Magnesium enhanced Porcine Bone particles by hydrothermal method for improved Cell Osteogenic Differentiation *In Vitro*

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Aim

This study sought to improve the biocompatibility of porcine bone grafts by incorporating magnesium oxide (MgO) ions to their surface via hydrothermal treatment. This MgO-coated porcine bone could be effectively employed in guided bone regeneration in dentistry.

Background

In dentistry, Guided Bone Regeneration (GBR) is widely used to assist new bone regeneration, often with different types of bone graft. Porcine bone is viable for GBR procedures such as sinus elevation and ridge augmentation. Moreover, magnesium (Mg) ions help stimulate pre-osteoblast osteogenic differentiation. Hydrothermal synthesis has also been proven beneficial in coating bone particles with MgO, such as β-TCP (Salamanca et al., 2022)

Materials and Methods

Processing MgO-coated porcine bone involves five stages:

1. Pre-treatment: oil was extracted from porcine bone.
2. Acid treatment: porcine bones were further purified.
3. Calcination: Fig. 2.
4. Screening
5. Hydrothermal treatment: Fig. 3.
6. Based on concentration of MgO, porcine bone powder was divided into 500-1000μm with three distinct concentrations of MgO.

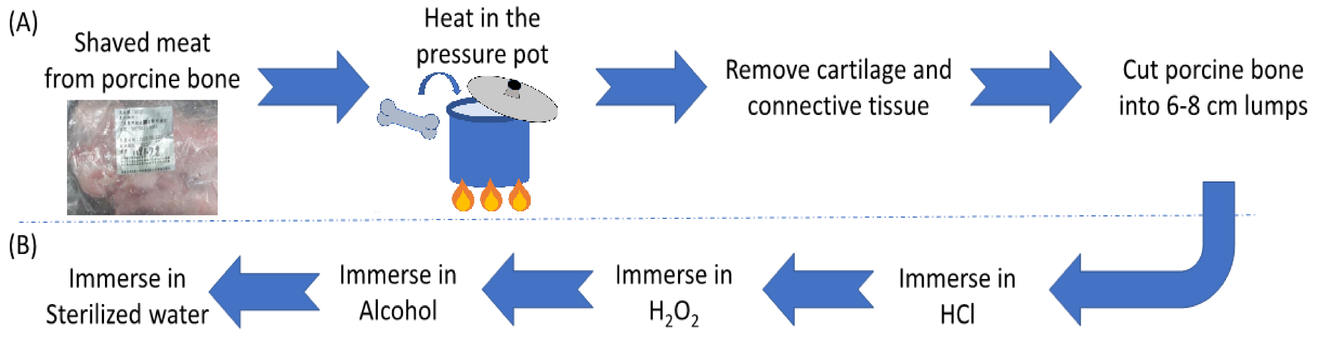


Fig. 1. (A) Pre-treatment (B) Acid treatment protocol

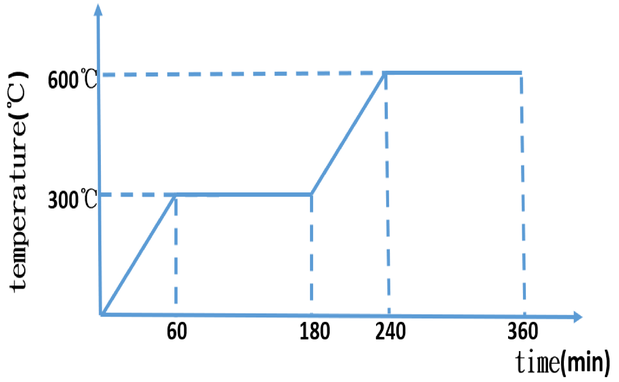


Fig. 2. Calcination process

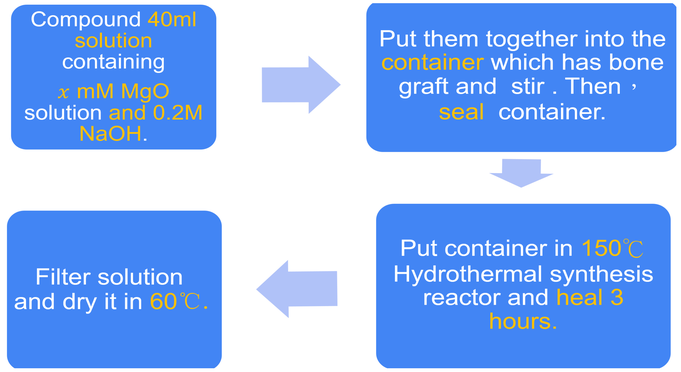


Fig. 3. Hydrothermal method process

Experiment Group

Hydrothermal method (500-1000 μm porcine graft)		
Porcine Bone	Porcine bone/2mM MgO	Porcine bone/5mM MgO

Results and Discussion

The pH value (**Fig. 4 & Table. 1**) indicated that porcine bone particles immersed in sterilized water had a minimal impact on the water's pH level. This observation confirmed that the porcine bone graft did not contain any residual contaminants.

