Journal of the Formosan Medical Association (2018) xx, 1–9

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfma-online.com

Original Article

Effects of bone morphogenic protein-2 loaded on the 3D-printed MesoCS scaffolds

Kuo-Hao Huang [a,b], Yen-Hong Lin [c,d], Ming-You Shie [d,e,f,1], Chun-Pin Lin [a,b,*,1]

[a] *Graduate Institute of Clinical Dentistry, School of Dentistry, National Taiwan University, Taipei, Taiwan*
[b] *Department of Dentistry, National Taiwan University Hospital, Taipei, Taiwan*
[c] *The Ph.D. Program for Medical Engineering and Rehabilitation Science, China Medical University, Taichung, Taiwan*
[d] *3D Printing Medical Research Center, China Medical University Hospital, Taichung, Taiwan*
[e] *School of Dentistry, China Medical University, Taichung, Taiwan*
[f] *Department of Bioinformatics and Medical Engineering, Asia University, Taichung, Taiwan*

Received 18 May 2018; received in revised form 8 June 2018; accepted 9 July 2018

KEYWORDS
Bone morphogenetic protein-2;
Mesoporous;
Calcium silicate;
3D printing;
Drug release

Background/Purpose: The mesoporous calcium silicate (MesoCS) 3D-printed scaffold show excellent bioactivity and can enhance the bone-like apatite formation. The purpose of this study aims to consider the effects of the different loading methods on the novel grafting materials which composed of bone morphogenetic protein-2 (BMP-2) loaded MesoCS scaffold by employing 3D-printing technique.
Methods: The MesoCS scaffold were fabricated by fused deposition modeling. In this study, there are two methods of loading BMP-2: (1) the pre-loading (PL) method by mixing MesoCS and BMP-2 as a raw material for a 3D-printer, and (2) the direct-loading (DL) method by soaking the 3D-printed MesoCS scaffold in a BMP-2 solution. The characteristics of MesoCS scaffold were examined by transmission electron microscopy (TEM), X-ray diffraction (XRD) and scanning electron microscopy (SEM). Their physical properties, biocompatibility, and osteogenic-related ability were also evaluated.
Results: The 3D MesoCS/PCL scaffolds showed excellent biocompatibility and physical properties. After soaking in simulated body fluid, the bone-like apatite layer of the PL and DL groups could be formed. In addition, the DL group released fifty percent more than the PL group at the end of the first day and PL showed a sustained release profile after 2 weeks.
Conclusion: The 3D MesoCS/PCL porous scaffolds were successfully fabricated via a 3D printing system and were tested *in vitro* and were found to show good cellular activity for cell behavior although the PL method was not favorable for clinical application in relation with the preservation of BMP-2. With regards to different growth factor loading methods, this study

* Corresponding author. School of Dentistry, National Taiwan University, Taipei, Taiwan.
E-mail address: chunpinlin@gmail.com (C.-P. Lin).
[1] Both authors contributed equally to this work.

https://doi.org/10.1016/j.jfma.2018.07.010

Please cite this article in press as: Huang K-H, et al., Effects of bone morphogenic protein-2 loaded on the 3D-printed MesoCS scaffolds, Journal of the Formosan Medical Association (2018), https://doi.org/10.1016/j.jfma.2018.07.010

demonstrated that PL of BMP-2 into MesoCS prior to printing will result in a more sustained drug release pattern as compared to traditional methods of scaffolds directly immersed with BMP-2.

Introduction

With an increasingly aging population, bone defects and disorders have become a global healthcare problem and have led to a tremendous clinical need for bone repairs.[1,2] However, bone destroyed in the orofacial region as a consequence of trauma or disease may compromise esthetics or functions and reduce patients' quality of life, where surgical intervention is a requirement for reconstruction of the hard tissues with the application of implants or grafts. The main choices of grafting material in dental surgery are allografts, xenografts, and alloplasts. These materials have good performance in space maintenance, which is known as osteoconductivity. Various types of synthetic bone grafts, such as calcium phosphate, calcium sulfate, Bioglass®, etc., have been used to fill bone defects over past decades.[3,4] However, these materials have some disadvantages, including difficulty related to manipulation and a lack of osteoinductive potential that limits their use in clinical settings.[5] Therefore, the current study has basically developed new bone substitutes with osteoconductive and osteoinductive potential.[6]

