Facile One-Pot Green Synthesis and Antibacterial Activities of GO/Ag Nanocomposites

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Abstract New materials with good antibacterial activity synthesized by simple, environmentally friendly method have attracted numerous research interests. Taking advantage of Ag and graphene oxide (GO), the GO/Ag nanocomposites were prepared by a facile, green “one-pot” reaction to achieve superior antibacterial properties. AgNO₃ is as a precursor of Ag nanoparticles (Ag NPs), and the grape seeds extract as an environmentally friendly reducing and stabilizing agent. The as-prepared GO/Ag nanocomposites were characterized by various technologies. The results indicated that the silver ion was reduced by the grape seeds extract and Ag NPs were decorated on the surface of GO nanosheets successfully. The average size of the Ag NPs anchored on the GO surface was 40–50 nm. In this study, we prepared GO/Ag nanocomposites with different Ag NPs to GO ratios and carefully investigated their antibacterial activities. We found that the GO/Ag nanocomposites show better antibacterial activity when the concentration of AgNO₃ is 2 mM. GO/Ag nanocomposites have a minimum inhibitory concentration 23.8 µg/mL, lower than bare Ag NPs 25.5 µg/mL. We try to explain the antibacterial mechanism based on optical microscopy observation.

KEY WORDS: GO/Ag nanocomposites; Green “one-pot” reaction; Superior antibacterial properties

1 Introduction

Graphene is a new carbon material with a special two-dimensional (2D) hexagonal lattice structure of *sp*²-hybridized carbon atoms [1–3]. Since its discovery, the study

on it has increased exponentially. Graphene oxide (GO) is a graphene-related material with a range of reactive oxygen functional groups on its basal plane, which can be chemically exfoliated from graphite oxide (GtO) [4, 5]. GO has attracted a great deal of attention, not only because it has a unique two-dimensional (2D) hexagonal lattice structure, but also it has excellent water dispersity, chemical stability and mechanical properties [6–11]. It holds great promise in many exciting applications, such as conductive thin film, supercapacitors, drug delivery, biochemical sensing and cell imaging [12–16].

As a nanomaterial, what attracts us is that the excellent antibacterial activity of GO firstly found by Hu et al. [17]. Since then, the antibacterial activity of GO was widely studied by many researchers [18–22]. Most groups indicated that GO has better antimicrobial effect than reduced GO (rGO) and the antibacterial mechanism from both the experimental and theoretical point of view has been explained. The antibacterial activity of GO is found to relate

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to destructive extraction of phospholipids from bacteria membranes, membrane puncture and oxidative stress [17–19, 22]. However, some groups such as Ruiz *et al.* put forward opposite views and their study indicated that GO films could not produce antibacterial properties and failed to produce growth inhibition zones around bacteria. Despite these conflicting reports about the antimicrobial activity of GO, GO can act as a nanoscale support to deposit Ag NPs, which is a widely used antibacterial nanomaterial without drug resistance [23, 24]. Ag NPs deposited on GO nanosheets can be synthesized by chemical reduction in AgNO_3 using different reducing agents, which have been reported by many groups due to the good dispersibility and stability of deposited Ag NPs [25–31]. GO has a lot of oxygen functional groups ($-\text{OH}$, $-\text{COOH}$, $-\text{C}=\text{O}$) on its basal plane [4, 5]. At the same time, some literature reported that cellulose nanocrystals (CNC) could be prepared by acid hydrolysis of dissolving pulp or cotton. The process can make the surface of CNC attach a large number of reducing groups hydroxyl group ($-\text{OH}$) and other groups, showing good dispersibility, stability and reducibility in a diluted system [32–35]. This is similar to the oxygen functional groups of GO. Therefore, CNC were often used to prepare CNC/Ag nanocomposites not only due to its good dispersing ability and stability but also its surface reducing groups, which exhibited excellent antibacterial activities [36–38]. However, the use of chemical agent is still harmful to the environment and human being. In our previous study, grape seeds extract, a food waste, can be used as reducing reagent and stabilizing reagent to synthesize Ag NPs with high stability [39]. Inspired by the results above, we synthesized GO/Ag nanocomposites by a simple, green and facile method using grape seeds extract as synergistic reducing agent in this work and discussed the antibacterial activity against four kinds of clinical pathogenic bacteria and four kinds of aquatic pathogenic bacteria in detail.

