Bone Formation With Discs or Particles of Natural Coral Skeleton Plus Polyglactin 910 Mesh: Histologic Evaluation in Rat Calvaria

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Several procedures have been used to regenerate localized bone defects around dental implants or to increase bone volume at an implant site, including bone grafting, placement of barrier membranes, and use of bone graft substitutes. This study sought to determine whether the bone graft substitute natural coral skeleton (NCS), with or without a protective polymer mesh, enhances bone formation in rat critical size craniotomy defects. The control group (1) had unfilled defects, while the defects in the four experimental groups (six rats each) were treated with: (2) an NCS disc of the size of the defect; (3) NCS granules; (4) NCS granules covered by a polyglactin 910 mesh; and (5) polyglactin 910 mesh alone. Undecalcified histologic sections were assessed by histomorphometric measurements 28 days later. The three NCS groups showed improved bone formation, which was statistically significant in groups (2) (NCS disc) and (4) (NCS granules covered by polyglactin 910 mesh). Group 4 had more bone formation than all the other groups. Polyglactin 910 mesh alone (group 5) produced no greater bone formation than the unfilled control. It is concluded that the bone formation obtained with NCS granules is enhanced when the particles are retained at the site of the defect with a protective mesh.
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Key words: bone graft substitute, bone regeneration, natural coral, critical size defects, polymer mesh

Considerable research is presently being conducted to find the ideal material to support bone repair or regeneration. The deficiencies of autogenous grafts and allogeneic bank bone have led to a search for synthetic alloplast alternatives. There is now good evidence that synthetic materials can promote bone repair and that both resorbable and nonresorbable alloplastic materials are osteoconductive with no osteoinductive effect. Calcium carbonate is now used as a bone substitute in periodontal surgery to regenerate lost periodontium, and has recently been used in association with implants.[1]

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The clinical responses to calcium carbonate grafts in adult periodontitis, as assessed by the filling of osseous defects, are similar to those obtained with other materials. Calcium carbonate yielded a fourfold to sevenfold better clinical result than debridement alone. It is thus a useful and beneficial bone replacement graft material when used with appropriate, thorough surgical techniques.

Natural coral porous calcium carbonate appears to be a clinically useful bone replacement graft material that gives essentially similar or slightly better responses in periodontal osseous defects than other bone replacement graft materials.[2–4] Other advantages include its better clinical handling, resorbability, and potential for improved bone regeneration. This material appears to be safe and clinically effective for treating periodontal osseous defects.

An ideal bone grafting material should be replaced by host bone; therefore, the implant needs to be both biodegradable and osteoconductive. The calcium carbonate skeleton of marine corals fulfills both requirements,[5–7] and direct contact between the bone and

calcite, without the interposition of soft tissue at the interface, was recently reported.[8,9]

Schmitz and Hollinger[10] defined a critical sized defect (CSD) as the smallest bone wound that is not healed by bone formation during the lifetime of the animal. A CSD is therefore a perfect model for testing a biomaterial, as the control defect heals more slowly than the experimental defect. Hollinger and Kleinschmidt[11] found that 8-mm-diameter defects in the calvaria of young rats (25 to 35 days old) did not heal spontaneously when monitored for 13 months.

This study evaluates bone formation in rat critical sized craniotomy defects using natural coral (NC) in the form of discs, granules, and granules covered by a polyglactin 910 mesh.

Materials and Methods

A total of 30 Long-Evans rats from the same genus, 40 to 45 days old and weighing 200 to 225 g, were used. They were fed a normal diet and housed in wire cages. The rats were anesthetized by intraperitoneal injection of 40 mg/kg Tiletamine and Zolazepam (Zoletil, Reading, France), which lasted 45 minutes. The cranial area was shaved and scrubbed with 1% iodine, and two lateral and one posterior transversal incisions were made. A flap was raised to allow direct access to the frontal and parietal bones. Using an 8-mm surgical trephine, an 8-mm-diameter circular defect was then made in the calvaria at the intersection of the coronal and sagittal sutures. Five groups of six rats each were then constituted. Those in group 1 (control) were untreated. The defects in group 2 (NCD) were filled with natural coral discs (8 mm diameter, 0.5 mm thick). Those in group 3 (NCG) were filled with 80 mg natural coral granules (Biocoral 450, Inoteb, Saint-Ouen, France). Those in group 4 (NCG + P910) were filled with natural coral granules and covered with a polyglactin 910 mesh (Vicryl, Ethnor S.A., Neuilly, France); and those in group 5 (P910) were covered with a polyglactin 910 mesh alone, which was fixed with lateral sutures.

