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The release of serum proteins and dye from glass ionomer (polyalkenoate) and acrylic cements: a pilot study

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Glass ionomer cements (GICs) are composite materials with the potential for use as improved bone substitutes and cements. The hydrophilic nature of the GIC matrix may confer the ability to release therapeutic agents after surgical implantation which would aid the development of GICs for wider biomedical application. Acrylic and GIC were loaded (5% w/w) with either a model dye or high molecular weight proteins and eluted *in vitro* over 84 days to study simulated drug release. Serum proteins were also adsorbed on to the surface of acrylic and two different GICs and desorption measured over six days. GIC was a suitable matrix for simple dye and protein release, protein release being greater from the GIC than from the acrylic cement. Selective desorption from the two different GICs studied was noted indicating GICs may be formulated to release specific drugs or proteins.

1. Introduction

Glass ionomer (polyalkenoate) cements (GICs) are formed from the reaction of a basic glass and an organic polyacid. The set cement is a reaction-bonded polymer composite consisting of glass particles embedded in an ion-rich, crosslinked, hydrogel matrix [1]. These materials are established in dentistry, where they form an important class of restorative material. They have recently found wider clinical application as bone substitutes, and as cements for non-load-bearing applications in head and neck surgery/otolaryngology [2–4]. The properties that make GICs suitable for wider biomedical application include a minimal exotherm on setting, adhesion to mineralized tissues and metal, excellent biocompatibility and osteoconductive activity following implantation into established bone tissue [2, 3, 5]. It has also been suggested that GICs may represent an alternative to established acrylic bone cements used in orthopaedic surgery [6]. The low exotherm and hydrophilic nature of GICs suggest that they could provide a suitable matrix for drug/protein delivery, which would be advantageous in all their current and potential biomedical applications.

Antibiotics have already been added to acrylic bone cements where their use has contributed to the reduction in post-operative infection rates associated with total hip replacement surgery [7–9]. More recently, experimental studies with growth hormone loaded acrylic cements have shown stimulation of interfacial bone following implantation [10]. Studies using loaded dental and orthopaedic acrylics have provided a better understanding of the drug release mechanisms that operate with these materials. Water soluble compounds are released from glassy polymers following development of small cracks and channels due to hydrostatic pressure and dissolution of drug particles [11, 12]. At low drug loadings release is essentially a surface phenomenon where only the outermost regions of the material are involved in the release process [13, 14]. In this case, a large proportion of the incorporated drug remains trapped in the bulk of the cement where it can have no therapeutic activity [10]. This problem may be overcome by loading greater concentrations of a drug into the matrix or by introducing polymer disrupters, although this is at the expense of the mechanical properties of the material [11, 12]. GICs have a hydrophilic matrix, and it is therefore possible that a different mechanism will operate in this system leading to improved drug release.

The aim of this study was to investigate the release of dye and serum proteins from GICs, simulating the release of drugs or biologically active peptides. The results of this study will determine the suitability of GICs as carrier materials for therapeutic agents, while a greater understanding of the release mechanisms involved will aid the design of improved materials for biomedical application.

2. Materials and methods

2.1. Materials

Two fluoroaluminosilicate glass-based GICs were used in the study. GIC1 (Ionocem®, Ionos GmbH and

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Co KG, Germany) was reacted with a copolymer of polyacrylic and polymaleic acid, while GIC2 (LG27, Department of Materials Science, University of Limerick, Eire) was formed using polyacrylic acid. The polymethylmethacrylate (PMMA) used was a peroxide/amine auto-polymerising acrylic resin (Simplex Rapid Clear®, Howmedica Ltd. UK).

