Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary Information

Competition among physical, chemical, and hybrid gelation mechanisms in biopolymers

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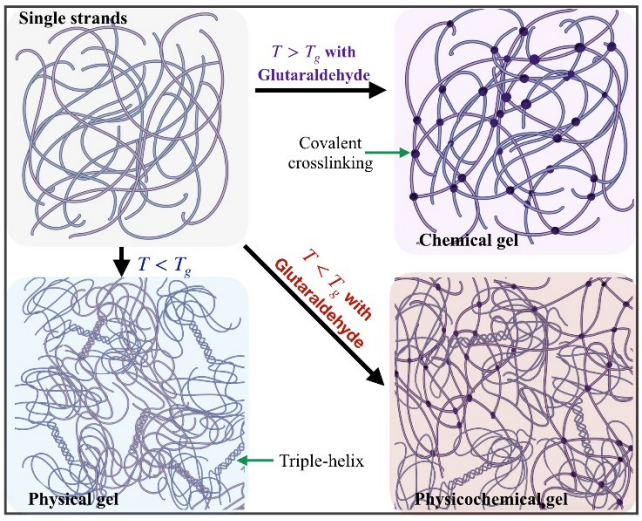


Figure SI1. Cartoon with three gelatin types: Physical gel, chemical, and physicochemical gels with glutaraldehyde as the crosslinking agent. T_g is the gelation temperature.

Estimation of gelation temperature for physical gels. An example of how T_g is obtained for gelatin solution at $C = 16\ Wt\ \%$ is presented in Fig. SI2a. For temperatures $\geq 40\ ^oC$, $G'(\omega)$ is proportional to ω^2 and $G''(\omega)$ to ω over a significant frequency domain, a behavior characteristic of polymer solutions.[1,2] As the temperature is lowered, both $G'(\omega)$ and $G''(\omega)$ get close and parallel, and eventually, when the temperature is equal to $T_g = 36.5\ ^oC$, both moduli are almost the same in almost four orders of magnitude of the frequency. A *log-log* plot of $G'(\omega)$ or $G''(\omega)$ *vs.* ω forms straight lines with the same slope; the system has reached the critical gel. For $T < T_g$, the viscoelastic behavior is solid-like. Also, at the gel point, $\tan(\delta)$ is independent of frequency, $\tan(\delta) = \tan(n\pi/2)$, as seen in the inset of Fig. SI2b for $36.5\ ^oC$ where the n value of Chambon & Winter criteria can be obtained, as well as the of stiffness, S. In this case, $n = 0.62$, which is equal to the results reported by Peyrelasse *et al.*[2] for a concentration range between 0.17 and $0.40\ g\ cm^{-3}$.

