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Improved intracellular uptake of self-targeting nanoparticles to cancer cells by combination of non-specific and receptor mediated endocytosis

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Using t-Boc as a protective group to deactivate the amino groups of folic acid (FA), we have synthesized FA-PEG conjugates and attached them to nanoparticles through chemical bonding. The self-crosslinking of FA and its conjugates did not occur. Monodisperse nanoparticles coated with FA-PEG conjugates were obtained and FA-PEG coating was on individual nanoparticles, resulting in high efficiency of intracellular nanoparticle uptake into cancer cells.

Key words: PEG-FA, nanoparticles, surface modification, monodisperse, intracellular uptake

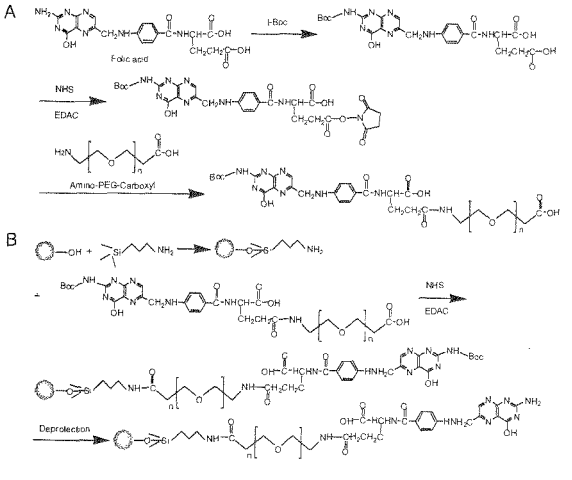
1. INTRODUCTION

Nanoparticles with functional properties have been extensively used in a wide range of bio-applications,[1] for examples, drug and gene delivery, cell and tissue engineering, medical imaging, etc., to serve diagnostic and therapeutic roles. Achieving good nanoparticle dispersivity and biocompatibility is the most crucial step towards the development of nanoparticles designed for intravenous delivery as contrast media or drug carriers. However, nanoparticles have a large surface area/volume ratio and tend to agglomerate or adsorb plasma proteins, and major problems yet to be solved include short circulation time in plasma, non-specific targeting, and low efficiency of nanoparticle internalization to target cells.[2] One possible approach to improving the dispersivity and biocompatibility of nanoparticles and their ability to target specific cells is to coat nanoparticles with biocompatible polymers and targeting agents. The protein-resistive, nonantigenic and biocompatible polyethylene glycol (PEG) has been coated on nanoparticle surfaces, to disperse the nanoparticles and reduce nonspecific protein adsorption and further clearance by macrophages.[3] To achieve the specific cancer cell recognition and increase efficiency of nanoparticle intracellular uptake via receptor-mediated endocytosis, low molecular weight targeting agent such as folic acid (FA) has been coated on nanoparticles because it can couple to the folate receptors overexpressed on cancer cell membranes.[4] Moreover, research has been performed to attach PEG-FA conjugates to the surfaces of nanoparticles through amide bonds to combine the advantages of both PEG and folic acid.[5] However, the protocols that have been developed so far to synthesize PEG-FA conjugates and immobilization of these conjugates on nanoparticles are mainly based on the reaction between amino and carboxyl groups to form amide bonds. The main drawback of these protocols is that the reaction between amino and carboxyl groups may cause self-crosslinking of the reactant when the reactant such as folic acid has amino group on one end and carboxyl group on another end. Some nanoparticles may also be crosslinked together by polymerized folic acid, which may cause severe agglomeration of nanoparticles. Currently, most researchers use gel filtration columns to remove highly polymerized folic acid during the synthesis of PEG-FA conjugates. However, these columns are extremely expensive. Furthermore, folic acid of low polymerization degree still exist in the solution and may polymerize to form bigger molecules during further surface modification process. In this work, a novel method was proposed to synthesize monodisperse nanoparticles immobilized with PEG-FA conjugates for biomedical applications.

2. EXPERIMENTS

PEG-FA conjugates and monodisperse nanoparticles of magnetite modified with PEG-FA conjugates, were synthesized using the protocol described in Scheme 1.

