

Regulating the local pH level of titanium via Mg-Fe layered double hydroxides films for enhanced osteogenesis

Qianwen Li ^{a, b}, Donghui Wang ^a, Jiajun Qiu ^{a, b}, Feng Peng ^{a, b} and Xuanyong Liu ^{a, *}

^a. State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Shanghai Institute of Ceramics, Chinese Academy of Sciences, Shanghai 200050, China.

^b. University of Chinese Academy of Sciences, Beijing 100049, China.

* Email: xyliu@mail.sic.ac.cn Tel: +86 21 52412409 Fax: +86 21 52412409

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1. Experimental Section

1.1. Surface characterization

The chemical compositions and chemical states of the sample surfaces were determined by X-ray photoelectron spectroscopy (XPS; PHI 5802, Physical Electronics Inc., Eden Prairie, MN, USA) with an Mg K α (1253.6 eV) source.

1.2. Water contact angle analysis

The hydrophobicity of the sample surface was evaluated by contact angle measurement (Automatic Contact Angle Meter Model SL200B, Solon, China) at room temperature. A sessile ultrapure water droplet with a volume of 2 μL was dropped onto the specimen surface and then the equipped camera system immediately captured the photograph. The measurement was conducted four times for each sample set and the results are expressed as means \pm standard deviation (SD).

1.3. Assessments of corrosion resistance

The corrosion resistance of the surfaces of the samples was assessed by dynamic potential polarization curves in a physiological saline solution using an electrochemical workstation (CHI760, Chenhua, China). The measurement was examined using a conventional three-electrode electrochemical cell with a saturated calomel electrode (SCE) as the reference electrode, a graphite rod as the counter electrode, and the sample with a 0.3 cm² exposed area as the working electrode. Prior

to the polarization test, the samples were stabilized in the solution for 10 min and the tests were conducted at room temperature at a scanning rate of 0.01 V s^{-1} .

1.4. Surface zeta potential measurements

The surface zeta potential of samples with size of $20 \text{ mm} \times 10 \text{ mm} \times 1 \text{ mm}$ were measured by a Surpass electrokinetic analyzer (Anton Parr, Austria) equipped with a special cell for membrane samples. A 0.001 mol/L potassium chloride (KCl) electrolyte solution was used as the medium.

1.5. Extracellular matrix (ECM) mineralization measurement

First of all, the rBMSCs were seeded on the samples (four replicates) placed into a 24-well plates with a density of 1×10^4 cells per well (cultured for 7 days) or 0.5×10^4 cells per well (cultured for 14 days). As for ECM mineralization measurement, the cells were fixed with 75% ethanol for 60 min and stained with $40 \times 10^{-3} \text{ mol/L}$ Alizarin Red S at pH 4.2 (ARS; Sigma-Aldrich, USA) for 10 min. Afterwards, the samples were washed out with ultrapure water several times and then images were taken by a fluorescence microscope.

1.6. Statistical analysis

Statistically significant differences (P) between the various groups were measured using the one-way analysis of the variance and Tukey's multiple comparison tests. A value of $P < 0.05$ was considered to be statically significant, and

was represented by the symbol “*”, a value of $P < 0.01$ was represented by “**”, and $P < 0.001$ was “***”. All statistical analysis was carried out using a GraphPad Prism 5 statistical software package. All data are expressed as means \pm standard deviation (SD).

2. Results and discussion

Fig. S1 gives the XPS spectra acquired from $\text{Mg(OH)}_2@\text{Ti}$, LDH-4@Ti and LDH-2@Ti samples. As shown in Fig. S1b, the peak around 50 eV is attributed to Mg 2p and the peak around 56.85 eV is attributed to Fe 3p. However, on the surface of $\text{Mg(OH)}_2@\text{Ti}$, no peak around 56.85 eV is found (Fig. S1a). The peaks around 726.0 eV and 711.2 eV are attributed to Fe 2p_{1/2} and Fe 2p_{3/2} respectively (Fig. S1c). In addition, no Fe element is found on the surface of $\text{Mg(OH)}_2@\text{Ti}$ (Fig. S1c). Fig. S1d exhibits two peaks centered around 464.25 eV (Ti 2p_{1/2}) and 458.55 eV (Ti p_{3/2}), corresponding to the bonding energy of Ti⁴⁺ in Ti(OH)_6^{2-} . The Ti(OH)_6^{2-} may come from the dissolution of titanium species which occurs in an alkaline environment. Mg(OH)_2 prepared surface of pure Ti may connect with titanium substrate by the Ti-O bond produced in the dissolution of titanium.

