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Organic and mineral networks in carapaces, bones and biomimetic materials

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Abstract

Skeletal tissues associate, in close interaction, an organic matrix and a mineral network. In crustaceans, chitin, the most frequent polysaccharide in invertebrate exoskeletons, is associated with calcite. In compact bone, collagen, major structural protein in vertebrate tissues is associated with hydroxyapatite. A parallel was evidenced in vivo between the three-dimensional assemblies of chitin and collagen matrices and molecular arrangements described in liquid crystals. The purified macromolecules, highly concentrated, assemble in vitro in ordered liquid crystalline phases. After stabilisation, they form biomimetic materials, presently investigated, in a pure state or in addition with inorganic phases to develop hybrid materials. ***To cite this article: M.-M. Giraud-Guille et al., C.R. Palevol 3 (2004).***
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Résumé

Réseaux organiques et minéraux dans la carapace, l'os et les matériaux biomimétiques. La trame organique et le réseau minéral des tissus squelettiques sont en interaction étroite. Chez les crustacés, la chitine, le polysaccharide le plus abondant chez les Invertébrés, est associée à la calcite. Dans l'os compact, le collagène, protéine structurale majeure des vertébrés, est associé à l'hydroxyapatite. Un parallèle est mis en évidence in vivo entre les structures tridimensionnelles des matrices de chitine ou de collagène et les arrangements moléculaires de cristaux liquides. Ces macromolécules purifiées donnent in vitro, en milieu condensé, des états cristallins liquides. Les matériaux biomimétiques obtenus sont étudiés après stabilisation, à l'état pur ou associés à des phases minérales. ***Pour citer cet article : M.-M. Giraud-Guille et al., C.R. Palevol 3 (2004).***
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Keywords: Carapace; Bone; Collagen; Chitin; Mineralisation; Liquid crystal; Biomimetism

Mots clés : Carapace ; Os ; Collagène ; Chitine ; Minéralisation ; Cristal liquide ; Biomimétisme

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1. Introduction

Skeletal materials found in living organisms offer a variety of complex and subtle architectures with various specific properties that inspired material scientists in physics and chemistry [6]. This explains, in relation with the recent focus on nanostructures and nanomaterials, the renewed interest for models described in palaeontology, ultrastructural anatomy, and marine biology. They correspond to natural composites that associate a more or less abundant organic matrix, synthesized by specialised cells, to a mineral phase occupying spaces left free within the fibrillar network. Mollusc shells, crustacean carapaces, fish scales, compact bones provide such remarkable examples. An essential characteristic of biological materials is their hierarchical organisation, at the nanometre-to-millimetre scale, or more, allowing responses to solicitations at all these levels. During the course of evolution, a limited number of structural macromolecules have been selected, sometimes adopting the same organisation principles, despite their biochemical diversity.

The elaboration of an organic matrix, followed by its impregnation by a mineral phase will be recalled here in two model materials, crab carapaces, and human compact bones. The first example, chosen among invertebrates, associates an organic network mainly made of polysaccharides to calcium carbonate crystals. The second example, more representative of vertebrates, associates a protein matrix to calcium phosphate crystals. These fibrillar networks often present similar three-dimensional arrangements. Interpreting the origin of the series of nested arcs observed, using transmission electron microscopy, in decalcified sections of these tissues, allowed to introduce the notion of 'liquid crystalline biological analogue' [10]. The underlying hypothesis is that some major biological macromolecules possess liquid crystalline assembly properties. Such self-assemblies would appear, during morphogenesis, at different moments and in different compartments, when molecular concentrations reach critical levels. Are concerned collagen and chitin in extracellular matrices, but also cellulose in plant cell walls and DNA in certain chromosomes. Many works, performed in vitro with purified biological macromolecules in concentrated states, have validated the liquid crystalline hypothesis [21]. The transition from ordered fluid states to stabilised colloidal gels by solvent evaporation or pH modification allows us to synthesize chitin- or collagen-based materials that mimic connective tissue organisations.

The aims of this mini-review are first to recall the structure of two exemplar composite materials, then to focus on results that allowed us to demonstrate the self-assembly properties of their organic matrix and finally to describe dense materials today reproduced in vitro, opening perspectives in tissue engineering.

2. Crab cuticle example

2.1. Chitin protein matrix

Crustaceans protect themselves from predators by secreting at their body surface an organic matrix, called carapace or cuticle, reaching several millimetres in thickness. Calcite crystals then appear and grow within the fibrillar organic network ending in a rigid acellular composite material forming an exoskeleton.

Continuous growth of the animal implies a periodic set aside of this rigid cuticle during moult. The different steps of matrix deposition and mineralisation are identical throughout the successive inter-moult cycles [13]. The organic matrix is formed by four superimposed layers (Fig. 1A). The epicuticle (ep) and pigmented (pi) layers are deposited before moult; the principal (pr) and membranous (mb) layers are deposited after moult. The matrix, composed of chitin associated with proteins, is produced by an underlying monolayer of epidermal cells (Fig. 1B). In a lower ratio the cells also secrete lipids mostly in the epicuticle and pigments in the pigmented layer. Enzymes, for example alkaline phosphatase and carbonic anhydrase, are also synthesized in relation with the control of calcification, which occurs in the two middle layers (pi and pr).

