Preparation of water-soluble and biocompatible graphene

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Graphene as a two-dimensional material is particularly attractive because of its excellent electrical conductivity, mechanical properties, large surface area, low coefficient of thermal expansion and very high aspect ratio. However, the water insoluble property of graphene restricts its application in biomedical fields. Therefore the objective of this reported work is to find an efficient way to synthesise water-soluble and biocompatible graphene for biomedical applications. A stable aqueous graphene oxide (GO) solution was first obtained in the presence of non-ionic Pluronic copolymer. The GO-tripolymer showed good solubility in phosphate buffered saline (PBS) and the same UV absorption peaks as GO. Since a traditional reducing agent such as hydrazine had large toxicity, reduced GO (RGO) obtained by hydrazine reduction has some toxicity. In this work, a non-toxic reducing agent of ascorbic acid, galactose or bovine serum albumin was used as a RGO-tripolymer solution. The RGO-tripolymer exhibited good solubility in PBS. Finally, the cytotoxicity of RGO-tripolymers was investigated. Any RGO-tripolymer showed low cytotoxicity.

1. Introduction: Graphene as a two-dimensional material has good properties of excellent electrical conductivity, mechanical properties, large surface area, low coefficient of thermal expansion and very high aspect ratio. Hence, it attracts more and more attention in many fields including biomedical fields such as tissue engineering, drug delivery, bioimaging and biosensing [1–5]. However, the typical π-conjugated structure leads graphene to aggregate through π–π stacking in water solution [6]. Graphene will lose its superior properties once they agglomerate. As is known, human tissue contains water, which inevitably comes into contact with the materials. Therefore water-soluble and biocompatible graphene is needed in biomedical applications.

Oxidation-reduction, as one of the methods to synthesise graphene, is very popular for large yield production [2, 7–9]. In this method, graphite is first oxidated to graphene oxide (GO), which is then reduced to graphene [8]. To widen the application of graphene in biomedical fields, much effort has been made to increase the dispersion of reduced GO (RGO) in water solution. Si and Samulski [10] prepared sulphonated graphene by sulphonation of graphite oxide. Wang *et al.* [11] synthesised hydrophilic graphene by reacting GO nanosheets with poly(sodium 4-styrene sulphonate) and simultaneously reducing them through hydrazine hydrate under hydrothermal conditions. Hao *et al.* [12] used 7,7,8,8-tetracyanoquinodimethane anion to stabilise graphene and obtained prepared aqueous dispersed graphene. Although hydrophilic graphene sheets are successfully prepared via these methods, hydrophilic graphene in a physiological environment has not been investigated and its biocompatibility has not been considered. Therefore a new method of preparing water-soluble graphene for biomedical applications is still needed. Furthermore, solubility of hydrophilic graphene in a physiological environment needs further investigation.

Pluronic copolymers are amphiphilic synthetic polymers, which can stabilise hydrophobic materials in water. Moreover, they are biocompatible, low toxic and FDA approved [13, 14]. Therefore we prepared a stable aqueous GO solution in the presence of non-ionic Pluronic copolymer.

Common reductants such as hydrazine and dimethylhydrazine derivatives are highly toxic and dangerously unstable [15, 16]. Thus, the reduction processes of GO bring some danger as well as environmental problems. Even the RGO would become toxic if the reductants were removed completely. It is known that L-ascorbic acid, monosaccharide and proteins are environment-friendly agents and have certain reducibility [15–17]. Moreover, biopolymers such as bovine serum albumin (BSA), and heparin, were used to functionalise RGO and the biopolymer functionalised RGO has good cytocompatibility [18]. Therefore we reduce GO-tripolymer using environment-friendly reductants of L-ascorbic acid, galactose and BSA to obtain the water-soluble RGO-tripolymer for biomedical applications. Finally, to inspect the potential application of RGO in biomedical fields, the cytotoxicity of RGO-tripolymer was investigated in this research. At the same time, considering GO had little cell toxicity according to some references, we did not characterise the cell viability of GO-polymer when we designed our experiments [19, 20].