2 Experimental

All chemicals, including graphite powder, silver nitrate (AgNO_3), potassium permanganate (KMnO_4), potassium perchlorate (KClO_4), sodium nitrate (NaNO_3), sulfuric acid (H_2SO_4 , 98%) and hydrochloric acid (HCl , 37%), were purchased from China National Pharmaceutical Group Corporation. All chemicals were of analytical grade and used as received. All solutions were prepared and diluted using ultrapure water ($18.2 \text{ M}\Omega \text{ cm}$).

2.1 Preparation of GO and GO/Ag Nanocomposites

GO was synthesized by the modified Hummers' method from graphite according to previous work [40]. Grape

seeds extract was obtained according to our previous report [39]. For the GO/Ag nanocomposites, in a typical assay, 260 μL of GO suspension (1.4 mg/mL) was added into different concentrations of AgNO_3 solution (1, 2, 3 mM) in a 50-mL round-bottom flask. Then, 1 mL of grade seeds extract was added to get a final volume of 20 mL and the mixture was heated at 95 $^\circ\text{C}$ for 20 min. Finally, the as-prepared nanocomposites were stored at 4 $^\circ\text{C}$ for further use.

2.2 Instrumentation

The morphology and microstructure of the nanocomposites were characterized by transmission electron microscopy (TEM, JEM-1230) at an acceleration voltage of 100 kV. The crystallographic structural analysis was carried out by using an X-ray diffractometer (XRD) (Rigaku D/max-2500VPC) with $\text{CuK}\alpha$ radiation from 5 $^\circ$ to 80 $^\circ$ at a scanning rate of 0.02 $^\circ \text{ s}^{-1}$. Ultraviolet–visible (UV–Vis) spectra were recorded over 200–800 nm using a Shimadzu UV-2550 UV–Vis spectrophotometer. The Fourier transform infrared (FT-IR) spectra were recorded over 4000–400 cm^{-1} using an FT-IR spectrophotometer (Nicolet Nexus 670) in a KBr pellet at room temperature. Zeta potential measurements were taken using a Zetasizer nano-ZS90 (Malvern Instruments), and all the aqueous samples were diluted before measurements.

2.3 Antimicrobial Activity Test

2.3.1 Zones of Inhibition Test

All disks and materials were sterilized in an autoclave before the experiment, and all antibacterial tests were performed in triplicate. The antibacterial activity of GO/Ag nanocomposites, Ag NPs (without GO), grape seeds extract and GO was evaluated by agar cup-plate method against the four kinds of clinical pathogenic bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*) and four kinds of aquatic pathogenic bacteria (*Vibrio anguillarum*, *Vibrio alginolyticus*, *Aeromonas punctata* and *Vibrio parahaemolyticus*). In detail, about 100 μL of the clinical pathogenic bacteria suspensions (10^6 CFU/mL) was spread evenly on Luria–Bertani (LB) agar plates, while the aquatic pathogenic bacteria suspensions with the same concentration were spread on nutrient agar plates. Oxford cups (8 mm in diameter) were gently placed on the inoculated agar plates, and 30 μL of as-prepared nanomaterial solution was added to the cups. Then, the clinical pathogenic bacteria and aquatic pathogenic bacteria were incubated overnight at 37 or 28 $^\circ\text{C}$, respectively. The zones of inhibition were measured and reported as an average.

2.3.2 Inhibition Kinetic Test

According to antimicrobial tests, we chose *Escherichia coli* as index bacteria for the inhibition kinetic test. The bacterium was incubated overnight at 37 °C, and then the bacterial suspension (10^6 CFU/mL) was incubated with 10 and 20 µg/mL of GO/Ag nanocomposites at 37 °C, respectively. Bacterial growth rates were obtained by measuring the absorption value at 600 nm (OD_{600}) at different time intervals. The normal saline was used as control.

2.3.3 Minimum Inhibitory Concentration Measurement

The minimum inhibitory concentration (MIC) of the synthesized Ag NPs and GO/Ag nanocomposites was determined by the tube double dilution method [39].

2.3.4 Thermostability Test

In order to further study the antibacterial activity of GO/Ag nanocomposites in different environments, we chose *Escherichia coli* as index bacteria for the thermostability test. After GO/Ag nanocomposites was heated for 1 h at 20, 40, 60, 80 and 100 °C, respectively, 30 µL of GO/Ag nanocomposites was added to the oxford cups (8 mm in diameter), which was on the LB agar plates. At last, the bacteria were incubated overnight at 37 °C. The zones of inhibition were measured and reported as an average.

