Is Sodium Alginate an Alternative Hemostatic Material in the Tooth Extraction Socket?

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SYNOPSIS
Alginate has been used in wound dressing and tissue engineering. In this study, our designed alginate sponge was examined for use as an optimal occlusion material. Alginate powder was mixed in double-distilled water (3.0% w/v), sterilized and lyophilized to become a sponge. Sixty Wistar rats were undergone the right mandibular incisor extraction and divided into 3 groups: the first group received an alginate sponge; the second received a gelatin sponge; and the third was untreated. The mandibles were retrieved after 1, 2, 4 and 8 weeks and subjected for bleeding measurement, radiographic and histological analyses.

Alginate and Gelatin groups showed significantly shorter bleeding time until hemostasis than control. In Alginate group, as well as the other groups, bone formation was observed from 2 weeks post operation. The extraction socket receiving alginate was finally filled with osteoblasts and bone, suggesting that alginate might be considered as a candidate for not only cartilage but bone generation.

Key words: alginate, tooth extraction socket, hemostasis

INTRODUCTION
Recently, the number of patients who are receiving oral anticoagulant treatments has been expanding because of increase of systemic or local vascular diseases such as cerebral infarction. However, when tooth extraction is required in such patients, the prescription of the medicine is generally reduced or stopped for several days before the dental extraction. On the other hand, it has also been suggested that extractions should be carried out without any interruption or diminution of the anticoagulant treatment but with emphasis on the efficiency against of the local hemostasis,¹,² which can definitely avoid risks caused by clogged blood flow by suspending the medicine. Currently, endovascular occlusion materials have been developed such as cyanoacrylate, polyvinyl alcohol, hyaluronic acid gels, and cellulose acetate; however, some of these polymers are inadequate and toxic because they adhere to the vessel by destroying the vessel surface leading...
to an unwanted immune response and inflammation. Alginate, as a candidate for embolization, is a natural polysaccharide extracted from brown sea algae. It is biocompatible, hydrophilic, and biodegradable under normal physiological conditions. The gel form of alginate provides a moist environment which promotes healing and epidermal regeneration. The sponge form is generally produced by chemical bridging of additional calcium ions and then rapidly releases the ions in exchange for sodium ions on contact with blood, by which alginate will possess a hemostatic effect when placed in a body. Therefore, alginate has been popular in many biomedical applications, such as wound dressing and tissue engineering.

In this study, our designed alginate matrix which was pursued for its manipulability was examined to produce a three-dimensional porous sponge and emerge as an optimal occlusion material after tooth extraction in dental treatments, using a rat model.

**MATERIALS AND METHODS**

**Preparation of alginate sponges**

Low viscous sodium alginate was purchased from Sigma (A2033 St. Louis, USA). To prepare alginate sponges, 0.6 g of alginate powder was first dissolved and thoroughly mixed in 20 ml of double-distilled water. The 3.0% w/v alginate solution was sterilized by autoclaving. Five hundreds micro litters each of the solution was distributed into 1.5 ml tubes and frozen at –80ºC for 6 hours, lyophilized overnight, and stored in a freezer at –40ºC until the operation.

**Animal surgery**

Male Wistar rats 10 weeks old, weighing 350 to 400 g, were obtained from Sankyo Laboratory (Tokyo, Japan). The exposed part of the right mandibular incisor was cut every three days three times so as to lose retention by periodontal ligament. At three days after the final cut, the incisor was pulled out under the anesthesia with intraperitoneal injection of the mixture of 2 ml/kg xylazine – ketamine – acepromazine (4, 50, and 1 mg/ml, respectively). After the extraction of the incisor, sixty rats were evenly divided into three groups: (1) the extraction socket was filled with an alginate sponge (Alginate); (2) the extraction socket was filled with a gelatin sponge (Gelatin); (3) the extraction socket was untreated (Control). At 1, 2, 4 and 8 weeks after the operation, the rats were sacrificed and the mandibles were dissected out for analyses. In this experiment, Spongel (Astellas, Japan), commercially used and well-established, was used as a gelatin sponge.

