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Hybrid biomaterials based on calcium carbonate and polyaniline nanoparticles for application in photothermal therapy

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ABSTRACT

Inorganic materials contain remarkable properties for drug delivery, such as a large surface area and nanoporous structure. Among these materials, $CaCO_3$ microparticles (CMPs) exhibit a high encapsulation efficiency and solubility in acidic media. The extracellular pH of tumor neoplastic tissue is significantly lower than the extracellular pH of normal tissue facilitating the release of drug-encapsulating CMPs in this area. Conducting polyaniline (PANI) absorbs light energy and transforms it into localized heat to produce cell death. This work aimed to generate hybrid CMPs loaded with PANI for photothermal therapy (PTT). The hybrid nanomaterial was synthesized with $CaCO_3$ and carboxymethyl cellulose in a simple, reproducible manner. The CMP-PANI-Cys particles were developed for the first time and represent a novel type of hybrid biomaterial. Resultant nanoparticles were characterized utilizing scanning electron microscopy, dynamic light scattering, zeta potential, UV–vis, FTIR and Raman spectroscopy. *In vitro* HeLa cells in dark and irradiated conditions showed that CMP-PANI-Cys and PANI-Cys are nontoxic at the assayed concentrations. Hybrid biomaterials displayed high efficiency for potential PTT compared with PANI-Cys. In summary, hierarchical hybrid biomaterials composed of CMPs and PANI-Cys combined with near infrared irradiation represents a useful alternative in PTT.

1. Introduction

Calcium carbonate ($CaCO_3$) has been widely used in the development of hybrid organic-inorganic materials [1,2]. Primarily due to the properties $CaCO_3$ of compound: biocompatibility, large specific area, hierarchical structure, and mesoporosity [3–5]. This inorganic material can adsorb or encapsulate compounds [6] and deliver them in areas with an acidic pH, which is found in solid neoplastic tissues [7,8]. Bioinorganic materials possess key properties for drug delivery, such as a large surface area and nanoporous structure, [9] which permit loading of $CaCO_3$ microparticles (CMPs) with various drugs [10,11]. Alternatively, polyaniline (PANI) based organic-inorganic hybrid materials have been synthetized with interesting results, exhibiting improved or novel characteristics in comparison to those of the base PANI polymer [12,13].

In recent years photothermal therapy (PTT) has been extensively studied and compared to conventional chemo- and radiotherapy. This therapy is minimally invasive and can be actively delivered to specific biological targets using conductive particles [14,15]. Considering these advantages, PPT has emerged as a promising therapeutic modality for treating oncological disease. This therapy utilizes light in the near infrared (NIR) wavelength range of 700 nm–1200 nm and nanomaterials with good heat conduct-

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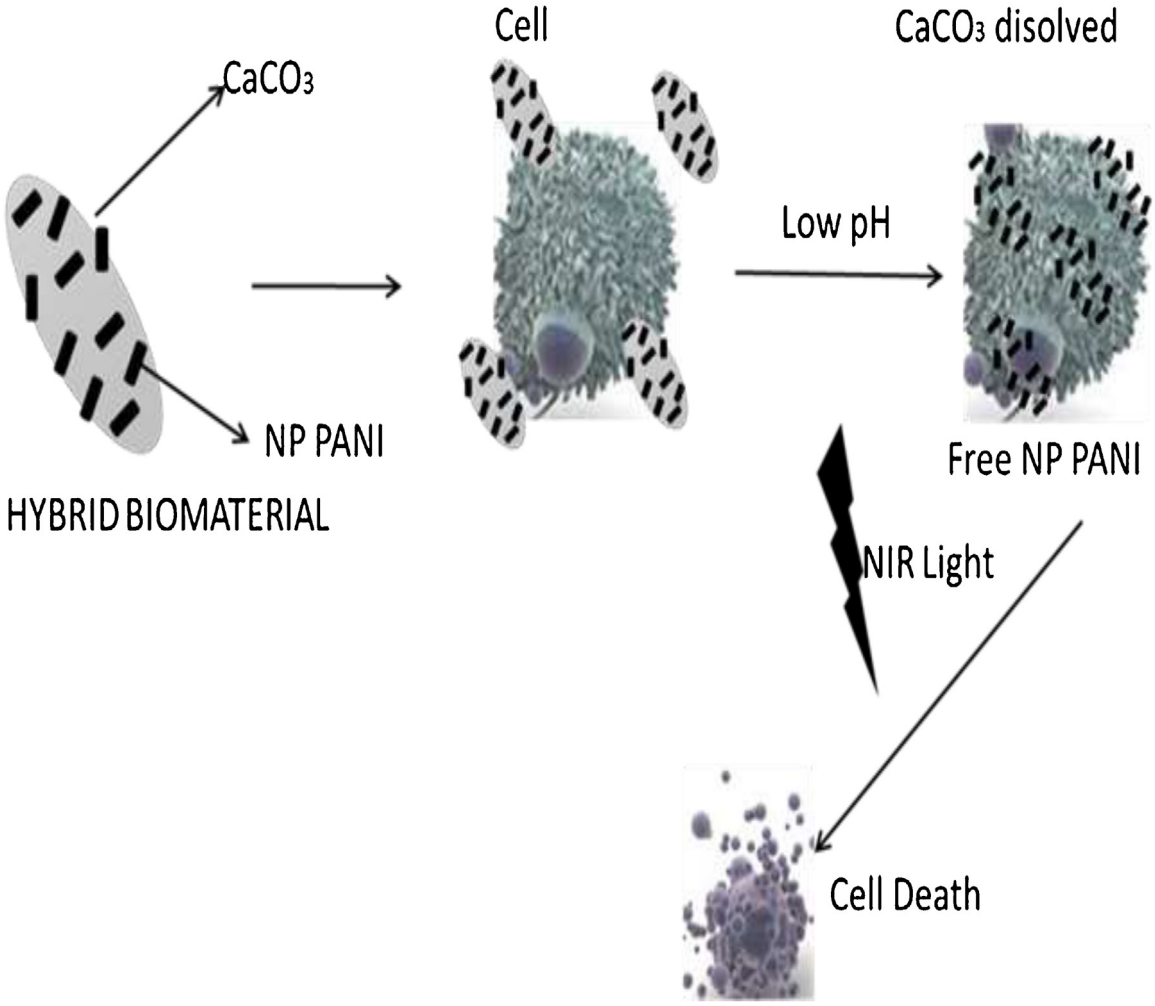

