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Preparation of magnetic graphene composites with hierarchical structure for selective capture of phosphopeptides

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Abstract

A novel graphene composite affinity material consisting of graphene scaffold, Fe₃O₄ nanoparticles for actuation and fully covered porous titania nanostructures as affinity coating has been designed and constructed. The obtained magnetic graphene composites have a saturation magnetization (Ms) value of 7.3 emu g⁻¹, a BET specific surface area of 111.8 m² g⁻¹ and an average pore size of 15.1 nm for the porous affinity coating. The multifunctional graphene composites can realize the selective capture and convenient magnetic separation of target phosphopeptides by taking advantage of the decorated magnetic nanoparticles, highly pure and well crystallized affinity coating, and unique porous structure. Sensitivity and selectivity of the affinity graphene composites were evaluated using digests of standard proteins and complex biosamples as well as by comparison with the widely used TiO₂ affinity microspheres. The results show that the affinity graphene composites can realize selective capture and rapid separation of low-abundance phosphopeptides from complex biological samples. Thus, this work will contribute to future applications in the purification and separation of specific biomolecules, in particular, low-abundance phosphopeptide biomarkers.

1. Introduction

As a type of important functional material, graphene and its derivatives graphene oxide (GO) have continued to draw considerable interests in both theoretical studies and practical applications in the past two decades.¹ By virtue of their ultrahigh surface area, excellent chemical and thermal stability, and remarkable electrical and mechanical properties, graphene has tremendous potential for applications in various fields.² Especially, graphene serves as a scaffold or substrate to form composites with other functional materials such as metals, oxides, and polymers, which has been extensively explored in biomedicine.³ For instance, many types of nanostructures functionalized graphene have been studied as multi-synergistic platform for cancer detection and therapy.⁴ In addition, the polymers integrated graphene also show excellent performance in intracellular delivery of drug, gene and RNA, etc.⁵ Recently, several graphene based composite materials have also been introduced in

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bioseparation for capture and enrichment of target cells and various biomolecules.⁶ Notably, besides their extremely high surface to volume ratio and the capabilities to incorporate functional nanostructures and prevent macroscopic aggregation,⁷ they can also provide more chances for target binding and be free from the hindrance of interaction with target peptides,⁸ due to their unique double-sided chemical structure and high flexibility.

Reversible phosphorylation, one of the most common and important post-translational modifications of proteins, plays pivotal roles in various biological processes such as signal-transduction, regulatory, and metabolic pathways.⁹ Many reports revealed that phosphopeptides arising from the abnormal phosphorylation in tissues or body fluids are potential biomarkers with high clinical relevance, which would provide a critical step toward understanding the signalling pathways in normal and disease states.¹⁰ Mass spectrometry (MS) is a powerful tool for the analysis of protein phosphorylation, because they can provide direct and intrinsic information of the peptides and screen multiple peptides simultaneously.¹¹ However, the identification and characterization of phosphopeptides remain challenging tasks in contemporary proteomics research, due to their small quantity, the low stoichiometry of phosphorylation and the suppression effect by non-target impurities.¹²

Nanomaterials have drawn considerable interests to improve the sensitivity of target biomolecule detection due to their high surface area and similar size to biomolecules.¹³ Some metal oxide particles have been demonstrated to offer selective and reversible chemisorption of phosphopeptides on their amphoteric surface.¹⁴ Further optimizing the structure of the materials and integrating with other functional composites would provide new opportunities for improving the capture efficiency and facilitate the separation process. Recently, a few graphene-metal oxide nanostructures have been introduced to capture phosphopeptide by taking advantage of the high surface area of graphene and specific affinity of metal oxides.¹⁵ Although promising, unfortunately, these efforts failed to obtain a pure interface of metal oxides; as the affinity sites for phosphopeptides, metal oxides are anchored on the graphene randomly while most of the surface area of the graphene is still exposed, which would result in nonspecific binding of impurities. Furthermore, only the solid sphere-like or spindle-like metal oxides integrated graphene composites are explored for selective capture target peptides, and therefore it is intriguing while very challenging to modify graphene with porous nanostructures such as metal oxides with unique porous nanostructures, which possess significantly enhanced affinity-binding capabilities than their simple solid counterparts. Alternatively, the separation of graphene composites with target peptides by centrifugation or filtration is time-consuming, difficult to control, and co-sedimentation of non-targets with large molecular-weight would also complicate the capture efficiency in phosphopeptide enrichment. Therefore, developing new methods to fabricate multifunctional graphene composites with highly pure and nanostructured surfaces of metal oxides is highly desirable.

