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Attachment of biomolecules (protein and DNA) to amino-functionalized carbon nanotubes

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Abstract: An efficient method for the attachment of biomolecules [e. g. bovine serum albumin (BSA) protein and deoxyribonucleic acid (DNA)] to amino-group-functionalized multiwalled carbon nanotubes (f-MWCNTs) was reported. MWCNTs were prepared by spray pyrolysis of a benzene-ferrocene solution in argon atmosphere at ~850 ℃ followed by functionalization with an amino group by chemical modification of carboxylic groups introduced on the nanotube surface. This process involves a direct coupling of ethylenediamine with carboxylic groups to introduce amino groups by amide formation. The as-synthesized MWCNTs, f-MWCNTs, and amino f-MWCNTs with BSA protein and DNA were characterized by scanning and transmission electron microscopy, and Fourier transform infrared spectroscopy, which confirm the attachment of biomolecules (BSA protein and DNA) to the amino f-MWCNTs.

Keywords: Carbon nanotubes; Functionalization; Protein; DNA
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1 Introduction

Carbon nanotubes (CNTs) exhibit interesting electrical, structural, and mechanical properties that make them highly promising nanoscale building blocks for the construction of novel functional materials[1-2]. There have been several recent investigations concerning the use of CNTs for biological purposes and the introduction of CNTs into biological systems[3-9]. A common technique to incorporate CNTs into such systems is through functionalization of the CNTs, which enables chemical bonding between the CNTs and the material of interest[10-11]. The functionalized carbon nanotubes (f-CNTs) are believed to be very promising in the field of biological technologies. For example, Hu et al.[4] have investigated the growth of neurons on functionalized multiwalled CNTs (f-MWCNTs). Pantarotto and coworkers[5] have demonstrated that gene expression through f-CNTs levels up to 10 times higher than those achieved with deoxyribonucleic acid (DNA) alone. The CNTs have been shown to cross the cell membranes easily and to deliver the peptides, proteins, nucleic acids, and medical drug into cells[12-13]. To realize these applications, biomolecules, such as proteins and DNAs, must be bound to CNTs. The biomolecules can be connected to CNTs via noncovalent or covalent bonding[14-20]. Williams et al.[15] developed a method to couple single-walled CNTs (SWCNTs) covalently to peptide nucleic acid (PNA, an uncharged DNA analogue) and to hybridize these macromolecular wires with complementary DNA. It was found that DNA attachment occurs predominantly at or near the nanotube ends. Maruyama et al.[16] reported the attachment of protein molecules to f-MWCNTs in an aqueous buffer solution. A chemical reaction using carbodi-imide forms chemical bonds between open-ended tips of MWCNTs and protein molecules. In another study, f-MWCNTs were attached to the protein via diimide activated amidation[17]. The good stability, accessibility, and selectivity, however, will be achieved through covalent bonding because of its capability to control the location of the biomolecule, improve stability, accessibility, and selectivity, and reduce leaching. In the present study, we report the preparation of amino f-MWCNTs and

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attachment of biomolecules [e. g. bovine serum albumin (BSA) protein and DNA] to the amino f-MWCNTs. The morphology of f-MWCNTs before and after the attachment of biomolecules has been analyzed by transmission electron and scanning electron microscopy. The functionalization of MWCNTs and attachment of biomolecule to the amino f-MWCNTs have been confirmed by fourier transform infrared spectroscopy.

2 Experimental

2.1 Synthesis of CNTs

The CNTs were synthesized by the spray pyrolysis of ferrocene and benzene solution under argon atmosphere at ~850 ℃[21-22]. The optimum flow rate and concentration of ferrocene in benzene were found to be ~2 mL/min and 50 mg/mL, respectively. The as-grown CNTs are multiwalled CNTs.

