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Unexpected Strength and Toughness in Chitosan-Fibroin Laminates Inspired by Insect Cuticle

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Unexpected strength and toughness in chitosan-fibroin laminates inspired by insect cuticle

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Materials and Methods

Materials Fabrication

Chitosan film: Chitosan (medium molecular weight, high degree of deacetylation; Sigma Aldrich) was dissolved at 2% w/v in 1% v/v acetic acid, 6 ml of the solution was poured on a 9 cm petri dish, and the solvent was then evaporated overnight at 37°C. The resulting crystallized film was submerged in NaOH 4% (w/v) for 10 min to neutralize the protonated amino groups and avoid further dissolution.[32] Films were intensely washed in deionized water to remove the remaining NaOH and dried at 37°C. The final thickness of the chitosan film was ~12µm.

Fibroin film: Degummed silk from *Bombyx Mori* (Mielke's Fiber Arts USA) was washed several times in deionized water and dried before being dissolved at 15% w/v in 80% (w/v) at 60°C for 6 hours. The dissolved silk was dialyzed against water for 4 days with constant water replacements in a tube with a 12-14 kDa molecular weight cut off (VWR Scientific, USA). The solution was filtered and centrifuged twice at 7500 rpm for 15 min to remove impurities, and final protein solution (5% w/v) was stored at 7°C to prevent formation of micelles and gelation. The fibroin solution was cast on a 9 cm petri dish and dried at 37°C for 12 hours to create a fibroin film.

Shrilk fabrication: To create Shrilk, a chitosan film was first formed and dried on a petri dish as described above, and then the fibroin solution was cast on top of this layer and dried at 37°C for 12 hours. The resulting laminate was immersed in methanol for 30 min to force the beta (i.e. insoluble) transition of the protein, and finally washed with deionized water and dried. To form thicker multilaminar structures (Fig. 3E), several Shrilk films were stacked together with a small amount of fibroin solution in between each layer. Trapped bubbles were removed by physically compressing the materials using a microscope slide, and

then the water was evaporated at 37°C followed by a methanol treatment to force the beta transition of the new fibroin.

Micromolding to Create Microtopographic Features

We micromolded the fibroin layer of Shrilk by casting the fibroin solution between a poly(dimethoxysilane) (PDMS) mold (fabricated by polymer casting on a structured Silicon surface) and a flat chitosan film. The water was evaporated at 37°C, treated in methanol, and the resulting structured Shrilk film was then easily peeled off from the mold.

Stress/Strain measurements:

Material characterization studies were carried out by cutting films of chitosan, fibroin, Shrilk or the chitosan-fibroin blend in strips (1.5 cm wide x 8 cm long) and measuring stress-strain relationships with an Instron 3342 instrument (500N, Instron, USA). The thickness of the samples was measured by microscopy (Axio Observer, Zeiss, Germany) as the average of 5 different points of the film, and results were confirmed by scanning electron microscopic analysis. For a realistic comparison between different hydration states, the thickness of water-saturated samples was determined before swelling.

From the Stress/Strain measures (Fig. S1), we determined the ultimate stress as the maximum ordinate, and the associated strain was defined as the ultimate strain. The Young Modulus (E) was determined by the average slope between the origin and the ultimate strain. The area under the curve, obtained by integration using a Riemann sum approximation between the zero and the ultimate strain, was used to determine the modulus of toughness.

Swelling:

Square (2 x 2 cm) samples of Shrilk with a constant chitosan thickness and variable amount of fibroin, were weighed before and after being immersed in deionized water at 37°C for 24h. The linear approximation in Fig. S3 was made with the supposition that both phases are independent, and the final weight of the Shrilk samples is given by:

$$W_{wet}=W_{cs}\ A_{cs}+W_{fib}\ A_{fib}$$

Where W_{wet} is the weight of the hydrated Shrilk, W_{cs} and W_{fib} are the dry weight of chitosan and fibroin in the sample, respectively, and A_{cs} and A_{fib} their associated water absorptions.