Herein, for the first time, magnetic nanoparticles decorated graphene were fully grafted with titania nanopetals via a facile solvothermal process. The multifunctional graphene composites consisting of magnetic particles, graphene scaffold and titania coating (denoted as MGT) were applied to selective enrichment and separation of phosphopeptides. Notably,

the MGT composites have the following advantages: (1) high content of pure and crystalline titania fully coated on the surface of graphene substrate to achieve remarkable affinity for phosphopeptides; (2) unique nanostructure assembled by nanopetals with open pore spaces offering a large exposed surface area being free of “shadow effect”¹⁶ and high mass transfer resistances; (3) decorated magnetic nanoparticles providing conveniently separation by the simple application of an external magnetic field. Therefore, it is expected that the novel multifunctional graphene composites will be highly beneficial for future applications in rapid and highly efficient analysis of protein phosphorylation.

2. Experimental Section

2.1. Materials

Graphite powder, ammonium bicarbonate (NH_4HCO_3), ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), ethylene glycol (EG), ethylenediamine (EN), trifluoroacetic acid (TFA), acetonitrile (ACN), ammonium bicarbonate (NH_4HCO_3), sodium acetate (NaOAc), tetrabutyl titanate (TBT), isopropanol (IPA), dimethylformamide (DMF), phosphoric acid (H_3PO_4), hydrogen peroxide (H_2O_2), sulfuric acid (H_2SO_4), hydrochloric acid (HCl) and ethanol (EtOH) were purchased from Alfa Aesar. Potassium permanganate (KMnO_4), 2,5-Dihydroxybenzoic acid (2,5-DHB), β -casein, trypsin (from bovine pancreas, TPCK treated), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Human serum samples were collected from healthy volunteers. All the chemical agents were used without further purification.

2.2. Preparation of magnetic graphene composites

Graphene oxide (GO) was synthesized from natural graphite powder by a modified Hummers method,¹⁷ while Fe_3O_4 nanoparticles were synthesized via a reported solvothermal approach.¹⁸ The magnetic graphene composites (MG) were prepared by the following method. 8 mg of GO was dispersed into 16 mL of IPA via ultrasonication for 1 hour to form GO suspension. 8 mg of prepared Fe_3O_4 nanoparticles were well dispersed into 16 mL of IPA via ultrasonication for 30 min. Then, the particle suspension was added dropwise into the GO solution under agitation, and the mixture was further stirred for 12 h at room temperature. Finally, the obtained magnetic composites were collected by a magnet, washed with IPA for three times, and then redispersed into 24 mL of IPA.

2.3. Preparation of magnetic graphene composites with fully coated TiO_2 layer (MGT)

The MGT precursor composites (MGTP) were first prepared via a facile solvothermal method. In brief, 8 mL of DMF was injected into the previous dispersion of MG composites under agitation, followed by the addition of TBT (0.8 mL). The mixture was transferred to a Teflon-lined stainless-steel autoclave and sealed to heat at 200°C for 24 h. The produced MGTP composites were collected by a magnet, washed with ethanol for several times, and finally dried in an oven at 60 °C for 12 h. To obtain the MGT products, the prepared MGTP composites were calcined in N_2 at 400°C for 2 h.

