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Unanticipated stabilization of zinc-silicate glasses by addition of lanthanum: Implications for therapeutic inorganic ion delivery systems

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ABSTRACT

Two series of lanthanum containing zinc-silicate glasses were developed (A: $0.51SiO_2$–$0.29Na_2O$–$(0.20\text{-}X)ZnO$–XLa_2O_3, $0 \le X \le 0.09$; B: $0.51SiO_2$–$0.35ZnO$–$(0.14\text{-}Y)Na_2O$–YLa_2O_3, $0 \le Y \le 0.14$). Glasses were characterized in order to examine composition–structure–property–function relationships with a view to their potential use as therapeutic inorganic ion delivery systems. Glasses were melt synthesized and the role of lanthanum was investigated with respect to (i) glass structure based on density, glass transition temperature (T_g) and ^{29}Si MAS-NMR, (ii) the effect on radiopacity (computed tomography) as a function of composition and (iii) the ion release characteristics under simulated physiological conditions. A linear increase in density and T_g was observed with the addition of La_2O_3 to each glass series. Generally, glasses demonstrated a constant molar volume, while density was observed to increase linearly with the addition of La_2O_3. The peak maxima of the ^{29}Si MAS NMR spectra remained relatively unchanged by the addition of La_2O_3 in both glass series, but the line shapes and widths reveal modifications in the local silica structure. Radiopacity increased with La_2O_3: series A ranged between 1770 ± 33 and 6590 ± 89 HU and B Series B ranged between 2340 ± 54 and 9120 ± 290 HU. Series A glasses were observed to release only Na, it was also noted that increased La_2O_3 caused a reduction in Na^+ release. Series B glasses released all constituent glass elements. Interestingly, in series B, La^{3+} concentration decreased over time indicating possible reprecipitation. Based on the structural data, lanthanum does not behave as a traditional network modifier and may in fact act to stabilize the network against degradation by the formation of mixed cation clusters.

1. Introduction

Over the last 30 years, bioactive glasses have been used in applications for hard tissue repair and regeneration because these glasses bond directly to bone, which is attributed, in part, to the partial dissolution and recrystallization of ions at the implant surface [1,2]. Therapeutic inorganic ions (TII) released from these glasses directly impact cellular functions by up-regulating gene expression [3], particularly genes involved with angiogenesis [4–6] and osteogenesis [7–9], both of which are crucial for effective implant integration. The therapeutic effect of different TIIs, summarized in two reviews by Lakhar et al. [10] and Hoppe et al. [11], shows that TIIs may modulate and/or improve osteogenesis, angiogenesis and inflammatory responses to bioactive glasses both in vivo and in vitro. Based on these data, a new design philosophy is emerging that aims to trigger specific host responses through localized delivery of TIIs from bioactive glasses [2,10,11]. In this context, bioactive glasses have the potential to act as delivery vehicles for a vast number of TIIs in applications outside the area of hard tissue repair and regeneration [12].

In the context of cancer therapy, the design paradigm shifts from one of tissue repair to one where localized tissue damage is the goal. A promising application for TII releasing glasses in cancer therapy is the treatment of hepatocellular carcinoma (HCC). Drug eluting bead-transarterial chemoembolization (DEB-TACE) is a minimally invasive procedure that has emerged as the standard of care for the majority of patients with HCC or metastatic colorectal cancer [13]. Briefly, in DEB-TACE, chemotherapy, typically doxorubicin (DOX), is loaded into microspheres composed of either sulphonated polyvinyl alcohol (s-PVA) via an ion exchange mechanism [14] or sodium acrylate/vinyl alcohol copolymer via absorption and ion exchange mechanisms [15]. Under fluoroscopic guidance, a catheter is inserted percutaneously into the femoral artery, and is guided into the blood vessels feeding the tumor. The drug-loaded microspheres are then deployed through the catheter and flow with the blood stream leading to vessel occlusion, causing tumor infarction with concurrent localized delivery of the chemotherapeutic

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agent. Presently, the available materials for DEB-TACE are limited by two major factors. Firstly, existing materials are radiolucent, which prevents direct imaging of the particles both during and after the procedure [16,17]. Secondly, existing particles must be drug loaded prior to the procedure, which requires significant logistical coordination between the pharmacy and the interventional oncology team to ensure proper drug loading [18,19]. Once loaded the microspheres have a limited shelf life of 4 h at room temperature and 24 h if refrigerated [20].

