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Chitin nanofibrils reinforced multifunctional monolith poly(vinyl-alcohol) cryogel

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Highly porous and spongy monolith cryogel was prepared by one freeze-thawing of a PVA solution containing chitin nanofibrils (CNF) as nanofiller and glutaraldehyde (GA) as cross-linker. The presence of CNF significantly reinforced the spongy structure of cryogel that repeatedly squeezing-releasing did not deteriorate its spongy structure. The *in situ* self-polymerized polydopamine was formed inside the spongy cryogel by soaking cryogel in a dopamine solution. The polydopamine modified cryogel (PVA-CNF-D) showed a very good antioxidant activity that approximately 49% of free radical of DPPH• was consumed within 2 min. Silver nanoparticles (SNP) could be spontaneously reduced from silver nitrate solution and deposited onto the polydopamine modified surface without exogenous reducing reagent. The SNP incorporated spongy cryogel demonstrated a very effective antibacterial activity against *E. coli.*

Introduction

Poly(vinyl alcohol) (PVA) is a hydrophilic, biocompatible polymer with various desired characteristics for biomedical applications. Cryotropic treatment (single or repeated cycles of freeze–thawing) of concentrated PVA aqueous solution is known to produce macroporous gels, so-called poly(vinylalcohol) cryogels (cryoPVA). The cryoPVA has a three-dimensional polymer network in which individual hydrophilic polymer chains are inter-connected by hydrogen bonding, its shows porous, spongy, rubbery and elastic properties[1, 2]. The cryoPVA is also a hydrogel that has been extensively evaluated as a potential matrix for protein, peptides[3] and ligand immobilization[4].

In recent years, various compounds such as salt[5], gelatin[6], egg albumin, bacterial cellulose[7], chitosan[8] and epichlorohydrin[9] have been added into the aqueous PVA solution for the preparation of a more robust and mechanically stable cryoPVA. It was also noticed that addition of a biological compound into the PVA solution was able to modify the PVA microstructure by affecting its crystallization process during the freeze–thawing procedure[10].

Recently, the utilization of bio-nanofibrils to produce environmentally-friendly materials, which avoid using or producing harmful substances, has drawn a greater research attention. It has been reported that chitin nanofibril (CNF) of width 3–10 nm can be easily obtained from squid pen by mechanical treatment under mild acidic conditions[11, 12]. Due to its appealing intrinsic bio-friendly properties such as biodegradability, biocompatibility and biofunctionality, the chitin nanofibrils has been found favorable utilization in wide range of applications such as biomedical engineering[13], cell growth[9], tissue engineering[14], wound dressing[15] and biosensing[16].

Dopamine, which contains the catechol and amine functional group in its structure recently has been shown to be able to oxidatively self-polymerize into a polydopamine layer firmly adhered on the surfaces of diverse organic, inorganic, polymeric materials surfaces[17, 18]. The surface adherent polydopamine active layer has been reported can act as a reducing agent, binding reagent and platform for secondary reactions[19-21]. In addition, the reductive character of polydopamine can induce *in situ* silver nanoparticles formation on the surface contacts with silver nitrate solution. The silver nanoparticles are known to have outstanding antibacterial properties[22]. Herein, we report our results on the reinforcement effect of CNF on the mechanical stability of glutaraldehyde cross-linked PVA cryogel. Furthermore, the antioxidant and antimicrobial activity of the polydopamine modified and silver nanoparticles deposited cryogels are also presented, respectively.

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Experimental

Material

Squid pen was collected from a local fish market, sodium hydroxide (NaOH), hydrochloric acid (HCl), Triton® X-100 ($C_{34}H_{62}O_{11}$), Tris-HCl, 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH•), methanol (CH_3OH) were obtained from ACROS, NJ, USA. Glutaraldehyde (GA, 25% w/v aqueous solution), Poly(vinyl)alcohol (PVA, MW 2000), dopamine hydrochloride ($(HO)_2C_6H_3CH_2CH_2NH_2$·HCl), and silver nitrate ($AgNO_3$) were obtain from Sigma-Aldrich. All other chemicals were analytical grade used without further purification.

Preparation of chitin nanofibrils (CNF) suspension

Chitin nanofibrils were produced from the purified squid pen by mechanical treatment according to the method described previously [11, 12]. Briefly, the collected squid pens were washed thoroughly with tap water and dried before ground into fine powder by a high speed blender. Powdered squid pen (30 g) was dispersed in 500 mL of 0.5% Triton X-100 by stirring for 24 h at room temperature to remove lipoproteins. After thoroughly washing with tap water, the wet cake was suspended in 500 mL 1 N NaOH solution and stirred for 24 h to eliminate the remaining proteins. After washing thoroughly with deionized water to neutral pH, the collected wet cake (water content ca 74%) of 2 g was dispersed in 100 mL deionized water and adjusted pH to 3–4 with dilute HCl. The transparent CNF suspension was obtained by ultrasonicating the dispersed chitin cake with power of 10 W for 30–60 min at 4 ◦C.

