Toughening, micromechanics, and multi-scale structural analyses of high-performance biotools

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NANYANG
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**TOUGHENING, MICROMECHANICS, AND MULTI-SCALE
STRUCTURAL ANALYSES OF HIGH-PERFORMANCE
BIOTOOLS**

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Toughening, Micromechanics, and Multi-scale Structural Analyses of High-performance Biotoools

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Abstract

Through millions of years of evolution, living organisms have refined a wide range of biomineralized tissues to meet the key functional requirements that are central to their survival. This includes load-bearing elements and protective armor that must display adequate stiffness and strength or sharp and cutting “biotools” that are key in predation and feeding. These mineralized tissues exhibit multi-scale hierarchical structures to meet their functional requirements, with a precise organization of their building blocks to optimize the combination of their mechanical properties including stiffness, strength, and fracture resistance.

A distinctive model system that has gathered recent interest is the dactyl club from stomatopods (mantis shrimps). In comparison to many biomineralized composites that play a passive (defensive) mechanical role, dactyl clubs are dynamically active, hammer-like devices which are used by stomatopods to shatter the hard shells of their prey through repetitive impact loading. The mantis shrimp appendage is one of the most fascinating multifunctional biological material “biotools” in the animal kingdom, which the animal uses for its aggressive predatory strategies. Its hierarchical structure helps to strike and catch its prey 50 times faster than the blink of an eye, while exhibiting exceptional damage tolerance properties.

In this research project, the multi-scale hierarchical structure and chemical composition of mantis shrimp appendages was comprehensively probed by various analytical techniques. It is demonstrated that dactyl impact surface consists of a finely-tuned mineral gradient, with fluorapatite substituting amorphous apatite towards the outer surface. Raman spectroscopy measurements show that calcium sulphate, previously not reported in mechanically active biotools, is co-localized with fluorapatite. Ab initio computations suggest that fluorapatite/calcium sulphate interfaces provide binding stability and promote the disordered-to-ordered transition of fluorapatite.

Hertzian contact partial loading-unloading indentation measurements revealed that the different layers of the club possess distinctive deformation and energy dissipation mechanisms. High-resolution electron microscopic studies show that sliding and rotation of fluorapatite crystallites leads to a quasi-plastic response in the outer, impact layer of

the club. On the other hand, the presence of micro-channels along mineralized chitin fibrils in the inner layer of the club results in a distinct contact mechanics response. Under hydrated conditions, densification of these micro-channels under compressive load leads to a strain hardening behavior in the inner layer of the club.

The work demonstrates that the macroscopic size of the club is below the critical size above which Hertzian cone fracture could be formed. Instead, the club's response remains in the quasi-plastic regime.

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Table Captions

- Table 3.1** List of hard tissues with a function relevant to wear and abrasion resistance. Indicated in the table are degree of mineralization and the function of the material
- Table 3.2** Comparison of materials properties and resistance against a blunt contact between Chiton's radula magnetite and geological magnetite
- Table 3.3** Type of gradients found near the surface of abrasion-resistant biological materials
- Table 4.1** Elemental composition (in atomic percent) determined by Electron Probe Micro Analysis (EPMA). Note the decrease of C from the periodic (bulk) to the impact region, and the 4 to 5 times higher content of S in the impact region as compared to the periodic region. Together with evidences from Raman spectroscopy (Figure 4.6), S is attributed to the hydrated calcium sulfate phase gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$). Neither Sr nor Ba was detected by EPMA.

Figure Captions

Figure 1.1 As exemplified by Nacre and Bone, mechanical properties of natural composites can exceed their constituents and their homogenous mixtures properties. Re-drawn from Ref [5].

Figure 1.2 (a) Spearer and (b) Smasher mantis shrimps raptorial appendages. Spearer appendages are optimized for impaling the soft preys (*e.g.* fish), while the smasher are evolved to shatter preys with a protective hard shells (*e.g.* snail).

Figure 2.1 **Figure 2.1. Contribution of Extrinsic and Intrinsic toughening mechanisms in bone** ¹ Intrinsic toughening mechanisms promote plasticity at the crack tips at sub-micron scales to dissipate the cracking energy. In contrast, extrinsic toughening mechanisms shield the crack tips and balance stress concentration to prevent stress localization and crack propagation.

Figure 2.2 **Representative indentation loading-unloading curve.** The indentation tip initially (load = 0) moves toward the surface of the sample. As soon as the force transducer senses a load that exceeds a defined pre-load, an initial contact point will be defined [0,0] (pre-load is normally set to 0.1 μN to 2 μN based on the rigidity of the sample). Immediately after the initial contact, the load increases up to the prescribed peak load and the indenter penetrates inside the sample, while the transducer records both the load and the displacement. Upon unloading, the load goes back to zero and the final contact depth is recorded. The mechanical response of the sample during the loading and unloading, as well as the final depth is used to infer the mechanical properties of the sample. The loading-unloading function is defined based on the nature of the sample, the indentation tip geometry, and the type of data that the user is interested in acquiring (elastic, elastic-plastic, fracture).

Figure 2.3 **Representative indentation loading unloading curves for fully elastic and elastic-plastic contacts.** For the fully elastic response, the unloading curve almost overlap onto the loading curve, while for the elastic-plastic response, the unloading curve follow a different path, with a residual indentation depth observed after a loading/unloading cycle.

Figure 2.4 **A schematic Hertzian contact.** h_{max} is the total depth of penetration under the indentation load P , h_a is the depth of the circle of contact from the specimen free surface, and h_c is the distance from the bottom of the contact to the contact depth

circle, a is the radius of the contact circle and R is the radius of the indenter.

Figure 2.5 A schematic Hertzian curve fitting on a loading-unloading indentation curve.

During loading, the initial response is elastic and is followed by plastic yielding, which can be detected as the curve deviates from the ideal Hertzian solution. The elastic response is used to calculate the elastic modulus using Eq. 2.3. Upon unloading, the response is fully elastic and can be extracted by a linear fitted curve. The residual impression is due to yielding occurring when the load exceeds the elastic regime.

Figure 2.6 Schematic of sharp indentation and corresponding loading-unloading curve.

By increasing the load from zero to the peak load, $P_{\max}$, the indenter penetrates in the specimen up to the maximum contact depth h_t . h_c is the contact depth beneath the contact (for materials with a fully plastic response, h_t would be equal to h_c)

Figure 2.7 Schematic of multiple curve plot of calibration indents and extracted data

(contact depths and areas) used for tip area calibration. The exact load range will depend on which type of probe is being used and where the user desires the calibration to be valid. The extracted contact depths define the range of calibration.

Figure 2.8 Schematic indentation residual impressions surrounded by plastic zones.

Indentation spacing should be designed to prevent any overlapping between these zones.

Figure 2.9 Schematic loading-unloading indentation curve on a thin polymeric film is

affected by its glass substrate. Upon loading, the indenter initially penetrates in the film and the loading curve corresponds to the mechanical response of the film. By exceeding 10% to 30% of the film thickness, the response is affected by the substrate and the loading curve exhibits a sharp deviation from the initial curvature, which is due to the substrate mechanical response.

Figure 2.10 Imperfect contact due to high surface roughness.

Upon loading, the initial applied load compresses surface roughness asperities. This leads to larger contact depths in loading-unloading curves and unrealistic measured elastic modulus and hardness.

Figure 2.11 Tilted contact and imperfect loading-unloading curve due to the tilted surface

². Although the loading curve seems normal, the unloading curve exhibits a sharp deviation in curvature due to the imperfect contact.

Figure 2.12 Inadequate tip contact depth.

The residual impression areas made by the very

shallow and very deep tip contact depth of the tips can deviate from the calibrated tip area function and cause significant errors in the measured elastic modulus and hardness.

Figure 2.13 The three modes of crack loading; Opening (Mode I), sliding (Mode II) and tearing (Mode III). Depending on the specimen loading direction and sample thickness, any of these modes can initiate the crack formations in the specimen.

Figure 2.14 Schematic representation of indentation fracture pattern of brittle materials using a cube corner indenter. Upon exceeding critical fracture loads, the plastic zones form, the cracks initiate and propagate to make crack paths.

Figure 2.14 Schematic representation of indentation fracture pattern of brittle materials using a cube corner indenter. When the load exceeds a critical value, a plastic zone forms within which cracks can nucleate and propagate. Because the residual stress field under the indenter upon unloading exhibits a strong decay with the distance, the crack will arrest at a finite length c .

Figure 2.15 Indentation residual impression/crack length scale diagram and schematic diagram of indentation fracture frequency. (a) The cracking nucleation load, P_c , corresponds to the intersection between the residual impression size scales as $P^{1/2}$ (continuous line) and while the crack length scales as $P^{2/3}$ (dotted curve). Depends on the material property, this intersect changes. **(b)** The x-axis is in log-scale to cover orders of magnitude of load. The y-axis is the fraction of cracked corners measured post-indentation. In this specific example, no fracture occurred up to load of 20 mN, whereas at loads above 700 mN, all indent corners are cracked. The critical load is defined as the load at which 50% of indentation corners are cracked.

Figure 3.1 Illustrative examples of biological materials with a function requiring a high tolerance against wear. Functional wear-resistant biological materials are divided into three broad categories, namely (1) protective structures, (2) supporting structures, and (3) mechanically-active “biotools”.

Figure 3.2 Damage mechanisms at a contact between an elastic indenter and an elastic-plastic material. (A) Yielding beneath a blunt contact. **(B)** Cracking at a blunt contact. **(C)** Yielding at a sharp contact. **(D)** Cracking at a sharp contact. Due to limited data available, the influence of friction is not considered here.

Figure 3.3 Selection chart of biological materials for resistance against yielding at a blunt contact. (A) Stiff indenter. (B) Finite stiffness indenter, with E' representing the plane-strain modulus of the indenter.

Figure 3.4 Selection chart of biological materials for resistance against cracking at sharp contact.

Figure 3.5 *L*-shape selection charts of biological materials against combined yielding and cracking at a blunt contact. In this type of plots, guidelines have an *L*-shape: vertical lines represent cracking loads and horizontal lines yielding loads. In this representation, a material located on the right and above a given guideline is predicted to resist damage at that specific load. Note that for the cracking mode, since the load scales with the radius of contact R , vertical guidelines are translated towards the right for higher radii of contact.

Figure 3.6 *L*-shape selection charts of biological materials against combined yielding and cracking at a sharp contact. See text and Figure 3.5 for explanation of *L*-shape plots. Note that the horizontal guidelines were constructed for a specific residual indentation depth (h_r) of 10 μm . For higher and lower values of h_r , the guidelines are translated downwards and upwards, respectively.

Figure 3.7 **Figure 3.7. Depth-sensing nanoindentation with a spherical punch (nominal radius 1mm) can be used to detected the first yielding event beneath the contact surface.** (A) Schematic representation of the indentation response. The dotted curve is the ideal Hertzian solution and the solid curve is the experimental load-depth curve. Deviation from the Hertzian solution is related to yielding, defined by the critical load P_y . When yielding occurs, a residual impression is observed, with indentation depth h_r . (B) Representative curves on bio-magnetite from Chiton's radula and geological magnetite. Despite a higher hardness, geological magnetite yields a lower load from a blunt contact in agreement with Eq. 3.2, while also exhibiting a larger residual depth. (C) Distribution plots of P_y ($n = 30$ and 25 for Chiton's radula and geological magnetite, respectively); (D) Distribution plots of h_r ($n = 25$ and 20 for Chiton's radula and geological magnetite, respectively) after maximum indentation loads of 2000 mN.

Figure 3.8 Indentation at a sharp contact can essentially be perceived as a competition between yielding and cracking from the indent cornerr. (A) The indent size

scales with the maximum applied load P as $P^{1/2}$, while the crack length scales as $P^{2/3}$. The cracking nucleation load, P_c , corresponds to the intersection between the two curves. Depending on the material property group, the crack-length line (dotted curve) shifts along the indentation size curve (continuous line), resulting in changes in P_c . **(B)** In giant sponge spicule, cracks in the lamellar, tougher region nucleate at loads about 100-fold higher than in the monolithic (non-lamellar) region. (B) Reproduced from Ref. ³ with authorization from Wiley VCH.

Figure 4.1 Stomatopod dactyl club and spear macro- and microstructures. **(a)** Anterior view of a “Smasher” type stomatopod with extended dactyl clubs. **(b)** Schematic of sample preparation before and after polishing. **(c)** Overview photograph of the actual sample. **(d)** Optical micrograph of a yy cross-section, showing the 2 sub-domains of the impact region. **(e)** Anterior view of a “Spearer” type stomatopod with a single visibly extended appendage. **(f)** Schematic of sample preparation for indentation and spectroscopic measurements. **(g)** Optical micrograph of a yy cross-section, illustrating the location of the impact region. **(h)** Actual sample after polishing. **(i)** Higher magnification micrograph of the impact region of (g). P_{ii} refer to nanoindentation experiments performed perpendicular to the ii -plane.

Figure 4.2 Optical micrographs of a sectioned and polished spearing appendage (yy cross-section). **(a)** Bright field image. **(b)** Polarized light image. **(c-e)** Polarized light micrographs at various sample rotations. Note the apex region appearing bright at 0° and 90° orientations, and in dark at a 45° orientation.

Figure 4.3 FESEM micrographs of the impact region fracture surface from a spearing appendage. **(a)** Low magnification side-view and **(b)** top view of the impact region. **(c)** High magnification view of the apex region that is subjected to the highest impact forces, showing the elongated bundle of crystallites between adjacent channel-like structures. Inset: Atomic Force Microscope (AFM) zoom-in illustrating the lamellar microstructure, with lamellae 50 to 200 nm thick. **(d)** and **(f)** High magnification views of the center of the impact region, showing the presence of equiaxed crystallites as opposed to elongated fibers observed in (c). Note that the crystallite size decreases towards the outer surface. **(e)** Sub-region of the impact region with a contour line highlighting regions where no crystallites are observed. All features described were consistently observed in various samples.

Figure 4.4 Energy Dispersive Spectroscopy (EDS) elemental maps of a sectioned and polished spearing dactyl segment. **(a)** Backscattered electron (BSE) micrograph,

revealing the local differences in electron density (yy cross-section). **(b)** Calcium. **(c)** Phosphorous. **(d)** Carbon. **(e)** Point spectrum acquired in the impact region. **(f)** Magnesium. **(g)** Sulfur. **(h)** Fluorine. All maps were background-subtracted.

Figure 4.5 EDS elemental maps of a sectioned and polished dactyl segment from a smashing stomatopod. The backscattered electron micrograph (BSE) reveals the local differences in electron density (yy cross-section). Elemental distributions are shown for calcium (Ca); phosphorous (P); carbon (C); magnesium (Mg); sulfur (S); and fluorine (F). All maps were background-subtracted.

Figure 4.6 Raman spectra (range 800–1200 cm^{-1}) profiles from bulk to impact surface. **(a)** spearing and **(b)** smashing dactyl segments. Broad band centered at 1080 cm^{-1} : Amorphous Calcium Carbonat (ACC). Band centered at 955 cm^{-1} : Amorphous Calcium Phosphate (ACP). Sharp band shifted towards 965 cm^{-1} : crystalline Fluorapatite (FAP). Comparison with fluorine EDS maps (Figures 4-4, 4-5) suggests that ACP is also predominantly fluorinated. Band at 1007 cm^{-1} (only found in the impact region): Calcium sulfate (CAS, gypsum). **(c)** Polarized Raman spectra (range 540–660 cm^{-1}) acquired in the apex region, with polarization angles of 0 and 90° relative to the free surface. **(d)–(h)** Raman mapping of the spear impact surface with a box in **(a)** highlighting the region where Raman mapping was carried out. **(d)** Carbonate intensity. **(e)** Sulfate intensity. **(f)** Phosphate intensity (full phosphate band, with center of Gaussian fit adjusted for integration). **(g)** Center of phosphate peak, showing a clear shift at the interface from ACP to FAP. **(h)** Full Width at Half Maximum (FWMH) of the phosphate band, with low values indicating crystalline FAP and a high value disordered, ACP phase. Note that high crystallinity of FAP is co-localized with the sulfate intensity in the impact region and vice versa.

Figure 4.7 High signal-to-noise ratios EDS spectra of both types of stomatopod's dactyl appendages, for both the impact (blue) and bulk (periodic, red) regions. Bottom spectra are zoom-in of the low-intensity region for better visualization of lower abundant elements. Note in particular the decrease of C intensity from bulk to impact, the detection of Sr in trace amount (with no distinguishable intensity differences between bulk and impact regions), as well as the detection of S solely in the impact region.

Figure 4.8 **(a)** Raman spectra acquired parallel to the yy and zz directions, with insets representing the Raman vibrational modes of the phosphate groups identified by ab

initio computations. (b) Comparison of spectra in hydrated and dry conditions in two regions of the spearer, indicating no difference in composition and peak location in the hydrated state.

Figure 4.9 Raman mapping of a sectioned and polished dactyl segment from a smashing stomatopod with box highlighting the zones where Raman mapping was carried out. The four types of maps are, from left to right: (1) phosphate intensity (full phosphate band, with center of Gaussian fit adjusted for integration); (2) center of phosphate peak, showing a clear shift at the interface from ACP (centered at 955 cm⁻¹) to FAP (peak center at 965 cm⁻¹); (3) Full Width at Half Maximum (FWMH) of the phosphate band, with low values indicating crystalline FAP and high value disordered (ACP) phase; (4) sulfate intensity.

Figure 4.10 High-resolution TEM observations in the impact region. (a) and (b) same regions (zoom-in in the bottom images) at various times, illustrating the formation of small crystalline domains under the beam (fringes in b not present at time 0). (c) Different regions observed after stabilization of the new phase under the beam. The fringes near the edge are 2.41 Å apart and correspond to the {200} planes of CaO as described in Ref. [51]. From the analysis of the upper part of the image in (c), a set of fringes 2.41 Å apart running parallel to the surface of the sample and at 90° with respect to those running perpendicular to the surface can be observed. This set of fringes can be univocally assigned to one of the <100> zone axes of CaO, forming from FAP by beam irradiation.

Figure 4.11 Low acceleration voltage TEM imaging and electron diffraction patterns. (a-b) Bright field images, with red arrows on the higher magnification micrographs indicating beam damage. **(c)** Electron diffraction pattern of crystalline domains with diffraction spots assigned to (left) FAP ($D = 2.80 \text{ Å} \rightarrow [121]/[211]$; $D = 2.28 \text{ Å} \rightarrow [122]$) and (right) gypsum ($D = 2.46 \text{ Å} \rightarrow [-231]$). The same pattern is shown in each image, with the assignment for each phase highlighted separately for clarity. **(d)** Electron diffraction pattern of an amorphous domain at $t = 0$ (top) and $t = 5 \text{ min}$ (bottom), illustrating crystallization of the amorphous domains after a few minutes of irradiation-induced damage.

Figure 4.12 Mechanical characterization of the impact region of a sectioned and polished spearing dactyl segment measured by nanoindentation. (a) Reduced modulus (E_r) and Hardness (H) maps (yy cross-section). Latin numbers correspond to Raman spectra displayed in Figure 4-6a. **(c-d)** High-resolution nanoindentation line

scans. Line 1 (in the center of the impact) spans from the ACC bulk with a sharp transition to the FAP/CAS regions, whereas line 2 (along the extruded edge) encompasses the bulk (blue), the localized disordered zones (yellow) and the ordered FAP/CAS regions near the outer surface. **(b)** E_r distribution plots measured in the impact region, normal to the three cross-sectional planes shown in Figure 4-1. **(e)** and **(f)** Average values of E_r of a spearer sample **(e)** (same data sets as in **(b)**) and of a smasher sample **(f)** in the impact region measured normal to the xx , yy , and zz directions, illustrating the mechanical anisotropy of the impact surface. * $p < 0.001$. **(g)** Schematic representation of FAP crystallite orientation in the impact layer.

Figure 4.13 Representative indentation fracture curves in impact regions measured with a high-load transducer and a cube-corner tip. Notice the lower load scale for the spearer appendage, illustrating the lower loads at which damage events occur. Corresponding post-indentation FESEM micrographs are shown in Figure 4-14.

Figure 4.14 **Figure 4-14. Indentation fracture analysis of the dactyl appendages impact regions.** **(a)** Cracking frequency at indent corners vs. peak load (cube corner tip). **(b)** Representative Raman spectra from 800 to 3200 cm^{-1} wavenumber acquired near the indents. Chitin is detected in the impact region of the smasher dactyl club, but not of the spearer dactyl appendage. **(c)** Scanning Probe Microscope (SPM) and SEM micrographs of residual indents on spearer samples after indentation peak loads of 3 mN (left) and 40 mN (right). **(d)** SEM micrographs of residual indents on smasher samples after indentation peak loads of 30 mN (left) and 500 mN (right).

Figure 4.15 **FAP/CAS interfaces computed by *ab initio* simulations.** Hydrogen bonding network is predicted to stabilize the interface along the [100] directions of both crystals. The binding energy in FAP/CAS is 2 kCal/mol stronger per hydrogen bond than in Hydroxyapatite (HAP)/CAS.

Figure 5.1 **(a)** Photographs of a fractured snail shell after impact from a stomatopod's dactyl club, showing the classical brittle cone cracks (Hertzian cracks) at the impact points that led to macroscopic fracture. **(b)** Schematic illustration of the impact depicting the cone shape fracture pattern in the snail shell. **(c)** Macro-photographs of a damaged and a pristine dactyl club. Arrows indicate eroded regions after

repetitive impacts. **(d)** Micro-CT image of a used club (left) and cross-sections of a used and a pristine club.

Figure 5.2 Indentation response of the smasher dactyl club in distinct layers. **(a)** Sample preparation for bi-directional probing of contact response and schematic cross section of a club, with color coding representing the location where indentation studies were carried out (this color coding is used throughout the manuscript). **(b)** Partial loading-unloading curves in dry (top) and hydrated (middle and bottom) conditions. Full unloading was performed in order to obtain the contact depth and contact radius at a given peak load. The water content in hydrated conditions is 4 -5 wt. pct in the outer layers and 10 wt. pct in the inner layers. **(c)** Indentation stress-strain curves in dry conditions computed from the partial loading-unloading curves obtained in (b). **(d)** Indentation stress-strain curves (dry conditions) in the yy and zz directions depicted in (a), illustrating the anisotropic response near the impact surface. **(e)** Indentation stress-strain curves (hydrated conditions) computed from the partial loading-unloading curves obtained in (b). The inset shows the strain-hardening behavior of the inner layers. **(f)** Partial loading-unloading curves (hydrated conditions) measured at 0.6 mN/s (thick line), 6 mN/s (thin lines), and 300 mN/s (dash lines), illustrating the influence of loading rate in various regions of the clubs.

Figure 5.3 Anisotropic response of the dactyl clubs as measured by partial loading-unloading curves in distinct layers. These curves were used to compute the indentation stress-strain curves shown in Figure 5.2d.

Figure 5.4 Schematic definition of the contact depth (h_c) and yield stress (s_y) point of the indentation stress-strain curve. **(a)** Schematic of partial loading-unloading curves of two cycles (i and $i+1$), with definition of contact load (p_i), residual depth (h_{ri}), contact depth (h_{ci}), and maximum depth (h_{ti}). The contact depth (h_c) at each load was extracted to calculate the contact radius (a) and the mean contact pressure (P_m) (Materials and Methods, Section 5.4). **(b)** Method used to identify the yield point. The initial linear portion of the stress-strain curve is obtained by curve fitting and the deviation with the indentation stress-strain curve represents the yield point.

Figure 5.5 Indentation response of the “spearer” mantis shrimp dactyl clubs, for distinct layers. **(a)** Schematic cross section of a spear sample, with color-coding

representing the location where indentation measurements were carried out. (b) A macro-photo of a spear sample with its characteristic radius of curvature. (c) Partial loading-unloading curves for the dry spear sample. Full unloading was performed in order to obtain the contact depth and contact radius at a given peak load. (d) Indentation stress-strain curves computed from the partial loading-unloading curves obtained in (b). (e-f) Partial loading-unloading curves and corresponding indentation stress-strain curves in hydrated conditions.

Figure 5.6 Indentation stress-strain curves for FAP, the spearer club outer layer, and the smasher club outer layer computed from partial loading-unloading indentation curves. While there is no difference for the FAP curves between dry and hydrated conditions, the yield strength (σ_y) in the outer layer of the spearer and smasher clubs decrease by 0.2 GPa and 0.9 GPa, respectively. This decrease is likely related to the presence of organic components in these layers, which is higher in the smasher sample.

Figure 5.7 Residual displacement after partial loading-unloading cycles in the inner layers of the dactyl clubs in hydrated conditions. Three cycles are highlighted (#11, #22, and #32) to emphasize the large residual displacements (h_r) upon unloading, which indicate that strain-hardening is due to a plastic deformation mechanism consisting of micro-channel densification.