Fluorescent labeling was used to evaluate bone formation. Calcein (20 mg/kg, Merck, Lyon, France) was given by intramuscular injection on day 0; Xylenol Orange (90 mg/kg, Sigma, Aldrich, France) was injected intramuscularly on day 27. Rats were sacrificed on day 28 by an intracardiac overdose of anesthetic. The calvaria were removed and fixed in 10% neutralized formalin, dehydrated in an ethanol series, and embedded in resin (Technovit 7200, Kulzer, Germany). Undecalcified serial sections were cut using the Exakt cutting-grinding system (Microm, Lyon, France) described by Donath and Breuner,[12] Takata and Donath,[13] and Donath.[14] A total of eight sections were randomly selected from the midsagittal plane of each calvaria and examined by fluorescent microscopy, contact microradiography prepared with an x-ray apparatus (Faxitron, Hewlett Packard, Paris, France) (exposure time 3 minutes at 30 kV), and Giemsa-Paragon or von Kossa's staining.

New bone formation was assessed using a microscope coupled with a high-resolution video camera and semi-automated image analysis (Quantimet, Leica, Rueuil-Malmaison, France). Care was taken not to include the natural coral surface when quantifying bone formation. The data were analyzed using Student's *t* test.

Results

Table 1 summarizes the histomorphometric results.

Group 1 (Control). The 8-mm osseous defect had a small amount of spontaneous bone formation at 28 days. Histomorphometric measurements were 0.8 ± 0.076 mm^2 (mean ± SD). Examination of fluorochrome-labeled stained sections showed that bone formation occurred in two directions: centripetal and in thickness (Figs 1 to 3).

Table 1 Histomorphometric Measurements of Each Treatment Group at 28 Days

Group	Treatment	n	New bone (mm^2) (mean ± SD)
1	Control	6	0.804 ± 0.076
2	Natural coral disc (NCD)	6	1.167* ± 0.342
3	Natural coral granules (NCG)	6	1.172 ± 0.447
4	Natural coral granules + P910 (NCG + P910)	6	1.474* ± 0.180
5	Polyglactin 910 mesh (P910)	6	0.713 ± 0.105

*Denotes significance at $P < .05$ as compared to the control group.

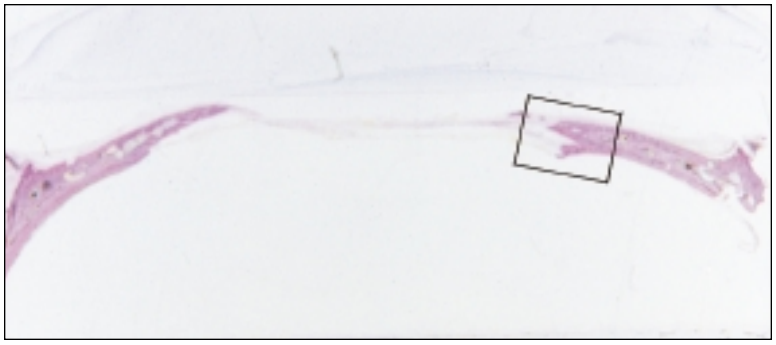

Fig 1 Low-magnification view of histologic section of control calvaria at 28 days (Giemsa-Paragon staining, magnification ×12).

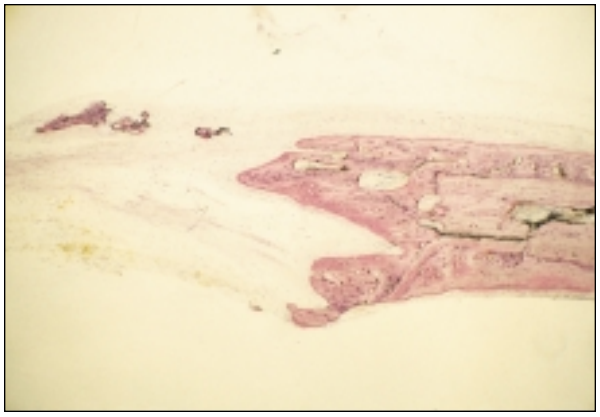

Fig 2 Higher-magnification view of bony edge shown in Fig 1. New bone formation is difficult to visualize clearly (Giemsa-Paragon staining, ×60).

