Electronic Supplementary Info

Biomimetic approach to forming chitin/aragonite composites

Natasha H. Munro and Kathryn M. McGrath*
MacDiarmid Institute for Advanced Materials and Nanotechnology, School of Chemical and Physical Sciences, Victoria University of Wellington, PO Box 600, Wellington, New Zealand
Fax: +64 4 463 5241; Tel: +64 4-4635963; E-mail: Kathryn.McGrath@vuw.ac.nz

Experimental

Materials

Chitosan (CS, degree of deacetylation = 82 ± 2%, M_w = 100,000-300,000 g mol^{-1}, Acros Organics), $CaCl_2.2H_2O$ (Sigma-Aldrich, ACS reagent), $NaHCO_3$ (Panreac, AR grade), $CaCO_3$ (Univar, AR grade), poly(acryclic acid) (PAA, Sigma-Aldrich, average M_w = 1,800), high molecular weight (HMW) PAA (Sigma-Aldrich, average M_w = 100,000), acetic acid (Univar, AR grade), acetic anhydride (AR grade, Acros Organics) and methanol (LR grade) were used as received. Poly(Aspartic acid) (PAsp) was prepared, following the procedure of Bennett[1], with an average M_w of 363,000 g mol^{-1}. Deionised water (18.2 MΩ cm resistivity, Sartorius arium 611UV purification system) was used in all solutions.

Chitosan templates

CS (0.400 g) was stirred in 19.8 mL H_2O, acetic acid (0.200 mL) was added and the mixture was stirred for 1 h. The 2% (wt/wt) CS solution in 1% (v/v) acetic acid solution (20 mL) was centrifuged at 3,000 rpm for 20 min, poured into a circular 9 cm diameter plastic Petri dish and dried at 50°C for 48 h. Films for mechanical testing were prepared with CS (1.000 g) in 45.5 mL H_2O, with acetic acid (0.500 mL). The solution was poured into a square 12 x 12 cm petri dish. All solutions used were scaled up to give the same ratio of solution to the polymer film.

Chitin templates

The chitosan templates were reacetylated as described elsewhere[2]. Acetic anhydride (5.0 mL) dissolved in methanol (25 mL) was applied to the chitosan template and left overnight at ambient temperature. The acetylated film was rinsed with ethanol and washed thoroughly with water. The degree of deacetylation = 40 ± 10%.

Mineralisation

Prior to mineralisation, the chitosan templates were treated with NaOH solution (30 mL, 1.00 M) and left to set for 1 h. The films were subsequently washed repeatedly until the solution was neutral. The chitin or chitosan templates were mineralised by the sequential soaking of the films in 60 mL aliquots of calcium chloride (500 mM), sodium bicarbonate (500 mM) and saturated calcium carbonate solutions, respectively. PAA was dissolved in the solution after bubbling with CO_2(g) prior to soaking the films. The optimal amount of PAA with respect to the organic template was found to be 2% (0.008 g). This resulted in the greatest nucleation of aragonite and vaterite for the chitin and chitosan templates, respectively. $CaCl_2$ and $NaHCO_3$ solutions were bubbled with CO_2(g) for 1 h. A saturated calcium carbonate solution was prepared following the procedure outlined by Kitano[3].

Briefly, 2.6 g of $CaCO_3$(s) was added to 1 L H_2O and was bubbled with CO_2(g) for 6 h. The suspension was filtered and the filtrate was rebubbled for 1 h. The films were soaked in $CaCl_2$ and $NaHCO_3$ solutions for 24 h and in the Kitano solution for five days.

