Bone Regeneration by Using Drug Delivery System Technology and Apatite Intelligent Materials

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Introduction

Artificial bone cements containing various pharmacological active materials were developed based on hydroxyapatite cement and apatite/collagen composite cement, and the device exhibited controlled drug release in response to plasma calcium levels for bone regenerative therapy.

Materials and Methods

Experimental Materials

The apatite cement bulk powder consisting of an equimolar mixture of tetracalcium phosphate (TECP, $Ca_4(PO_4)_2O$), dicalcium phosphate dihydrate (DCPD, $CaHPO_4\ 2H_2O$) was prepared by grinding at 90 strokes per second for 10 min in an agate vibration mixer. The 0.5% b-estradiol (ES)-loaded apatite cement obtained from the mixed powder of TECP and DCPD and ES after storing at 37°C and 100% relative humidity.

Self-setting apatite/collagen cement system

The apatite/collagen composite cement bulk powder was ground apatite cement bulk powder with 20% type I bovine collagen for 0 and 10 min. Apatite/collagen composite cement bulk powder was mixed with phosphoric acid for 1 min to form a paste, then 0.5% VK2 were mixed with this paste, and stored at 37°C and 100% relative humidity.

In-vitro drug release test.

In-vitro drug release test was measured in simulated body fluid (SBF) comprised of various kinds of inorganic ions and bovine albumin, (pH 7.25) was used as dissolution media. During the release tests, the entire dissolution medium was replaced with fresh buffer at various intervals. All drug concentrations were determined by HPLC.

Animal experiments

(1) Animal experiments for in-vivo ES release were performed as follows: Vitamin D-deficient female Wistar rats were performed ovariectomy. Bone mineral density (BMD) of lumbar vertebrae of diseased and healthy rats was measured by bone mineral densitometry. Blood samples were collected at suitable time intervals from the tail artery. Plasma ES was measured by a radioimmunoassay using an oestradiol radioimmunoassay kit.

(2) For in-vivo apatite/collagen cement study, two of the cements were implanted in the left and right sides of the intramuscular tissue on the backs of female Wistar rats under anesthesia. After 72 days the implanted apatite/collagen cements were excised with the surrounding soft tissues.

In vivo cement mineral mass (CMM) measurement.

The two-dimensional soft-X-rays transmission image in the cement-implanted rats were measured by using a bone mineral densitometry under anesthesia at 0, 6, 23, 62 and 72 days.

Histology

Transverse sections were cut from the central part of the cement samples, and the sections were stained by Masson's trichrome stain method.

Results and Discussion

Plasma calcium level responsive estradiol release form apatite bone cement[1),2)]

The effects of plasma calcium levels on estradiol release from a self-setting apatite bone cement containing estradiol and on the BMD of ovariectomized rats were investigated. Apatite cement was consisting of TECP, DCPD and ES. The *in-vitro* release profiles from the cements in simulated body fluid containing 0, 5 and 10 mg/100mL calcium indicated that ES release rate decreased with increasing calcium concentration in the dissolution media. This *In-Vitro* result accounts for the calcium level responsive nature of drug release from the apatite cement matrix. On the other hand, after subcutaneous implantation of the cement, *in-vivo* ES release in diseased rats (ovariectomized rats on a low calcium diet) was significantly higher than that in normal rats. The diseased rats maintained a low calcium level during drug release. The *in-vivo* ES release in osteporosis rats supported the hypothesis that the drug release behavior of the apatitic cement drug delivery system was responsive to plasma calcium levels.

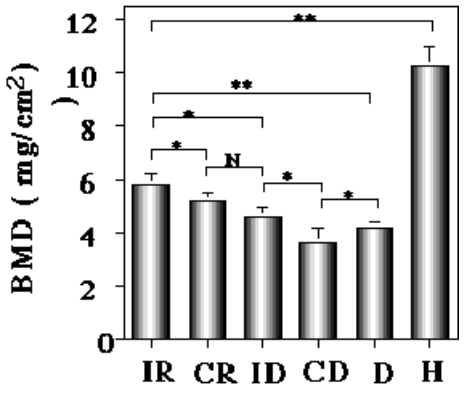

Fig. 1. The bone mineral density (BMD) of the diseased rats after estradiol release for 22 days. Each bar represents the mean and standard deviation. Significantly different by ANOVA: * $p<0.05$, ** $p<0.01$ (n=4). CD, Control of diseased rat, ID, Cement Implanted diseased rat, CR, Control of recovery rat, IR, Implanted recovery rat, H, Control of healthy rat, D is the group CD before cement implantation.

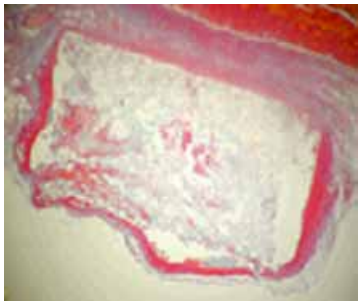

Fig. 2. Microphotographs of cross-sections of apatite/collagen cements containing VK2 after implantation for 72 days. (The cement containing 20% collagen with grinding for 20 min)

As shown in Figure 1, the BMD of the recovery model rat was greater after the experiment than before. The results suggested that the severity of osteoporosis in this animal model is decreased by implantation of the ES-loaded apatite cement.

Menatetrenone release from Apatite/collage composite bone cement[3)]

Apatite cement and collagen were combined by a mechanochemical method to create a new self-setting apatite/collagen composite cement, and menatetrenone (VK2) was loaded into a drug delivery system to test biocompatibility in rats. Powder X-ray diffraction analysis (XRD), scanning electron microscopy (SEM) and electron probe microanalyzer (EPMA) were performed to characterize the physicochemical properties of apatite/collagen composite cements. The XRD results suggested that ground apatite/collagen cement was completely transformed into bone-like hydroxyapatite, but that without grinding was incomplete. The SEM and EPMA results suggested that ground apatite/collagen cement was a homogeneously dispersed of nanoapatite crystals in collagen metrics, similar to natural bone. In contrast, the cement without grinding was heterogeneously distributed. In order to evaluate in-vivo cement density (CMM), microradiograms were measured for 72 days after implanting apatite/collagen composite cements in intramuscular tissue on the backs of rats, and cross sections of the cements and surrounding soft tissues were observed by microscope. The CMM results of the apatite/collagen composite cements suggested that the biodegradation rate was dependent on the cement quality and nano-geometrical structure. The CMM result of VK2 loaded apatite/collagen cements suggested that the biodegradation rates of the cements were significantly dependent on their formulation. The CMM of ground apatite/collagen cement increased until 7 days and then decreased, and bone-like cells penetrated deeply in the center. The microphotograph and CMM results of apatite/collagen without grinding indicated that a lot of bone-like cells penetrated into the cement and the cement shape was totally deformed as shown in Figure 2.

On the other hand, in order to develop artificial bone system with drug delivery ability as a cell scaffold the apatite cement and collagen are combined to create apatite/collagen composite cement containing VK2. The microphotograph result of apatite/collagen indicated that a lot of bone-like cells penetrated into the cement.

References

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