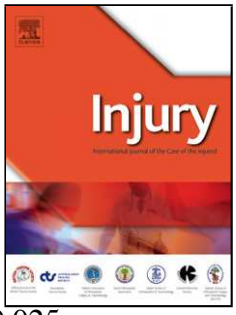
The authors review the literature on use of coral materials in bone repair.
The find that hydroxyapatite-based coral materials perform well. These are the GraftIT-type materials.
But Calcium Carbonate-based materials perform poorly.

Article:
Pountos, I and Giannoudis, PV (2016) Is there a role of coral bone substitutes in bone repair? Injury, 47 (12). pp. 2606-2613. ISSN 0020-1383

https://doi.org/10.1016/j.injury.2016.10.025



Title: Is there a role of coral bone substitutes in bone repair?

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PII: S0020-1383(16)30675-1
DOI: http://dx.doi.org/doi:10.1016/j.injury.2016.10.025
Reference: JINJ 6952

To appear in: *Injury, Int. J. Care Injured*

Please cite this article as: Pountos Ippokratis, Giannoudis Peter V.Is there a role of coral bone substitutes in bone repair?.*Injury* http://dx.doi.org/10.1016/j.injury.2016.10.025

Is There a Role of Coral Bone Substitutes in Bone Repair?

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Abstract

Xenogeneic bone graft materials are an alternative to autologous bone grafting. Among such implants, coralline-derived bone grafts substitutes have a long track record as safe, biocompatible and osteoconductive graft materials. In this review, we present the available literature surrounding their use with special focus on the commercially available graft materials. Corals thanks to their chemical and structural characteristics similar to those of the human cancellous bone have shown great potential but clinical data presented to date is ambiguous with both positive and negative outcomes reported. Correct formulation and design of the graft to ensure adequate osteo-activity and resorption appears intrinsic to a successful outcome.

Keywords: Corals, mesenchymal stem cells, scaffold, bone healing, growth factors

Introduction

Bone grafting is the most common transplant procedure performed today. It is estimated that approximately 450,000 bone transplantation procedures are performed annually in the USA and 2.2 million worldwide.[1] Autologous bone grafting has all the properties of the ideal graft material, being an osteoinductive and osteoconductive scaffold with no immunogenicity and containing significant numbers of osteoprogenitor cells.[2,3] However, its use has several drawbacks including limited availability, variable graft quality, increased operative time and donor site morbidity.[4] To overcome the increasing need for bone graft materials, research has focused on the development of novel bone graft substitutes.[5,6] A large number of substitutes have been developed and a significant number are commercially available for clinical use.

Bone graft biomaterials derived from mineralizing marine organisms have been vividly investigated over the last 50 years. Several marine species produce mineralized structures within their anatomy that resembles the human bone.[7] Examples of such species include sponges (Porifera), red algae (Rhadophyta), corals (Cnidarians) and a range of other organisms like snails (Mollusca), starfish

(Echinodermata) etc.[7] Among such marine derived biomaterials, corals are one of the most studied in the field of bone tissue engineering. The aim of the herein manuscript is to present the available literature on coral bone substitutes.

Corals as graft material

Corals are marine invertebrates belonging in the class Anthozoa of phylum Cnidaria. They are approximately 7 thousand species and can be classified as soft corals (without an inorganic structure) and hard corals or stony corals. The hard corals typically live in compact colonies of many identical individual polyps. The polyps reside in a centripetal exoskeleton. The outer layer of the corals is inhabited by calcicoblasts, which like the osteoblasts they produce a hard outer skeleton composed of calcium carbonate which, strengthens and protect the organism.