Figure 5.8 Influence of the loading rate on the Hertzian indentation response. (a) Partial loading-unloading curves. (b) Computed indentation stress-strain curves in various layers. Loading rates ranged from 6 mN/s to 300 mN/s.

Figure 5.9 Dynamic Finite Element Analysis of an impact against an stiff target (steel, $E = 200$ GPa). (a) Geometry of the club used for the simulation as obtained from SEM images. The clubs was divided into six layers with constitutive relationships obtained by fitting the experimental indentation stress-strain curves shown in Figure 5.2. (b) Maximum stress at the impact point. (c) Maximum strain and (d) maximum strain rate, which are reached in the innermost layers. The details of the computations are given in Materials and Methods, Section 5.4.

Figure 5.10 Contact deformation and damage mechanisms of the dactyl clubs revealed by post-indentation FESEM observations of the planes beneath the contact points. (a) **Blunt contact, inner layers:** (i) low magnification micrograph illustrating both the plastic and elastic regions; (ii) higher magnification micrograph depicting the

elastic-plastic boundary; (iii) and (iv) high magnification micrographs of closed channels in the plastic region and open channels in the elastic region, respectively, illustrating micro-channels densification under contact. **(b) Sharp contact, inner layers:** (i) low magnification micrograph illustrating the plastic region near the sharp indenter edge and the surrounding elastic region; (ii) micro-channels before indentation viewed under two different orientations; (iii) and (iv) boundary between the plastic and the elastic regions, with green shading area in (iv) representing the closed, densified structure. **(c) Native impact blunt contact, outermost layer:** (i) low magnification micrograph comprising both the plastic and the elastic regions; (ii) high magnification micrograph of the plastic region near the contact revealing highly misoriented FAP crystallites; (iii) high magnification of the elastic region showing the textured structure of FAP crystallites; (iv) fracture surface of the impact region (undeformed sample) confirming the textured structure of FAP; (v) schematic of misoriented FAP crystallites near the contact point, drawn by following the contour of actual crystallites on high magnifications micrographs (quantification of crystallites orientation is shown in Figure 5.11). **(d) Sharp contact, outer layers:** (i) top view of shear-induced lateral cracks emerging on the free surface; (ii) high magnification of an indent corner revealing fibril bridging between the two sides of a lateral crack; (iii) low magnification side view showing lateral and lateral cracks nucleated by the sharp contact; (iv) high magnification micrograph of the radial crack tip region, showing the formation of a micro-cracking process zone surrounding the main crack.

Figure 5.11 Quantitative analysis of crystallite orientation under a native contact and influence of crystallite rotation on the elastic modulus. (a, b) Distribution of crystallite orientation in the same locations as shown in Figure 5.10c of the main text, obtained using Image J for both the oriented (a) and the misoriented (b) regions. (c, d) Average elastic moduli after native impact on regions with preferentially oriented crystallites (left) and misoriented crystallites (right).

Figure 5.12 Energy absorption by plastic yielding during a Hertzian contact cycle. (a) Definition of the elastic energy (W_e), the plastic energy (W_p), and total contact energy (W_t). (b) (W_p/W_t) vs. strain during a contact cycle in various layers and for two loading rates.

Figure 5.13 Multi-scale indentation studies on the dactyl clubs. (a) Schematic indent locations. **(b-d)** Post-indentation FESEM micrographs corresponding to the

locations shown in (a). Outer layer, peak load of 10 mN (b); outer layer, peak load of 100 mN (c); inner layer, peak load of 3000 mN (d). (e) Characteristic indent sizes shown in (b), (c), and (d) at the same scale. (f) Indentation curves corresponding to indents in (b) and (c). Dotted lines are fitting of the initial elastic region, with deviation from the experimental curves used to detect pop-in events (shear-induced cracks associated with the pop-in events shown in (c)). (g) High load (3000 mN) indentation curve of the dactyl club inner region, illustrating no pop-in events. The maximum loads from (b) and (c) are shown for comparison.

Figure 5.14 Sharp contact indentation and residual stress fields in the dactyl clubs and comparison with geological FAP. (a) Indentation curves for FAP, the dactyl club outer layer (DOL) and the dactyl club inner layer (DIL). The shaded areas under the curves correspond to the energy released during cracking event at the contact point. (b) Released energy vs. indentation load for the 3 samples. (c-e) Post-indentation FESEM micrographs of FAP (c); DOL (d), and SIL (e). (f) Indentation curves on FAP and DOL samples used for visualization of residual stress fields. (g-i) Raman mapping for visualization of residual stress fields around indents of FAP and DOL. Relative intensity of the ν_1 band of FAP (g); peak shift of the ν_1 band (h); and residual stress fields as computed from the relative peak intensity and peak shift (i).

Figure 5.15 Influence of the anisotropy and loading rate during sharp tip indentation. (a) Released cracking energy vs. indentation load in the zz and yy directions, illustrating a slightly higher absorption by plastic dissipation in the zz direction (lower cracking energy released). (b) Released energy vs. indentation load at low and high loading rates, showing no statistical differences between the two rates. Representative indentation curves on the outer (c) and inner (d) layers at low and high loading rates. In both regions, the loading rate had no significant influence on the sharp contact indentation response.

Figure 6.1 Selection chart of biological materials against yielding at a blunt contact (See Chapter 3).

Figure 6.2 L-shaped selection charts of biological materials against combined yielding and cracking at a sharp contact. Note that the absolute values of cracking loads (vertical guidelines) strongly depend on the indenter geometry. Here the chart was constructed for the cube-corner geometry employed in this study, for which the external load to nucleate indentation cracks is about one order of magnitude lower

than for the classical Vickers geometry. The location of the materials on the chart, on the other hand, only depends on the intrinsic materials properties.

Figure 6.3 Schematic cartoon of the hierarchical structure of the distinct layers of the dactyl clubs. The outer layer of the dactyl club is composed of oriented FAP crystallites while the inner layer is composed of sheets of chitin fibrils arranged in a helicoidal fashion.

Abbreviations

EDS	Energy Dispersive X-ray Spectroscopy
EPMA	Electron Probe Microanalysis
HRTEM	High Resolution Transmission Electron Microscopy
SEM	Scanning Electron Microscopy
FESEM	Field Emission Scanning Probe Microscope
TEM	Transmission Electron Microscopy
DSI	Depth Sensing Indentation
FAP	Fluorapatite
CAS	Calcium Sulfate
ACP	Amorphous Calcium Phosphate
ACC	Amorphous Calcium Carbonate
DFEA	Dynamic Finite Element Modeling
DOL	Dactyl club Outer Layer
DIL	Dactyl club Inner Layer

Chapter 1 - Introduction

Over millions of years, living organisms have refined the process of biomineralization to design hard tissues with optimized structural properties that are adapted to specific evolutionary pressures.^{4,5} These functions can be divided into three main categories: (i) protective armors (*e.g.* mollusk shells, fish scales and invertebrate cuticles); (ii) supporting structures (*e.g.* bone); and (iii) mechanically active biotools (*e.g.* tooth, invertebrate mandibles and claws)⁶. All these biomaterials share a central commonality: they are assembled from nanoscale building blocks with rather weak intrinsic mechanical properties, but their multi-scale hierarchical structures confer them with much higher macroscopic properties. Figure 1.1 shows that natural materials can challenge the well-known role of mixture of composites materials and present superior properties in comparison to their individual constituents and homogenous mixtures.¹

From an engineering perspective, these structures provide a wide range of opportunities to design bio-inspired composites with superior structural properties.⁷ Thus, bio-inspired processing of these natural models through various assembling strategies and design principles provide us with novel guidelines to develop high-performance engineering composites⁸⁻¹⁰. Nacre¹¹⁻¹³, bone¹⁴, tooth^{15,16}, antler^{17,18}, fish scale¹⁹, and crustacean mandibles²⁰ are such natural materials that have been studied intensively in the past two decades, with the goal to elucidate and eventually mimic their structural and mechanical design concepts.

In this project we have studied an ultra damage tolerant biological structure, namely the mantis shrimp (stomatopod) dactyl club. We focused the research on characterizing the chemical composition and spatial distribution of the constitutive phases that make up the club. Combined with multi-scale structural investigations and micro- and nano-mechanical studies, this work establishes the fundamental toughening strategies endowing stomatopods' clubs with their remarkable damage tolerance.

Figure 1.1. As exemplified by Nacre and Bone, mechanical properties of natural composites can exceed their constituents and their homogenous mixtures properties. Re-drawn from Ref [5].

The stomatopods are an ancient group of marine tropical and sub-tropical crustaceans with a fossil record that dates back more than 300 million years.²¹ These species are well known for their complex visual systems²² and their fast punches²³ –used in their aggressive predatory strategies– which are delivered through their raptorial appendages called “dactyl clubs”. Based on the type of raptorial appendages, they are further divided into two main categories: the smasher (*e.g. Odontodactylus scyllarus*) and the spearer (*e.g. Lysiosquilla maculata*) species. In the smasher species, the dactyl clubs strike their prey’s hard shell (Figure 1.2) therefore destroying their protective armor. Remarkably, many of these hard shells, such as crab exoskeletons, are themselves considered as tough biological materials.²⁴ The spearer dactyl is not only used for striking, but also for accurately spearing passing soft-body preys.

Dactyls can be propelled at accelerations up to 10 g's, reaching speeds up to 23 m/s and generating forces of up to 1000 N, more than 100 times the animal's body weight.²³ Despite applying these large repetitive loads, the dactyl clubs are extremely damage tolerant and can withstand thousands of high-energy strikes with minimal internal damage, before being eventually replaced during molting cycles.²⁵ Therefore, this structure serves as an intriguing bioinspired model for enhancing current impact tolerant structural materials. This thesis focuses on the micro-scale architectural and biomechanical features of mantis shrimp clubs, and on the role of the microstructural design and toughening mechanisms in imparting the clubs with their high impact tolerance.

Figure 1.2. (a) Spearer and (b) Smasher mantis shrimp raptorial appendages¹. Spearer appendages are optimized for impaling the soft preys (*e.g.* fish), while the smasher are evolved to shatter preys with a protective hard shells (*e.g.* snail).

¹ <http://arthropoda.southernfriedscience.com/?p=801> , Accessed 19th Jan 2015

1.1 Hypotheses

According to the functionality of the mantis shrimp appendages, we are assuming that these biotools are designed to fulfill two main functions, namely providing an offensive weapon during hunting (striking), while at the same time minimizing internal damage. According to these criteria, we reason that the clubs possess a multi-scale hierarchical structure, with specific structural designs allowing to maximize impact forces, while at the same time toughening the clubs to dissipate impact energy during a strike.

1.2 Objectives

To address our hypotheses, the following objectives were established:

1. To conduct chemical and structural characterization of the clubs across various length scales.
2. To measure micro- and nano-scale mechanical properties of the clubs, including modulus, contact mechanics response, and fracture toughness.
3. To evaluate possible anisotropy in the mechanical response.
4. To establish structure-property relationships of the dactyl club structure.
5. To identify the damage and toughening mechanisms under impact loading and to correlate them with the mechanical response.
6. To evaluate the role of loading rate on the mechanical response of the clubs.

Chapter 2 - Literature Review

2.1 Biological materials and bioinspiration

Biological materials are made from a limited amount of chemical elements (mostly C, N, O, H, Ca, P, S, Si) to construct their building blocks^{26,27}. These elements are available in large quantities from their natural surroundings (such as oceans for marine organisms), and living organisms are able to process natural composites at ambient temperature and pressure²⁸, a feat that man-made materials are still far from realizing. Over millions of years, Nature has evolved countless methods to ensure its survival⁴. Biological systems have optimized strategies to fulfill their multi-purpose functionalities such as mobility or protective and supportive elements^{4,5,29}. While bone³⁰⁻³² and exoskeleton^{33,34} are designed to play supportive roles in vertebrate and invertebrate creatures, nacre shell^{11,35}, urchin spines³⁶ and fish armor³⁷ are designed for protective roles⁴.

Based on their mineral content, these materials can be arbitrarily divided into three main categories: high mineral content, low mineral content and unmineralized⁶. Among these, biomineralized tissues have been optimized to resist intense mechanical loads¹. In biological composites, the mineralized components provide rigidity while the organic components act as flexible components, structural templates, and binders between the mineral phases¹. The trade-offs between strength and toughness, and stiffness and density, lead to optimization in mineralized/unmineralized ratio in biological materials³⁸. Materials with a higher degree of mineralization exhibit higher load bearing capability (strength and stiffness) whereas non-mineralized materials display higher viscoelastic damping and toughness properties. However, recent studies have revealed that the constituents of the organic matrix as well as the spatial arrangement of mineral and organic phases have a much deeper effect on mechanical properties of biomineralized systems^{39,40}.

Based to the scope of this research, this chapter is focused on highly biomineralized hard tissues, *i.e.* their structural properties and their strengthening and toughening strategies, and on the relevant characterization methods. In last few decades, several of these biological materials have been studied intensively, leading to established structure-properties relations. Nacre^{11,13,41,42}, conch⁴³, human and shark tooth enamel^{16,44-}

⁴⁸, chiton tooth^{49,50}, sea urchin tooth^{51,52}, biosilica³, fish armour¹⁹ and crayfish mandibles²⁰ are just a few examples of these biological models that have been investigated from a structural and mechanical point of view. Over the last two decades, materials scientists and engineers have tried to mimic these natural models, coming up with novel bioinspired solutions to fabricate tough organic/inorganic composites ^{7,53}.

2.2 Damage resistance and toughening mechanisms in biological materials

Biomaterialized hard tissues such as bone^{31,54-56}, tooth^{15,46,57} and seashells^{13,58,59} utilize multi-scale design strategies to enhance their damage resistance and fracture toughness. These materials are designed to fulfill the contradictory requirements of being sufficiently stiff for load-bearing, while at the same time tough enough to prevent crack initiation and propagation. Their mineral phases (*e.g.* apatite or calcite phases) exhibit strong chemical bonds, leading to a high elastic modulus. In contrast, non-mineral phases observed in fibrous biopolymeric structures (*e.g.* collagen and chitin) are able to deform plastically and to dissipate a higher amount of energy before failure, thereby preventing catastrophic failure and enhancing fracture toughness.^{1,60}

Several toughening strategies have been reported in biomaterialized hard tissues. Hydrogen bond breakage in pure collagen (molecular uncoiling), fibrillar sliding of mineralized collagen fibrils, fibrillar sliding of collagen fiber arrays and microcracking are considered as “intrinsic toughening mechanisms”. Constrained microcracking, crack deflection and crack bridging, on the other hand, are referred as “extrinsic toughening mechanisms”^{1,61} (Figure 2.1). Intrinsic mechanisms enhance the fracture energy by locally dissipating fracture energy via damage mechanisms such as nano- and micro-fiber cracking and interfacial debonding. In comparison, extrinsic toughening mechanisms operate on a larger scale to shield the structure and micro-cracks against the applied loads. Such mechanisms have been investigated in depth in bone⁵⁸, deer antler¹⁷, nacre⁵⁸ and teeth¹⁵. For instance in nacre, they include inelastic deformation by a sliding-locking mechanism of the bricks and mortar structure and viscoelastic dissipation of the organic phase, which both contribute to increasing the fracture toughness⁶². Conch shells exploit spatially-distinct energy dissipation mechanisms, such as multiple micro-cracking in the outer layer and crack bridging in the middle layers⁴³ of their multi-layer structure. Other notable examples include deformation twinning an inelastic deformation in inorganic layers in the shell of *P. placenta*⁵⁹, or multi-layer structures with functionally graded properties in fish scale armors^{19,63} that better distributes contact energy.

Figure 2.1. Contribution of Extrinsic and Intrinsic toughening mechanisms in bone ¹

Intrinsic toughening mechanisms promote plasticity at the crack tips at sub-micron scales to dissipate the cracking energy. In contrast, extrinsic toughening mechanisms shield the crack tips and balance stress concentration to prevent stress localization and crack propagation.

2.3 Mechanical characterization of Biogenic Composites

Biological materials exhibit a wide variety of hierarchical and multi-layer structures, leading to distinct anisotropic and graded properties^{19,25,64,65}. Thus, a common characteristic of these structures is the presence of meso-, micro-, and nano-scale length scale constituents. In order to evaluate the mechanical properties of these distinct sub-domains, various techniques are potentially required. However, nano- and micro-mechanical instrumentation have rapidly evolved in the past decade, allowing to conduct mechanical characterization over a wide range of length scales sometimes using a single instrument. In this work, we employed Depth-Sensing Indentation (DSI) to probe the mechanical response of the clubs across a wide range of forces, from hundreds of nano-Newton (nN) to tens of Newton. We employed various contact geometries, namely blunt and sharp tips to investigate elastic, elasto-plastic, and indentation fracture properties. A concise overview of these methods and their data analysis is first summarized since these constitute the core of experimental methods used in this work.

2-3-1 Depth sensing indentation studies

Mechanical properties characterization of biological materials have been the topic of intense interest in materials science over the last decade. Among these properties, the elastic modulus (E) and hardness (H) are the most commonly measured in order to compare and report mechanical properties of biological materials, and indentation methods are routinely employed to probe these properties. Early indentation methods were developed to measure the size of the residual plastic impression on the specimen as a function of the indenter load using post-indentation optical observations, with indentation sizes typically in the mm range. In nanoindentation experiments, the sizes of the residual impressions are in the micro- or nano-meter range, and observing the residual impression via optical methods is not possible.

Indenter tips with known geometries (including pyramid tips and cono-spherical tips) allows precise measurements of residual impression areas from load-axial displacement data. In this method, a stiff tip (indenter, typically made of diamond) penetrates onto an ideally flat free surface, with either the force or the displacement

imposed. After the initial contact between the tip and the surface (generally defined by a pre-load to sense the surface), the load is increased up to a prescribed value, and is then returned back to zero. With modern equipment the loading and unloading rates can be precisely defined. The applied load and depth of penetration are simultaneously measured using a transducer that measures both the force and the displacement. The extracted loading-unloading curve is used to measure the mechanical properties such as the modulus and hardness. Figure 2.2 shows a schematic indentation loading-unloading cycle used to evaluate the mechanical response of materials.

The loading part of the indentation cycle consists of an initial elastic contact, followed by plastic flow, or yielding, as the load increases. Upon unloading back to zero force, if yielding has occurred the load-displacement unloading curve follows a different path and a residual impression is left on the specimen surface. In other words, after a loading/unloading cycle, the displacement fully goes back to zero for a perfectly elastic contact or there is a residual displacement for an elastic-plastic contact (Figure 2.3). Blunt tip geometries usually lead to a smooth transition from elastic to elastic-plastic contact (Section 2.3.1.1), whereas with sharp tip geometries, high stresses are generated at the contact point, giving rise to plastic deformation with a residual permanent indent.

Figure 2.2. Representative indentation loading-unloading curve. The indentation tip initially (load = 0) moves toward the surface of the sample. As soon as the force transducer senses a load that exceeds a defined pre-load, an initial contact point will be defined [0,0] (pre-load is normally set to 0.1 μN to 2 μN based on the rigidity of the sample). Immediately after the initial contact, the load increases up to the prescribed peak load and the indenter penetrates inside the sample, while the transducer records both the load and the displacement. Upon unloading, the load goes back to zero and the final contact depth is recorded. The mechanical response of the sample during the loading and unloading, as well as the final depth is used to infer the mechanical properties of the sample. The loading-unloading function is defined based on the nature of the sample, the indentation tip geometry, and the type of data that the user is interested in acquiring (elastic, elastic-plastic, fracture).

Figure 2.3. Representative indentation loading/unloading curves for fully elastic and elastic-plastic contacts. For the fully elastic response, the unloading curve almost overlap onto the loading curve, while for the elastic-plastic response, the unloading curve follow a different path, with a residual indentation depth observed after a loading/unloading cycle.

2.3.1.1 Hertzian contact

In his seminal work, Hertz found that the indentation contact radius (a) is related to the applied load (P), the indenter radius (R) and the elastic properties of the material⁶⁶ (Eq. 2.1). This theory has been used since the late 1980s to probe elastic properties of materials under contact load. In this method, blunt tips (mainly a rigid spherical indenter) are used to impose a mostly elastic response to the sample, rather than plastically deform it (which happens with sharp tips due to the stress localization). Figure 2.4 shows a schematic Hertzian contact.

Figure 2.4. A schematic Hertzian contact. $h_{\max}$ is the total depth of penetration under the indentation load P , h_a is the depth of the circle of contact from the specimen free surface, and h_c is the distance from the bottom of the contact to the contact depth circle, a is the radius of the contact circle and R is the radius of the indenter.

According to Hertzian theory:

$$a^3 = \frac{3PR}{4E} \quad (\text{Eq. 2.1})$$

For rigid indenter and non rigid specimens:

$$h = \delta = \frac{a^2}{R} \quad (\text{Eq. 2.2})$$

where h is the contact depth and δ is the distance of mutual approach. Then we can express the load as a function of contact depth:

$$P = \frac{4}{3}ER^{\frac{1}{2}}h^{\frac{3}{2}} \quad (\text{Eq. 2.3})$$

In analyzing loading-unloading curves exhibiting an elastic regime followed by a plastic regime (Figure 2.5), the elastic modulus of the sample can be extracted from the initial response of the ‘loading’ segment of the loading-unloading curves by curve fitting the data according to Eq. 2.3.

Figure 2.5. A schematic Hertzian curve fitting on a loading-unloading indentation curve. During loading, the initial response is elastic and is followed by plastic yielding, which can be detected as the curve deviates from the ideal Hertzian solution. The elastic response is used to calculate the elastic modulus using Eq. 2.3. Upon unloading, the response is fully elastic and can be extracted by a linear fitted curve. The residual impression is due to yielding occurring when the load exceeds the elastic regime.

2.3.1.2 Oliver and Pharr method

In 1992, Oliver and Pharr⁶⁷ introduced a versatile depth sensing method to obtain the size of indents without imaging them, and to calculate both E and H values from indentation loading-unloading curves (Figure 2.6). This method is valid for a wide range of indentation tip geometries and allows measuring elastic modulus and hardness of materials ranging from the KPa to the GPa range^{20,68}. In contrast to Hertzian contacts, the E and H values are extracted from the elastic *unloading* portion of a loading-unloading curve. A wide range of indentation tips from sharp (cube corner and Berkovich) to blunt (cono-spherical) geometries can be used for such measurements.

Figure 2.6. Schematic of sharp indentation and corresponding loading-unloading curve.

By increasing the load from zero to the peak load, P_{max} , the indenter penetrates in the specimen up to the maximum contact depth h_t . h_c is the contact depth beneath the contact (for materials with a fully plastic response, h_t would be equal to h_c)

Base on this theory, the elastic modulus (E) and hardness (H) can be calculated as:

$$E = \frac{dP}{dh} \frac{1}{2} \frac{\sqrt{\pi}}{\sqrt{A}} \quad (\text{Eq. 2.4})$$

and

$$H = \frac{P}{A} \quad (\text{Eq. 2.5})$$

where A is the contact area and $\frac{dp}{dh}$ is the slope of the unloading curve. The tip area can be calculated by curve fitting of the extracted data from indentation curves using a standard

sample with known elastic modulus (Figure 2.7). The contact area can then be fitted with as a mathematical function of the contact depth (h_c) by:

$$A = C_0 h_c^2 + C_1 h_c + C_2 h_c^{\frac{1}{2}} + C_3 h_c^{\frac{1}{4}} + C_4 h_c^{\frac{1}{8}} + C_5 h_c^{\frac{1}{16}} \quad (\text{Eq. 2.6})$$

where the first term represents the ideal area function of the indenter (2.598 for a cube corner and 24.5 for a Berkovich probe).

Figure 2.7. Schematic of multiple curve plot of calibration indents and extracted data (contact depths and areas) used for tip area calibration. The exact load range will depend on which type of probe is being used and where the user desires the calibration to be valid. The extracted contact depths define the range of calibration.

2.3.1.3 Common error sources in depth sensing methods

Due to the contact depth length scales in nanoindentation studies (micrometer and nanometer ranges), there are some common sources of error, which can potentially affect the indentation data. Here we briefly describe the most common of these errors:

1. Indenter tip area calibration

To calculate the contact area from resulted indentation curves, appropriate tip calibration (based on extracted contact depths) should be used. Inappropriate tip calibration ranges can lead to errors in calculated E and H values in orders of magnitude⁶⁶.

2. Sample fixation

Unstable sample can freely move under contact loads and lead to inaccurate measured contact depths. Samples should thus be completely fixed to the stage. For soft materials (*e.g.* hydrogel films), one must ensure that no bubble is trapped under the probing area. This error leads to lower calculated elastic modulus and hardness values.

3. Spatial resolution of indents

Upon post-yield indentation loadings, plastic zones (plastic flows) are created around the residual impressions (Figure 2.8). Performing indentation in the vicinity of these plastic zones result in measurements that are affected by this plastic deformation, which can lead to unrealistic mechanical response of the samples. The minimum indent spacing that has been suggested is more than three times the contact depth⁶⁶.

