Applied Clay Science 165 (2018) 179–187

Contents lists available at ScienceDirect

Applied Clay Science

journal homepage: www.elsevier.com/locate/clay

Research paper

The prospect of layered double hydroxide as bone implants: A study of mechanical properties, cytocompatibility and antibacterial activity

Feng Peng[a,b], Donghui Wang[a], Dongdong Zhang[a,b], Huiliang Cao[a], 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

ARTICLE INFO

Keywords:
Layered double hydroxide
Bone implants
Elastic modulus
Cytocompatibility
Antibacterial activity

ABSTRACT

With the ability to host anions or neutral drugs, layered double hydroxide (LDH) are hot spots in the field of biomaterials. Intensive works so far only have focused on their application in surface modification or injectable medicine. Yet, little literatures report the potential of LDH as bone implants. In the present study, four types of LDH (Mg—Al, Mg—Fe, Zn—Al and Zn—Fe) were prepared via a coprecipitation method. Our results showed that the synthetic LDH powders can be pressed into discs in the absence of binder. The discs can maintain their shape after immersed in culture medium for 10 days, not including Mg—Al LDH disc. The elastic modulus of all discs was between 9 and 35 GPa, which was close to cortical bone (5–23 GPa). Zn—Al LDH and Zn—Fe LDH showed higher cytotoxicity than Mg—Al LDH and Mg—Fe LDH whether in the form of suspension or extract. Furthermore, even up to 10 mg/mL, the extract of Mg—Al LDH and Mg—Fe LDH showed no cytotoxicity, while cells were totally died in the extract of Zn—Al LDH and Zn—Fe LDH. Compared with cells, bacterial showed an even lower tolerance concentration to Zn—Al LDH and Zn—Fe LDH. This study indicates that Mg-containing LDH show better cytotocompatibility, while Zn-containing LDH show better antibacterial property. With proper elastic modulus and controllable biological effect, LDH are promising to be used as bone implants.

1. Introduction

Tissue engineering is a rising emerging technology, which is a powerful weapon for human to win the battle against diseases. Various material and methods were used for the preparation of tissue engineering implants (Chen et al. 2017; Kankala et al. 2018a, 2017b,c, 2018b; Ma et al. 2017). Among all the tissue engineering implants, the demand for bone implants are growing significantly, because the increase of natural and man-made calamities, especially aging populations. Over the past few decades, bioactive ceramics based implants were regarded as one of the most promising biomaterials in bone repair for their bioactive properties towards bone tissue regeneration (Bal and Rahaman 2012; Islam et al. 2017; Rahaman et al. 2007). Nevertheless, the poor intrinsic osteoinductivity is the pivotal drawback of bioactive ceramics in regenerating large bone defects and growth factors are needed to trigger the healing process at injured sites (Mourino and Boccaccini 2010; Parent et al. 2017). To solve the problem, researchers fabricate a series of bioactive implants as delivery platform of proteins, growth factors or hormones to promote osteogenesis, angiogenesis, cell proliferation and differentiation (Araujo et al. 2017; Kankala et al. 2017a, 2015b; Macias-Andres et al. 2017; Parent et al. 2017). However, the implants can only load with limited amount of active pharmaceutical ingredient (API) to maintain its mechanical strength. Furthermore, the API would experience a burst release at the initial implantation and it is difficult to control. Preparation of bone implants with good biocompatibility and could act as a controllable carrier for a large amount of API remains a significant challenge.

Layered double hydroxide (LDH), also known as anionic clays, consist of positively charged brucite-like layers and negatively charged interlayer area containing anions and solvation molecules (Mishra et al. 2018; Wang and O'Hare 2012). On one hand, the surrounding anions or molecules can be exchanged into its interlayer via the so called ion exchange process. On the other hand, LDH structure can be destroyed in high temperature (< 500 °C) and recover its original layered structure in solution, this is the so called memory effect (Lv et al. 2006; Ni et al. 2007; Peng et al. 2018). Anions and molecules can be stocked in LDH interlayer via memory effect. Profiting from these two properties, LDH could host a large amount of drugs in its interlayers, and are widely studied in drug delivery field (Kankala et al. 2015a; Kuthati et al. 2015; Wei et al. 2015). For instance, our group developed a butyrate-inserted

