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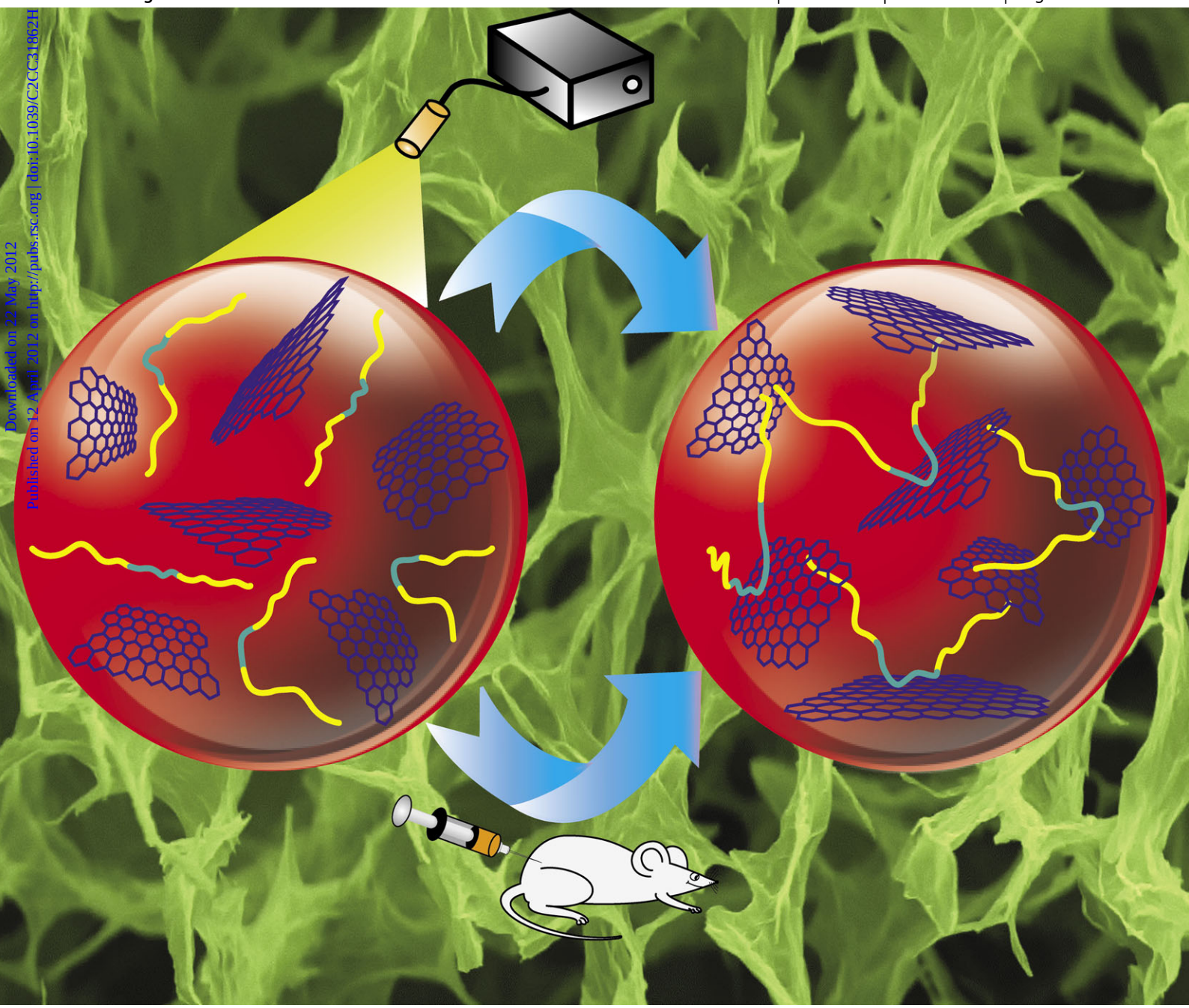

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A stimuli-sensitive injectable graphene oxide composite hydrogel†

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We report the formation of a self-assembled hydrogel of graphene oxide nanosheets by physically crosslinking in a low concentration Pluronic solution without any chemical modification of GO. This hydrogel undergoes a sol–gel transition upon exposure to various stimuli, such as temperature, near-infrared light, and pH. Since the thermosensitive gelation occurs at near body temperature, this system can be applied as an injectable gel system. NIR laser irradiation can also trigger very rapid gel formation by photo-thermal effect. An *in vivo* mouse experiment confirms the stable gel formation after injection with no severe acute cytotoxicity.

