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L. Placek

A. W. Wren

A. Coughlan

Mark R. Towler
Missouri University of Science and Technology, mtowler@mst.edu

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Gallium Containing Glass Polyalkenoate Bone Cements: Ion Release and *E. coli* Inhibition

L. Placek, A.W. Wren, A. Coughlan, M.R. Towler
Inamori School of Engineering, Alfred University
Alfred, NY, USA
e-mail: lmp2@alfred.edu

Abstract- The Glass Polyalkenoate Cement (GPC) series (LCon., LGa-1 and LGa-2) containing gallium (Ga) and a 50 wt% addition of E11 polyacrylic acid (PAA), previously developed, was evaluated for ion release, specifically Si, Ca, Zn and Ga, and *E. coli* inhibition. The maximum inhibition was observed in the t = 0 samples and was 0.35 mm for LCon. and 0.65 mm for LGa-1 and LGa-2.

Keywords-glass polyalkenoate cement, gallium, ion release, E. coli inhibition

I. INTRODUCTION

Glass polyalkenoate cements were originally developed to replace conventional dental filling materials, specifically, amalgam, silicate cements, and zinc oxide cements [1]. They are now used in a wide range of dental applications [1, 2] and have significant potential for use in orthopedic applications [3] due to the ability to tailor their properties based on the specific application [1, 4].

Previous research shows that the Ga GPC series: LCon., LGa-1 and LGa-2 have suitable handling and mechanical properties for use as orthopedic bone cements [5]. This work focuses on evaluating the therapeutic potential of two specific elements, zinc (Zn) and Ga, within this system that have shown therapeutic activity in other biological applications.

Zinc is a proven antibacterial agent and is currently used in a wide variety of applications that employ this characteristic [6]. Gallium is known to have a therapeutic effect in treating bone cancer [7] and is currently used as an agent in cancer treatment [8]. The gallium ion also has anti-inflammatory and immunosuppressive activity in animal models of human disease [8]. Potential ion release from these cements may have anti-bacterial or chemotherapeutic effects on the surrounding biological environment.

II. MATERIALS AND METHODS

A. Glass and GPC Preparation

Three Ga containing glass compositions (*LCon., LGa-1, LGa-2*) were formulated for this study and are listed in Table 1. Glasses were prepared by weighing out appropriate amounts of analytical grade reagents and ball milling (1 h). The mix was then oven dried (100ºC, 1 h) and fired (1500ºC, 1 h) in a platinum crucible and shock quenched into water. The resulting frit was dried, ground and sieved to retrieve a glass powder with a maximum particle size of 45µm.

Cements were prepared by thoroughly mixing the glass powders (<45µm) with E11 polyacrylic acid (PAA – M*w*: 210,000, <90 µm) and de-ionized (DI) water on a glass plate. The cements were formulated in a P:L ratio of 2:1.5 with a 50 wt% addition of PAA.

TABLE I. GLASS COMPOSITIONS (MOL FRACT.)

	LCon.	LGa-1	LGa-2
SiO_2	0.48	0.48	0.48
Ga_2O_3	0.00	0.08	0.16
ZnO	0.40	0.32	0.24
CaO	0.12	0.12	0.12

B. Ion Release

Cement disks (~9 mm Ø x 2 mm) were prepared in triplicate and immersed in sterile, DI water for a period of 1, 7 and 30 days. The amount of water used was normalized to the surface area (SA) of each disk according to equation 1. After extraction of the disk, the solution was filtered and the ion release profile was measured using Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) on a Perkin-Elmer 5300UV (Perkin Elmer, Ma, USA) with Si, Ca, Zn and Ga calibration standards prepared from stock solutions and a control of de-ionized H_2O.

$$\boldsymbol{mL\ H_2O = \frac{SA}{10}} \quad (1)$$

C. E. coli Inhibition Studies

Cement disks were made and stored in a manner identical to that described above for ion release (n = 3). The cement disks were tested immediately after preparing (t = 0) and after 1, 7 and 14 days of immersion in DI water. Bacterial stock solution was made by swabbing frozen *E. coli* from a glycerol stock onto a lysogeny broth (LB) agar plate and incubating for 24 hrs at 37°C, then removing one colony into 5 mL of LB broth and incubating again. To prepare the sample plates: disks were placed, spaced out, in triplicate with 20 mL of liquid LB agar per petri dish. Once the dishes solidified, sterile swabs were used to spread the diluted bacterial stock (50 µL LB broth/*E. coli* cells, 950 µL DI water) over the surface. The plates were then incubated in a 37°C oven for 36 hrs; subsequently the disk diameter (D) and inhibition halo (Z) were measured and equation 2 was then used to compute the inhibition zone (IZ) of each disk.

$$\boldsymbol{IZ = \frac{Z-D}{2}} \quad (2)$$

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III. RESULTS

Ion release of silica (Si) and calcium (Ca) is shown in figure 1 and of Zn and Ga in figure 2. The columns are cumulative over each progressive time period. For Si, Ca, and Zn there is a significantly large release of ions in the first day relative to the following two periods of 6 and 23 days. It also can be seen that there is no significant difference between the measured values for Ca and Zn release over each time period with the exception of the 1 and 30 day values for LCon. (Ca only) and LGa-2. Release of Ga does not occur in the control cement, or significantly in LGa-1 and LGa-2 until 30 days. At 30 days the maximum cumulative release of Ga is 2.4 µg/mL.

