Shape-controlled synthesis of protein-conjugated silver sulfide nanocrystals and study on the inhibition of tumor cell viability†

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Stable protein–conjugated silver sulfide nanoparticles, nanorods and nanowires have been prepared by an aqueous chemistry method and the study results showed they had potential applications for tumor treatment.

There is a keen interest in the synthesis of bioactive and biocompatible nanomaterials for a variety of applications in recent years, including fluorescent labeling, pathogen and toxin detection, drug delivery, and so on.[1–3] For example, inorganic nanocrystals are bioconjugated with the attachment of DNA, peptides, and proteins.[4–7] A typical route in the preparation of conjugates involves mixing biomolecules with modified nanoparticles in solution.[8] However, the direct conjugation of biological species to inorganic nanoparticles has not been a common practice despite some obvious advantages, due to generally incompatible experimental conditions required for biological species and for the formation and stabilization of nanoparticles. Silver sulfide (Ag_2S) is a good prospective photoelectric and thermoelectric material. Hence, studies on Ag_2S nanomaterials focused mostly on the preparation of the non-bioconjugated Ag_2S nanocrystals and applications of photoelectric materials, and there have been very few reports on the preparation of bioconjugated Ag_2S nanocrystals.[9] Moreover, it is noted that there is also no report of bioactivity of Ag_2S nanocrystals, although many silver compounds have important bioactivity, such as silver sulfadiazine (SSD), silver sulfadiazine/chlorhexidine, and SSD with cerium nitrate for use in burns, open wounds and chronic ulcers as an antibacterial agent.[10] Herein, we report the facile and controllable synthesis of protein–conjugated Ag_2S nanoparticles, nanorods, and nanowires, and study their possible application in tumor treatment. The as-prepared Ag_2S nanocrystals (especially nanoparticles) showed good inhibition of C6 glioma cell viability.

Our synthesis method involves aqueous chemistry at ambient conditions and does not require rigorous conditions, such as high-temperature and high-pressure apparatus, which were used previously in the synthesis of protein-conjugated Ag_2S nanoparticles.[9] The syntheses of Ag_2S nanoparticles were performed by a one-step procedure, *i.e.* aqueous solution of $AgNO_3$ and thioacetamide (TAA) solution were added simultaneously into the BSA solution with stirring. Then it was kept static under nitrogen protection for 72 h to form the Ag_2S nanoparticles. TAA was comparatively unstable and slowly hydrolyzed to release S^{2-} ions into the reaction solution. The synthesis of Ag_2S nanorods was performed by a two-step procedure according to our previous experiment.[11] The first step was the generation of the silver(I)–BSA complex by mixing of the $AgNO_3$ and BSA solutions for 6 h. The second step was the formation of Ag_2S nanorods by adding TAA into the above mixing solution at ambient condition. The only difference of the two above mentioned syntheses lies in the fact that there was no chelation time between Ag^+ and BSA for the former, but there was in the latter. The synthesis of Ag_2S nanowires were performed by the AAO template-based method. The AAO tubular films were prepared by a two-step aluminium anodic oxidation process as the reference.[12] Then the AAO tubular membrane with a pore size of 50 nm and a thickness of over 1000 nm was mounted between the two halves of a H-tube cell, each of which contains a part of the insoluble salt (Ag_2S), *i.e.*, Ag^+ on one side and S^{2-} on the other side. When the two solutions met in the channel of the AAO tubular membrane, Ag_2S nanowires would be formed. A set of control experiments was carried out in the aqueous solution without BSA, in which all other conditions were the same as the nanoparticles synthesis. As a result, bulk crystals were obtained. In addition, in order to investigate the possible application of these nanocrystals in inhibiting tumor cells, a C6 glioma cell line was used as the model to study the inhibition effects of Ag_2S bulk crystals and nanocrystals at the same content of 0.05 mg ml^{-1}, and cell viability was measured by an MTT assay based on succinic dehydrogenase activity at OD 490 nm.

