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Nacre, a Natural Biomaterial

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1. Introduction

Nacre, or mother-of-pearl, is a calcium carbonate structure produced by bivalves, gastropods, and cephalopods as an internal shell coating. Because of its highly organized internal structure, chemical complexity, mechanical properties and optical effects, which create a characteristic and beautiful lustre, the formation of nacre is among the best-studied examples of calcium carbonate biomineralization. In this chapter we will detail the structure of nacre and its growth mechanism. We will summarize the several results obtained on the *in vivo* and *in vitro* biological activity of nacre and its organic molecules.

2. Nacre structure

The interdigitating brickwork array of nacre tablets (Fig.1), specific, in bivalves ("sheet nacre") is not the only interesting aspect of nacre structure. Nacre is an organo-mineral composite at micro- and nano-scale. The bio-crystal itself is a composite. It has not only the mineral structure of aragonite but possesses intracrystalline organic material (Watabe, 1965). The primary structural component is a pseudohexagonal tablet, about 0.5 μm thick and about 5 to 10 μm in width, consisting primarily (97%) of aragonite, a polymorph of CaCO_3 , and of organics (3%). Nacre can be worked as pieces and powder.

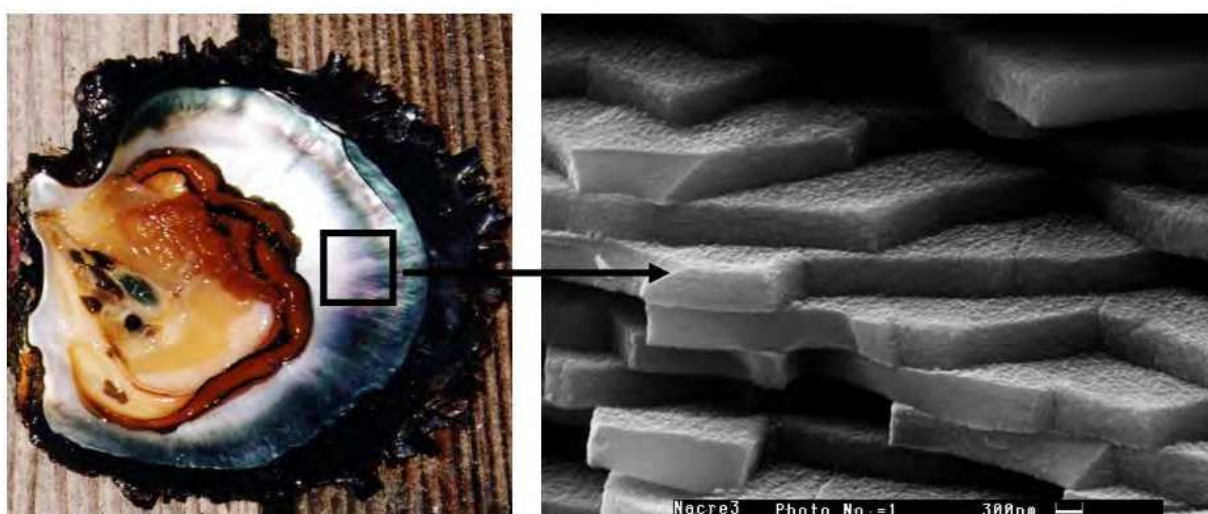


Fig. 1. Characteristic brick and mortar structure of the nacreous layer of *Pinctada*.

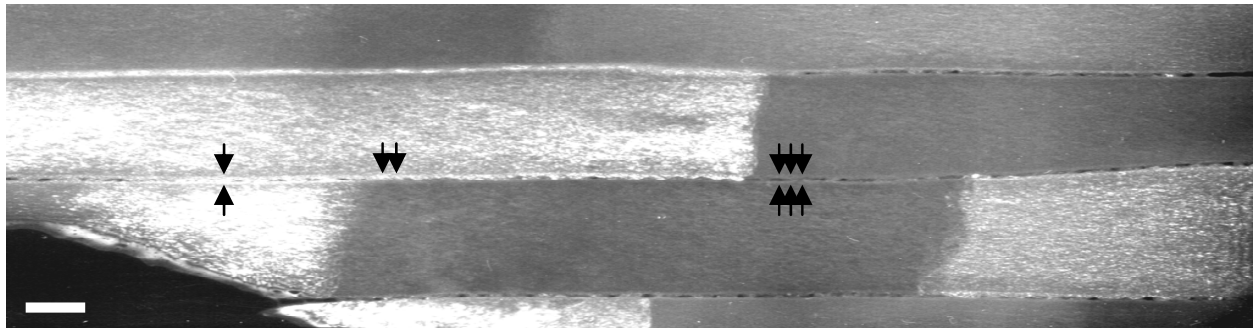


Fig. 2. Darkfield TEM image of nacre evidencing the crystalline structure of the organic matrix (bar is 100nm). Organic matter is in contrast when under Bragg conditions whilst the mineral phase remains systematically extinguished.

Transmission electron microscopy performed in the darkfield mode evidences (Fig.2) that intracrystalline matrix is highly crystallised and responds like a 'single crystal'. The organic matrix is continuous inside the tablet, mineral phase is thus finely divided but behaves in the same time as a single crystal.

The tablet of nacre, the biocrystal, does diffract as a single crystal but is made up of a continuous organic matrix (intracrystalline organic matrix) which breaks the mineral up into coherent nanograins (~45 nm mean size, flat-on) which share the same crystallographic orientation. The single crystal-like mineral orientation of the tablet is supposedly created by the heteroepitaxy of the intracrystalline organic matrix. This is a strong hypothesis because this work demonstrates at the same time that this intracrystalline matrix is well crystallized (i.e. periodic) and diffracts as a "single crystal" too (darkfield TEM mode, Fig. 2). However these "organic crystals" do not show the same orientation in adjacent tablets.

Neighbouring tablets above and below can maintain a common orientation, which again raises the issue of the transmission of mineral orientation from one row to the next. Bridges are well identified in *Pinctada* between successive rows in the pile. This implies that an organic template is controlling the orientation of the aragonite.

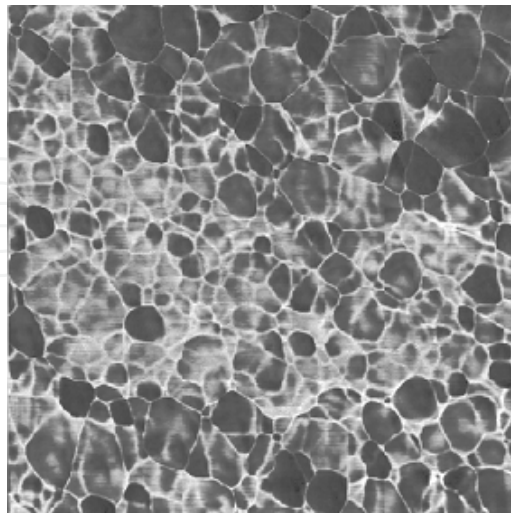


Fig. 3. AFM picture in Phase Contrast ($1 \times 1 \mu\text{m}^2$) at the nanometer (nm) length scale, the aragonite component inside individual tablets is embedded in a crystallographically oriented foam-like structure of intra-crystalline organic materials in which the mean size of individual aragonite domains is around 50 nm.

Intermittent-Contact Atomic Force Microscopy with phase detection imaging reveals a nanostructure within the tablet (Fig.3). A continuous organic framework divides each tablet into nanograins. Their mean extension is 45nm. It is proposed that each tablet results from the coherent aggregation of nanograins keeping strictly the same crystallographic orientation thanks to a hetero-epitaxy mechanism (Rousseau et al., 2005a).

3. Nacre growth mechanism

Formation of nacre (*mother-of-pearl*) is a biomineralization process of fundamental scientific as well as industrial importance. However, the dynamics of the formation process is still not all understood. Scanning electron microscopy and high spatial resolution ion-microprobe depth-profiling have been used to image the full three-dimensional distribution of organic materials around individual tablets in the top-most layer of forming nacre in bivalves. Nacre formation proceeds by lateral, symmetric growth of individual tablets mediated by a growth-ring rich in organics, in which aragonite crystallizes from amorphous precursors (Fig.4). The pivotal role in nacre formation played by the growth-ring structure documented in this study adds further complexity to a highly dynamical biomineralization process.

