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ChemComm

Cite this: *Chem. Commun.*, 2012, **48**, 8389–8391

www.rsc.org/chemcomm

COMMUNICATION

Influence of pristine SWNTs in supramolecular hydrogelation: scaffold for superior peroxidase activity of cytochrome c†

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Received 18th May 2012, Accepted 4th July 2012
DOI: 10.1039/c2cc33593j

The influence of pristine SWNTs in supramolecular hydrogelation of amphiphilic dipeptide carboxylates is delineated. The developed SWNT-nanohybrids with notable mechanical strength act as a scaffold for superior peroxidase activity of cytochrome c in organic media.

Carbon nanotubes (CNTs) have extended their tentacles in almost every discipline of science owing to their unique physicochemical properties.[1] Recently, attempts have been made to prepare soft nanocomposites by hybridizing this pseudo one-dimensional allotrope of carbon with low molecular weight gels (LMWGs).[2,3] In a few instances, functionalized CNTs have been tied up with LMW organogels to develop CNT–gel composites.[2] However, literature on the inclusion of pristine CNTs in LMW hydrogels are very scarce.[2] The extreme insolubility of CNTs in water impedes the involvement of this carbonaceous nanomaterial within molecular hydrogels. Therefore, development of CNT–hydrogel composites is of great importance because of its huge prospects in material science to biomedicine.[4] Hence, to develop a CNT–hydrogel composite, ideally the features of neither of the components should be compromised, rather they must be complementary to each other in synergistic reciprocity. In this regard, small molecules have been exploited for solubilizing CNTs in water by forming a supramolecular self assembly of amphiphiles on a nanotube surface.[5] Thus, it will be intriguing to find a molecular structure that would not only facilitate debundling of CNTs, but also dispersed CNTs will participate in gelation through capillary forces and physical cross linking between self-assembled fibrillar networks (SAFINs) leading to the formation of SWNT–hydrogel composites.

The present work reports the influence of pristine SWNTs in development of soft composites using amphiphilic peptide carboxylates (**1–4**, Fig. 1) with much improved mechanical strength (10-fold higher compared to native hydrogel). Interestingly, the gelation efficiency of amphiphiles distinctly improved 2-fold due to inclusion of SWNTs within the SAFIN. Importantly, the fitting fusion of dispersed CNTs within the SAFIN of hydrogels was exploited to improve the peroxidase activity of immobilized cytochrome c (cyt c) in toluene (120 times greater than that in water). Moreover, cyt c entrapped within SWNT–hydrogel exhibited superior activity (1.7-fold higher) compared to that of the native hydrogel.

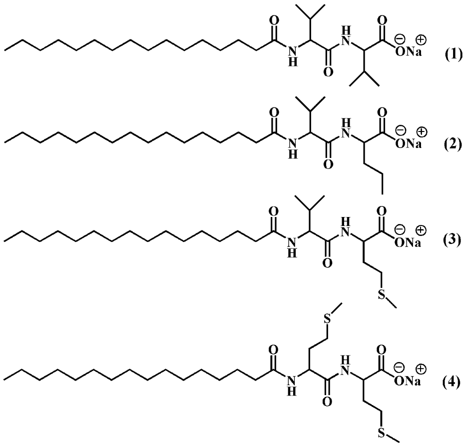

Fig. 1 The structure of amphiphiles (**1–4**).

Among the different dipeptide carboxylates synthesized (**1–4**, Fig. 1), amphiphile **1**, comprised of a C-16 alkyl tail and two L-valine residues, formed a viscous solution in water at 5% *w*/*v*. Interestingly, replacement of L-valine with other aliphatic amino acids, like L-norvaline and L-methionine (devoid of any branching at a side chain), resulted in excellent hydrogelation ability for amphiphiles **2–4** with comparable minimum gelation concentration (MGC) = 0.7–1.0% *w*/*v* (Fig. S1a and Table S1, ESI†) due to the attainment of the optimum hydrophilic–lipophilic balance (HLB) required for gelation.[3,6] The gel-to-sol transition temperature (T_{gel}) of these thermoreversible gels (**2–4**) was ~40–46 °C at their MGC and progressively increased with increasing gelator concentration (Fig. S2, ESI†). Debundling of SWNTs in water by exogenous dispersing agents is primarily driven by the hydrophobic interactions between nanotube and amphiphile.[1,5] Hence, we investigated the SWNT dispersion efficacy of these amphiphilic carboxylates in water. The amphiphiles (**1–4**) showed moderate SWNT dispersion (~40–50%) that was quantified following a reported protocol.[5] These results encouraged us to explore the

