CYTIDINE FUNCTIONALIZATION PROMOTES SYNERGISTIC MECHANICAL PROPERTIES IN NACRE-MIMETIC NANOCOMPOSITES

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1 Introduction

Natural nanocomposites, such as nacre, insect cuticle, spider silk, teeth, bone, and antler [1] show low density and synergistic mechanical properties. They have exceptionally high toughness, strength, and stiffness compared with the properties of the component materials [2]. The excellent properties originate from the sophisticated self-assembled structures, which often is a combination of high fraction of aligned reinforcing and small fraction of soft energy dissipating domains [3]. But the biological materials are difficult and expensive to scale up, and to engineer from the first principles. Biomimetics [2] aims to achieve some of the properties of natural structural materials, using rationally engineered and scalable components.

Nacre, also known as mother-of-pearl, is a hard biological composite produced by mollusces such as abalone and oyster as an inner layer of shells. It is composed of 95 vol% inorganic platelet-shaped aragonite (an orthorhombic polymorph of CaCO₃), which are bound together by a small fraction of biopolymers [4]. Nacre is stiff (Young’s modulus = 60 – 90 GPa) and strong (60 – 140 MPa), like ceramics usually are, but it has remarkably high toughness [4-7]. Under wet conditions it has a toughness of thousand times higher than pure aragonite [4, 5]. The mechanical performance of nacre per density is as good as some metals and alloys [8-10].

Several energy dissipation mechanisms have been recognized for nacre to explain its toughness [11-13]. Frictional platelet sliding [5, 7, 14] has been suggested as one of the key mechanisms, where the organic phase acts like a lubricant by allowing controlled movement between the platelets thus providing energy dissipation. Other nanoscale mechanisms, such as stiffening of biopolymer [5, 15-17] limit the mutual sliding of the aragonite platelets. Therein sacrificial bonds and hidden length scales have characteristic role in the biological materials [15, 18, 19].

The nacre-like balanced design is extremely difficult to transfer into synthetic materials. It is especially challenging because, in order to achieve the same mechanical behavior, the highly ordered structure of nacre needs to be replicated at several length scales. There are several different approaches to mimic nacre. Maybe the most conventional method is layer-by-layer technique [20-24]. The method is tedious and time consuming, as typical adsorption times per layer is in the scale of minutes [20] and a few micrometer thick film can be produced in days or weeks. Ice-templating followed by sintering [25-27] results in tough nacre-mimetic composites but it also suffers from tedious multistep processing.

Recently a large-scale one step method of mimicking nacre [8,28] was obtained. The key element of the method [8,28] is based on individually coated core-shell colloidal nanoplatelets, that is, nanoclay coated by a polymer layer. To control the mutual sliding, the core-shell colloidal sheets had to be interlocked. Polyvinylalcohol (PVA) coated clay platelets were covalently crosslinked with boric acid [8]. It resulted in stiff and strong composite but it was unfortunately brittle as well. The next step was to the change covalent interactions into weaker supramolecular interactions to facilitate larger mutual sliding: PVA was changed to a polyelectrolyte, poly(diallyldimethylammonium chloride) (PDADMAC) and the colloidal platelets were linked by ionic crosslinks [28]. The hypothesis
was that breaking of the ionic bonds would dissipate energy and result in tougher material. However, the composite behaved characteristically in the same way: ionic crosslinking increased the tensile strength and Young’s modulus, but reduced the strain at failure, as very little energy dissipation was allowed by the "rigid” structure. This encouraged to study whether even weaker bonds, like hydrogen bonds, could act as dynamic sacrificial bonds providing energy dissipation mechanism and synergistic mechanical properties.

There are benefits using cationic polyelectrolytes (like PDADMAC) instead of PVA. Part of the original counter-ions of the polyelectrolyte remains intact upon binding onto the anionic nanoclay. In this study we coat the clay platelets with cationic PDADMAC to create core-shell colloidal nanoplatelets. Then we exchange part of the the original chloride counter-ions of PDADMAC to 2' deoxycytidine 5'-monophosphate (dCMP), which makes strong hydrogen bonds with well defined hydrogen bonding acceptors and donors, as well as it involves phosphate groups allowing ionic complexation to the cationic PDADMAC polymers.

