HEALTH AND MEDICINE

Magnetoelectric dissociation of Alzheimer’s β-amyloid aggregates

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The abnormal self-assembly of β-amyloid (Aβ) peptides and their deposition in the brain is a major pathological feature of Alzheimer’s disease (AD), the most prevalent chronic neurodegenerative disease affecting nearly 50 million people worldwide. Here, we report a newly discovered function of magnetoelectric nanomaterials for the dissociation of highly stable Aβ aggregates under low-frequency magnetic field. We synthesized magnetoelectric BiFeO₃-coated CoFe₂O₄ (BCFO) nanoparticles, which emit excited charge carriers in response to low-frequency magnetic field without generating heat. We demonstrated that the magnetoelectric coupling effect of BCFO nanoparticles successfully dissociates Aβ aggregates via water and dissolved oxygen molecules. Our cytotoxicity evaluation confirmed the alleviating effect of magnetoelectrically excited BCFO nanoparticles on Aβ-associated toxicity. We found high efficacy of BCFO nanoparticles for the clearance of microsized Aβ plaques in ex vivo brain tissues of an AD mouse model. This study shows the potential of magnetoelectric materials for future AD treatment using magnetic field.

INTRODUCTION

Magnetoelectric materials exhibit rigidly coupled magnetic and electric polarization (1, 2), which is the basis for their unique optical (3), mechanical (4), and thermal properties (5). According to the origin of magnetoelectric coupling, two different types of magnetoelectric materials exist: (i) single-phase multiferroic oxides with spin-orbit interactions and (ii) heterogeneous multiphase oxides with individual magnetostrictive and piezoelectric composites linked via interfaces (6). Because of the d-electrons of constituent metal ions (e.g., Fe²⁺, Fe³⁺), which are essential for magnetism (partially occupied d-orbitals) (7), many limitations have been imposed on designing multifunctional single-phase magnetoelectric materials. In contrast, heterogeneous multiphase materials are dominantly influenced by the piezo-magnetoelectric effect occurring at the interfaces between two different composites. Thus, interface engineering strategies, including core-shell structure fabrication, have been used to improve magnetoelectric coupling of heterogeneous multiphase materials through the donation of an additional lattice strain (8).

Bismuth ferrite (BiFeO₃, BFO) and cobalt ferrite (CoFe₂O₄, CFO) are the representative single-phase magnetoelectric materials that have been widely used to construct modern electronics, such as sensors, transducers, and spintronic devices (9). Compared with their single phase structure, the heterogeneous BFO-CFO system shows a much stronger magnetoelectric coupling based on their well-matching lattice structure and induced piezo-magnetoelectric effect as well (10). Recently, Mushtaq et al. (11–13) reported that BFO-coated CFO (BCFO) can efficiently generate excited charge carriers in response to low-frequency magnetic field (e.g., below 1 kHz). Furthermore, the magnetic field–responsive BCFO is biocompatible with human cells [e.g., osteoblasts (12) and neuronal cells (14)] and mouse models (15), hinting at its potential for medical applications.

Here, we report magnetoelectric dissociation of Alzheimer’s β-amyloid (Aβ) aggregates and neurotoxicity mitigation by BCFO nanoparticles under a low-frequency magnetic field. Alzheimer’s disease (AD) is the primary cause of age-related dementia with progressive decline in cognitive functions (e.g., attention, memory, and language), ultimately leading to complete dependency and death (16). According to the amyloid hypothesis (17), overproduction of Aβ peptides and accumulation of their insoluble aggregates (e.g., Aβ fibrils and plaques) in the brain is the major pathological event resulting in synaptic dysfunction and neurodegeneration. As depicted in Fig. 1, we hypothesized that magnetoelectric BCFO nanoparticles dissociate neurotoxic Aβ aggregates into oxidized nontoxic Aβ debris under a low-frequency magnetic field.

