Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
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Electronic Supplementary Material

A dual-inhibitor system for effective antifibrillation of Aβ40 peptides by biodegradable EGCG-Fe(III)/PVP nanoparticles

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1.Materials and methods

Materials.

Polyvinyl alcohol (PVA, Mw=16000~20000), polyvinyl pyrrolidone (PVP, Mw=10kDa), epigallocatechin gallate (EGCG), gallic acid (GA), glutathione (GSH), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Thioflavine T (ThT), and hexafluoroisopropanol(HFIP)were purchased from Sigma. Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), gadolinium(III) chloride hexahydrate ($GdCl_3 \cdot 6H_2O$), manganese chloride, glutaraldehyde, sodium dihydrogen phosphate, disodium hydrogen phosphate, NaOH and ethanol were purchased from Sinopharm Chemical Reagent Co., Ltd. Dimethylsulfoxide (DMSO) was purchased from Bioengineering (Shanghai) Co., Ltd. 2,2,2-trifluoroethanol was purchased from Aladdin. RPMI 1640, fetal bovine serum (FBS) were obtained from Xiamen Lulong Biotechnology Development Co., Ltd. All chemicals were used without purification.

Synthesis of EGCG-Fe/PVP (EFPP) and EGCG-Fe/PVA (EFPA) NPs

Typically, 100 mg PVP was dissolved in 10 mL of water at room temperature under vigorous stirring. $FeCl_3$ aqueous solution (0.2 mL, 100 mg mL^{-1}) was then added to the PVP solution. After stirring for 1 h, an EGCG aqueous solution (1 mL, 3.36 mg mL^{-1}) was added to the above reaction mixture and stirred overnight. The resulting nanoparticles were dialysed (MWCO= 8000-14,000) against distilled water for 24 h.

For the synthesis of EFPA NPs, the PVP was replaced by PVA.

Synthesis of EGCG-Mn/PVP (EMPP) and EGCG-Gd/PVP (EGPP) NPs.

Synthesis of EMPP and EGPPNPs was consistent with EFPP NPs. Simply, ferric chloride was replaced with manganese chloride or gadolinium chloride.

Synthesis of GA-Fe/PVP (GFPP) NPs.

Synthesis of GA-Fe/PVP NPs was consistent with the method to EFPP NPs. First, 100 mg PVP was dissolved in 10 mL of water at room temperature under vigorous stirring. Then 0.2 mL 100 mg mL^{-1} $FeCl_3$ aqueous solution was added to the solution. After stirring for 1 h, 1 mL 3.36 mg mL^{-1} GA solution was added to above reaction and

further stirred 12 h. The resulting nanoparticles were dialysed (MWCO = 8000-14,000) against deionized water for 24 h.

Synthesis of GA-Fe/PVA (GFPA) NPs.

The method of synthesis of GA-Fe/PVA NPs was consistent with GA-Fe/PVP. Simply, just replace the PVP with PVA.

2.Characterization

2.1 Determining quantification of EGCG in EFPP NPs.

The quantification of EGCG in as-synthesized EFPP was measured by UV-vis. First, the ultraviolet absorption of a series of concentration of EGCG was measured, and then a standard curve of EGCG was made (Figure S5A). To determine the quantification of EGCG in EFPP NPs, the supernatant and washing solutions in the EGCG NPs prepared process were all collected and analyzed by UV–vis spectroscopy at wavelength of 276 nm. The concentration of free EGCG in collected solution was calculated according to the following equation:

$$\mathbf{y = 0.022x + 0.047} \qquad (1)$$

Where y is the absorbance value at 276 nm and x is the concentration of unreacted EGCG in collected solution.

Therefore, the concentration of EGCG in EFPP NPs was calculated according to the following equation:

$$\mathbf{m_{EGCG}/m_{EFPP}} = \frac{m-xV}{m} \qquad (2)$$

Where m is the quality of EGCG in the whole action，v is the volume of collected solution and x is the concentration of unreacted EGCG in collected solution.