Studies on the coralline structure revealed significant similarities to that of cancellous bone.[8] The coralline material is characterized by a uniform network of interconnected channels and pores similar to those in osteon-evacuated bone grafts.[8,9] When implanted in-vivo was found to be biocompatible. It allowed vascular ingrowth and inhabitation of cell lineages found in bone. The new bone formation occurred without an intervening endochondral phase.[8] Resorption of the corals is carried out by osteoclastic activity and the actions of the carbonic anhydrase enzyme.[10] Resorption is linked to bone apposition and can be influenced by the systemic administration of acetazolamide, a diuretic inhibiting carbonic anhydrase.[10,11] Among the different coral species, significant structural differences exist. This could have direct implications to their bone forming capacity. It has been previously proposed that the larger the porosity volume, the greater was the coral resorption as well as the new bone apposition.[12] Three main species have been investigated as bone graft substitutes: Acropora sp., Goniopora sp., and Porites sp. Porites sp. have a homogeneous structure and consistent pore size while Goniopora sp. have a bimodal pore size and a strongly disordered structure.[12,13] Acropora have oriented

pores, irregular pore size and the largest permeability compared to Goniopora and Porites sp.[13] Their transverse section however, was closed and the useful size was limited because of its habitat type.[13] Porites had the smallest pore size and had the lowest permeability. Other coral genera have been previously investigated but with very limited use.[14,15,16] Among them, Dichocoenia stokes were found to trigger a foreign-body reaction when implanted in rabbits.[14] These corals were also found to have slow resorption rates.[15] Facites and Lobophyllia and Pocillopora have a skeletal structure similar to the diaphysis of compact bone with a dense and compact outer wall (theca) surrounded by a thin inner septa (closed porosity).[16,17] Other coral genera exist like the Montipora, Fungia, Polyphyllia, Acanthastrea, and Turbinaria but our current available evidence on these corals is rather poor or non-existent.

In the early 70s, observations suggesting that porous structures have improved bone integration sparkled a race towards the ideal bone graft substitute.[18] The foundations of stony corals as biomaterials have been set a few years later by the work of White et al.[19] White et al. proposed the replamineform technique (replicated life forms) which could be used to duplicate the coral carbonate microstructure and convert it to ceramic, metal, or polymer materials. Utilizing this technique the unique coral pore structures composed of the brittle calcium carbonate could be preserved and copied to produce an alternative material with the same structure but converted to hydroxyapatite. In addition to the converted form, corals have been used in their natural form i.e. as calcium carbonate. The bone formation of both calcium carbonate and hydroxyapatite occurred initially on surface of the pore regions and progressed toward the center of the pore and was linked to graft resoption.[20] At present there are two commercially available corals: the Biocoral ® composed of corals on their natural form and Pro Osteon™ composed of coralline material converted to hydroxyapatite.

Experimental Studies

I. In-vitro studies

The vast majority of the available in-vitro studies have analysed the biocompatibility between the corals and the osteoprogenitor cells. Scaffolds derived from corals should be able to support the attachment, proliferation and differentiation of Mesenchymal Stem Cells (MSCs) and osteoblasts.[21] The available studies showed that the corals are not cytotoxic and promote cell growth.[22] When cells were seeded on coral granules revealed good attachment, spread, and proliferation on the material surface.[23] Comparing cryopreserved bone allograft, coralline hydroxyapatite and demineralized freeze-dried dentin revealed that coralline hydroxyapatite was the most potent promoter of the long term cellular attachment.[24] In a similar study including commercially available graft products, Doherty et al. compared the levels of cellular attachment of rat bone, Surgibone® , Ostilit® , Biocoral® and Tisseel®.[25] The results showed that rat bone and Tisseel® (fibrin glue) had the greatest cell affinity followed by Biocoral® and Surgibone®, while Ostilit® did not facilitate cellular attachment.

