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

C. Brekken[1], I. Sandvig[1], O. Haraldseth[1], G. Skjåk-Bræk[2], and Y. Mørch[2]
[1]Circulation and Medical Imaging, NTNU, Trondheim, Norway, [2]Biotechnology, NTNU, Trondheim, Norway

INTRODUCTION

Manganese-enhanced MRI (MEMRI) of brain will benefit from controlled local delivery of Mn^{2+}. In the present study we aimed at designing alginate beads for slow release of Mn^{2+} in the perspective to develop new MEMRI applications. The gelling properties of alginates are highly dependent on the total composition and arrangement of the monomers in the polymer chains. However, as alginate's affinity to divalent ions differs greatly, the choice of ions is also of importance (Ref.1). The binding and release of Mn^{2+} from distinct alginate types using different combinations of divalent ions was studied.

METHODS

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) or a strictly alternating (polyMG) alginate (47% G) into solutions containing divalent cations. The solutions contained either 100mM $MnCl_2$ + 1mM $BaCl_2$ or 100mM $MnCl_2$ + 10mM $CaCl_2$. Beads were washed twice in saline to remove excess ions. Elemental analysis by ICP-MS was used to measure the content of Mn, Ba and Ca in the gel after dissolution in Na-citrate. Gel stability towards ion-exchange (hence manganese release) was studied by monitoring the dimensional swelling of the gel beads upon incubations in saline (0.9% NaCl). The saline solution was exchanged hourly before measuring the diameter of the gel beads.

MRI at 7T on a Bruker Biospec 70/20 AS with BGA-12 400mT/m gradients and a 72mm volume resonator T/R. The MRI protocol consisted of tri-axial 2D scout scans, and an evolutionary 2D T1-w sequence 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. Key parameters for MRI: 2D MSME (T1) scout scan: TE.=11ms, TR=200ms, FOV=50x50mm, Matrix=128x128, Sl.thickness=1mm (3 orthog. slices), NEX=1, AT=25s. 2D MSME (T1) evolution: TE.=8.1ms, TR=500ms, FOV=30x40mm, Matrix=128x64, Sl.thickness=1mm (1 slice), NEX=1, 6 frames pr. hr AT=24hrs. The MR images were analyzed on the MRI console (PV4.0). SNR in one ROI inside and one ROI outside and close to the rim of the beads were calculated and averaged for each distinct alginate bead design.

RESULTS AND DISCUSSION

Both elemental analysis and swelling studies of the alginate gel beads showed great differences in the ion binding properties of alginate to manganese: Not surprisingly, an alginate with high content of G and with added barium in the gelling solution was most stable against ion exchange, followed by the high-G Mn/Ca alginate. Gels of high-M and polyMG alginate, however, all dissolved immediately in saline, indicating that for these gels most of the manganese is released quickly in exchange for sodium ions (data not shown).

Elemental analysis of the different gels showed that for all three alginates the combination of ions in the gelling reservoir was of importance for the final amount of manganese in the gel: After a two-step wash in saline the polyMG Mn/Ca alginate contained only half the amount of manganese compared to that of high-G Mn/Ca alginate with 2.2 and 4.2 mg Mn/ml alginate, respectively. High-M and polyMG Mn/Ba alginate contained manganese levels of approximately 3 mg/ml (TABLE 1).

TABLE 1. *Amount of manganese, calcium and barium bound in alginate gels of various compositions. Alginate concentration was 1.8% (w/v). The gels were washed 2 times in physiological saline solution (3 ml 0.9% for each) to remove excess gelling solution and slightly dried on a paper cloth before they were dissolved in Na-citrate. The values are means ± stdev of 3 parallels and corrected for concentration of ions in the solvent.*