Figure 2.8. Schematic indentation residual impressions surrounded by plastic zones. Indentation spacing should be designed to prevent any overlapping between these zones.

4. Substrate effects

In thin film indentations, the extracted loading-unloading curve can be affected by the substrate., especially for very thin films. It has been suggested that the contact depth should not exceed 10% of the film thickness ⁶⁶. Figure 2.9 shows how the indentation curve of a polymeric film is affected by its glass substrate. In these cases the extracted E and H values would be significantly higher than the real values of the film.

Figure 2.9. Schematic loading-unloading indentation curve on a thin polymeric film is affected by its glass substrate. Upon loading, the indenter initially penetrates in the film and the loading curve corresponds to the mechanical response of the film. By exceeding 10% to 30% of the film thickness, the response is affected by the substrate and the loading curve exhibits a sharp deviation from the initial curvature, which is due to the substrate mechanical response.

5. Surface roughness

Rough surfaces can prevent a perfect contact between the tip and the sample, resulting in artificially larger contact depths (due to the roughness flattening) and in turn to lower calculated elastic modulus and hardness (Figure 2.10).

Figure 2.10. Imperfect contact due to high surface roughness. Upon loading, the initial applied load compresses surface roughness asperities. This leads to larger contact depths in loading-unloading curves and unrealistic measured elastic modulus and hardness.

6. Tilted surfaces

Tilted samples can cause inappropriate contact between the tip and the surface and lead to incorrect elastic modulus and hardness ². It has been suggested that for any samples with undefined surface roughness/surface angle, the topographical profiles shall be obtained before the indentation experiments.

Figure 2.11. Tilted contact and imperfect loading-unloading curve due to the tilted surface ². Although the loading curve seems normal, the unloading curve exhibits a sharp deviation in curvature due to the imperfect contact.

7. Inappropriate tip contact depth

Based on the tip geometry and radius, it is recommended to keep the contact depth from 1/10 to 1/3 of the tip radius. Generally measurements at very shallow and very deep depths do not follow the area function of indentation tips (Figure 2.12)

Figure 2.12. Inadequate tip contact depth. The residual impression areas made by the very shallow and very deep tip contact depth of the tips can deviate from the calibrated tip area function and cause significant errors in the measured elastic modulus and hardness.

8. Temperature drift effects

Due to the high depth sensing resolution in nanoindentation experiments (down to 0.1 nm), the temperature changes (*e.g.* the temperature difference between the tip and the sample) can affect the measuring depths.

2-3-2 Fracture toughness measurements

Fracture resistance is one of the most vital design criteria in biomineralized hard tissues. Materials scientists and mechanical engineers have investigated and quantified the fracture resistance of these materials, which then serves as a benchmark to evaluate synthetic mimic analogs. Two interrelated quantities – the energy release rate (Eq. 2.7) and the critical stress-intensity factor (Eq. 2.8) – and three types of loadings (figure 2.13) have been used to analyze cracks extensions and fracture toughness⁶⁹.

$$G_c = \frac{\pi \sigma_f^2 \alpha_c}{E} \quad (\text{Eq. 2.7})$$

and

$$K_{Ic} = \sigma_f \sqrt{\pi \alpha_c} \quad (\text{Eq. 2.8})$$

where G_c is the critical energy release rate, σ_f is the failure stress, α_c is the half-crack length and K_{Ic} is the critical stress intensity factor (Mode I).

Figure 2.13. The three modes of crack loading; Opening (Mode I), sliding (Mode II) and tearing (Mode III). Depending on the specimen loading direction and sample thickness, any of these modes can initiate the crack formations in the specimen.

Depending on the crack loading modes and fracture toughness criteria used, several techniques have been developed to evaluate the fracture resistance of biological materials, which include methods such as impact, tensile, flexural (3 points and 4 points) and indentation testing^{17,70}. Except for indentation fracture studies, the other methods require standard sample geometries, which involve machining the sample and/or

introducing a notch in order to generate pre-cracks that act as stress concentrators during fracture testing. Unfortunately, biological materials are faced with several challenges because of their sample size and complex shape ^{67,68}, in turn leading to technical challenges in machining and preparing standardized samples that often precludes the use of these standard geometries ⁷¹. Hence the first three methods are limited when the samples are small (sub-cm scale) and/or irregularly shaped. From that perspective, indentation methods have become popular due to the relative simplicity of sample preparation and relative ease of testing, making them versatile to probe local and bulk properties ⁷¹. Indentation fracture toughness of brittle materials are largely derived from the Lawn equation ^{72,73}(Eq. 2.7) obtained from tension induced median/radial cracks (Figure 2.6).

$$K_{Ic} = \alpha \sqrt{\frac{E}{H} \frac{P}{c^{\frac{3}{2}}}} \quad (\text{Eq. 2.7})$$

where K_{Ic} is the critical stress intensity factor (Mode I), α is an empirically determined “calibration” constant, E is the elastic modulus, H is the hardness, P is the applied load, and c is the length of the surface trace of the half penny crack measured from the center of the indent.

This method has been used extensively to measure fracture toughness of biomineralized materials such as bone^{74,75} and tooth^{15,16,57}. In this method Vickers, Berkovich, or cube corner tip geometries can be used. However the cube corner is the most convenient, because the external loads to nucleate cracks at tip corners are smaller than for the Vickers and Berkovich geometries by roughly 2 orders of magnitude ⁷¹. Upon loading, the region immediately below the indenter is subjected to a hydrostatic core stress field. As the local stress exceeds the yield criterion, the core expands to form a confined plastic zone, within which flaws can be nucleated. Surrounding the plastic deformation, there is an elastic field ⁷⁶, which leads to an elastic/plastic mismatch, and eventually to residual internal stresses upon unloading. This residual stress field drives crack propagation, which will extend until a given crack length c . Figure 2.14 presents a schematic model of indentation cracking in a brittle material.

Figure 2.14. Schematic representation of indentation fracture pattern of brittle materials using a cube corner indenter. When the load exceeds a critical value, a plastic zone forms within which cracks can nucleate and propagate. Because the residual stress field under the indenter upon unloading exhibits a strong decay with the distance, the crack will arrest at a finite length c .

For biological materials exhibiting a non-brittle mechanical response (for instance those with a low degree of mineralization), median/radial cracks do not nucleate under an indentation load and the method is not suitable. In 2008, Miserez *et. al.*⁶⁵ introduced a semi-quantitative model for materials that do not exhibit radial crack patterns during indentation. In this method, the critical load (P_c) is defined as the external load required to nucleate cracks at the indentation corners. Below P_c , only plastic deformation occurs and the indentation size a scales as $a \sim P^{1/2}$. If a crack is nucleated, on the other hand, its length c scales as $P^{2/3}$ (Eq. 2.7). P_c is then defined as the point where the crack length c is equal or larger than the indent size a , which can be represented as shown in Figure 2.15a (*i.e.* if $c < a$, no cracked are formed). Using this simple criterion, the fracture toughness is obtained as⁶⁵:

$$P_c = \frac{1}{\alpha^3 \chi^4} \frac{K_c^4}{E^2 H} \quad (\text{Eq. 2.8})$$

where P_c is the critical load, α is a non-dimensional geometric parameter ($\alpha = 2$ for the four-sided Vickers indenter and 1.30 for the three-sided Berkovich and cube-corner indenters), χ is non-dimensional constant (0.022 for Vickers and Berkovich indenters and

0.040 for cube-corner indenters), K_c is the fracture toughness, E is the elastic modulus and H is the hardness.

Figure 2.15 represents a typical plot used to obtain the critical load. The plot depicts the fraction of crack corners as a function of the applied load, with the number of cracked corners obtained by SEM observations post-indentation. For instance at 30 mN, no indentation cracks were formed, whereas at 800 mN, all corners were cracked. P_c is then defined as the value for which 50% of indent corners are cracked. It should be mentioned that this method provides only semi-quantitative values of the fracture toughness and is only valid to measure the initiation toughness. Thus, R -curve behavior is not accounted for with this method.

Figure 2.15. Indentation residual impression/crack length scale diagram and schematic diagram of indentation fracture frequency. (a) The cracking nucleation load, P_c , corresponds to the intersection between the residual impression size scales as $P^{1/2}$ (continuous line) and while the crack length scales as $P^{2/3}$ (dotted curve). Depends on the material property, this intersect changes. Re-plotted from Ref. [3] with authorization from Wiley VCH. (b) The x-axis is in log-scale to cover orders of magnitude of load. The y-axis is the fraction of cracked corners measured post-indentation. In this specific example, no fracture occurred up to load of 30 mN, whereas at loads above 800 mN, all indent corners are cracked. The critical load is defined as the load at which 50% of indentation corners are cracked.

Chapter 3 - Wear and Abrasion Resistance of Biological Materials*

3.1 Introduction

Protection against wear and abrasion is a critical requirement for the survival of many living organisms. Teeth, jaws and mandibles which equip the mouths of organisms from diverse Phylae are subjected to intense cyclic mechanical loading during feeding and mastication, and must sustain a high tolerance to wear in order to fulfill this critical function^{57,77}. Mollusk shells, turtle carapaces, or insect cuticles all have a primary function of shielding the animal's body against severe environmental pressures and predators, and a critical criterion to succeed in this task is to minimize loss of material by wear in the armor or protective structure.

From the engineering perspective, examination of hard tissues that have evolved to fulfill functions demanding a high wear tolerance provide unique insights for the design of novel wear-resistant materials. In restorative medicine, wear damage is indeed recognized as a major reason for implant loosening and failure⁷⁸⁻⁸⁰. For instance, metallic alloys give rise to large elastic incompatibilities at articular joints, while many synthetic polymers suffer from bio-compatibility issues or from excessive abrasion damage, leading to rapid degradation of the prostheses⁸¹. Given these issues, the need to develop biocompatible synthetic materials that are optimized for wear damage is critical. Ultimately, the goal is to apply the identified design concepts of biological hard tissues at both the microstructural and biomolecular level for the development of materials with optimized combinations of properties against wear damage.

A wide range of structure-property relationships have been established for biological materials in recent years, especially regarding their elastic, plastic, and fracture properties. Comprehensive books and reviews exist on the topic^{4,7,42,82-84}, whereas compilation of mechanical properties through materials selection charts have been presented by Wegst and Ashby^{85,86}. Ashby plots are designed to display two or more

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material properties on a single chart with the purpose of visually comparing their property performance for given loading modes. They provide a convenient way to select materials according to specific performance indexes. The plots display a given material property versus another one in log-log scale to cover a large range of materials. A criterion for a given loading regime is then derived (for instance tension, bending, or buckling), leading to property indexes such as (E/ρ) , $(E^{1/2}/\rho)$, or $(E^{1/3}/\rho)$, where E is young's modulus and ρ is the density. On a log-log scale, materials exhibiting an equivalent performance against a given index are located along linear "guidelines", whose slopes correspond to the power-law exponent of the performance index (for instance slopes of 1, 1/2, or 1/3 for the examples described above). Materials located in the same performance index possess the same performance while materials in the same "bubbles" present a similar range of properties.

Much less has been done regarding the specific theme of their tribological behaviour. Major reasons for this apparent shortcoming are that: (1) A single metric unit for wear damage does not exist. It depends on a combination of other fundamental properties such as hardness (H), elastic modulus (E), or fracture toughness (K_{Ic})^{87,88} and on the type of wear mechanisms⁸⁹. In fact, even for engineering materials, predicting the tribological performance of a material remains rather elusive⁸⁹⁻⁹¹. (2) Although standardized methods exist, wear tests are often carried out without fully controlled or comparable variables from one study to the other⁹². (3) An implication of the latter issue is that wear is strongly system-dependant: the type of mechanical sollicitation, the wear environment or presence of a third body all strongly affect the wear response. For biological materials, the wear response strongly depends on the biological function of the structure: whether it is employed for food grasping, attacking prey and feeding, for shielding of a soft body, and whether it must function in a hydrating environment will play a fundamental role in the chemistry and evolution of the structural organization of these materials.

Following a previous work where predicted wear map resistance of various engineering materials were presented⁸⁸, in this chapter these maps in the context of biological materials are expanded. In comparison to the comprehensive databases existing for engineering materials, much less data are currently available for natural materials. Yet,

this number has rapidly increased in the past years, allowing us to gather a substantial amount of data from the literature. Combined with those compiled in recent reviews^{4,84,86}, these data allow general trends to be drawn, providing guidelines on materials property groups that provide tailored resistance against wear and abrasion.

3.2 Biological structures with wear resistance–oriented functions

We may divide hard tissues into three broad categories based on their mineral content: *(i)* those with a high mineral content; *(ii)* those with a medium to low mineral content; and *(iii)* those that are unmineralized. This classification, albeit arbitrary, is justified from a materials perspective by the clear contrast in hardness and modulus of the minerals compared to that of organic materials. We also define as “highly” mineralized those tissues containing at least 75 vol. pct of mineral phase. With such categorization, bones can belong to two categories^{93,94}: they can be highly mineralized as in the case with many vertebrate bones, or they can be classified as having a medium mineral content like as with antler which contains approximately 35 vol. pct of minerals^{17,95}. Crustacean exoskeletons which are composites of chitin and calcium carbonate, are considered to belong to tissues with a medium mineral content. Additionally three functions of hard tissues are defined: *(i)* protective armor (*e.g.* mollusk shells); *(ii)* supporting structures (*e.g.* bone); and *(iii)* mechanically-active biotools (*e.g.* teeth and horse hoof). Table 3.1 provides a list of tissues whose functions dictate that they must exhibit a high tolerance against wear and for which relevant mechanical properties data have been found in the literature, whereas illustrative examples of such structures are displayed in Figure. 3.1.

3.3. Selection of properties and map construction

3.3.1 Properties

The choice of relevant mechanical properties are based on available analysis on abrasion resistance of engineering materials⁸⁸, which essentially assumes that wear and abrasion tolerance is largely governed by the onset of damage at the contact between two solid bodies. A few additional remarks are necessary. When *R*-curve behaviour was

mentioned in the literature, *i.e.* an increase of fracture energy with crack propagation due to energy-dissipating processes in front of the crack tip, in this study we used the fracture energy (or its equivalent critical stress intensity factor) at *initiation* of crack propagation. This choice is justified by the fact that the analysis considers wear to be governed by nucleation of the first damage event. Conversions between the fracture toughness K_{Ic} and its equivalent fracture energy at initiation G_c (or the J -integral at initiation, J_{Ic} , for elasto-plastic cases) is obtained through the relation:

$$G_c = \frac{K_{Ic}^2 (1 - \nu^2)}{E} \quad (\text{Eq. 3.1})$$

where $E/(1 - \nu^2)$ is the plane-strain modulus and ν the Poisson's ratio, assumed to be 0.3 in all cases. The hardness H is one of the key material parameters appearing in previous analyses. In this study we used published hardness values when those were available; however in cases where this property was not reported, we used the well-known approximation between H and yield strength σ_y , $H \approx 3\sigma_y$ ^{96,97} to convert σ_y into H values. We are aware of the limitation of this relation, but for the present purpose of comparing a wide variety of tissues, with H values spanning over two orders of magnitude or more, it provides a reasonable estimation⁹⁸. Although relevant for biological materials, the lack of systematic data on the friction coefficient for biological materials did not allow us to include this parameter in our maps, although the significance of friction measurements on the abrasion tolerance is mentioned in the discussion.

Table 3.1. List of hard tissues with a function relevant to wear and abrasion resistance. Indicated in the table are degree of mineralization and the function of the material.

Biological materials	DOM ^{a)}	Function	Ref
Nacre	High	Protective armour	11,62,99
Conch	High	Protective armour	100
Human tooth (enamel)	High	Tool	15,16
Shark tooth (enameloid)	High	Tool	48
Chiton tooth	High	Tool	49
Sea Urchin tooth	High	Tool	101
Biosilica	High	Support	3
Bone (cortical/trabecular)	High	Support	14,54
Dactyl club mantis shrimp	High	Tool	25
Fish armour (ganoine)	High	Protective armour	37
Turtle shell	Medium	Protective armour	102
Human tooth (dentin)	Medium	Tool	15,16
Lobster exoskeleton (outer/bulk)	Medium	Protective armour	24
Crab claw (tip/inner)	Medium	Tool	103
Antler	Medium	Tool	17,18
Tucan beak (shell/foam)	Medium	Tool	104
Glycera jaw (outer/bulk)	Medium	Tool	105
Horse hoof	Low/No	Tool	106
Spider fang	Low/No	Tool	107
Human nail	Low/No	Tool	108,109
Squid sucker ring	Low/No	Tool	110
Nereis jaw (with/without metal ions)	Low/No	Tool	111
Insect cuticle	Low/No	Support	112,113
Cephalopod beak	Low/No	Tool	114
Black coral	Low/No	Support	115
Mussel thread (inside/coating)	Low/No	Tool/Protection	116

^{a)}DOM= Degree of mineralization,

Figure 3.1. Illustrative examples of biological materials with a function requiring a high tolerance against wear. Functional wear-resistant biological materials are divided into three broad categories, namely (1) protective structures, (2) supporting structures, and (3) mechanically-active “biotools”.

3.3.2 Abrasion resistance map construction

A comprehensive treatment of the material property combinations that are predicted to govern abrasion was previously presented by Zok and Miserez⁸⁸. A summary of how the subsequent abrasion maps are constructed and of their significance is shortly summarized.

First, two limiting cases of contact geometry are considered: blunt contacts, in which $a \ll R$ where a is the contact circle radius and R the radius of curvature of the abrasive media; and sharp contacts, where $R \rightarrow 0$. Second, abrasion tolerance is assumed to be governed by the incipient damage event occurring at the contact, which is either yielding or cracking. The damage modes for the two types of contact geometry are

schematically illustrated in Figure 3.2. Third, for each case, relationships between a critical normal load for yielding or cracking are derived from established contact mechanics theory. Typically, a critical load P_i for a given geometry and damage mode is obtained (with $i = y$ for yielding and $i = c$ or cracking), providing a relationship between P_i and basic materials properties. The obtained equations of interest for the current analysis are the following:

(i) Yielding from a blunt contact:

$$\frac{P_y}{R^2} = C_1 \left(\frac{H^3}{E^2} \right) \cdot \left(1 + \frac{\bar{E}}{E'} \right)^2 \quad (\text{Eq.3.2})$$

where E is the plane-strain modulus of the abraded material and E' that of the abrasive material; and C_1 a constant equal to 0.8.

(ii) Cracking from a blunt contact:

$$\frac{P_c}{R^2} = C_2 \left(\frac{G_c}{R} \right) \cdot \left(1 + \frac{\bar{E}}{E'} \right) \quad (\text{Eq.3.3})$$

where C_2 is a constant best obtained by experimental calibration, and G_c is the critical energy release rate (fracture energy). Using a material with well-known properties (soda-lime glass), C_2 has been found to be ≈ 9000 .

(iii) Yielding from a sharp contact:

$$\frac{P_y}{h_r^2} \approx C_4 H \quad (\text{Eq.3.4})$$

where h_r is a prescribed residual penetration depth after contact, and C_4 a constant that depends on the projected area of contact. For well-defined geometries used in depth-sensing indentation techniques, C_4 is known and equal to 24.5 for Vickers and Berkovich geometries and 2.6 for the Cube-Corner geometry⁶⁶.

(iv) Cracking from a sharp contact:

$$P_c \approx C_6 \frac{K_c^4}{H^3} \quad (\text{Eq.3.5})$$

where C_6 is calibration constant dependent on the geometry of the contact, and which is obtained through experiment. For the commonly-used Vickers geometry $C_6 \approx 1.5 \times 10^4$, while it is about two orders of magnitude lower for the cube-corner geometry^{117,118}.

Figure 3.2. Damage mechanisms at a contact between an elastic indenter and an elastic-plastic material. (A) Yielding beneath a blunt contact. (B) Cracking at a blunt contact. (C) Yielding at a sharp contact. (D) Cracking at a sharp contact. Due to limited data available, the influence of friction is not considered here.

From these relationships we note, for instance, that yielding from a blunt contact is governed by the property group $H^3/\bar{E}^2$ times an elastic mismatch parameter $(1 + \bar{E}/E)^2$, or that cracking damage from a sharp contact is predicted to scale with K_c^4/H^3 . Additional relations exist to take friction into account. As explained above these are not considered in the present study.

Next, following Ashby's material selection charts¹¹⁹, two types of maps can then be constructed. First, for a given damage mode and contact geometry "traditional" maps with relevant property combinations are plotted on logarithmic scales, with guidelines added having slopes corresponding to the derived power-law relationships in Eqs. 3.2-3.5. Second, maps taking into consideration the two failures modes (yielding and cracking) can also be prepared. In those, critical loads for yielding are plotted as a family of *horizontal* lines corresponding to given values of the material property group. Similarly

and using identical values of critical loads, family of *vertical* lines corresponding to cracking are plotted. Both sets of curves are truncated where they meet, hence yielding “L-shaped” curves. With this representation materials performing equivalently against abrasion appear along a specific *L*-curve. Materials laying above or on the right of a given guideline curve are predicted to perform better than those situated below or on the left of the *L*-shaped guideline curve.

3.4 Property Maps and Discussion

3.4.1 Abrasion property maps

The property map for resistance against a blunt contact is shown in Figure 3.3A. As expected, highly mineralized materials are found on the upper right corner of the map (high modulus and hardness), unmineralized materials are found in the lower-left corner, while moderately-mineralized materials are broadly located in the middle. The outer layer of Chiton radula’s stands out as featuring the highest resistance against yielding of all biological materials, a characteristic previously discussed by Weaver *et al.* ⁴⁹, while biosilica also performs quite well thanks to its relatively low modulus ³ which is associated with partial hydration of amorphous biosilica. The outer layer of the bloodworm’s jaw ^{120,121} is predicted to resist best among moderately-mineralized materials, where the mineral phase of this peculiar material is the copper-based atacamite ¹²². Interestingly, the map indicates that some non-mineralized materials can perform as well as highly mineralized materials under this loading mode, for instance black coral and squid beak, which are both heavily cross-linked polysaccharide/protein composites.

The map displayed in Figure 3.3B takes into account the finite stiffness of the abrasive material, represented by E' on the map. The distinction with Figure 3.3A is that a given guideline (equal material performance) flattens at higher elastic modulus values, and in a more pronounced fashion at lower values of E' . The key conclusion of the map is that materials with very different moduli –which were predicted to performance equivalently against an infinitely stiff abrasive– now feature distinct behaviors. The stiffer the material the better their predicted resistance against a blunt abrasive because they are more likely to lie above a given guideline, especially for more compliant abrasive materials. As an illustrative example, spider fang and conch are predicted to

perform equivalently against an infinitely stiff abrasive. For abrasive materials having a finite stiffness in the range ~ 30 to 100 GPa, on the other hand, conch is situated above the associated guidelines, indicating that it now has a better performance metric than spider fang.

Figure 3.3. Selection chart of biological materials for resistance against yielding at a blunt contact. (A) Stiff indenter. (B) Finite stiffness indenter, with E' representing the plane-strain modulus of the indenter.

Resistance against cracking from sharp contact is illustrated in the selection chart displayed in Figure 3.4. Before commenting on its main features, it should be noted that the property map relies on fracture toughness values (here the critical stress intensity fracture factor, K_{Ic}). However, perhaps with some exceptions such as bone,^{32,54} nacre⁴², or more recently enamel¹²³, there remains a paucity of comprehensive K_{Ic} values for many biological materials, which is due to the difficulty in performing reliable fracture mechanics experiments on irregularly shaped and small-scale biological structures¹²³. This challenge, while recognized, has still not been solved and there remains a lot of room to develop testing procedures at small scales, using sample preparation methods such as Focus Ion Beam (FIB)¹²⁴. In many cases, researchers rely on indentation fracture experiments^{71,76,77}.

Although there are controversies^{125,126} regarding the absolute value of fracture toughness obtained with these experiments, the technique provides valuable and quantitative information, especially regarding initiation of fracture events which is our prime focus for wear and abrasion. The trends that emerge from the map are the following. Overall, unmineralized and weakly mineralized materials are expected to resist better against cracking from sharp abrasives. This is not surprising given that the critical load to induce cracking scales with K_{Ic}^4 , and unmineralized materials in general have higher K_{Ic} values. Because of this power-law scaling relationship, even small changes in the critical stress intensity factor have dramatic effects on damage initiation. This is clearly illustrated for horse hoof, which is mostly made of highly cross-linked β -keratin¹²⁷. This material exhibits the highest fracture toughness reported among biological materials, which translates into a high predicted resistance against cracking. Antler, which is the toughest materials among moderately-mineralized materials, is another material that stands out on the map. Heavily mineralized materials, owing to their generally lower K_{Ic} values, appear to be more prone to wear damage from sharp contact. However, mineralized structures that have high toughness, such as nacre, can compete with some weakly mineralized materials.

Figure 3.4. Selection chart of biological materials for resistance against cracking at sharp contact.

