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Controlled gentamicin- strontium release as a dual action bone agent: Combination of the porous titanium scaffold and biodegradable polymers

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ABSTRACT

Titanium is considered a promising biomaterial for load bearing implantation in orthopedics. However, its biologically inert behavior makes the surface treatment essential to improve the bioactivity. In this study, a porous titanium scaffold was fabricated using the space holder method and its surface was treated by the direct oxidation method. Strontium containing gelatin micro spheres (SrGMS) were synthesized using the water in oil method and deposited on the surface of the surface treated porous titanium scaffold (Ti-SrGMS). A thin layer of gentamicin containing poly vinyl alcohol (PVA) was deposited on the surface of Ti-SrGMS for two purposes: first, to postpone strontium release and second, to initially release gentamicin. Raman spectroscopy revealed rutile formation due to the thermal oxidation of titanium at 600 °C. Scanning electron microscope observation also indicated that SrGMS particles were deposited uniformly on the surface of the titanium scaffold. Fourier transform infrared (FTIR) spectra showed that strontium ion was loaded in the gelatin micro sphere successfully. *In vitro* drug release measurement using inductively coupled plasma-mass spectrometer (ICP-MS) also revealed that by using a layer of PVA with different concentrations on SrMGS particles, it would be possible to control the strontium release as it was needed for the surrounding tissue.

1. Introduction

Recent approaches addressing bone reconstruction in the field of bone tissue engineering have developed promising remedies facilitating the use of allografts and auto grafts limited by complications arising from immunological rejection and disease transmission. The main purpose of bone tissue engineering is designing a biocompatible interconnected porous scaffold that can provide appropriate strength, stiffness and appropriate waste removal, nutrient delivery [1], and cell attachment and bone ingrowth abilities [2].

Titanium and its alloys have been widely utilized for *in vivo* load bearing implantation, due to their high specific strength, excellent biocompatibility and superior corrosion resistance in physiologic mediums [3]. However, it should be noted that titanium and its alloys are essentially bioinert materials [4] and do not have bone bonding ability, such that after implantation in the bone defects of the living body, they will be merely encapsulated by a fibrous tissue. To alleviate this problem, some surface treatment techniques have been introduced to improve the bioactivity of the surface of titanium. Direct oxidation is a simple and effective method used to improve apatite inducement ability of titanium implant; this is because at high temperatures, titanium reacts with the surrounding oxygen and a thick layer of bioactive titanium oxide (anatase or rutile) is formed at the surface of titanium [3].

Because of the high corrosion resistance of titanium, no significant ion is released in the human body during implantation. However, some ions or drugs are useful and should be released during implantation to improve bone formation [1] or inhibit future infections [5,6]. Huang et al. designed a new titanium base composite with controlled Si/Ag ion release ability, to improve the biofunctionalization of the titanium implant [7].

Strontium ion is an effective agent for osteoporosis; it increases the rate of new bone formation by triggering the osteoblast cells and also decreasing bone resorption through triggering the osteoclast cells (the simultaneous dual action in bone formation).

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Strontium increases pre-osteoblast proliferation, osteoblast differentiation, type I collagen synthesis and bone matrix mineralization, while osteoclast differentiation and activation are inhibited, so it is used as a well-known dual action bone agent to improves bone density and strength. Actually controlled strontium release improve bone quality through changes in bone matrix properties and bone mineral density (BMD) in patients [8,9]. So, many studies have been done on strontium release [5,10]; however, according to the previous researches, strontium has some burst release during the first 12 h of implantation [5,10]. The problem is the fast release of strontium, which makes the period of strontium release much shorter than that of bone healing and complete formation [11]. Also, an antibiotic agent may be released at the first step of implantation such that the controlled release of a bone forming agent can work better. Because bacterial infection may start at the time of surgery and during implantation, the implanted bone graft or scaffold can behave as an avascular region in the early stage of implantation, so systematic antibiotic delivery cannot act effectively, and local antibiotic release, especially in the early stage of implantation, is more recommended [5,7]. So, many researchers have tried to study bioceramics [12] and biopolymers [13] for load bearing implants. However, because of the superior mechanical properties of metals and metallic base composites [14] in comparison to polymers and ceramics, biometallic materials are more applicable and reliable options for load bearing implantations [15]. So a combination of metals and biodegradable polymers (as drug carrier) seems to be essential for load bearing implants, ensuring *in vivo* degradation, as well as controlled drug delivery and gene therapy.

Gelatin is a denatured protein from the triple helix of collagen that behaves as a gel. Ideal characteristics such as biodegradability, biocompatibility, ease of processing and its low antigenic activity in physiological environments have resulted in the wide use of gelatin in pharmaceutical and medical applications. It also provides hemostasis and facilitates cell adhesion and proliferation during tissue regeneration [16]. Glutaraldehyde is well known as an agent for gelatin crosslinking [17], but it is a toxic material causing inflammation *in vivo* [18,19]; so, according to the literature, vacuum heating [20] is recommended for gelatin crosslinking, but, because of its high temperature and protein denaturation at high temperatures, this process cannot be used for protein loaded gelatin cross linking.

