Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary information

Materials and Methods

Figure S1 to S9

References (*47, 48)*

Materials and Methods

Materials

Synthetic microcrystalline graphite (<20μm, Product No. 282863), lithocholic acid (>97%), and dichlorobenzene (anhydrous) were purchased from Sigma-Aldrich. Synthetic graphite (<5μm, Product No. 4827) was purchased from Asbury Carbon. All reagents are used as received.

Preparation of the dispersed graphene solution

The dispersed nanosize graphene was prepared from synthetic microcrystalline graphite (<20μm) according to the previous work [47]; Synthetic microcrystalline graphite (1.5g) was added to 300mL dichlorobenzene. The mixture was homogenized at 2400 rpm for 1 hr (IKA-RW20, Stage II), followed by cuphorn sonication for 30 mins (Power%= 50 %, Pulse = 20 sec. (On), 10 sec (Off)). Finally the slurry was centrifuged at 4400 rpm for 30 minutes. The supernatant was obtained in ODCB.

The dispersed microsize graphene was prepared from synthetic graphite (<5μm); Synthetic graphite (1.5g) was added to 300mL dichlorobenzene. The mixture was homogenized at 1000 rpm for 1 hour without cuphorn sonication. Finally the slurry was centrifuged at 4400 rpm for 30 minutes. The supernatant was obtained in ODCB.

Synthesis of starting material (*N-(2-aminoethyl)-3α-hydroxy-5β-cholan-24-amide (LCA)*)

LCA was synthesized by excess of the diaminoethane to react with the methyl lithocholate according to the previous work[33]; The methyl lithocholate was dissolved in methanol and excess of diaminoethane (20-30 times) was added. The solution was heated with an oil bath

(80 – 90 °C) for 2 days. The resulting solution was poured into the water and the precipitate filtered. The product was recrystallized from acetonitrile and dried in a vacuum.

Synthesis of Methyl lithocholate

Methyl lithocholate was synthesized to the previous work[48]; To a stirred suspension of lithocholic acid (2.53 g, 6.27mmol) in methanol (25mL) was added Acetyl chloride (0.24mL, 3.38mmol) dropwise at room temperature for 3.3hrs. And then, di-water (25mL) was added to the mixture. The resulting precipitate was collected by filtration and dried at 50°C to give crude products (2.68g, quantitatively) as a colorless powder. Analytically pure methyl lithocholate was obtained by recrystallization from acetonitrile.

Synthesis of *N-(2-Hydroxyethyl)-3-hydroxy-5-cholan-24-amide*

N-(2-Hydroxyethyl-3-hydroxy-5-cholan-24-amide was synthesized by the reaction of methyl lithocholate with 2-aminoethanol according to the previous work[3]; 2-Aminoethanol (3.5 g, 57.6mmol) was dissolved in methanol (25mL) and methyl lithocholate (0.5g 1.28mmol) was added. The resulting mixture was stirred at room temperature for 5 days, then poured over ice-water (100mL). The white solid obtained was filtered off, washed with water, dried in a vacuum, and analyzed without further purification.

Preparation of Graphene nanoscrolls (GNS)

An amount of LCA in the range from 0.001 to 0.1mmol was dissolved in ODCB (1ml) with heat treatment at 60°C. Then, the dissolved LCA solution was immediately poured into the dispersed graphene solution (5ml). The solution with precipitates was stored at room temperature for 24 hours and the complex of LCA-graphene was observed. The resulting

precipitate was obtained by filtering with a PTFE membrane, affording LCA induced Graphene nanoscrolls (GNS).

1. **Synthesis of Nanosize-graphene scrolls (N-GNS)**

To obtain N-GNSs, LCA of 0.02mmol was dissolved in ODCB (1mL) and heated to 60°C. Then, the dissolved LCA solution was immediately poured into the dispersed nanosize graphene solution (5ml). The solution with the precipitates was stored at room temperature for 24 hrs. The precipitate was obtained by filtering with a PTFE membrane washed with methanol followed by centrifugation at 4000 rpm for 20 minutes and by decantation. This procedure was at least repeated five times. The smaller solid core N-GNSs were obtained. To remove the included LCAs inside GNSs, N-GNSs were washed with methanol and followed by centrifugation at 4000 rpm for 20 minutes and by decantation. Additionally, the obtained N-GNSs were stored in methanol for few days in order to have long time enough to dissolve LCAs. The hollow core N-GNSs were obtained.

