

MgO implanted in rat tibia bone marrow is osteoinductive through the formation of a matrix, containing hydroxyapatite

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Abstract

Healing of rat tibia after intramedullary implantation of MgO was analysed by Environmental Scanning Electron Microscopy (ESEM) and Energy-Dispersive X-ray spectroscopy (EDX). The results indicated the formation of hydroxyapatite in the entire intramedullary space after 1 week of healing. Then corroded Mg, MgO and MgCO₃ were incubated with DMEM in vitro for 16 h and the surface of the material was analysed by EDX and Time-of-Flight Secondary Ion Mass (ToF-SIMS). The chemical analysis of the Mg corrosion products indicate that hydroxyapatite is formed at the material surface and that MgCO₃ was an efficient catalyzer of hydroxyapatite formation

Introduction

The discovery of the induction of bone formation by metallic magnesium was made early in the last century [1]. The exploitation of this discovery, through application of biodegradable implants in orthopedic surgery, was hampered by the release of hydrogen gas during the corrosion of Mg to MgO. This effect can be avoided by implanting MgO [2], transforming to MgOH and MgCO₃ in the tissue, and still inducing bone formation [2, 3]. The aim of the present study was to investigate the fate of implanted MgO over time and to elucidate the mechanism of bone induction.

The results obtained indicate a possible pathway for the healing of MgO into bone, starting with a catalytic formation of hydroxyapatite at the MgCO₃ surface in contact with body fluid.

Materials and Methods

Chemicals: MgO and MgCO₃ were from Sigma Chem. Co (99.9% PA). MgO was also prepared by corrosion of pure Mg (Sigma Chem Co) in water. The samples were washed consecutively in hexane, acetone, ethanol and water. A paste of 0.1 g of dry metal oxide and 0.1 ml of sterile saline was prepared.

Experimental: The metal oxide paste was implanted into rat tibia as described previously [3]. During bone healing in rat tibia, samples were taken after 1 week. The bones were frozen in ethanol, freeze substituted for 1 week and sectioned with a diamond saw and stored in absolute ethanol until analysis.

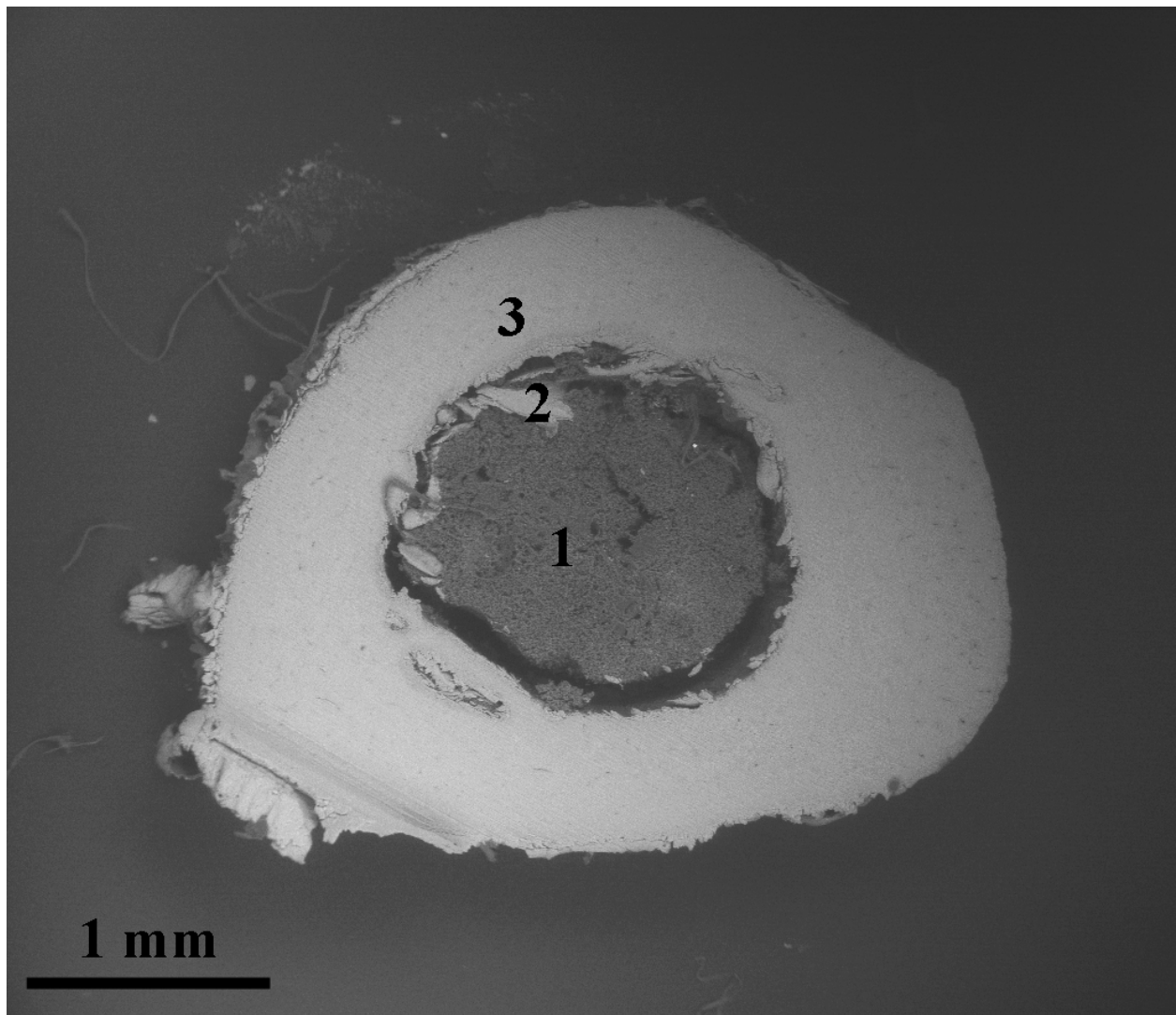
The MgO-paste corroded MgO and MgCO₃ were also incubated in cell culture medium (DMEM Fischer Scientific) in a humidified chamber at 37°C for 16h and then rinsed 3 times in distilled water and dried at 60°C. The volume of DMEM was stable within 0.1% per 24h of incubation and the pH was stable as indicated by the phenol-red indicator.

