Biomed Eng Lett (2016) 6:10-15
DOI 10.1007/s13534-016-0214-6

REVIEW ARTICLE

Graphene and Thermo-responsive Polymeric Nanocomposites for Therapeutic Applications

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Received: 14 December 2015 / Revised: 27 January 2016 / Accepted: 10 February 2016

Abstract
Functional nanomaterials are of great benefit for various therapeutic applications. Recently, the advanced emerging nanotechnology enables the synthesis of drug-loaded multi-functional graphene and thermo-responsive polymeric nanomaterials. Given the physical and biochemical properties of multi-functional graphene and thermo-responsive polymeric nanomaterials, they hold the powerful potential for therapeutic applications. In this paper, we review various graphene and thermo-responsive poly(*N*-isopropylacrylamide) (PNIPAM) nanocomposites and highlight their therapeutic applications.

Keywords Graphene, Thermo-responsive polymer, Nanocomposite, Therapy

INTRODUCTION

The emerging nanotechnology is of tremendous potential to embody the conceptual designs of nanomaterials for various theranostic applications [1-3]. In particular, the nanotechnology offers great opportunity to synthesize multi-functional nanocomposites that can enhance the capability of imaging, diagnosis, and therapy [4]. In this review, we focus on development of graphene and poly(*N*-isopropylacrylamide) (PNIPAM) nanocomposites for various therapeutic applications. Graphene, a two-dimensional (2D) monolayer containing honeycomb lattice structures of carbon atoms, has widely been employed for various electronic, photonic, and energy harvest applications, because it shows excellent physical and chemical properties (e.g., high electrical conductivity, thermal conductivity, and mechanical strength) [5]. In particular, graphene is of great potential for drug delivery applications due to large surface area and easy chemical functionalization [6]. Despite its great prospective for biological applications, the major limitation of graphene is poor solubility in aqueous solution due to its hydrophobicity. In contrast, graphene oxide, the oxidized form of graphene, can be soluble in aqueous solution. The graphene oxide is also an insulating material, because the oxygen functional group of graphene oxide breaks sp^2 bonding networks. To improve the electrical property of graphene oxide, the chemical reduction treatment is required [7]. There are a number of chemical reduction treatment methods (e.g., hydrazine [8], hydroxylamine [9]) of graphene oxide. Although these chemical reduction treatments enable the increase of electrical conductivity [10], the chemical agents are toxic and explosive. Thus, the non-toxic method with high electrical conductivity needs to be developed. Recently, L-ascorbic acid has been employed for reduction of graphene oxide sheets [11]. The alkaline solution (e.g., sodium hydroxide) has also been used for rapid deoxygenation of exfoliated graphite oxide [12]. It demonstrated that the reduction speed of dispersed graphite oxide was proportional to concentrations of alkali solutions. Although the chemical reduction treatments of graphene oxide enhance the electrical conductivity and dispersion, the oxygen functional groups are not completely removed from surfaces of graphene oxides [13]. To efficiently remove the impurities on the graphene oxide surface, the chemical reduction treatment has combined with thermal annealing method [14]. Recently, stimuli-responsive smart nanomaterials have been synthesized for theranostic applications, such as cellular imaging, drug delivery, and tumor therapy [15, 16]. In particular, thermal- and pH-responsive polymers have

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employed for biological applications [17]. PNIPAM, one of thermo-responsive smart polymers, hold excellent properties, such as lower critical solution temperature (LCST) and phase transition. PNIPAM is generally hydrophilic below LCST, whereas it undergoes phase transition into hydrophobic above LCST [18, 19]. The hydrophilic monomers enable the increase of LCST in PNIPAM. The hydrophilic PNIPAM becomes hydrophobic state in hyperthermic tumor tissues, showing high efficiency of cellular uptake and controlled drug release. Given this thermo-responsive property, PNIPAM polymers are of great potential for tumor targeting and therapy applications.

