148. Leonor, I.B., Ito, A., Onuma, K., Kanzaki, N. and Reis, R.L. (2003) In vitro bioactivity of starch thermoplastic/hydroxyapatite composite biomaterials: an in situ study using atomic force microscopy, *Biomaterials* 24, 579-585.

149. Pashkuleva, I., Marques, A., Vaz, F. and Reis, R.L. (2004) Surface modification of starch based blends using potassium permanganate-nitric acid system and its effect on the adhesion and proliferation of osteoblast-like cells, *J. Mat. Sci.: Mat. in Med.* Accepted.

150. Pashkuleva, I., Marques, A., Vaz, F. and Reis, R.L. (2003) Surface modification of starch based biomaterials can simultaneously enhance cell adhesion and proliferation and induce bioactivity, *18th conference of European Society of Biomaterials, Stutgart, Germany*, T103.

151. Kawashita, M., Nakao, M., Minoda, M., Kim, H.M., Beppu, T., Miyamoto, T., Kokubo, T. and Nakamura, T. (2003) Apatite-forming ability of carboxyl group-containing polymer gels in a simulated body fluid, *Biomaterials* 24, 2477-2484.

152. Kokubo, T., Hanakawa, M., Kawashita, M., Minoda, M., Beppu, T., Miyamoto, T., and Nakamura, T. (2004) Apatite formation on non-woven fabric of carboxymethylated chitin in SBF, *Biomaterials* 25, 4485-4488.

153. Mann, S. (1993) Molecular tectonics in biomineralization and biomimetic materials chemistry, *Nature* 365, 499-505.

154. Weiner, S., Traub, W. (1984) Macromolecules in mollusk shells and their functions in biomineralization, *Phil. Trans. R. Soc.* B304, 425-434.

155. Iwatsubo, T., Sumaru, K., Kanamori, T., Yamaguchi, T., Sinbo, T. (2004) Preferential mineralization of CaCO₃ layers on polymer surfaces from CaCl₂ and water-soluble carbonate salt solutions superstaurated by poly(acrylic acid), *J. Appl. Pol. Sci.* 91, 3627-3634.

156. Sugawara, A., Ishii, T., Kato, T. (2003) Self-organized calcium carbonate with regular relief structures, *Angew. Chem. Int. Ed.* 42, 5299-5303.

157. Kato, T., Suzuki, T., Amamiya, T., Irie, T., Komiyama, M., Yui, H. (1998) Effects of macromolecules on the crystallization of CaCO3 the formation of organic/inorganic composites, *Supramol. Sci.* 5, 411-415.

158. Wierzbicki, A., Cheung, H.S. (2000) Molecular modeling of inhibition of hydroxyapatite by hposphocitrate, *J. Mol. Str. (Theochem)* 529, 73-82.

159. Mao, C., Li, H., Cui, F., Ma, Ch., Feng, Q. (1999) Oriented growth of phosphates on polycrystalline titanium in a process mimicking biomineralization, *Journal of Crystal Growth* 206, 308-321.

160. Shiba, K., Honma, T., Minamisawa, T., Nishiguchi, K. and Noda, T. (2003) Distinct macroscopic structures developed from solutions of chemical compounds and periodic proteins, *Embo Reports* 4, 148-153.

161. Asai, S., Koumoto, K., Matsushita, Y., Yashima, E., Morinaga, M., Takeda, K., Iritani, E., Tagawa, T., Tanahashi, M., Miyazawa, K. (2003) Advances in nature-guided materials processing, *Sci. Techn. Adv. Mat.* in press.

LEARNING FROM MARINE CREATURES HOW TO DESIGN MICRO-LENSES

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1. Introduction

The increasing technological requirement for a new generation of optical devices with novel architectures, tunability and tailored properties, provides a potent stimulus to the academic study of optical systems in living organisms. Mimicking Nature's methods of biological manufacture is proving to be a major step forward in modern technology [1-7]. In general, a successful bio-inspired engineering effort includes three key objectives: (i) To search for smart biological solutions in design, synthesis, and integration of complex materials and systems; (ii) To learn about their structure, properties, tunability and mechanisms of formation; (iii) To identify new bio-inspired synthetic strategies and to apply this knowledge to the fabrication of novel, superior materials and devices.

We are interested in discovering natural optical systems, whose hierarchical architecture and hybrid character offer outstanding optical properties and enable multi-faceted roles for these units. Biology provides a multitude of varied, new paradigms for the development of adaptive optical networks. Recently, we have shown that biologically formed optical systems are often unique in their ability to perform multiple functions – optical and structural. For example, the study of light-sensitive ophiuroids showed the skeletal dorsal arm plates of some species not only afford protection [8,9], but also form highly efficient micro-lens arrays [10]. Other research has concentrated on the exceptional fiber-optical properties of siliceous spicules of certain hexactinellid sponges, whose primary function is structural (either skeletal or anchoring) [11-13].

This review describes the design and optical features of the natural ophiuroid micro-lenses, summarizes their advantageous properties, and shows our first, bio-inspired engineering efforts in the fabrication of new optical structures.

