Carbohydrate Polymers 153 (2016) 482–491

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Structural characterization and anti-cancerous potential of gallium bioactive glass/hydrogel composites

T.J. Keenan[a,*], L.M. Placek[a], A. Coughlan[c], G.M. Bowers[a,b], M.M. Hall[a], A.W. Wren[a]

[a] Inamori School of Engineering, Alfred University, Alfred, NY, USA
[b] College of Liberal Arts & Sciences, Dept. of Chemistry, Alfred University, Alfred, NY, USA
[c] Dept. of Bioengineering, The University of Toledo, Toledo, OH, USA

ARTICLE INFO

Article history:
Received 26 January 2016
Received in revised form 20 July 2016
Accepted 23 July 2016
Available online 26 July 2016

Keywords:
Hydrogel
Bioactive glass
Composites
Characterization
Anti-cancerous

ABSTRACT

A bioactive glass series ($0.42SiO_2$-$0.10Na_2O$-0.08CaO-$(0.40-X)$ZnO-$(X)Ga_2O_3$) was incorporated into carboxymethyl cellulose (CMC)/dextran (Dex) hydrogels in three different amounts (0.05, 0.10, and 0.25 m^2), and the resulting composites were characterized using transmission electron microscopy (TEM), differential scanning calorimetry (DSC), and ^{13}C Cross Polarization Magic Angle Spinning Nuclear Magnetic Resonance (CP MAS-NMR). Composite extracts were also evaluated in vitro against MG-63 osteosarcoma cells. TEM confirmed glass distribution throughout the composites, although some particle agglomeration was observed. DSC revealed that glass composition and content did have small effects on both T_g and T_m. MAS-NMR revealed that both CMC and Dex were successfully functionalized, that cross-linking occurred, and that glass addition did slightly alter bonding environments. Cell viability analysis suggested that extracts of the glass and composites with the largest Ga-content significantly decreased MG-63 osteosarcoma viability after 30 days. This study successfully characterized this composite series, and demonstrated their potential for anti-cancerous applications.

1. Introduction

Bone is an extremely complex, dynamic, composite tissue, in which defects can occur due to trauma, infection, or tumor resection which are too large for the body to repair without assistance (Killion et al., 2013), and in order to correct these flaws, complex materials must also be synthesized to perform in a dynamic manner. One such class of materials is known as bioactive glasses (BG), which were first formulated by Hench and Paschall (1973), and these materials possess a low glass network-former and high glass network-modifier content, which upon implantation into the body, allow for a series of surface reactions to occur in order for the material to form a strong chemical bond with host tissue, and then the subsequent dissolution of the material over time. BGs are of interest in defect-filling applications because their dissolution products, which are released over time from the eroding glass network, can stimulate positive responses in the host tissue, such as bone-promoting properties (Xynos, Edgar, Buttery, Hench, & Polak, 2001), and can also provide additional therapeutic effects, such as anti-bacterial (Bellantone, Williams, & Hench, 2002; Kawashita et al., 2000), and potentially, anti-cancerous abilities. In the current study, a BG series ($0.42SiO_2$-$0.10Na_2O$-0.08CaO-$(0.40-x)$ZnO-$(x)Ga_2O_{3)}$ was formulated, in which ZnO was substituted with up to 16 mol% Ga_2O_3. This glass composition has been previously shown to allow for dissolution of the glasses upon submersion in aqueous media over periods of up to one year, to allow for calcium (Ca)/phosphorous (P)-containing depositions to form on the surface, to induce only minor pH increases after 30 days of incubation in ultrapure water, and to promote the survival of MC3T3-E1 osteoblasts (Keenan et al., 2016). Additionally, this glass series did not exhibit any statistically significant fluctuations in pH in a separate study by the authors, which evaluated the ionic dissolution and pH of liquid extracts obtained after incubation periods of up to 30 days in phosphate-buffered saline solution (PBS) (Keenan, Placek, Hall, & Wren, 2016). Each constituent of this series has been shown to play a role in either bone metabolism, or the establishment of an interfacial bond between implant and bone, including: (1) silicon (Si), which not only acts as the primary glass network-former, but is also a key element in the formation and calcification of bone tissue (Carlisle, 1970; Carlisle, 1981); (2) sodium (Na), which acts as the primary glass network-modifier and exchanges with H_3O^+ ions in solution, beginning the process of forming a chemical bond between implant

