Methods for fabricating and characterizing a new generation of biomimetic materials [1]

Nils Almqvist [a,d,*], Neil H. Thomson [a,2], Bettye L. Smith [a], Galen D. Stucky [b], Daniel E. Morse [c], Paul K. Hansma [a]

[a] *Department of Physics, University of California, Santa Barbara, CA 93106, USA*
[b] *Department of Chemistry, University of California, Santa Barbara, CA 93106, USA*
[c] *Departments of Molecular, Cellar, and Developmental Biology, University of California, Santa Barbara, CA 93106, USA*
[d] *Department of Physics, Luleå University of Technology, S-971 87 Luleå, Sweden*

Abstract

Bringing together current ideas in the fields of biomineralization and composite laminate materials, we have attempted to fabricate model materials that mimic abalone nacre through the rapid assembly of inorganic tablets, such as talc. Several physical methods were tested to aid the orientation of the talc tablets in fluid suspensions with a low percentage, 10% by dry weight, of organic binding material. The orientation of talc tablets in the synthesized composites was characterized by X-ray diffraction and scanning electron microscopy. The modulus of rupture of the materials was measured in a three-point bending test. We demonstrate that the alignment of tablets increases by the use of physical methods and from chemical surface treatment. Important factors to consider in making materials that mimic abalone nacre are discussed. Important factors to consider in making materials that mimic abalone nacre are discussed. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: Biomineralization; Talc; Polyvinylacetate; Orienting; X-ray; SEM; Biomimetics

1. Introduction

Molluscs, such as the abalone, live in shells that they construct from elements in the surrounding environment. These shells are highly structured composites of crystallized inorganic material concentrated from seawater and organic material secreted by the animal [1,2].

The structure of the shell is controlled through interactions between inorganic and organic materials in a process generally known as biomineralization [3]. Proteinaceous material secreted by the animal controls the crystal phase and orientation of the inorganic material [4–6]. The inorganic material in abalone shells is calcium carbonate and exists in one of two polymorphs, calcite or aragonite [4–8]. Abalone nacre, the mother of pearl part of the shell, consists of single crystal tablets of aragonite 0.4 to 1.2 μm thick and 5 to 10 μm across arranged in a highly regular array, creating an interlocking 'brick wall' structure, which is cemented together by thin layers (10 to 50 nm thick) of the organic material [9–11]. The structure of nacre has been extensively studied using electron microscopy [5–7,9–16] and atomic force microscopy [2,17]. The organic matrix between the tablets is made up chiefly of polysaccharides and proteins [18]. Nacre is stiffer, stronger and tougher than lightly-filled synthetic composites. It requires 3000 times more work to fracture abalone nacre than geological aragonite [19,20]. The high degree of order over long distances coupled with the energy absorbing properties of the organic matrix is thought to be the basis for their fracture resistance [21]. Amazingly enough, this increase in strength is a result of organic material present in only 1 to 5% by weight.

Much research has been conducted in order to elucidate the biochemical processes involved in shell growth [3]. It has been shown that flat pearls of nacre can be grown on

---

* Corresponding author. E-mail: nilsalm@physics.ucsb.edu
[1] The first and second authors contributed equally to this work.
[2] Also corresponding author. E-mail:
paznht@pan1.pharm.nottingham.ac.uk

inorganic substrates by inserting the substrate between the mantle and the shell of the abalone [8]. These pearls give the same mechanical properties as the abalone nacre, but grow at the same rate as the shell, a maximum of about 5 μm per day [5]. As a result of this large body of information, a new paradigm for how abalone nacre growth occurs has recently been put forward [22]. This experimentally verified model is based on the growth of mineral bridges between successive aragonite tablets. The organic material coats the tablets as thin sheets but allows the mineral to grow through nanometer-sized pores that function like molecular stencils.

Clearly, the nacre gets its high strength by a slow growth process in which the tablets become highly oriented and tightly imbedded by the interlamellar organic sheets. Therefore, if materials mimicking the abalone shell are desired in large quantities, then we need to speed up the assembly of tablets on a vastly faster time-scale than occurs in the animal. In our approach, we attempted to mimic the abalone shell growth by investigating methods for the rapid assembly of inorganic tablets into a structured microlaminate composite.