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† Electronic supplementary information (ESI) available: synthesis and characterization of amphiphiles, gelation results, UV-vis-NIR spectra, SEM and TEM images, peroxidase activity data. See DOI: 10.1039/c2cc33593j

complementary fusion of dispersed SWNTs with a self-assembled gel to develop SWNT–hydrogel composites. To this end, SWNT (1 mg) was added to an aqueous solution (1 mL) of amphiphiles (**1–4**) at their MGC followed by probe sonication (10 min at 30% power output) and then this was kept undisturbed at room temperature. Initially, a homogeneous suspension of SWNTs in water was obtained for all amphiphiles. In the case of SWNT-**1**, SWNTs precipitated out from the aqueous medium after some time, probably due to disparate interactions between SWNTs and **1**, which itself is a non-gelator. Upon incorporation of 0.1% *w*/*v* SWNTs at their MGC (0.7% *w*/*v*) for **2** (featuring L-valine and L-norvaline), a highly viscous material was obtained, which was, however, not stable to inversion of the glass vial. This could be due to the difference in HLB of the amphiphile that determines the extent of dispersion of the nanotubes[5] and subsequently self-assembly of the resulting nanocomposites. Importantly, inclusion of sulfur-containing amino acid L-methionine within the gelator's structure (**3**) led to the formation of a hybrid gel comprised of 0.1% *w*/*v* SWNT and 0.8% *w*/*v* gelator and the composite gel was stable to inversion of the glass vial (Fig. S1b, ESI†). Strikingly, the gelation efficiency of the SWNT-**3** composite was enhanced 2-fold as the MGC of **3** (0.8% *w*/*v*) reduced to 0.4% *w*/*v* in presence of only 0.01% *w*/*v* SWNT (Table S2†). To the best of our knowledge, this is the first example where not only were pristine SWNTs integrated within a LMW hydrogel, but also a supramolecular gelation phenomenon was triggered by the presence of SWNTs. This result indicates the efficient SWNT dispersion is associated with higher polarizability of sulfur atoms.[6] At the same time, non-covalent interactions between dispersed SWNTs and gelator **3** play a crucial role in improving the gelation ability of the SWNT-**3** composite.[6] To further probe the involvement of SWNTs in supramolecular gelation, we used amphiphile **4** comprised of two L-methionine residues. SWNT-incorporating (0.1% *w*/*v*) molecular hydrogel (SWNT-**4**) formation was observed at MGC (1.0% *w*/*v*). Importantly, a similar improvement in gelation ability was also observed in presence of 0.01% *w*/*v* SWNT (MGC decreased from 1% *w*/*v* to 0.6% *w*/*v*, Table S2). These results clearly indicate the influence of SWNT in self-assembled gelation due to matching integration of dispersed SWNTs within the SAFIN of the hydrogel. These soft nanocomposites were thermoreversible and stable over a long period of time. The T_{gel} for SWNT-**3** composite was higher than the native gel at comparable concentration and increased with gelator concentration at fixed SWNT concentration (0.1% *w*/*v*, Fig. S3, ESI†). Also at fixed gelator concentration (0.5% *w*/*v*), T_{gel} steadily improved with increase in the amount of SWNT up to an optimum concentration (0.05% *w*/*v*, Fig. S4, ESI†). Such a steady increase in T_{gel} suggests physical reinforcement of the hydrogel in presence of SWNTs.