2.2. Dye elution

Acridine Orange base (AOB) (Sigma Ltd. Poole, UK) was added to the glass component of GIC1 or polymer component of the PMMA at a concentration of 5% (w/w). Both were mixed well and the aqueous copolymer or monomer added, the ratio of powder to liquid being 2.6:1 for GIC1 and 2:1 for PMMA. Discs of cement 12 mm in diameter and 2.5 mm thick were produced in a poly(tetrafluoroethylene) mould. GIC1 discs were allowed to set for 40 min at 37 °C and 100% humidity. PMMA discs were allowed to set for 15 min at room temperature. Discs were then eluted in duplicate batches of five into 10 ml of agitated phosphate-buffered saline (PBS) at 37 °C. Samples were taken on changing the PBS after 5, 10, 15, 20 and 25 min, then every hour between 17 and 23 h, every 3 h between 41 and 50 h, after 64 h and after 7, 11, 18 and 84 days. The amount of dye released as percentage absorption at 470 nm was measured in a Phillips PU 8700 series spectrophotometer.

2.3. Protein elution

Discs of GIC1 and PMMA were prepared as described above, but incorporating 5% w/w of Bovine Serum Albumin (BSA) (Sigma Ltd. Poole, UK). Elution was then carried out sampling at five minute intervals for the first hour, after 20, 23, 26, 29 and 44 h, and after 6, 10 and 17 days. The released albumin was assayed by the Bio-Rad protein method (Bio-Rad Laboratories GmbH and Co GG, Watford, UK).

2.4. Protein absorption and desorption

Plain discs of GIC1, GIC2 and PMMA were soaked in duplicate batches of five in 10 ml of a 0.01% solution of BSA for 46 h at 37 °C or in 10 ml of foetal calf serum (FCS) (Sigma Ltd. Poole, UK) for 30 h. Discs were rinsed three times with PBS, and eluted as described above, duplicate samples being taken after 17, 19, and 21 h and after 6 days for analysis of protein content. Statistical analysis was undertaken using the unpaired Student's t-test.

2.5. Release

The cumulative release values were plotted versus the square root of time. Diffusion coefficients were then deduced from the initial gradients of these desorption curves, as described by Crank [15]. Thus:

$$D = \frac{\pi l^2 R^2}{16}$$

where D is the diffusion coefficient, l is the thickness of the disc and R is the gradient of the linear plot.

Theoretical release curves were also generated, using the mathematical function derived by Barnes [16]:

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2}\exp\left[\frac{-\pi^3 R^2 t}{16}\right]$$

where M_t is the cumulative release at time t and M_∞ is the equilibrium value after infinite time. Comparing the ideal curves obtained using this formula with the actual release curves provided a greater understanding of the diffusion processes.

3. Results and discussion

3.1. Dye elution

There was a difference in release behaviour of AOB from glass-ionomer and acrylic cements. Both matrices released the dye rapidly initially by diffusion at a rate proportional to the square root of time (Fig. 1a). The release rate from PMMA being $0.21 \pm 0.03\%$ ml min^{-1} (calculated using linear regression analysis on the first five time points, average correlation coefficient = 0.97, $n = 2$) (Table I) was higher than the release rate from GIC1 $0.14 \pm 0.04\%$ ml min^{-1} (calculated using linear regression analysis on the first five time points, average correlation coefficient = 0.98, $n = 2$) (Table I). The diffusion coefficient for GIC1 was calculated as $1.2 \pm 0.5 \times 10^{-6}$ cm^2 s^{-1} ($n = 2$) (Table I). The cumulative release from GIC1 at longer time periods approached a constant value, whereas there was still some ongoing release from PMMA (Fig. 1b). It is likely that the difference in release profiles was due to disruption of the matrix of the PMMA cement by the particulate AOB powder, thus increasing the cements porosity and leading to crack/channel forma-