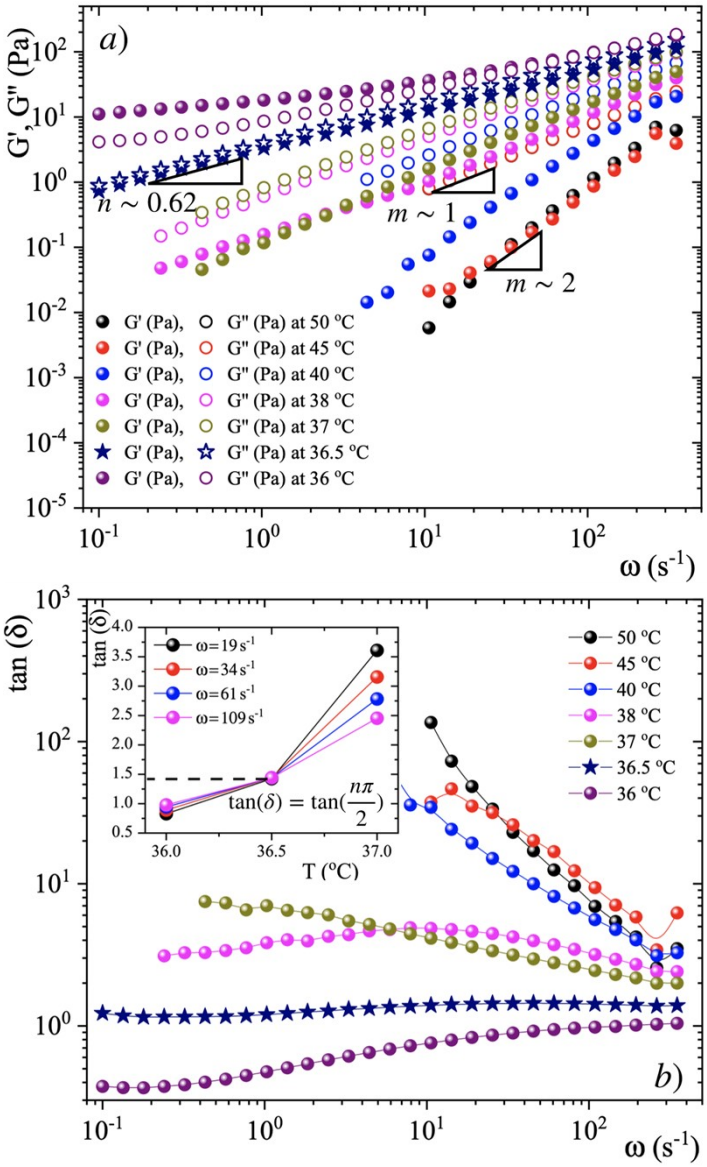


Figure SI2. a) Linear viscoelastic spectra (SAOS protocol) at different temperatures for a gelatin solution (*16 Wt %)* to obtain a physical gel. b) *tan (δ) vs.* ω at different temperatures; inset *tan (δ)* as a function of the temperature for different frequencies.

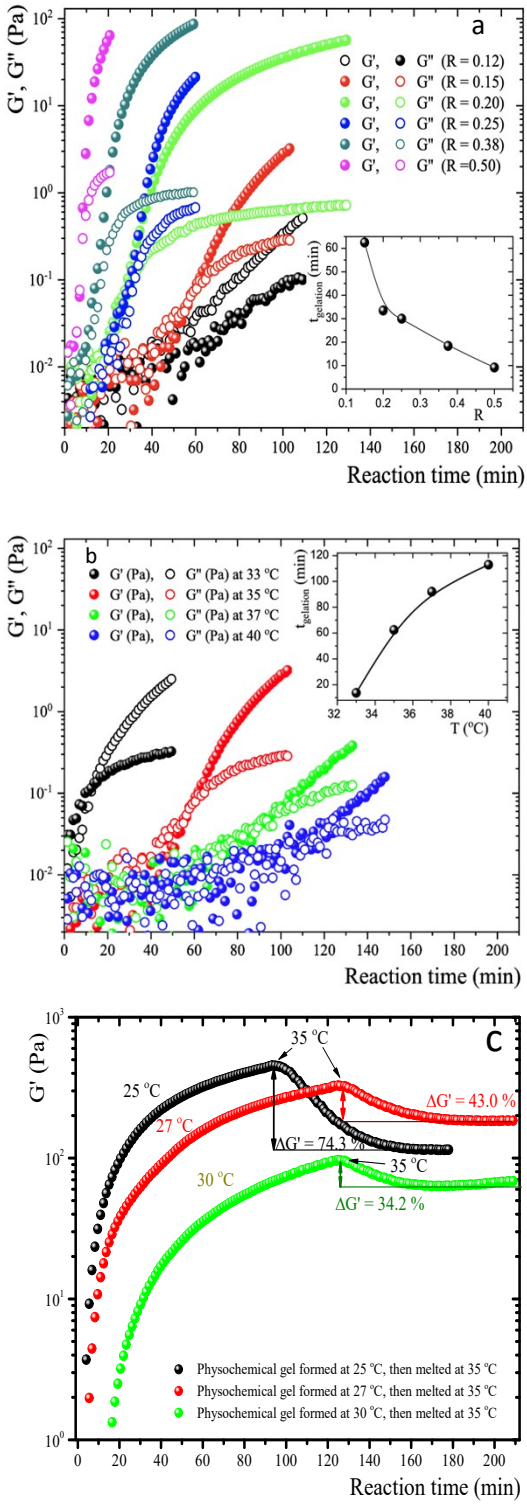


Figure SI3. a) Isothermal time sweep curves for a gelatin solution under chemical gelation at C =*5 Wt %* with different *R* values at *35 °C*. Experiments were performed at $\omega = 0.5\ s^{-1}$ and a strain of $\gamma = 0.8\ \%$. Insert: Gelation time as a function of R. b) Isothermal time sweep curves for a gelatin solution under chemical gelation at different temperatures with C = *5 Wt %* and *R* = 0.15. Insert: Gelation time as a function of the temperature. c) Isothermal time

sweep curves for a gelatin solution under hybrid gelation (*5 Wt %* and $R = 0.15$). The hybrid gel was formed at three different temperatures $\leq T_g$, and subsequently, the gel was melted to destroy the triple helices melted at 35 °C (indicated by an arrow), showing the remaining G' due to the chemical scaffold. As the temperature of hybrid gel formation is lower than T_g, the $\Delta G'$ is larger, showing a greater quantity of triple helices than at temperatures closer to T_g.