Hierarchical structures of the organic matrix are evidenced in sections analysed in optical microscopy or in transmission electron microscopy (TEM). At low magnifications, sections transverse to the body surface show regular laminae in all the chitin-containing layers (pi, pr, mb) (Fig. 1A). At higher magnifications, the matrix appears formed of regularly disposed fibrils seen in longitudinal, oblique, or transverse views (Fig. 1C); each lamina corresponds to a 180° rotation of the fibrillar directions (Fig. 1D). At high resolution

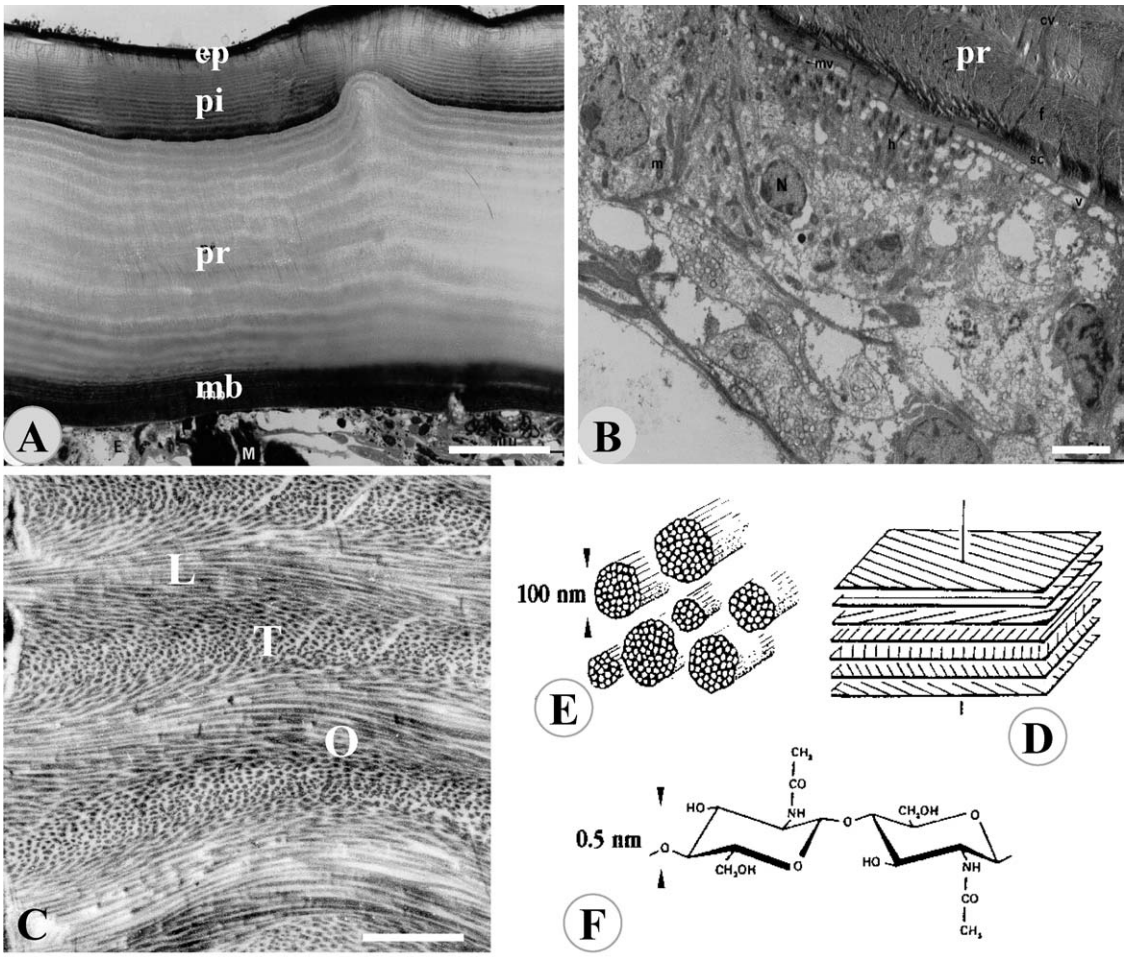

Fig. 1. Organic chitin–protein matrix of the crab cuticle. (**A**) Semi-thin section normal to the carapace, from top to bottom four layers are visible: **ep** (epicuticle), **pi** (pigmented layer), (**pr**) principal layer, (**mb**) membranous layer. Optical microscopy, bar = 50 µm. (**B**) Ultrathin section of the principal layer of the cuticle and the underlying epidermal cells. TEM, bar = 5 µm. (**C**) Ultrathin section of the principal layer, the chitin-protein fibrils are seen in longitudinal, oblique or transverse view forming a regular stratification. TEM, bar = 1 µm. (**D**) Plywood representation where the fibrillar directions in successive plans turn from a small and constant angle. (**E**) Aspect of the chitin protein fibrils observed at high magnification in TEM; the central electron light chitin crystals are surrounded by a sheath of contrasted proteins. (**F**) Linear chain of chitin, polymer of *N*-acetyl glucosamine.
Fig. 1. Matrice organique de chitine et protéines de la cuticule de crabe.

in TEM, in domains where the section plan is perfectly transverse to the fibril axis, a honeycomb pattern appears which reveals the elementary chitin protein units. The central clear rods, a few nanometres in diameter, correspond to chitin crystallites; the dark sheath corresponds to the surrounding proteins (Fig. 1E) [6,27]. Each chitin crystallite is composed of about 20 linear chains of *N*-acetyl-glucosamine, based upon the rod diameter and crystalline lattice dimensions (Fig. 1F). Keeping the same basic structure, different modes of aggregation exist, giving fibrils of various diameters, or reticulated morphologies, precisely disposed from top to bottom of the cuticle [18].