Excessive t-Boc (*tert*-butyloxycarbonyl) was first used to protect the amino groups of folic acid and prevent folic acid from being crosslinked. The N-hydroxysuccinimide (NHS) ester of folic acid (Boc-FA-NHS), which binds to amino groups specifically, was synthesized by esterification of folic acid with N-hydroxysuccinimide in dry dimethylsulfoxide (DMSO) in the presence of catalyst, N-Ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC). The product was recrystallized from dichloromethane and centrifuged down and washed with ethanol, after dialyzed in Spectra/Por CE tubing (MWCO 500) against saline (50 mM, 2×2000 mL) and water (2×2000 mL). Boc-FA-NHS was purified on a silica gel (70-200 mesh, 60 Å) column using a stepwise gradient of methanol (10 to 80%) in chloroform and then chloroform/methanol/water (60:30:10) for the elution of the pure product. The Boc-FA-NHS was further attached to amino-PEG-carboxyl (MW 5000) in the presence of EDAC to form Boc-FA-PEG-carboxyl and the final product was purified in similar procedure. The products and intermediates were characterized by TLC to show a single spot. The magnetite nanoparticles were modified by amino-silane to provide amino groups on the nanoparticle surfaces, which bond to the Boc-FA-PEG-carboxyl through the formation of amide bond. The t-Boc protective group was removed under mild condition,[6] to avoid the dissolution or degradation of nanoparticles in strong HCl acid solution that is usually used for amino group deprotection.



Scheme 1: Synthesis of PEG-folic acid conjugates and surface modification of nanoparticles.

3. RESULTS & DISCUSSION

Due to the protection of amino group on one end of FA and FA-PEG conjugate during the whole process of conjugate synthesis and further surface modification on nanoparticles, reactions only occurred on another end of FA and FA-PEG conjugate and thus avoid the self-crosslinking of FA and FA-PEG. For AFM sample preparation, a small drop of FA-PEG-nanoparticle solution was dropped onto mica surface and air-dried. The AFM and TEM images in Fig. 1 showed that the FA-PEG conjugated nanoparticles look very monodisperse and the average size of nanoparticles was around 10 nm. Therefore, the FA-PEG coatings were on individual nanoparticles.

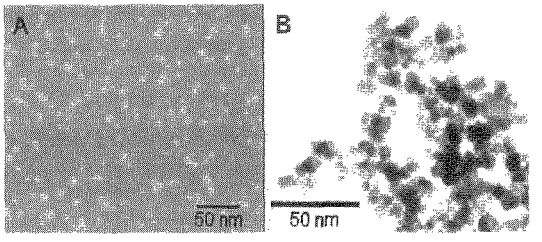


Fig. 1. AFM (A) and TEM (B) images of monodisperse PEG-folic acid conjugated nanoparticles.

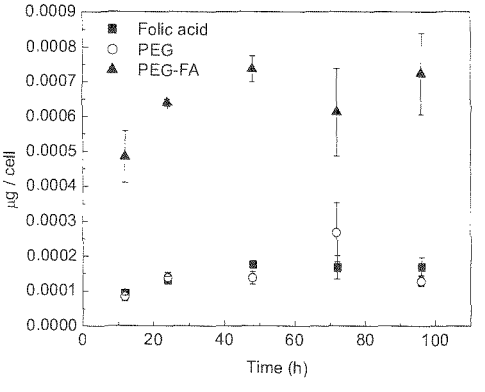


Fig. 2. Intracellular uptake of nanoparticles after surface modified with folic acid, PEG, and PEG-folic acid conjugates.

The nanoparticles coated with only FA, only PEG and FA-PEG were used in intracellular uptake test to clarify the advantages of attaching FA-PEG conjugate to nanoparticles over only FA or PEG. The nanoparticles were dispersed in cell media and fed to breast cancer BT20 cells and nanoparticles inside cells were collected after each interval and quantified, in a similar procedure as reported before.[4] In Fig.2, it was seen that cells took up much more FA-PEG

coated nanoparticles over the whole cell culture period, which showed that the contributions of both FA and PEG to the intracellular nanoparticle uptake were very obvious. It is worthy noting that to coat FA-PEG on individual nanoparticles is critical since some small cells won't take up big particles or nanoparticle agglomerates. Furthermore, more FA-PEG conjugates can be coated on nanoparticles than on nanoparticle agglomerates and higher intracellular uptake efficiency can be achieved no matter based on non-specific endocytosis or receptor mediated endocytosis.

4. CONCLUSION

PEG and FA have good potential for use in a variety of applications in bio-related fields and there is great need to combine them together and bind to nanoparticles. However, nanoparticles are in a high energy status, not stable and easy to agglomerate. Furthermore, FA has functional amino and carboxyl groups on each end which may cause self-crosslinking. Thus appropriate chemical approaches to modifying nanoparticles with PEG and FA conjugates should be selected carefully. In this study we have synthesized samples of monodisperse nanoparticles coated with FA-PEG conjugates through t-Boc protection of amine and deprotection in mild condition. Use of this method can also be extended to the attachment of FA or FA conjugates to other nanoparticles.

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