We have so far discussed both types of damage initiation, *i.e.* yield and fracture, separately. In order to draw a broader picture, it is useful to represent both types of damage on single maps. These *L*-shaped curves (the reader is referred to Section 3.3.2 for more details on their significance) are displayed in Figure 3.5 (blunt contact) and 3.6 (sharp contact). Let us first discuss abrasion resistance against a blunt abrasive, where x and y coordinates on the property map are G_c (the critical energy release rate) and H^3/H^2 , respectively. The sub-class of heavily mineralized materials is grouped in a comparatively larger subset on the property map. In comparison, the moderately mineralized subset is shifted to the right and to the bottom, whereas unmineralized materials are found in a narrow band along the x -axis, which is elongated along the y -axis. Biosilica emerges as the best performing material (among those with a complete set of known properties) in terms of abrasion against a blunt contact, notably lamellar biosilica,

which is situated on the right and above the guideline with normalized load $P_c/R^2 = 10$ MPa. Because the critical load P_c for cracking scales with the abrasive radius R for this mode⁸⁸, the vertical portion of an L -shape guideline translates towards the right at higher radii (here plotted for R values of 1 and 5 mm, respectively), in turn lowering the critical load for cracking and possibly switching the governing failure mode. For instance for $R = 1$ mm, bulk biosilica is predicted to resist cracking, but at $R = 5$ mm, it is predicted to fail by cracking. It is noteworthy that there exist some interesting mineralized materials that have recently been described, such as Chiton teeth⁴⁹ or the stomatopod dactyl club²⁵, and for which fracture toughness data are currently still lacking. It may well be that these materials could surpass biosilica in terms of abrasion against a blunt contact. It is also noteworthy that the critical load for cracking in this case is governed by the fracture stress energy, G_c . Because of the classical relation between G_c and the critical stress intensity factor, K_{Ic} (Eq. 3.1), materials with a lower modulus can somehow counterbalance their lower fracture toughness value K_{Ic} , thus exhibiting a better performance.

Figure 3.5. L-shape selection charts of biological materials against combined yielding and cracking at a blunt contact. In this type of plots, guidelines have an L-shape: vertical lines represent cracking loads and horizontal lines yielding loads. In this representation, a material located on the right and above a given guideline is predicted to resist damage at that specific load. Note that for the cracking mode, since the load scales with the radius of contact R , vertical guidelines are translated towards the right for higher radii of contact.

Moderately mineralized materials are all situated on the right of the L-shape guidelines and intercept the horizontal sections of the latter. The inference is that cracking is unlikely to occur at the contact and that these materials are governed by contact yielding, with crab claw emerging as having the best abrasion resistance in this sub-class. Non-mineralized tissues are also clearly predicted to fail by yielding at a static blunt

contact. Indeed, even for normalized contact pressures as high as 100 MPa and large contact radius of 5 mm chosen here to draw the *L*-shape guidelines, the map indicates that these materials will yield well before critical loads for cracking are reached. Hence, their resistance against wear is again governed by the material property group H^3/E^2 , with squid beak being the most abrasion resistant against this contact mode, even exceeding dentin or crab claw.

Let us now turn our attention to the *L*-shape abrasion map against sharp contacts (Figure 3.6), which is defined by the material parameters K_{Ic}^4/H^3 for cracking (*x*-axis) and H for yielding (*y*-axis). Here, because yielding is governed by the simple hardness parameter, each sub-class of material is located in a narrow region along the *y*-axis of the map space. On the other hand, since resistance to contact cracking is linked to the intrinsic materials properties (K_{Ic} and H) through a power-law type relationship, small variations in these properties have a much more pronounced effect on the critical cracking parameter, such that the sub-classes expand over a broader range along the *x*-axis. In turn, the various sub-classes of materials are grouped in elongated “bubbles”. Inspecting the map further using the *L*-shape guidelines, a few characteristics are noteworthy.

First, nacre and conch shells are predicted to provide the highest resistance against sharp contact abrasion (note their location on the right and above the 3N guideline) among all biological materials. Second, highly mineralized materials are more likely to fail by contact cracking because their critical loads for cracking are usually lower or close to their critical loads for yielding. This feature, however, is not universal. In dentin and bone for instance, which in comparison to shells or biosilica are less mineralized, contact damage is likely to be initiated by yielding. Third, in materials that are moderately mineralized or unmineralized, on the other hand, the critical loads for cracking are exceedingly higher than the critical loads for yielding. This can be realized by following the 2N guideline, which indicates that all unmineralized materials shown on the plot would exhibit yielding (here defined as a residual impression depth of 10 mm) well before cracking could occur.

Figure 3.6. L-shape selection charts of biological materials against combined yielding and cracking at a sharp contact. See text and Figure 3.5 for explanation of L-shape plots. Note that the horizontal guidelines were constructed for a specific residual indentation depth (h_r) of 10 μm . For higher and lower values of h_r , the guidelines are translated downwards and upwards, respectively.

Thus, it can be concluded that against a sharp abrasive contact cracking is the prevailing damage mode in highly mineralized tissues, whereas in unmineralized materials contact yielding is the dominant mode. Furthermore the best performing materials against sharp contact abrasion are mineralized materials that display relatively high fracture toughness values.

3.4.2 Comparison with experimental data

There are only a few experimental studies on the tribological response of biological materials and such data are often not directly comparable from one study to another. In order to test some of the predictions described here, controlled depth-sensing indentation experiments^{66,128} provide valuable insights on incipient wear damage of biological materials. For blunt contact, the critical load for yielding is proportional to the

material property group H^3/E^2 and this incipient yielding event can be monitored during contact-depth experiments with a spherical punch. Here, the load response initially follows the elastic Hertzian contact ¹²⁹:

$$P = \frac{4}{3} \cdot \frac{E}{(1-\nu^2)} \cdot h^{3/2} \quad (\text{Eq. 3.6})$$

Deviation from Eq. 6 indicates yielding beneath the contact, and this can be experimentally captured by comparing the experimental load-depth curve with the Hertzian solution ^{130,131} of the indented material with modulus E . The point of deviation on the load axis is then taken as the onset of yielding, P_y , as schematically depicted in Fig. 3.7A.

Figure 3.7. Depth-sensing nanoindentation with a spherical punch (nominal radius 1mm) can be used to detect the first yielding event beneath the contact surface. (A) Schematic representation of the indentation response. The dotted curve is the ideal Hertzian solution and the solid curve is the experimental load-depth curve. Deviation from the Hertzian solution is related to yielding, defined by the critical load P_y . When yielding occurs, a residual impression is observed, with indentation depth h_r . **(B)** Representative curves on bio-magnetite from Chiton's radula and geological magnetite. Despite a higher hardness, geological magnetite yields a lower load from a blunt contact in agreement with Eq. 3.2, while also exhibiting a larger residual depth. **(C)** Distribution plots of P_y ($n = 30$ and 25 for Chiton's radula and geological magnetite, respectively); **(D)** Distribution plots of h_r ($n = 25$ and 20 for Chiton's radula and geological magnetite, respectively) after maximum indentation loads of 2000 mN.

In order to assess the validity of the scaling $P_y \propto H^3/E^2$, we compared the elastic-plastic indentation response of the external layer of Chiton's radula (*Cryptochiton stelleri*) with that from geological magnetite. These measurements provide a direct comparison since both materials have a very similar chemistry consisting iron oxide magnetite^{50,132}, the main difference being the small organic content in Chiton's magnetite, which leads to

lower elastic modulus⁴⁹. These latter values were first verified by indentation with a sharp indenter and are indicated in Table 3.2. A series of indentations were then carried out with a cono-spherical indenter of nominal curvature $R = 1$ mm, at maximum loads ranging from 1 to 4 mN as described in by Weaver *et al.*⁴⁹, with representative load-depth indentation curves shown in Figure 3.7B. The distribution plot of P_y was then plotted for each indent, which is shown in Figure 3.7C. The results confirmed that because of its higher H^3/E^2 value (~ 2.5 fold), Chiton's magnetite has a higher P_y value of 417.5 ± 59.4 mN vs. 207.2 ± 17.2 mN for geological magnetite (see also Table 2). Furthermore, we also plotted the value of the residual depth, h_r , after indentation with the cono-spherical tip. Since geological magnetite starts yielding at lower indentation loads, the sub-contact plastic zone starts forming and expanding at lower applied loads, which should result in higher residual depth upon unloading. This is indeed what was observed in the experiments, as indicated by the h_r distribution plot shown in Figure 3.7D, which was obtained at maximum indentation loads of 2000 mN.

Table 3.2. Comparison of materials properties and resistance against a blunt contact between Chiton's radula magnetite and geological magnetite.

Material	H [GPa]	E [GPa]	H^3/E^2 [GPa]	P_y [mN]	h_r [nm]
Chiton radular's magnetite	10.5 ± 1.5	107.5 ± 17.5	0.100 ± 0.01	417.5 ± 59.4	28.2 ± 4.0
Geological magnetite	10.5 ± 0.2	175 ± 4	0.0378	207.2 ± 17.2	58.7 ± 3.6

For sharp contact, indentation cracking experiments on giant sponge spicule silica provide experimental support for the predictions. This system was chosen as a model system to study the influence of a lamellar microstructure on the cracking behavior of biosilica³. In this work, the critical load (P_c) for initiation of cracks at a cube-corner tip was compared between lamellar silica and a bulk silica standard. An analysis of cracking at a sharp contact indicated that in this case, the initiation threshold for cracking scales as:

$$P_c \propto \frac{K_{Ic}^4}{E^2 \cdot H} \quad (\text{Eq. 3.7})$$

which is a slightly refined version of Eq. 3.5, and whose schematic representation is illustrated in Figure 3.8A. Because the critical load scales with K_{Ic}^4 and since lamellar silica exhibited a significant higher toughness (together with slightly lower modulus and hardness), it was observed that P_c was about 100-fold higher for lamellar silica vs. bulk silica (see Figure 3.8B) which is in agreement with the map displayed in Figure 3.6. Since irreversible damage is delayed in the lamellar structure, a higher wear tolerance would thus be expected.

Figure 3.8. Indentation at a sharp contact can essentially be perceived as a competition between yielding and cracking from the indent corner. (A) The indent size scales with the maximum applied load P as $P^{1/2}$, while the crack length scales as $P^{2/3}$. The cracking nucleation load, P_c , corresponds to the intersection between the two curves. Depending on the material property group, the crack-length line (dotted curve) shifts along the indentation size curve (continuous line), resulting in changes in P_c . (B) In giant sponge spicule, cracks in the lamellar, tougher region nucleate at loads about 100-fold higher than in the monolithic (non-lamellar) region. (B) Reproduced from Ref. ³ with authorization from Wiley VCH.

Among unmineralized materials, a few limited tribological studies have been undertaken and include nano- and micro-scale friction measurements on insect pads ¹³³, as well as nano-friction and nano-wear measurements on marine worm jaws ^{121,134}. Such measurements are particularly relevant, since with current nanomechanical

instrumentation direct comparison between various materials can be undertaken under reproducible conditions, including contact geometry or force magnitude. Indeed one challenge that emerges when assessing the abrasion of biological materials is that even among the materials that have been investigated, different methods have been used to quantify their wear resistance, making comparisons problematic. Systematic studies with standardized procedures are thus critical in order to obtain meaningful comparisons of wear tolerance. In this respect, measurements of the nano-scale friction coefficient (as measured by controlled scratching experiments) have been shown to be convenient in assessing local variations in wear resistance at small scales. Such nanoscratch measurements were carried out by Pontin *et al.*¹²¹ on marine worm jaws, allowing these authors to correlate local changes in chemical composition and mechanical properties at the micro-scale to wear resistance. Given the universal presence of microscopic structural and chemical gradients discussed above, the method could prove very useful as a way to quantitatively compare the biological materials presented in Figures. 3.3-3.6.

Systematic instrumented indentation studies such as described above, namely elastic-plastic contact with a spherical punch or indentation cracking with a sharp indenter, could be very useful in the future as a systematic method to evaluate the wear resistance of a broader spectrum of biological materials.

3.4.3 Microstructural considerations

The current analysis does not attempt to correlate the abrasion resistance property to the actual wear mechanisms occurring in biological materials. Such structure/property relationships for wear and abrasion will require quantitative experimental data which are currently still lacking for most biological materials. Indeed, despite the growing amount of mechanical properties available for biological materials (see for instance the recent reviews by Wang and Gupta⁴², Chen *et al.*⁸⁴, or Studart⁷), there are few experimental studies available regarding their tribological behavior. One notable exception is nacre, for which recent nanotribology investigations have revealed that wear mechanisms are various and complex, and depend on many environmental parameters¹³⁵⁻¹³⁸. Specifically, these studies have shown that (i) wear mechanisms are highly correlated to hydration, with the organic matrix influencing the nanofracture behaviour through physico-chemical

interactions ¹³⁶, and (ii) that the energy-absorption capability of the organic matrix is strongly reduced during dynamic frictional loading, resulting in propagation of microcracks through the mineral tablets ¹³⁸. Other intriguing phenomena in nacre are associated with friction-induced increase in temperature at the contact point during dynamic friction testing ¹³⁷. Such localized temperature increases are high enough to thermally degrade the matrix and even induce aragonite-to-calcite phase transformation, leading to a drastic increase in wear damage. Clearly, wear mechanisms are materials-specific and depend highly on the physico-chemical interactions between the components of the biological composites.

Furthermore, variations in structural and chemical compositions at various length scales have not been discussed in our analysis, although the variation in local properties are reflected in the “size” of the bubble on the material property charts. However, it is well-established that most organisms optimize the performance of their load-bearing tissues by fine-tuning their microstructure and chemical composition in order to respond to local environmental pressures ¹³⁹⁻¹⁴¹. Biological tissues that feature wear and abrasion resistance functionality are emblematic examples of this adaptation, which can take various forms of biomolecular or microstructural gradients from the bulk to the surface, including mineral gradients, microstructural gradients, porosity gradients, chemical gradients, and hydration gradients, which all translate into mechanical gradients (Table 3.3).

A comprehensive mechanical analysis of how these gradients affect localized damage by yielding and cracking is beyond the scope of the present paper. Nevertheless, it is worth mentioning at least two important mechanisms by which they increase the damage tolerance. First, gradients lead to stress redistribution beneath the contact, leading to reduction in stress concentration near the contact, and in turn to improved tolerance against contact yielding ^{142,143}. Such a mechanism has been supported by Finite Element Modeling of contact stress distribution in various protective armors, in which elastic modulus and hardness gradients are due to mineral gradients ^{37,144}. Second, sharp gradients such as those established at organic/inorganic interfaces or at regions with abrupt variations in the mineral content have the ability to impede crack propagation to deflect crack along the interface according to mechanisms theoretically described by

Hutchinson and co-workers^{145,146} and confirmed experimentally in various biological materials, *e.g.* sponge spicules,^{3,147} dentin/enamel junction^{15,57} or stomatopod hammer²⁵. It is clear that microstructural and biomolecular gradients are nearly ubiquitous among wear-resistant materials, and that attempts at mimicking abrasion-resistant materials will require their incorporation into syntheses and processing efforts.

Table 3.3. Type of gradients found near the surface of abrasion-resistant biological materials.

Type of gradient	Species/Region	Ref
Mineral gradient	Dentin-enamel junction (DEJ)	15,16
	Glycera jaw	148
	Sea urchin tooth	101
	Chiton radular	49
	Stomatopod club	25
		55
Porosity gradient	Bone	149
	Crab exoskeleton	110
	Squid sucker ring teeth	
Microstructural gradient (layer thickness, fiber orientation)	Sponge spicule	150
	Stomatopod club	25
	Gastropod shell	144
Hydration gradient	Squid beak	114
	Horse hoof	106
	Insect cuticle	112,113
Chemical/Cross-linking gradient	Squid beak	114
	Mussel thread	116
	Stone crab claw	103
	Nereis jaw	111
Metal ions gradient	Glycera jaw	148
	Nereis jaw	111
	Spider fang	107
	Scorpion sting	151

3.4.4 Biological function

From a biological standpoint, there are several implications regarding whether a material is predicted to perform strongly or weakly against a particular damage mode and contact geometry. Clearly, a high damage tolerance is intimately associated with specific selective pressures. The *L*-shape map for sharp abrasives predicts that the best overall abrasion tolerance should be obtained for nacre and conch shells (Figure 3.6). This is not surprising given that these structures, in addition to their protective function against predators, have also evolved to protect the animal against the wear-aggressive sandy environment. Similarly, Chiton forage by scraping algae from rocky substrates with their radula, meaning that their survival largely depends on a high abrasion resistance of the radula material. Inspecting the abrasion resistance map against a blunt contact (Figure 3.3), we discussed that Chiton radula exhibits the highest wear tolerance of any biological materials against this specific wear mode (note that fracture data of chiton are not available hence the material is absent on *L*-shape maps) and that the animal has even optimized the abrasion tolerance of magnetite in comparison to geological magnetite (Figure 3.7).

If, on the other hand, the material exhibits a low resistance against a particular damage mode, it may be that the latter is not critical for the biological function of the material. For instance, the maps predict that horse hoof has the lowest tolerance against yielding against a blunt contact, but the highest resistance against sharp contact cracking. The implication is that sub-surface yielding occurs routinely but does not impede the hoof's function. Instead, the material seems to have been optimized to prevent damage by cracking. At the opposite side of the spectrum, biosilica appears to have been optimized to resist against blunt contact, but is weakly designed against sharp contact, likely because the latter is not frequently encountered by static structures such as sponge skeleton where biosilica is found.

Multi-functionality is also a key parameter of many biological materials. For instance, in structures that must sustain large elastic deformations, a continuous mineral phase is not a viable option since it would lead to premature catastrophic fracture under tensile loading. Thus collagenous mussel elastic threads, which are used by the animal to

thether itself to hard substrates, are surrounded by a discontinuous protective layer (the cuticle) made of a specific protein this is cross-linked and hardened through complexation with metal ions^{152,153}. Under fully hydrated conditions, this cuticle is much harder than the interior core, yet can be extended to fairly large strains until ultimate failure¹¹⁶. Hence, mussels have evolved a composite strategy whereby the extensible interior is coated with an semi-hard, yet extensible protein/metal network. Such multifunctionality characteristics are common to many materials displayed in the combination of abrasion resistance maps presented.

3.5 Conclusions

Many biological materials fulfill a protective function or are mechanically-active biotools. A key requirement for these functions is to minimize loss of materials at contact points during repetitive loading, which requires a high tolerance against wear and abrasion in various environments. Whereas elastic, plastic, or fracture properties of many biological materials have been studied over the past decades, our understanding of the tribological properties of these materials remains sparse. Here, we have presented a simplified treatment of abrasion damage in order to provide an overview and compare the wear-tolerance of a variety of biological materials. This analysis is based on the assumption that wear tolerance is largely governed by the nucleation of first irreversible damage at a contact point, in the form of localized yielding or fracture. Using this hypothesis, abrasion resistance maps were constructed in the spirit of Ashby's materials selection maps for biological materials with documented elastic, plastic, and fracture properties, allowing a comparison of their predicted wear and abrasion resistance.

The analysis shows that the abrasion tolerance of biological materials is highly dependant on the load regime and contact geometry, in turn implying that Nature has evolved environmentally-dependant specific solutions against wear resistance. The maps also provide general guidelines of biological tissues that are able to sustain the highest tolerance against wear damage, which provides guidelines for the selection of interesting model systems for subsequent biomimetic materials synthesis and processing. Wear mechanisms in biological materials are perhaps even more more complex than in engineering materials. As such, a next step will be to link these mechanisms with

intrinsic mechanical properties, including elastic, plastic, and fracture properties, as well as with the physico-chemical characteristics of nano-scale interfaces present in biological materials, such as interfacial strength. Such studies will undoubtedly fill an important gap in our current understanding of the functional properties of biological materials, and will provide key lessons for bio-inspired materials to be used in restorative applications.

Chapter 4 - Chemo-Mechanical Characterization*

4.1 Introduction

Stomatopods, or “mantis shrimp”, are crustaceans that have evolved a pair of powerful raptorial appendage used during their aggressive hunting strategies. These intriguing structures can take two forms depending on the species: a “smasher” (Figure 4.1a) which is used as an impact hammer to shatter hard shells and incapacitate their prey, or a “spearer” which serves as a grasping and holding device for passing prey (Figure 4.1e). In addition to being used to grasp and impale their prey, at close proximities, the spear-like modification can be used in a hammer-like fashion and possesses a distinctive flattened impact surface. Both limbs are deployed at a remarkably high velocity of up to 23 m/s, which represents one of the fastest appendage movements in the animal kingdom²³. Recent investigations by Weaver *et al.*²⁵ have revealed the complex interplay between microstructure, mechanical properties, and impact mechanics of the dactyl club from *Odontodactylus scyllarus*. These findings have showed that the club is divided into three distinct regions: (i) the impact surface, made of highly crystalline apatite and which is near or in direct contact with the external environment, (ii) the periodic region made of a rotated plywood of chitin/amorphous calcium carbonate (ACC) whose main role is to protect the tool against internal damage, and (iii) the striated region made of a mineralized chitin fibrils running in a nested fashion around the club, which presumably prevents expansion during impact.

The impact surface is particularly critical for the hunting success of stomatopods, since its role is to inflict an impact force as high as possible during a single blow. In order to assist in this function, species have developed both chemical and microstructural strategies in their evolutionary toolkit. Ultra-high mineralization^{154,155} is an obvious choice to optimize the hardness and stiffness near free surfaces, and most species indeed fine-tune the biomineralization process such that the mineral phase at the free surface exhibits a combination of mechanical properties that is optimized for impact or abrasion tolerance^{6,156}. Illustrative examples of this strategy include, among others, protective

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scales of fish ³⁷, the chiton radular teeth ⁴⁹, or the external protective armor of deep sea gastropods ¹⁴⁴.

Figure 4.1. Stomatopod dactyl club and spear macro- and microstructures. (a) Anterior view of a “Smasher” type stomatopod with extended dactyl clubs. (b) Schematic of sample preparation before and after polishing. (c) Overview photograph of the actual sample. (d) Optical micrograph of a yy cross-section, showing the 2 sub-domains of the impact region. (e) Anterior view of a “Spearer” type stomatopod with a single visibly extended appendage. (f) Schematic of sample preparation for indentation and spectroscopic measurements. (g) Optical micrograph of a yy cross-section, illustrating the location of the impact region. (h) Actual sample after polishing. (i) Higher magnification micrograph of the impact region of (g). P_{ii} refer to nanoindentation experiments performed perpendicular to the ii -plane.

Microstructural adjustments are also commonly employed by species as a way to provide an extra level of control over the properties of their extra-cellular tissues and tools, with specific examples including porosity gradients in bones ⁵⁵ or the preferred crystalline orientation in both vertebrate and invertebrate teeth ²⁰. Stomatopods employ both strategies in their dactyl clubs. Moving from the bulk of the club to its surface, it

was found that amorphous calcium carbonate is gradually replaced by amorphous calcium phosphate, which is itself progressively substituted by hyper-mineralized calcium phosphate in the form of hydroxyapatite, with nanoindentation data distinctly showing that this chemical gradation correlates with elastic modulus and hardness ones. Synchrotron *x*-ray mapping indicated that the apatite crystallites are preferentially oriented with their long axis perpendicular to the surface or equivalently parallel to the loading direction during impact.

Here we investigated the impact surface in more detail using confocal Raman microscopy, polarized light microscopy, Energy dispersive spectroscopy (EDS) mapping, Electron Probe Micro Analyzer (EPMA), multi-directional depth-sensing nanoindentation, and indentation fracture studies with the goal to elucidate the relationships between mineral phase distribution and texture with the micro-scale mechanical properties. Only through the use of these combined techniques, were we able to identify two new impact-region specific crystalline phases that were not previously detected in stomatopod dactyl segments, namely Fluorapatite (FAP) and Calcium Sulfate (CAS), with the former progressively substituting amorphous calcium phosphate (ACP) closer to the impact surface. *Ab initio* computations of the FAP/CAS interface suggest that the two minerals are stabilized by a network of hydrogen bonding, with a greater binding energy than computed for Hydroxyapatite (HAP)/CAS interfaces. Nanomechanical data also reveals clear evidences of elastic modulus and hardness anisotropy in the impact region for both types of species (the smashers and the spearers), with the highest values measured normal to the impact surface. Our results imply that fluorine substitution for hydroxyls in the apatite crystal lattice is an important parameter in endowing stomatopod hunting appendages with optimal mechanical and surface chemistry properties, that CAS provides additional mechanical and chemical stability, and that the preferred crystallite orientation correlates with stiffness and hardness anisotropy. Finally, indentation fracture studies indicate a higher damage tolerance for the smasher type of stomatopods, which is attributed to residual organic chitin phase in the impact region that is absent in the spearer species.