2.2 Functionalization of MWCNTs and attachment of biomolecules to the amino-functionalized MWCNTs

The as-synthesized MWCNTs (0.2 mg) were dispersed in mixture of conc. H_2SO_4 and conc. HNO_3 (3∶1, vol.) using ultrasonication bath at room temperature for 8 h. The suspension was exposed to 1 mol/L HCl and sonicated for 30 min for the generation of carboxylic (COOH) groups on the side walls and ends of CNTs. The resulting suspension was filtered and washed with distilled water and dried at 80 ℃ for 4 h under vacuum. The MCWNTs (~0.1 mg) with carboxylic groups were sonicated in thionyl chloride ($SOCl_2$) (~ 50 mL) for 30 min at room temperature. The suspension was refluxed under magnetic stirring at room temperature for 48 h and then filtered. The filtered powder was washed with tetrahydrofuran (THF) and dried at room temperature for 20 min under vacuum. The thionyl chloride-treated MWCNTs (~10 mg) were added to excessive ethylenediamine [EDA; $NH_2(CH_2)_2NH_2$] under a magnetic stirring at room temperature for 10 h. The mixture was washed with THF and filtered. The filtered powder was dried at 80 ℃ for 10 h under vacuum. This process leads to the replacement of thionyl chloride (COCl) with amide group (CO—NH). This approach introduces amino group on the side walls and ends of MWCNTs via amide formation.

The process for attaching protein (e. g. BSA) to the amino f-MWCNTs surface is detailed as follows: in the typical process, amino f-MWCNTs (~ 2 mg) were dispersed in 10 mL of a 50 mmol/L phosphate (Na_2HPO_4) buffer solution (pH = 6.1), and 1.0 mL of BSA protein buffer solution was added. The optimum concentration of BSA protein in phosphate buffer was found to be 5 mg/mL. The amino f-MWCNTs-BSA protein solution was shaken at 100 r/min for 4 h at room temperature. The mixture suspension was centrifuged at 12,000 r/min for 5 min and washed with 50 mmol/L phosphate buffer solution (pH = 6.1) for four times to remove unbound protein. The washed amino f-MWCNTs-BSA protein sample was dispersed in deionized water. In another experiment, the amino f-MWCNTs (~1 mg) were dispersed in deionized water (~5 mL) and few drops of DNA (calf thymus) were added. The mixture solution was sonicated for 1 h in the ultrasonicator. The amino f-MWCNTs-DNA mixture was washed with deionized water for three times to remove unbound DNA.

2.3 Characterization

The as-prepared samples were examined by scanning electron microscopy (SEM) (Philips XL 20) and transmission electron microscopy (TEM) (Tecnai $G^2$20). The as-prepared MWCNTs were sonicated in ethanol for 20 min. A few drops of the resulting suspension were transferred onto a carbon-coated copper grid. The as-prepared samples such as f-MWCNTs and amino f-MWCNTs-BSA/DNA liquid samples were dropped directly onto the carbon-coated copper grid. Fourier transform infrared spectroscopy (FTIR) was used to check the presence of reactive groups on the MWCNTs surface after the acid treatments and attachment of biomolecules. FTIR spectrum of the samples was recorded using Perkin-Elmer (spectrum 100, USA) spectrometer. In sample preparation for FTIR measurement, as prepared samples were mixed with potassium bromide (KBr) powder and casted into pellets.

3 Results and discussion

3.1 Fourier transform infrared spectroscopy

To covalently bond molecules to the CNTs, it first requires the formation of functional groups on the CNTs. The carboxylic group is often the best choice because it can undergo a variety of reactions and is easily formed on CNTs via oxidizing treatments. It is reported that the presence of carboxylic group at the nanotube ends and at defects on the sidewalls has advantages to perform acid base chemistry and to introduce on the nanotube amide, ester linkage, and so on[9-10,18]. The control of reactants and/or reaction conditions may control the locations and density of the functional groups on the CNTs, which can be used to control the locations and density of the attached biomolecules.