Graphene oxide (GO) is an oxidized derivative of graphene which has attracted a lot of interest due to its extraordinary electronic, thermal, mechanical and optical properties.[1] The hydrophilic groups of GO make it water dispersible and provide sites for further chemical modification.[1] 3-D assembly of GO has potential applications in various fields such as electronic devices, energy storage, biosensors and drug delivery.[2] Several studies have reported 3-D assembly of GO solution in water, forming hydrogels.[3] Hydrogels formed by physical or chemical cross-linking of polymers have been extensively explored as functional soft material for numerous biomedical applications.[4] In previous reports, GO based hydrogels were prepared by adding either polymers (*e.g.* PVA, PEI), macromolecules (*e.g.* hemoglobin, DNA), small organic molecules, or cations (CTAB, $CaCl_2$) into GO solution.[3] In these cases, the main driving force for gelation was hydrogen bonding, π–π interaction, or electrostatic interaction.[3] Chemical attachment of cyclodextrin (CD) with GO and supramolecular host–guest complex formation have also been employed to form graphene based hydrogels,[5] where Pluronic block-copolymers served as a surface stabilizer to disperse graphene sheets in aqueous solution and were further utilized to form physical networks through complexation between Pluronic and CD. In this case, hydrogel was formed by Pluronic and CD, and the presence of graphene had no role in gelation.[5a] All these graphene/GO hydrogels formed instantaneously after mixing at room temperature except GO–DNA hydrogel, which required heating to 90 °C for gelation.[3c] In contrast, there is no report of temperature dependent sol–gel transition of GO solution, where gelation was mediated by GO without any chemical modification. Thermosensitive hydrogels are useful for biomedical application as an injectable hydrogel if the sol–gel transition can be achieved at near body temperature (*i.e.* 37 °C).[4b,c]

In this study, we report a temperature responsive physical hydrogel of GO obtained by adding a small amount of Pluronic into GO solution. This mixture remains in a sol state at low temperature but forms a hydrogel when the temperature is raised to body temperature. It is worth mentioning that the hydrogel described herein is not the same gel formed by Pluronic itself. Pluronic block-copolymers are well known to form thermo-sensitive hydrogels at a concentration of 15 wt% or higher,[4c] whereas we used less than 1 wt% of Pluronic in this study. Appropriate concentrations of both GO and Pluronic are necessary to form this thermo-sensitive hydrogel unlike only Pluronic-mediated gelation or Pluronic–CD hydrogel containing graphene.

Three different types of Pluronic, namely F68, F127, and P105, were used for the evaluation of hydrogel formation with GO. We found that F127 and P105 can form hydrogels with GO whereas F68 cannot. Among them, F68 is more hydrophilic than F127 and P105 (Fig. S1, ESI†). So, it appears that hydrophobicity of Pluronic is important for the formation of GO–Pluronic hydrogel. We have examined different concentrations of Pluronic and GO that can induce the gel formation and found that the concentrations of GO and Pluronic both are important for gelation. No gel formation occurred at GO concentration below 0.3 wt%, irrespective of Pluronic concentration. For a particular concentration of GO (*i.e.* 0.4 wt%) gel formation occurred with Pluronic concentration ranging from 0.25 to 1 wt% (Fig. 1A). Below or above these concentrations of Pluronic, gel did not form. This phenomenon is similar to the previous finding of Bai *et al*, where they demonstrated the gelation of GO in the presence of certain low concentrations of PVA.[3a]

We believe that both hydrophobic association and hydrogen bonding are responsible for the thermosensitive gelation of GO and Pluronic. Pluronic chains can adsorb over GO *via* interaction of the PPO block with the hydrophobic basal plane of a GO sheet. This interaction will increase at the elevated temperature due to the increase in hydrophobicity of PPO at higher temperature. At the same time, PEO chains can also interact with the oxygen containing groups of GO by H-bonding. We think, at a critical Pluronic concentration range that induces the gel formation, some Pluronic chains that are adsorbed onto a GO sheet *via* hydrophobic association can also interact with nearby GO sheets *via* H-bonding, thus acting as a physical cross-linker among GO sheets (Fig. 1B). At higher concentration of Pluronic,

