Available online at www.sciencedirect.com
SCIENCE DIRECT®
C. R. Palevol 3 (2004) 493–501
COMPTES RENDUS PALEVOL

General Palaeontology (Palaeobiochemistry)

Conservation of signal molecules involved in biomineralisation control in calcifying matrices of bone and shell

Christian Milet *, Sophie Berland, Meriem Lamghari, Lucilia Mouries, Cécile Jolly, Sandrine Borzeix, Dominique Doumenc, Évelyne Lopez

UMR CNRS 5178 'Biologie des organismes marins et écosystèmes', département des milieux et peuplements aquatiques USM 0401, Muséum national d'histoire naturelle, 7, rue Cuvier 75231 Paris cedex 05, France

Received 15 October 2003; accepted after revision 12 July 2004

Available online 02 October 2004

Written on invitation of the Editorial Board

Abstract

Bone and nacre feature highly orchestrated biomineralised structures. They are calcified structures that share an intervening organic matrix laid by specialized cells, bone cells in vertebrates and epithelial mantle cells in molluscs. Organic framework provides a scaffold for crystallisation and directs mineralisation. Bone and shell likely undergo self-reparation when damaged, clues to the presence in these mineralised structures of signal molecules, targeting the cells involved in their renewal. In molluscs, calcium carbonate crystallise under two mineral polymorphs, aragonite or calcite. Sudo et al. [22] reported two exclusive proteins in the shell of a bivalvia molluscan, *Pinctada fucata*, MSI 60 in aragonite and MSI 31 in calcite. We built up specific antibodies and experimented immunohistochemistry in Haliotis. These tools revealed the presence of Pinctada-like proteins within Haliotis mantle cells, in a zonation consistent with shell matrix mineralisation control involvement. Another facet of matrix-mediated biomineralisation control arises from bone and nacre interactivity. In vivo experiments of nacre implantation in sheep and rabbit established the biocompatibilty of nacre and bone. The nacreous part from the shell of Pinctada, a bivalve mollusc, was implanted as bone device in sheep and showed that nacre passes bone acceptance. Nacre implants were not subjected to intolerance reaction and the recipient bone provided with nacre underwent a sequence of bone regeneration within an osteoprogenitor-rich cell layer. The water-soluble organic matrix was extracted from powdered nacre by a gentle non-decalcifiying process. Three mammalian cell types, fibroblasts (human), bone marrow stromal cells (rat) and pre-osteoblasts (mouse) were used to characterize in vitro the effect of nacre water-soluble matrix on mammal bone cell lineage. In vitro studies provided evidence for the presence, in nacre organic matrix, of signal molecules responsible for the recruitment of mammal cells in the osteogenic pathway and bone cell activation undergoing a complete sequence of mineralisation. Retrieving like-proteins in shell matrix of molluscs from distant taxa and bone nacre interactivity provides convergent data supporting the conservation of molecular signals for biomineralisation control within the organic framework of biomineralised structures. ***To cite this article: C. Milet et al., C. R. Palevol 3 (2004)***.

* Corresponding author.
E-mail addresses: milet@mnhn.fr (C. Milet), berland@mnhn.fr (S. Berland), lopez@mnhn.fr (Lopez).

1631-0683/$ - see front matter © 2004 Académie des sciences. Published by Elsevier SAS. All rights reserved.
doi:10.1016/j.crpv.2004.07.010

Résumé

L'os et la nacre, deux modèles d'étude de la conservation de molécules de contrôle des biominéralisations dans les matrices minéralisantes. Des anticorps dirigés contre des protéines spécifiques de la nacre, MSI 31 et MSI 60 (respectivement associées au dépôt de carbonate de calcium sous forme de calcite ou d'aragonite) ont été préparés. Ils révèlent par marquage immunohistochimique les régions de l'épithélium du manteau contenant les populations cellulaires impliquées dans la synthèse des couches minéralisées de la coquille. Par ailleurs, des expériences d'implantation de nacre dans l'os in vivo démontrent la bonne tolérance de ce matériau et une réaction locale d'ostéogenèse aboutissant à la soudure entre l'os néoformé et la nacre. La présence dans la nacre de molécules ayant une fonction de signalisation sur les ostéoblastes est discutée d'un point de vue phylogénétique et fonctionnel. ***Pour citer cet article : C. Milet et al., C. R. Palevol 3 (2004)***.

Keywords: Biomineralisation; Matrix proteins; Bone cells; Mantle cells; Nacre; Signal molecules

Mots clés : Biominéralisations ; Protéines matricielles ; Cellules osseuses ; Cellules palléales ; Nacre ; Molécule signal

Version française abrégée

Tissu osseux et nacre sont des structures composites, organiques et minérales, résultant de la calcification d'un réseau matriciel, compétent pour la minéralisation. La trame organique joue un rôle prépondérant dans le guidage et le contrôle de la cristallisation du minéral.