Talc was chosen as the model inorganic template, because it consists of crystal sheets of similar dimensions as the aragonite tablets formed in the abalone shell. Additionally, talc is an abundant naturally occurring mineral that is resistant to heat, electricity and acids. Talc is one of the softest minerals (defined as hardness 1 on the Mohs' hardness scale) and belongs to the class of layered phyllosilicates. The chemical formula of talc is $Mg_3(Si_4O_{10})(OH)_2$. The crystal structure consists of stacked layers of magnesium hydroxide in a sandwich between silicate layers [23]. Two tetrahedral silica molecules share an octahedral sheet of magnesium hydroxide. There is a gap between adjacent silica layers, which are held together via weak van der Waals bonds and therefore talc cleaves easily in this plane.

Much work has been carried out investigating properties of talc and synthetic polymer mixtures [24–27]. In these systems, the inorganic material is used in quantities below 40% (wt.) as a filler to moderate the mechanical properties of the polymer, changing ductile behavior to quasi-brittle. It has been shown that the talc particles in the mixtures are highly oriented after compression molding or extrusion/coextrusion [28,29]. These materials are widely used as industrial materials, such as structural elements in interiors of automobiles. Related work involves making polymer nanocomposites using layered silicates as reinforcing agents [30,31], with the inorganic content below 25% (wt.).

Our work attempts to bridge the gap between biomineralization and composite materials by using methods to create biomimetic materials based on talc and organic material. In contrast to the talc/polymer work, the materials we fabricated contained inorganic material as the main constituent (90% wt.). The special focus was: (i) to get the content of organic material close to that in abalone nacre, and (ii) to physically orient the talc tablets. Talc was suspended with either polyvinylacetate (PVA) or epoxy silane in mixtures of ethanol and water and the resulting slurry was subjected to simple physical processing to orient the talc tablets. In dense suspensions, plate-like particles are forced into parallel alignment by shearing [32]. We developed a number of physical methods that all were promising enough to be examined. They were sedimentation, centrifugation, spinning, shearing (in various configurations) and dipping (illustrated in Fig. 1). X-ray diffraction and scanning electron microscopy (SEM) were used to characterize the extent of orientation we achieved in a given direction. The mechanical strength of each composite material was determined in a three-point bend-
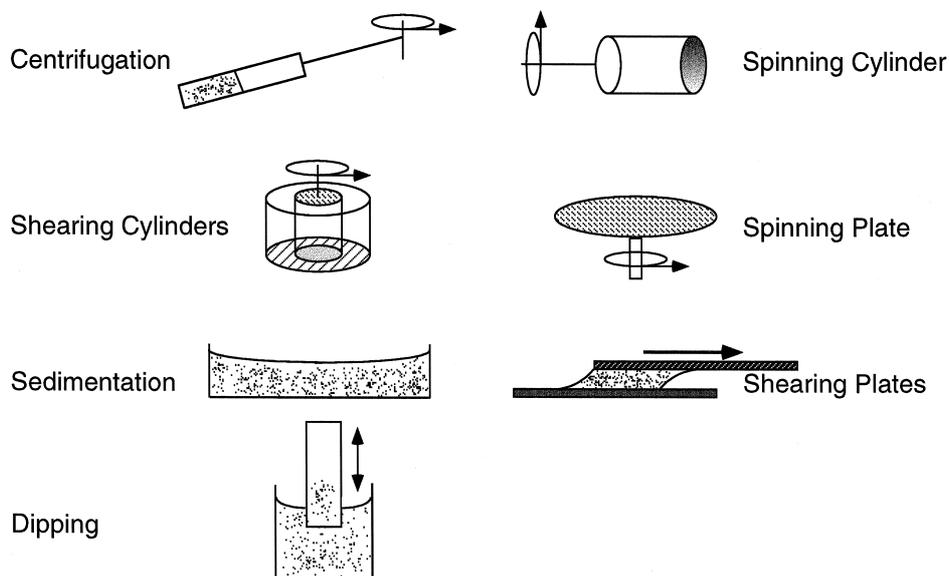

Fig. 1. Schematic illustrations of the different physical methods used to increase the orientation of the talc tablets.

ing test and the strength as a function of the tablet orientation was investigated.