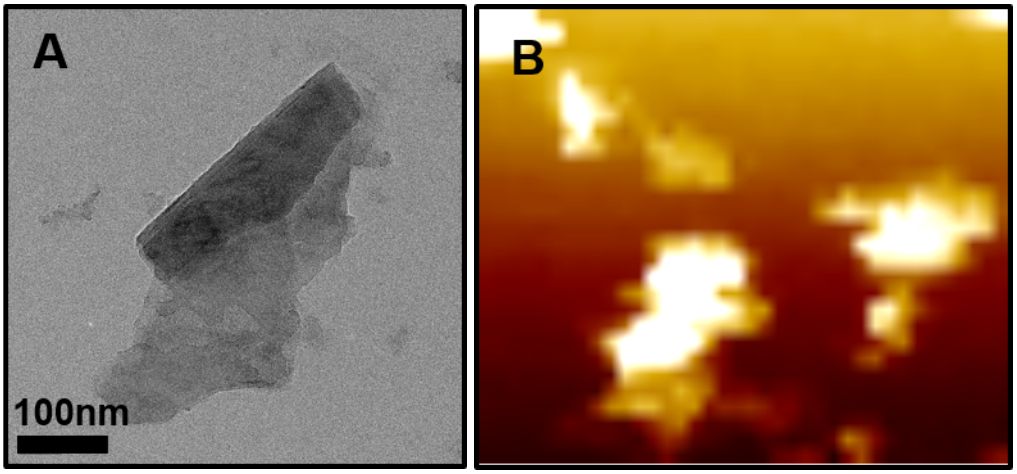
2. **Synthesis of Microsize-graphene scrolls (M-GNS)**

To obtain M-GNS, LCA of 0.003mmol was dissolved in ODCB (1mL) and heated to 60°C. Then, the dissolved hot solution was stored at room temperature for 24hrs. The dissolved hot solution was stored at room temperature for 24hrs. And then LCA fiber solution was poured into the dispersed microsize graphene solution (5mL) at RT and the precipitates were obtained by filtering with a PTFE membrane, followed by centrifugation at 4000 rpm for 20 minutes and by decantation. Here, the LCA fiber as a template interacts with graphene and then larger solid core M-GNSs were obtained. To remove the LCA fiber inside GNSs, M-GNSs were sufficiently washed with methanol several times and followed by centrifugation at 4000 rpm for 20 minutes and by decantation. The hollow core M-GNSs were obtained.

Characterization

The purified solid sample was re-dissolved in methanol (2ml). A drop of the sample by a microsyringe was placed on a holey carbon grid and then was allowed to evaporate methanol at 18°C. TEM measurements were carried out on JEM-2100F with an accelerating voltage of 200 kV. Optical micrographs (OM) were taken with an OLYMPUS BX51 polarized optical microscope and AcquCAM II digital camera. A TOMORO AcquPro 2005TM (Image partnership Co., Ltd.) was used for image capture. Fluorescence microscope equipped with a mercury lamp and a filter (U-MWU2) was used. And Atomic Force Microscopy (AFM) measurements were made with PSI AFM (XE-100). All the topography images were realized in a noncontact mode using a PPP-NCHR (PointProbe® Plus Non-Contact High Resolution Frequency-Reflex Coating) silicon probe with a tip radius of less than 10 nm (NanosensorsTM). System control and data acquisition were performed by XEP software (Park Systems Corp.), and data analysis was done with a XEI software (Park Systems Corp.). Scanning Electron Microscopy (SEM) measurements were carried out on Nova nano SEM 450 with an accelerating voltage of 15 kV. Micro-Raman spectrometer (JASCRO NRS-3100) analysis was carried out with an excitation laser wavelength of 532nm using the 100 X objective. Micro confocal-Raman spectrometer (MonoRa-750i/ELT 10000) analysis and mapping were carried out with an excitation laser wavelength of 488 nm argon-ion laser. Samples were prepared by drop casting of the dispersion on SiO_2/Si substrates and the solvent was evaporated at RT. The TGA curves are obtained by thermogravimetric Analyzer TGA7 (PERKIN ELMER) with N_2 purging at a heating rate of 10°C/min.