Following osteogenic induction, mineralized matrix and alkaline phosphatase activity was noted within the coral particles.[23,26] DNA content, ALP activity, Ca content were significantly higher in osteoblasts seeded in coral scaffold in comparison to other materials.[26] Mineralized nodules formation (both in area and number) was more predominant on the coral surface than in glass disk.[26] Gene expression analysis of osteoblasts loaded on coral Porites sp. scaffolds showed an increased expression of the RUNX2, osteopontin, alkaline phosphatase and osteocalcin genes. The authors concluded that coral is a favourable carrier for osteogenetically competent cells to attach and remain viable.[27] In another study significantly higher levels of osteogenic differentiation markers, namely alkaline phosphatase (ALP), Osteocalcin (OC) levels, and Osteonectin and Runx2, Integrin gene expression were detected in the cultures on corals (Porites sp) in comparison to bone.[28]

A number of authors have tried to expand corals properties with the addition of an osteoinductive element. Coral particles are capable to absorb and subsequent elute transforming growth factor beta 1 (TGF-beta1) in vitro.[29,30,31] TGF-beta1 release was also found to vary with particle size, higher release being obtained with the smaller particles.[29] In a study by Zhang et al. a coral/chitosan composite was

combined with a plasmid encoding platelet-derived growth factor B (PDGF-B) gene. The resulted scaffold found to upregulate the proliferation and the PDGF-B expression of the seeded cells.[30] Combinations of platelet-rich plasma (PRP), marrow stromal cells (MSCs) and porous coral have shown to exert a higher osteogenic effect.[31]

II. Animal Studies

The available evidence based on experimental animal studies which explore the potential of coralline grafts to support bone healing can be subdivided in three distinct methodologies; studies where the coralline grafts have been implanted in ectopic places, studies where coralline material implanted on bone in cases of fracture healing or bony defects site and finally composite coralline grafts preloaded with growth factors in applications including bone defects spinal fusion.

Ectopically implanted coral material seem to be biocompatible but inner without inducing an osteogenic response.[32] Once an osteoinducing signal is added either in the form of osteogenic cells or growth factors, bone formation is initiated.[32,33,34] The structural characteristics and the degree of bone formation was found to be linked to the resorption of the calcium carbonate corals.[32,35] Such approach can result in the construction of material of predesigned shape with structure similar to the native bone.[33,36] This strategy can be utilized to fabricate pre-vascularized tissue engineered bone grafts.[37] Such grafts can have a predetermined shape, organized internal vascular network with a vascular pedicle attached to the graft.[37] Furthermore, comparative studies have highlighted that new bone formation was higher in the Porites coral and Acropora coral than in either the beta-tricalcium phosphate or the banked bone constructs.[32] Analyzing further the way that bone formation occurs within the corals it is of interest to mention the work of Ripamonti et al. group.[38,39] A partially converted corral, composed of 7% hydroxyapatite and calcium carbonate was preloaded with verapamil (calcium channel blocker) or bipsosphonate zoledronate (osteoclast inhibitor) and implanted intramuscularly in baboons.[38] The

results showed that the inhibition of movement of calcium and osteoclastic functions strongly inhibited the induction of bone formation. BMP-2 downregulation with the up-regulation of Noggin genes was noted indicating that the induction of bone formation by coral-derived macroporous constructs is via the BMPs pathway. The same group, has also shown that if the same coral material is loaded with hTGF-β3 both the adjacent muscle and the macroporous bioreactor show upregulation of BMP-2 up-regulation.[39] This finding correlates with the observation of bone formation occurring at the periphery of the graft but also could be the result of the recruiting of osteoprogenitor cells from the adjacent soft tissues.

Coralline graft material implanted adjacent to bone in the treatment of bone defects has been analysed by a number of authors.[40,41,42] Intra-bony defects in dogs treated with either coralline calcium carbonate graft (Biocoral®) or autologous bone showed no difference in terms of healing.[41] In osteochondral defects, application of Biocoral ® resulted in bone ingrowth associated with graft resorption and noticeably enhance the overall healing of the defect. Intra-articular defects filled with coralline hydroxyapatite had no adverse effects to the joint environment in comparison to other graft materials that can generate inflammation of the synovium and damage the cartilage when their particles are released in the joint.[40] The coralline hydroxyapatite graft was found to be surrounded by new bone but there was minimal resorption of the graft. In another study, the bone ingrowth of a coralline hydroxyapatite material (Interpore 500) at 1 years post-implantation was found to be limited to 66.5 % of the surface of the graft raising concerns over its overall resorption.[43] Poor results have been also reported when hydroxyapatite granules (Pro Osteon 200™) were used around porous coated metal implants.[7] The results showed that the grafted implants were largely encapsulated in fibrous tissue and the addition of concentrated autologous bone marrow did not change the outcome.