4.2 Results

4.2.1 Microstructural features and preferred fiber orientation

Optical micrographs of a spearer sample (yy cross-sectional plane, Figures 4.2a-b) show a clear distinction between the bulk material and the impact region, which can also be discerned on low magnification FESEM images (Figures 4.3a-b). The bulk material is made of successive layers about 10 to 50 μm thick with an orientation that follows the outer contour of the cross-section. The impact region is about 50 mm thick at the center of the region but decreases on the sides. In the smasher sample, the impact region is divided into two sub-domains (Figure 4.1d). Sub-domain I, which is adjacent to the periodic region of the bulk, is about 500-700 μm thick, and exhibits elastic modulus (E) and Hardness (H) values of 45 and 1.8 GPa²⁵, respectively. Sub-domain II is thinner (about 50 μm thick) and expands towards the impact surface, with E and H values sharply increasing from sub-domain I.

Figure 4.2. Optical micrographs of a sectioned and polished spearing appendage (yy cross-section). (a) Bright field image. (b) Polarized light image. (c-e) Polarized light micrographs at various sample rotations. Note the apex region appearing bright at 0° and 90° orientations, and in dark at a 45° orientation.

Optical micrographs further reveal that the spear impact region exhibits two symmetrical extruded edges on each side of the cross-section. Because of this extruded geometry, these edges are subjected to the highest contact forces during impact or grasping. Polarized light microscopy imaging reveal that the edges are birefringent, as revealed by the bright and dark contrast at sample/analyzer orientations of 0 and 90°, and 45°, respectively, which is suggestive of a fibrous or lamellar micro- and nano-structure in biological materials ¹⁵⁷. FESEM studies of a spear fracture surface confirm this hypothesis. At high magnifications (Figure 4.3c), a fibrous/lamellar microstructure is observed, with a preferred lamellar orientation perpendicular to the outer surface. Further evidence of the fibrous/lamellar structure of the edges was obtained by imaging a polished sample by AFM (inset), which confirm the fibrous structure of the edge with fibrous/lamellar features measuring 50-200 nm thick. This is stark contrast with the center of the impact region (Figures 4.3d and f), which exhibits equiaxed crystallites with no preferred orientation. A noteworthy feature of these crystallites is that their size clearly decreases towards the outer surface by nearly 10-fold, with an average size of 100-200 nm near the bulk down to 10-30 nm at the surface. Investigations by synchrotron *x*-ray diffraction on the smasher clubs had previously indicated that the apatite crystallites are preferentially oriented perpendicular to the surface near the impact region.

Figure 4-3. FESEM micrographs of the impact region fracture surface from a spearing appendage. (a) Low magnification side-view and (b) top view of the impact region. (c) High magnification view of the apex region that is subjected to the highest impact forces, showing the elongated bundle of crystallites between adjacent channel-like structures. Inset: Atomic Force Microscope (AFM) zoom-in illustrating the lamellar microstructure, with lamellae 50 to 200 nm thick. (d) and (f) High magnification views of the center of the impact region, showing the presence of equiaxed crystallites as opposed to elongated fibers observed in (c). Note that the crystallite size decreases towards the outer surface. (e) Sub-region of the impact region with a contour line highlighting regions where no crystallites are observed. All features described were consistently observed in various samples.

4.2.2 Chemical mapping and gradients

EDS elemental mapping (Ca, P, F, S, Mg, C) of a spear sample cross-section (Figure 4.4) show that Ca and Mg are all present in significant concentrations, whereas C is detected in relatively small amounts. These results are analogous to previous data reported for *Odontodactylus scyllarus* club samples, where the bulk region was found to be mainly comprised of ACC with Mg playing a role of stabilizing the amorphous over the crystalline carbonate¹⁵⁸. Mapping in the impact region over much longer time durations, however, provides further important findings. In addition to the high phosphorous concentration, sulfur and fluorine signals are also detected, with the S mapping showing enriched regions along the contour of the impact region. S and F are similarly distributed in the club samples (Figure 4.5). Since EDS is only semi-quantitative for light elements, we used EPMA in order to obtain quantitative elemental analysis (Table 4.1). The C content decreases from ca. 17 at. pct in the bulk to 7 at. pct in the impact regions. In contrast, the S content is 4 to 5-fold higher in the impact regions, suggesting that sulfur is not associated with residual organic material. This finding is confirmed further by the Raman spectroscopy data.

Figure 4.4. Energy Dispersive Spectroscopy (EDS) elemental maps of a sectioned and polished spearing dactyl segment. (a) Backscattered electron (BSE) micrograph, revealing the local differences in electron density (yy cross-section). **(b)** Calcium. **(c)** Phosphorous. **(d)** Carbon. **(e)** Point spectrum acquired in the impact region. **(f)** Magnesium. **(g)** Sulfur. **(h)** Fluorine. All maps were background-subtracted.

Figure 4.5. EDS elemental maps of a sectioned and polished dactyl segment from a smashing stomatopod. The backscattered electron micrograph (BSE) reveals the local differences in electron density (yy cross-section). Elemental distributions are shown for calcium (Ca); phosphorous (P); carbon (C); magnesium (Mg); sulfur (S); and fluorine (F). All maps were background-subtracted.

Table 4.1. Elemental composition (in atomic percent) determined by Electron Probe Micro Analysis (EPMA). Note the decrease of C from the periodic (bulk) to the impact region, and the 4 to 5 times higher content of S in the impact region as compared to the periodic region. Together with evidences from Raman spectroscopy (Figure 4.6), S is attributed to the hydrated calcium sulfate phase gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$). Neither Sr nor Ba was detected by EPMA.

Region \ Element		O	Ca	P	C	F	Na	S	Mg	Cl	K	Total
Spearer	Impact region	53.8	19.4	10.8	7.5	6.1	1.5	0.82	0.1	0.04	0.01	100
	Periodic region	54.5	16.6	7.7	16.2	3.9	0.2	0.15	0.6	0.2	0.01	100
Smasher	Impact region	58.8	17.4	10.9	6.8	4.1	1.2	0.40	0.2	0.03	0.01	100
	Periodic region	54.3	14.6	6.8	18.1	4.2	0.1	0.10	1.6	0.1	0.01	100

Raman spectroscopy profiles (range $800\text{--}1200\text{ cm}^{-1}$) from the bulk to the surface region (Figure 4.6a, spear sample) indicate the following salient features. In the bulk, ACC is the main band detected (trace *i*), but the ACC intensity decreases towards the impact region (trace *ii*). Amorphous calcium phosphate (ACP) exhibits an opposite trend, namely a low intensity peak appearing deep in the bulk and becomes the dominant peak close to the bulk-impact surface interface. Approaching the free impact surface, the carbonate band diminishes significantly, whereas the phosphate peak sharpens significantly –indicative of higher crystallinity– and shifts towards 965 cm^{-1} , which is associated with FAP¹⁵⁹. A sulfate band (1007 cm^{-1}) is also observed in the impact surface region. Raman spectra profiles of the club sample (Figure 4.6b) show identical trends. EDS and EPMA elemental data as well as Raman spectroscopy analysis indicate that the outer layer is composed of FAP instead of Hydroxyapatite (HAP), which was previously not distinguishable by *x*-ray diffraction analysis of the (002) reflection, and that a sulfate phase is also present in the appendages.

Figure 4-6. Raman spectra (range 800–1200 cm^{-1}) profiles from bulk to impact surface. (a) spearing and **(b)** smashing dactyl segments. Broad band centered at 1080 cm^{-1} : Amorphous Calcium Carbonat (ACC). Band centered at 955 cm^{-1} : Amorphous Calcium Phosphate (ACP). Sharp band shifted towards 965 cm^{-1} : crystalline Fluorapatite (FAP). Comparison with fluorine EDS maps (Figures 4-4, 4-5) suggests that ACP is also predominantly fluorinated. Band at 1007 cm^{-1} (only found in the impact region): Calcium sulfate (CAS, gypsum). **(c)** Polarized Raman spectra (range 540–660 cm^{-1}) acquired in the apex region, with polarization angles of 0 and 90° relative to the free surface. **(d)–(h)** Raman mapping of the spear impact surface with a box in **(a)** highlighting the region where Raman mapping was carried out. **(d)** Carbonate intensity. **(e)** Sulfate intensity. **(f)** Phosphate intensity (full phosphate band, with center of Gaussian fit adjusted for integration). **(g)** Center of phosphate peak, showing a clear shift at the interface from ACP to FAP. **(h)** Full Width at Half Maximum (FWMH) of the phosphate band, with low values indicating crystalline FAP and a high value disordered, ACP phase. Note that high crystallinity of FAP is co-localized with the sulfate intensity in the impact region and vice versa.

Known sulfated biominerals include the hydrated CAS gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), barite (barium sulfate, BaSO_4) and celestite (strontium sulfate, SrSO_4)¹⁶⁰. In EDS area elemental analyses, Ba is not found anywhere in the clubs, whereas trace amount of Sr is detected, but with little variation for both the bulk and the impact regions (Figure 4.7). With EPMA neither Ba nor Sr is found (Table 4.1), whereas the S concentration is 4- to

5- fold higher in the impact regions. These data suggest that trace amount of Sr is substituted for Ca in the apatite lattice, but not associated with sulfate. A further confirmation of the source of the sulfate phase is obtained by inspecting the Raman shifts associated with the SO_4^{2-} n_1 symmetric mode. For gypsum, barite, and celestite, these bands are centered at 1008, 989, and 1000 cm^{-1} , respectively¹⁶¹⁻¹⁶³. In our measurements, the Raman shift is located at 1007-1008 cm^{-1} . Taken together, these spectroscopic data lead us to conclude that the sulfated compound in the clubs is CAS. The n_1 mode also provides additional information about the level of hydration of the CaSO_4 phase: partially dehydrated bassanite ($\text{CaSO}_4 \cdot 0.5\text{H}_2\text{O}$) and anhydrite (CaSO_4) are centered at 1015 and 1017 cm^{-1} , respectively¹⁶¹, thus implying that the CAS phase is the hydrated gypsum.

Figure 4.7. High signal-to-noise ratios EDS spectra of both types of stomatopod's dactyl appendages, for both the impact (blue) and bulk (periodic, red) regions. Bottom spectra are zoom-in of the low-intensity region for better visualization of lower abundant elements. Note in particular the decrease of C intensity from bulk to impact, the detection of Sr in trace amount (with no distinguishable intensity differences between bulk and impact regions), as well as the detection of S solely in the impact region.

Polarized Raman measurements in the impact region at 0 and 90° polarization angles relative to the free surface were also conducted. Salient features of these spectra in the range 540-660 cm^{-1} (Figure 4.6c) are the presence of two maxima at 582 and 592 cm^{-1}

attributed to the n_4 phosphate vibration mode ¹⁶⁴ and displaying opposite intensity trends. The 582 cm^{-1} peak is the most intense at 90° , whereas at 0° the maximum intensity shifts toward the 592 cm^{-1} peak. Finally, Raman spectra of the impact region were also acquired on three mutually perpendicular surfaces prepared according to Figure 4.1f (which are identical to the surfaces probed by nanoindentation described in the next section). The relative intensity of the main FAP peak centered at 965 cm^{-1} is slightly lower when probed in the zz direction as compared to the yy direction, whereas the relative intensity of the 1073 cm^{-1} peak exhibits an opposite trend with a higher intensity in the zz direction (Figure 4.8).

Figure 4.8. (a) Raman spectra acquired parallel to the yy and zz directions, with insets representing the Raman vibrational modes of the phosphate groups identified by ab initio computations. (b) Comparison of spectra in hydrated and dry conditions in two regions of the spearer, indicating no difference in composition and peak location in the hydrated state.

In order to better visualize the distribution of the various phases, we performed confocal Raman imaging (Figures 4.6d-h and Figure 4.9). Not surprisingly, the carbonate (Figure 4.6d) is the dominant mineral phase in the bulk, mostly in an amorphous form. Interestingly the carbonate map also indicates a periodicity that follows the layered structure observed by optical microscopy and SEM. The overall phosphate distribution

(Figure 4.6f) unambiguously shows a higher relative intensity of phosphate in the impact region. The spatial discrimination between ACP and FAP was assessed by mapping the spectral position of the center of the phosphate band (Figure 4.6g). The resulting map clearly shows that the phosphate band is centered at 955 cm^{-1} throughout the bulk, whereas the entire impact region is centered at 965 cm^{-1} , corresponding to FAP. The interface between the two regions appears to be steep as visually illustrated by the sudden color change from green to red. The Full Width at Half Maximum (FWMH) of the phosphate band, which can be related to phase crystallinity (Figure 4.6h), further indicates that the peak is sharper in the impact region, indicating higher crystallinity of the FAP phase. Sulfate band integration was also performed (Figure 4.6e) and shows that sulfate is only present in the impact region and is co-localized with the FAP.

Further subtle details emerge by comparing the sulfate with the carbonate and phosphate distributions: a higher relative intensity of the sulfate peak is observed in a strip region approximately $10\text{ }\mu\text{m}$ thick directly adjacent to the bulk, and this correlates with an almost zero intensity of the carbonate band. In addition, depleted zones (contour highlighted in Figure 4.6e) of the sulfate distribution within the impact region correlate with a local decrease of crystallinity of the phosphate phase (highlighted green/yellow zone of FWHM map in Figure 4.6h). These regions are also characterized by a higher content of carbonate (Figure 4.6d) and Mg (Figure 4.4). Under high resolution FESEM (Figure 4.3e,) this zone also features a clear microstructural distinction in comparison to the regions that are characterized by a sharp FWHM (Figure 4.3f), with no crystallites observed where the CAS intensity is low. Taken together, the data suggest that enriched sulfate concentration directly correlates with higher crystallinity of the FAP and that inversely, depleted sulfate patches together with higher carbonate and Mg content, decrease FAP crystallinity.

Figure 4.9. Raman mapping of a sectioned and polished dactyl segment from a smashing stomatopod with box highlighting the zones where Raman mapping was carried out. The four types of maps are, from left to right: (1) phosphate intensity (full phosphate band, with center of Gaussian fit adjusted for integration); (2) center of phosphate peak, showing a clear shift at the interface from ACP (centered at 955 cm⁻¹) to FAP (peak center at 965 cm⁻¹); (3) Full Width at Half Maximum (FWMH) of the phosphate band, with low values indicating crystalline FAP and high value disordered (ACP) phase; (4) sulfate intensity.

Further electron diffraction analyses by Transmission Electron Microscopy (TEM) also confirmed the presence of both crystalline and amorphous domains in the impact regions (See Materials and Methods, Transmission Electron Microscopy (TEM))

observations), with crystalline reflections that could be assigned to both FAP and CAS. TEM investigations are useful to distinguish crystalline from amorphous areas of biominerals in the sub-micrometer range ^{36,165}; however these materials, including bioapatites, are often prone to damage during observation, including irradiation-induced crystallization. In the present study, high resolution imaging at 200 kV acceleration voltage led to sample damage within a few seconds of observation (Figure 4.10). At lower acceleration voltage, beam damage was slower but still noticeable (Figure 4.11). Thus, confocal Raman proved a more suitable method to distinguish ordered versus disordered domains in the present work, especially for imaging microstructural features larger than ~ 5 nm.

Figure 4.10. High-resolution TEM observations in the impact region. (a) and (b) same regions (zoom-in in the bottom images) at various times, illustrating the formation of small crystalline domains under the beam (fringes in b not present at time 0). (c) Different regions observed after stabilization of the new phase under the beam. The fringes near the edge are 2.41 Å apart and correspond to the $\{200\}$ planes of CaO as described in Ref. [51]. From the analysis of the upper part of the image in (c), a set of fringes 2.41 Å apart running parallel to the surface of the sample and at 90° with respect to those running perpendicular to the surface can be observed. This set of fringes can be univocally assigned to one of the $\langle 100 \rangle$ zone axes of CaO, forming from FAP by beam irradiation.

Figure 4.11. Low acceleration voltage TEM imaging and electron diffraction patterns. (a-b) Bright field images, with red arrows on the higher magnification micrographs indicating beam damage. (c) Electron diffraction pattern of crystalline domains with diffraction spots assigned to (left) FAP ($D = 2.80 \text{ \AA} \rightarrow [121]/[211]$; $D = 2.28 \text{ \AA} \rightarrow [122]$) and (right) gypsum ($D = 2.46 \text{ \AA} \rightarrow [-231]$). The same pattern is shown in each image, with the assignment for each phase highlighted separately for clarity. (d) Electron diffraction pattern of an amorphous domain at $t = 0$ (top) and $t = 5 \text{ min}$ (bottom), illustrating crystallization of the amorphous domains after a few minutes of irradiation-induced damage.

4.2.3 Nanoindentation and indentation fracture

Reduced modulus (E_r) and hardness (H) mapping on a yy cross-sectional plane of a spear sample (Figure 4.12a) clearly indicate the presence of a steep mechanical gradient from the bulk, periodical region to the impact surface region, as previously found in dactyl smasher samples. E_r values abruptly increase from 25 – 30 GPa in the bulk up to 65 – 70 GPa in the impact region, with peak values of 75 GPa measured immediately next to the outer surface. These gradients are directly correlated to variations in the phase chemistry: the softer bulk region is made of ACC (see associated Raman spectra in Figure 4.6a), the intermediate region corresponds to the transition from ACC to ACP

(green color on the indentation map), whereas the stiffest and hardest regions close to the impact surface are composed of FAP with small amounts of CAS (compared Figure 4.12a with Figure 4.6a). The hardness mapping features identical trends, with H increasing from 1 – 1.5 GPa in the bulk up to 6 – 6.5 GPa in the impact region. In order to correlate further the mechanical properties with the local crystallo-chemistry, high-resolution nanoindentation linescans were conducted across regions comprising the localized disordered zones (displayed in Figures 4.3e and 4.6h) as well as their adjacent crystalline domains. These scans with corresponding SEM images (Figures 4.12b-c) indicate that in the localized disordered zones (highlighted in yellow, line 2), the Young's modulus gradient is less pronounced (E_r increasing from 50 to 55 GPa) than along line 1, where only crystalline domains are probed and where the modulus rapidly increases to maximum values of 75 GPa. Along line 2, as soon as the indents were placed on the crystalline regions, a sudden increase in E_r is noticed, leading to values near the outer surface that are identical to those measured along line 1.

Figure 4-12. Mechanical characterization of the impact region of a sectioned and polished spearing dactyl segment measured by nanoindentation. (a) Reduced modulus (E_r) and Hardness (H) maps (yy cross-section). Latin numbers correspond to Raman spectra displayed in Figure 4-6a. **(c-d)** High-resolution nanoindentation line scans. Line 1 (in the center of the impact)

spans from the ACC bulk with a sharp transition to the FAP/CAS regions, whereas line 2 (along the extruded edge) encompasses the bulk (blue), the localized disordered zones (yellow) and the ordered FAP/CAS regions near the outer surface. **(b)** E_r distribution plots measured in the impact region, normal to the three cross-sectional planes shown in Figure 4-1. **(e)** and **(f)** Average values of E_r of a spearer sample (e) (same data sets as in (b)) and of a smasher sample (f) in the impact region measured normal to the xx , yy , and zz directions, illustrating the mechanical anisotropy of the impact surface. * $p < 0.001$. **(g)** Schematic representation of FAP crystallite orientation in the impact layer.

Given the preferred orientation of mineralized fibers in the impact region detected in both types of samples, the mechanical response was probed by indentation across three mutually perpendicular planes, with all indents performed in the extruded edge discussed above. A distribution plot and average values of a spear sample (Figures. 4.12d and e) show that the modulus is clearly anisotropic, with average values of 77.6 +/- 4GPa normal to the free surface (zz plane), 67.4 +/- 2.9GPa normal to the yy plane, and 64.2 +/- 2.3GPa normal to the xx plane. The anisotropic response for a smasher sample was also probed (Figure 4.6f). While the absolute values are lower than the spearer sample, a similar anisotropic response is obtained and found to be statistically significant. Normal to the impact surface (zz plane), the modulus is 61.1 +/- 3.1 GPa, whereas normal to the yy and xx planes, the modulus are 55.5 +/- 2.8 GPa and 51.1 +/- 3.6 GPa, respectively. In summary, stiffness anisotropy is detected in both types of stomatopod's dactyl segments.

Fracture toughness values were evaluated using a methodology we previously developed for sponge biosilica³. A sharp cube corner tip was selected for these experiments because of its ability to induce cracking at external loads one to two orders of magnitude lower than the Berkovich geometry. In addition a high-load transducer allowing to perform indents from a few mN up to the N range was employed. A few indents were placed at each load (representative indentation fracture curves are provided in Figure 4.13), which were subsequently observed by FESEM in order to determine the fraction of cracked corners.

Figure 4-13. Representative indentation fracture curves in impact regions measured with a high-load transducer and a cube-corner tip. Notice the lower load scale for the spearer appendage, illustrating the lower loads at which damage events occur. Corresponding post-indentation FESEM micrographs are shown in Figure 4-14.

With this method, a nucleation load P_c corresponding to the critical load for sharp indentation cracking is obtained (defined as the fraction of cracked corners of 0.5), from which a value of fracture toughness *at initiation* can be evaluated using a micromechanical model presented in Ref. [166]. In comparison to the H and E values combination, a clearer contrast between the species is observed, with a P_c value for the smasher club of 145 mN *vs.* 20 mN for the spearer appendage (Figure 4.14). This translates into fracture toughness values of $2.02 \text{ MPa m}^{1/2}$ and $1.47 \text{ MPa m}^{1/2}$, respectively. In order to complement these fracture data, we also acquired Raman spectra in the impact regions. A Raman shift doublet near 3000 cm^{-1} corresponding to chitin, as well as minor bands in the range $1300\text{--}1400 \text{ cm}^{-1}$ also attributed to chitin [167], were detected in the smasher dactyl club impact region but not in the spearer species (Figure 4.14b).

Figure 4-14. Indentation fracture analysis of the dactyl appendages impact regions. (a) Cracking frequency at indent corners vs. peak load (cube corner tip). **(b)** Representative Raman spectra from 800 to 3200 cm⁻¹ wavenumber acquired near the indents. Chitin is detected in the impact region of the smasher dactyl club, but not of the spearer dactyl appendage. **(c)** Scanning Probe Microscope (SPM) and SEM micrographs of residual indents on spearer samples after indentation peak loads of 3 mN (left) and 40 mN (right). **(d)** SEM micrographs of residual indents on smasher samples after indentation peak loads of 30 mN (left) and 500 mN (right).

4.3 Discussion

The predatory success of stomatopods is intimately linked to the mechanical efficiency of their dactyl spear and club-like dactyl modifications, and the impact surface is especially important in inflicting large forces during feeding and defense. The present data provide important refinements to the crystallo-chemistry of the phases present in the impact region of stomatopod dactyl segments. Raman spectroscopy provides clear evidences, in both types of appendages, of the amorphous-to-crystalline transition of the apatite phase, which from the peak location (965 cm^{-1}) (Figure 4.6a-b) appears to be FAP¹⁵⁹ as opposed to HAP. This Raman shift of the phosphate peak location towards FAP in the impact region is clearly visualized in the map displayed in Figure 4.6g. Finally, the presence of FAP as the dominant crystalline phase is corroborated by high resolution EDS mapping (Figure 4.4) as well as by EPMA quantitative elemental analysis (Table 4.1), which show fluorine enrichment in the impact region that correlates with higher phosphorous and calcium distributions. These variations in phase chemistry and ordering are directly correlated to mechanical gradients.

As the main phase from the bulk to the impact surface transitions from ACC to ACP to FAP, the dactyl clubs become stiffer and harder. Localized disordered zones in the impact region, however, appear significantly softer and less stiff (Figures 4.12b-c). Given that maximum principal stresses during impact are predicted to be located near the impact/periodic region interface²⁵, this may imply a mechanical damping role for these localized, more compliant disordered zones. The usage of phosphate over carbonate, which is the common mineral building block synthesized by many invertebrates for most of their protective structures, is unusual. In fact, the occurrence of apatite in general is uncommon among invertebrates^{132,168}, although FAP was recently reported in the outer crown of crayfish mandibles²⁰, which also displayed a carbonate-to-phosphate transition followed by an amorphous-to-crystalline conversion of the apatite phase. These observations suggest that the synthesis of apatite in the biotools or teeth of invertebrates, especially crustaceans, may be more widespread in Nature than previously recognized.

From a mechanical and physicochemical perspective, there are several advantages for the animal to process FAP for their mechanically-active appendages. First, as a consequence of its more compact structure and higher mechanical stability, FAP

appears to be slightly stiffer and stronger than HAP, as reported by Enax *et al.*⁴⁷ who measured higher values of E_r and H in FAP single crystals in comparison to HAP. Other evidences of higher modulus in FAP have been obtained using nanomechanical¹⁶⁹ and acoustic emission microscopy techniques¹⁷⁰. Furthermore, fluoride ions have been reported to enhance the matrix protein-mineral interaction and to facilitate packing and organization of apatite crystal arrays¹⁷¹. *Ab initio* computational studies of various apatites have also suggested that FAP has a slightly higher elastic and bulk modulus than HAP¹⁷². Perhaps more significant, fluorination is well established to decrease the solubility of apatite in water by several orders of magnitude^{173,174}, resulting in its extensive incorporation in the jaws of aggressive marine predators, such as sharks^{175,176}. Likewise, the impact surface of stomatopod dactyl segments must be shielded from chemical dissolution in order to fulfill their function, and thus it is not surprising that FAP is chemically favored over HAP.