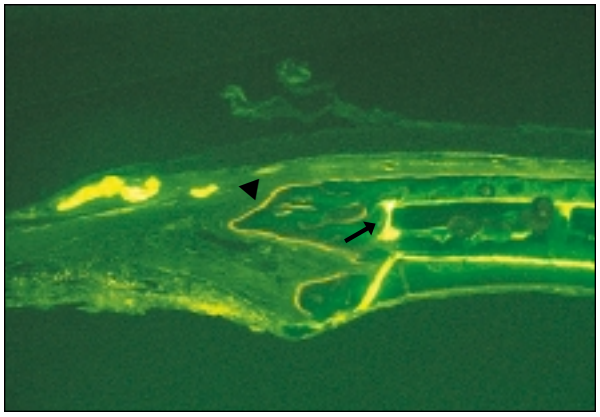

Fig 3 View of same area as shown in Fig 2, using fluorescent labeling. Yellow fluorescence *(arrow)* corresponds to calcein administered at day 0; orange fluorescence *(triangle)* corresponds to Xylenol administered at day 27, one day before histology. Bone formation is thus visible between the two markers (×60).

Group 2 (NCD). The interconnecting pores of coral were infiltrated by a vascularized connective tissue matrix. Bone formation occurred at the periphery of the disc, and never in the central zone. There was little or no resorption of the biomaterial at 28 days. Bone formation was more extensive when there was an initial contact between the natural coral disc and the surrounding bony walls. New bone formation after 28 days of implantation was 1.167 ± 0.342 mm^2 ($P = .029$ when compared to control).

Group 3 (NCG). The bone defect healed as in group 2. There were often bony bridges between the bone edges and the granules. Although the quantity of biomaterial was the same in all animals, the amount of granules left in the defect at 28 days varied considerably. The granules had spread into the surrounding connective tissue. This was confirmed by the variations in the amount of new bone in individual specimens in this group (1.172 ± 0.447 mm^2). This difference was not statistically significant ($P > .05$) when compared with the control group (Fig 4).

Group 4 (NCG + P910). Almost all of the granules remained within the defect at 28 days. The Vicryl membrane was not completely resorbed (Figs 5 and 6). New bone formation was 1.474 ± 0.18 mm^2, which was significantly greater than in the control group ($P < .001$).

Group 5 (P910). The craniotomies were covered by polyglactin 910 mesh to test its influence on new bone formation. Small amounts of the mesh were still apparent at 28 days. Mean bone formation was 0.713 ± 0.105 mm^2, which was not statistically significant as compared to the control group ($P > .05$) (Fig 7).

Discussion

This investigation shows that an osteoconductive material moderately enhances bone formation in a cranial defect that does not heal spontaneously. The bone formation is clearly related to the natural coral, since bone formation occurred in all groups in which it was

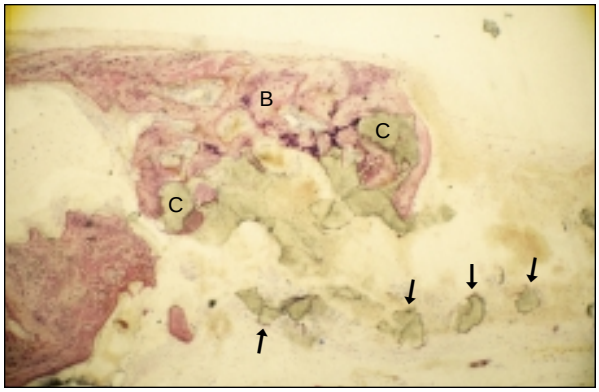

Fig 4 Histologic section of a group 3 calvaria. Bone formation (B) occurred around the coral granules (C) located near the bony edges. A few more granules *(arrows)* can be observed in the connective tissue (Giemsa-Paragon staining, ×45).