Production of monolith cryoPVA-CNF from PVA-CNF mixture

The cryoPVA monoliths were prepared according to freeze–thawing method described by Plieva et. al[1] with minor modification. The PVA (2%, w/v) and CNF (2%, w/v) were dissolved in deionized water by stirring at elevated temperature (90°C). After cooling to room temperature, the pH of the solution was adjusted to 1.0–1.2 with 5 M HCl. Glutaraldehyde was then added to a final concentration of 1.0% (w/v). The well-mixed and deaerated solution was poured into a 24-well microplate and frozen at -18 °C overnight. The frozen monoliths (cryoPVA-CNF) formed in the wells were defrosted and washed with deionized water until the pH reached neutral.

Polydopamine modified cryoPVA-CNF

The thoroughly washed cryoPVA-CNF monolith was squeezed to remove most of water and immersed in a dopamine solution (5 mg/mL in 10 mM Tris–HCl, pH 8.5) with slow magnetic stirring for 18 h at room temperature. The obtained dark-brown cryoPVA-CNF monolith was rinsed thoroughly with pH 8.5 Tris–HCl buffer to remove any polydopamine nanoparticles attached on the outer surface of monolith. The obtained spongy cryogel monolith was designated as cryoPVA-CNF-D and kept in deionized water for further use.

In situ formation of silver nanoparticles in cryoPVA-CNF

The reducing capability of the polydopamine was utilized to synthesize silver nanoparticles in the cryoPVA-CNF-D from silver nitrate solution. In a typical procedure, the squeezed cryoPVA-CNF-D was immersed in 50 mM of $AgNO_3$ solution for 18 h at room temperature under mild stirring. After washing thoroughly with deionized water, the obtained grey monolith gel was designated as cryoPVA-CNF-Ag and stored in a brown glass vial at 4°C for later use.

Free radical scavenging activity of cryoPVA-CNF-D

Spectrophotometric assay using the stable radical (DPPH•) as a reagent was employed for measuring the free radical scavenging activity of polydopamine modified cryogel. Briefly, 0.1 mM DPPH• solution was prepared in methanol and 170 µL of this solution was added to 830 µL of methanol for measuring the initial absorbance of DPPH•. To this DPPH• solution, 1 mg of dried cryoPVA-CNF-D was added and well-mixed instantly. The absorbance from 430 to 600 nm was scanned using DPPH• solution as a blank. The free radical scavenging activity (ScA) of the sample was calculated using equation 1:

$$ScA = [(OD_{m,\,t=0} - OD_{m,\,t=t})/\ OD_{m,\,t=0}] \times 100\% \quad \ldots\ldots\ldots (1)$$

where OD_m is the peak adsorbance detected at different time in the range 430 to 600 nm.

Antimicrobial activity of cryoPVA-CNF-Ag

E. coli grown in LB medium was employed to examine the antibacterial effect of cryoPVA-CNF-Ag. *E.coli* grown overnight at 37 °C were harvested by centrifugation and washed twice with pH 6.5, 0.9% NaCl solution. After resuspending in phosphate buffer saline solution to a concentration of OD_{600} =1, 10 µL of the *E. coli* solution was uniformly plated on LB nutrient agar plates. A piece of cryoPVA-CNF, cryoPVA-CNF-D and cryoPVA-CNF-Ag with thickness about 3.5 mm were placed on the agar plate, respectively. The plates were incubated at 37 °C overnight. The antibacterial activity of the samples was measured based on the width of clear zone formed around the samples.

Characterization

Field emission scanning electron microscopy (FE-SEM) images of the samples were taken using JOEL, JSM-6500F electron microscope. Transmission electron microscopy (TEM) images were taken using a Hitachi H-800 transmission electron microscope (Japan). Critical point drying (Samdri PVT-3D) was employed to prepare all the samples for FE-SEM observation. The samples were first coated in a sputter coater with a Pt layer in vacuum condition. The X-ray diffraction (XRD) measurement was performed on Rigaku D/MAX-B X-ray diffractometer by using Copper K-alpha (Cu Kα) radiation with 2θ in the range from 5 to 70° at a scan rate of 2° min^{-1}. The operation voltage and current were kept at 40 kV and 100 mA, respectively.Thermal gravimetry analysis (TGA) was carried out by using Perkin Elmer, Diamond TG/DTA. Sample (6–8 mg, dry weight) was heated from ambient temperature to 600 °C under nitrogen flow (20 mL /min) at a constant heating rate of 10 °C/min. The polydopamine and silver nanoparticle content in the samples were estimated by the percentage of weight loss from the corresponding TGA curves. Mechanical strength of the as-prepared cryoPVA-CNF was evaluated by a modular compact rheometer (MCR 102, Austria) using holder PP 25 performed under ambient conditions. UV-Vis spectroscopy (V-550-JASCO) was employed for measuring the absorbance for free radical scavenging activity of cryoPVA-CNF-D.