2.3.5 Optical Observation

The bacterial suspension (10^6 CFU/mL) was incubated with 10 µg/mL of GO/Ag nanocomposites for 8 h, and the

normal saline in the same volume was as a control. The morphology change of bacteria was observed under an optical microscope by crystal violet staining.

3 Results and Discussion

3.1 Characterization of GO/Ag Nanocomposites

Figure 1a is the photograph of GO and as-prepared GO/Ag nanocomposites with different concentrations of $AgNO_3$. As shown in Fig. 1a, the GO was ultrasonically exfoliated in water to form GO suspension. Upon adding the grade seeds extract to the mixture of $AgNO_3$ and GO, the mixture turned yellow after it was heated at 95 °C for 20 min, which indicates the formation of Ag NPs. With increasing concentration of $AgNO_3$, the color of the aqueous solution turned more and more deep, which is similar to the previous report [30]. In the reaction process, it is likely that the reduction in Ag first occurs on the defects of the GO sheets and small Ag nucleation centers form, from which AgNPs are grown.

To verify the formation the Ag NPs, UV–Vis absorption spectra of grape seeds extract, the GO suspension and GO/Ag nanocomposites with different concentrations of $AgNO_3$ were recorded. As shown in Fig. 1b, GO shows a maximum absorption band at about 230 nm and a shoulder peak at 300 nm, due to the excitation of the π – π^* transition of aromatic C–C and the n – π transition of C=O, respectively [26]. A clear peak at about 432 nm appears for GO/Ag nanocomposites, which is the characteristic peak of Ag NPs due to the surface plasmon resonance (SPR) [27, 28]. In addition, with increasing $AgNO_3$ concentration, the SPR absorption increases and a very little redshift appears in the maximum

Fig. 1 a Photograph of aqueous suspension of as-prepared GO nanosheets and synthesized different concentrations of GO/Ag nanocomposites (from left to right, the concentration of $AgNO_3$ is 1, 2 and 3 mM, respectively). b UV–Vis spectra of grape seeds extract, the GO suspension and GO/Ag nanocomposites with different concentrations of $AgNO_3$

absorption peak, which suggests the formation of higher concentrations of Ag NPs and increased particle size.

The presence of Ag NPs in the nanocomposites was also confirmed by XRD (Fig. 2). Figure 2a is the XRD pattern of GO. A sharp reflection peak at 10.2° (Fig. 2a)

Fig. 2 XRD patterns of the GO *a* and GO/Ag samples with 1 mM AgNO_3 *b*, 2 mM AgNO_3 *c*, 3 mM AgNO_3 *d*

corresponds to the feature diffraction peak (001) of exfoliated GO [41]. For the GO/Ag nanocomposites (Fig. 2b–d), there are four main peaks at $2\theta = 38.2^\circ$, 44.4° , 64.5° , 77.5° , which correspond to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) planes of the cubic Ag crystal (JCPDS No. 04-0783), respectively, indicating that the metallic Ag NPs are formed after reduction. However, the peak at 10.2° disappeared after the GO was decorated with Ag NPs, which may be explained that anchoring of Ag NPs on the surface of the GO sheets prevents the stacking of the GO layers [42].

To further validate the formation of Ag NPs and map their distribution on the surface of GO, TEM was used to observe the morphologies of the GO/Ag hybrid materials directly. As shown in Fig. 3, Ag NPs are deposited on GO nanosheets successfully and the average size of Ag NPs is about 40–50 nm. The loading amount of Ag NPs on GO nanosheet increases with increasing AgNO_3 concentration, which is consistent with the increased absorption intensity of SPR of the hybrid material. Moreover, the size of Ag NPs becomes a little bit larger when the concentration of AgNO_3 increases, but the morphology and size of Ag NPs do not change largely. On the surface of the GO and GO/Ag, some wrinkles are observed, which may be important for preventing aggregation of GO and keeping high surface

Fig. 3 TEM images of GO nanosheet, and as-prepared GO/Ag nanocomposites with different concentrations of AgNO_3 . *a* GO nanosheet, *b–d* as-prepared GO/Ag nanocomposites (the concentration of AgNO_3 is 1, 2 and 3 mM, respectively)

Fig. 4 FTIR spectra of the GO/Ag samples with 1 mM AgNO₃ *a*, 2 mM AgNO₃ *b*, 3 mM AgNO₃ *c*, the grape seeds extract *d*

area with a particular advantage of attaching Ag NPs on the GO sheets.

