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Supporting information for manuscript:

Flexible, microstructured surfaces using chitin-derived biopolymers

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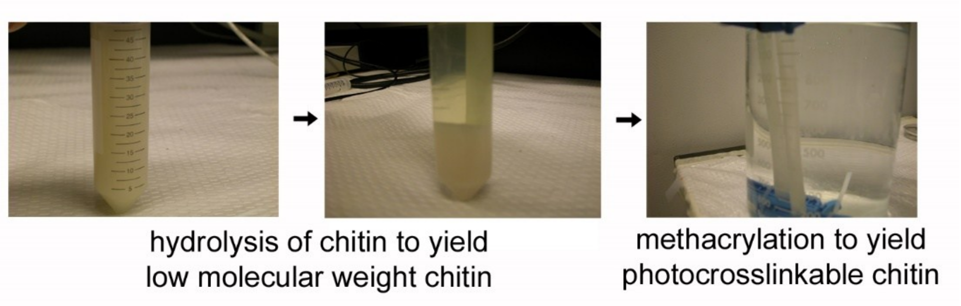


Steps showing the formation of photocrosslinkable chitin.

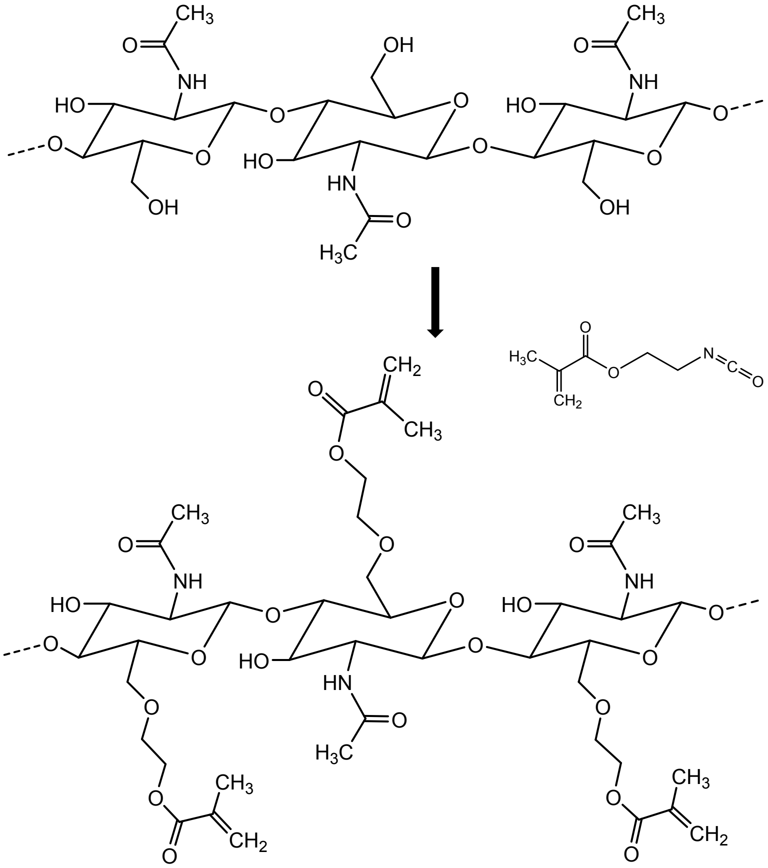


Figure S1: Reaction scheme of chitin with 2-isocyanatoethylmethacrylate to form photoreactive chitin.

