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Neuroprotective effect of single-wall carbon nanotubes with built-in peroxidase-like activity against β -amyloid-induced neurotoxicity^{†‡}

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Carbon nanotubes (CNTs) have emerged as a leading nanomaterial for biomedical applications because of their extraordinary properties, which make them useful as delivery vehicles for drugs, proteins, and DNA into cells. However, the numerous applications of carbon nanotubes inevitably increase the potential risk of this nanomaterial. To address this issue, it is necessary to develop protocols for the effective and safe degradation of CNTs. In this study, we demonstrate a self-degradation route for single-wall carbon nanotubes mediated by the built-in peroxidase-like activity of bacterial magnetic nanoparticles (BMPs). Bio-compatible BMPs which originated from *Magnetospirillum* sp. AMB-1 were directly conjugated through covalent bonding to functionalized SWNTs (f-SWNTs) without any additional functionalization processes. This SWNT-BMP hybrid was proven to exhibit highly synergetic peroxidase-like activity, and BMPs act as a highly effective intrinsic peroxidase for the self-degradation of BMP-decorated SWNTs. Moreover, it was shown to be an inhibitor that reduces the formation of β -amyloid ($A\beta$) fibrils, which are considered a key element in Alzheimer's disease. Thereby the SWNT-BMP hybrid exerts neuroprotective effects against β -amyloid ($A\beta$) fibrillation-induced neurotoxicity in SH-SY5Y human neuroblastoma cells. These results suggest that the SWNT-BMP hybrid could offer a new approach for treating or preventing neurodegenerative diseases.

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Introduction

The mechanism of β -amyloid ($A\beta$)-induced neurotoxicity is complex and remains unclear. Nevertheless, the aggregation of $A\beta$ in the extracellular space is a hallmark in the pathogenesis of Alzheimer's disease (AD). Some studies suggest that $A\beta$ can cause cell death in AD by evoking a cascade of oxidative damage to neurons.^{1–4} Therefore, the prevention of $A\beta$ protein aggregation and use of antioxidant compounds have been actively discussed as beneficial for AD therapy.⁵

It was recently shown that nanoparticles can play an important role in forming protein aggregates, including amyloid fibrils, *via* inducing or preventing protein misfolding.^{6–9} Polyoxometalates (POMs) or graphene oxides (GOs) can inhibit $A\beta$ aggregation or dissociate $A\beta$ fibrils depending on their hydrophobic surface's physicochemical properties.^{10,11} It was found that magnetic nanoparticles (Fe_3O_4) possess intrinsic

peroxidase-like catalytic activity, thus opening the door for the development of nanoscale materials in the biochemical field.¹² Since this discovery, various inorganic nanomaterials have been reported to have peroxidase-like catalytic activities.^{13–15} Subsequently, several hybrid complexes with inorganic nanomaterials incorporated within different matrices have been constructed and proved to be highly efficient catalyst systems in various organic transformation reactions.^{16,17} Some recent reports showed that carboxyl-modified graphene oxide (GO) and single-wall carbon nanotubes (SWNTs) display intrinsic peroxidase-like activities, such that they could catalyze 3,3',5,5'-tetramethylbenzidine (TMB) to produce a blue-colored product in the presence of H_2O_2 .^{18–20} The results clearly showed that the observed "catalytic" effect could be attributed to the intrinsic properties of these materials rather than any metal residues.²¹

Several methods have been reported for attaching various types of nanoparticles onto carbon nanotubes (CNTs) to combine the unique properties of CNTs with the innovative properties of novel nanoparticles in one discrete structure.^{22,23} In this study, biocompatible bacterial magnetic nanoparticles which originated from *Magnetospirillum* sp. AMB-1 were directly conjugated through covalent bonding to functionalized SWNTs (f-SWNTs) without any additional functionalization processes. This SWNT-BMP hybrid was proven to exhibit highly synergetic peroxidase-like activity; BMPs act as a

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highly effective intrinsic peroxidase for the self-degradation of BMP-decorated SWNTs. Moreover, the BMP–SWNT hybrid was shown to be an inhibitor that reduces the formation of A β fibrils, which are considered a key element in Alzheimer's disease. Our findings could offer a new approach for treating or preventing neurodegenerative diseases.