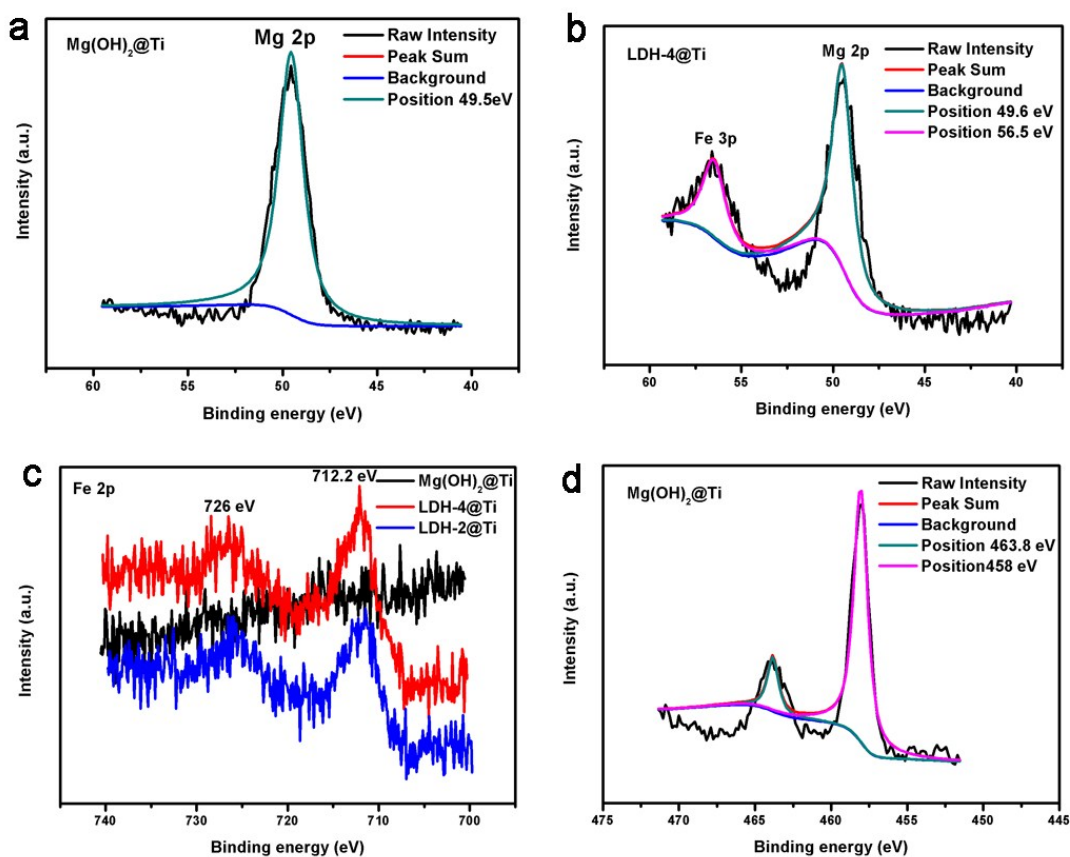


Fig. S1 XPS spectra acquired from Mg(OH)₂@Ti, LDH-4@Ti and LDH-2@Ti samples: XPS spectra of Mg 2p acquired from Mg(OH)₂@Ti (a) and LDH-4@Ti (b), XPS spectra of Fe 2p (c), XPS spectra of Ti 2p acquired from Mg(OH)₂@Ti (d).

The water contact angles of various surfaces are displayed in Fig. S2. It can be observed that modified with the Mg(OH)₂ film, and Mg-Fe LDHs films with different Mg/Fe atomic ratio, the contact angles of pure titanium surface significantly decrease. Modified with Mg(OH)₂ films, the surface of titanium almost became super hydrophilic. In addition, the water contact angles of LDH-4@Ti and LDH-2@Ti are around 30°.

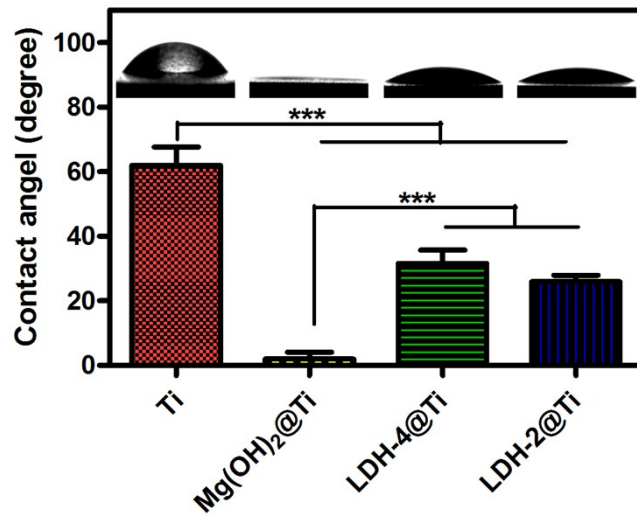


Fig. S2 Water contact angles of various samples. ***P < 0.001.

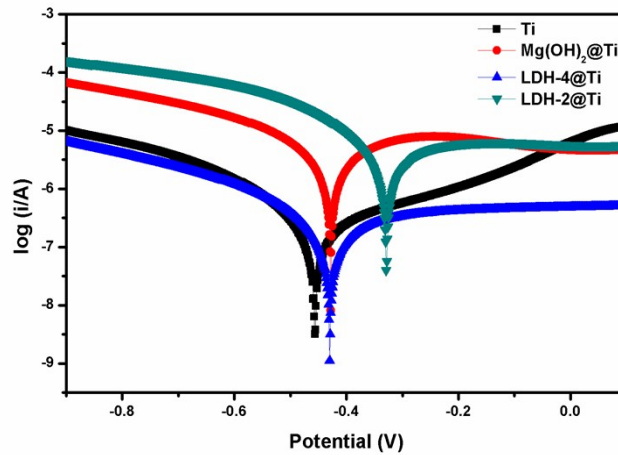


Fig. S3 Polarization curves of various samples in 0.9% NaCl solution.

