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Research paper

# Targeting of fluorescent palygorskite polyethyleneimine nanocomposite to cancer cells

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ABSTRACT

Palygorskite (Pal), a natural clay mineral, grafted by functional macromolecule polyethyleneimine (PEI) via the coupling grafting method, has further been modified by introducing both fluorescein isothiocyanate (FI) and folic acid (FA), with the aim of specifically targeting and detecting cancer cells. The formed Pal-PEI–FI–FA nanocomposite was nontoxic up to a concentration of 300 $\mu g \cdot mL^{-1}$, and further could be specially taken up by HeLa cells via FA receptor-mediated endocytosis as shown by confocal microscopy and inductively coupled plasma optical emission spectroscopy (ICP-OES) data. Therefore, it is concluded that the novel Pal-based nanocomposite has great potential for biomedical sensing, diagnosis, and therapeutics.



## 1. Introduction

Progress in utilizing inorganic nanoparticles for biomedical applications has advanced rapidly due to multifunctional nanocomposites in which two or more components are incorporated to give multifunctional capabilities (Gao et al., 2004; Lee et al., 2007; Liong et al., 2008; Valeria et al., 2011; Tamara et al., 2012; Chun and John, 2013; Singh et al., 2013). For example, by controlling the morphology of gold nanoparticles and conjugating their surface with antibodies, the nanocomposites with the ability of both selective imaging and photothermal killing of cancer cells by using light with longer wavelengths could be allowed (Wang et al., 2011; Yallapu et al., 2011). Similar success was also demonstrated with polymer-coated carbon nanotubes, by conjugating multiple components such as fluorescent molecules, tumor-targeting moieties, not only could these multifunctional nanotubes target human cancers, they could also be bound with hydrophobic cancer drugs for targeted cancer chemotherapy (Shi et al., 2009; François et al., 2011; Li et al., 2011).

Palygorskite (Pal) is a hydrated magnesium aluminum silicate, a fibrous natural clay mineral with reactive hydroxy groups on its surface (Gard and Follett, 1968; Myriam et al., 1998; Liu, 2007; Cai et al., 2009; Feng et al., 2012; Zhao et al., 2012). Its fibrillar single crystal is the smallest structure unit with a length of 200–500 nm and 10–25 nm in diameter (VanScoyoc et al., 1979; Jones and Galan, 1988; Ma et al., 2009). Due to the special nano structure, low price and large specific surface area, it can be a promising nanoplatform for biomedical applications including, but not limited to, protein drug and gene delivery, drug carriers, and cancer targeting and therapeutics. However, only a few examples of clay-based nanocomposites have been reported, focused on seeking stable fluorescent nanocomposites with biomedical and biotechnological applications (DellaGuardia and Thomas, 1983; Windsor and Tinker, 1999; Aguzzi et al., 2007; Aloisi et al., 2010).

Branched polyethyleneimine (PEI) is a kind of water-soluble polyamine, and there are a great number of primary amines on its macromolecular chains, which can be efficiently tailored for spatial distribution of various moieties (Bae et al., 2011; Date et al., 2011; Kim et al., 2011; Knowles et al., 2012; Siu et al., 2012). Because of these properties, interest has been focused recently toward modifying inorganic nanoparticles with PEI to clay polymer nanocomposite (CPN), then used for selective removal of cadmium ions from blood, efficient gene delivery in cells, nano-drug delivery systems, and so on (Lee et al., 2011; Jin et al., 2012), however, most of these products were designed and focused on enhancing one specific function, or rather, functionalizing the branched PEI with one specific small biomolecule.

Herein, the assembly of a multifunctional Pal-based nanocomposite with the aim of specifically targeting cancer cells is described. As shown in Fig. 1, Pal nanoparticles were grafted by functional macromolecule PEI, via the coupling grafting method, further modified by both fluorescein isothiocyanate (FI) and folic acid (FA), with the aim of specifically targeting and detecting cancer cells. To our best knowledge, none of the literature reports has been related to the use of one natural material