Participation of SWNTs in self-assembled hydrogelation was investigated by different spectroscopic and microscopic techniques. Efficient exfoliation of SWNTs was evident from the distinctive van Hove peaks in the UV-vis-NIR spectra of a diluted aqueous dispersion of SWNT-**3** and SWNT-**4** hybrids (Fig. S1c, ESI†). The well-resolved peaks in the absorption spectra centered at 550–900 nm (S_{22} transition) and 1200–1600 nm (S_{11} transition) arise from interband transitions between the mirror spikes in the density of states of individualized SWNTs, revealing the superior quality of dispersion.[5]

The zeta (ζ) potential for SWNT-**3** and SWNT-**4** composites in water were found to be −107 and −85 mV, respectively, which is notably higher compared to that of SWNT-**2** hybrid (−63 mV). Such high ζ potential value suggests strong binding of gelator molecules on the nanotube surface, resulting in the efficient debundling of SWNT.[5]

The hydrophobic interaction between the amphiphilic gelator and SWNTs was probed by FTIR spectra of xerogels of **3** and SWNT-**3** composite. The C–H bending vibration peaks were slightly shifted to a lower frequency from 2919 and 2851 cm^{-1} in gelator **3** to 2918 and 2849 cm^{-1}, respectively, in SWNT-**3** composite (Fig. S5, ESI†). Also, the carboxylate stretching frequency shifted from 1585 and 1405 cm^{-1} in the native gel to 1581 and 1401 cm^{-1} in the composite, respectively. The observed decrease in both C–H bending and carboxylate stretching frequencies further specify the integration of dispersed nanotubes within the gel matrix due to the hydrophobic interactions between gelator and SWNT.[2]

The FESEM image of hydrogel **3** shows the presence of a 3D SAFIN with a diameter of ~50–70 nm (Fig. 2a) and length of several micrometers. The SWNTs suspended in water were found to be in an aggregated state (Fig. 2b). Interestingly, SWNT-**3** composite (0.1% *w*/*v* SWNT and 0.8% *w*/*v* of **3** (at MGC)) exhibited interconnecting fibrillar networks (Fig. 2c) with comparable dimension (~50–70 nm) to that of gelator **3**. A magnified view of the SEM image of SWNT-**3** revealed the presence of SWNTs (~5 nm diameter and a length of a few micrometers), which cross-link the gel fibers (Fig. S6a, ESI†). This evidently proves the integration of SWNTs within the gel matrix, facilitating supramolecular hydrogelation. Formation of a similar intertwined fibrillar network by physical cross-linking of SWNTs within the SAFIN of the hydrogel was observed in the FESEM images of SWNT-**4** hybrid (SWNT (0.5% *w*/*v*)-**4** (1% *w*/*v*)) with ~60–90 nm fiber diameter (Fig. S6b,c, ESI†). Similarly, transmission electron microscopic (TEM) images of gelator **3** showed the presence of thin fibers, while SWNTs were found in a bundled state (Fig. S7a,b, ESI†). In the presence of **3**, debundled SWNTs were observed with 5–7 nm dimension (Fig. S7c, ESI†). Importantly, SWNT-**3** composite exhibited

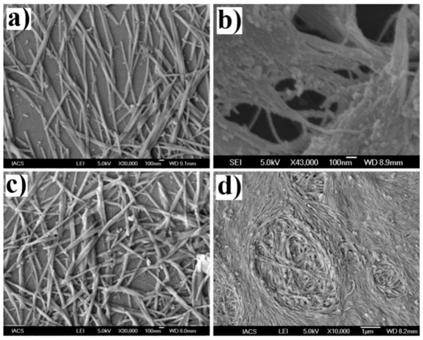

Fig. 2 FESEM images of (a) hydrogel **3**, (b) pristine-SWNT, (c) SWNT-**3** composite and (d) SWNT-**3** hybrid gel containing cyt c.

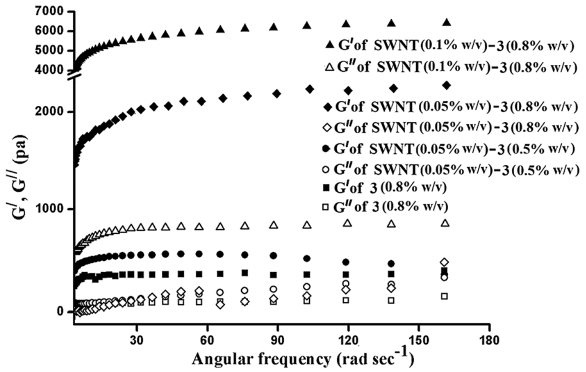

Fig. 3 Plot of storage modulus (G') and loss modulus (G'') of **3**, SWNT-**3** hybrid gel as a function of angular frequency.