2. Structural and Optical Characterization of Biologically Formed Micro-Lenses

Echinodermata, the group of animals including sea stars and sea urchins, show various levels of photosensitivity. An especially interesting example is presented by brittlestars (Ophiuroidea) in the genus *Ophiocoma*, which exhibit strong light responses. It has been generally believed that many echinoderm's have an extraocular sensitivity to light that is supported by unspecialized, "diffuse," or dermal receptors [14-16]. The absence of specialized "eyes" is not, however, readily reconciled with such reactions as the color-change and negative phototactic behavior observed in certain species. The variations in color and escape mechanisms are most striking in a brittlestar *Ophiocoma wendtii* (Fig. 1) [17, 18]. The individuals are a dark brown color during the day, and are strikingly banded with gray and black during the night. It has been documented that *O. wendtii* is able to escape from predatory fish by hiding in coral crevices, and that it may be able to sense shelter at a distance [18,19]. Recently, we suggested that these strong responses to light might be related to the presence of a characteristic lensar extension of the brittlestar skeleton, which may constitute an advanced photoreception system [10,17,18].

Echinoderms use calcium carbonate for their skeleton construction. Each skeletal element (ossicle) is a single crystal of calcite [8,9,20,21]. Unlike biogenic calcite of other phyla or abiologically formed calcite crystals, echinoderm skeletons are composed of a unique, intricately shaped, three-dimensional, single-crystalline meshwork with smooth and curved surfaces lacking crystal faces (so-called, stereom) (Fig. 2). In the living animal, the calcite meshwork is filled with soft tissue (so-called, stroma), and the ossicles are

Figure 1. The same individual of the brittlestar *O. wendtii*, photographed during the day (top) and during the night (bottom).
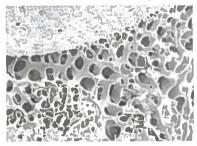

Figure 2. Typical stereom structure in echinoderm skeletons. The entire elaborate mesh is a single calcite crystal.

attached to one and other by connective tissue and muscle. Every joint of a brittlestar arm is composed of five major ossicles, including two lateral arm plates which support several spines and tentacle scales , one dorsal and one ventral arm plate. These surround the arm

and enclose a large internal skeletal ossicle (vertebra) that is adapted for articulation. The analysis of the ultrastructure of these skeletal units showed that while the stereom in the vertebrae, spines, tentacle scales, and ventral arm plates generally share a typical design (Fig. 2), the dorsal arm plates (DAP) and portions of the lateral arm plates in certain species of *Ophiocoma* exhibit an unusual extension of the stereom – a thick, transparent, layer (~ 40 μm thick) that covers the external surface of the plate [10,17]. It is composed of a close-set array of hemispherical calcitic structures (reaching 40-50 μm in diameter) with a characteristic double-lens design (Fig. 3) [10]. The lenses are uniform in shape and appear as the scaled replicas of each other. The ratio between the
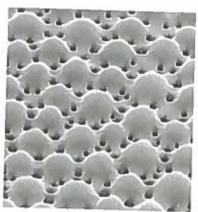

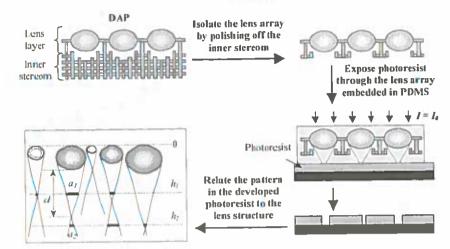*Figure 3.* Array of microlenses on the surface of the dorsal arm plate in *O. wendtii*.

lens thickness (t) and the lens diameter (L) estimated for a statistically significant set of micro-lenses was:

$$t/L = 0.9 \pm 0.05.$$

The degree of development of the lensar layer seems to correlate with the photosensitivity among several ophiocomid brittlestars that were studied. The layer is particularly pronounced in a highly photosensitive species, *Ophiocoma wendtii*, and it is absent in the light indifferent species, *Ophiocoma pumila* [10,17].

The optical properties of the microlens array were tested, using photolithography [10]. In these experiments, a film of a photosensitive material (positive photoresist) was illuminated through the isolated lensar layer (Scheme I). The illumination dose (I) was fixed slightly below the sensitivity level of the photoresist (I_0). As a result, the developed photoresist film was only affected precisely where the illumination dose increased due to the focusing activity of the lenses. The photoresist was placed at different distances h from the array. The photoresist films appeared to be selectively exposed under each micro-lens. Thus, the lensing effect was confirmed and mapped, and its quantitative characteristics (such as the position of the focal plane, d; the coefficient of the intensity enhancement by the micro-lenses, E; the angular selectivity, ϕ; the size of the focused beam at the focal plane, a_0; etc.) were experimentally determined from the values of h, L and the sizes of the spots in the photoresist, a, using basic equations of the geometric optics for a thick lens [22].

Scheme 1.

Our analysis showed that the average position of the focal plane is located at the distance d = 4–7 µm below the micro-lenses. The size of the spot in the focal plane a_0 is approximately 3 µm. The intensity of the incoming light is enhanced at the focal point by a factor of $E \sim$ 50. The angular selectivity ϕ is about $10°$. These results presented the first experimental evidence that the micro-lens arrays are effective optical elements capable of significantly enhancing and guiding the light inside the tissue [10]. Calcitic micro-lenses of analogous structure were also reported in trilobite eyes [23-25].

3. Advantages of the Natural Micro-Lens Design

The optical characteristics of the external DAP layer obtained in the imaging experiments showed that brittlestar micro-lenses are in many aspects superior to their man-made analogs. This bio-optical system presents a spectacular example of Nature's ability to evolve sophisticated solutions even to complex technological problems.