2 Experimental

2.1 Materials

The clay was an anionic natural montmorillonite (MTM), Cloisite Na+ from Southern Clay Products/ROCKWOOD Clay Additives GmbH, (Moosburg, Germany) with a cation-exchange capacity of 92.6 meq/100 g and an interlayer spacing of 1.17 nm. A clay dispersion (0.5 wt %) was prepared. The clay was added little by little into the water, and intensively stirred for 24 h to guarantee delamination of the hydrophilic MTM platelets. The dispersion was allowed to sediment for 24 h and the supernatant was used in the experiments.

Poly(diallyldimethylammonium) chloride (PDADMAC, \( M_w = 200,000 - 350,000 \) g/mol, 20 wt% aqueous solution, Sigma-Aldrich) and 2' deoxycytidine 5'-monophosphate sodium salt hydrate (dCMP, ≥99%, Sigma-Aldrich) were used as received. High purity deionized MilliQ water was used in all experiments.

2.2 Film Preparation

PDADMAC and dCMP were separately dispersed in water at 1.0 wt % and 3 - 4 wt % concentrations, respectively. The anionic clay was coated by cationic PDADMAC, and part of the peripheral chloride counter-ions were exchanged to dCMP (Fig 1). Two batches were prepared: one with dCMP and other batch without dCMP as a reference. Finally, the disc-shape composite films were obtained by vacuum filtration and additional drying at 40°C at least for 48 h and then further dried in vacuum, while applying a slight weight to maintain the planar shape.

2.3 Mechanical testing

The samples were conditioned and measured under controlled humidity, as listed in Table 1. The samples denoted as “dry” were vacuum dried at least 36 h. The tensile testing was carried out on a DEBEN Microtest N tensile tester equipped with a MT 10250 controller (Deben UK Ltd, Suffolk, UK). A nominal strain rate of 0.1 mm/min, 200 N load cell and gauge length of 10 mm were used. The strain was determined optically by Digital Speckle Photography (DSP) system, which is a non-contact optical deformation measuring system. Software recognizes a surface pattern of the specimen from the digital images and allocates the coordinates to the image pixels. After measurement the software allows to compare the images and to calculate the cross-correlations for the expected displacements and to choose the best value, which represent the strain at that point.

The speckle pattern was sprayed on the samples by airbrush and then ribbons (average size of 18 mm x 2.2 mm x 0.11 mm) were glued on sandpaper from their ends. Aramis 6.2 (GOM mbH, Germany) was used for digital image correlation. The images of the specimen were recorded by two high-resolution cameras (5 MB) with same time step as the tensile tester (2 images/s). Specimens, which broke outside the DSP detection area or near the clamps, were neglected, if their stress-strain curves differed significantly from other specimens of the same film. Also the specimens that contained visible cracks or other defects were neglected if they showed uncharacteristic behavior.

The width and thickness of the specimens were measured with digital slide gauge and linear gage
(LGF-01100L-B transmission-type photoelectric linear encoder, Mitutoyo) respectively. Each specimen was measured three times before the speckle patterning and conditioning. The average cross-sectional area was used for calculating stress.

The width, thickness, force, and strain values were imported into MATLAB for further processing. The engineering stress (i.e. force/initial cross-sectional area), Young's modulus and work-to-failure (area under the curve) were calculated. The Young's modulus was determined normally till 0.04 % strain where the samples behaved mostly linearly. The linear correlation coefficients were also calculated for those points used to identify the Young's modulus to see how well the linear estimation describes the behavior. Usually the linear correlation coefficients were in the range of 0.98 - 1.00. Average stress-strain curves (Fig. 4) were obtained by linear interpolation and extrapolation till the average maximum strain of the specimens.