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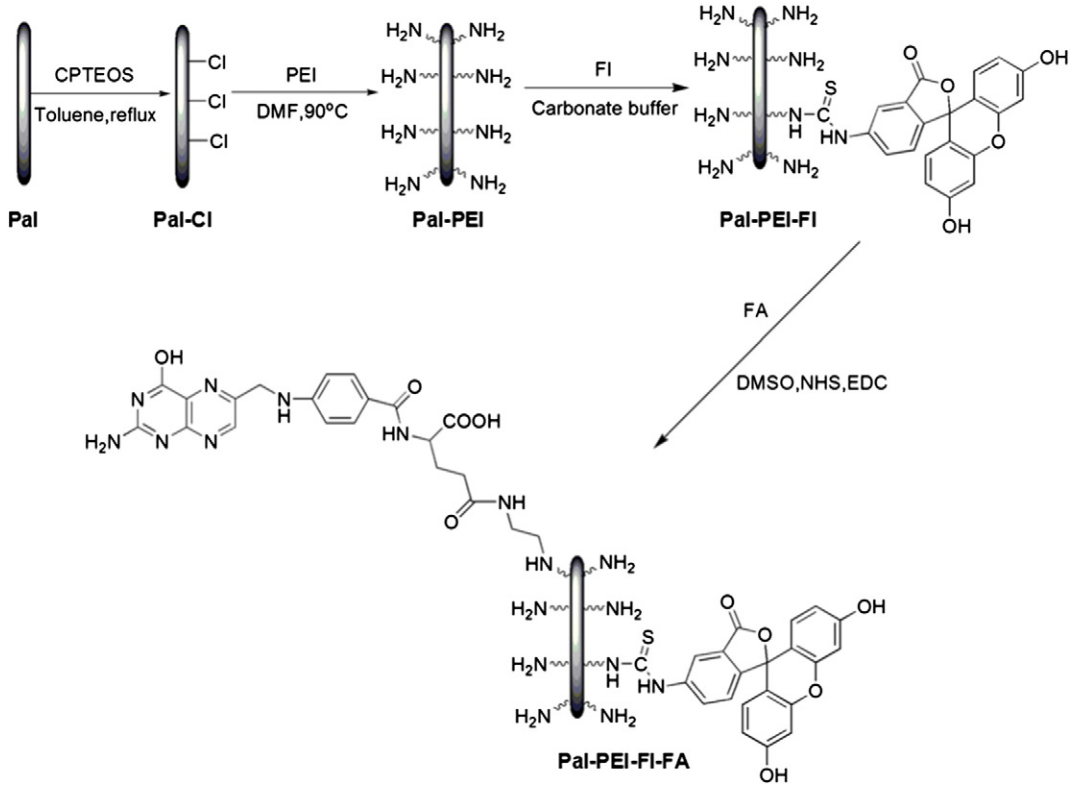


**Fig. 1.** Schematic representation of reactions to modify Pal with specific biomolecules for cancer cell targeting.

as the inorganic matrix for cancer targeting and imaging studies, which is the subject of the present research.

## 2. Experimental section

### 2.1. Materials

Pal (Jiangsu Autobang Co., Ltd., China) was purified through dispersion into $(NaPO_3)_6$ aqueous solution, followed by treating with HCl and $H_2O_2$, with the aim of removing the impurities and activating hydroxyl groups on the surface (Suárez et al., 1995; Frini-Srasra and Srasra, 2010). Fluorescein isothiocyanate (FI) and branched PEI with molecular mass 25 kDa was purchased from Shanghai Aladdin Reagent Co. γ-Chloropropyl triethoxysilane (CPTEOS, 99%) is a reagent of Acros Organics. Folic acid (FA), triethylamine, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), and all other chemicals and solvents were obtained from Aldrich and used as received.

### 2.2. Grafting of PEI to Pal matrix

Grafting of PEI onto the surface of Pal was carried out according to the method described previously with some modifications (An and Gao, 2008; Pang et al., 2011). Briefly, the purified Pal was firstly reacted with coupling agent of CPTEOS at 80 °C, then, added into a 10% PEI solution and reacted at 90 °C for 6 h to obtain Pal PEI nanocomposite. The reaction processes to prepare the Pal PEI nanocomposite can be seen in Fig. S1 (Supporting information). A quantitative measure was carried out by elemental analysis as shown in Table S1 (Supporting information), where the increase in nitrogen content is a clear indicator for the attachment of PEI. The increase of nitrogen content in the last reaction means that 18.2 wt.% of PEI was bound to the Pal surface.

### 2.3. FI conjugation on the Pal PEI nanocomposite

The Pal PEI nanocomposite, was labeled with FI by dispersing 25 mg of nanoparticles in carbonate buffer (pH = 9.0), to which 250 μL of an ethanolic FI solution (1 mg $mL^{-1}$) was added and stirred for 30 min. After this, the FI functionalized CPN (Pal-PEI–FI) was collected by centrifugation, washed with deionized water repeatedly, and dried under vacuum.

### 2.4. Folic acid modification

A 50 μg portion of folic acid was sonicated in DMSO, to which 20 L of a 1 μg $mL^{-1}$ EDC solution was added to activate the carboxylic acid groups of FA. In a separate flask containing the Pal-PEI-FI DMSO dispersion, the folate solution was added, after which the mixture was stirred for 20 h at room temperature. The obtained Pal-PEI-FI-FA nanocomposite was recovered by centrifugation, washed with copious amounts of deionized water and ethanol, dried in vacuo and stored at 277 K.

### 2.5. Methods

The morphologies of the products were observed by transmission electron microscopy (TEM). Nitrogen ($N_2$) adsorption-desorption isotherms were measured by an ASAP 2010 analyzer with nitrogen. CHN elemental analyses were measured on an Elementar Vario EL analyzer. The chemical composition analysis of Pal was conducted by SHIMADZU XRF-1800 X-ray fluorescence (XRF) spectrometer. Powder X-ray diffraction patterns (PXRD) were performed with Rigaku-Dmax 2400 diffractometer using Cu-Kα radiation over the 2θ range of 5–70°. UV–vis spectra were collected using a Perkin-Elmer Lambda 20 UV–vis spectrometer. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to quantify the viability of the cells. Confocal microscopic