2.2. Calcium carbonate network

The new cuticle calcifies after each moult by nucleation and growth of calcium carbonate in the form of calcite crystals [9,21,22]. The first crystalline germs appear in the most external part of the pigmented layer

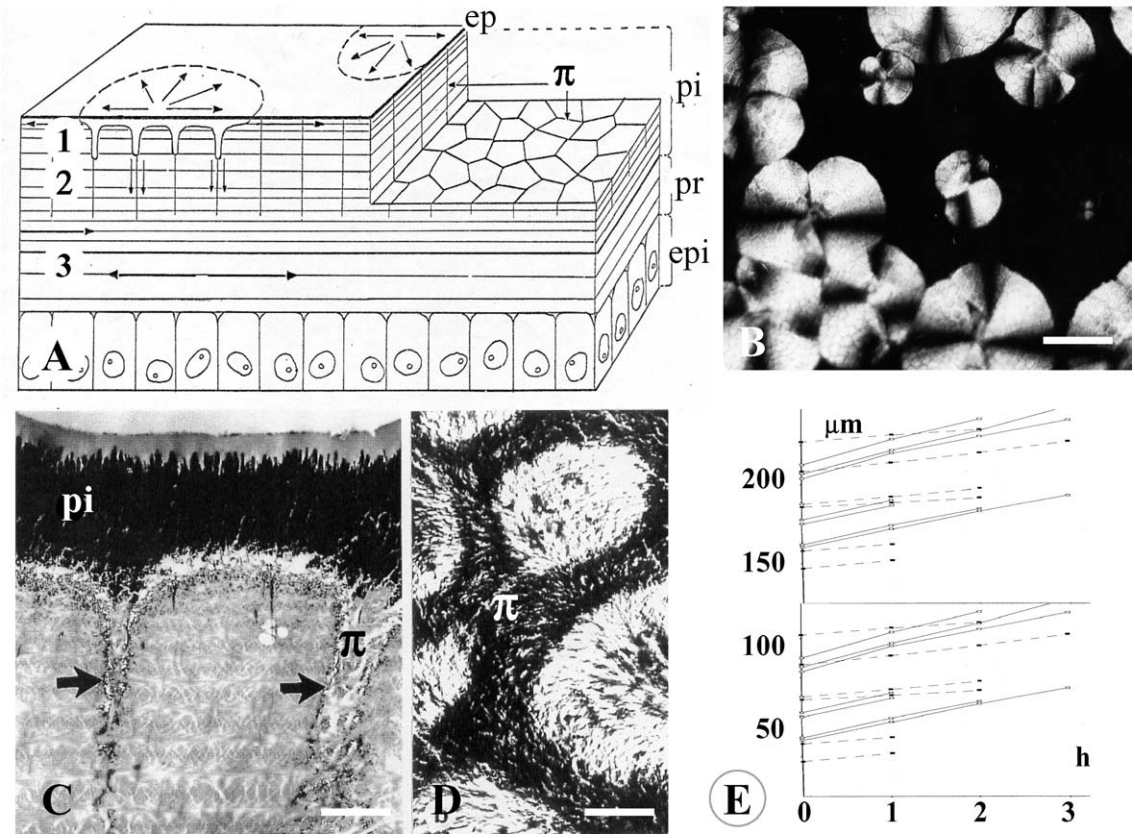

Fig. 2. Calcite growth in the crab cuticle. (**A**) Diagram of the crab carapace at the first stages after moult. Pre-exuvial layers (**ep**, **pi**) are present and the principal layer (**pr**) is in formation. The limits (**π**) of the underlying epidermal cells (**epi**) are shown. The mineral phase first appears as calcite spherulites just under the epicuticle (**1**), then progresses along the cell imprints (**π**) and radially fills the space between the organic network (**3**). (**B**) Nucleation of calcite spherulites in a newly secreted cuticle that fuse to form a crystalline mosaic. Polarised light microscopy, bar = 100 µm. (**C**, **D**) Ultrathin sections of the cuticle at the start of calcification in transverse and tangential view. The crystalline domains are located in the exterior lamellae of the pigmented later (**pi**), small calcite crystals first appear along, and then inside the cell imprints (**π**). TEM, bar = 5 µm. (**E**) Growth of calcite spherulites in absence (continuous line) or presence (dotted line) of diamox. The crystal growth rate is reduced from 20 to 10 µm h^{-1}.

Fig. 2. Croissance de calcite dans la cuticule de crabe.

(Fig. 2A) and grow in the form of flat discs or spherulites that fuse laterally to form a crystalline mosaic (Fig. 2B). The mineral further grows along the interprismatic septae that correspond to imprints left in the cuticle by the underlying epidermal cells (Fig. 2C and D). Calcification then progresses radially from these vertical axes in the pigmented and principal layers, with various speeds as a function of the animal body curvature and antero-posterior axis [13]. When the space in between the organic fibrils is lower than 300 Å, as in the membranous layer, mineralisation does not occur [9].

Crab cuticles are complex structures where initiation, growth, and orientation of calcite domains are controlled by organic intra- and extracellular elements. Pore canals, which are thin cytoplasmic expansions of epidermal cells, play a role in the transport of calcium ions [32]. Carbonic anhydrase activity, producing carbonate ions, is demonstrated in the crab cuticle during the inter-moult cycle and shows a maximum during the first stages of calcification just after the moult. The enzyme enhances the precipitation of calcium carbonate; it was shown that diamox, its inhibitor, decreases by a factor 2 the growth rate of calcite crystals in the

cuticle (Fig. 2D) [16]. Alkaline phosphatase is present in calcified tissues, namely in crustacean carapaces, as it removes phosphate ions, which are considered as inhibitors of calcification [31]. The presence of cation-binding glycoproteins in the sites where calcification is initiated, the interprismatic septae, plays a role in calcium carbonate nucleation [18]. The twisted fibrillar matrix of chitin generates screw dislocations, which are detected in scanning electron microscopy by the presence of spiral steps on the surface of the crystals. These defects favour crystal growth by introducing privilege sites for further crystalline units to grow [22]. All these actors of mineralisation show a precise distribution both in space, over the cuticle structure, and time, during the inter-moult cycle.

