Development of Poloxamer Bone Wax and Evaluation of Hemostatic Performance

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ABSTRACT

Development of Poloxamer Bone Wax and Evaluation of Hemostatic Performance

Bone wax is an essential material for bone hemostasis in orthopedic surgery, thoracic surgery, and neurosurgery, and is generally defined as a substance used to physically control bleeding caused by bone fractures. Especially, when physically pressed on the fractured or cut surface of a bone, it forms a physical barrier on the bone surface to prevent bleeding. Bone wax can be divided into non-absorbable and absorbable products according to their characteristics. Non-absorbable bone wax products are not metabolized or absorbed in the body and remain indefinitely at the application site, which causes many complications. In the case of absorbable bone wax products, poloxamer multi-block copolymer (CAS No. 9003-11-6, synonym; Pluronic, Polyoxypropylene-polyoxyethylene Block Copolymer, PEG-PPG-PEG, PEO-PPO-PEO) was used to overcome the shortcomings of non-absorbable bone wax products in order to achieve the characteristics of ease of topical application, physical barrier formation, biocompatibility, and possibility of absorption and excretion in the body. However, existing absorbable bone wax products still have several limitations that have not been overcome (limited physical properties, poor bone adhesion, poor hemostatic quality, and interference with bone union), so that when applied to the affected area, they dissolve too quickly in blood and body fluids, and the physical barrier cannot be maintained for a certain period, which reduces the hemostatic effect. In this study, we developed a new type of absorbable bone wax (Hans Bone Wax; HBW) using two poloxamer multi-block copolymers with different molecular weight ranges and compared it with existing absorbable bone wax products (OSTENE, NOVOSEAL) in terms of physicochemical properties, efficacy evaluation through animal testing, biological safety, etc., to demonstrate the possibility of a new product that improves the limitations of existing absorbable bone wax products. In vitro, the adhesive strength, yield load, and solubility of each product were compared and evaluated, and in vivo, the hemostasis of the bone amputation site and the absorption/degradation were evaluated through animal experiments. In addition, a biological safety (Cytotoxicity) evaluation according to the ISO 10993 guideline was conducted on the newly developed HBW. In conclusion, HBW, a

new type of absorbable bone wax, was superior in the evaluation of hemostasis of the bone amputation site and biological response through histological analysis. It is thought that this can improve the clinical environment by providing convenient usability, excellent hemostatic performance, and biocompatibility in stopping bleeding at the bone amputation site.

Keywords: Bone defect, Bone bleeding, Bone wax, Absorbable, Poloxamer, Hemostasis

1. Introduction

Bones have rich channels for blood and marrow, and when they are surgically incised or traumatically fractured, bleeding can remain a difficult problem in the area of bone bleeding (especially in highly vascularized bones such as the spine and sternum). Bone wax is an essential material for bone hemostasis in orthopedic surgery, thoracic surgery, and neurological surgery, and is generally defined as a substance used to physically control bleeding due to bone fractures. In particular, when physically compressed on the fractured or cut surface of the bone, it forms a physical barrier on the bone surface to stop bleeding[1-3].

Bone wax can be divided into non-absorbable and absorbable products according to their characteristics. In the case of non-absorbable bone wax products, it was first described by Parker and Horsley in 1892 and consisted of beeswax softened with isopropyl palmitate and paraffin wax. Beeswax is an insoluble substance and is not metabolized or absorbed in the body, so it remains in the application site indefinitely, causing many complications. The most common complications are inhibition of bone formation, infection, and foreign body reaction[3-9].

For absorbable bone wax products, an experimental absorbable bone wax was developed in 1950 by Geary and Frantz by combining Carbowax, poly(ethylene glycol) (PEG), and oxidized cellulose. From 1980 to 2000, numerous bone wax substitute prototypes were reported in the literature, such as fatty acid salts, fibrin/collagen pastes, gelatin pastes, glycolic or lactic acid/glycerol oligomers, partially deacetylated chitin hydrochloride, PEG/microfibrillar collagen pastes, polydioxanone/natural oils, and polyorthoesters[4, 5, 10]. Unfortunately, none of these formulations reached widespread use or the market, suggesting the difficulty of combining the beneficial properties of traditional bone waxes with the advantages of absorbable materials.

In the 2000s, an ideal absorbable bone wax product was proposed as a material that was easy to use and effective like traditional beeswax, completely absorbable, non-inflammatory, and biocompatible, and an absorbable product composed solely of alkylene oxide block copolymers was developed in 2001. Based on this, a commercially available water-soluble alkylene oxide copolymer-based bone wax was developed in 2006, which was reported to be smooth by manual manipulation, adhere well to bleeding bone, and dissolve within 24 to 48 hours at the grafted site, and be absorbed and excreted in

the early stage of bone healing, thus not inhibiting bone formation[4, 5, 10].

The properties required for absorbable bone wax products include ease of topical application, physical barrier formation, biocompatibility, and the possibility of absorption and excretion in the body. Poloxamer multiblock copolymer (CAS No. 9003-11-6, synonym; Pluronic, Polyoxypropylene-polyoxyethylene Block Copolymer, PEG-PPG-PEG, PEO-PPO-PEO) is a material that can satisfy these properties. Poloxamer multiblock copolymers can control the length of structurally repeating hydrophilic or hydrophobic blocks. Accordingly, the sol-gel transition temperature, the temperature range where the gel exists, and the minimum gel formation concentration can be controlled. The commercially available ABA triblock copolymer series of poloxamers provides a pool of more than 50 amphiphilic, water-soluble, and polymorphic materials (A = hydrophilic block (PEG or PEO) and B = hydrophobic block (PPG or PPO)). The physical and chemical properties of poloxamer copolymers can be finely tuned by modifying the molar mass ratio between the PEO and PPO blocks (from 1:9 to 8:2), and recent reviews on the physicochemical studies of these aqueous solutions have been reported. This offers great potential for directly modifying the in vivo properties and interactions with cells and cell membranes, and for designing innovative nanomedicines and novel biomaterials [26, 27]. According to these research results, when comparing the critical micelle concentration for the overall composition and temperature, the amphiphilic ABA triblock copolymer shows higher conditions than the diblock (AB) and lower conditions than the BAB triblock copolymer where both sides are hydrophobic [27]. At 4 to 30 ℃, a poloxamer aqueous solution transitions into a hydrogel when the temperature is increased because a transition occurs from a single molecule to a micelle. Therefore, the poloxamer hydrogel is a structure formed by micelles being packed in a certain structure, and because of this, the hydrogel easily erodes in the presence of an excessive amount of water[11].

Absorbable bone wax products are slowly dissolved in the body, absorbed, and excreted based on the principle that they are transferred to the sol phase by body temperature and body fluids. According to Grindel J.M. research team, poloxamer is excreted through the kidneys in feces by approximately 85% within 48 hours after being applied to the body and is completely purified within a week[12, 13].

Currently, major bone wax products on the market are provided by several manufacturers such as Aesculap, CP medical, Covidien, Ethicon, and Surgical Specialties. Among them, the use of non-absorbable products is decreasing due to side effects and complications caused by beeswax, and the use of absorbable products developed in the

2000s is predominant. A representative example of an absorbable bone wax product is Ostene, which is a product approved by the U.S. Food and Drug Administration. It is softened by manual manipulation before use, applied to bleeding bones, and causes hemostasis. It dissolves within 24 to 48 hours, and the dissolved alkylene oxide copolymer is removed from the body without being metabolized. In addition, since this copolymer is hydrophilic, it is known to adhere well to wet surfaces and is suitable for hemostasis of bones. According to these characteristics, the range of indications is wide, and it is mainly applied to the bleeding area of bones during orthopedic surgery, thoracic surgery, and neurosurgery[5].

As a clinical case of absorbable bone wax products, a clinical case was reported to evaluate the efficacy in reducing postoperative bleeding and transfusion rates in total knee arthroplasty (TKA). The patient group that received hemostasis using absorbable bone wax products after total knee arthroplasty was compared with the patient group that received hemostasis using electrocautery, and the estimated blood loss, hemoglobin level, and transfusion rate were compared between the groups. The results of these studies were reported to have obtained results that the use of absorbable bone wax products was safe and effective in reducing overall bleeding and blood loss and maintaining higher hemoglobin levels[14, 15].

In the case of existing absorbable bone wax products, they have secured many clinical use cases for a long time based on the advantages of low cost, easy handling, malleability, suture ability, and bone adhesion. However, there are still several limitations that have not been overcome (limited physical properties, poor bone adhesion, insufficient hemostatic quality, and interference with bone union). When applied to the affected area, they are dissolved too quickly by blood and body fluids, and the physical barrier cannot be maintained for a certain period, which reduces the hemostatic effect.

In this study, we studied the mixing and dissolution method of poloxamer multi-block copolymer (PEG-PPG-PEG) to develop a new type of absorbable bone wax product (Hans Bone Wax; HBW). HBW was developed by mixing and dissolving two poloxamer multi-block copolymers with different molecular weight ranges. We conducted a comparative evaluation of the developed HBW with existing products, including analysis of physical and chemical properties, biological safety, and efficacy evaluation through stability and animal testing, in order to verify the possibility of a new product that improves the limitations of existing absorbable bone wax products.