We have identified a number of unique, advantageous properties and design strategies found in the brittlestar microlenses, which could be emulated in synthetic micro-lens arrays:

   a) High fill factor lens array with characteristic micron-scale porous structure
   b) Individually addressed crystalline lenses
   c) Wide range transmission adjustment using pigment rearrangement
   d) Characteristic lens geometry that minimizes spherical aberration
   e) Fracture toughness due to the formation of an inorganic-organic composite
   f) Crystallographic orientation along the optical axis of the lens crystal to eliminate birefringence
   g) Angular selectivity
   h) Cross-talk suppression by pigment distribution in pores

   i) Potential wavelength selectivity due to the pigment involvement
   j) Potential refractive index modification by gradient of specialized intracrystalline biomolecules

The importance of these properties in the improvement of the optical performance of micro-lenses is detailed below.

3.1. SIGNAL RECEPTION

It has been shown that the remarkable focusing properties of the bio-micro-lenses are used by the brittlestars in their natural habitat for survival purposes [18]. In a series of transmission electron microscopy (TEM) studies, Hendler and Byrne have identified neural bundles under the lenses, and suggested these to act as primary photoreceptors in the light signal detection [17]. This hypothesis was further confirmed in our lithographic experiments [10]. The nerve bundles are positioned in the pores of the stereom at the distance of about 5 µm from the lens, which corresponds well to the location of the experimentally identified focal point d. Moreover, the diameter of the nerve fibers correlates with the size a_0 of the focused beam in the focal plane.

Therefore, the arrays of lenses appear to form a sophisticated optical device, in which each component (a single micro-lens) is individually addressed by the detector positioned at the focal point of the lens. Due to the observed angular selectivity of these lenses and their different orientation, such device could act as a compound eye [26] that provides a wide field of view due to the non-planar arrangement of the lenses, which can be tuned on demand when the arm moves. The ways in which the signals detected by each receptor are processed and integrated remains unknown, and worthy of further investigation.

3.2. TRANSMISSION TUNABILITY

One of the most noticeable reactions to light in certain species of ophiocomid brittlestars is diurnal color change (Fig. 1) [18]. The change from a dark color during the day to a light color at night cannot be explained by camouflage, as has been shown experimentally [18]. Hendler and Byrne have shown that the channels in stereom contain chromatophore cells filled with pigment granules [17]. They proved that the diurnal color change is caused by the migration of these cells. The light microscopic sections of DAPs prepared from night and day samples showed that during the day, pigment-filled processes of the chromatophore beneath the epidermis cover the lenses. During the night the chromatophores withdraw into the stereom channels surrounding the lenses, deeper within the DAP. As a result, the intensity of light reaching the receptors is regulated by chromatophores depending on the illumination conditions; a function performed by an iris in a human eye. Similar process is utilized in so-called "transition" sunglasses. The behavior of $O.$ $wendtii$ suggests that the transmitted light signal is tuned to match the sensitivity level of the neural bundle and to optimize the reception [18].

The intensity-adjustment function of the chromatophore cells as well as the involvement of the sub-lens nerve bundles in photoreception (as opposed to a "diffuse" dermal reception), were corroborated by neurophysiological [27-28] data. Cobb and Hendler studied the

reaction of *O. wendtii* to light stimuli by direct recording from the nerve cord in brittlestar arms [29]. Nervous response of light-adapted, dark brown pigmented brittlestars has been monitored after the chemical disruption (by bleaching) of successive layers of the tissue. The removal of epidermal layer only (bleaching for less than 10 s) did not result in any change in the response compared to untreated, control specimens. Thus, "diffuse" photoreception by epithelial cells was ruled out. The removal of the pigment layer (bleaching for ~30 s) caused an approximately ten-fold increase in the response to light "ON", confirming the involvement of pigment in photoreception and its "sunglasses" function. The disruption of the sub-lens layer (bleaching for >45 s) resulted in no photic responses, consistent with the suggestion that the sub-lens neural bundles are the primary photoreceptors (Fig. 4).
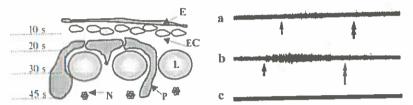

Figure 4. Left: Schematic presentation of the structure of the epidermis and dermis in the DAP. Lenses (L), pigment (P), cuticle of the epidermis (E), epithelial cells (EC) and nerve bundles (N) are indicated. Right: Recordings of the nervous response (right). Single arrows represent "Light 'ON'". Double arrows represent "Light 'OFF'". a) Response to light in a control, untreated specimen or specimen treated for <10 s. b) Response to light when the arm was bleached for 30 s. c) Response to light when the arm was bleached for >45 s. Adapted from [27].

In technical terms, the brittlestar micro-lenses is a tunable optical device that exhibits a wide-range transmission tunability achieved by controlled transport of radiation-absorbing intracellular particles. Other functions of the chromatocyte pigment may include the diaphragm action, and therefore, numerical aperture tunability; wavelength selectivity; minimization of the "cross-talk" between the lenses, and therefore, the improved angular selectivity.

3.3. ABERRATIONS

In an ideal lens, all rays of light would converge to the same point in the focal plane, forming a clear image (Fig. 5). The influences that cause different rays to focus to different points in real optical systems are called aberrations [22]. For a most common lens type, i.e. for lenses made with spherical surfaces, rays that are parallel to the optic axis but at different distances from the lens center do not converge on the same point. Rays that strike the outer edges of the lens are focused closer to the lens than rays that strike the inner portions of the lens (Fig. 5). This effect, called spherical aberration, results in a considerable image blurring and presents a serious technological problem in lens fabrication.

Figure 5. Ray tracing in an ideal, thin lens (left) and in a spherical, thick lens (right) that illustrates the concept of spherical aberration.