Characterisation

The chitosan and reacetylated chitin films were characterised by FT-IR spectroscopy. The powdered samples were prepared as KBr discs and the spectra were acquired from five scans with 4 cm^{-1} resolution on a Perkin-Elmer Spectrum One Ft-IR Spectrometer. The degree of deacetylation (DD) was calculated using the method of Brugnerotto *et al.*[4] The mineralised films were characterised by SEM, EDS, powder XRD and microRaman spectroscopy. SEM samples were mounted on aluminium stubs with double sided carbon tape. Cross-sectional samples were prepared by mounting a piece of the sample against a carbon-tape coated cut stub, with the sample protruding above the top of the stub. A folded over piece of carbon tape was applied, level to the top of the stub. A second piece of carbon tape was pressed onto the tape and the sample. The whole stub was frozen in liquid nitrogen, and the tape and sample were fractured while frozen, then thawed. The samples were coated with two coats of carbon using a Quorum Q150T coater. SEM images were acquired using a JEOL JSM-6500F field emission SEM in SEI and BEI mode. An accelerating voltage of 10.00 kV and a probe current of 9.0 μA were typically used. The instrument was generally operated at a working distance of approximately 10 mm. EDS imaging was carried out in BEI mode, with an accelerating voltage of 15.0 kV and a probe current of 17 μA. Samples were prepared for X-ray diffraction (XRD) by grinding of the composites in liquid nitrogen to form a powder. XRD patterns were acquired with a PANalytical X'Pert Pro powder difffractometer using Cu/Kα radiation. The data were obtained with generator settings of 45 kV and 40 mA, and scans were acquired with 2θ from 5 to 80°. The diffraction patterns were compared to diffraction patterns of known compounds from the database, using the software X'Pert Highscore 2.2c. Calculations of the various amounts of the three anhydrous polymorphs of calcium carbonate were performed using the software or from the peak areas for the peaks at 25.0° (vaterite), 29.5° (calcite) and 45.9° (aragonite) as detailed elsewhere[5]. Raman spectra of individual crystals were obtained using a Renishaw RM2000 ramascope using 633 nm excitation with a circularly polarized laser and a Renishaw edge-laser filter. A linear polarizer was not used. The incident laser beam of a Jobin-Yvon LabRam spectrometer was centred on a crystal and spectra were taken using a 633 nm HeNe laser centred at 800 cm^{-1}, with the pinhole set at 1000 μm. A beam intensity of 3 mW and a N_2(l) cooled CCD were used. The spectrometer was calibrated using a silicon standard, setting the major peak to 520.5 cm^{-1}. The tensile elongations to break (TEB) were measured using an Instron Universal Testing Machine Model 1122 (Instron Corporation, Canton, MA.) with a cross-head speed of 2 mm min^{-1}. The test bars were 79 mm long, with a gauge size of 28 mm long by 4 mm wide. The thickness of each test bar was measured at three points and the average taken. Five test bars were tested for each sample and the average TEB was calculated. The error bars indicate the standard error of the values from the mean, with a 95% confidence interval.

Figures and Tables

ESI Table 1 - Extent of mineralisation and polymorph proportions for polysaccharide/calcium carbonate composites

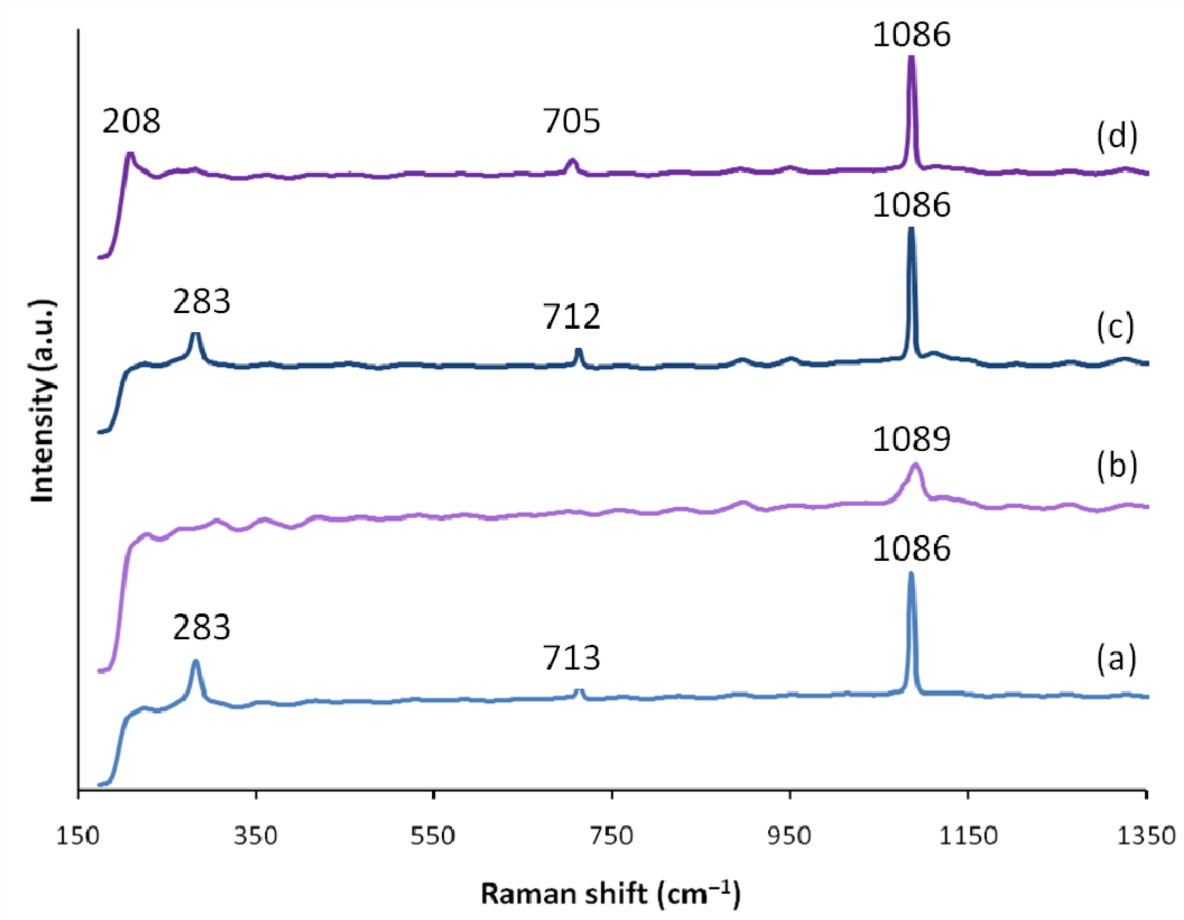
Template	Additive present	% Mineral	% Calcite[a]	% Aragonite[a]	% Vaterite[a]
Chitosan	None	1 ± 8	100	—	—
Chitosan	LMW PAA	6 ± 6	52 ± 2	—	48 ± 8
Chitosan	HMW PAA	11 ± 1	58 ± 2	—	42 ± 8
Chitosan	PAsp	2 ± 1	100	—	—
Chitin	None	8 ± 2	100	—	—
Chitin	LMW PAA	20 ± 1	22 ± 2	78 ± 5	—
Chitin	HMW PAA	15 ± 2	13 ± 2	87 ± 5	
Chitin	PAsp	11 ± 1	100	—	—