In recent years, there have been several studies on calcium silicate (CS)-based bone cement intended to overcome the deficiencies of traditional bone cement, aiming at the development of new inorganic bone cements that have both enhanced bioactivity and degradability.[7,8] CS-based materials have also shown great ability for bone-regeneration as bioactive and biodegradable grafting materials.[9] Most importantly, CS-based materials release Si ions during degradation that has been proven to be associated with bone regeneration.[10] However, traditional CS powder can only serve an osteoconduction role, and the potential of drug-delivery is limited by its large particle size and limited nanopore structure. Therefore, we developed a mesoporous CS nanoparticle with a diameter between 2 and 50 nm via a simple method applied in a previous study.[11] MesoCS not only acts as the carrier for loading and controlled release of biomolecules but also increases the reactionary surface area within the tissue.[12]

There are several growth factors that have been used in clinics that have been approved by the FDA (the United States Food and Drug Administration) as well as the EMA (European Medicines Agency). In terms of hard tissue regeneration, bone morphogenetic proteins (BMP) are a group of growth factors found during the development of bone and cartilage.[13] The roles and functions of BMP during bone formation have been widely recognized in past decades. For therapeutic use, recombinant human BMP-2 (rhBMP-2) has been approved by the FDA since 2002.[14] Studies have also shown the capacity of rhBMP-2 in bone regeneration in orthopedic and dental surgery. However, the release pattern of BMP-2 in an implant site became an issue because the traditional application exhibits a burst release within a couple of days after implantation and attenuates rapidly.[15]

Over the past several years, additive manufacturing, also known as a three-dimensional printing (3D-printing) technique has been broadly used in the preparation of highly porous scaffolds.[16] The major advantage of 3D-printing scaffolds is that they provide an environment much closer to *in vivo* conditions for cell infiltration.[3] Furthermore, the scaffolds can also be fabricated using biomaterials as grafts with osteoconductivity to repair bone defects.[17] Consequently, the combination of a MesoCS scaffold and BMP-2 might serve as ideal bone grafts that have osteoconductivity and are osteoinductive. By means of a 3D-printing technique, there are two methods of loading BMP-2 on MesoCS nanoparticles: (1) the pre-loading (PL) method by mixing MesoCS and BMP-2 as a raw material for a 3D-printer, and (2) the direct-loading (DL) method by soaking the 3D-printed MesoCS scaffold in a BMP-2 solution. The PL scaffold can theoretically adsorb more BMP-2 into mesoporous channel and has exhibited good odontogenesis in a previous study. However, the PL method was not favorable for clinical applications in relation to the preservation of BMP-2. Hence, the main purpose of this study is to evaluate the effects of the different loading methods on novel grafting materials composed of a BMP-2-loaded MesoCS scaffold by employing a 3D-printing technique.

Materials and methods

Synthesis and characterization of mesoporous calcium silicate nanoparticles

The MesoCS nanoparticles were prepared using a sol–gel technique that has been described elsewhere.[11] Initially, 3.3 g cetyltrimethylammonium bromide (CTAB, Sigma–Aldrich, St. Louis, MO) was mixed with 6 mL $NH_3 \cdot H_2O$ in double-distilled water (ddH_2O, 300 mL) and then stirred for 15 min at 60 °C. Next, 15 mL tetraethyl orthosilicate (TEOS, Sigma–Aldrich) and 15.6 g calcium nitrate were added with vigorous stirring for 3 h at room temperature. Then, the solution was filtrated, and the precipitates were washed with 1 N hydrochloric acid and absolute alcohol three times. The collected powders were placed in a 60 °C oven overnight for drying and sintered at 800 °C for 2 h to remove the remaining traces of CTAB. The micro-structure of the MesoCS nanoparticles was characterized using transmission

Please cite this article in press as: Huang K-H, et al., Effects of bone morphogenic protein-2 loaded on the 3D-printed MesoCS scaffolds, Journal of the Formosan Medical Association (2018), https://doi.org/10.1016/j.jfma.2018.07.010