2.4. Materials characterization

Scanning electron microscopy (SEM) images were performed on a field emission scanning electron microscope (FESEM; NanoSEM 630, NOVA) equipped with an energy-dispersive X-ray analysis system (EDXA). Transmission electron microscopy (TEM) and high-resolution TEM (HRTEM) images were taken with a JEOL-2010 microscope at the accelerating voltage of 200 kV. Powder X-ray diffraction (XRD) patterns were collected on a PANalytical Empyrean X-ray powder diffractometer (Cu K α radiation, 45 kV, 40 mA) and the detective range from 5 to 80 degree. Fourier transform infrared spectra were determined on a Bruker Vertex V70 FTIR spectrometer over a potassium bromide pellet and then scanned from 400 to 4000 cm⁻¹ at a resolution of 6 cm⁻¹. Nitrogen adsorption isotherms were measured at a liquid nitrogen temperature (77 K) with a Micromeritics ASAP 2020 apparatus. The specific surface area was determined by the Brunauer-Emmett-Teller (BET) method. The total pore volume was evaluated by the t-plot method, and pore size distribution was analyzed with the supplied BJH software package from the adsorption branches of the isotherms. Magnetization measurement was carried out with a superconducting quantum interface device (SQUID) magnetometer at 300 K.

2.5. Tryptic digest of standard proteins

1 mg of protein (β -casein or BSA) was dissolved in 1 mL of 50 mM NH₄HCO₃ solution (pH=8.0), and then trypsin was added into the solution with a mass ratio of 1:50 (trypsin/substrate) for 18 h at 37°C. Finally, the obtained tryptic digests were diluted to the target concentration and stored at -20 °C for further use.

2.6. Selective capture of phosphopeptides

The obtained MGT composites were washed with ACN solution for twice, and then dispersed into an aqueous solution of 0.5% TFA and 65% ACN at a final concentration of 10 mg mL⁻¹. The amount of MGT composites for phosphopeptide enrichment could be optimized, and 2.5 μ L of suspension of MGT composites is enough for the enrichment. 100 μ L of protein digest solution was mixed with 2.5 μ L above MGT suspension and then shaken for 5 min at room temperature. Then, the MGT composites with captured phosphopeptides were separated from the mixture with the help of a magnet. The obtained microspheres were washed twice with 100 μ L aqueous solution of 0.5% TFA and 65% ACN. After that, the captured phosphopeptides were eluted with 10 μ L 3% ammonium hydroxide for further MS detection. For enriching and isolating the phosphopeptides from human serum, the pristine human serum (1 μ L) was dissolved in 100 μ L aqueous solution of 0.5% TFA and 65% ACN, and then treated according to the same procedure as the standard protein digests. The MGT composites can be easily regenerated by washing with 3% ammonium hydroxide for 3 times and then 0.5% TFA and 65% ACN for twice. The process of enrichment using recycled MGT composites is similar to that of new MGT composites.

2.7. MALDI-TOF mass spectrometry analysis

The above products obtained from the elution step were deposited on MALDI plates, and then 1 μ L of 20 mg mL⁻¹ DHB (in 50% ACN/water containing 1% H₃PO₄ aqueous solution, v/v) was further deposited. MALDI-TOF mass spectrometry analysis was performed on an

AB SCIEX MALDI-TOF/TOF 5800 mass spectrometer (Foster City, CA, USA) in positive ion mode with 355 nm Nd:YAG laser, 200 Hz repetition rate, and 20 kV acceleration voltage.

2. Results and discussion

2.1. Synthesis and characterization of MGT composites

In this work, well-dispersed and $-NH_2$ functionalized Fe_3O_4 nanoparticles (Figure S1a-b) were prepared via a simple solvothermal method which would play the role of actuation material for magnetic separation in practical applications. The graphene oxide (GO) was obtained according to a modified Hummer's method (Figure S1d-f), which would act as the scaffold to carry all the functional components. As shown in Scheme 1a, the magnetic nanoparticles were easily assembled on the surface of the graphene oxide through electrostatic and coordinate interactions, thereby forming the magnetic graphene oxide composites (denoted as MG). The MG composites were dispersed into a tetrabutyl titanate solution to fully adsorb the organic precursor molecules. After solvothermal treatment, the precursor composites (denoted as MGTP) were constructed at the high temperature in the closed autoclave; and subsequent calcination process was introduced to obtain pure crystalline titania and remove possible residual organics, resulting in the multifunctional MGT composites.