Magnetic field allows for superior tissue penetration depth suitable for noninvasive medical treatments of the adult human brain. For example, magnetic resonance imaging (MRI) can map the entire brain of patients and diagnose the early stage of AD using a magnetic field and MRI contrast agents (e.g., gadolinium and magnetite) without any surgical procedure (18, 19). In addition, irradiation of low-frequency magnetic field on the scalp—known as “transcranial magnetic stimulation”—can alleviate depression or pain symptoms by inducing intracranial electric current that causes neuronal depolarization without any brain tissue damage (20). As such, a low-frequency magnetic field (e.g., below 1 kHz) with a nonthermal effect is medically acceptable for treating neural tissue (21), in contrast to a high-frequency magnetic field inducing a substantial amount of heat generation (22). In addition, low-frequency magnetic field has a much lower risk of causing damage to the outer tissues (e.g., scalp and skull) for the deep brain treatment in patients than other physical stimuli, including mechanical vibration (ultrasound), heat radiation (infrared light), and ionizing radiation (x-ray) (23, 24). Herein, we propose a previously unknown biomaterial platform using low-frequency magnetic field that can overcome the potential risk of the conventional approaches [e.g., sonodynamic (25, 26), photodynamic (27–29), radiodynamic (30), and magnetic hyperthermia platforms (31)]. In the current study, we unveil the potential of magnetoelectric materials for low-frequency magnetic field-induced dissociation of toxic Aβ aggregates, a major pathological hallmark of AD.
RESULTS AND DISCUSSION

We synthesized magnetoelectric BCFO nanoparticles by applying sol-gel chemistry to CFO nanoparticles (Fig. 2A). First, we prepared CFO nanoparticles through a facile hydrothermal treatment of metal ion precursors at 130°C for 15 hours according to the literature (11). The as-prepared CFO nanoparticles were black powders exhibiting a spherical morphology (Fig. 2B and fig. S1) with a size of 26.5 ± 6.2 nm (n = 810) (fig. S2). To make a BFO shell structure on CFO nanoparticles, we applied wet chemical coating and calcination processes to the as-prepared CFO nanoparticles with bismuth and ferric ions under different conditions. The as-synthesized BCFO nanoparticles were dark brown solids showing a CFO-like morphology (Fig. 2C and fig. S1) with a size of 33.2 ± 8.2 nm (n = 680) (fig. S2).

We attribute the color and size changes from CFO to BCFO nanoparticles to the BFO shell structure (approximately 3.4 nm thickness) on CFO nanoparticles. Our x-ray photoelectron spectroscopy results confirmed that BCFO nanoparticles were composed of Co^{2+}, Fe^{3+}, O^{2−}, and Bi^{3+} (fig. S3). We investigated x-ray diffraction (XRD) patterns to examine the existence of crystalline BFO in BCFO nanoparticles. According to the XRD spectra (Fig. 2D), CFO nanoparticles exhibited a cubic spinel structure with the Fd3m space group (a = b = c = 8.384 Å). On the other hand, BCFO nanoparticles had not only the cubic spinel structure but also a rhombohedral perovskite structure with the R3c space group (a = b = 5.5934 Å and c = 13.887 Å), which is in accordance to the crystalline structure of magnetoelectric BFO (32). Furthermore, our scanning transmission electron microscopy (TEM) images displayed the BFO shell structure on BCFO nanoparticles based on the evenly distributed constituent elements on their surface as well as its interplanar lattice spacing of 0.391 nm (Fig. 2E), corresponding to the (012) facet of the rhombohedral perovskite BFO structure (32). These results were in contrast to those of CFO nanoparticles with a different interplanar lattice spacing of 0.294 nm (fig. S4). Together, we concluded that BCFO nanoparticles had a crystalline BFO shell structure on the surface of CFO nanoparticles.

The BCFO nanoparticles produced charge carriers in response to alternating magnetic fields (Fig. 3A). To investigate BCFO’s magnetic behavior, we collected magnetic hysteresis loops (from −5 to +5 T of magnetic field) at ambient conditions. As shown in Fig. 3B, CFO nanoparticles exhibited saturation magnetization (M_s) and remnant magnetization (M_r) at 113.3 and 35.4 electromagnetic units (emu g^{-1}), respectively, indicating their room temperature (RT) ferromagnetism. BCFO nanoparticles had more enhanced ferromagnetic characteristic than CFO nanoparticles (M_s = 123.8 emu g^{-1} and M_r = 39.4 emu g^{-1}). We attribute the increased ferromagnetism to the magnetoelectric coupling of the BFO shell structure (33). The core-shell structure of BCFO nanoparticles is known to enhance the magnetic field–driven electric polarization due to the lattice strain at the interface between two different magnetoelectric materials.
Thus, we envisioned that the magnetoelectric catalysis triggered by thermia) of high-frequency magnetic field (e.g., 5 to 500 kHz) (22). Under low-frequency magnetic field (below 1 kHz) occurs regardless of the induction heating effect (also known as “magnetic hyperthermia”) of high-frequency magnetic field (e.g., 5 to 500 kHz) (22). Thus, we envisioned that the magnetoelectric catalysis triggered by low-frequency magnetic field is suitable for the medical treatment of extremely delicate soft tissues such as the brain without any adverse thermal effect.

