Dynamic Properties of Manganese-Alginate Gels for Controlled-Release of Mn^{2+}

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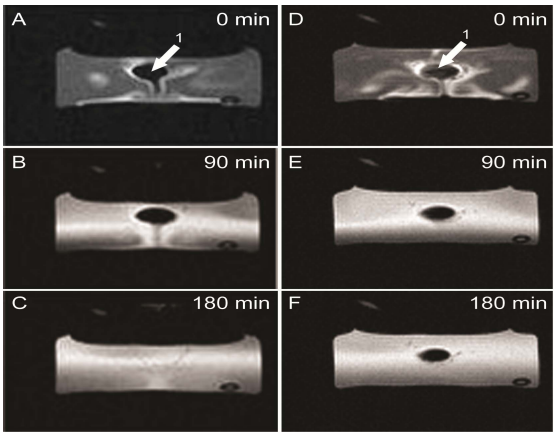

Figure 1: MRI of PolyMG Ba^{2+} (A-C) and High G Ba^{2+} (D-F). The high concentration of Mn^{2+} in the alginate gels (1) causes signal loss (T2* effect), note however that Mn^{2+} concentration remains high in High G Ba $^{2+}$ alginate (D-F) whereas PolyMG Ba^{2+} seems to release Mn^{2+} faster (A-C).

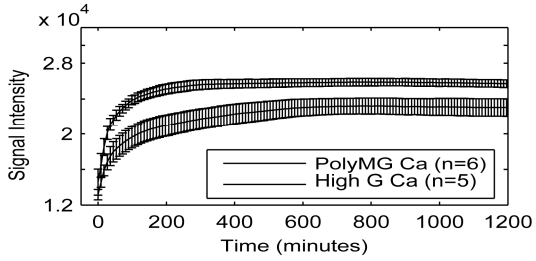

Figure 2: Dynamic MEMRI ±SE of PolyMG Ca^{2+} (upper curve) and High G Ca^{2+} up to 20h after immersion in 0,9% NaCl.

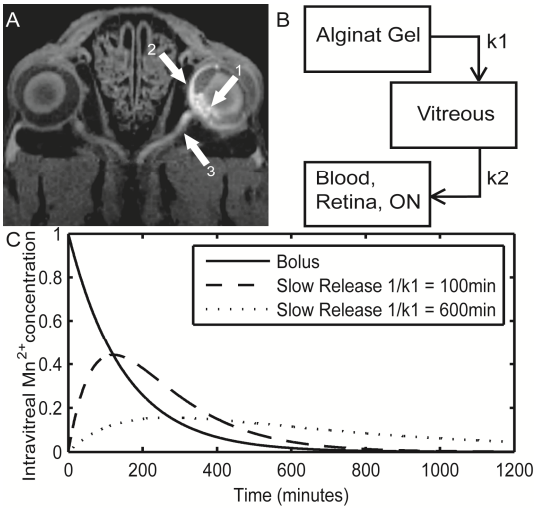

Figure 3: A: MEMRI of the rat eye and ON 24h after *ivit* injection of High G Ba^{2+} alginate beads (1), note the enhancement of the retina (2) and ON (3).
B: Compartment model of *ivit* Mn^{2+} gel transport. Mn^{2+} is released from the alginate gel into the vitreous with rate k1 and cleared from the vitreous with rate k2.
C: Simulation of *ivit* Mn^{2+} concentration using bolus injection and slow release alginate Mn^{2+} gel.

Introduction

Manganese-enhanced MRI (MEMRI) is a versatile technique for imaging of the central nervous system. However, high concentration of manganese (Mn^{2+}) is neurotoxic[1] but could be managed with slow release of Mn^{2+}. A candidate for slow release is alginate beads, which can be given different gelling properties by altering the composition and arrangement of the monomers in the polymer chains and by selecting different divalent ions[2]. The aims of the present study were to 1) Reveal time-constants for Mn^{2+} release for six different types of Mn^{2+}-alginate gels, 2) demonstrate optic nerve (ON) Mn^{2+} enhancement after Mn^{2+} alginate bead injection and 3) simulate intravitreal (*ivit)* Mn^{2+} concentration after Mn^{2+} alginate bead injection.

