Electronic Supplementary Information

Preparation of Hydroxyapatite Nanoparticle-Hyaluronic Acid Hybrid Membranes through Citric Acid Molecular Mediation

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Experimental procedure S1

Optical transmittance of the membranes was characterized by using UV-Vis diffuse-reflectance absorption. The transmittance spectra of membranes were recorded, and their average transmittance in the visible light region between 380−780 nm was calculated. Moreover, the haze value measurement was performed with an integrating sphere, and was calculated according to our previous report.[S1, S2]

The hydration layer states in/on the membranes were investigated by the FT-IR spectrometer (FT/IR-4600, JASCO Co., Ltd.) spectral deconvolution technique at the wavenumber region between 3800–2400 cm^{-1} under the accumulation times of 100 and the resolution of 4 cm^{-1}. Using the transmittance method, the spectra were recorded after subtracting a spectrum of pristine KBr as the background, respectively. In particular, the O–H stretching band was deconvoluted into six components of *(i)* 2915 ± 20 cm^{-1}, *(ii)* 3105 ± 5 cm^{-1}, *(iii)* 3200 ± 20 cm^{-1}, *(iv)* 3300 ± 20 cm^{-1}, *(v)* 3400 ± 20 cm^{-1} and *(vi)* 3600 ± 20 cm^{-1}, belonging to *(i)* C–H symmetry, C–H_2 asymmetry, *(ii)* N–H stretching vibration of HYA, and *(iii)* free water molecules, *(iv)* O–H stretching vibration of HYA, *(v)* intermediate water molecules and *(vi)* nonfreezing water molecules, respectively.[S3–5] The deconvolution was performed by fitting with the Voigt function, and has been examined by our previous paper.[S6] Of the six components, three components derived from the hydration layer (specifically, *(iii)*, *(v)*, and *(vi)*) were extracted and their relative ratios were discussed. Based on the difference of the measurement technique, the evaluation and discussion of the hydration layer using the attenuated total reflection (ATR) method were discussed. The spectra were recorded after subtracting a spectrum of pristine air as the background, respectively.

The thermal behavior of the membranes with changing the temperature was measured by TG-DTA curves under N_2 gas atmosphere, and the weight concentration of hydration layer was evaluated. The temperature change in the membrane in an aluminum pan was in two steps; first, the temperature was held at 37°C for 90 mins to completely remove the extra waters weekly adsorbed on the membranes. Then, the temperature was changed from 37 to 275 °C at the heating rate of 10 °C/min (sampling time: 1 sec). The weight loss in the region between DTA peak tops was calculated as the hydration layer.

References

S1 Z. Liu, T. Kataoka, S. Samitsu, D. Kawagoe and M. Tagaya, *J Mater Chem B*, 2022, **10**, 396–405.

S2 Z. Liu, T. Kataoka, D. Kawagoe, D. Noda, Y. Chai and M. Tagaya, *Colloid Interface Sci Commun*, 2020, **39**, 100316.

S3 S. Morita, M. Tanaka and Y. Ozaki, , DOI:10.1021/la0625998.

S4 K. Haxaire, Y. Maréchal, M. Milas and M. Rinaudo, *Biopolymers*, 2003, **72**, 10–20.

S5 R. Gilli, M. Kacuráková, M. Mathlouthi, L. Navarini and S. Paoletti, *Carbohydr Res*, 1994, **263**, 315–326.

S6 S. Yamada, M. Tagaya, S. Yamada and S. Motozuka, *J Mater Chem B*, 2020, **8**, 1524–1537.