The sequential steps of functionalization were examined by FTIR. Concentrated acids are known to introduce acidic groups to the sidewalls and ends of CNTs[23]. The FTIR spectra of as-synthesized MWCNTs and after functionalization with carboxylic and amino groups are shown in Fig. 1(a)-(c). The peak at ~3 430 cm^{-1} is attributed to the presence of OH group on the surface of the as-synthesized MWCNTs [Fig. 1(a)]. The peak at 1 595 cm^{-1} is associated with the vibration of carbon skeleton (C═C) of the CNTs[23]. The peak at 1 720 cm^{-1} is due to C═O stretching of the carboxylic (—COOH) group [Fig. 1(b)][10, 24]. Another peak at 1 400 cm^{-1} corresponds to the O—H bond in carboxylic group[24]. A peak at 1 200 cm^{-1} is assigned to C—O bond stretching. The results indicate that carboxylic groups have been attached to the MWCNTs. The MWCNTs with carboxylic acid group (MWCNTs-COOH) were further treated with thionyl chloride and ethylenediamine to form amide-terminated MWCNTs. The FTIR spectrum of the amino f-MWCNTs sample (MWCNTs-CO-NHCH$_2$CH$_2$NH$_2$) [Fig. 1(c)] shows the disappearance of the peak at 1 720 cm^{-1} and appearance of new peak with lower wave number (1 650 cm^{-1}), which is known to correspond to amide carbonyl (C═O) stretch. The peak around 1 585 cm^{-1} is associated with the N—H plane stretch and also the vibration of carbon skeleton of CNTs[10]. The peak at 1 330 cm^{-1} is identified as C—N bond stretching. Two peaks around 2 920 cm^{-1} and 2 837 cm^{-1} show the C—H stretching mode of methylene in ethylenediamine molecule. The small peak at 3 540 cm^{-1} may be due to the N—H stretch of the amine (NH_2) group. From the FTIR spectrum 1(c), the existence of amine groups attached to the MWCNTs is clear.

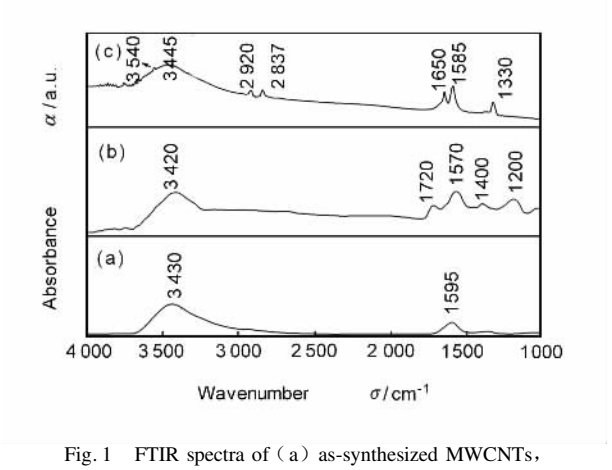

Fig. 1　FTIR spectra of (a) as-synthesized MWCNTs, (b) carboxylic f-MWCNTs and (c) amino f-MWCNTs

The attachment of BSA protein and DNA to the amino f-MWCNTs was verified by comparing the FT-IR spectrum of as-prepared amino f-MWCNTs and amino f-MWCNTs-BSA/DNA samples. FTIR spectrum for amino f-MWCNTs-BSA protein and amino f-MWCNTs-DNA samples are shown in Fig. 2(a) and (b). The biomolecules contain both amine and carboxylic groups. In the present experiment, the carboxylic groups of biomoelcules (such as BSA protein and DNA) react with the free amine groups of the amino f-MWCNTs. As a result, the carboxylic bonds in biomolecules have been converted into amide bonds (—NH— C═O). The interaction between amino f-MWCNTs and biomolecules (BSA protein and DNA) is noticed by the shift of the amide bond (C═O) peak (1 650 to 1 642 and 1 650 to 1 645 cm^{-1} for amino f-MWCNTs-BSA and amino f-MWCNTs-DNA samples, respectively) in the FTIR spectrum 2(a) and (b) [Fig. 2]. The peaks appeared at 1 320 cm^{-1} in spectrum 2(a) and 1 325 cm^{-1} in 2(b) are attributed to the C—N bonds of amide group. As can be seen that new peak has been observed at 1 465 cm^{-1} in spectrum 2(a) and 1 460 cm^{-1} in spectrum 2(b), which is associated with the N—H bond of amide group. The peaks at 1 727, 1 730 and 1 160, 1 180 cm^{-1} are associated with C═O and C—O stretching vibrations of the carboxylic groups, respectively. The peaks at 1 355 cm^{-1} in spectrum 2(a) and 1 365 cm^{-1} 2(b) correspond to the O—H bond in carboxylic groups. This observation indicates the existence of the COOH groups in the biomolecules-treated MWCNTs samples, providing further evidence of BSA protein and DNA attached to the amino f-MWCNTs. In addition, the peaks at 2 930 and 2 855 cm^{-1} in spectrum 2(a) and at 2 945 and 2 840 cm^{-1} in 2(b) correspond to different C—H bond stretching vibrations associated with the biomolecules (BSA protein and DNA).