We hypothesized that a low-frequency magnetic field (e.g., below 1 kHz) triggers catalysis at the surface of BCFO nanoparticles through the as-generated charge carriers. To examine the advent of the magnetoelectrically driven chemical reactions, we tested dihydroethidium (DHE) and terephtalic acid (TA) assays; DHE assay detects the occurrence of 2-hydroxyethidium through the electron transfer reactions and TA assay probes the generation of 2-hydroxyterephthalic acid based on the hole migration process (34). We found that the excited charge carriers were transferred from the surface of BCFO nanoparticles to the reactants (i.e., DHE and TA) approximately 4.2 times more than that of CFO nanoparticles under a low-frequency magnetic field (1 kHz and 13.6 mT) (Fig. 3C). The charge transfer rate of BCFO nanoparticles was proportional to the strengths (4.6, 9.2, and 13.6 mT) and frequencies (0.25, 0.5, and 1 kHz) of the alternating magnetic fields applied to the solutions (fig. S8). The BCFO nanoparticles generated a negligible amount of heat upon the irradiation of low-frequency magnetic field (Fig. 3D and fig. S9). Insignificant heat release by BCFO nanoparticles suggests that the magnetoelectric catalysis under low-frequency-magnetic field (below 1 kHz) occurs regardless of the induction heating effect also known as “magnetic hyperthermia” of high-frequency magnetic field (e.g., 5 to 500 kHz) (22). Thus, we envisioned that the magnetoelectric catalysis triggered by low-frequency magnetic field is suitable for the medical treatment of extremely delicate soft tissues such as the brain without any adverse thermal effect.

We hypothesized that the drastic morphological changes of Aβ fibrils were caused by the damage of the Aβ fibrils’ secondary structure that builds up their structural robustness. We applied thioflavin T (ThT) assay and circular dichroism (CD) analysis to evaluate the change of β sheet structure in Aβ fibrils. ThT is a gold standard dye for assessing the degree of amyloid aggregation because it emits
strong fluorescence upon it, binding to the β sheet structure (see fig. S12 for representative fluorescent images of ThT assay) (38). As shown in Fig. 4C and fig. S13, ThT fluorescence intensity decreased markedly from 1.00 to 0.36 after irradiation of low-frequency magnetic field in the presence of BCFO nanoparticles for 6 hours, implying the dissociation of the β sheet structure in Aβ fibrils. We further collected CD spectra of Aβ fibril solutions to spectroscopically support the ThT assay results. As shown in Fig. 4D, characteristic peak intensities of the β sheet structure (at 196 and 217 cm$^{-1}$) in Aβ fibrils were reduced by almost half when the Aβ fibrils were subjected to BCFO nanoparticles under low-frequency magnetic field. According to Beta Structure Selection (BeStSel) algorithm analysis results of the CD spectra, the magnetoelectric treatment not only decreased β sheet structure in Aβ fibrils but also increased random structure (fig. S14). Note that neither BCFO nanoparticles nor low-frequency magnetic field alone could affect the native β sheet structure of Aβ fibrils. Furthermore, we observed that the decreased β sheet structure in the Aβ debris did not recover even after 72 hours of incubation at 37°C without any stimulation (fig. S15). These results imply that magnetoelectrically excited BCFO nanoparticles dissociated β sheet–rich Aβ fibrils into thermodynamically stable, amorphous amyloid aggregates, according to the literature (39).