2.2 Quantification of iron in EFPP. First, a series of iron solutions with different concentration gradients were prepared, and then the standard curve was made by ICP-AES (Optima 7000, PerkinElmer, Figure S5B).With the oxidative degradation by HNO_3 (2%) for 2 h, the quantities of Fe in EFPP was measured by ICP-AES and was calculated based on the standard curve.

$$Y = 322644.175C_{Fe} - 77526.49 \quad (3)$$

The quantification of Fe in EFPP NPs was calculated according to the following equation:

$$m_{Fe}/m_{EFPP} = \frac{c(Fe)\times V1}{c(EFPP)\times V} \quad (4)$$

Where V1 is the solution volume of iron and v is the solution volume of EFPP NPs.

2.3 Quantification of PVP in EFPP.

The quantification of PVP in EFPP was measured by Elemental analyzer (Vario EL Cube,Elementar) and was calculated according to the following equation:

$$m_{PVP}/m_{EFPP} = \frac{Mw\times N\%}{14n} \quad (5)$$

Where N% is the percentage of N in EFPP NPs from elemental analysis results, Mw is the molecular weight of PVP and n is the number of PVP polymerization degree.

2.4 Fluorescence intensity ratio.

The fluorescence intensity ratio of nanoparticles on Aβ40was calculated according to the following equation:

$$\text{fluorescence intensity ratio} = \frac{Ymax\ (A\beta 40) - Ymax(NP)}{Ymax\ (A\beta 40)} \times 100\% \quad (6)$$

Where Y_{max} (Aβ40) is the maximum fluorescence intensity of Aβ40 alone in the ThT assay, and Y_{max}(NP) is the maximum fluorescence intensity of Aβ40 with NPs.

2.5 Characterization of Aβ40 Secondary Structure.

CD analysis was used to study the secondary structure changes of Aβ40 during aggregation. As shown in Fig. S7, the CD spectra of Aβ40 alone exhibited one prominent negative peak at220 nm, which corresponded to typical β-sheet structure. However, after introduction of EFPP NPs with various concentrations, the intensity of this representative negative peak around 220 nm gradually decreased, especially when addition of 50μg mL^{-1} EFPP NPs. This result indicated that the formation of β-sheet

structure was much less than that of Aβ40 alone sample and secondary structures became random coils or amorphous aggregates.

3. Supplementary figures and tables

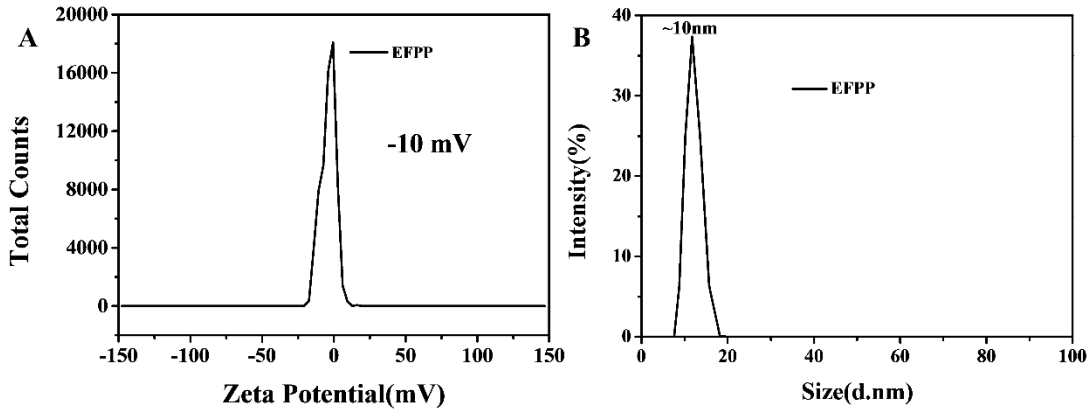

Fig.S1 (A) Zeta potential characterization of EFPP NPs; (B) hydrodynamic size of EFPP NPs.