Figure S1. (A) TEM and (B) AFM images of the disperesd graphene.



The graphite in dichlorobenzene (ODCB) was homogenized at 2400 rpm for 1 hr, followed by cuphorn sonication for 30 mins. (Power%= 50 %, Pulse = 20 sec.(On), 10 sec (Off)). The slurry was centrifuged at 4400 rpm for 30 minutes and collected from the supernatant ODCB dispersion. The concentration of the final dispersed graphene solution was calculated using the vacuum filter through a weighed membrane. The dispersed graphene solution had a concentration of 0.3mg/mL. A TEM image of the dispersed graphene is shown (fig.S1A). The few-layers graphene, consisting of single layer and graphene flakes, is observed. Additionally, an AFM image (fig.S1B) shows the thickness of graphene layers. The thickness of thinnest graphene layer is below 1nm, which is graphene monolayer or bilayers.

Figure S2. TEM images on confirmation of intact graphene without interaction between graphene and other bile acid derivatives; (A) cholic acid, **(B)** deoxycholic acid, **(C)** lithocholic acid, **(D)** methyl lithocholate and **(E)** N-(2-aminoethyl)-3α-hydroxy-5β-cholan-24-amide with only one or two functions.

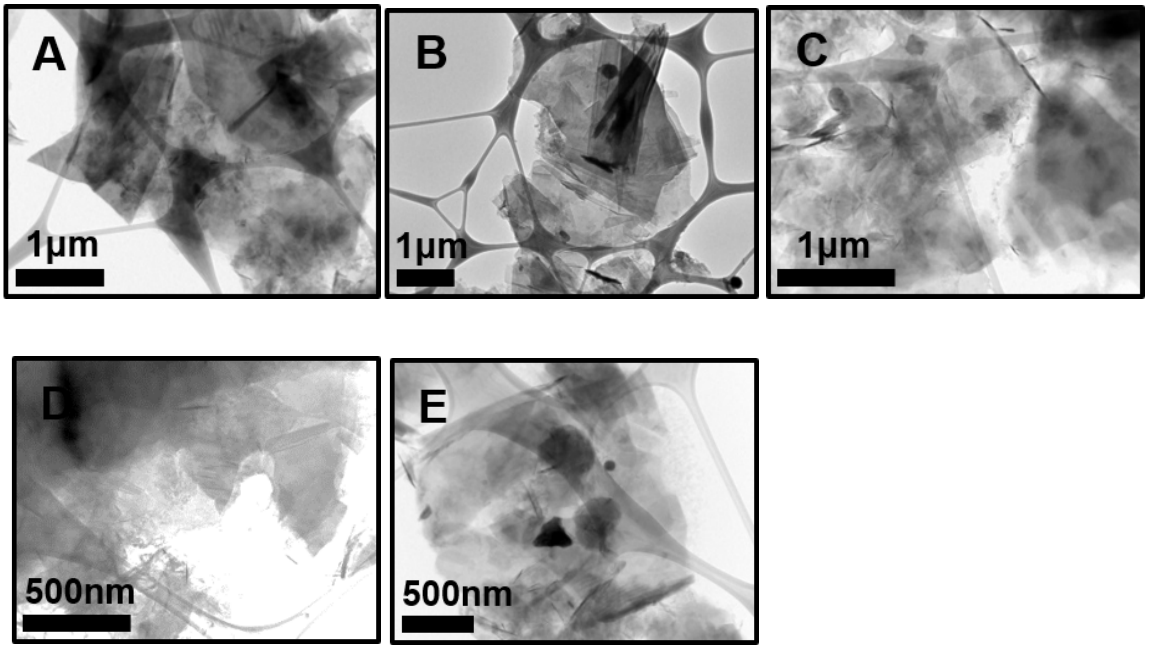
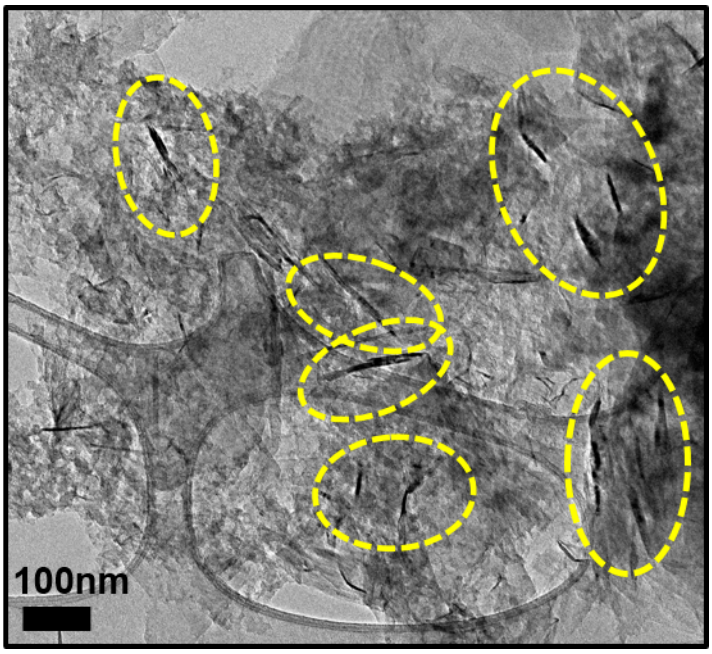
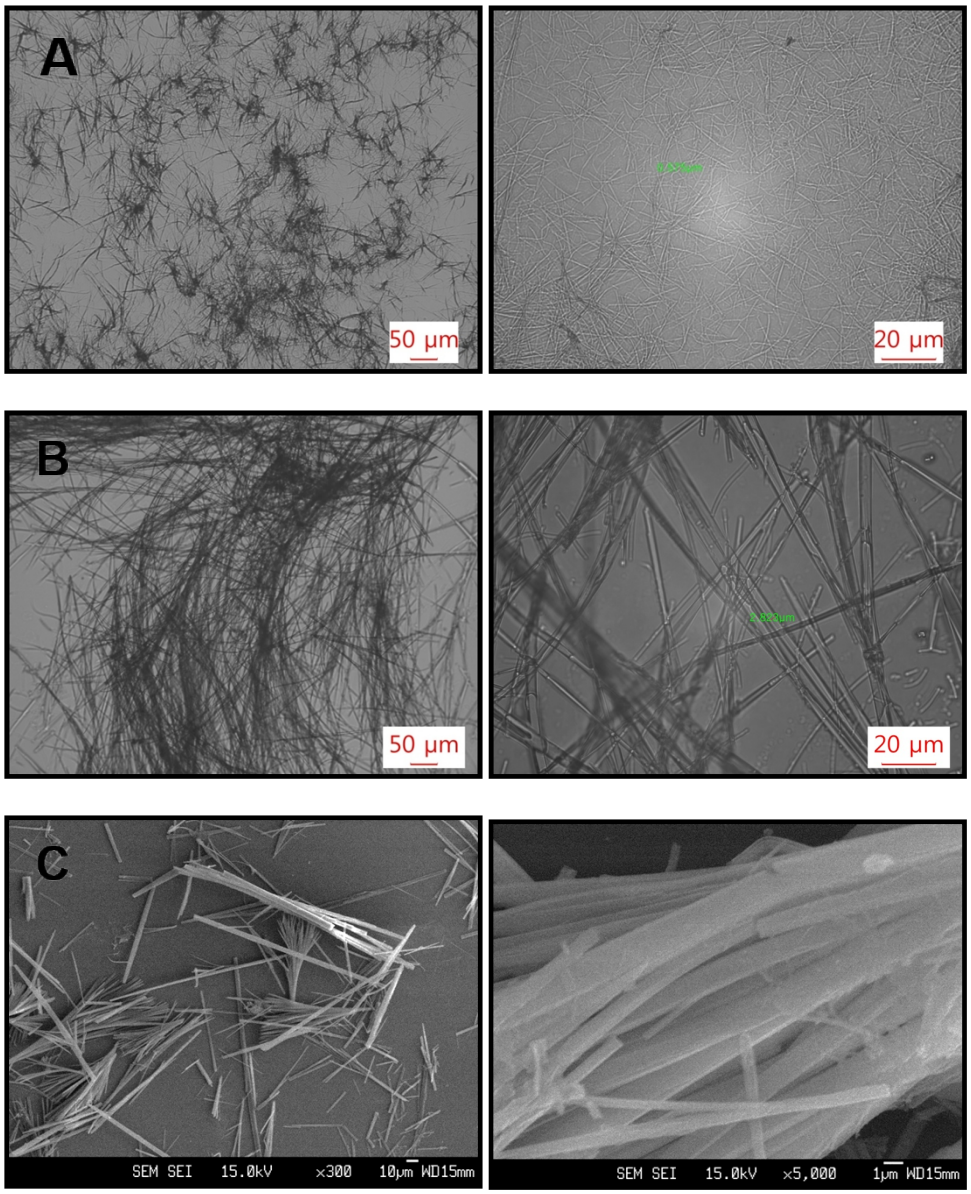


