Applied Surface Science 255 (2008) 336–339

Contents lists available at ScienceDirect

Applied Surface Science

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

Induction of osteoconductivity by BMP-2 gene modification of mesenchymal stem cells combined with plasma-sprayed hydroxyapatite coating

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ARTICLE INFO

Article history:
Available online 3 July 2008

PACS:
87.68.+z

Keywords:
Plasma-sprayed
Coatings
Mesenchymal stem cells
Osteoconductivity

ABSTRACT

Success in bone implant depends greatly on the composition and surface features of the implant. The surface-modification measures not only favor the implant's osteoconductivity, but also promote both bone anchoring and biomechanical stability. This paper reports an approach to combine a hydroxyapatite (HA) coated substrate with a cellular vehicle for the delivery of bone morphogenetic protein-2 (BMP-2) synergistically enhancing the osteoconductivity of implant surfaces. We examined the attachment, growth and osteoinductive activity of transfected BMP-producing bone marrow mesenchymal stem cells (BMSCs) on a plasma-sprayed HA coated substrate. It was found that the HA coated substrate could allow the attachment and growth of BMP-2 gene modified BMSCs, and this combined application synergistically enhanced osteoconductivity of the substrate surface. This synergistic method may be of osseointegration value in orthopedic and dental implant surgery.

1. Introduction

The osseointegration of bone implants is related to their composition and surface feature [1]. Surface-modified implants favor not only the osteoconductivity, but also promote both bone anchoring and biomechanical stability [2,3]. The osteoconductivity of implant surfaces has been markedly improved during the past two decades by various physical and chemical means. Osteoconductive plasma-sprayed hydroxyapatite (HA) coatings can successfully promote bone healing and apposition, leading to the rapid biological fixation of the implants [4–6]. More recently, attempts have been made to further promote local bone-formation by conferring the implant surfaces with osteoinductive properties, namely, by functionalizing them with an osteogenic agent, such as bone morphogenetic protein-2 (BMP-2) [7–10]. Nevertheless, in order to optimize the desired effects, BMP-2 must be delivered in a more physiological-like manner, viz., released from and functionalized to the local cells inlaying the lattice work of a bone-matrix-like material (calcium–phosphate coating). So this work was conducted to a novel approach using a hydroxyapatite coated substrate combined with a cellular vehicle for the delivery of bone morphogenetic protein-2, with which synergistically enhancing the osteoconductivity of implant surfaces, thus achieving osseointegration.

2. Materials and methods

2.1. Fabrication of HA coating materials

The substrates for the coating were 1 cm^2 Ti6Al4V rectangular substrates for the using in vitro test. The substrates were grit-blasted before plasma spraying. Then, the substrates were cleaned ultrasonically in petroleum ether and in alcohol. Pure crystalline HA powder having a spherical shape with an average diameter of 52 μm was used for plasma spray coating. The plasma spray process was performed using a METCO MN plasma-spraying system with AR2000 Robot. The thickness of HA coating controlled was about 100 μm. These substrates were sterilized under UV light for 24 h before in vitro experiment. The HA coated substrates were characterized by scanning electron microscopy (SEM) and energy dispersive X-ray analysis (Fig. 1).

2.2. Preparation of BMP-2 gene modified BMSCs

A Percoll (GibcoBRL, USA) density gradient centrifugation and the different adherence rate method was used to isolate rat bone marrow mesenchymal stem cells (rBMSCs) as previously described

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doi:10.1016/j.apsusc.2008.06.160

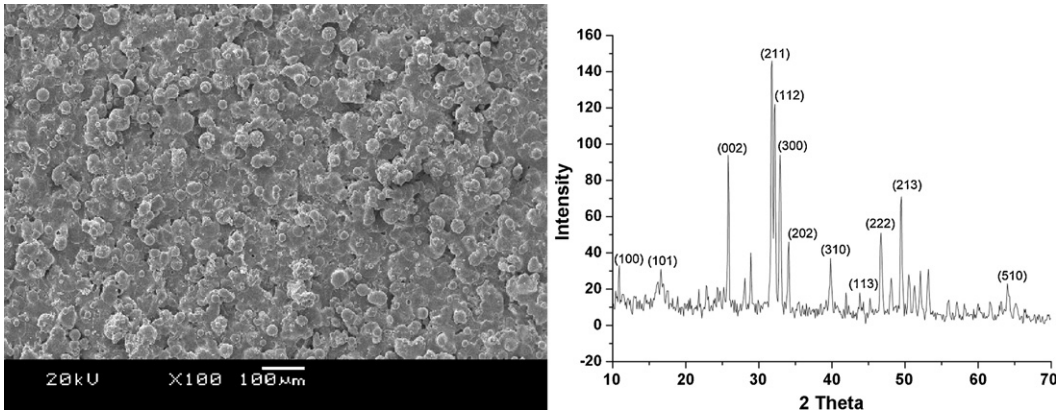

Fig. 1. HA coated substrates were characterized by scanning electron microscopy (left panel) and energy dispersive X-ray analysis (right panel).