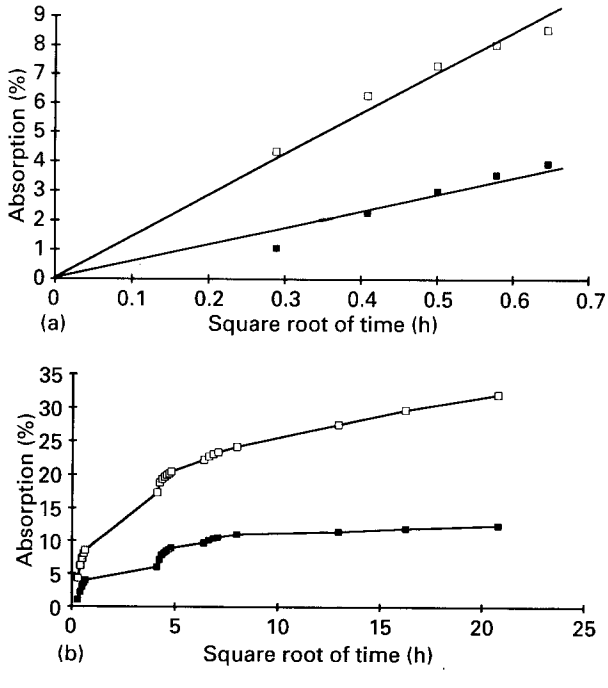

Figure 1 Cumulative acridine orange base (AOB) dye release following a loading of 5% w/w to the powder phase: (a) release within the first 25 min, (b) release over 18 days. (■) = GIC1, (□) = PMMA. Initial release rate of dye from GIC1 = (0.14 ± 0.04) %/ml min^{-1} and from PMMA = (0.21 ± 0.03) %/ml min^{-1}. Diffusion coefficient of albumin for GIC1 = $(1.2 \pm 0.5) \times 10^{-6}$ cm^2 s^{-1}.

TABLE I Initial release rates and diffusion coefficients of acridine orange base (AOB) dye and albumin incorporated into cements

	Initial release rate		Diffusion coefficients	
	AOB ($\%\,ml^{-1}\,min^{-1}$)	Albumin ($\mu g\,ml^{-1}\,min^{-1}$)	AOB ($10^{-6}\,cm^2\,s^{-1}$)	Albumin ($10^{-6}\,cm^2\,s^{-1}$)
GIC	0.14 ± 0.04	13 ± 3	1.2 ± 0.5	2.4 ± 0.5
PMMA	0.21 ± 0.03	8 ± 3	–	1.6 ± 0.6

tion through which dye could diffuse leading to a higher value of release rate (Table I). Another explanation for a higher release could be the chemical interaction of AOB molecules during the setting process of the acrylic, e.g. by inhibiting the catalyst and thus leading to a less dense network between PMMA molecules. In the GIC1 it appeared that the AOB became dispersed in the hydrogel matrix from where it diffuses over a longer time period. The ionic AOB also have become fixed in the ionomeric GIC matrix.

3.2. Protein elution

The release curves of both matrices were similar, showing rapid initial release by diffusion (Fig. 2a). GIC1 releasing albumin at a rate of $13 \pm 3\,\mu g\,ml\,min^{-1}$ (calculated using linear regression analysis on the first six time points, average correlation coefficient = 0.98, $n = 2$), and PMMA releasing albumin at a rate of $8 \pm 3\,\mu g\,ml\,min^{-1}$ (calculated using linear regression analysis on the first six time points, average correlation coefficient = 0.98, $n = 2$) (Table I). At later time points, release from both materials slowed down, the release from GIC1 was however superior to PMMA at all time points resulting in 1.5 times as high a final amount released (Fig. 2b). Comparison between the actual release curve for GIC1 and the calculated ideal curve revealed good correlation at early time periods. However, at later time points release was reduced, probably by build-up of protein in the elutant (Fig. 3). The diffusion coefficients are given in Table I. The eluted discs all maintained their structural integrity and there was no evidence that the protein molecules disrupted the cement matrices. These results are in agreement with those reported previously for the release of albumin and drugs from PMMA [14].

