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Drug/ion co-delivery multi-functional nanocarrier to regenerate infected tissue defect

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ABSTRACT

Regeneration of infected tissues is a globally challenging issue in medicine and dentistry. Common clinical therapies involving a complete removal of infected areas together with a treatment of antimicrobial drugs are often suboptimal. Biomaterials with anti-bacterial and pro-regenerative potential can offer a solution to this. Here we design a novel nanocarrier based on a mesoporous silicate-calcium glass by doping with Ag ions and simultaneously loading antimicrobial drugs onto mesopores. The nanocarriers could controllably release multiple ions (silver, calcium, and silicate) and drugs (tetracycline or chlorohexidine) to levels therapeutically relevant, and effectively internalize to human dental stem cells (~90%) with excellent viability, ultimately stimulating odontogenic differentiation. The release of Ag ions had profound effects on most oral bacteria species through a membrane rupture, and the antibiotic delivery complemented the antibacterial functions by inhibiting protein synthesis. Of note, the nanocarriers easily anchored to bacteria membrane helping the delivery of molecules to an intra-bacterial space. When administered to an infected dentin-pulp defect in rats, the therapeutic nanocarriers effectively regenerated tissues following a complete bacterial killing. This novel concept of multiple-delivering ions and drug can be extensively applied to other infectious tissues that require relayed biological functions (anti-bacterial then pro-regenerative) for successful healing.

1. Introduction

Regeneration of infected tissues has been challenging in medicine and dentistry [1]. Clinical therapies generally involve a surgical removal of infected tissues and then a post-treatment with antimicrobial drugs [2]. However, complications are often encountered - infected tissues are not completely removed due to anatomical complexities or insufficient function of administered antibiotic drugs – which significantly limit the regenerative process of the infected tissues that largely lack innate healing capacity [3].

For example, infected dental pulp tissues rarely regenerate themselves and thus a preventive approach of removing a whole pulpal tissue followed by a tight sealing is considered a gold standard [4]. To treat bone infection, osteomyelitis, a prolonged antibiotic administration over weeks to months in conjunction with a surgical local debridement or amputation is necessary, even which shows a poor regeneration capacity of hard tissues [5]. Therefore, along with the surgical treatments, the defects have often been reconstructed through a proper implantation with biomaterials to augment the poor regenerative ability of infected degenerative tissues [6–8].

Antibacterial drugs have been administered to the infected tissues as a major therapy through systemic uptakes or local injections, which can inhibit the synthesis of cell wall, protein, DNA, RNA, or other important constitutional molecules in pathogenic bacteria [9]. However, administration of free drugs induces insufficient delivery to target sites due to a short half-life and a possible systemic circulation; to overcome this, drugs are often overdosed leading to significant side effects [10]. The use of delivery vehicles is

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thus an optimal solution to this; biomaterials that can load drugs efficiently and then deliver them in a sustained and controlled manner potentiate the therapeutic efficacy of drugs in infected sites [11].

On the other stream of therapies for infection, specific types of therapeutic materials have been used. Metallic compounds (*e.g.,* Ag, Mn, Mg, or Cu based), carbon nanomaterials (*e.g.,* carbon nanotube and graphene oxide), and some polymers are studied as the representative antibacterial materials [12–14]. Among them, Ag-based compounds are an essential part of the antimicrobial materials available in clinics [15]. Although their antibacterial effects have been recognized for centuries, the possible mechanisms have only recently been disclosed, *i.e.,* inhibition of a bacterial growth through membrane rupture, enzymatic deactivation, and DNA denaturation [16]. For the case of nano-sized Ag materials, even with their excellent antibacterial effects, tissue toxicity (biocompatibility issue) has frequently been encountered [17], necessitating further development of other Ag-incorporated nanomaterial formulations.

Here we aim to construct a unique nanomaterial system that can effectively treat infectious tissues and consequently regenerate them while preserving excellent biocompatibility of tissues. For this, we focus on mesoporous bioglass nanospheres (BGn) which are structured to efficiently deliver not only silver ions and antimicrobial drugs to bacteria but also hard tissue regenerative ions (calcium and silicate) to host stem cells [18–20]. Therefore, ions/drug delivering BGn are considered to play a bi-functional role (*i.e.,* anti-bacterial and pro-regenerative) in infected tissues.

Ag ions are doped to the glass structure of BGn (replacing Ca partially in silica networks) whereas anti-bacterial drugs are loaded onto the mesopore channels of nanoparticles (NPs); thus different loading and release strategies for multiple therapeutic molecules are envisaged. Although some studies have also incorporated Ag ions within the bioactive glass structure they are mainly in the microparticulate form [21–26]. Unlike the microparticles, the nanoparticles can be used for the intracellular delivery of therapeutic molecules. Some unique properties of BGn reported thus far include ease of ionic doping, highly mesoporous nature, degradability, excellent biocompatibility, and tissue regeneration capacity [27–31]. In particular, the BGn chemistry flexible for ionic modification enables the NPs to deliver target ions; here Ag ions, among others, are to induce anti-bacterial effects [32].