Fig. S1

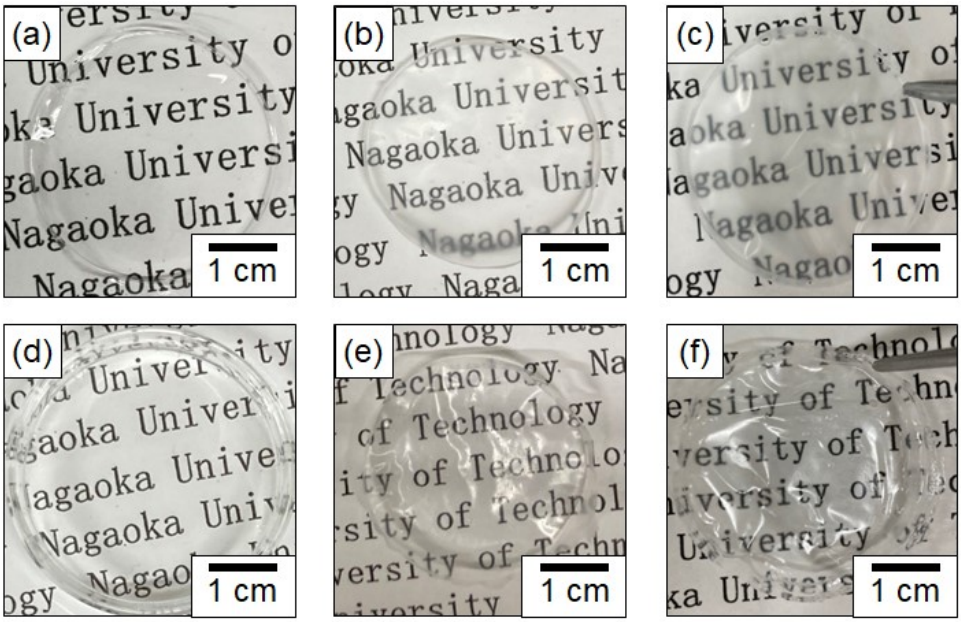

Fig. S1 Photographs of (a, d) HYA, (b, e) HAp-HYA, and (c, f) Cit/HAp-HYA membranes after the washing by (a−c) ethanol and (d−f) water.

Fig. S2

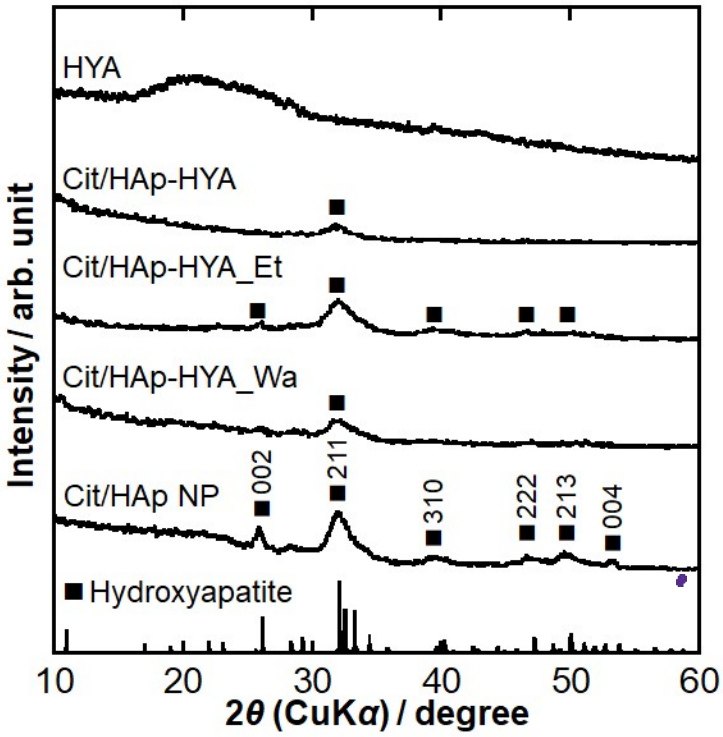

Fig. S2 XRD patterns of the HYA and Cit/HAp NPs alone and the membranes with the washing processes. The samples including the NPs exhibited the single phase due to hydroxyapatite (■: ICDD No.: 00-009-0432). The crystallite size (D_{211}) is 16.3 nm (the NPs alone), 9.6 nm (Cit/HAp-HYA), 13.6 nm (Cit/HAp-HYA_Et) and 10.9 nm (Cit/HAp-HYA_Wa).