La biominéralisation, sous forme de carbonate de calcium dans la nacre et de phosphate de calcium dans l'os, est liée, dans les deux cas, à la présence d'un polymère support, de type fibroïne pour le carbonate et collagène pour le phosphate de calcium. Cette trame adsorbe des molécules intervenant dans le contrôle et la régulation de la minéralisation. La matrice organique est secrétée par des cellules spécialisées : cellules osseuses ou cellules épithéliales du manteau. La nacre comme l'os sont susceptibles d'auto-réparation, indice de la présence, dans la matrice de la nacre, de signaux morphogènes ciblant les cellules aptes à engager le processus de régénération, comme il en existe dans la matrice osseuse. Les résultats présentés ici apportent des éléments en faveur de la conservation au cours de l'évolution de molécules intervenant dans le contrôle de la cristallisation et de facteurs signaux de recrutement des cellules impliquées dans le processus de biominéralisation.

Un premier volet de cette étude concerne la conservation des molécules impliquées dans le contrôle du réseau de cristallisation. Dans la coquille des Mollusques, le carbonate de calcium cristallise dans le réseau rhomboédrique sous forme de calcite, ou dans le réseau orthorhombique sous forme d'aragonite (constituant cristallin de la nacre). Sudo et al. [22] ont caractérisé deux protéines, constitutives exclusivement de la matrice organique de la calcite ou de l'aragonite chez Pinctada, respectivement MSI 31 et MSI 60. Nous avons synthétisé des anticorps spécifiquement dirigés contre l'une ou l'autre de ces molécules. Une étude immunohistochimique, mise en place chez l'Ormeau *(Haliotis tuberculata)*, a mis en évidence la présence, dans le manteau, de protéines apparentées aux protéines MSI 31 et MSI 60 de Pinctada. La localisation du marquage obtenu dans l'épithélium du manteau est cohérente avec l'implication de la population cellulaire marquée dans la synthèse de la matrice des couches minéralisées de la coquille.

Un second volet s'intéresse à la conservation des molécules impliquées dans le recrutement et la différenciation de cellules squelettogènes. Nos travaux antérieurs établissent que la nacre recèle un potentiel ostéogénique. Des fragments de nacre, implantés in vivo, en site endo-osseux chez l'animal comme implant prothétique induisent une activité ostéogénique de la part du tissu osseux receveur [2,11,12]. Le premier résultat notable est la parfaite biocompatibilité de la nacre implantée. Aucune réaction inflammatoire chronique en réaction à un corps étranger n'est entraînée par l'implant. Le second point fort des résultats de cette expérimentation est l'ostéointégration de la nacre dans le tissu osseux néoformé. L'analyse en microscopie électronique par spectrométrie de rayons X de l'interface os/nacre montre que l'ancrage direct de l'os sur la nacre est précédé par une phase d'activation

cellulaire dans le tissu hôte et par la mise en place, à la surface de la nacre, d'un micro-environnement ionique riche en calcium et en phosphate [2]. Durant cette phase, l'implant de nacre subit une érosion de surface. Se fondant sur ces résultats, nous avons mis au point une méthode d'extraction par laquelle seule la composante hydrosoluble de la matrice organique de la nacre est extraite, en n'appliquant aucun traitement déminéralisant ou dénaturant. La présence des molécules actives sur l'ostéogenèse dans l'extrait hydrosoluble de la matrice de la nacre de Pinctada est vérifiée in vitro sur les cellules formatrices d'os et sur leurs précurseurs : des cellules stromales de la moelle osseuse (rat), des fibroblastes (humains) et des ostéoblastes (murins) à différents stades de maturation [13,18]. Les résultats obtenus montrent que le potentiel ostéogénique des molécules signal stockées dans la matrice organique de la nacre s'exprime, sur les cellules de la lignée osseuse, des cellules souche aux ostéoblastes, mais également en amont, sur des cellules fibroblastiques, lors d'une phase de recrutement cellulaire.

La présence de protéines matricielles apparentées chez deux mollusques phylogénétiquement distant et la compatibilité de molécules signal provenant de la nacre avec les cellules mammaliennes impliquées dans l'ostéogenèse constituent des indices forts en faveur de la conservation moléculaire des signaux de contrôle des biominéralisations.

1. Introduction

Bone and shell are biomineralised structures that share a matrix supported and controlled calcification process. They are the result of a mineral crystallization deposited on an organic matrix scaffold: calcium phosphate in bone and calcium carbonate in molluscan shell. This matrix is laid by special cells: osteoblasts in vertebrates and epithelial mantle cells in molluscs. Epithelium is the first provider for the macromolecules involved in shell construction as bone cells are in osteogenesis.