The biological and therapeutic effects of the ion/drug co-delivering BGn are evidenced in a bacteria infected tooth, a model considered to represent one of the most common and significant pathogens demanding public healthcare and thus can find future clinical applications. Some of the key findings also include multi-targeting anti-bacterial pathways provided by the delivered Ag ions and antibacterial drug, and the unique role of nanocarriers through interactions with bacteria membrane in potentiating intra-bacterial delivery of both ion/drug molecules. This approach is envisaged to extend the applications to many infected tissues that require repair and regeneration process after compromising the infectious conditions.

2. Experimental section

2.1. Materials and chemicals

Calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$), tetraethyl orthosilicate (TEOS; $C_8H_{20}O_4Si$), silver nitrate, ($AgNO_3$), poly(-ethylene glycol) (PEG; $(C_2H_4)nH_2O$, Mn = 10000), anhydrous methanol (CH_3OH), ammonium hydroxide (NH_4OH, 28.0%), tris-hydroxymethylaminomethane (Tris–buffer, 99.8%), 1 N hydrochloric acid (HCl), nitric acid (HNO_3, 70%), phosphate buffered saline (PBS) tablets, highly pure chemicals for the preparation of simulated body fluid (SBF), tetracycline hydrochloride as an antibiotic, and all other chemicals used in this study, unless otherwise specified, were all purchased from Sigma-Aldrich. Ultrapure deionized water with 18.2 MΩ.cm (DW, Millipore Direct-Q system) was used throughout all experiments.

2.2. Sol-gel synthesis of Ag-doped nanoparticles

Silver free and silver-doped mesoporous bioactive glass NPs, namely, BGn and Ag-BGn, respectively, based on $85SiO_2 - (15\text{-}x)CaO - x\,AgO$ glass composition (x = 0 and 5 wt%), were prepared by the ultrasound-assisted sol-gel method using PEG as a structural template [33]. Typically, 0.5 mmol of PEG and 0.8 mmol of $Ca(NO_3)_2{\cdot}4H_2O$ (for BGn), or 0.5 mmol of PEG, 0.53 mmol of $Ca(NO_3)_2{\cdot}4H_2O$ and 0.135 mmol of $AgNO_3$ (for Ag-BGn) were solubilized in 150 ml of anhydrous methanol and then the pH was adjusted to around 12.5 using NH_4OH. Another solution containing TEOS was prepared by diluting 4.3 mmol of TEOS in 30 ml of anhydrous methanol. Detailed procedures for the preparation of nanoparticles were described elsewhere [34].

2.3. Nanoparticle characterizations

The morphology and elemental composition of the nanoparticles were examined by high resolution transmission electron microscopy (HR-TEM; JEM-3010, JEOL, Japan) and TEM-equipped energy dispersive spectroscopy (EDS; Oxford). The N_2 adsorption-desorption was recorded at −196.2 °C using a Quadrasorb SI (Quantachrom instruments Ltd., USA). The Brunauer-Emmett-Teller (BET) equation was used to estimate the specific surface area, and pore size and volume were determined on the basis of the Non-Local Density Functional Theory (NLDFT). The ζ-potential of nanoparticles was measured by a laser Doppler electrophoresis instrument (Zetasizer Nano ZS, Malvern Instruments, UK) in DW (25 °C, pH 7.4).

The *in vitro* hydroxyapatite forming ability of the nanoparticles was tested at 37 °C in simulated body fluid (SBF). The SBF was prepared according to the formulation, as described elsewhere [35]. The nanoparticles (50 mg) were immersed in SBF (10 ml) for up to 28 days, and SBF was refreshed every 2 days. The phase of the nanoparticles was analyzed by X-ray diffraction (40 mA and 40 kV, Rigaku, Ultima IV, Japan) with CuKα radiation (λ = 1.5418 °A) from 4 to 70° with a step size of 0.02°. The chemical bonds were analyzed by attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR; Varian 640-IR, Australia) with GladiATR diamond crystal accessory (PIKE Technologies, USA).

The cumulative release of Si, Ca, and Ag ions from the nanoparticles was recorded for up to 28 days. Nanoparticles (50 mg) were immersed in Tris-HCl buffered solution (10 ml, pH 7.4) at 37 °C under 120 rpm. At predetermined time points, nanoparticles were centrifuged (15000 rpm, 10 min) and the supernatants were used for the determination of released ions using inductively coupled plasma atomic emission spectrometry (ICP-AES; OPTIMA 4300 DV, Perkin-Elmer, USA) [33]. Three replicate samples were tested and data were averaged.

2.4. In vitro test using hDPSCs

Multipotent stem cells were isolated and established from the human dental pulp (hDPSCs) 3rd molar tooth as described previously in detail [18], after an approval by Dankook University Dental Hospital (IRB No. H-1407/009/004) [36]. Passages below 10 were used throughout the experiments. The cells were maintained in a humidified atmosphere of 5% CO_2 at 37 °C with a normal