To overcome the first of these issues, Sharma et al. proposed that "image-able" microspheres (i.e. radiopaque microspheres) should be developed in order to provide intra-procedural feedback regarding their temporal and spatial distribution in the target tissues. Such information would allow physicians to personalize embolization treatments and ensure optimal embolization and localized chemotherapy delivery [17]. To address the issue of image-ability, Kehoe et al. recently developed a radiopaque zinc-silicate based glass proposed for use in transarterial embolization (TAE) of uterine leiomyaomas [21,22]. However, due to its high temperature synthesis, this glass cannot be loaded with traditional organic chemotherapies during production. An alternative approach to loading embolic microspheres with traditional chemotherapies is to produce bioactive glasses composed of radiopaque elements, which are also capable of releasing TII's that may permit localized tissue damage. In this way, the bioactive glass would be provided "drug-loaded" thereby eliminating existing logistical issues while concurrently extending the shelf life as compared to existing material technologies.

Selection of an appropriate TII within the framework of a DEB-TACE treatment requires that, upon release, the TII will cause cellular damage through either a necrotic and/or apoptotic pathway. Research into new metal-based drugs containing lanthanum is fueled by an increase in cisplatin-resistant cancers and has led to the development of a number of lanthanum-based therapies [23,24]. This growing body of literature indicates that lanthanum (La^{3+}) is a good target TII for treating a number of different cancers. Soluble lanthanum citrate induces anoikis in human cervical cancer cell line (HeLa) by cleavage of caspase-9, the principal initiator of most apoptosis pathways [25]. In a study using SiHa cells (a human cervical cancer), lanthanum citrate caused apoptosis through an oxidative stress mediated pathway [26]. In a chicken lymphoblastoid cell line, Zhang et al., demonstrated that $LaCl_3$ induced apoptosis, reduced the number of cells in G2/M phase, increased the number of cells in G0/G1 phase and also inhibited telomerase activity [27]. Durgo et al. showed that complexation of quercetin with lanthanum increased the cytotoxicity of quercetin as well as its stability [28]. Complexes of lanthanum with natural melophlins have demonstrated significant in vitro cytotoxicity to renal cancer A-498, however the mechanism underlying this observation remains unclear [24]. Since lanthanum, in a simple salt or organometallic compound, causes cell death in a number of different cancer cell lines, human and animal, it is an excellent target TII for use in HCC.

Kehoe et al. have demonstrated that the incorporation of La_2O_3 into zinc-silicate glasses increases radiopacity of embolic particles [22]. In addition, the growing body of literature revealing the anti-cancer properties of lanthanum makes it an excellent target TII for use in bioactive glasses for HCC therapy. However, designing a La^{3+} releasing glass depends on understanding its structural role and impact on ion release. Coon and Shelby suggest that lanthanum behaves as a network intermediate in a sodium-silicate glass, supported by the large increase in glass transition temperature (T_g) with increased La_2O_3 loading [29]. However, in a Na–Ca–Si based glass, Branda et al. conclude that lanthanum behaves as a network modifier due to a shift to lower wavenumbers and broadening of the SiO_4 FTIR peak with La_2O_3 addition [30]. In a borosilicate glass, Molieres et al. showed that La_2O_3 addition caused network depolymerization using Raman spectroscopy. Generally network depolymerization results in increased ion release; unexpectedly Molieres et al. found that La_2O_3 addition slowed dissolution [31]. Schaller et al. demonstrated increased network polymerization with addition of La_2O_3 to Na_2O or K_2O containing silicate glasses as well as production of O^{-2} species [32]. It is clear that La_2O_3 has an impact on the controlled release of ions from glasses, however the literature pertaining to the impact of La_2O_3 on zinc-silicates is limited.