Figure S3. TEM images of N-GNSs at a low magnification.



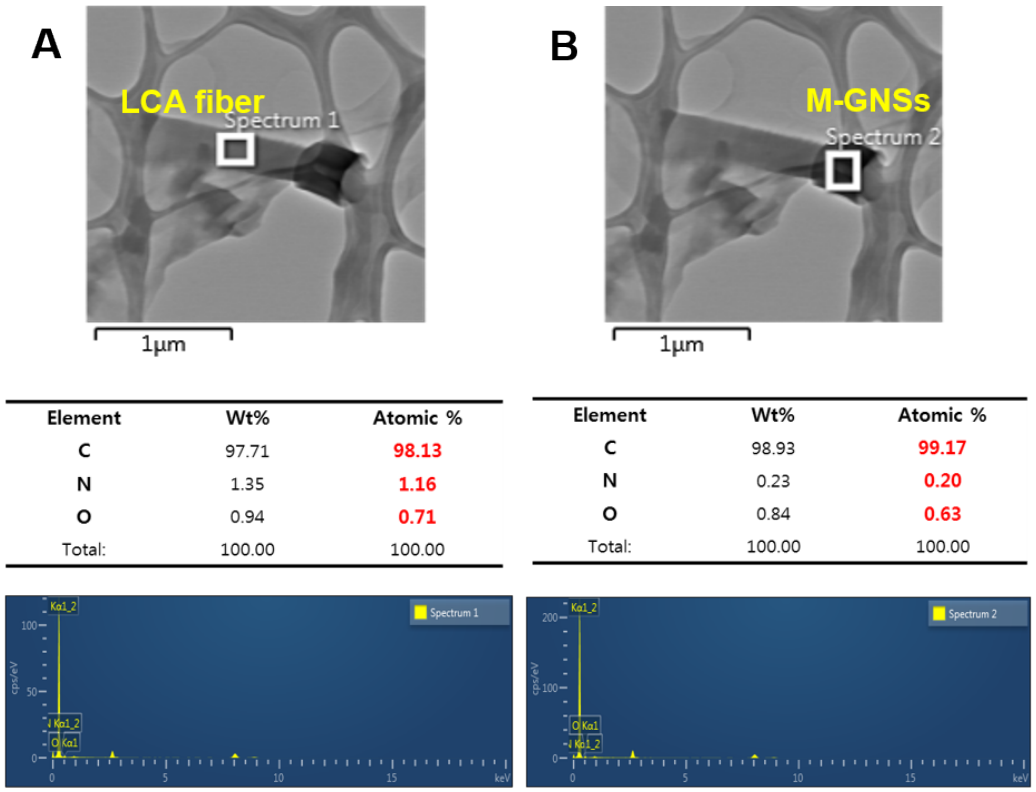
The tube-like morphology scrolled by interaction of graphene with LCAs. The TEM images at a low magnification (fig.S3) represent the produced nanosize GNSs (N-GNS).

Figure S4. Optic microscopy images of LCA in two solvents, (A) ODCB and (B) Xylene and (C) SEM images of LCA in ODCB.



