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Bioabsorbable bone plates enabled with local, sustained delivery of alendronate for bone regeneration

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ABSTRACT

We prepared a bone plate enabled with the local, sustained release of alendronate, which is a drug known to inhibit osteoclast-mediated bone resorption and also expedite the bone-remodeling activity of osteoblasts. For this, we coated a bone plate already in clinical use (PLT-1031, Inion, Finland) with a blend of alendronate and a biocompatible polymer, azidobenzoic acid-modified chitosan (i.e., Az-CH) photo-crosslinked by UV irradiation. As we performed the in vitro drug release study, the drug was released from the coating at an average rate of 4.03 μg/day for 63 days in a sustained manner. To examine the effect on bone regeneration, the plate was fixed on an 8 mm cranial critical size defect in living rats and the newly formed bone volume was quantitatively evaluated by micro-computed tomography (micro-CT) at scheduled times over 8 weeks. At week 8, the group implanted with the plate enabled with sustained delivery of alendronate showed a significantly higher volume of newly formed bone (52.78 ± 6.84%) than the groups implanted with the plates without drug (23.6 ± 3.81%) ($p < 0.05$). The plate enabled with alendronate delivery also exhibited good biocompatibility on H&E staining, which was comparable to the Inion plate already in clinical use. Therefore, we suggest that a bone plate enabled with local, sustained delivery of alendronate can be a promising system with the combined functionality of bone fixation and its expedited repair.

1. Introduction

Bone fixation systems made of biodegradable polymers, such as poly(lactic-co-glycolic acid) (PLGA), poly(lactic acid) (PLA) or poly(glycolic acid) (PGA), have attracted a great deal of interest as they would not need a secondary removal surgery due to biodegradability [1,2]. The major compartments of the bone fixation systems are plates and screws, where the plate is positioned and fixed on a fractured bone by screws. In this way, the undesired motion of the fractured bone can be prevented until complete healing.

However, the bone fixation systems currently in clinical use do not have the functionality to treat patients with bone loss or diseases, such as osteoporosis. It was reported that approximately 11.4% of comminuted fractures induce bone loss [3], which would impair the stability of the bone fixation systems, thereby often needing a secondary surgery [3,4]. Such complications would also include infection, which was reported to occur in approximately 13.9% patients with fractured bone [5]. The osteoporotic patients often show low bone density, which delays bone healing [6] and causes severe complications, such as microfracture, malunion or a loosening of the fixation system even with adequate fixation of fractured bone [7,8]. It has been reported that the failure of bone fixation systems occurs in 2 to 10% of fractures related to osteoporosis [8,9]. For these reasons, a strategy to facilitate bone healing is needed to properly treat fractured bone without a failure of the fixation system [10].

In this sense, alendronate can be a good candidate therapeutic agent to prevent the failure of the fixation system. Alendronate has been widely used for treatment of bone diseases, such as osteoporosis, Paget's

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disease and inflammation-related bone loss [11–13]. The drug is a significant and potent inhibitor of bone resorption as it prevents the recruitment and differentiation of osteoclasts [13,14]. Moreover, it has been recently reported that the drug can improve the recruitment, differentiation and bone-remodeling activity of osteoblasts, thereby expediting bone repair [15–17]. Alendronate has been shown to increase bone mineral density, which is especially effective in the treatment of bone loss and osteoporosis [4,18,19].

Alendronate is often administered orally or via injection; however, when being orally administered, alendronate may induce irritation in the gastrointestinal (GI) tract, as well as abdominal pain and nausea [20]. Given these drawbacks, the local delivery of alendronate can be a promising approach to therapy. Because a bone fixation system needs to be placed locally onto a fractured bone, a combined entity of bone fixation and drug delivery should be advantageous. In addition, the sustained delivery of alendronate can benefit from the continuous inhibition of bone resorption during bone healing [21–23], which should improve bone density.

In this work, therefore, we prepared a bone fixation plate with the added functionality of the local, sustained delivery of alendronate. For this, we employed a bone plate already in clinical use (PLT-1031, Inion, Finland) and coated it with 4-azidobenzoic acid-modified chitosan (i.e., Az-CH) loaded with alendronate. Az-CH was crosslinked via UV irradiation to serve as a drug diffusion barrier for the sustained delivery of alendronate [24,25]. In addition, crosslinked Az-CH can form covalent bonds with poly(lactic acid), one of the major constituents of the Inion plate [26,27], and thus, the Az-CH based coating could be stably attached on the surface of the Inion plate. Az-CH is also proven to be biocompatible to a large extent [27,28].

We characterized the coating with Fourier transform infrared spectroscopy (FTIR), and we examined its morphology by scanning electron microscopy (SEM). We also performed the in vitro drug release study in phosphate buffered saline (PBS; pH 7.4) at 37 °C with the plate coated with both Az-CH and alendronate. For the in vivo evaluation, the plates were fixed on a craniotomy defect, 8 mm in diameter, created on the skull of living rats [29]. The degree of reconstructed bone volume was quantitatively measured using micro-computed tomography (micro-CT) at a predetermined schedule for 8 weeks following implantation. The histopathologic analyses were also carried out with the tissue including the implanted plate 8 weeks after implantation with hematoxylin and eosin (H&E) staining.