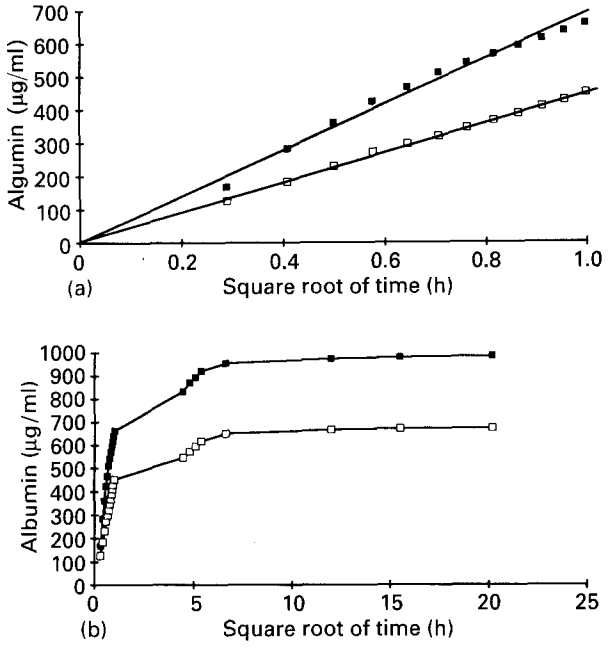

Figure 2 Cumulative bovine serum albumin release following a loading of 5% w/w to the powder phase: (a) release within the first hour, (b) release over 17 days; (■) = GIC1, (□) = PMMA. Initial release rate of albumin from GIC1 = $(13 \pm 3)\,\mu g\,ml^{-1}\,min^{-1}$ and from PMMA = $(8 \pm 3)\,\mu g\,ml^{-1}\,min^{-1}$. Diffusion coefficient of albumin in GIC1 = $(2.4 \pm 0.5) \times 10^{-6}\,cm^2\,s^{-1}$ and in PMMA = $(1.6 \pm 0.6) \times 10^{-6}\,cm^2\,s^{-1}$.

3.3. Protein absorption and desorption

The desorption of albumin from GIC2 was three times as great as from GIC1 ($p < 0.02$) and both GICs released significantly more albumin than PMMA ($p < 0.02$) with GIC2 desorbing more albumin by a factor of ten ($p < 0.01$) (Fig. 4). The desorption of FCS from GIC1 and GIC2 was also greater than from PMMA ($p < 0.02$). In addition, the desorption of FCS from GIC2 was greater than from GIC1 at all the time points studied ($p < 0.02$). For the mixed proteins greater desorption was recorded compared to albumin alone, however, this difference decreased over time from 2.5 times after 17 h to 1.5 times after 144 h. Differences in the measured desorption between individual materials were less than for the same material following absorption/desorption of the two types of protein. GIC1 desorbed about 85% of the amount of FCS proteins compared to GIC2 and PMMA desorbed about 61% of the amount of FCS proteins compared to GIC2 this relationship holding for all time points (Fig. 5).

The results suggest that preferential adsorption of proteins had taken place at the surface of the two GICs. It was also possible that the hydrogel matrix of

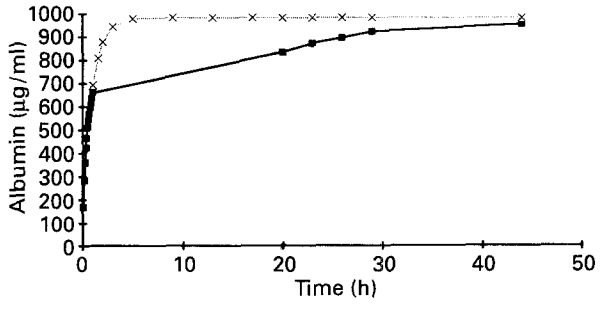

Figure 3 Comparison between the theoretical cumulative release curve (×) and the actual release of albumin from GIC1 ■ within the first 45 h.

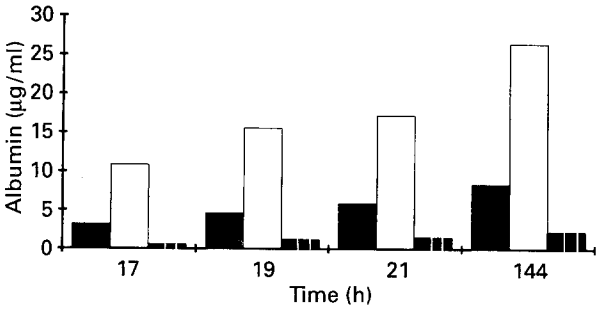

Figure 4 Cumulative albumin desorption following loading by soaking in 0.01% albumin for 46 h; ■ = GIC1, □ = GIC2, ◫ = PMMA.