Fig. 1a–b, c–d and e–f are representative TEM images and SAED pattern of the as-prepared Ag_2S nanoparticles, nanorods and nanowires, respectively. The nanoparticles exhibited a sharp particle distribution with an average particle diameter of 65 nm (Fig. 1a), which was composed of smaller particles 10 nm in diameter, as shown in Fig. 1b. The nanorods and nanowires had mean sizes of 40 and 50 nm in diameter and 220 nm and over 1000 nm in length, respectively (Fig. 1c and e). All of these nanocrystals are obviously well dispersed and uniform (SEM images are shown in ESI†). In order to observe the details of conjugation of BSA-nanocrystals, the as-

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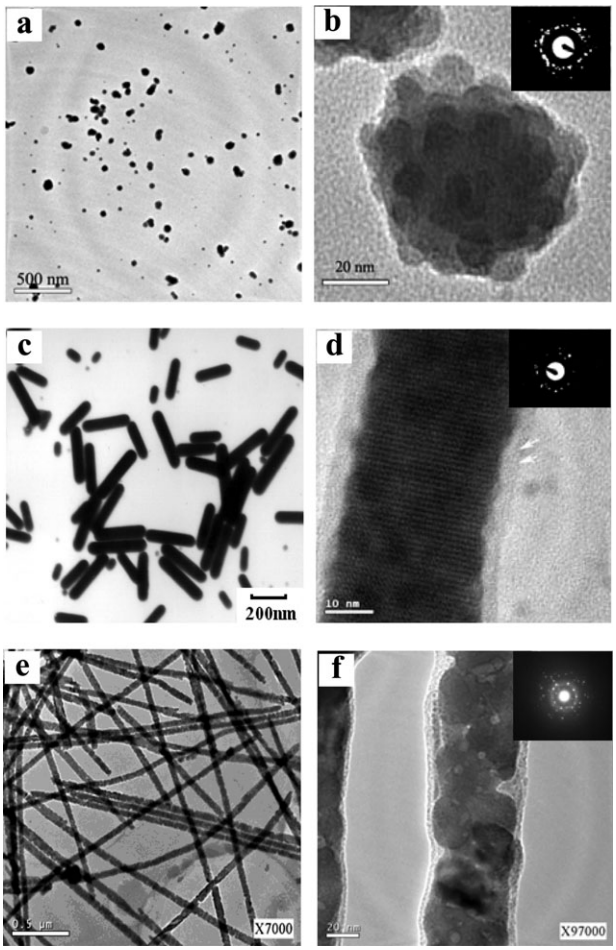

Fig. 1 TEM images of silver sulfide nanoparticles (a and b), nanorods (c and d) and nanowires (e and f). The insets in (b), (d) and (f) show the corresponding SAED pattern.

prepared nanocrystals were dyed by using phosphotungstic acid hematoxylin stain for TEM experiments. As shown in Fig. 1b, d and f, the edges of the nanocrystals look fuzzy and amorphous after being stained, showing the nanoparticles, nanorods and nanowires are uniformly coated with BSA, just like the arrangement that would be expected if each nanoparticle, nanorod or nanowire was immersed in a small pot of BSA.

Moreover, the FTIR spectra of pure BSA and BSA-conjugated Ag_2S nanocrystals were determined. The FTIR peaks of pure BSA at 3308 cm^{-1}, 3068 cm^{-1}, 1656 cm^{-1} and 1539 cm^{-1} are assigned to the stretching vibration of the hydroxyl group, amide A′, amide I and amide II, respectively. Comparing the spectra of BSA-conjugated nanocrystals with that of pure BSA, the characteristic peaks of the hydroxyl group and amide A′ shifted to a high wavenumber of about 100 cm^{-1} and 120 cm^{-1}, respectively, which hints the strong coordination between Ag_2S surfaces and –OH and –NH groups in BSA. The further study by thermogravimetry-differential thermal analysis (TG/DTA) indicated that the protein content in nanoparticles, nanorods and nanowires was 6%, 11% and 12%, respectively. Furthermore, the nanorods exhibit good crystalline and clear lattice fringes from the HRTEM. The insets in Fig. 1b, d and f are the corresponding SAED patterns, revealing that the nanorods are crystalline and can be indexed to monoclinic Lt–Ag_2S. But the nanoparticles and nanowires have a polycrystalline structure. On the basis of these results, it can be hypothesized that the nanocrystals were conjugated with the BSA protein. And the result showed that the presence of BSA was a key factor in controlling and regulating the shape of the Ag_2S crystals. In the control experiment, bulk Ag_2S crystals were obtainted. From the SEM images (see ESI†), the bulk crystals show a ruleless bulk morphology, and the size is very inhomogeneous.