In general, it has been known that LCAs grow to be fibers in several solvents and SEM and OM images show LCAs grow to fibers in ODCB.

Figure S5. TEM images and Energy dispersive spectrometer (EDS) analysis of M-GNSs included by a LCA fiber. (A) Detection of LCA fiber and **(B)** solid core M-GNSs at the center point by EDS.



Element	Wt%	Atomic %
C	97.71	98.13
N	1.35	1.16
O	0.94	0.71
Total:	100.00	100.00

Element	Wt%	Atomic %
C	98.93	99.17
N	0.23	0.20
O	0.84	0.63
Total:	100.00	100.00

LCAs and solid core M-GNSs at the center point were detected by the elementary analysis of Energy dispersive spectrometer (EDS) images of M-GNSs. These data show LCAs and M-GNSs with corresponding EDS mapping for C, N and O.

Figure S6. Fluorescence images on attachment effects of LCA fiber, graphene and M-GNSs with inclusion of LCA fiber on pyrene as a fluorescent probe. (A) LCA fiber added with pyrene of 0.025mmol and **(B)** the dispersed microsize graphene attached with pyrene. **(C)** M-GNSs attached by pyrene at low magnification and **(D)** M-GNSs emitting fluorescence under excitation light.

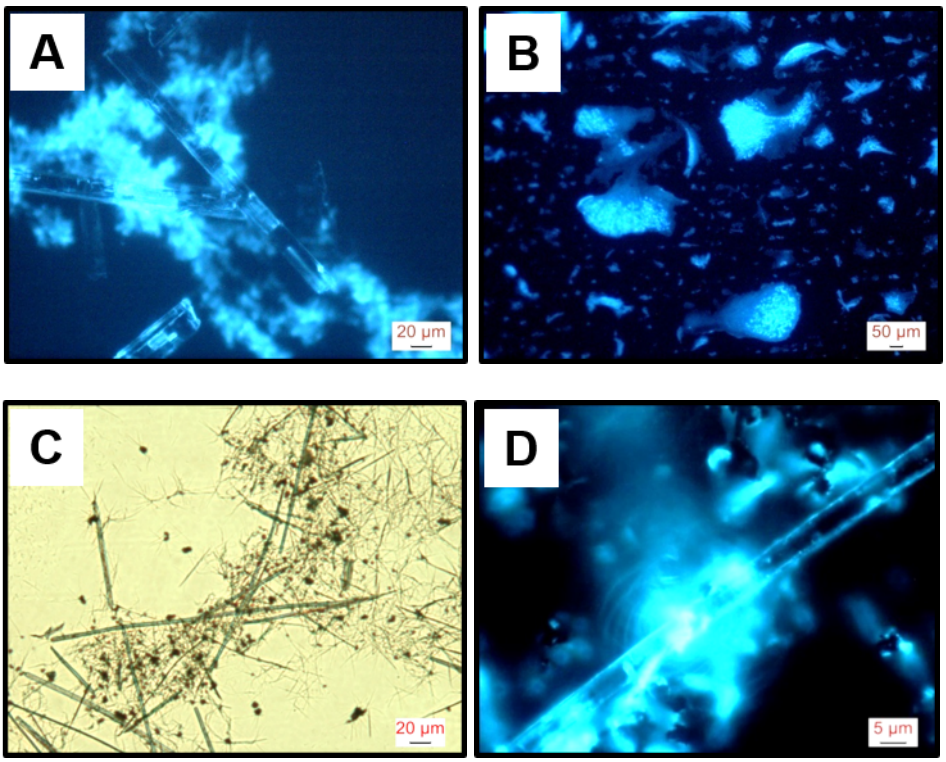
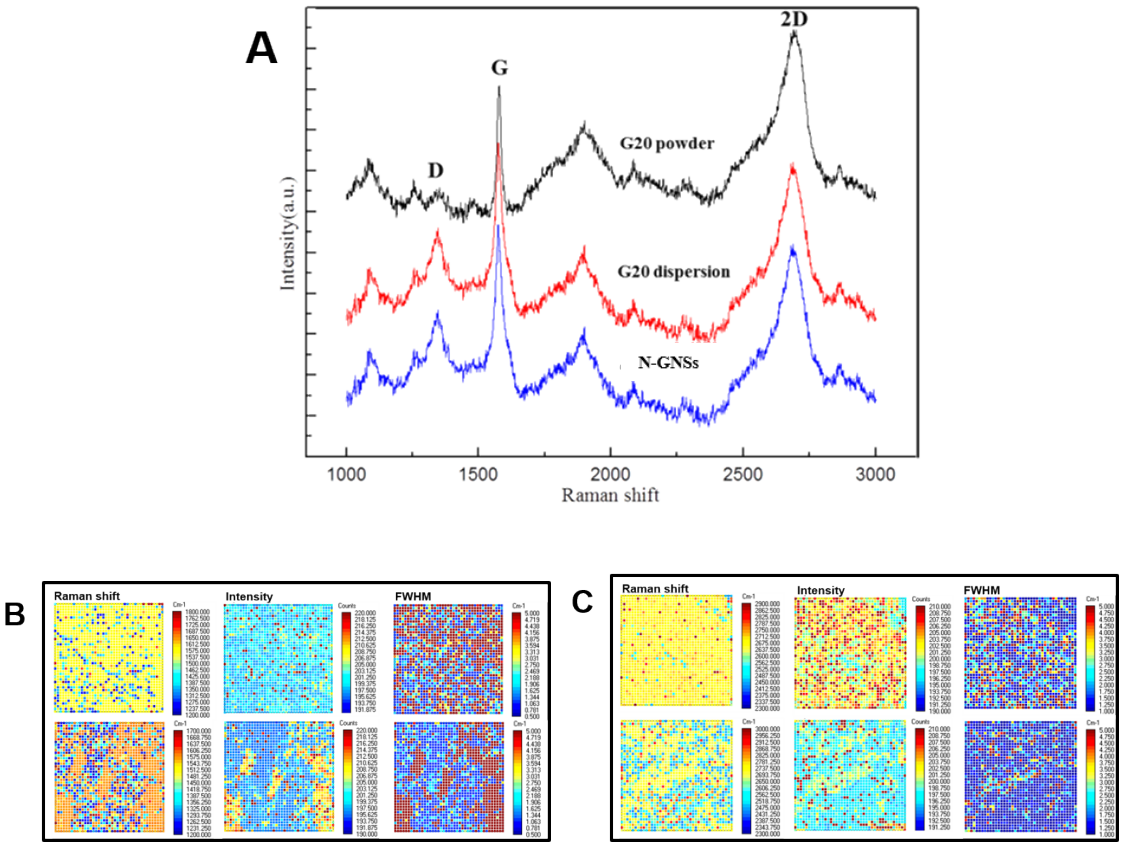
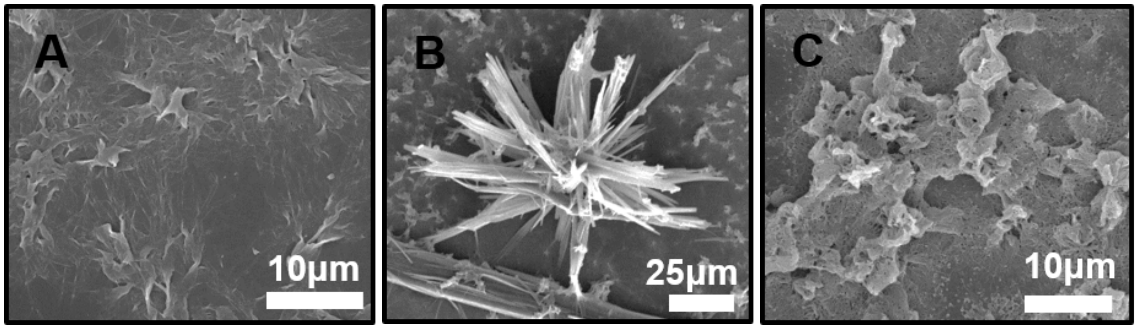


Figure S7. Raman spectra amd mapping images of Graphene powder, the dispersed graphene and N-GNSs with 532nm wavelength of excitation laser. (A) Raman spectra of Graphene (<20μm) powder (G20P), the dispersed graphene (G20D) and N-GNSs. Raman mapping images of G20D at an upper level and N-GNSs at a bottom level **(B)** for G band (~1500cm^{-1}) and **(C)** G20D and N-GNSs for 2D band (~2700cm^{-1}) .