2. Materials and method

2.1. Preparation of test materials

An absorbable bone wax product, Hans Bone Wax (HBW) was produced by mixing and dissolving two poloxamer multiblock copolymers with different molecular weight ranges. Liquid poloxamer (Poly(ethylene glycol-ran-propylene glycol), 438200, Sigma-Aldrich, USA) and powder poloxamer (Kolliphor® P 338 Geismar, 50424591, BASF Corporation, USA) were mixed at a certain ratio and then dissolved to synthesize the product. For the thermal synthesis method, refer to "Biocompatible composition for bone hemostasis material, treatment method using same, and method for preparing same" (Korean Patent No. 10-2133503). Briefly, the manufacturing method is as follows: liquid poloxamer: powder poloxamer were mixed at a certain ratio, heated to 80℃ to dissolve for 30 minutes, and the dissolved material was poured into a mold for molding and cured at room temperature (Table 1). For the experimental comparison, OSTENE (Baxter Healthcare Corporation, United Kingdom) and NOVOSEAL (CG Bio Co. Ltd., Korea), which are existing absorbable bone wax products whose raw materials are composed of poloxamer multiblock copolymers, were selected as reference materials.

Table 1. Confirmation of curing status (block formation) according to mixing and dissolution conditions of liquid and powder poloxamer.

		PEG-ran-PPG* (%)	P 338** (%)	Melting conditions	Curing conditions	Curing status
Mixing ratio	Group A	15	85	80 ℃ / 30 min	Room temperature (15 ~ 25 ℃)	Solid block
	Group B	25	75			Solid block
	Group C	35	65			Solid block
	Group D	45	55			Solid block
	Group E	55	45			Solid block
	Group F	65	35			Solid block (Sticky)
	Group G	75	25			Solid block (Very Sticky)
	Group H	85	15			Solid block (Very Sticky)

*PEG-ran-PPG : Poly(ethylene glycol-ran-propylene glycol) / Liquid Poloxamer

**P 338 : Kolliphor® P 338 Geismar / Powder Poloxamer

2.2. Physical properties by mixing and dissolving conditions of poloxamer

The formation of various solid blocks was confirmed under the conditions of mixing and dissolving liquid poloxamer and powder poloxamer at a ratio of 15 to 85 wt%, respectively. In order to measure the minimum force applied to the dough after the formation of the solid block, it was placed on the three-axis bending jig of a tensile tester (LLOYD's Long travel extensometer), and the upper jig was loaded at a speed of 5 mm/min in the vertical direction to measure the maximum force when the test material was bent or broken. In addition, in order to measure the adhesive force to the surface to achieve physical hemostasis, 1 g was removed after the formation of the solid block, kneaded into a sphere shape, and placed between the grips of the compression/tensile tester to adhere with a constant load (50 ± 5 N), and then the two grips were moved at a speed of 50 mm/min to measure the maximum force at the moment of falling.

2.3. Analysis of molecular weight distribution and thermal properties according to poloxamer synthesis

In addition, to confirm the stability of liquid poloxamer and powder poloxamer for thermal synthesis, the molecular weight distribution and thermal property changes were measured by Gel Permeation Chromatography (GPC) and Differential Scanning Calorimetry (DSC), respectively, after synthesis. Molecular weight analysis was performed using EcoSEC HLC-8420 GPC (RI-Detector) from Tosoh, after completely dissolving the sample in THF and injecting it at a concentration of 3 mg/mL. Thermal property changes were measured using DSC 204 F1 Phoenix from NETZSCH, by heating the sample at 10℃/min and measuring the thermal property changes at 30 to 300℃.

2.4. Comparative analysis of physical properties of selected bone wax and commercialized bone wax products

To analyze the physical characteristics of absorbable bone wax products, the adhesive strength, yield load, and solubility were compared and evaluated for each test material. Each test was conducted by considering the environment of the clinical indication of absorbable bone wax products, comparing and evaluating the adhesive strength to the bone surface, the force required to knead the test material before use in the body, and the time taken for dissolution when reacting with body fluids in the body, etc. to confirm the physical characteristics of each test material. For the adhesive strength test, 1 g of each test material was taken out and kneaded into a sphere shape, and this was placed between the grips of a compression/tensile tester and adhered with a constant load (50 ± 5 N). Then, both grips were moved at a speed of 50 mm/min to measure the maximum force at the moment of separation. For the yield load test, each test material was placed on the three-axis bending jig of the tensile tester, and a load was applied to the upper jig at a speed of 5 mm/min in the vertical direction to measure the maximum force when the test material was bent or broken. For the solubility test, 2 g of each test substance was taken, kneaded into a spherical shape, and prepared. These were completely immersed in 10 ml of phosphate-buffered saline (PBS), stored in a 37°C incubator, and the dissolution pattern and weight change over time were measured.

2.5. Cytotoxicity evaluation of selected bone wax

Cytotoxicity was evaluated qualitatively using the Extraction method based on the ISO 10993-5 and ISO 10993-12. Briefly, Hans Bone Wax (HBW) was extracted an elution rate of 0.2 g/ml in polar (Sodium Chloride) and non-polar (Dimethyl sulfoxide) elusion solvent at 37 ± 1 °C for 72 ± 2 h. Following the extraction, monolayers of L-929 mouse fibroblast cells were dosed with: The HBW extracts eluted with polar solvents were treated at 10%, the HBW extracts eluted with nonpolar solvents were treated at 0.5% and incubated at 37 ± 1 °C, 5 ± 1% CO2, 95% humidity for 48 ± 2 h. Following the incubation, Cells were observed using an optical microscope. Finally, Observe each well of the HBW extract, blank test solution, negative control solution, and positive control solution with a microscope and determine the grade as follows.

Table 2. Cytotoxicity evaluation grades.

Grade	Reaction	Culture conditions
0	None	No cell lysis, no reduction in cell growth
1	Slight	Not more than 20% reduction in cell growth
2	Mild	Not more than 50% reduction in cell growth
3	Moderate	Not more than 70% reduction in cell growth
4	Severe	Nearly complete of complete destruction of cell layers

2.6. Evaluation of the site of bleeding after bone amputation using animals

Each test substance was applied to the bone amputation bleeding site, and usability/spreadability evaluation, hemostasis performance evaluation, and hemostasis maintenance evaluation were performed[16-20]. The experimental animals used were Sprague Dawley (SD) rats (male) over 8 weeks of age, and the animal experiments were conducted in compliance with the provisions of the “Animal Protection Act” and the “Act on Laboratory Animals” of the Republic of Korea, and in compliance with the animal experiment methods of the Animal Experiment Ethics Committee of Konkuk University (Animal Experiment Approval Number: KU19201). For respiratory anesthesia of the rat, it was placed in the induction chamber, and anesthesia was induced using isoflurane (Oxygen flowmeter 0.9 L/min, 3.5% isoflurane with 100% O2). After induction of anesthesia, isoflurane anesthesia is maintained using a mask (oxygen flowmeter 0.4 ~ 0.8L/min, 1.5% isoflurane with 100% O2), and the core body temperature is maintained at 37 ~ 38℃ throughout the entire test period using a warming mat. The skin and muscles of the femoral area of the rat are incised, the bone is exposed, and the circular defect site (1 site/femur) is marked. While injecting and aspirating saline into the exposed area, drill intermittently using a hand drill to prepare a 2-mm circular defect graft site. The location of the circular defect is kept constant at the diaphysis adjacent to the gluteal tuberosity. Each test substance prepared in advance by kneading 10 mg each is evenly filled or spread onto the circular defect area using sterilized surgical gloves or surgical tools and attached (Figure 1). Commercially available bone waxes, OSTENE and NOVOSEAL, were used as the comparative group, and selected bone wax, Hans Bone Wax (HBW) was used as the experimental group.

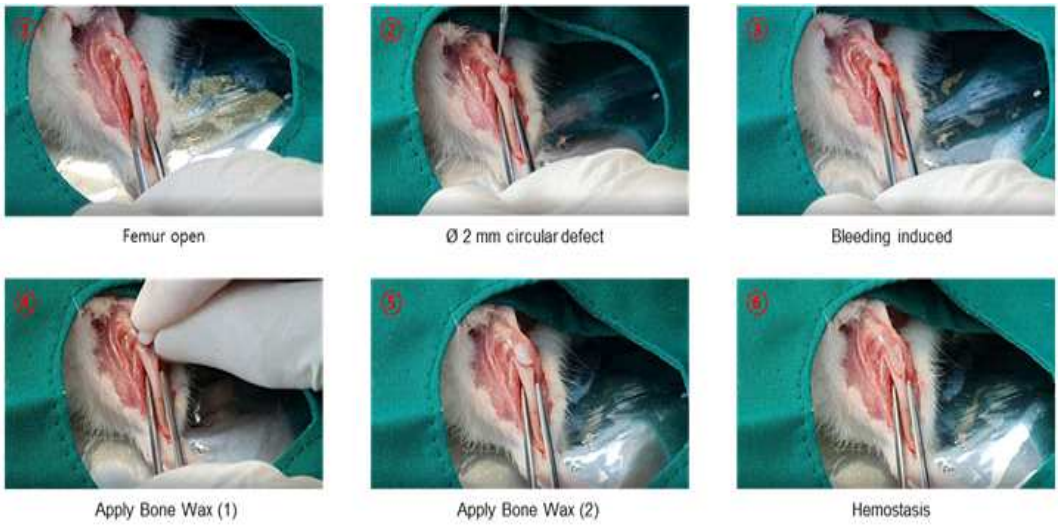


Figure 1. Schematic diagram for animal experiment of bone wax(Usability/Spreadability, Hemostatic performance, Hemostasis maintenance, Absorption/Degradability, Histopathological evaluation).

2.6.1. Usability/Spreadability evaluation

When applying each test substance, the adhesion to the tissue, spreadability, and ease of application of the hemostatic agent were evaluated according to the evaluation grade. The control group was evaluated without any treatment.

Table 3. Usability/Spreadability evaluation grades.