Materials & methods

Carboxyl functionalization of SWNTs

A quantity of 10 mg of SWNTs (ASP-100F) was added to a mixture of concentrated sulfuric acid (15 ml) and nitric acid (5 ml) and ultrasonicated for more than 24 h. A volume of 200 ml of deionized (DI) water was then added to the mixture. The diluted mixture was filtered using a 70 μ m filter to eliminate any unnecessary SWNT aggregates. A second filtering step was performed using 2 μ m filter paper to harvest f-SWNTs. Any remaining toxic ingredients and additives were removed by washing several times using excess DI water. After sufficiently washing the filter paper, the remaining f-SWNTs on the filter paper were placed into 1 ml of DI water and vortexed for 3 min to disperse the f-SWNTs in the solution.

BMP extraction and purification

BMPs were obtained from *Magnetospirillum* sp. AMB-1 (ATCC® 700264) that were cultured in magnetic spirillum growth medium (MSGM) for 5 days in a shaking incubator at 30 °C under anaerobic conditions. The cultured *Magnetospirillum* sp. AMB-1 were centrifuged for 25 min at 5000 rpm and then lysed by sonicating (VCX500, Sonics & Materials, USA) for 30 min. BMPs were collected using a neodymium–iron–boron (NdFeB) magnet and then washed 5 times with 1 $\times$ PBS. The collected BMPs were dispersed in 1 $\times$ PBS and sterilized by autoclaving (121 °C, 15 min). The concentration of the extracted BMPs was determined using an inductively coupled plasma-atomic emission spectrometer (ICP-AEX, ICPS-7500, Shimadzu, Japan).

Conjugation of f-SWNTs to BMPs

A quantity of 4 mg of *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC, Sigma-Aldrich, Korea) and 11 mg of *N*-hydroxysulfosuccinimide sodium salt (Sulfo-NHS, Sigma-Aldrich, Korea) were dissolved in 100 μ l of 0.5 M and 0.1 M MES buffer (2-(*N*-morpholino)ethanesulfonic acid, pH = 8.3), respectively. The mixture was then added to 1 ml of 1 mg ml⁻¹ carboxylated SWNTs for reaction with a carboxyl group for 15 min to form an amine-reactive *O*-acylisourea intermediate. After adjusting the pH (ranging from 7 to 8) by adding 30 μ l of 20 $\times$ PBS buffer, the solution was mixed with 10 μ l of 1 mg ml⁻¹ BMPs contained in 1 $\times$ PBS and placed on a shaker at room temperature for 2 h to accelerate the binding reaction. Unconjugated f-SWNTs were separated from the SWNT–BMP conjugates three times in 1 $\times$ PBS buffer under a strong magnetic field.

Raman microscopy of the SWNT–BMP hybrid

Raman spectra were collected using a LabRam Aramis (Horiba Jobin-Yvon, France) spectrometer with an Ar-ion laser (514.532 nm) excitation source. A drop of each sample was loaded onto clean glass wafers and dried before analysis. The samples were focused using an optical microscope with a spot size of approximately 1 μ m. The excitation power was 1 mW, and the spectra were calibrated prior to acquisition using a glass wafer.

Catalytic activity of the SWNT–BMP hybrid

Catalytic experiments were performed using 20 μ l of 1 mg ml⁻¹ SWNT–BMP hybrid ($\sim 7.2 \times 10^9$ nanoparticles in a reaction volume of 200 μ l) in a 25 mM NaH₂PO₄ buffer solution with a 1.6 mM TMB substrate and 50 mM H₂O₂. The samples were allowed to react for 20 min at 40 °C and centrifuged at 15 000g for 10 min to collect the precipitate at the bottom of the tube. The supernatant solution from each tube was then transferred into a 96-well plate. A volume of 20 μ l of 0.18 M H₂SO₄ was added to each sample to stop the catalytic reaction, and the optical absorbance was read at 450 nm (yellow color). For kinetic studies, the optical absorbance was read every 30 s at 650 nm for 30 min at room temperature (blue color). To prevent unexpected signals from particles, we placed small magnets beside each well to hold the particles near the magnets.

Degradation of f-SWNTs

To demonstrate BMP-induced degradation, both f-SWNTs and the SWNT–BMP hybrid were simply incubated in 200 μ M H₂O₂ for 24 h at 37 °C. For hMPO-induced degradation, a quantity of 5 μ g of human myeloperoxidase (hMPO) was mixed with 100 μ l of 1 mg ml⁻¹ f-SWNTs suspended in 50 mM phosphate buffer containing 140 mM NaCl and 100 μ M diethylenetriaminepentaacetic acid (DTPA), a transition metal chelator. Hydrogen peroxide was added at a rate of 200 μ M per hour for 5 h. Due to the loss of hMPO activity in the incubation system, the enzyme was replenished after 5 h, and the reaction mixture was maintained at 37 °C for 24 h. Sodium hypochlorite was added every hour to give concentrations of 200 μ M for 24 h at 37 °C.