[a] The uncertainty values were calculated according to the method of Dickinson and McGrath[5].

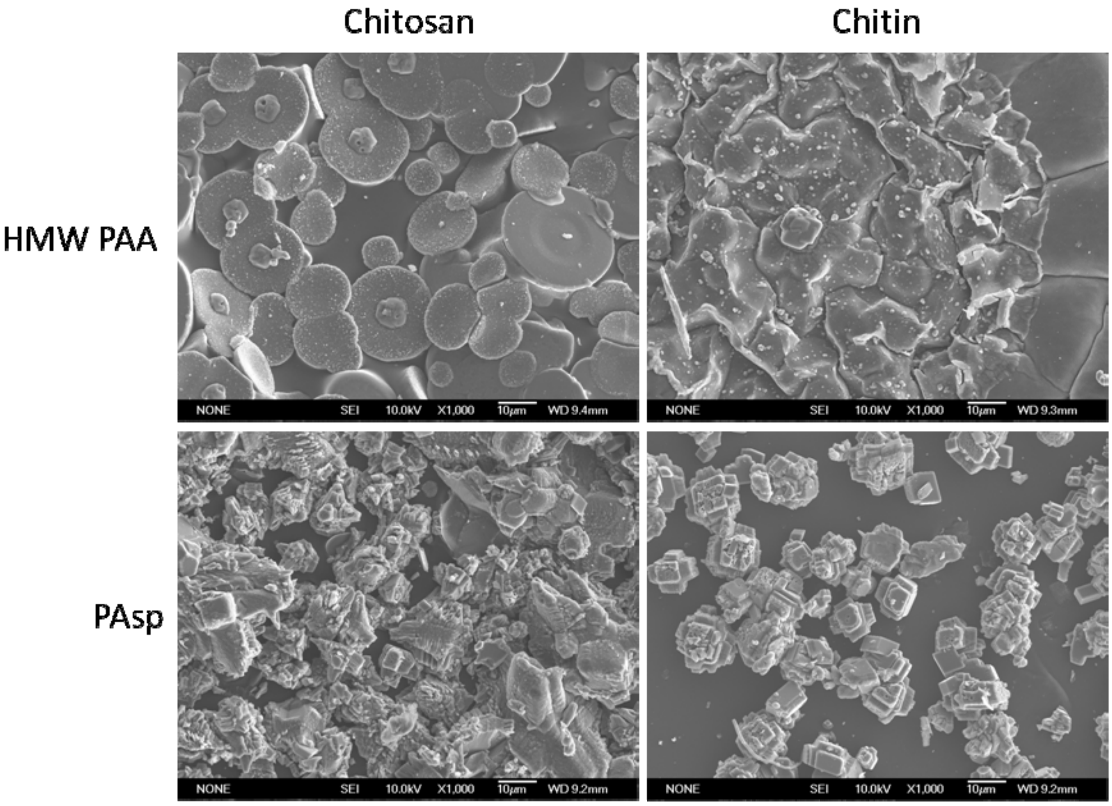
ESI Table 2 - Peak positions and assignments for the various polymer/calcium carbonate composites