In molluscan shell, calcium carbonate crystallizes under two mineral polymorphs: aragonite or calcite. The mineral is impregnated in biopolymers within an extracellular space between the mantle cells layer and the shell. This open space is filled with an extrapallial fluid in which the organic crystals are laid down in orderly arrays in intimate association with a matrix of organic macromolecules [21]. Crystal ingrowth and polymorphism is mediated by organic matrix proteins [4,8,23]. In Pinctada shell, calcite and aragonite are arranged in separated layers, the innermost one, a nacreous layer, being pure aragonite. Haliotis shell also contains an inner coverage of nacre, the unique $CaCO_3$ crystal form being aragonite and a prismatic outer layer where $CaCO_3$ crystallized under calcite with interspersed aragonite inclusion [7,16]. Thermal analysis of bivalvia shell show that the organic matrix in aragonite differs from that of calcite [9] and the proteic pattern of the matrix partly supports this specificity. After Crenshaw [6] first characterized Ca-binding glycoprotein in the matrix extracted from the shell of *Merceneria merceneria*, subsequent studies have focused on fractionation, purification, and characterization of the peptidic components of the matrix. Advanced results are convergent with a general feature of highly acid composition of the soluble matrix [17,19] related to a general capacity to bind calcium ions. Moreover, it has been clarified in vitro that the organic matrix is involved in accurate crystallization process control in aragonite or calcite [8]. Signal candidate to support this control must be exclusive of calcite or aragonite matrix. Sudo et al. [22] reported two such exclusive proteins in the shell of a bivalvia molluscan, *Pinctada fucata*, MSI 60 in aragonite and MSI 31 in calcite. We built up specific antibodies and experimented immunohistochemistry in Haliotis. Pertinent specific shell matrix proteins producing zones were tagged within Haliotis mantle epithelium.

Another facet of matrix-mediated biomineralisation control arises from results that we obtained on bone and nacre interactivity. A series of *in vivo* experiments of nacre implantation in sheep and rabbit established the biocompatibility of nacre and bone. Bone provided with nacre undergoes osteogenesis and remodelling leading to bone and nacre welding. The organic matrix was extracted from nacre, especially the water-soluble fraction, and its biological effects were experimented on the mammalian cells involved in bone regeneration. Cell culture modelling allowed us to establish that the nacre water-soluble matrix activated bone cells to maturation and recruited bone precursors cells, stromal cells, to bone-cell differentiation.

Bone and shell likely undergo self reparation when damaged, clues to the presence in these mineralised

structures of signal molecules, targeting the cells involved in bone or shell renewal.

Both the presence of like-proteins in the shell matrix of molluscs from distant taxa and the bone nacre interactivity provide convergent data in support of the conservation of molecules involved in biomineralisation control.

Retrieving a stain with antibodies run on specific protein from a Bivalvia (Pinctada) and a Gasteropoda (Haliotis) highlights the importance of the sequences that we held in the biomineralisation process and support the hypothesis of phylogenic conservation of sequences playing an important role in the control of the biomineralisation process.

2. Material and methods

2.1. Immunohistochemical study in Haliotis mantle cells

The Haliotis specimen reported in this study were reared in seawater. They all belong to an age class from 3 to 6 years old. This range was chosen to ensure a high growing capacity. Two thirds were collected between March and April, during the annual growing phase and one third was collected, late October, during the resting period [5].

2.1.1. Sample preparation

The whole mantle margin that lines the shell was dissected out and fixed in a 4% solution of paraformaldehyde fluid buffered in PBS adjusted to pH 7.6 for 12 h at 4 °C then embedded in paraffin. Serial 6-µm sections were cut for immunohistochemical studies.

2.1.2. Construction of antibodies

The antibodies were constructed using the sequences of the framework proteins for the nacreous (MSI 60) and prismatic (MSI 31) layers of *Pinctada fucata* reported by [22]. These sequences fulfilled three requirements: potent immunogenecity, sequence specificity, and a pertinent ratio of acidic amino acids, this criteria being a general feature of proteins involved in the biomineralisation process. The designed sequences were checked by screening generalist sequence database in order to avoid immunological cross reactivity with metabolic proteins.

2.1.3. Immunochemistry procedure

After washing and blocking of a non-specific protein attachment site using normal rabbit serum (1:50) during 20 min at room temperature, sections were probed for peroxidase or fluorescence MSI 31-like and MSI 60-like immunolabelling. Sections were incubated in diluted (1:2000) MSI 31 or MSI 60 antibody in PBS at 4 °C overnight.

The nuclei were also visualized by DAPI (4',6-diamidino-2-phenylindole, Sigma) treatment, 1 h in 1:100 PBS at room temperature.

2.1.4. Controls

Nonimmune rabbit serum at the same dilution was used instead of the primary antisera. Antibody specificity was checked by incubating with the respective antigen for 1 h prior to the immunochemical procedure. Cross reactivity was also checked by incubating MSI 31 antigen to the constructed anti MSI 60 antibody and vice-versa prior to the immunocytochemical procedure.

2.2. Bone nacre biocompatibility

Mother of pearl was obtained from the inner shell layer of Pinctada, Molluscan, bivalvia.

