Properties of novel bone hemostat prepared using sugar-modified hydroxyapatite, phosphoryl oligosaccharides of calcium and thermoplastic resin

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Abstract. A novel hemostatic agent was prepared using phosphoryl oligosaccharides of calcium (POs-Ca), hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂; HAp) obtained by the hydrolysis of POs-Ca or sugar-containing HAp (s-HAp; 60.3 mass% calcium-deficient HAp and 39.5 mass% organic materials, Ca/P ratio = 1.56) and thermoplastic resin (the mixture of random copolymer of ethylene oxide/propylene oxide (EPO) and polyethylene oxide (EO); EPO : EO : water = 25 : 15 : 60 (mass ratio); 25EPO-15EO). The gel formed by mixing 25EPO-15EO with water (25EPO-15EO/water mass ratio: 0.20) was flash frozen at -80°C, freeze-dried at -50°C for 15 h and then ground using mixer. The consistency conditions of hemostats mixed with POs-Ca or s-HAp were optimized for the practical uses. The mean stanching times of hemostats were: s-HAp/25EPO-15EO (8.2 h; s-HAp/25EPO-15EO = 0.20) > 25EPO-15EO (5.3 h) > POs-Ca/25EPO-15EO (4.7 h; POs-Ca/25EPO-15EO = 0.20). The gentamicin, a typical antibiotic agent, loaded s-HAp/25EPO-15EO composite hemostat showed the steady state releasing in phosphate buffered saline till 10 h immersion at 37.0°C.

1. Introduction
When bones are cut open during a surgery, a large amount of bleeding occurs from the bone’s vessel. Most useful hemostat used currently is a bone wax, whose major component is beewax. Unfortunately, the hemostats practically used are not fully biocompatible and sometimes cause infection, because it is derived from animals [1]. The random copolymer of ethylene oxide and propylene oxide (EPO), which has excellent flexibility/adhesion performances, is one of the promising materials as a novel hemostat. Furthermore, the properties of EPO seem to be possible to be controlled by the addition of polyethylene oxide (EO), because it has the characteristics of water-soluble, nontoxic, and anti-immune/suppresses protein/cell adhesions [2].

The phosphoryl oligosaccharides of calcium (POs-Ca), which is available for edulcorant, is extracted from the potato starch [3,4]. The advantages of POs-Ca may be that it has groups of Ca²⁺ and PO₄³⁻, which is the potential ability of re-calcification, and that it is easily dissolved in water. The present authors [5] have prepared a sugar-containing hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂; s-HAp) from POs-Ca solution through the hydrothermal treatment. Thus the POs-Ca, as well as its hydrolyzed materials (s-HAp), may be available as biomaterials.

As shown above, the ideas of designing novel hemostatic agents are based upon the flexibility, adhesion, biocompatibility and infection prevention, as well as the

![Figure 1. Molecular structures of (a) POs-Ca and (b) EPO](image)
stanching effect. Taking these performances into account, the authors paid attention to the development of a novel hemostat through the combination of POs-Ca, s-HAp and thermoplastic resin. The present paper describes the optimized preparation conditions of novel materials for stanching the blood, using POs-Ca, s-HAp and thermoplastic resin of EPO and EO.

2. Experimental procedures

The starting s-HAp was obtained by hydrothermally treating 10 mass% POs-Ca solution at 100ºC for 5 h. The thermoplastic resin was prepared by mixing EPO and EO with the mass ratio being EPO : EO : water = 25 : 15 : 60 (abbreviated as 25EPO-15EO); the mixing ratio was determined on the basis of preliminary investigation. The resulting 25EPO-15EO was mixed with de-ionized water to form gel (mass ratio of 25EPO-15EO to water: 1.0), frozen at -80ºC and dried at -50ºC for 9 to 20 h. The freezedried materials were ground and was sieved into 1.0 ~ 2.0 mm. The operation performance of the hemostat was examined by the consistency, i.e., the measurement of the spread size, after it was set between two plates and loaded with a mass of 500 g. The stanching of blood was evaluated by checking the time that the paper on the hemostat started to be wet by the simulated blood coming out the top of a porous HAp cube (porosity of 70% and mean pore size of 200 µm), due to the capillary force. The crystalline phases were checked using an X-ray diffractometer (XRD; RINT2100V/P, Rigaku, Tokyo, Japan) with CuKα radiation (40 kV, 40 mA). The particle morphology and chemical composition were checked using a field-emission scanning electron microscope (FE-SEM; S-4500, Hitachi, Tokyo), an energy-dispersive X-ray microanalyzer (EDX) and a scanning transmission electron microscope (STEM). The cylindrically-shaped hemostats with the diameter of 4.1 mm and height of 13 mm were implanted for 17 weeks in the femur and tibia of Japanese white rabbits. The release amount of gentamicin from the hemostat in the phosphate buffered saline (PBS(-)) at 37.0ºC was evaluated by o-phthalaldehyde method [6], using a spectrophotometer (BioSpec-1600, Shimadzu, Kyoto) at the wavelength of 332.0 nm.

