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Structural and mechanical properties of the coral and nacre and the potentiality of their use as bone substitutes

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ABSTRACT

The main objective of this work is to develop resistant compact material samples with different porosities from coral and nacre adapted to the filling of bone cavities. The characterization of materials was conducted using scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS) and laser granulometry. The micro-hardness and the influence of porosity on the mechanical behavior of these biomaterials under compression as well as three-points bending tests were also assessed. Both materials showed similar particles size ranging from 50 to 100 μm in diameter, distributed according to the Gauss curve. The modal particle size, the median D_{50} and $D_{90}-D_{10}$ are the most important parameters which allow for the distinction between coral and nacre samples. The two biomaterials showed a micro hardness (138–167 HV for coral and 261–340 HV for nacre) higher than that of bovine bones (55–70 HV). The maximum compression stresses were 32.82 MPa for coral and 37.06 MPa for nacre at 50% of porosity. *S–N* curve with ASME format is constructed to predict the fatigue life extended from 10^1 to 10^6 cycles, which reveals an endurance limit at a compression stress ratio of about 10.

1. Introduction

One of the major concerns of orthopedic surgery and traumatology is the substitution of bone deficiencies [1,2].

During the last 20 years, a tremendous increase in the bone synthetic substitution has been noticed. Such substitutes are subdivided into three groups: phosphocalcic cements, calcium phosphate ceramics and gels of hydroxylapatite nanoparticles (HAP). Recently, coral and nacre had attracted the attention of many orthopedists to replace some synthetic substitutes often exhibiting toxicity, incompatibility or mechanical strength problems [3–7]. The coral had been used for opening osteotomies, replacement of proximal tibia during a total hip arthroplasty and also in the treatment of benign tumors or traumatology. This material simplifies also the surgical procedure [8–13]. Nacre has been recently proposed as a biocompatible and osteoinductive biomaterial for possible use in periodontal and implant surgery [14].

Fatigue is the most frequent problem and most dangerous mechanisms that cause sudden failures of biomaterials implants. Although there are many factors that affect the endurance limit of biomaterials (i.e. geometry, grain size, porosity, compaction quality), and the resistance to final cracks, is directly related to the applied stress of these biomaterials [7].

The present study assesses the physico-chemical properties of the Tunisian coral and nacre and represents the first step to recommend their use as bone substitutes.

The transformation of a powdered or granular product from nacre and coral into a compact material with mechanical properties similar to those of bones could be done by two methods: the dry and the liquid techniques [15]. For application as bone substitution, the materials porosity should be between 50 and 70% [16]. In order to achieve the consolidation of the powder and obtain a proper bone reconstruction that could resist to mechanical stress; we used the technique of cold compression with a binder because sintering is not possible for these two biomaterials. Sintering is a risky process for coral and nacre mainly because of the possible loss of the organic percentage since temperature of 200 °C and elimination of proteins that are essential for the bone regeneration [17,18].

The nacre is known for its excellent mechanical properties, in particular hardness. Therefore, it becomes an important parameter

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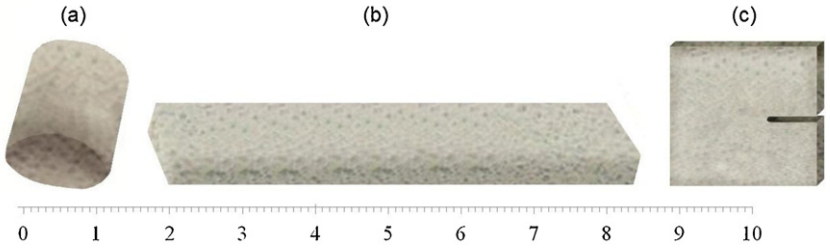

Fig. 1. Photograph of the tested specimens: (a) circular cylinders for compression (b) prismatic for three-point bend (c) compact fatigue compression types.

for a successful design of hardened synthetic ceramics. Binding and compression tests were necessary to ensure adequate resistance and compatibility with bone resistance [19–21].

