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ScienceDirect

Procedia Chemistry 19 (2016) 75 – 82

Procedia Chemistry

5th International Conference on Recent Advances in Materials, Minerals and Environment (RAMM) & 2nd International Postgraduate Conference on Materials, Mineral and Polymer (MAMIP), 4-6 August 2015

Human mesenchymal stem cells response to magnesium-based biomaterials

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Abstract

Implants have been used as replacement materials for lost tissues and are mainly from metals such as stainless steel and titanium. Metals implants may release toxic elements, and alternative such as magnesium-based implants are suggested but its high degradation rate in human bio-environment limit its uses. Thus, we proposed alloying the magnesium with zinc atoms and dispersing bioactive hydroxyapatite (HAp) within the magnesium and zinc matrix and assess the effects of the conditioned-medium from the Mg-Zn-HAP on human bone marrow mesenchymal stem cells (hMSC) viability. The Mg with and without Zinc powders were purchased from Merck and Alfa Aesar. The precursor powder has particle size of 0.06-0.3 mm. Powder mixtures with different composition i.e., 100 weight percent (wt%) Mg, 9:1 Mg-Zn, 90:9:1 Mg-Zn-Mn, 9:1 Mg-Zn + 10 wt% HAp and 90:9:1 Mg-Zn-Mn + 10 wt% HAP were sintered at 300 °C. Then, the powder was incubated with culture medium (1.0 mg/ml and 2.0 mg/ml) and placed in an incubator shaker for 4 hours. The medium was filtered using 0.2 µm syringe filter and kept at 4 °C. The conditioned-medium was supplemented with 10% (v/v) fetal bovine serum and 1% Pen/Strep and incubated overnight at 37 °C in a CO_2 incubator prior to use on hMSC. The cell viability was assessed using Alamar Blue assays at Days 1, 2, 4 and 7. The hMSC showed increased proliferation when cultured in MgZn- and MgZnMn-conditioned medium when compared to Mg-HAp conditioned medium. These metallic ions may play role in stimulating cell proliferation. The presence of metallic ions such as Mg, Mn and Zn promote hMSC proliferation and may play role as an important elements for regeneration of hard tissue during implantation.

Peer-review under responsibility of School of Materials and Mineral Resources Engineering, Universiti Sains Malaysia

Keywords: Magnesium-based biomaterials; Implants; Body fluids

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doi:10.1016/j.proche.2016.03.118

1. Introduction

Increasing demand for implants has seen the development of bio-implants composite materials incorporating various types of compatible materials. For load bearing applications, metal is commonly used which includes stainless steels, titanium alloys and cobalt-chromium based alloys[1]. Nevertheless, corrosion products from these alloys may be considered harmful to human tissues. Thus, alternative must be available to ensure safety and benefits for patients. Magnesium has growingly gain attention as potential implants for load bearing areas; cheaper option and beneficial ionic release to human body. Magnesium (1.7 g/cm^3) has almost similar density to human calvarial bone (1.75 g/cm^3), and an elastic modulus of 45 GPa similar to human bone (40-57 GPa)[1]. Magnesium is commonly present within human bone and muscle, and lacks of it may induce osteoporosis[2]. Hence, magnesium may influence hard tissue mineralization by binding strongly to phosphates through formation of hydroxyapatite[1].

In term of magnesium-based biomaterials, many studies incorporated magnesium within matrices or scaffold during fabrication, since magnesium is known as an important cofactor for broad number of enzymes such as transferase, oxidoreductase and isomerase. Ions present within the biomaterials such as magnesium (Mg), zinc (Zn), calcium (Ca), manganese (Mn), strontium (Sr) and cobalt (Co) are important in bone regeneration and essential cofactors of enzyme, collectively known as metallic ions for therapeutic application (MITA)[3]. Magnesium alloys, on the other hand, may have a few issues if it used solely as implant materials such as corrosion products from these alloys, timing of corrosion process upon implantation and also its effects to the surrounding tissue.

Although magnesium alloys has gained acceptance for its light weight properties, however, the corrosion behavior need proper understanding prior to the use of magnesium as potential implant for load bearing areas. Magnesium alloys may undergo many types of corrosion process such as galvanic and intergranular corrosion[4]. To harness magnesium alloys for biomedical applications, a few elements such earth metal alkali and alkaloids can be incorporated within the structure of magnesium alloys to enhance its properties. Strontium (Sr), zinc (Zn), manganese (Mn) and calcium (Ca) have been used previously to reduce the corrosion effects and slows down biodegradation[1]. Other element such as copper (Cu) and aluminum (Al) has been used as alloying elements in Mg. However, excessive copper amounts have been linked to neurodegenerative diseases like Alzheimer‘s and in high doses of aluminum has been shown to increase estrogen-related gene expression in human breast cancer cell when cultured in a laboratory setting[5].

In the current work, we propose alloying the magnesium with zinc atoms and dispersing bioactive hydroxyapatite (HAp) in the Mg-Zn alloy matrix by mechanical alloying forming a Mg-Zn-HAp composite. First, Zn atoms are added to the Mg through mechanical alloying mechanism in ball and mill setting. Then HAP powder is added to the Mg-Zn matrix. Problems within the Mg-Zn-HAp composite may exist such as inhomogeneous dispersion of each particles and presence of globules within the matrix. Different phases in HAp dispersed Mg-Zn composite would provide different corrosion mechanism in body fluid. For example, high level of Zn can dramatically increase the corrosion rate as a result of the formation of intermetallic particles in comparison to low level of Zn. At the same time, dispersed HAp particles would provide different corrosion rate as it is the noblest phase amongst the Mg alloy and Mg-Zn intermetallic phases.

Since the composite is multi-phase, the objectives of this work are to propose mechanical alloying mechanism of magnesium and zinc, to investigate interfacial bonding of the composite and to study compatibility of the composite with human bones from the aspect of mechanical integrity and biodegradation rate. However, in this part of the paper, we reported the dissolution properties of Mg-Zn-HAp composite powder in cell culture medium and then used the medium on human mesenchymal stem cells (hMSCs) viability. We hypothesized that addition of other elements such as Zn, Mn and HAp within the Mg may reduce the degradation rate and the presence of HAp may enhance osseointegration. Currently, novel strategies based on biodegradable metals, such as magnesium alloys and iron, which are dissolved *in vivo* when no longer needed have potential in bone regeneration[6].

2. Materials and Method

2.1 Synthesis of Mg-Zn alloys