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# Rational design of a stable, effective, and sustained dexamethasone delivery platform on a titanium implant: An innovative application of metal organic frameworks in bone implants

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ABSTRACT

Endowing titanium implants with the capability of delivering dexamethasone in a sustained and controlled manner to promote osseointegration and osteogenesis has become an emerging field. However, most of the currently developed dexamethasone/titanium implants are far from satisfactory because of some inherent defects. In this work, we constructed a stable, effective, and sustained dexamethasone delivery platform on titanium disc by immobilizing dexamethasone@zeolitic imidazolate framework-8 nanoparticles into the micrometer-scale artificial etch pits on titanium substrate using methanol-induced regenerated silk fibroin membrane encapsulation. In addition, the titanium substrate was aminated and covalent interactions were established between the titanium substrate and the silk fibroin membrane through genipin crosslinking. Briefly, owing to the synergistic barrier effect of the zeolitic imidazolate framework-8 shell and the silk fibroin membrane, the silk fibroin-dexamethasone@zeolitic imidazolate framework-8-titanium could release dexamethasone in a controlled manner over 30 days. Moreover, the pit walls together with the silk fibroin membrane protected the nanoparticles from detaching from the titanium substrate in the case of mechanical wear. The covalent interactions between the silk fibroin membrane and the titanium substrate prevented the silk fibroin membrane from self-peeling from the titanium substrate in a moist environment. *In vitro* cell culture indicated that the as-prepared titanium disc had good cytocompatibility with MC3T3-E1 cells. Furthermore, the cells cultured on the titanium disc demonstrated higher differentiation, calcium deposition, and expression of osteogenic genes than the cells cultured on the silk fibroin-zeolitic imidazolate framework-8-titanium and pristine titanium. In this work, dexamethasone was utilized as a drug model and could be replaced by other osteogenic drugs or growth factors. Thus, we believed that our design philosophy could inspire future research work on the development of Ti implants.

## 1. Introduction

In the past few decades, titanium (Ti) and Ti alloys have been widely used as bone substitutes for clinical bone reconstruction because of their desirable properties [1]. However, the naturally formed oxide thin film on Ti surface causes pristine Ti implants to lack desirable bioactive properties [2]. Surface modification and surface functionalization can endow Ti implants with specific surface structure or surface chemistry and therefore influence initial cell behavior, but tissue-engineered bone regeneration still requires the regulation of additional growth factors or osteogenic drugs [3–8]. A trace amount of growth factors or osteogenic drugs can greatly facilitate the osteogenic differentiation of implanted cells as well as osteogenesis, but the uncontrolled release of these reagents *in vivo* can cause adverse side effects [9,10]. Thus, endowing Ti implants with the capability of controlled delivery of growth factors/osteogenic to stimulate specific cell/gene responses at the molecular level and therefore promote osteointegration and osteogenesis has become an emerging field and has drawn tremendous attention [11].

Dexamethasone (DEX), a widely used osteogenic drug, supports the

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osteogenic differentiation of human mesenchymal stem cells (hMSCs) and, compared with growth factors, has a relatively longer half-life, a higher cost-effectiveness, and a more sufficient availability [9,12]. Thus, a number of DEX/Ti implants have been developed and utilized as substitutes for bone defect repair, but most of them are far from satisfactory because of some inherent defects.

The physical absorption-based drug loading/release strategy (*e.g.,* π-π stacking and hydrophobic interaction) cannot guarantee long-term and controlled DEX delivery [13,14]. In addition, the direct contact of the tissues surrounding the bone defect site with naked DEX molecules may have negative effects on cell behavior and metabolism of the implant recipient [9,15]. Immobilizing DEX nanocarriers onto the surface of Ti implants can endow those implants with desirable DEX delivery capabilities and avoid the local supersaturation of DEX molecules at bone defect site. However, how to protect the DEX nanocarriers from detaching from the Ti implants in the case of mechanical wear and the inherent cytotoxicity and biotoxicity of the nanocarriers are still major challenges [16,17]. Artificially constructed nanoporous inorganic membranes or coatings on the surface of Ti implants can also act as ideal reservoirs for DEX preservation and delivery, but the fabrication processes of these membranes or coatings are relatively complicated and time-consuming [18–20]. In addition, substantial efforts should be made to enhance the bonding strength of the inorganic membranes or coatings with the Ti substrate. Encapsulating drugs into Ti implants using a polymeric membrane is another effective drug loading strategy, but the as-prepared Ti implants are not ideal drug delivery platforms. The DEX molecules encapsulated by a hydrophilic polymer membrane in DEX/Ti implants are exhausted within a few days [21]. DEX/polypyrrole composite membrane-coated Ti discs can release DEX molecules under electrical stimulation. However, when sweep voltages were applied to the Ti disc, 80% of the loaded DEX molecules were subsequently released [22]. In addition, the self-peeling and fracture of the polymeric membranes coated on the Ti implants are other fatal defects for successful drug loading and drug delivery [17]. Moreover, the cytocompatibility, bioaffinity, biodegradability, and long-term stability in a moist environment of the polymeric membranes should also be taken into consideration [23].

