Elucidating the secondary structure of differently congealed, mineralised fibroin hydrogels & derivatives by confocal Raman-μ-spectroscopy

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Introduction

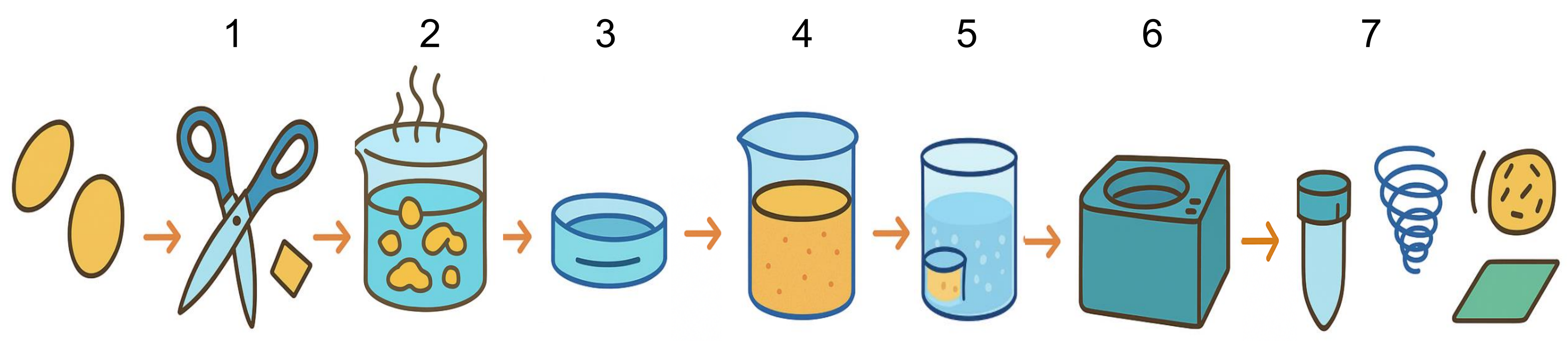
Silk fibroin (SF), derived from the cocoons of *Bombyx mori*, is a natural protein increasingly used in biomedical applications due to its biocompatibility, biodegradability, and tunable mechanical properties. The molecular structure of SF is primarily composed of various secondary structural motifs, including β-sheets, α-helices, and random coils. In its raw, aqueous solution form, SF predominantly exhibits a random coil conformation. Through different physical gelation mechanisms, these structural features can be modified to form stable hydrogels with distinct properties.[1]

Research Question

How do different physical gelation methods influence the secondary structure of SF hydrogels, based on analysis by Raman spectroscopy?
How do these structural modifications affect the extent and success of subsequent calcification?

