JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 13 (2002) 509–515

Physical and mechanical properties evaluation of *Acropora palmata* coralline species for bone substitution applications

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The search for ideal materials for bone substitution has been a challenge for many decades. Numerous natural and synthetic materials have been studied. For this application, exoskeletons of coral have been considered a good alternative given its tendency to resorption, biocompatibility and similarity to the mineral bone phase. Very few studies of these materials consider a detailed analysis of the structure–property relationship. The purpose of this work was to carry out the microstructural characterization of a coralline species named *Acropora palmata* and the determination of the mechanical and physico-chemical properties. Measurements of hardness, compressive strength, bulk density and apparent porosity were performed. From these results it was determined that this marine coral species could be an alternative xenograft due to its mechanical properties and osteoconductive nature.

1. Introduction

During the last 20 years, the search for satisfactory mineral materials to be used as bone substitutes has increased: Hydroxyapatite, tricalcium phosphate, bioactive glasses, among others. The search has been oriented to materials that exhibit a good physiological response and offers the additional advantage of excellent biocompatibility, endurability and osteoconductive properties. The ideal material should be susceptible to total resorption by the ossean cells and biocompatible with the natural bone. This material should be free of any substance of biological origin able to cause an inmunological response [1]. Thus, according to Bajpai [2], ''an ideal bone graft or substitute should be a material that is biologically inert, readily available, easily adaptable to the site in terms of shape and size, and replaceable by the host bone. Replacement of the scaffold by the host bone necessitates that the substitute be biodegradable''.

Exoskeletons of certain coralline species have been studied by Guillemin *et al.* [3–5] as a bone graft, acting as a scaffold for direct osteoblastic apposition showing high biocompatibility, and consequently presenting an interesting alternative as a xenograft.

Papacharalambous *et al.* [6] studied coral skeleton of *Acropora* and *Pocillopora* species, as onlay graft material for contour augmentation of the face. Patients were treated with coral blocks or granules. The implants were totally resorbed and in every case it was observed a new bone apposition. Roux *et al.* [7] used coral fragments *madrepora genera porites* as a bone graft substitute to repair skull defects. These study indicated that these materials appear to be very promising for use in cranial reconstructive surgery. All the implants were totally resorbed.

Quintana *et al.* [8] used hydroxiapatite obtained by chemical transformation of natural coral genera *Porites* to fill the alveolar cavities generated by post-dental

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extractions, and for contour mandibular augmentation. In 100% of the cases radiological osseointegration was observed.

Escobar *et al.* [9] experimented with two coral species genera *Porites* in guinea pigs as implant material for bone reconstruction. A rapid and satisfactory integration was observed, determining that these species are a good alternative as bone graft for large osseous defects; besides they exist in large amounts along the Venezuelan marine coast.

The purpose of this study was to evaluate the physico-chemical and mechanical properties, and to characterized the microstructure of a coralline *Acropora palmata* species.

2. Materials and methods

2.1. Chemical analysis

The corals were obtained from Turiamo Bay, Aragua, Venezuela. The content of calcium, magnesium and sodium was determined by atomic absorption spectroscopy (AAS) with a 1200 Varian Techtron Spectrometer. The sulfur and carbon concentration was estimated by Leco Combustion (LC: CS200) and the presence of minority elements was investigated by inductively coupled plasma spectrometry (ICPMS). The spectrometer used was a Perkin Elmder Optima 3000.

To perform the chemical analysis the samples were selectively cut with an abrasive cutter using a diamond disk. Samples from the external porous part and from the inner zone of the coral were analyzed. These specimens were identified as ''porous'' and ''compact'' samples, respectively. Both samples, the porous and the compact were ground in amortarand sieved with a 150 Tyler sieve.

2.2. Macro- and microstructural characterization

Macrographs of the coralline specimens were taken using a 50 mm macro lens. Transverse unpolished sections of the *Acropora* coral were coated with Pd–Pt in an Ion Sputter Hitachi E102 for examination in a scanning electron microscope (Hitachi–S2400 equipped with an EDS Kevex-IV) at an accelerating voltage of 20 kV.

The phases present were determined by the X-ray powder diffraction method, using a CuK_α radiation and a nickel filter, at 40 kV and 20 mA. The diffractometer used was a Siemens D5005.

2.3. Density and porosity tests

Bulk density was obtained following the method described in the standard ASTM C373-77 [10].

True density of the coralline samples was determined following the method specified in the standard ASTM C135-76 [11]. Three tests were performed for each value reported.

The apparent porosity was determined by the saturation method described in the standard ASTM C830-88 [12] which is based in the Archimedes' principle. This property was also measured using a helium porosimeter Core Labs. Inc., and a Hg porosimeter Ruska S-25 on cylindrical samples of 2.54 cm diameter and 3.80 cm height. The He pressure was in the range of 0.34–1.37 MPa and the confined pressure was 0.7 MPa. Both tests were performed at 25 °C.