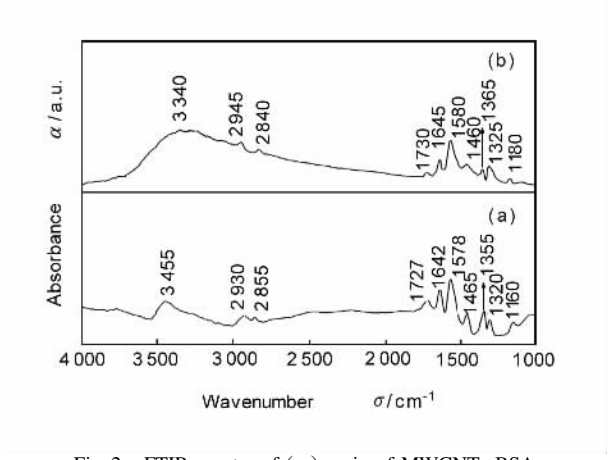

Fig. 2　FTIR spectra of (a) amino f-MWCNTs-BSA protein and (b) amino f-MWCNTs-DNA

Taken together, all these evidence confirm the binding of BSA protein and DNA to the amino f-MWCNTs.

3.2 Microstructural characterizations

The SEM images of as-synthesized MWCNTs and carboxylic group f-MWCNTs are shown in Fig. 3(a) and (b). The as-synthesized MWCNTs are held together into bundles via Van der Waals forces [Fig. 3(a)]. The f- MWCNTs are discrete and shorter than that of as-synthesized MWCNTs due to the acid treatments [Fig. 3(b)]. The TEM image of as-synthesized MWCNTs is illustrated in Fig. 3(c). It is evident from Fig. 3(c) that the nanotubes are entangled and randomly oriented. The outer surface of MWCNTs is smooth [Inset of Fig. 3(c)]. The diameter and length of MWCNTs are ~30-80 nm and 10-20 μm, respectively. After the acid treatment, the MWCNTs are dispersed and most of the nanotubes are shortened (length of MWCNTs ~1-5 μm) [Fig. 3(d)]. The magnified TEM image of carboxylic group f-MWCNTs is shown in inset of Fig. 3(d). Fig. 4 shows the typical TEM image of amino f-MWCNTs. It can be observed from the TEM image of Fig. 4 that the nanotubes surface is rough compared with the nanotubes without functionalization treatment [Inset of Fig. 3(c)]. TEM studies confirmed the success of the attachment of BSA protein molecules and DNA to amino f-MWCNTs, as shown in Fig. 5 and 6. The BSA protein molecules densely decorate the side walls of the MWCNTs [Fig. 5(a)]. The location of the BSA protein is representative of where the amine groups were present. The magnified TEM image of amino f-MWCNT-BSA protein is shown in Fig. 5(b). The attachment of BSA protein on the surface of amino f-MWCNTs is uniform. The homogeneous positioning of BSA on the surface of nanotubes is similar to the attachment of ferritin protein onto carbon nitrogen nanotubes that is reported by K. Jiang et al.[18]. The TEM images of the amino f-MWCNTs- DNA are shown in Fig. 6(a) and (b). The images vividly reveal the modified surface of the nanotubes with DNA. Fig. 6(c) is the magnified TEM image of the MWCNTs with DNA. It can be clearly seen from a comparison of Fig. 6(c) with Fig. 4 that the surface of the CNTs has been modified with DNA after attachment. The DNA attachment to amino f-MWCNT is revealed through the presence of diffuse hazy contrast at the walls of CNTs. It may be mentioned that the present type of study based on FTIR and TEM on the binding of BSA protein and DNA to amino-functionalized MWCNTs appears to be first of its type. To confirm the BSA/DNA molecules attachment to MWCNTs, we carried out several experiments without using amino f-MWCNTs. The as-synthesized (without functionalization treatment) MWCNTs were dispersed in deionized water and few drops of DNA solution were added. The TEM studies showed that the MWCNTs were free of DNA molecules on their sidewalls. Similar result has been observed for MWCNTs-BSA experiment.