Results and discussion

Characterization of monolithic cryoPVA-CNF

Under intensive ultrasonication in a mild acidic condition, the suspended squid pen powder was dissolved and resulted in a transparent solution with significantly increased viscosity. The increase of viscosity is mainly resulted from the entanglement of nano-sized chitin fibers of 3–10 nm in width and several micrometers in length[12].

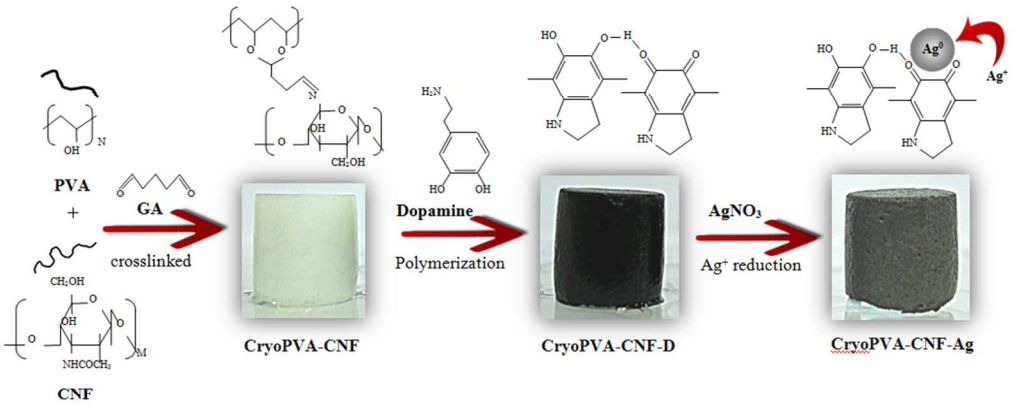

Fig. 1 Schematic preparation and chemical structure of cyoPVA-CNF, cryoPVA-CNF-D and cryoPVA-CNF-Ag

These chitin nanofibrils (CNF) were used as a nanofiller to reinforce the mechanical strength of PVA cryogel cross-linked by GA. The schematic diagram and chemical structure for the preparation of CNF reinforced cryogel (cyoPVA-CNF), polydopamine modified cryogel (cryoPVA-CNF-D) and Ag nanoparticles containing cryogel (cryoPVA-CNF-Ag) is shown in Fig.1.

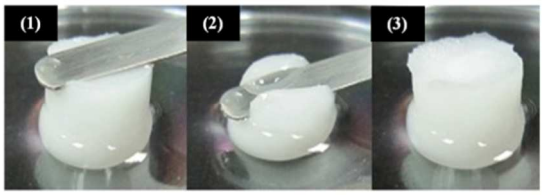

Fig. 2 Images of spongy cryoPVA-CNF monolith (1) before compression; (2) under compression; (3) release of compression

The cryogel (cryoPVA) prepared by cross-linking 2% (w/v) PVA solution with GA but without adding CNF turned out to be a brittle gel. When gently compressed by a finger to squeeze out water, the cryoPVA monolith ruptured. In contrast, the PVA cryogel prepared in the presence of 2% (w/v) CNF turned out to be very robust. As shown in Fig. 2, the cryoPVA-CNF monolith can be pressed down by a spatula to squeeze out water easily without damaging its structure. Once the spatula was released, the squeezed monolith returned back to its original shape by sucking back the discharged water. The compression and release were repeated at least 5 times without observing any damage to the monolithic cryogel. Also shown in Fig. S1 (ESI), cryoPVA-CNF monolith returned back to its original shape after a 1 kg standard calibration weight was unloaded. Evidently, the presence of CNF not only enhances the mechanical stability of cryoPVA-CNF but also endues its resilience. Probably, the resilient CNF also participates the cross-linking of PVA because an appreciable amount of amine groups on CNF is available for reacting with GA as shown in Fig. 1. FE-SEM images of cryoPVA-CNF (Fig. 3) taken from the surface of monolith shows the macroporous structure of cryogel consisted of nanofibrils along with irregular flakes. Apparently, during the freezing process water was transformed into ice-crystals that also leaded to the increase of local concentration of PVA and CNF.