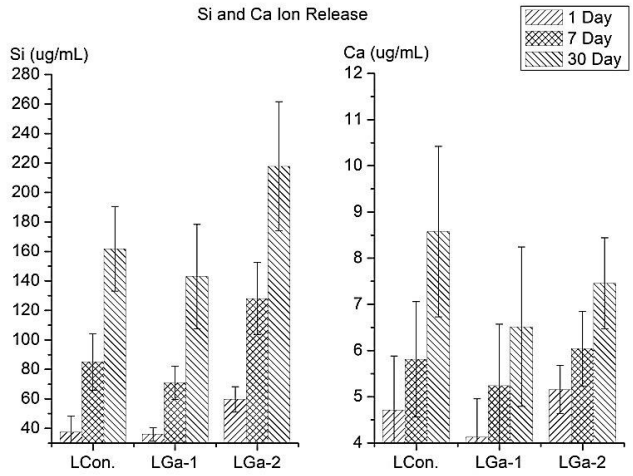

Figure 1. Cumulative ion release profiles of Si and Ca for each cement over 1, 7 and 30 days.

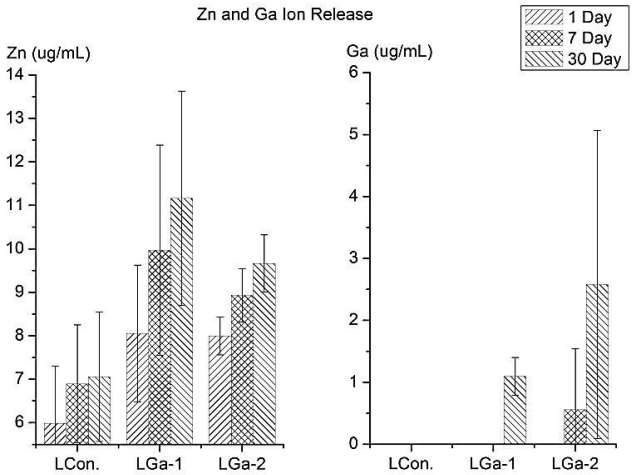

Figure 2. Cumulative ion release profiles of Zn and Ga for each cement over 1, 7 and 30 days.

E. coli inhibition zones are shown in figure 3. The inhibition zones for LCon. cement over each time period is not significantly different than that of the others but are all centered around 0.35 mm. LGa-1 and LGa-2 cements both exhibit a significant trend of decreasing inhibition with a maximum of 0.65 mm at t = 0 to a minimum at 14 days, with the exception of the 7 and 14 day values for LGa-2 which follow the trend but are not significantly different. This trend is expected with the general reduction in ion release over time.

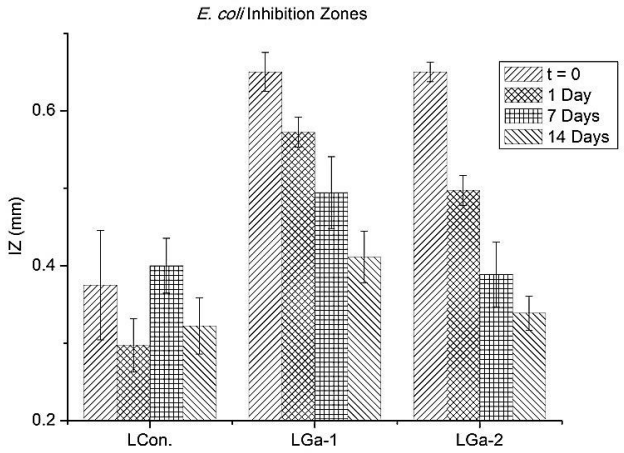

Figure 3. Inhibition of *E. coli* growth by each cement over 0, 1, 7 and 14 days.

IV. CONCLUSION

The cement series exhibits significant ion release and, in the case of zinc, related *E. coli* inhibition behavior, enhancing their candidacy for use as therapeutic bone cements. Future work to develop these cements will include simulated body fluid (SBF) and cell culture studies to determine the structural and cellular effects resulting from interaction between the cement and biological environment.

REFERENCES

[1] P. Nagaraja Upadhya and G. Kishore, "Glass Ionomer Cement – The Different Generations," *Trends Biomater. Artif. Organs,* vol. 18, pp. 158-165, Jan 2005.

[2] O. Clarkin, D. Boyd and M. R. Towler, "Strontium-based glass polyalkenoate cements for luting applications in the skeleton," *J. Biomater. Appl.,* vol. 24, pp. 483-502, 2010.

[3] S. M. Kenny and M. Buggy, "Bone cements and fillers: A review," *J. Mater. Sci.: Mater. Med.,* vol. 14, pp. 923-938, 2003.

[4] A. Oliva, F. D. Ragione, A. Salerno, V. Riccio, G. Tartaro, A. Cozzolino, S. D. Amato, G. Pontoni and V. Zappia, "Biocompatibility studies on glass ionomer cements by primary cultures of human osteoblasts," *Biomaterials,* vol. 17, pp. 1351-1356, 1996.

[5] A. W. Wren, A. Coughlan, L. Placek, M. R. Towler, "Gallium containing glass polyalkenoate anti-cancerous bone cements: glass characterization and physical properties," *J Mater. Sci: Mater. Med.,* vol. 23, pp. 1823-33, 2012.

[6] O. Yamamoto, "Influence of particle size on the antibacterial activity of zinc oxide," *Int J Inorganic Mater,* vol. 3, pp. 643-6, 2001.

[7] R. Ortega, A. Suda, G. Devès, "Nuclear microprobe imaging of gallium nitrate in cancer cells," *Nuclear Instruments and Methods in Physics Research Section B: Beam Inter Mater Atoms,* vol. 210, pp. 364-367, 2003.

[8] C. R. Chitambar, "Medical applications and toxicities of gallium compounds," *Int J Environ Res Public Health,* vol. 7, pp. 337-361, 2010.