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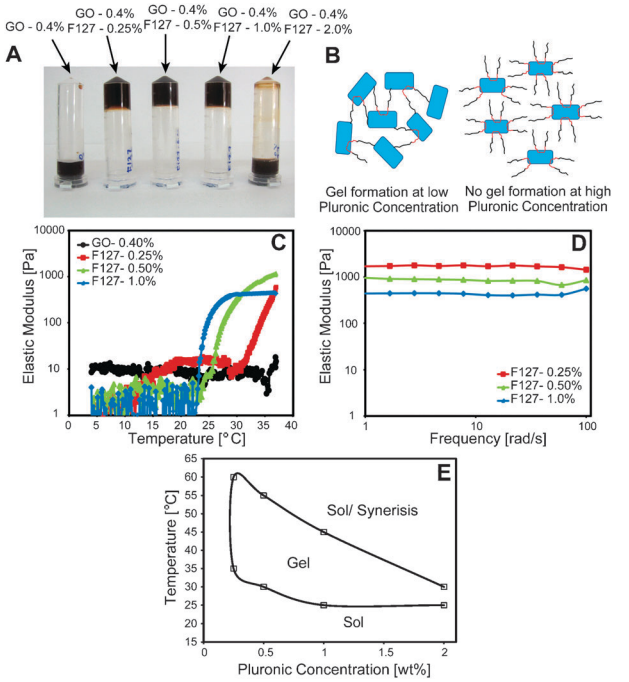

Fig. 1 (A) Photos of GO and F127 mixture showing gel formation after incubation at 37 °C. No gelation of GO solution occurred without Pluronic or with 2 wt% Pluronic. (B) Gelation scheme of GO at critical low Pluronic concentration and no gelation at higher Pluronic concentration. (C) Temperature dependent gelation profile of GO–F127 hydrogels. (D) Frequency sweep measurement of GO–F127 hydrogels. (E) Phase diagram of GO–F127 hydrogels (GO size ~500 nm).

many polymer chains adsorbed onto GO and covered the whole surface, thus preventing H-bonding between GO and PEO chains. This resulted in reduced crosslinking effect by Pluronic among GO sheets and no gel formation occurred (Fig. 1B).

The temperature dependent gelation process of GO–Pluronic gels was characterized by using a rheometer (Fig. 1C). GO itself did not form any gel state. When the concentration of F127 was 1 wt%, gel formation started at 22 °C and reached saturation at 30 °C. In the cases of 0.5 wt% and 0.25 wt% of F127, the gelation started at 24 °C and 30 °C, respectively. The highest elastic modulus (1700 Pa) was observed when the concentration of F127 was 0.25 wt%. The elastic modulus of the hydrogel decreased to 880 Pa and 430 Pa by increasing the concentration of F127 to 0.5 wt% and 1.0 wt%, respectively (Fig. 1D). GO–P105 hydrogels showed similar gelation profiles as GO–F127 gels but the elastic moduli of GO–P105 gels were little higher than those of GO-F127 gels at similar Pluronic concentrations (data not shown).

Unlike the previous reports, where GO based hydrogels formed only with micro-sized GO, we used nano-sized GO sheets (size ~500 nm) (Fig. S2, ESI†) for gel formation. We further reduced the size of GO to ~100 nm by sonication, but no significant difference was observed in the gelation profile and elastic modulus of the gels between the smaller GO (~100 nm) sheets and the larger size GO (~500 nm) sheets (Fig. S3, ESI†). We also investigated the effect of the degree of oxygen functionality of GO on gel formation. GO with less oxygen (10–20 wt%) did not form a gel with Pluronic. Low oxygen functionality may reduce the chances of crosslinking with Pluronic by H-bonding. This supports our understanding that H-bonding between PEO chains and O-containing groups of GO is crucial for gel formation. The phase diagram of GO-F127 hydrogels (Fig. 1E) shows that, when F127 concentration was 0.25 wt%, gel formation started only after 35 °C. At higher F127 concentrations, *i.e.* 0.5 wt% and 1.0 wt%, gel formation occurred at 30 °C and 25 °C, respectively. When F127 concentration was further increased to 2 wt%, gel formation was observed at 25 °C, but it formed a sol state again after increasing the temperature to 30 °C (Fig. 1E). With increasing temperature, we observed syneresis in these gels, which might be due to the combined effect of higher heat capacity of GO and temperature-induced hydrophobicity increase of Pluronic. Gels prepared with P105 show synerisis at lower temperature than gels prepared with F127 (Fig. S3C, ESI†).