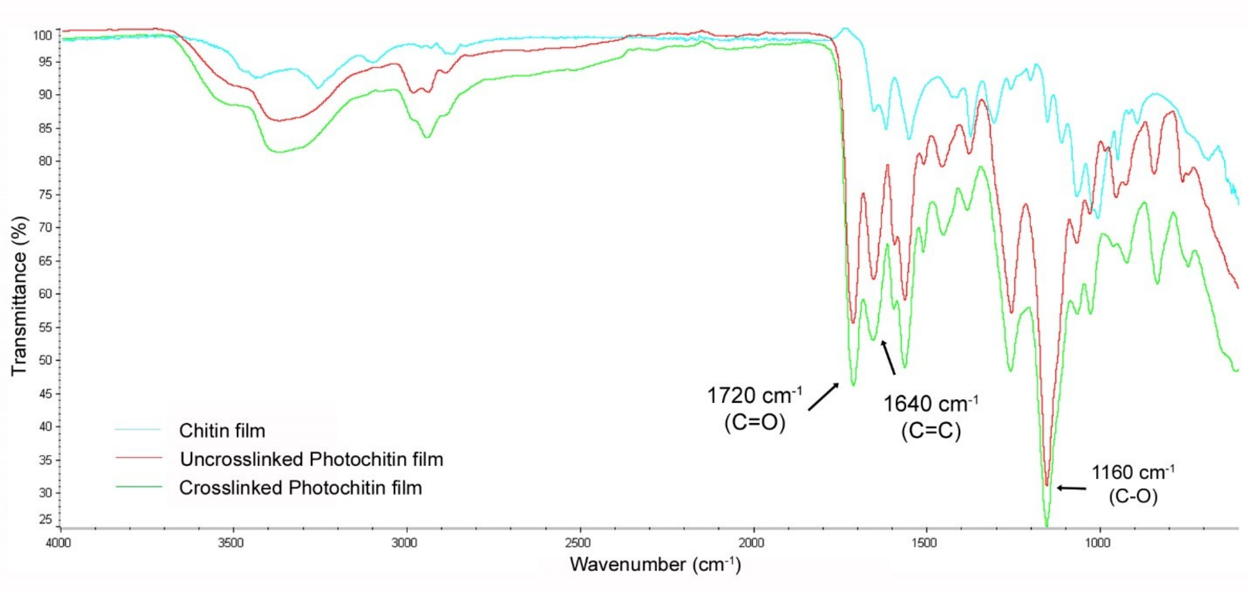


Figure S2: FTIR analysis to confirm the conjugation of photoreactive groups on the chitin.

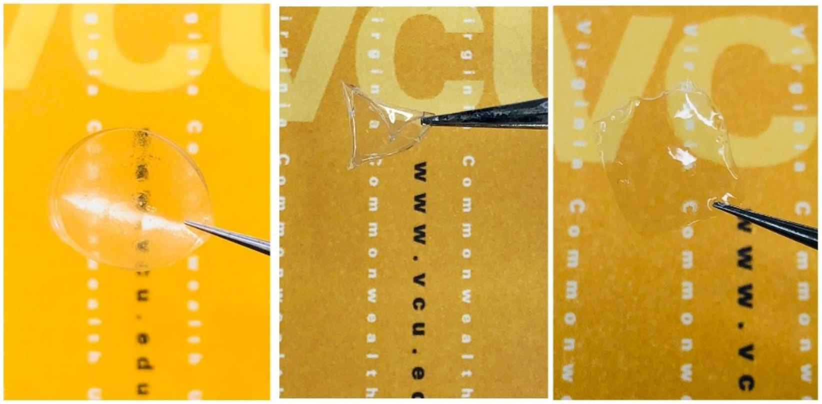

Chitosan film Photochitin (PC) film PC + 0.1% PEG-DA film

Figure S3: Photochitin films are optically transparent and strong (middle). However, the addition of a small amount (0.1% w/w) of PEG-DA enabled the formation of robust films which are easier to handle.