IR Spectrometry:

IR spectra (Fig. 4 & Fig. S6) were obtained with a resolution of 2 cm^{-1} between 4000 and 500 cm^{-1} (Vertex 70, Bruker, Germany) and analyzed with Essential FTIR (Operant LLC, USA). The FTIR spectrum of the fibroin in Shrilk was obtained by employing as background the single beam spectrum for a chitosan film with characteristics similar to that of the chitosan phase in Shrilk. The ratio of acetyl groups per glucosamine residue (i.e. degree of acetylation) was calculated as the relative intensity of the absorption bands at both sides of the rocking of the methyl group band (1379cm^{-1}),[33] which are situated at 1321 and 1417 cm^{-1} in Fig. S4. In the chitosan used in this experiments, 20.3±0.7% of the glucosamine groups were found to be acetylated.

SEM analysis:

The Scanning Electron Microscope images were taken with a Zeiss field emission Ultra55 SEM (Carl Zeiss SMT GmbH, Germany). Dry samples, immobilized on an aluminum holder, were introduced without modification in the chamber and examined under a 5 to 15KeV electron beam.

Table S1. Mechanical Properties

Material	Thickness (µm)	Ultimate Stress (Mpa)	Ultimate Strain	Modulus of toughness (J/cm^3)	Young Modulus (Gpa)
Chitosan	12.24±2.48	59.14±5.23 (w) 3.48±0.37	0.062±0.013 (w) 0.370±0.039	1.74±0.33 (w)0.75±0.01	0.95±0.28 (w)0.008±0.001
Fibroin	32.04±4.3	3.14±0.65 (w)4.17±1.02	0.017±0.004 (w) 0.211±0.079	0.037±0.008 (w) 0.91±0.18	0.19±0.08 (w)0.019 ±0.002
Blend (1:2)	34.38±2.9	12.36±2.47	0.032±0.007	0.11±0.023	0.38±0.11
Shrilk (1:1)	20.47±1.17	71.55±15.21 (w) 3.42±0.69	0.025±0.011 (w)0.195 ±0.058	1.37±0.21 (w) 0.51±0.14	2.63±1.72 (w) 0.017±0.009
Shrilk (1:2)	27.34±2.23	119.67±14.02 (w) 3.25±0.72	0.035±0.014 (w) 0.230±0.068	2.62±0.18 (w) 0.47±0.06	5.73±1.12 (w) 0.014±0.004
Shrilk (1:3)	41.98±2.25	51.29±6.06 (w) 3.07±0.81	0.026±0.005 (w) 0.191±0.045	1.15±0.11 (w) 0.51±0.09	1.99±0.66 (w) 0.016±0.008
Shrilk (1:4)	51.74±7.71	46.39±7.90 (w) 3.18±0.72	0.033±0.008 (w) 0.155±0.007	1.06±0.12 (w) 0.34±0.07	1.40±0.60 (w) 0.020±0.005
Shrilk (1:5)	67.16±6.95	32.88±0.86 (w) 2.28±0.86	0.030±0.002 (w) 0.146±0.050	0.63±0.26 (w) 0.21±0.06	1.07±0.34 (w) 0.016±0.011

*All errors are standard deviation. The number in brackets corresponds to the chitosan:fibroin weight ratio and (w) refers to water saturated samples.

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[33] J. Brugnerotto, J. Lizardi, F. M. Goycoolea, W. Argüelles-Monal, J. Desbrières, M. Rinaudo, *Polymer* **2001**, *42*, 3569-3580.
[34] U. G. K. Wegst, M. F. Ashby, *Philosophical Magazine* **2004**, *84*, 2167-2186