Grading scale	Usability/Spreadability Evaluation
Grade 0	After attachment, detachment occurs due to bleeding at the defect site or attachment is not possible
Grade 1	The degree of adhesion to the defect site does not exceed 50%, and more than 50% of the adhesion is to the user's glove.
Grade 2	The degree of adhesion to the defect site does not exceed 80%, and more than 20% is adhered to the user's glove.
Grade 3	More than 80% adhered to the defect site or does not stick to the user's glove

2.6.2. Evaluation of hemostatic performance

After applying each test substance, the hemostatic performance was evaluated according to the evaluation grade for 5 minutes. The control group was evaluated without any treatment.

Table 4. Hemostatic performance evaluation grades.

Grading scale	Hemostatic Performance Evaluation
Grade 0	The bleeding does not stop
Grade 1	Blood oozing occurs when the bleeding site is stopped for less than 1 minute.
Grade 2	Blood oozing occurs when the bleeding site is stopped for less than 3 minutes.
Grade 3	Blood oozing occurs when the bleeding site is stopped for less than 5 minutes.
Grade 4	Bleeding site stops bleeding for more than 5 minutes and there is no oozing

2.6.3. Evaluation of maintenance of hemostasis

After completing the usability/spreadability evaluation and hemostasis performance evaluation, the maintenance of hemostasis (1 hour) was evaluated. The maintenance of hemostasis was recorded over time (6 times, 10 minutes each). If blood oozing occurred during the evaluation of maintenance of hemostasis, the time was recorded, and the amount of bleeding was measured by absorbing the blood using pre-weighted paper, and the amount of bleeding was quantitatively evaluated and recorded.

2.7. Evaluation of absorption/degradability after transplantation of the bone amputation bleeding site

After implanting each test substance into the site of hemorrhage after bone amputation, the pattern and extent of absorption/degradability in the body were evaluated[16-21]. 10-week-old Sprague Dawley (SD) rats (male) were used as experimental animals. Animal experiments were conducted in compliance with the provisions of the “Animal Protection Act” and the “Act on Laboratory Animals” of the Republic of Korea, and in compliance with the animal experiment methods of the Animal Experiment Ethics Committee of Konkuk University (Animal Experiment Approval Number: KU21007). The preparation process for implanting the test substance into the femur of the animal was the same as in section 2.6. Each test substance, prepared in advance by kneading into circular defects in amounts of 50 mg, was evenly filled or spread onto the circular defect site using sterilized surgical gloves or surgical tools and attached. To evaluate absorption/degradability, the incised muscles and skin of the implantation site were sutured to prevent the sample from coming out. The transplant site was sutured and observed for 1, 3, 7, and 14 days. When the observation period ended, the animals were euthanized, and the degree of absorption/decomposition and biological response of the transplant site were evaluated. The surrounding tissues were removed to evaluate the degree of inflammation. The control group was evaluated without any treatment. For the comparison group, a commercialized bone wax, OSTENE, was used, and for the experimental group, a selected bone wax, Hans Bone Wax (HBW) was used.

Table 5. Absorption/degradability evaluation grades.

Grading scale	Absorption/degradability evaluation
Grade 0	No absorption/decomposition at all around the defect
Grade 1	Absorbed/decomposed by 2/4 or more, 50% or more around the defect
Grade 2	Absorbed/decomposed by 3/4 or more and 75% or more around the defect
Grade 3	Completely absorbed/decomposed and not observed

2.8. Histopathological evaluation

Specimens were prepared for histopathological analysis. The femurs and surrounding tissues were fixed in 10% neutral buffered formalin and decalcified using Solution Lite (Sigma-Aldrich) prior to routine tissue processing. Each specimen was then embedded in paraffin, and 4-μm thick tissue sections were obtained through the grafted area and stained with hematoxylin and eosin. The types and degrees of inflammatory responses and bone regeneration at the cortical bone defect site were compared between control, comparative, and experimental groups according to international standard guidelines (ISO 10993-6) at each time point.

2.9. Statistical analysis

Statistical analyses were conducted using Prism 4.02 (GraphPad Software, San Diego, CA, USA). A one-way analysis of variance (ANOVA) was employed to assess significant differences between groups, followed by Bonferroni's post hoc test for multiple comparisons of parametric data. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Physical properties by mixing and dissolution conditions of poloxamer

Liquid poloxamer (Poly(ethylene glycol-ran-propylene glycol), 438200, Sigma-Aldrich, USA) and powder poloxamer (Kolliphor® P 338 Geismar, 50424591, BASF Corporation, USA) were mixed at a ratio of 15 to 85 wt% as shown in Table 1, and then dissolved and cured to prepare the mixture. The degree of synthesis at room temperature during curing was observed. When the mixing ratio of liquid poloxamer exceeded a certain range (65% or more), block formation was difficult, and a viscous shape was formed. In order to obtain a poloxamer mixture in the form of a solid block after curing, the mixing ratio of liquid poloxamer (15 to 55%) and powder poloxamer (85 to 45%) was appropriate. When the solid block with this mixing ratio was kneaded and spread, it was confirmed that the usability and adhesiveness were maintained (Figure 2). In addition, when the hardness and stickiness of the poloxamer synthesized in this way were measured, the correlation with the mixing ratio of the liquid poloxamer and the powder poloxamer was confirmed (Figure 3). As the mixing ratio of the liquid poloxamer increased, the hardness of the poloxamer in the solid block state hardened after synthesis decreased (Figure 3A), and the stickiness did not increase significantly above a certain mixing ratio (35% or more) (Figure 3B). Among the mixing ratios of the liquid poloxamer and the powder poloxamer, the condition with similar physical properties to the commercialized bone wax Group B((PEG-ran-PPG : P 338 = 25% : 75%) = Selected Bone Wax = Hans Bone Wax(HBW)) was selected.

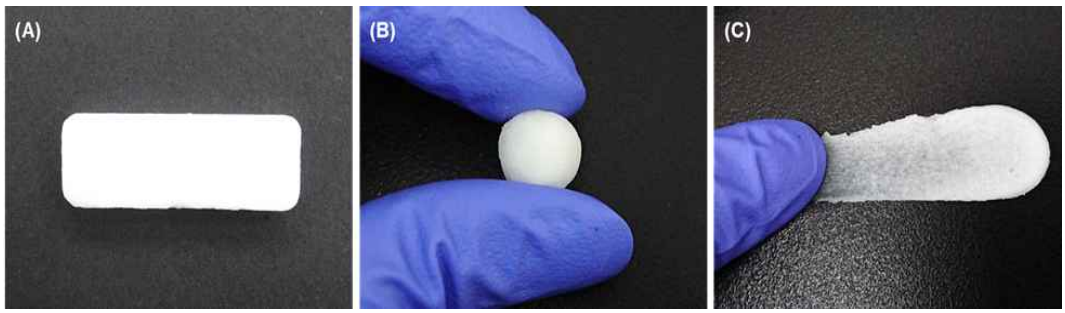
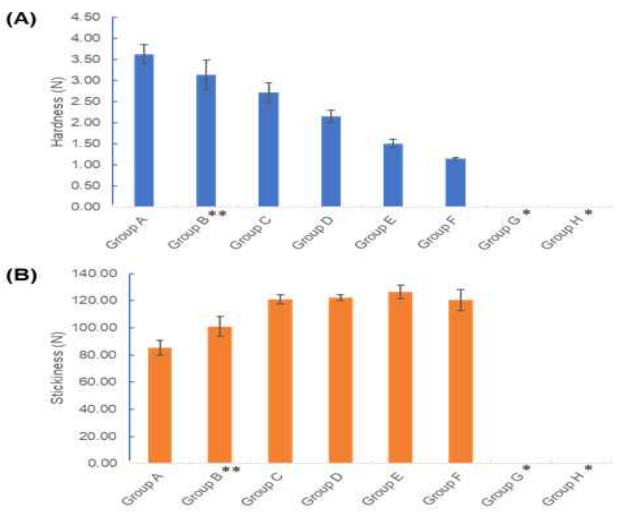


Figure 2. The results of mixing and dissolving liquid and powder poloxamer according to conditions and kneading. (A) Condition of the hardened solid block after mixing and dissolving poloxamer. (B) Solid block kneaded by hand 10 to 20 times. (C) The state of spreading the kneaded form.



		PEG-ran-PPG* (%)	P 338** (%)
Mixing ratio	Group A	15	85
	Group B	25	75
	Group C	35	65
	Group D	45	55
	Group E	55	45
	Group F	65	35
	Group G	75	25
	Group H	85	15

Figure 3. Confirmation of physical properties (hardness, stickness) according to mixing and dissolution conditions of liquid and powder poloxamer. Liquid poloxamer and powder poloxamer were mixed and dissolved according to conditions, 1 g was taken, kneaded into a spherical shape 10 to 20 times, and then measured. (A) Measures the hardness of a substance. (B) Measures the stickiness of a substance.