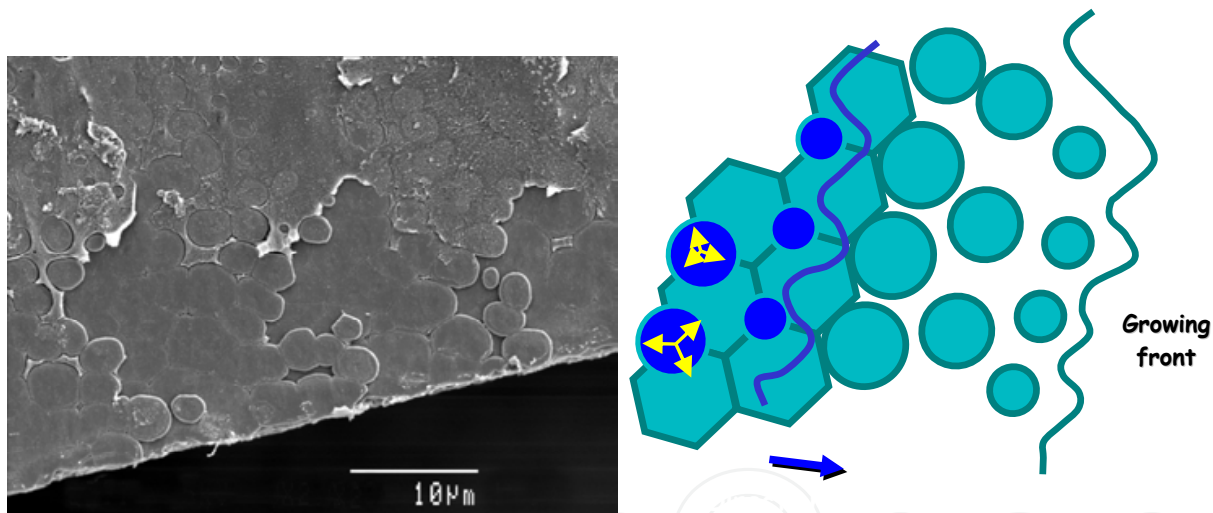


Fig. 4. Nuclei (future nacre tablets) grow circularly in a film, get in contact and form polygons.

Figure 5 shows the surface membrane, observed from the point of view of the nacre layer, and corresponding features in the growing nacre surface, respectively. Several important observations can be made. i) For each nacre tablet, there exists in the membrane a circular structure (Fig. 5a, c). The circular membrane structure displays higher topography than the adjacent surface membrane. We refer to this circular structure as the 'ring'. ii) This ring makes direct physical contact with the growing nacre tablets. Each nacre tablet features a similar ring, several hundred nm wide, with a complementary (i.e. lower) topography (Fig. 5b, d). iii) Neighbouring tablets can merge during growth by lateral expansion that leads to a 'collision'. In this process, ring structures surrounding each tablet first merge together, then disappear (Fig. 5d).

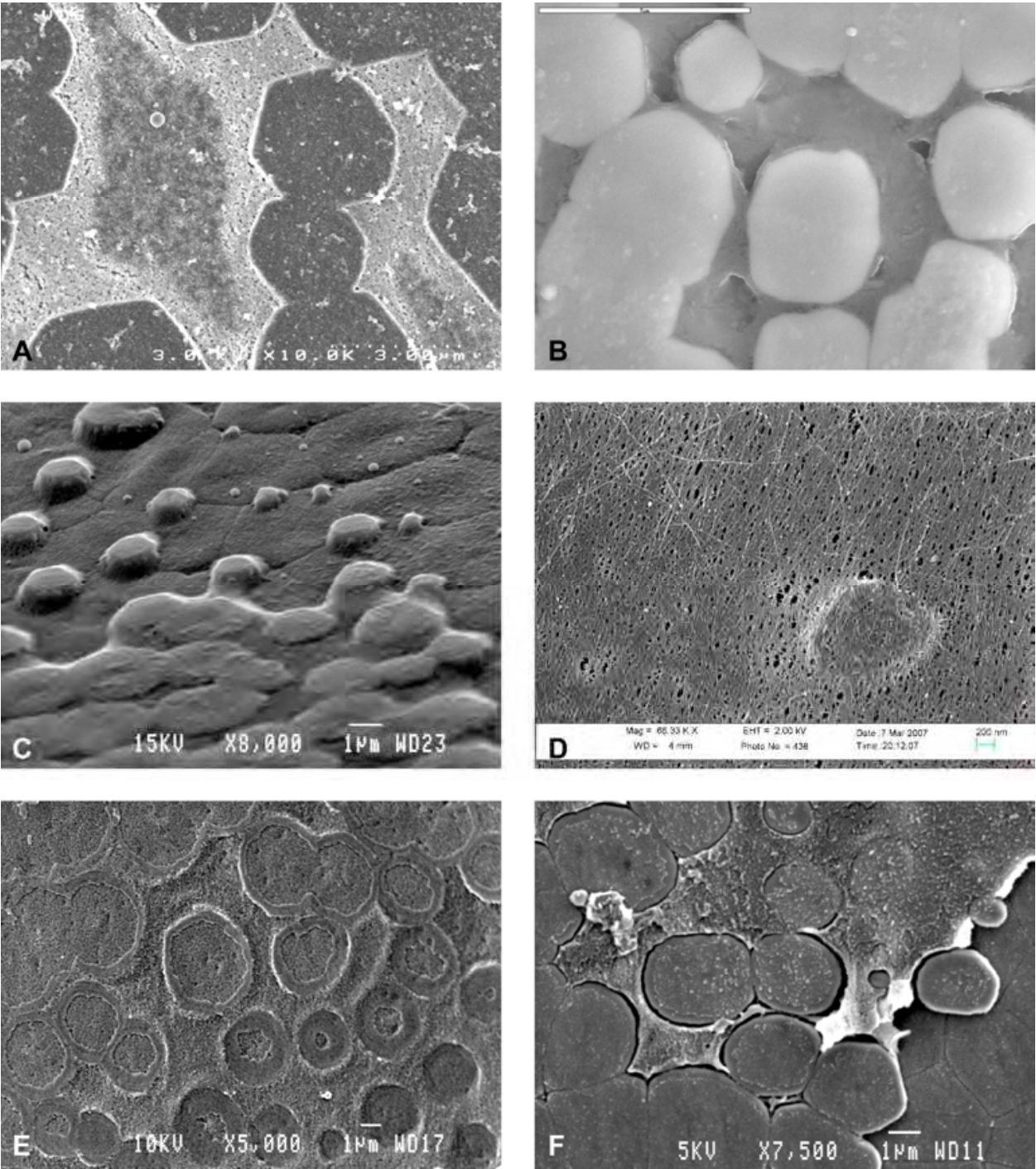


Fig. 5. Direct observation of the nacre surface of the same sample series with different SEM secondary electron detectors : A: in-lens detector at 3kV, B: same but at 15kV both with an Hitachi 4500-FEG microscope, C, E, F: lower detector of the JEOL JSM-840A at 15, 10 and 5kV, D: with a high-resolution Ultra 55 Zeiss using an in-lens secondary electron detector at 2kV.