Thioflavin T assay for A β fibril formation

First, a 2.5 mM ThT stock solution was prepared by adding 8 mg of ThT to 10 ml of 1 $\times$ PBS buffer and filtering through a 0.2 μ m syringe filter. This solution was stored in the dark. This stock solution was diluted into the 1 $\times$ PBS buffer on the day of analysis to generate a 20 μ M ThT working solution and covered with aluminum foil. Then, 1 mg of A β 25–35 (Sigma Aldrich, Korea) was dissolved in DMSO to the final concentration of 10 mM. This A β stock solution was diluted into 1 $\times$ PBS buffer to generate 10, 50, and 100 μ M A β solutions and incubated at RT with gentle shaking for an appropriate amount of time. On the day of analysis, 5 μ l of the ThT

working solution was mixed with 95 μL of each A β fibril solution in a black 96-well plate and measured using a microplate reader (Victor3, Perkin Elmer, USA). The fluorescence intensity was measured by excitation at 450 nm and emission at 485 nm.

MTS assay for cell viability

The 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay was performed to evaluate the hybrid's cytotoxicity and cell viability. A human-derived cell line, SH-SY5Y (neuroblastoma), was used for biocompatibility evaluation. The cells (5×10^3 cells per well) were seeded into a 96-well plate with 120 μL of culture medium. After the cells were sufficiently cultured, 25 μL of the CellTiter 96® Aqueous One Solution reagent (Promega, USA) was added to each well. The plate was then incubated at 37 °C for 1–4 h in a humidified 5% CO₂ atmosphere, and the absorbance was recorded at 490 nm using a microplate reader (Sunrise, Tecan, Switzerland). All assays were performed in three separate experiments. The absorbance of the control cells was defined as 100% viability in every assay.

Statistical analysis

All data are presented as means $\pm$ STD. Statistical analysis was performed using one-way analysis of variance (ANOVA). A probability value of less than 0.05 was considered significant.

Results & discussion

Synthesis of the f-SWNT-BMP hybrid

The BMPs from the *Magnetospirillum* sp. AMB-1 used in our approach are inherently biocompatible, can be effectively

conjugated to other biomolecules, and disperse well in aqueous solutions because of their stable lipid membrane surrounding the magnetic core (see the ESI†).^{24,25} The lipid membrane is rich in phosphatidyl-ethanolamine (approximately 29%), which provides amine-binding sites, making the BMPs easy to disperse and conjugate to other biomolecules, such as antibodies, enzymes, and polypeptides.

Therefore, we could make our hybrid *via* a very simple method. We used the lipid bilayer functional groups to directly link with the CNTs *via* a common conjugation method without any functionalization process involving BMPs. SWNTs (Hanwha Chemical Co. Ltd., Korea) were functionalized through covalent modification by attaching carboxyl groups (–COOH) to the SWNT surfaces in acidic solutions.²⁶ The BMPs were conjugated to the f-SWNTs with 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC; Thermo Fisher Scientific Inc., USA), which facilitates carboxyl-to-amine conjugation (Fig. 1(a)).

Direct evidence of the conjugation of BMPs to the f-SWNTs was obtained by magnetic attraction (Fig. 1(b) and (c)). In general, the magnetic susceptibility is induced in CNTs by metal-filled particles or metallic impurities entrapped during the fabrication process. After functionalization of the SWNTs, an SEM-EDX analysis confirmed that most metal catalysts such as Ni, Co, and Fe disappeared in the f-SWNTs (see the ESI†). After functionalization, the f-SWNTs and the SWNT-BMP hybrid were fully dispersed in DI water. However, the SWNT-BMP hybrid was attracted by a magnet within one minute unlike the f-SWNTs. Unconjugated f-SWNTs were separated from the SWNT-BMP conjugates three times in 1 $\times$ PBS buffer under a strong magnetic field (Fig. 1(c)). In this process, we are confident that most of the unconjugated f-SWNTs have been removed and only the SWNT-BMP hybrid has been purified over 90%.

