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Preparation and biocompatibility of diphasic magnetic nanocomposite scaffold

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ABSTRACT

We describe the study of a new type of diphasic magnetic nanocomposite scaffold (PLGA/Col-I-PLGA/n-HA/Fe_2O_3) and its preparation using a novel low-temperature deposition manufacturing (LDM) technology. In order to study the biocompatibility of this scaffold, we evaluated and explored its feasibility as a scaffold for tissue engineering. Diphasic magnetic nanocomposite scaffolds (PLGA/Col-I-PLGA/n-HA/Fe_2O_3) were prepared using LDM technology. The mechanical properties of the scaffold were tested using an electronic testing machine, electron microscopy was utilized to observe the ultrastructure, and a medium (ethanol) immersion method was used to determine the porosity of the scaffold. The scaffold was co-cultured with bone mesenchymal stem cells (BMSCs) and was induced to differentiate. The biocompatibility of the scaffold was then tested. The mechanical test results of the diphasic magnetic nanocomposite scaffold demonstrated good mechanical properties. Electron microscopy studies revealed two layers of pore sizes each with a uniform distribution, with the upper cartilage pore size observed to be small while the middle continuous phase was found to be in a good integration. Pore size and porosity test results demonstrated a cartilage layer pore size of 186 μm, with a porosity measured to be 89.5%. The pore size and porosity of the bone layer were 394 μm and 86.1%, respectively. These properties met the design requirements of double layer scaffolds. Co-culture of the diphasic magnetic nanocomposite scaffold and bone mesenchymal stem cells (BMSCs) exhibited good proliferation of bone mesenchymal stem cells (BMSCs), and the scaffold was found to be able to promote differentiation of the differentiation-oriented cells. These results demonstrated a good biocompatibility of the diphasic magnetic nanocomposite scaffold. The diphasic magnetic nanocomposite scaffold (PLGA/Col-I-PLGA/n-HA/Fe_2O_3) was found to have suitable mechanical properties as well as cell compatibility. The measured pore size and porosity met the requirements for cell adhesion and cell growth, which matched more closely to that of the physiological structure of normal articular cartilage and subchondral bones. We expect this to represent new technology for improved repair of cartilage and subchondral bone lesions caused by osteoarthritis or trauma.

1. Introduction

It has been demonstrated that clinical diseases are often accompanied by subchondral bone injuries, including arthritis or injury-caused articular cartilage injuries. Therefore, the joint repair of cartilage and subchondral bones represents a better method than the repair of cartilage alone. Scaffolds in bone tissue engineering are typically designed in a three-dimensional porous form, and must enable cell adhesion, intercellular regulation, and interactions. These scaffolds have defined mechanical properties and the ability to release bioactive molecules. In addition, they possess the ability to promote the differentiation and proliferation of seeded cells. These properties enable the

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formation of microenvironment where bone mesenchymal stem cells can differentiate into chondrocytes and osteoblasts, in order to promote bone or cartilage tissue formation [1]. The main principles behind the use of diphasic engineering scaffolds for bone tissues include combining two different layers of scaffolds in three-dimensions, inoculating the same or different seed cells onto the scaffolds in two different layers, and finally verifying the different types of bone tissues through histopathologic examination. Therefore, the use of diphasic engineering scaffolds for bone tissues can better simulate the different physicochemical properties and biological activity representative of normal bone tissues. In addition, it can properly fit subchondral bones, enabling the achievement of a good fix and repair of defects in lesions [2].

Rapid prototyping technology is the primary method utilized for the production of three-dimensional scaffolds. This includes melting deposition, selective laser sintering, and three-dimensional printing. Because this provides the advantage of rapid preparation, digitization, the ability to adjust in material production, commercialization potential, and the ability to control through 3D printing, it is a topic that is intensively studied [3]. Low-temperature deposition manufacturing (LDM) is based on the method of separating combination phases. This enables rapid prototyping, easy operation, and is controllable during the formation of scaffolds. Under low temperature conditions, the biological activity of numerous materials has been demonstrated to be well maintained, while the running and porosity of the scaffolds has been shown to be able to be controlled in three-dimension throughout the printing process. This has been used widely for the preparation of engineering scaffolds for bone tissues [4]. In this study, we demonstrate a new type of diphasic magnetic nanocomposite scaffold (PLGA/Col-I-PLGA/n-HA/Fe_2O_3) that was prepared using LDM technology, and report on its measured biological indexes.

2. Materials and methods

2.1. Main experimental materials, reagents and instruments

Polylactic-*co*-glycolic acid (PLGA), type I collagen (Col-I), nano hydroxyapatite (n-HA), magnetic ferric oxide (Fe_2O_3) (Shenzhen Xincheng Biotechnology Co., Ltd.); low temperature rapid prototyping instrument (Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences), mechanical testing machine CTM4000, (Research Institute of Tsinghua University in Shenzhen), electron microscope (MIRA3 TESCAN, Research Institute of Tsinghua University in Shenzhen).