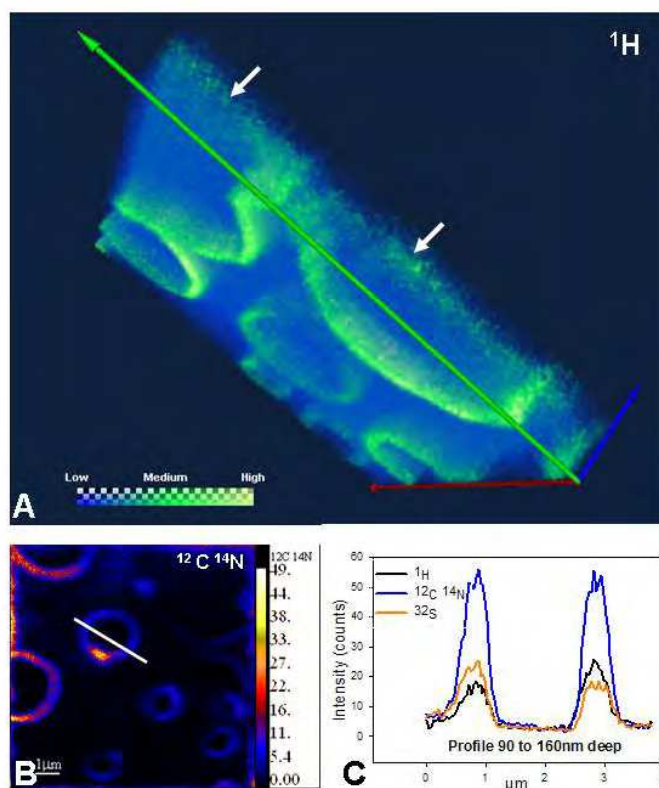


Fig. 6. Distribution of organics around nacre tablets. a) A 3-dimensional reconstruction of the H distribution at different depths in the surface layers of the forming nacre. The data were obtained with the NanoSIMS ion microprobe. The direction of sputtering is indicated by a white arrow. The sputtered volume is about $10\mu\text{m} \times 10\mu\text{m} \times 0.2\mu\text{m}$. For greater clarity, the thickness of this volume (blue axis) has been expanded by a factor of 6. The surface membrane is visible in the first images (white arrow) but deeper in the structure organics are concentrated only in the growth-rings surrounding each nacre tablet. (Full 3-D reconstructions of the H, N and S distributions are available in SD as movies) b) NanoSIMS image showing the distribution of N, which is also preferentially concentrated in the growth-ring around each nacre tablet. c) Line-profile (track indicated in b) showing the enhanced concentrations of H, N, and S in the growth-ring. See text for discussion.

The NanoSIMS allows chemical composition to be imaged with extremely high spatial resolution (on the order of 100 nm), while depth-profiling through the inter-lamellar matrix and into the nacreous structure with a depth resolution of about 10 nm per image (Meibom et al., 2004, 2007). The 3-dimensional distribution of H, N and S are diagnostic of organic compounds in which their concentration is greatly increased over that in the aragonitic nacre tablets. Figure 6a shows the 3D reconstruction of a depth-sequence of H images from the top-most layers (in the membrane), via intermediate levels to an estimated depth of around $0.2\mu\text{m}$ in the tablet structure, where the overlying membrane has been sputtered away completely and the distribution of organics around individual tablets becomes visible. The distribution of organics in the ring surrounding each nacre tablet corresponds to the characteristic ring in the overlying mantle; see Fig. 5d. Figure 6c shows in greater detail the distribution of organics around individual tablets. Importantly, concentrations of H, N and S

are clearly confined to the rim around each tablet. In the ring, the signal from N is almost two orders of magnitude higher than in the surrounding aragonitic tablets. Sulfur and Hydrogen are enriched by factors of about 10 and 7, respectively over the signal observed from inside the tablet. We proposed a model illustrated in Fig. 7. A growth-ring structure, rich in organic materials, surrounds each growing nacre tablet during formation of sheet nacre in *Pinctada margaritifera* bivalves. This structure disappears as nacre tablets grow laterally and collide with adjacent tablets. It is conceivable that this organic ring structure acts to nucleate aragonite into the highly oriented nano-crystals (~ 50 nm in size) that make up the meso-crystal (i.e. μm sized) nacre tablets (Wohlrab et al., 2005; Kulak et al., 2007). This adds support for a highly dynamic biomineralization process during which organic materials and carbonate precursor phases (likely amorphous) are delivered to the site of nacre tablet formation from the overlying mantle with a high degree of spatial control.

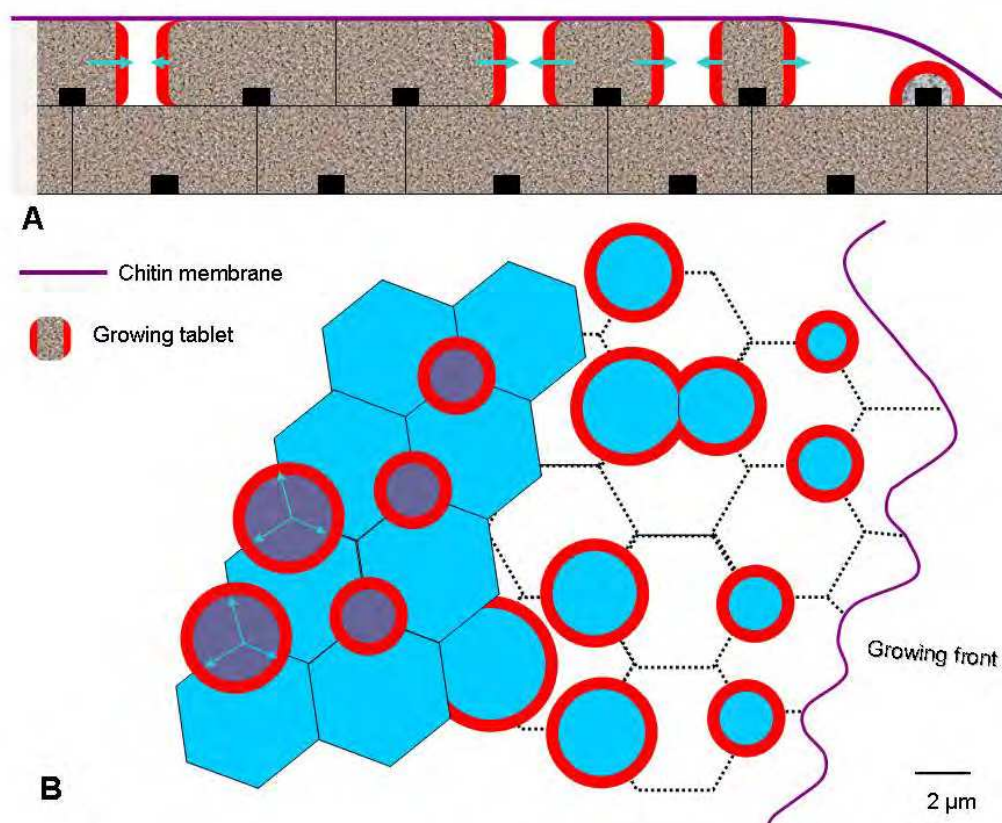


Fig. 7. Schematic representation of the nacre tablet growth-model. a) Side-view of the top-most tablet-layers. Underneath the top membrane, which is in direct contact with the overlying mantle of the animal, individual tablets grow laterally from a carbonate-charged silk-phase through crystallization mediated by organics in the nucleation sites (black rectangles) and in the growth-rings (shown in red). The nucleation sites are generally placed above the junctions of three tablets in the underlying layer of tablets. When two tablets collide during growth, the growth-rings first merge and subsequently disappear, allowing adjacent tablets to make physical contact. b) Top view of the nacre growth front. Individual tablets nucleate and grow concentrically reflecting the shape of the surrounding organic growth-ring. Only after collision and disappearance of the growth-rings do individual tablets take the form of hexagons. The hexagonal geometry of individual tablets is therefore the result of the distribution of nucleation sites, which inherit their distribution from the underlying layer.

4. Biological action of raw nacre

Nacre biological action. A major breakthrough was done in 1992, when E. Lopez et al. discovered that natural nacre from the pearl oyster *Pinctada maxima* is simultaneously biocompatible and osteoinductive. Nacre shows osteogenic activity after implantation in human bone environment (Silve et al., 1992). Raw nacre pieces designed for large bone defects were used as replacement bone devices in the femur of sheep. Over a period of 12 months, the nacre blocks show persistence without alteration of the implant shape. A complete sequence of osteogenesis resulted from direct contact between newly formed bone and the nacre, anchoring the nacre implant (Atlan et al., 1997). Furthermore, when nacre is implanted in bone, new bone formation occurs, without any inflammatory reaction and fibrous formation. We observed an osteoprogenitor cellular layer lining the implant, resulting in a complete sequence of new bone formation (Fig.8). Results showed calcium and phosphate ions lining the nacre within the osteoprogenitor tissue (Atlan et al, 1999).