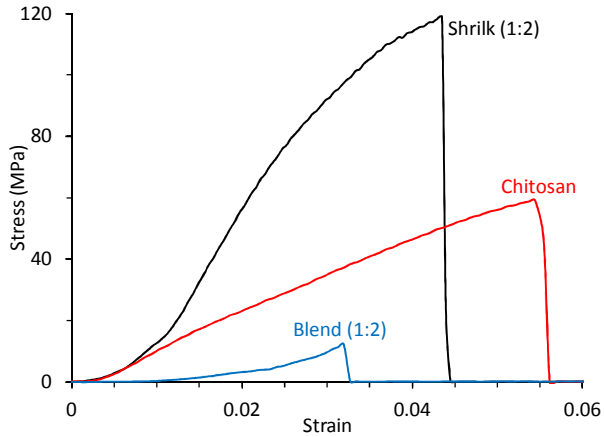

Fig. S1 – Representative stress-strain curves of Shrilk (blue line, 1:2 weight ratio chitosan:fibroin), chitosan (red line) and the 1:2 blend of chitosan:fibroin.

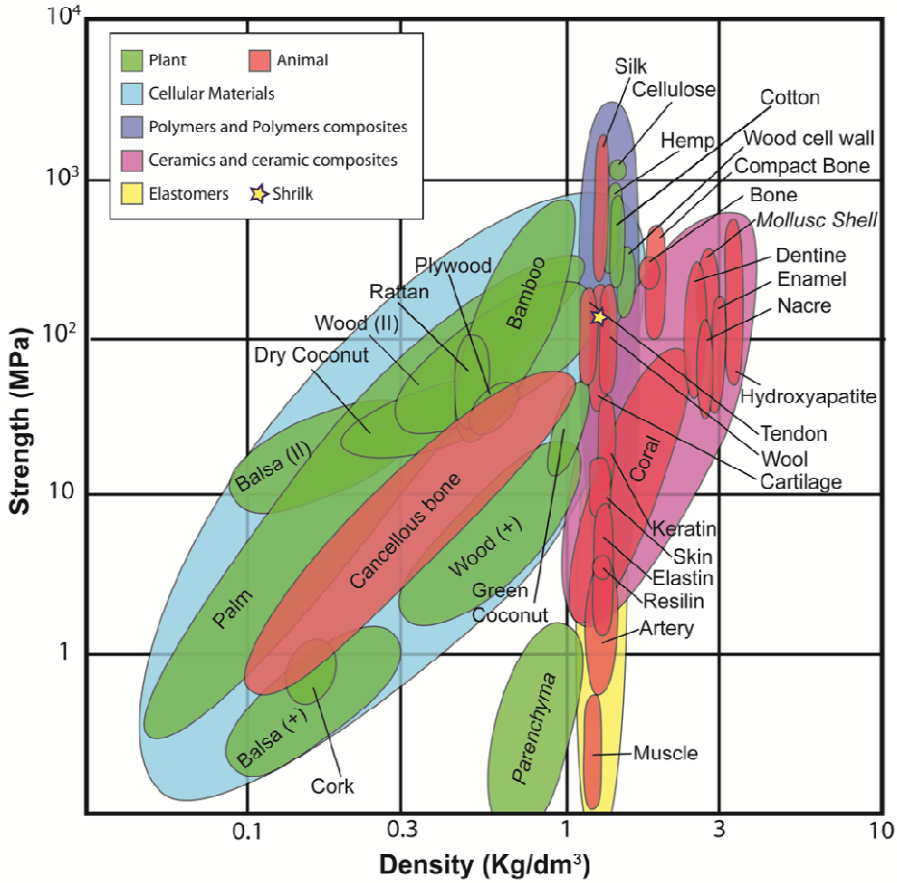

Fig. S2 – Ashby plot of natural materials, with the star indicating properties of Shrilk. The diagram represents approximate values of yield and ultimate strength for several groups of natural materials. (II) and (+) are values for a force applied parallel or perpendicular to the main molecular direction respectively. Italic names are values from a compression test. Diagram reproduced from [34]