2. Experimental

2.1. Materials and physical processing

The talc suspensions were prepared by initially mixing polyvinylacetate (E-372NR, Borden, Columbus, OH) or 2-(3,4-epoxycyclohexyl)-ethyltrimethyloxysilane (Gelest, Tullytown, PA) with ethanol/water mixtures (1/2 v/v) and then adding nine weight equivalents of talc (Sigma, St. Louis, MO) relative to the organic polymer, while slowly stirring. The premixing of the organic with a solvent (ethanol/water mixture) ensured better wetting of the talc. The typical premixture was 10 g of talc per 30 ml of solvent–polymer mixture. However, for a few of the physical processing methods we used higher viscosity fluids, up to 10 g talc per 12 ml of solvent–polymer mixture. After stirring for a few minutes, the slurries were ready to use.

To investigate the effect of the surface chemistry of the talc tablets on the ease of making an ordered assembly, optimal silanisation of the talc powder was carried out by pretreating with a mixture of 3% (vol.) epoxysilane in ethanol (adjusted with acetic acid to pH 5), for several hours, before physical processing. The total silane to talc concentration was 10% (w/w).

We investigated seven different physical fabrication methods. The samples were physically processed and then left to cure at 70°C for one day. The following physical methods, schematically shown in Fig. 1, were used.

(i) Centrifugation—The talc suspensions were loaded in plastic tubes (diameter 14.5 mm) and centrifuged for 10 min at 180–1500 × g. The results shown herein are from centrifugation at about 180 × g. Water separated in the centrifugation step was carefully removed.

(ii) Shearing cylinders—Viscous (10 g talc per 12 ml solvent–polymer mixture) talc suspensions were confined between a glass beaker (diameter 46.4 mm) and a rigid plastic tube (diameter 28.4 mm), the tube being concentric with the beaker. The beaker was rotated with variable speed by a small dc motor. The slurry was sheared between the cylinders for several hours, with heating to about 60°C from a heating lamp, until the suspensions were completely solidified. The rotation speed of the beaker was initially 1 rpm, but it was successively decreased during the process.

(iii) Sedimentation—The talc suspensions (10 g talc per 15–30 ml solvent–polymer mixture) were poured into plastic petri-dishes and left to stand for one day without further manipulation.

(iv) Dipping—A glass slide was mounted onto a stage for repeated dipping into the suspension of talc and was left after each dip at a 45° angle for 1 min to partially dry under a heat lamp.

(v) Spinning cylinder—The talc suspensions (10 g talc per 20 ml solvent–polymer mixture) were continuously dripped into a plastic cylinder (diameter 35 mm) that was spinning at approximately 500 rpm.

(vi) Spinning plate—The talc suspensions were spin-coated onto a glass plate by continuously dripping onto the rotating plate (300–700 rpm) with a home-made variable speed spin-coater.

(vii) Shearing plates—Viscous talc suspensions were sheared by hand between two glass slides.

2.2. Materials characterization

The amount of orientation of the talc tablets in the materials was characterized with SEM and X-ray diffraction. Fracture surfaces of the material specimens were sputter-coated with gold and examined in the scanning electron microscope (JSM-5300LV, JEOL, Tokyo, Japan). We avoided polishing of the surfaces, since it appeared that it creates a falsely oriented surface layer of talc.

The X-ray diffraction patterns were recorded with a Scintag PADX diffractometer, using K_α-radiation from a copper target. The X-ray spectra presented herein are representative for the sample under investigation.

The modulus of rupture (MOR) was measured in a three-point bending test with a span length, S, of 4.83 mm. Rectangular beams of the composites were prepared with varying dimensions. The widths, W, were 3.8–6.4 mm and the depths, D, were 1.8–5.0 mm. The representative sample, for the data in this paper, had a W of approximately 4.5 mm and a span–depth ratio (S/D) of about 2. If P is the load for fracture, the modulus of rupture, σ_r, is evaluated as:

$$\sigma_r = \frac{3}{2} \frac{PS}{WD^2}. \tag{1}$$

3. Results

It was difficult to wet the talc tablets with an organic content of only 5%. Therefore, we chose to work with systems containing 10% by weight. Fig. 2 shows the X-ray diffraction (XRD) patterns obtained for different physical processing of the composite material made of talc mixed with 10% (w/w) PVA, suspended in the mixture of ethanol in water (1/2 v/v). The highest intensity X-ray reflections of talc are indicated in the uppermost graph. The crystallographic c-axis of talc is normal to the tablet surfaces. Hence, the (002) reflection, and multiples thereof, in Fig. 2 are from talc tablets oriented parallel to the specimen surface. The peaks from (020) and (132) also include negligible contributions from other crystallographic planes. To quantify the amount of ordering parallel to the sample surface, we define an orientation factor as the intensity-ratio between the (006) and (132) peaks at
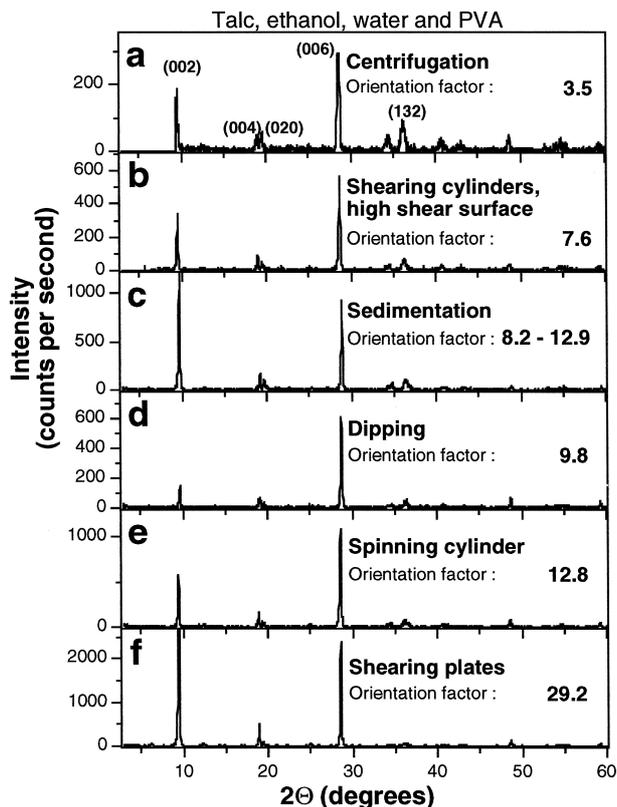
Fig. 2. X-ray diffraction patterns for the composite materials made of talc, ethanol, water and PVA fabricated with different physical methods.
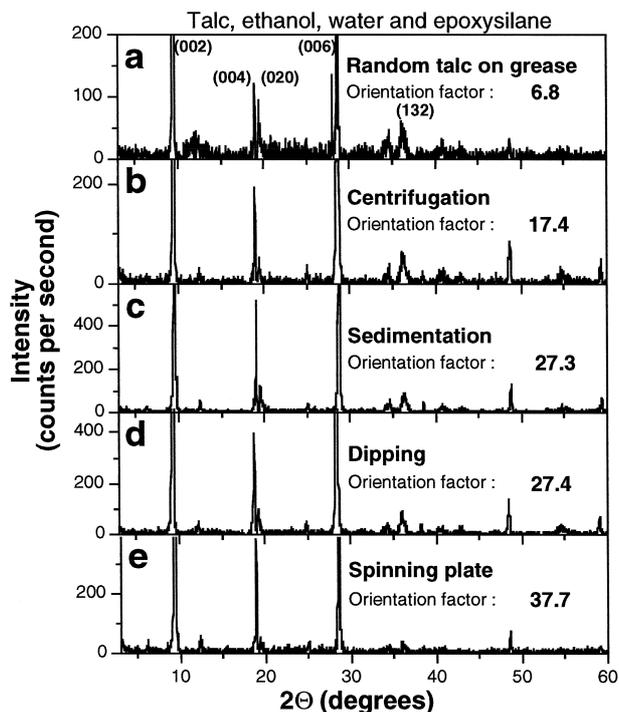

Fig. 3. X-ray diffraction patterns for the composite materials made of talc, ethanol, water and epoxysilane fabricated with different physical methods.

angles of incidence 28.6° and 36.2°, respectively. The (006) peak is chosen since it is high-intensity, isolated and well-defined. We rejected the use of the (002) peak since its intensity can be affected by the surface roughness at such a low angle of incidence. There are no well-defined peaks in the pattern that are from tablets planes exactly transverse to (006). Hence we choose to use the (132) peak, i.e., planes that are tilted about 60° from the (006) planes.