Even more peculiar than FAP is the occurrence of a sulfated biomineral in the impact region in both types of dactyl segments. To the best of our knowledge, a sulfated mineral had not previously been reported in load-bearing biological materials. In comparison to phosphates and carbonates, sulfates are not commonly synthesized by living organisms and when they are, they serve highly specific function such as in the statoliths of jelly fish ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, gypsum) or in certain algae (BaSO_4 , barite)¹⁷⁷. This rare occurrence of CAS begs the question of its role in stomatopod dactyl segments. Confocal Raman imaging provides key insights to answer this question. A higher sulfate concentration in the form of gypsum is concomitantly accompanied with a higher crystallinity of the fluoroapatite phase (compare Figure 4.6e and Figure 4.6h). Thus, the data suggest that there is a direct correlation between sulfate content and FAP crystallinity, indicating that CAS may play a role in the disordered-to-ordered transition of FAP. In order to test this hypothesis, we performed *ab initio* computations of interfacial FAP/CAS relationships. Using the COMPASS force field package¹⁷⁸, the three structures were first constructed and verified against established crystal structure lattices¹⁷⁹. Various interfacial crystallographic relationships were tested, and the $[100]_{\text{FAP}}//[100]_{\text{CAS}}$ gave the best interfacial lattice matching (Figure 4.15).

Figure 4-15. FAP/CAS interfaces computed by *ab initio* simulations. Hydrogen bonding network is predicted to stabilize the interface along the $[100]$ directions of both crystals. The binding energy in FAP/CAS is 2 kCal/mol stronger per hydrogen bond than in Hydroxyapatite (HAP)/CAS.

Computations indicated that the interface can be stabilized by a network of interphase hydrogen bonding between FAP and gypsum, suggesting that hydrogen bonding plays an important role in providing binding strength at the interface. The computations, which are based on the density functional theory whose capabilities have been used extensively in order to predict H-bonding network^{180,181}, also show that the hydrogen bond network is mediated through the gypsum water molecules, suggesting that the hydration state of sulfate plays a specific stabilization role at the interface. In order to compare the influence of fluoride ions on interface stability, we computed the bond length and bond energies of both FAP/CAS and HAP/CAS interfaces. For CAS/FAP, we found that the H---F hydrogen bond length interface is 1.95 Å versus 2.06 Å for the H---OH bond length of the CAS/HAP interface, and that the binding energy of the FAP/CAS interface is 2 kCal/mol stronger per hydrogen bond. Taken together, these data imply that CAS could play a templating role for the disordered-to-ordered transition of FAP, thus explaining the co-localization of crystalline FAP with CAS. One possible two-steps mechanism for this phase transition would be for CAS to first stabilize ACP, which could

then crystallize using the $[100]_{\text{CAS}}$ plane as a template that lowers the driving force for crystal nucleation and leads to a lower total free energy of the system. Epitaxial relationships between FAP and hydrated CAS have previously been observed for synthetically grown FAP crystals¹⁸². Moreover, the stronger binding energy of the FAP/CAS interface suggests that fluorine ions play a role to further optimize the stiffness and strength of the biomineralized complex near the outer impact surface.

In addition to this chemical strategy, the mechanical response of dactyl segments impact regions is further tailored through preferred crystalline orientation, and this strategy is detected in both species. In the spearing type, polarized light and electron microscopy images indicate that FAP exhibits a polycrystalline texture, with the long crystallite axes oriented perpendicular to the impact surface. Further evidence of the preferred orientation of FAP crystallites in the vicinity of the impact surface is supported by polarized Raman spectra acquired at 0 and 90° polarization angles relative to the free surface. The relative maxima of the ν_4 phosphate vibration mode (582 and 592 cm^{-1}) match those measured on single HAP and FAP crystals (Figure 4.6c)¹⁶⁴, namely the intensity ratio (I_{582}/I_{592}) is less than unity at 90° and higher than unity at 0°, indicating that the c -axes of FAP crystallites are oriented perpendicular to the impact surface. A final line of evidence confirming the preferred orientation of the FAP crystallites was obtained by inspecting the Raman spectral response from different directions, which showed variations in relative intensities between the major (965 cm^{-1}) and the minor (1075 cm^{-1}) phosphate ν_1 bands when probed in the zz and yy orientations, respectively (Figure 4.8). These variations in relative intensities were further corroborated by computing the Raman vibration modes using *ab initio* computations (See Materials and Methods, *Ab initio* Raman computations). In the dactyl clubs from the smashing species, synchrotron x-ray diffraction data previously showed that the apatite crystals were preferentially oriented with their c -axis perpendicular to the impact surface.

FAP has a hexagonal crystal structure with an anisotropic stiffness tensor, resulting in a higher modulus in the direction normal to the basal plane (parallel to the lattice c -axis) than normal to the prismatic planes (parallel to the lattice a - and b -axes). Stiffness constants of FAP have been computed by *ab initio*¹⁷² and force field¹⁸³ simulation methods. The *absolute* values of computed FAP stiffness constants, in the

range 126-150 GPa for C_{11} and 167-180 GPa for C_{33} –which would correspond to the moduli if the measurements were ideally carried out normal to the basal and prismatic crystal planes, respectively– are clearly higher than the modulus values of stomatopod's dactyl segments. This is likely related to the polycrystalline nature of the FAP crystals and to the presence of a thin organic matrix in the dactyls, which can lead to inter-crystalline sliding during indentation measurements. Meaningful comparisons between computations and experiments, however, can still be obtained by comparing the stiffness constant ratio C_{33}/C_{11} . Menendez-Proupin *et al.*¹⁷² reported a C_{33}/C_{11} ratio of 1.33, while Mkhonto and De Leeuw¹⁸³ obtained a ratio of 1.17. For the spearer sample, the ratio of the reduced modulus normal to the impact surface over the reduced modulus parallel to the impact surface E_{zz}/E_{xx} is equal to 1.21, whereas for the smasher sample the ratio is $E_{zz}/E_{xx} = 1.18$ (Figures. 4.12d-e). The moduli ratios, close to 1.20 for both species, are within the range predicted by computer simulations on single crystals, confirming the textured orientation of FAP crystals in the impact region with the basal plane oriented parallel to the impact free surface.

Experimental measurements of the C_{33}/C_{11} ratio of FAP single crystals have also been carried out using an ultrasound technique¹⁸³, and values in the range 1.22–1.25 were reported, again close to the present experimental data.

It should be mentioned that the ratios E_{zz}/E_{yy} are significantly smaller (1.15 and 1.10 for the spearer and the smasher, respectively), which can be attributed to two main sources. First, the xx -plane in our experiments is perpendicular to the yy -plane, whereas in the hexagonal crystal structure the prismatic planes are oriented at 60° from each other. Even if the FAP crystals were perfectly oriented with the c -axis normal to the zz measurement plane, either the xx or the yy planes would not coincide to a main prismatic crystal plane. Second, it is likely that not all grains are perfectly oriented with the c -axis normal to the impact surface, resulting in less elastic asymmetry during measurements because indentation are actually carried out at an angle from the principal crystal axes.

While optimized hardness and modulus are important for maximizing impact forces during prey knocking, an additional biomechanical requirement is a fracture resistance in the impact region that is high enough to prevent permanent micro-damage to the appendages. Although the microscopic size of the impact region (< 100 μ m)

precludes the usage of standard fracture toughness specimens, as has for instance been done for other biominerals such as conch shells¹⁰⁰ or bone³² (even “miniaturized” samples with sizes in the mm range are orders of magnitude larger than the dactyl appendages’ impact regions), fracture toughness was assessed by indentation fracture³, which is also deemed suitable as a comparative method (comprehensive fracture toughness studies could perhaps be conducted by machining micro-scale beam specimens, and subsequently testing them by bending with a nanoindenter or AFM. However, nanoscale sample machining of biological samples with techniques such as focus ion beam currently does not allow to prepare damage-free specimens of suitable length scale).

The fracture data, summarized in Figure 4.14, indicate a better tolerance to damage for the smasher species with fracture toughness values of $\sim 2 \text{ MPa m}^{1/2}$ versus $1.5 \text{ MPa m}^{1/2}$ for the spearer species. The data should be considered with a certain level of caution because the *R*-curve behavior, namely the increase in fracture resistance with crack propagation due to the building up of a fracture process zone⁴², is not captured by indentation fracture. Hence, the present values should be regarded as fracture toughness at *initiation*. Nevertheless, the contrast in the critical nucleation load P_c between the two species is evident (125 mN for the smasher vs. 20 mN for the spearer) and strongly suggests a relation with evolution pressures and hunting strategies. Whereas the spearer species does in some instance use its dactyl segments to punch its preys, its main approach is to grab them with the dactyl barbs, meaning that the appendage is more often loaded in a bending regime. In contrast, the smasher exclusively uses hard blows, which imposes a higher tolerance to impact damage for its club. Chitin was detected in the impact region of the smasher dactyl segments by Raman spectroscopy, but not in the spearer dactyl segments (Figure 4.14b). Given the well-known role of thin organic phases in biocomposites to enhance the fracture toughness^{42,155}, the data suggest that over evolution timeline the smasher species, which evolved from the spearer ones²¹, has incorporated residual chitin in the impact region in order to increase the damage tolerance of its dactyl club. The presence of a small amount of chitin also explains the slightly lower hardness and modulus of the smasher clubs (Figures 4.12e-f).

Stomatopods have evolved a dual chemical and microstructural strategy for the outer most region of their dactyl segments in order to inflict high impact damage to their

prey. The impact region of these remarkably robust appendages is composed of calcium phosphate with a finely-tuned phase transformation and chemistry. FAP crystallites in the impact region are oriented in a textured manner near the contact points in order to optimize impact forces perpendicular to the surface. The spatial correlation between CAS and a higher degree of FAP crystallinity, together with coherent lattice matching between the two phases as predicted by atomistic computations, suggest that FAP crystallization is enabled by the presence of CAS, an unusual biomineral that had not previously been reported in load-bearing biological materials. *Ab initio* computations indicate the FAP/CAS interface is mediated by H-bonding, which is stronger than the HAP/CAS interfacial energy. This dual presence of FAP and CAS in the impact region, coupled with the increase in mechanical properties associated with higher crystallinity, denotes a composite strategy for strengthening appendages that are subjected to high impact forces. Our data also reveal that the smasher species has optimized its tolerance to fracture damage in the impact region through incorporation of thin inter-crystallite chitin as a toughening phase. This study provides insights for the synthetic fabrication of impact and wear resistant fluoroapatite-based crystalline materials.

4.4 Materials and Methods

Sample preparation: Live smashing (*Odontodactylus*) and spearing (*Lysiosquilla*) stomatopods were acquired from commercial sources in Singapore. The second thoracic appendages from each species were removed and stored in a -20°C freezer. The dactyl segments from each appendage were embedded in an acrylic cold mounting resin (ClaroCit, Struers), sectioned with a slow speed diamond saw, and polished with P1200/4000 grade of SiC paper and then 1 and 0.25 mm diamond paste. After each step, diamond paste particles and polishing debris were removed from samples in an ultrasonic bath for 5 min. For anisotropic indentation studies, embedded samples were cut across two perpendicular planes using a slow speed diamond saw (Figure 4.1). In order to ensure that the exposed surfaces were from the impact region (namely less than a couple of microns from the original free surface), three mutually perpendicular planes were directly polished with fine diamond paste without any coarse pre-polishing. The planes were oriented such that the normal to the *zz*-plane was perpendicular to the impact surface, whereas the normal to the *xx*- and *yy*-planes were parallel to the impact surface (Figure 4.1). Energy Dispersive Spectroscopy (EDS) and Raman mapping (described below) were acquired on the second halves of the *yy*-planes.

Microstructural imaging and characterization: Polished cross-sections were imaged using a ZEISS Axio Scope A1 optical microscope in bright field, dark field, and polarized light modes. The polarizer and analyzer were fixed in cross-orientation and the images were acquired at sample rotation of 0, 45, and 90°. For FESEM, the dactyl segments were immersed in liquid Nitrogen and immediately fractured in the lateral direction and the cross-sections of were coated with 5 nm Pt. Samples were examined with a FESEM (JEOL, 7600F) at a low 1KV accelerating voltage. In order to prevent surface charging, Lower Secondary Electron Image (LEI) as well as Gentle-Beam (GB) modes were used. EDS measurements were conducted on polished and carbon coated dactyl samples (*yy*-planes), with a Bruker X-Flash energy dispersive spectrometer. EDS compositional maps were acquired at a 20 kV accelerating voltage. AFM images were acquired in the AC mode in air on polished cross-sectional samples using an Asylum MFP3D-Bio. Nanoworld SSS-NCH cantilevers with nominal resonant frequencies

between 250-390 kHz were used. Cantilevers were driven slightly below resonance at a frequency of ~286 kHz and samples were scanned at a rate of ~1 Hz in closed loop.

Transmission Electron Microscopy (TEM) observations: Preliminary attempts to prepare TEM samples by Focus Ion Beam (FIB) proved unsuccessful as the beam heavily burned the samples, even when using the minimum beam current necessary to machine the material. Thus, TEM analyses were done on a powder sample ground from the impact region of the clubs, which was dispersed in ethanol and then carefully placed on copper TEM grids. Calcium phosphates (bioapatites) have previously been analyzed by TEM, e.g. Refs. [^{184,185}]; however it is also well-known that TEM can readily lead to irradiation damage of calcium phosphates and that subtle differences in apatite chemistry leads to variation in sample stability under the electron beam ¹⁸⁶. Two types of measurements were thus performed, using either high or low acceleration voltage apparatus. High-resolution imaging was carried out on a JEOL 2100F TEM at 200 KV. During these observations, the samples were very unstable within the first few seconds of beam irradiation. In particular for initially amorphous domains, localized crystallization occurred under the beam as shown in Figure 4.10, which represents images acquired at various time points on the same location. Interference fringes were detected after 20 seconds, which were absent in the first few seconds of observation. These fringes are associated with CaO, which can form by crystallization of amorphous FAP regions as previously reported, for instance by Meldrum et al. ¹⁸⁷ or Wang et al. ¹⁸⁸.

In order to reduce sample damage, additional analyses were carried on Carl Zeiss Libra 120 Plus BioTEM operating at an acceleration voltage of 120 KV. Electron diffraction was carried out on particles initially observed in the bright field mode. To minimize sample damage, diffraction patterns were immediately recorded (Figure 4.11). Additionally in some cases, the patterns were acquired at regular intervals in order to verify any sample damage or crystallization.

Both crystalline and amorphous particles were detected (Figures 4.11c-d), confirming confocal Raman observations that both ordered and disordered regions are present in the impact region of the clubs. It should be noted that on average, more particles displayed crystalline patterns. However, even under these lower acceleration voltage conditions, sample damage was observed in the bright field images (Figures

4.11a-b) and crystallization of the initially amorphous particles was also observed under the beam, with crystalline reflections appearing after a few minutes of beam irradiation (Figures 4.11d).

For the initially crystalline particles, because the apparatus is not equipped with a sub-micron selected area diffraction aperture, diffraction arises from multiple particles leading to a powder-like diffraction pattern. Diffraction spots corresponding to FAP were clearly detected (Figure 4.11c, left). Other spots could unambiguously be assigned to gypsum and not to FAP (Figure 4.11c, right), confirming the presence of both phases in the impact region of the dactyl segments. In summary, low acceleration voltage TEM observations confirmed the presence of amorphous and crystalline regions, as well as of both FAP and gypsum. However, the spatial discrimination between amorphous and crystalline domains was not possible on these powder samples. For such information, confocal micro-Raman imaging (Figure 4.6) proved a much more suitable technique due to ease of sample preparation, lack of sample damage, and unambiguous band assignment. The ability to rapidly detect preferred orientation of FAP crystallites using the polarized mode (Figure 4.6c) is another advantageous feature of Raman spectroscopy over TEM observations in the present samples.

EPMA Analyses: Quantitative Wavelength Dispersive Spectroscopy (WDS) analyses were performed using a JXA-8530F Field Emission Gun Electron Probe X-ray Microanalyzer (FEG-EPMA). Point analyses were acquired using a 10 μm diameter defocused beam at a probe current of 10 nA and an accelerating voltage of 15 kV. Results were quantified using external calibration standards and a ZAF matrix correction procedure. Error on repeat analysis of standards was <2% of measured values.

Raman spectroscopy and imaging: Raman spectrometry of embedded and polished cross-sections were acquired with a Horiba LabRAM HR ($\lambda = 785 \text{ nm}$) equipped with an X100 microscope objective lens. Line mapping studies were performed along a straight line from the outermost layer of the bulk to the impact surface. Each Raman spectrum was acquired for 90s and smoothed using the Horiba LabSpec and OriginPro 8 software. Raman imaging was performed using a confocal Raman microscope (alpha300,

WITec, Ulm, Germany) equipped with a frequency doubled Nd:YAG laser excitation ($\lambda=532$ nm) and a piezoscanner. The laser beam was focused on the sample through an objective (60X, NA = 0.8) with a spot size of $0.61 \lambda/\text{NA}$. For imaging, an integration time of 0.3 s was used for acquisition and pixels were separated by 0.5 μm . The spectra were acquired using a CCD detector (DU401A-BV, Andor, UK) placed behind the spectrometer (UHTS 300; WITec). The ScanCtrlSpectroscopyPlus software (WITec) was used for measurements and WITec Project Plus for spectrum processing. The images were generated by integrating the intensity of the signal for the wavenumber ranges of phosphate (920–990 cm^{-1}), sulphate (990–1025 cm^{-1}), and carbonate (1040 – 1125 cm^{-1}). Regions that were subsequently characterized by indentation fracture were also probed by Raman spectroscopy in the range 800–3200 cm^{-1} , selected in order to detect the potential presence of organic components. For these measurements, the average and baseline subtraction functions of the software were used to extract the average spectra and detect the possible presence of chitin

Nanoindentation: Indentation studies were performed on three mutually perpendicular cross sections (Figure 4.1) using a Triboindenter TI-950 Nanomechanical tester (Hysitron, Minneapolis, MN, USA), equipped with a Berkovich diamond tip. A peak force of 2 mN was used for the mapping mode and 1 mN for the 3D anisotropy studies. Each indentation experiment consisted of a 5 sec. loading and 5 sec. unloading cycle. In the mapping mode, indentations were performed on the xx cross-sectional plane of the impact region with indentation spacing of 15 μm . For the 3D anisotropy study, a series of 30 indents with a 10 μm interval were carried out. Indents were placed on the three cross-sectional planes defined above (xx , yy , and zz), with P_{ii} corresponding to indents perpendicular to the ii -plane.

Identical samples preparations were used for indentation fracture experiments. These tests were performed using a high-load 10 N transducer and a cube-corner diamond tip. Peak indentation loads were varied from 1 to 1200 mN (1 to 200 mN for spearers and 10 to 1200 mN for smashers based on preliminary observations). A total number of 46 indents and 64 indents for the spearer and smasher, respectively, were placed on the most-outer regions of the samples. Following indentation, the indents were observed by

FESEM with a JEOL 7600F microscope in order to visualize chipped and cracked corners. For the lowest load indents ($< 5\text{mN}$) the scanning probe microscopy (SPM) mode of the nanoindenter was used to image the indents using a low-load (10mN) transducer and a cube corner diamond tip. The force point was set at 2mN in the imaging mode. High-resolution line scan indentations were conducted using a cube corner tip with 5mm indent spacing and a “5-2-5” sec. load function (loading-holding-unloading), with a maximum load of $1500\text{ }\mu\text{N}$. The tip was calibrated on fused quartz and polycarbonate standard samples for shallow and deep contact depths, respectively.

Ab initio computations: CAS, HAP and FAP structures were constructed using the COMPASS force field method¹⁷⁸, which yielded good agreement with tabulated crystal structure data. Several hydrogen types were introduced, based on the polar strength of the atom that the hydrogen was attached to. Due to a strong polarization effect produced by hydrogen bonding, the *ab initio* charge bond increment scheme was adopted to describe the hydrogen bonding with good accuracy. Various interfacial models between CAS and FAP (or HAP) were constructed and the (100) planes gave the best lattice matching, thus leading to a coherent epitaxial interface. Raman vibration modes for the FAP crystals were performed using the CASTEP software package¹⁸⁹, with a Norm-conserving pseudo-potential, a kinetic energy cutoff of 630eV in, and $1\times 1\times 2$ K-sampling. The phonon modes were obtained by diagonalizing the dynamical matrix, which the mass-reduced Fourier transformed into the force constant matrix. The force constants were computed by evaluating the response wavefunction with the perturbation theory. The Raman tensor was computed by the finite-difference method, *i.e.* the mode displacement derivatives of the polarizability tensor. The relative intensity and polarization dependent computed data were used to identify the modes responsible for the experimental peaks.

In order to explain the orientation dependence of the Raman spectra for the sample probed in the yy and zz direction (Figure 4-8 a), we performed *ab initio* computations of Raman vibration modes of a FAP crystal using the CASTEP software package 47. The computations indicate that the maximum intensity peak originates from the bond stretching mode of PO_4^{3-} . In this this mode the O-P-O bond has a weak in-plane

(zy plane) angular variation and a slightly higher out-of-plane (xy plane) angular variation, resulting in a computed Raman tensor with moderate anisotropy and smaller zz component. Experimentally, this translates into a slightly smaller intensity in the zz direction if the crystallites are preferentially oriented, as observed in Figure 4-8 a. The minor peak at 1075 cm⁻¹ is predicted to result from cooperative bending of PO₄³⁻ bonds and exhibits an opposite trend. The O-P-O bond in this mode exhibits a large in-plane angular variation, leading to more pronounced changes in the polarizability in the zy plane, and thus to a stronger enhancement of the Raman tensor component in the zz direction, which is also captured experimentally.

Raman tensor components of FAP bands

PO₄³⁻ stretching (centered at 965 cm⁻¹) PO₄³⁻ collective bending (centered at 1075 cm⁻¹)

$$P_{ij} = \begin{bmatrix} 1.9101 & 0 & 0 \\ 0 & 1.9101 & 0 \\ 0 & 0 & 1.833 \end{bmatrix} \quad P_{ij} = \begin{bmatrix} 0.0782 & 0 & 0 \\ 0 & 0.0782 & 0 \\ 0 & 0 & 0.8866 \end{bmatrix}$$

In the dactyl clubs from the smashing species, synchrotron x-ray diffraction data previously showed that the apatite crystals were preferentially oriented with their *c*-axis perpendicular to the impact surface.

Chapter 5 - Fracture Resistance and Energy Dissipation*

5.1 Introduction

Structure-property relationships of a wide range of biological hard tissues have been investigated in the past two decades ^{6,84}, *e.g.* nacre ⁶², mollusk shells ^{59,100}, or fish and gastropod armors ^{37,144}, providing a source of inspiration to fabricate artificial tough organic/inorganic composites ^{7,53}. In comparison to these biomineralized composites that play a passive (defensive) mechanical role, the stomatopod's (commonly called "mantis shrimps") dactyl clubs are dynamically active, hammer-like devices. Exploiting their outstanding vision ¹⁹⁰ to accurately target their prey, mantis shrimp deliver fast and powerful punches with their dactyl clubs to shatter the prey's hard shells through repetitive impact loading. Remarkably, these shells are themselves considered a benchmark of super tough biocomposites (Figure 5.1). Recent studies ^{25,191} have correlated the key chemical, as well as the nano- and micro-structural features of stomatopod dactyl clubs with their nanomechanical response, revealing that the club is essentially made of two distinct regions, an outer ("impact") region and an internal ("periodic") region. In chapter 4 we presented that the outer region exhibits spatially-inverted gradients of amorphous calcium carbonate and calcium phosphate in the form of fluorapatite (FAP)¹⁹¹. We also found that FAP gradually changes from an amorphous to a crystalline state towards the outer surface, and this transition is facilitated by templating from calcium sulphate¹⁹¹. In addition, crystalline FAP is preferentially oriented towards the outermost club surface in order to maximize impact forces. On the other hand, the inner region is made of a helicoidal structure composed of partially-mineralized chitin fibrils, where the mineral phase is mostly amorphous calcium carbonate.

While compelling details of the structural design and mechanical behavior of the stomatopod clubs have recently emerged, the main contact damage mechanisms and their relative contributions to the contact resistance of the clubs have not been explored. Here, we sought to establish and quantify the contact damage tolerance of stomatopod dactyl

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clubs using complementary nanomechanical and spectroscopic methods. Notably, our data show that the outer impact layers exhibit a quasi-plastic response related to interfacial sliding and rotation of FAP crystals, whereas the inner soft layers feature strain-hardening behavior associated with micro-channel densification. These mechanisms synergistically combine to enhance the overall damage tolerance of stomatopod dactyl clubs. Critically, brittle Hertzian cracks that would lead to catastrophic cracking of the clubs are prevented during impact because the club size remains below a critical size, below which the contact mechanics response is in the quasi-plastic regime.

