactive tissue able to undergo a sustained state of remodeling may be necessary to maintain osseointegration over time. A wide variety of grafted materials have been proposed for bone regeneration, but it is not clear which is the material of choice because most of them seem to possess some negative properties.

Formation of 100% living bone within the extraction socket using MGCSH was evident by histologic examination in all the specimens analyzed in our study. This is in agreement with other studies supporting calcium sulfate as a valid bone substitute. The almost complete absence of graft remnants indicate that MGCSH underwent an almost complete resorption and that it was replaced by newly formed bone.

To determine the healing pattern of newly formed tissue in relation to the presence of grafted material and to evaluate the influence of socket depth, cross-sections along tissue cores from the socket sites were examined histomorphometrically. From the most superficial to the deeper sections there were no statistically significant differences in trabecular percentage area. A histologic study of natural healing of extraction socket in humans showed very little osteogenetic activity in the superficial bone fraction where osteoblasts were only occasionally observed. This seems to be related to tissue composition in the healing process of conventional non-grafted sockets that heal by secondary intention. The presence of MGCSH during the healing process in the most superficial area of the socket seems to promote osteogenic activity; indeed, there were no differences in trabecular area percentage between coronal and apical sections. Payne et al. reported that calcium sulfate, in comparison with polytetrafluoroethylene and polylactic acid, offers greater potential of guided tissue regeneration in the surgical sites where primary wound healing cannot be obtained. In a wound healing model, the presence of inflammation, there was compromised bone formation, most of which was a woven type. In another study, in which the healing process of grafted extraction socket was investigated in comparison with other materials, the authors reported a different lamellar/woven ratio especially in superficial cut sections. Artzi et al., using a cancellous porous bovine bone, found a mean lamellar area fraction of 15.9% in the superficial sections and a mean connective tissue area fraction of 52.4%. In another study, a similar woven bone dominance was reported in demineralized freeze-dried bone grafts sites in dogs.

In a human study, Froum et al. reported the percentage of vital bone and residual implanted material using DFDBA and bioactive glass as a filler material in extraction sockets. The mean vital bone measurements for bioactive glass and DFDBA-treated sockets were, respectively, 59.5% and 32.4%, while in the control unfilled sockets, it was 34.7%. The authors also reported residual grafted material of 13.5% with DFDBA and 5.5% with bioactive glass.

In the calcium sulfate-grafted extraction sockets, at 3 months, we found a good bone consistency and an almost completely preserved volume, which is fundamental to achieve an ideal implant placement. This clinical observation is very important in relation to the histologic data because we did not find grafted material in any of the coronal and middle specimens examined.

This is in agreement with other studies in which the authors demonstrated that calcium sulfate is a completely and rapidly resorbable material with the ability to guide new bone formation occurring in association with its resorption. Only in the apical portions of the examined section did we find an eosinophilic substance that was detected very close to the bone trabeculae and,
when colored with Von Kossa stain, showed a high calcium ion content. Based on its composition, as well as position, this substance was interpreted as a product or a residue of the grafted material. This seems in agreement with an in vitro study in which the authors described the presence of a calcium phosphate trellis left by calcium sulfate resorption process.\textsuperscript{32}

The presence of this residual grafted material in the apical sections seems to be connected to the longer resorbable time of pre-hardened granular MGCSH (G 170) we used in this portion of the extraction sockets.

If compared with other studies, the present data seem to be very interesting because the most important negative characteristic of other grafted materials is the resorption time. Different data have been reported regarding the resorbable capability of other grafted materials. In other reports,\textsuperscript{41-43} bovine bone-derived material was reported resorbable in nature; however, contradictory findings were reported in other studies.\textsuperscript{44-47} In a clinical and histological study in human grafted extraction sockets, Artzi et al.\textsuperscript{23} found between 26.4\% to 35.1\% residual cancellous porous bovine bone at 9 months. Since osteoclasts could not be identified, these authors suggested that partial degradation, rather than resorption of the grafted particles, probably occurred and that the biocompatibility of bovine bone substitute is enough to obtain vital new bone. This is an issue that deserves further investigation, since the presence of a reactive peri-implant bone may be required to maintain osseointegration over time. In a histologic study, Becker et al. placed microscrews into extraction sockets treated with xenogenic bovine bone, DFDBA, and intraoral autologous bone.\textsuperscript{25} Biopsies from the bovine bone- and DFDBA-implanted sockets revealed dead particles entrapped within dense connective tissue. They concluded that neither xenogenic bovine bone, nor DFDBA, nor autogenous bone contribute to bone-microscrew contact and are not recommended for enhancement of vital bone-implant contact.\textsuperscript{25}

Although many variables, including type and size of defect and time of healing response, as well as differences in host response, make comparisons and
conclusions difficult, the results of our study suggest that MGCSH seems to be an ideal graft material in extraction sockets due to its characteristics; i.e., it is readily available, completely and rapidly resorbable, biocompatible, and well tolerated by the tissues. Further studies with greater number of sites are indicated to determine osteogenic activity of calcium sulfate.

ACKNOWLEDGMENT
This work was partially supported by the National Research Council (CNR), Rome, Italy, by the Ministry of Education, University and Research (MIUR), Rome, Italy, and by the Research Association for Dentistry and Dermatology (AROD), Chieti, Italy.

REFERENCES


Correspondence: Dr. Adriano Piattelli, Via F. Sciucchi 63, 66100 Chieti, Italy. Fax: 39-0871-3554076; e-mail: apiattelli@unich.it.

Accepted for publication October 30, 2003.