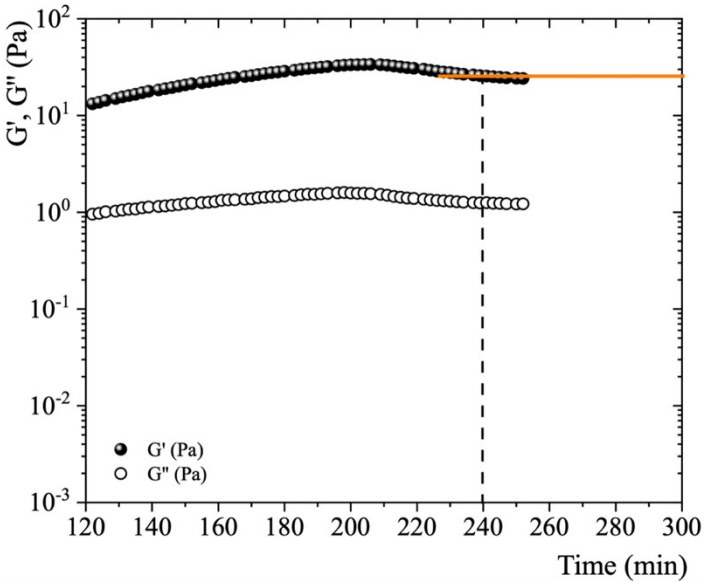
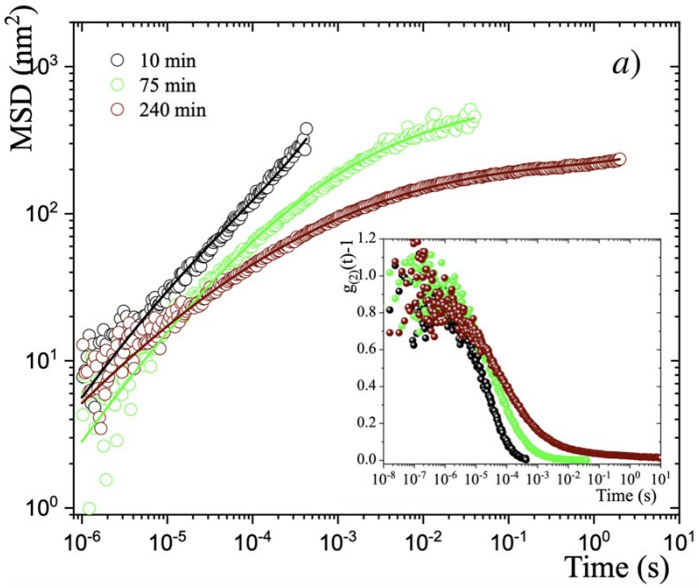


Figure SI4. Isothermal sweep curves for physical gelatin gels with *5 wt %* at $T = 29$ *°C*. Experiments were performed at $\omega = 0.5\ s^{-1}$ and strain of $\gamma = 0.8$ *%*.