3. Results and discussion

3-1 Properties of starting materials

The commercial POs-Ca contains 5.0 mass% Ca and 3.7 mass% P, and Ca/P molar ratio is estimated to be 1.02 [5]. Such POs-Ca powder was composed of spherical particles with diameters of approximately 30 µm (FE-SEM). The properties of other starting materials have been reported elsewhere; s-HAp contains 60.3 mass% calcium-deficient HAp (XRD; Ca/P molar ratio: 1.56 (EDX)) and 39.5 mass% organic materials (thermogravimetry) [7]. The resulting s-HAp powder contained agglomerates of particles with sizes of below 50 nm (STEM). The consistency of 25EPO-15EO was 17.0 mm, which was a little lower than that of commercial hemostat (18.2 mm). The implantation of 25EPO-15EO hemostat in a tibia of the Japanese white rabbits for 17 weeks is shown in Fig. 2. The noted bone regeneration, as well as the excellent biocompatibility and no toxicity, was confirmed from the implanted tibia of Japanese white rabbits.

3.2 Fabrication of 25EPO-15EO hemostats with POs-Ca and s-HAp addition

In order to enhance the bioabsorbability, as well as biocompatibility, POs-Ca and s-HAp were incorporated into EPO hemostat. The effect of POs-Ca and s-HAp addition on the consistency of 25EPO-15EO is shown in Fig. 3. Note that the ratio of POs-Ca/25EPO-15EO and s-HAp/25EPO-15E was fixed at 0.20. The consistency of 25EPO-15EO hemostat increased from 14.2 to 19.3 mm with increasing mass ratio of water to 25EPO-15EO from 1 to 4 (Fig. 3(a)). The consistency behavior of
POs-Ca/25EPO-15EO hemostat was divided into two, according to the mass ratio of water to POs-Ca/25EPO-15EO, i.e., 14.9 to 20.1 mm (mass ratio of water to POs-Ca/25EPO-15EO: 0.8 to 2.0) and 20.1 to 25.5 mm (2.2 to 4.6) (Fig. 3(b)). The consistency of s-Hap/25EPO-15EO hemostat increased from 14.4 to 18.7 mm with increasing mass ratio of water to 25EPO-15EO from 1.0 to 3.6 (Fig. 3(c)). The addition of POs-Ca notably contributes to enhancing the consistency of 25EPO-15EO composite at the initial stage. This phenomenon is explained by assuming that the high dissolution of POs-Ca in water may enhance the fluidability of EPO, thereby showing the higher consistency. On the other hand, the retarded increase in consistency of s-Hap/25EPO-15EO hemostat is interpreted as the reduced fluidability, due to the presence of Hap being hardly soluble in water.

The stanching times of 25EPO-15EO, POs-Ca/25EPO-15EO and s-Hap/25EPO-15EO hemostats are shown in Fig. 4. The stanching times of the composites were: 8.17 ± 0.76 h (s-Hap/25EPO-15EO) ≥ 5.33 ± 0.29 h (25EPO-15EO) > 4.67 ± 0.29 h (POs-Ca/25EPO-15EO). The time for surgery operation is estimated to be 2 to 3 h, indicating that the present time for stanching the blood may be satisfactory for the utilization as a hemostat. Since the POs-Ca and EPO are water soluble, the stanching performances of EPO and POs-Ca/25EPO-15EO composite hemostats seem to be reduced by the penetration of simulated blood in the space formed with the dissolution of POs-Ca and 25EPO-15EO.

3.3 Drug releasing behavior of 25EPO-15EO hemostats with POs-Ca and s-Hap addition
We incorporated gentamicin, a typical antibiotic agent, into these hemostats, and examined the releasing behavior.
behavior of it in the PBS(-). The releasing behavior of gentamicin from the hemostats in the PBS(-) is shown in Fig. 5. The released amounts of gentamicin after 10 h immersion from the hemostats were: POs-Ca/25EPO-15EO (482.5 g cm\(^{-3}\)) > 25EPO-15EO (327.0 g cm\(^{-3}\)) > s-HAp/25EPO-15EO (178.2 g cm\(^{-3}\)). Moreover, the steady state of gentamicin releasing was found in the case of s-HAp/25EPO-15EO hemostat. The relationship between amount of gentamicin releasing (\(Q_t\) at time = \(t\)) and time (\(t\)) is expressed as \(Q_t = 17.7t + 13.3\). The steady state of gentamicin releasing from the 25EPO-15EO in PBS(-) solution may thus be controlled by HAp within s-HAp particles, probably due to the interaction of gentamicin with Ca\(^{2+}\)/PO\(^{4-}\)[8] in HAp.

4. Conclusion

Novel hemostatic agent was prepared mixing biocompatible POs-Ca, s-HAp and thermoplastic resin (25EPO-15EO). The results obtained were summarized as follows:

(1) The consistency conditions for the practical uses as hemostats were examined with changing mass ratio of water to hemostat, i.e., 14.2 to 19.3 mm (25EP-15EO), 14.9 to 25.5 mm (POs-Ca/25EPO-15EO) and 14.4 to 18.7 mm (s-HAp/25EPO-15EO). The mean stanching times of hemostats were: s-HAp/25EPO-15EO (8.2 h; s-HAp/25EPO-15EO = 0.20) > 25EPO-15EO (5.3 h) > POs-Ca/25EPO-15EO (4.7 h; POs-Ca/25EPO-15EO = 0.20).

(2) The releasing behavior of gentamicin, a typical antibiotic, from the hemostats in the PBS(-) was examined. The amounts of gentamicin from the hemostats were: POs-Ca/25EPO-15EO (482.5 g cm\(^{-3}\)) > 25EPO-15EO (327.0 g cm\(^{-3}\)) > s-HAp/25EPO-15EO (178.2 g cm\(^{-3}\)). The steady state of gentamicin releasing was found in the case of s-HAp/25EPO-15EO hemostat.

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