The total porosity was calculated from Equation 1, [13]:

$$P_T = 1 - \frac{\rho_b}{\rho_t} \tag{1}$$

where P_T is the total porosity, ρ_b the bulk density and ρ_t the true density.

2.4. Permeability tests

Permeability tests of cylindrical cores of 2.54 cm diameter and 3.80 cm height were carried out in a pore permeability chamber PDPK 400, to determine the permeability values along the cylinder axis. The tests were performed using a N_2 pressure of 0.25 atm, and a N_2 flow of 80 cm^3/s, at a temperature of 22 °C.

Permeability of the corals samples was calculated using Darcy's law as expressed in Equation 2 [14]

$$k = \frac{QL\mu}{\Delta PA} \tag{2}$$

where Q is the volumetric flow rate, L is the specimen length, μ is the kinematic fluid viscosity, ΔP is the established pressure gradient across the specimen, and A is the specimen cross-sectional area. Three tests were carried out for each value reported.

2.5. Mechanical tests

Vickers micro hardness test were performed on six specimens, in a Shimadzu, M Type. At least five measurements were obtained from each sample in two perpendicular directions. The load used was 50 gf, applied during 15 s. The surfaces of the samples were mechanically polished with abrasive papers (No. 200–600) and alumina powder (0.3 μm was used for the final polishing.

Compression tests were performed using a Wykeham Farrance testing machine, following the method described in the standard ASTM C773-88 [15]. Eight cylindrical cores, each about 2 cm in length and 1 cm in diameter, were axially loaded to fracture. The applied

TABLE I Chemical analysis of porous and compact samples(% wt/wt)

Sample	Ca	Na	Mg	S	C	O	Minority elements
Porous	38.800	0.608	0.114	0.156	11.500	48.734	0.088
Compact	39.000	0.794	0.090	0.156	11.500	48.373	0.087

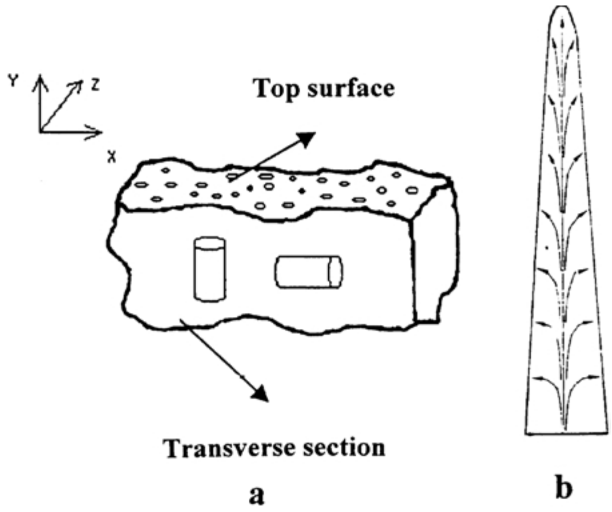

Figure 1 (a) Schematic drawing showing the *X*, *Y* directions of the compression test specimens from transverse section of the coral. (b) Skeletal architecture and direction of polyps growth as indicated by arrows.

load range was 200–400 kg at a strain rate 0.02 mm/min. The samples were taken in the *X* and *Y* directions from the transverse section of the coral, as shown in Fig. 1. Cores were machined to a tolerance of 0.01 mm to insure that the ends of the cores were flat and parallel enough to give meaningful results. The surfaces of the cylinders were analyzed by scanning electron microscopy (SEM) before and after the compression test.

3. Results and Discussion

3.1. Chemical analysis

Chemical analysis of ''porous'' and ''compact'' samples are shown in Table I and Table II. Both samples have a very similar composition. As expected, the chemical composition of the coral has as principal elements, O, Ca and C, and as minority elements Na, S and Mg. Trace elements were also determined as shown in Table II. The total amount of trace elements is less than 0.1 wt %. According to these results the coral should be considered free of harmful elements.

TABLE II ICP analysis of porous and compact samples

Element	Porous sample (% wt/wt)	Compact sample (% wt/wt)
Se	< 0.0010	< 0.0010
Mo	< 0.0010	< 0.0010
Zn	< 0.0010	< 0.0010
Cr	< 0.0010	< 0.0010
Sb	< 0.0010	< 0.0010
B	0.0334	0.0364
P	0.0021	< 0.0010
Pb	< 0.0010	< 0.0010
Ni	< 0.0010	< 0.0010
Bi	< 0.0010	< 0.0010
Cu	0.0021	< 0.0010
Co	< 0.0010	< 0.0010
Cd	< 0.0010	< 0.0010
Ba	< 0.0010	< 0.0010
Fe	0.0135	0.0082
Si	0.0059	< 0.0130
Mn	< 0.0010	< 0.0010
Ag	< 0.0010	< 0.0010
Ti	< 0.0010	< 0.0010
Al	0.0033	0.0009
Sr	> 0.010	> 0.010
Li	< 0.0010	< 0.0010
K	0.0024	0.0019
Σ	0.0877	0.0874

3.2. Density and porosity measurements

The mean values of the bulk density and true density of the *Acropora palmata* coral were $1.89 \pm 0.16\ g/cm^3$ and $2.64 \pm 0.02\ g/cm^3$, respectively. These values are in good agreement with those published by Hughes [16]. The value of bulk density reported [17] for the compact bone was $2.1\ g/cm^3$.