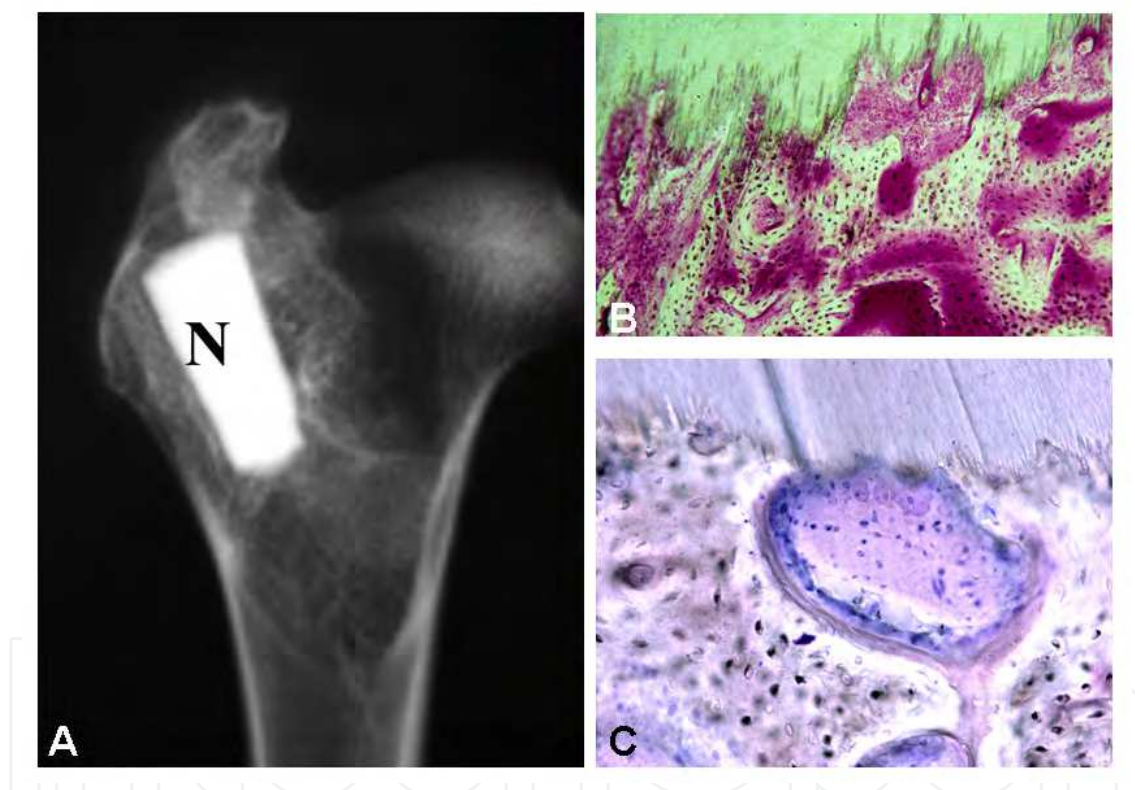


Fig. 8. Radiographic image of the femur epiphysis harvested 10 months after implantation of a block of nacre (N) showing the preserved size and shape of the implant and the close contact between the implant and surrounding cancellous bone (A). After erosion of the implant surface nacre is in direct contact with the new formed bone (B). At the interface bone forming cells are stimulated (C) (Atlan et al, 1999).

Osteogenesis thought to begin with the recruitment of mesenchymal stem cells, which differentiate to form osteoblasts in response to one or more osteogenic factors. In previous studies (Atlan et al., 1997; Silve et al., 1992), as reviewed in Westbroek and Marin (1998), it has been shown by means of *in vivo* and *in vitro* experiments that nacre can attract and activate bone marrow stem cells and osteoblasts.

Other authors have demonstrated the same activity of nacre. Liao et al., in 2000 have published results about the implantation in back muscles and femurs of rats of nacre pieces coming from the shell of the freshwater *Margaritifera*. They concluded that their nacre was biocompatible, biodegradable and osteoconductive material. They confirmed the results obtained with *Pinctada* nacre. More recently Shen et al. (2006) have demonstrated the *in vitro* osteogenic activity of pearl. Hydroxyapatite can be formed on pearl surface in Simulated Body Fluid based on a dissolution-binding-precipitation mechanism. Cell culture shows that pearl has the same osteogenic activity as shell nacre.

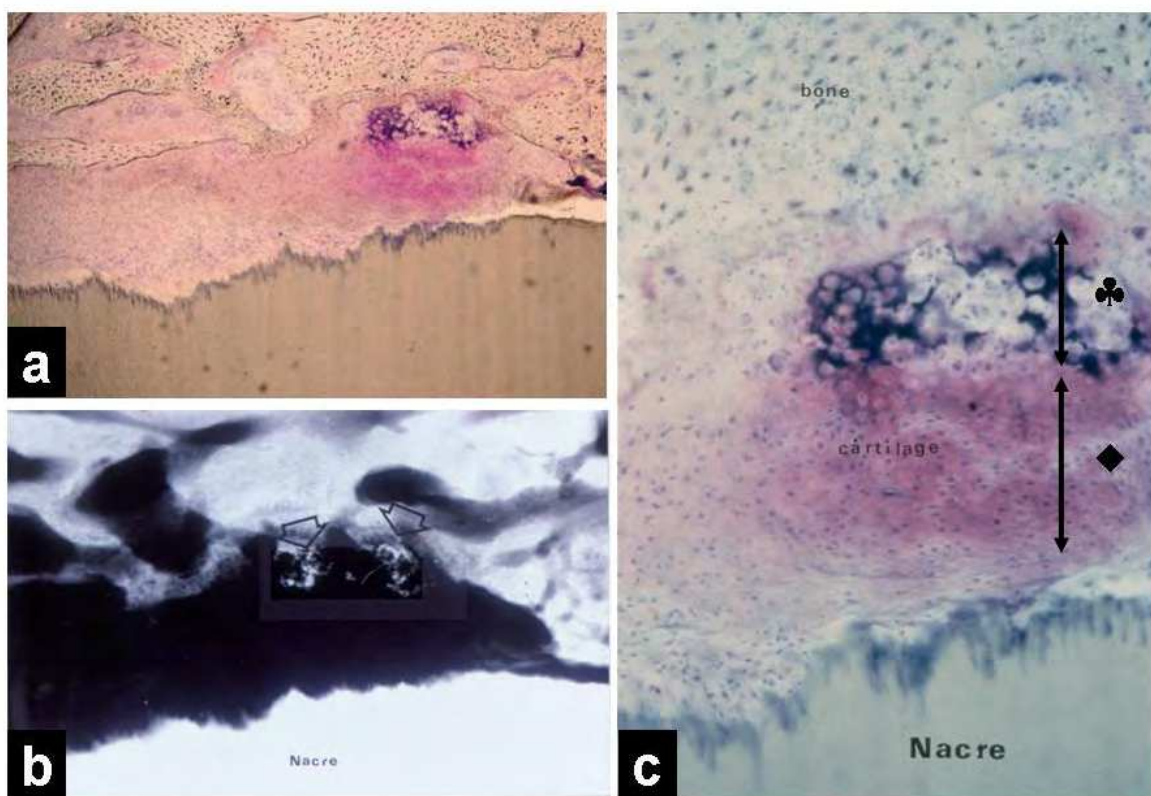


Fig. 9. Histological study of the nacre/ bone interface, lateral to the nacre trochlea, after 6 months of implantation: longitudinal section of nacre subchondral implant, islets of ossification at the interface between nacre and bone (a: x8; b: microradiography of the same area), showing the sequence of cell differentiation from nacre to spongy host bone, chondrocytes (♦) and hypertrophic chondrocytes (♣), after basic fuchsin and toluidine bleu staining (c: x 16).

Nacre has also been implanted in the subchondral bone area in the sheep knee. We implanted nacre blocks in sheep trochlea by replacing the half of the femoral trochlea (nacre group). For comparison we used complete cartilage resection (resection group) down to the subchondral bone. In the “nacre group”, implants were well tolerated without any synovial inflammation (Fig.9). This complementary study was the first one designed to analyze the behaviour of nacre *in vivo*, in the intra articular cavity after implantation into the subchondral bone in the sheep’s knee (Delattre, 2000). After 6 months of implantation, a new tissue was formed on the articular surface of nacre. This tissue is composed of new formed bone and articular cartilage, partially covering the

subchondral implant placed in the defect. The presence of nacre can progressively stimulate the regrowth of a tissue, reproducing the functional osteochondral structure in which the subchondral bone sends stimulating messages to the cartilage layer. The regrowth reaction seems to be a well regulated physiological process, demonstrating the efficacy and good tolerance to nacre. Laterally to the nacre implant we observed islets of endochondral ossification. Endochondral ossification is initiated by the formation of cartilage templates of future bones, built by mesenchymal progenitor cells, which condensate and differentiate into chondrocytes. During the repair process the events of endochondral ossification are recapitulated (Fig.9).