The X-ray diffraction revealed structural ordering with the talc tablets oriented parallel to the material surfaces of the physical processing device. The spectra shown in Fig. 2 are collected with the samples mounted to have their tablets oriented in the same plane and the graphs are sorted according to their orientation factor. It is evident from the figure that the degree of orientation within the samples depends upon the physical method used to fabricate them. Centrifugation was not successful in orienting the tablets. Apparently this process is too quick and separates the inorganic from the organic matrix. Shearing between cylinders is a promising method, although our samples showed only slightly more orientation than the corresponding non-sheared samples. The use of an inflatable inner cylinder instead of our rigid plastic tube would most likely improve the results. The remaining physical methods (dipping to shearing plates), all increased the ordering of tablets, with the spinning and shearing plates proving to be the best

methods. The use of ethanol in the mixtures improved the ease of creating order, especially in the methods with no or low shear rate (i.e., sedimentation and dipping). This finding seems to be directly related to enhanced wetting of the relatively inert talc tablets.

We found that surface modification with epoxysilane gives a better degree of orientation for a given physical method employed. The silane condenses with hydroxyl groups of the talc and alters its surface chemistry. Fig. 3 shows the XRD patterns derived from the system of talc mixed with 10% (w/w) epoxysilane. A powder diffraction pattern obtained from talc tablets sprinkled on a layer of vacuum grease is included as an example of an unordered sample. The results confirm our earlier findings that the spinning plate and dipping methods are successful in orienting the tablets. The increased orientation can be seen, in addition to the orientation factor, in the drop of unwanted
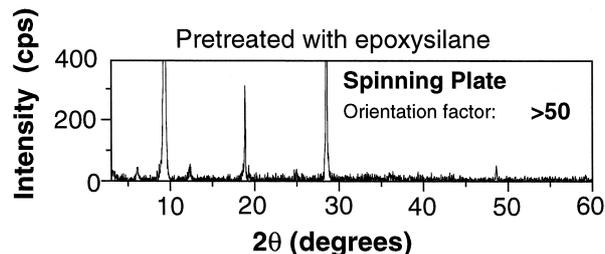

Fig. 4. X-ray diffraction pattern of the talc, ethanol, water composite pretreated with epoxysilane.

diffraction peaks as, for example, (020). Furthermore, the orientation for each physical method is significantly improved by the silane treatment. Again, this is a direct consequence of the difference in the ability of PVA/ethanol and epoxysilane to wet talc. Fig. 4 shows the XRD pattern obtained when a suspension of talc, that had been pretreated with epoxysilane under optimal silanisation conditions (see Section 2) was oriented on the spinning plate. The sample shows excellent structural ordering, higher than for the other samples. The orientation factor is greater than 50, since the *non-orientation* peak at (132) drops into the background noise. Thus, in addition to the physical method used, the surface chemistry of the tablets plays an important role for the amount of orientation.

As revealed by SEM, the characteristic dimensions of the talc tablets are about 3–10 μm across and a few hundred nanometers thick. Their aspect ratios vary considerably. SEM imaging of the composites revealed that although the tablets were generally aligned in a unidirectional manner by the physical treatment, they did not exhibit a very high microscopic degree of structural ordering. The main ordering was found on the 50–200 μm length scale. However, the silane treatment facilitates ordering at the smaller length scales. The SEM image in Fig. 5 reveals the ordering on a spinning plate sample mixed with epoxysilane. The composites of talc, ethanol and PVA are fairly brittle. The modulus of rupture, evaluated as the average from at least ten measurements, shows only a weak correlation between orientation factor and strength, especially since the variances in the measurements are large. The MOR for the spinned cylinder sample is 6.4 MPa and slightly lower for the methods yielding smaller orientation factor. The centrifuged composites stand out as the exceptionally weakest samples with a strength an order of magnitude lower than of the other samples. We could not observe any differences in strength for different

Fig. 5. SEM image (from above) of a spinning plate sample, with orientation factor 37.7, of talc, water/ethanol and epoxysilane. The individual tablets form a larger-scale overall orientation.

loading directions or any dependencies on the samples' S/D ratios, although the measured values varied extensively. The densities of the samples are 1.0–1.1 g/cm^3 but have no measurable correlation to the strength or to the orientation factor. In comparison, the samples fabricated with silane are more elastic but have a lower modulus of rupture. Moreover, the MOR across the tablets of dry abalone nacre at a S/D ratio of 1.4, was measured to be larger than 300 MPa. However, at a S/D ratio of 5 we measured the modulus to 110–185 MPa, both along and across the tablets.

