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Hemorheological characteristics of red blood cells exposed to surface functionalized graphene quantum dots

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ABSTRACT

Graphene quantum dots (GQDs) are potential candidates for various biomedical applications such as drug delivery, bioimaging, cell labeling, and biosensors. However, toxicological information on their effects on red blood cells (RBCs) and the mechanisms involved remain unexplored. To the best of our knowledge, our study is the first to investigate the toxicity effects of three GQDs with different surface functionalizations on the hemorheological characteristics of human RBCs, including hemolysis, deformability, aggregation, and morphological changes. RBCs were exposed to three different forms of GQDs (non-functionalized, hydroxylated, and carboxylated GQDs) at various concentrations (0, 500, 750, and 1000 μg/mL) and incubation times (0, 1, 2, 3, or 4 h). The rheological characteristics of the RBCs were measured using microfluidic-laser diffractometry and aggregometry. Overall, the hemolysis rate and rheological alterations of the RBCs were insignificant at a concentration less than 500 μg/mL. Carboxylated GQDs were observed to have more substantial hemolytic activity and caused abrupt changes in the deformability and aggregation of the RBCs than the non-functionalized or hydroxylated GQDs at concentrations >750 μg/mL. Our findings indicate that hemorheological assessments could be utilized to estimate the degree of toxicity to cells and to obtain useful information on safety sheets for nanomaterials.

1. Introduction

Graphene quantum dots (GQDs) have drawn a lot of research interest in the biomedical fields owing to their superiority over conventional semiconductor-based quantum dots; their advantages include low cost, high chemical stability, high photostability, low toxicity, and excellent biocompatibility (Geim, 2009; Shen et al., 2012; Wen et al., 2015). GQDs have been investigated for many different applications such as photovoltaic devices (Gupta et al., 2011), biosensing (Ran et al., 2013), cellular imaging (Sun et al., 2015), drug delivery, and cancer therapy (Ge et al., 2015).

For using GQDs in biomedical applications, the main criteria are their toxicity profiles. Literature reviews have reported that nano-sized GQDs could be internalized into cells quicker than micro-sized graphene oxide nanomaterials. Nurunnabi et al. showed that the GQDs showed no acute toxicity and did not cause any morphological changes in different types of cancer cells and their in vivo study revealed that the GQDs did not cause significant toxicity to the treated animals (Nurunnabi et al., 2013c). However, it should be noted that most of the reported cytotoxicity studies are based on MTT assays, and very few systematic cytotoxicity evaluations have been conducted for assessing the biocompatibility of GQDs. Xu and his group reported that different types of GQD internalizations did not induce significant cytotoxicity, as proved by the high viability, low LDH release, low internal cellular ROS levels,

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and negligible amount of cell apoptosis (Jiang et al., 2015). In addition, extended use of GQD-based nanotechnology has resulted in concerns regarding the risk of overexposure in humans (Yokel and MacPhail, 2011). In fact, a recent epidemiological study showed a high correlation between exposure to nanoparticles and the incidence of life threatening cardiovascular events (Buzea et al., 2007). Blood, which directly or indirectly encounters any hazardous materials, is adept to transport foreign substances to cells, tissues, and organs. In this case, it is very important to study the toxicity of GQDs on blood, particularly erythrocytes (Li et al., 2008). Accidental exposure by different routes of administration can potentially lead to the translocation of nanomaterials into the bloodstream (Smock et al., 2014). In particular, many of the proposed biomedical applications require direct injection of nanomaterials through different routes of administration, which will most probably also lead to direct contact of the nanomaterials with blood components. Therefore, evaluation of GQD biocompatibility with blood is a necessary part of early development of nanotechnology-derived medical devices and drug carriers.

Several studies have been reported on the toxicity of graphene on red blood cells (RBCs) by measuring hemolysis, but the deformability and aggregation of RBCs has not been studied. Hemolysis (or haemolysis) is defined as the rupture of red blood cells which occurs due to cell membrane damage, resulting in the release of hemoglobin. Deformability is defined as the ability of RBCs to change shape in response to an external force, without rupturing. When human RBCs undergo large deformations under external stresses, they can pass through capillaries that are narrower than the diameter of a resting RBC (Kim et al., 2015). RBCs under low shear rate or at stasis have a unique phenomenon that forms rouleaux formation similar to a stack of coins. RBC aggregation is the main determinant of blood viscosity and sedimentation rate at low shear rate (Shin et al., 2009). Cai et al. (Cai et al., 2015) improved the hemolytic effect of graphene oxide nanosheets on RBCs by grafting bovine serum albumin (GO-g-BSA), where the whole blood was collected from a healthy rabbit. The BSA-modified GO-treated RBCs had typical biconcave shapes, while most of the GO-treated RBCs showed significant alterations in their morphologies, such as echinocytic or acanthocytic shapes. Chowdhury et al. (Chowdhury et al., 2013) also investigated the morphology and hemolysis of whole human blood after treatment with dextran-functionalized graphene nanoplatelets (GNP-Dex), which results from reactive oxygen species (ROS) generation. Liao et al. (Liao et al., 2011) evaluated the hemolysis of human RBCs incubated with various concentrations of graphene oxide (GO) and graphene sheets (GS). Their results showed that GO, with the smallest size is higher hemolyitic than aggregated graphene sheets. However, GO coated with chitosan showed significantly the lowest hemolytic activity. These results suggest that particle size, particulate state, and oxygen content of graphene are main contributors to hemorheological responses to RBCs.