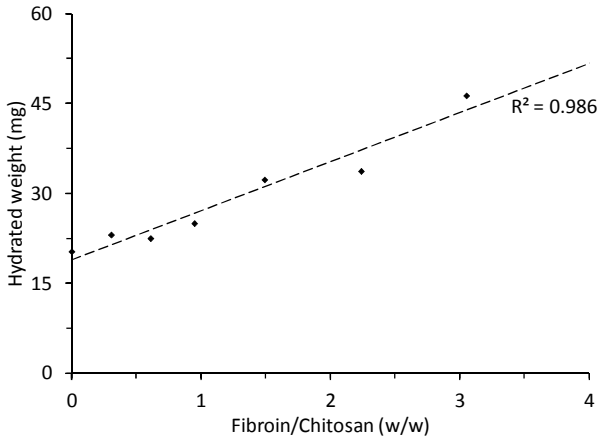

Fig. S3. – Weight of the hydrated samples with respect to fibroin:chitosan content. The linear fit presumes an independent absorption of each phase and neglects the effect of the interaction. Slope of the linear fit is 8.20 mg and the y-intercept is 18.89 mg.

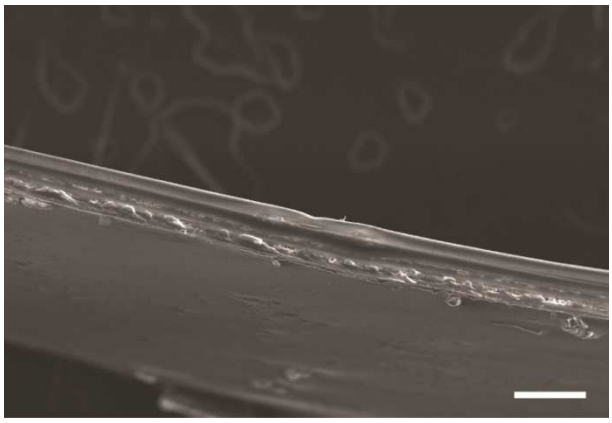

Fig. S4. – A scanning electron micrograph of a cross section of a microfabricated multi-laminate Shrilk, similar to **Figure 3e** (bar, 100 µm)

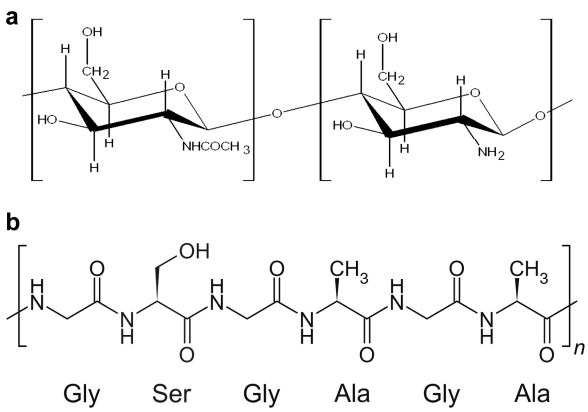

Fig. S5 – **(a)** Chitosan/chitin typical structure, where the acetyl-glucosamine (left) and the glucosamine (right) monomers, are representatives of chitin and chitosan respectively. **(b)** Fibroin molecule.

