Development of a new biodegradable operative clip made of a magnesium alloy: Evaluation of its safety and tolerability for canine cholecystectomy

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Background. *Operative clips used to ligate vessels in abdominal operation usually are made of titanium. They remain in the body permanently and form metallic artifacts in computed tomography images, which impair accurate diagnosis. Although biodegradable magnesium instruments have been developed in other fields, the physical properties necessary for operative clips differ from those of other instruments. We developed a biodegradable magnesium-zinc-calcium alloy clip with good biologic compatibility and enough clamping capability as an operative clip. In this study, we verified the safety and tolerability of this clip for use in canine cholecystectomy.*

Methods. *Nine female beagles were used. We performed cholecystectomy and ligated the cystic duct by magnesium alloy or titanium clips. The chronologic change of clips and artifact formation were compared at 1, 4, 12, 18, and 24 weeks postoperative by computed tomography. The animals were killed at the end of the observation period, and the clips were removed to evaluate their biodegradability. We also evaluated their effect on the living body by blood biochemistry data.*

Results. *The magnesium alloy clip formed much fewer artifacts than the titanium clip, and it was almost absorbed at 6 months postoperative. There were no postoperative complications and no elevation of constituent elements such as magnesium, calcium, and zinc during the observation period in both groups.*

Conclusion. *The novel magnesium alloy clip demonstrated sufficient sealing capability for the cystic duct and proper biodegradability in canine models. The magnesium alloy clip revealed much fewer metallic artifacts in CT than the conventional titanium clip. (Surgery 2016;■:■-■.)*

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THE OPERATIVE CLIP IS ONEof the most common instruments for vessel ligation in abdominal operation. Especially in the case of an endoscopic operation, it often is used instead of suture. Operative clips are usually made of pure titanium (Ti) or Ti alloys, which are strong enough to ligate vessels. However, Ti instruments have several drawbacks in this field. As Ti instruments remain in the body permanently, there may be complications associated with foreign-body reaction.[1-3] In addition, they can cause allergic symptoms. Ti can induce clinically relevant hypersensitivity in patients who are exposed to Ti chronically.[4] They

also form artifacts in computed tomography (CT), which makes it difficult to diagnose the area around the clips accurately. To overcome these disadvantages, biodegradable polymer clips were developed in the early 1990s.[5-8] However, the application of these clips was limited because of their large size and weaker strength.

Unlike Ti, magnesium (Mg) has good biodegradability and is an essential element for the human body. However, pure Mg showed poor ductility and corrodes too rapidly in a physiologic environment,[9] which results in excess production of hydrogen gas.[10-13] To compensate for this weakness, a Mg alloy is being developed as a material for biodegradable instruments. By adding some elements and removing trace impurities, an Mg alloy can become sufficiently biodegradable for use in operative instruments. Recently, treatment results of biodegradable instruments made of Mg alloy including bone screws, ligaments in orthopedics, and dental implants in oral maxillofacial operation were reported.[14-18] However, the physical properties of the Mg alloy necessary for use as operative clips differ from those of other instruments. Besides suitable biodegradability, it requires the sufficient ductility and mechanical strength for applying tissue ligation. This is the reason why the existing Mg alloy could not be used for operative clips. In this regard, by choosing the proper additive elements through first-principles calculations followed by experimental evaluation,[19] removing trace impurities, controlling the microstructure of the alloy, and a finite element analysis for occluding a blood vessel in a model, we have successfully developed a new Mg alloy clip.[20]

The aim of this study was to verify the safety and the tolerability of our newly developed Mg alloy clip for use in canine cholecystectomy.

METHODS

The study protocol was approved by the Institutional Animal Care and Use Committee of Shin Nippon Biomedical Laboratories (Approval ID: IACUC747-005). The study was carried out according to Shin Nippon Biomedical Laboratories animal experimentation regulation.

Materials. Selection of the alloying elements was based on our previous study using first-principles calculations followed by experimental evaluation.[19] As alloying elements, calcium and zinc promoted the deformation of magnesium in a relatively isotropic manner and enhanced the fracture toughness, resulting in good deformability at ambient temperature. The manufacturing method of the new Mg alloy clip has been described previously.[20] In brief, this new alloy consists of Mg-0.2 at .% zinc (Zn)-0.1 at .% calcium (Ca) and has sufficient mechanical strength and ductility for applying tissue ligation by controlling its microstructure.[20] In the previous study, the equivalent plastic strain distribution of the clip during occlusion was evaluated by finite element analysis using the material data of Mg-0.2 at .% Zn-0.1 at .% Ca alloy. The alloying of magnesium with essential elements, zinc and calcium, and the control of the microstructure by hot extrusion and annealing were conducted. Mechanical characterization revealed that the Mg-Zn-Ca alloy obtained by hot extrusion at 623 K followed by annealing at 673 K for 2 hours possessed a fracture strain >0.40. Details of the mechanical properties have been reported in the previous literature.[20] The Mg alloy clip used in this study is shown in Fig 1, *A*. The Ti clip (LIGACLIP Extra Ligating Clip, Medium, Ethicon Endo-Surgery, LLC, Cincinnati, OH) was used for a control (Fig 1, *B*). The reusable clip applier (Multi-Patient Single-Clip Applier, Ethicon Endo-Surgery, LLC) was used to apply the Ti and Mg alloy clips.

Animal preparation and anesthesia. Nine 1-year-old female beagles (Kitayama Labes Co Ltd, Yamaguchi, Japan) each weighing between 7.9−10.7 kg were used in this study. They were fed a standard diet and observed for a week to ensure good health. They were divided into 2 groups as follows: group I was treated with Mg alloy clips ($n = 6$) and group II was treated with Ti clips ($n = 3$). Three of the dogs in group I were observed for 3 months after operation, and the other 3 were observed for 6 months. All of the dogs in group II were observed for 6 months after operation.

After premedication with antibiotics and analgesics, medetomidine (0.08 mg/kg) and midazolam (0.3 mg/kg) were administered intramuscularly for tracheal intubation. Anesthesia was maintained by inhaled isoflurane for the duration of the procedure.

Operative procedure. The animals were placed in a supine position, and laparotomy was performed by upper median incision. The gallbladder was dissected from the liver bed from its fundus to the cervix, and the cystic artery was dissected using an electrical scalpel. After the gallbladder was retracted laterally to expose the triangle of Calot, the cystic duct was trimmed and ligated twice by Ti or Mg alloy clips. The cystic duct was dissected and the gallbladder was removed. After the procedure, complete hemostasis and no bile leakage was confirmed. The abdominal incision was closed in 2 layers.