Similarity between the bulk density of a xenograft and bone is very desirable in order to keep the weight of the implant material as close as possible to that of the original bone.

The total porosity value was 28.65% calculated from Equation (1) using the values of bulk and true density reported above. The apparent porosity values were in the range of 27.71% to 30.56% when determined by the saturation method, in the range of 25.76% to 28.89% by the helium method, and $21.12 \pm 1.78\%$ by the Hg method. The values are, therefore, quite similar for the different methods. Values of porosity of 10% for the cortical bone and 50–90% for the trabecular bone have been reported [18]. Although the values of porosity for *Acropora palmata*, obtained in this work, are smaller than those of the trabecular bone, they still seem to be reasonable for trabecular bone substitution.

According to Guillemin *et al.* [3] coral resorption and new bone apposition depend mainly on porosity, their results show that the higher volume of porosity, the greater coral resorption and the new bone apposition.

3.3. Permeability tests

The permeability indicates the conductivity of a fluid through a porous material. This property is important since it is related to the degree of osteoconductivity of the material. Grimm *et al.* [19] determined the absolute permeability in 16 samples of trabecular human bone

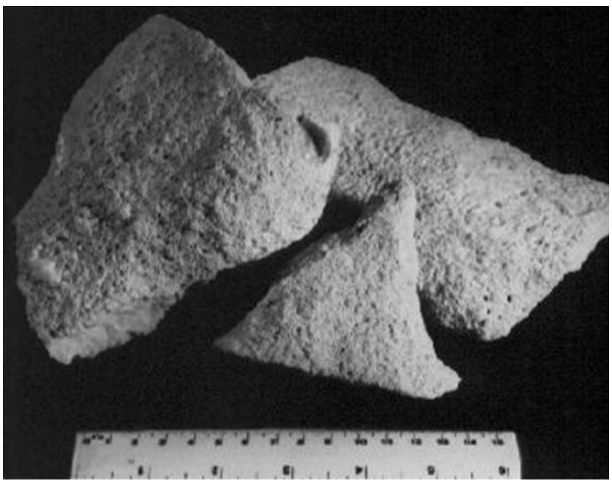

Figure 2 Macrograph from the external surface of the coral showing the porous aspect.

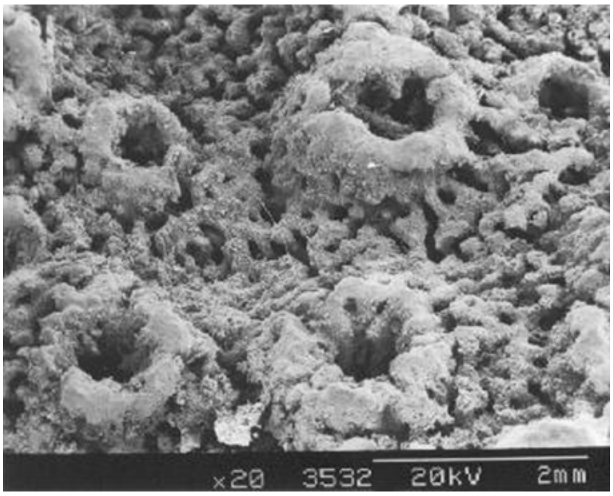

Figure 3 SEM micrograph of the externalsurface showing macroporous and interconnected channels.