The formation of network structure in the hydrogels was evaluated under SEM after lyophilization. No network structure was found in the lyophilized solution of GO, and only aggregated GO sheets were observed (Fig. 2A). All the hydrogel samples showed network structures (Fig. 2B–D). It could be clearly observed that the density of the network changed depending on Pluronic concentration. A hydrogel with an F127 concentration of 0.25 wt% showed denser network formation (Fig. 2B) compared to that of 0.5 wt% and 1.0 wt% (Fig. 2C and D). These observations are consistent with the rheological characterization of the gels (Fig. 1C and D) *i.e.* the gels with higher elastic modulus showed higher network structure. Similar SEM results were observed for GO–P105 hydrogels (Fig. S4, ESI†). As supporting data for the effect of Pluronic concentration on the network structure of GO–Pluronic hydrogel as well as a potential application of the hydrogel, we characterized FITC–dextran release from hydrogels. As expected, gels having higher elastic moduli and denser network structure showed slower release of loaded FITC–dextran (Fig. 2E and F). The molecular weight of

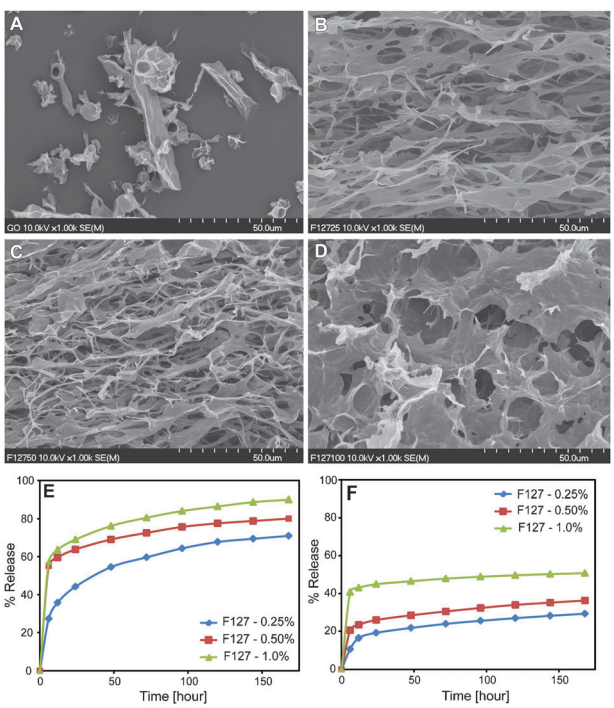

Fig. 2 SEM images of GO (A) and GO–F127 (B–D) gels with F127 concentration of 0.25, 0.5 and 1.0 wt%, respectively. *In vitro* release of 4 kDa (E) and 70 kDa (F) FITC–dextran from GO–F127 hydrogels.

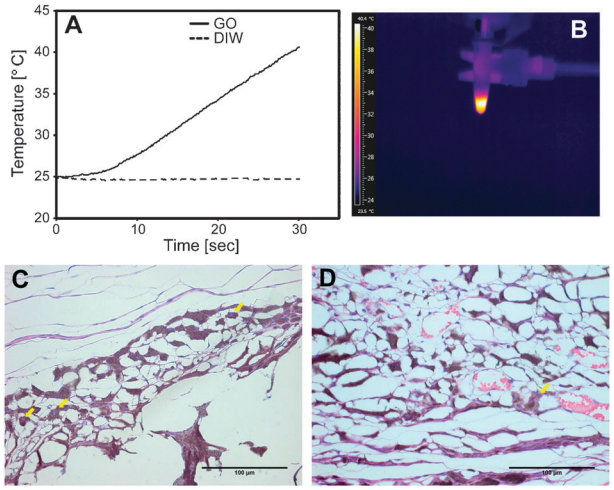

Fig. 3 Temperature increase of GO solution by NIR laser irradiation in comparison to DIW. (B) Thermal imaging of NIR laser induced temperature increase in GO–Pluronic sol. H&E staining of tissue samples collected from mice after 3 weeks (C) and 8 weeks (D) of injection of GO–F127 0.25 wt% gel. Yellow arrows point to the migrated macrophages.