5.2 Results

5.2.1 Hertzian contact response

Visual inspection of a pristine (freshly-produced) club following molting, and of a repeatedly impacted club are shown in Figure 5.1c and indicate clear differences in their surface appearance. The impact surface of used clubs exhibited significant erosion, whereas fresh ones were much more even. We subjected clubs to X-ray microtomography (micro-CT), which distinctly show a high surface roughness for the heavily used club (Figure 5.1d). On micro-CT sections of clubs that were heavily impacted, irregularities and depressions were noticed on the surface, whereas cross-sections of pristine samples indicated a smooth and regular curvature.

Figure 5.1. (a) Photographs of a fractured snail shell after impact from a stomatopod's dactyl club, showing the classical brittle cone cracks (Hertzian cracks) at the impact points that led to macroscopic fracture. (b) Schematic illustration of the impact depicting the cone shape fracture pattern in the snail shell. (c) Macro-photographs of a damaged and a pristine dactyl club. Arrows indicate eroded regions after repetitive impacts. (d) Micro-CT image of a used club (left) and cross-sections of a used and a pristine club.

Stomatopod clubs were probed by nanoindentation with a blunt (cono-spherical) tip in the zz and yy directions normal and parallel to the free impact surface, respectively (Figure 5.2a). Critically, spots in the immediate vicinity from one another (± 2 mm) of the same sample were probed across mutually orthogonal directions, as described in Materials and Methods (Section 5.4). Load-displacement curves from the impact surface to the bulk (inner layers) are displayed in Figure 5.2b (yy direction) and in Figure 5.3

(comparison between both directions). Stress-strain curves were obtained by conducting full unloading cycles at regular load increments according to the procedure described by He *et al.*¹⁹² (Figure 5.4).

Figure 5.2. Indentation response of the smasher dactyl club in distinct layers. (a) Sample preparation for bi-directional probing of contact response and schematic cross section of a club, with color coding representing the location where indentation studies were carried out (this color coding is used throughout the manuscript). (b) Partial loading-unloading curves in dry (top) and hydrated (middle and bottom) conditions. Full unloading was performed in order to obtain the contact depth and contact radius at a given peak load. The water content in hydrated conditions is 4 -5 wt. pct in the outer layers and 10 wt. pct in the inner layers. (c) Indentation stress-strain curves in dry conditions computed from the partial loading-unloading curves obtained in (b). (d) Indentation stress-strain curves (dry conditions) in the yy and zz directions depicted in (a), illustrating the anisotropic response near the impact surface. (e) Indentation stress-strain curves (hydrated conditions) computed from the partial loading-unloading curves obtained in (b). The inset shows the strain-hardening behavior of the inner layers. (f) Partial loading-unloading curves (hydrated conditions) measured at 0.6 mN/s (thick line), 6 mN/s (thin lines), and 300 mN/s (dash lines), illustrating the influence of loading rate in various regions of the clubs.

The stress values hereafter reported thus correspond to the *mean contact pressure* P_m and the indentation stress-strain response derived from this analysis are plotted in

Figure 5.2c (dry conditions, yy direction) and 5.2d (comparison between zz and yy direction). In the outer region the club exhibits a clear elastic-plastic transition with a gradual drop in yield stress (σ_y) moving from the outer layers towards the inner (bulk) layers. In the vicinity of the free impact surface, the indentation response is distinctly anisotropic. Here both the slope (elastic modulus) and the yield stress σ_y are higher in the zz (5.0 ± 0.1 GPa) than in the yy direction (3.5 ± 0.1 GPa). The anisotropic response is less marked further inside the outer layers, and was not observed in the softer bulk layers (Figure 5.2d). Quantitatively, σ_y decreases to 2.1 ± 0.1 GPa (zz direction) and to 1.6 ± 0.2 GPa (yy directions) near the interface with the inner region. Within the inner layers, the indentation stresses are much lower and remain below 1.0 GPa. In addition, the elastic-plastic transition becomes less obvious, possibly because surface yielding is initiated early at very low contact depth, even though a relatively large tip radius was used.

Figure 5.3. Anisotropic response of the dactyl clubs as measured by partial loading-unloading curves in distinct layers. These curves were used to compute the indentation stress-strain curves shown in Figure 5.2d.

Figure 5.4. Schematic definition of the contact depth (h_c) and yield stress (σ_y) point of the indentation stress-strain curve. (a) Schematic of partial loading-unloading curves of two cycles (i and i+1), with definition of contact load (p_i), residual depth (h_{ri}), contact depth (h_{ci}), and maximum depth (h_{ti}). The contact depth (h_c) at each load was extracted to calculate the contact radius (a) and the mean contact pressure (P_m) (Materials and Methods, Section 5.4). **(b)** Method used to identify the yield point. The initial linear portion of the stress-strain curve is obtained by curve fitting and the deviation with the indentation stress-strain curve represents the yield point.

Dental enamel is one of the few biomineralized hard tissues for which indentation stress-strain curves have been generated^{192,193}. It is composed primarily of hydroxyapatite (HAP), which is chemically very similar to FAP from the dactyl club, making enamel a very convenient benchmark for comparison with the outer layers of the clubs. The indentation yield stress of enamel has been reported to be approximately 3 GPa, which is in the same range as the outermost layer of the club. We also carried out indentation measurements on the “spearer” stomatopod species (Figure 5.5) and obtained a higher yield stress in the yy direction of 4.4 +/- 0.3 GPa, which is consistent with the absence of the softer chitin organic phase in the outer layers of the spearer club (chapter 4)¹⁹¹. The data are also in agreement with control experiments on an organic-free geological FAP (Figure 5.6) where an indentation yield stress of close to 9 GPa was measured.

Figure 5.5. Indentation response of the “spearer” mantis shrimp dactyl clubs, for distinct layers. (a) Schematic cross section of a spear sample, with color-coding representing the location where indentation measurements were carried out. (b) A macro-photo of a spear sample with its characteristic radius of curvature. (c) Partial loading-unloading curves for the dry spear sample. Full unloading was performed in order to obtain the contact depth and contact radius at a given peak load. (d) Indentation stress-strain curves computed from the partial loading-unloading curves obtained in (b). (e-f) Partial loading-unloading curves and corresponding indentation stress-strain curves in hydrated conditions.

Figure 5.6. Indentation stress-strain curves for FAP, the spearer club outer layer, and the smasher club outer layer computed from partial loading-unloading indentation curves. While there is no difference for the FAP curves between dry and hydrated conditions, the yield strength (σ_y) in the outer layer of the spearer and smasher clubs decrease by 0.2 GPa and 0.9 GPa, respectively. This decrease is likely related to the presence of organic components in these layers, which is higher in the smasher sample.

Under hydrated conditions (loading/unloading cycles, Figure 5.2b, bottom curves and derived stress-strain curves, Figure 5.2e) the mechanical contrast between the various layers becomes even more evident. Indeed, whereas the elastic-plastic behavior of the outermost layer is moderately affected by hydration (y direction, $\sigma_{y,hydrated} \approx 2.6 \pm 0.2$ GPa, $\sigma_{y,dry} \approx 3.5 \pm 0.1$ GPa) a sharp decrease in indentation stresses is noticed in the inner layers of the clubs and these differences amplify as we move deeper into the bulk. Here, the indentation stress-strain response features a strain hardening type behavior, with a rapid rise of stress when the indentation strain exceeds 20 to 30 pct. (Figure 5.2e, inset). Since indentation curves exhibit large residual displacements upon full unloading (Figure 5.7), strain-hardening can be attributed to plastic deformation as opposed to an elastic mechanism which would involve very small residual displacements. Partially mineralized

biocomposites and biopolymers are well-known to be strongly susceptible to hydration⁸⁴ and the inner layers of the dactyl clubs thus represent a compelling illustration of the contrast in mechanical properties between dry and hydrated conditions (Figure 5.2e and Figures 5.5e-f) in these materials. It should be noted that in native conditions, the dense outer region acts as an efficient barrier against water infiltration, thus the stress-strain response of the inner region in native conditions is likely to stand between the boundaries of the dry (Figure 5.2c) and the fully hydrated curves (Figure 5.2e).

Figure 5.7. Residual displacement after partial loading-unloading cycles in the inner layers of the dactyl clubs in hydrated conditions. Three cycles are highlighted (#11, #22, and #32) to emphasize the large residual displacements (h_r) upon unloading, which indicate that strain-hardening is due to a plastic deformation mechanism consisting of micro-channel densification.

Given the presence of a visco-elastic organic phase, we also verified the indentation response at higher loading rates under hydrated conditions. In the outer regions no differences were observed when the loading rate was increased up to 500-fold (Figure 5.2f and Figure 5.8). In the layers just below the impact surface, the influence of loading rate remained negligible (minor shift of the curves towards the left, shown in red). In the bulk inner layers, a small loading rate effect was detected, which is consistent with the larger content of viscoelastic chitin in this region. However, the spatial location had

the most dominant influence on the indentation response (compare the cyan green curves on the far right, measured on a location 50 μm away from the green (middle) curves).

Figure 5.8. Influence of the loading rate on the Hertzian indentation response. (a) Partial loading-unloading curves. **(b)** Computed indentation stress-strain curves in various layers. Loading rates ranged from 6 mN/s to 300 mN/s.

The impact velocities generated by the clubs at the contact point are extremely high (up to 20 m/sec). In order to assess the level of strain rates that such high velocity impacts generate in the club, we conducted Dynamic Finite Element Analysis (DFEA) simulations of the contact. We used a stiff steel target ($E = 200$ GPa) and separated the clubs into six distinct layers, with the constitutive laws of each layer obtained by fitting the indentation stress-strain curves presented in Figure 5.2e (details of the simulation provided in Materials and Methods, Section 5.4). At an impact velocity of 20 m/sec, a maximum strain rate of $8.9 \times 10^4 \text{ sec}^{-1}$ was reached in the innermost layers (Figure 5.9), which compares with a strain rate on the order of $\sim 3.3 \text{ sec}^{-1}$ for the highest loading rate (300 mN/sec) in our experiments. Hence, it cannot be completely excluded that such high strain rates would affect the indentation response more significantly than observed in Figure 5.2f. However, the maximum strain values in the innermost layers as obtained by simulation are about 10 pct (Figure 5.9), which according to the indentation stress-strain curves (Figure 5.2e, inset), correspond to moderate stresses that are lower than 50 MPa.

Thus, it is anticipated that strain rate effects will not drastically affect the stress levels during an impact.

Figure 5.9. Dynamic Finite Element Analysis of an impact against a stiff target (steel, $E = 200$ GPa). **(a)** Geometry of the club used for the simulation as obtained from SEM images. The club was divided into six layers with constitutive relationships obtained by fitting the experimental indentation stress-strain curves shown in Figure 5.2. **(b)** Maximum stress at the impact point. **(c)** Maximum strain and **(d)** maximum strain rate, which are reached in the innermost layers. The details of the computations are given in Materials and Methods, Section 5.4.

5.2.2 Micro-mechanisms of deformation

The stress-strain response of the inner layers is reminiscent of the compressive macroscopic response of lobster endocuticle³⁴, which is also a chitin/amorphous CaCO_3

composite exhibiting a helicoidal microstructure, and whose strain-hardening in hydrated conditions has been attributed to densification of the pore channels running along the mineralized chitin fibrils. Such channels are also clearly present in the inner layers of the dactyl clubs (Figure 5.10a) and we hypothesized that strain-hardening is also due to micro-channel densification. We carried out high-load Hertzian indentation and devised a method to observe the plane directly under the contact point (Materials and Methods, Section 5.4). The observations reveal clear morphological differences between the plastic region located directly beneath the indent compared to the surrounding elastic region. Whereas open channels are noticed in the elastic region, these pores are closed in the plastic region. The disparities between both areas are even more apparent at higher magnification (Figures 5.10a-iii and iv), with no open channels in the plastic region. Further confirmation was achieved by conducting similar experiments using a sharp indenter (Figure 5.10b). Here the maximum plastic strains are located near the side of the indenter pyramidal faces. Closed channels are detected in the plastic region and are unambiguously distinct from the open channels of the elastic region (Figures 5.10b-ii to 5.10b-v). These observations thus corroborate the notion that micro-channel densification plays a significant role in absorbing the impact energy. To the best of our knowledge, micro-scale densification has not previously been detected by depth-sensing nanoindentation.

Figure 5.10. Contact deformation and damage mechanisms of the dactyl clubs revealed by post-indentation FESEM observations of the planes beneath the contact points. (a) Blunt contact, inner layers: (i) low magnification micrograph illustrating both the plastic and elastic regions; (ii) higher magnification micrograph depicting the elastic-plastic boundary; (iii) and (iv) high magnification micrographs of closed channels in the plastic region and open channels in the elastic region, respectively, illustrating micro-channels densification under contact. **(b) Sharp contact, inner layers:** (i) low magnification micrograph illustrating the plastic region near the sharp indenter edge and the surrounding elastic region; (ii) micro-channels before indentation viewed under two different orientations; (iii) and (iv) boundary between the plastic and the elastic regions, with green shading area in (iv) representing the closed, densified structure. **(c) Native impact blunt contact, outermost layer:** (i) low magnification micrograph comprising both the plastic and the elastic regions; (ii) high magnification micrograph of the plastic region near the contact revealing highly misoriented FAP crystallites; (iii) high magnification of the elastic region showing the textured structure of FAP crystallites; (iv) fracture surface of the impact region (undeformed sample) confirming the textured structure of FAP; (v) schematic of

misoriented FAP crystallites near the contact point, drawn by following the contour of actual crystallites on high magnifications micrographs (quantification of crystallites orientation is shown in Figure 5.11). **(d) Sharp contact, outer layers:** (i) top view of shear-induced lateral cracks emerging on the free surface; (ii) high magnification of an indent corner revealing fibril bridging between the two sides of a lateral crack; (iii) low magnification side view showing lateral and lateral cracks nucleated by the sharp contact; (iv) high magnification micrograph of the radial crack tip region, showing the formation of a micro-cracking process zone surrounding the main crack.

Using the same methodology, we set out to elucidate the micro-mechanisms of deformation under blunt, native contacts of the impact surface (Figure 5.10c). Such observations were thus conducted in the immediate vicinity of naturally impacted surfaces such as displayed in Figures 5.1c and d. Two distinct regions can again be detected: a first region directly beneath the contact which was heavily and irreversibly deformed during the high-load impact, and a second region away from the impact point that mostly sustained elastic deformation. In the latter, we observed an elongated columnar-like texture (middle to lower part of Figure 5.10c-i), which can be better observed at higher magnification, both on the finely-polished sample (Figure 5.10c-iii) as well as on a fracture surface (Figure 5.10c-iv). Previous investigations have established that the club's outermost layers consist of oriented FAP rods with small amount of chitin (chapter 4).

In the heavily deformed region right beneath the contact, on the other hand, we observed misaligned FAP crystallites, visualized at higher magnification in Figure 5.10c-ii. The microstructural morphology of this region is clearly distinct from the elastic region (compare with Figure 5.10c-iii). These observations indicate that the FAP crystallites were subjected to intense sub-micron sliding and rotation, triggered by shear-band localization of the contact stress field ¹⁹⁴. For better visualization, we drew the preferred orientation of FAP crystallites detected in the plastic region on high-magnification FESEM images (Figure 5.10c-v, lower right panel), which compellingly illustrates the re-arrangement of FAP crystallites in the plastic region. Further quantification of the crystallite orientation shown in Figures 5.10c-ii and 5.10c-iii was carried out by image analysis with the Image J software (Figure 5.11a,b). In the elastic

region, we observed that most FAP crystallites were oriented at 60° with respect to the horizontal axis of the image. In the heavily deformed region, on the other hand, the crystallites exhibited a much broader orientation distribution with many crystallites oriented in the range -20° to 20° . We then verified whether FAP crystallite sliding and rotation leads to localized damage by conducting a series of indents in both regions (aligned and misaligned crystallites). We found no statistically significant differences for the average elastic modulus of both regions (Figure 5.11c,d).

Figure 5.11. Quantitative analysis of crystallite orientation under a native contact and influence of crystallite rotation on the elastic modulus. (a, b) Distribution of crystallite orientation in the same locations as shown in Figure 5.10c of the main text, obtained using Image J for both the oriented (a) and the misoriented (b) regions. (c, d) Average elastic moduli after native impact on regions with preferentially oriented crystallites (left) and misoriented crystallites (right).

We also evaluated the relative contribution of plastic dissipation (W_p) to the total contact energy ($W_p + W_e$), where W_e is the elastic energy (Figure 5.12). We found that in the outermost layer 20 to 25 pct. of the total contact energy was dissipated by plastic yielding. Plastic dissipation was slightly higher in the next outer layer (30 - 35 pct.) and reached up to 50 pct. in the inner layers, indicating that micro-channel densification can absorb larger relative amounts of contact energy.

Figure 5.12. Energy absorption by plastic yielding during a Hertzian contact cycle. (a) Definition of the elastic energy (W_e), the plastic energy (W_p), and total contact energy (W_t). **(b)** (W_p/W_t) vs. strain during a contact cycle in various layers and for two loading rates.

5.2.3 Indentation fracture

To complement our understanding of the dactyl club damage tolerance, we investigated the fracture response of the dactyl club using indentation fracture. For this purpose, we selected the sharp cube-corner indenter geometry for its ability to nucleate cracks, for a given material, at much lower external loads than the standard Berkovich geometry^{3,195}. Indents were performed across a wide range of peak loads (from 10 mN to 3000 mN, Figure 5.13).

Figure 5.13. Multi-scale indentation studies on the dactyl clubs. (a) Schematic indent locations. (b-d) Post-indentation FESEM micrographs corresponding to the locations shown in (a). Outer layer, peak load of 10 mN (b); outer layer, peak load of 100 mN (c); inner layer, peak load of 3000 mN (d). (e) Characteristic indent sizes shown in (b), (c), and (d) at the same scale. (f) Indentation curves corresponding to indents in (b) and (c). Dotted lines are fitting of the initial elastic region, with deviation from the experimental curves used to detect pop-in events (shear-induced cracks associated with the pop-in events shown in (c)). (g) High load (3000 mN) indentation curve of the dactyl club inner region, illustrating no pop-in events. The maximum loads from (b) and (c) are shown for comparison.

In the outer layers of the club, the crack nucleation load P_c as detected by the first pop-in event on the load-displacement curve was between 15-25 mN. This compared to P_c values as low as 4-8 mN on a control geological FAP sample (Figure 5.14). In the inner layers, on the other hand, no cracks nucleated up to values as high as 3000 mN, which corresponded to the maximum load compatible with the club size (Figure 5.13d). Post-indentation FESEM micrographs revealed the initiation of three cracks in the immediate vicinity of the indent (Figure 5.13c). A close inspection of load-displacement curves associated with the indent patterns shown in Figure 5.13c revealed three pop-in events (Figure 5.13f), as detected by discontinuities in the loading curve, which can be

attributed to perturbations beneath the contact point ¹³¹. At low loads, the pop-in events were precisely identified by fitting the initial portion of the curve with a power-law relationship and by comparing it with the experimental curve. At higher loads, the pop-in events were evident from the curves. These correlations strongly suggest that the pop-in events are due to micro-cracking around the indents. However, in comparison to classical radial cracks that are formed in purely brittle materials ¹⁹⁴, the crack patterns indicated shear-induced cracking, nucleated by shear band localization generated by the contact stress field. We probed the damage micro-mechanisms at sharp indents (Figure 5.10d) and identified at least two types of toughening mechanisms by observing the crack tip regions. First, we detected evidences of chitin micro-fiber bridging at shear-induced crack corners (Figure 5.10d-ii). Second, we also observed the formation of a process zone consisting of micro-cracks surrounding the main radial crack (Figure 5.10d-iii). Both mechanisms parallel key dissipative energy processes identified in bone ^{17,42}, indicating a similar synergistic action of multiple toughening mechanisms in the mantis shrimp's clubs.

Figure 5.14. Sharp contact indentation and residual stress fields in the dactyl clubs and comparison with geological FAP. (a) Indentation curves for FAP, the dactyl club outer layer (DOL) and the dactyl club inner layer (DIL). The shaded areas under the curves correspond to the energy released during cracking event at the contact point. (b) Released energy vs. indentation load for the 3 samples. (c-e) Post-indentation FESEM micrographs of FAP (c); DOL (d), and SIL (e). (f) Indentation curves on FAP and DOL samples used for visualization of residual stress fields. (g-i) Raman mapping for visualization of residual stress fields around indents of FAP and DOL. Relative intensity of the ν_1 band of FAP (g); peak shift of the ν_1 band (h); and residual stress fields as computed from the relative peak intensity and peak shift (i).

In order to quantify the released energy during crack events at the indentation edges, we correlated the pop-in events with the energy released during cracking. For sharp contact indentation, where yielding can be followed by cracking events at the contact point ⁷⁶, the area under a loading-unloading cycle corresponds to the sum of two energies: the energy dissipated by plastic yielding and the elastic energy released during cracking events (a third contribution, the elastic energy restored during the unloading portion of the cycle, can be considered negligible here). Thus, if no cracking occurs 100 pct. of the input energy is dissipated by plastic yielding and this value decreases when cracks nucleate from the contact point and propagate. This quantity can be regarded as a “damage index (D)”, which is related to the capacity to absorb contact energy by yielding prior to cracking.

The area under pop-in events due to the elastic energy released during cracking was integrated as illustrated in Figure 5.14a, which was done for the outer layer, an FAP geological control, and the inner region. The total energy released during cracking as a function of the applied load was also computed (Figure 5.14b). For instance, at an applied load of 100 mN, the released elastic energy by cracking relative to the total energy during the indentation process ($1-D$) is 45 ± 4 pct. for FAP and 12 ± 3 pct. for the club outer layer. In other words, the club outer layer is able to absorb almost four times more contact energy by yielding prior to cracking in comparison to the geological FAP crystal. Figure 5.14b also indicates that the released energy during crack propagation exhibits a power-law dependence with the load in FAP, whereas for the club outer layer the correlation is almost linear and lower in absolute values than in FAP. A slightly higher absorption energy was also noticed in the zz direction (Figure 5.15a). For the inner layer, $1-D = 0 \pm 0$ pct for the entire applied load range meaning that no cracking occurs during the indentation process.

Hence, a high amount of plastic yielding occurs in the bulk region before any cracks are nucleated, which is not surprising considering its strain-hardening indentation stress-strain response. To further quantify damage localization, the high load indents were inspected with FESEM (Figure 5.14 c-e) and the results were compared with the control geological FAP crystal. We found that the ratio of damage size (r_d) to indent size (r_i) in the outer layer of the dactyl club sample is about 50 pct. smaller compared to the FAP

crystal sample (1.50 ± 0.04 and 2.90 ± 0.34 respectively). For the inner layer no crack or external damage was observed around the indents, which also corroborates the indentation curves for this layer (Figures 5.14a and b, blue curves). We also assessed loading rate effects during sharp indentation and found no statistically significant differences on the indentation profiles and released energies for both the inner and the outer layers (Figures 5.15b-d).

Inhibition or delay of cracking at sharp contacts in ceramics can be attributed to the ability of the material to undergo localized shear deformation¹⁹⁶. This mechanism gives rise to contact stress redistribution under the contact point^{194,196}, in principle resulting in the formation of a significant residual stress field. To test that this mechanism applies to the dactyl clubs, we used high resolution Raman micro-spectroscopy to detect the shift of the ν_1 band of FAP (Figure 5.14f-i) around the indents, according to a methodology developed by He *et al.*¹⁹³ The experiments were performed on indents generated with a 15 mN peak load, on both the outer layer of the dactyl club and a FAP geological crystal. We observed that the residual stress area in the dactyl club outer layer was almost four times larger than in FAP (Figure 5.14i). This further confirmed that in the geological FAP a high fraction of indent energy is released by cracking, thus leaving a small residual stress field. In the club outer layer, on the other hand, a much larger yielding zone developed around the contact point, building-up a complex residual stress field where a high amount of contact energy was absorbed.

Figure 5.15. Influence of the anisotropy and loading rate during sharp tip indentation. (a) Released cracking energy vs. indentation load in the zz and yy directions, illustrating a slightly higher absorption by plastic dissipation in the zz direction (lower cracking energy released). **(b)** Released energy vs. indentation load at low and high loading rates, showing no statistical differences between the two rates. Representative indentation curves on the outer **(c)** and inner **(d)** layers at low and high loading rates. In both regions, the loading rate had no significant influence on the sharp contact indentation response.