The morphology of the resulting products was investigated by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). As shown in Figure S1, compared with pure GO sheets, many Fe_3O_4 particles were grafted on the surfaces of the GO sheets. The Fe_3O_4 particles were well distributed on the GO sheets, indicating a successful negative-positive electrostatic attraction mechanism. TEM images further provide the evidences of their assembly. According to the HRTEM image (Figure S1i, inset), the lattice fringe spacing (0.296 nm) agrees well with the lattice spacing of (220) planes of cubic magnetite. Figure 1 presents the SEM images of the prepared MGT composites. Interestingly, relatively large-scale flower-like film structures were formed, due to the template-function of the magnetic graphene hybrids. Apparently, almost no naked GO and Fe_3O_4 nanoparticles can be observed, indicating the magnetic graphene composites completely covered with the TiO_2 nanostructure layer. It should be noted that the morphology and unique structure is still similar to their precursor graphene composites (Figure S2), although the high temperature calcination process was applied to transfer the phase structure of the coated affinity layer. High-magnification SEM images further reveal that the thickness of the nanostructured layer is about 200 nm, and it is assembled by lots of nanopetals, and many open pores were also constructed. TEM images of the MGT composites further confirm that the porous layer is constructed from loosely stacked nanopetals. Furthermore, the magnetic nanoparticles with unchanged morphology can be clearly observed, while the graphene substrate is still hard to identify owing to their low contrast and the presence of high content of TiO_2 layer. According to high resolution TEM (HRTEM) images, the nanopetals are very thin with a thickness about 5 nm only, and the lattice fringe with a spacing of 0.354 nm of the nanopetal (Figure 1f) exhibit the crystalline nature that can be indexed to the (101) plane of the anatase TiO_2 .

The energy dispersive X-ray (EDX) spectra were recorded to identify the element composition of the graphene composites. As shown in Figure 2a, for the MG composites, C, O and Fe are the three main elements. After further modification with titania layer, new peaks of the element Ti are presented in the EDX spectrum of MGT composites. Notably, the relative intensity of element Ti is very high, indicating the high content of TiO_2 in the composites. Furthermore, detailed elemental mapping analysis (Figure S3) have been applied to analyze the elemental components and distributions of the MGT composites. The homogeneity of the distribution of the element Ti support that the magnetic graphene composites were fully and evenly coated with the titania nanostructures.

The phase structures of the prepared magnetic particles and graphene composites were investigated by wide-angle XRD analysis (Figure 3). Apparently, the XRD patterns of Fe_3O_4 nanoparticles show well-defined diffraction peaks which be assigned to the characteristic diffraction peaks from Fe_3O_4 (JCPDS card No. 65-3107). These diffraction peaks also present in the XRD patterns of all the magnetic graphene composites, indicating that the magnetic nanoparticles were successfully attached on the graphene and the modification process would not affect the crystal structure of Fe_3O_4 . Besides the diffraction peaks of Fe_3O_4 , a broad peak at 10.3° can also be found in the MG composites, which can be attributed to the (002) reflection of the GO. After solvothermal treatment for introduction of the titanic resource, several new peaks are clearly observed and become the main peaks in the XRD patterns of MGTP composites, which can be assigned to the characteristic diffraction peaks of protonic titanate $\text{H}_2\text{Ti}_2\text{O}_5 \cdot \text{H}_2\text{O}$ (JCPDS card No. 47-0124).¹⁹ More importantly, the strongest peak around 9.0° in the XRD patterns of MGTP composites corresponds to the (002) planes of protonated titanate, representing the typical layered crystal structure of the protonic titanate coated on the graphene composites, which agrees well with the TEM results. Compared with those of the precursor MGTP composites, except for those assigned to Fe_3O_4 , all the characteristic diffraction peaks of protonic titanate disappeared, while new diffraction peaks appeared and they can be satisfactorily indexed to crystalline anatase TiO_2 phase (JCPDS card no. 21-1272). These results indicate that layered protonic titanate was converted to TiO_2 upon calcination.

