Original Article

Development and Evaluation of a Novel Bone Hemostatic Agent That Does Not Inhibit Bone Repair

Takahide Sunakawa[1)*] Takao Kaneko[1)] Tomohiro Umeda[2)]
Keisuke Ito[3)] Hiroyasu Ikegami[1)] and Yoshiro Musha[1)]

[1)]Department of Orthopaedic Surgery (Ohashi), School of Medicine, Faculty of Medicine, Toho University
[2)]Nara Women's University Social Cooperation Center
[3)]Spine and Spinal Cord Center, Toho University Ohashi Medical Center

ABSTRACT

Background: Standard bone wax (wax) is often used during operations on bones and is widely utilized in orthopedics. However, it has suboptimal biocompatibility and inhibits bone formation in osseous tissue in the region of application. We attempted to address these limitations by developing a new material.

Methods: We produced a random copolymer of ethylene oxide (EO) and propylene oxide (PO) to produce a new material (EPO gel), which was combined with hydroxyapatite (HAp) obtained from hydrothermally processed phosphoryl oligosaccharides of calcium (POs-Ca) to produce POs-Ca・HAp-EPO. Then, the adhesive strength and duration of hemostasis *in vitro*, bleeding time (a measure of hemostatic function), biocompatibility, and bone repair *in vivo* of EPO and POs-Ca・HAp-EPO were compared with those of wax.

Results: Adhesive strength was 54.0±1.0 N for EPO and 65.0±1.21 N for POs-Ca・HAp-EPO ($p<0.001$). Although the adhesive strengths of EPO was lower than that of wax (68.7±3.56 N), the strength of POs-Ca・HAp-EPO and wax did not significantly differ. Duration of hemostasis after application was 4.5±0.25 h for EPO and 6.0±0.23 h for POs-Ca・HAp-EPO ($p<0.001$). Because the results of the animal experiments showed that hemostasis was achieved with both EPO and POs-Ca・HAp-EPO during the intraoperative observation period (≥30 min), the hemostatic function of these agents was deemed sufficient. In histologic observation, both EPO and POs-Ca・HAp-EPO exhibited favorable biocompatibility, and neither material inhibited bone repair.

Conclusions: When hemostatic function and ease of handling based on adhesiveness to bone were considered, the characteristics of POs-Ca・HAp-EPO were superior to those of EPO.

J Med Soc Toho 60 (3): 159–167, 2013

KEYWORDS: bone hemostatic agent, bone repair, phosphoryl oligosaccharides of calcium, ethylene oxide, propylene oxide

Standard bone hemostatic wax (wax) is used almost exclusively to control bleeding from bone tissue during surgery, because ligation of blood vessels and coagulation are difficult to achieve by other means.[1–3)] The principal ingredient in wax is beeswax, which is obtained by melting and filtering honeycombs. Wax has a clay-like consistency and

1, 3) 2–17–6 Ohashi, Meguro, Tokyo 153–8515
2) Kitauoya Nishimachi, Nara 630–8506
*Corresponding Author: tel: 03(3468)1251
e-mail: takahide.sunakawa@gmail.com

Received Oct. 3, 2012: Accepted Mar. 21, 2013
Journal of the Medical Society of Toho University
60 (3), May 1, 2013. ISSN 0040–8670, CODEN: TOIZAG

is applied to a bleeding surface after being sufficiently softened for manual molding. Wax is widely used because it is easy to handle and has good hemostatic effects, making it essential for orthopedic surgeries. However, wax is not hydrophilic and therefore does not dissolve easily and has poor biocompatibility. Thus, it can inhibit callus formation and synostosis in treated bone tissue.[4] In addition, because it is derived from an animal source, immunoreactions and infections are potential problems. A previous study found that macrophages, foreign body giant cells, polynuclear leukocytes, and lymphocytes, among other cell types, accumulate at the site of wax application, resulting in inflammation and/or edema. Foreign-body granulomas may also form at the site, via accumulation of granulation-tissue cells and fibroconnective tissue.[4] The present study aimed to address these limitations by developing a more advanced bone hemostatic agent that would enhance rather than inhibit osteogenesis, including callus formation and synostosis, and would not cause inflammatory reactions.

Polyethylene glycol (PEG) is a high-molecular-weight compound comprising polymerized ethylene oxide (EO) monomers. PEG is highly biocompatible and nontoxic, resulting in its widespread use.[5–7] Interactions between PEG and hydrophobic molecules may produce nonionic surfactants, which are used as emulsifying agents in cosmetics and have been shown to be safe.[8, 9] When used as an emulsifying agent, these surfactants have low viscosity, which sometimes results in the need to increase viscosity. The Pluronic® (BASF SE, Ludwigshafen, Germany) brand of surfactants includes agents that are manufactured by block copolymerization of propylene oxide (PO) to PEG from EO and are currently marketed for their role in increasing viscosity. Some Pluronic® surfactants are used to enhance drug solubility and decrease drug elimination. Pluronic® surfactants are also reported to have roles in enhancing the effects against cancer cells of some anticancer drugs.[10–12] Block copolymerization is the regular polymerization of monomers (*e.g.*, AABBAABB), whereas random copolymerization involves irregular polymerization (*e.g.*, AABABBBABAA). Random copolymerization allows for easy viscosity adjustment, providing the necessary handling requirements for a bone hemostatic material. Thus, we prepared a random copolymer of EO and PO (EPO) gel. Furthermore, hydroxyapatite (HAp), another highly biocompatible material,[13, 14] was added with the expectation of improving osteoconduction of EPO gel. The HAp that was used in this study was a highly absorbent material obtained from hydrothermally processed phosphoryl oligosaccharides of calcium (POs-Ca).[15] These 2 types of novel bone hemostatic agents were compared both *in vitro* and *in vivo* with a conventional bone hemostatic agent and analyzed with respect to adjustment methods, operability, hemostatic function, biocompatibility, and bone repair.