2.2.1. In vivo studies

Pinctada mother of pearl pieces were implanted in sheep, under sterile surgical conditions within experimental bone defects. Solid nacre implants were placed in the femur epiphysis [2]. Nacre, grounded to 50–150-µm particles was used to fill cavities prepared in lumbar vertebrae [12].

A histological study of the bone provided with nacre was performed on undecalcified samples after methyl methacrylate embedding [2]. Ca and P X-ray mapping at the nacre/bone interface was undertaken on 100-µm thick undecalcified sections using a scanning electron microscope (Leica Stereo Scan 260) coupled to an energy dispersive X-ray Analyser (Kevex Delta Analyser). X photons were detected and analysed using a boron nitrite ultra thin window detector to show the distribution of the atomic elements in the sample.

2.2.2. Extraction of water-soluble matrix (WSM)

Nacre reduced to particles was suspended according to the procedure described in the Patent

No. FR951225, and the resulting supernatant, WSM, was added to the culture media.

2.2.3. In vitro studies

The biological effects of nacre WSM were evaluated on the mammalian cells involved in bone regeneration process, the bone cells, and their recruitable precursors. We used MRC5, human foetal lung fibroblasts, obtained from the Institute for Medical Research (London); primary cultures of bone marrow cells from femurs of young male Wistar rats, MC3T3-E1, preosteoblasts cell line from mouse calvaria (Dr Kumeggawa's kind supply, Josai Dental University, Sakado, Japan) and MG63 cell line (human osteosarcoma, European Collection of Cell Cultures, Salisbury, UK) model of immature osteoblasts. Cultures were processed as described in [18]. Bone-cell differentiation was evaluated by measurement of alkaline phosphatase activity, osteocalcine levels and detection of mineralised nodule using Von Kossa staining. WSM activity was compared to that of standards osteogenesis inducers, dexamethasone (DEX), BMP-2, β-glycerol-phosphate (BGP), and ascorbic acid (AA).

3. Results

3.1. Immunohistochemistry on molluscan mantle cells (Fig. 1)

The presence of *Pinctada fucata* MSI 31 and MSI 60-like proteins was observed in the mantle epithelium of *Haliotis tuberculata*. The immunohistological labelling was only detected in the spring batch specimen and exclusively in the anterior right quadrant of the mantle. Our results highlighted three distinct limited zones of MSI31 and MSI60-like proteins distribution in the anterior right lobe of the Haliotis mantle: the pad of outer epithelium in the periostracal groove, the tubular zone and a limited zone of the folded external epithelium. The specimens collected in autumn, the low-growing period, did not reveal any immunohistological labelling. Molecules are present in a time range related to the shell growth period.

Controls checked for the specificity of the reaction. Controls for antibodies specificity with the antigen–antibody preincubation step underwent a significant fading of the labelling.

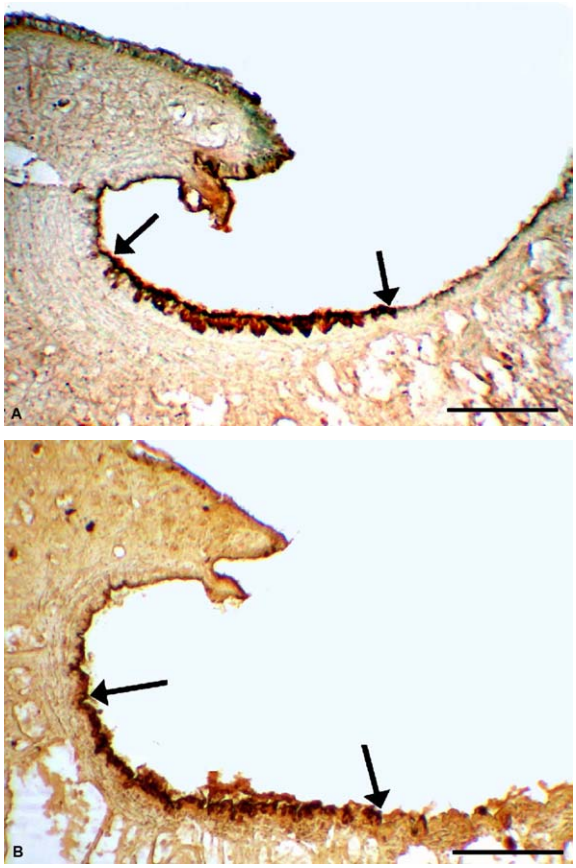

Fig. 1. Light microscope observation showing the zone for MSI31-like **(a)** and MSI60-like **(b)** labelling (dark DAB reaction product) within the external epithelium in Haliotis (→). Bar = 200 μm.
Fig. 1. Observation en microscopie optique de la zonation des protéines apparentées à MSI-31 et MSI-60 (réaction chromatique de la DAB) dans l'épithélium externe d'Haliotis (→). Échelle = 200 nm.

The designed antibodies were also specific to either MSI 31 or MSI 60 antigenic sequences, since there was no outcome difference in the results after a preincubation of the antibody with the opposite synthetic antigen.