2.4 Microscopy

Transmission Electron Microscopy (TEM) of the microtomed sections was performed on a JEOL JEM-3200FSC Cryo-TEM, operating at the liquid nitrogen temperature. Freeze-dried composite samples were sectioned at -40 °C using a Leica UC7 ultramicrotome. The thin (~70 nm) sections were deposited on lacy carbon grids and rehydrated with water for 5 min. The samples were then blotted and subsequently vitrified in a mixture of liquid ethane and propane (-180 °C). Zero-loss imaging of vitrified samples was carried out with JEOL JEM-3200FSC 300 keV TEM operated at liquid nitrogen temperature.

Scanning Electron Microscopy (SEM) was carried out on a JEOL JSM7500FA field emission microscope using an acceleration voltage of 1.5 - 2 keV. The cross-sections were obtained as a result of tensile test or prior to mechanical testing simply by bending by hand until a specimen ruptured. A thin platinum, Pt, layer was sputtered onto the samples (Emitech K950X/K350, Quorum Technologies Ltd, Kent, UK).

Atomic force microscopy, AFM, was performed on a Veeco Dimension 5000 Scanning Probe Microscope equipped with Nanoscope V controller (Veeco, Inc. Santa Barbara, CA, USA). Tapping mode was used to analyze the clay platelets with and without polymer. The samples were obtained from the MTM and MTM-PDADMAC dispersions. All samples were deposited from dilute aqueous dispersion onto freshly cleaved mica.

2.5 Elemental Analysis

Elemental analyses, EA, were performed by Mikroanalytiches Labor Pascher (Remagen-Bandorf, Germany). Hydrogen, carbon, nitrogen, chlorine and phosphorus were determined (Table 2) at least two parallel samples were used.

3 Results and Discussion

3.1 Structure and composition

The AFM imaging of the clay platelets prior dCMP functionalization, confirmed that the individual platelets were covered with thin layer of PDADMAC (Fig. 2). The coating increased the thickness of the platelets approximately by 1.2 nm, from 1.2 nm to 2.4 nm (Fig. 2 C and F). The PDADMAC coating is clearly seen in phase image (Fig. 2E) as a rough softer phase covering the platelets. Without the PDADMAC only the edges of the MTM platelets are seen in the phase image, while surface of the MTM look the same as the mica substrate (Fig. 2 B).

The amount of nitrogen, based on EA, showed that films without dCMP contain 23 ± 1 wt % of PDADMAC. The chlorine/nitrogen ratio indicated that 39 ± 2 % of the amino groups of PDADMAC have still the original chloride counter-ions, which means that they are not bound on the clay surface and therefore they are available for the supramolecular functionalization. The dCMP treated films showed a lower chlorine content and phosphorus was detected. After dCMP treatment only 14 ± 2 % of amino groups had still chloride indicating that the most of the counter-ions had been exchanged to dCMP. The weight percentages of PDADMAC and dCMP were calculated from the nitrogen, phosphorous and chlorine contents. The dCMP-functionalized films have 20.9 ± 1.2 wt% of PDADMAC and 7.1 ± 0.7 wt% of dCMP. The two types of compositions are denoted as MTM/PDADMAC and MTM/PDADMAC/dCMP.

The SEM images of fractured surfaces show the aligned layered nacre-mimetic structure in both compositions MTM/PDADMAC and
MTM/PDADMAC/dCMP in microscale (Fig. 3 A and D). The high-resolution TEM revealed the nanoscale structure, where the stacks of alternating clay (dark) and soft polymer layers (light) can be seen (Fig. 3 B, C, E and F). Both layers are approximately 1 nm thick.

4 Mechanical properties

The stress-strain curves for at least 5 parallel specimens and the standard deviation in different relative humidities are shown in Fig 4. Under dry conditions (Fig. 4A) the strength and stiffness clearly increase upon addition of dCMP and at higher humidities (Fig. 4B and C) they remain at least at the same level upon adding dCMP but the strain become increased. This indicates that dCMP takes active part on binding as adding low molecular weight molecules could lower the strength and stiffness due to the plasticization effect, however, here it serves us an indirect evidence that hydrogen bonding takes place and allows simultaneous sliding and binding. The mechanical properties based on the tensile measurements are shown in Fig 5 and the actual values with their standard error are collected in Tab. 1.