Methods

1. Synthesis of EPO gel and its chemical changes over time

Granulated EPO was provided as a starting material by Meisei Chemical Works, Ltd. (Kyoto, Japan) and was mixed with purified water and heated to increase its concentration. The resultant material was frozen at −80℃ and freeze-dried at −50℃ for 15 h. The resulting product was then pulverized using a mixer to obtain powdered EPO. The prepared powdered EPO was mixed with enough water to match the consistency of wax. The resultant EPO gel (0.1 g) was immersed in 50 cm^3 of simulated body fluid (SBF),[16] which has a composition very similar to that of plasma, and pH was serially measured for 10 h in a temperature-controlled incubator maintained at 37℃.

2. Preparation of HAp-added EPO and assessment of adhesive strength and duration of hemostasis of the novel hemostatic agents

A 10% mass solution of POs-Ca (Oji Cornstarch, Co., Ltd., Tokyo, Japan) was used as a starting ingredient for hydrothermal synthesis. The POs-Ca solution was placed in a reactor and heated at 100℃ for 5 h.[15] The precipitate that was obtained after being allowed to stand was vacuumed, filtered, and rinsed with distilled water before being dried to obtain HAp (POs-Ca・HAp) powder. POs-Ca・HAp-supplemented EPO complex gel (POs-Ca・HAp-EPO) was prepared at a ratio of 0.2 POs-Ca・HAp per EPO. The following measurements were performed to compare gelatinous EPO alone, POs-Ca・HAp-EPO, and Nestop® (Alfresa Pharma Corp., Osaka, Japan) wax; 5 samples of each were investigated in each test.

1) Adhesive strength

Commercially available HAp porous blocks (width, 17 mm; height, 10 mm; depth, 21 mm; porosity, 70%; mean pore diameter, 200 μm) (HOYA Corp., Tokyo, Japan) were used. The samples (0.1 g) were placed on the HAp porous blocks and compressed with a load of 78.4 N・min^{-1} by an autograph strength tester (AGS-J; Shimadzu Corp., Kyoto, Japan). The test materials were then pulled at a crosshead speed of 10 mm・min^{-1} from the block (Fig. 1). The adhe-

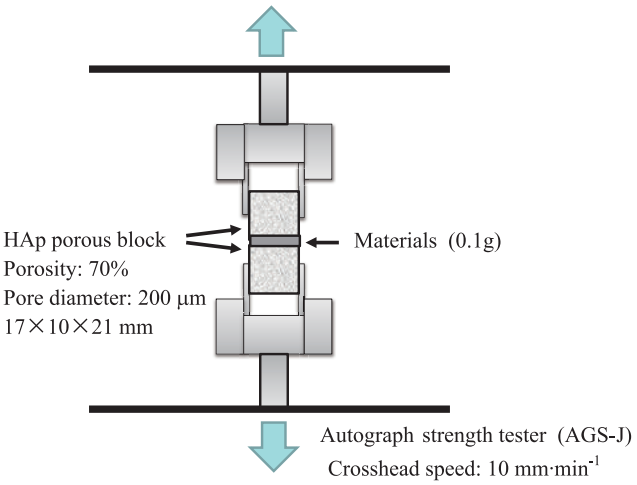


Fig. 1 Measurement of adhesive strength

The samples were placed on hydroxyapatite (HAp) porous blocks and compressed with a load of 78.4 N·min^{-1} by an autograph strength tester. The test materials were then pulled at a crosshead speed of 10 mm·min^{-1} from the block. Adhesive strength was defined as the maximum load recorded on the stress-strain curve.