Fig. S3

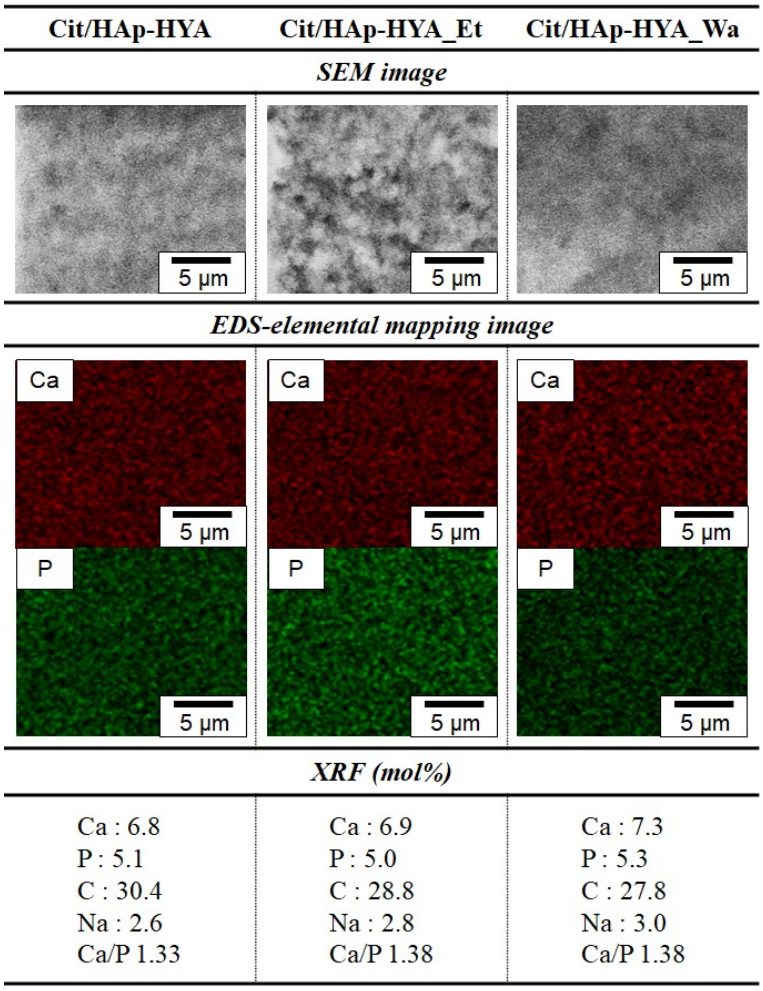

Fig. S3 SEM and EDS-elemental mapping images, and the chemical elements (mol%) measured by XRF of the membranes. The chemical elements of Ca and P measured by the EDS mapping were detected in all the membranes, showing the relative Ca and P concentrations of 8.1 and 5.0 mol% (Cit/HAp-HYA), 9.2 and 5.4 mol% (Cit/HAp-HYA_Et), and 8.3 and 4.9 mol% (Cit/HAp-HYA_Wa).