SYNTHESIS OF GRAPHENE-BASED PNIPAM NANOCOMPOSITES

To synthesize graphene-based PNIPAM nanocomposites, a number of methods (e.g., covalent bonding, frontal polymerization, *in situ* free-radical polymerization, and click chemistry) have previously been explored [20-23]. The major problem of the graphene is still poor dispersion. To overcome this limitation, PNIPAM can be incorporated into graphene to make graphene oxide-based PNIPAM nanocomposites. The graphene oxide-based PNIPAM nanocomposite shows the dispersion and thermo-responsive properties. The dispersion property of graphene oxide-based PNIPAM nanocomposites is of great importance for drug delivery and controlled release applications. The thermal- and pH-responsive graphene oxide interpenetrating PNIPAM hydrogel networks have been synthesized by covalent bonding between graphene oxide and PNIPAM [20]. The cross-linking reaction enabled the increase of the mechanical strength in graphene oxide interpenetrating PNIPAM-based hydrogel networks, showing that the graphene oxide was dispersed within PNIPAM hydrogels in a homogeneous manner. The graphene oxide interpenetrating PNIPAM-based hydrogel networks showed pH-sensitive property due to their residual carboxyl groups. The graphene-based PNIPAM nanocomposite hydrogels have been synthesized by frontal polymerization [21]. It demonstrated that the graphene enabled the increase of the swelling ratio [21] and mechanical property of nanocomposites