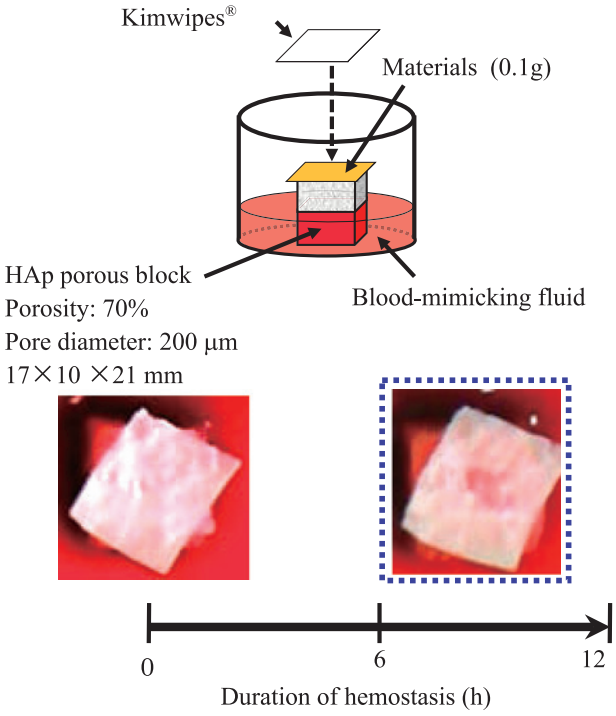


Fig. 2 Measurement of hemostasis duration

Each test material was applied to the upper surface of a hydroxyapatite (HAp) porous block. The block was placed in a dish containing a blood-mimicking fluid and was allowed to absorb this solution. Duration of hemostasis was defined as the time between the placement of a Kimwipes® on the test material and the detection of a change in its color.

sive strength between the test materials and the HAp block was defined as the maximum load recorded on the stress-strain curve.

2) Duration of hemostasis

Each test material (0.1 g) was applied to the upper surface of a HAp porous block (described above). The HAp porous block was placed in a dish containing a blood-mimicking fluid, *i.e.*, Blood-Colored Concentration (Leardal Medical Japan Co., LTD., Tokyo, Japan), and was allowed to absorb this solution. Duration of hemostasis was defined as the time between the placement of a tissue (Kimwipes®; Nippon Paper Cresia Co., Ltd., Tokyo, Japan) on the test material and the detection of a change in the color of the tissue (Fig. 2).

Statistical analysis was performed using the Student *t*-test, with $p<0.05$ regarded as significant.

3. *In vivo* assessments (animal study)

Male Japanese white rabbits, under general anesthesia, were used for this study. In each of 10 rabbits, a circular hole (diameter, 4 mm) was drilled into the cortical bone on the medial aspect of the cranial ends of both tibiae (the circular hole group). The bone defects were filled with (1) EPO, (2) POs-Ca・HAp-EPO, or (3) wax, which was formed into a cylinder with a diameter of 4 mm and a height of 7 mm (Fig. 3a). In another group of 8 rabbits, the cortical bone was scraped with a 3-mm diameter steel bar to expose a 15 mm×3 mm length of cancellous bone, parallel to the long axis of the bone (the longitudinal groove group). The grooves were filled with 1 of the 3 test materials, using an identical volume of material (135 mm^3) (Fig. 3b). In each group (4), control (sham) holes were similarly prepared, but not filled. Fillings (1) and (2) or (3) and (4) were used in the right and left tibias, respectively.

1) Hemostatic function

To assess hemostatic function, bleeding time was measured at 5 control holes in 5 rabbits with circular holes, where hemostasis was induced by fingertip occlusion of the hole. In addition, in 10 rabbits with circular holes, a 23-Ga needle was inserted into the auricular artery and immediately removed to measure the bleeding time needed to achieve spontaneous hemostasis without applying compression. In all cases, the external appearance of the holes or grooves were observed for at least 30 min each for 27 defects filled with (1) to (3), and the surgical wounds were closed after absence of bleeding had been confirmed.

2) Biocompatibility and bone repair

Four and 8 weeks after surgery, 4 rabbits—2 receiving

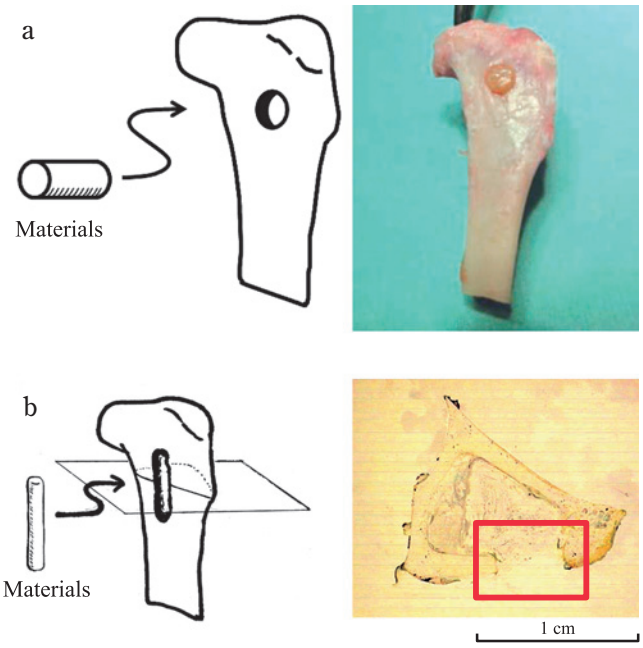
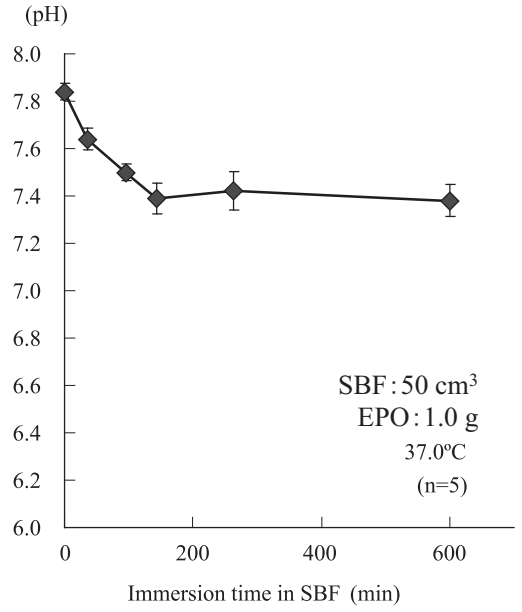