*Group G, H: Not measurable

** Group B (PEG-ran-PPG : P 338 = 25% : 75%) = Selected Bone Wax = Hans Bone Wax(HBW)

3.2. Analysis of molecular weight distribution and thermal properties according to poloxamer synthesis

In order to confirm the unique characteristic changes according to thermal synthesis through the 80℃ dissolution process under the selected synthesis conditions of Group B ((PEG-ran-PPG : P 338 = 25% : 75%) = Selected Bone Wax = Hans Bone Wax(HBW)), molecular weight distribution and thermal property analysis were performed (Figures 4, 5). When the unique molecular weight distributions of liquid poloxamer and powder poloxamer were confirmed before mixing, each unique molecular weight peak was confirmed. When checking the detection time, the liquid poloxamer detected a peak at 11.3 min (Figure 4A, red arrow), while the powder poloxamer showed two peaks at 11.3 min and 12.0 min (Figure 4B, blue arrow). In HBW, both above peaks (Figure 4C, green arrow) were confirmed, confirming that thermal synthesis was stably performed without molecular weight change. In addition, when the change in characteristics due to heat during synthesis was confirmed, liquid poloxamer: in the range of 30 to 55 ℃ / powder poloxamer: in the range of 57 to 65 ℃ / HBW: in the range of 55 to 68 ℃, an endothermic phenomenon occurred in all samples, and the thermal behavior confirmed the melting point, and after this melting point range, in the range of 68 to 300 ℃, a constant thermal flow was shown without any change in thermal characteristics (Figure 5).

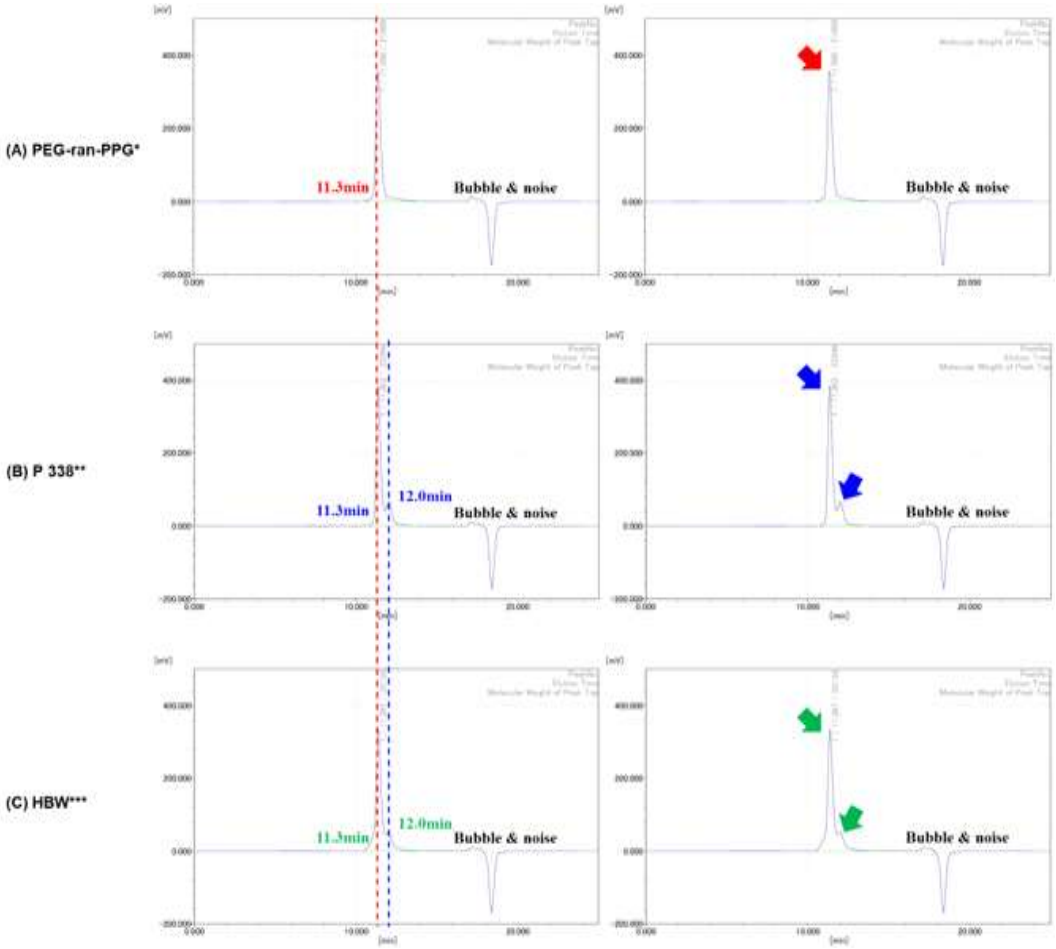


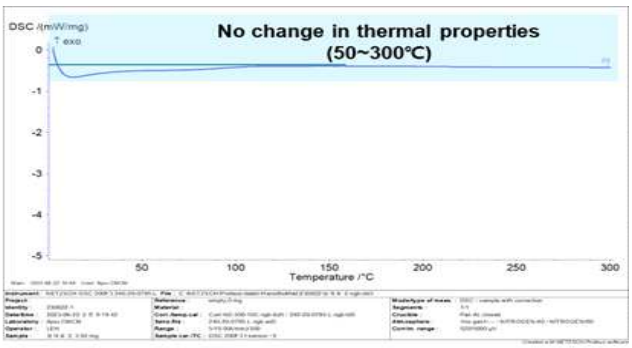
Figure 4. Confirmation of molecular weight distribution according to synthesis conditions of liquid and powder poloxamer. (A) Native molecular weight peak of PEG-ran-PPG (red). (B) Native molecular weight peak of P 338 (blue). (C) Native molecular weight peak of HBW (green) according to synthesis conditions.

*PEG-ran-PPG : Poly(ethylene glycol-ran-propylene glycol) / Liquid Poloxamer

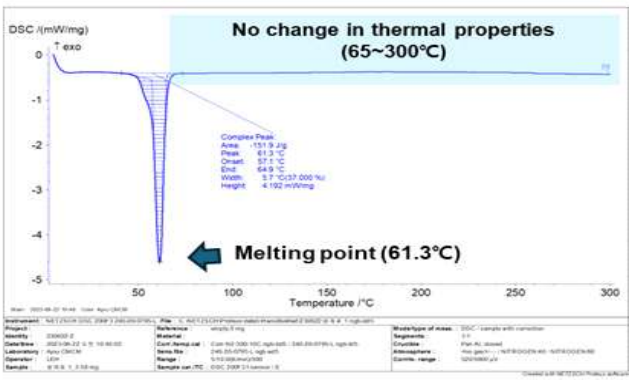
**P 338 : Kolliphor® P 338 Geismar / Powder Poloxamer

***HBW(Hans Bone Wax) = Selected Bone Wax = Group B (PEG-ran-PPG : P 338 = 25% : 75%)

(A) PEG-ran-PPG*



(B) P 338**



(C) HBW***

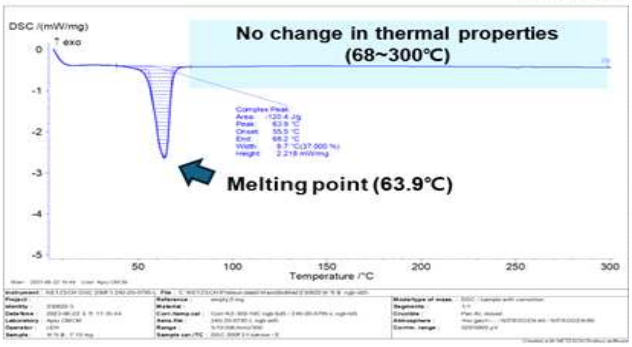


Figure 5. Confirmation of changes in thermal properties according to synthesis conditions of liquid and powder poloxamer. (A) Changes in thermal properties of PEG-ran-PPG. (B) Changes in thermal properties of P 338. (C) Changes in thermal properties of HBW according to synthesis conditions.

*PEG-ran-PPG : Poly(ethylene glycol-ran-propylene glycol) / Liquid Poloxamer

**P 338 : Kolliphor® P 338 Geismar / Powder Poloxamer

***HBW(Hans Bone Wax) = Selected Bone Wax = Group B (PEG-ran-PPG : P 338 = 25% : 75%)

3.3. Physical properties (adhesion, yield load, solubility) of HBW and commercialized bone wax

Considering the clinical use environment of Hans Bone Wax(HBW) and commercialized bone wax, the physical properties of each test material were analyzed by comparing and evaluating the adhesive strength to the bone surface (adhesion), the force required to knead the test material before use in the body (yield load), and the time required to dissolve when reacting with body fluids (solubility). As a result of measuring the adhesive strength under the same conditions, the commercialized bone waxes OSTENE and NOVOSEAL were 74.25 ± 2.92 N and 77.24 ± 5.38 N, respectively, while HBW had a higher adhesive strength than the commercialized bone wax at 100.24 ± 8.27 N (Table 6 & Figure 6). As a result of the yield load measurement, the commercialized bone waxes, OSTENE and NOVOSEAL, were 4.07 ± 0.33 N and 1.47 ± 0.12 N, respectively, and HBW was 3.17 ± 0.19 N (Table 7 & Figure 7). As a result of the solubility measurement, the commercialized bone waxes, OSTENE and NOVOSEAL, showed almost similar solubility in the dissolution patterns and weight changes over time, and it was confirmed that HBW had a later dissolution time than the commercialized bone wax (Figure 8).

Table 6. Comparison of physical properties (adhesion) of developed HBW and commercialized Bone wax.