Fig. 2 shows the photoluminescence (PL) spectra of different nanocrystals with an excitation wavelength of 280 nm. The pure BSA (curve 1) has an emission peak of 346 nm, however, the emission peaks of all the nanocrystals appear at 425 nm (curve 2, 3, 4), shifted to higher wavenumber by about 79 nm. The results suggested that there may be conjugate bonds between the Ag_2S nanocrystals and BSA.

Fig. 3 shows the inhibition rate of the nanocrystals on glioma cell viability. The Ag_2S bulk crystals show no significant effect on C6 glioma cell. However, the inhibition rate of nanoparticles, nanorods and nanowires reach 35.9 ± 5.6%, 20.1 ± 4.3%, and 16.5 ± 4.2% compared with the control group (without nanocrystals, $n = 6$, $p < 0.01$), respectively. Neuroblastoma is a frequently occurring disease. For example, neuroblastoma accounts for about 9% of all childhood cancers. The research showed nanoparticles could provide a new way to kill the tumor cells directly by inducing cell physiological functional disorder.[13] The biological activity of Ag_2S nanoparticles, nanorods, and nonowires on C6 glioma cells might suggest their use in curing glioma disease or other tumors. Therefore, tumor treatment in *in vivo* models by a local injection method should be examined, and also the mechanism of the effect of the silver sulfide nanoparticles, nanorods and nanowires on tumor cells needs to be studied.

This paper offers a new way to synthesize the bioactive inorganic nanoparticles directly conjugated with biological

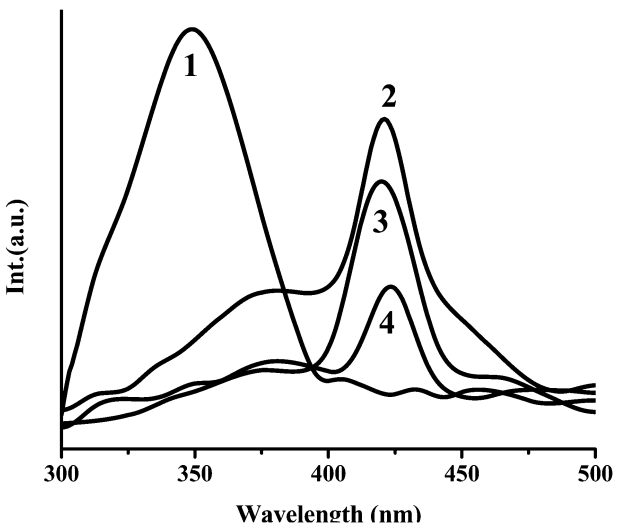

Fig. 2 Photoluminescence (PL) spectra of different nanocrystals with an excitation wavelength of 280 nm, (1): pure BSA; (2): nanoparticles; (3): nanorods; (4): nanowires.

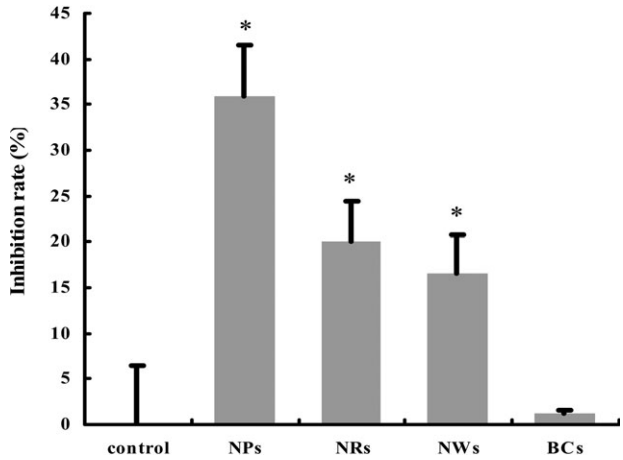

Fig. 3 The inhibition rate of Ag_2S nanoparticles (NPs), nanorods (NRs), nanowires (NWs) and bulk crystals (BCs) on the proliferation of C6 glioma cells measured by the MTT method. "*" means significant difference compared with control, $n = 6$, $p < 0.01$.

species, and shows that nanomaterials with different shapes have different bioactivity. This work was financially supported by the National Basic Research Program of China (Grant No. 2005CB724306) and the National Science Foundation of China (Grant No. 20771036).

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