Fig. 3 Animal study using Japanese white rabbits
(a) Circular hole group, for observation of the cortical bone surface of tibia.
(b) Longitudinal groove group, for histologic assessment of the axial section of a longitudinal groove on the tibia.
The bone defects were filled with (1) EPO, (2) POs-Ca・HAp-EPO, (3) wax, or (4) control (sham).
EPO gel: random copolymer of ethylene oxide (EO) and propylene oxide (PO), POs-Ca: phosphoryl oligosaccharides of calcium, HAp: hydroxyapatite



Time (min)	0	36	96	144	264	600
pH	7.84 ±0.035	7.64 ±0.045	7.50 ±0.036	7.39 ±0.064	7.42 ±0.081	7.38 ±0.066

Fig. 4 Changes in pH of EPO gel specimens with immersion in the SBF
Powdered EPO was easily converted into a gel by adding only water. After EPO gel was adjusted to the consistency of wax and immersed in SBF, no changes in chemical composition over time were seen. EPO gel remained neutral until it was completely dissolved.
EPO gel: random copolymer of ethylene oxide (EO) and propylene oxide (PO), SBF: simulated body fluid

treatments (1) and (2), and 2 receiving treatments (3) and (4)—were killed to assess filler absorption and bone repair. Additional observations were also performed in 2 of the rabbits with circular holes, 4 months after treatment. For the histologic assessments, axial section samples were prepared from the 8 rabbits in the longitudinal groove group and stained with Villanueva bone stain for observation (Fig. 3b).

Rabbits were treated according to the guidelines for the protection of experimental animals of Toho University School of Medicine. The protocol was approved by the ethics board of Toho University (A12-54-108).

Results

1. Chemical changes in EPO gel over time

Powdered EPO was easily converted into a gel by adding only water. After EPO gel was adjusted to the consistency of wax and immersed in SBF, no changes in the chemical composition were observed over time. The pH of EPO gel remained neutral until the EPO was completely dissolved (Fig. 4).

2. Assessment of adhesive strength and duration of hemostasis

1) Adhesive strength

Fig. 5 shows the results of the comparison of adhesive strength between EPO (Fig. 5a), POs-Ca・HAp-EPO (Fig. 5b), and wax (Fig. 5c). Adhesive strength was 54.0±1.0 N for EPO and 65.0±1.21 N for POs-Ca・HAp-EPO ($p<0.001$). Although the adhesive strength of EPO was lower than that of wax (68.7±3.56 N), the strength of POs-Ca・HAp-EPO and wax did not significantly differ.

2) Duration of hemostasis

Fig. 6 shows that duration of hemostasis after application of EPO (Fig. 6a) was 4.5±0.25 h, whereas the duration after application of POs-Ca・HAp-EPO (Fig. 6b) was 6.0±0.23 h ($p<0.001$). The insolubility of wax resulted in no evi-

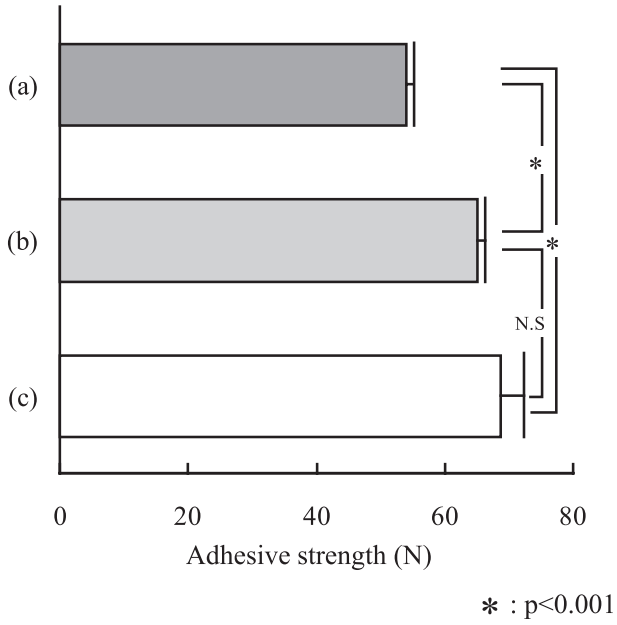


Fig. 5 Results of adhesive strength test
(a) EPO, (b) POs-Ca·HAp-EPO, (c) wax. Adhesive strength was 54.0±1.0 N for EPO and 65.0±1.21 N for POs-Ca·HAp-EPO, indicating higher strength for the latter. Although the adhesive strength of EPO was lower than that of wax (68.7±3.56 N), the strength of POs-Ca·HAp-EPO and wax did not significantly differ.
EPO gel: random copolymer of ethylene oxide (EO) and propylene oxide (PO), POs-Ca: phosphoryl oligosaccharides of calcium, HAp: hydroxyapatite