		OSTENE	NOVOSEAL	HBW
Adhesive strength (N)	1	71.26	73.70	91.03
	2	74.32	74.64	99.57
	3	79.08	84.78	95.90
	4	73.32	80.95	113.20
	5	73.27	72.14	101.50
Mean ± SD		74.25 ± 2.92	77.24 ± 5.38	100.24 ± 8.27

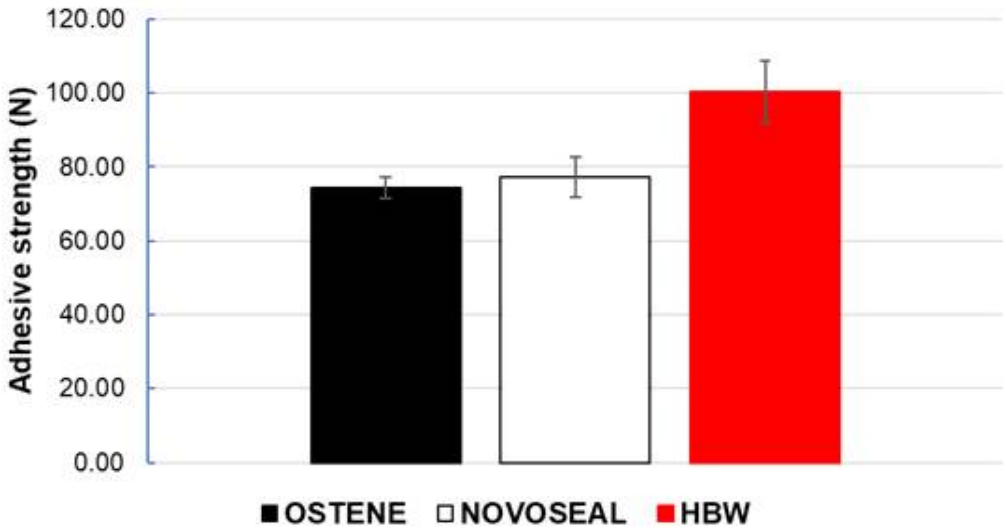


Figure 6. Comparison of physical properties (adhesion) of bone wax.

Table 7. Comparison of physical properties (yield load) of developed HBW and commercialized bone wax.

		OSTENE	NOVOSEAL	HBW
Yield load (N)	1	3.50	1.40	3.22
	2	4.11	1.67	2.89
	3	4.17	1.41	3.26
	4	4.32	1.50	3.12
	5	4.26	1.38	3.38
Mean ± SD		4.07 ± 0.33	1.47 ± 0.12	3.17 ± 0.19

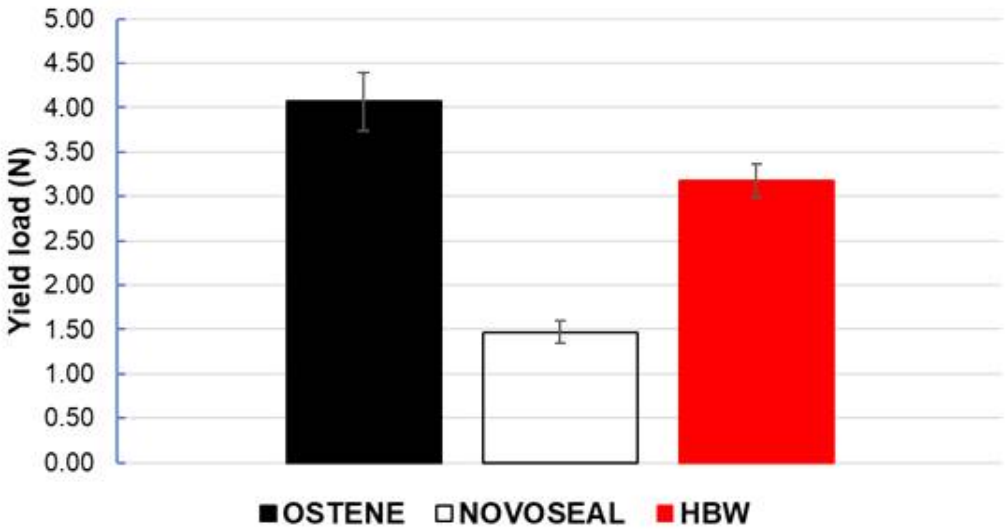


Figure 7. Comparison of physical properties (yield load) of bone wax.

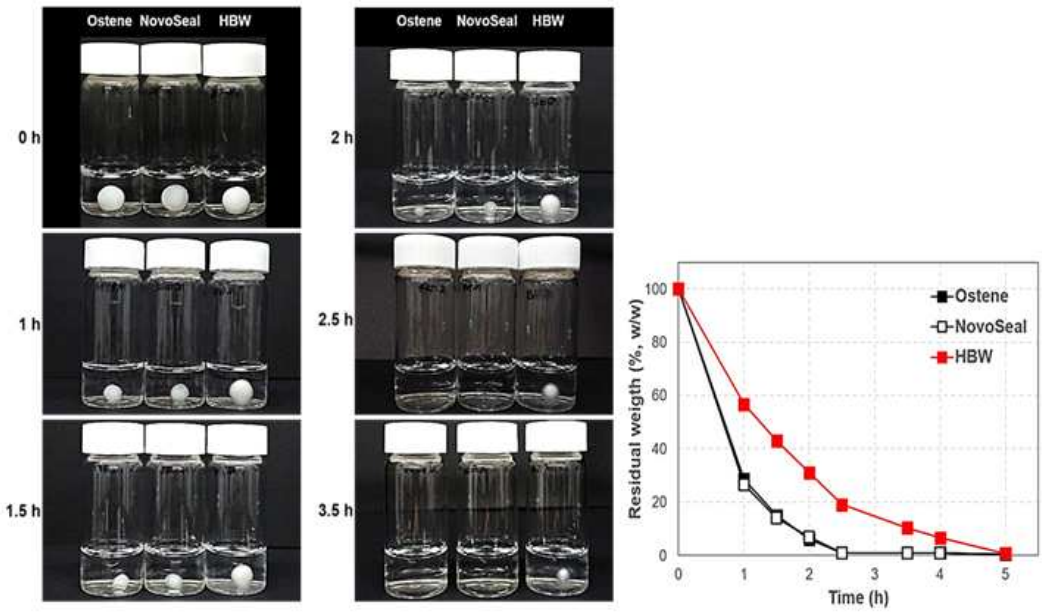


Figure 8. Comparison of physical properties (solubility) of bone wax.

3.4. Cytotoxicity evaluation of HBW

Since the negative control group and positive control group produced test results suitable for the validity criteria, it was confirmed that this test was properly conducted. In addition, it was confirmed that there was no separation of granules in the cytoplasm of the cultured cells, cell lysis, or cell growth inhibition under all conditions treated with the test substance. Therefore, the qualitative morphological reactivity of cells exposed to the test substance eluted with a polar solvent was judged as 'None', and the qualitative morphological reactivity of cells exposed to the test substance eluted with a non-polar solvent was judged as 'None'. In summary of the above results, the cytotoxicity response grade of Hans Bone Wax(HBW) under the conditions of this test was judged to be 'Grade 0' (Table 8).

Table 8. Results of cytotoxicity test among biocompatibility evaluation of HBW.

Extract(Test substance)						Control substance								
Polar solvent			Non-polar solvent			Blank test			Negative control			Positive control		
0	0	0	0	0	0	0	0	0	0	0	0	4	4	4

3.5. Evaluation of the bone excision bleeding area

3.5.1. Usability/Spreadability evaluation

In the usability/spreadability evaluation when applying bone wax, no specimens were observed to show Grade 0 in either the comparative OSTENE, NOVOSEAL, or the test group Hans Bone Wax (HBW). In the case of NOVOSEAL, 5 out of 8 specimens were observed to show Grade 2, and in the case of OSTENE, 1 each of Grade 1 and Grade 2 were observed, and 6 specimens were observed to show Grade 3. In the test group HBW, all 8 specimens were observed to show Grade 3. In the case of NOVOSEAL, bone wax mostly got on the glove, which limited the molding and quantitative application of bone wax, while in the test group HBW, it did not get on the glove in any specimen, making molding easy and quantitative attachment possible (Table 9 & Figure 9).

Table 9. Results of usability/spreadability performance evaluation of developed HBW and commercialized bone wax through animal experiments.

	Grade 0[a]	Grade 1[b]	Grade 2[c]	Grade 3[d]
OSTENE	0/8*	1/8	1/8	6/8
NOVOSEAL	0/8	1/8	5/8	2/8
HBW	0/8	0/8	0/8	8/8

a; After attachment, detachment occurs due to bleeding at the defect site, or no attachment

b; The degree of attachment to the defect site does not exceed 50%, and more than 50% of the material is absorbed into the user's glove

c; The degree of attachment to the defect site does not exceed 80%, and more than 20% of the material is absorbed into the user's glove

d; More than 80% of the material is absorbed into the defect site, or no material is absorbed into the user's glove

*; Number of animals by grade/Total number of animals

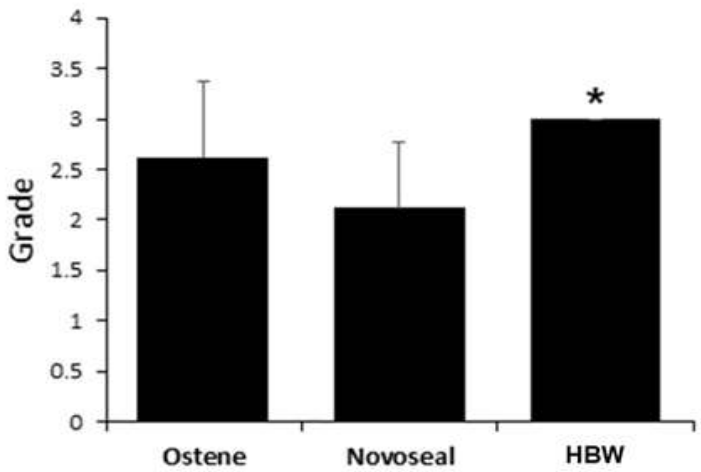


Figure 9. Results of usability/spreadability evaluation results of bone wax through animal experiments(*, $p<0.05$: compared to NOVOSEAL).