* Corresponding author at: Inamori School of Engineering, Alfred University, Alfred, NY 14802, USA.
E-mail addresses: tjk2@alfred.edu, booyakaucsme619@gmail.com (T.J. Keenan).

http://dx.doi.org/10.1016/j.carbpol.2016.07.100
0144-8617/

and host (Hench, 1991); (3) calcium (Ca), which can increase the expression of insulin-like growth factors, which are responsible for osteoblast proliferation (Marie, 2010), (4) zinc (Zn), which was included for its osteostimulative (Holloway, Collier, Herbst, Hodge, & Nicholson, 1996), and anti-bacterial (Peters, Jackson, Iwano, & Smith, 1972) properties; and (5) gallium (Ga), which was incorporated in an attempt to harness some of the bone-promoting (Bockman et al., 1995; Matkovic, Apseloff, Shepard, & Gerber, 1990; Warrell, Alcock, & Bockman, 1987), anti-bacterial (Kaneko, Thoendel, Olakanmi, Britigan, & Singh, 2007; Olakanmi, Britigan, & Schlesinger, 2000), and anti-cancerous (Hart & Adamson, 1971; Pro et al., 2004; Seligman & Crawford, 1991; Warrell, Coonley, Straus, & Young, 1983) properties exhibited by the pharmaceutical gallium nitrate ($Ga(NO_3)_3$), from the ionic form (Ga^{3+}), upon dissolution from the glass network.

Despite the ability of BGs to chemically bond to tissue, and to release therapeutic ions into the local environment over time, the challenge of developing a sufficient mechanism to deliver these materials to a defect site, and maintain their positioning for extended periods of time, is still an area which requires much more work. Some progress has been made with the development of glass-ceramic scaffolds (Deb, Mandegaran, & Di Silvio, 2010; Handel, Hammer, Nooeaid, Boccaccini, & Hoefer, 2013; Valiathan & Krishnan, 1999) and glass-ionomer/polyalkenoate cements (Boyd & Towler, 2005; Boyd, Clarkin, Wren, & Towler, 2008; Wren, Boyd, & Towler, 2008; Wren, Coughlan, Placek, & Towler, 2012), however, these methods still suffer major drawbacks, such as the requirement for pre-implant machining, extended setting times (Ber, Hatton, & Stewart, 2007), and potentially necrosis-inducing exothermic reactions during the setting process (Chang, Duan, Cai, & Zhang, 2010). Ideally, one would like to obtain a matrix material which can not only allow for easy delivery of BG particles to a bone defect site of any shape without prior shaping, and maintain that position for a long period of time, but also act as a scaffold upon which bone-forming osteoblast cells can attach and deposit new host tissue, without the risks posed by long setting times and exothermic reactions. One class of materials which can potentially be manipulated to satisfy each of these requirements are called polymer hydrogels, which are defined as three-dimensional networks of hydrophilic polymers which can absorb and retain significant amounts of water (Chang, Zhang, Zhou, Zhang, & Kennedy, 2010). Polymer hydrogels have been used in a wide range of applications, including drug-delivery, cosmetics, contact lenses, corneal implants, and in the replacement of tissues such as skin, tendons, ligaments, and bone, because of the excellent hydrophilicity, permeability, and biocompatibility they have displayed (Chang, Duan et al., 2010; Chang, Zhang et al., 2010; Calvert, 2009; Chan, Whitney, & Neufeld, 2009; Liu & Fan, 2005; Yamamoto, Takashi, & Tabata, 2003). Of particular interest for biomaterials applications in the modern age, are hydrogels made from natural polymers due to their natural abundance, biocompatibility, and biodegradability (Cavalieri et al., 2006; Prabaharan & Mano, 2006; Yu, Lu, & Xaio, 2007; Zhai, Yoshii, Kume, & Hashim, 2002). Of the many natural polymers, cellulose and dextran are of particular interest for several reasons. Cellulose is the most abundant polysaccharide available worldwide (Klemm, Heublein, Fink, & Bohn, 2005), which translates to a relatively low cost compared to some other materials, consisting of many β-1,4 linked D-glucose units (Crawford, 1981; Updegraff, 1969), and has proven effective as a constituent of composite materials, such as hydroxyapatite/cellulose composites for enhanced osteoconductivity (Fang, Wan, Tang, Gao, & Dai, 2009). Dextran (Dex) is a homopolysaccharide consisting of straight chains of D-glucose units with α-1,6 linkages, with branches most commonly stemming from α-1,3 linkages (Kwak & Lafleur, 2003), and has also proven to be an effective component in composite materials like Dex gels seeded with bone morphogenic protein (BMP)-loaded polyethylene glycol (PEG) microspheres for BMP-delivery (Chen et al., 2007). Additionally, prior work has been conducted by Hudson et al., in which the water-soluble cellulose derivative sodium carboxymethyl cellulose (CMC) was functionalized with hydrazine groups, and cross-linked via hydrazone bond formation to conjugates of Dex, which was first modified with aldehyde groups, and the anti-fungal Amphotericin B (AmB), to obtain hydrogel composites which were shown not to cause tissue toxicity in mice, and also exhibited anti-fungal efficacy against *Candida albicans* (*C. albicans*) (Hudson, Langer, Fink, & Kohane, 2010).