Position /°2Theta	**d-spacing/Å**	**Assignment**	**Face**
9.37	9.44	*Chitin*[a]	*(001)*
19.56	4.54	*Chitin*[a]	*(021)*
23.16	3.84	Calcite	(012)
26.35	3.38	Aragonite	(111)
27.33	3.26	Aragonite	(021)
29.53	3.03	Calcite	(104)
31.31	2.86	Calcite/Aragonite	(006) and (002)
33.28	2.69	Aragonite	(012)
36.26	2.48	Calcite/Aragonite	(110) and (200)
38.03	2.37	Aragonite	(112)
38.75	2.32	Aragonite	(022)
39.55	2.28	Calcite	(113)
41.30	2.19	Aragonite	(211)
43.15	2.10	Calcite/Aragonite	(202) and (122)
45.99	1.97	Aragonite	(221)
47.60	1.91	Calcite	(024)
48.59	1.87	Calcite/Aragonite	(116) and (202)
50.38	1.81	Aragonite	(132)
52.59	1.74	Aragonite	(113)
57.56	1.60	Calcite	(122)
59.42	1.56	Aragonite	(311)
60.83	1.52	Calcite	(214)
63.35	1.47	Calcite/Aragonite	(125) and (151)
64.84	1.44	Calcite/Aragonite	(300) and (004)
66.36	1.41	Aragonite	(330)

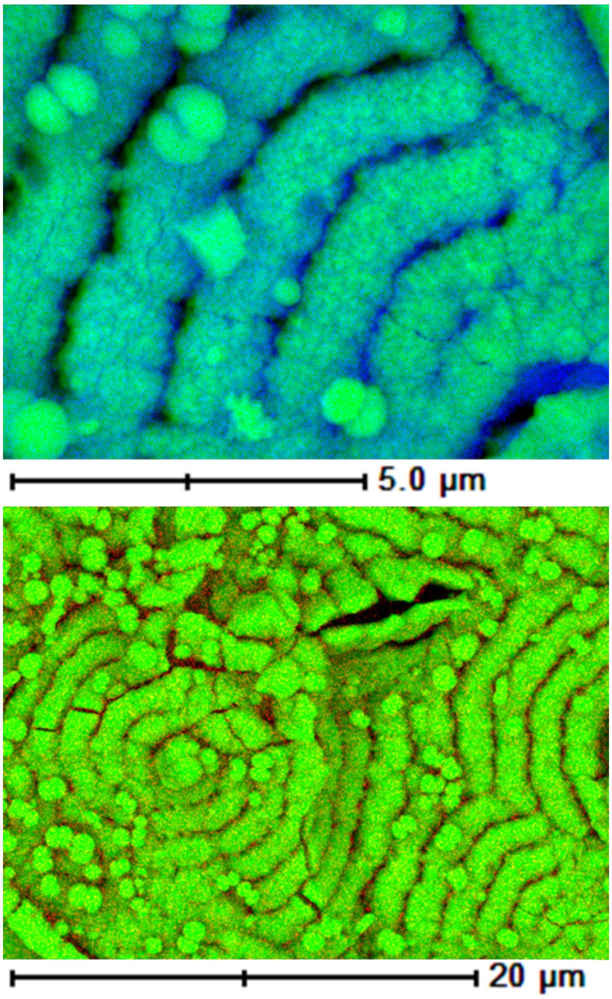
[a] The chitin peaks were assigned to anhydrous β-chitin as described by Saito *et al.*[6]