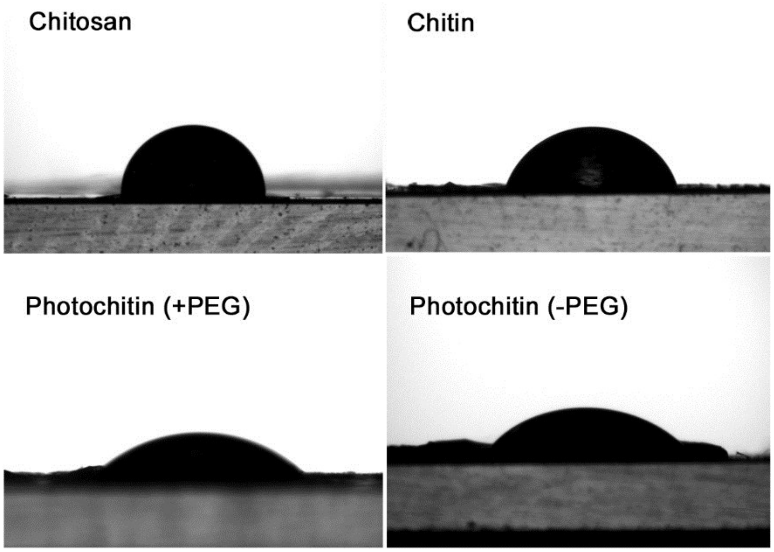


Figure S4: Contact angle measurements showing droplet on top: chitosan and chitin films.

Bottom: photochitin (with 0.1% PEG-DA) films and photochitin (with no PEG-DA)

Comments	Tensile Strength	Young’s Modulus	% Elongation	Ref.
Chitosan films with 25% glycerol added to the composition, prepared in 1% acetic acid solution.	17.4 MPa	-	60.3	[7]
Chitosan films using 1% acetic acid	70.3 MPa	-	6.2	[8]
Chitosan films using a 0.5% acetic acid solution (paper also studies the effect of crosslinking on mechanical properties using crosslinking reagents)	37.6 MPa	-	-	[9]
Chitosan films using 1% acetic acid	6.53 MPa			[10]
Chitosan films in Lactic Acid. Studied the effect of addition starch in the composite.	~26.0 MPa		~32	[11]
Chitosan films using 1% acetic acid solution		1.24 GPa	~5.5	[12]
Chitin films using KOH/urea solution	~30-80 MPa	3.9 GPa	11	[1]
Chitin films using FA-DCA solution. method developed by [2]	29.0 MPa		3.0	[3]
Mixture of βchitin and PEG. The properties of 3:1 βchitin/PEG is reported. The tensile strength decreases with the increase in PEG	35.0 MPa			[4]
Chitin films using 5% DMAc/ LiCl solution	38.34-77.21 MPa	1.2 – 3.6 GPa		[5]
Chitin films using ionic liquids	59.42 MPa	1.50 GPa		[6]

Table S1: Prior work on mechanical testing of chitin and chitosan films using different solvents and preparation techniques.