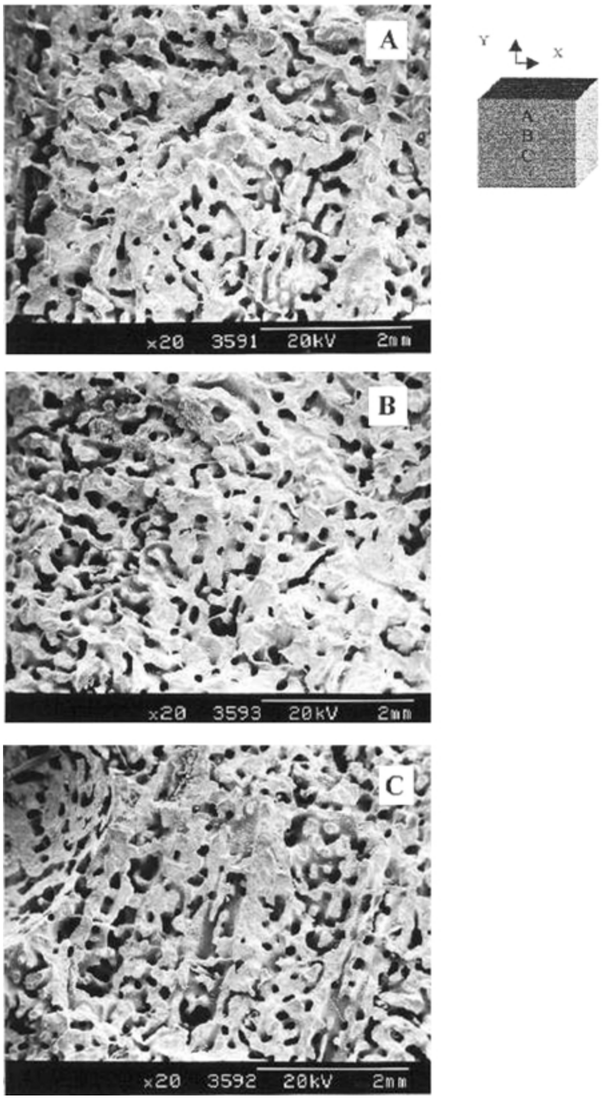

Figure 4 SEM photomicrographs showing a microstructural profile of the cross-section (A,B,C) of the *Acropora palmata* coral line species.

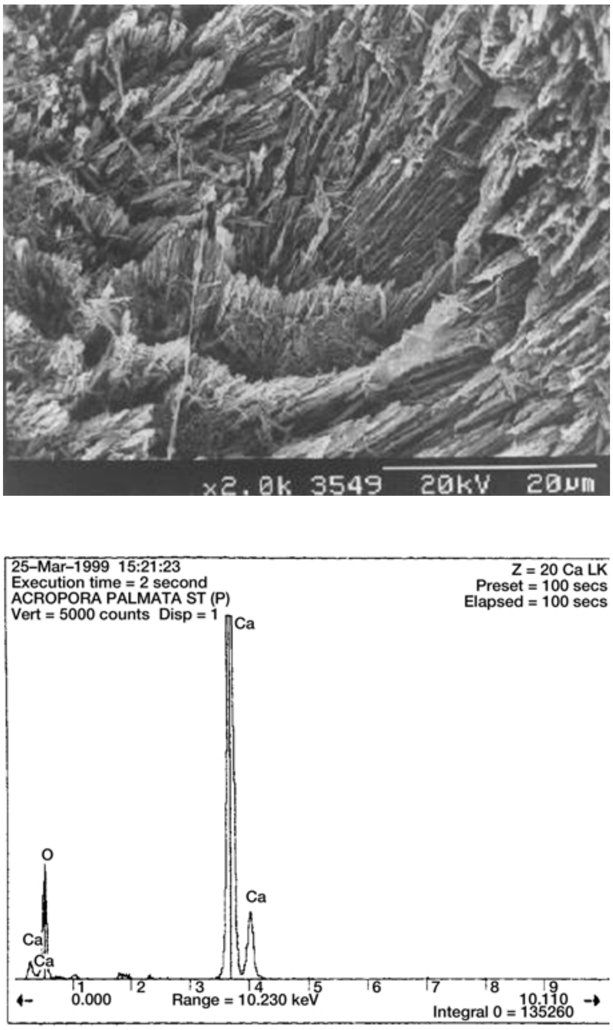

Figure 5 SEM micrograph from the transverse sections showing crystal orientation, and its corresponding EDS microanalysis.

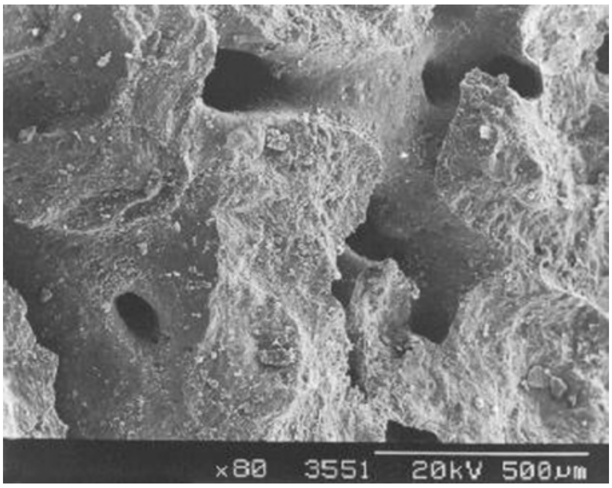

Figure 6 SEM micrograph from the transverse sections. The ''apparent compact'' zone around the pores is observed.