2. Materials and methods

2.1. Materials

4-azidobenzoic acid was obtained from Tokyo Chemical Industry (Tokyo, Japan). Chitosan (Mw; <200 kDa, degree of deacetylation; 75–85%), N,N,N′,N′-tetramethylethylenediamine (TEMED), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), alendronate sodium trihydrate, acetic acid solution, o-phthaldialdehyde (OPA), 2-mercaptoethanol (2 ME) and egg-white lysozyme were all purchased from Sigma-Aldrich (MO, USA). Phosphate buffered saline (PBS, pH 7.4) was obtained from the Seoul National University Hospital Biomedical Research Institute. Bioabsorbable bone fixation plates (PLT-1031), composed of poly(trimethylene carbonate), polylactide and polyglycolide [30], were purchased from Inion (Finland). Zolazepam and tiletamine (0.3 ml/kg; Zoletil®) were supplied from Virbac (France). Xylazine (0.1 ml/kg; Rompun®) was obtained from Bayer (Germany). Betadine was obtained from Hyundai Pharm (Korea). The absorbable sutures (Vicryl 3–0; 6–0) used for animal surgery were supplied from Ethicon (NJ, USA).

2.2. Synthesis of an azidobenzoic acid-modified chitosan (Az-CH)

Az-CH was synthesized as described in a previous study. In brief, a chitosan solution was first prepared with 200 mg chitosan dissolved in 15 ml distilled water adjusted to pH 4.75 using acetic acid solution. TEMED (116.2 mg) was dissolved in 1 ml distilled water, which was then added to the chitosan solution. To this resulting solution, a mixed solution of EDC in 1 ml distilled water and 40 mg 4-azidobenzoic acid in 1 ml dimethyl sulfoxide was then added. After adjusting the pH to 5 with 1 M HCl, the reaction was carried out at room temperature overnight. The solution was filtered via a 0.22 μm-pore filter (GSWP04700, Millipore, Bedford, MA), which was then freeze-dried for 3 days to give a dry powder of Az-CH.

2.3. Preparation of bone plate samples

We prepared the three different types of bone plate samples in this work:

(1) UP: unmodified bone plates with no treatment
(2) Az-CH_P: bone plates coated with Az-CH only
(3) AL–Az-CH_P: bone plates coated with Az-CH and alendronate

As shown in Fig. 1, we first cut a whole piece of an Inion bone plate (PLT-1031, mesh type: 14 × 14 holes) into square-shaped pieces (6 × 6 mm), each with a screw hole at the center. These pieces were each used as the UP samples without further treatment. To prepare the coated samples, i.e., Az-CH_P or AL–Az-CH_P, the coating solution was first prepared: 20 mg Az-CH or a blend of 20 mg Az-CH and 25 mg alendronate was dissolved in 1 ml of 2% v/v acetic acid solution. Then, four drops of the coating solution (3 μl per drop) were added on top of the unmodified plate around the screw hole at the center, as described in Fig. 1(A). The coated samples were each placed under UV irradiation (100 W; 365 nm, Blak-Ray, UVP, USA) for 5 min to crosslink Az-CH, which were then dried at room temperature for 24 h in a dark room (Fig. 1(B)). In this work, we coated only one side of the plate that should be faced toward the fractured bone after fixation. We also avoided the screw holes from coating, which would be under severe frictional stress during fixation. For the in vivo experiments and the mechanical property evaluation, we cut the Inion plate into a piece containing three screw holes, as depicted in Fig. S1 in the Supplementary Information. The outer two holes were used to suture and fix the plate on a bone for the in vivo experiments or employed as sites for clamping for the mechanical property evaluation. The coatings were made in the same way as described above, only around the screw hole in the middle.

2.4. Characterizations of Az-CH

To confirm the formation of Az-CH with the method employed in this work [28], we performed a spectrophotometric analysis. For this, the solutions of Az-CH, chitosan and 4-azidobenzoic acid were each prepared: 20 mg Az-CH or 20 mg chitosan was completely dissolved in 1 ml of 2% acetic acid solution, and 4-azidobenzoic acid (4 mg) was dissolved in 1 ml methanol. We obtained the UV spectra of the resulting three solutions at wavelengths from 250 nm to 400 nm (UV-1800, Shimadzu, Japan). To further confirm the formation of Az-CH, we also performed a Fourier transform infrared (FTIR) analysis. For this, Az-CH, chitosan and 4-azidobenzoic acid were each milled with potassium bromide (KBr) to produce a fine power, which was then compressed into a thin pellet.

2.5. Plate characterizations

The surface of the coating on the plate samples (i.e., AL–Az-CH_P) was examined and compared with that of the non-coated, intact surface, using a scanning electron microscope (SEM; 7501F, Jeol, Japan). Prior to imaging, the sample was placed on the SEM specimen mount and sputter coated with platinum for 5 min (208HR, Cressington Scientific, England). We also conducted FTIR analysis (JASCO 6100, Japan). To do this, each plate sample was milled with potassium bromide (KBr) to produce a fine powder and then compressed into a thin pellet for analyses. The intact Az-CH and alendronate were also analyzed for comparison.