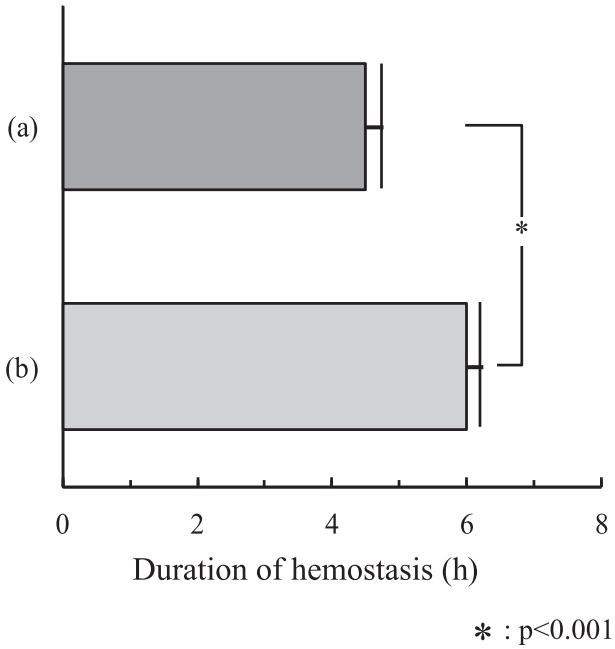


Fig. 6 Duration of hemostasis
(a) EPO, (b) POs-Ca·HAp-EPO. Duration of hemostasis after application of EPO was 4.5 h, while duration after application of POs-Ca・HAp-EPO was 6.0 h ($p<0.001$).
EPO gel: random copolymer of ethylene oxide (EO) and propylene oxide (PO), POs-Ca: phosphoryl oligosaccharides of calcium, HAp: hydroxyapatite

dent changes 12 h after application.

3. *In vivo* assessments (animal study)

1) Hemostatic function

The 5 control (sham) circular holes demonstrated a bleeding time ranging from 9 min 35 sec to 19 min (mean ± SD, 14 min 3 sec ± 3 min 29 sec). In the 10 rabbits with punctured auricular arteries, bleeding time ranged from 1 min 45 sec to 2 min 45 sec (mean ± SD, 2 min 16 sec ± 18 sec). In bone defects (27 defects in 18 rabbits) filled with EPO, POs-Ca・HAp-EPO, or wax, no bleeding was observed during the 30-min observation period.

2) Biocompatibility, osteoconduction, and bone repair

Fig. 7 shows the cortical bone surfaces in the circular hole group 8 weeks after EPO (Fig. 7a), POs-Ca・HAp-EPO (Fig. 7b), or wax (Fig. 7c) was used to fill the bone defects. In all the EPO, POs-Ca・HAp-EPO, and control (sham) cases (Fig. 7d), the defects were completely repaired. However, wax remained unchanged in all the defects after its application, and the defects in cortical bones retained their original shape in all cases.

The histologic findings of axial section samples taken from longitudinal groove defects are shown in Fig. 8. In the EPO (Fig. 8a) and POs-Ca・HAp-EPO groups (Fig. 8b), the grooves were entirely bridged by new bone and almost fully repaired by 8 weeks, similar to the control (sham) cases (Fig. 8d). Four weeks after surgery, EPO and POs-Ca・HAp-EPO were dissolved and completely absorbed. The lack of evidence of inflammatory reactions around the bone defects indicated that these materials had good biocompatibility and did not inhibit bone repair. In contrast, the bone surfaces in the wax group (Fig. 8c) remained unchanged, and the defects persisted, even after 4 months.

Discussion

This study revealed that powdered EPO can be easily converted into a gel by adding water, which reduced the time needed to adjust and mass-produce EPO gel. The chemical composition of EPO did not change after it was mixed with water, and the gelatinous EPO remained essentially inert until it was completely dissolved. These features indicate that EPO does not affect the long-term chemical composition of added substances. Thus, it can be