2. Experiment: Graphite oxide was prepared by the modified Hummer method [6]. Graphite oxide was then dispersed in water and sonicated for 10 h, into which Pluronic F-127 was added. After the mixture had been stirred for 30 min, GO-tripolymer solution was obtained. Then, GO-tripolymer was reduced by L-ascorbic acid, galactose or BSA at 90°C for 8 h before RGO-tripolymer solution was obtained. The resultant solution was dialysed in water for three days. Pure GO was reduced with the same methods. The dispersion of all GO and RGO sheets in phosphate saline buffer (PBS) were observed after they had stood for 1 h. RGO-tripolymer solutions were characterised by UV spectroscopy (UV1101M054). RGO-tripolymer solutions were dropped to the surface of a copper network, which was dried by an infrared lamp. Then, transmission electron microscopy (TEM) images were obtained using a Tecnai 12 TEM operating at 200 kV. Cell viability was measured using a cell counting kit-8 (CCK-8, Dojindo, Rockville, MD, USA). RGO-tripolymer was added into each well of a 96-well plate containing 3T3 cells. The cells were incubated in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. After 24 or 72 h exposure, the cells were washed with serum-free Dulbecco's Modified Eagle Medium (DMEM) and 10 µl of CCK-8 solution was added to each well. After 1 h incubation, the absorbance of the above solutions was measured at 450 nm.

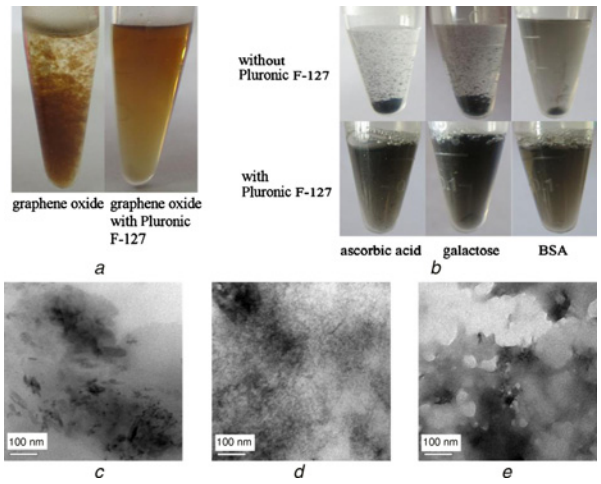

Figure 1 *Digital pictures*
a GO and GO-tripolymer suspensions in PBS
b RGO and RGO-tripolymer suspensions in PBS reduced by different reductants
TEM images
c RGO-tripolymer reduced by ascorbic acid
d RGO-tripolymer reduced by galactose
e RGO-tripolymer reduced by BSA

3. Results and discussion: GO was obtained by oxidation and ultrasonication. Although GO is highly hydrophilic and well dispersed in water, it tends to aggregate in PBS because of the screening effect of ions. To stabilise GO in PBS, non-ionic amphiphilic Pluronic F-127 was added as a surface-modifying agent. A homogeneous brown solution was obtained after being modified by Pluronic F-127 and is shown in Fig. 1*a*, which was denoted as GO-tripolymer solution. Good solubility of the graphene is defined as: the graphene can be dispersed well in water and the graphene solution is a transparent and homogeneous solution. The stabilised effect of Pluronic F-127 comes from a PEO-PPO-PEO triblock structure, in which hydrophobic PPO segments bind to the hydrophobic surface of GO via the hydrophobic effect, whereas hydrophilic PEO chains extend into water. Hence, the aggregation of GO is inhibited. Fig. 1*b* shown pictures of RGO and RGO-tripolymer suspensions in PBS reduced by ascorbic acid, galactose and BSA. From Fig. 1*b*, it is seen that all RGO and RGO-tripolymer suspensions were black, which indicated that all GOs and GO-tripolymers were successfully reduced. When unmodified GO sheets were reduced by ascorbic acid or galactose, they aggregated badly. Some aggregates were also found at the bottom of the centrifuge tube after the unmodified GO sheets had been reduced by BSA, although some part of them dispersed well in PBS. As is known, BSA itself is an amphiphilic molecule, hence it can stabilise RGO sheets in the reducing process. Hence, RGO sheets which were reduced by BSA showed better dispersivity than those which were reduced by ascorbic acid or galactose. A black homogeneous RGO-tripolymer