Recently, metal organic frameworks (MOFs), a rapidly growing class of hybrid crystalline porous materials, have found applications in diverse fields including catalysis [24], gas storage [25,26], and chemical sensing [27]. Specifically, in the biomedical field, MOFs have been extensively used as alternative drug delivery platforms because of their high porosity, easy functionalization, structural diversity, reproducible drug loading/release capability, and potential biodegradability [28,29]. Zeolitic imidazolate framework-8 (ZIF-8), which is constructed from $Zn^{2+}$ and 2-methylimidazole, features a nanoscale size, a high surface area, outstanding chemical and thermal stability, excellent biocompatibility, and pH-sensitivity and therefore has been extensively used as a nanocarrier for the delivery of proteins, DNA, enzymes, and drugs [30,31]. However, until now, MOFs have seldom been used in bone implants or bone scaffolds, and dexamethasone@zeolitic imidazolate framework-8 (DEX@ZIF-8) nanoparticles have never been reported before. Compared with other DEX carriers, the DEX@ZIF-8 nanoparticles were assumed to be able to retain DEX bioactivity and deliver DEX in a long-term and controlled manner. In addition, the pH-sensitivity of ZIF-8 might facilitate the renal excretion of the degradation products of the DEX@ZIF-8 nanoparticles *in vivo* [29].

In our recent work, it was found that acid-etched Ti implants had a large number of uniform micron-scale etch pits on their surface; these pits could serve as ideal depots for nanosized drug carriers. Silk fibroin (SF), a natural protein obtained from spider or silk worm, has ample $NH_2$ groups and has been extensively used in the biomedical fields. Furthermore, methanol-induced regenerated SF membranes comprise massive water-insoluble SF micelles, in which the larger terminal hydrophilic blocks (*i.e.,* random coil structure) define the outer edges, while the smaller hydrophilic blocks (i.e., α-helix structure) and hydrophobic blocks (*i.e.,* β-sheet structure) constituted the inner part. Thus, methanol-induced SF membranes can not only provide good preservation and local delivery of drugs but also maintain good structural integrity in a moist environment [32–35]. In addition, the specific hydrophilic surface and abundant Arg-Gly-Asp (RGD) sequences of the SF membrane facilitate cell adhesion [36]. In this regard, the nanosized DEX@ZIF-8 particles were immobilized into the artificial etched pits on the Ti substrate using methanol-induced regenerated SF membrane encapsulation. Presumably, the synergistic barrier effect of the ZIF-8 shell and the SF membrane could endow the resultant Ti implant with the capability of delivering DEX in a sustained and controlled manner. In addition, the pit walls together with the SF membrane protected the DEX@ZIF-8 nanoparticles from detaching from the Ti substrate in the case of mechanical wear.

To prevent the SF membrane from self-peeling from the Ti substrate in a moist environment, covalent interactions were established between the SF membrane and the Ti substrate by utilizing piranha solution, (3-aminopropyl)triethoxysilane (APTES), and genipin (GNP) together [3,37–39]. The as-prepared Ti disc was termed silk fibroin-dexamethasone@zeolitic imidazolate framework-8-titanium (SF-DEX@ZIF-8-Ti). Fig. 1 shows schematic diagrams of the process of preparing the SF-DEX@ZIF-8-Ti, the conformation transition of the regenerated SF fiber with methanol treatment, and the DEX release from the SF-DEX@ZIF-8-Ti/osteogenic differentiation of adherent cells. Fig. 2 displays a schematic diagram showing the construction of covalent interaction between the SF membrane and the Ti substrate.

In this work, the SF-DEX@ZIF-8-Ti was investigated in detail, but DEX was utilized only as a drug model and could be replaced by other osteogenic drugs or growth factors. Thus, we hoped that our design philosophy could inspire future research work on the development of Ti implants.

## 2. Experimental section

### 2.1. Materials

Zinc nitrate hexahydrate ($Zn(NO_3)_2{\cdot}6H_2O$), sodium carbonate ($Na_2CO_3$), lithium bromide (LiBr), ethanol, methanol, hydrochloric acid, sulfuric acid, and hydrogen peroxide were purchased from Sinopharm Chemical Reagent Co, Ltd (Shanghai, China). Pristine Ti discs were bought from Weiye Metal Materials Co., Ltd. (Wuhan, China). The *bombyx mori* silkworm cocoons were purchased from Nanyang, Henan, China. GNP was supplied by Chengdu ConBon Biotech Co., Ltd. (Chengdu, China). APTES was bought from Aladdin Industrial Co., Ltd. (Shanghai, China). DEX was obtained from Wuhan Keri Biological Technology Co., Ltd. (Wuhan, China). All chemicals were used without any purification. Deionized ultrapure water was used throughout the experiment.

### 2.2. Preparation of the DEX@ZIF-8 nanoparticles

150 mg of $Zn(NO_3)_2{\cdot}6H_2O$ was dissolved in 5 mL of deionized water. 330 mg of 2-methylimidazole and 5 mg of DEX were dissolved into 10 mL of methanol. Then the two solutions were mixed and stirred for 5 min. Afterwards, the resultant solution was centrifuged at 12,000 rpm for 15 min. Next, the precipitated DEX@ZIF-8 particles were washed with methanol for three times and then resuspended in methanol (10 mL) by sonification. The ZIF-8 nanoparticles were also prepared and used as control in subsequent assays.

### 2.3. Preparation of the regenerated SF solution

Silkworm cocoons were degummed in 0.5% (w/v) $Na_2CO_3$ aqueous solution at 100 °C for 30 min and then thoroughly washed with deionized water. The resultant SF fibers were dissolved in 9.3 M LiBr solution (liquor ratio = 5/1) at 37 °C, and the as-prepared solution was