The FTIR spectra of GO/Ag nanocomposites and the grape seeds extract are shown in Fig. 4. It can be seen that GO/Ag nanocomposites and the grape seeds extract have very similar absorption peaks centered at 3435, 2920, 2852 and 1615 cm⁻¹. The strong and wide band at 3435 cm⁻¹ is referred as the strong stretching vibrations of O–H functional group. The corresponding peaks at 2920 and 2852 cm⁻¹ are due to the C–H stretching vibration of aliphatic CH, CH₂ and CH₃, and peak at 1615 cm⁻¹ is attributed to the stretching vibration of cyclohexene. This can be explained that the functional groups on grape seeds extract were recombined with as-prepared GO/Ag nanocomposites successfully, improving the stability of the nanocomposites.

3.2 Antibacterial Activity

Before we studied the antibacterial activity in detail, two kinds of common pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus* were used to study the

Table 1 Mean zone of inhibition of GO/Ag with different GO/Ag ratios against *Escherichia coli* and *Staphylococcus aureus*

Zone of inhibition (mm)		
Sample	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
GO/Ag (1 mM AgNO ₃)	14.30	14.10
GO/Ag (2 mM AgNO ₃)	15.90	14.90
GO/Ag (3 mM AgNO ₃)	15.10	13.70

antibacterial effect of the hybrid material with different GO/Ag ratios. The results are listed in Table 1. We can see that when the concentration of AgNO₃ is 2 mM, the hybrid material shows the maximum inhibition zone. The reason may be that when the concentration of AgNO₃ increases from 1 to 2 mM, the concentration of Ag NPs increases (Fig. 1b) and leads to higher antibacterial effects. When the concentration of AgNO₃ increases further, the loading amount of Ag NPs on GO sheet increases further but aggregation degree of AgNPs increases (Fig. 3), leading to the slight decline of antibacterial activity. So we select 2 mM AgNO₃ as a model to study the antibacterial activity later in detail.

The antibacterial activity of GO/Ag nanocomposites was studied by using eight bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Vibrio anguillarum*, *Vibrio alginolyticus*, *Aeromonas punctata* and *Vibrio parahaemolyticus*, which were investigated by means of agar diffusion method. The antibacterial activity of the GO suspension, grape seeds extract and biosynthesized bare Ag NPs was also evaluated as a control. As shown in Fig. 5, the GO suspension and grape seeds extract show no antibacterial activity and no inhibition haloes for the bacterial strains tested, while the GO/Ag nanocomposites and bare Ag NPs both show obvious inhibition zone against all the tested pathogens (Fig. 5). It can be seen from Table 2 that the prepared hybrid material exhibits higher antibacterial activity against two kinds of clinical pathogenic bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and two kinds of aquatic pathogenic bacteria *Vibrio anguillarum* and *Vibrio parahaemolyticus*, indicating potential application in clinical diagnosis and aquaculture. The diffusion of the GO/Ag sheets over the agar surface prevents the bacteria growth in the specific area occupied by the GO/Ag nanocomposites. In addition, GO/Ag nanocomposites show a little larger inhibition zone than green synthesized bare Ag NPs for almost all bacteria (Fig. 5). Several combined reasons are as follows: First, when the Ag NPs are deposited on the surface of GO, Ag NPs is not easily aggregated, leading to higher stability and the better bacterial activity. Second, the ligand molecules from the grape seeds extract have interacted with silver surface to further improve the stability of Ag NPs. The zeta potential of GO/Ag hybrid material in water is -25.3 mV (2 mM AgNO₃, Fig. 6), which confirms the strong repulsion among the nanocomposites and prevents the aggregation of Ag NPs. As indicated above, GO has a unique two-dimensional (2D) hexagonal lattice structure and a lot of ionizable (-OH, -COOH) edge groups on the surface, which makes it have excellent water dispersity and chemical stability. Some literature reported that the zeta potential value of

Fig. 5 Antimicrobial effect of different samples on eight different microorganisms (1, GO/Ag, 2, Ag NPs, 3, GO alone, 4, the grape seeds extract)

GO was negative in the pH range 2–10 [43, 44]. And the negative charges of GO are a consequence of the ionization of the different groups present [45]. The zeta potential of the bare biosynthesized AgNPs by grape seed extract in our previous report is -28.4 mV due to the existence the functional component in grape seeds extract on the surface of AgNPs [39]. In this study, we also measured the zeta potential of GO and biosynthesized bare Ag NPs, which was -20.9 and -27.9 mV, respectively (Fig. 6). The results indicated that when Ag NPs were deposited on GO nanosheets, GO/Ag hybrid materials have high zeta potential of -25.3 mV due to the synergistic effects of GO and biosynthesized bare Ag NPs.