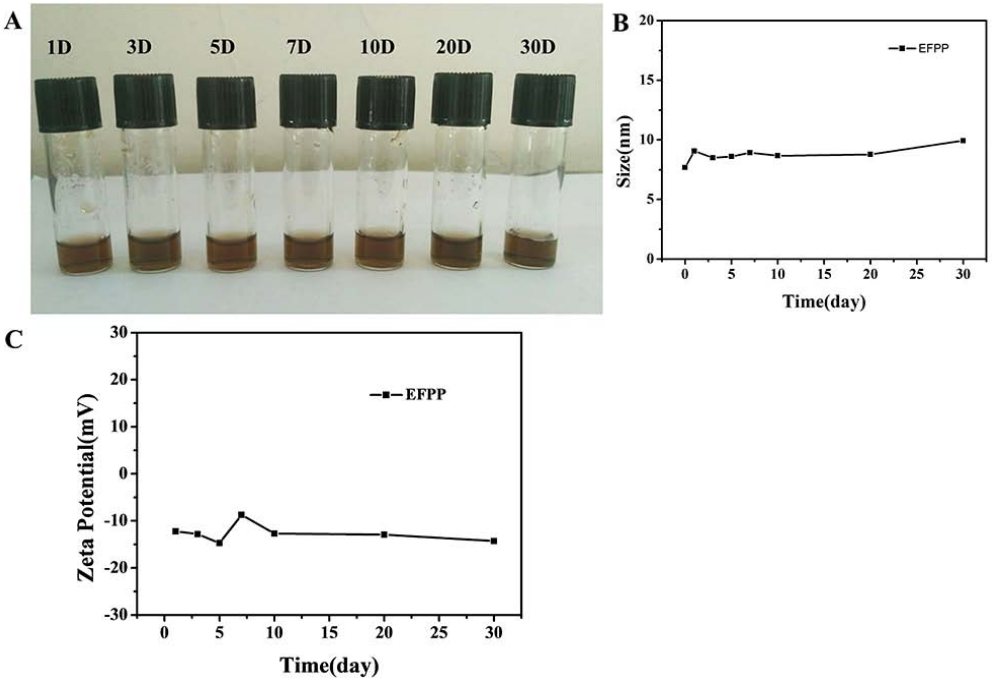

Fig. S2 Stability characterization of EFPP NPs：(A) the digital photograph of EFPP NPs during different times；(B) hydrodynamic size of EFPP NPs during 30 days.

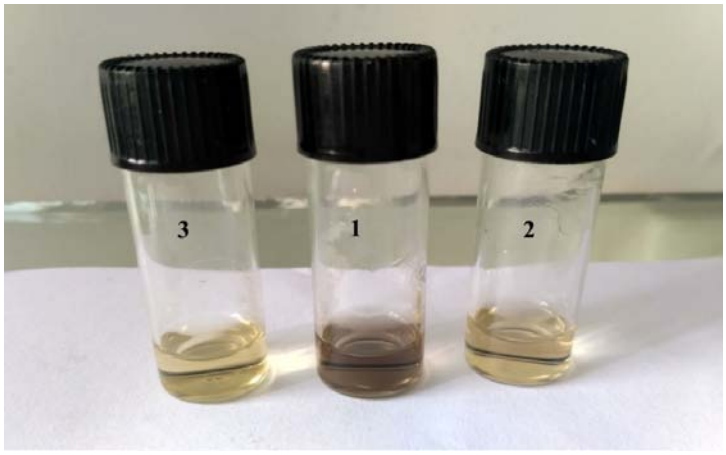

Fig. S3The stability characterization of EFPP NPs in different buffer for 24 h：
(1) pH=7.4 PBS； (2) pH=5.0 PBS；(3) pH=7.4 PBS +10 mM GSH.