Composite grafts composed of coralline material and growth factors or cells has been utilized by a number of authors.[44-52] Combinations of coral graft, BMPs and osteoprogenitor cells have shown potent bone healing potential which was comparable to the autologous bone grafting.[44,45,46,48,49] The

cellular component of the composite graft originate in the vast majority from bone marrow. However, osteogenically induced adipose tissue stem cells have been utilized with favorable results.[45] Transfected cellular lines with vascular endothelial growth factor resulted in enhanced vascularization and resorption of the coralline graft and a higher osteogenic response.[52] In single-level posterolateral lumbar arthrodesis performed in 48 adult New Zealand White rabbits, the combination of BMPs and coralline hydroxyapatite resulted in 100% fusion rates.[53] This was in contrast to the groups receiving coralline hydroxyapatite with bone marrow (0% union rates) and the coralline hydroxyapatite with autogenous iliac crest bone (50% union rates). The authors concluded that when coralline grafts were combined with autogenous iliac crest bone graft served as a graft extender yielding results comparable to those obtained with autograft alone.[53] In addition to BMPs other molecules have been investigated. In a comparative study of Insulin growth factor-1 (IGF-1) and BMP-2, IGF-1 was more potent inducer of bone regeneration when loaded on a coralline hydroxyapatite scaffold for the treatment of proximal tibial defects.[50] Platelet rich plasma was found to significantly upregulate the bone healing process when loaded on corals for the treatment of radial diaphyseal critical size defects.[47,51] Cylindrical calcium carbonate implants loaded with bovine-derived bone proteins were used in the treatment of a canine segmental bone defects.[54] The results revealed healing of the defect with total resorption of the coralline material at 12 weeks following implantation. It also highlighted the absence of union in the control group representing the coralline implants alone. Contradictory results though have been reported. In segmental tibial defects in sheep, composite grafts composed of calcium carbonate (Biocoral), BMP and IV collagen resulted in a large amount of callus compared to the coral alone with no significant difference in the mechanical strength of the resulted bone.[55] This study however highlighted a statistically significant increase in the detectable ant-BMP antibody, suggesting an underlying immunogenic reaction.

Commercially available Corals

i **Pro Osteo ™ (former Interpore, Biomet, USA)**

Pro Osteon™ is a graft substitute derived from Goniopora or Porites corals. It is fabricated utilizing a replamineform process, which involves the conversion of the calcium carbonate exoskeleton to a crystalline hydroxyapatite replica. In this process all the organic material of the corals are extracted and the microarchitecture is preserved. The result is a graft material with longitudinal pores of 500-600 microns and interconnecting pores of 220-260 microns in diameter.[56] Pro Osteon™ comes in two varieties; Pro Osteon 500 ™ and 200 with the number following the trade name designating the nominal pore diameter.

More recently, a resorbable version of this graft has been developed. This new product utilizes the replamineform process producing a composite of calcium phosphate and calcium carbonate. This composite graft has an outer layer of calcium phosphate while the core of the material remains as calcium carbonate. Therefore, due to the fact that calcium carbonate can be resorbed faster than calcium phosphate, the graft can facilitate the remodeling allowing more effective bone ingrowth within the graft material.

There have been a number of clinical studies analyzing the effectives of Pro Osteon™ in a range of clinical applications **[Table 1].**[56-73] The majority of the studies involve cases of periodontal and maxillofacial defects. These studies revealed the presence of new bone formation, integration of the implant with reduction in the defect size.[57,58,59,60] A poor resorption of the implant was highlighted in some studies.[61]

In 10 cases of hindfoot arthrodesis the application of Pro Osteon 500™ had satisfactory results with one case of nonunion.[62] The group reported on the poor resorption of the graft and the difficulties they faced to contain the graft material and the asymptomatic extrusion of the graft in all the cases. In tibial plateau fractures, no difference was noted between the cases treated with Pro Osteon™ and those with autologous bone graft.[63]