Scheme 1. Preparation of hybrid CMP-PANI-Cys microparticles and NIR laser irradiation of HeLa Cells.

ing properties. [16] Some nanomaterials absorb light in the NIR region, dissipating the radiation as heat [17,18]. In these nanomaterials absorbed photons are transformed into phonons in a process that involves rapid electron–phonon relaxation followed by phonon–phonon relaxation, resulting in increased system and, by conduction, surrounding temperature producing local heat [19]. NIR-induced PTT is particularly attractive because it is minimally absorbed by normal tissue and has relatively deep tissue penetration [20]. PANI particles absorb light energy then transform it into localized heat to produce controlled, local necrosis of neoplastic cells, which is termed photoablation [21].

This unique property of certain nanomaterials has been exploited to kill cancer cells. The hybrid materials used to synthesize advanced microparticles must contain numerous characteristics, such as a particular size and uniform shape, good solubility in an aqueous dispersion or solution, an excitation wavelength must be between 650 nm and 950 nm to avoid damaging the surrounding tissues, high photostability, and non-cytotoxic effects [22,23]. To locate these properties, different materials with conductive properties have been studied, such as graphene oxide [24], metallic nanoparticles [25], nanocomposites [26,27], and polymeric nanomaterials [14], among others [28]. Of the latter variety, PANI has generated great interest due to a low weight, mechanical flexibility, low cost, especially high electrical conductivity [29], low cytotoxicity [30], and biocompatibility [31], which are ideal for preparing hybrid microparticles utilized in PTT [21,22]. However, PANI exhibits low solubility in common solvents and it is not soluble in water medium, due to the balance hydrophilic/hydrophobic groups in the molecule [32]. Therefore, PANI modification with hydrophilic molecules such as L-cysteine can improve solubility in water-based media, maintaining both the conductivity and stability of the polymer [33]. Furthermore, research has demonstrated that the L-cysteine molecule improves biocompatibility to different materials. [34–37] Yslas et al. demonstrated that the modification of a PANI substrate with L-cysteine enhances cell attachment and growth in regard to unmodified PANI which is relevant to tissue engineering applications [34]. Liu et al. synthesized cysteine-coated CuS nanoparticles to operate as highly efficient PTT agents [35]. The Cys-CuS nanoparticles displayed high biocompatibility due to the biocompatible cysteine coating [35]. Moreover, Kesarkar et al. developed a facile method for impregnating a bi-functional linker L-cysteine onto gold nanoparticles [36]. The half-maximal cytotoxic concentration value of functionalized gold nanoparticles increased after conjugating with L-cysteine, improving biocompatibility [36]. The covalent immobilization of L-cysteine onto graphene oxide was recently demonstrated to improve uptake in zebrafish embryos, which showed no tissue defects, malformation, significant hatching delay, or death [37].

In view of the preceding information, this study aimed to generate biocompatible, hierarchical hybrid nanomaterials, composed of PANI modified with L-cysteine (PANI-Cys) and $CaCO_3$, which is able to incorporate the active photothermal nanomaterial (PANI) and deliver the loaded materials to an area with acidic pH near the cancer cells. (Scheme 1).