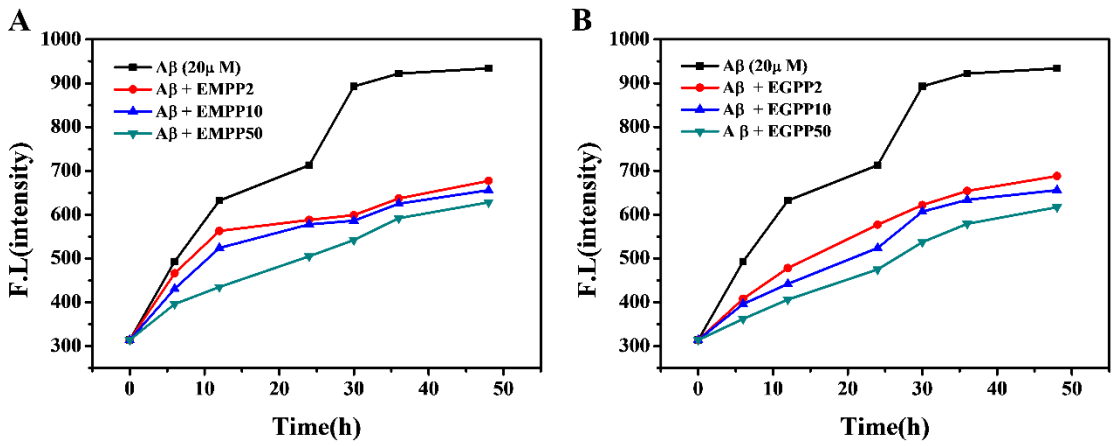

Fig. S4 ThT assay for the inhibition effect of Aβ40 fibrillation by EMPP (A) and EGPP (B).

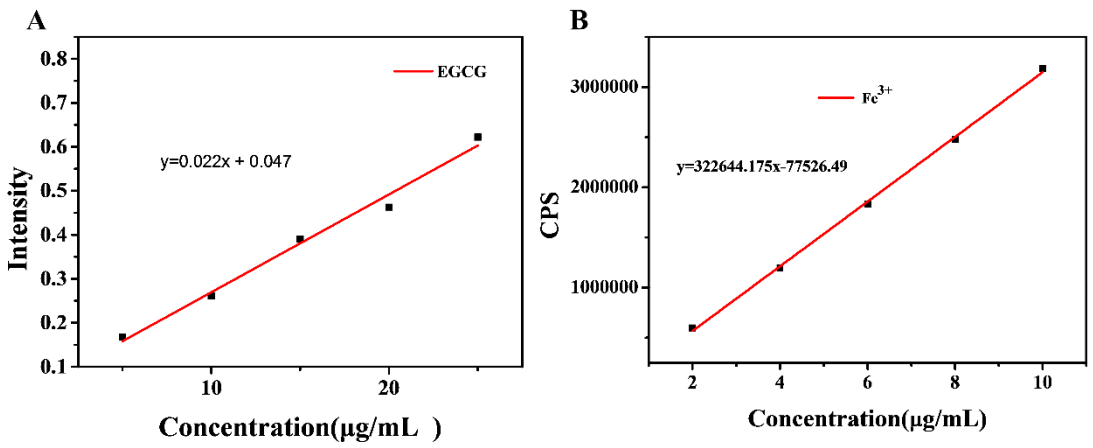

Fig. S5 (A) the standard curve of EGCG measured by UV-vis; (B) the standard curve of Fe measured by ICP-AES.