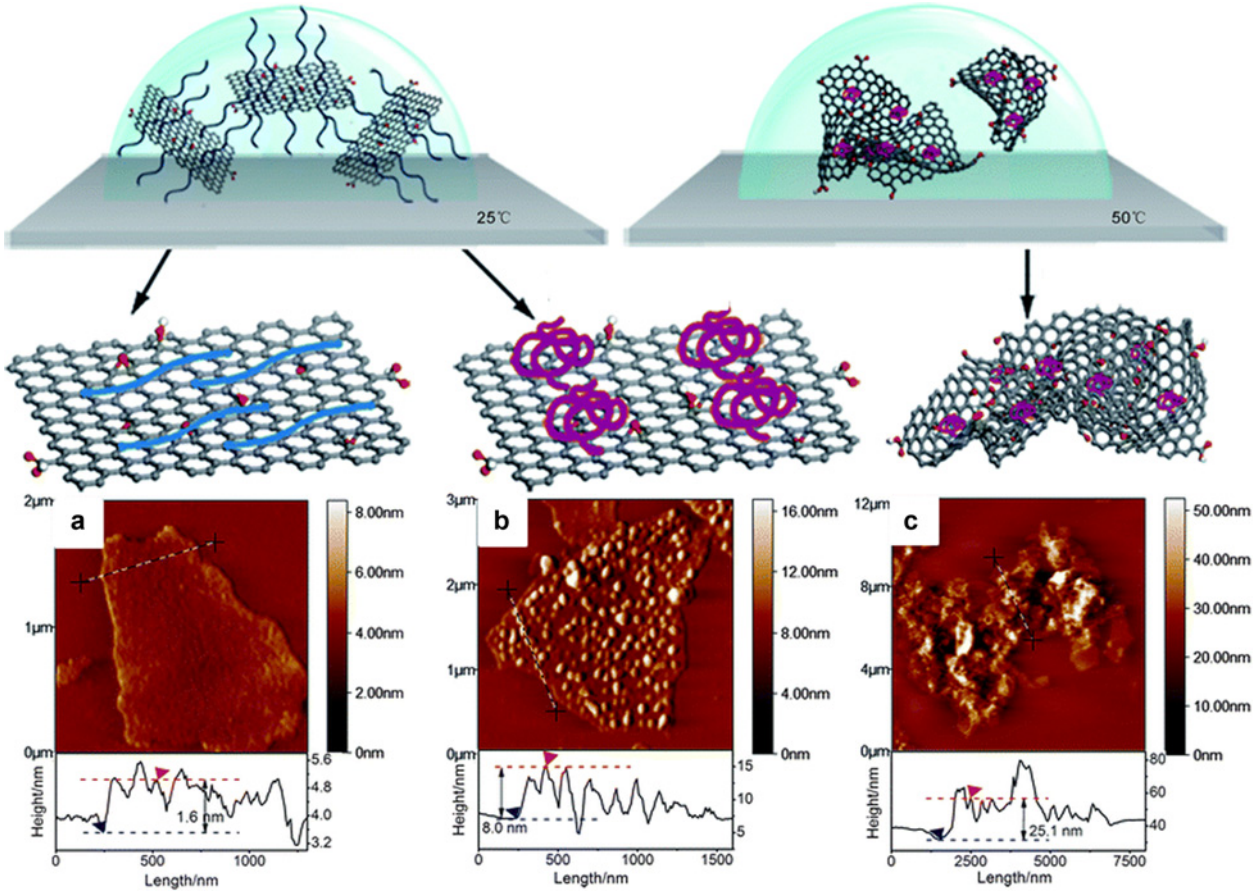


Fig. 1. AFM image analysis of a droplet of graphene oxide-based PNIPAM nanocomposite suspension at different conditions: (a) fully dried at 25°C, (b) partially dried at 25°C and heated at 50°C, and (c) heated and fully dried at 50°C. (reprinted with permission from ref. 23, copyright 2012 Royal Society of Chemistry).

[22]. In contrast, it confirmed that the graphene did not affect LCST of PNIPAM. *In situ* free-radical polymerization has been employed to synthesize graphene oxide-based PNIPAM hybrid nanocomposites [23]. It showed that temperature enabled the control of structural reorganization from random-coil to globule of graphene oxide-based PNIPAM hybrid nanocomposites. Atomic force microscopy (AFM) image analysis showed that the structural transform of graphene oxide-based PNIPAM was affected by water content of graphene oxide-based PNIPAM nanocomposites (Fig. 1) [23]. PNIPAM showed linear structure, as the graphene oxide-based PNIPAM solution was dried under vacuum at 25°C (Fig. 1a). When graphene oxide-based PNIPAM solution was partially dried under vacuum at 25°C and was subsequently heated at 50°C, PNIPAM on graphene oxide surfaces could transform into globule structure due to hydrogen bonding between C=O and N–H groups of PNIPAM (Fig. 1b). It resulted in phase change of PNIPAM from hydrophilic to hydrophobic state. In contrast, graphene oxide-based PNIPAM allowed for crumpling structures containing macroscopic aggregation, as graphene oxide-based PNIPAM solution was heated and was subsequently dried at 50°C (Fig. 1c). This process could reduce the interfacial surface energy between graphene oxide and water. The wettability of graphene oxide-based PNIPAM nanocomposite film was controlled by reversible switching of near-infrared (NIR) laser irradiation, showing that the contact angle of graphene oxide-based PNIPAM hybrid nanocomposite films exposed to a NIR laser irradiation increased to become hydrophobic surfaces. Furthermore, PNIPAM-grafted graphene sheets have been synthesized via click chemistry [24]. The amide linkage-mediated alkyne groups were used on graphene oxide surfaces. It demonstrated that PNIPAM-grafted graphene sheets showed non-toxic, high solubility, and stability in aqueous solutions to maintain the long-term dispersion. The camptothecin anticancer drug was highly loaded in graphene sheet conjugated with PNIPAM, because of π-π stacking and hydrophobic interactions. Although the dispersion of graphene oxide-based PNIPAM nanocomposite is stable in water, the graphene oxide is not completely dissolved in phosphate-buffered saline (PBS). In contrast, graphene oxide-based PNIPAM nanocomposites are dissolved in both water and PBS solutions due to high solubility [24].