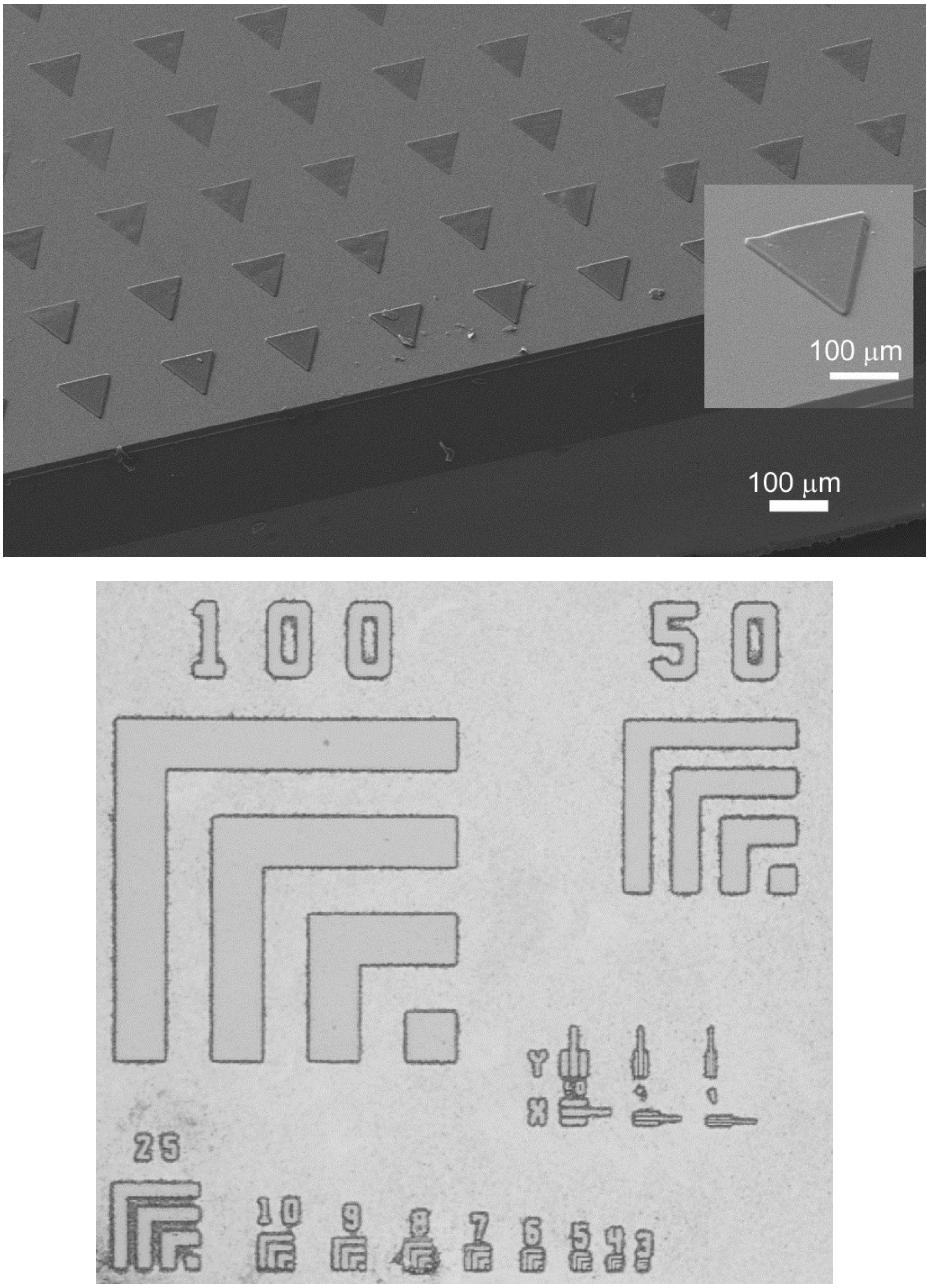


Figure S5: High resolution structures that can be formed using photolithography. The lower panel shows a test mask showing that features down to 10 µm can be easily formed.

Figure S6: Mechanical robust and flexible films formed by photocrosslinking.

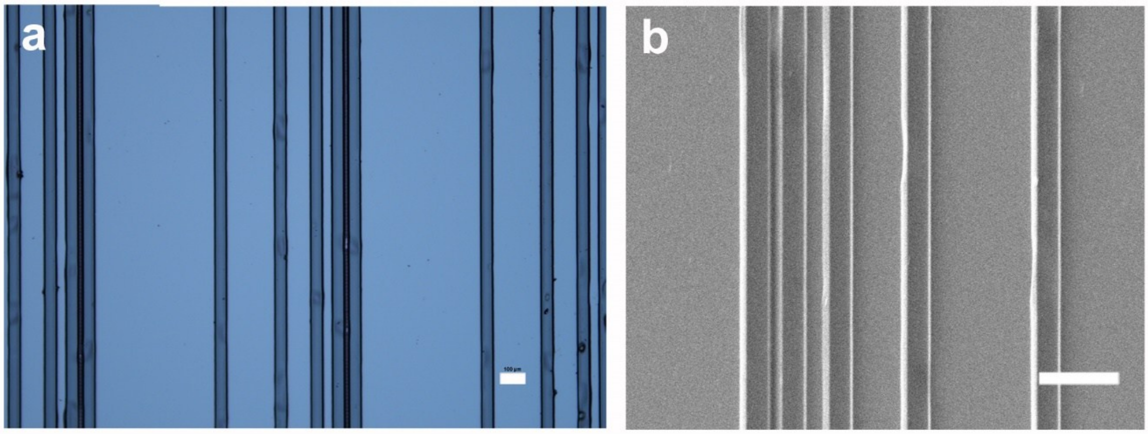


Figure S7: Gradient architectures used in the cell culture experiments. (L) Optical image and (R) SEM image. Scale bar = 100 µm on both images.