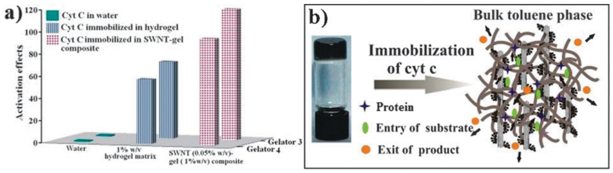

Fig. 4 (a) Variation of cyt c activity entrapped in the hydrogel and SWNT–gel composite in toluene. [Pyrogallol] = 10 mM; [H_2O_2] = 30 mM; [cyt c] = 5 μg mL^{-1}. Experimental errors are within ±5–10%. (b) A schematic depiction of the SWNT–hydrogel network as a scaffold for biomolecular catalysis in toluene.

densely packed interconnecting fibers of 40–50 nm dimensions (Fig. S7d, ESI†).

Further we investigated the mechanical properties of the developed nanohybrids by rheology.[2,7] In a typical oscillatory frequency sweep experiment for hydrogel **3** (at 0.8% *w*/*v*) at a fixed strain (γ, 0.01%), the storage modulus (G') was higher than the loss modulus (G'') over the entire angular frequency (ω) range (0.1–200 rad sec^{-1}, Fig. 3). Interestingly, ~4 and 10-fold increases in G' were observed across the same ω range in the presence of 0.05% and 0.1% *w*/*v* SWNT within the hydrogel of **3** at MGC, denoting the mechanical reinforcement of the nanocomposite. In both cases, G' and G'' ($G' > G''$) showed a plateau region (Fig. 3). The SWNT-**3**, at two times lower concentration compared to its MGC (0.4% *w*/*v*) and SWNT (0.05% *w*/*v*) showed similar plateau region which is a characteristic fearure of viscoelastic materials. Both G' and G'' were also higher compared to that of native hydrogel **3** at 0.8% *w*/*v*. Thus, the poor rigidity of the molecular hydrogel was significantly strengthened by physical cross-linking of pristine SWNTs with the SAFIN of the hydrogel.

Development of SWNT–gel hybrids would be further justified if the nanocomposites offer superior applications with respect to its native constituents. Previously, amphiphilic networks of molecular gels have been explored to increase the enzyme activity in organic solvents.[8] Accordingly, the activity of cyt c immobilized in hydrogels of **3**, **4** and composites of SWNT-**3**, SWNT-**4** was studied following the oxidation of pyrogallol by H_2O_2 in toluene. The immobilized cyt c in the hydrogel of **3** and **4** exhibited 70- (K_{obs} = 455 s^{-1}) and 58-fold (K_{obs} = 375 s^{-1}) improvement in its activity, respectively, compared to the activity of cyt c in water (K_{obs} = 6.5 s^{-1}) (Fig. 4a). Interestingly, cyt c immobilized within SWNT-**3** composite exhibited 120-fold (K_{obs} = 800 s^{-1}) improvement in its activity (~1.7-fold higher than hydrogel **3**, Fig. 4a). Similarly, 95-fold (K_{obs} = 625 s^{-1}) activation was observed for cyt c entrapped within SWNT-**4**. A marked improvement in the activity of cyt c immobilized within the nanocomposites can be attributed to the presence of amphiphobic SWNTs in amphiphilic networks and increased contact area between cyt c and substrate in small hydrogel particles. Hydrophobicity of SWNTs within SAFIN also facilitated mass transfer efficiently across the interface. Presumably, hydrophobic purpurogallin rapidly diffused out to bulk toluene from the hydrophilic domain resulting in higher activation of cyt c (Fig. 4b). The FESEM image shows entrapped cyt c within an amphiphilic network of the composite (Fig. 2d). A similar spectral pattern and position of the soret band (390–420 nm) of cyt c in water, as well as entrapped in **3** and SWNT-**3** hybrid indicated no structural change in the heme moiety (Fig. S8, ESI†).[8]

In summary, pristine SWNTs were effectively infused within a LMW hydrogel without compromising the intrinsic features of either of the components. Notably, the mechanically reinforced SWNT–hydrogel composites were found to be a scaffold for hosting superior biomolecular catalysis in toluene.

We are grateful to DST, India (SR/S1/OC-25/2011) for financial assistance and CSIR for fellowship to TK and SKM.

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