3. Compact bone example

3.1. Bone matrix

Mineralised compact bone is composed of specialised cells, a dense organic matrix, and inorganic phosphate ions. Skeletal tissues have three functions, a mechanic one supporting the body weight, a protective one of essential body organs, a metabolic one as reservoir of mineral ions, mostly calcium and phosphate. The major part of the organic fraction consists in type-I collagen, a structural protein to which are associated non-collageneous proteins (osteonectin, osteopontine, osteocalcin, sialoproteins...). The organic fraction is first synthesized by cells called osteoblasts, whose cycle of development and interaction with the environment is under genetic control, actively studied by gene manipulation and genetic mutations [25]. In a second step, the organic matrix calcifies by the deposit of calcium phosphate in the form of small crystals. Skeletal tissues are remodelled through lifetime by highly polarised cells called osteoclasts. The physiological process of remodelling alternates synthesis and resorption phases and is controlled by mechanical and hormonal stimuli. The action of osteoclasts through complex biochemical events, will both dissolve the mineral fraction by creating very low pH domains through the action of Na^+/H^+ pumps, and disrupt the organic matrix by secreting specialised enzymes, namely metalloproteases [2].

The general structure of compact bone consists of adjacent cylindrical structures of 0.2 mm in diameter, called osteons, laying parallel to the bone long axis. Each osteon is formed by coaxial layers of collagen fibrils running helically around a haversian canal containing nerve and blood vessels. It is generally admitted that collagen fibrils are organised in distinct lamellae, fibrils all running parallel in one lamella and showing a strong change of orientation from one lamella to the next [16]. Three osteon types are described in the literature, depending on their aspect in polarized light microscopy respectively described as bright, intermediate or dark type (Figs. 3A′, A″, 3B′) [1]. The very dense collagen network in decalcified sections of compact bone shows collagen fibrils arranged either in orthogonal sheets (Fig. 3C), or in nested arcs (Fig. 3D) [15]. Interpreting the origin of the arced patterns has introduced the hypothesis of an analogy between, notably, the fibrillar collagen distribution in bone matrix and the macromolecular arrangements in cholesteric liquid crystals (cf. § 4). From these observations, it has been proposed that two main osteon models coexist in a compact bone; the first obeys the classical description of osteons following an *orthogonal plywood* model (Fig. 3A), the second obeys the *twisted plywood* model, where the collagen fibrils follow small and regular changes in their orientation (Fig. 3B). In this last case, the structure is continuous, but stratification, i.e. a lamellar aspect, appears in microscopy sections at each 180° rotation of the fibrillar directions [19].

3.2. Calcium phosphate network

The bone mineral fraction is made of apatite with the following general composition $Ca_{10}(PO_4)_6(OH)_2$. However, the mineral phase is heterogeneous, lacunae exist, as well as substitutions of Ca^{2+} by other divalent ions (Mg^{2+}, Ba^{2+}, Sr^{2+}), substitutions of PO_4^{3-} by HPO_4^{2-}, or of OH by H_2O [27]. The mineral composition in vertebrate bones has been shown to vary in function of species, age, and maturation stage. X-ray diffraction diagrams of bone mineral samples appear different from those taken with standard hydroxyapatite crystals. Data correspond to rather amorphous phases for young bones, with a transition toward more crystallized samples as a function of age [23].

Electron micrographs of bones at the beginning stages of calcification show the initial locus of mineralisation at the ultrastructural level. The very first calcium phosphate crystals appear in the gap zones of the