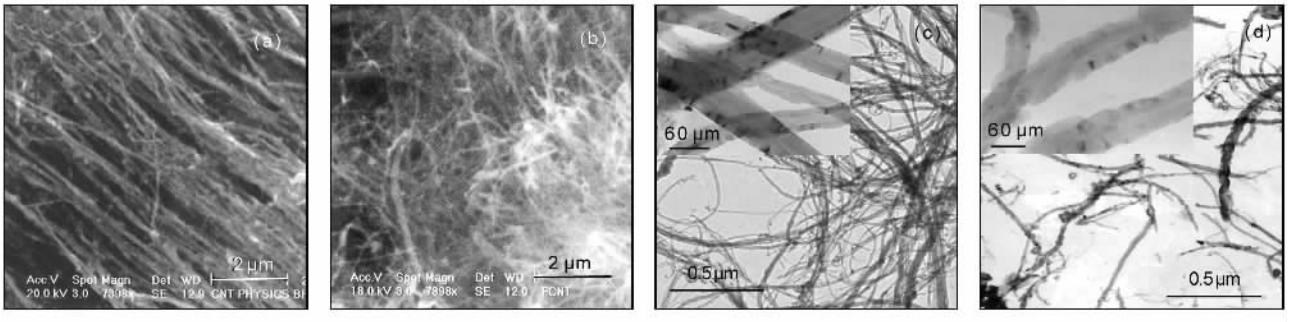

Fig. 3　SEM images of (a) as-synthesized MWCNTs and (b) carboxylic f-MWCNTs. TEM images of (c) as-synthesized MWNTs and (d) carboxylic f-MWCNTs. Insets of (c) and (d) show the magnified TEM image of as-synthesized MWCNTs and carboxylic f-MWCNTs, respectively

4 Conclusions

The biomolecules (BSA protein and DNA) have been attached to the MWCNTs through interaction between amino f-MWCNTs and biomolecules. The MWCNTs have been prepared by spray pyrolysis method and then functionalized with amino group. The process follows three steps, (i) the MWCNTs were functionalized with carboxylic groups using acid oxidation treatments, (ii) these carboxylic groups f-MWCNTs were further treated with ethylenediamine to introduce amino groups via amide formation, and (iii) the amino f-MWCNTs were treated with BSA protein and DNA. The TEM observations clearly confirm the attachment of BSA protein and DNA to the

amino f-MWCNTs. The chemical linkage of f-MWCNTs and binding of biomolecules（BSA protein and DNA）to the amino f-MWCNTs have been confirmed by FTIR spectroscopy. The FTIR results show the presence of carboxylic （at 1 720 cm^{-1} C═O ）and amino groups（at 3 540 cm^{-1} N—H）in the f-MWCNTs. The attachment of biomolecules（BSA protein and DNA）to amino f-MWCNTs is confirmed by the shift of the C═O （amide bond）peak in the amino f-MWCNTs-BSA protein/DNA samples.

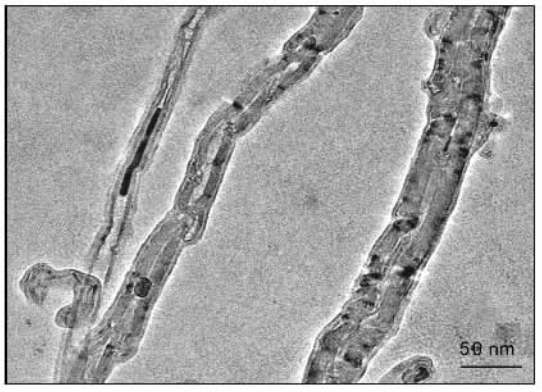

Fig. 4　TEM image of amino f-MWCNTs. Notice the change in microstructure of nanotubes at the outer surface