**Bleeding time measurement**

Bleeding time was measured in seconds right after the tooth extraction until established hemostasis, which was recognized using the gauze blotted near the forming the blood clot without disturbing its formation. Observations were made every 15 seconds until staining no longer occurred.

**Radiographic and histological analyses**

The specimens were first denuded of soft tissue. Bone mineral density of rat right mandible was measured with a dual energy X-ray absorptometry (DEXA, DCS-600EX : ALOKA). CT scans in rat mandibles were taken on MICRO FOCUS X-RAY CT SYSTEM (SMX-90CT : SHIMADZU). Furthermore, the samples were subjected for histology. They were fixed in 10% neutral buffered formalin, demineralized in 10% EDTA solution, dehydrated in gradient alcohol (70 – 100%) and embedded in paraffin. Five µm thick longitudinal sections were cut and stained with hematoxylin-eosin to assess morphological progression of tissue regeneration within the extraction
cavity. The double-staining with alizarin red and alcian blue was also performed for bone and cartilage on another set of the sections.

**Statistical analysis**

Results of bleeding time and bone mineral density were presented as mean ± standard deviation. The comparisons of individual group means were performed with Kruskal-Wallis test. Values of p < 0.05 were regarded as statistically significant.

**RESULTS**

**Bleeding time**

Control group took 391 ± 80 seconds to achieve the hemostasis when the extraction socket was untreated. Both Alginate and Gelatin groups showed significantly shorter bleeding time (180 ± 41, 185 ± 75 seconds, respectively) than Control group. However, between Alginate and Gelatin group, there was no difference of the bleeding time. (Fig 1)

**Radiographic analysis**

The bone mineral density (BMD) of the rat mandibles increased in a time dependent manner in all groups. Changes of BMD of all groups were traced similar over time. BMD summed over all groups elevated in 16% from 1 week to 8 weeks post operation. (Fig 2)

The µCT scans were performed to observe frontal sections of the extraction socket at 1 mm intervals. Scans at the medial plane of the first molar were compared among all groups. In all

![Figure 1](image1.png)

**Figure 1**  Bleeding time after incisors extraction. Compared with Ext group, Gel and Alg groups significantly decreased the bleeding time. However, there was no difference between Gel and Alg groups. (Alg: Alginate, Gel: Gelatin, Ext: Control)

![Figure 2](image2.png)

**Figure 2**  Bone mineral density of rat mandibles measured with DEXA. BMD increased with time during the experimental period in all groups. BMD summed over all groups was 16% higher at 8 weeks after incisor extraction than that at 1 week. (Alg: Alginate, Gel: Gelatin, Ext: Control)
groups, a bone-like tissue appeared around the cortical bone at 2 weeks after extraction, gradually grew toward the center of the extraction socket, and filled the socket by 8 weeks. (Fig 3)

**Histological analysis**
When the extraction socket was untreated, it was filled with blood clot accompanied with inflammatory cells at 1 week. At 2 weeks this clot was in part replaced with connective tissues. The formation of new bone from the host cortical bone began. After 8 weeks, blood clot was entirely replaced with the newly formed bone. The formation of blood clot was less in Gelatin group than that in Control group. In the extraction socket filled with the gelatin sponge, the bone formation was observed at 2 weeks. Though the gelatin sponge was remained until 4 weeks, it was completely absorbed by 8 weeks. Similar to Control group, the extraction socket was replaced with bone marrow and newly formed bone tissue. In Alginate group, as well as the other groups, the formation of bone tissue was observed from 2 weeks post operation; notably, absorption of the alginate scaffold had not yet been completed by 8 weeks, when mature woven bone started to take place of lamellar bone and bone marrow. However, Alginate group demonstrated that bone formation was observed not only from the cortical bone but also in peripheral of the residual alginate. (Fig 4, 5)

![Figure 3](image-url) Cross sectional images of rat mandibles by µCT. In all groups, a bone-like tissue appeared around the cortical bone at 2 weeks after extraction, gradually grew toward the center of the extraction socket, and filled the socket by 8 weeks. (Alg: Alginate, Gel: Gelatin, Ext: Control)
**Figure 4**  Cross sections of extraction sockets at 4 weeks and 8 weeks, stained with hematoxylin and eosin. In Ext group, blood clot was maintained till 4 weeks after extraction. At 8 weeks, it was replaced with the newly formed bone. Both Alg and Gel groups were represented in the sockets at 4 weeks later, however, the foreign body reaction could not be observed. Although Alg had remained till 8 weeks, the new bone formation around residue of Alg began to be observed.

