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Poly aspartic acid peptide-linked PLGA based nanoscale particles: Potential for bone-targeting drug delivery applications

Tao Jiang [a,b,c], Xiaohua Yu [a,b,e], Erica J. Carbone [a,b,c], Clarke Nelson [a,b], Ho Man Kan [a,b,e], Kevin W.-H. Lo [a,b,c,d,*]

[a] Institute for Regenerative Engineering, University of Connecticut Health Center, School of Medicine, Farmington, CT 06030, United States
[b] The Raymond and Beverly Sackler Center for Biomedical, Biological, Physical and Engineering Sciences, University of Connecticut Health Center, School of Medicine, Farmington, CT 06030, United States
[c] Department of Medicine, Division of Endocrinology, University of Connecticut Health Center, School of Medicine, Farmington, CT 06030, United States
[d] Department of Biomedical Engineering, University of Connecticut, School of Engineering, Storrs, CT 06268, United States
[e] Department of Orthopedic Surgery, University of Connecticut Health Center, School of Medicine, Farmington, CT 06030, United States

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ABSTRACT

Delivering drugs specifically to bone tissue is very challenging due to the architecture and structure of bone tissue. Poly(lactic-*co*-glycolic acid) (PLGA)-based nanoparticles (NPs) hold great promise for the delivery of therapeutics to bone tissue. The goal of the present research was to formulate a PLGA-based NP drug delivery system for bone tissue exclusively. Since poly-aspartic acids (poly-Asp) peptide sequence has been shown to bind to hydroxyapatite (HA), and has been suggested as a molecular tool for bone-targeting applications, we fabricated PLGA-based NPs linked with poly-Asp peptide sequence. Nanoparticles made of methoxy – poly(ethylene glycol) (PEG)-PLGA and maleimide-PEG-PLGA were prepared using a water-in-oil-in-water double emulsion and solvent evaporation method. Fluorescein isothiocyanate (FITC)-tagged poly-Asp peptide was conjugated to the surface of the nanoparticles via the alkylation reaction between the sulfhydryl groups at the N-terminal of the peptide and the C=C double bond of maleimide at one end of the polymer chain to form thioether bonds. The conjugation of FITC-tagged poly-Asp peptide to PLGA NPs was confirmed by NMR analysis and fluorescent microscopy. The developed nanoparticle system is highly aqueous dispersible with an average particle size of ~80 nm. *In vitro* binding analyses demonstrated that FITC-poly-Asp NPs were able to bind to HA gel as well as to mineralized matrices produced by human mesenchymal stem cells and mouse bone marrow stromal cells. Using a confocal microscopy technique, an *ex vivo* binding study of mouse major organ ground sections revealed that the FITC-poly-Asp NPs were able to bind specifically to the bone tissue. In addition, proliferation studies indicated that our FITC-poly-Asp NPs did not induce cytotoxicity to human osteoblast-like MG63 cell lines. Altogether, these promising results indicated that this nanoscale targeting system was able to bind to bone tissue specifically and might have a great potential for bone disease therapy in clinical applications.

1. Introduction

Drugs administrated systematically for bone disorders, such as osteoporosis or osteosarcoma, are often problematic because they are rapidly cleared from the body due to the body's excretory system, sometimes even before they are able to fully affect their target sites such as diseased bone. To combat this issue, drugs are generally administered in high dosages and/or frequently which can lead to detrimental systemic side effects (Rizzoli and Reginster, 2013). For instance, the osteoporosis drug Forteo® (generic name: teriparatide) must be administered daily via injection and is associated with severe systemic side effects such as headaches, orthostatic hypertension, hypercalcaemia, and potentially tumors (Cipriani et al., 2012). It would be safer and more effective if the drug could be delivered specifically to bone tissue via a controllable, sustained release delivery system (Aoki et al., 2012). Therefore,

* Corresponding author at: University of Connecticut Health Center, School of Medicine, Farmington, CT 06030, United States. Tel.: +1 860 679 2949; fax: +1 860 679 1553.
E-mail address: wlo@uchc.edu (K.W. -H. Lo).

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there exists a strong need for developing a targeted delivery system specific to bone tissue by using a sustained release drug delivery device.

Delivering drugs specifically to bone tissue is very challenging due to the architecture and structure of bone tissue. Bone possesses one of the most complex hierarchical micro- and nano-structures in the human body, and these unique characteristics call for the proper selection of drug carriers that can target specific diseased portions of the bone. Novel delivery devices must provide exposure of the drug to the complex mineralized structure of bone before being excreted by the body. Nanoscale particles are promising therapeutic delivery devices because of their relatively high drug encapsulation capacity and their small size, which makes them compatible with various administration routes including intravenous injection (Tautzenberger et al., 2012). Moreover, the ability of nanoparticles to traverse the nanostructure of bone allows for them to reach bone fracture sites and promote drug release more easily than larger particles. One of the most common biodegradable polymers used and studied for nanoscale drug delivery is the copolymer poly(lactide-*co*-glycolide) (PLGA) (Vert et al., 1998; Buescher and Margaritis, 2007; Xiao et al., 2010). This polymer has demonstrated excellent host biocompatibility, variable physicochemical properties, and predictable degradation rates (Mooney et al., 1996; Sabir et al., 2009). Furthermore, it has been FDA-approved for a number of biomedical applications and is currently used clinically in surgical sutures and drug delivery devices (Jain, 2000; Jain et al., 1998; Lu et al., 2009). Another advantage of using PLGA-based particles is that PLGA can be easily modified and functionalized to allow the covalent attachment of biological molecules. Surface hydrolysis, aminolysis, and oxygen plasma treatment have been proven to be effective methods to attach reactive groups such as carboxyl, amine, hydroxyl, or peroxyl groups to PLGA marcromolecular chains (Croll et al., 2004; Wan et al., 2004). These modifications lead to enhanced material hydrophilicity, creation of cell recognition sites, as well as introduction of functional groups that are readily activated to covalently bind with peptides or growth factors. On the other hand, functionalities can also be introduced into PLGA by synthesizing PLGA-based block copolymers. Such block copolymers can be synthesized by copolymerization of lactide and glycolide in the presence of other monomers or polymers containing desired functional groups (Zhao et al., 2005; Lin et al., 2010). In certain cases, a second functional polymer can be linked to PLGA macromolecules through the terminal carboxyl groups (Nam et al., 2003; Cao et al., 2010). With the introduction of various functionalities, it is then possible to conjugate biological molecules, such as peptides, to the PLGA-based particles for target specificity. For example, the peptide sequence cyclo(1,12)Pen-ITDGEATDSGC (cLABL) was conjugated to the surface of PLGA nanoparticles to target human umbilical cord vascular endothelial cells (HUVECs) which upregulated intercellular cell-adhesion molecule-1 (ICAM-1) expression (Zhang et al., 2008). Therefore, it is believed that the use of short bone-targeting peptide linked PLGA nanoparticles will provide exciting possibilities for bone therapies.