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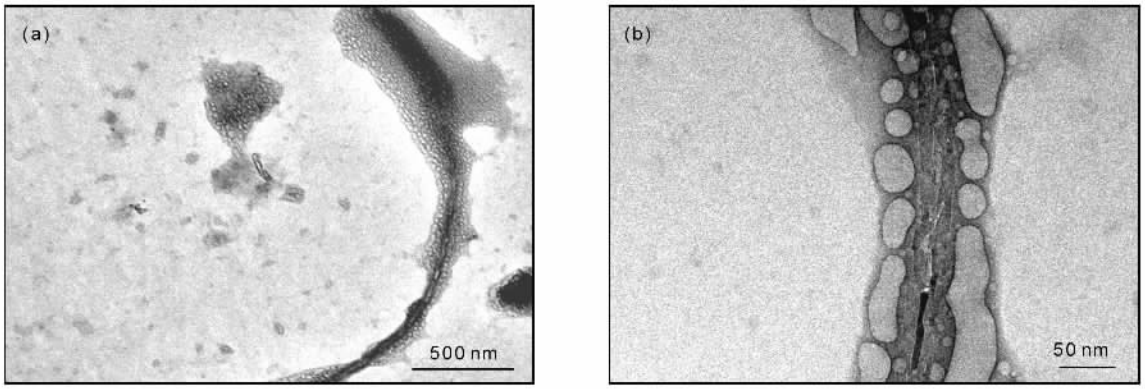

Fig. 5　（a）and（b）TEM images of as prepared amino f- MWCNT-BSA protein samples，which show that the globular and elongated species are attached on the surface of nanotubes

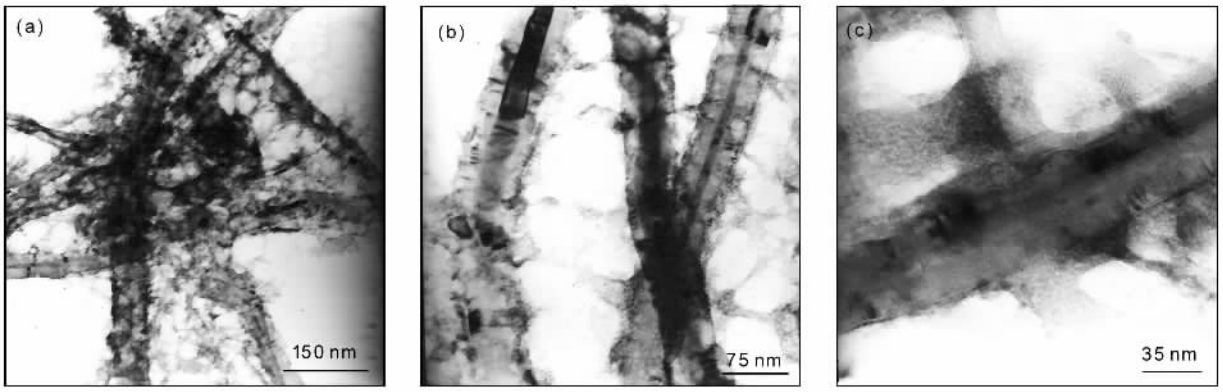

Fig. 6　（a）and（b）TEM images of as prepared amino f- MWCNT-DNA sample.（c）magnified TEM image of the amino f-MWNT-DNA

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氨化碳纳米管接枝生物分子(蛋白质和 DNA)

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摘 要: 报告一种氨基功能化处理的多壁碳纳米管(f-MWCNTs)用于生物分子(如牛血浆蛋白素(BSA)蛋白质及脱氧核醣核酸(DNA))的接枝处理高效方法。以苯—二茂铁喷入氩氛炉于~850 ℃裂解制得 MWCNTs,,而后向纳米管表面导入羧基化合物,再以氨基基团对 MWCNTs,进行功能化处理。该过程包括:乙二胺与羧基基团直接结合,经酰胺化引入胺基基团。对制得的 MWCNTs、f-MWCNTs以及由 BSA 蛋白质和 DNA 接枝的氨化 f-MWCNTs等样品,采用 SEM、TEM、FTIR 进行表征。结果表明:生物分子(BSA 蛋白质和 DNA)接枝于氨化的 f-MWCNTs。

关键词: 碳纳米管;功能化处理;蛋白质;DNA

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