Graphene & Graphene Oxide and amyloid peptide binding and its implications in Alzheimer's disease

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Introduction

Alzheimer's disease is a neurodegenerative disease caused by the incorrect cleaving of the transmembrane Amyloid Precursor Protein into the neurotoxic $A\beta_{40}$ and $A\beta_{42}$ fragments[2]. These fragments are soluble oligomers with a random coil conformation that can impair synapses or neurotransmission; they may also aggregate into parallel and antiparallel beta sheets to form amyloid plaques, which can block or distort signaling between neuronal pathways[7].

Aβ fibrils self-assemble into parallel and antiparallel beta sheets on hydrophobic graphite, but not on hydrophilic mica[5,6]. Aβ fibrils also assemble on graphene, which irreversibly captures fibrils[3], suggesting graphene might have a role in the study of Alzheimer's amyloid plaque.

These studies characterize binding between amyloid beta peptide fibrils and graphene using Raman spectroscopy, scanning electron microscopy (SEM), and circular dichroism (CD). The goal is to provide evidence that graphene can attract free floating Aβ fibrils and Aβ plaque. Both studies currently use diphenylalanine peptide, a self-assembling model peptide for Aβ fibrils.

Methods

-For the SEM, a stock solution of diphenylalanine dissolved in 1,1,1,3,3,3-hexafluouro-2-propanol (HFIP) (100 mg/mL) was added to graphene dispersed in 7x TE (1 mg/mL). All samples were dried on aluminum stubs coated with gold. A total of fourteen samples were analyzed. -Samples 1 and 2 were the stock graphene and diphenylalanine solutions. Samples 3-14 were three sets of four samples each. The 1st of a set was not shaken; the 2nd was hand shaken; the 3rd was vortex shaken; the 4th was sonicated. -Samples 2-6 had a 1:1 ratio of graphene and diphenylalanine; samples 7-10 had a 2:1 ratio; samples 11-14 had a 1:2 ratio. FF nanotubes structures are characterized by two strong, positive bands, with one located around 200nm and the second appearing around 220nm. A local, positive minimum is located between the two strongly positive bands and occurs around 209nm. Finally, a strong negative band is located below 195nm, also representing a global spectral minimum. Changes in the ellipticity intensity would therefore represent a modulation of nanotubes character, either in an increasing or decreasing capacity.

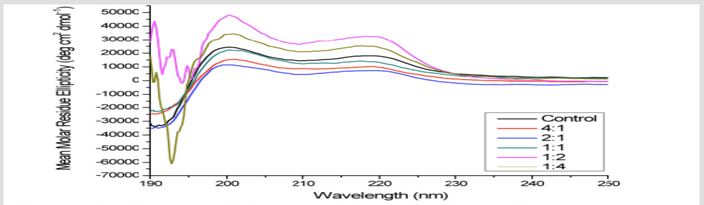
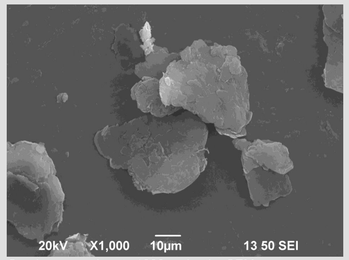


Figure 4-10A. CD spectra of 0.04 mg/ml (128 µM) of pre-formed FF nanotubes titrated with increasing concentrations of graphene-oxide (0.01, 0.02, 0.04, 0.08, 0.16 mg/ml GO). Ratios are expressed in the form [FF]:[GO]. Samples with [FF]:[GO] ratios less than 1 showed an increase in ellipticity at 200.8 nm and 219 nm relative to the FF control. Samples with [FF]:[GO] ratics greater than 1 showed a decrease in ellipticity at 200.8 nm and 219 nm relative to FF control. A 1:1 ratio of [FF]:[GO] produced a signal with ellipticities similar to the FF control. Changes in ellipticity at 200.8 nm and 219 nm as a function of [GO] are plotted in figure 4-10B.

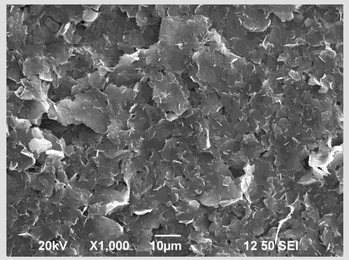
Results

Experiment 1

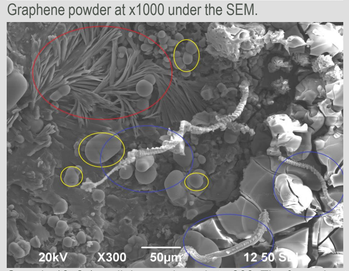
Signs of binding include fanned out or twisted cylinders with graphene "scales" or "ridges" on them. All samples were prevalently graphene with no observable effect from diphenylalanine.