ranging in age from 32 to 89 years, and found that the values were between 0.4 and 11×10^{-9} m^2 (405–11145 D), which were strongly correlated to the specimen porosity (78–92%). The values of absolute permeability and Klinkerberg permeability obtained for the *Acropora palmata* were 0.376×10^{-11} and 0.369×10^{-11} m^2 (3.76 and 3.69 D), respectively. These values are significantly smaller than those reported for the bone. However, this was expected, taking into account that the values of the coral porosity (21–28%) are smaller than those of the trabecular bone. Even then, these values are still reasonable for bone graft, since the

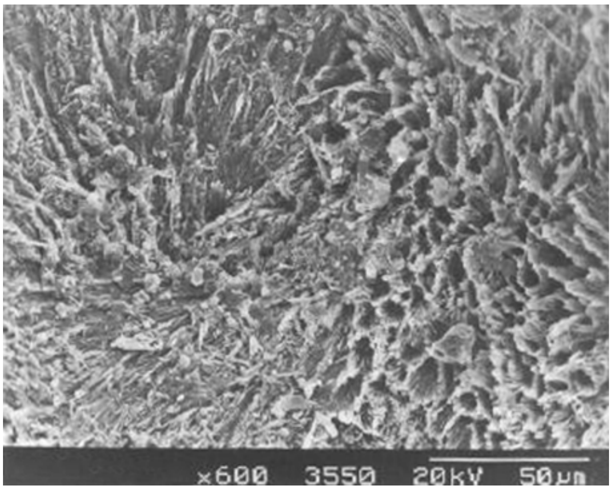

Figure 7 SEM micrograph showing a magnified detail of ''apparent compact'' zone. Small pores and crystals that grow in different directions are observed.

bone replacement acts only as a scaffold for direct osteoblastic apposition and only needs some degree of porosity to activate the bone apposition process.

3.4. Macro and microstructural characterization

Macrographs from the external surfaces of the coral are shown in Fig. 2, where the porous aspect of the coral is observed. The density number of macropores was between 12–16 pores/cm^2 for both, the superior and inferior faces and the maximum pore size observed was 3000 μm. These surfaces were also examined by SEM. Fig. 3 corresponds to a magnified region showing macropores surrounded by small interconnected channels.

A microstructural profile of the transverse section of the coral is shown in Fig. 4. Pores of different sizes and morphologies are observed: spherical (150–175 μm) and elongated (350–500 μm). The values obtained are very