In cases of spinal fusion the results were mixed. In one study of idiopathic scoliosis surgery the utilization of coralline hydroxyapatite resulted in fusion in all 27 patients.[64] The authors reported on the 'marbilized' appearance of the grafts. In another study of 40 cases of posterolateral lumbar fusion augmented with Pro Osteon 500™, a union rate of 92.5% was noted.[65] In 13 cases of revision following spinal surgery where hydroxyapatite was used, foreign-body like giant cells and the development of inflammatory granulation tissue around graft was noted.[66] In a study of 60 cases of instrumented posterolateral lumbar and lumbosacral fusion using either Pro Osteon 500 R™ or iliac bone graft or both, there were no cases of non-union with complete resorption 1 year postoperatively.[67] It was also highlighted that the incorporation of coralline hydroxyapatite mixed with local bone and bone marrow needs adequate bleeding bone surface. Pro Osteon 500R™ use was found to be inappropriate for intertransverse posterolateral fusion, because the host bone in this area is little. However, the use of hydroxyapatite over the decorticated laminae that represents a wide host area was followed by solid dorsal fusion within the expected time.[67]

ii **Biocoral ® (Inoteb, Saint-Gonnery, France)**

Biocoral ® is a coral-derived bone graft in its natural pure form composed of 99% calcium carbonate and the remaining ~1% includes simple amino acids.[11] It undergoes minimal processing to remove potential contaminants and preserves the original morphology and chemistry. Acropora genera obtained from the French part of the Great Barrier Reef in New Caledonia is used for this product.[67]

Clinical studies utilizing Biocoral ® have shown mixed results.[74-83] Early studies have utilized Biocoral ® in the treatment of bony maxillofacial defects. Of interest is the study of Roux et al. presented the outcome of this product in 183 patients.[74] They reported that the coral block moved or was partly resorbed and split into pieces after 7 to 36 months in 20% of cases. At 1 year 40 to 50% resorption rate was noted and the overall infection rate was 4%. In another study when Biocoral® was implanted in the anterior maxilla a high revision rate was observed (83% revision rate) in contrast to posterior maxilla

and mandible (6% revision rate). [75] In cervical fusion a poor fusion rate of 45 % and 60% has been reported in two studies of 48 and 40 patients.[76,77] In scaphoid fractures, the utilization of a composite graft composed of Biocoral®, BMP and collagen resulted in a high failure rate of 80%.[78] The use of the same implant however, in 4 diaphyseal and one olecranon ulnar non-union resulted in successful consolidation in all cases.[79] Finally, in iliac crest defects treated with Biocoral®, a poor bone ingrowth was observed only in biopsies at one year of follow-up.[80]

Discussion and Future directions

An ideal bone graft substitute should be osteoconductive, inert, readily available and adaptable in terms of size and shape. It should also be biodegradable, to allow bone ingrowth and provide structural support. Corals pose several of the aforementioned properties. Coral structure is similar to cancellous bone and one of the few xenogeneic materials that can form chemical bonds with bone in-vivo. Coral based biomaterial could overcome the drawbacks of autologous bone grafting.

Coralline calcium carbonate based materials were considered to have a high-resolution rate, poor longevity and stability. They rely on bone ingrowth for structural support and predominately they were used to fill well-contained voids. The available literature utilizing calcium carbonate grafts for fracture healing is rather limited. Their resorption is unpredictable with some authors reporting full resorption while in other studies the resorption was poor. In cases of scaphoid fracture non-union, treated with composites of calcium carbonate coral, collagen and bone morphogenetic protein, poor results have been documented.[78] The authors stated that in such avascular conditions the coral did not resorb adequately and acted as a barrier between the two bone parts obstructing the healing process. In a later study by the same group, complete union was achieved utilizing the same composite graft in 5 ulnar non-unions.[79]