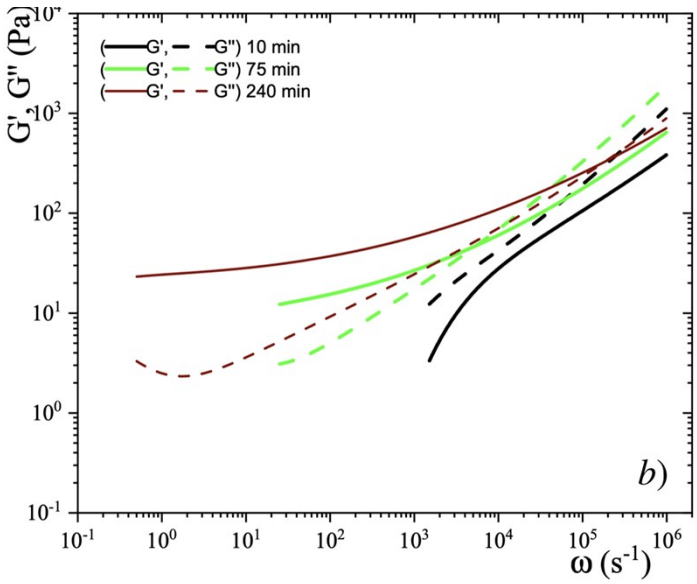
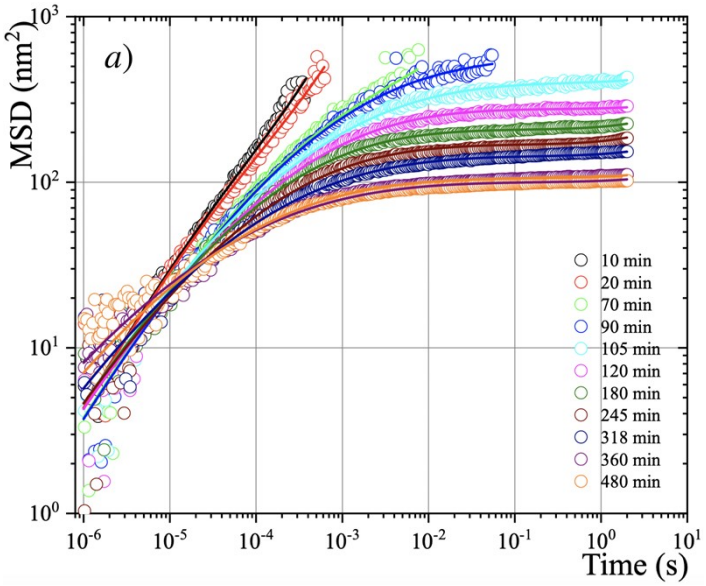


Figure SI5. a) *MSD vs. time* for polystyrene microspheres (*dia. 500 nm* and *vol. fraction 0.025*) embedded in a *5 Wt %* gelatin solution three times after quenching to *25 ºC*. *10 min*, *75 min*, and *240 min*. Open circles correspond to experimental MSD data, and the continuous lines correspond to best fitting using the Bellour *et al*. model. [3] Inset: Intensity correlation functions that produced curves in (a). b) Viscoelastic spectra for different times after quenching temperature (*10 min, 75 min,* and *240 min*) through the generalized Stokes-Einstein relationship [4,5] using the MSD adjusted by Bellour *et al*. model. [3]



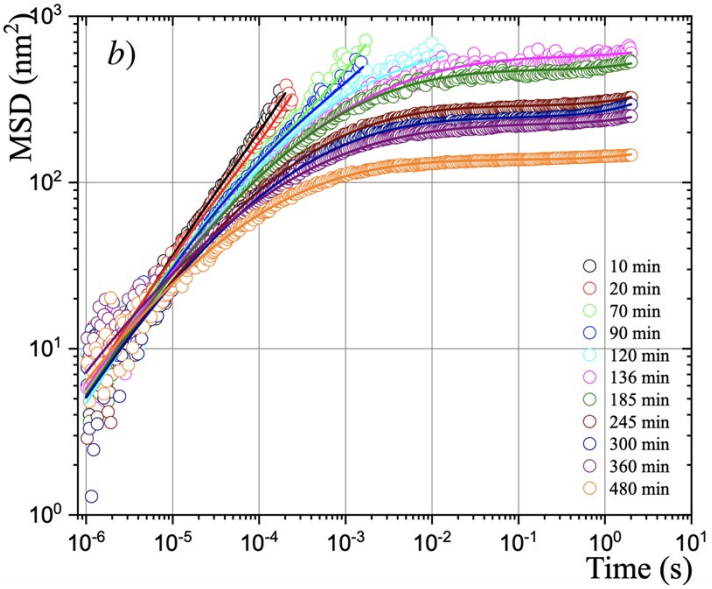


Figure SI6. *MSD vs. time* for microspheres embedded in gelatin solutions. a) Physicochemical gel formation for C = *5 Wt %* (*dia. 500 nm* and *vol. fraction 0.025)* after adding glutaraldehyde (*R = 0.15*) and simultaneously quenching to *27 ºC. b)* Physicochemical gel formation for C = *5 Wt %* (*dia. 500 nm* and *vol. fraction 0.025)* after adding glutaraldehyde (*R = 0.15*) and simultaneously quenching to *30 ºC.* Open circles correspond to experimental MSDs data, and continuous lines correspond to best fitting using the Bellour *et al*. model. [3]