Then, the MIC value was determined to verify the biocidal activities. *Staphylococcus aureus* was chosen as a model and the normal saline as control. Equal volumes of *Staphylococcus aureus* suspensions were incubated with different concentrations of GO/Ag nanocomposites. Growth or no growth of bacteria was determined by visual observation, and the MIC value is 23.8 $\mu\text{g/mL}$ (Table 3). After inoculation on the LB agar plates with *Staphylococcus aureus* suspension for additional 24 h, the MBC

values at 48 h are higher than MIC at 24 h, which is 47.7 $\mu\text{g/mL}$ (Table 3). The MIC and MBC values of GO/Ag hybrid materials are lower than bare biosynthesized Ag

Table 3 MIC and MBC tests of biologically synthesized GO/Ag nanocomposites

	Concentration of GO/Ag ($\mu\text{g/mL}$)	24 h	48 h
1	190.6	—	—
2	95.3	—	—
3	47.7	—	—
4	23.8	—	+
5	11.9	+	+
6	5.95	+	+
7	2.98	+	+
8	1.49	+	+

Table 2 Mean zone of inhibition of GO/Ag against various pathogenic bacteria ($n = 8$)

Bacteria strains	Zone of inhibition (mm) GO/Ag
<i>Escherichia coli</i>	15.90
<i>Pseudomonas aeruginosa</i>	15.50
<i>Staphylococcus aureus</i>	14.90
<i>Bacillus subtilis</i>	13.50
<i>Vibrio anguillarum</i>	15.50
<i>Vibrio alginolyticus</i>	13.25
<i>Aeromonas punctata</i>	14.75
<i>Vibrio parahaemolyticus</i>	17.50

Fig. 7 Bacterial inhibition growth kinetics of normal saline, 10 $\mu\text{g/mL}$ GO/Ag and 20 $\mu\text{g/mL}$ GO/Ag nanocomposites

Fig. 6 Zeta potential of GO/Ag hybrid material in water (2 mM AgNO_3) **a**, GO **b**, bare Ag NPs **c**

Fig. 8 Mean zone of inhibition of GO/Ag nanocomposites (2 mM AgNO₃) after treatment at different temperatures against *Escherichia coli*

NPs [39], indicating higher biocidal activity of GO/Ag nanocomposites.

Further experiments were conducted to characterize the bacterial inhibition growth kinetics of the GO/Ag nanocomposites. We selected the most common clinic pathogenic bacterium *Escherichia coli* as the model to study the bacterial inhibition growth kinetics by monitoring the optical absorption at 600 nm (OD₆₀₀). As shown in Fig. 7, the OD value at 600 nm for the normal saline increased gradually with increasing incubation time, which indicates the growth of *Escherichia coli*. In comparison, the growth of *Escherichia coli* was completely inhibited by 10 µg/mL GO/Ag. These results

demonstrate that the prepared GO/Ag nanocomposites have excellent antibacterial activities, which makes it have good potential applications in antibacterial materials.

As everyone knows, the thermostability of the nanocomposites in different environments is very important. After heat treatment of the GO/Ag nanocomposites at different temperatures for 1 h, the antibacterial activity was studied and compared. In the test, *Escherichia coli* was chosen as the index bacteria. As shown in Fig. 8, except for 100 °C treatment, all GO/Ag nanocomposites after heat treatment exhibit better antibacterial activity than that without heat treatment. The reason may be that heat treatment can further induce formation of more Ag NPs. These results illustrate that GO/Ag nanocomposites have a good thermostability.

To explain the antibacterial mechanism of the prepared GO/Ag nanocomposites, the structural and morphological changes of bacteria were observed by optical microscopy with crystal violet staining. As shown in Fig. 9, the number of *Escherichia coli* decreased greatly after incubation with GO/Ag nanocomposites, indicating the powerful antibacterial activity. It was also found that the shape of bacterium was damaged and its membrane was penetrated by the as-prepared nanocomposites. It may be explained that when GO/Ag nanocomposites contact with microbial cells, GO can lead to destructive extraction of phospholipids from *Escherichia coli* membranes, resulting in destabilization of the membrane and cellular wall [22]. Moreover, the Ag ions can change the partial pressure of oxygen in cells and result in the oxidation of substance in the cell, which destroys the composition to cause cell death.

