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3D printing of biomaterials with mussel-inspired nanostructures for tumor therapy and tissue regeneration

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ABSTRACT

Primary bone cancer brings patients great sufferings. To deal with the bone defects resulted from cancer surgery, biomaterials with good bone-forming ability are necessary to repair bone defects. Meanwhile, in order to prevent possible tumor recurrence, it is essential that the remaining tumor cells around bone defects are completely killed. However, there are few biomaterials with the ability of both cancer therapy and bone regeneration until now. Here, we fabricated a 3D-printed bioceramic scaffold with a uniformly self-assembled Ca-P/polydopamine nanolayer surface. Taking advantage of biocompatibility, biodegradability and the excellent photothermal effect of polydopamine, the bifunctional scaffolds with mussel-inspired nanostructures could be used as a satisfactory and controllable photothermal agent, which effectively induced tumor cell death *in vitro*, and significantly inhibited tumor growth in mice. In addition, owing to the nanostructured surface, the prepared polydopamine-modified bioceramic scaffolds could support the attachment and proliferation of rabbit bone mesenchymal stem cells (rBMSCs), and significantly promoted the formation of new bone tissues in rabbit bone defects even under photothermal treatment. Therefore, the mussel-inspired nanostructures in 3D-printed bioceramic exhibited a remarkable capability for both cancer therapy and bone regeneration, offering a promising strategy to construct bifunctional biomaterials which could be widely used for therapy of tumor-induced tissue defects.

1. Introduction

Primary bone cancer brings patients great sufferings and put an end to normal activities in life. Generally, surgical intervention is the most common treatment for primary bone cancer in clinic [1]. To deal with the bone defects resulted from cancer surgery, biomaterials with good bone-forming ability should be implanted to repair bone defects. Meanwhile, in order to prevent possible tumor recurrence, it is essential that the remaining tumor cells around bone defects are completely killed. However, to our knowledge, there are few biomaterials with the ability of both cancer therapy and bone regeneration until now. It remains a significant challenge to achieve a new biomaterial that can kill the bone tumor cells by employing an effective and safe protocol, and at the same time possess the ability to stimulate bone regeneration after surgical intervention of bone tumor.

In recent years, photothermal therapy of tumors has attracted considerable attention owing to its minimal invasiveness and high selectivity [2–4]. However, most of the current photothermal agents are limited by on long-term safety because of their non-degradation and/or potential toxicity. Inspired by biomineralization, many nanostructures have been successfully synthesized by biomimetic methods using natural biopolymers. Interesting, polydopamine is a polymer imitating melanin that occurs naturally in organs and tissues, such as hair, skin, brain medulla, iris of eyes, and brain medulla [5]. Owing to its satisfactory biocompatibility and

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biodegradability, polydopamine has greater potential for biomedical application [6,7]. Furthermore, its absorption spectrum can extend to near-infrared regions (NIR) and has a high photothermal conversion efficiency of 40%, suggesting that dopamine is a favorable photothermal agent for tumor therapy [8]. Moreover, inspired by strong adherence of mussels to surfaces even in wet marine conditions owing to the reactive catechol-containing compound lysine and 3,4-dihydroxyphenyl-L-alanine [9], it has been demonstrated that polydopamine can effectively enhance the surface roughness and hydrophilicity as well as modulate specific cellular responses to biomaterials, such as cell adhesion, morphology, proliferation and differentiation[10–13]. Inspired by previous studies, we speculated that polydopamine could combine the functions of photothermal therapy and tissue engineering.

3D porous scaffolds are one of the most promising biomaterial forms for the bone defect regeneration as the interconnected macropores are essential to supply sufficient space for cellular activity, nutrient transport and cell-cell interactions [14]. Meanwhile, the biomaterial surface with specific nanostructures directly affects cell-scaffold interactions, tissue formation and function. Thus, it is of great interest to prepare a 3D porous scaffold with nanostructured surface for promoting bone formation [15–18].

Here, we reported the fabrication of a 3D-printed bioceramic scaffold with a uniformly self-assembled Ca-P/polydopamine nanolayer surface. Taking advantage of the satisfactory photothermal effect of polydopamine, the bifunctional scaffolds could effectively induce tumor cell death *in vitro*, and significantly inhibited tumor growth in mice. In addition, owing to the mussel-inspired nanostructured surface, the prepared polydopamine-modified bioceramic (DOPA-BC) scaffolds could support the attachment and proliferation of rabbit bone mesenchymal stem cells (rBMSCs), and significantly facilitated the formation of new bone tissues in rabbit bone defects even under photothermal treatment. Therefore, the prepared scaffolds with Ca-P/polydopamine nanolayer surfaces could effectively kill tumor cells and regenerate large bone defects, showing great potential for treating tumor-related bone defects.

2. Materials and methods

2.1. Materials

Tetraethyl orthosilicate (TEOS), triethyl phosphate (TEP) and calcium nitrate tetrahydrate ($Ca(NO_3)_2$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Sodium alginate was purchased from Alfa Aesar (USA), and Pluronic F-127 was purchased from Sigma- Aldrich (USA).