For a thick calcitic lens that has the size of the brittlestar micro-lenses and is formed by two spherical surfaces, the expected value of the light enhancement factor at the distance d from the lens would be $E_0 = 3\text{-}4$. The experimental value of E determined in the lithographic experiments was 15 times higher than the latter [10]. Therefore, the brittlestar micro-lenses must be significantly compensated for spherical aberration. Indeed, a close examination of the design of the brittlestar micro-lenses showed that they have a very peculiar shape. Only the top surface is spherical. The bottom surface has a characteristic aspherical form. The calculated shape of the ideal calcitic lens that is totally compensated for spherical aberration appeared to completely coincide with the shape of the bio-lens (Fig. 6) [10]. Moreover, the presence of pigment-filled chromatophores in the pores around the lenses would presumably block the light striking the outer portion of the lens, thus improving the operational size of the lens (L_0).
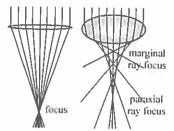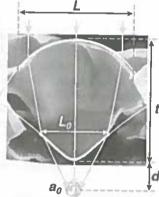

Figure 6. Cross-section of the brittlestar lens, showing its doublet structure delineated by one spherical and one aspherical surface. The bio-lens is superimposed with the calculated profile of an ideal lens that is compensated for spherical aberration (white contour). The match is striking.

Technological ways to minimize spherical aberration include: (i) the use of lens doublets instead of the single lens; (ii) bending one lens of the doublet into its best, aspherical (usually parabolic) form; (iii) the use of screens that disable the most problematic, exterior portions of the lens; and (iv) the use of high-index materials. Brittlestars seem to employ all of the above four approaches in their lens design. As a result, a remarkable level of

compensation for spherical aberration is achieved and significantly enhanced light converges into one point where the photoreceptor is positioned.

3.4. BIREFRINGENCE

Birefringence is the division of light into two components, which is found in materials that have two different indices of refraction in different crystallographic directions [22]. It is observed in crystals that have a crystallographic axis of higher symmetry, so-called uniaxial crystals. The direction of the axis of higher symmetry is optically unique, in that the propagation of light in this direction is independent of its polarization. This direction is called the optic axis of the material. Light propagating in any other direction will split into two beams that travel at different speeds.

Calcite is the classical example of a doubly refracting material. Its birefringence is extremely large, with indices of refraction of 1.66 and 1.48 along the optic axis and in the perpendicular direction, respectively. The use of calcite in the construction of optical lenses is, therefore, disadvantageous, as it will result in the formation of two images, unless the crystal is precisely oriented in the direction of the optical axis (Fig. 7a). Amazingly, in the brittlestar dorsal arm plate, it is indeed the case – the optical axis of the constituent calcite is oriented parallel to the lens axis and perpendicular to the plate surface (Fig. 7b). Since only the light striking the array perpendicular to the plate surface is effectively detected (rays striking the surface at an angle are stopped by pigment), the negative effect of birefringence is corrected and the receptor receives one clear signal.
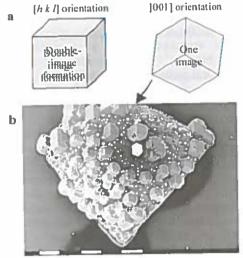

Figure 7. a) Schematic illustration of birefringence in calcite crystals. Double image is formed when the crystal is oriented in the general, [h k l] crystallographic direction. One image is formed when the crystal is oriented in the [001] direction, i.e. along the optical axis. b) Decoration of the DAP with synthetic calcite crystals that grow epitaxially on the surface. The experiment visualizes the crystallographic orientation of the biogenic substrate. The overgrown calcite crystals and therefore the lenses are oriented in the optic axis direction.

3.5. MECHANICAL PROPERTIES

Calcite is typically used in echinoderms for structural purposes [6,8,9]. This material is, however, highly brittle: cracks propagate easily along the {104} cleavage planes (Fig. 8, left). Organisms have evolved several sophisticated means to reinforce this intrinsically brittle material for skeleton construction. To that end, living organisms commonly employ organic-inorganic composite structures [8,9]. Addadi and Weiner suggested that specialized, intracrystalline macromolecules found inside biogenic calcite crystals of different origin are involved in the control of their mechanical properties [6,8,30,31]. These macromolecules are often adsorbed onto selective crystallographic planes in the crystal. It has been shown that in echinoderm calcite, the intracrystalline macromolecules interact specifically with crystallographic planes approximately parallel to the optic axis [6,8,30-32]. Positioned oblique to the cleavage planes (the optic axis forms an angle of about 45° with the cleavage planes), these macromolecules provide an effective crack-stopping mechanism by absorbing and deflecting the advancing cracks (Fig. 8, right). Such reverse fiber-reinforced composite exhibits reduced brittleness, increased plasticity, and fracture toughness [6,31].

As a result, the ossicles and associated micro-lenses formed by brittlestars are mechanically strong. It is also conceivable that intracrystalline macromolecules are involved in the regulation of the refractive index of calcite, thus further reducing the aberrations in the lens.
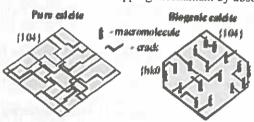

Figure 8. Schematic presentation of the crack-arresting function of specific intracrystalline macromolecules in biogenic calcites. See text for details.