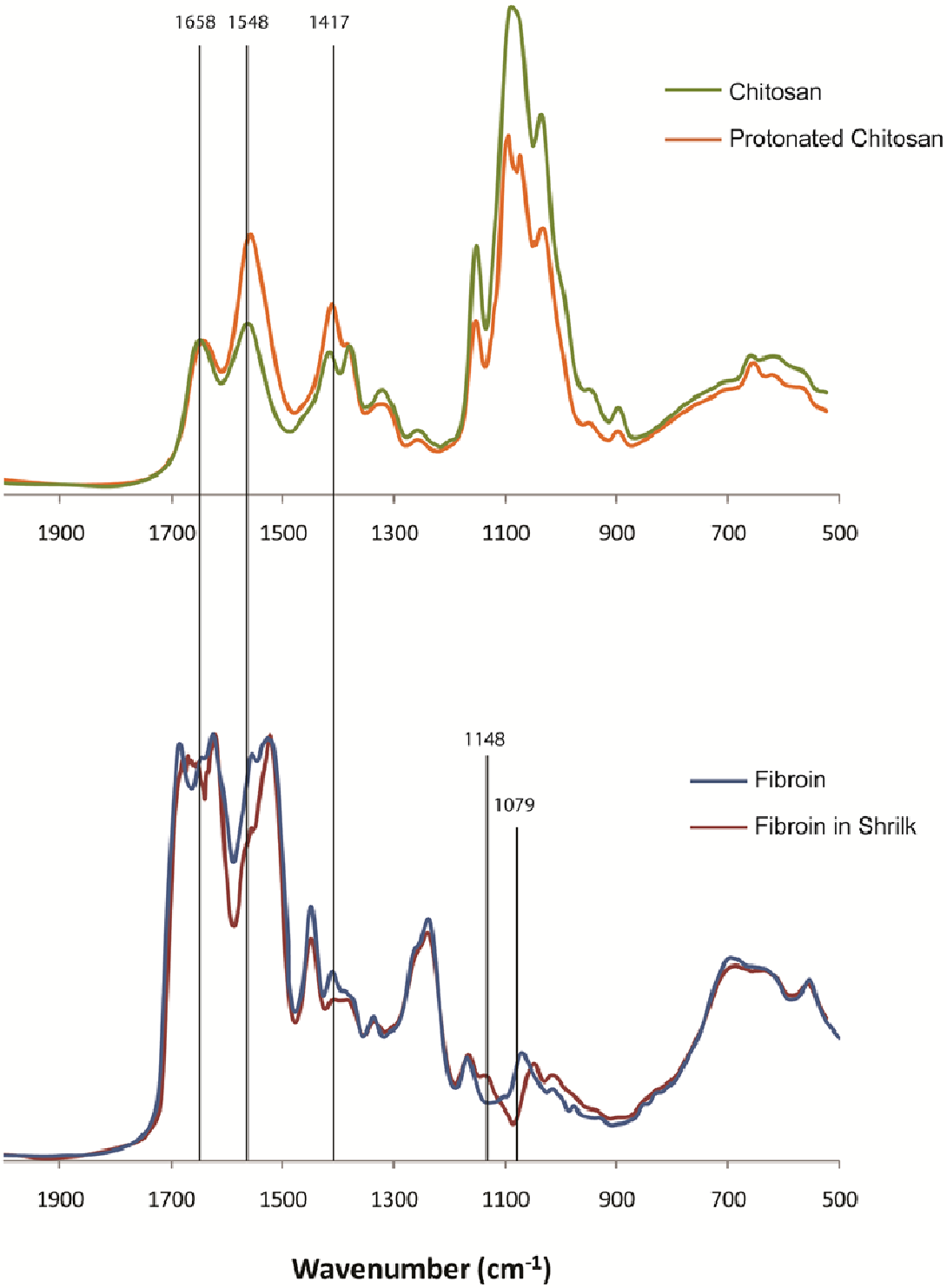

Fig. S6 – Magnified spectrum from Figure 4. Peaks with relevance to the discussion are marked.

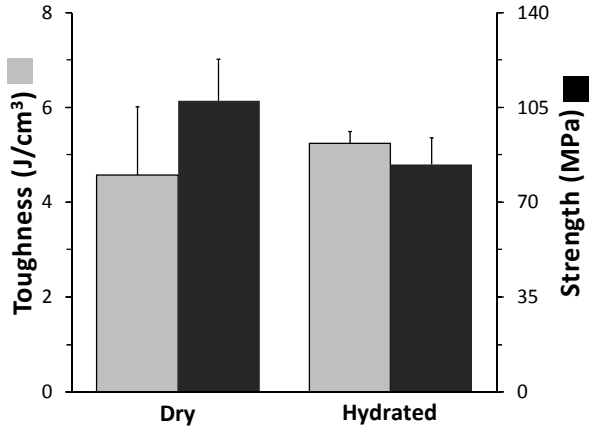

Fig. S7 – Mechanical properties of Shrilk samples coated with a very thin (i.e.1µm) Parylene-C layer. Hydrated samples were submerged in 37°C water for 24h before the mechanical test. In this conditions samples retain 80% of their original strength, demonstrating the suitability of the coating, even in extreme condition, to prevent moisture absorption (Error bars indicate SD)