Fig. S4

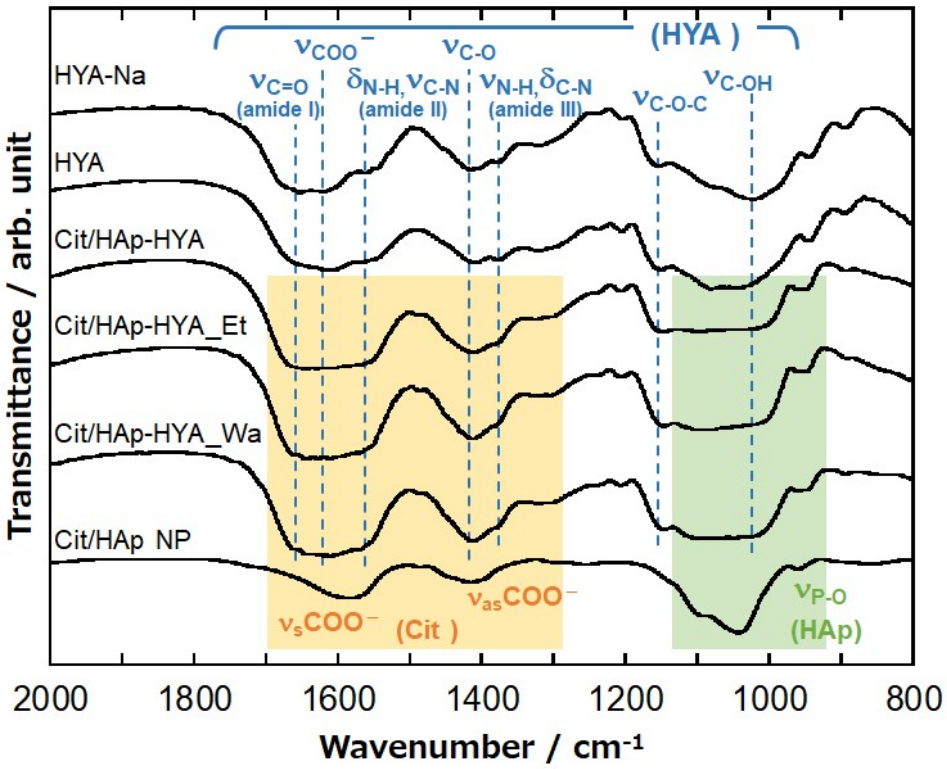

Fig. S4 FT-IR spectra of the membranes in the measured region between 2200−800 cm^{-1}.