Following hydrothermal synthesis, MgO was deposited on the surface of porcine bone particles, as confirmed by SEM images (**Fig. 5A**) and EDS results (**Fig. 6 & Table. 3**). Moreover, the mass of Mg element was observed increased after hydrothermal treatment. Additionally, processed SEM images (**Fig. 5B & Table. 2**) showed that hydrothermal synthesis-treated porcine bone exhibited a rougher surface compared to untreated porcine bone. FTIR results **(Fig. 7)** showed that it didn't change porcine bone's structure a lot after hydrothermal treatment.

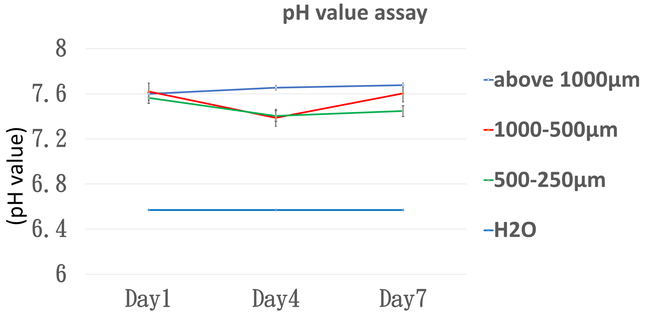


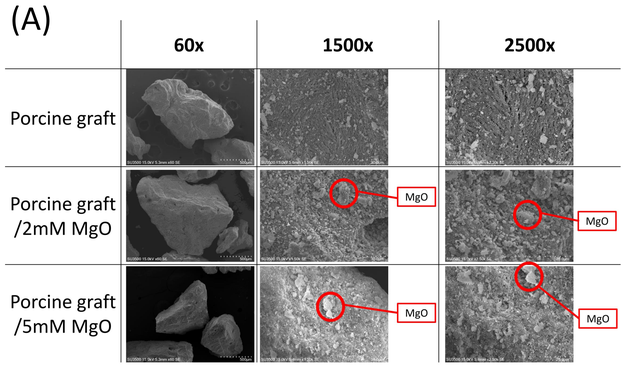
Fig. 4. pH value from all porcine groups.

pH value (AVG)	Day1	Day4	Day7
> 1000um	7.6	7.65	7.68
500-1000um	7.62	7.39	7.60
250-500um	7.56	7.40	7.45

Table.1. pH value assay.

	Rq	Ra	Rt	Rc
porcine graft	24.2577	18.0928	204.8304	-1.016
porcine graft / MgO	49.7454	40.3499	265.2163	2.1405

Table.2. Roughness parameter.



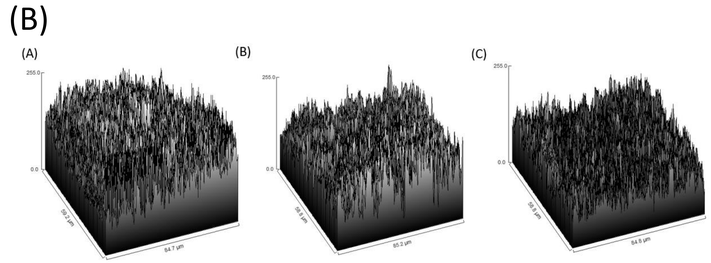
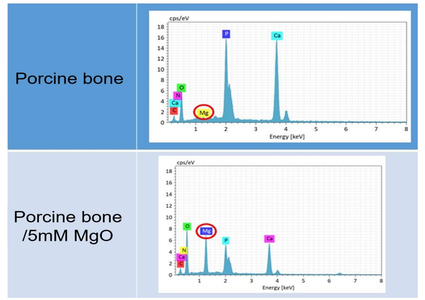


Fig. 5. (A) SEM image, its magnifications are 60x, 1500x, 2500x. **(B)** SEM images after image processing; (A) Porcine Graft / 5mM MgO (B) Porcine graft / 2mM MgO (C) Porcine Graft .