* Corresponding author.
E-mail address: xyliu@mail.sic.ac.cn (X. Liu).

https://doi.org/10.1016/j.clay.2018.08.020
Received 1 May 2018; Received in revised form 31 July 2018; Accepted 21 August 2018

Ni—Ti LDH film on the surface of nitinol alloy for H_2O_2-mediated tumor and bacterial killing (Wang et al. 2017). Li et al., used Mg—Al LDH to simultaneously deliver an anticancer drug 5-fluorouracil and Allstars Cell Death siRNA (CD-siRNA) for effective cancer treatment (Li et al. 2014). Furthermore, the release of API from LDH is sensitive to pH values. Combining the advantage of ease preparation, low cost, good biocompatibility, low cytotoxicity and full protection of the loaded API, LDH might be more desirable act as bone implants than bioactive ceramics (Rives et al. 2013). Nevertheless, there are relatively few studies devoted to explore the possibility of LDH as bone implants.

The general chemical formula of LDH is $[M^{2+}_{1-x}M^{3+}_x(OH)_2][A^{n-}]_{x/n}{\cdot}zH_2O$, where M^{2+} represent bivalent cations and M^{3+} represent trivalent cations. Monovalent and tetravalent metal cations also might form LDH and common layer ions of binary LDH were concluded in Table S1 (Zhao et al. 2012). From the view of biomaterials, it would be more meaningful to synthetize LDH with biological functional elements. Mg^{2+} involves into a series of life activities and is an essential element for bone regeneration (Ko et al. 2013; Neri et al. 1997; Rahamimoff 1977). Zhang et al. found that implant-derived Mg could improve bone-fracture via inducing local neuronal production (Zhang et al. 2016). Zn^{2+} is essential to skeletal development and lack of Zn^{2+} might cause bone growth retardation (Eberle et al. 1999; Prasad 1991; Qiao et al. 2014). Moreover, Zn ions possess excellent antibacterial ability and were widely used as inorganic antibacterial agents (Huo et al. 2013; Wang et al. 2016). Among all the alternative high valent cations, Al^{3+} and Fe^{3+} show the lowest toxicity than others (Hallab et al. 2002; Mochizuki et al. 2016; Poljak-Blazi et al. 2011). Furthermore, Mg, Zn, Al, Fe-based LDH are extensively studied by researchers. Thus, Mg^{2+} and Zn^{2+} were selected as bivalent cations, and Al^{3+} and Fe^{3+} were selected as trivalent cations. To evaluate the feasibility of LDH as bone implants, we have herein prepared four different LDH (Mg—Al, Mg—Fe, Zn—Al and Zn—Fe) via a coprecipitation method. The obtained LDH powders were pressed into disc and the mechanical properties were measured. Furthermore, the cytocompatibility of LDH suspension and LDH extract were evaluated using two different cell lines (MC3T3-E1 and HUVEC), and the effects of LDH suspension and LDH extract to bacteria (*E.coli* and *S.aureus*) were also evaluated.

2. Materials and methods

2.1. Preparation and characterization of Mg—Al, Mg—Fe, Zn—Al and Zn—Fe LDH

LDH powders were prepared by a coprecipitation method. Briefly, 10 mL $MgCl_2$ (600 mM) and 10 mL $AlCl_3$ (200 mM) were mixed, and then added with 80 mL ultrapure water. The pH value of the mixed solution was adjusted with sodium hydroxide (pH value = 9, 10, 11, 12). After magnetic stirring, the reaction solution was kept at 80 °C for 2 h. The precipitates were rinsed with ultrapure water for three times, and then isolated by centrifuging (6000 rpm, 15 mins). The obtained precipitates were dried at 80 °C and the obtained Mg—Al LDH powders were denoted as MgAlL. Mg—Fe, Zn—Al, Zn—Fe LDH were prepared via the same processes and denoted as MgFeL, ZnAlL and ZnFeL, respectively.

To prepare LDH discs, the obtained powders were uniaxially pressed at 10 MPa in a mold 10 mm in diameter. Binder, such as polyvinyl alcohol (PVA-124), is often used in preparation of ceramic discs, and following a sintered process (up to 1000 °C) to get rid of it. Because the structure of LDH would be destroyed in such a high temperature, binder is abandoned in the present study.

