Advanced Nanoparticle Drug Delivery System Targeting *Staphylococcus aureus* within Osteoblasts

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ABSTRACT: Osteomyelitis is a serious complication in orthopaedic surgery. One possible reason for chronic osteomyelitis is that certain bacteria can be internalized into human cells and can escape from antibiotic treatments as well as host immune systems. Nanoparticle drug delivery systems can be taken up by infected human cells and could be a promising approach to deliver drugs into infected human cells to eliminate intracellular infection[1]. In this study, a novel copolymer of polyethyleneimine-conjugated poly(lactic-co-glycolic acid) (PEI-PLGA) was synthesized. Polymeric nanoparticles were fabricated using a surfactant-free solvent evaporation method. Zeta potential and particle size analyzer were used to determine surface charge, particle size and distribution. Gentamicin incorporated into PEI-PLGA nanoparticles could be released in a sustained manner. The nanoparticles possessed a tolerable cytotoxicity for osteoblast cells (viability higher than 90%) with a high dosage up to 150 μg/well. *Staphylococcus aureus* (*S. aureus*)-infected osteoblasts were used as a model to evaluate the ability of antibiotic-incorporated nanoparticles in killing intracellular bacteria. Our developed biocompatible and biodegradable nanoparticles have the potential to serve as a sustained drug delivery system for preventing intracellular infection.

Keywords: intracellular infection, nanoparticles, antibiotic-incorporated, uptake

HYPOTHESIS: Antibiotic-incorporated polymeric nanoparticles will be uptaken by infected osteoblasts and will be effective in killing bacteria inside osteoblasts thereby preventing intracellular infection.

AIMS: (i) Develop biocompatible and biodegradable nanoparticles with sustained drug delivery properties; (ii) Determine the effects of nanoparticle properties (e.g. size and surface charge) on the uptake of nanoparticles by osteoblasts; (iii) Evaluate the efficacy of antibiotic-PLGA nanoparticles in elimination of intracellular bacteria.

MATERIALS AND METHODS:

PEI was used for PLGA aminolysis. PEI-PLGA copolymer was synthesized using an aminolysis approach[2]. Polymeric nanoparticles with incorporated antibiotic were fabricated using a solvent evaporation method. Rhodamine B-labeled gentamicin was used as an indicator to determine its release from nanoparticles *in vitro* and the uptake of nanoparticles. The cytotoxicity of nanoparticles for human osteoblast cell line (CRL-11372) was evaluated using MTT assay. *S. aureus* was internalized by human osteoblasts after co-culturing for 45 min. Antibiotic-nanoparticles were introduced in the medium of infected osteoblasts for 4 h. The intracellular infection ratio was evaluated using fluorescence microscopy. Flow cytometry was used to analyze the internalization efficiency of nanoparticles.

RESULTS & DISCUSSION:

Surfactant-free particle systems have attracted attention recently because they can avoid the use of toxic surfactants and reduce rapid uptake by phagocytic cells. In this study, PEI-PLGA copolymers were synthesized utilizing poly(hydroxy acid) aminolysis by an amine compound (i.e. PEI). The produced PEI-PLGA nanoparticles possessed a positive zeta potential of 39 mV, and a mean diameter of 120 nm. Gentamicin was incorporated into nanoparticles during synthesis. A sustained release, up to 2 weeks, of gentamicin from the nanoparticles was obtained (Fig. 1). Cytotoxicity study showed that osteoblast viability remained up to 90% with a concentration of PLGA nanoparticles up to 150 μg/well (24 well plate).

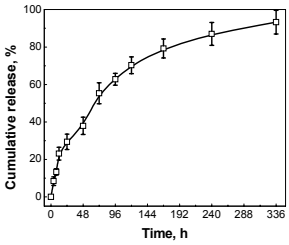

Fig. 1. (a) Typical release profile of gentamicin from PLGA nanoparticles

Moreover, an *S. aureus* infected osteoblast model was developed (Fig. 2a) to evaluate the prevention of intracellular bacteria. Nanoparticles were found to be uptaken by 80% of the osteoblasts. Bacterial colony counting studies showed that gentamicin can be released from PLGA nanoparticles and gentamicin nanoparticles can eliminate *S. aureus in vitro* (Fig. 2b). Our developed nanoparticles could be an advanced therapy for prevention of intracellular infection.

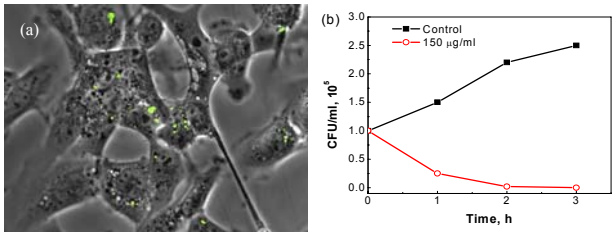

Fig. 2. (a) *S. aureus*-osteoblast co-culture model; (b) Bacterial killing curve of control and gentamicin-nanoparticles (gentamicin concentration: 150 μg/ml).

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