A process of nacre powder preparation has been patented (Lopez et al., 1995). The obtained nacre powder has been experimented in injectable form in vertebral and maxillar sites of sheep (Lamghari et al., 1999a, 1999b). This work has demonstrated that nacre powder is resorbable and that this resorption induced the formation of normal bone. The nacre powder filled the whole experimental cavity after 1 week post-surgery. There was no inflammatory or foreign body reaction in the cavity area. Samples taken at 8 weeks after injection showed dissolution of the nacre within the cavity. Angiogenesis had began by that time and the cavity was invaded by a network of capillaries. The cavity contained newly formed woven bone (Fig.10). The vertebral bone adjacent to the cavities contained interconnected bone lamellae. They were also bone remodelling units with central lacunae rich in bone marrow. This new formed Bone was functional as normal bone.

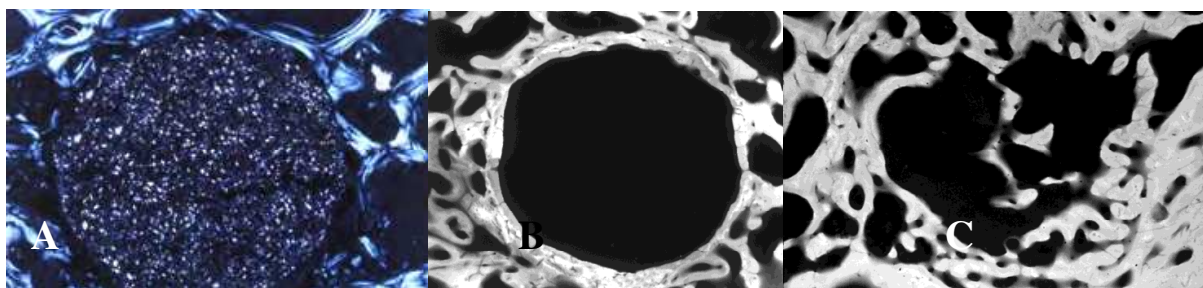


Fig. 10. Nacre injected into the sheep vertebrae. Polarized light image showing transverse section through a bone defect. The cavity is filled with nacre (A, x25). In the empty cavity after 8 weeks, the bone was organised into concentric rings (B). 8 weeks after injection the nacre powdered has disappeared and been replaced by new formed bone (C) (Lamghari et al, 2001).

In the *in vivo* studies nacre has been shown to be resorbable. Osteoclast activity was therefore studied *in vitro* on nacre (Fig.11). The idea was to assess the plasticity of bone resorbing cells and their capacity to adapt to a biomineralized material with a different organic and mineral composition. Osteoclast stem cells and mature osteoblasts were cultured on nacre substrate and osteoclast precursor were shown to differentiate into osteoclasts capable of resorbing nacre (Duplat et al, 2007).

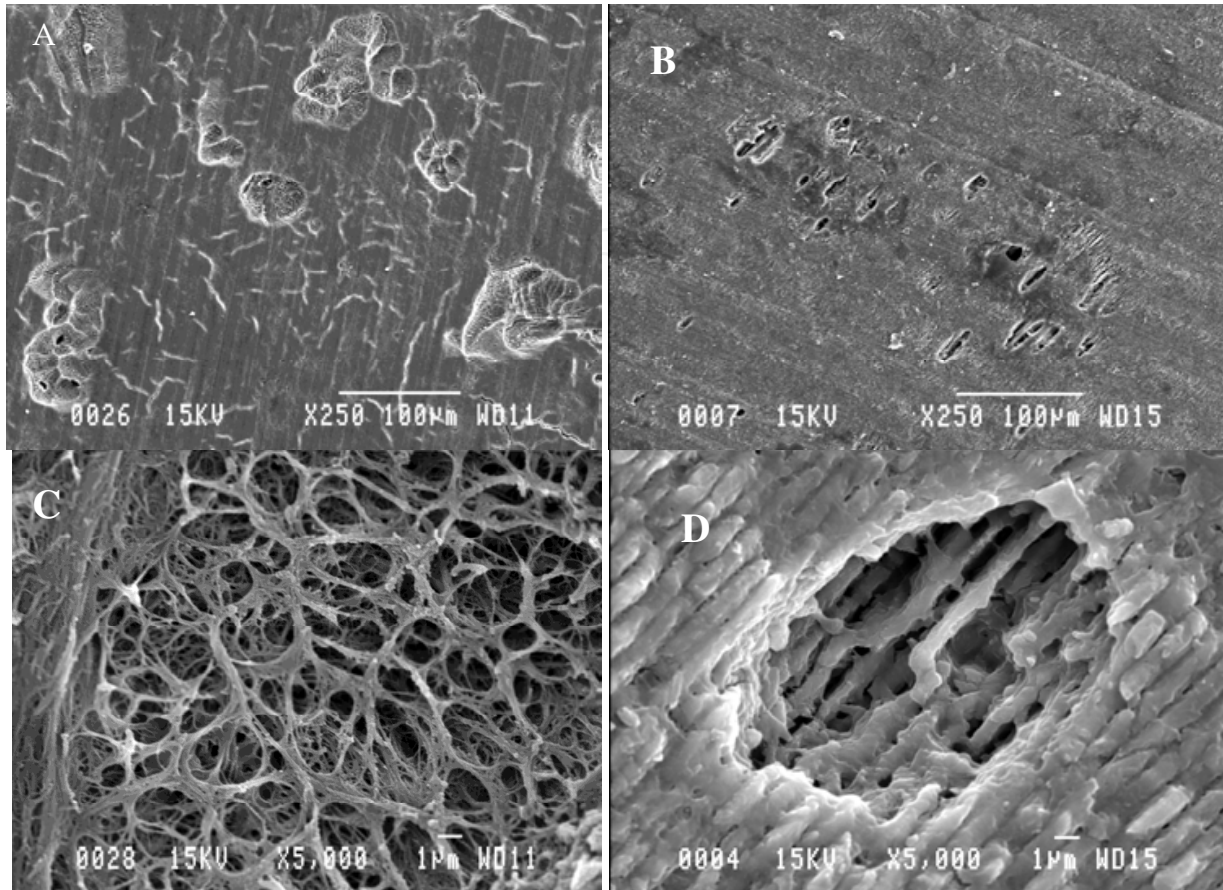


Fig. 11. Resorption process of osteoclasts differentiated from human CD14⁺ monocytes on bone (A, C) and on nacre (B, D). SEM examination of resorption lacunae. The form of the lacunae is quite different and can be explained by the different organic and mineral compositions as well as by the mineral density of bone and nacre.

5. Biological action of nacre extracts

Nacre is composed of aragonite crystal tablets covered by an organic matrix (3%) (Bevelander & Nakahara, 1969). This organic phase is composed of chitine, polysaccharides, proteins, peptides, lipids and other small molecules lower than 1000 Da. The organic molecules can be extracted with aqueous and organic solvents. The water-soluble organic matrix dictates which calcium carbonate crystal structure is formed and when it is deposited (Cariolou & Morse, 1988). The bulk of the watersoluble biopolymer is thought to consist of a complex mixture of proteins and peptides (Kono et al., 2000; Weiss et al., 2000). Nacre possess in its organic matrix molecular signals that have the ability to trigger bone cell commitment. Efforts were undertaken in order to identify these active molecules and to determine their mode of action on mammalian bone cells. A particular attention was paid to the water-soluble matrix (WSM) molecules of nacre. We supposed that this fraction contains molecules which are released when the nacre implants are placed in living systems. Molecules extracted from nacre of the pearl oyster *P. maxima* with water have been shown to have a biological activity on various mammalian pre-osteogenic cell types (Rousseau et al., 2003).