We attribute the results of the ThT and CD analyses to the magnetoelectric coupling effect of BCFO nanoparticles. To examine the action of magnetoelectric catalysis on the dissociation of Aβ fibrils, we used different scavengers for trapping electrons, holes, hydroxyl radicals (•OH), and superoxide ions (•O$_2^-$) (table S1). As shown in fig. S16, the dissociation of Aβ fibril’s β sheet structure was suppressed by the depletion of not only the excited electrons and holes but also •OH and •O$_2^-$ from the surface of BCFO nanoparticles. Note that the scavengers themselves did not change the amount of Aβ fibril’s β sheet structures (fig. S16). Our results imply that the dissociation of Aβ fibrils was caused by the as-generated free radical species (•OH and •O$_2^-$), which were generated by the charge transfer to dissolved oxygen molecules in the solution (fig. S17) (40).

According to the literature (41–43), free radical species can post-translational modify the amino acid sequence of Aβ fibrils by depriving electrons from Aβ peptides to induce structural instability. For example, histidine (His$^b$) in Aβ peptides can be converted to 2-oxo-histidine by the action of •OH and •O$_2^-$ (fig. S18), and methionine (Met$^{35}$) also can be oxidized to methionine sulfoxide by the free radical species (fig. S19) (44). As histidine and methionine of Aβ peptides construct outer β strands and axial structure in Aβ fibrils, respectively (45–48), the oxidation of these amino acid residues can deconstruct Aβ fibril structure. To investigate the occurrence of oxidation on Aβ fibrils, we collected mass-to-charge (m/z) distributions of Aβ fibril solutions to spectroscopically support the ThT assay results (fig. S20). In the absence of either BCFO nanoparticle or low-frequency magnetic field, Aβ fibrils exhibited a strong peak at m/z 4516 (Aβ$_{12}$ + H$^+$), indicating the typical molecular weight of the pristine Aβ peptide. In contrast, Aβ fibrils after the magnetoelectric dissociation showed oxidation signals at m/z 4531 (Aβ$_{12}$ + O). In addition, our additional MALDI-TOF-MS analysis results with laser-induced fragmentation suggested that the oxidized amino acid residue is Met$^{35}$, and 28.7% of Met$^{35}$ was converted to methionine sulfoxide after the magnetoelectric treatment (fig. S21). The amount of as-produced methionine sulfoxide (28.7%) is very similar to the amount of oxidized Aβ peptides (30.0%) constituting Aβ fibrils after the magnetoelectric dissociation (fig. S22). Together, our results show that magnetoelectrically excited BCFO nanoparticles can dissociate β sheet structures of Aβ fibrils by causing the Met$^{35}$ oxidation of constituent Aβ peptides.

To examine the neural biocompatibility of BCFO nanoparticles, we tested LIVE/DEAD and Cell Counting Kit-8 (CCK-8) assays for the SH-SY5Y cell line, a common neuroblastoma model for studying neurodegenerative diseases (49). The LIVE/DEAD assay visually distinguishes live (green) and dead cells (red) using the fluorescent probes [calcine acetoxymethyl ester (calcine-AM) and ethidium homodimer-1 (EthD-1)] (50), and CCK-8 assay is a colorimetric method for assessing the mitochondrial dehydrogenase activity of live cells based on the water-soluble formazan product (26). We incubated SH-SY5Y cells with BCFO nanoparticles and applied the LIVE/DEAD assay to the cells after 1 and 7 days of cultures, respectively. As displayed in the LIVE/DEAD assay results (Fig. 5A), most SH-SY5Y cells emitted green fluorescence (live state, normal activity) after 7 days of culture. In addition, the cell confluence (i.e., the percentage of green color area in the captured image) was increased from 8.9% (at day 1) to 58.7% (at day 7) in the presence of BCFO nanoparticles (fig. S23), corresponding to the doubling time (approximately 2 days) of SH-SY5Y cells in the
results at day 1 and day 7. (B) Bright-field image at day 7. The black dots in the

bright-field image at day 7 were captured at the same spot. (C) Cell viability

and bright-field images at day 7 were captured at the same spot. (D) Alle-
viating effect of BCFO nanoparticles on Aβ neurotoxicity under irradiation of

low-frequency magnetic field (13.6 mT and 1 kHz) for 6 hours. All values were

statistically analyzed by means of one-way analysis of variance (ANOVA) (n = 4)

(***P < 0.001; n.s., not significant).

