Supplementary Information

Guanidinylated Nanochitins: Guanidinylated Chitin Nanocrystal is Dispersiable at Neutral pH

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S1. ^{13}C NMR analysis of the surface chitosan on GChNC

The GChNC (30 mg) was added to 2% DCl-D_2O (1.0 mL) and heated at 80°C with stirring for 24 h. The GCNC suspension was centrifuged and supernatant was applied to ^{13}C NMR measurement (**Figure S1**). In the spectrum, the signals attributed to the quaternary carbon in the guanidine groups are observed at around 158 ppm (**b**).[S1] This result also supports the presence of guanidino group on GChNC surface.

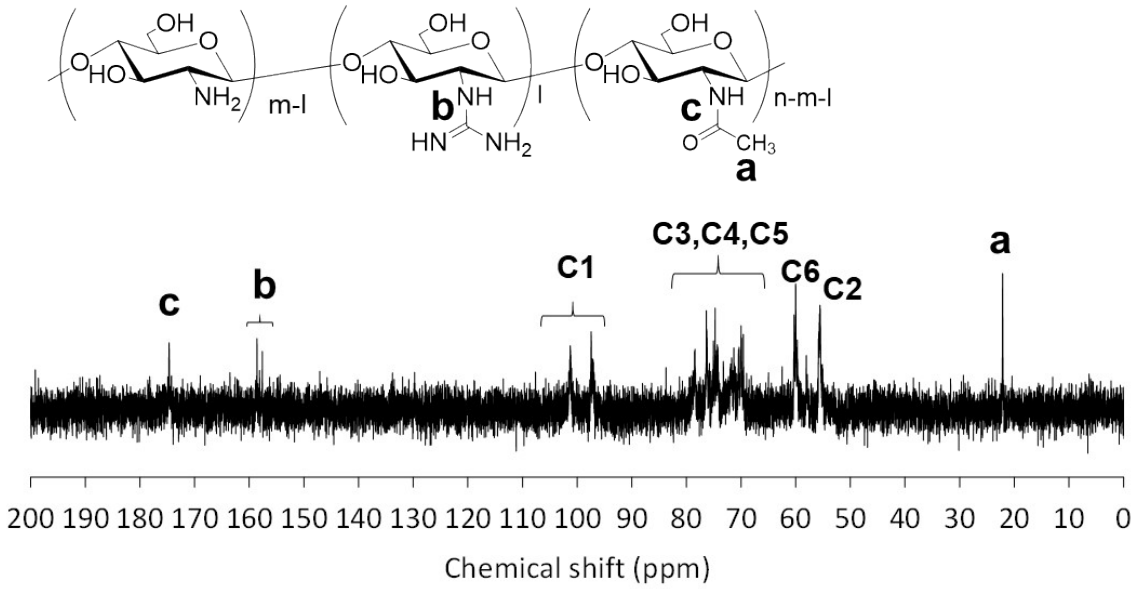

Figure S1. ^{13}C NMR spectrum of the soluble chitosan/chitin fraction extracted from GChNC surface in 2% DCl-D_2O.

S2. Dispersibility of GChNC in simulated body fluid

Figure 2S shows photo image of a 0.1 wt% GChNC dispersion prepared in simulated body fluid (SBF) using an ultrasonic homogenizer.[S2] The GChNC particles were homogeneously dispersed by sonication. However, after 30 min, visible macroscopic aggregates were observed. This aggregation is likely caused by ionic interactions between guanidino groups and CO_3^{2-}, PO_4^{3-}, and SO_4^{2-} anions.

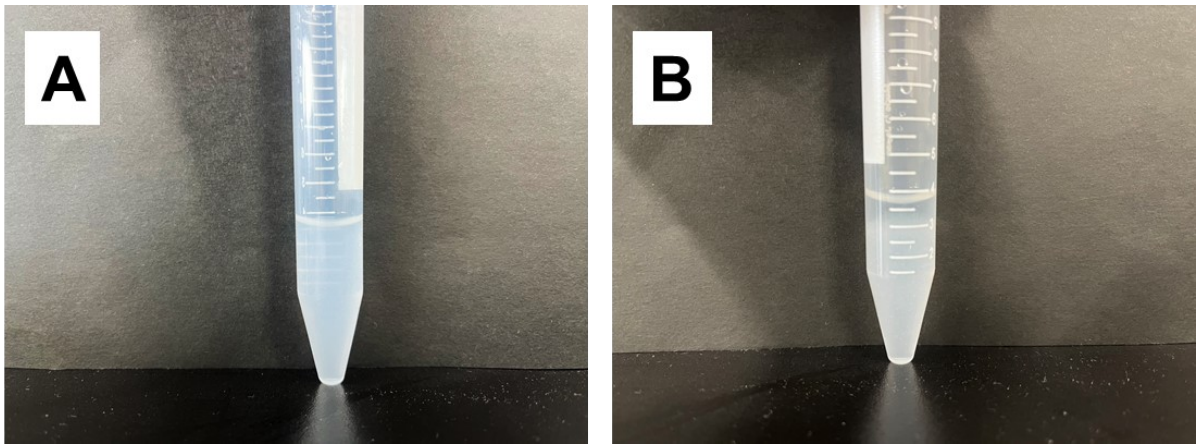

Figure S2. Photo image of the 0.1% GChNC dispersion prepared with the SBF after sonication (**A**) and after 30 min (**B**).