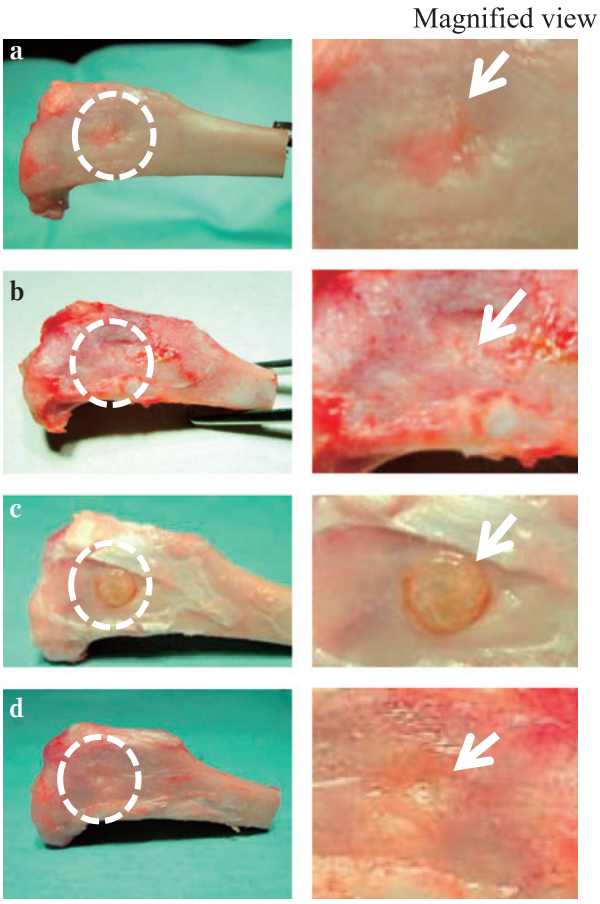


Fig. 7　Appearance of the cortical bone surface at 8 weeks
(a) EPO, (b) POs-Ca･HAp-EPO, (c) wax, (d) control (sham) in the circular fenestration group. In all EPO, POs-Ca･HAp-EPO, and control (sham) cases, the defects were completely repaired. In contrast, the wax remained unchanged in all defects. EPO gel: random copolymer of ethylene oxide (EO) and propylene oxide (PO), POs-Ca: phosphoryl oligosaccharides of calcium, HAp: hydroxyapatite

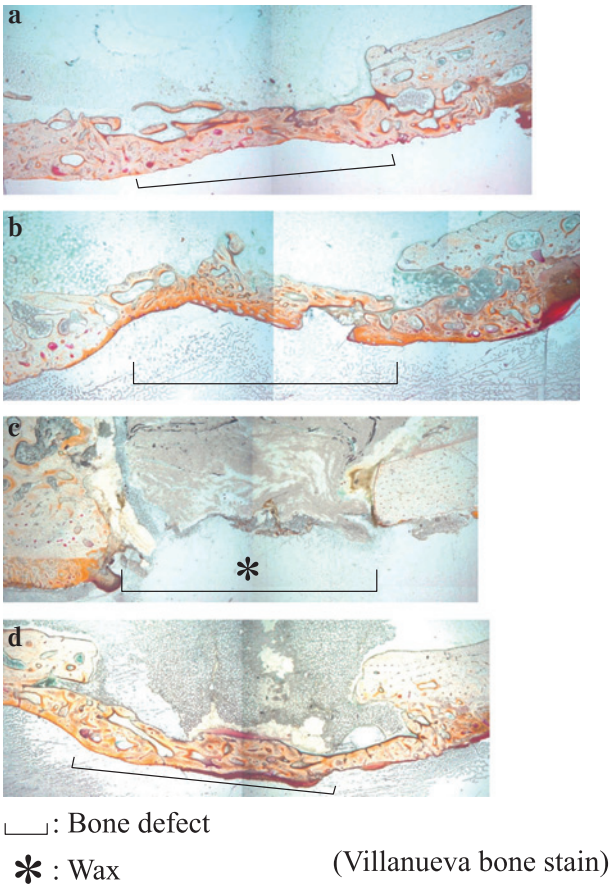


Fig. 8　Histologic findings at 8 weeks (×40)
(a) EPO, (b) POs-Ca･HAp-EPO, (c) wax, (d) control (sham) in the longitudinal groove group. In the EPO and POs-Ca･HAp-EPO cases, the grooves were entirely bridged by new bone and almost fully repaired at 8 weeks, similar to the control (sham) cases. In contrast, the wax remained unchanged (✻).
EPO gel: random copolymer of ethylene oxide (EO) and propylene oxide (PO), POs-Ca: phosphoryl oligosaccharides of calcium, HAp: hydroxyapatite

combined with any material or drug irrespective of polarity and may be used as a drug delivery system.[17, 18] EPO biopolymers can also be combined with various substances to provide additional value in a bone hemostatic agent. The current study was designed to produce an unconventional bone hemostatic agent that could promote hemostasis without inhibiting bone repair. Calcium phosphate was added to EPO to enhance adhesive strength and osteoconduction. However, because solubility is extremely important for osteoconduction,[19–22] low-temperature synthesis was successfully attempted via hydrothermal synthesis, using POs-Ca at 100℃. The HAp, derived from POs-Ca through this method, produced carbonated apatite with Type A sites, which was superior in solubility to stoichiometrically equivalent HAp. Thus, we expected that this novel HAp would enhance osteoconduction and bone repair.

We assessed whether the 2 bone hemostatic agents produced from this novel material were suitable for clinical use. The adhesive strength of POs-Ca・HAp-EPO was stronger than that of EPO and equivalent to that of wax, suggesting that the marked viscosity of the saccharide contained in the POs-Ca contributed to efficient adhesion.[23] Enhanced adhesion to bone provided better hemostasis and easier handling characteristics. Hemostasis after application of POs-Ca・HAp-EPO persisted up to 6 h and was longer than that recorded with EPO, perhaps because the POs-Ca・HAp in the gel physically inhibited infiltra-

tion of blood-mimicking fluid. The characteristics of POs-Ca・HAp-EPO were superior to those of EPO.