4. Discussion

Surprisingly, not many attempts to fabricate microlaminate materials by orienting inorganic tablets and binding them together with an organic matrix can be found in the literature. Phyllosilicates, such as talc, mica and clays are an obvious choice for the microlaminate inorganic material, since they form layered structures and, in the case of talc and clay, exist as small crystal tablets with characteristic dimensions close to those of abalone nacre. In general, the face basal planes of phyllosilicates have a slightly negative charge, which is balanced by cations in the interlayer space. In suspension, the edge charges vary with pH. This polar nature causes difficulties in dispersion and wetting of the mineral. The use of buffers, surface coatings and coupling agents are important to overcome these difficulties. For example, it is well known that for clays like kaolin, edge–face interactions are negligible in phosphate buffers at neutral or high pH [32]. Organic cations can be used to render the normally hydrophilic silicate surface organophilic and, therefore, improve the wetting by polymers. In this work with talc, we explored the use of several coupling agents, intercalation compounds and organic matrices. We got significant improvements with ethanol and silane in the mixtures. Talc differs from the other phyllosilicates in the sense that it is relatively uncharged and hydrophobic. Therefore, the improved ease of creating order with the water/ethanol and epoxysilane mixture is most likely due to enhanced wetting.

The strengths of different mollusc nacres are significantly different. Some nacres have a work of fracture that is very different in different loading directions, i.e., along or across the crystal aragonite tablets [21]. Other nacres have more isotropic mechanical properties [19]. It is believed that the precise and regular arrangement of the tablets in nacre plays an important role for its superior mechanical properties [21]. The tablets are thought to be of critical thickness for preventing crack propagation and the structural arrangement is effective in stopping cracks. In addition, cracks that propagate through the nacre travel mostly around the tablets, which increases the required work for fracture. The mechanisms of fracture in dry nacre can be described by pull-out of the tablets. In addition, wet

nacre often has a significantly higher fracture resistance [19,20]. This is due to large-scale delaminations of the tablets and, therefore, additional plastic work during fracture. Fibrils of the organic matrix stretch and bridge the delamination cracks and spaces between the tablets. The fibrils can be stretched hundreds of percent before they detach from the calcium carbonate. If the mechanisms of energy absorption during fracture include large-scale delaminations, the strengths are expected to increase for samples with smaller S/D ratios [19].

Our talc composites show structural ordering. However, they have not the high degree of ordering as in a nacre. Since the composites are constituted by 90% wt. of the inorganic, we count the measured densities as relatively low. Furthermore, each talc tablet is extremely easily cleaved and the strength of binding between organic and inorganic is unknown but probably weak. Therefore, it is likely that these facts together are responsible for our mechanical results: the composites are fairly isotropic, with only modest improvements in strength resulting from increased orientation of tablets. It is probable that the talc tablets do not stop cracks effectively, that the organic–inorganic interface is weak and that the porosity is influencing the mechanical properties.

Major issues in the fabrication of a high strength laminate materials are: to wet and align the suitable inorganic tablets, bind the tablets to the organic matrix and to each other, and finally, evaporate excess solvent without cracking the composite. We have succeeded in wetting and aligning talc tablets. Ultimately, self-assembly of monodisperse tablets may yield the highest degree of order. It is, however, clear from this study that it is necessary to control their surface chemistry and that physical processing is valuable to finish the processing. Apparently, to fabricate a laminate material with the high strength of nacre, we would need to use stronger inorganic tablets and achieve stronger binding to the organic matrix. To achieve higher performance, we believe that the organic material should be more closely related to the fibrils in the abalone nacre [33]. We have started to synthesize microlaminates from ground muscovite mica. Our preliminary results are promising and highly ordered microlaminate mica composites have been fabricated. Future work will include further treatments, refined mechanical measurements and additional characterization methods.

5. Conclusions

We have synthesized microlaminate composites of an inorganic material mixed with 10% (w/w) of an organic material to mimic abalone nacre. We have demonstrated that the alignment of tablets in the composite is increased by the use of physical processing methods. The methods in Fig. 1 are sorted, from centrifugation to shearing plates, according to their effectiveness in aligning tablets. Addition of ethanol to the mixtures improved the ease of orienting the tablets. Additionally, talc mixed with epoxysilane gave a better degree of orientation for each physical method employed. The improvement in tablet orientation gave only modest improvements in the mechanical properties, measured simply as the modulus of rupture. The results in this study are encouraging as the first step in the process towards fabricating a material that mimics both the structure and mechanical properties of the abalone nacre.