S3. Preparation of homogeneous GChNF dispersion by a high pressure homogenization treatment

The GChNF dispersion prepared by the ultrasonic treatment was additionally defibrate by the high pressure homogenization treatment (star burst system, sugino machine limited, Japan). The transparency was clearly increased (**Figures S3A and B**). The homogenously defibrated GChNF was observed by the SEM analysis (**Figure S3C**). The viscosity of the 1.0% GChNF dispersion prepared with the high pressure homogenization treatment was 47,650 mPa·s, which was higher than that of the 1.0% GChNF dispersion prepared by the sonication treatment (30,243 mPa·s). This result suggests that defibration was enhanced by the high pressure homogenization treatment.

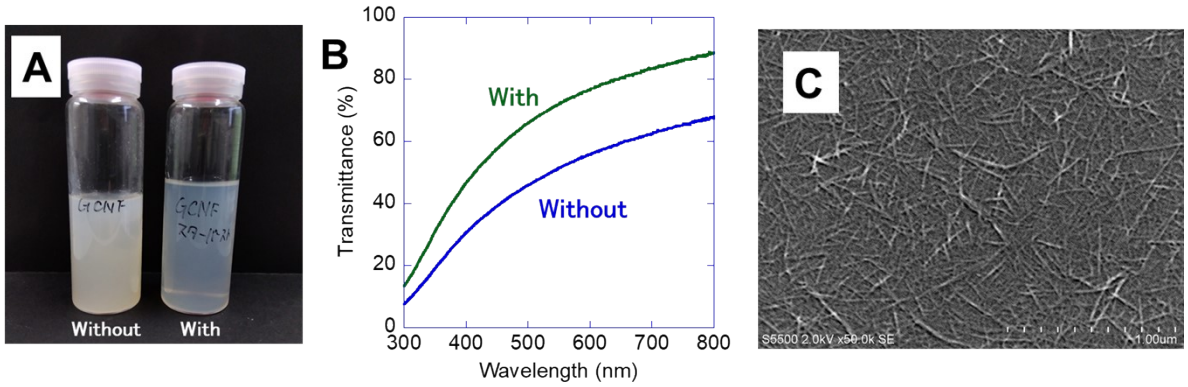

Figure S3. Photo image (**A**) and transmittance spectra (**B**) of the 1.0% GChNF dispersion prepared with or without the high pressure homogenization treatment. SEM image of the GChNF dispersion prepared with the high pressure homogenization treatment (**C**).

S4. Injectability of gelatin-GCNC composite hydrogel.

The gelatin-GChNC solution prepared by heating at 60°C was loaded into a syringe. The gelatin-GChNC solution in the syringe was gelled upon cooling to room temperature (**Figure S4A**). The gelatin-GChNC gel was successfully ejected from the syringe by extrusion while maintaining its gel state, indicating its injectability (**Figure S4B**).

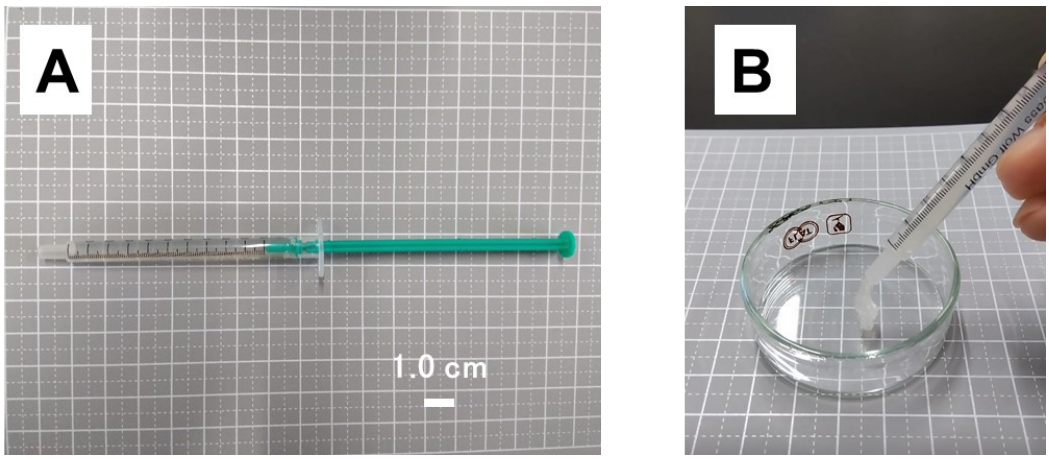

Figure S4. Photo images of the syringe with the gelatin-GChNC composite hydrogel (**A**) and its injection (**B**).

Supplementary references

S1. H. Izawa, M. Kinai, S. Ifuku, M. Morimoto and H. Saimoto, *Int J Biol Macromol*, 2019, **125**, 901-905.

S2. T. Kokubo, H. Takadama, *Biomaterials*, 2006, **27**, 2907-2915.