Fig. S3 shows the Tafel plots of different samples tested in physiological saline, suggesting the tendency of corrosion potential of various samples are $Ti < LDH-4@Ti$, $Mg(OH)_2@Ti < LDH-4@Ti$, $Mg(OH)_2@Ti < LDH-2@Ti$.

Fig. S4 presents the average zeta potential variation versus pH of the potassium chloride solution acquired from the various samples. At the investigated pH range, the potentials of all samples are negative and reveal a descending trend with increasing pH values. Furthermore, the average zeta potential of samples can be ranked $Ti <$

LDH-2@Ti < Mg(OH)₂@Ti < LDH-4@Ti. The variation in the zeta potential patterns may be attributed to the dissolution of films which will cause the release of metal ions.

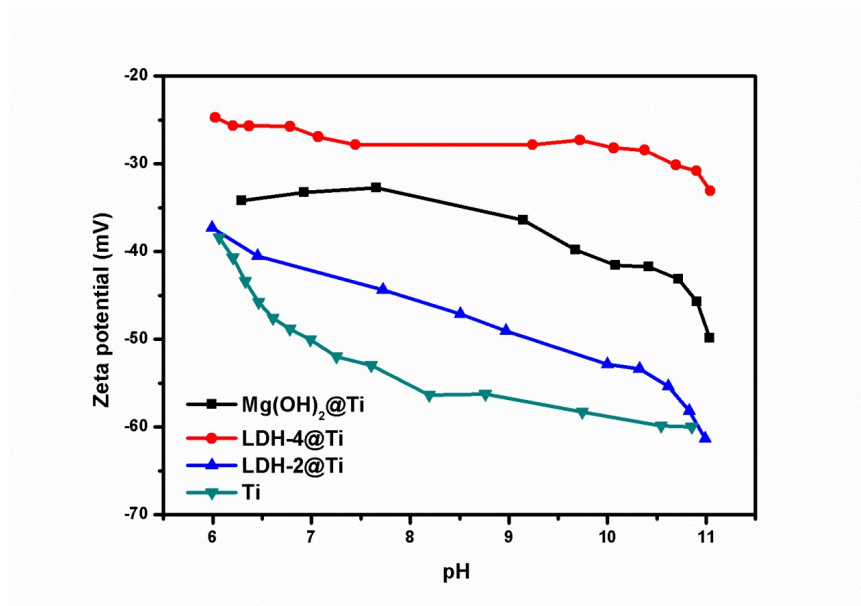


Fig. S4 Zeta potential variations versus pH acquired from the various samples.

Fig. S5 shows the qualitative results of ECM mineralization of rBMSCs cultured on samples for 7 days and 14 days. After culturing for 7 days, the ECM mineralization results have no significant difference among different samples. However, after culturing for 14 days, LDH-4@Ti shows the highest amount of ECM mineralization, followed by LDH-2@Ti, Mg(OH)₂@Ti and Ti.

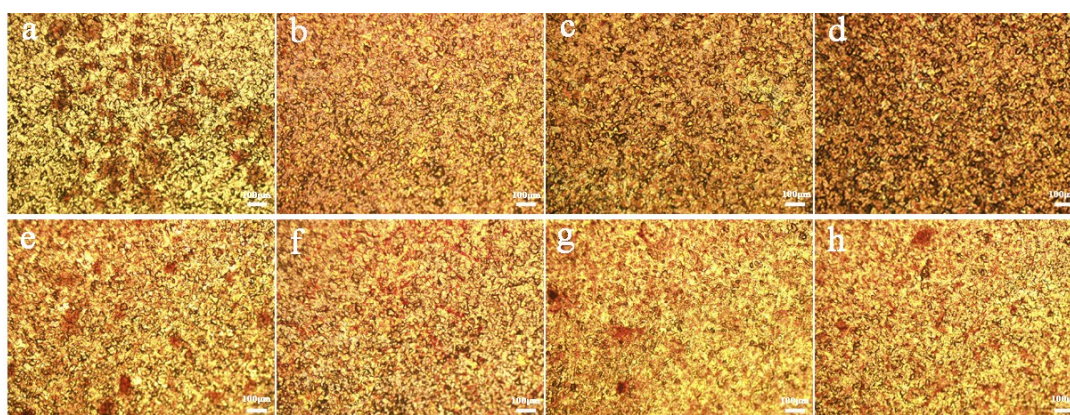


Fig. S5 Qualitative results of ECM mineralization of rBMSCs cultured on Ti (a), Mg(OH)₂@Ti (b), LDH-4@Ti (c) and LDH-2@Ti (d) for 7 days and cultured on Ti (e), Mg(OH)₂@Ti (f), LDH-

4@Ti (g) and LDH-2@Ti (h) for 14 days.