Fig. 1 (a) Illustration of SWNT-BMP hybrid formation. EDC and sulfo-NHS are used to activate the carboxyl groups of f-SWNTs. BMPs are directly conjugated to f-SWNTs through their primary amine groups. Magnetic attraction of (b) f-SWNTs and (c) SWNT-BMP hybrid.

The arrangement of BMPs on the f-SWNT surfaces were confirmed with electron microscopy images and the binding properties of the SWNT-BMP hybrid were confirmed by Raman spectroscopy and XPS analysis (see the ESI†).

Catalytic activity of the SWNT-BMP hybrid as a peroxidase

We analyzed the mimetic peroxidase activity of the BMPs and the SWNT-BMP hybrid by measuring the extent of oxidation of TMB, a peroxidase substrate, by H_2O_2 treatment, to the oxidized product, indicated by a change to blue color observed at a wavelength of 650 nm. As shown in Fig. 2(a), the TMB/ H_2O_2 system without the SWNT-BMP hybrid and BMPs was colorless (control), while the BMPs/TMB/ H_2O_2 system showed a deep blue color (BMP). Furthermore, when the SWNT-BMP hybrid was added to the system, an even deeper blue color was observed (Hybrid). These results confirm that BMPs show peroxidase-like activity toward TMB, and the hybrid enhances such activity.

To further characterize the peroxidase-like activity of the hybrid, a series of experiments were performed by changing

the concentration of BMPs while maintaining a constant concentration of the SWNTs (0.1 mg ml^{-1}) for the formation of the hybrid (Fig. 2(b)). Interestingly, we found that the peroxidase-like activity was significantly enhanced upon the formation of the SWNT-BMP hybrid (Fig. 2(b) and the ESI†). These results confirm that BMPs show peroxidase-like activity toward TMB, and the SWNT-BMP hybrid improves this activity.

Fig. 2(c) shows the time-dependent peroxidase-like activity changes against BMPs and the SWNT-BMP hybrid. The color change for the hybrid solution was much faster, and resulted in a higher peroxidase-activity than that from the BMPs alone. SWNTs possess a high surface-to-volume ratio, as well as high affinity for hydrophobic molecules, such as TMB, that may increase the local concentration of the substrate and improve the catalytic effect. We also found that the catalytic activity of the hybrid is dependent on the concentration of H_2O_2 . Like the natural enzyme-catalyzed reaction, the hybrid-catalyzed reaction is inhibited at high H_2O_2 concentrations (Fig. 2(d)). Moreover, the SWNT-BMP hybrid quickly catalyzed the oxidation of the peroxidase substrate TMB and induced a rapid initial reaction rate and a subsequent

Fig. 2 (a) Peroxidase-like activity of Hybrid. Color change of the TMB substrate in TMB + H_2O_2 (control), TMB + H_2O_2 + BMPs (BMP), and TMB + H_2O_2 + SWNT-BMP hybrid (Hybrid) showing the synergetic peroxidase-like activity of Hybrid. (b) Synergetic peroxidase-like activity of the SWNT-BMP hybrid. (c) Comparison of the time-dependent peroxidase-like activity changes with the SWNT-BMP hybrid and BMPs. (d) The catalytic activity of the SWNT-BMP hybrid is dependent on the concentration of H_2O_2 . (e) Moreover, the SWNT-BMP hybrid quickly catalyzed the oxidation of the peroxidase substrate TMB and induced a rapid initial reaction rate and a subsequent stable stage depending on the concentration of H_2O_2 .

stable stage depending on the concentration of H_2O_2 (Fig. 2(e)).

To further analyze the catalytic mechanism, the catalytic activity of the hybrid was studied by kinetic theory of enzyme using H_2O_2 and TMB as substrates. A series of experiments were performed by changing the concentration of one substrate and keeping the concentration of the other constant. The hybrid-catalyzed reaction is inhibited at high H_2O_2 concentrations like the enzyme catalyzed reaction, however, within a suitable range of H_2O_2 concentrations, typical Michaelis–Menten curves were observed. The maximum initial velocity (V_{max}) and Michaelis–Menten constant (K_{m}) were obtained using the Lineweaver–Burk plot and are shown in the ESI.† These results support the idea that the SWNT–BMP hybrid exhibits peroxidase-like behavior toward typical peroxidase substrates.