Fig. S5

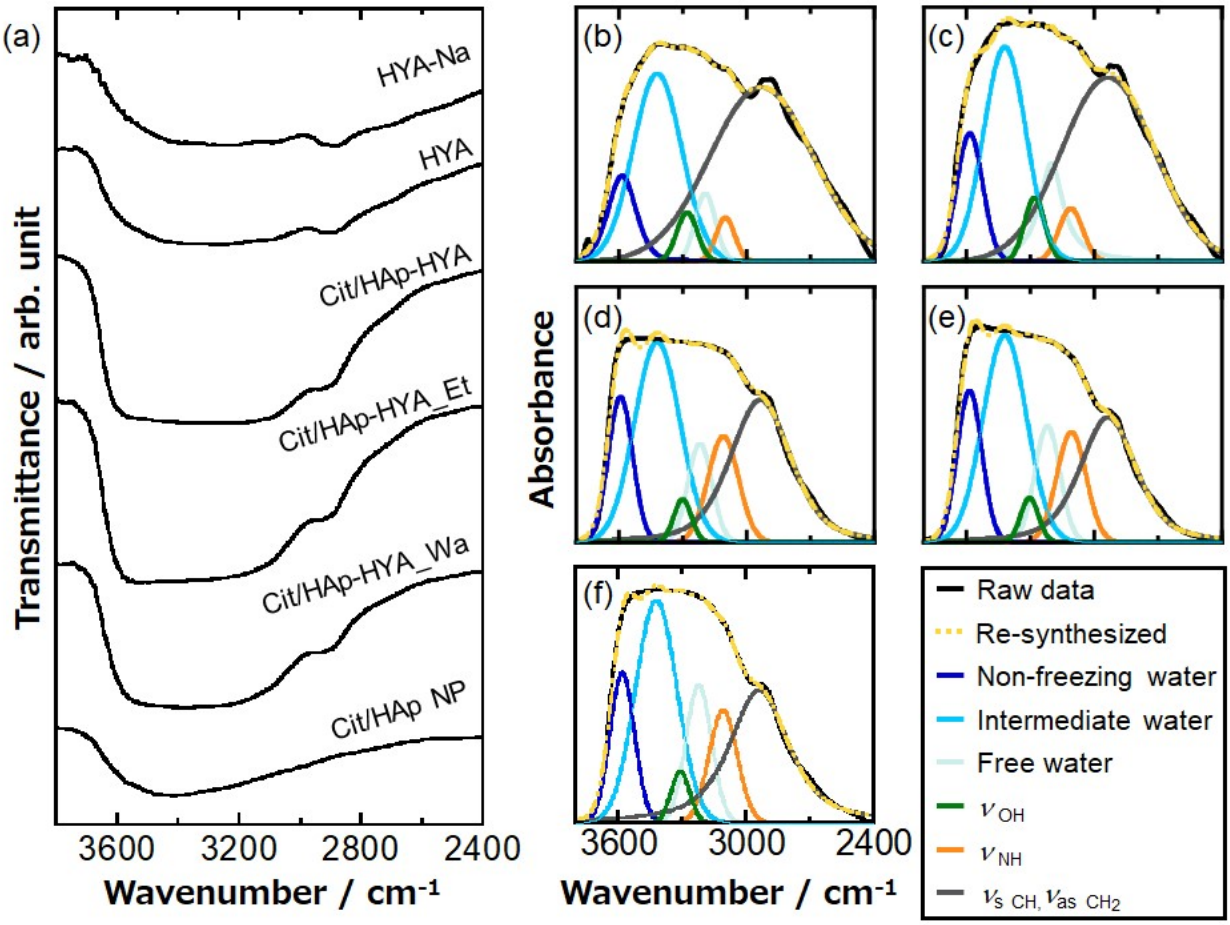

Fig. S5. FT-IR spectra of the membranes in the measured region between (a) 3800−2400 cm^{-1}, and (b−f) are the curve fitting and peak separation results ((b) HYA-Na, (c) HYA, (d) CitHAp-HYA, (e) CitHAp-HYA_Et, and (f) CitHAp-HYA_Wa).

Fig. S6

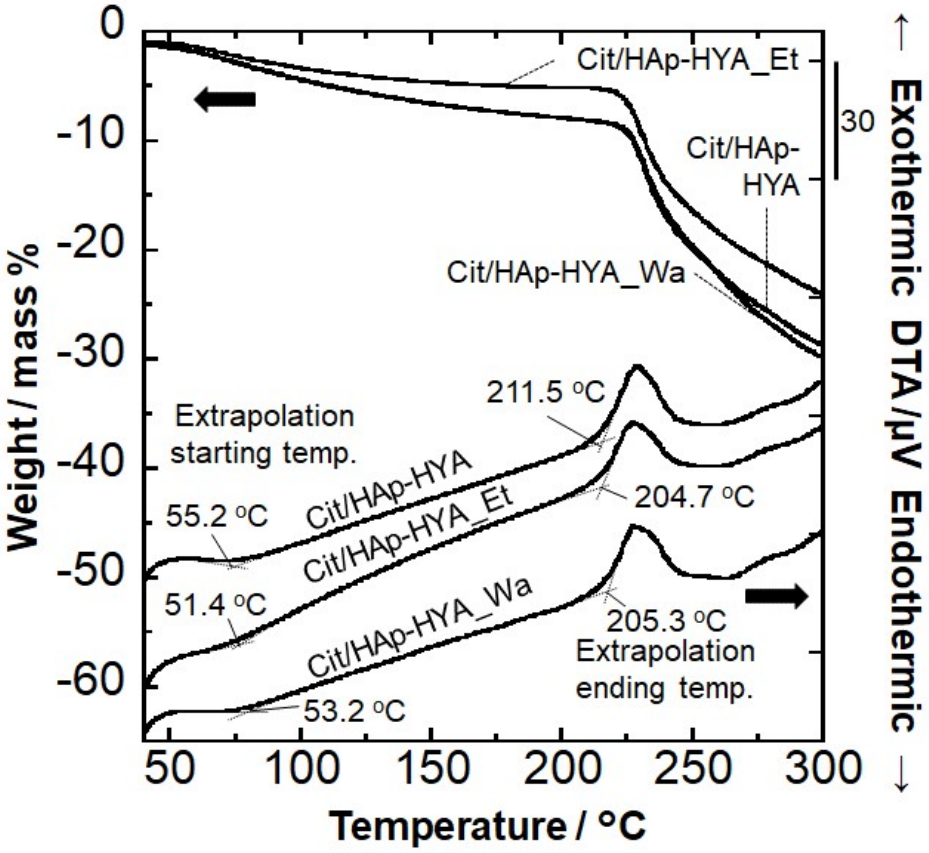

Fig. S6 TG-DTA curves of the membranes, and the weight loss is 6.5 wt% (Cit/HAp-HYA), 4.1 wt% (Cit/HAp-HYA_Et), and 6.5 wt% (Cit/HAp-HYA_Wa).