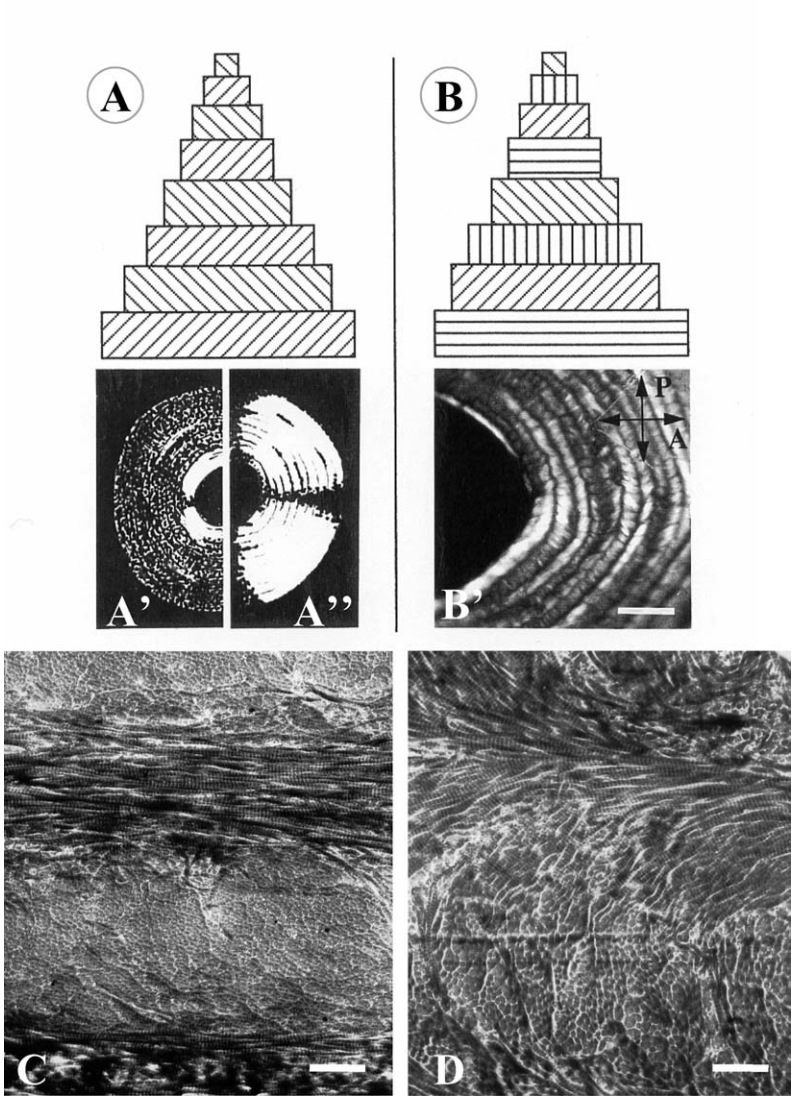

Fig. 3. Collagen matrix in compact bone osteons. Two alternate directions of fibrils (**A**) will give rise in polarised light microscopy either to dark-type (**A′**) or bright-type (**A″**) osteons as a function of their marked transverse or longitudinal orientation with respect to the osteon axis. Multidirections of fibrils (**B**) regularly changing from a small and constant angle will give rise, in polarised light microscopy, to intermediate-type osteons (**B′**) ; bar = 5 μm. (**C**, **D**) Decalcified compact bone osteons observed in thin sections. Two situations exist with either: two main directions of collagen fibrils, here appearing transverse or normal to the section plane (**C**), or regularly varying directions of collagen fibrils that form arced patterns in oblique view with respect to the osteon axis (**D**). TEM, bar = 0.1 μm.
Fig. 3. Matrice de collagène d'ostéones de l'os compact.

collagen fibrils, following a regular 67-nm periodicity [24]. Mineralisation then continues within intra- and inter-fibrillar spaces, more or less completely along the fibrils. The crystals appear as flat platelets in the size range of nanometres with their long axis making a small angle with the direction of the collagen fibrils [33]. The same observation was made in fish scales by small-angle X-ray scattering; the information that collagen fibrils display preferential orientations introduces a finite number of collagen orientations [5].

4. Extracellular matrices as analogues of liquid crystals

4.1. The model

When observed in transmission electron microscopy, ultra-thin sections of the organic matrix, both in crab carapaces and in compact bone osteons, reveal typical series of arced patterns [7,19]. Such figures frequently evidenced in sections of extracellular tissues do not result from authentic curved filaments. In an ideal representation, the molecular directions are drawn as parallel and equidistant straight lines on a series of rectangles and from one card to the next, the lines turn by a small and constant angle (Fig. 4A). Series of nested arcs appear on oblique sides of the model, just as they appear in microscopy after sectioning of the material (Fig. 4B). Another consequence of the twisted plywood arrangement is the presence of periodic extinctions when the sections are viewed in polarized light microscopy, with a planar disposition observed in the crab cuticle (Fig. 4C) and coaxial circles in intermediate type osteons (Fig. 3B′).

4.2. Liquid crystalline assemblies

Geometric analysis demonstrates that the three-dimensional organisation of major biological macro-

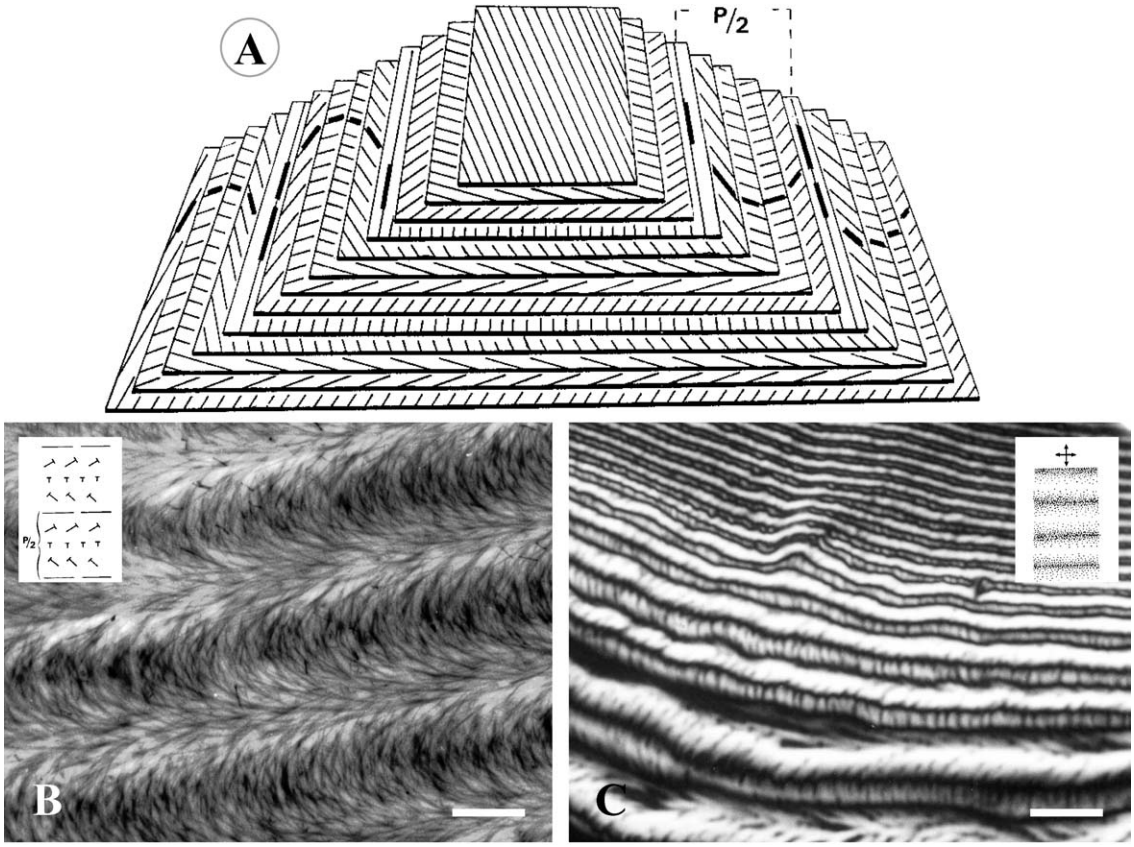

Fig. 4. Extracellular matrices biological analogues of liquid crystals. (**A**) The twisted plywood model consists of successive discrete plans superposed to form a pyramid. On each card, the straight lines represent the molecular directions, all parallel in one plane and turning by a small angle from one card to the next. On oblique sides of the model the successive molecular directions draw superposed series of nested arcs, with P/2 corresponding to a 180° rotation. (**B**) Materials following the twisted plywood model show periodic extinctions in sections observed in polarised light microscopy between crossed polars; extinction positions correspond to macromolecules lying normal to the section plane. PLM, bar = 10 µm. (**C**). A major consequence of the helicoidal geometry is the production of series of arced patterns in sections observed in microscopy, due to the superposition of the successive molecular orientations within the section plane. TEM, bar = 1 µm.
Fig. 4. Matrices extracellulaires analogues biologiques de cristaux liquides.

molecules is analogous to that of molecules in cholesteric liquid crystals. The formation of connective tissues such as crab cuticles and compact bones is thus suggested, at initial stages of their elaboration, to imply liquid crystalline states of matter [8]. A main difference is that, unlike liquid crystals, the final state exhibits no fluidity. The rigidity of the extracellular matrices is progressively established by molecular cross-links and/or mineralisation.