Fig. 5. In vitro evaluation results with SH-SY5Y cell line. (A) LIVE/DEAD assay

results at day 1 and day 7. (B) Bright-field image at day 7. The black dots in the

bright-field image represent BFCO nanoparticles. Note that the LIVE/DEAD assay and

bright-field images at day 7 were captured at the same spot. (C) Cell viability

with different concentrations (from 0 to 3 mg ml⁻¹) of BCFO nanoparticles. (D) Alle-
viating effect of BCFO nanoparticles on Aβ neurotoxicity under irradiation of

low-frequency magnetic field (13.6 mT and 1 kHz) for 6 hours. All values were

statistically analyzed by means of one-way analysis of variance (ANOVA) (n = 4)

(***P < 0.001; n.s., not significant).

healthy state (see fig. S24 for LIVE/DEAD assay with control conditions) (51). Bright-field images demonstrated BCFO nanoparticles as black dots (Fig. 5B and fig. S25) that made contact with the intact neurite outgrowths of SH-SY5Y cells (fig. S26). The neural biocompatibility of BCFO nanoparticles was also observed in differentiated SH-SY5Y cells with their long neurite outgrowths (fig. S27). In addition, CCK-8 assay results exhibited more than 90% viability of SH-SY5Y cells at up to 500 

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m l⁻¹ of BCFO nanoparticles (see Fig. 5C and fig. S28 for differentiated SH-SY5Y cells) and the negligible cytotoxic effect of BCFO nanoparticles upon the irradiation of low-frequency magnetic field as well (fig. S29). These biochemical assay results imply that the magnetoelectric treatment did not cause significant damage to the function of cellular proteins, such as intracellular esterase and mitochondrial dehydrogenase (fig. S30). Collectively, our LIVE/DEAD and CCK-8 assay results suggest neural biocompatibility of magnetoelectric BCFO nanoparticles.

We hypothesized that BCFO nanoparticles may mitigate the neurotoxicity of Aβ aggregates. To investigate the alleviating effect of BCFO nanoparticles, we applied CCK-8 assay to SH-SY5Y cells (Fig. 5D). When we incubated SH-SY5Y cells with mature Aβ fibrils (20 

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M), cell viability was reduced to 53.2%. The similar low viability was shown when the Aβ fibrils were added into the cell culture plate after treatment of either BCFO nanoparticles (100 

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g ml⁻¹) or a low-frequency magnetic field (1 kHz and 13.6 mT). On the other hand, we observed a significant recovery of the cell viability up to 85.0% after incubation with Aβ fibrils that were dissociated by magnetoelectrically excited BCFO nanoparticles. We attribute this result to the disassembly of the Aβ fibril structure. According to the literature (52, 53), accumulation of insoluble Aβ fibrils on the neuronal plasma membrane induces action potential desynchronization and membrane permeation, which may trigger pathological downstream consequences including mitochondrial dysfunction and neuronal apoptosis. Our results suggest that BCFO nanoparticles and a low-frequency magnetic field may have the potential to treat not only Aβ fibrils but also neurotoxic Aβ plaques in AD brain tissue.

Aβ plaques are the highest-order, micrometer-sized aggregates formed by complex assembly processes between Aβ fibrils and biological components (e.g., lipids, protein, and metal ions) in the extracellular space of brain tissue (37). To explore Aβ plaque clearance efficacy of magnetoelectrically excited BCFO nanoparticles, we used ex vivo brain slices of a transgenic AD mouse model (5xFAD, 4 months of age). We applied BCFO nanoparticles (100 