Currently, composites have been synthesized consisting of degradable CMC/Dex hydrogels impregnated with various loadings of Ga-BG particles, which have been characterized by the authors to determine their swelling characteristics, ionic dissolution rates, and their compatibility with fibroblasts and osteoblasts (Keenan, Placek, Keenan, Hall, & Wren, 2016). Additionally, the authors have conducted studies with both this glass series, and this series of composites, to determine their potential as anti-bacterial and anti-fungal agents (Keenan, Placek, Hall et al., 2016) This study aims to structurally characterize these composites through the use of transmission electron microscopy (TEM), differential thermal analysis (DTA), and ^{13}C cross-polarization magic angle spinning nuclear magnetic resonance (CP/MAS-NMR), and to determine the anti-cancerous potential of liquid extracts obtained from these composites against MG-63 osteosarcoma.

Table 1
Glass compositions (mol. fr.).

	Control	*TGa-1*	*TGa-2*
SiO_2	0.42	0.42	0.42
Ga_2O_3	0.00	0.08	0.16
ZnO	0.40	0.32	0.24
Na_2O	0.10	0.10	0.10
CaO	0.08	0.08	0.08

2. Materials & methods

2.1. Glass synthesis

Three glasses were formulated for this study: 2 Ga-containing glasses (*TGa-1*, *TGa-2*), and 1 Ga-free SiO_2-Na_2O-CaO-ZnO glass (*Control*). The Ga-containing glasses contained incremental substitutions of Ga_2O_3 at the expense of ZnO (Table 1). The powdered mixes of analytical grade reactants (Fisher Sci., PA, USA) were mixed using silica beads, and then oven dried (100 °C, 1 h) and melted (1500 °C, 1 h) in platinum crucibles, and then shocked quenched in water. The resulting frits were dried, ground using a Gy-Ro Mill (Glen Creston Ltd, South West London, UK) in 10 s intervals at 3400 rpm, and sieved, to retrieve glass powders with a maximum particle size of 4 μm (Keenan, Placek, Keenan et al., 2016).

2.2. Polymer synthesis

2.2.1. Materials

Carboxymethyl cellulose, sodium salt (CMC), adipic acid dihydrazide (AAD), *N*-hydroxysulfosuccinimide, sodium salt (Sulfo-NHS), dextran (150,000 MW.) (Dex), and dimethyl sulfoxide (DMSO) were obtained from Acros Organics (NJ, USA). *N*-(3-Dimethyl laminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC), sodium (meta) periodate ($NaIO_4$), ethylene glycol (EG), 0.1 M sodium hydroxide (NaOH), and 0.1 M hydrochloric acid (HCl) were obtained from Thermo Fisher Scientific (MA, USA).