The demonstration was made in vitro with purified molecules dispersed in aqueous solutions at a proper concentration. Anisotropic distributions of elongated polysaccharide crystallites or protein monomers were evidenced in polarized light microscopy [20,29]. The strong birefringence observed between crossed polars arises from spontaneous ordering of the molecules when a critical concentration is achieved. Both systems are intrinsically chiral, thus the nematic order due to parallel alignment of molecules has a long-range helical distortion, termed 'twist'. This helical arrangement is revealed by the fingerprint patterns typical of cholesteric liquid crystals (Figs. 5A and C). The distance between two dark bands corresponds to a 180° rotation of the molecular orientations and corresponds to the half-cholesteric pitch P/2. The first-order nature of the transition from a disordered isotropic state to a highly ordered cholesteric phase is demonstrated by the existence of a concentration domain in which the

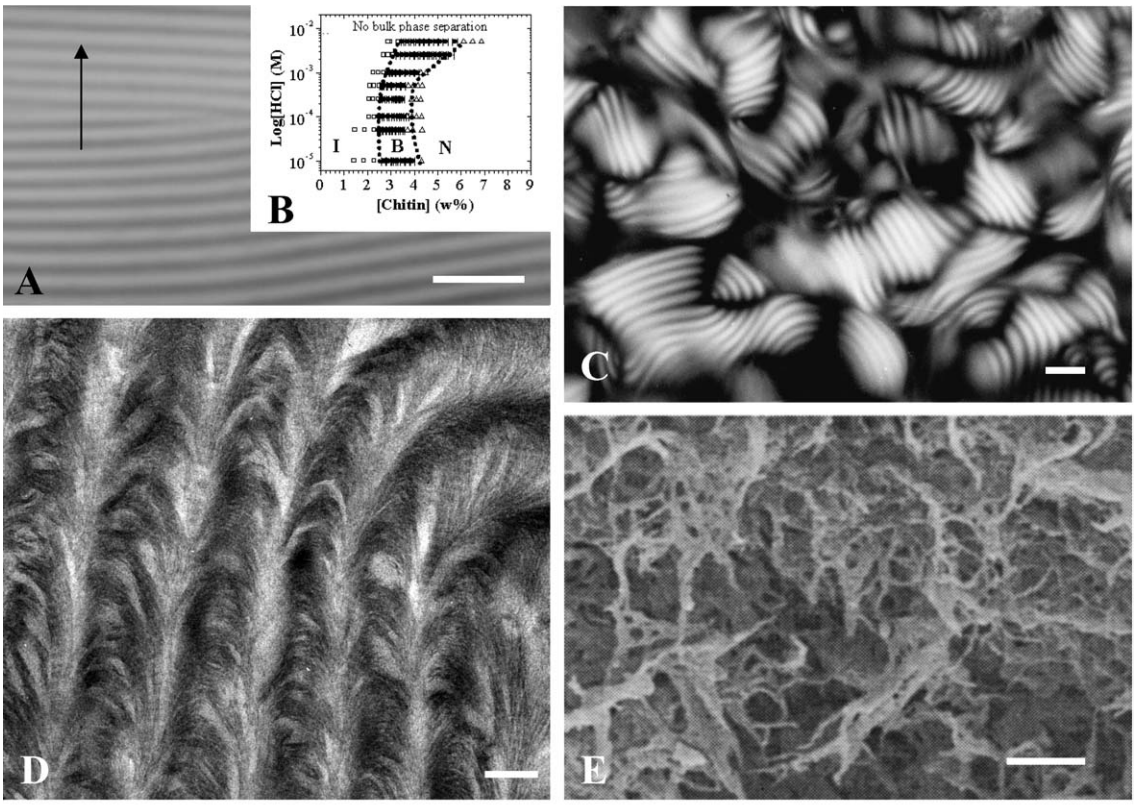

Fig. 5. Liquid crystalline phases and biomimetic materials. (**A**) Suspension of chitin crystallites at a concentration of ~40 mg ml^{-1}. Fingerprint patterns observed in polarized light between crossed polars are characteristic of the cholesteric phase. The arrow indicates the direction of the cholesteric axis. PLM, bar = 100 μm. (**B**) Phase diagram obtained for chitin particles as a function of concentration and pH. Open square: isotropic phase, solid circle: biphasic phase, open triangle: chiral nematic phase. (**C**) Cholesteric phase obtained in concentrated acid-soluble collagen solutions above 80 mg ml^{-1}. The half cholesteric pitch P/2 measures from 1 to 4 μm. PLM, bar = 10 m. (**D**) Stabilised cholesteric phase obtained by pH modification inducing the formation of cross-striated collagen fibrils. A continuous twist between the fibrils is observed. TEM, bar = 1μm. (**E**) Collagen silica hybrids at a respective concentration of 5 and 15 mg ml^{-1} analysed by SEM after lyophillisation. SEM, bar = 50 μm.