Fig. 9 Optical microscopy images for *Escherichia coli* stained with crystal violet without **a**, with **b** GO/Ag

4 Conclusion

An environmentally friendly, facile, simple method was developed to synthesize GO/Ag nanocomposites. A food waste, grape seeds extract was as a synergistic reducing agent with GO itself to reduce AgNO₃ and grow Ag NPs on GO nanosheet. GO/Ag nanocomposites displayed higher biocidal activity than bare Ag NPs with the MIC value 23.8 µg/mL, which is due to the better dispersion and stability after deposition on GO nanosheets. The prepared hybrid material exhibits higher antibacterial activity against two kinds of clinic pathogenic bacteria and two kinds of aquatic pathogenic bacteria, indicating a wide application in future. Optical microscopy observation confirms that the antibacterial mechanism is due to the damage of cell membrane. However, further efforts are still needed to study the antibacterial mechanisms of GO/Ag at molecular levels.

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References

- [1] K.S. Novoselov, A.K. Geim, S.V. Morozov, D. Jiang, Y. Zhang, S.V. Dubonos, I.V. Grigorieva, A.A. Firsov, *Science* **306**, 666 (2004)
- [2] A.K. Geim, K.S. Novoselov, *Nature Mater.* **6**, 183 (2007)
- [3] A.K. Geim, *Science* **324**, 1530 (2009)
- [4] S. Park, R.S. Ruoff, *Nature Nanotechnol.* **5**, 217 (2010)
- [5] O.C. Compton, S.B.T. Nguyen, *Small* **6**, 711 (2010)
- [6] D. Li, R.B. Kaner, *Science* **320**, 1170 (2008)
- [7] D. Li, M.B. Müller, S. Gilje, R.B. Kaner, G.G. Wallace, *Nature Nanotechnol.* **3**, 101 (2008)
- [8] D.A. Dikin, S. Stankovich, E.J. Zimney, R.D. Piner, G.H.B. Dommett, G. Evmenenko, S.B.T. Nguyen, R.S. Ruoff, *Nature* **448**, 457 (2007)
- [9] S. Gilje, S. Han, M.S. Wang, K.L. Wang, R.B. Kaner, *Nano Lett.* **7**, 3394 (2007)
- [10] S. Park, J. An, I. Jung, R.D. Piner, S.J. An, X. Li, A. Velamakanni, R.S. Ruoff, *Nano Lett.* **9**, 1593 (2009)
- [11] C.N.R. Rao, A.K. Sood, K.S. Subrahmanyam, A. Govindaraj, *Angew. Chem. Int. Ed.* **48**, 7752 (2009)
- [12] S. Lee, S.H. Kang, *Langmuir* **27**, 9174 (2011)
- [13] Y. Wang, Z. Li, J. Wang, J. Li, Y. Lin, *Trends Biotechnol.* **29**, 205 (2011)
- [14] L. Feng, Z. Liu, *Nanomedicine* **6**, 317 (2011)
- [15] T. Kuila, S. Bose, P. Khanra, A.K. Mishra, N.H. Kim, J.H. Lee, *Biosens. Bioelectron.* **26**, 4637 (2011)
- [16] K.R. Ratina, W. Yang, J.J. Gooding, P. Thordarson, F. Braet, *Electroanalysis* **23**, 803 (2011)
- [17] W. Hu, C. Peng, W. Luo, M. Lv, X. Li, D. Li, Q. Huang, C. Fan, *ACS Nano* **4**, 4317 (2010)
- [18] O. Akhavan, E. Ghaderi, *ACS Nano* **4**, 5731 (2010)
- [19] S. Liu, T.H. Zeng, M. Hofmann, E. Burcombe, J. Wei, R. Jiang, J. Kong, Y. Chen, *ACS Nano* **5**, 6971 (2011)