The humidity has a strong effect on the mechanical properties of both compositions, though they act very differently when humidity is increased. The strength and Young’s modulus of MTM/PDADMAC reduce due to the plasticization effect, while the maximum strain remains roughly unchanged, which results in a low work-to-failure. The water plasticizes the MTM/PDADMAC/dCMP as well, however, the strain increases significantly leading to higher work-to-failure. At relative humidity of 60 % the maximum strain of MTM/PDADMAC/dCMP is 69 % higher than that of MTM/PDADMAC.

We observed the most interesting behavior at the relative humidity of 60 % where the tensile strength, Young’s modulus, maximum strain and work-to-failure were 58 ± 2 MPa, 12.4 ± 0.6 GPa, 2.28 ± 0.16 % and 0.98 ± 0.08 MJ/m^2, respectively. In contrast to the previous studies, adding molecular interactions (due to dCMP) increased the tensile strength and the strain without compromising the elastic modulus. This led to the improved toughness seen as 123 % increase of work-to-failure. The change in the mechanical properties is not drastically large, but it is conceptually remarkable as the synergistic mechanical properties are thought to be the landmark in biomimetic composites, thus encouraging to identify in more depth related concepts.

It comes obvious that water is needed to plasticize the structure. At RH 40 % there is a crossover area, where there starts to be just enough water molecules in the structure to allow some lubricative sliding in dCMP-containing films. Note that nacre itself too is only tough under wet conditions.

5 Conclusions

At 60 % relative humidity, the tensile strength, Young’s modulus, maximum strain, and work-to-failure changed from 43 ± 2 MPa, 13.7 ± 1.1 GPa, 1.35 ± 0.10. % and 0.44 ± 0.05 MJ/m^2 to 58 ± 2 MPa, 12.4 ± 0.6 GPa, 2.28 ± 0.16 % and 0.98 ± 0.08 MJ/m^2, respectively, upon adding dCMP. The tensile strength and maximum strain increased 34 % and 69 %, respectively, as a result of dCMP treatment, while Young’s modulus remain at the same level, leading to a 123 % higher work-to-failure, which indicates higher toughness. We suggest that dynamic hydrogen bonds allow slippage and sacrificial bonds between self-assembled nanoplatelets, thus promoting toughness, still providing dynamic intercolloidal interactions between the sheets also promoted by water molecules.

The composite materials prepared in this study have the strength in the same range with nacre and even higher strain than nacre but they do not have as high stiffness. In dry conditions, Young's modulus of the resulted films was about half of the one reported for the nacre. However the resulting films even without any modification had high stiffness and strength properties in dry state even if they were brittle. Usually high performance composites require energy-intensive preparation; our composites are instead produced in the mild conditions. Also the supramolecular process gives a possibility to tune the mechanical properties on demand by functionalization the structure with other molecules.

The resulting materials had more than 70 wt % of inorganic material and therefore, they are expected to have excellent flame and heat shield capabilities, as mentioned in previous studies [8, 28]. These properties together with lightweight structure and the good mechanical properties, suggest to identify
hydrogen bonded self-assembled inorganic/organic nacre-mimetic materials towards applications.

Acknowledgements
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References


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**Fig. 1.** The preparation of the nacre-mimetic structure. First the nanoclay platelets were coated with polyelectrolyte via adsorption. Then excess polymer was washed away. Ionic binding was done by exchanging the original peripheral chloride counter-ions to hydrogen bonding dCMP.