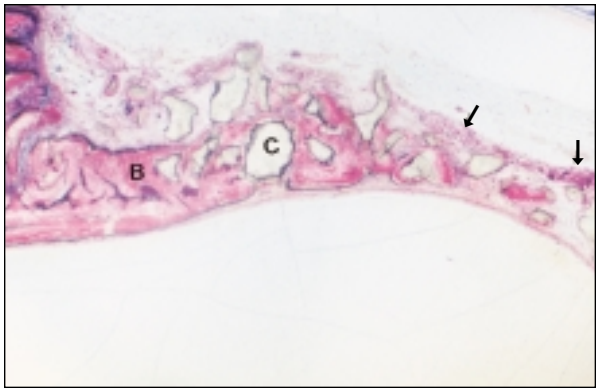

Fig 5 Giemsa-Paragon staining of a group 4 calvaria, coral + polyglactin 910. Bone formation (B) starts at the bony edges and invades coral granules (C). The polyglactin 910 mesh covering the granules is still clearly visible at 28 days *(arrows)* (×30).

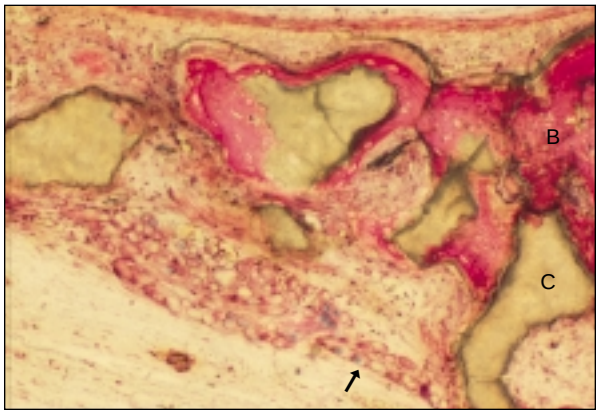

Fig 6 Higher-magnification view of coral granules shown in Fig 4. Bone formation has already connected coral granules. Note osteocytes in this newly formed bone. Polyglactin 910 mesh shows almost undisturbed continuity *(arrow)* (×100).

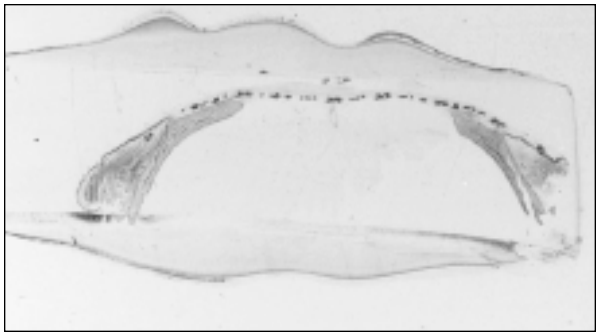

Fig 7 Low-magnification view of group 5 calvaria, showing the position of Polyglactin 910 mesh and minimal bone repair (×10).

used. Bone growth started at the bony edges, establishing bridges with the granules as in other models.[15]

The amount of bone formation is independent of the form of the biomaterial; discs and granules were ossified to the same extent. However, the defects repaired with a natural coral disc varied less, probably because the granules were not retained within the defect. This has been described with other granular biomaterials. Pepelassi et al[16] used plaster of Paris to prevent particle scatter and loss of graft material. Nilveus et al[17] suggested that the loss of graft material during the healing phase can contribute to the failure of bone fill in periodontal defects. Our study indicates that the polyglactin 910 mesh held the granules within the defects and enhanced bone formation in group 4 (Fig 8). The P910 mesh alone does not promote bone formation.

Reynolds and Bowers[18] reviewed the histologic results of early block sections of defects grafted with demineralized freeze-dried bone allografts (DFDBA) and concluded that intrabony sites harboring residual graft particles had significantly more new bone, cementum, and periodontal ligament formation than sites without graft matrix. Early loss of biomaterial particles during wound healing hampered regeneration: the authors concluded that graft containment is essential for optimal healing. The results presented here confirm that a soft mesh can retain the biomaterial particles in the defect, allowing better results.