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g ml⁻¹) and a low-frequency magnetic field (13.6 mT and 1 kHz) to ex vivo brain slices for 6 hours (Fig. 6A; see fig. S31 for experimental setup). To highlight Aβ plaques, we stained the treated brain slices using thioflavin S (ThS), a fluorescent probe for labeling β sheet–dominant, neurotoxic Aβ plaques (54). As displayed in Fig. 6B, the cerebral cortex region of the brain slice before any treatment was covered with a significant amount of Aβ plaques with a density of 133.1 mm⁻². The brain slices after treatment of either BCFO nanoparticles or low-frequency magnetic field also exhibited a similar distribution of Aβ plaques to the nontreated brain slice (fig. S32). However, we found that the density of Aβ plaques in the cerebral cortex region drastically decreased from 133.1 to 26.4 mm⁻² after adopting magnetoelectrically excited BCFO nanoparticles (Fig. 6C). Our immunohistochemistry analysis results also displayed the Aβ clearance efficacy of magnetoelectrically excited BCFO nanoparticles to ex vivo brain slices of AD mouse model (fig. S33). Furthermore, we observed that the magnetoelectrically treated brain slices did not show significant changes in their morphology and neuron distribution as well according to the Nissl staining results (fig. S34). The results show that the magnetoelectrically excited BCFO nanoparticles, which are capable of dissociating nanometer-thick Aβ fibrils, can also be used to clear micrometer-sized Aβ plaques, the major pathological hallmark of AD.

Accumulation of Aβ plaques in the brain of AD animal models and patients begins in the neocortex and gradually extends to the deep brain regions (e.g., hippocampus, basal ganglia, and pons) (17, 55). However, “noninvasive” treatment of the deep brain regions is limited due to the thickness of the surrounding tissue (56). Magnetic field can pass through the deep brain regions with negligible scattering and absorption losses. Our results demonstrated that relatively weak magnetic field (strength of several mT) compared to MRI scanners (strength of several tesla) can eliminate Aβ aggregates from the brain tissue by stimulating BCFO nanoparticles. Nevertheless, magnetoelectrically excited BCFO nanoparticles are able to induce not only dissociation of self-assembled Aβ aggregates but also slight secondary structure changes of single plasma proteins such as bovine serum albumin and lysozyme (fig. S35). It implies that the introduction of Aβ-targeting motifs into BCFO nanoparticles is necessary to prevent an unexpected adverse effect. We expect that targeted delivery of BCFO nanoparticles in the Aβ-accumulated specific brain regions may be achieved by coating the nanoparticle with different motifs, such as LK7 and H102 peptides (57, 58). Our spectroscopic analysis result demonstrates that BCFO nanoparticles are capable of surface functionalization for conjugating the motifs (fig. S36). To test the clinical feasibility of the magnetoelectric
platform in the future, we plan to conduct in vivo studies using AD mouse models for the recovery of their cognitive and behavior functions.

We report BCFO nanoparticle’s newly discovered function to dissociate Aβ peptide aggregates under a low-frequency magnetic field. We synthesized piezo-magnetoactive BCFO nanoparticles having a core-shell structure based on two different magnetoelectric materials. The BCFO nanoparticles triggered magnetoelectric catalysis under a low-frequency magnetic field by transferring excited charge carriers to the surrounding environment with negligible heat generation. Magnetoelectrically excited BCFO nanoparticles converted neurotoxic Aβ fibrils to nontoxic amorphous globular debris by oxidizing the primary structure of the constituent Aβ peptides and subsequently destabilizing the β sheet secondary structure of self-assembled Aβ fibrils. We demonstrated the clearance capability of magnetoelectrically excited BCFO nanoparticles for Aβ fibril–dominant, micrometer-sized Aβ plaques that were accumulated in the brain slices. This proof-of-concept study unveils the hidden function of magnetoelectric nanoparticles for future treatment to the brain slices. This proof-of-concept study unveils the hidden function of magnetoelectric nanoparticles for future treatment.

Fig. 6. Ex vivo evaluation results with the brain slices of the SxFAD AD mouse model. (A) Schematic illustration of the disassembly of Aβ plaques in the brain slices by the use of BCFO nanoparticles and low-frequency magnetic field. (B) Representative microscope images and (C) Aβ plaque densities of the brain slices in the zoomed area after the treatment of BCFO nanoparticles (100 μg ml⁻¹) and low-frequency magnetic field (1 kHz and 13.6 mT) for 6 hours (see fig. S20 for brain slice images of the other two conditions). White dotted boxes in the entire brain slice images (left) represent the zoomed area of the brain slice (right). White dotted circles in the zoomed area (right) indicate the representative Aβ plaques. All values in Aβ plaque densities were statistically analyzed by means of one-way ANOVA (n = 3) (**P < 0.001).