**Fig. 2.** Uncoated MTM (A-C) and PDADMAC coated MTM (D-F) platelets on freshly cleaved mica observed by AFM. A and D show the height images with corresponding phase images B and E, and height profiles C and F, respectively. The polymer coating is easily seen in phase image E.
Fig. 3. SEM (A, D) and high-resolution TEM (B, C, E, F) micrographs of fracture surfaces of the films: A-C) for MTM/PDADMAC and D-F) for MTM/PDADMAC/dCMP.

Fig. 4. The effect of humidity on tensile stress-strain curves for MTM/PDADMAC (dashed black line) and MTM/PDADMAC/dCMP (solid red line) at various humidities: A) Vacuum dried, B) RH 40\%, and C) RH 60\%. Average curves are presented with standard deviation for at least 5 samples.
Fig. 5. The effect of humidity on the mechanical properties for MTM/PDADMAC (hatched) and MTM/PDADMAC/dCMP (white): A) tensile strength, B) maximum strain, C) modulus and D) work to failure per volume (integral of the stress-strain curve), which is an indication of toughness of the material, with standard deviation. At the dry state, the dCMP treated films are stronger and stiffer than polymer-clay films without the treatment. At higher relative humidities (40 and 60%), the dCMP treatment increases the strain and toughness meanwhile the strength and stiffness remain at same level compared with the untreated film.

Table 1. The tensile properties of nacre-mimetic nanocomposites with standard error.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tensile Strength (MPa)</th>
<th>Young's modulus (GPa)</th>
<th>Max. Strain (%)</th>
<th># of specimens</th>
</tr>
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<tbody>
<tr>
<td>Dry: Vacuum dried, measured at RH: 12-17 %, T: 22-24 °C</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Without dCMP</td>
<td>120±5</td>
<td>21.9±0.6</td>
<td>1.17±0.11</td>
<td>8</td>
</tr>
<tr>
<td>With dCMP</td>
<td>162±9</td>
<td>38.1±2.6</td>
<td>0.77±0.09</td>
<td>7</td>
</tr>
<tr>
<td>RH 40%: after vacuum drying conditioned 4 days at RH: 44-50 %, measured at RH: 16-18 %, T: 21-24 °C</td>
<td></td>
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<td></td>
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<tr>
<td>Without dCMP</td>
<td>64±4</td>
<td>16.3±0.7</td>
<td>0.81±0.07</td>
<td>14</td>
</tr>
<tr>
<td>With dCMP</td>
<td>68±4</td>
<td>14.6±0.7</td>
<td>1.03±0.12</td>
<td>5</td>
</tr>
<tr>
<td>RH 60%: conditioned at RH: 53-67 %, measured at RH: 53-67 %, T: 20-21 °C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without dCMP</td>
<td>43±2</td>
<td>13.7±1.1</td>
<td>1.35±0.10</td>
<td>9</td>
</tr>
<tr>
<td>With dCMP</td>
<td>58±2</td>
<td>12.4±0.6</td>
<td>2.28±0.16</td>
<td>13</td>
</tr>
</tbody>
</table>

Table 2. The results of elemental analysis in wt% with standard error.

<table>
<thead>
<tr>
<th>Element</th>
<th>MTM (2 parallel samples)</th>
<th>MTM/ PDADMAC (4 parallel samples)</th>
<th>MTM/ PDADMAC/ dCMP (2 parallel samples)</th>
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<tbody>
<tr>
<td>C</td>
<td>0.265 ± 0.005</td>
<td>15.3 ± 0.4</td>
<td>17.61 ± 0.05</td>
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<tr>
<td>H</td>
<td>0.76 ± 0.03</td>
<td>3.02 ± 0.08</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td>N</td>
<td>0.27 ± 0.03</td>
<td>2.26 ± 0.05</td>
<td>3.21 ± 0.02</td>
</tr>
<tr>
<td>Cl</td>
<td>0.14 ± 0.00</td>
<td>2.25 ± 0.09</td>
<td>0.81 ± 0.10</td>
</tr>
<tr>
<td>P</td>
<td>0.021 ± 0.002</td>
<td>0.0185 ± 0.0012</td>
<td>0.73 ± 0.05</td>
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