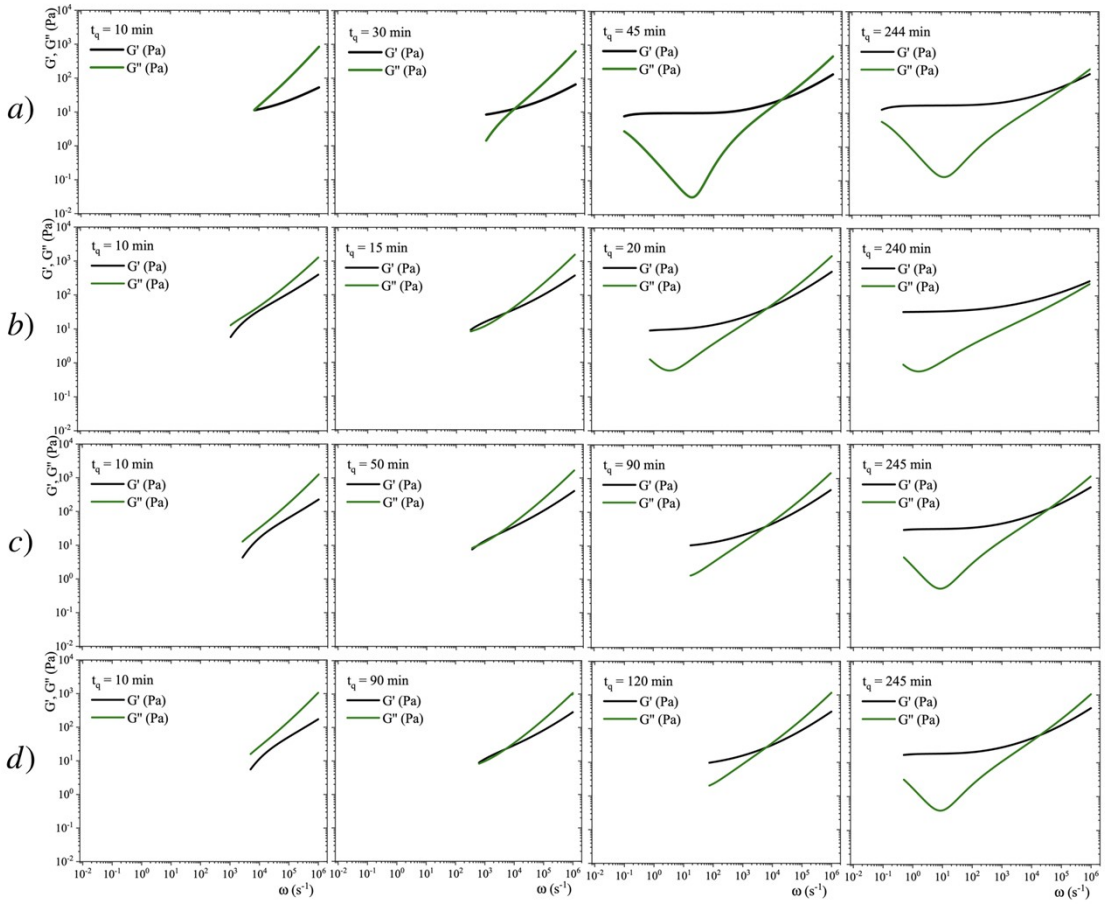


Figure SI7. Time evolution of viscoelastic spectra for gelatin solution. a) Chemical gel formation for *C = 3 Wt %* at different t_q after adding glutaraldehyde (*R = 0.25*).Physicochemical gel formation for *C = 5 Wt %* at different t_q after adding glutaraldehyde (*R = 0.15*) and simultaneously quenching: b) *25 ºC,* c) *27 ºC*, and c) *30 ºC.*

References

1 M. Doi, S. F. Edwards and S. F. Edwards, *The Theory of Polymer Dynamics*, Oxford University Press, 1988, vol. 73.

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