In summary, photosensitive brittlestars are impressively armed for light sensing. They form an array of nearly perfect optical lenses that are micron scale, lightweight, mechanically strong, aberration-free, birefringence-free, individually-addressed; that show a unique focusing effect, signal enhancement, intensity adjustment, angular selectivity, and photochromic activity. Together with neural receptors and intraskeletal chromatophores, these micro-lenses form an effective optical device that may function as a compound eye. Given appropriate neural integration, each DAP would have an effective visual field of ~10° surveying a different part of the object space [26]. Since *O. wendtii* has a large number of differently oriented DAPs, it could potentially extract a considerable amount of visual information about its environment.

4. Bio-Inspired Engineering

For the purpose of advancing the state-of-the-art optical technology striving towards the construction of a new generation of open space devices with variable field of view, tunable

transmission, wavelength selectivity, etc., the brittlestar micro-lens arrays represent an inspirational example. We believe that the above biological principles, if understood correctly and creatively applied in technology, could well revolutionize our ability to make tunable, lightweight, porous micro-lens arrays for a wide variety of applications.

The following fabrication strategies were inspired by the principles involved in the formation of echinoderm calcitic structures.

4.1. IN VITRO NANOTECHNOLOGY EXPERIMENTS INVOLVING AMORPHOUS CALCIUM CARBONATE

A fascinating feature of echinoderm skeleton is that it is a multifunctional material composed of large single crystals with finely-tuned shape, micro-ornamentation, crystallographic orientation, and composition [8,9,20,21]. Single crystals with controlled micro-pattern are widely used in technology as components of various electronic, optical and sensory devices [33,34]. Their fabrication is, however, a complex, multistep process that could be potentially improved by learning from nature and introducing biological crystal growth techniques.

We have approached the mechanisms of the shape regulation in echinoderm calcite in the study of sea urchin larval spicules [35,36]. We have shown that the formation of final crystalline structures occurs through the transformation from the transient amorphous calcium carbonate (ACC) phase (Fig. 9).

For the purposes of bio-inspired crystal engineering, we identified two major elements of the larval spicule formation and used them in our synthetic effort:

(i) Amorphous calcium carbonate stabilized by specialized macromolecules is deposited in a preformed space and adopts its shape;

(ii) Oriented nucleation then occurs at a well-defined, chemically modified intracellular site and the crystallization front propagates through the amorphous phase, resulting in the formation of a single crystal with controlled orientation and predetermined microstructure.

Figure 9. Sea urchin *Paracentrotus lividus* larval spicule (25 h embryo). Triradiate spicule is first deposited in an ACC form within a membrane-delineated compartment, inside a syncitium formed by specialized mesodermal cells. Within 20 h, an oriented calcite with the crystallographic *a*-axes parallel to the three radii nucleates (see a rhombohedral-shaped crystal at the center). The subsequent amorphous-to-crystalline transition results in the formation of a single crystal of the predetermined triradiate shape and crystallographic orientation [35].

4.1.1. *Oriented Nucleation of Calcite Controlled by Organically Modified Surfaces*

The crystallographically oriented growth of calcite is common in biological environments [6,8,20,35,37]. It is generally believed that crystal nucleation is templated by membranes in the form of vesicles, syncitia, and cells, which are usually "primed" with specialized, acidic macromolecules (rich in Asp, Glu, Ser, Thr, often sulfonated or phophorylated) [8,30,32,37]. The control of crystallographic orientation is achieved by stereochemical recognition at the organic-inorganic interface, conceivably by virtue of a match between the structures of the organic surface and that of a particular crystal plane [3,6,38].

In our effort to mimic the biological process of oriented crystallization, we templated calcite nucleation by constructing specially-designed organic surfaces. Self-assembled monolayers (SAMs) of alkanethiols terminated in functional groups

that have a direct biological relevance (CO_2^-, SO_3^-, PO_3^{2-}, OH) were used as substrates for calcium carbonate deposition [38,39]. We showed that such templates induced a remarkable level of control of crystallographic orientation of calcite crystals. The crystallographic orientations of the crystals were distinct and homogeneous for each surface, but different on different surfaces. An even higher level of control was achieved in the experiments involving micropatterned SAMs [40]. By adjusting the geometry and sizes of the features in the SAM pattern, the concentration of the solution, and the functionality of the surface of the SAM, we could fabricate arrays of oriented crystals with the controlled density of nucleation in defined locations (Fig. 10) [7].

Figure 10. Oriented nucleation of an array of calcite crystals templated by a patterned organic substrate. Islands of $HS(CH_2)_{15}CO_2H$ SAM supported on Ag were used. Crystals grow uniformly in the [012] direction.

4.1.2. *Stabilization of Amorphous Calcium Carbonate*

Amorphous calcium carbonate (ACC) is highly unstable. If not stabilized, this phase rapidly transforms into crystalline polymorphs [41-44]. The formation of ACC is, however, common in biological systems [35,36,45,46]. We have shown that biogenic ACC is stabilized by means of specialized macromolecules rich in hydroxyamino acids, glycine, glutamate, phosphate and polysaccharides [45, 46]. These macromolecules extracted from the biological ACC tissue and introduced as an additive into saturated calcium carbonate solution induced the formation of stabilized ACC *in vitro*.

The stabilization of ACC can also be achieved using surface-mediated processes. Our results show that significantly disordered organic substrates bearing phosphate and hydroxyl groups, suppress the nucleation of calcite and induce the formation of a metastable ACC layer from highly saturated solutions [47]. Such disordered organic surfaces can be generated using a mixture of alkanethiols of different lengths, functionalized with PO_3H, OH and CH_3 groups and supported on metal substrates (we will refer to this surface as to "ACC-inducing"). The difference in the length of the carbon chain in the thiol molecule

prevents the formation of an ordered interfacial layer, and therefore, the formation of ordered nucleation sites. The deposited ACC layer is stabilized for 1-2 hrs and then transforms into a polycrystalline film.