The activity of the nacre water-soluble matrix (WSM) from *P. maxima* was measured on the clonal osteogenic cell-line MC3T3-E1 established from newborn mouse calvaria. These cells have the capacity to differentiate into osteoblasts, and form calcified bone tissue *in vitro*. The WSM increased alkaline phosphatase activity (Pereira-Mouriès et al., 2002b) and induced the formation of bone nodules (Rousseau et al., 2003). On bone marrow stromal cells, WSM stimulated the proliferation, the differentiation and the early mineralization of osteoprogenitor cells (Lamghari et al., 1999a).

Recently, a great number of low molecular weight molecules was identified in WSM as main components, whereas proteins are minor (15 % w/w). It was supposed that the signal-molecules of nacre might to be low molecular weight molecules. This feature may facilitate their diffusion in the host tissues from nacre implants. The hypothesis that nacre low MW molecules are active in bone cell differentiation was evaluated. Water-soluble molecules from nacre were fractionated according to dialysis, solvent extraction and reversed-phase HPLC. The two sub-fractions ESM and F1 were tested on MC3T3-E1 cell culture. Mass spectrometry analysis showed that the F1 sub-fraction contains around 30 polar molecules ranging from 50 to 300 Da (Fig.12). Peptides were not identified in this fraction. However, the aggregative property of these molecules during chromatography precludes the obtaining of a more purified active fraction.

The presence of BMP-like molecules in WSM was supposed but not demonstrated (Rousseau et al., 2007). Molecules isolated from nacre induced mineralization of the preosteoblasts extracellular matrix after 16 days of culture that was analyzed as hydroxyapatite by Raman spectroscopy. This study indicated that the nacre molecules efficient in bone cell differentiation are certainly different from proteins, and probably more related to peptides (Rousseau et al., 2003, 2007). Molecules isolated from nacre, ranging from 50 to 235 Da, induced red alizarin staining of the preosteoblasts extracellular matrix after 16 days of culture. The treatment of cells with nacre molecules accelerated expression of collagen I and increased mRNA expression of Runx2 and osteopontin (Fig.13). Raman spectroscopy demonstrated the presence of hydroxyapatite in samples treated with this molecules. Scanning electron microscopy pictures showed at the surface of the treated cells the occurrence of clusters of spherical particles resembling to hydroxyapatite (Fig. 14). Nacre low molecular weight molecules stimulate the early stages of bone differentiation and the formation of an extracellular matrix able to initiate hydroxyapatite nucleation.

The water-soluble matrix of nacre is composed of at least 110 molecules ranging from 100 to 700 Da, with a low content (10 %) of peptides (Bédouet et al., 2006). On the other hand, the protein content of the WSM represents 15 % (w/w) of the extract (Pereira-Mouriès et al., 2002a). Dialysis fractionation of the water-soluble matrix of nacre indicated that low molecular mass molecules lower than 1kDa were important components. They represent 0.14% of the nacre weight, i.e. 60% of the water soluble matrix itself, and contained a huge chemical diversity, since 100 molecules with close molecular weight were gathered after MS analyses of only half of the recovered fractions lower than 1kDa. Some of the low molecular weight molecules were glycine-rich peptides (9%) whereas the chemical nature of the others remains unidentified. These molecules could correspond either to degradation product of biopolymers or to metabolites accumulated in nacre during the growth of the oyster shell.

The low molecular weight fraction contained specific inhibitors of cysteine protease in addition to proteinase K inhibitors (Bédouet et al., 2007). The specificity of the proteinase

inhibitors Found in the nacre water-soluble fraction is quite interesting. Indeed, the cysteine proteinases, particularly cathepsin B and L, are involved in different pathologies, such as arhtrosis and cartilage degradation. The organic matrix of nacre contains both general and more specific inhibitors of cysteins protease in addition to proteinase K inhibitors. The mollusc shell may be used as a new source of natural inhibitors, particularly for the cystein proteinases. The proteinase inhibitors present in the shell may play a major role in the regulation of biomineralization, and they may protect the nacre layer against proteolytic digestion during the perforation of the shell by worms.

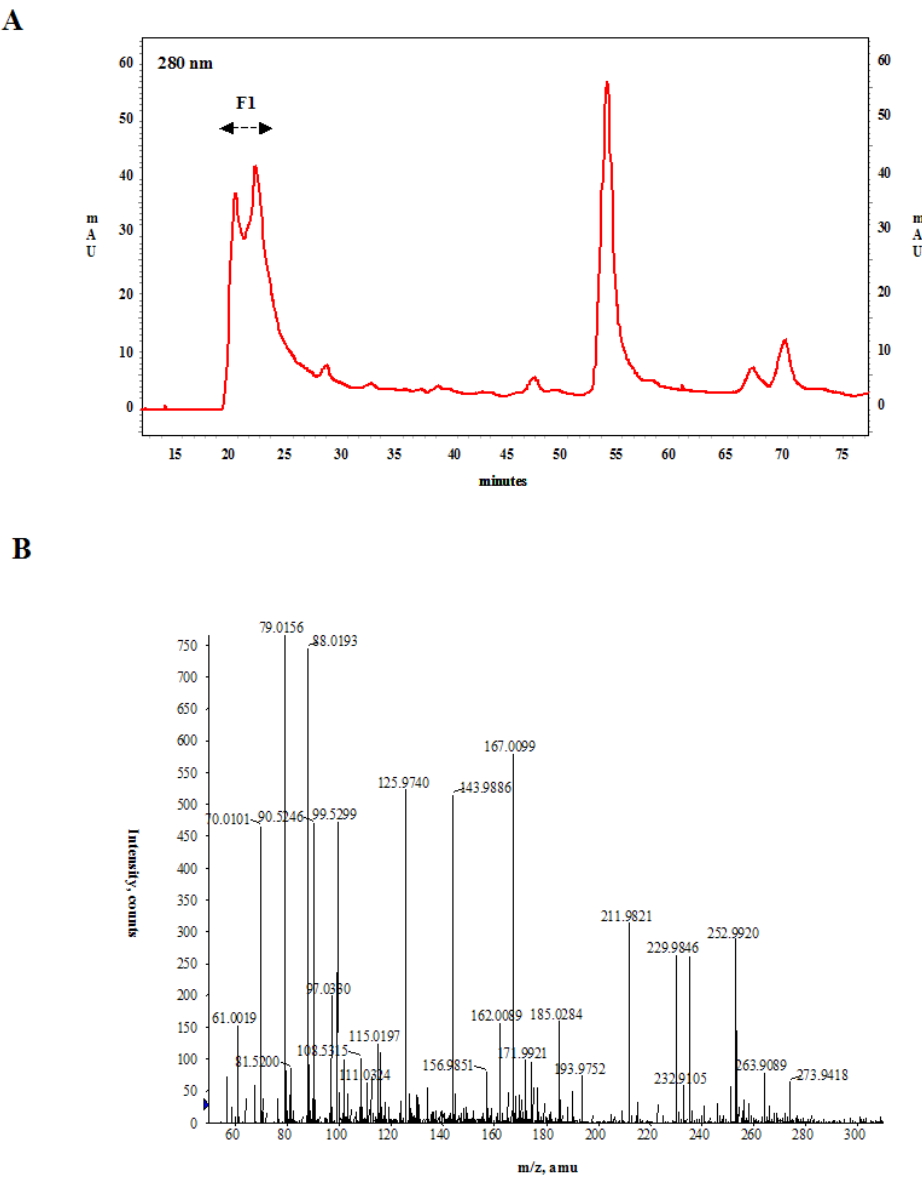


Fig. 12. Isolation of active fraction F1 from the nacre low molecular weight molecules using reversed-phase chromatography. The ESM fraction (75 mg) was loaded onto a C₄ column (2.2 x 25 cm) before elution with a linear acetonitrile-0.1 % TFA gradient. The flow rate was 3 mL/min and fractions (5 mL) eluted between 16 and 25 min were pooled giving the fraction F1 (A). Analysis using electrospray mass spectrometry (ion-positive mode) of the fraction F1. Labels indicate [M+H]⁺ ion species (B).