Analysis: Samples were analysed by Environmental SEM/EDX analysis, using the backscattering detector and ToF-SIMS analysis were made as described [3].

Results

ESEM images made with the backscattered electron detector, detecting the compositional contrast of the bone are shown in Figure 1, together with EDX-analysis of the elements of the tissue.

In untreated rat bone (Figure 1a), the Mg content is 0.1% in the bone marrow and 0.35% in the cortical bone. The content of Ca and P in the bone marrow is 3.1 and 2.7 % respectively. The content of Ca in cortical bone 17% and the content of P is 10%.

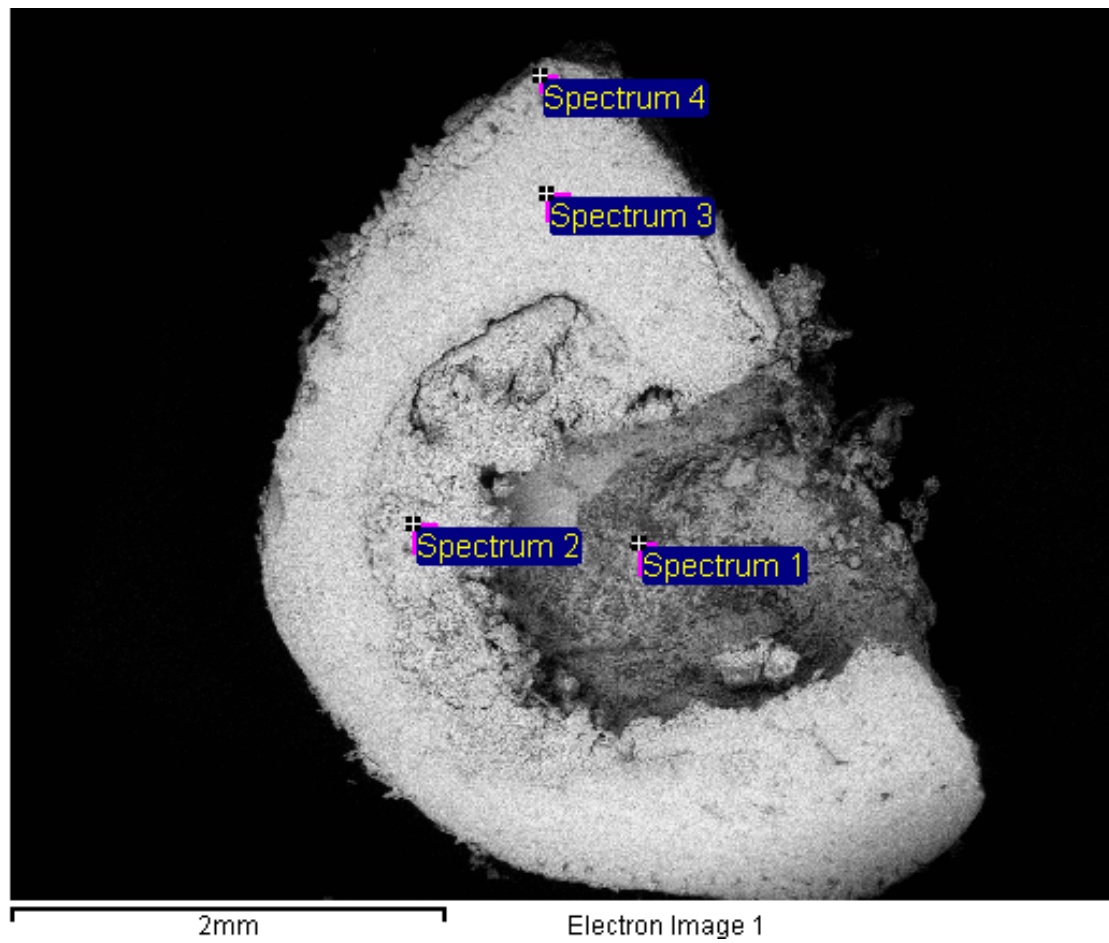


Spectrum	In stats.	C	N	O	Na	Mg	P	Cl	K	Ca	Total
Spectrum 1	Yes	46.36	14.83	32.11	0.30	0.13	2.72	0.06	0.40	3.08	100.00
Spectrum 2	Yes	27.29	5.50	40.33	0.40	0.36	9.43	0.12	0.21	16.35	100.00
Spectrum 3	Yes	26.44	4.57	40.91	0.41	0.34	9.82	0.10	0.11	17.29	100.00

Figure 1a. ESEM image of rat tibia using the backscattering detector and EDX analysis of the bone and bone marrow.