The aim of this study is to examine the fundamental composition–structure–property–function relationships in a new class of bioactive glasses containing La_2O_3, Na_2O, ZnO and SiO_2 in order to design a bioactive glass capable of controlled release of La^{3+} for use in DEB-TACE. To support this objective, this paper will evaluate the role of lanthanum in a novel quaternary glass system using theoretical structure analysis identified by Brow and Bunker. The theoretical data will be compared to the ^{29}Si MAS-NMR, density and T_g data for glasses in this system as a function of La_2O_3 replacement for either ZnO or Na_2O. In addition, the ion releasing characteristics of the glasses will be determined after exposure to simulated physiological conditions. Finally, the clinical utility of the glasses with respect to diagnostic imaging visualization will be assessed using CT radiopacity. The findings will be used to better understand the fundamental role of lanthanum in zinc-silicates as well as determine if these compositions have potential for use in DEB-TACE.

2. Materials and methods

2.1. Glass synthesis and processing

Two series of glasses (A and B) were synthesized as in Table 1. Briefly, the composition of series A was $0.51SiO_2$–$0.29Na_2O$–$(0.20\text{-}X)ZnO$–XLa_2O_3, where $0 \le X \le 0.09$. The composition of glass series B was $0.51SiO_2$–$0.35ZnO$–$(0.14\text{-}Y)Na_2O$–YLa_2O_3, where $0 \le Y \le 0.14$.

The appropriate mass of each analytical grade reagent: silicon dioxide (SiO_2), sodium carbonate (Na_2CO_3), zinc oxide (ZnO) and lanthanum oxide (La_2O_3) (Sigma Aldrich, Canada) were weighed using an analytical balance and combined in plastic mixing containers. Powders were homogenously mixed for 1 h using a mechanical mixer, packed into a platinum crucible (50 mL) and fired at 1520 °C (Carbolite High Temerature Box Furnace RK-33,859–03, Cole Parmer) for 1 h, then quenched, dried over night at 120 °C. Glass frits were ground using an agate planetary ball mill (Pulverisette 7, Laval Labs Inc., Canada) for 180 s and sieved to retrieve particles with the following size ranges: 150–300 μm and <45 μm. The <45 μm powders were subsequently used for basic material characterization. The 150–300 μm fractions were used for experiments with direct clinical relevance (ion release properties and radiopacity) since this range is commonly used during TACE procedures [33].

2.2. Characterization of glass powders

2.2.1. Particle size analysis

Particle size distribution was evaluated using a Mastersizer 3000 (Malvern Instruments, Canada). Background measurements of deionized water were used to zero the instrument. Obscuration value was set between 5 and 8%. The particle size was measured ($n = 5$) using both a blue ($\lambda = 470$ nm) and red ($\lambda = 632.8$ nm) laser with values

Table 1
Glass composition, reported as mole fraction of raw materials used.

	Glass ID	SiO_2	Na_2O	ZnO	La_2O_3
Series A	A.1	0.51	0.29	***0.20***	***0***
	A.2	0.51	0.29	***0.1775***	***0.0225***
	A.3	0.51	0.29	***0.155***	***0.045***
	A.4	0.51	0.29	***0.1325***	***0.0675***
	A.5	0.51	0.29	***0.11***	***0.09***
Series B	B.1	0.51	***0.14***	0.35	***0***
	B.2	0.51	***0.0933***	0.35	***0.0467***
	B.3	0.51	***0.07***	0.35	***0.07***
	B.4	0.51	***0.056***	0.35	***0.084***
	B.5	0.51	***0.0467***	0.35	***0.0933***
	B.6	0.51	***0***	0.35	***0.14***

Bold and italic values indicate significance at which components of the glass composition are changing.