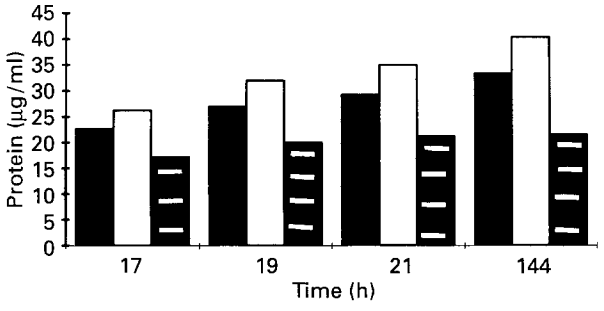

Figure 5 Cumulative protein desorption from discs following loading by soaking in foetal calf serum (FCS) for 30 h; ■ = GIC1, □ = GIC2, ⊟ = PMMA.

the set GICs provided a more favourable environment for maintenance of protein stability than PMMA.

The differences between the two GICs could reflect the different formulation of these materials. The great variety of glasses which can be incorporated into various organic acid matrices may enable GICs to be designed for selective adsorption and desorption of proteins.

4. Conclusions

GICs are capable of releasing dyes or high molecular weight proteins incorporated into their matrix during setting of the cement. In addition, set GICs are capable of absorbing and then desorbing high molecular weight proteins. The GICs evaluated in this study released proteins but not dye (AOB) more efficiently than PMMA. GICs may be suitable for use as release matrices for certain drugs and growth factors, and further study is warranted.

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References

1. P. V. HATTON and I. M. BROOK, *Brit. Dent. J.* **173** (1992) 275.
2. I. M. BROOK, G. T. CRAIG, P. V. HATTON and L. M. JONCK, *Biomaterials* **13** (1992) 721.
3. R. T. RAMSDEN, R. C. D. HERDMAN and R. H. LYE, *J. Laryngol. Otol.* **106** (1992) 949.
4. G. BABIGHIAN, *ibid.* **106** (1992) 954.
5. P. V. HATTON, G. T. CRAIG and I. M. BROOK, *Adv. Biomater.* **10** (1992) 331.
6. L. M. JONCK and C. J. GROBBELAAR, *Clin. Mater.* **6** (1990) 323.
7. H. W. BUCHOLZ, R. A. ELSON and H. LODERKAMPER, in "Recent advances in orthopaedics", Vol. 3, edited by B. McKibbin (Churchill Livingstone, Edinburgh, 1979). p. 61.
8. A. S. BAKER and L. W. GREENHAM, *Amer. J. Bone Joint Surg.* **70** (1988) 1551.
9. G. JOSEFSSON, G. GUDMUNDSSON, L. KOLMERT and S. WIJKSTROM, *Clin. Orthop. Rel. Res.* **253** 174.
10. S. DOWNES, D. WOOD, A. J. MALCOLM and S. Y. ALI, *ibid.* **252** (1990) 293.
11. I. M. BROOK and R. VAN NOORT, *Biomaterials* **6** (1985) 281.
12. I. M. BROOK, R. VAN NOORT and D. J. LAMB, in "Biological and biomechanical performance of biomaterials", Vol. 6, edited by P. Christel, A. Meunier and A. J. C. Lee (Elsevier Science Pub, Amsterdam, 1986) p. 251.
13. S. DOWNES and P. A. MAUGHAN, *Clin. Mater.* **4** (1989) 109.
14. S. DOWNES, *ibid.* **7** (1991) 227.
15. J. CRANK, "The mathematics of diffusion" (Oxford University Press, London, 1975) p. 238, 244.
16. C. BARNES, *Physics* **5** (1934) 4.