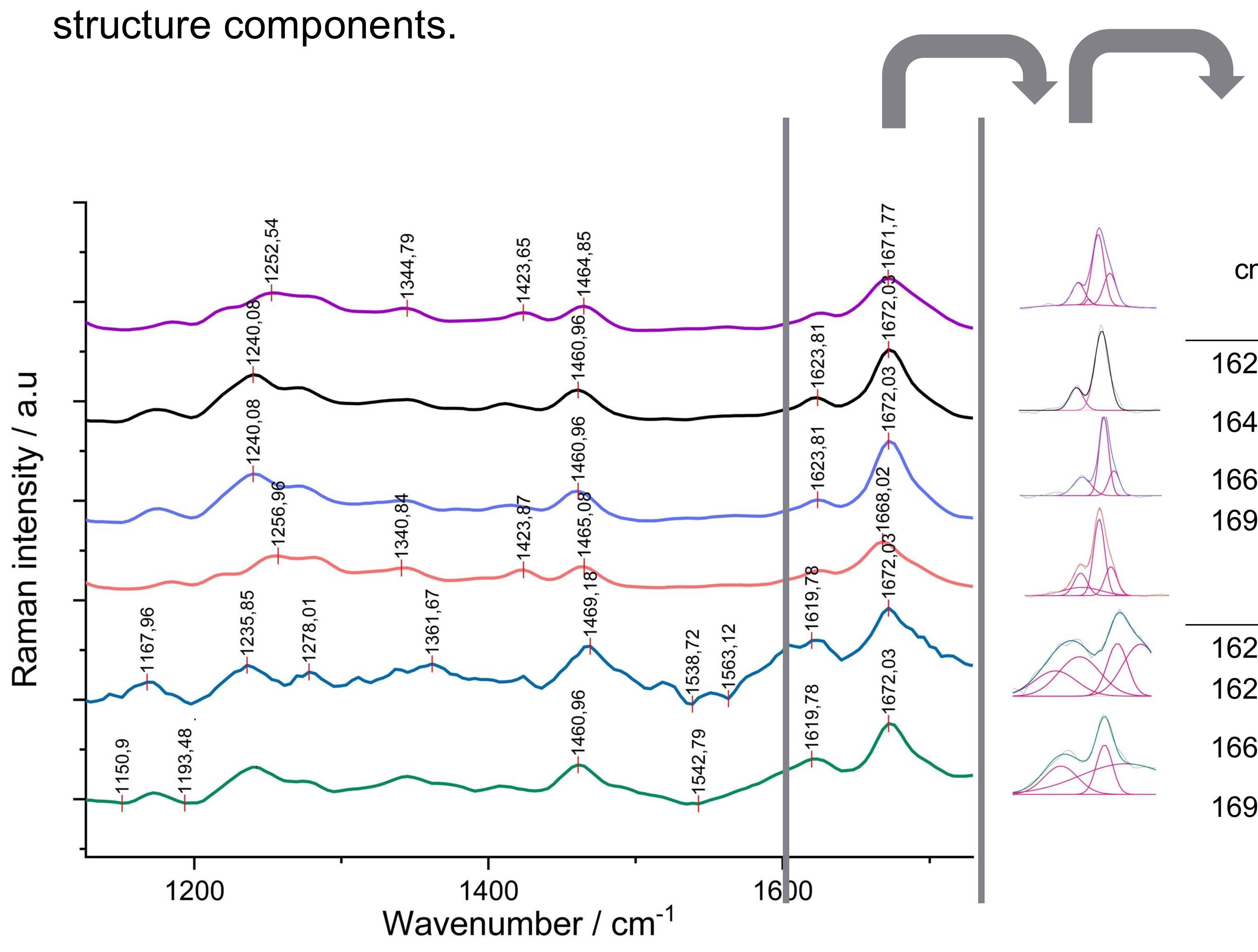
Synthesis

1 Cut cocoons in pieces
2 Degumming for 30 minutes in Na_2CO_3
3 Drying
4 Dissolution in LiBr for 5 hours at 69°C
5 Dialysis for 48h against water
6 Centrifugation
7 Hydrogel and derivative formation: Vortex, Sonication, Sponge and Film