To overcome the weaknesses of calcium carbonate, conversion of the calcium carbonate to hydroxyapatite has been performed. This procedure preserves the porous structure of the corals and in

theory delays the resorption of the graft. Unfortunately, this new material is either slowly resorbed or considered by some as permanent. In animal models, White et al. highlighted that the resorption rate varies between 0 to 5% per year.[56] Several authors have also raised concerns in terms of the slow resorption of coralline hydroxyapatite.[62] Coughlin et al. analysed the clinical outcome of 10 patients treated with hindfoot arthrodesis with the application of Pro Osteon 500™.[62] The authors reported a case of nonunion but satisfactory results in the remaining patients, and highlighted the difficulties to contain the graft material with asymptomatic extrusion of the graft in all the cases. They also raised concerns regarding the slow resorption rates and the presence of the graft material 6 years following implantation.

The permanent nature of the coralline hydroxyapatite has triggered the development of a 'resorbable' version of the implant. For the fabrication of this implant the same replamineform process was utilized, however, only partial with conversion.[90] The new implant is composed of coralline hydroxyapatite limited to 2 to 10 microns on the outer surface and has an unconverted inner core which remains as calcium carbonate.[90] The aim theoretically is a more resorbable implant but also represents a concern as this is an unpredictable factor in terms of the graft properties and overall function in-vivo. The available literature is limited and the full potential of this construct is yet to be elucidated.

Coralline graft substitutes have several other disadvantages. Their effectiveness seems to be influenced by the anatomic site of implantation. As mentioned before in areas of poor blood supply they seem to produce poor results. The anatomic location also seems to influence the results possibly related to the overall local vascularity.

Another major disadvantage of the coral material is the initial mechanical weakness. Once bone in-growth occurs the mechanical stability improves. It is characteristic that the compressive strength of corals could be as low as 2.62MPa when the one of bone is between 131 and 283 MPa.[13] In this context, FDA has issued warnings for one of the commercially available corals.[91] Briefly the Pro Osteon™ use is contraindicated in segmental defects, fracture of the growth plate, in patients with systemic or

metabolic disorders affecting bone healing, in vascularly impaired bone, in infected sites or in cases when soft tissue coverage is not possible and finally cases where stabilization of the fracture cannot be attained. [91] In addition, FDA database clearly indicates that Pro Osteon™ does not pose sufficient mechanical strength to support fracture reduction and relies on bone ingrowth to stabilize the defect site. [91]

Even if the above-mentioned issues are addressed, corals can be considered a viable solution as a bone graft material only if they are sustainable and with minimal environmental impact.[92] Porities and Goniopora corals that are used for the commercially available products derive from corals of the Pacific and Indian Oceans. These corals are not classed as endangered, however, their overexploitation together with the environmental changes, ocean warming and acidification could put them at risk. Furthermore, some authors highlighted the negative effect or even complete cessation of the overall calcification that the rising water temperature and acidity has on these corals.[13,93,94] In addition, a substantial decrease in the coral reefs has been noted since 1990 and it is expected that approximately 50% of the reefs will be destroyed by 2030.[13] These data add to the overall uncertainty when planning to explore the utilization of the corals further.

Despite all the aforementioned concerns, we believe that some coral derived biomaterials are good void fillers with distinct role in our armamentarium. Their utilization should be performed with prior knowledge of the properties of each different product. The fact that they are inner osteoconductive material, safe from a disease transmission point of view, and also the need to incorporate an osteoinductive signal to safeguard the overall success, is an undisputable strength. As far as the coralline hydroxyapatite is concerned, this should be considered as a permanent implant, the effectiveness of the partially converted analogue would require further investigation in terms of their overall effectiveness and properties in clinical applications. Tissue engineering approaches with graft supplementation with different osteogenic cells, bone marrow, platelet rich plasma and a number of growth factors is

promising but the ideal combination enhancing the neoangiogenesis and osteogenesis needs further clarification.

