In Vitro Biological Evaluation (Cytocompatibility and Degradation Products) of Germanium-containing Ionomer Glasses

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Introduction:

Aluminum free glass-ionomer cements (GICs) show promise for use in vertebroplasty, but are limited by an inability to match required injectability with suitable mechanical properties. Recently, we have shown that cements modified with a germanium containing glass phase overcome this limitation[1]. However, little is known about the biocompatibility of germanium containing glasses. In order to evaluate the impact of germanium modification on biocompatibility it is necessary to study the glass phase in isolation. As such, this study evaluates and correlates the *in vitro* cytocompatibility of germanium-containing glasses for ionomer bone cements with their degradation by-products as a function of time (up to 30 days). Regression analysis is used to model composition-property relationships[1].

Materials and Methods:

12 multicomponent zinc-based glasses were melt-quenched and ground to sub-45 micron powder (Table 1). 0.1g of each glass powder (n=3) were incubated in 10mL tissue culture water at 37°C for 1, 7 and 30 day periods. An MTT assay was performed using NIH 3T3 mouse fibroblast cells to quantitatively evaluate the cell viability of filtered extracts against a tissue culture water control. Inductively coupled plasma optical emission spectrometry (ICP-OES) was used to determine zinc, strontium and germanium ion levels for each extract.

Table 1: Glass molar compositions

	Zn	Sr	Si	Ge	Zr	Na	Ca
DG 200	0.36	0.04	0.48 0	0	0	0	0.12
DG 201	0.36	0.04	0	0.447	0.0335	0.0335	0.087
DG 202	0.36	0.04	0	0.48 0	0	0	0.12
DG 203	0.36	0.04	0.215	0.215	0.05	0.05	0.07
DG 204	0.36	0.04	0.48 0	0	0.05	0.05	0.02
DG 205	0.36	0.04	0	0.38	0.05	0.05	0.12
DG 206	0.36	0.04	0.447	0	0.0335	0.0335	0.087
DG 207	0.36	0.04	0.38 0	0	0.05	0.05	0.12
DG 208	0.36	0.04	0	0.48	0.05	0.05	0.02
DG 209	0.36	0.04	0.215	0.215	0.025	0.025	0.12
DG 210	0.36	0.04	0.223	0.223	0.0335	0.0335	0.087
DG 211	0.36	0.04	0.24	0.24	0.025	0.025	0.07

Results:

Table 2 lists cell viability results for all twelve glass extracts at three experimental time points. Figure 1 displays germanium ion extract levels for three time points. Germanium, zinc and strontium extract levels were recorded up to 400, 1 and 20 parts per million (ppm), respectively. Potential composition-property relationships were identified through regression analysis, using a design of mixtures approach[3].

Table 2: Cell viability of glass extracts over all time periods

Composition	Cell viability (%)		
	1 d extracts	7 d extracts	30 d extracts
Blank	100	100	100
DG 200	98 ± 8	106 ± 8	104 ± 8
DG 201	105 ± 6	106 ± 5	105 ± 11
DG 202	104 ± 6	113 ± 10	100 ± 7
DG 203	104 ± 12	114 ± 13	110 ± 12
DG 204	105 ± 16	116 ± 13	107 ± 14
DG 205	113 ± 17	124 ± 15	116 ± 22
DG 206	106 ±16	107 ± 11	108 ± 16
DG 207	106 ± 17	108 ± 14	102 ± 13
DG 208	108 ± 13	111 ± 16	103 ± 13
DG 209	102 ± 9	109 ± 8	106 ± 9
DG 210	94 ± 6	100 ± 8	103 ± 13
DG 211	92 ± 5	97 ± 5	96 ± 10

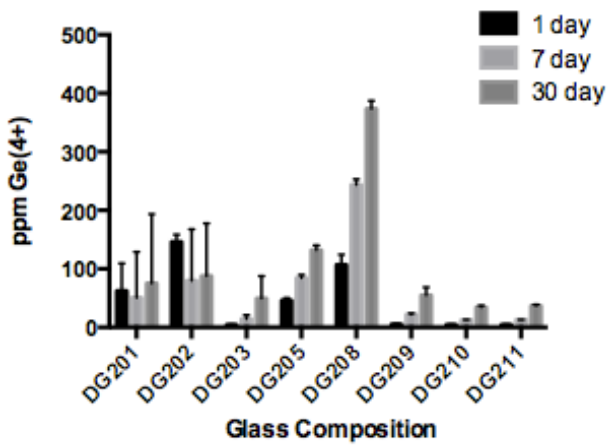

Figure 1: Ge^{4+} glass extract concentrations

Discussion:

No glasses extracts significantly exceeded or fell short of 100% cell viability, indicating cytocompatibility over all time periods. Zinc and strontium divalent ion levels resulting from aqueous degradation of the solid glasses were minimal. Much higher levels of germanium (IV) were discovered in the extracts; significantly varying levels of germanium release depend on glass composition. Interestingly, germanium release appears not to plateau at 30 days for certain compositions (eg. DG208). The levels of Sr^{2+} released by 30 days are in the reported therapeutic range for metabolically compromised bone[2].

References:

1. Dickey, B.T., Kehoe, S., Boyd, D., *paper under review*, Journal of Mechanical Behaviour of Biomedical Materials (January 24, 2013).
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3. Kehoe, S. *et al*. Journal of Non-Crystalline Solids (2012), 358(23): 3388-3395.