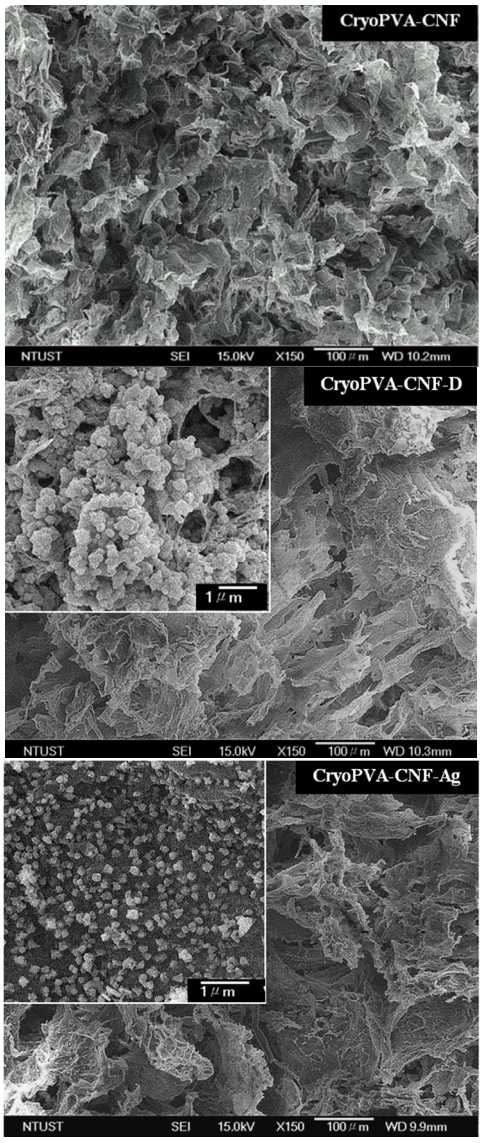

Fig. 3 FE-SEM images of CryoPVA-CNF, CryoPVA-CNF-D and CryoPVA-CNF-Ag.

With increased PVA and CNF, the cross-linking between PVA itself or with CNF by GA will be facilitated. Once thawed, the melt of ice crystals thus left wide pores in the cross-linked gel[6]. The macroporous structure of cryoPVA-CNF with pore size up to 0.5–100 μm was observed. The high water holding capacity along with fast water discharge rate upon squeezing cryoPVA-CNF is mainly resulted from its resilient and macroporous structure.

The monolith cryoPVA-CNF was further functionalized by immersing in the dopamine solution to have self-polymerized polydopamine formed inside the macroporous spongy structure. As expected, the white opaque cryoPVA-CNF turned into dark brown monolith after polydopamine functionalization. The detailed mechanism of oxidative self-polymerization of dopamine is not clear yet but explanations have been tried by several investigators[23, 24]. The self-polymerized polydopamine has been reported to have strong adhesion to surface of many materials[25, 26]. It will not only deposit on the surface as an adlayer but also form as nanoparticles in the solution. Since dopamine solution was sucked into the squeezed cryoPVA-CNF, polydopamine nanoparticles as well as its adlayer were expected to form inside the macroporous structure and also on the interior surface of macroporous cryoPVA-CNF. Both polydopamine nanoparticles and adlayer should provide the reported functionalities, such as reactive oxygen species scavenging (ROS) and metal cations sequestering to the cryoPVA-CNF-D. It has been reported that the catechol group of dopamine or DOPA can strongly interact with metal such as titanium or iron surface at acidic pH which prevents catechol from oxidation. DOPA grafted polymers have been used for surface modification of metal surfaces [27,28]. However, alkaline pH (pH 8.5) employed in this work is very favorable for dopamine oxidation to form polydopamine aggregates. Therefore, dopamine itself is not expected to react with the structural PVA or CNF only oxidatively self-polymerized polydopamine nanoparticles and adlayer are in macroporous structure of cryoPVA-CNF. As shown in Fig. 3, after polydopamine functionalized cryogel (cryoPVA-CNF-D) has a smoother surface as compared with that of cryoPVA-CNF. Aggregated nanoparticles with sizes ranging from 0.11 to 0.35 μm can also be clear observed in cryoPVA-CNF-D. Recently, polydopamine surface has been demonstrated to be very effective for *in situ* reducing silver ions to silver nanoparticles without the need of an exogenous reducing agents[29-31]. A uniform silver nanoparticles deposition was achieved on the surface of polydopamine. Macroporous structure of cryoPVA-CNF can provide a large interior surface area as well as void for the formation of polydopamine adlayer and nanoparticles. The polydopamine functionalized cryoPVA-CNF was further functionalized with silver nanoparticles by immersing cryoPVA-CNF-D into a silver nitrate solution. An apparent color change from dark brown to grey was noticed after immersion. As observed by FE-SEM and TEM (Fig. S2; ESI), quite uniform nanoparticles with size about 50 nm appeared on the smooth surface of cryoPVA-CNF-Ag.