When the substrate is engineered in such a way that ordered SAMs that induce oriented calcite nucleation are patterned *into* the ACC-inducing background, the formation of the ACC layer is followed by the controlled oriented nucleation at the defined locations of the SAM and the transformation of the patterned ACC layer into an array of oriented calcite crystals (Fig. 11).
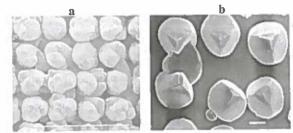
Figure 11. Surface-induced formation of ACC (a) and its transformation into an array of oriented calcite crystals (b). Scale bars are 100 μm and 10 μm, respectively. An ACC-inducing surface was patterned with a square array of 1 μm dots of $HS(CH_2)_{11}PO_3H_2$ serving as nucleation sites. The c-axes of the crystals form a constant angle of ~20° with the surface normal, as expected for the oriented growth of calcite on a PO_3-terminated SAM.

4.1.3. *Fabrication of Large Single Calcite Crystals with Controlled Micropattern and Orientation*

The above studies show that ACC may be successfully used as the transient phase for calcite crystallization in an artificial system. Moreover, if nucleation sites are imprinted onto the ACC-inducing surface, the ACC transforms into oriented crystals whose orientation is controlled by the nucleating region of the template.

In our new approach to the fabrication of micropatterned single crystals, we apply the biomineralization principles summarized in Chapter 4.1; that is, the use of the controlled amorphous-to-crystalline transition initiated at a well-defined nucleation site and taking place within an ornate reaction volume that determines the shape of the mineral [47]. Figure 12 outlines the experimental procedure. An arbitrary photoresist micropattern (usually an array of isolated posts with feature sizes <10 μm) was formed on a glass slide using routine photolithographic procedures. The micropatterned substrate was primed with a disordered phosphate-, methyl- and hydroxy-terminated monolayer that induces the formation of amorphous $CaCO_3$. One nanoregion of the SAM of $HS(CH_2)_nA$ (A = OH, CO_2H) serving as calcite nucleation site was integrated into each template (Fig. 12a). When placed in a supersaturated solution of calcium carbonate, these organically modified templates induced the deposition of a micropatterned ACC mesh in the interstices of the framework. Oriented nucleation of calcite then occurred at the SAM nanoregion, followed by the propagation of the crystallization front through the ACC film (Fig. 12b).
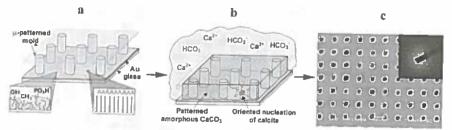
Figure 12. a)-b) Schematic presentation of the fabrication procedure of micropatterned crystals (see text for details). c) Magnified scanning electron micrograph of the resulting crystal, showing its microstructure. Inset: large-area transmission electron diffraction, confirming that the section is a single crystal of calcite oriented along the optic axis.

This new bio-inspired crystal engineering strategy made it possible, for the first time, to directly fabricate millimeter-size single crystals with a predetermined sub-10-micron pattern and controlled crystallographic orientation (Fig. 12c). We showed that in addition to their "shaping" function and the control of the ACC formation and nucleation, organically modified 3D templates act as the stress release sites as well as discharge sumps for excess water and impurities during crystallization [47]. The described mechanism of the amorphous-to-crystalline transition may have direct biological relevance and important generic implications in the fabrication of defect-free, micropatterned crystalline materials for a wide variety of applications.

The design of these synthetic calcite crystals is remarkably similar to the perforated stereom structure of echinoderm skeletal elements – their biological analogs. We are currently exploring the ways to synthesize single crystalline micro-lens arrays using the above strategy. Towards this goal, we are creating the micropatterned frameworks with the top surfaces bearing arrays of concave structures that will define the shape and the curvature of the lenses.

4.2. FABRICATION OF TUNABLE POROUS MICRO-LENS ARRAYS USING INTERFERENCE LITHOGRAPHY

An interesting feature of the brittlestar micro-lens arrays is the presence of pores surrounding the lenses. We discussed in Chapter 3.2 that these pores are important functional elements of the biological optical device, as they are used for the transport of pigment that regulates the illumination dose reaching the lens.

We have developed a novel, simple approach that uses multi-beam interference lithography to create porous hexagonal micro-lens (1-8 μm in diameter) arrays from photoresist materials [48]. In our experiments, we used a continuous wave diode-pumped solid state laser to photopolymerize a negative tone resist SU8. The optics setup was similar to that described by Turberfield *et al.* [49]. The physical basis for the process is detailed below. When the interference light is transferred into a negative tone photoresists during exposure, a periodic pattern of strongly and weakly exposed regions is generated. The highly exposed regions are then polymerized and the unexposed regions are dissolved away to reveal the holes. When the difference between the adjacent strongly and weakly exposed regions is close to a certain threshold value, the gradual change in the intensity between the regions produces the lens-like topography in the photoresist film combined with holes.