Materials and Methods

A total of six different alginate gel beads (diam ~400 microns) were formed by dripping a 1.8% (w/v) solution of **high G** alginate (from *Laminaria hyperborea*, 67% G), **high M** alginate (*from Macrocystis pyrifera*, 40% G) and **polyMG** alginate (47% G) into solutions containing either 100mM $MnCl_2$+1mM $BaCl_2$ or 100mM $MnCl_2$+10mM $CaCl_2$. The beads were washed twice in saline to remove excess ions. MRI was obtained every 10 minutes starting immediately after washing of beads and continuing up to 24 hrs after immersion of single alginate gel beads into containers with 5 ml of 0.9% NaCl solution inside the magnet using a custom-made fluid transfer line. Fisher rats (n=2) were injected intravitrally with Mn^{2+} -containing alginate gel spheres (10-15 spheres in 0.9% NaCl).
MEMRI of alginate beads: 7T Bruker Biospec 70/20 AS with BGA-12 400mT/m gradients. T1w MSME: TE/TR=8.1/500ms, matrix=128x64, FOV=30x40mm^2, SI.thickness=1mm, NEX=1, 1 frame every 10 min for 24h.
MEMRI of rat eye and ON: 2.35T Bruker Biospec Avance DBX-100T, 200mT/m gradients, T1w 3D FLASH with TR/TE=15/4.2ms, FA= 25º, matrix =256×256×128, FOV = 50x50x20mm^3, 8 averages, acquisition time 65.5min.
Data analysis was performed using in-house developed software and the Curve Fitting Toolbox™ (Matlab v.7.8.0 Mathworks Inc. Natick MA, USA). Intensity curves were calculated form a ROI placed close to the rim of the container but excluding the alginate beads in each frame. Time constants were extracted from a mono-exponential fit to the intensity curves where the first 4 data points were excluded to avoid the rapidly released Mn^{2+} precipitated by the initial washing in 0.9% $NaCl_2$. *Ivit* Mn^{2+} concentration from a slow release contrast agent was simulated based on a compartment model. The time constant for vitreal clearance (bolus injection) (k2, figure 3B) was adapted from [3].

Results and discussion

Table 1.*Time-constants (minutes) for Mn^{2+} release after exponential fit to intensity curves (95% confidence intervals in brackets).*

	Poly MG	High M	High G
Ca^{2+}	84 (82, 86) n=6	197 (193, 202) n=2	200 (192, 208) n=5
Ba^{2+}	171 (133, 208) n=2	243 (237, 249) n=6	597 (530, 665) n=5

As expected, alginate with high G and Ba^{2+} released Mn^{2+} most slowly since high amounts of G-blocks and Ba^{2+} stabilize the gel. Generally, High M and High G gels are more stable with Ba^{2+} than Ca^{2+}, as also demonstrated by our results. However, Ba^{2+} binds poorly to Poly MG alginate and was not expected to increase the time constant as much as our results indicated. This issue should be addressed in future studies.
Ivit injection of High G Ba^{2+} alginate beads lead to significant enhancement of the retina and ON 24h post *ivit* injection. This demonstrates that Mn^{2+} was released in exchange for sodium ions in the vitreous body and subsequently taken up by retinal ganglion cells (RGC) and transported anterogradely within their axons. Simulations based on a compartment model (figure 3B) showed that the maximum *ivit* Mn^{2+} concentration was reduced to 45% and 16% compared to a bolus injection (100%) by using slow release contrast agents with time constants 100 min and 600 min, respectively. In addition, the simulation showed that slow release increases the time Mn^{2+} is available for uptake by RGC, which is postulated to increase MR contrast in the Mn^{2+} enhanced ON[3].
In conclusion, we have demonstrated that the release rate of Mn^{2+} from alginate gel beads can be controlled by adjusting the type of alginate and by introducing different combinations of divalent ions. Furthermore, simulation shows that the use of Mn^{2+} alginate gel may reduce the maximum Mn^{2+} concentration by ~85% in the vitreous, thus limiting potential toxic effects of Mn^{2+} on RGC.

References

[1] Thuen et al. (2008), JMRI, 28: 855–865.
[2] Mørch et al. (2006), Biomacromolecules 7(5): 1471-1480
[3] Olsen et al. (2010), JMRI, 32: 551–560