3.5.2. Evaluation of hemostatic performance

Hemostatic performance was evaluated based on whether oozing occurred and the time of occurrence for 5 minutes after applying bone wax. In the case of NOVOSEAL, 4 out of 8 specimens were observed as Grade 4, and the remaining specimens were observed to exhibit various degrees of hemostatic performance from Grade 0 to Grade 3. In the case of OSTENE, almost all specimens were observed as Grade 4 (7/8). In the case of the test group Hans Bone Wax (HBW), all 8 specimens were observed as Grade 4. In the case of NOVOSEAL, hemostatic performance was observed to be low in specimens with low usability/spreadability evaluation grades, and in the case of the test group HBW, hemostasis was maintained in all specimens, confirming that hemostatic performance was like that of the control group OSTENE (Table 10 & Figure 10).

Table 10. Results of hemostatic performance evaluation of developed HBW and commercialized bone wax through animal experiments.

	Grade 0[a]	Grade 1[b]	Grade 2[c]	Grade 3[d]	Grade 4[e]
OSTENE	0/8*	0/8	0/8	1/8	7/8
NOVOSEAL	2/8	0/8	1/8	1/8	4/8
HBW	0/8	0/8	0/8	0/8	8/8

a; no hemostasis
b; bleeding site stops bleeding for less than 1 minute, blood oozing occurs
c; bleeding site stops bleeding for less than 3 minutes, blood oozing occurs
d; bleeding site stops bleeding for less than 5 minutes, blood oozing occurs
e; bleeding site stops bleeding for more than 5 minutes, no oozing occurs
*; number of animals in grade/total number of animals

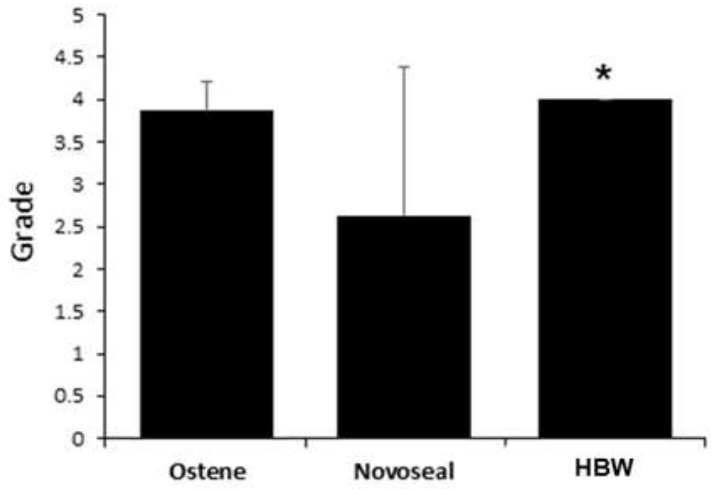


Figure 10. Results of hemostatic evaluation results of bone wax through animal experiments(*, $p<0.05$: compared to NOVOSEAL)

3.5.3. Evaluation of maintenance of hemostasis

Hemostatic maintenance time was measured by recording the bleeding time for 1 hour after applying bone wax. The average hemostasis maintenance time of NOVOSEAL was observed to be 18.6 minutes ± 26.0 minutes, and the average hemostasis maintenance time of OSTENE was observed to be 22.5 minutes ± 19.1 minutes. In the test group Hans Bone Wax (HBW), the hemostasis state was observed to be maintained for 56.8 minutes ± 8.8 minutes. In the case of NOVOSEAL, it was observed that bone wax was detached from the defect site after oozing occurred, whereas in the case of OSTENE and HBW, each bone wax was observed to be maintained within the defect site for a considerable period even after oozing occurred (Figure 11).

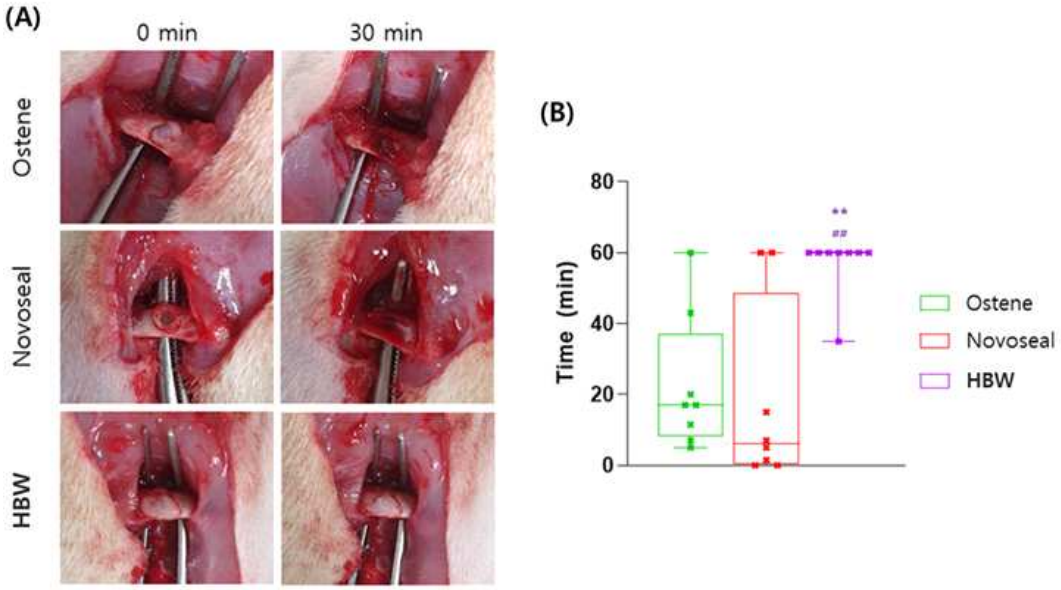


Figure 11. Results of hemostasis maintenance evaluation of bone wax through animal experiments. (A) Hemostasis maintenance pattern by time after application of bone wax. (B) Results of hemostasis maintenance by time after application of bone wax (*, $p<0.05$; **, $p<0.01$: compared to NOVOSEAL, #, $p<0.05$: compared to OSTENE)

3.6. Evaluation of absorption/degradability after transplantation of the site of bone excision bleeding

After 1, 3, 7, and 14 days of bone wax application, autopsies were performed, and the application site was visually evaluated. One day after bone wax application, both OSTENE and Hans Bone Wax (HBW) were observed to remain in the defect (Figure 12, yellow arrows) only in trace amounts (Grade 2). While the group to which OSTENE was applied showed little or no test material in the surrounding tissues, HBW showed a coagulated blood clot (Figure 12, red arrow) above the defect without adhesion to the surrounding tissues. The blood clot observed in the group to which HBW was applied was observed until 3 days after application of the test material, but it was completely absorbed and not observed in the visual observation on the 7th day. On days 7 and 14 after bone wax application, the defect was observed to disappear in all groups due to granulation tissue or bone regeneration within the bone defect (Table 11 & Figure 12).

Table 11. Results of absorption/degradation evaluation of developed HBW and commercialized bone wax through animal experiments.

		Day 1	Day 3	Day 7	Day 14
OSTENE	Grade 0[a]	0/3*	0/3	0/3	0/3
	Grade 1[b]	0/3	0/3	0/3	0/3
	Grade 2[c]	3/3	0/3	0/3	0/3
	Grade 3[d]	0/3	3/3	3/3	3/3
HBW	Grade 0	0/3	0/3	0/3	0/3
	Grade 1	0/3	0/3	0/3	0/3
	Grade 2	3/3	0/3	0/3	0/3
	Grade 3	0/3	3/3	3/3	3/3

a; No absorption/decomposition at all around the defect

b; Absorbed/decomposed by 2/4 or more, 50% or more around the defect

c; Absorbed/decomposed by 3/4 or more, 75% or more around the defect

d; Completely absorbed/decomposed, not observed

*; Grade Number of animals/Total Number of animals

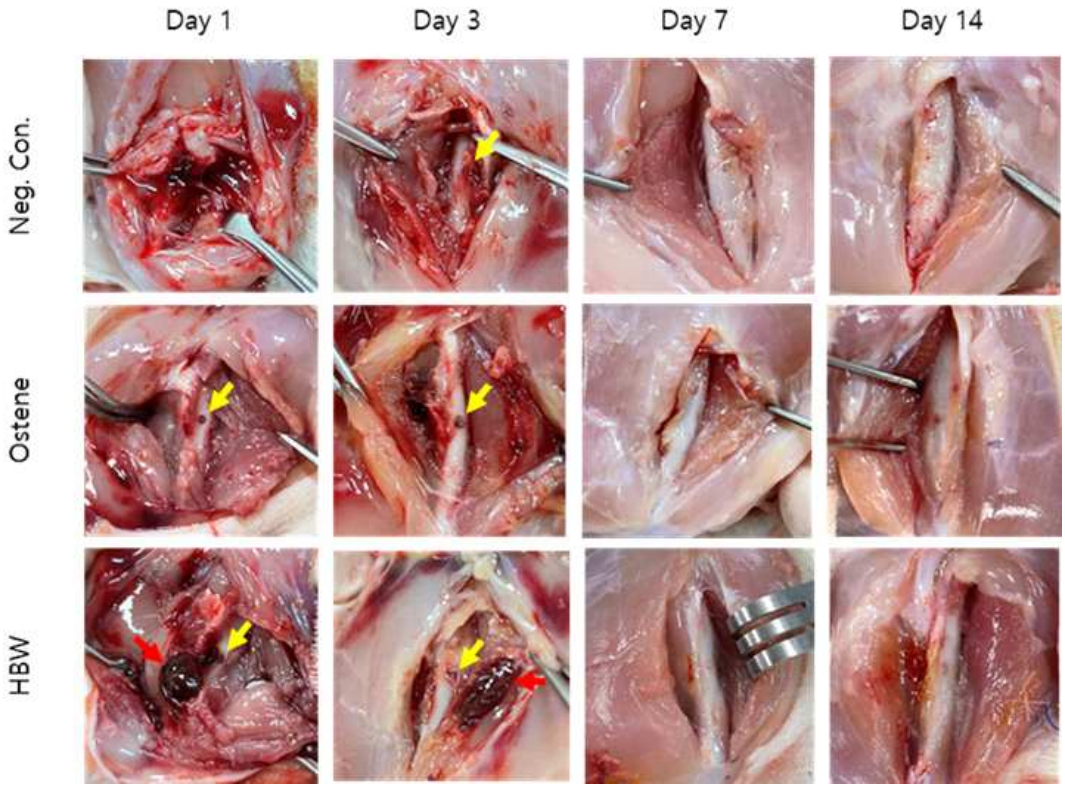


Figure 12. Results of in vivo absorption/degradation evaluation in a bone wax animal transplantation model.