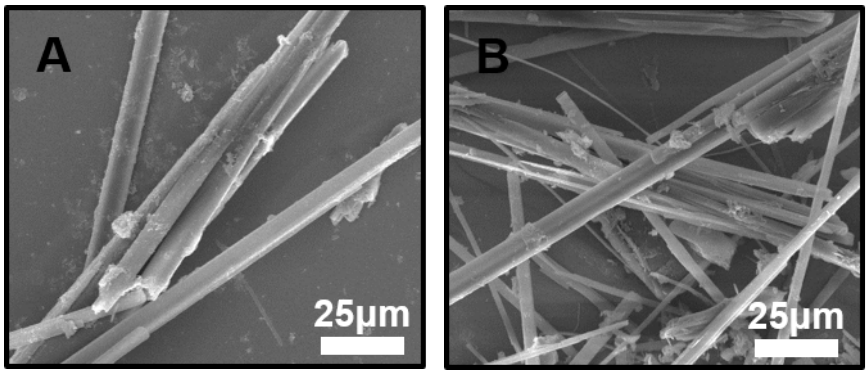
Raman spectra of Graphene (<20μm) powder (G20P), the dispersed graphene (G20D) and N-GNSs is shown. Raman spectrum of the dispersed graphene before being scrolled shows G (~1578cm^{-1}) and 2D bands (~2678cm^{-1}) for G20P and G (~1578cm^{-1}) and 2D bands (~2687cm^{-1}) for G20D (fig.S7A). In the case of those of N-GNSs, The remarkable changes in the range of G (~1573cm^{-1}), 2D bands (~2687cm^{-1}) and I_D/I_G intensity value of 0.38, which is indicated the sp^2 carbon defects and phonon dispersion at D band, are observed. Previously, the red shift of G band indicated the increasing number of graphene layers and disoriented stacked layers of graphene. We suggest that the reasons about the red shift of N-GNSs at G band are the same in our case. Likewise, the shape of 2D band at 2700cm^{-1} is used to classify monolayer graphene and few-layer graphene. In our sample, N-GNSs show the blue shift of 9cm^{-1} at 2D band because of increasing π- π stacking for the scrolled graphene layers compared with that of G20D. In addition, Raman shift, intensity and full width at half-maximum (FWHM) distribution range of G and 2D bands are compared with those of G20D and N-GNSs through Raman mapping images (fig.S7, B and C). At G band, it is noticed that Raman shift and intensity of N-GNSs are larger than those of G20D and FWHM changes from ~20cm^{-1} to ~25cm^{-1} with the red shift of N-GNSs position to about 1500cm^{-1}. Also, at 2D band, the relation of Raman mapping images with the blue shift of Raman spectra confirms scrolling of graphene, that is, formation of GNSs.

Figure S8. SEM images of self-assembled LCAs stored at (A) 4°C, (B) 18°C and (C) 28°C for 24hrs in ODCB.



To obtain the LCA fibers, we create favorable conditions for growing to LCA fiber by controlling temperature. The SEM images show the different morphologies of LCAs dependent on the temperature and there is no growing to LCA fiber at 4°C and 18°C, except only growing to LCA fiber at 18°C.

Figure S9. SEM images of the supernatant precipitates before purification. LCA fibers are dominantly observed. Thus it is necessary to wash excess LCA fibers with methanol in order to observe graphene scrolls**. (A)** N-GNSs and **(B)** M-GNSs before purification.



References

47. Christopher E. Hamilton, Jay R. Lomeda, Zhengzong Sun, James M. Tour. *Nano letters,* 2009, 9, 10, 3460-3462.

48. Valkonen, A., Lahtinen, M., Virtanen, E., Kaikkonen, S., Kolehmainen, E. *Biosensors and Bioelectronics,* 2004, 20, 6, 1233-1241.