THERAPEUTIC APPLICATIONS OF NANOCOMPOSITES

The most challenging requisite of graphene and graphene oxide for clinical applications is the biosafety and biocompatibility [25-27]. However, the issues about the toxicity and biocompatibility of graphene-based materials have not been completely resolved [28]. Although many previous reports have indicated the promise of graphene-based material as a drug delivery carrier, toxicity issues are still conflicting [29]. To improve biocompatibility, gelatin-functionalized graphene nanosheets have previously been developed [30]. This gelatin–graphene nanosheet showed high solubility, stability, and drug-loading efficiency. It demonstrated that gelatin enabled the control of the aggregation of graphene nanosheets and allowed for controlled release of anticancer drugs. To improve the solubility and biocompatibility of graphene-based materials, the surface functionalization is generally performed by two methods, such as covalent bonding and non-covalent bonding (e.g., π-π stacking interaction) [31]. The organic functionalization of graphene-based materials is conducted by covalent bonding between free radicals and C=C bonds of graphene or between oxygen groups of graphene oxide and organic functional group [30]. The folic acid-conjugated graphene oxide has been employed to load doxorubicin via strong π-π stacking interaction of non-covalent bonding [32]. This π-π stacking interaction allowed for high drug loading efficiency and controlled release of anticancer drugs [32]. Furthermore, thermo-responsive PNIPAM-based nanocomposites have previously been used for tumor apoptosis and stem cell differentiation applications [33, 34]. Breast cancer cells were cultured with doxorubicin-loaded PNIPAM-*co*-acrylic acid nanocomposites (Fig. 2a) [33]. Cancer spheroids were spontaneously generated in microwells containing different diameters (e.g., 300-700 μm). Doxorubicin anticancer drug was released from PNIPAM-*co*-acrylic acid nanocomposites at 37°C due to size shrinkage of nanocomposites and phase transition from hydrophilic to hydrophobic. It demonstrated that the smallest cancer spheroids treated with doxorubicin-loaded PNIPAM-*co*-acrylic acid nanomaterials were severely disrupted (Fig. 2b). In contrast, the largest cancer spheroids almost maintained their spheroid size for 96 h, because they showed strong cell-cell interaction and tight junction. Another study represented that retinoic acid-loaded PNIPAM-*co*-acrylamide nanocomposites has been employed to direct human induced pluripotent stem cell (iPSC)-derived neuronal differentiation (Figs. 3a-3d) [34]. It demonstrated that the human iPSC treated with 1-2 μg/mL retinoic acid-loaded PNIPAM-*co*-acrylamide nanocomposites was highly viable and significantly enhanced to Tuj1-positive neuronal differentiation (Figs. 3e-3g). This study could be potentially powerful tool for future clinical treatment of neuronal disorder diseases.

Graphene-based PNIPAM nanocomposites are of great interesting nanocarrier for applications of NIR laser irradiation-mediated drug delivery and controlled release. Compare to other external stimuli (e.g., pH, magnetic field, and ultrasound), NIR laser (808 nm wavelength) irradiation-mediated imaging and therapy showed various advantages due to its