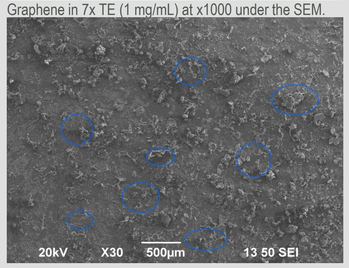
Graphene powder at x1000 under the SEM.



Graphene in 7x TE (1 mg/mL) at x1000 under the SEM.



Sample 10: 2:1 gr:diphe, sonicated at x300. The fanned out cylinders are circled in red. The twisted cylinders are circled in blue. Some droplets of gold left by SEM preparation are circled in yellow. The rest is graphene.



Sample 8: 2:1 gr:diphe, hand shaken at x30. There are twisted cylinders all over the surface, some circled in blue.

Experiment 2

The diphenylalanine and GO samples self-assembled into nanotube.

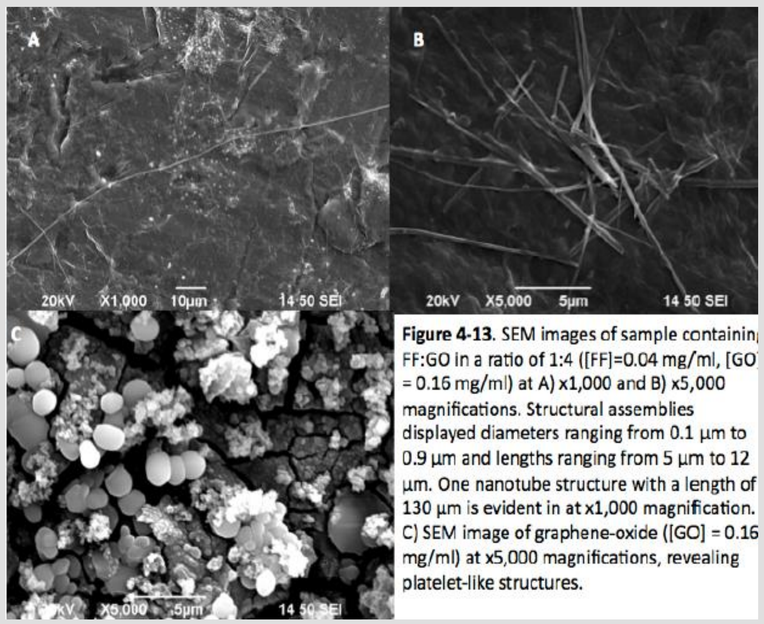


Figure 4-13. SEM images of sample containin FF:GO in a ratio of 1:4 ([FF]=0.04 mg/ml, [GO = 0.16 mg/ml) at A) x1,000 and B) x5,000 magnifications. Structural assemblies displayed diameters ranging from 0.1 µm to 0.9 µm and lengths ranging from 5 µm to 12 µm. One nanotube structure with a length of 130 µm is evident in at x1,000 magnification. C) SEM image of graphene-oxide ([GO] = 0.16 mg/ml) at x5,000 magnifications, revealing platelet-like structures.

Conclusions

Experiment 1 explored if diphenylalanine easily bound to graphene dispersed in 7xTE, which is ideal for keeping graphene powder in an even suspension within a solution. Graphene did bind to diphenylalanine, with a higher binding rate in a 2:1 gr:diphe ratio, but otherwise the binding efficacy was seemingly random and unreliable. Furthermore, the reference article[4] found ideal binding occurred in the 3.7-5.4 pH range. This study's samples had a 7.3 pH to mimic cerebrospinal fluid.

Experiment 2 CD spectral scans with the increase in the GO concentration reveal a modulation of nanotubes character relative to FF control samples (0.4mg/ml FF, 0mg/ml GO)suggestive of FF-GO nanocomposite formation. Samples with FF:GO ratios of 1:2 and 1:4 demonstrated an increase in ellipticity intensity at both diagnostic wavelengths. These increase in the quantity of nanotubes upon addition of GO, suggests the formation of peptide-GO nanocomposite.

These experiments have demonstrated that there are binding capabilities between graphene & graphene oxide and diphenylalanine, even in less than ideal situations. Diphenylalanine was chosen because its two phenylalanines mimic those in amyloid beta peptides.

Future Directions

The next step is to recreate an aqueous graphene dispersion using a modified Hummers method[4] and determine if it is a better medium for diphenylalanine binding than 7x TE, since 7x TE is a possible deterrent of binding, and ddH_20 is a possible enabler. Once the more productive dispersion solution is determined, we will experiment the conditions of binding between graphene and amyloid beta peptide, whether in random coil or beta sheet conformation. The pH will remain comparable to that of cerebrospinal fluid.

Afterwards, experimentation of amyloid beta peptide to graphene will be done in artificial cerebrospinal fluid to determine how the extracellular fluid in the brain may change the reactions between Aβ and graphene. I

Citations

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