Autopsy was performed 1, 3, 7, and 14 days after each application of bone wax, and the application site was visually evaluated. On days 1 and 3 after application of bone wax, only a small amount of bone wax was observed to remain in the defect(yellow arrow), and in the HBW application group, coagulated blood clots without adhesion to the surrounding tissue were observed(red arrow). After 7 days of application of bone wax, the defect was confirmed to disappear in all groups due to granulation tissue or bone regeneration within the bone defect.

3.7. Histopathological evaluation

To compare the type and degree of inflammatory response between the control and comparison groups and the experimental group for cortical bone defects at each autopsy time point, the results were analyzed according to the international standard guideline (ISO 10993-6). The test group, Hans Bone Wax (HBW), was evaluated at 8 points 14 days after application, which was observed to be lower or like the control group's 9 points. There was no statistically significant difference in the tissue regeneration response among the HBW group, test group, control group (Neg. Con.), and comparison group (OSTENE), indicating that HBW does not inhibit bone regeneration (Figure 13 & Figure 14 & Table 12).

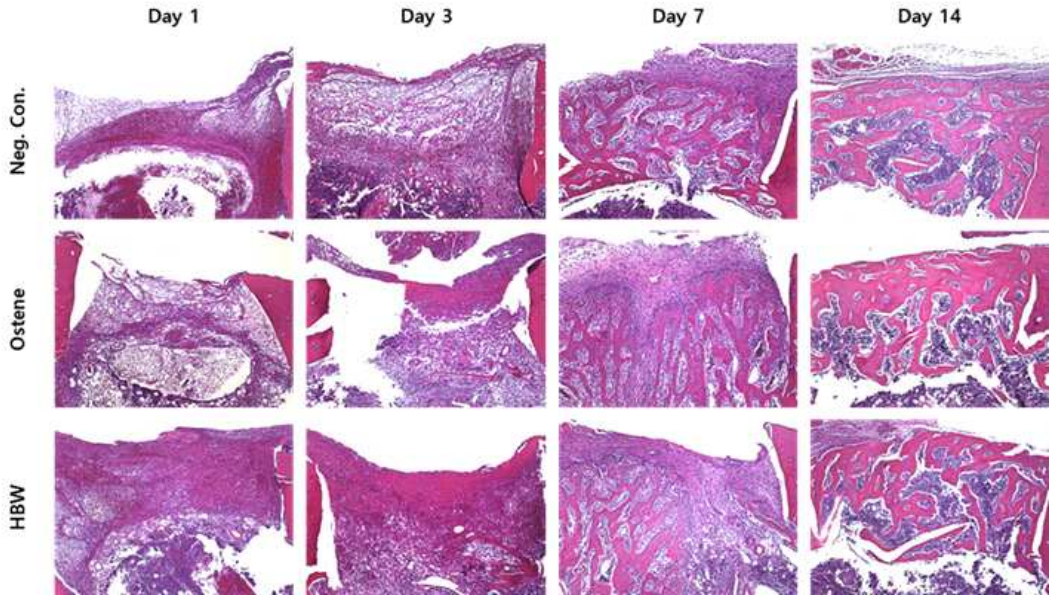


Figure 13. Histological evaluation of cortical bone defects in a bone wax animal transplantation model.

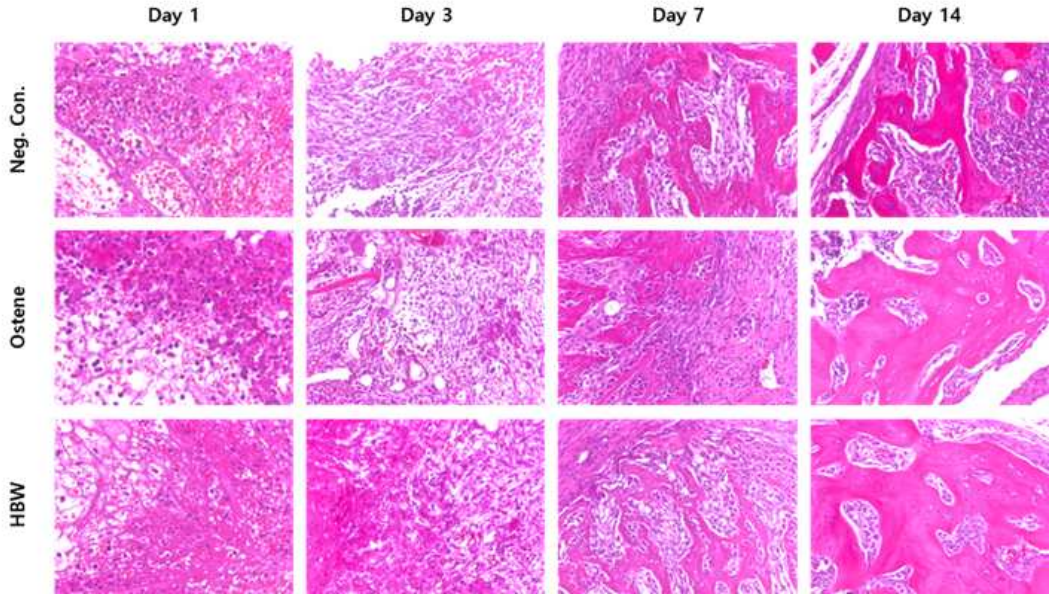


Figure 14. Histological evaluation of inflammation and bone regeneration in cortical bone defects in a bone wax animal transplant model.

Table 12. Histological evaluation of inflammation and bone regeneration in cortical bone defects 14 days after bone wax animal transplantation.

Animal No.	Negative Control			OSTENE			HBW		
	1	2	3	1	2	3	1	2	3
PMN cell[a]	0	0	0	1	0	0	0	0	0
Lymphocytes	2	1	1	3	2	1	2	1	1
Plasma cells	0	0	0	0	0	0	0	0	0
Macrophages	1	1	1	1	1	1	1	1	1
Giant cell	0	0	0	0	0	0	0	0	0
Necrosis[b]	0	0	0	0	0	0	0	0	0
SUB TOTAL(X2)	**6**	**4**	**4**	**10**	**6**	**4**	**6**	**4**	**4**
Neovascularisation[c]	2	2	2	1	2	2	2	2	2
Fibrosis[d]	1	1	2	3	1	1	1	1	2
Fatty Infiltrate[e]	0	0	0	0	0	0	0	0	0
SUB TOTAL	3	3	4	4	3	3	3	3	4
TOTAL	**9**	**7**	**8**	**14**	**9**	**7**	**9**	**7**	**8**
GROUP TOTAL		**24**			**30**			**24**	
Test average [8] – Negative control average [9] = 0									

a: 0, none; 1, rare; 2, 5-10/hpf; 3, heavy infiltrate; 4, packed

b: 0, none; 1, minimal; 2, mild; 3, moderate; 4, severe

c: 0, none; 1, minimal capillary proliferation; 2, groups of 4-7 capillary with supporting fibroblastic structures; 3, broad band of capillaries with supporting structures; 4, extensive band of capillaries with supporting structures

d: 0, none; 1, narrow band; 2, moderately thick band; 3, thick band; 4, extensive band

e: 0, none; 1, minimal amount of fat associated with fibrosis; 2, several layers of fat and fibrosis; 3, elongated and broad accumulation of fat cells about the implant site; 4, extensive fat completely surrounding the implant.

4. Discussion

In this study, we aim to improve the shortcomings of existing commercially available absorbable bone wax by thermally synthesizing liquid poloxamer and powder poloxamer to present an absorbable bone wax with improved usability and physical properties.

In the case of absorbable bone wax, it is known through animal experiments and clinical trials that it has many advantages over non-absorbable bone wax. A representative advantage is that it is absorbable in the body, so it is absorbed in the body after hemostasis of the bone amputation site, and does not inhibit bone regeneration, and can significantly reduce the risk of bone nonunion and infection [16-20]. There were many development directions according to the raw materials of absorbable bone wax, and among them, it was confirmed through in vivo animal experiments that absorbable materials such as oxidized regenerated cellulose, microfibrillar collagen, and gelatin were absorbed at the application site after application. Oxidized regenerated cellulose was decomposed and disappeared from the application site within 6 weeks to 1 year, and microfibrillar collagen and gelatin were found to be absorbed within 14 to 90 days [3]. In contrast, in the case of absorbable bone wax that has been recently commercialized, the decomposition period in the body was significantly shortened to 48 to 72 hours by using poloxamer, an alkylene oxide copolymer. Additionally, the degraded/absorbed poloxamer was eliminated through renal or hepatic excretion [20, 22].

