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Evaluation of solubility and cytotoxicity of lanthanum-doped phosphate glasses nanoparticles for drug delivery applications

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ABSTRACT

This paper examined the preparation and characterization of lanthanum-doped phosphate glasses nanoparticles designed for effective management of bone regeneration and associated infections. Glasses incorporated different La_2O_3 contents (0, 5 and 10 mol%) were prepared successfully by a modified alkoxide sol-gel method. They were encoded as P0, PL5 and PL10. Specific surface area and the total pore volume of the glasses were found to decrease by the addition of La_2O_3. TEM photos showed that glasses were spherical nanoparticles and incorporated uniform mesopores which increased in size by the increase of La_2O_3 contents. On the other hand, the dissolution of glasses nanoparticles was tested in different media (distilled H_2O, SBF and tris-HCl buffer). The results showed that lanthanum-modified glasses demonstrated faster ionic release profiles of phosphate species relative to the base glass with faster dissolution from PL5 relative to PL10 in both distilled H_2O and tris-HCl buffer. Furthermore, the possibility of using such glasses as a drug carrier was examined by loading the ciprofloxacin onto samples and studied its release profile. A sustained drug release profile from all phosphate glasses was achieved. Additionally, the incorporation of La_2O_3 in the modified glasses led to a prolonged drug release pattern relative to the base glass.

The cytotoxicity test against BHK fibroblast cells showed that all phosphate nanoparticles were biocompatible. The viability of incubated cells with PL10 was > 97% suggesting that PL10 was not toxic up to 2.5 mg dose. However, a mild reduction of cell viability (93.3%) occurred at 5 mg, and at this dose, the cell viability increased in the following order P0 → PL5 → PL10, as it was 80, 87.4 and 93.3%.

In conclusion, the long-term sustained pattern provided merely by the lanthanum-modified phosphate glasses nanoparticles implied the possibility of their application for localized osteomyelitis treatment.

1. Introduction

Phosphate based glasses are known to be biodegradable in aqueous environments besides having good biocompatibility, low toxicity and minimal inflammatory response [1,2]. Recently, they have been evaluated for drug delivery applications and bone tissue engineering. They showed highly positive and promising results [3–5]. More recently, phosphate-based glasses have been used successfully as nerve guides [6]. Additionally, they were widely used to deliver some antibacterial ions (silver, zinc and copper) to treat local infections [7,8].

Lately, the biodegradation of phosphate glass particles have been improved by the addition of some effective dopants like vanadium or molybdenum oxides [9]. Results showed that the rate of glass dissolution was reduced via stronger hydrogen bonding with high valence vanadium. Better surface attachment and consequent lower rate of drug release was related to hydrogen bonding between the amino-functional groups of vancomycin and the hydrated P—O—H groups in the glass network [9].

Previously, La_2O_3 nanoparticles were prepared and characterized [10]. The anti-bacterial activity of those nanoparticles was evaluated. They showed positive inhibitory effect against gram-positive bacteria such as *Staphylococcus aureus* [10,11]. Moreover, lanthanum calcium manganate nanoparticles were synthesized. They had shown promising antibacterial efficacy against *Pseudomonas aeruginosa* [11].

Lanthanum-containing apatite with variable lanthanum content was prepared by solid state reaction [12]. The addition of lanthanum was found to improve some physicochemical properties of modified apatite such as higher thermal stability beside higher flexural strength. In

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addition, they had better biocompatibility and less cytotoxicity against osteoblast [12]. Moreover, lanthanum-doped hydroxyapatite nanorods were fabricated and loaded with amoxicillin [13]. The result indicated that lanthanum addition had extended the drug release from nanorods. Also, drug loaded lanthanum-doped hydroxyapatite showed an inhibitory results against both Gram positive and Gram negative bacteria [13]. Furthermore, lanthanum substituted β-tricalcium phosphate were developed employing the precipitation technique. The anti-bacterial efficiency of modified β-tricalcium phosphate was confirmed against both *Staphylococcus aureus* and *Escherichia coli* [14]. Synthesis of composite materials based on hydroxyapatite and lanthanum phosphate composite was also reported. Positive bioactivity in simulated body fluid, alongside good biocompatibility toward osteoblasts was proved for those composites [15]. Lanthanum incorporating hydroxyapatite coatings on Ti substrates was carried out, and showed to improve both the cell proliferation and osteogenic differentiation [16].

More recently, lanthanum ions have shown promising tendency to promote the proliferation of human adipose derived mesenchymal cells and to induce their osteogenic differentiation earlier than using a powerful osteoinductive agent such as dexamethasone [17]. Likewise, the osteogenic differentiation potential of lanthanum ions on both rat osteoblasts and murine primary bone marrow stromal cells has been confirmed [18,19]. Finally, the effect of addition of lanthanum oxide to iron phosphate glasses have shown to improve some properties of those glasses, such as glass transition temperature T_g, and thermal stability [20].