(A)

Chemical element	Porcine graft	Porcine graft / 2mM MgO	Porcine graft / 5mM MgO
	Wt %	Wt %	Wt %
Mg	0.16±0.02	1.1±0.26	4.92±1.53
Ca	30.20±8.70	10.37±1.94	15.71±2.45
P	12.66±2.72	2.34±0.3	7.4±2.09
O	32.15±6.03	37.74±7.37	41.38±2.21
C	4.54±0.62	4.43±1.07	7.18±0.25

(B)



(A) Table. 3. Control and samples weight ratio percentages from EDS. **(B) Fig. 6.** EDS element analyze.

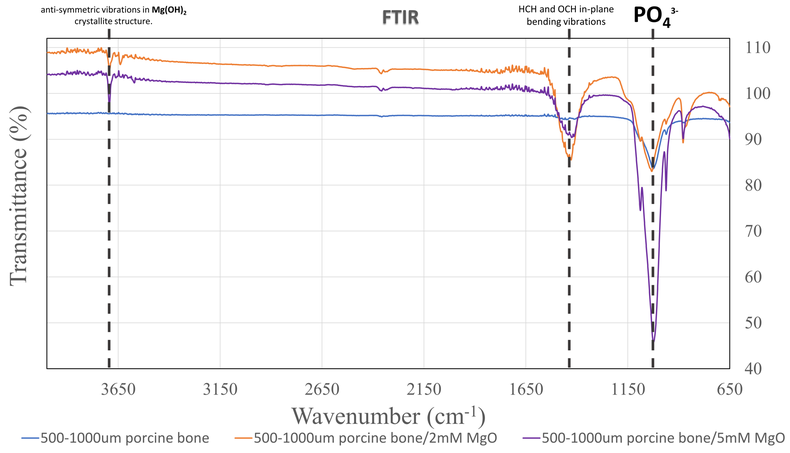


Fig. 7. Analysis of atomic peaks and functional groups via infrared spectroscopy (FTIR) spectra.

Results and Discussion

The MTT, immunofluorescence, ALP results (**Fig. 8 & Fig. 9**) indicated that porcine bone coated with 5mM MgO exhibited a high rate of cell proliferation, cell attachment and ALP. The results of qPCR**(Fig. 10)** showed that porcine bone coated with 5mM MgO has the highest expression of osteogenesis-related genes at Day 14.

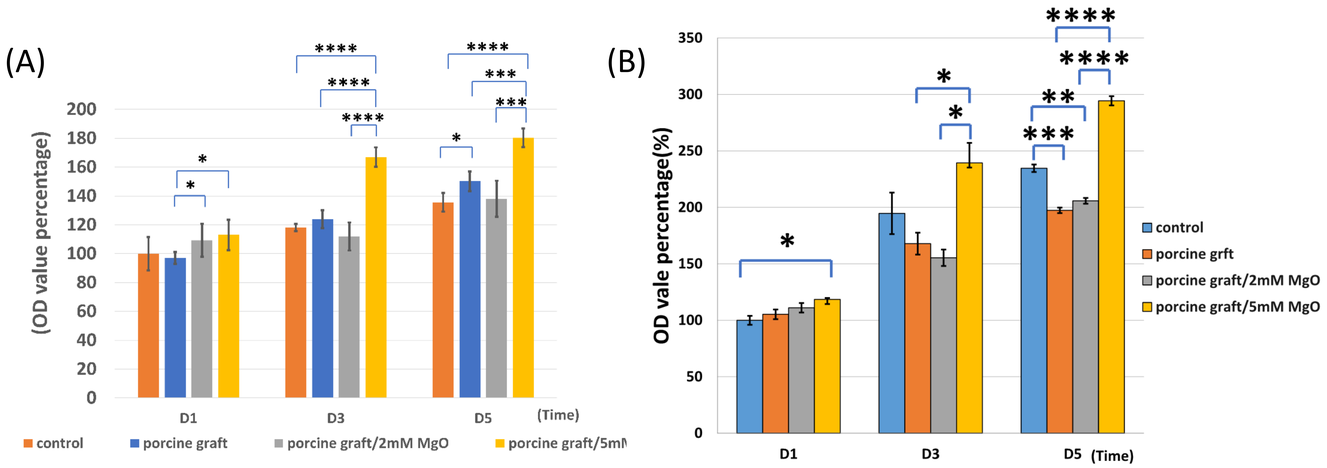


Fig. 8. (A) Cell proliferation was determined by the MTT assay of MG-63. (B) Alkaline phosphates activity detection was for all porcine graft biomaterials
* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001 (significant difference used by student's t-test)