3.2. Nacre bioactivity on bone cells

When implanted as replacement bone device, Pinctada nacre pieces showed persistence in bone tissue. Bone supplied with nacre implant underwent osteogenesis process. Histological analysis of undecalcified samples showed that osteogenesis began within an intervening activated cell layer. Bone ingrowth re-

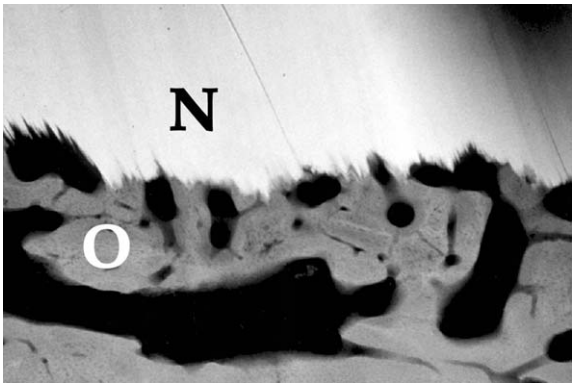

Fig. 2. Light microscopy of an undecalcified section of a trabecular bone provided with a nacre implant. Microradiography showing the welding between bone trabeculae **(O)** and nacre **(N)**.
Fig. 2. Observation en microscopie optique d'une coupe d'os trabéculaire non déminéralisé à l'interface d'un implant de nacre. La microradiographie montre la soudure entre l'os **(O)** et la nacre **(N)**.

sulted in a direct contact between newly formed bone and nacre, providing the anchoring of the implant (Fig. 2).

X-ray diffraction electron microscopy analysis established that the first step of nacre osteogenic effect occurred via the establishment of a calcium and phosphorus front (Fig. 3). Ca and P, constitutive elements of the bone mineral phase, show a dense distribution pattern at the edge of nacre within the host tissues. The Ca–P-rich cell layer indicates that nacre cellular activation targets osteogenesis.

The organic matrix of nacre was supposed to be the source of signal molecules diffusible from the implanted nacre. The water-soluble components of the organic matrix were extracted from nacre following a gentle patented process without preliminary demineralisation step. The proteic fraction of the organic matrix released by this process exhibits a highly hydrophobic, glycine- and alanine-rich pattern.

To test the hypothesis that WSM was responsible for nacre bioactivity on bone, in vitro experiments were