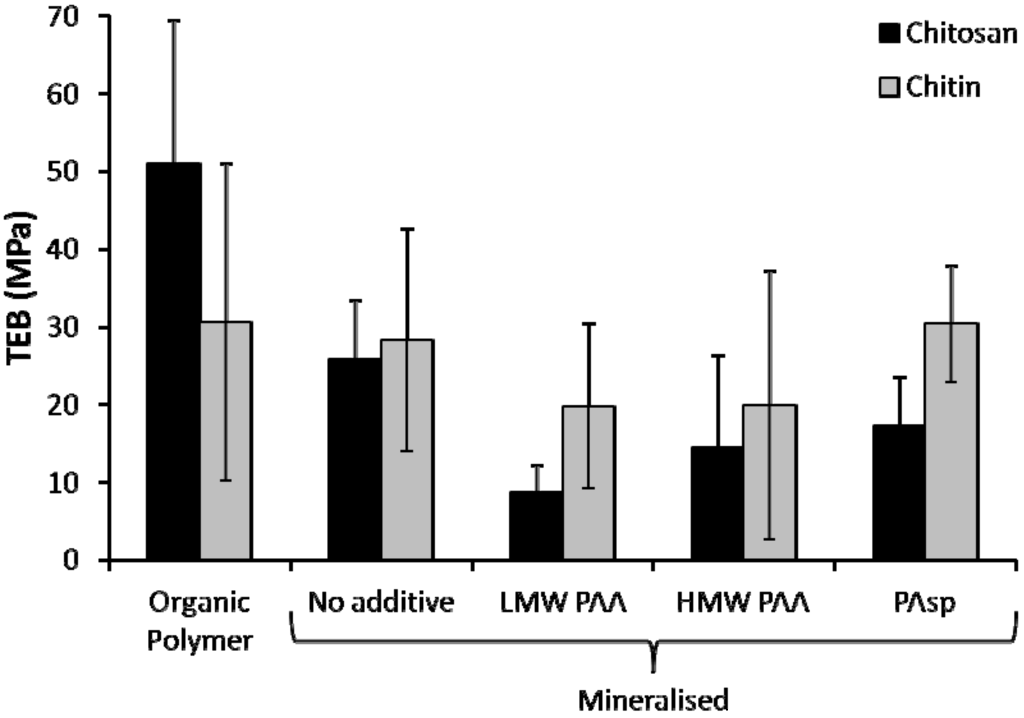
ESI Figure 1 – MicroRaman single-crystal spectra of (a) chitosan/$CaCO_3$ (b) chitosan/PAA/$CaCO_3$ (c) chitin/$CaCO_3$ and (d) chitin/PAA/$CaCO_3$ composites. The data are consistent with the results from XRD analysis and confirm the presence of aragonite in the chitin/PAA/$CaCO_3$ composites.



ESI Figure 2 – SEM images of chitin/$CaCO_3$ composite materials prepared with HMW PAA or PAsp. The cubic crystals of calcium carbonate prepared with PAsp are similar to those prepared in the absence of additive, showing the weak influence of this polymeric acidic additive on the composite structure. Variation of the molecular weight of PAA did not significantly change the ratio of the polymorphs nucleated (Table 1) or the morphology of the crystals; flat, smooth aragonite and calcite crystals were formed in the case of PAA or HMW PAA additives.



ESI Figure 3 – EDS images of chitin/PAA/$CaCO_3$ composite materials showing the back-scatter image and Ca. These images confirm that the ordered structures are compromised of $CaCO_3$ crystals, and are consistent with the data from other techniques.



ESI Figure 4 – Tensile elongation to break (TEB) of the composite materials. The chitosan composites were significantly weaker than the chitosan polymer film alone and the chitin composites were the same strength as the chitin polymer film. These data indicate that at the levels of mineralisation studied, the polymer scaffold itself determines the work of fracture of the composite materials.

References

(1) G. D. Bennett *J. Chem. Educ.* 2005, **82**, 1380.
(2) K. Kurita, S. Ishii, K. Tomita, S. I. Nishimura and K. Shimoda *J. Polym. Sci. A* 1994, **32**, 1027.
(3) Y. Kitano *Bull. Chem. Soc. Jpn.* 1962, **35**, 1973.
(4) J. Brugnerotto, J. Lizardi, F. M. Goycoolea, W. Argüelles-Monal, J. Desbrières and M. Rinaudo *Polymer* 2001, **42**, 3569.
(5) S. R. Dickinson and K. M. McGrath *Analyst* 2001, **126**, 1118.
(6) Y. Saito, H. Kumagai, M. Wada and S. Kuga *Biomacromol.* 2002, **3**, 407.