The appearance of thus synthesized micro-lens arrays (Fig. 13) is strikingly similar to their biological prototype shown in Fig. 3. The lens size, shape, symmetry and connectivity are controlled by beam wave vectors and their polarizations; while the pore size is adjusted by laser intensity, exposure time and the concentration of a photosynthesizer in the resist. We showed that these synthetic micro-lenses are capable of focusing light. The incorporation of holes in the lens array provides means for the transport of photoradiation-absorbing liquids and, therefore, for transmission and numerical aperture tunability and wavelength selectivity.
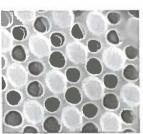

Figure 13. Micro-lens array with integrated pores fabricated by multi-beam lithography.

5. Conclusion

Multidisciplinary groups involving materials scientists, chemists, physicists, biologists work together trying to understand the mechanisms controlling the formation of elaborate structures of biological minerals. We believe that further studies of biological systems will increase our understanding of how organisms evolved their sophisticated optical structures for survival and adaptation and will provide additional materials concepts and design solutions. Ultimately, these biological principles will improve our current capabilities to fabricate optical elements and contribute to the construction of novel, adaptive, micro-scale optical devices.

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References

1. Alper, M., Calvert, P.D., Frankel, R., Rieke, P.C. and Tirrell, D.A. (1991) *Materials synthesis based on biological processes*, Materials Research Society, Pittsburgh.
2. Braun, P.V., Osenar, P. and Stupp, S.I. (1996) Semiconducting superlattices templated by molecular assemblies, *Nature* **380**, 325-328.
3. Mann, S., Archibald, D.D., Didymus, J.M., Douglas, T., Heywood, B.R., Meldrum, F.C. and Reeves, N.J. (1993) Crystallization at inorganic-organic interfaces - Biominerals and biomimetic synthesis, *Science* **261**, 1286-1292.
4. Heuer, A.H., Fink, D.J., Laraia, V.J., Arias, J.L., Calvert, P.D., Kendall, K., Messing, G.L., Blackwell, J., Rieke, P.C., Thompson, D.H., Wheeler, A.P., Veis, A. and Caplan, A.I. (1992) Innovative materials processing strategies - a biomimetic approach, *Science* **255**, 1098-1105.
5. Mann, S. and Ozin, G.A. (1996) Synthesis of inorganic materials with complex form, *Nature* **382**, 313-318.
6. Addadi, L. and Weiner, S. (1992) Control and design principles in biological mineralization. *Angew. Chem.-Int. Edit. Engl.* **31**, 153-169.

7. Aizenberg, J., Black, A.J. and Whitesides, G.M. (1999) Control of crystal nucleation by patterned self-assembled monolayers, *Nature* **398**, 495-498.
8. Lowenstam, H. A. and Weiner, S. (1989) *On Biomineralization*, Oxford Univ. Press, Oxford.
9. Wainwright, S.A., Biggs, W.D., Currey, J.D. and Gosline, J.M. (1976) *Mechanical design in organisms*, John Wiley and Sons, New York.
10. Aizenberg, J., Tkachenko, A., Weiner, S., Addadi, L. and Hendler, G. (2001) Calcitic microlenses as part of the photoreceptor system in brittlestars, *Nature* **412**, 819-822.
11. Cattaneo-Vietti, R., Bavestrello, G., Cerrano, C., Sara, M., Benatti, U., Giovine, M. and Gaino, E. (1996) Optical fibres in an Antarctic sponge, *Nature* **383**, 397-398.
12. Sundar, V.C., Yablon, A.D., Grazul, J.L., Ilan, M. and Aizenberg, J. (2003) Fiber-optical features of a glass sponge, *Nature* **424**, 899-900.
13. Sarikaya, M., Fong, H., Sunderland, N., Flinn, B.D., Mayer, G., Mescher, A. and Gaino, E. (2001) Biomimetic model of a sponge-spicular optical fiber - mechanical properties and structure, *J. Mater. Res.* **16**, 1420-1428.
14. Hyman, L.H. (1955) *The invertebrates: Vol. 4, Echinodermata*, McGraw-Hill, New York.
15. Yoshida, M., Takasu, N. and Tamotsu, S. (1984) Photoreception in echinoderms, in M.A. Ali (eds.), *Photoreception and vision in invertebrates*, Plenum, New York, pp. 743-771.
16. Millot, N. (1975) The photosensitivity of echinoids, *Adv. Mar. Biol.* **13**, 1-52.
17. Hendler, G. and Byrne, M. (1987) Fine structure of the dorsal arm plate of *Ophiocoma wendti*: Evidence for a photoreceptor system (Echinodermata, Ophiuroidea), *Zoomorphology* **107**, 261-272.
18. Hendler, G. (1984) Brittlestar color-change and phototaxis (Echinodermata: Ophiuroidea: Ophiocomidae), *PSZNI Mar. Ecol.* **5**, 379-401.
19. Cowles, R.P. (1910) Stimuli produced by light and by contact with solid walls as factors in the behavior of ophiuroids, *J. Exp. Zool.* **9**, 387-416.
20. Donnay, G. and Pawson, D.L. (1969) X-ray diffraction studies of echinoderm plates, *Science* **166**, 1147-1150.
21. Ameye, L., Hermann, R., Wilt, F. and Dubois, P. (1999) Ultrastructural localization of proteins involved in sea urchin biomineralization, *J. Histochem. Cytochem.* **47**, 1189-1200.
22. Hint, H.T. (1936) *Geometrical optics*, Methuen and Co, London.
23. Clarkson, E.N.K. and Levi-Setti, R. (1975) Trilobite eyes and the optics of Des Cartes and Huygens, *Nature* **254**, 663-667.
24. Gal, J., Horvath, G., Clarkson, E.N.K. and Haiman, O. (2000) Image formation by bifocal lenses in a trilobite eye?, *Vision Res.* **40**, 843-853.
25. Towe, K.M. (1973) Trilobite eyes: Calcified lenses in vivo, *Science* **179**, 1007-1010.
26. Land, M.F. (1981) Optics and vision in invertebrates, in H. Autrum (eds.), *Comparative physiology and evolution in invertebrates B: Invertebrate visual centers and behavior I*, Springer, Berlin, pp. 471-592.
27. Cobb, J.L.S. and Hendler, G. (1990) Neurophysiological characterization of the photoreceptor system in a brittlestar, Ophiocoma wendtii (Echinodermata: Ophiuroidea), *Comp. Biochem. Physiol.* **97A**, 329-333.
28. Stubbs, T.R. (1982) The neurophysiology of photosensitivity in ophiuroids, in J.M. Lawrence (eds.), *Echinoderms: Proceedings of the International Conference, Tampa Bay*, Balkema, Rotterdam, pp. 403-408.
29. Johnsen, S. (1997) Identification and localization of a possible rhodopsin in the echinoderms *Asterias forbesi* (Asteroidea) and *Ophioderma brevispinum* (Ophiuroidea), *Biol. Bull.* **193**, 97-105.
30. Berman, A., Addadi, L., Kvick, Å., Leiserowitz, L., Nelson, M. & Weiner, S. (1990) Intercalation of sea urchin proteins in calcite: Study of a crystalline composite material, *Science* **250**, 664-667.
31. Addadi, L., Aizenberg, J., Albeck, S., Berman, A., Leiserowitz, L. & Weiner, S. (1994) Controlled occlusion of proteins - a tool for modulating the properties of skeletal elements, *Mol. Cryst. Liq. Cryst. Sci. Technol. Sect. A-Mol. Cryst. Liq. Cryst.* **248**, 185-198.
32. Albeck, S., Aizenberg, J., Addadi, L. & Weiner, S. (1993) Interactions of various skeletal intracrystalline components with calcite crystals, *J. Am. Chem. Soc.* **115**, 11691-11697.
33. Gonis, A. (2000) *Nucleation and Growth Processes in Materials*, Materials Research Society, Boston.
34. Vere, A.W. (1988) *Crystal Growth: Principles and Progress*, Plenum, New York.
35. Beniash, E., Aizenberg, J., Addadi, L. and Weiner, S. (1997) Amorphous calcium carbonate transforms into calcite during sea urchin larval spicule growth, *Proc. R. Soc. Lond. Ser. B-Biol. Sci.* **264**, 461-465.