- [20] S. Liu, M. Hu, T.H. Zeng, R. Wu, R. Jiang, J. Wei, L. Wang, J. Kong, Y. Chen, *Langmuir* **28**, 12364 (2012)
- [21] J. Chen, X. Wang, H. Han, *J. Nanopart. Res.* **15**, 1 (2013)
- [22] Y. Tu, M. Lv, P. Xiu, T. Huynh, M. Zhang, M. Castelli, Z. Liu, Q. Huang, C. Fan, H. Fang, *Nature Nanotechnol.* **8**, 594 (2013)
- [23] J.S. Kim, E. Kuk, K.N. Yu, J.H. Kim, S.J. Park, J.L. Hu, S.H. Kim, Y.K. Park, H.P. Yong, C.Y. Hwang, *Nanomed. Nanotechnol. Biol. Med.* **3**, 95 (2007)
- [24] C. Marambio-Jones, E.M.V. Hoek, *J. Nanopart. Res.* **12**, 1531 (2010)
- [25] Q. Bao, D. Zhang, P. Qi, *J. Colloid Interface Sci.* **360**, 463 (2011)
- [26] J. Shen, M. Shi, N. Li, B. Yan, H. Ma, Y. Hu, M. Ye, *Nano Res.* **3**, 339 (2010)
- [27] M.R. Das, R.K. Sarma, R. Saikia, V.S. Kale, M.V. Shelke, P. Sengupta, *Colloids Surf. B* **83**, 16 (2011)
- [28] C. Xu, X. Wang, *Small* **5**, 2212 (2009)
- [29] A.F.D. Faria, D.S.T. Martinez, S.M.M. Meira, A.C.M.D. Moraes, A. Brandelli, A.G.S. Filho, O.L. Alves, *Colloids Surf. B* **113**, 115 (2014)
- [30] T. Jia, C. Qian, L. Xu, Z. Shuai, L. Feng, C. Liang, H. Xu, L. Zhuang, P. Rui, *ACS Appl. Mater. Interfaces* **5**, 3867 (2013)
- [31] M.R. Das, R.K. Sarma, S.C. Borah, R. Kumari, R. Saikia, A.B. Deshmukh, M.V. Shelke, P. Sengupta, S. Szunerits, R. Boukherroub, *Colloids Surf. B* **105**, 128 (2012)
- [32] H. Yu, B. Sun, D. Zhang, G. Chen, X. Yang, J. Yao, *J. Mater. Chem. B* **2**, 8479 (2014)
- [33] H. Liu, D. Wang, S. Shang, Z. Song, H. Liu, D. Wang, S. Shang, Z. Song, *Carbohydr. Polym.* **83**, 38 (2011)
- [34] M.D. Xue, T. Kimura, J.F. Revol, D.G. Gray, *Langmuir* **12**, 2076 (1996)
- [35] S. Shafiei-Sabet, W.Y. Hamad, S.G. Hatzikiriakos, *Cellulose* **21**, 1 (2014)
- [36] K. Liu, H. Liang, J. Nasrallah, L. Chen, L. Huang, Y. Ni, *Carbohydr. Polym.* **142**, 183 (2016)
- [37] D. Ye, Z. Zhong, H. Xu, C. Chang, Z. Yang, Y. Wang, Q. Ye, L. Zhang, *Cellulose* **23**, 749 (2015)
- [38] H. Yu, Z. Qin, B. Sun, C.F. Yan, J. Yao, *J. Nanopart. Res.* **16**, 2202 (2014)
- [39] H. Xu, L. Wang, H. Su, L. Gu, T. Han, F. Meng, C. Liu, *Food Biophys.* **10**, 12 (2015)
- [40] W.S. Hummers, R.E. Offeman, *J. Am. Chem. Soc.* **80**, 1339 (1958)
- [41] P. Lian, X. Zhu, S. Liang, Z. Li, W. Yang, H. Wang, *Electrochim. Acta* **55**, 3909 (2010)
- [42] J. Li, C.Y. Liu, *Ber. Dtsch. Chem. Ges.* **2010**, 1244 (2010)
- [43] X. Hu, Y. Yu, W. Hou, J. Zhou, L. Song, *Appl. Surf. Sci.* **273**, 118 (2014)
- [44] P. Borthakur, P.K. Boruah, N. Hussain, B. Sharma, M.R. Das, S. Matic, D. Řeha, B. Minofar, *J. Phys. Chem. C* **120**, 14088 (2016)
- [45] D. Li, M.B. Müller, S. Gilje, R.B. Kaner, G.G. Wallace, *Nat. Nanotechnol.* **3**, 101 (2008)