Self-degradation of BMP-decorated f-SWNTs

Previously, it was shown that the human neutrophil enzyme myeloperoxidase (hMPO) catalyzes the biodegradation of single-walled carbon nanotubes *in vitro*.²⁷ Gao *et al.* found that magnetic Fe_3O_4 nanoparticles could be used as an intrinsically active catalyst for some oxidation reactions.¹² BMPs with magnetic cores composed of Fe_3O_4 can also act as an effective intrinsic peroxidase. Based on these findings, we attempted to demonstrate the degradation of BMP-decorated f-SWNTs using the intrinsic mimetic enzyme activity of the BMPs, which is similar to that found in magnetic nanoparticles.

The f-SWNTs and SWNT–BMP hybrid were incubated with 200 μM H_2O_2 for 24 h at 37 °C. TEM was used to track the morphological changes of the resulting BMP-induced degradation (Fig. 3(a)–(d)). Most of the f-SWNTs remained bundled under H_2O_2 conditions after 24 h of incubation

(Fig. 3(b)). Remarkably, the TEM images revealed that the majority of the hybrid bundles were degraded, leaving residual BMPs and carbonaceous materials in the BMP-induced degradation samples under the same conditions as those for f-SWNTs (Fig. 3(d)). These results strongly suggest that BMPs can act as a highly effective intrinsic peroxidase for SWNT degradation. The Raman spectra of the f-SWNTs and hybrid samples underscore the key function of the BMPs in the degradation process. After 24 h of H_2O_2 treatment, the $I_{\text{D}}/I_{\text{G}}$ ratio for the hybrid drastically increased compared with that for the f-SWNT sample due to the BMP-induced degradation (Fig. 3(e) and (f)).

To compare the effect of BMP-induced SWNT degradation with other degradation methods, we also performed hMPO-induced SWNT degradation according to a previous method.²⁷ As shown in Fig. 4, the intensity of the D-band for the hybrid under BMP-induced degradation was higher than that for the f-SWNTs under hMPO-induced degradation. The relative degradation ratio ($I_{\text{D}}/I_{\text{G}}$) for the BMP-induced degradation (Fig. 4(1)) was also higher than that for the hMPO-induced degradation (Fig. 4(2)). In addition, in the Raman spectra for the BMP-induced degradation, the hybrid showed clear disappearance of the radial breathing mode (RBM) at 169 cm^{-1} , indicating the destruction of the tubular structures in the f-SWNTs.

Inhibition of amyloid beta fibrillation with SWNT–BMP hybrid

The effect of the Hybrid on the aggregation of $\text{A}\beta$ peptides was investigated using a ThT assay (Fig. 5). ThT fluorescence is considered a highly sensitive marker for the amyloid state of various aggregating proteins and peptides. ThT in solution displays weak fluorescence in its free form, whereas its fluorescence is strong when bound to amyloid fibrils.²⁸

Fig. 3 TEM images (a)–(d) and Raman spectra (e and f). Most of the f-SWNTs remained bundled under H_2O_2 conditions after 24 h of incubation (b), whereas the majority of the SWNT–BMP hybrid bundles were degraded, leaving residual BMPs and carbonaceous materials in the samples after the BMP-induced degradation (d). Scale bars are 200 nm. In the Raman spectra, the $I_{\text{D}}/I_{\text{G}}$ ratio for the hybrid drastically increased compared with that for the f-SWNT sample due to the BMP-induced degradation (e and f).

Fig. 4 Raman spectra of the hMPO-induced and BMP-induced degradation of f-SWNTs. The intensity of the D-band for the hybrid under BMP-induced degradation (1) was higher than that for the f-SWNTs under hMPO-induced degradation (2). The relative degradation ratio (I_D/I_G) for the BMP-induced degradation was also higher than that for the hMPO-induced degradation. In addition, in the Raman spectra for the BMP-induced degradation, the hybrid showed clear disappearance of the radial breathing mode (RBM) at 169 cm⁻¹, indicating the destruction of the tubular structures in the f-SWNTs.

Fig. 5 Effect of SWNTs, BMPs, and Hybrid on the inhibition of β -amyloid fibrillation (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

A β 25–35 (100 μ M) was mixed with 10 μ g mL⁻¹ SWNTs, 0.5 μ g mL⁻¹ BMPs, or 10 μ g mL⁻¹ SWNT-BMP hybrid (SWNT: BMP = 20:1). After 3 h, both SWNTs and SWNT-BMP hybrid showed inhibition of A β fibrillation, whereas BMPs did not show such behavior. After 48 h, however, the fluorescence intensity in the presence of BMPs was also considerably decreased compared with that of only A β . Additionally, A β fibril formation was extremely inhibited by the SWNT-BMP hybrid. There was no interference in ThT fluorescence by f-SWNTs, BMPs, or the SWNT-BMP hybrid used in this study (see the ESI†).