Alginate	Gelling solution	Moles divalent ion/ mole uronic acid			Bound divalent ion(in mg/ml alginate)		
		Mn^{2+}	Ca^{+2}	Ba^{2+}	Mn^{2+}	Ca^{+2}	Ba^{2+}
Macrocystis pyrifera (M.pyr)	0.1M Mn^{2+} and 10mM Ca^{2+}	0.82 ± 0.07	0.15 ± 0.01	0	4.03 ± 0.34	0.55 ± 0.036	0
	0.1M Mn^{2+} and 1mM Ba^{2+}	0.66 ± 0.01	0	0.04 ± 0.002	3.27 ± 0.05	0	0.54 ± 0.02
Laminaria hyperborea	0.1M Mn^{2+} and 10mM Ca^{2+}	0.84 ± 0.02	0.21 ± 0.002	0	4.17 ± 0.11	0.75 ± 0.008	0
	0.1M Mn^{2+} and 1mM Ba^{2+}	0.81 ± 0.05	0	0.14 ± 0.006	4.01 ± 0.24	0	1.71 ± 0.07
Polyalternating alginate (polyMG)	0.1M Mn^{2+} and 10mM Ca^{2+}	0.44 ± 0.01	0.10 ± 0.003	0	2.19 ± 0.04	0.37 ± 0.011	0
	0.1M Mn^{2+} and 1mM Ba^{2+}	0.63 ± 0.04	0	0.01 ± 0	3.12 ± 0.20	0	0.11 ± 0.005

Dynamic T1-weighted MRI of single alginate beads immersed into NaCl-solution showed that the release rate differed by a factor of up to ~100% between the 4 differently designed beads imaged so far (Ref.2). Also, the MRI examination accurately depicted the dissolving alginate beads as the SNR inside the bead rapidly increased due to equilibration with the surrounding reservoir (FIGURE 1). Furthermore, the SNR differed between the different beads which can be correlated to the elemental analysis data (TABLE 1) in terms of molar amount of manganese per alginate bead.

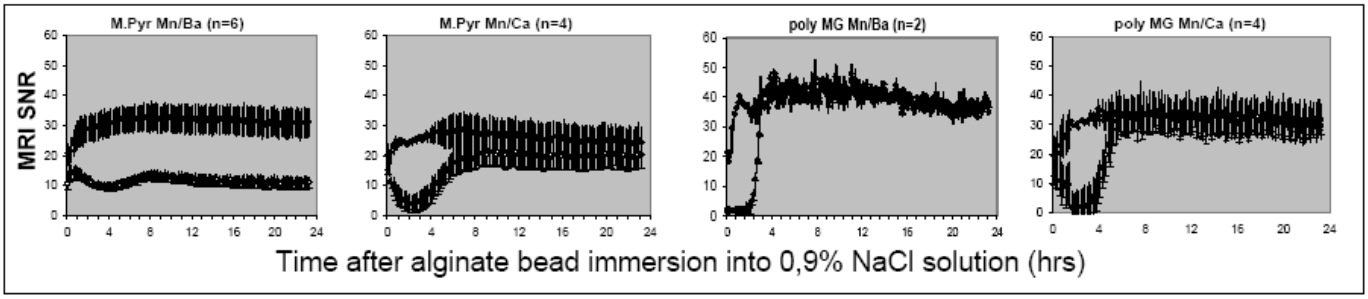

FIGURE 1. *Dynamic MEMRI Signal-to-Noise ratios (Mean±SEM) of 4 distinct alginate bead types up to 24hr after immersion in 0.9% NaCl solution at Time=0. Upper curves (closed circles) represent SNR in a ROI around and outside the rim of the alginate beads. Lower curves (open circles) represent SNR in a ROI placed inside the alginate beads.*

CONCLUSIONS

The total amount of manganese in gels as well as the release rate of Mn^{2+} out of gels can be controlled by the choice of alginates and combination of cross-binding ions. For a high-G alginate, addition of Ba^{2+} will lead to stable gels towards ion exchange and thereby a slow release rate of manganese, whereas a polyMG alginate will give the opposite. The results indicate that nano-fabrication of Mn-alginates can tailor-make biocompatible manganese delivery systems and hopefully help in introducing MEMRI in targeted contrast-enhanced MRI of otherwise toxicity-limited organs, such as the brain.

REFERENCES

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