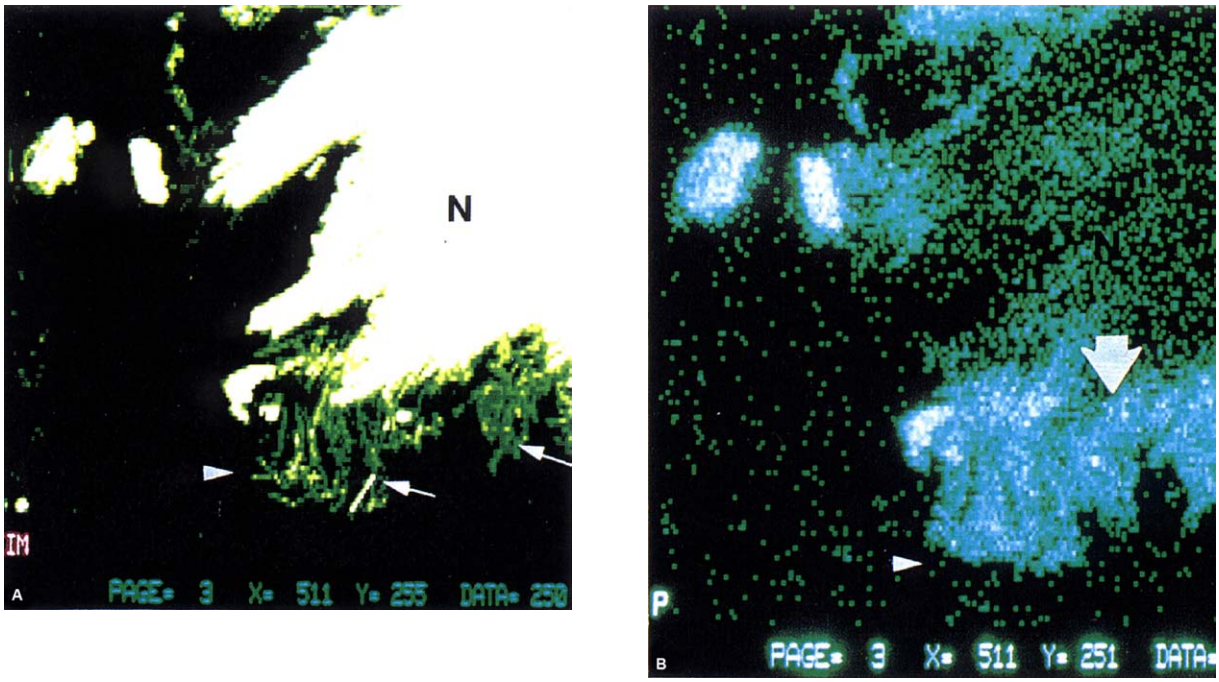

Fig. 3. Electron microscopy and energy dispersive X-ray analysis at the interface of a bone replacement nacre implant (× 1200). **(A)** Scanning image showing a nacre implant **(N)** and mineralisation clusters in the host tissue (➤) and at the edge of nacre (→). **(B)** Phosphorus-rich front (⇨) at the edge of the nacre implant (**N**) and within the host tissues (➤).
Fig. 3. Microscopie électronique à balayage et analyse aux rayons X par dispersion d'énergie à l'interface de l'os et de l'implant de nacre (× 1200). **(A)** Image de l'implant de nacre **(N)** îlots de minéralisation dans le tissu hôte (➤) à l'interface de la nacre (→). **(B)** Zone riche en phosphore (⇨) au bord de l'implant de nacre **(N)** et dans le tissu hôte (➤).

done using the mammalian cell types involved in bone regeneration. Cell types were chosen to provide a set of osteogenic lineage, from precursors to pre-differentiated bone cells. Bone marrow cells, MRC5 fibroblasts, MC3T3-E1 and MG63, respectively preosteoblasts and immature osteoblasts were used.

Bone marrow cells supplemented with WSM started to mineralise after 14 days of culture (Fig. 4). In the meanwhile, osteocalcine level increased consistently, corroborating the maturation in bone forming cells of WSM treated bone marrow cells (Fig. 4).

MRC5 fibroblasts response to WSM treatment was an early increase in alkaline phosphatase activity when compared with differentiating factors (DEX and BMP-2) effects. Alkaline phosphatase activity and osteocalcine measurements showed that osteoblastic cells were induced to maturation (MG63), up to mineralisation in MC3T3-E1 cultures.

4. Discussion

The shell is a composite structure, constituted of mineral and organic components, in which peptides and proteins play an important role in achieving crystallization control [24].

We constructed antibodies raised against shell matrix proteins potentially involved in the mineralisation control and processed for the proteins localization in the epithelium mantle cells, source for the matrix framework for shell construction. Sudo et al. [22] characterized, in the shell of Pinctada, two matrix proteins, MSI 31 and MSI 60, specific to the organic matrix of the calcitic and the aragonitic layer respectively. The peptidic sequences of those proteins were used in this study for specific antibody construction. We chose sequences liable to cause implication in biomineralisation. Previous studies have suggested that protein with $(ASP\text{-}X)_n$ repeating sequence, comprising regular repeating negative charges, could bind Ca^{2+} ion and thus perform an important function in mineralisation [10,24]. We can assume that this high content of $(ASP\text{-}X)_n$ repeating sequence could be retrieved in Haliotis species. *Haliotis tuberculata* shell matrix proteins contain a high proportion of acidic amino acids [1,3,20]. Characterization of shell matrix components, such as nacrein [15], MSI 31, and MSI 60 [22] in Pinctada, mucoperlin [14] in Pina, showed that those matrix

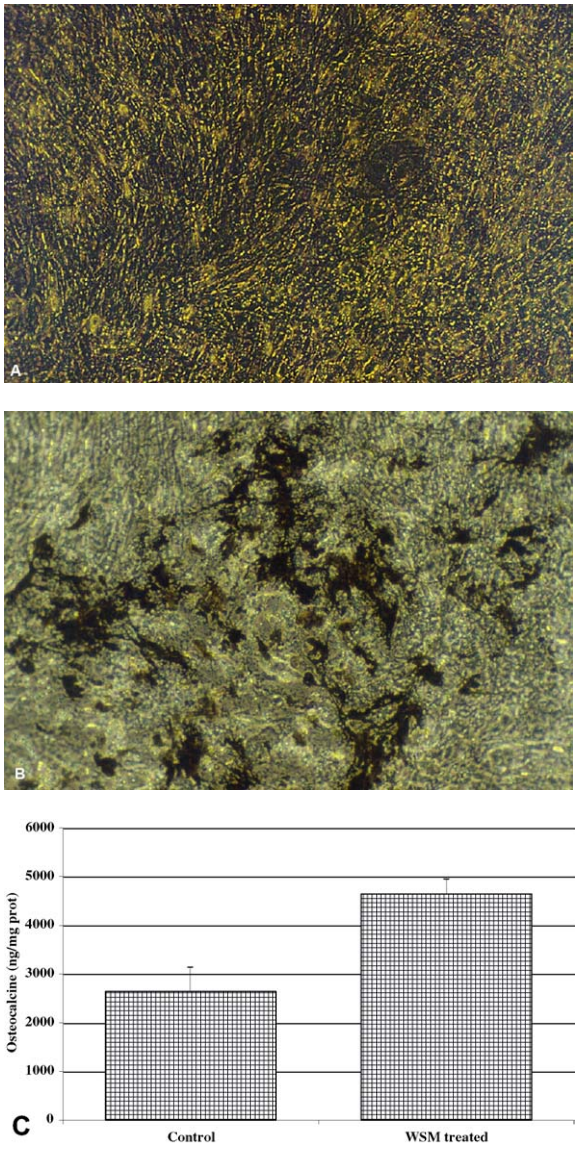

Fig. 4. WSM effect in bone marrow stromal cells cultures. (**A**–**B**) Calcium staining at day 14 using the Von Kossa technique: **(A)** diffuse low-level staining in control culture without WSM; **(B)** mineral clusters – dark staining – in WSM-treated cells. **(C)** Osteocalcin level at day 14.