XRD was also employed to analyze the presence of silver nanoparticles. As shown in Fig. 4, the intense peaks for the crystallographic plane (020) at 9.0° and (110) at 20 ° of β-chitin[32] were observed for cryoPVA-CNF. These specific peaks disappeared in cryoPVA-CNF-Ag, instead three major peaks at 38.2 °, 44.4 °, and 64.6 ° corresponding to the crystallographic planes (111), (200) and (220), respectively were observed. The appearance of these peaks indicates the face centered cubic (fcc) structure of the silver nanoparticles (JCPDS No. 87-0720). This result provides further insight evidence of silver nanoparticles formation in cryoPVA-CNF-Ag due to the presence of polydopamine as observed by FE-SEM and TEM (Fig.3 and Fig. S2).

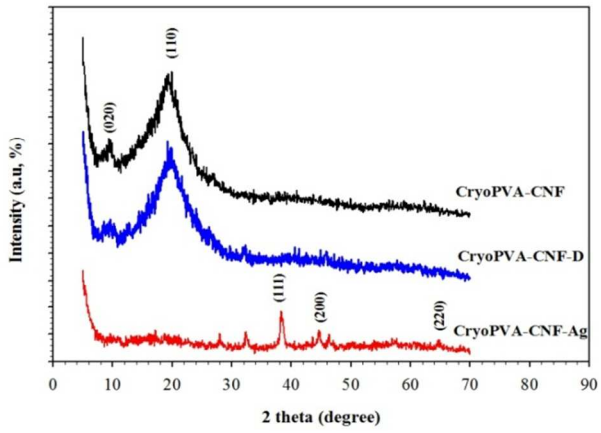

Fig. 4 XRD patterns of CryoPVA-CNF, CryoPVA-CNF-D and CryoPVA-CNF-Ag

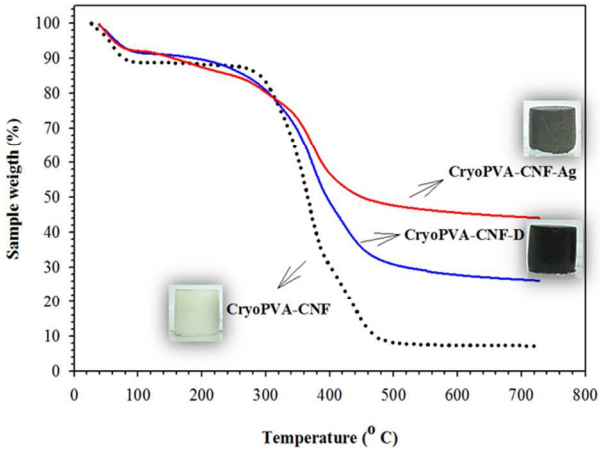

Fig. 5 TGA curves of CryoPVA-CNF, CryoPVA-CNF-D and CryoPVA-CNF-Ag

The silver nanoparticles content in cryoPVA-CNF-Ag was analyzed by TGA. As shown in Fig. 5, the 1st stage approximately 8-10% weight loss occurred at a temperature below 100 °C for all the samples is mainly due to the evaporation of water in the samples. A significant second stage weight loss was observed at about 370 °C for all the samples (Fig. S3, ESI) which was resulted from the degradation of PVA and CNF[12]. Under N_2 atmosphere with temperature increased to 720 °C, 7.25%, 26.50%, 44.36% weight remained for cryoPVA-CNF, cryoPVA-CNF-D and cryoPVA-CNF-Ag, respectively. In comparison with cryoPVA-CNF, the excess amount of remaining

weight of cryoPVA-CNF-D and cryoPVA-CNF-Ag are evidently resulted from the presence of polydopamine and silver nanoparticles. The cryoPVA-CNF-Ag has the highest remaining weight because polydopamine was first formed then used to induce silver nanoparticles formation. Based on these remaining weight percentages, polydopamine and silver nanoparticles content in cryoPVA-CNF-D and cryoPVA-CNF-Ag are estimated to be 19% and 18% (w/w), respectively.