Conclusions

Research is ongoing on strategies how to enhance and optimize bone repair strategies.[95-100] Ongoing research Coralline-derived bone grafts are safe, inert osteoconductive material, which are readily available in nature. Their highly porous structure is similar to cancellous bone. Raw coralline graft products are brittle, lack mechanical strength and are resorbed by the host fast. The conversion to hydroxyapatite diminishes the resorption of the graft making it a permanent implant. Our current clinical evidence is limited to well-contained voids in dental and maxillofacial surgery. Some authors report good clinical results, yet others reported devastating poor outcomes. Until further clarification and development of new coral based implants that address the short-comings of the current materials the utilization of such material should be limited to well contained, well vascularized defects, bearing into consideration the potential permanent nature of the this graft material.

Conflict of Interest:
No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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Table 1. Clinical studies analyzing the outcome of Pro Osteon™ in patients.

Study/ Year	Participants	Condition site	Study characteristics	Outcome
Krejci et al., 1987 [56]	12 pts	Periondontal angular osseous defects	Each patient had 3 defects, one filled with Pro Osteon 200™, one with OrthoMatrix HA-500 and one unfilled	• While the defect sites improved with respect to plaque index, probing depth measurements, and defect fill, only those treated with the nonporous OrthoMatrix HA-500 hydroxyapatite revealed a statistically significant improvement treatment modalities.
Bucholz et al., 1989 [63]	49 pts	Closed Tibial plateau fractures	RCT, 20 pts treated with Pro-Osteon™, 20 patients with autograft from Iliac crest, 9 lost in FU	• No significant differences in the two groups • Interporous hydroxyapatite is a safe, effective alternative to autogenous cancellous bone for the filling of metaphyseal defects associated with Tibial plateau fractures.
Salyer et al., 1989 [70]	25 pts	Maxillofacial deformities	Non-randomised, 17 pts treated with Pro Osteon 200™, 8 pts with autograft	• No difference in length of stay, clinical function, complications and aesthetic performance
Oreamuno et al., 1990 [57]	24 pts	Periondontal angular osseous defects	The defects were randomly filled with either Pro Osteon™ or decalcified freeze-dried bone	• Pro Osteon produced greater reduction in pocket depth and higher attachment levels and defect fill
Hjorting-Hansen et al., 1990 [59]	22 pts	Periondontal osseous defects	Bone biopsies and histologic examination of Interpore 200™	• New bone formation was notes within the grafts.
Small et al., 1993 [60]	27 pts	Maxillary sinus augmentation	Graft material composed of Interpore 200™ and demineralized cortical bone	• Integration noted in all implants
Byrd et al., 1993 [61]	43 pts	Craniofacial bone augmentation	52 sites in 43 patients for the aesthetic correction of congenital or posttraumatic deformities	• Resorption not occurred, no cases of infection, 2 patients required revision
Nicolaides et al., 2000 [72]	2 pts	Open supracondylar osteotomies	Treatment of valgus deformities using coral wedge	• No complications with complete incorporation of the graft
Irwin et al. 2001 [71]	71 pts	Bone defects derived from excision of tumours	Retrospective review of consecutive patients managed with coralline hydroxyapatite Pro-Osteon 500™	• Complications encountered in 12 patients (3 major and 9 minor complications) • Pro-Osteon 500™ is a viable option for the management of bone defects in orthopaedic oncology.