The average bleeding time for circular control (sham) holes was 14 min 3 sec, with a maximal bleeding time of 19 min, while rabbits with a punctured auricular artery had an average bleeding time of 2 min 16 sec and a maximal bleeding time of 2 min 45 sec. The intraoperative observation of 30 min or longer exceeded these average and maximal bleeding times and revealed that hemostasis was also achieved in filled circular holes and longitudinal grooves in all cases (novel materials, 18 defects). In the *in vitro* experiments, duration of hemostasis was approximately 4 h 30 min for EPO and approximately 6 h for POs-Ca・HAp-EPO. On the basis of these results, both novel materials were deemed to have adequate hemostatic function.

The capability of these novel materials to repair bone defects was also investigated. When section samples were prepared, we were unable to consistently cut the center of circular bone defects in the circular hole group. Thus, only the appearance of the cortical bone surface was observed. In contrast, when preparing the longitudinal groove group, we were able to cut the treated bone defects into consistent sections by cutting vertically along the longitudinal axis of the tibiae (Fig. 3). For the histologic assessments, axial section samples were prepared from the longitudinal groove group. Observations of the cortical bone surfaces of the circular holes revealed that the defects were completely repaired in the control (sham), EPO, and POs-Ca・HAp-EPO groups. Histologic assessment showed that the cortical bone defects were repaired and replaced with new bone in the control (sham), EPO, and POs-Ca・HAp-EPO groups. In contrast, wax inhibited osteogenesis and repair for a prolonged period. Both EPO and POs-Ca・HAp-EPO had good biocompatibility and did not inhibit bone repair. Therefore, these materials might be useful in overcoming the drawbacks of wax.

Before starting this study, we expected that POs-Ca・HAp-EPO would be superior to EPO in improving bone repair; however, no apparent differences in histologic findings were seen. A possible reason for this unexpected result is the lack of quantitative and statistical analyses for new bone formation in cortical bone defects. Further animal studies are needed to determine whether POs-Ca・HAp-EPO is superior to EPO alone. We evaluated bone repair at only 2 time-points (4 and 8 weeks), which might also be a reason for the negative result. Future studies will require time-course histologic evaluation.

In summary, addition of POs-Ca increased viscosity and adhesive strength to levels equivalent to those of wax. The results of the animal study showed that EPO and POs-Ca・HAp-EPO had good biocompatibility and did not inhibit bone repair. The superior adhesive strength and hemostatic effects of POs-Ca・HAp-EPO indicate that it has better characteristics than EPO as a novel bone hemostatic agent.

References

1) Wellisz T, Armstrong JK, Cambridge J, et al.: Ostene, a new water-soluble bone hemostasis agent. *J Craniofac Surg* **17**: 420–425, 2006
2) Anfinsen OG, Sudmann B, Rait M, et al.: Complications secondary to the use of standard bone wax in seven patients. *J Foot Ankle Surg* **32**: 505–508, 1993
3) Finn MD, Schow SR, Schneiderman ED: Osseous regeneration in the presence of four common hemostatic agents. *J Oral Maxillofac Surg* **50**: 608–612, 1992
4) Harjula A, Järvinen A: Postoperative median sternotomy dehiscence. *Scand J Thorac Cardiovasc Surg* **17**: 277–281, 1983
5) Hume PS, He J, Haskins K, et al.: Strategies to reduce dendritic cell activation through functional biomaterial design. *Biomaterials* **33**: 3615–3625, 2012
6) Navarra CO, Goracci C, Breschi L, et al.: Influence of post type on degree of conversion of a resin-based luting agent. *Am J Dent* **25**: 17–20, 2012
7) Biswas A, Liu Y, Liu T, et al.: Polyethylene glycol-based protein nanocapsules for functional delivery of a differentiation transcription factor. *Biomaterials* **33**: 5459–5467, 2012
8) Mano SS, Kanehira K, Sonezaki S, et al.: Effect of polyethylene glycol modification of TiO_2 nanoparticles on cytotoxicity and gene expressions in human cell lines. *Int J Mol Sci* **13**: 3703–3717, 2012
9) Altintas H, Odemis M, Bilgi S, et al.: Long-term complications of polyethylene glycol injection to the face. *Aesthetic Plast Surg* **36**: 427–430, 2012
10) Hosseinzadeh H, Atyabi F, Dinarvand R, et al.: Chitosan-Pluronic nanoparticles as oral delivery of anticancer gemcitabine: Preparation and *in vitro* study. *Int J Nanomedicine* **7**: 1851–1863, 2012
11) Kabanov AV, Batrakova EV, Alakhov VY: Pluronic block copolymers as novel polymer therapeutics for drug and gene delivery. *J Control Release* **82**: 189–212, 2002
12) Kabanov AV, Alakhov VY: Pluronic block copolymers in drug delivery: From micellar nanocontainers to biological response modifiers. *Crit Rev Ther Drug Carrier Syst* **19**: 1–72, 2002
13) Hama C, Umeda T, Musha Y, et al.: Fabrication of hydroxyapatite, alginate and thermoplastic resin and its evaluation. *Arch Bio-Ceram Res* **9**: 327–330, 2009
14) Hama C, Umeda T, Musha Y, et al.: Preparation of novel hemostatic material containing spherical porous hydroxyapatite/alginate granules. *JCS- Japan* **118**: 446–450, 2010
15) Umeda T, Itatani K, Mochizuki H, et al.: Properties of calcium phosphate powder prepared from phosphoryl oligosaccharides of calcium. *Key Eng Mater* **309-311**: 515–518, 2006
16) Kokubo T, Kushitani H, Sakka S, et al.: Solutions able to reproduce *in vivo* surface-structure changes in bioactive glass-ceramic