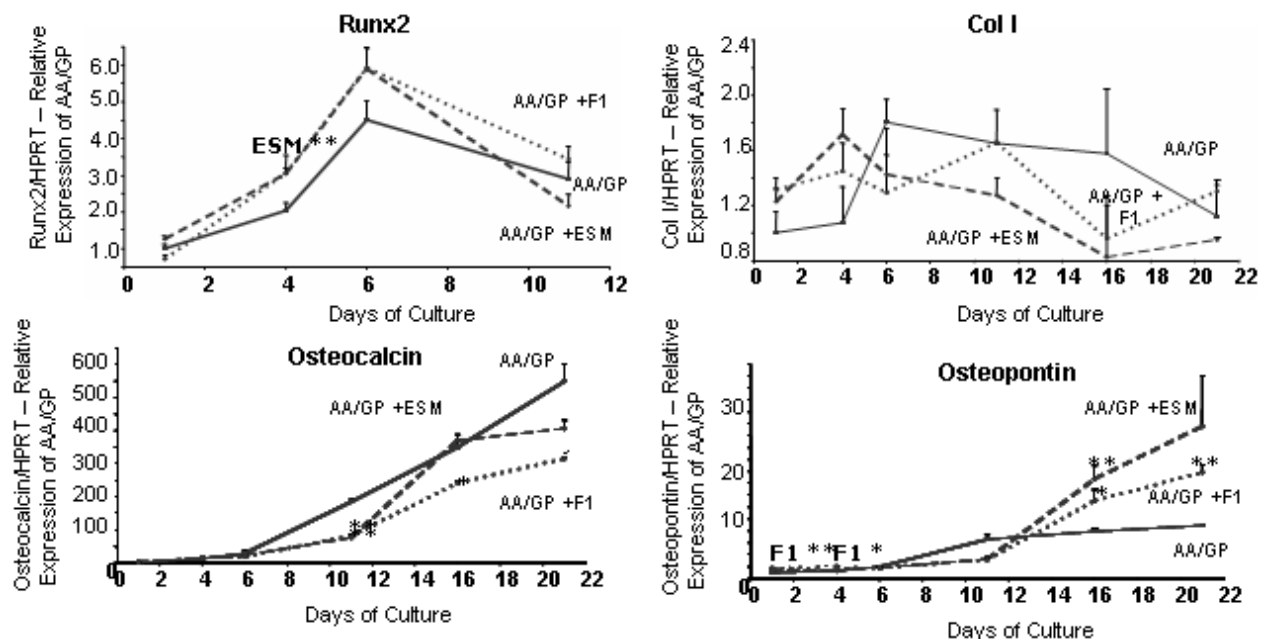


Fig. 13. Effects of ESM and F1 fractions on Runx2, type I collagen, osteopontin and osteocalcin gene expression in MC3T3-E1 cells analyzed by quantitative RT-PCR. The MC3T3-E1 cells were cultured during 1, 4, 6, 11, 16 and 21 days in the absence or presence of ESM and F1 fraction (200 $\mu\text{g}/\text{mL}$) in α -MEM medium supplemented with 10 mM GP (β -glycerophosphate) and 50 $\mu\text{g}/\mu\text{L}$ AA (ascorbic acid). Total cellular RNA was isolated and expression of Runx2, type I collagen, osteopontin and osteocalcin were determined by quantitative RT-PCR relative to the HPRT expression. Results are the means + SEM of 5 experiments for Runx2 and 3 experiments for col I, osteopontin and osteocalcin. The reference is day 1 from AA/GP samples. Data were corrected for initial template quantity variations by normalizing values by HPRT. A minimum significant difference (* $p < 0.05$, ** $p < 0.01$) between control and the samples ESM and F1 was determined.

Lipids presence was discovered in the so-called “ethanol soluble matrix” (ESM). The extract contains fatty acids, triglycerides, cholesterol and ceramides in low abundance (Fig.15). Application of ESM on human skin explants previously partially dehydrated induced an overexpression of filaggrin (responsible for hydration of stratum corneum) and a decrease of transglutaminase expression (overexpressed in inflammatory skin diseases). We also showed that ESM extracted from the mother of pearl of *P. margaritifera* induced a reconstitution of the intercellular cement of the *Stratum corneum* (Fig.16) (Rousseau et al., 2006). If the physiological and structural functions of the lipids in the nacre remain elusive, their biological activity on delipidated human skin explants is more accurate. Two lipids preparations containing respectively 0.5 and 1% of nacre lipids in weight were prepared with an excipient. According to the immunolabelling of filaggrin in skin explants, it has been showed that the nacre lipids induced on the delipidated skin an expression of filaggrin higher to the the level found in untreated skin. On the other hand, the lipid formulation reduced the expression of TGase 1 in the cornified envelope in a dose dependent manner. It seemed that the lipids extracted from nacre had brought to the stratum corneum the elements necessary to a rapid reconstruction of the intercellular cement, by acting on the expression levels of transglutaminase and filaggrin.

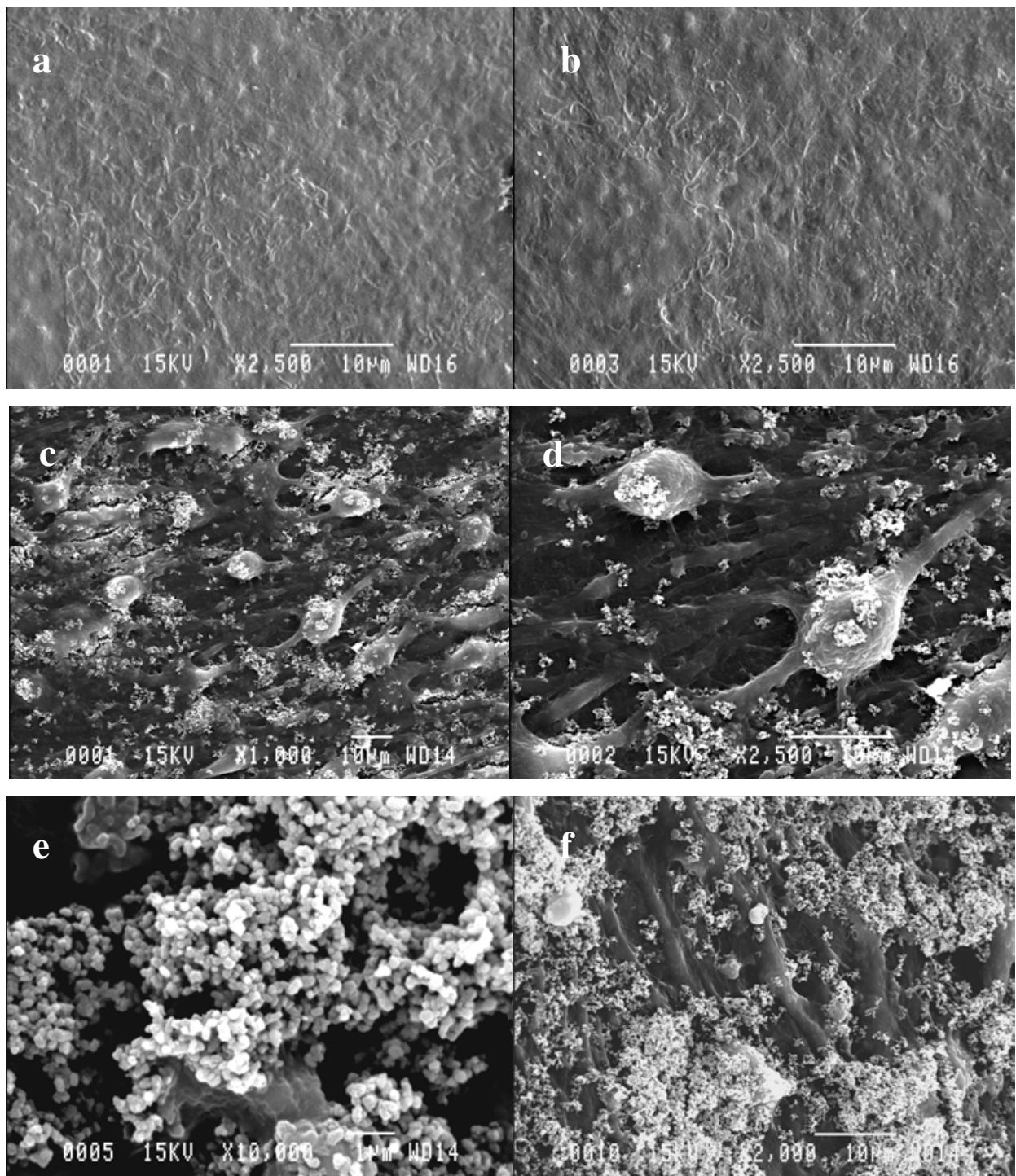


Fig. 14. Scanning electron microscopy observation of the MC3T3-E1 cells extracellular matrix treated with ESM for 16 days. Control cell (a), cells treated with AA/GP (b), cells treated with ESM (200 µg/mL) in presence of AA/GP (c, d, e, f) at different magnifications.