To visualize A β fibrils, FITC-labeled A β antibodies were added to the samples after 7 days of A β peptide incubation in the presence and absence of the hybrid (see the ESI†). The fluorescence image of the A β fibrils without the hybrid shows a strong signal compared with the A β fibril sample in the presence of the hybrid.

Nanoparticles have high surface-to-volume ratios, and this enormous surface means that proteins may be bound onto the particle surface. As demonstrated in previous studies,^{29,30} the interaction between the hydrophobic groups of the A β peptide and SWNTs could affect the stability of the electrostatic network of the A β peptide and lead to conformational changes in the peptide.²⁰ BMPs covered with a stable lipid membrane containing lipids and proteins could also interfere with the fibrillation of A β peptides through electrostatic interactions and hydrogen bonding.^{31,32} We thought that hybrids composed of SWNTs and BMPs may act as effective inhibitors of the formation of A β fibrils.

Effect of the SWNT-BMP hybrid on the A β -induced neurotoxicity

The cytotoxicity of the Hybrid against SH-SY5Y cells, which are human-derived neuroblasts, was evaluated using the MTS assay and normalized to the viability of the control cells without treatment with the SWNT-BMP hybrid (100% viability).

The cytotoxic effect of the SWNT-BMP on the viability of SH-SY5Y cells was insignificant, even at a high concentration of 20 μ g mL⁻¹ for 1×10^4 cells within 3 days, which is ideal for use in practical applications in the biomedical field (Fig. 6(c) and Fig. S2†). Optical images captured by inverted microscopy also show that there is no significant difference between the survival of SH-SY5Y cells without (Fig. 6(a)) and with (Fig. 6(b)) the SWNT-BMP hybrid (10 μ g mL⁻¹) after 3 days.

For the A β fibril-induced neurotoxic conditions in SH-SY5Y cells, 200 μ M A β 25–35 with and without 10 ng mL⁻¹ of the SWNT-BMP hybrid was introduced into the SH-SY5Y cells and incubated at 37 °C for 3 days. As shown in Fig. 7,

Fig. 6 Optical microscopy images of cultured SH-SY5Y cells (a) without and (b) with the Hybrid. The scale bars are 200 μ m. (c) Cytotoxic effects of the Hybrid on the viability of SH-SY5Y cells.

Fig. 7 The effect of the SWNT-BMP hybrid on SH-SY5Y cell growth under β -amyloid-induced neurotoxic conditions by the (a) MTS assay and (b) ATP level assay. Control: untreated cells, A β : 200 μ M A β -treated cells, A β + HYB: 200 μ M A β and 10 ng mL⁻¹ SWNT-BMP hybrid-treated cells. Both the MTS and ATP assay results show that the cell viabilities of the SWNT-BMP hybrid-treated cells increased by more than 30% (***p* < 0.001, *n* = 6).

A β mixed with the SWNT-BMP hybrid treated SH-SY5Y cells showed an approximately 30% increase of cell viability compared with the A β injured cells. We suppose that the hydrophobic surfaces of our SWNT-BMP hybrid may interact with the A β peptides and inhibit A β fibril formation. In addition, A β is associated with reactive oxygen species (ROS) generation, which leads to mitochondrial dysfunction, lipid peroxidation, and apoptosis.³³ Previous studies have provided convincing evidence that A β -associated free-radical damage to neurons is a fundamental process in connection with AD.³⁴ Thereby, we believe that our SWNT-BMP hybrid with the built-in peroxidase-like activity of BMPs effectively protects SH-SY5Y cells against A β -induced damage by suppressing the generation of ROS. Our results suggest that the SWNT-BMP hybrid has protective effects against A β fibrillation-induced neurotoxicity, which makes this material a potential therapeutic agent for treating or preventing neurodegenerative diseases.

Conclusions

Biocompatible BMPs originating from *Magnetospirillum* sp. AMB-1 were directly conjugated through covalent bonding to f-SWNTs without any additional functionalization processes. This hybrid exhibited highly synergetic peroxidase-like activity, and moreover, was shown to be an inhibitor that reduces the formation of A β fibrils, which are considered a key element in Alzheimer's disease. Our findings could offer a new approach for treating or preventing neurodegenerative diseases.

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