To further investigate the transformation of the surface property of the prepared nanomaterials, the FT-IR spectra were recored. As shown in Figure 4 and Figure S4, besides the characteristic absorption peak of Fe_3O_4 (stretching vibration of Fe-O bond) at around 560 cm^{-1} , stretching vibrations of O-H at 3434 cm^{-1} , C=O in carbonyl groups at 1741 cm^{-1} and C=C inaromatic rings at 1631 cm^{-1} , and deformation of C-O in epoxide moiety at 1090 cm^{-1} from graphene oxide in MG composites can also observed, which is in accordance with the previous reported results.²⁰ Furthermore, the presence of new bands at 1436 and 1383 cm^{-1} indicates the formation of either a monodentate complex or a bidentate complex between the carboxyl group and iron atoms on the surface of the magnetic nanoparticles.²¹ For the spectrum of MTGP precursor composites, the presence of new bands at 682 cm^{-1} can be ascribed to the formation of Ti-O-Ti and Ti-O-O, and in the region of $1000\text{-}1200\text{ cm}^{-1}$ confirms the formation of Ti-O-C and Ti-O-O-C species.¹⁹ Apparently, the organic residuals were removed after calcination, as most of the characteristic peaks at $1000\text{-}1500\text{ cm}^{-1}$ were absence. However, the strong and broad band at around 3430 cm^{-1} can still be obviously observed, indicating the existence of many hydroxyls on the surface of MGT

composites, which would contribute to their water dispersibility in practical applications. Above results evidently demonstrated the successful construction of the multifunctional graphene composites.

The surface structure of the final MGT affinity composites was characterized by N₂-sorption measurement (Figure 5a). The adsorption/desorption isotherms of the MGT composites have type IV curves with an H1 hysteresis loop at relatively high P/P₀ according to the IUPAC classification,²² suggesting the composites possess the porous structure. The average Brunauer-Emmett-Teller (BET) specific surface area and pore size of the MGT affinity composites are calculated to be 111.8 m² g⁻¹ and 15.1 nm, respectively. Figure 5b displays the pore-size distribution derived from the adsorption branch using the Barrett-Joyner-Halenda (BJH) method. A narrow peak in the mesoporous range can be clearly observed, which is possibly attributed to the interspace between aggregate small titania crystallites. More importantly, lots of macrospores were also presented in the MGT composites, which is also evidenced by previous TEM results. It suggests that these macrospores arise from the stacking of titania nanopetals during formation of affinity titania layer on the graphene substrate, thereby leading to the formation of open pores. It is noteworthy that the porous structure of the affinity composites are beneficial to enhance their performance in practical applications owing to their high surface areas and numbers of affinity sites. Especially, affinity composites with unique architecture consist of highly open pore spaces with a large exposed surface area, short diffusion pathways with reduced resistance and low tortuosity, which facilitate improved mass transport compared with a tortuous structure.²³ In other words, the unique porous structure can avoid the “shadow effect” arising from small and deep pores which would hamper the capture and release of target biomolecules.^{24, 25}