36. Beniash, E., Addadi, L. and Weiner, S. (1999) Cellular control over spicule formation in sea urchin embryos: A structural approach, *J. Struct. Biol.* **125**, 50-62.

37. Aizenberg, J., Hanson, J., Koetzle, T.F., Leiserowitz, L., Weiner, S. & Addadi, L. (1995) Biologically induced reduction in symmetry - a study of crystal texture of calcitic sponge spicules, *Chem.-Eur. J.* **1**, 414-422.

38. Aizenberg, J., Black, A.J. and Whitesides, G.M. (1999) Oriented growth of calcite controlled by self-assembled monolayers of functionalized alkanethiols supported on gold and silver, *J. Am. Chem. Soc.* **121**, 4500-4509.

39. Han, Y.-J. and Aizenberg, J. (2003) Face-selective nucleation of calcite on self-assembled monolayers of alkanethiols: Effect of the parity of the alkyl chain, *Angew. Chem. Int. Ed.* **42**, 3668-3670.

40. Xia, Y.N. and Whitesides, G.M. (1998) Soft lithography, *Annu. Rev. Mater. Sci.* **28**, 153-184.

41. Koga, N., Nakagoe, Y.Z. and Tanaka, H. (1998) Crystallization of amorphous calcium carbonate, *Thermochim. Acta* **318**, 239-244.

42. Gower, L.B. and Odom, D.J. (2000) Deposition of calcium carbonate films by a polymer-induced liquid-precursor (PILP) process, *J. Cryst. Growth* **210**, 719-734.

43. Raz, S., Weiner, S. and Addadi, L. (2000) Formation of high-magnesian calcites via an amorphous precursor phase: Possible biological implications, *Adv. Mater.* **12**, 38-41.

44. Sawada, K. (1997) The mechanisms of crystallization and transformation of calcium carbonates, *Pure Appl. Chem.* **69**, 921-928.

45. Aizenberg, J., Lambert, G., Addadi, L. and Weiner, S. (1996) Stabilization of amorphous calcium carbonate by specialized macromolecules in biological and synthetic precipitates, *Adv. Mater.* **8**, 222-225.

46. Aizenberg, J., Lambert, G., Weiner, S. and Addadi, L. (2002) Factors involved in the formation of amorphous and crystalline calcium carbonate: A study of an ascidian skeleton, *J. Am. Chem. Soc.* **124**, 32-39.

47. Aizenberg, J., Muller, D.A., Grazul, J.L. and Hamann, D.R. (2003) Direct fabrication of large micropatterned single crystals, *Science* **299**, 1205-1208.

48. Yang, S., Megens, M. and Aizenberg, J. (2003) Fabrication of biomimetic microlens arrays with integrated pores by interference lithography, *Unpublished data.*

49. Campbell, M., Sharp, D.N., Harrison, M.T., Denning, R.G. and Turberfield, A.J. (2000) *Nature* **404**, 53-55.