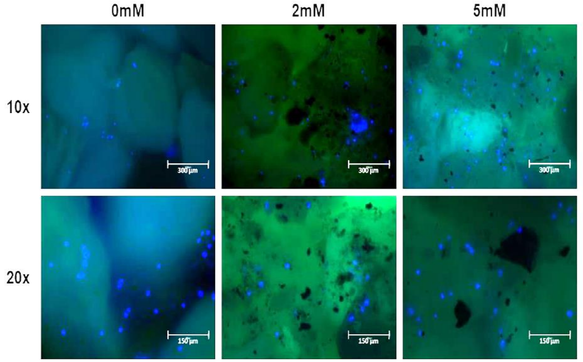


Fig. 9. 24-hours cell culture with DAPI/phalloidin immunofluorescent imaging, indicating MG-63 cell attaches on the all porcine graft surface.

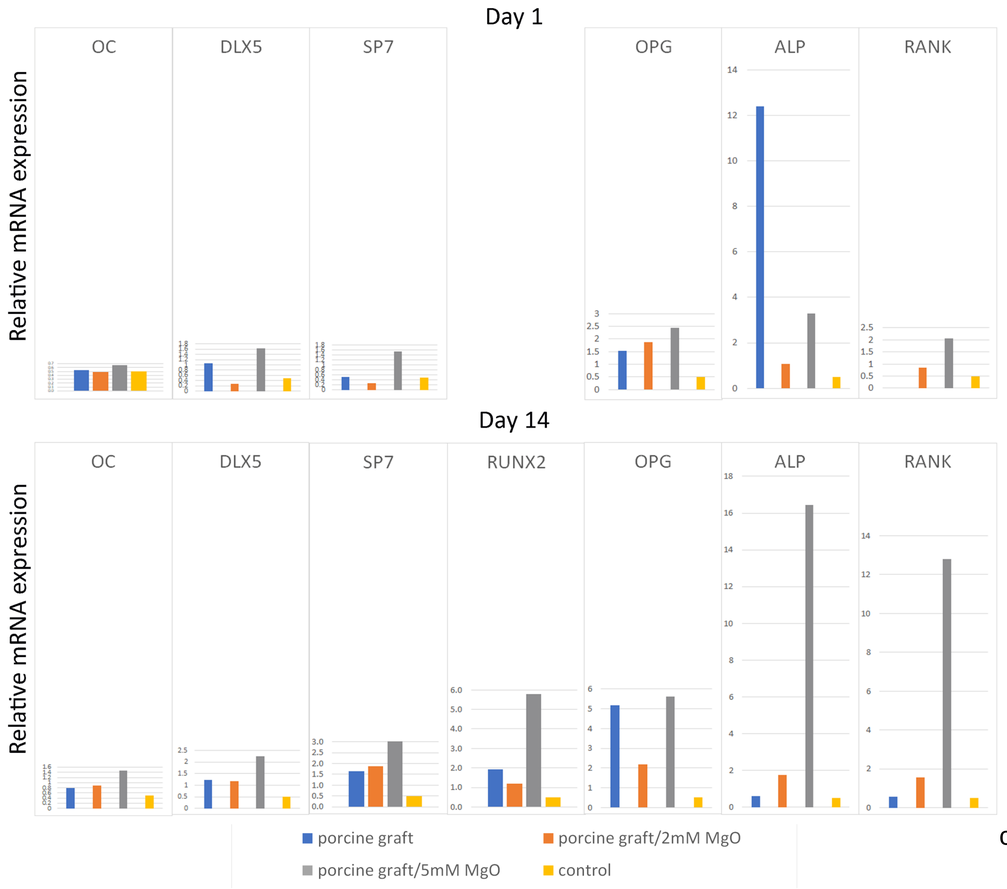


Fig. 10. Cells cultured with 5mM MgO graft showed higher relative qPCR osteoblast gene expression than control and 2mM MgO .

Conclusions

Using SEM and EDS, we verified that hydrothermal method coated porcine bone grafts with MgO, creating a rough surface. MTT, ALP and qPCR results showed the highest cell proliferation and ALP for 5mM MgO-coated porcine bone with higher osteoblast gene expression. Immunofluorescence figures showed that all porcine bone had good attachment. Future research will explore this enhanced graft material and its potential GBR treatment applications through additional analyses such as using Human Mesenchymal Stem Cells (hMSCs) to do further test and *in vivo* research.