Fig. 5. Phases cristallines liquides et matériaux biomimétiques.

two phases coexist. In chitin suspensions, a biphasic domain, i.e. an isotropic phase containing cholesteric droplets, appears at concentrations ranging from 20 to 40 mg ml^{-1}. At higher volume fractions, the samples are completely chiral nematic (Fig. 5A). The half-cholesteric pitch varies as a function of concentration and ionic strength, and ranges between 10 to 60 µm in our experiments (Fig. 5B) [3]. In collagen solutions, at concentrations around 80 mg ml^{-1}, cholesteric globules can spontaneously emerge within the isotropic phase. As the concentration is further increased, they merge and fuse, leading to homogeneous cholesteric domains with P/2 values ranging from 0.1 to 4 µm [Fig. 5C]. The general features of the liquid crystal phases obtained with collagen solutions and chitin dispersions are mostly in agreement with theoretical models originally proposed by Onsager to describe the self-assembly of rod-like particles [28].

5. Biomimetic materials

The liquid crystalline ordered phases obtained at high concentrations in vitro are viscous, and occur at the level of individual chitin crystallites or collagen triple helical molecules. In biological tissues, the stabilized cholesteric order is observed between larger entities, which are at the level of fibrils having diameters of 100 nm or more. The stabilisation of the fluid phases was a necessary step to establish the relevance with physiological assembly mechanisms and obtain biomimetic materials.

5.1. Stabilised collagen matrices

The passage from the molecular to the fibrillar level in vitro was extensively studied with collagen in dilute solutions, fibrillogenesis being initiated by pH modification [11]. In concentrated systems, collagen liquid crystals are obtained at acidic pH, where repulsions between positively charged residues are large enough to maintain a liquid state.

When the solutions are exposed to ammonia vapours, NH_3 dissolution induces a pH rise without diluting the sample. As the net charge of collagen monomers is strongly decreased, hydrophobic interactions dominate, causing the triple helices to aggregate and form fibrils. This step corresponds to a sol/gel transition and the resulting fibrillar matrices mimic collagen connective tissue organisations [4]. The stabilized samples can be prepared for studies using Transmission Electron Microscopy by classical fixation, dehydration and embedding procedures. The ultrastructure of fibrillar gels obtained in vitro is influenced by the physicochemical parameters of the solution. Regular cholesteric geometries giving superposed series of arced patterns, similar to those observed within compact bones, are now obtained in a well-controlled manner over large domains [Fig. 5D].

5.2. Stabilised chitin matrices

Ordered chitin suspensions are obtained in slightly acidic solutions, with electrostatic repulsions that originate from ionised amine groups present on the surface of the rigid crystallites. The suppression of these repulsions by a pH increase results in the precipitation of large aggregates; however, no gelation occurs that would hold the three-dimensional organisation as observed with collagen. Solids have nevertheless been obtained with chitin cholesteric suspensions. Complete evaporation of the solvent results in the formation of rigid films that retain the helical symmetry of the liquid phase, but with much smaller cholesteric pitch values [30]. The macromolecular organisation of the chiral nematic phase was also stabilised by addition of polyacrylamide monomers at concentrations compatible with the liquid crystals and formation of a polyacrilamide gel that blocks the crystallite orientations [3].

5.3. In vitro mineralisation

Ordered gels and films of collagen and chitin are currently under investigation to form organic-mineral hybrids. An example inspired by biological processes consists of ions that diffuse and precipitate in contact with the organic matrix. Chitin matrices, prepared from decalcified crustacean shells, when soaked in solutions containing appropriate concentrations of calcium and carbonate ions, are covered with rhombohedric calcite crystals that exhibit in Scanning Electron Microscopy chiral features in the form of screw dislocations [22].

Another approach is to co-polymerise silica precursors, namely sodium silicates, with collagen mono-

mers in solution. When mixed in various proportions, the condensed monomers form different morphologies: condensed mineral flakes of silica, polymerised collagen ribbons, or collagen fibrils surrounded by condensed silica (Fig. 5E) [12].

6. Conclusion

In conclusion, the study of hierarchical ordered biocomposites, like compact bone and crustacean carapaces, reveals the contribution of both cellular and acellular processes in the morphogenesis of complex skeletal tissues.

Specialized cells synthesize and export the necessary components in a time-controlled manner, allowing complex physicochemical transformations and associations, the final structures selected by evolution being the most efficient in a given context. This includes, in the case of extracellular matrices, large structural molecules, as proteins, polysaccharides, and glycosaminoglycans, small molecules implied in the initiation steps of mineralisation, and ionic species forming the mineral phase.

In the absence of cells, the basic principles of interaction between the organic matrix and the mineral phase are now better understood at the molecular level and explained in terms of epitaxial growth or oriented nucleation. The ability to orientate the formation of the mineral through specific polypeptide sequences is currently investigated by material science chemists. At a supramolecular level, macromolecules self assemble into an organized scaffold ordered at different scales, which serves as a macroscopic mould for the growth of a reinforcing mineral phase.

The possibility to reproduce compact and ordered matrices experimentally is interesting for two purposes:

- to produce new materials, close to biological tissue architectures, proposed as soft or hard tissue substitutes;
- to inform on in vitro cell expression in response to cell interaction in a three-dimensional context.

Future steps in experimental investigations of biomineralisation will be to study the growth of calcium and phosphate mineral phases in confined environment, i.e. In the bulk of matrices that mimic biological organisations. Various degrees of complexity can be explored: add to the system peptide-like sequences known to initiate crystal formation in vivo or add cells in the three-dimensional scaffolds in search of matrix-molecule synthesis and control of mineralisation.