Fig. 4. Effet de la matrice hydrosoluble de la nacre (WSM) sur des cultures de cellules de moelle osseuse. (**A**)–(**B**) Mise en évidence du dépôt de calcium à J14 par la réaction de Van Kossa. **(A)** Réaction diffuse et de faible intensité dans le lot contrôle. **(B)** Îlots de minéralisation dans les cultures supplémentées en WSM. **(C)** Dosage d'estéocalcine dans les cultures témoin et en présence de WSM à J14

proteins share calcium-binding domains. The sequences that we held were selected for their specificity and their high ratio in anionic or acidic amino acids, respectively 57% and 81% in the selected MSI 31 and MSI 60 sequences. Our work demonstrated the in situ immunolocalization of MSI 31 and MSI 60-like proteins in outer mantle epithelium of Haliotis. Sudo et al. [22] reported in situ hybridisation results showing in what limited-extent region the mantle expressed two shell specific matrix proteins, MSI 31 and MSI 60 mRNA in Pinctada. The two regions are contiguous and exclusive, MS1 31 being expressed within a more external area at the mantle edge and MSI 60 in the adjacent folded external epithelium. We obtained quite a similar pattern of expression of these proteins in Haliotis with a complete overlap of the two zones described in Pinctada.

Retrieving a stain with antibodies run on specific protein from a Bivalvia (Pinctada) and a Gasteropoda (Haliotis) highlights the importance of the sequences that we held in the biomineralisation process and support the hypothesis of phylogenic conservation of sequences playing an important role in the control of the biomineralisation process.

Nacre and bone interactivity also raises argument for signal molecule conservation. In vivo and in vitro experiments demonstrated that soluble signals can diffuse from nacre and induced bone cell activation [2,11,13]. In an attempt to characterize the molecules involved, soluble matrix was extracted from nacre, without demineralisation step, to avoid molecular denaturation. Nacre water-soluble matrix encloses diffusible signal molecules that can target bone-forming cells [18]. The water-soluble matrix isolated from *Pinctada maxima* nacre contains the signals responsible for the biological activity of the whole nacre. Indeed, this matrix acts in particular on bone-cell differentiation, up until the final step of mineralisation.

Bone and nacre can result in an interactive hybrid system. In vivo studies established that not only bone and nacre can weld [2], but also nacre implantation into bone can induce new bone formation following an endochondral process in the same way as an autologous bone implant does [11].

The guidelines for the reasons why nacre can pass bone acceptance arise from nacre matrix bioactivity on bone cell lineage and on cell recruitment in the osteogenesis pathway.

Because the interactions resulting in controlled crystallization are tailored to an essential biological function, the skeletal modelling, the understanding of the matrix character components underlying this control would therefore be of interest in basic and applied studies. Biominerals, highly organized organic and mineral materials hide clues in bioactivity supply, as demonstrated in bone nacre interactivity. Nature generally performs interaction in biological systems through high-specificity receptor-recognition route. Finding conservative molecules in biomineralised structures among distant taxa can provide data for running the knowledge of biomineralisation control facets underlying related signal molecules.

Acknowledgments

The authors wish to thank Francine Lallier for her kind help in the manuscript preparation.

References

[1] S. Albeck, S. Weiner, L. Addadi, Polysaccharides of intracrystalline glycoproteins modulate calcite crystal growth in vitro, Chem. Eur. J. 2 (1996) 278–284.

[2] G. Atlan, O. Delattre, S. Berland, A. Le Faou, G. Nabias, D. Cot et al., Interface between bone and nacre implants in sheep, Biomaterials 20 (1999) 1017–1022.

[3] L. Bedouet, M.-J. Schuller, F. Marin, C. Milet, E. Lopez, M. Giraud, Soluble proteins of the nacre of the giant oyster *Pinctada maxima* and of the abalone *Haliotis tuberculata*: extraction and partial analysis of nacre proteins, Comp, Biochem. Physiol. 128 (2001) 389–400.

[4] A.-M. Belcher, X.-H. Wu, R.-J. Christensen, P.-K. Hansma, G.-D. Stucky, D.-E. Morse, Control of crystal phase switching and orientation by soluble mollusc-shell proteins, Nature 38 (1996) 56–58.