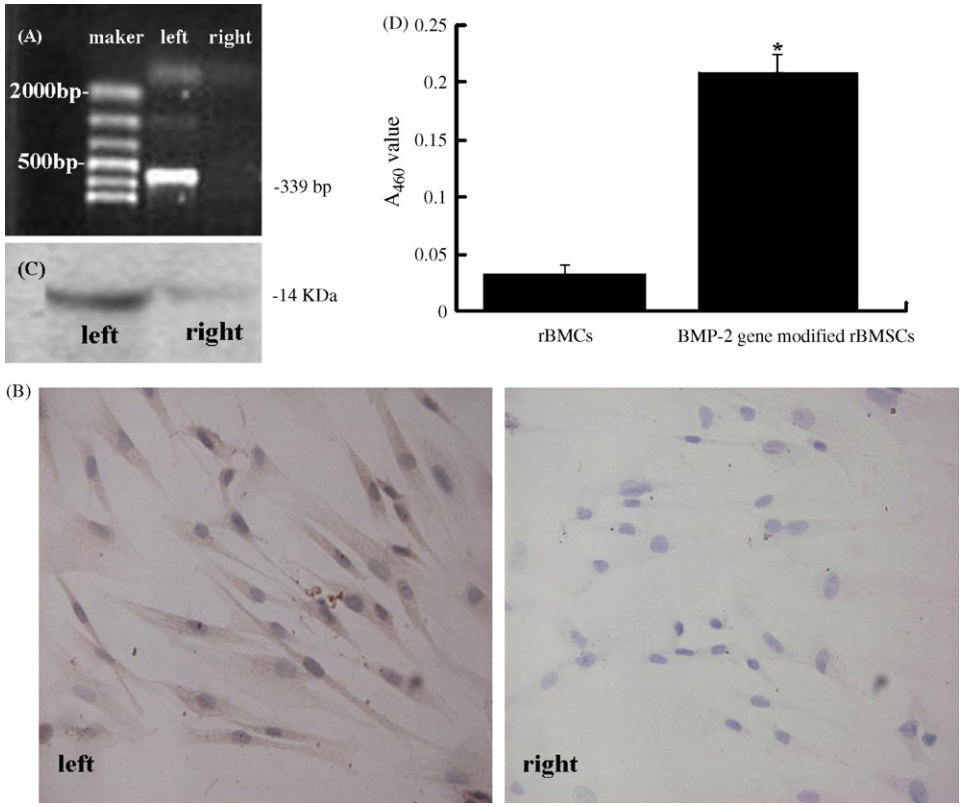

Fig. 2. BMP-2 modification results detecting in gene modified rBMSCs (left) and rBMSCs alone (right). (A) RT-PCR; (B) immunocytochemistry, magnification 200×; (C) Western blot; (D) ELISA (A_{460} value, $n = 6$, $^*p < 0.01$ vs. the rBMSCs group).

[11]. BMP-2 gene was gained by RT-PCR and was cloned into plasmid pcDNA3.0. The rBMSCs of passage four after transfection with the BMP-2 gene, were analyzed for BMP-2 mRNA expression in the endochylema using reverse transcription polymerase chain reaction (RT-PCR, Fig. 2A), and detected protein secretion of the modified cells by immunocytochemistry (Fig. 2B), Western blot assay (Fig. 2C) and enzyme-linked immunosorbent assay (ELISA) (Fig. 2D). The experiment was then divided into four groups of treatment as in Table 1 for in vitro osteoconductive effect observations.

2.3. Culturing and observation of BMP-2 producing cells on HA coated substrates

For in vitro bioactivity observation, rBMSCs or BMP-2 gene modified rBMSCs were seeded on the HA spray-coated and uncoated substrates, respectively. Briefly, cells were plated on the substrate at the density of 3×10^5 cells per substrates, and grown in Dulbeco's modified essential medium (DMEM) supplemented with 10% fetal calf serum and cultured at 37 °C and 5% CO_2. The cells were grown on the substrates for up to 2 weeks. Cell attachment morphology and growth behavior were examined

Table 1
The experiment was divided into four groups as followed

Groups	Cells	Surface modification
Group 1	rBMSCs	HA uncoated
Group 2	BMP-2 gene modified rBMSCs	HA uncoated
Group 3	rBMSCs	HA coated
Group 4	BMP-2 gene modified rBMSCs	HA coated