Thalgott et al., 2001 [65]	40 pts	Lumbar fusion	Retrospective series of 40 patients undergoing instrumented autogenous posterolateral lumbar fusion augmented with Pro Osteon 500 ™	• An overall fusion rate of 92.5% was achieved • Coralline hydroxyapatite is an effective bone graft extender in difficult-to-fuse patients
Thalgott et al., 1999 [69]	26 pts	Cervical fusion	Retrospective, 26 patients anterior discectomy and reconstruction from C3 to T1	• No evidence of plate breakage, screw breakage, resorption of the implant, or pseudarthrosis. • There was no evidence of nonunion.
Mashoof et al., 2002 [64]	27 pts	Adolescent idiopathic scoliosis	Consecutive patients, 70/30 ratio of coralline hydroxyapatite to autograft	• All patients achieved solid fusion at an average follow-up of 27 months. • Coralline hydroxyapatite is safe, biocompatible, and effective in augmenting autogenous bone graft
Korovessis et al., 2002 [66]	13pt	Cervical, thoracic, lumbar spine fusion	Biopsies during revision surgery	• Foreign-body like giant cells & development of inflammatory granulation tissue around hydroxyapatite • Bone formation was observed in 11/15 cases
Korovessis et al., 2005 [67]	60	Lumbar spine fusion	Prospective randomized study, 3 Groups: Pro Osteon 500 R™ vs Iliac Crest graft vs both	• No radiological evidence of non-union • The resorption of hydroxyapatite was completed 1 year postoperatively.
Coughlin et al., 2006 [62]	10	Hindfoot arthrodesis	Retrospective review, 6 years FU	• One case of non-union • Extrusion of the graft from the joint occurred in all patients
Wasielewski et al. 2008 [71]	17 pts	Complex acetabular reconstruction	Retrospective review of patients who underwent acetabular revision using Pro Osteon 500™	• No cups required re-revision, but 1 had failed. • Radiographic evidence of bone incorporation was observed in every coralline hydroxyapatite graft. • No graft resorption was observed.

RCT: Randomised Controlled Study, FU: Follow-up, Pts: Patients

Table 2. Clinical studies analyzing the outcome of Biocoral® in patients.

Study/ Year	Participants	Condition site	Study characteristics	Outcome
Marchac et al., 1994 [11]	36 pts	Craniofacial osseous contour defects	36 consecutive patients requiring correction of 54 minor bony contour defects	• 5 sites of clinically evident resorption • 2 incidences of wound irritation • 1 case of infection
Roux et al., 1995 [74]	183 pts	Cranial base reconstruction	587 Madreporic Coral grafts as bone substitute	• In 20% of cases the coral block moved or was partly resorbed and split into pieces after 7 to 36 months • 40 to 50% resorption of their volume after a year or more • The local infection rate was only 4%
Piattelli et al., 1996 [82]	6 pts	Deficient alveolar ridges	Biocoral® gel particles in connection with expanded polytetrafluoroethylene membranes	• At 6 months Biocoral® particles were still present and almost all were completely surrounded by mature bone
Yukna et al., 1998[75]	21 pts	Dento-alverolar defects	48 augmentation sites (Biocoral® or bone graft)	• 2 implants failed to osseo-integrate • One case of infection with resorption of coral granules was observed in the anterior maxilla. • When Biocoral® placed in anterior maxilla a high revision rate was observed (83% revision rate) in contrast to posterior maxilla and mandible (6% revision rate)
Bizette et al., 1999 [76]	48 pts	Cervical fusion	Retrospective review of cases	• Clinical improvement in 52% of pts • Fusion rate 60%
Vuola et al., 2000 [80]	10 pts	Iliac crest defects	Biopsies performed at 1 year	• All the blocks still detectable at 2.1 years. • Bone ingrowth could be observed only in two out of seven biopsies. • One implant had to be removed after 1.7 years due to infection.
Kujula et al., 2002 [78]	10	Scaphoid fractures	BMP/coral/collagen composite implant	• 80% failure of union
Kujala et al., 2004 [79]	5 pts	4 Diaphyseal and one olecranon ulnar non-unions	BMP/coral implant combined with internal fixation. Additional autografting was used in three cases.	• Solid union was achieved in all cases. • No adverse effects were encountered.
Scarano et al., 2006 [81]	94 pts	Maxillary sinus Augmentation	Histological examination of biopsy performed 6 months after implantation.	• No inflammatory cell infiltrate was present • Graft particles appeared to be fused by newly formed bone • Areas of resorption were present at the surface of some graft particles
Ramzi et al., 2008 [76]	40 pts	Cervical fusion	Prospective study, Anterior cervical fusion	• 45% fusion rate at 44 months (22 out of 40 patients not fused)

RCT: Randomised Controlled Study, FU: Follow-up, Pts: Patients