(“: Alginate; #: Gelatin, +: blood clot. Bar = 300 µm)

**Figure 5**  Cross sections of extraction sockets at 8 weeks, stained with alizarin red and alcian blue. The newly formed bone around remaining Alg appeared dark blue. (”: Alginate. Bar = 300 µm)
DISCUSSION
Many kinds of wound dressings are commercially available and their effectiveness has been reported. In this study, the alginate sponge promoted the hemostasis after tooth extraction as well as the gelatin sponge, which has been widely used for the hemostasis of bleeding wounds.\(^1\)

The bleeding time in Alginate group was almost 54% less than that in Control group. Alginate, represented by Kaltostat, is an absorbent hemostatic embolizing material for skin wounds and is also licensed for use as an absorbent hemostatic cavity dressing in soft tissues. Jarvis et al. found calcium alginate rapidly released calcium ions in exchange for sodium ions on contact with blood, pushing up platelet activation.\(^4\)
Calcium alginate needs to be crosslinked with divalent cations in order to form stable hydro gel. On the other hand, the alginate sponge without calcium ions, prepared in this experiment, was not crosslinked; however, it expressed the hemostatic effect, which might be elicited by its porous structure easy to hold the hemorrhage as a reservoir.

The process of the healing of an extraction socket is composed of three phases: 1) formation and maturation of blood clots, 2) infiltration of fibroblasts to replace the coagulum and 3) bone tissue formation.\(^10\) From the results of radiographic and histological analyses, there was no difference in the bone healing in the tooth extraction socket among the three groups. Matthew IR et al. showed when Kaltostat was implanted to tooth extraction socket, it delayed wound healing and elicited a foreign body reaction,\(^11\) and when this calcium alginate was implanted between bone and periosteum, immunoreactions persisted up to 12 weeks after surgery.\(^5\) Rosdy and Clauss found Kaltostat was cytotoxic to fibroblasts and epidermal cells by direct contact or via the extraction medium in vitro.\(^12\) Because high calcium concentration was reported to inhibit the growth of cells in vitro,\(^13\) the cytotoxicity of Kaltostat might be considered partially due to the high Ca\(^{2+}\) release. Consequently, Kaltostat, which consists of non-woven sodium calcium alginate fibers, is well known to give rise to a florid foreign body giant cell body reaction in vivo.\(^5,6,11\) Therefore, the cytotoxicity by Ca\(^{2+}\) might cause a long time foreign body reaction. Since the alginate sponge prepared in this study contained less or almost no calcium, it did not induce a chronic inflammation and the socket filled with alginate sponge looked healing normally. However, the retained remnants of alginate sponge were found even at 8 weeks after surgery. It remains unexplored to manipulate to adapt biodegradation of this material.

Generally, alginate has given an optimal condition to cartilagenic cells to grow and has been used extensively as the scaffold for cartilage tissue engineering.\(^14,15\) It was noteworthy that our extraction socket receiving alginate was finally filled with osteoblasts and bone rather than cartilage tissue, suggesting that there was less or no cartilagenic cells in the socket and that alginate might be considered as a candidate for not only cartilage but also bone generation. This finding indicated that the progenitor cells of osteoblasts could migrate into alginate sponge and differentiate.

In conclusion, success of local hemostasis is indeed required for the patients under oral anticoagulant treatments. Our alginate sponge demonstrated the potentiality of an alternative embolization material for tooth extraction socket and extensive studies of the material are required.

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REFERENCES