Analysis of a MgO-treated bone after 1 w of healing is shown in Figure 1b. The composition of cortical bone (spectrum 3) shows 0.3% Mg content, 22% Ca and 12%P. The composition of the bone marrow (spectra 1 and 2) shows 0.3% Mg, 15-20% Ca and 7-10% P. Thus, the composition of the soft tissue areas, appearing white in back scattering analysis, is similar to the composition of the compact bone areas. The conclusion is that the bone marrow contains Mg and Ca and P similar in composition as the hydroxyapatite of bone, after 1 w of healing.

The results indicate that MgO implanted into bone marrow tissue transforms to form a hydroxyl-apatite containing matrix that induces bone formation.



Spectrum	In stats.	C	N	O	Na	Mg	P	S	Cl	K	Ca	Total
Spectrum 1	Yes	31.78	8.02	37.98	0.37	0.25	7.33	0.25	0.11	0.15	13.76	100.00
Spectrum 2	Yes	24.37	5.72	38.86	0.45	0.32	10.24	0.17	0.19	0.24	19.45	100.00
Spectrum 3	Yes	24.50	4.20	37.45	0.41	0.33	11.27	0.09	0.08	0.06	21.60	100.00
Spectrum 4	Yes	37.63	4.31	33.13	0.34	0.27	8.30	0.16	0.18	0.15	15.51	100.00

Figure 1b. ESEM image of rat tibia and EDX analysis, at 1 week of healing after intramedullary implantation of MgO-paste.

Then experiments were performed to elucidate the ability of Mg corrosion products to induce formation of HA after incubation in DMEM.

Table 1. EDX data on MgO paste after incubation in water or DMEM +/- 5% CO₂

Sample/atom	C	O	Mg	Ca	P
Mg+H ₂ O	32.2	18.1	49.7	<0.05	<0.05
MgO+DMEM	16.1	22.0	59.3	1.2	1.3
MgCO ₃ /+H ₂ O	38.9	49.6	11.4	<0.05	<0.05
MgCO ₃ +DMEM	45.1	44.7	10.0	2.17	1.0
MgO+ DMEM + 5%CO ₂	9	61	24.5	4.1	1.7

Incubation of pure metallic Mg in water for 24 h, to form MgO, (Mg + H₂O), analysed by EDX, contained Mg 50%, O 18% and C 32%. Pure Mg incubated in water for 24 h followed by incubation in DMEM (MgO + DMEM) for 24 h contained Mg 59%, O 22%, C 16.1% Ca 1.2% and P 1.3%. Pure Mg incubated in DMEM + 5%CO₂ for 48 h (MgO+DMEM+5%CO₂) contained Mg 25%, O 61%, C 9%, Ca 4% and P 1.7%.

The data suggest that there is a significant effect on the amount of Ca uptake when incubating Mg in the presence of CO₂. Thus, incubations were made with commercially available MgCO₃ in air for 24 h. The EDX analysis shows that the binding of Ca to the material (2.2%) is higher than the corresponding uptake of Ca (by MgO+DMEM; 1.2%).

A molecular analysis of the elements Ca and P bound to the Mg corrosion products was made by ToF-SIMS. Table II.

Table II. ToF-SIMS data on MgO paste after incubation in DMEM +/- 5% CO₂

Sample/ion	CaO 56u	CaOH 57u	Ca ₂ PO ₄ 174.9u	Ca ₃ PO ₅ 230.9u	Ca ₅ PO ₇ 342.8u
MgO+ DMEM + 5%CO ₂	10523	83757	8602	3193	389
MgO +DMEM	1131	4036	175	231	343

The ToF-SIMS analysis showed higher levels of secondary ions specific for hydroxyl-apatite on the material incubated in the presence of 5% CO₂. The conclusion drawn from the presented data is that Mg-corrosion products bind Ca and P to their surfaces and that MgCO₃ seems to catalyse the formation of HA.

The effect of HA-exposure in the tissue will be a binding of osteopontin to the mineral, inhibiting further mineralization. The HA-osteopontin complex may then stimulate stemcells to differentiate to osteoblasts and form bone.

Metal oxides with a capacity to induce bone formation through catalysis of HA formation at their surface may serve as bone-inducing fillers of bone defects or activators of healing in compromised bone. Thus, active HA-catalysing metal oxides may be come to serve as a complement to bone morphogenetic protein (BMP-2).

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