2. Materials and methods

2.1. Materials

Two Tunisian marine biomaterials: red coral (*Corallium rubrum*) and nacre (*Pinctada radiata*). The coral well represented all along the Northern West coast of Tunisia was taken in Tabarka region and known for its use mainly for artisanal small scale production. Its use as a bone substitute has recently drawn the interest of many orthopedists to replace some synthetic substitutes. Coral samples (branches) were washed with fresh water and then grinded in a blender. Nacre is a hard, with white layer and iridescent reflection. It is formed in the shell of many shellfish species, and secreted by the epithelial cells of the mantle tissue of various mollusks [22]. In the present study we used the nacre (*P. radiata*) collected from the Gulf of Gabes (South East of the Tunisia).

To make nacre usable, the rigid outer layer of the shell is removed using a grinding wheel to mechanically abrade it. The nacre is then polished to bring out its iridescent appearance, as the exposure to the sunrays can obliterate this iridescent. At the end, the nacre layer is grinded in a blender to obtain a fine powder.

2.2. Scanning electron microscopy and energy dispersive X-ray spectroscopy

The microstructure of coral and nacre was assessed using the Scanning Electron Microscopy (SEM). The observations were performed on gold-coated specimens and investigated at an acceleration voltage of 15 kV for both samples.

The chemical characterization of powder of samples was performed using energy dispersive X-ray spectroscopy (EDS). EDS allows the determination of the type of calcium phosphate present in samples. EDS was recorded in constant pass energy mode and expressed as intensity on the *Y*-axis versus binding energy on *X*-axis.

2.3. Particle size distribution of powders

According to ISO13320-1 standard, the granulometer (Mastersizer 2000) has been used to determine the particle size distribution of powders by means of laser diffraction principle. The result is reported as volume percent. Coral and nacre samples were milled using a ball-mill in order to reduce the particle size and cause the break-up of any strongly bound aggregates. The method is based on the application of Stokes's Law settling, and assumes the particles to be spherical (hence the results expressed in equivalent spherical diameter). The next step is to dilute the powder. The apparatus will then estimate the background noise in order to record the diffraction phenomena caused by the water-solvent. Sample solutions were injected into the measuring cell of the granulometer. Each particle that passes through the radiation beam deviates the light, which is then analyzed by detectors. The results are then handled with matrices calculations [23–25].

2.4. Compact specimen's preparation

We conducted the consolidation of the coral and nacre powder to obtain porous compact specimens (Fig. 1). The thickness of our oysters (*P. radiada*) ranged between 1 and 3 mm where the range of pearl oysters (*P. maxima*) is between 5 and 20 mm [11]. The nacre layer thickness value suggests its use as a porous compact since the grain size was between 50 and 100 μm after crushing.

A hot uniaxial and hot isostatic pressing process (50 kN) was used to consolidate the powder. Three types of specimens have been implemented for the uniaxial compression, the three-points bending and fatigue tests. Different porosities 50, 60 and 70% were made controlled by the value of pressure and time.

The technique used to obtain microporous samples consists in the application on the powder of a pressure of 20 kN during approximately 2 h at ambient temperature. The powder is being mixed with an organic chemical compound (povidone, 2-oxopyrrolidinyl, gelatinous cornstarch) for pasting between grains.

2.5. Experiment design and mechanical tests

Micro-hardness tests have been conducted on specimens in natural form. Samples were mounted in plastic holder to fix them during preparation and testing. We used the Vickers indentation fracture toughness test as a simple method to implement. The tested samples had a smooth surface and were held perpendicular to the indenter. Micro-hardness testing as per ASTM standards E384 provides an allowable range of loads for testing with a diamond indenter. The resulting indentation was measured and converted to Vickers hardness (*HV*) value. Each print was performed three times on the same load, so that the average of measurement is the most significant. The following equation has been used for the calculation of *HV*:

$$HV = (2F\ \sin(136^\circ/2))/d^2 \cong 0.18544\,F/d^2 \quad (1)$$

where *F* is the load applied to the diamond in kgf and is the average length of the diagonal left by the indenter in mm.

The used machine is of the type Mini Bionix servo-hydraulic. The axial load is ranging between ±25 kN with standard displacement of ±50 mm. The computer controlling the machine determines the uni-axial compression, the three-point flexural loading and the fatigue performance [20]. Specimens used for compression test were cylindrical in shape with circular flat ends, 12.7 mm in diameter and 25.4 mm in length as instructed in ASTM standards D695. During the test, the specimen is placed between compressive plates parallel to the surface and compressed at a uniform loading speed. Compressive stress and strain were calculated and plotted as a stress–strain diagram. Young's modulus and maximum compression stress were determined.