The purpose of our study was to investigate the hemorheological alterations in human RBCs exposed to various concentrations of three GQDs (non-functionalized, hydroxylated, and carboxylated) with varying exposure times. We experimentally measured the hemolysis, deformability, and aggregation of the RBCs exposed to GQDs and observed the RBC shapes using a scanning electron microscope. The novelty of this study lies in the introduction of a hemorheological approach to evaluate GQD toxicity on RBCs. To the best of our knowledge, there are no previous quantitative studies on GQD toxicity on RBCs using hemorheological methods. Our study shows that toxicity evaluations of various nanomaterials can be performed by investigating the hemorheological properties of RBCs exposed to the said nanomaterials.

2. Materials and methods

2.1. Preparation of GQDs

2.1.1. Synthesis of non-functionalized GQDs

The non-functionalized GQDs were synthesized as described previously with some modifications (Nurunnabi et al., 2013a). First, 100 mg of carbon fiber was dissolved in 40 mL mixture of acid solutions (sulfuric acid: nitric acid = 3:1). The carbon fiber-containing acid solution was then subjected to bath sonication for 1 h, followed by ultrasonication for 10 min. The mixture was then placed in a 3-neck round bottom flask, which was placed on a heating mantle at 90 °C with stirring for 12 h; this was followed by exposure to N_2 gas. After completion of the reaction, the required amount of distilled water was added. Sodium hydroxide and sodium carbonate were added in the specified amounts to make it neutralized (pH 7). The solution was slowly stirred at 0–4 °C for a certain period to remove the precipitated salts. Non-functionalized GQDs were then collected by decantation and freeze-dried for 48 h.

2.1.2. Synthesis of hydroxylated GQDs

We prepared the hydroxylated GQDs using a previous method with some modifications (Nurunnabi et al., 2013b). First, we prepared GQDs through exfoliation of carbon fibers in a strong acidic mixture for 12 h at 95 ± 5 °C. Simultaneously, polydopamine was formed from dopamine hydrochloride through vigorous stirring at pH 8.5 for 3 h. Next, specific amounts of both GQDs and polydopamine were dissolved in PBS separately and mixed together properly. The mixture was then vortexed for 10 min, followed by incubation for 1 h at room temperature.

2.1.3. Synthesis of carboxylated GQDs

The carboxylated GQDs were synthesized from carbon fiber as described in a previous report (Nurunnabi et al., 2013c). In brief, 100 g of carbon fiber was added to 40 mL of sulfuric acid, and the solution was sonicated for 1 h at room temperature. The solution was then injected slowly into a mixture of nitric acid (20 mL) and sulfuric acid (450 mL) at 95 °C. The reaction was carried out for 12 h to exfoliate carbon fiber into monolayer graphene to make GQDs. To introduce carboxyl groups on the surface of the GQDs, citric acid was added, and stirring was continued for 1 h at 60 °C. After completion of the reaction, an excess amount of water was added to the solution. Sodium hydroxide and sodium carbonate were then added to the solution to bring the pH to 8. Next, the solution was placed in an ice bath, and the temperature was maintained at 0–4 °C with slow stirring. The precipitate was then separated from the solution by decantation. The final product was freeze-dried for 3 days to obtain a fine powder.

2.2. Characterization

The UV absorbance of the GQDs with different functional groups was measured by UV spectroscopy (Mecasys. Co. Ltd, South Korea) using a 1 cm-wide quartz cuvette. The fluorescence intensity of the GQDs with the different functional groups was measured by photoluminescence spectroscopy (Sinco, South Korea). The hydrodynamic size of the GQDs was measured by Dynamic light scattering (DLS) at a fixed scattering angle of 175° at 25 °C using a Malvern Instruments Zetasizer Nano S 90 (ZEN1690) (UK) equipped with a 633 nm He-Ne gas laser.

2.3. Preparation of blood samples and GQD toxicity assessment

Human venous blood was drawn from three healthy male donors using 21G butterfly infusion into a Vacutainer® (BD, Franklin