Several authors have questioned the usefulness of placing a biomaterial beneath a barrier membrane. Caffesse et al[19] compared membrane alone and membrane + DFDBA in beagle dogs, and concluded that the bone graft did not enhance regeneration any

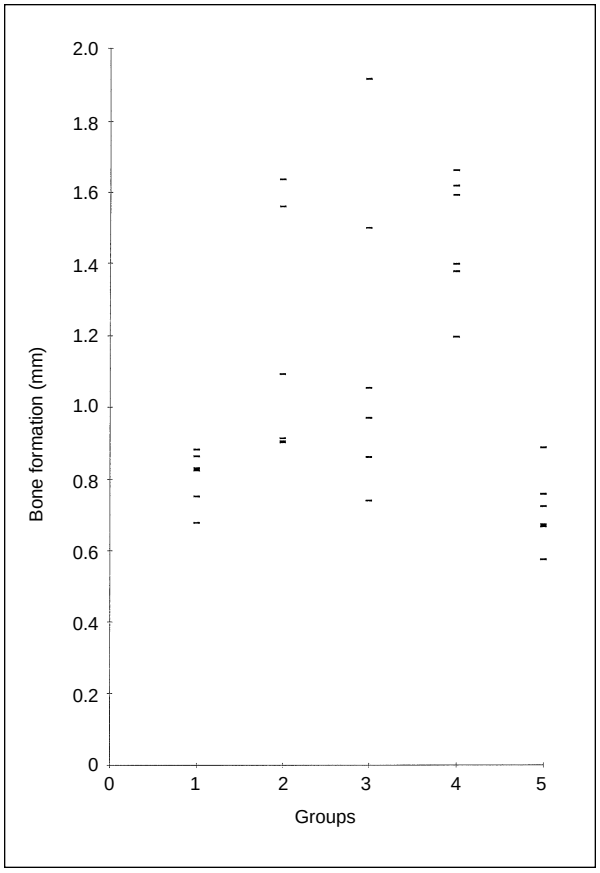

Fig 8 Bone formation obtained in the different animals and experimental groups. The amount of newly formed bone is greatest in group 4. This difference is statistically significant compared to the control group (group 1).

more than the membrane alone. But as they had no group with bone graft alone, the effect of the graft and membrane separately and together cannot be assessed. Lekovic and Kenney[20] demonstrated the same amount of mean gain in bone height and in attachment in furcation defects treated with tricalcium phosphate covered by four types of membranes. Here again, the lack of a control site filled with tricalcium phosphate alone or of a membrane alone precludes a clear demonstration of the role played by each component. This is the case in all of the studies that seek to measure the benefit of placing grafting materials under a membrane.[21–25]

The membrane is used to create and maintain a secluded environment in which osteogenesis may take place relatively unimpeded. This space can be preserved with a rigid membrane (reinforced expanded polytetrafluoroethylene membranes) to resist mechanical collapse, or with screws placed in such a way that they support the membrane like a tent pole[26] or to add a biomaterial that acts as a scaffold.

The authors used an osteoconductive material that is gradually resorbed during its substitution by new bone formation from osteogenic cells migrating from bony edges. The polyglactin mesh is used only to prevent the loss of bone graft substitute particles. Resorbable materials have the advantage of avoiding a second surgical procedure and ensuring bone formation without any remnants of particles. This should be a true advantage in implant surgery, where bone (and not a composite bone-hydroxyapatite tissue) is needed for osseointegration.

Natural coral skeleton (NCS) is a resorbable bone graft substitute that has been tested experimentally[5,6] and clinically for treating infrabony periodontal defects.[3,4] Chemically, NCS is 98% calcium carbonate and 1% oligo elements in the form of aragonite crystals.[5] It is biocompatible, highly osteoconductive, and its gradual resorption is accompanied by bone formation.[5,6,9] Polyglactin 910 is a resorbable polymer made of 10% lactic acid and 90% polyglycolic acid. It is synthesized by copolymerization of a mixture of purified lactide and glycolide. As a suture material, it is completely absorbed within 60 days, while membranes are reported to be broken down in 30 to 60 days. Lundgren et al[27] showed that a membrane barrier of polyglactin 910 woven mesh began to disintegrate in 14 days, and limited the epithelial downgrowth to the coronal third of periodontal bone defects in dogs. However, the time required for this material to disintegrate when used as a protective mesh for a bone graft substitute remains to be determined.

Conclusion

Rat calvaria bone formation is slightly enhanced by grafting with natural coral in a critical sized defect when compared to an untreated control defect. Bone formation is significantly enhanced when the graft particles are retained within the defect with a protective net of polymer mesh. This approach could be used in clinical situations such as dehiscences around implants, bone regeneration before implantation, and repair of periodontal infrabony defects.

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