A series of oscillatory dynamic mechanical measurements were performed to study the viscoelastic properties of the functionalized cryogels. As shown in Fig. 6, both storage (G') and loss (G") modulus increase slightly with angular frequency for all samples. The storage modulus G' is considerably higher than the loss modulus G" that indicates the elastic solid-like behaviour of the as-prepared cryogels. The cryoPVA-CNF-D has the highest storage modulus, which indicates the presence of polydopamine enhances the mechanical strength of the cryogel. Probably, the *in situ* formed polydopamine plays the role as nanofiller to cross-link the PVA and/or CNF chains in cryoPVA-CNF. The cross-linker role of polydopamine diminished when silver ions appeared to be reduced by polydopamine surface into silver nanoparticles that leaded to an appreciable decrease of storage modulus of cryoPVA-CNF-Ag.

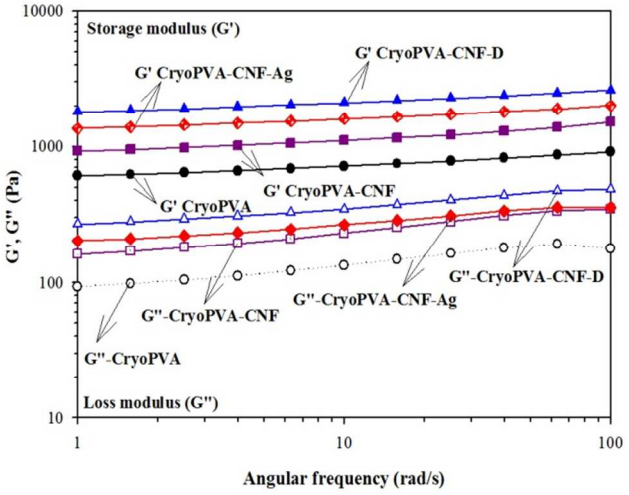

Fig. 6 Rheological measurement as a function of angular frequency with storage (G') and loss (G") modulus for CryoPVA, CryoPVA-CNF CryoPVA-CNF-D and CryoPVA-CNF-Ag.

Activities of functionalized cryoPVA-CNF

The free radical scavenging activity of polydopamine functionalized cryoPVA-CNF-D was evaluated by measuring its DPPH• free radical scavenging activity. The absorbance of original purple colored DPPH• solution will decrease when free radical DPPH• accepts a proton from an antioxidant to form a stable DPPH molecule[33]. As can be seen in Fig. 7, the absorption peak at 516 nm decreased with time when cryoPVA-CNF-D was incubated in DPPH• solution. Approximately 49% of free radical of DPPH• was consumed within 2 min and up to 75% was scavanged after 12 min. The original dark purple color of DPPH• reagent turned into light yellowish solution. Evidently, the polydopamine formed inside the macroporous structure of cryoPVA-CNF-D not only can facilitate the silver nanoparticles formation but also eliminate free radical[20].

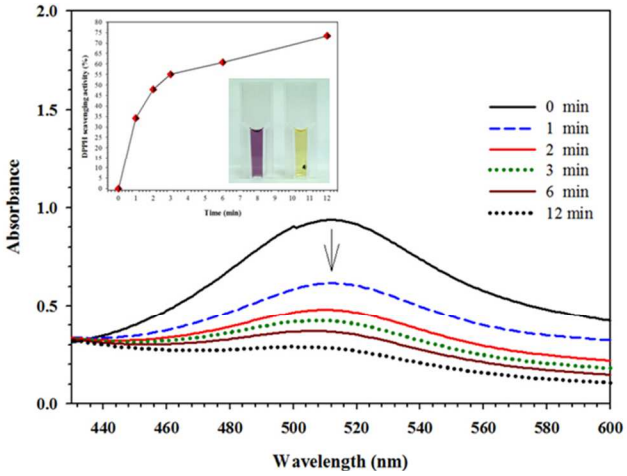

Fig. 7 Overlay of UV-Vis spectra of DPPH• antioxidant assay of cryoPVA-CNF-D in methanol and the calculated scavenging activity (inset).

When silver nanoparticles were formed *in situ* in cryoPVA-CNF-D, the gel was expected to have antimicrobial activity. The antibacterial activity of cryoPVA-CNF-Ag for *E. coli* was measured by the disc diffusion method. It was found that the silver nanoparticles containing gel disc exhibited about 4 mm inhibition zone.

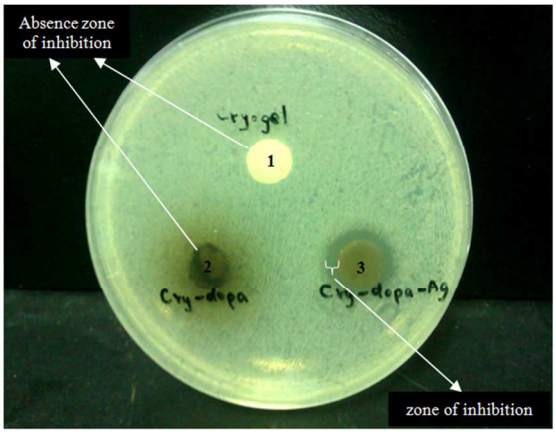

Fig. 8 Photograph showing absence and zone of inhibition of *E. Coli* growth within nutrient agar for CryoPVA-CNF (1), CryoPVA-CNF-D (2) and CryoPVA-CNF-Ag (3) .