Fig. S7

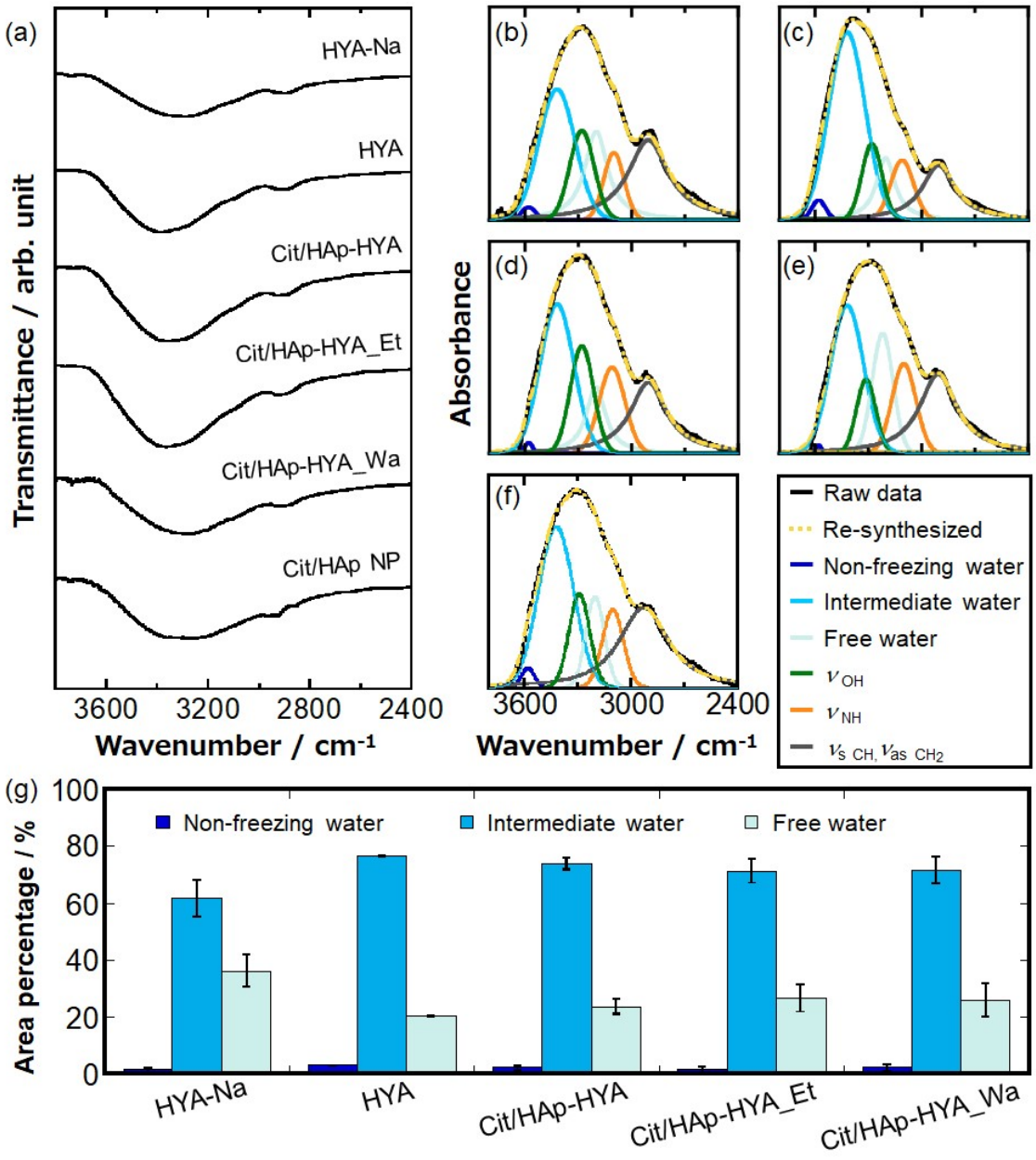

Fig. S7 FT-IR spectra of the membranes with the washing in the measurement region between 3800−2400 cm^{-1}, which was measured by the ATR method, and (b−f) is the curve fitting and peak separation results ((b) HYA-Na, (c) HYA, (d) CitHAp-HYA, (e) CitHAp-HYA_Et, and (f) CitHAp-HYA_Wa. (g) is the area percentages based on the peak separation results.