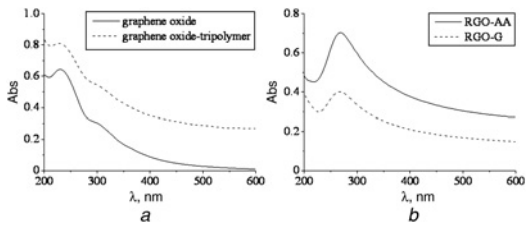

Figure 2 *UV spectra*
a GO and GO-tripolymer solutions
b RGO-tripolymer solutions

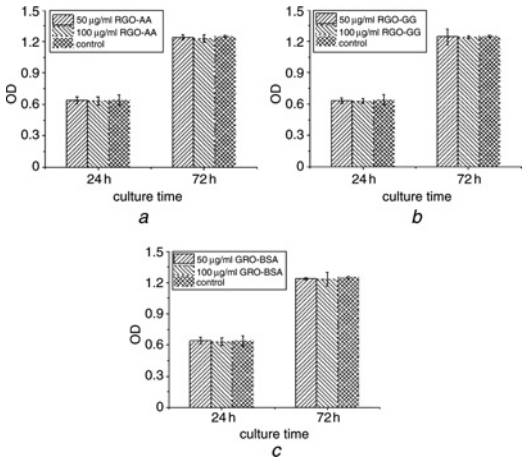

Figure 3 *Cell viability of 3T3 cells after 24 h exposure and 72 h exposure of RGO-tripolymer reduced by ascorbic acid, galactose, and BSA*
a Ascorbic acid
b Galactose
c BSA

solution in PBS was obtained after the GO-tripolymer was reduced by ascorbic acid, galactose or BSA. However, little aggregates were also found in the RGO-tripolymer solutions which were reduced by ascorbic acid or galactose. TEM images of RGO-tripolymer reduced by ascorbic acid (Fig. 1*c*), galactose (Fig. 1*d*) and BSA (Fig. 1*e*) depicted flake-like transparent sheets. From Fig. 1*c*, little small aggregates scattered in the image, which was consistent with the result of Fig. 1*b*.

Fig. 2*a* shows the UV–vis absorbance spectra of GO and GO-tripolymer solution. GO showed a strong absorption peak at 230 nm belonging to the π–π^* of the C=C plasmon peak and a shoulder peak about 300 nm belonging to n–π^* transitions of the carbonyl groups, which indicated that GO was successfully prepared [15]. GO-tripolymer showed nearly the same peaks as GO, which suggested that Pluronic F-127 modification did not bring any transition of functional groups such as C=C and carbonyl groups in the GO sheets. The peak at 230 nm disappeared and a new peak around 265 nm emerged in the UV spectra of the RGO-tripolymers (Fig. 2*b*), which suggested that sp^2 carbon was restored and atoms were possibly rearranged within RGO-tripolymers [16]. The results implied that GO-tripolymer could be reduced and the aromatic structure could be restored. However, as for RGO-tripolymer reduced by BSA, UV spectroscopy could not characterise the biggest absorption of graphene because of the interference of BSA, which had strong absorption peaks at 215 and 280 nm. This was because the molecular weight of BSA was so large that BSA could not be removed by dialysis.

To explore further the cytotoxicity of RGO-tripolymer with different concentrations, the WST-8 assay was employed. In this case, the cytotoxicity of RGO-tripolymer on the mitochondrial activity of cells was investigated, see Fig. 3. In Fig. 3*a*, the cell viability with 50 or 100 μg RGO-tripolymer was nearly the same as that without RGO-tripolymer after cells had been cultured for 24 h. When the culture time was prolonged, the cell viability increased and no dose-dependent effects on RGO-tripolymer were detected. The cytotoxicity of RGO-tripolymer reduced by galactose (Fig. 3*b*) or BSA (Fig. 3*c*) showed the same trends as Fig. 3*a*. These results indicated that the cells were not influenced by any RGO-tripolymer at any of the employed concentrations.

4. Conclusion: Pluronic F-127 functionalised GO (GO-tripolymer) was successfully prepared to improve the solubility of GO in PBS. GO-tripolymer showed the same UV absorption peaks as GO. GO-tripolymer can be reduced by ascorbic acid, galactose or

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BSA. The RGO-tripolymers excited good solubility in PBS. RGO-tripolymer reduced by ascorbic acid or by galactose showed a strong UV absorption peak about 265. Any RGO-tripolymer showed low cytotoxicity. In conclusion, the prepared RGO-tripolymers had good solubility in a physiological environment and no cytotoxicity.

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6 References

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