No inhibition zone was observed for the cryoPVA-CNF and cryoPVA-CNF-D as controls (Fig. 8). Evidently, the observed antibacterial activity was resulted from the release of silver ions from cryoPVA-CNF-Ag. In other words, the robust and spongy cryoPVA-CNF-Ag has a long lasting antimicrobial capacity due to the silver nanoparticles embedded in its macroporous structure.

Conclusions

The glutaraldehyde cross-linked cryogel prepared from the mixture of PVA and CNF solution yields a robust, macroporous, spongy, and elastic cryogel. The presence of CNF significantly reinforced the mechanical structure of the cryogel. The antioxidant and antimicrobial functionalization of cryogels were successfully prepared by immersing in dopamine solution followed by silver nitrate solution. Silver nitrate was effectively reduced to form Ag nanoparticles on the polydopamine surface and incorporated into the macroporous structure of cryoPVA-CNF. This facile preparation of a polydopamine functionalized durable and macroporous cryogel not only can generate an antimicrobial spongy hydrogel but may also be used for biomolecules immobilization for the applications of enzymatic reaction and bioseparations.

Acknowledgements

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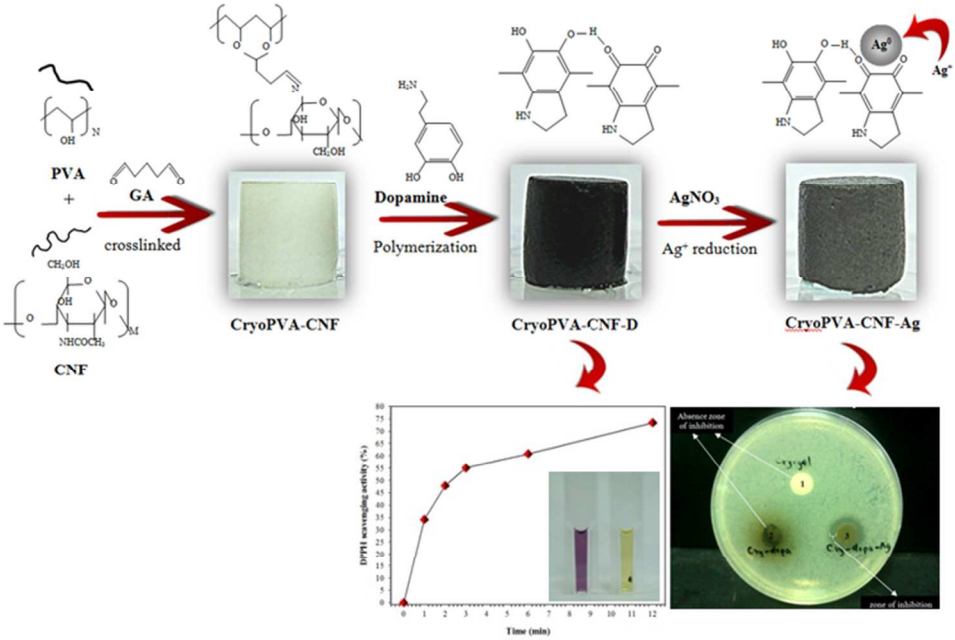
References