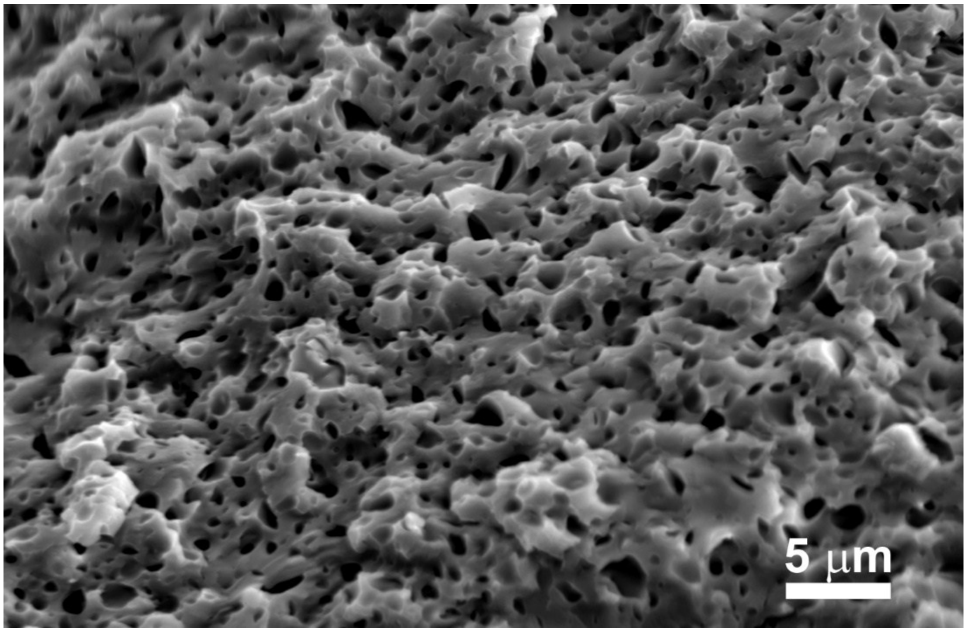


Figure S8: SEM image showing the porous surface of the 3D photochitin hydrogels.