However, some problems are also emerging with absorbable bone wax based on poloxamer. Among them, in the case of usability-related parts that occur when applied to the affected area in a clinical environment, it is related to the physical properties of absorbable bone wax based on poloxamer. Due to the characteristics of poloxamer, it has low mechanical strength and is rapidly decomposed in a short period of time in the body, making it difficult to maintain hemostasis [23-25]. In addition, there is an inconvenience of attaching to surgical gloves when kneading before application to the affected area due to poor molding texture [17].

To improve this, in this study, two types of poloxamers were thermally synthesized to improve the molding texture and decomposition period. First, the hardness of the developed Hans Bone Wax (HBW) was adjusted to be similar to that of the existing commercially available absorbable bone wax by minimizing the force required for the kneading process when used in a clinical environment. In order to improve the molding

texture, the content ratio of the hydrophobic block PPG (=PPO) was changed by using liquid poloxamer and powder poloxamer. According to the review of Booth et al., in amphiphilic ABA triblock copolymers, it was reported that the critical micelle concentration can be controlled depending on the type, position, and length of the B block (hydrophobic block). It can be confirmed that the critical micelle concentration increases overall when the hydrophobic block is increased [27]. The developed HBW was thermally synthesized using liquid poloxamer and powder poloxamer. It can be confirmed that the melting point is higher than that of powder poloxamer due to the increase in hydrophobic block. Through this, it can be confirmed that the adhesion and solubility of the developed HBW are greatly increased while maintaining the hardness. This shows that the overall critical micelle concentration increases when liquid poloxamer and powder poloxamer are thermally synthesized compared to when poloxamer is used alone. In conclusion, it can be judged that the content of the hydrophobic block PPG (=PPO) was changed through thermal synthesis, and the overall critical micelle concentration increased, which increased the adhesiveness and solubility.

As a result, the stickiness was controlled to have sufficient adhesiveness on the bone tissue surface, and the range that does not adhere to surgical gloves was studied. This is thought to have improved the inconvenience of the method of adding pre-gelatinized starch to improve the molding texture in the study by J. Suwanprateeb et al.

In addition, through animal experiments, it was confirmed that after creating a defect in the femur of a rat and inducing bleeding, hemostasis was maintained by applying absorbable bone wax and ultimately bone regeneration was not inhibited. The existing commercially available absorbable bone wax was evaluated as a comparative group, and the factors that should be considered when applying it to the affected area in a clinical environment, such as usability/spreadability, hemostatic performance, hemostasis maintenance, and absorption/decomposition of bone wax in the body, were compared through animal experiments.

As a result of the animal experiments in this study, it was observed that the test group, HBW, was superior to the commercially available OSTENE and NOVOSEAL in both usability/spreadability and hemostatic performance. In the evaluation of the usability/spreadability of bone wax, NOVOSEAL was observed to have mostly 5/8 individuals indicating Grade 2, and OSTENE was observed to have mostly 6/8 individuals indicating Grade 3, whereas HBW was observed to have all individuals (8/8) indicating Grade 3, confirming that the adhesion within the tissue, spreadability, and user

convenience were improved compared to the control group. In the 5-minute hemostatic performance evaluation, NOVOSEAL was observed to exhibit various degrees of hemostatic performance from Grade 0 to Grade 4, OSTENE exhibited Grade 4 hemostatic performance in most of the 7/8 cases, and HBW exhibited Grade 4 hemostatic performance in all cases. In the 60-minute hemostatic maintenance time evaluation, NOVOSEAL showed an average of 18.6 minutes and OSTENE 22.5 minutes, showing that while the control group showed a hemostatic time of about 20 minutes, the test group HBW showed a hemostatic maintenance time of 56.8 minutes, showing an increase in the hemostatic maintenance time of about 3 times compared to NOVOSEAL and about 2.5 times compared to OSTENE. In conclusion, the test substance HBW was observed to have excellent usability/spreadability and tissue adhesion compared to the control groups OSTENE and NOVOSEAL, and as a result, it was observed to be excellent not only in the 5-minute hemostatic performance evaluation but also in the 60-minute hemostatic maintenance time evaluation. In addition, in the evaluation of the absorption/degradability of bone wax, HBW and OSTENE were both observed to have been absorbed/degraded by more than 75% (Grade 2) 1 day after the application of the hemostatic agent, and HBW and OSTENE were confirmed to have been absorbed/degraded to a level that could not be observed with the naked eye (Grade 3) 3 days after the application of bone wax. In the evaluation of inflammatory response and tissue regeneration response through histopathological evaluation, HBW, OSTENE, and the negative control group were all observed to have similar degrees of inflammatory response 1 and 3 days after the application of bone wax, and all three groups were confirmed to have similar granulation tissue formation and bone regeneration responses 7 and 14 days after the application of bone wax. These results are similar to those of many studies including the study by J. Suwanprateeb et al. [16-20].

The absorbable bone wax (Hans Bone Wax; HBW) developed through this study can effectively adhere to the surface of bone bleeding and amputation sites to exhibit a physical hemostatic effect, and has improved performance compared to existing commercialized bone wax through results such as usability/spreadability, hemostatic performance, and hemostasis maintenance. In addition, it was confirmed through histopathological evaluation after transplantation that it does not inhibit granulation tissue formation and bone regeneration reaction, and it is judged that the possibility of clinical application has been secured.

5. Conclusion

The absorbable bone wax (Hans Bone Wax; HBW) developed through this study was improved in molding texture and mechanical properties by thermally synthesizing liquid poloxamer and powder poloxamer, and through animal testing considering the clinical use environment, it was demonstrated that the performance characteristics regarding hemostasis were improved by comparing it with the existing commercialized bone wax in terms of usability/spreadability, hemostatic performance, and hemostasis maintenance, etc. In addition, it was confirmed through histopathological evaluation after transplantation that it did not inhibit bone regeneration reaction, confirming that it is a suitable material for the treatment of bone bleeding and amputation sites. The absorbable bone wax (Hans Bone Wax; HBW) developed in this way is expected to be able to increase user satisfaction and achieve rapid hemostasis of the affected area in clinical use environments. Additionally, it is judged that research is necessary to verify the clinical applicability of HBW.

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Abstract in Korean

폴록사머 본 왁스의 개발 및 지혈 성능 평가

뼈 왁스는 정형외과 수술, 흉부 수술 및 신경 수술 등 뼈 지혈에 필수적인 재료로 일반적으로 뼈 골절로 인한 출혈을 물리적으로 제어하는 데 사용되는 물질로 정의되고 있습니다. 특히 뼈의 골절 또는 절단 표면에 물리적으로 압착하였을 때, 골 표면에 물리적인 차단막을 형성함으로써 출혈을 막아줍니다. 뼈 왁스는 특성에 따라 비흡수성 제품과 흡수성 제품으로 나눌 수 있습니다. 비흡수성 뼈 왁스 제품의 경우, 체내에서 대사되거나 흡수 되지 않아 도포 부위에 무기한 남아 있기 때문에 많은 합병증을 기인합니다. 흡수성 뼈 왁스 제품의 경우, 국소 적용의 용이성, 물리적인 차단막 형성, 생체 적합성, 체내 흡수 및 배출 가능성 등의 특성을 이루기 위해 폴록사머 다중 블록 공중합체(CAS No. 9003-11-6, 동의어; Pluronic, Polyoxypropylene-polyoxyethylene Block Copolymer, PEG-PPG-PEG)를 사용하여 비흡수성 뼈 왁스 제품의 단점을 극복했습니다. 하지만, 기존 흡수성 뼈 왁스 제품의 경우, 아직도 몇 가지 극복하지 못한 한계점들(제한된 물리적 성질, 아쉬운 뼈 부착력, 지혈 품질 부족, 뼈 결합 방해)이 있어 환부 적용 시 혈액 및 체액에 의해 너무 빠르게 용해되어 물리적 장벽을 일정 시간 유지하지 못해 지혈작용이 저하되고 있습니다. 본 연구에서는 분자량 범위가 서로 다른 두 가지의 폴록사머 다중 블록 공중합체를 사용하여, 새로운 형태의 흡수성 뼈 왁스(Hans Bone Wax; HBW)를 개발하였고, 기존 흡수성 뼈 왁스 제품들(OSTENE, NOVOSEAL)과 물리화학적 특성 분석, 동물실험을 통한 유효성 평가, 생물학적 안전성 등의 비교평가를 진행하여, 기존 흡수성 뼈 왁스 제품의 한계점을 개선한 새로운 제품의 가능성을 입증하고자 합니다. In vitro 상에서 각 제품의 접착력, 항복하중, 용해도를 비교 평가하였고, 동물실험(In vivo)을 통하여 골 절단 부위의 지혈에 대한 평가 및 흡수/분해에 대한 평가를 하였습니다. 그리고 새로 개발된 HBW에 대해 ISO 10993 가이드라인에 따른 생물학전 안전성(세포독성) 평가도 진행하였습니다. 결론적으로 골 절단 부위의 지혈 및 조직학적 분석을 통한 생물학적 반응 평가에서 새로운 형태의 흡수성 뼈 왁스인 HBW가 우수하였습니다. 이는 골 절단 부위의 출혈을 지혈하는데 있어, 편리한 사용성과 뛰어난 지혈 성능 및 생체적합성을 제공하여 임상환경을 개선할 수 있을 것으로 생각됩니다.

핵심되는 말: 뼈 결손, 뼈 출혈, 뼈 왁스, 흡수성, 폴록사머, 지혈