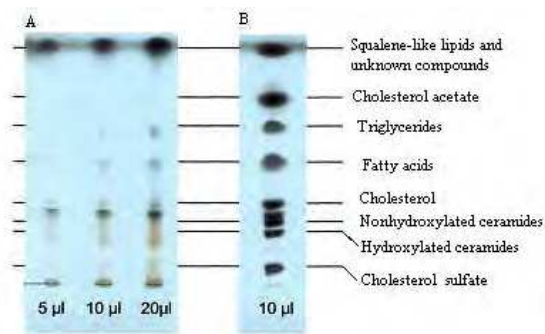


Fig. 15. TLC analysis of lipids extracted from the nacre of *P. margaritifera* with hexane/diethylether/acetic acid (60,25,15, v/v/v) as a mobile phase. Samples of 5, 10 and 20 µl of the lipid extracts at 20 mg/ml and a standard lipid mixture loaded and stained with copper sulfate reagent.

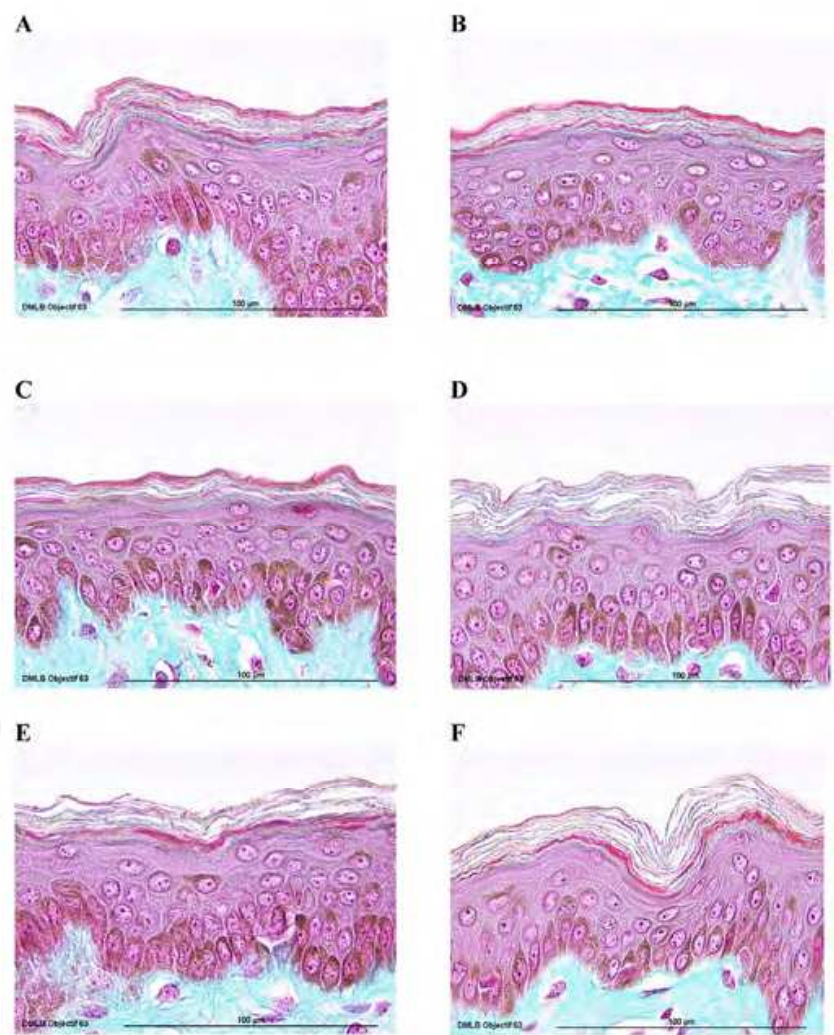


Fig. 16. Histological observation of the skin explants morphology. Control at T₀ (A), in dehydrated skin at T₀ (B), in dehydrated skin at T + 3 h (C), in dehydrated skin at T + 3 h treated with excipient (D) and in dehydrated skin treated with 0.5 % (E) and 1 % (F) of nacre lipids at T + 3 h. Staining was performed according to the Masson's Trichrom.

6. Conclusion

Nacre, or mother-of-pearl, is a calcium carbonate structure produced by bivalves, gastropods, and cephalopods as an internal shell coating. Because of its highly organized internal structure, chemical complexity, mechanical properties and optical effects, which create a characteristic and beautiful lustre, the formation of nacre is among the best-studied examples of calcium carbonate biomineralization. A major breakthrough was done in 1992, when E. Lopez et al. discovered that natural nacre from the pearl oyster *Pinctada* is simultaneously biocompatible and osteoinductive. A complete sequence of osteogenesis resulted from direct contact between newly formed bone and the nacre, anchoring the nacre implant. Furthermore, when nacre is implanted in bone, new bone formation occurs, without any inflammatory reaction and fibrous formation. In previous studies as reviewed in Westbroek and Marin, it has been shown by means of *in vivo* and *in vitro* experiments that nacre can attract and activate osteoblasts. Nacre has been implanted in the subchondral bone area in the sheep knee. A process of nacre powder preparation has been patented. The obtained nacre powder has been experimented in injectable form in vertebral and maxillar sites by sheep. Nacre implant induced no inflammatory reaction. Nacre is biocompatible, osteogenic and osteoinductor.

Nacre is composed of aragonite crystal tablets covered by an organic matrix. The water-soluble organic matrix dictates which calcium carbonate crystal structure is formed and when it is deposited. The bulk of the watersoluble fraction is thought to consist of a complex mixture of proteins and peptides.

The water soluble matrix (WSM) increases alkaline phosphatase activity and induces formation of bone nodules of the clonal osteogenic cell-line MC3T3-E1. WSM, Dexamethasone and BMP-2 all stimulate alkaline phosphatase activity in a manner corresponding to a differentiation into an osteoblast phenotype. On bone marrow stromal cells, WSM stimulates the proliferation, the differentiation and the early mineralization of osteoprogenitor cells. Small molecules isolated from nacre induce mineralization of the preosteoblast extracellular matrix after 16 days of culture. Raman spectroscopy revealed as hydroxyapatite the crystals formed extracellularly. We also extracted proteinase inhibitors from the nacre of *Pinctada*. The low molecular weight fraction contained specific inhibitors of cysteine protease in addition to proteinase K inhibitors. We also discovered the presence of lipids in nacre and we showed that their lipids induced a reconstitution of the intercellular cement of the human *Stratum corneum*.

Nacre has been mostly studied the last two decades for its action on bone and skin (Table 1).

Biocompatibility

- Nacre is not toxic for the human body after maxillary implantation
- When nacre is implanted in bone, no inflammatory reaction nor fibrous formation was observed.

Effects on Bone

- Osteogenic activity was observed in sheep after implantation in bone environment
- Nacre powder is resorbable and this resorption induced the formation of normal bone *in vivo*
- Nacre can attract and stimulate osteoblasts activity *in vitro*
- The nacre molecules involved in bone cell differentiation are small, certainly different from proteins, and probably peptides

Effects on Cartilage

- Nacreous trochlea is covered with new non fibrous cartilage after implantation in sheep

Anti-proteases effect

- Presence of cysteine proteases and proteinase K inhibitors in nacre organics

Effect on Skin

- Nacre lipids are able to reconstitute the epiderm intercellular cement

Table 1. Summary of the biological actions of nacre

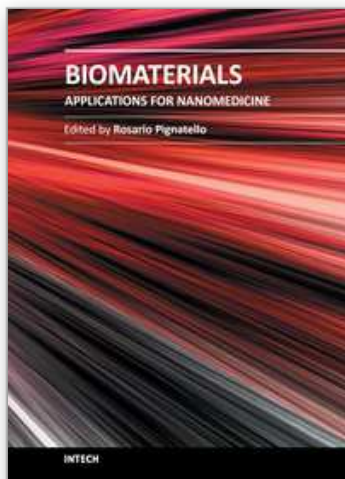
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