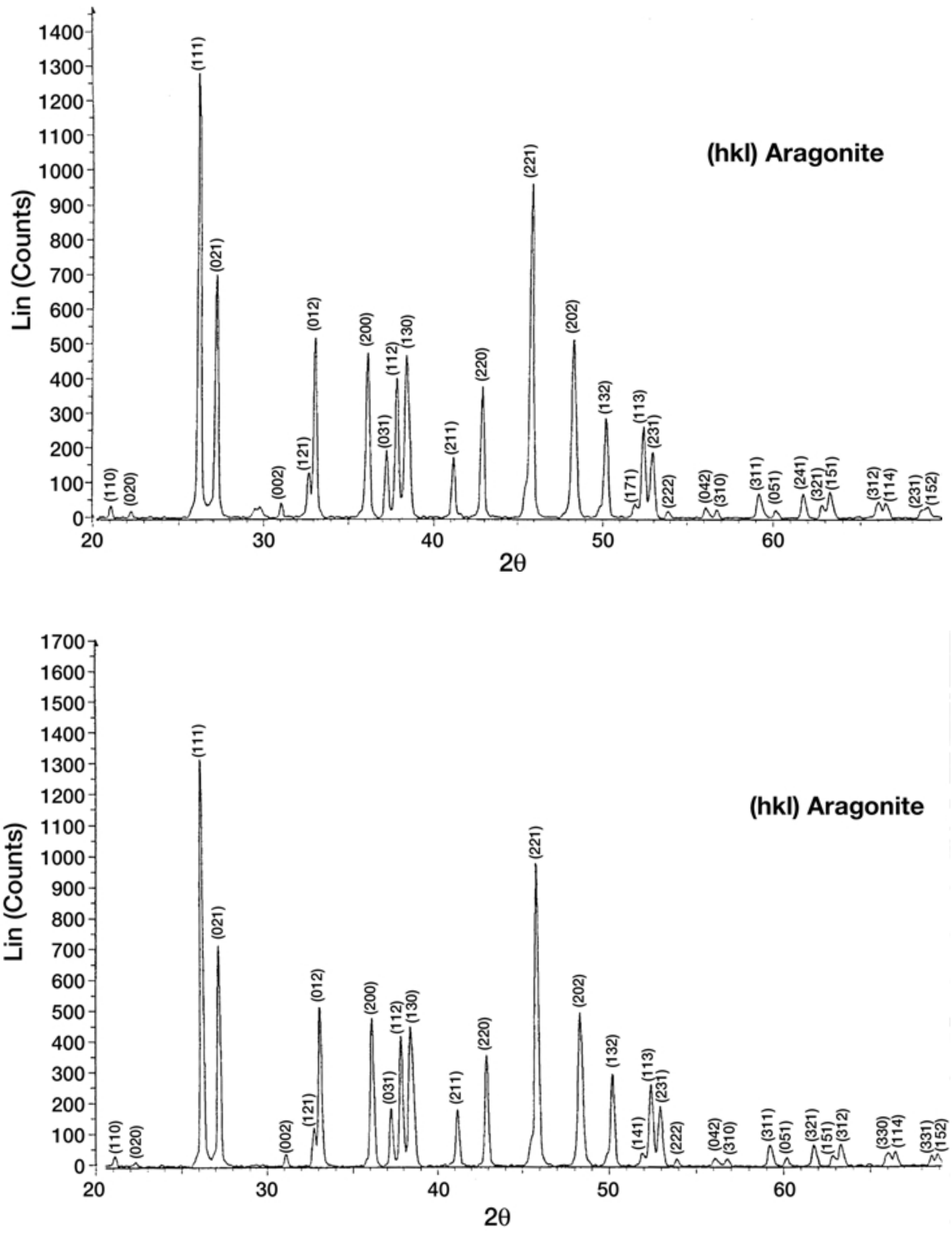

Figure 8 (a) X-ray diffraction pattern from the coral external ''porous zone''. (b) X-ray diffraction pattern from the coral internal ''compact zone''.

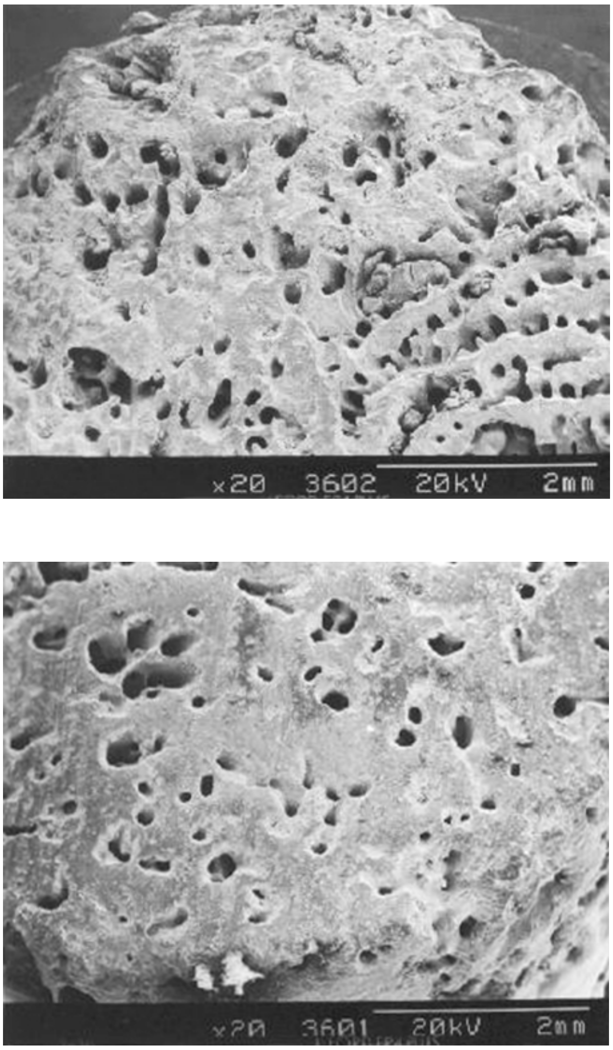

Figure 9 (a) General view of sample surface taken from the *X* direction before the compression test. (b) General view of the sample surface taken from the *Y* direction before the compression test.