According to the previous mentioned findings, the evaluation of lanthanum-doped phosphate glasses nanoparticles might be promising for biomedical applications. Therefore, the main aim of the present study was the preparation and characterization of lanthanum modified phosphate glasses nanoparticles designed to treat severe bone infection. The effect of incorporating different lanthanum oxide contents on the in vitro dissolution and biocompatibility of those nanoparticles was assessed. The ability to use modified nanoparticles for control delivery of ciprofloxacin was evaluated as well.

2. Materials and methods

2.1. Preparation of phosphate glasses nanoparticles

Phosphate glasses nanoparticles incorporating different lanthanum oxide contents were prepared using a modified alkoxide sol-gel technique. The prepared glass compositions were based on the chemical formula $(La_2O_3)_x(P_2O_5\text{-}CaO\text{-}Na_2O)_{(100-x)}$, whereas, x was assigned a value of 0, 5 and 10 mol%. The chemical composition of prepared glass nanoparticles (mol%), beside their code are shown in Table 1. Also, both [O/P] and network connectivity of samples were provided.

The phosphate glass nanoparticles were prepared using chemical grade materials without further purification included *n*-butyl phosphate (1:1 M ratio of mono $OP(OH)_2$(OBun) and di-butyl phosphate $OP(OH)(OBun)_2$ (Alfa Aesar, 98%)), Na-methoxide (30 wt% in methanol, Acros organics), Ca-hydroxide 99% (Acros organics), Lanthanum (III) nitrate hexahydrate (Alfa Aesar), glycerol (Fisher scientific) and absolute ethanol.

Firstly, *n*-butyl phosphate was added dropwise to a beaker that contained Na-methoxide solution while continuous stirring was carried out. Meanwhile, Calcium hydroxide was dissolved in a mixture of glycerol and ethanol until complete dissolution. Afterword, calcium containing solution was added to the previously prepared sodium phosphate solution and stirring was continue over night to prepare the base phosphate glass (P0) in the form of gel. On the other hand, lanthanum doped phosphate glass gels were prepared by the addition of the respective amounts of La_2O_3 in the form of dissolved lanthanum nitrate. The prepared modified samples were also left for stirring overnight. The resulted base and modified phosphate gels were dried at 65 °C for several weeks. Finally, the dried gels were subjected to heat-treatment program as represented in Table 2. This program was chosen based on thermal analysis and was carried out to achieve full glass stabilization by converting the prepared gels to phosphate glass nanoparticles.

Table 1
The chemical compositions of different phosphate glasses in mol%, and the network connectivity of P0, PL5 and PL10 glasses.

Glass code	P_2O_5	CaO	Na_2O	La_2O_3	[O/P]	Network connectivity
P0	50	25	25	0	3	2
PL5	47.5	23.75	23.75	5	3.16	1.68
PL10	45	22.5	22.5	10	3.34	1.34

Table 2
Heat-treatment program (± 1 °C) for sintering of dry gel to transform it to glass.

Temperature (°C)	Rate °C/min	Holding time (h)
0–120	5	10
120–150	5	10
150–180	5	10
180–260	5	10

2.2. Characterization of different glass nanoparticles

2.2.1. X-ray diffraction analysis (XRD)

X-ray diffraction analysis was used to evaluate the amorphous nature of the prepared glasses using the X-ray diffractometer model BRUKER axs, D8ADVANCE adopted with Ni-filter and Cu radiation target with a tube voltage of 40 kV and a current of 40 mA.

2.2.2. Thermal analysis

The thermogravimetric (TGA) and differential scanning calorimetric (DSC) analyses were employed in order to evaluate the thermal characteristics of the investigated gel powders. The weight losses of the different phosphate glasses were recorded using a computerized 7 series USA PerkinElmer thermal analysis system (± 0.001 °C). Scans were performed in an air atmosphere at a temperature range of 50–400 °C at a rate of 10 °C min^{-1}. The materials were analyzed using aluminum oxide powder as a reference.

2.2.3. Transmission electron microscopy (TEM) and energy dispersive X-ray spectroscopy (EDX)

The morphology of the synthesized glass nanoparticles was studied using transmission electron microscope (TEM) (model Jeol-JEM 2100, Japan, microscope) operating at an accelerating voltage of 80 kV. The powder samples were ultrasonically dispersed in ethanol to form diluted suspension, and then few drops were deposited on the carbon-coated copper grid. The suspension was then left for 20 min until dryness prior to imaging. Moreover, the elemental analysis of different glass powders was performed using energy dispersive X-ray spectroscopy (EDS) (HR scanning EM-FEG Quanta 25a) to examine the degree of homogeneity and distribution of oxide components throughout the phosphate glass network.

2.2.4. Textural analysis of phosphate glass nanoparticles

Nitrogen adsorption-desorption isotherms were measured with a high-speed gas sorption analyzer (NOVA 2000 series, chromatic, UK), at 77 K (± 5%). Before the measurements, the samples were out-gassed at 150 °C in vacuum for 6 h. The pore volume, average pore diameter and pore-size distribution were calculated from the adsorption branches of the isotherms using the Barrett-Joyner-Halanda (BJH) method. The total pore volume was estimated from the gas amount adsorbed at a maximum relative pressure. The Barrett-Emmett-Teller (BET) approach was utilized to estimate the specific surface areas.