2.2. Method for preparing PLGA/Col-I-PLGA/n-HA/Fe_2O_3 composites

2.2.1. Preparation of cartilage phase scaffold solution

Organic solvent (1,4-dioxane) was added into PLGA, followed by uniform mixing; Col-I powder was then added in order to prepare a solution with a mass ratio of 20%. The solution was then put on a shaker for 15 min, followed by electromagnetic stirring for 12 h in order to make the mixture uniform (see Fig. 1A).

2.2.2. Preparation of bone phase solution

Organic solvent (1,4-dioxane) was added into PLGA, followed by uniform mixing; n-HA and Fe_2O_3 were added according to the mass ratio in order to obtain a 20% solution. The solution was put on a shaker for 15 min, followed by electromagnetic stirring for 12 h to ensure a uniform mixture (see Fig. 1B).

2.2.3. PLGA/Col-I-PLGA/n-HA/Fe_2O_3 preparation

PLGA/Col-I of the cartilage phase and PLGA/n-HA/Fe_2O_3 of the bone phase were printed successively using a low-temperature rapid prototyping instrument at −4 °C. First, the prepared solution was transferred to the LDM instrument to print initial three-dimensional products with a specified parallel space. At this time, the products had some pores. Next, a biological composite solution was added into the pores of the three-dimensional materials. After filling, the material surfaces were finished and smoothed in order to obtain a uniform distribution of the composite. The above steps were then repeated (see Fig. 2A).

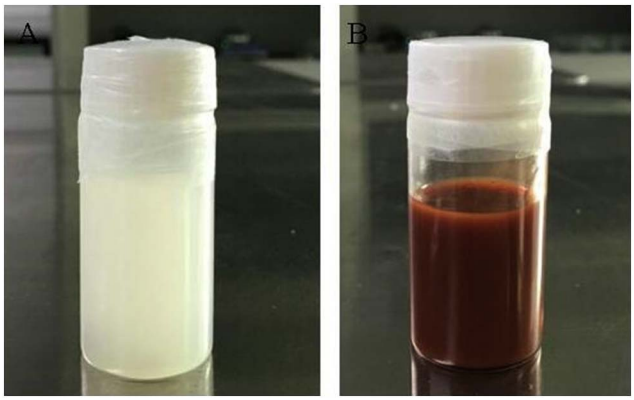

Fig. 1. A: Cartilage phase, PLGA/Col-I, B: bone phase, PLGA/n-HA/Fe_2O_3.

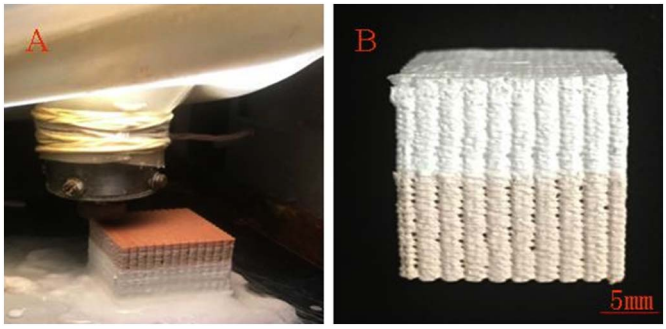

Fig. 2. A: PLGA/Col-I-PLGA/n-HA/Fe_2O_3 composites 3D printing process, B: PLGA/Col-I-PLGA/n-HA/Fe_2O_3 composites (10×).

Storing after forming. The free space generated by the melting ice in the composites acted as the initial space pore of the magnetic nano porous artificial bone scaffolds. The organic solvent was removed by freeze-drying under vacuum. Following two days of freeze-drying, the space pore of the three-dimensional composite scaffolds was increased further after sublimation of the organic solvent. At this point, the desired PLGA/Col-I-PLGA/n-HA/Fe_2O_3 composites were obtained (see Fig. 2B).

2.3. Test of biological properties of PLGA/Col-I-PLGA/n-HA/Fe_2O_3 composites

2.3.1. Mechanical property test

The tensile strength, flexural strength, and flexural modulus of the PLGA/Col-I-PLGA/n-HA/Fe_2O_3 composites were measured using a CTM4000 Sans Universal Testing Machine in a bending and tensile laboratory with the method of three-point bending. For these tests, the pointing span was 20 mm and the loading rate was 5.00 mm/min.

2.3.2. Microstructure observation

Fresh fractures of PLGA/Col-I-PLGA/n-HA/Fe_2O_3 composites were scanned and analyzed following a metal spraying treatment. A Field Emission Scanning Electron Microscope (FE-SEM, Czech Tescan) was used to generate secondary electron images and backscattered electron images. Morphological and microscopic characteristics of the specimen's cross-sections were observed and analyzed.

2.3.3. Determination of porosity

The medium (ethanol) immersion method was used to determine