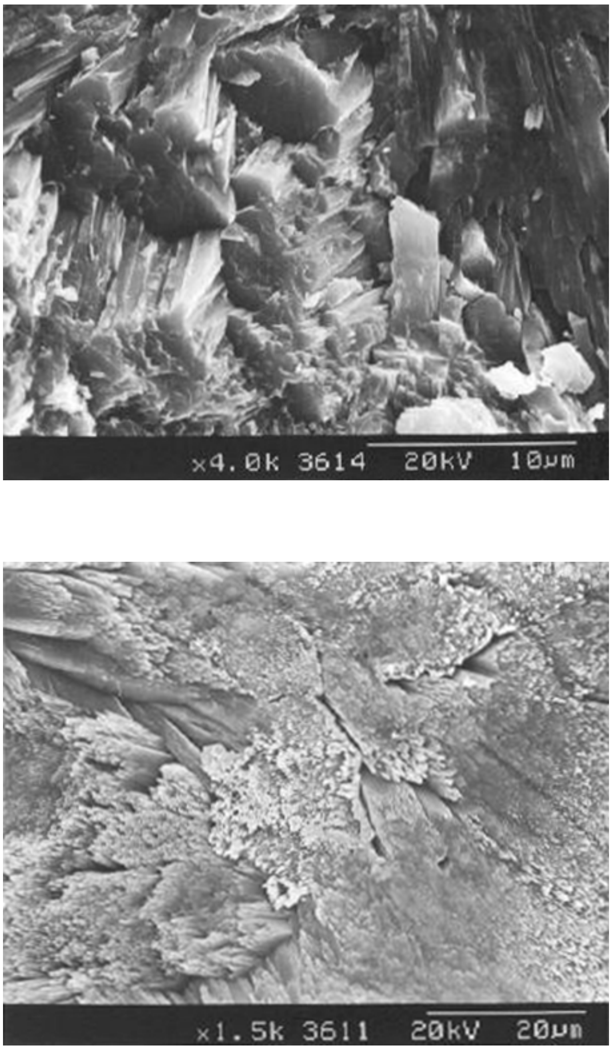

Figure 10 (a) Fracture surface from the specimen taken from the *X* direction showing the step-like appearance. (b) Fracture surface from the specimen taken from the *Y* direction.

similar to those reported for synthetic materials that have been successfully implanted [20–23]. Figs. 5–7 show magnified areas of Fig. 4. The area shown in Fig. 7, comes from the apparently ''compact'' zone around the pores. These areas are formed bysmall pores (2–10 μm) and crystals that grow in different directions. The EDS analysis showed (Fig. 5) only the presence of Ca, O and C, as expected.

Evidences that pore size is a major determinant of the nature of tissue ingrowth, have been found by different researchers [3, 20–23]. Guillemin [3] studied coral implants with different porosity and reported that implants with small pore sizes, 50–100 μm, showed a higher rate of degradation and bony in growth than implants with large pore sizes 200–400 μm. Frayssinet *et al.* [20] studied calcium phosphate macroporous ceramics having their pores filled with a highly soluble calcium cement and a 500–1000 μm pore size range. They found that the cement in the ceramic pores was progressively replaced by bone. Klawitter *et al.* [22] observed that interconnecting pores of 40–100 μm allowed the ingrowth of osteoid tissue and pores of 15–40 μm permitted fibrous tissue penetration of the material.

X-ray diffraction (XRD) analysis are shown in Fig. 8(a) and (b) from the ''porous'' zones (external surface) and the ''compact'' zones (internal) respectively. In both diffractrograms only the aragonite phase was found.

3.5. Mechanical tests

The mean value of Vickers microhardness obtained for the *Acropora palmata* coral was 3.31 GPa (329.48 $\pm$ 3.44 HV). Rohl *et al.* [24] reported a value of hardness for the trabecular bone in the transverse direction of 0.56 GPa and in the longitudinal direction of 0.59 GPa although this values are variable between individuals and depend on several factors such as age, sex, race, etc.

The hardness in the Mohs scale was 4, which indicates an intermediate value of hardness between minerals.

The values of the ultimate compressive strength (compressive strength at fracture) of the samples oriented in the *Y* direction of the coral (applied stress parallel to the polyps growth direction) was in the range of 45–50 MPa and in the range of 20–23 MPa in the *X* direction (applied stress perpendicular to the polyps growth direction). These results can be related to the polyps growth direction, which is strongly vectorial. In consequence, there is a strong structure anisotropy and therefore, mechanical properties anisotropy. Figs 9(a) and (b) present general views of the samples surfaces before fracture taken from the *X* and *Y* direction, respectively. As can be seen in those figures, the *X* direction seems to be more porous than the *Y* direction. This is in fact a consequence of the directionality of the polyps growth. In this way, there are more effective sites for the crack to develop in the *X* direction than in the *Y* direction. Therefore, it was expected that the samples oriented in the *X* direction failed at lower stress levels.

Fig. 10(a) (*X* direction) and Fig. 10(b) (*Y* direction) are fracture surfaces from the samples after compression tests. In the *X* direction the surface has a step-like appearance. Each step represents a surface through which many crystals have fractured at the same time. In the *Y* direction a different mechanism of fracture was operating. The fracture surface is formed by packets of crystals that have been plucked from adjacent clusters as suggested by Chamberlain [25]. Røhl [26] studied 60 specimens of trabecular bone and determined that the compression strength was in the range of 0.51–5.60 MPa with a mean value of 2.22 MPa. The *Acropora palmata* compressive strength was higher than these values.