[5] J. Clavier, O. Richard, Étude sur les ormeaux dans la région de Saint-Malo, Association pour la mise en valeur du littoral de la côte d'Emeraude, 1985, pp. 1–285.

[6] M.A. Crenshaw, The soluble matrix from *Mercenaria mercenaria* shell, Biomineralisation 6 (1972) 6–11.

[7] Y. Dauphin, J.-P. Cuif, H. Mutvei, A. Denis, Mineralogy, chemistry and ultrastructure of the external shell layer in ten species of Haliotis with reference to *Haliotis tuberculata* (Mollusca: Archaeogastropoda), Bull. Geol. Inst. Univ. Uppsala NS 15 (1989) 7–38.

[8] G. Falini, S. Albeck, S. Weiner, L. Addadi, Control of aragonite or calcite polymorphism by mollusk shell macromolecules, Science 271 (1996) 67–69.

[9] I. Kobayashi, Data on the differential thermal analyses of bivalvian shells, Bull. Fossil. Club Tokyo 7 (1973) 1–4.
[10] M. Kono, N. Hayashi, T. Samata, Molecular mechanisms of the nacreous layer formation in *Pinctada maxima*, Biochem. Biophys. Res. Commun 264 (2000) 213–218.
[11] M. Lamghari, P. Antonietti, S. Berland, A. Laurent, E. Lopez, Arthrodesis of lumbar spine transverse processes using nacre in rabbit, J. Bone Miner. Res. 16 (12) (2001) 2232–2237.
[12] M. Lamghari, S. Berland, A. Laurent, H. Huet, E. Lopez, Bone reactions to nacre injected percutaneously into the vertebra of sheep, Biomaterials 22 (2001) 555–562.
[13] E. Lopez, B. Vidal, S. Berland, S. Camprasse, G. Camprasse, C. Silve, Demonstration of the capacity of nacre to induce bone formation by human osteoblasts maintained in vitro, Tissue Cell 24 (5) (1992) 667–679.
[14] F. Marin, P. Corstjens, B. de Gaulejac, E. de Vrind-De Jong, P. Westbroek, Mucins and molluscan calcification. Molecular characterization of mucoperlin, a novel mucin-like protein from the nacreous shell layer of the fan mussel *Pinna nobilis* (Bivalvia, Pteriomorpha), J. Biol. Chem. 275 (27) (2000) 20667–20675.
[15] H. Miyamoto, T. Miyashita, M. Okushima, S. Nakano, T. Morita, A. Matsushiro, A carbonic anhydrase from the nacreous layer in oyster pearls, Proc. Natl Acad. Sci. USA 93 (1996) 9657–9660.
[16] H. Mutvei, Y. Dauphin, J.-P. Cuif, Observations sur l'organisation de la couche externe du test des Haliotis (Gastropoda) : un cas exceptionnel de la variabilité minéralogique et microstructurale, Bull. Mus. Nat. Hist. Nat. Paris 7 (1) (1985) 73–91 ser. 4.
[17] H. Nakahara, M. Kakei, G. Bevelander, Studies on the formation of the crossed lamellar structure in the shell of *Strombus gigas*, Veliger 23 (3) (1981) 207–211.
[18] L. Pereira-Mouries, M.-J. Almeida, C. Milet, S. Berland, E. Lopez, Biactivity of nacre water-soluble organic matrix from the bivalve mollusk Pinctada maxima in three mammalian cell types : fibroblasts, bone marrow stromal cells and osteoblasts, Comp. Biochem. Physiol. 132 (2002) 217–229.
[19] T. Samata, Ca-binding glycoproteins in molluscan shells with different types of ultrastructure, Veliger 33 (2) (1990) 190–201.
[20] T.-E. Schäffer, C. Ionesco-Zanetti, R. Proksch, M. Fritz, D.-A. Walters, N. Almqvist et al., Does abalone nacre form by heteroepitaxial nucleation or by growth trough mineral bridges?, Chem. Mater. 9 (1997) 1731–1740.
[21] K. Simkiss, K.-M. Wilbur, Molluscs - Epithelial control of matrix and minerals, in: K. Simkiss, K.M. Wilbur (Eds.), Biomineralization: Cell Biology and Mineral Deposition, Academic Press, New York, 1987, pp. 230–260.
[22] S. Sudo, T. Fujikawa, T. Nagakura, T. Ohkubo, K. Sakaguchi, M. Tanaka et al., Structures of mollusc shell framework proteins, Nature 387 (1997) 563–564.
[23] J.-B. Thompson, G.-T. Paloczi, J.-H. Kindt, M. Michenfelder, B.-L. Smith, G.-D. Stucky, D.-E. Morse, P.-K. Hansma, Direct observation of the transition from calcite to aragonite growth as induced by abalone shell proteins, Biophysics 79 (2000) 3307–3312.
[24] S. Weiner, L. Hood, Soluble protein of the organic matrix of mollusk shells: a potential template for shell formation, Science 190 (1975) 987–989.