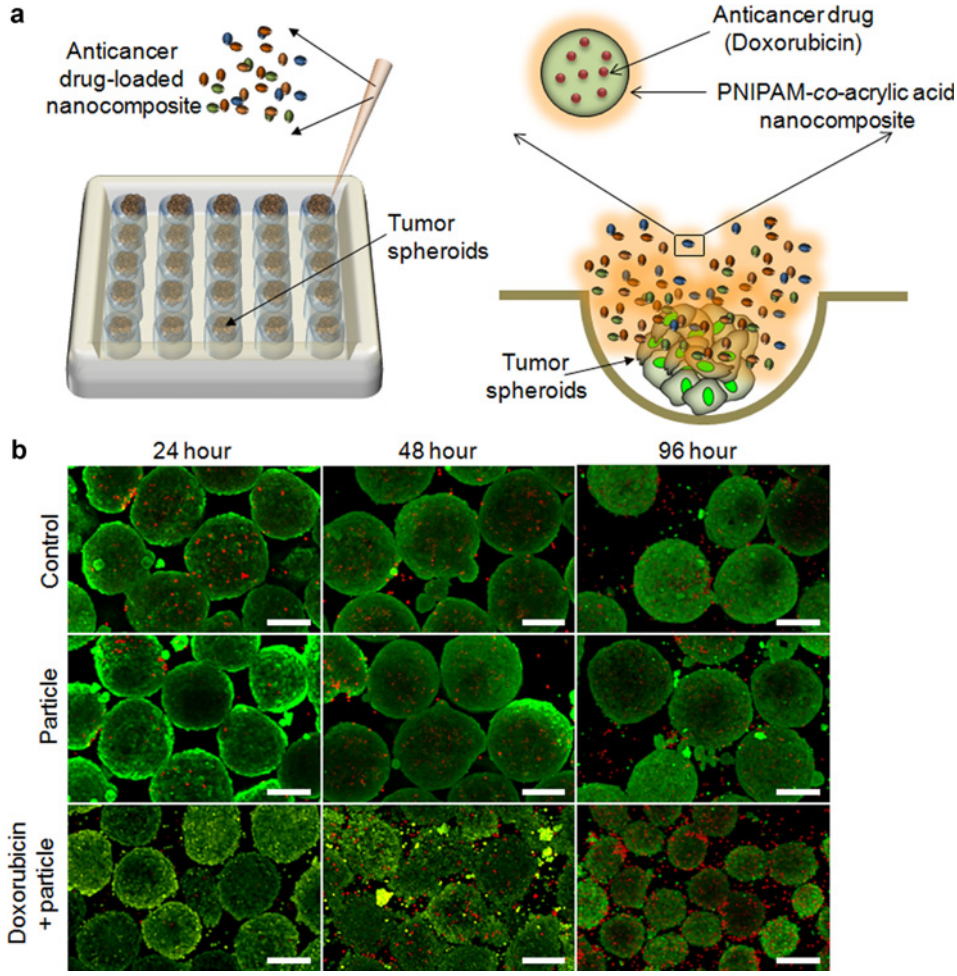
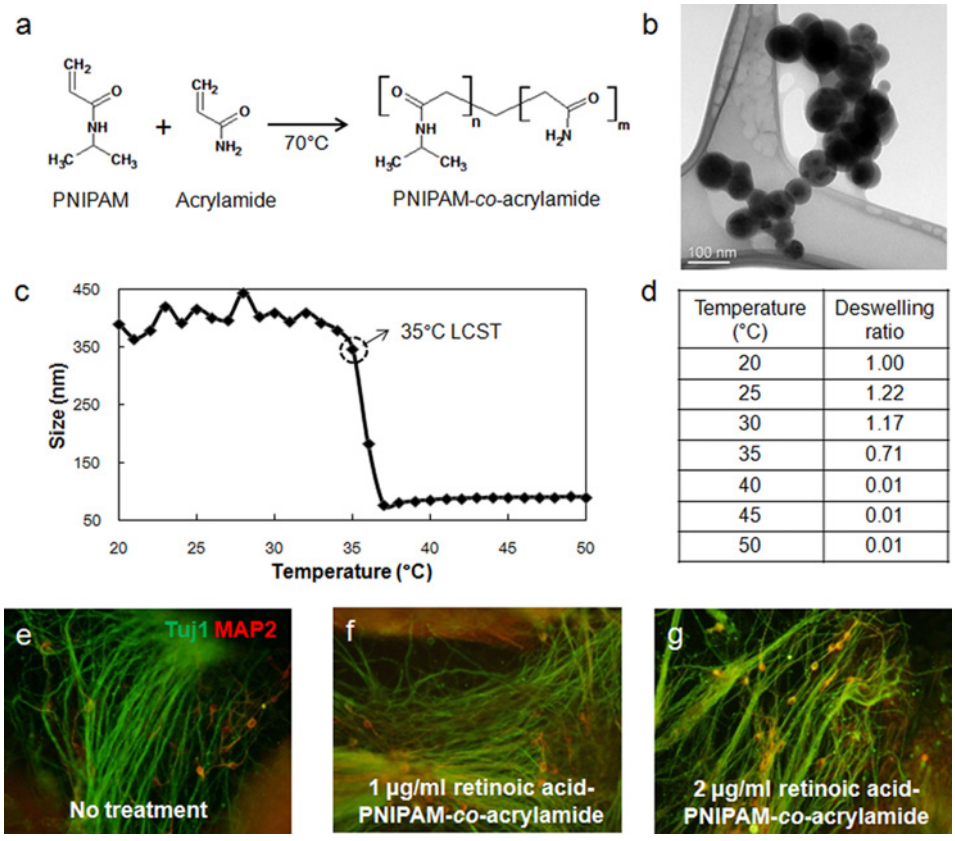