Magnetic separation is a rapid and convenient means for extraction of targets from complex mixture in practical application. Magnetic properties of the Fe₃O₄ nanoparticles, MG and MGT composites were examined at 300 K via a superconducting quantum interface device (SQUID) magnetometer. As displayed in their hysteresis loops (Figure 6a and Figure S5), the magnetic nanoparticles and composites show relatively strong magnetism with negligible coercivity and remanence at room temperature. The saturation magnetization values of Fe₃O₄ nanoparticles, MG and MGT composites are 74.7, 42.0 and 7.3 emu g⁻¹, respectively. As expected, the saturation magnetizations of the graphene composites are less than that of the naked magnetite particles because of the introduction of nonmagnetic species. Especially, after further functionalization of porous TiO₂ affinity layer, the saturation magnetization of MGT composites decreased dramatically, indicating the modification of high content of titania species. These results agree well with the above MGT characterization results. Notably, they are strong enough for effective magnetic separation. As shown in Figure 6b, they are easily dispersed by shaking in the absence of a magnetic field, but can be rapidly separated from the mixture within 1 minute with the help of a permanent magnet.

2.2 Enrichment of phosphopeptides using MGT composites

Titania is one of the most popular materials to selectively separate phosphopeptides, thanks to the high affinity of titania towards phosphoric acid groups of the phosphopeptides via

bridging bidentate bonds which has been revealed.^{24, 26} Because of their fully coated TiO₂ affinity layer, unique nanostructure and excellent magnetic response, the as-prepared MGT composites were applied to rapid capture and separation of phosphopeptides. As shown in Scheme 1b, in a typical enrichment procedure, the MGT composites were mixed in the sample solution, and then the composites with captured target peptides were easily separated with the help of a magnet. After washing to remove the impurities, the captured phosphopeptides were released with the alkaline solution and then ready for detection.

The feasibility of the MGT composites for selective capture of phosphopeptides was investigated using digests of a model phosphoprotein (β -casein) which contains three phosphopeptides with molecular masses of 2061.8 Da, 2556.0 Da and 3122.2 Da. As shown in Figure 7a, for direct analysis of the β -caseindigests using the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry analysis, the obtained spectrum was dominated by nonphosphopeptides and only two phosphopeptides (marked with '☆') with weak intensity can be detected. However, after selective enrichment with the MGT composites, all the expected phosphopeptides were detected with strong intensity while almost no nonphosphopeptides were observed. Table S1 list the detailed information of the detected phosphopeptides from β -caseindigests by MALDI-TOF mass spectrum analysis. For comparison, enrichment experiment of target peptide using the magnetic graphene oxide MG composites were also conducted. As shown in Figure 7c, a mass spectrum with poor quality was obtained, and only two peaks of nonphosphopeptides with weak intensity can be observed, due to their poor affinity for target phosphopeptides. These results indicate that the fully coated affinity layer play a pivotal role in selective capture of phosphopeptides. The MGT composites can selectively capture phosphopeptides. Furthermore, the ability of MGT composites to extract low-abundance phosphopeptides was evaluated using the highly diluted β -caseindigests (Figure S6). As shown in Figure S6b, two phosphopeptides can still be detected even at the concentration of original digests as low as 5×10^{-10} M, indicating the high sensitivity and selectivity of the MGT composites for enrichment of phosphopeptides.

To further test their selectivity to capture phosphopeptides in complex samples, large amounts of tryptic digests of BSA spiked tryptic digests of β -casein (the molar ratio of BSA to β -casein is 100:1) were used to mimic a relative complex sample with a high level of contaminants. Figure 8 a shows the MALDI-TOF mass spectrum of the complex tryptic digests. Apparently, it is difficult to observe the target phosphopeptides, due to the presence of high-abundance non-phosphopeptides arising from BSA digests. However, after treatment using the MTG composites, all the target phosphopeptides could be detected with a clean background in the mass spectrum (Figure 8b). As a comparison, a commercial TiO₂ product was also used to enrich the phosphopeptides from the peptide mixture. As presented in Figure 8c, although the three phosphopeptides could be detected, the nonphosphopeptides dominated the mass spectrum with high intensities. These results further demonstrated the ability of MGT composites for selective enrichment of phosphopeptides. Notably, the great improvement of the selectivity of the MGT composites should be ascribed to the fully coated affinity layer, large surface area and unique open porous structure.