References

[1] A. Ascenzi, E. Bonucci, The compressive properties of single osteons, Anat. Rec. 161 (1968) 377–392.
[2] R. Baron, L'ostéoclaste et les mécanismes moléculaires de la résorption osseuse, Médecine/Sciences 17 (2001) 1252–1259.
[3] E. Belamie, P. Davidson, M.-M. Giraud-Guille, Structure and chirality of the nematic phase in α-chitin suspension, J. Phys. Chem. B 108 (2004) (in press).
[4] L. Besseau, M.-M. Giraud-Guille, Stabilization of cholesteric phases of collagen to ordered gelated matrices, J. Mol. Biol. 251 (1995) 197–202.
[5] A. Bigi, M. Burghammer, R. Falconi, M.H.J. Kock, S. Panzavolta, C. Riekel, Twisted plywood pattern of collagen fibrils in teleost scales: an X-ray diffraction investigation, J. Struct. Biol. 136 (2001) 137–143.
[6] J. Blackwell, M.-A. Weih, Structure of chitin-protein complexes: ovipositor of the ichneumon fly, Megagrhyssa, J. Mol. Biol. 137 (1980) 49–60.
[7] Y. Bouligand, Sur une architecture torsadée répandue dans de nombreuses cuticules d'Arthropodes, C. R. Acad. Sci. Paris, Ser. D 261 (1965) 3665–3668.
[8] Y. Bouligand, Sur l'existence de « pseudomorphoses cholestériques » chez divers organismes vivants, J. Phys. 30 (C4) (1969) 90–103.
[9] Y. Bouligand, Aspects ultrastructuraux de la calcification chez les Crabes, in: 7e Congrès int. Microsc. Électr., Grenoble, France, 3, 1970, pp. 105–106.
[10] Y. Bouligand, Twisted fibrous arrangements in biological materials and cholesteric mesophases, Tissue Cell 4 (1972) 189–217.
[11] W.D. Comper, A. Veis, The mechanism of nucleation for in vitro collagen fibril formation, Biopolymers 16 (1977) 2113–2131.
[12] T. Coradin, M.-M. Giraud-Guille, C. Helary, J. Livage, C. Sanchez, A novel route to collagen silica biohybrids, Mater. Res. Soc. Symp. Proc. 726 (2002) Q5.2.1–Q5.2.5.
[13] P. Drach, Mue et cycle d'intermue chez les crustacés décapodes, Ann. Inst. Océanogr. Monaco 19 (1939) 103–391.
[14] R. Frank, P. Frank, M. Klein, R. Fontaine, Microscopie électronique de l'os humain, Arch. Anat. Microsc. Morph. Exp. 44 (1955) 191–206.
[15] W. Gebhardt, Ueber funktionell wichtige Anordnungsweisen der gröberen und feineren Bauelemente der Wilbertiecknochens, Roux Arch. Entw. Mech. 20 (1906) 187–322.
[16] M.-M. Giraud-Guille, Carbonic anhydrase activity in the integument of the crab *Carcinus maenas* during the intermolt cycle, Comp. Biochem. Physiol. 69 (1981) 381–387.
[17] M.-M. Giraud-Guille, Calcification initiation sites in the crab cuticle: the interprismatic septae, Cell Tissue Res. 236 (1984) 413–420.

[18] M.-M. Giraud-Guille, Fine structure of the chitin-protein system in the crab cuticle, Tissue Cell 16 (1984) 75–92.
[19] M.-M. Giraud-Guille, Twisted plywood architecture of collagen fibrils in human compact bone osteons, Calcif. Tissue Int. 42 (1988) 167–180.
[20] M.-M. Giraud-Guille, Liquid crystallinity in condensed type-I collagen solutions: a clue to the packing of collagen in extracellular matrices, J. Mol. Biol. 224 (1992) 861–873.
[21] M.-M. Giraud-Guille, Twisted liquid crystalline supramolecular arrangements in morphogenesis, Int. Rev. Cytol. 166 (1996) 59–101.
[22] M.-M. Giraud-Guille, Y. Bouligand, Crystal growth in a chitin matrix: the study of calcite development in the crab cuticle, in: Z.S. Karnicki et al. (Eds.), Chitin World, Wirtschaftsverlag, 1995, pp. 136–144.
[23] M. Glimcher, Recent studies of the mineral phase in bone and its possible linkage to the organic matrix by protein-bound phosphate bonds, Phil. Trans. R. Soc. Lond. 304 (1984) 479–508.
[24] M.J. Glimcher, The nature of the mineral phase in bone: biological and clinical applications, in: L.V. Avioli, S.M. Krane (Eds.), Metabolic bone disease and related disorders, Academic Press, 1998, pp. 23–50.
[25] P. Marie, Différenciation, fonction et contrôle de l'ostéoblaste, Médecine/Sciences 17 (2001) 1252–1259.
[26] G. Montel, G. Bonel, J.-C. Heughebaert, J.-C. Trombe, C. Rey, New concepts in the composition, crystallisation and growth of the mineral component of calcified tissues, J. Cryst. Growth 53 (1981) 74–99.
[27] A.-C. Neville, Biology of the arthropod cuticle, Springer-Verlag, Berlin, 1975.
[28] L. Onsager, The effects of shape on the interactions of colloidal particles, Ann. N.Y. Acad. Sci. 51 (949) 627–659.
[29] J.-F. Revol, In vitro nematic ordering of chitin crystallites, Int. J. Biol. Macromol. 15 (1993) 329–335.
[30] C. Sanchez (Ed.), Biomimétisme et Matériaux, Observatoire français des techniques avancées (OFTA), Paris, 2001.
[31] K. Simkiss, Phosphates as crystal poisons, Biol. Rev. 39 (1964) 487–505.
[32] D.F. Travis, Structural features of mineralization from tissue to macromolecular levels of organization in the decapod Crustacea, Ann. N.Y. Acad. Sci. 109 (1963) 177–245.
[33] S. Weiner, T. Arad, V. Ziv, W. Traub, The mineralized collagen fibril: a building block for many vertebrate skeletal tissues, in: H. Slavkin, P. Price (Eds.), Chemistry and Biology of Mineralized Tisssues, Elsevier, 1992, pp. 93–102.