A-W. *J Biomed Mater Res* **24**: 721–734, 1990
17）Yoon H, Kim G: A three-dimensional polycaprolactone scaffold combined with a drug delivery system consisting of electrospun nanofibers. *J Pharm Sci* **100**: 424–430, 2011
18）Tabata Y: Nanomaterials of drug delivery systems for tissue regeneration. *Methods Mol Biol* **300**: 81–100, 2005
19）Itatani K, Tsuguwa T, Umeda T, et al.: Preparation of submicrometer-sized porous spherical hydroxyapatite agglometrates by ultrasonic spray pyrolysis technique. *JCS- Japan* **118**: 462–466, 2010
20）Musha Y, Umeda T, Yoshizawa S, et al.: Effects of blood on bone cement made of calcium phosphate: Problems and advantages. *J Biomed Mater Res B Appl Biomater* **92**: 95–101, 2010
21）Umeda T, Itatani K, Endo H, et al.: Effect of blood addition on the biocompatibility of calcium phosphate paste. *J Eur Ceram Soc* **26**: 525–531, 2006
22）Musha Y, Abe M, Umeda T, et al.: Properties of calcium-phosphate paste with hollow spherical β-calcium-orthophosphate agglomerates. *PRB* **20**: 149–154, 2006
23）Nagasawa Y, Nakagawa Y, Kenmochi J, et al.: Microscopic viscosity of aqueous solution of saccharides: A study by ultrafast pump-probe spectroscopy. *Cryobio Cryotech* **49**: 87–95, 2003

骨修復を阻害しない新規骨止血剤の開発と評価

砂川　隆英[1)]*　金子　卓男[1)]　梅田　智広[2)]
伊藤　圭介[3)]　池上　博泰[1)]　武者　芳朗[1)]

[1)]東邦大学医学部整形外科学講座（大橋）
[2)]奈良女子大学社会連携センター
[3)]東邦大学医療センター大橋病院脊椎脊髄センター

要約

背景：骨蝋（wax）は，骨に対する手術時には必要不可欠であり整形外科領域では広く使用されている．しかし生体親和性に乏しく塗り込められた部分の骨組織では仮骨形成や骨癒合を阻害する．新素材の使用によりこの問題点の解決を目指した．

対象および方法：エチレンオキサイド（ethylene oxide：EO）とプロピレンオキサイド（propylene oxide：PO）をランダム共重合させたEPOゲル（random copolymer of EO and PO：EPO gel）と，糖修飾リン酸カルシウム（phosphoryl oligosaccharides of calcium：POs-Ca）を水熱合成して得た水酸アパタイト（hydroxyapatite：HAp）をEPOに複合化したPOs-Ca・HAp-EPOを作成した．EPOおよびPOs-Ca・HAp-EPOの接着強度，止血持続時間と動物実験における止血機能，生体親和性および骨修復能を従来のwaxと比較した．

結果：EPOの接着強度は54.0±1.0 N，POs-Ca・HAp-EPOでは65.0±1.21 Nであり，より高かった（$p<0.001$）．EPOはwaxの強度（68.7±3.56 N）には及ばなかったが，POs-Ca・HAp-EPOはwaxと同等の強度を有し有意差はなかった．止血持続時間はEPOの4.5±0.25時間に比し，POs-Ca・HAp-EPOは6.0±0.23時間と長かった（$p<0.001$）．動物実験の結果から，術中観察時間（30分以上）でEPOとPOs-Ca・HAp-EPOのいずれも止血が得られていたことから，両者とも止血機能は十分と評価できた．EPOおよびPOs-Ca・HAp-EPOの組織学的観察では，生体親和性は良好で，骨伝導，骨修復を阻害しなかった．

結論：接着強度と止血効果も含めると，POs-Ca・HAp-EPOがより有用と評価できた．

東邦医会誌　60(3)：159–167，2013

索引用語：骨止血剤，骨修復，リン酸オリゴ糖カルシウム，エチレンオキサイド，プロピレンオキサイド

1, 3）〒153–8515　東京都目黒区大橋2–17–6
2）〒630–8506　奈良県奈良市北魚屋西町

東邦医学会雑誌　第60巻第3号，2013年5月1日
ISSN 0040–8670，CODEN: TOIZAG