Some researchers have tried to use biodegradable polymers or hydrogels (in the form of a microsphere or a thin layer) on the surface of metallic implants to release some ion/drugs such as silver [5] and gentamicin sulphate [10] and to combat bacterial infection [5].

Water-in-oil approach is the most used method for gelatin microsphere fabrication, while the exact water/oil ratio has not been reported. For example, some researchers have used 2/60 (0.03) [20], 10/60 (0.15) [5] and 20/60 (0.3) [18] water/oil ratio for gelatin microsphere fabrication. So, the effect of water/oil ratio on the particle size distribution of gelatin microspheres needs to be investigated.

As the most recent studies have been done on the controlled drug release, the main purpose of this study was to improve the osseoconductivity of porous titanium scaffolds using a new design of composite on the surface of titanium scaffold. In this design, the initial release of strontium (as the bone forming agent) was postponed and controlled; also, gentamicin (as antibiotic agent) was released at the initial time of implantation to reduce the chance of surgical infection.

In the designed composite, strontium containing gelatin microspheres were deposited on the surface of the porous titanium scaffold (noted as Ti-SrGMS: Titanium + Strontium containing Gelatin Micro Sphere); then, a thin layer of PVA containing gentamicin was deposited on the surface of Ti-SrGMS composite and noted as Ti-SrGMS-GenPVA (Fig. 1).

Also, while gelatin, PVA and titanium are generally biologically inert by themselves [5,13,21], the surface of the porous titanium scaffolds was treated (to improve the bioactivity of titanium) before microsphere deposition.

2. Materials and methods

2.1. Scaffold fabrication

2.1.1. Titanium scaffold fabrication and surface modification

The space holder technique was used to manufacture disc shaped (with the diameter of 13 mm and the height of 2 mm) titanium scaffolds containing 70 vol% nominal porosity, as described previously [22]. Briefly, raw materials including the commercially pure titanium powder (grade 2, the mesh size of 325, OSAKA Titanium Technologies Co., Japan) was blended with Sodium chloride (the mesh size of 35–40, Wako Pure Chemical Industries, Japan) as the spacer agent. Of course, in the case of solid samples, no spacer agent was used; instead, they were cold compacted at the pressure of 200 MPa and then sintered under vacuum (10^{-5} mbar) at the temperature of 800 °C for 2 h and 1200 °C for 2 h (the heating rate was adjusted at 5 °C/min). Finally, the sintered samples were cooled down in the furnace to room temperature [22]. For salt leaching, samples were rinsed in distilled water for 35 h [23].

Subsequently, porous and solid samples were heated up to 600 °C, using an electric furnace at a rate of 5 °C/min; they were kept at 600 °C for 240 min [24] and finally, allowed to cool to room temperature in the furnace.

2.1.2. Gelatin microsphere (GMS) preparation

To synthesize gelatin microspheres (GMS), 10 wt% gelatin (Type A: Wako Ltd, Osaka, Japan) and 0.5 wt% strontium hydroxide (as a bone formation drug) were dissolved in doubly distilled water at 40 °C; then 1, 5 and 10 ml of the aqueous solution were added to the 30 ml olive oil (which had been preheated to 40 °C) in a drop wise manner, and stirred at a stirring rate of 700 rpm by a mechanical stirrer. Actually, the aqueous/oil phase ratio was considered to be 0.03, 0.15 and 0.3 v/v, separately, because these ratios had been used by previous researchers [5,18,20]. After stirring water/oil emulsions for 15 min, the emulsions were cooled down to 5 °C rapidly and stirring was continued for 30 min; then microspheres were dehydrated by the addition of 15 ml acetone and stirring was continued for further 30 min; finally, microspheres were centrifuged, washed with cold acetone, and then dispersed in ethanol (0.1 wt%). Under a mild stirring condition, surface modified titanium porous scaffolds were immersed in the suspension of ethanol + strontium containing gelatin microspheres (SrGMS) for 1 h. Then, the surface modified porous titanium scaffolds were coated with SrGMS, air dried at 4 °C for 24 h, lyophilized (Freeze drier: EYELA, FDU-2200, Tokyo, Japan) for 24 h, and noted as Ti-SrGMS.

Then, Ti-SrGMS were heated in a vacuum oven at 140 °C and 0.08 Torr for 48 h to ensure the dehydrothermal crosslinking of SrGMS.

2.1.3. Polyvinyl alcohol (PVA) deposition

At this stage, the surface of Ti-SrGMS was coated by dipping in a solution including 10 mg/ml gentamicin+ 1 and 2 or 4 wt% polyvinyl alcohol (PVA) at room temperature for 10 min. Finally, the samples were dried at 37 °C for 24 h, chemically crosslinked by dipping in a glutaraldehyde solution (glutaraldehyde 5 M) for 1 h, dried at 37 °C for 24 h, and noted as Ti-SrGMS-GenPVA1, 2 and 4,