The crystal phases of different LDH were characterized by X-ray diffraction (XRD; D2PHASE, BRUKER, USA) using Cu Kα radiation (40 kV, 30 mA) selected by a nickel monochromator in the diffracted beam. The surface morphology and elemental compositions of the discs were observed by scanning electron microscopy (SEM; Hitachi-S3400 N, Hitachi, Japan) and energy dispersive spectrometry (EDS; IXRF-550i, IXRF SYSTEMS, USA). The chemical bonds of LDH were recorded using a Fourier transform infrared (FTIR) spectrometer (FTIR-7600, Lambda Scientific, Australia) between 500 and 4000 cm^{-1}.

2.2. Evaluation of mechanical properties

To test the stability of the pressed discs, all the samples were immersed in α-MEM (Minimum Essential Medium alpha-Medium) at 37 °C in a humidified atmosphere of 5% CO_2 in air for 10 days. The changes of the samples' shape over time were recorded using a digital camera. The mechanical properties of pressed discs before and after immersion were assessed using a nanoindentation machine (G200, Agilent Nano Indenter, USA) with a surface approach of 10 nm/s, a frequency target of 45 HZ and a poisons ratio of 0.3. The elastic modulus and nanohardness were measured according to Oliver-Pharr method.

2.3. Evaluation of cytocompatibility

Two different cell lines were used to evaluate the cytocompatibility of different LDH. Osteoblast-like cells (MC3T3-E1, Cells Resource Center of Shanghai Institute for Biological Science, Shanghai, China) were cultured with α-MEM supplemented with 10 fetal bovine serums (FBS, Hyclone, USA) and 1% antimicrobial of penicillin/streptomycin mixture (P/S, Hyclone, USA). Human umbilical vein endothelial cells (HUVECs, ScienceCell, USA) were cultured with ECM medium (ScienCell, USA) supplemented with 5% FBS and 1% P/S.

Herein, we take MgAlL sample and MC3T3-E1 cells as an example. Firstly, 100 μL cell suspensions with a cell density of 5×10^4 cell/mL were added to each well of a 96-well culture plate. 40 mg MgAlL sample was added into 4 mL α-3^{M}EM culture medium, and then triple dilution for eight times. After culturing for 24 h, the culture medium was replaced with 100 μL different diluted sample suspensions and culturing for another 24 h. Culture medium without MgAlL powder as the control group. Cell viability was tested by the alamarBlue assay (AbD Serotec Ltd., UK) according to the manufacturer's instruction, and calculated using the following equation:

Viability = $\frac{F_S}{F_C}\times 100\%$where F_s is the fluorescence intensity of the sample group and F_c is the fluorescence intensity of the control group.

The cytocompatibility of sample' extracts was also evaluated. The aforementioned diluted suspensions were kept in cell incubator for 24 h and then centrifuged (3000 rmp, 5 mins) to obtain the extracts. Following the same procedures mentioned above, the cell viability cultured in different extracts can be measured.

2.4. Evaluation of antibacterial activity

Gram-positive *Staphylococcus aureus* (*S.aureus*, ATCC 25923) and Gram-negative *Escherichia coli* (*E.coil*, ATCC 25922) with a concentration of 10^7 CFU/mL were used in the evaluation of antibacterial activity of different LDH in the form of suspension and extract. The detailed procedures were the same as the evaluation of cytocompatibility.

3. Results

The XRD patterns of MgAlL, MgFeL, ZnAlL and ZnFeL samples prepared in different pH values are depicted in Fig. 1. The measured XRD patterns of MgAlL samples fitted well to lattice planes (003), (006), (012), (015) and (018), and MgFeL samples to planes (003), (006), (012) and (015). No byproducts were observed of MgAlL and MgFeL sampels, indicating the formation of well crystallized LDH structure. It is worth noting that the peak of plane (003) of MgFeL samples shifted to a higher degree as the pH values rising, suggesting a higher pH value would lead to a narrower interlayer spacing. For ZnAlL samples, except for LDH structure, ZnO was also detected, which is a common impurity in the synthetic of Zn—Al LDH (Seftel et al. 2008;