Fig. 2. PNIPAM nanocomposite-mediated apoptosis of cancer spheroids. (a) Cancer spheroid cultured with anticancer drug-loaded PNIPAM-*co*-acrylic acid nanocomposites in a microwell. (b) The viability of cancer spheroid cultured with anticancer drug-loaded PNIPAM-*co*-acrylic acid nanocomposites. (reprinted with permission from ref. 33, copyright 2015 Elsevier).

biocompatibility and deep tissue penetration (e.g., NIR light travels at least 10 cm through breast tissue) [35, 36]. The photo- and thermo-responsive composites containing reduced graphene oxide nanoparticles and PNIPAM hydrogels has previously been generated [37]. The photo-sensitive property of composites was significantly affected by high optical absorbance and thermal conduction of reduced graphene oxide. The concentrations of reduced graphene oxide enabled the control of photo- and thermo-responsive properties. The reduced graphene oxide-based PNIPAM composite was largely affected by temperature as small contents of reduced graphene oxide were used. In contrast, the thermal treatment did not significantly influence on composites with large contents of reduced graphene oxide, suggesting that this photo- and thermo-responsive property of reduced graphene oxide-based PNIPAM composite could be useful for drug release in a controlled manner. The reduced graphene oxide nanosheet-based PNIPAM nanocomposite with mesoporous silica shell has also been fabricated to enhance loading capacity of anticancer drug [38]. It demonstrated that reduced graphene oxide converted NIR laser irradiation into heat energy for rapidly enhancing the diffusion of anticancer drugs, showing hyperthermia-based synergistic effects of chemo-photothermal therapy. It is probably because the reduction of graphene oxide not only restores the electro-conductivity of graphene oxide but also improves optical absorbance at NIR wavelength [39]. The combined treatment of chemotherapy and NIR-mediated photo-thermal therapy could minimize several damages, such as drug side effect and non-specific drug delivery [40]. Therefore, these graphene-based PNIPAM nanocomposites could be powerful tools for chemo-photothermal therapy applications of tumors.

CONCLUSIONS

The graphene has great potential for photo-sensitive effect due to its high optical absorbance. PNIPAM biomaterials also



Temperature (°C)	Deswelling ratio
20	1.00
25	1.22
30	1.17
35	0.71
40	0.01
45	0.01
50	0.01

Fig. 3. PNIPAM nanocomposite-mediated human iPSC differentiation. (a) Polymeric nanocomposites containing PNIPAM and acrylamide. (b) TEM image of PNIPAM-*co*-acrylamide nanocomposites. (c-d) Size distribution and deswelling ratio of PNIPAM-*co*-acrylamide nanocomposites. (e-g) Human iPSC-derived neuronal differentiation using retinoic acid-loaded PNIPAM-*co*-acrylamide nanocomposites. (reprinted with permission from ref. 34, copyright 2015 Elsevier).

enable the control of thermo-responsive structural change and phase transition, showing controlled release of anticancer drugs. The graphene-based PNIPAM nanocomposites have recently developed to enhance anticancer drug loading efficiency for photo-thermal therapy applications. In this paper, we discuss various graphene-based PNIPAM nanocomposites and their photo-thermal therapy applications. Therefore, the graphene-based PNIPAM nanocomposite could be a powerful carrier for therapy and diagnosis.

ACKNOWLEDGEMENTS

This research was supported by BioNano Health-Guard Research Center funded by the Ministry of Science, ICT & Future Planning (MSIP) of Korea as Global Frontier Project (Grant number H-GUARD_2014M3A6B2060503), Republic of Korea. This work was supported by Leading Foreign Research Institute Recruitment Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (MSIP) (2013K1A4A3055268). This work was also supported by Sogang University Research Grant of 2014 (Grant number 201410005.01).

CONFLICT OF INTEREST STATEMENTS

Seo HI declares that she has no conflict of interest in relation to the work in this article. Cheon YA declares that she has no conflict of interest in relation to the work in this article. Chung BG declares that he has no conflict of interest in relation to the work in this article.

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