1. F. M. Plieva, M. Karlsson, M.-R. Aguilar, D. Gomez, S. Mikhalovsky, I. Y. Galaev and B. Mattiasson, *Journal of Applied Polymer Science*, 2006, **100**, 1057–1066.
2. S. J. Kim, S. J. Park and S. I. Kim, *Reactive and Functional Polymers*, 2003, **55**, 53-59.
3. C. M. Hassan and N. A. Peppas, *Advances in Polymer Science*, 2000, **153**, 37-65.
4. A. Kumar, A. Rodríguez-Caballero, F. M. Plieva, I. Y. Galaev, K. S. Nandakumar, M. Kamihira, R. Holmdahl, A. Orfao and B. Mattiasson, *Journal of Molecular Recognition*, 2005, **18**, 84-93.
5. S. Pat¸achia, C. Florea, C. Friedrich and Y. Thomann, *Polymer Letters*, 2009, **3**, 320–331.
6. A. Bajpai and R. Saini, *Polym Int* 2005, **54**, 1233–1242.
7. L. E. Millon and W. K. Wan, *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 2006, 245-253.
8. M. Kumar, B. P. Tripathi and V. K. Shahi, *Journal of Hazardous Materials*, 2009, **172**, 1041-1048.
9. C. Chang, S. Chen and L. Zhang, *Journal of Materials Chemistry*, 2011, **21**, 3865-3871.
10. M. G. Cascone, B. Sim and D. Sandra, *Biomaterials*, 1995, **16**, 569-574.
11. Y. Fan, T. Saito and A. Isogai, *Biomacromolecules*, 2008, **9**, 1919-1923.
12. I. F. Nata, S. S.-S. Wang, T.-M. Wu and C.-K. Lee, *Soft Matter*, 2012, **8**, 3522-3525.
13. N. S. Rejinold, A. Nair, M. Sabitha, K. P. Chennazhi, H. Tamura, S. V. Nair and R. Jayakumar, *Carbohydrate Polymers*, 2012, **87**, 943-949.
14. H. Tamura, T. Furuike, S. V. Nair and R. Jayakumar, *Carbohydrate Polymers*, 2011, **84**, 820-824.
15. R. A. A. Muzzarelli, P. Morganti, G. Morganti, P. Palombo, M. Palombo, G. Biagini, M. Mattioli Belmonte, F. Giantomassi, F. Orlandi and C. Muzzarelli, *Carbohydrate Polymers*, 2007, **70**, 274-284.
16. Z. Shervani, Y. Taisuke, S. Ifuku, H. Saimoto and M. Morimoto, *Advances in Nanoparticles*, 2012, **1**, 71-78.
17. H. Lee, S. M. Dellatore, W. M. Miller and P. B. Messersmith, *Science*, 2007, **318**, 426-430.
18. B. P. Lee, J. L. Dalsin and P. B. Messersmith, *Biomacromolecules*, 2002, **3**, 1038-1047.
19. M. Sureshkumar and C.-K. Lee, *Carbohydrate Polymers*, 2011, **84**, 775-780.
20. B. Fei, B. Qian, Z. Yang, R. Wang, W. C. Liu, C. L. Mak and J. H. Xin, *Carbon*, 2008, **46**, 1795-1797.
21. N. Holten-Andersena, M. J. Harrington, H. Birkedal, B. P. Lee, P. B. Messersmith, K. Y. C. Lee and J. H. Waite, *PNAS*, 2011, **108**, 2651-2655.
22. M. Sureshkumar, D. Y. Siswanto and C.-K. Lee, *Journal of Materials Chemistry*, 2010, **20**, 6948-6955.
23. Y. Liaoa, Y. Wang, X. Feng, W. Wanga, F. Xua and L. Zhang, *Materials Chemistry and Physics 121*, 2010, **121**, 534–540.
24. L. Zhang, J. Shi, Z. Jiang, Y. Jiang, S. Qiao, J. Li, R. Wang, R. Meng, Y. Zhu and Y. Zheng, *Green Chemistry*, 2011, **13**, 300-306.
25. M. Guvendiren, P. B. Messersmith and K. R. Shull, *Biomacromolecules*, 2008, **9**, 122-128.
26. H. Y. Son, J. H. Ryu, H. Lee and Y. S. Nam, *Macromolecular Materials and Engineering*, 2013, **298**, 547-554.
27 B. P. Lee, J. L. Dalsin, and P. B. Messersmith**,** *Biomacromolecules*. 2002**, 3**, *1038-1047*
28 N. Holten-Andersen, M. J. Harrington, H. Birkedal, B. P. Leed, P. B. Messersmith, K.Y.C. Lee, and J. H. Waite, *PNAS* 2011, **108**, 2651–2655
29. J. Jiang, L. Zhu, L. Zhu, B. Zhu and Y. Xu, *Langmuir*, 2011, **27**, 14180-14187.
30. Y. Liao, Y. Wang, X. Feng, W. Wang, F. Xu and L. Zhang, *Materials Chemistry and Physics*, 2010, **121**, 534-540.
31. A. Postma, Y. Yan, Y. Wang, A. N. Zelikin, E. Tjipto and F. Caruso, *Chemistry of Materials*, 2009, **21**, 3042-3044.
32. R. L. Lavall, O. B. G. Assis and S. P. Campana-Filho, *Bioresource Technology*, 2007, **98**, 2465-2472.
33. B. Matthäus, *Journal of Agricultural and Food Chemistry*, 2002, **50**, 3444-3452.



The antioxidant and antimicrobial functionalization of chitin nanofibrils (CNF) reinforced poly(vinyl alcohol) cryogel prepared by immersing in a alkaline dopamine solution followed by reducing $AgNO_3$ into Ag nanoparticles onto the macroporous structure of spongy cryogel.