Results and Discussion

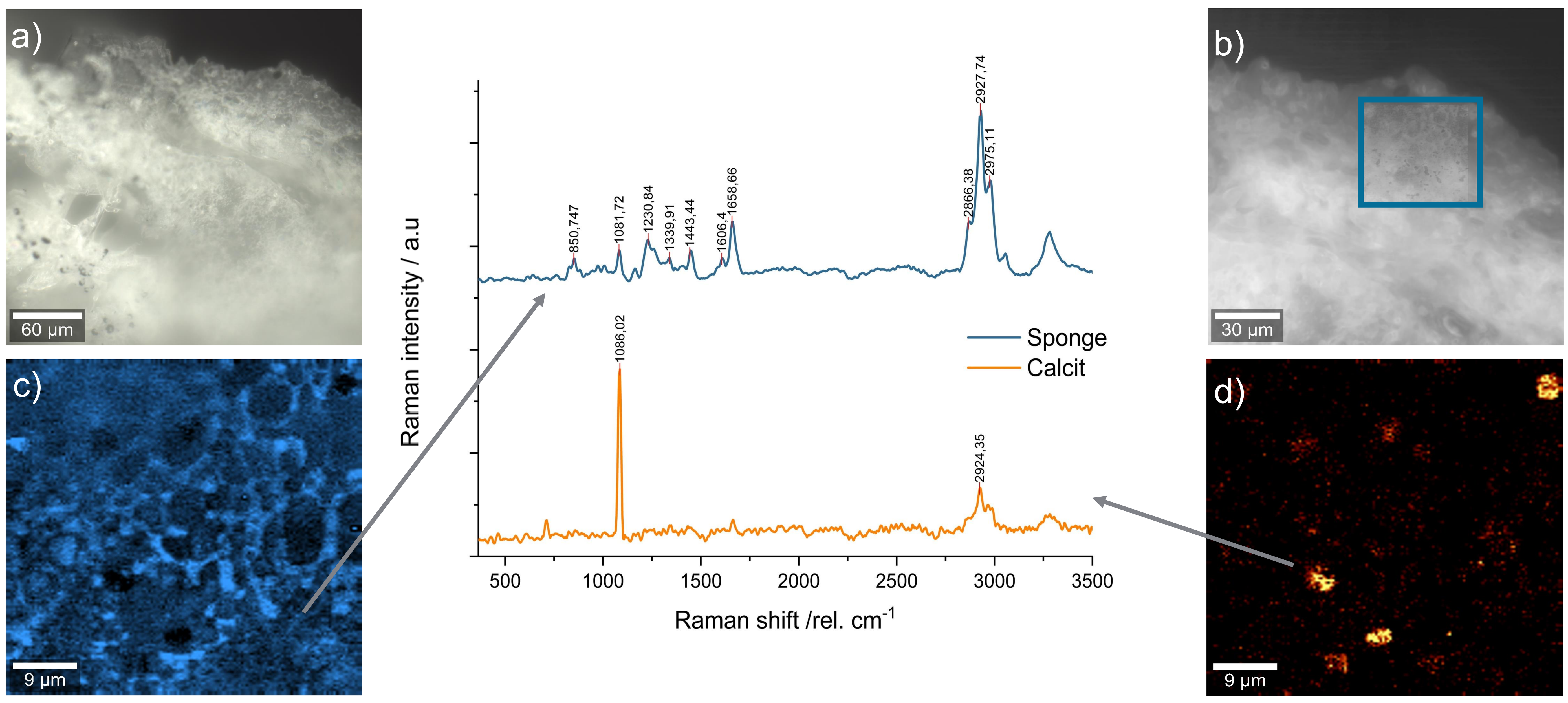
Raman spectroscopy was employed to investigate differences in secondary structure, with particular emphasis on variations arising from distinct gelation mechanisms. The spectral profiles reveal clear differences in characteristic bands, indicative of structural variations.
Peak deconvolution was performed for each gelation method to resolve the Amide I band, enabling detailed assessment of secondary structure components.



Amide Band shifts

cm^{-1}	Assignment	% content	cm^{-1}	Assignment	% content	cm^{-1}	Assignment	% content
	Solution			Vortex			Sonication	
1625.96	β-sheet	18.08	1621.09	β-sheet	21.59	1624.71	β-sheet	23.47
1648.91	Random coils	1.84	1666.08	Turns	0	1651.11	Random coils	2.07
1669.72	Turns	53.93	1674.14	Turns	78.40	1671.77	Turns	54.51
1695.40	Turns	26.14				1693.71	Turns	19.94
	Film			Sponge			Self gelation	
1626.04	β-sheet	16.51	1599.27	Side chains	20.34	1611.56	Side chains	24.92
1628.74	β-sheet	17.16	1626.48	β-sheet	31.96	1674.26	Turns	20.91
1666.93	Turns	46.99	1647.96	Random coils	0.16	1704.29	β-sheet (weak)	54.18
1692.90	Turns	19.33	1669.68	Turns	21.31			

True Component Analysis (TCA) Raman imaging of the calcified sponge including a microscopic image (a), the highlighted region of the area scan (b), component 1 representing the unmineralized hydrogel matrix (c), and component 2 corresponding to the mineralized hydrogel (d), yields succesfull calcification confirmed through Component Analysis. The resulting spectra in which component 2 (orange) exhibits a pronounced carbonate peak at 1086.02 cm^{-1} $v(CO_3^{2-})$, thereby also confirming calcite formation and β-sheet stabilization during mineralization.



References

[1] Zheng H., Zuo B., Functional silk fibroin hydrogels: preparation, properties and applications, J. Mater. Chem. B, 2021, 9, 1238-1258

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