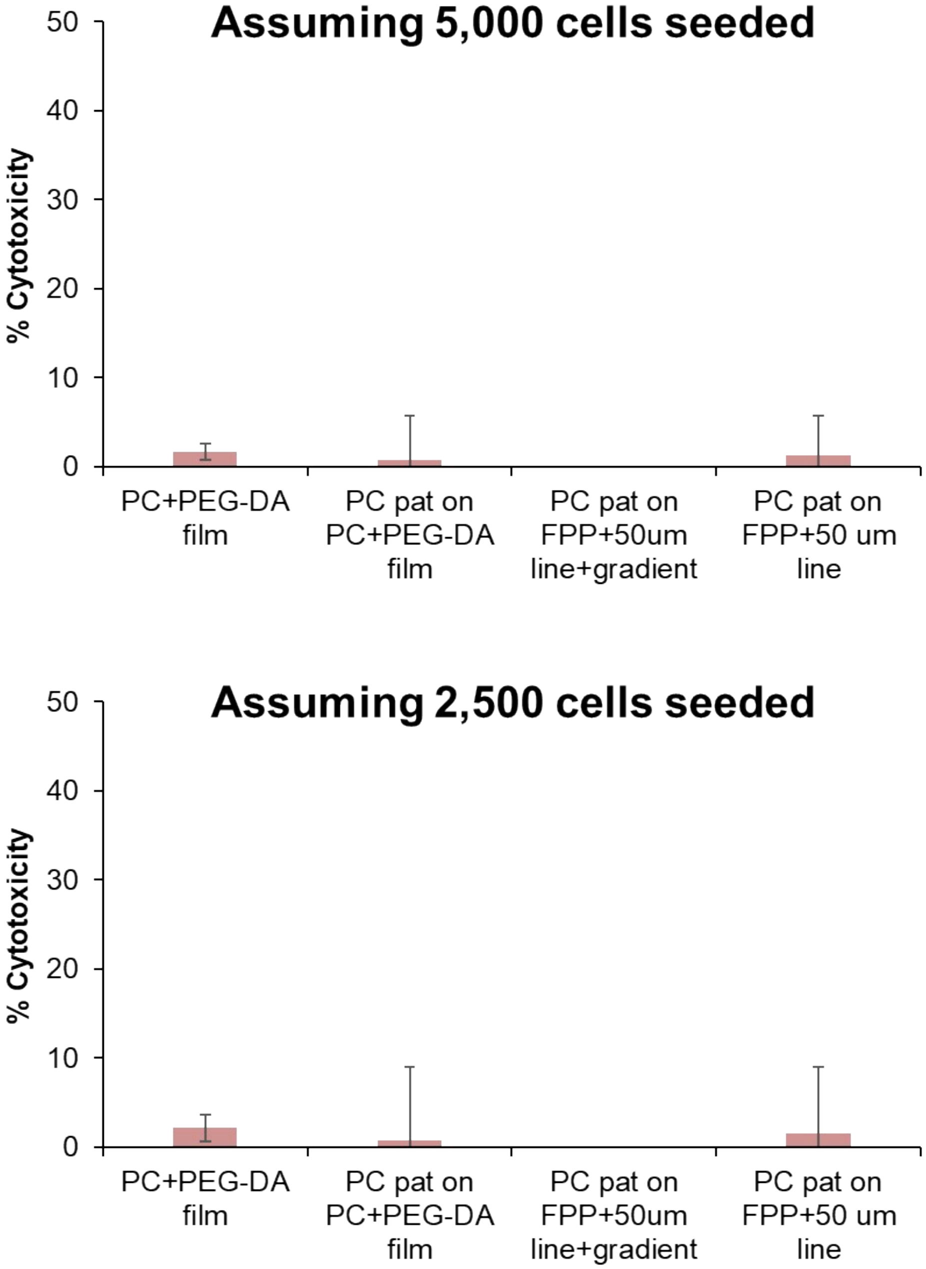


Figure S9: NIH 3T3s seeded for 48 hrs. Samples compared to tissue culture treated polystyrene (TCPS) (a 96 well plate). % Cytotoxicity, which was determined by normalizing to lysed TCPS and multiplying by 100%. Data presented as mean ± s.d.

Determination of degree of deacetylation

The degree of deacetylation (DD), which determines the content of free amino groups in the polysaccharides is used to differentiate between chitin and chitosan, as well as determine the chemical nature of the photochitin synthesized. Typically, chitin with a high degree of deacetylation is classified as chitosan. (Note that the term degree of acetylation (DA), which is also used is simply 100-DD). Various methods have been proposed for the determination of the DD. Several methods are tedious and expensive (e.g. NMR) or destructive to the sample (ninhydrin test). Therefore we used two analytical procedures for the DD determination – viz. infrared spectroscopy and UV-spectrophotometry for confirmation of the results.[13]

Determination of degree of deacetylation using infrared spectroscopy (IR)

The degree of deacetylation (DD) of the starting chitin, a commercially available chitosan as reference and the synthesized photocrosslinkable chitin (PC) were evaluated by FTIR using a technique proposed by Moore and Roberts.[14] Baseline corrections were made using the improved method suggested by Sabnis and Block.[15] Films of PC, chitin and chitosan were obtained using the protocols described above and dried to ensure no moisture.

The ratio of the absorbance at 1655 cm^{-1} to that of the absorbance at 3450 cm^{-1} was recorded and the DD was established using the equation:

$$DD = A_{1655}/A_{3450} \text{ x } 100/1.33 \text{ [14]}$$

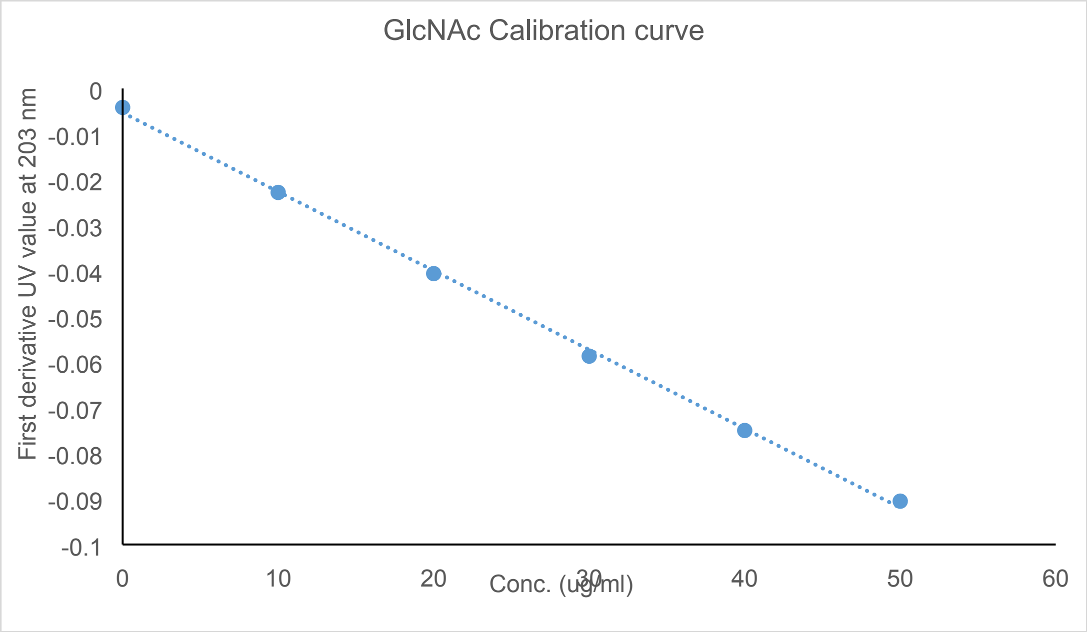
The absorbance peak at 1655 cm^{-1} corresponding to the amide I band was used as the probe and the absorbance at 3450 cm^{-1} corresponding to the *hydroxyl* stretch was used as the reference band. The *hydroxyl* band was used as an internal reference for the correction of variables such as film thickness and concentration. The “1.33” in the equation is the ratio of A_{1655}/A_{3450} for fully acetylated chitosan. The DD of commercially available chitin (Alfa Aesar) and chitosan (Sigma Aldrich) was evaluated using FTIR and compared with the values reported by the manufacturer in order to validate the results.

Sample	**Reported DD%**	**Calculated DD% (FTIR)**
Chitin (Alfa Aesar)	20-30	28.0
Chitosan (Sigma Aldrich)	75-85	79.4
PC	n/a	39.3

Determination of degree of deacetylation using infrared spectroscopy (IR)

We used previously reported and well established protocols for the spectrophotometric verification of the results obtained above.[13, 16] This method uses First Derivative UV-Spectrophotometric Analysis to obtain the DD. Initially, a standard curve was formed using solutions of GlcNAc of concentrations 0, 10, 20, 30, 40 and 50 µg/ml were made in 0.85% phosphoric acid. A calibration curve was obtained using the first derivative UV values at 203 nm as a function of GlcNAc concentration. A linear regression fit to the data was obtained ($R^2 = 0.99$)

GlcNAc (µg/ml)	A (203nm)
0	-0.00416
10	-0.0228
20	-0.0406
30	-0.0587
40	-0.075
50	-0.0905



Solutions in concentrated phosphoric acid at concentration 5 mg/ml was prepared by mixing chitin and photochitin (PC) in 85% phosphoric acid at 60^{O}C for 40 mins. The solution was then diluted 100 times using DI water and placed in the oven at 60^{O}C for 40 mins. The first derivative values at 203 nm of the samples were obtained. Chitosan was not tested in this method since the GlcNAc is very low (as expected). The amount of GlcNAc was determined using the calibration graph:

Sample	**First derivative (203 nm)**	**Amount of GlcNAc (µg/ml)**	**Calculated DD %**
Chitin	-0.0275	13.058	21.9
PC	-0.0443	22.94	40.2

It can be observed that both methods give a very close estimate to each other as well as the validation from the manufacturer’s data.

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