5.3 DISCUSSION

5.3.1 Quasi-plasticity versus brittle fracture

The results from both elastic-plastic (blunt Hertzian contact) and fracture (sharp contact) investigations provide clear insight into the overall damage tolerance of the club. The indentation process –and thus resistance to contact– of hard materials represents a close competition between yielding and cracking beneath the contact point^{76,88}. For blunt contacts, Lawn and co-workers¹⁹⁷ have suggested the following “brittleness” index ratio P_y/P_c :

$$\frac{P_y}{P_c} = \left(\frac{D}{A}\right) \left(\frac{H}{E'}\right) \left(\frac{H}{T}\right)^2 r' \quad (\text{Eq. 5.1})$$

where P_y and P_c are the external loads to initiate yielding and cracking, respectively, D and A are constants equal to 0.848 and 8.63×10^3 , respectively, H is the hardness, E' is the effective modulus, T is fracture toughness, and r' is the contact radius of curvature. When $P_y/P_c > 1$, the contact response of the material is brittle, whereas if $P_y/P_c < 1$, the behavior is referred as “quasi-plastic”. Therefore for quasi-plastic materials, significant yielding beneath the contact precedes the nucleation and rapid propagation of a large crack triggered by the Hertzian stress field¹⁹⁴, with yielding occurring along localized shear bands. Using the values of H , E' , and a contact radius of 10 mm, we obtained for the outer layer a P_y/P_c ratio of *ca.* 4.9×10^{-4} and 3.9×10^{-4} in the zz and the yy directions, respectively in both dry and hydrated conditions. This strongly suggests that the critical load for brittle cone fracture at the contact point is much larger than the critical load for quasi-plasticity. Thus incipient quasi-plasticity occurs within a confined sub-surface yield zone governed by the shear components of the Hertzian stress field^{197,198}.

Confined yield beneath the contact point in impact resistant, quasi-plastic ceramics can be achieved by preparing coarse grain microstructures, with grains typically larger than one micron^{194,199}. In stark contrast, the dactyl club’s outer layer is made of FAP nano-rods, and our post-impact observations (Figure 5.10c) reveal that confined yield is achieved by a distinct deformation micro-mechanism consisting of sub-micron inter-crystalline sliding and rotation of initially oriented FAP nano-rods, a mechanism that is gradually building up during each impact and which we are not aware of in other hard biological composites. This behavior is likely facilitated by the viscoelastic nature of

the organic phase surrounding the FAP crystalline rods, similar to axial tablet sliding observed in nacre^{62,200} albeit at a smaller length scale.

Furthermore, there also exists a transition radius r^* , corresponding to $P_y/P_c = 1$, above which the response shifts from quasi-plastic to brittle. This transition radius for the outermost layer of the dactyl club was found to be about 20 mm (zz direction). Since the average radius of curvature of the clubs is 2.1 ± 0.3 mm (Figure 5.2a, $n = 12$), a key implication is that during impact on hard shells the macroscopic size of the clubs precludes the formation of cone cracks in the clubs. On the other hand, such catastrophic cracks will be formed on the prey's hard shells (Figure 5.1). We also calculated the transition radius for the spearer species and obtained a value of 6 mm. Interestingly, the curvature radius of the impact (apex) region in the spearer is $30 \mu\text{m}$ (Figure 5.5b), again much smaller than the transition radius, suggesting a curvature that prevents catastrophic cracking at the impact point in all mantis shrimp species.

Mantis shrimps deliver very high impact velocities in the wild, which generate loading rates at the contact point that are larger than the highest loading rates imposed in our experiments. Strain-rate effects are well-known to influence the mechanical response of structural materials, especially ductile metallic alloys or structural polymers²⁰¹. In the extreme case, the so-called ductile-to-brittle transition occurs as the strain-rate increases, with concomitant changes in the dominant micro-mechanisms of failure²⁰¹. We reason that such ductile-to-brittle transition is unlikely in the dactyl clubs. Indeed, micro-plasticity under native blunt contact was clearly identified in native specimens (Figures 5.1d and 5.10c), which would be incompatible with a brittle response. We did not observe any catastrophic brittle fracture in the clubs, nor has it been documented in the past. The dactyl club represents a central component of stomatopods evolutionary survival and must have thus evolved to prevent such a ductile-to-brittle transition, which would completely undermine their hunting strategies. The inner layers, on the other hand, must exhibit a stronger strain rate dependence given their higher content of visco-elastic phase.

Although our DFEA simulations (Figure 5.9) indicate that the maximum strain rates attained in the inner layers following impact are clearly higher than those imposed in our indentation experiments, the maximum strains in these layers correspond to relatively low stresses, where strain-rate effects are expected to remain moderate.

Nevertheless, it remains to be determined to what extent higher loading rates than employed in our indentation experiments would affect the stress-strain response, in particular in the inner layers.

5.3.2 Outlook

To the best of our knowledge, indentation stress-strain curves with micrometer spatial resolution on multi-layer biological composites have previously not been reported, especially in hydrated conditions. In addition, our multi-scale sharp contact indentation measurements provide quantitative insights into the relative contributions of energy dissipated by yielding or released by cracking, respectively. Together, these data indicate that the outer region of the club exhibits a graded, quasi-plastic compressive response, resulting in significant energy dissipation by localized yielding before shear-induced circumferential cracks are nucleated. Mechanical anisotropy is largely restricted to the impact surface in the form of enhanced resistance against yielding in the direction perpendicular to the impact surface. On the other hand, the inner layers composed of chitin and amorphous minerals exhibit strain-hardening, with lower compressive yield strengths but much higher yield strains, a design which is efficient in absorbing additional impact energy. The deformation mechanisms associated with each response are markedly different. Whereas quasi-plasticity occurs by localized interfacial sliding and gradual rotation of apatite nano-rods during consecutive impacts, strain-hardening results from micro-channel densification.

We conclude that the macroscopic size of the clubs is smaller than the transition radius of a blunt contact above which the contact mechanics response switches from quasi-plastic to brittle, a key criteria in preventing catastrophic macroscopic failure of the clubs during impact against prey. These findings provide useful guidelines for designing synthetic multi-material structures that must resist repetitive impact loading: a compromise in absolute hardness and stiffness values near the impact surface would be beneficial through the incorporation of a residual soft phase that can promote localized interfacial sliding and gradual rotation of the initially textured hard phase, in turn leading to a quasi-plastic response at the impact point. Within the bulk of such components, on

the other hand, the use of materials with strain-hardening response via micro-densification would provide additional mechanical dissipation.

5.4 Materials and Methods

Materials: Club samples were dissected from stomatopods (“*Odontodactylus scyllarus*”) acquired from commercial sources in Singapore. The clubs were kept at -80°C before cutting and mounting. The samples were embedded in an acrylic cold mounting resin (ClaroCit, Struers), sectioned using a linear precision diamond saw IsoMet 4000 (Buehler, USA), ground with P1200/4000 grade SiC paper, and finally polished with 1 and 0.25 µm diamond paste. A 5 minutes ultrasonic bath was used to remove diamond paste particles and polishing debris from the samples surfaces after each step.

Field Emission Electron Microscopy: Samples were examined with a FESEM (JEOL, 7600F) at a low 5 kV accelerating voltage. To prevent surface charging, the samples were coated with 5 nm Pt and a lower secondary electron detector (LEI) was used.

Optical and micro CT imaging: the damaged and intact dactyl clubs were captured using an EOS 700D camera equipped with an EF 100 Macro lens (Canon, Japan). The micro CT micrographs were imaged using an inspeXio SMX-90CT Plus (Shimadzu, Japan) with a 90 kV voltage and Voxel size 0.012 mm/pix. The 3D model was rendered using VGStudio Max.

Observation of deformation and fracture mechanisms: High-load indents were placed with both blunt and sharp tips. Next, the indented samples were polished with fine (40 nm) colloidal silica suspension along a plane-oriented parallel to the loading axis, until the plane directly located underneath the indenter axis was exposed. The samples were thoroughly inspected at regular intervals by optical microscopy in order to avoid over-polishing. Once the plane parallel to the indenter axis was reached, FESEM observations were conducted on the deformed zones directly beneath the indents. Similar procedures were carried out on native surfaces whose visual inspection (Figure 5.1c) revealed intense impact by the animal.

Nanoindentation: Indentation studies were performed using a Triboindenter TI-950 Nanomechanical tester (Hysitron, Minneapolis, MN, USA). Hertzian contact studies were done using a 30 mN standard transducer with a 10 µm nominal cono-spherical fluid cell tip at loading rates ranging from 0.5 mN/sec to 300 mN/sec. For the dried conditions,

80 partial loading/unloading cycles were conducted from 50 mN up to 30 mN. For the hydrated conditions, a custom-made fluid cell was filled with water. In order to minimize the drift effects due to long-term loading/unloading cycles, the tip was engaged with the specimen in the imaging mode for 30 to 40 minutes before each experiment. 80 partial loading/unloading cycles from 10 mN to 30 mN were conducted. For the indentation fracture studies, a 10 N high-load transducer was used, and all indents were performed with a cube-corner diamond tip at loading rates ranging from 0.5 mN/s to 100 mN/s.

For multi-directional indentation studies, the response of the free surface was first obtained in the zz direction, which was possible because the free surface exhibited a remarkable small surface roughness (in the range of 20-30 nm). Next the sample was polished such that the cross-sectional plane perpendicular to the free impact surface was exposed (as depicted in Figure 5.2a). A series of loading/unloading cycles was conducted on this plane in the yy direction at well-defined distances (y_0, y_1, y_2 , etc), starting from the outermost layer ($y_0 = 0$). The samples were then finely polished normal to the zz direction from the free impact surface (thus exposing a plane perpendicular to the previous polishing plane) according to the y_i distances previously defined. After each polishing step, indentation cycles were conducted, such that the stress-strain response in the zz direction was obtained at distances equivalent to the measurements conducted in the yy direction, but orthogonal to the latter. Once a set of indentation cycles was obtained in the zz direction, the procedure was repeated for the location y_{i+1} .

Raman Spectroscopy: High resolution Raman imaging of post-indentation residual stress fields was obtained by using a confocal Raman microscope (alpha300, WITec, Ulm, Germany) equipped with a 633 nm laser source and a 20X objective lens. An integration time of 0.5 s and pixel size 0.3 μm was used for acquisition. The spectrometer was calibrated using a silicon wafer sample. WITec Project 2.08 was used for filtering, analysis, and plotting the data.

Tip Calibration and Stress-Strain derivation: To calibrate the 10 mm cono-spherical tip, a wide range of forces (50 μN to 12000 μN , total of 125 indents) were applied to standard polycarbonate and fused quartz samples, allowing to cover 35 to 1700 nm of contact depth range. Using the extracted stiffness (S) values and known Young's moduli E of polycarbonate and fused quartz, the contact radii were calculated from:

$$a = \frac{S}{2E}$$

$a-h_c$ values were subsequently plotted and fitted with the following expression:

$$a = 13.879 \times (h_c + 68.00775)^{0.78572}$$

The tip radius R at each contact depth can then be calculated as:

$$\frac{a}{R} = \frac{1}{\sqrt{(\tan^{-2} \theta + 1)}}$$

where $a = f(h_c)$ is the contact radius, and θ is the tangent angle.

The indentation stress-strain points for each cycle was finally calculated using:

$$\varepsilon = \frac{a}{R}$$

$$\sigma = P_m = \frac{P}{\pi a^2}$$

Dynamic Finite Element Analysis (DFEA): To simulate the impact event between the dactyl club and a rigid target, a 2D Planar model of the club and target was designed and analyzed using the commercial finite element software Abaqus/Explicit 6.15 under plane-strain conditions. The geometrical shape and dimensions of the club were acquired from FESEM micrographs, with the dimensions in the range 5 to 6 mm as shown in Figure 5.9a. The club model consisted of six regions, as defined from the indentation experiments. The interfaces between the layers were assumed to be perfectly bonded through tie interface condition. A hemi-spherical stainless steel barrier ($E = 200$ GPa, density = 8000 kg/m^3) of radius 10 mm was selected as a target. The contact between the club and target was defined as frictionless (tangential) and hard (normal). The club and steel target were discretised using plane-strain elements with reduced integration elements. After conducting mesh sensitivity analysis, three different mesh sizes of $1 \text{ } \mu\text{m}$, $10 \text{ } \mu\text{m}$ and $50 \text{ } \mu\text{m}$ were chosen for the outermost, the outer and the inner layers, and the target respectively. The finite element mesh contained a total of 23,615 nodes and 23,285 elements. The hollow-section in the club inner region was modeled as a stress-free with boundary.

The constitutive laws for each region obtained by fitting the experimental Hertzian stress-strain curves in hydrated conditions. For the outer layers with the

experimentally-obtained elastic-plastic behavior, J2 plasticity with isotropic hardening behavior was used. For the inner layers, a strain stiffening hyper elastic behavior (Ogden constitutive model with $n = 3$) was employed. Simulations were carried through finite strain by including non-linear geometry option in the software. The hemi-spherical steel target was fully constrained and a 20 m/s velocity was applied to the club along the normal direction. A total impact time of 30 μs was imposed, consisting of both the impact and the retraction movement. Upon impact compressive waves travels in both the deformable target and club. Von-Mises effective stress, longitudinal logarithmic strain, and strain-rates were monitored. It is to be noted that the properties across the club are not uniformly graded, rather there is a step change in the properties from the outer stiff and strong inorganic mineral layer to inner protein rich layer. The banded stress contours in Figure 5.9b in the club is due to very different Young's moduli in the outer layers and inner layers. The maximum effective stress of 951 MPa is seen in the outer layers of the club beneath the contact point at 2.25 μs .

Data analysis and plotting: Statistical analysis and data plotting were done using the OriginPro 9.1 software. Polynomial curve fitting (2nd order) was used for detecting shallow depth pop-in events (Figure 5.13f). A total number 24 dactyl club samples were tested in the entire study. All samples were from molted clubs, which were fully mineralized and also exhibited fully-formed inner layers. All conditions for indentation studies (spatial location, hydrated and dry, orientation, and loading rates) were repeated three times and all curves presented are representative for each set of conditions.

Chapter 6 – Conclusions and Future Work

6.1 Conclusions

Referring to our hypotheses and objectives (Chapter 1), we have performed a comprehensive study of the mantis shrimp raptorial appendages, including chemical, structural, and mechanical characterizations. These ultra damage tolerant biomineralized hard tissue represent a fascinating model to engineering composite materials with a high damage resistance, such as restorative implants, armors, and protective barriers. Chemo-mechanical correlations, toughening strategies and damage mechanisms of mantis shrimp dactyl clubs were elucidated using a wide range of microscopic, elemental, structural and mechanical characterization methods.

In Chapter 2, a recent research and overview of biological materials science and structural design strategies found in Nature, as well as related mechanical characterization methods and relevant concepts were briefly reviewed. In this Chapter we provided a concise presentation on bioinspiration and biomineralized materials as well as the mechanical characterization methods used in the next Chapters. Some of the concepts are described in greater depth in subsequent Chapters.

In Chapter 3, we presented a thorough study on wear and abrasion resistance of biological materials, from fully mineralized to fully organic-based biomaterials and discussed their tribological performance using a contact mechanics framework. We built selection maps to predict the wear and abrasion resistance of around 30 studied biological models in the spirit of Ashby's selection maps. On the basis of these materials selections maps, we can categorize the outer layer and the inner layer of the mantis shrimp dactyl club as belonging to the class of highly mineralized content medium mineralized content, respectively (Figure 6.1). For blunt contact, placing the stomatopod dactyl clubs on the maps using our experimental data reveals that the damage resistance of the impact region of the club is above that of nacre and human enamel and shark enameloid (Figure 6.1).

Figure 6.1 Selection chart of biological materials against yielding at a blunt contact (See Chapter 3).

The performance of the dactyl clubs against sharp contact can be assessed using the concept of “*L*-shaped material properties selection charts”. These charts consist in plotting the material hardness (H) against the cracking resistance K_{Ic}^4/H^3 in logarithmic scale, as previously presented for a wide range of hard engineering and biological materials (Chapter 3). Using the relevant values for the outer layer dactyl clubs measured in Chapter 3 and 4, we see on the materials property chart (Figure 6.2) that the dactyl club performs better than nacre, conch (inner and outer layer) and enamel, which among hard biological materials were predicted to exhibit the highest tolerance against damage at sharp contact.

Figure 6.2 L-shaped selection charts of biological materials against combined yielding and cracking at a sharp contact. Note that the absolute values of cracking loads (vertical guidelines) strongly depend on the indenter geometry. Here the chart was constructed for the cube-corner geometry employed in this study, for which the external load to nucleate indentation cracks is about one order of magnitude lower than for the classical Vickers geometry. The location of the materials on the chart, on the other hand, only depends on the intrinsic materials properties.

In Chapter 4, structural and chemo-mechanical properties of the different layers of the mantis shrimp dactyl club were analyzed. Our high resolution confocal Raman scanning as well as EDS elemental mapping in the inner layers demonstrated a gradual transition from ACC to ACP. By moving from the inner layer to the outer layer we found a step-wise transition from ACP to the crystalline FAP. We observed the presence of CAS wherever FAP exists. *Ab initio* modeling suggested that CAS acts as a template for FAP stabilization and formation. The presence of FAP and CAS has not been reported in pervious studies on mantis shrimps dactyl clubs. Our 3D nanoindentation studies as well as polarized Raman and optical microscopy studies revealed that the FAP crystallites are oriented toward the free surface of the impact region. The high load indentation studies on the impact regions of the spearer and smasher dactyl clubs showed the fracture toughness of the smasher dactyl club was higher than the spearer. According to Raman spectra we collected for these regions, we suggested that this difference was related to the

presence of chitin fibrils in the impact region of the smasher dactyl club. A schematic image of these hierarchical structures (for both smasher and spearer clubs) is presented in Figure 6.3.

Figure 6.3 Schematic cartoon of the hierarchical structure of the distinct layers of the dactyl clubs. The outer layer of the dactyl club is composed of oriented FAP crystallites while the inner layer is composed of sheets of chitin fibrils arranged in a helicoidal fashion.

In Chapter 5, we analyzed the energy dissipation and the toughening strategies of the dactyl clubs in order to elucidate how this biomineralized biotool can dissipate extremely high impact energy without sustaining internal damage. To investigate the mechanical response and deformation behavior of the dactyl club, we used a wide range of indentation geometries and force ranges. Both sharp and sphero-conical indentation tip geometries were employed and the contact forces ranged from the nano- to the milli Newton. Our indentation stress-strain studies in both dry and hydrated conditions (Figure 5.2) revealed the outer layers exhibit an elastic-plastic response, whereas the inner layers display a characteristic strain hardening behavior. We conducted these studies for different loading rates (up to 300X faster) and directions (yy , zz planes) in order to study

the effect of the loading rate and anisotropy on the mechanical response. We found that the applied loading rates did not have any significant effect on the contact response (Figure 5.2f and 5.8). On the other hand, multi-directional indentation studies of the impact region indicated a strong mechanical anisotropy response (Figure 5.2d), with the *zz*-direction (perpendicular to the impact surface) significantly stiffer and having a higher yield stress than the other direction (*yy*-direction, parallel to the impact surface).

Due to geometrical and technological limitations (see Section 5.2 and 5.3), higher loading rates were not experimentally assessed (the strike of the club is much faster in comparison to the highest loading rates that could be applied in the experiments). However, a 2D planner model of the dactyl club was modeled and dynamically analyzed using DFEA methods (Figure 5.9). The results revealed that although the strain rates during a strike were significantly higher than the applied strain rates, the maximum strain in the innermost layer of the dactyl club did not exceed 10 percent. According to the experimentally derived indentation stress-strain curves, these values correspond to moderate stresses in the inner layer. Nevertheless it remains to be determined to what extent this would affect the indentation stress-strain response.

High load indentation studies (with both sharp and blunt tips, Figure 5.10) revealed that densification of the micro-channels plays an important role on the mechanical behavior (strain-hardening response) of the inner layers. Fibril bridging, micro-cracking and induced shear cracking mechanisms were also detected during sharp high load indentations. Blunt high load indentation studies as well as high-resolution FESEM observations on the native impacted zones revealed a mechanism by which the preferred oriented crystallites slide and rotate, and eventually misalign beneath the contact points (Figure 5.10c and 5.11a,b). This mechanism has not previously been reported in other biological materials.

By taking a closer look at the sharp high load indentation curves and the related indent micrographs we correlated the pop-in events to the shear induced cracks, which had a key role in localizing damage (Figure 5.13). We calculated the released energy during cracking events as well as the damage size post-indentation fracture in the outer layer, the inner layer, and the geological FAP sample. We found that the geological FAP released much more contact energy by cracking damage in comparison to the outer layer

of the dactyl club, which was able to absorb a relatively high amount of energy by micro-plasticity. On the other hand, no cracking was observed in the inner layers, implying that almost all of the contact energy in these layers is absorbed by micro-channel densification. This Chapter revealed the key structural strategies and toughening mechanisms that confer the clubs with their very high contact damage tolerance.

6.2 Future Work

6.2.1 Finite element modeling on deformation mechanisms in the smasher dactyl club

Our findings on the toughening and deformation mechanisms of the dactyl clubs' impact region (Chapter 5) have shown that the quasi-plastic behavior occurs under blunt contact due to sliding and rotation of preferentially-oriented FAP crystallites. We suggest that this deformation mechanism could be related to the rupture of organic bridges, which would lead to sliding as well as to misalignments of the FAP rods under shear loads.

However due to geometrical and technological limitations, the study of this deformation mechanism at high-speed impact is impractical (Chapter 5). Finite element studies of this deformation mode could provide a solution to analyze the formation of these damage-localizing mechanisms, which could be an area of interest for aerospace and medical applications. To provide a realistic model, the mechanical properties of the mineral and organic phases at the nanoscale level could first be acquired by nano DMA indentation studies²⁰².

6.2.2 Biomineralization studies of the dactyl clubs

Our preliminary studies of the dactyl club molting process, which occurs every 3 to 6 months and takes 7 to 10 days to reach full mineralization, have shown that CAS is initially deposited on the club outer region. This deposition probably acts as the first step of mineralization and defines the overall geometry of the club. In the next step, the chitin fibrillar network is deposited, together with FAP to stiffen the club. Our work has shown that CAS acts as a template for FAP crystallization (Chapter 4). Preliminary FESEM, Raman spectroscopy and indentation data at various time points have indicated that there is a gradual stiffening of the clubs over the first few days after molting.

Taking a closer look at the formation of the clubs at different stages of the dactyl club formation (from larva to fully mature mantis shrimp), from a structural and mechanical points of view, will be an area of interest for bottom-up synthesis of mineralized biomaterials. The outcome of this research can provide useful inspiration to synthesize tough complex ceramic structures for bio-implants such as tooth and hip joints.

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List of Publications

Journal Papers

1. Cai, P., et al. Bio-inspired Mechanotactic Hybrids for Orchestrating Traction-mediated Epithelial Migration, **Advanced Materials**, Accepted, 2016
2. Alam, P., et al. Properties and architecture of the sperm whale skull amphitheater, **Zoology**, Accepted, 2016
3. **Amini, S.**, et al., The role of quasi-plasticity in the extreme contact damage tolerance of the stomatopod dactyl club, **Nature Materials**, 14, 943-950, 2015, doi:10.1038/nmat4309
4. Petrone, L. et al. Mussel adhesion is dictated by time-regulated secretion and molecular conformation of mussel adhesive proteins. **Nature Communications** 6, (2015).
5. Tadayon, M., et al., The Mantis Shrimp Saddle: A Biological Spring Combining Stiffness and Flexibility, **Advanced Functional Materials**, 25, 6437-6447, 2015
6. Alam, P. et al. l-Lysine templated CaCO₃ precipitated to flax develops flowery crystal structures that improve the mechanical properties of natural fibre reinforced composites. **Composites Part A: Applied Science and Manufacturing** 75, 84-88, (2015)
7. Rubin, D.*, **Amini, S.***, et al., Structural, nanomechanical and computational characterization of D,L-cyclic peptide assemblies, **ACS Nano** 2015, 9(3): 3360-3368. (*Co-first author).
8. Latza, V. et al., Multi-Scale Thermal Stability of a Hard Thermoplastic Protein-Based Material, **Nature Communications**, 6, 2015
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10. Guerette, P.A., et al., Nanoconfined β -Sheets Mechanically Reinforce the Supra-Biomolecular Network of Robust Squid Sucker Ring Teeth. **ACS Nano**, 2014. 8(7): p. 7170-7179.
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14. Pan, J., et al., Fabrication of a 3D hair follicle-like hydrogel by soft lithography. **Journal of Biomedical Materials Research. Part A**, 2013.
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Oral presentation

1. Chemical gradient and crystal anisotropy in the Stomatopods dactyl club, MRS 2013, Boston, Massachusetts, USA

Poster presentations

1. The role of quasi-plasticity on the extreme contact damage tolerance of the stomatopod dactyl clubs, ICMOBT, 2015, Hawaii, USA
2. Mechanical anisotropy in impact surface of mineralized “Biotools”. ICYRAM Conference, 2013, Singapore (**Best poster award**)
3. Mechanical design of mechanically-active and mineralized biotools. MRS Conference, 2012, Singapore