References

[1] D.E. Morse et al., in: C. Viney, S.T. Case, J.H. Waite (Eds.), Materials Research Society Symposium Proceedings, Vol. 292, 1993, p. 59.
[2] R. Giles, S. Manne, S. Mann, D.E. Morse, G.D. Stucky, P.K. Hansma, Biol. Bull. 188 (1995) 8.
[3] S. Mann, D.A. Archibald, J.M. Didymus, T. Douglas, B.R. Heywood, F.C. Meldrum, N.J. Reeves, Science 261 (1993) 1286.
[4] A.M. Belcher, X.H. Wu, R.J. Christensen, P.K. Hansma, G.D. Stucky, D.E. Morse, Nature 381 (1996) 56.
[5] C.M. Zaremba, A.M. Belcher, M. Fritz, L. Youli, S. Mann, P.K. Hansma, D.E. Morse, J.S. Speck, G.D. Stucky, Chem. Mater. 8 (1996) 679.
[6] D.A. Walters, B.L. Smith, A.M. Belcher, G.T. Paloczi, G.D. Stucky, D.E. Morse, P.K. Hansma, Biophysical Journal 72 (1997) 1425.
[7] G. Falini, S. Albeck, S. Weiner, L. Addadi, Science 271 (1996) 67.
[8] M. Fritz, A.M. Belcher, M. Radmacher, D.A. Walters, P.K. Hansma, G.D. Stucky, D.E. Morsem, S. Mann, Nature 371 (1994) 49.
[9] N. Watabe, J. Ultrastructure Research 12 (1965) 351.
[10] H. Mutvei, Stockholm Contr. Geol. 20 (1969) 1.
[11] H. Mutvei, SEM 2 (1979) 457.
[12] K.M. Towe, G.H. Hamilton, Calif. Tissues 1 (1968) 306.
[13] H. Nakahara, G. Bevelander, M. Kakei, Venus Jpn. J. Malac. 41 (1982) 33.
[14] K. Wada, Biomineralization 4 (1972) 141.
[15] S.W. Wise, Science 167 (1970) 1486.
[16] S.W. Wise, Eclogae Geol. Helv. 63 (1970) 775.
[17] S. Manne, C.M. Zaremba, R. Giles, L. Huggins, D.A. Walters, A.M.

Belcher, D.E. Morse, G.D. Stucky, J.M. Didymus, S. Mann, P.K. Hansma, Proceedings of the Royal Society of London B 256 (1994) 17.

[18] S. Weiner, W. Traub, FEBS Letters 111 (1980) 311.

[19] A.P. Jackson, J.F.V. Vincent, R.M. Turner, Proceedings of the Royal Society of London B 234 (1988) 415.

[20] A.P. Jackson, J.F.V. Vincent, Journal of Materials Science 25 (1990) 3173.

[21] J.D. Currey, Proceedings of the Royal Society of London B 196 (1977) 443.

[22] T.E. Schäffer, C. Ionescu-Zanetti, R. Proksch, M. Fritz, D.A. Walters, N. Almqvist, C.M. Zaremba, A.M. Belcher, B.L. Smith, G.D. Stucky, D.E. Morse, P.K. Hansma, Chemistry of Materials 9 (1997) 1731.

[23] C.S. Hurlbut Jr., K.C. Dana, in: Manual of Mineralogy (after J.D. Dana), 20th edn., Wiley, New York, 1985, p. 418.

[24] J.I. Velasco, J.A. De Saja, A.B. Martinez, Fatigue Fract. Eng. Mater. Struct. 20 (1997) 659.

[25] M.A. Ramos, M.S. Berna, J.P. Vigo Matheu, Polymer Engineering and Science 31 (1991) 245.

[26] .

[27] J.L. White et al., J. Non-Newtonian Fluid Mech. 69 (1997) 15.

[28] C.H. Suh, J.L. White, Polymer Engineering and Science 36 (1996) 2188.

[29] C.D. Mueller, S. Nazarenko, T. Ebeling, T.L. Schuman, A. Hiltner, E. Baer, Polymer Engineering and Science 37 (1997) 355.

[30] E.P. Giannelis, Advanced Materials 8 (1996) 29.

[31] R.A. Vaia, K.D. Jandt, E.J. Kramer, E.P. Giannelis, Chemistry of Materials 8 (1996) 2628.

[32] S. Jogun, C.F. Zukoski, J. Rheol. 40 (1996) 1211.

[33] X. Shen, A.M. Belcher, P.K. Hansma, G.D. Stucky, D.E. Morse, J. Biol. Chem. 272 (1997) 32472.