4. Conclusions

1. The skeleton of the *Acropora palmata* coral species was composed by Aragonite crystals preferentially oriented in the polyps growth direction. This species does not present harmful elements, which indicates that it can be considered as a potential biomaterial.
2. The pore diameter of the coralline species *Acropora palmata* strongly varies from the external (200–3000 μm) to the internal surfaces (2–300 μm). The pores appear rounded, elongated and interconnected by channels.
3. The values of total porosity obtained (28.65%) were between the values reported for the cortical and trabecular bone, while the absolute ($0.376 \times 10^{-11} m^2$) and Klinkerberg permeability values measured ($0.369 \times 10^{-11} m^2$) were low but still acceptable for bone graft.
4. The mean microhardness value of 3.31 GPa of the coral was higher than those reported for the trabecular bone. The mechanical properties of the coral are anisotropic, having an average ultimate compressive strength of 48 MPa in the polyp growth direction and 23 MPa perpendicular to it. Two different fracture surface patterns were observed in both directions. One with a step-like appearance (*X* direction) and another formed by packets of crystals (*Y* direction) which are related to the crystal orientation.

Acknowledgments

The authors wish to thank Dr F. Lozano for performing the coral species classification.

References

1. E. MARTZ, V. GOEL, M. POPE and J. PARK, *J. Biomed. Mater. Res. (Appl. Biomater.)* **38** (1927) 267.
2. P. K. BAJPAI, in ''Biomaterials in Reconstructive Surgery'', (L. R. Rubin, M. O. St Louis : Mosby, C. V., 1983) p. 312.
3. G. GUILLEMIN, A. MEUNIER, P. DALLANT and P. CHRISTEL, *J. Biomed. Mater. Res.* **23** (1989) 765.
4. G. GUILLEMIN, J. FOURNIÉ, J.-L. PATAT and M. CHÉTAIL, *C.R. Acad. Sci. Paris* **293** (1981) 371.
5. G. GUILLEMIN, J.-L. PATAT, J. FOURNIÉ, M. CHÉTAIL, *J. Biomed. Mater. Res.* **21** (1987) 557.
6. S. K. PAPACHARALAMBOUS and K. I. ANASTASOFF, *Int. J. Oral Maxillofac. Surg.* **22** (1993) 260.
7. F. X. ROUX, D. BRASNU, B. LOTY, B. GEORGE and G. GUILLERMIN, *J. Neurosurg.* **69** (1988) 510.
8. J. C. QUINTANA, *Acta Odontol. Venezol.* **36** (1998) 25.
9. H. ESCOBAR and H. FREITES, L. Ruiz, M. Dr. Thesis, Hospital Dr. Domingo Luciani, Caracas, Venezuela, 1998.
10. ASTM C373-77 (Standard Test Method for water absorption bulk density, apparent porosity and apparent specific gravity of fired whiteware products, 1997) p. 311.
11. ASTM C135-76 (Standard Test Method for true specific gravity of refractory materials by water immersion, 1976) p. 78.
12. ASTM C830-88 (Standard Test Method for apparent porosity, liquid absorption, apparent specific gravity and bulk density of refractory shapes by vacuum pressure, 1988) p. 251.
13. J. T. JONES and M. F. BERARD, in ''Ceramics Industrial Processing and Testing'' (The Iowa State University Press, Ames, Iowa, 1972) p. 93.
14. C. COLLINS, in ''Flow of fluids through porous materials'' (Reinhold Chemical Engineering Series, New York, 1961) p. 13.
15. ASTM C773-79 (Standard Test Method for compressive strength of fired whiteware materials, 1979) p. 818.
16. T. P. HUGHES, *Mar. Ecol. Prog. Ser.* **35** (1987) 259.
17. M. FARAG, in ''Selection of Materials and Manufacturing Process for Engineering Design'' (Prentice Hall, New York, 1989) p. 486.
18. J. A. BUCKWALTER, M. J. GLIMCHER, R. R. COOPER and R. RECKER, *J. Bone Joint Surg.* **77A**(8) (1995) 1256.
19. M. J. GRIMM and J. L. WILLIAMS, *J. Biomech.* **7** (1997) 743.
20. P. FRAYSSINET and N. ROUQUET, in ''11th Conference of the ESB'', Tolouse, France, July 8–11, 1998.
21. J. F. PIECUCH, *J. Dent. Res.* **61**(12) (1982) 1458.
22. J. J. KLAWITTER, Doctoral Thesis, Clemson University, Clemson, 1970.
23. R. T. CHIROFF, E. W. WHITE, J. N. WEBER and D. M. ROY, *J. Biomed. Mater. Res.* **6** (1975) 29.
24. J. Y. RHO, T.Y. TSUI and G.M. PHARR, *Biomaterials* **18**(20) (1997) 1325.
25. J. A. CHAMBERLAIN, *Paleobiology* **4** (1978) 419.
26. L. ROHL, E. LARSEN, F. LINDE, A. ODGAARD and J. JORGENSEN, *J. Biomech.* **24**(12) (1991) 1143.

Received 22 February 2000
and accepted 26 September 2001