### MATERIALS AND METHODS

#### Chemicals

Alzheimer’s Aβ (1–42; human) peptide was obtained from AnaSpec (Fremont, CA, USA). Dulbecco’s modified Eagle’s medium (DMEM), fetal bovine serum (FBS), and antibiotic-antimycotic (AA) were purchased from Gibco (Carlsbad, CA, USA). The LIVE/DEAD assay kit was purchased from Invitrogen (Carlsbad, CA, USA). CCK-8 assay reagent was acquired from Dojindo Corp. (Japan). Antibody for immunohistochemistry was bought from Abcam (Cambridge, UK). All of the other chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

#### Synthesis of CFO nanoparticles

CFO and BCFO nanoparticles were synthesized by following the literature (11, 59) and modifying minor steps. To prepare CFO nanoparticles, iron(III) chloride hexahydrate (0.995 g) and cobalt(II) chloride (0.239 g) were dissolved in aqueous solution (35 ml) containing hexadecyltrimethylammonium bromide (2.041 g) under vigorous stirring. Next, sodium hydroxide solution (6 M) was added to the mixture to adjust pH 11.0 under continuous stirring. After additional ultrasound stimulation for 30 min, the hydrothermal treatment was applied to the mixture at 180°C for 24 hours with a 50-ml Teflon-lined stainless steel autoclave. The resulting black-colored precipitates were washed with deionized (DI) water and ethanol several times after cooling down to RT. Afterward, the precipitates were collected by vacuum filtration with a 0.2-μm nylon membrane (Whatman, UK).

#### Synthesis of BCFO nanoparticles

To synthesize BCFO nanoparticles, the sol-gel treatment was applied to the as-prepared CFO nanoparticles. Briefly, fully dried CFO nanoparticles (50 mg) were dispersed into ethylene glycol solution (30 ml) containing bismuth(III) nitrate pentahydrate (0.160 g) and iron(III) nitrate nonahydrate (0.121 g). After 2 hours of sonication, the sol-state mixture was moved to a vacuum oven and was dried at 80°C for 24 hours. Next, the resulting gel-state mixture was preheated at 400°C for 30 min to eliminate organic compounds and successively calcined at 500°C for 90 min. The resulting BCFO nanoparticles were washed several times with DI water and ethanol on the nylon membrane and collected by a neodymium permanent magnet after ultrasound treatment.

#### Characterization of CFO and BCFO nanoparticles

Morphologies of CFO and BCFO nanoparticles were analyzed by SEM (S-4800, Hitachi, Japan) at 10 kV and TEM (Talos F200X, FEI Company, USA) at 200 kV. Crystallinity of nanoparticles was examined by an x-ray diffractometer (SmartLab, Rigaku Co., Japan) with Cu Kα radiation (λ = 1.5418 Å). Chemical states of nanoparticles were obtained by an x-ray photoelectron spectrometer (Sigma Probe, Thermo Scientific, USA) with C 1s calibration to 284.6 eV. Magnetic properties of nanoparticles were analyzed by a superconducting quantum interference device-vibrating sample magnetometer (SQUID-VSM) (MPMS, Quantum Design, UK). Relative amount of as-generated holes and electrons from nanoparticles was evaluated by TA assay (3 mM) (λex = 310 nm and λem = 430 nm) and DHE assay (20 μM) (λex = 510 nm and λem = 590 nm), respectively, using a spectrophotometer (JASCO FP6500). The magnetic field reactivity of nanoparticles was recorded by an oscilloscope (DSOX3012T, Keysight, CA, USA) under the effect of an electromagnet operated by a waveform generator (33512B, Keysight, CA, USA).
Low-frequency magnetic field stimulation

Experimental setup for magnetic field stimulation was prepared with electromagnets, which are operated by external electrical bias generated from two waveform generators (33512B, Keysight, CA, USA). Strength of applied alternating magnetic field was measured by a gaussmeter (GM07, Hirst, UK). To evaluate the magnetoelectric catalysis of nanoparticles, aqueous solution (400 μl) containing nanoparticles was subjected to alternating magnetic field under constant agitation.