To explore the effectiveness of the MGT composites for enrichment of phosphopeptide in practical biomedical applications, human serum was applied as a real complex sample. As the most important and complex body fluid, human serum containing various informative endogenous peptides including the phosphopeptides released by diseased tissue. These peptides have gained considerable interests for the disease biomarker discovery.²⁷ However, as shown in Figure 9a, it is difficult to directly detect the peptides by mass spectrum, let alone the phosphopeptides. This is mainly caused by the presence of high-abundance impurities (e.g. salts, highabundance proteins) and the low concentrations of phosphopeptides. By taking advantage of their high affinity for phosphopeptides and the convenient magnetic separation base on their rapid magnetic response, the MGT composites were applied to extract phosphopeptides from human serum. Figure 9b present the MALDI-TOF spectrum of the enriched phosphopeptides using the MGT composites at the mass range from 1000 to 3500. Apparently, four phosphopeptides could be detected and identified with strong intensity from clear background. Table S2 lists the detailed information of the identified phosphopeptides. The above results clearly demonstrate that the MGT affinity composites materials are highly capable for the selective enrichment of phosphopeptides from complex real-world biological samples. In virtue of the stability of the graphene composites and the convenient magnetic separation, the MGT composites have been regenerated and reused for 5 times. Fig. S7 shows the mass spectra of human serum after enrichment using the regenerated MGT composites for the third and fifth time. It is clear that the four target phosphopeptides can still be detected with high quality mass spectra, even after the MGT composites have been reused up to 5 times. These results demonstrate the good recyclability of the MGT composites.

Conclusions

In summary, novel graphene composites consisting of the graphene scaffold, magnetic nanoparticle and affinity coating layer have been prepared via a two-step strategy. The affinity outer layer with unique open porous structures is assembled by the highly pure and crystalline titania nanopetals, which fully cover the magnetic graphene substrate. The multifunctional graphene composites can realize the selective capture and convenient magnetic separation of target phosphopeptides from complex biomedical samples. Digests of model proteins and human serum were used to evaluate their effectiveness for phosphopeptide enrichment. The performance of MGT graphene composites is superior to those of commercial TiO₂ affinity materials being tested. Therefore, this work would contribute to future applications in rapid enrichment and separation of phosphopeptides for phosphoproteomics. Furthermore, it is also expected that the multifunctional graphene composites can also extend to other applications (e.g. catalysis, water purification) because of their functional components, unique structure and high surface area.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
The SEM (a and b) and TEM (c-f) and high resolution TEM (f inset) images of the affinity MGT composites.

Figure 2.
EDX spectra of the prepared MG and MGT composites.

Figure 3.
XRD patterns of the prepared Fe₃O₄, MG, MGTP and MGT composites.

Figure 4.
FTIR spectra of the prepared Fe₃O₄, MG, MGTP and MGT composites.

Figure 5.
(a) N_2 adsorption-desorption isotherm and (b) pore size distribution of the MGT composites.

Figure 6.

(a) Magnetic hysteresis curves of the Fe₃O₄ nanoparticles, MG and the MGT composites at 300 K, and (b) dispersion and separation process of the MGT composites.

Figure 7. MALDI-TOF mass spectra of the tryptic digests of β - casein: (a) direct analysis, (b) analysis after enrichment using MGT composites and (c) analysis after treatment using MG composites. * indicates phosphorylated peptides, ● indicates the corresponding dephosphorylated peptides.

Figure 8. MALDI-TOF mass spectra of the tryptic digests mixture of β -casein and BSA: (a) direct analysis, (b) analysis after treatment using MGT composites and (c) analysis after enrichment using commercial TiO₂ microspheres.

Figure 9.
MALDI-TOF spectrum of human serum: (a) direct analysis and (b) analysis after treatment using MGT composites.

Scheme 1.

Schematic illustration of (a) the synthesis strategy of MGT composites and (b) selective capture and magnetic separation of phosphopeptides.