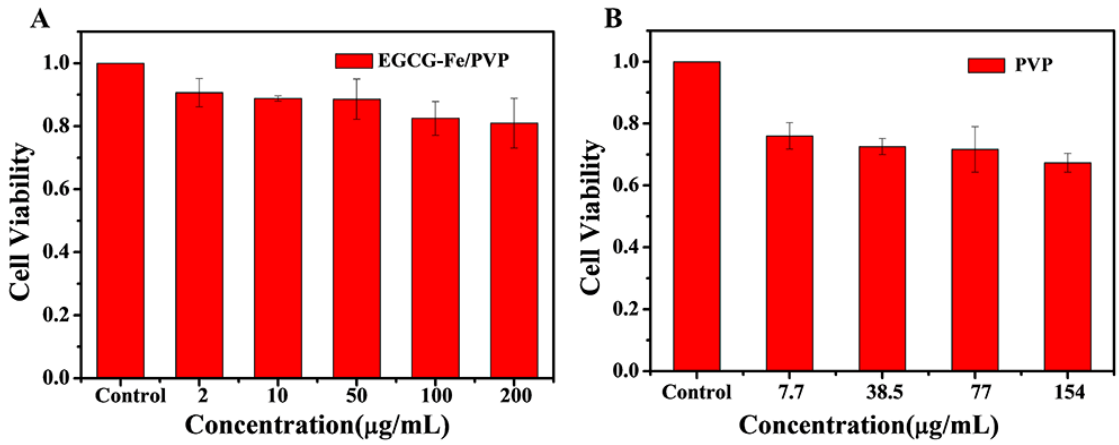

Fig.S6 In vitro cell viabilities of PC12 cells incubated with different concentrations of EFPP (A) and free PVP (B).

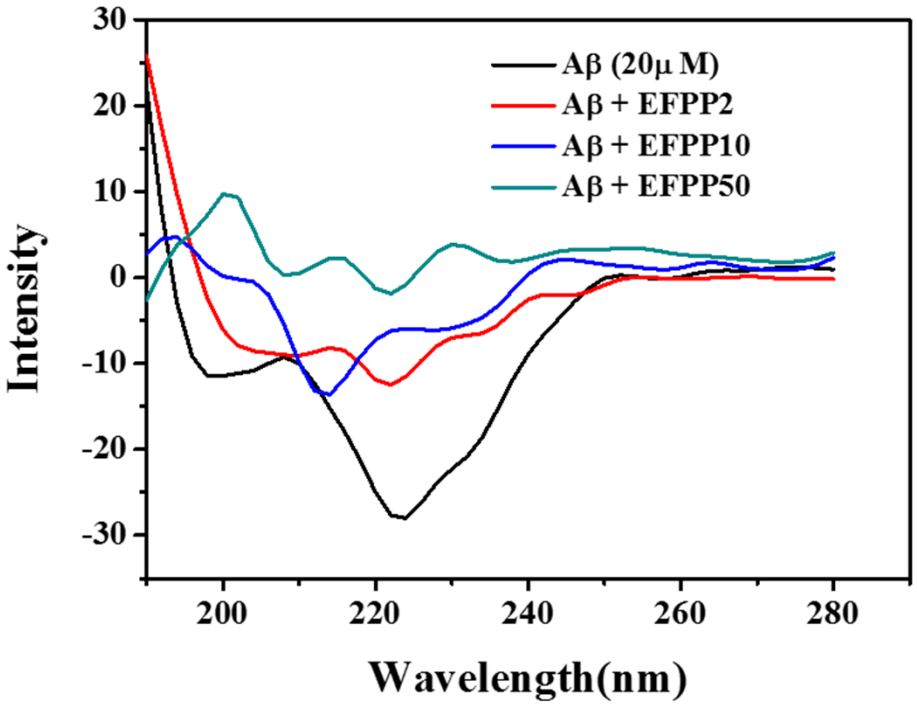

Fig. S7 CD spectra of Aβ40 with different concentrations of EFPP NPs (0, 2, 10, 50 μgmL^{-1}).

Table.S1 Quantitative elements determination in EFPP NPs.

Element	Weight %
N	9.66
C	52.34
H	8.36
O	27.19
Totals	97.55