Hydroxyapatite (HA) binding domains have been identified from several noncollagenous proteins, including osteocalcin (OCN) and osteopontin (OPN), and have demonstrated high affinity to bone tissue (Fujisawa and Kuboki, 1991; Oldberg et al., 1986, 1988; Gorski, 1992). Interestingly, a short peptide sequence of repetitive aspartic acid (Asp) amino acids has been shown to interact exclusively with hard tissues (bone and teeth) *in vitro* and *in vivo* (Kasugai et al., 2000; Yokogawa et al., 2001; Ouyang et al., 2009; Murphy et al., 2007; Ogawa et al., 2013). In fact, aspartic acid peptide sequence has been applied previously by several groups to target drugs to the bone tissue. For instance, it has been reported in pre-clinical animal studies to promote bone accumulation of small molecular weight agents, such as radiogallium-labeled bone imaging agent (Ogawa et al., 2013). These observations prompted us to fabricate PLGA-based NPs linked with poly-Asp peptide sequence so that the poly-Asp peptide will promote the NPs to interact with bone tissue specifically. To facilitate the imaging of the peptide-linked NPs, fluorescein isothiocyanate (FITC) was tagged to the C-terminus of the poly-Asp peptide sequence. A series of *in vitro* and *ex vivo* binding assays were performed to show the exclusive binding affinity of these FITC-poly-Asp NPs to bone tissue.

2. Materials and methods

2.1. Reagents

FITC tagged AspAspAspAspAspAspAspCys peptide sequence was synthesized by LifeTein (South Plainfield, NJ). Poly(lactide-*co*-glycolide)-b-poly(ethylene glycol)-maleimide (maleimide-PEG-PLGA) and methoxy poly(ethylene glycol)-b-poly(lactide-*co*-glycolide)(methoxy-PEG-PLGA) were purchased from Polyscitech (West Lafayette, IN). HA gels, 10X TBS buffer (Tris buffered saline), and 10X PBS buffer (phosphate buffered saline) were purchased from Bio-Rad (Hercules, CA).

2.2. Fabrication of the peptide-linked nanoparticles

Nanoparticles made of 9:1 (w/w) ratio of methoxy-PEG-PLGA and maleimide-PEG-PLGA were prepared using a water-in-oil-in-water (W/O/W) double emulsion and solvent evaporation method as described by Luo et al. (2010). In brief, approximately 108 mg of methoxy-PEG-PLGA and 12 mg of maleimide-PEG-PLGA were dissolved in 4 ml of methylene chloride. Two hundred microliters (200 μl) of 0.22 μm filtered DI water was added to the polymer solution and emulsified using a probe sonicator (Misonix Sonicator 3000, Farmingdale, NY) at 9 W for 30 s on ice. Eight milliliters (8 ml) of 1% sodium cholate solution was then added to the primary emulsion and sonicated at 15 W for 30 s on ice. The formed w/o/w emulsion was subsequently poured into 152 ml 0.5% sodium cholate solution and stirred vigorously for 2 h to allow the evaporation of methylene chloride. Nanoparticles were then washed with DI water and collected by centrifuging at 25,000 rpm with an Optima™ LE-80 K Ultracentrifuge equipped with a SW 28 rotor (Beckman Coulter, Inc.) for 20 min.

FITC-tagged AspAspAspAspAspAspAspCys (FITC-DDDDDDDC or FITC-poly-Asp) peptide sequence was conjugated to the surface of the nanoparticles via the alkylation reaction between the sulfhydryl groups at the N-terminal of the peptide and the C=C double bond of maleimide at one end of the polymer chain to form thioether bonds. Briefly, the peptide solution was mixed with the aqueous nanoparticle suspension at a molar ratio of 1.3:1 and incubated at neutral pH and room temperature overnight. The peptide conjugated nanoparticles were washed extensively with DI water and were either re-suspended in DI water or collected by centrifugation and lyophilization for future uses. Fig. 1 shows the schematic diagram for the synthesis of FITC-poly-Asp-conjugated nanoparticles.

2.3. Characterization of the peptide-linked nanoparticles

The morphology of the methoxy-PEG-PLGA/maleimide-PEG-PLGA nanoparticles was evaluated by transmission electron microscopy (TEM). In brief, a drop of the nanoparticle solution (10 mg/ml) was deposited onto a PELCO® grid and then imaged using a FEI Tecnai T12 S/TEM at an accelerating voltage of 80 kV (FEI, Hillsboro, OR).