2.2. Fabrication and characterization of DOPA-BC scaffolds

Bioceramic powders (Nagel) with specific composition ($Ca_7Si_2P_2O_{16}$) were first synthesized by a sol-gel method using tetraethyl orthosilicate, triethyl phosphate and calcium nitrate tetrahydrate according to our previous study. Then pure Nagel bioceramic (BC) scaffolds were fabricated via a 3D-Printing method [19]. The printable bioceramic ink was prepared by mixing 5 g of BC powders with 0.3 g of sodium alginate powders and 2.8 g of Pluronic F-127 (20 wt%) aqueous solution. After stirred homogeneously, they were put in a tube and printed via a needle (needle standard: 22G) according to computer assist design model. After dried at room temperature overnight, they were sintered at 1100 °C for 3 h to obtain pure BC scaffolds. To prepare self-assembled Ca-P/polydopamine nanolayers on bioceramic scaffolds (named as 2 mg/mL, 4 mg/mL, 6 mg/mL DOPA-BC), dopamine dydrochloride was first dissolved in Tris-HCl (pH: 8.5) at three different concentrations (2, 4, 6 mg/mL), and then pure BC scaffolds were soaked in Tris-dopamine solution for 72 h and then dried at 40 °C overnight.

The overall morphologies of pure BC and DOPA-BC scaffolds were investigated by optical microscopy (S6D, Leica, Germany). The surface microstructure and composition of pure BC and DOPA-BC scaffolds were characterized by scanning electron microscopy (SEM, SU8220, HITACHI, Japan), X-ray photoelectron spectroscopy (XPS, ESCAlab250, USA), and energy-dispersive spectroscopy (EDS). The sectional microstructure and morphology of the DOPA-BC scaffolds were also analyzed by scanning electron microscopy (SEM, SU8220, HITACHI, Japan) and linescan energy-dispersive spectroscopy (EDS).

2.3. Photothermal effects of DOPA-BC scaffolds

The photothermal effects of the DOPA-BC scaffolds were investigated in dry environment (air) and in wet environment (PBS) in a container, obtained by cutting a 48-well cell culture plate. Pure BC and DOPA-BC scaffolds were irradiated under an 808 nm NIR laser in dry environment (air) and in wet environment (PBS), respectively. Laser irradiation induced temperature increase of the DOPA-BC scaffolds with different polydopamine concentrations (2, 4, 6 mg/mL), and laser power densities (0.26, 0.30, 0.34 W/cm^2) were monitored real-timely by an infrared thermal imaging system. We also investigated the depth-dependent photothermal effect by covering the scaffolds with different thickness of pork (5 mm, 7.5 mm and 10 mm) to mimic the *in vivo* photothermal effect in rabbits. The highest temperature of the scaffolds, was exported to obtain the heating curves by FLIR R&D software. The distance of the output end of the NIR laser and the scaffolds was 25 cm, and the diameter of the laser spot was 0.6 cm, and the area was 1.13 cm^2.

2.4. Photothermal effects of DOPA-BC scaffolds on the viability of tumor cells

Saos2 cells (osteosarcoma cells) and MDA-MB-231 (breast cancer cells, the most frequent bone metastasis tumor cells) were cultured in MEM medium supplemented with 10% fetal bovine serum (FBS) under 5% CO_2 at 37 °C. To test cell viability, 1.0×10^4 of Saos2 cells or MDA-MB-231 cells were cultured with each pure BC scaffold or 4 mg/mL DOPA-BC (diameter: 10 mm, height: 2 mm) on 48-well culture plates for 48 h, respectively. Then, the tumor cells in pure BC scaffolds and DOPA-BC scaffolds were irradiated under NIR with the power density of 0.38 W/cm^2 for 10 min. As control, cells were cultured in pure BC and DOPA-BC scaffolds without NIR irradiation. After additional 12 h incubation, the viability of Saos2 cells or MDA-MB-231 cells in scaffolds was tested by a standard methyl thiazolytetrazolium (MTT) assay according to our previous study protocol, after extraction of the medium, 0.5 mg/mL of MTT solution was added into each samples for 4 h, followed by dissolving with dimethyl sulfoxide (DMSO). And then 100 μL of DMSO was extracted from each sample and measured at the absorbable wavelength of 490 nm by a SpectraMax Microplate Reader (Molecular Devices, Inc., USA) [20].

To further investigate the photothermal effect of DOPA-BC scaffolds on the viability of tumor cells in the surrounding area, 1.0×10^4 Saos2 and MDA-MB-231 cells were cultured on glass slices, respectively, in 48-well culture plates for 48 h. Then pure BC or DOPA-BC scaffolds were put onto the glass slices gently, and the cells on glass slices with pure BC and DOPA-BC scaffolds were irradiated by NIR laser (0.38 W/cm^2) for 10 min. As control, two kinds of tumor cells were cultured on glass slices with pure BC and DOPA-BC scaffolds at same condition without NIR irradiation. After additional 12 h incubation, the viability of tumor cells was also measured by MTT assay as above. Four samples of each group were