Preparation and characterization of Aβ fibrils

Preparation of Aβ fibril solution (40 μM) was carried out with a biocompatible Hepes buffer (pH 7.4) following previous studies (26, 28). Morphologies of Aβ fibrils were recorded by an AFM instrument (NNOVA-LABRAM HR800, Horiba, Japan) (with an area of 5 μm by 5 μm and a line number of 512), SEM (S-4800, Hitachi, Japan) at 10 kV, and TEM (Talos F200X, FEI Company, USA) at 200 kV. To prepare AFM samples, Aβ fibril solution (10 μl) was dropped onto an AFM mica and dipped into DI water to remove excess amount of Aβ fibrils on the surface of mica after allowing Aβ fibril solution for 30 min. AFM profile analysis was performed on three independent Aβ fibrils. To collect SEM images, Aβ fibril solutions (50 μl) were coated on a gold-coated silicon wafer (1 cm by 1 cm) for 30 min before washing three times with DI water. To gather TEM images, Aβ fibril solutions (5 μl) were incubated on a TEM grid for 1 min, and a centrifuged lead citrate solution was applied to the grid to stain Aβ fibrils for 5 min before washing the grid with DI water. Protein secondary structure of Aβ fibrils was analyzed by ThT assay and CD spectroscopy. ThT assay results were collected through a spectrofluorometer (JASCO FP6500) by recording the fluorescence intensity (λex = 440 nm and λem = 485 nm) of mixture solution, containing ThT solution (480 μl of 20 μM) and Aβ solution (20 μl of 30 μM). ThT fluorescence intensity at time zero for each condition was set up as 1.0 to compare the relative amount of change in the ThT fluorescence intensity. CD spectra of Aβ fibrils (30 μM) were acquired through a CD spectropolarimeter (Jasco-815-150 L, Jasco Inc., Japan) with a quartz cell (0.5-mm thickness) containing nanoparticles was subjected to alternating magnetic field under constant agitation.

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Cell culture

SH-SY5Y cell line (American Type Culture Collection, Manassas, VA, USA) was adapted for investigation on the biocompatibility and alleviating effect of BCFO nanoparticles. All SH-SY5Y cells were grown in complete growth medium (DMEM, 10% FBS, and 1% AA) for proliferation at a humidified environment with 37°C and 5% CO2. To prepare BCFO nanoparticles, all SH-SY5Y cells were differentiated for 7 days and further incubated with Aβ fibrils (20 μM) for 3 days before conducting the CCK-8 assay. All values in the CCK-8 assay were presented as the means ± SD with statistical analysis using one-way analysis of variance (ANOVA).

Ex vivo evaluations

To conduct ex vivo evaluations, an AD mouse model (5xFAD, aged 4 months) was euthanized so that its brain may be used. The extracted brain was molded with frozen section medium (FSC 22, Leica, Germany) and sliced in 30 μm thickness using a cryostat (CM1860, Leica, Germany) under −19°C. The sliced brain was fixed on a histobond slide glass for further experiments. Afterward, the brain slices were treated with BCFO nanoparticle solution (100 μg ml−1) and alternating magnetic field (13.6 mT and 1 kHz) for 6 hours. BCFO nanoparticles were uniformly distributed across the entire brain slices during the application of the magnetic field. Then, ThS solution [1 mM in phosphate-buffered saline (PBS) buffer] was applied to the brain slices to stain Aβ plaques for 1 hour and washed with a fresh PBS buffer for three times (28). Fluorescence images of ThS-stained brain slices were collected by a fluorescence microscope (Eclipse 80i, Nikon, Japan) equipped with a digital camera (DS-R2, Nikon, Japan). The large-size fluorescent images of the entire brain slices were collected by automatically reassembling multiple small-size snapshots through NIS-Elements software (Nikon, Japan). The fluorescent images were analyzed using Imagej software (U.S. National Institutes of Health, MD, USA) following the method in our previous study (28). The density of Aβ plaques was presented with statistical analysis using one-way ANOVA. Immunohistochemistry analysis was conducted by the adoption of Alexa Fluor 488 anti-Aβ1–42 antibody to ex vivo brain tissue of an AD mouse model for 1 hour staining after diluting 1:100 in PBS buffer. Nissl staining was demonstrated by the use of 0.1% cresyl violet solution in DI water for 15 min after dehydration processes with ethanol solutions.

SUPPLEMENTARY MATERIALS

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View/request a protocol for this paper from Bio-protocol.

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