FITC–Dextran also influenced the release rate, high molecular weight FITC–dextran (70 kDa) released slowly compared to the low molecular weight FITC–dextran (4 kDa). Therefore, it was evident that the release of FITC–dextran from the hydrogels was affected by diffusion and the network structure in the gel influenced the release.

This GO–Pluronic hydrogel also showed pH-responsive behaviour. When pH was higher than 8.5, no gelation occurred at 37 °C. Adding base to a preformed gel also transformed it into a sol. This process was reversible, so when pH was adjusted back to 7.0, gel formation occurred again (Fig. S6, ESI†), similar to the case of GO–PVA hydrogel.[3a] This pH dependency presumably resulted from the protonation–deprotonation of carboxylic groups (–COOH) present in GO. At higher pH, –COOH groups become deprotonated to $–COO^-$, inferring a very high negative charge on GO surface and electrostatic repulsion among GO sheets.[3a] The repulsive force seems to overcome the crosslinking effect of Pluronic and hinder the gel formation.

GO absorbs near-IR (NIR) light and can produce heat to increase the temperature of surroundings, applicable for removal of tumor from mice.[6a] Also, GO incorporated poly(*N*-isopropylacrylamide) hydrogels were reported to undergo a large volumetric change in response to NIR light.[6b,c] But, there was no report of NIR light induced sol–gel transition of GO solution. The photothermal property of GO in turn can be utilized for the light-driven, rapid formation of GO–Pluronic hydrogel. Upon irradiation of NIR laser (808 nm) for 30 s, the temperature of GO solution increased from 25 °C to 40 °C (Fig. 3A). Normally, when GO–Pluronic solution was incubated at 37 °C, it took about 5 min for the gelation. However, upon NIR light irradiation, it was possible to form a gel much rapidly (in less than 30 s). The IR imaging clearly showed that the temperature increase was localized and limited within the GO–Pluronic solution (Fig. 3B). This fast and localized increase of temperature by NIR light resulted in the rapid gelation.

There have been conflicting reports about the biocompatibility and toxicity of graphene and graphene oxide based materials. Most of the researchers have reported GO as a non-toxic material without any significant toxicity towards *in vitro* cultured cells or mice, whereas some reports showed toxic effects of GO.[7] We have examined the *in vivo* fate of our GO–Pluronic hydrogel after subcutaneous injection into mice. *In vivo* temperature-induced gelation was successfully achieved by injecting GO–Pluronic sol. H&E staining of the samples at 3 weeks (Fig. 3C) showed the migration of a few macrophages into the gel, suggesting mild chronic inflammation. However, after 8 weeks, the number of macrophages decreased in the site (Fig. 3D), indicating a time dependent healing of the early sign of inflammation. We did not notice any sign of acute inflammation, tissue necrosis, haemorrhaging or hyperaemia in the tissue samples. No noticeable degradation was observed in the gel samples 8 weeks post-injection, showing stable but non-biodegradable gel state *in vivo* (Fig. S7, ESI†). Thus, the present *in vivo* histopathological investigation suggests that GO–Pluronic hydrogels might be non-toxic and biocompatible for future *in vivo* applications.

In conclusion, we have developed a simple method to prepare a thermosensitive hydrogel system based on nano-sized graphene oxide by adding a small amount of Pluronic block copolymer as physical crosslinker without any chemical modification of GO. The sol–gel transition occurs at near body temperature and thus it can be applied as an injectable system. The strength of the gel can be tuned both by the concentration and the type of Pluronic. The photothermal effect of GO can be used to achieve the light-sensitive, rapid gelation of the system. We also demonstrated the long-lasting, stable gel formation by subcutaneous injection in mice, and the histological assay revealed that this gel does not show any severe chronic inflammatory response.

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