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# Effectiveness of the sodium alginate as surgical sealant materials

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ABSTRACT

Developing biocompatible tissue adhesives is a highly desired goal of the tissue engineering due to adverse effects of the sutures. Sodium alginate is a natural linear polysaccharide and has biocompatibility, non-toxicity, non-immunogenicity, biodegradability, antimicrobial activity, and can be simply gelled with divalent cations and used in a number of biomedical applications. So, in this study, we use simple Na-alginate solution as surgical sealant for wound close. We prepared 0.3% (w/w) alginate hydrogel solution and in vivo and in vitro experiment was carried out. Surgical incision along with bleeding was made on mouse dorsal surface and then closed the wound using either alginate solution or traditional suture materials. After 1 week and 2 weeks we found that alginate sample effectively sealed the bleeding wound and promote tissue regeneration without the aid of other surgical/dressing tools. We hypothesis that this suture-free wound closure may be very useful for those wounds on which sutures are hard to be placed and where aesthetic appearance are concerned. The convenient handling procedures, tissue adhesion, and aesthetic view of the wound surface, make alginate a promising materials for sealing applications of surgical practice.

## 1. Introduction

Wound healing is a complex biological process, which comprises blood coagulation, inflammation, proliferation, and remodelling [1,2]. Current technologies for reconnecting and sealing tissues after surgical procedures such as sutures, wires, and staples have several limitations, particularly in minimally invasive procedures. For example, the use of suture for wound closure is time-consuming, may cause further tissue damage, result in infection, and excessive tension on the suture line and the surrounding tissue leads to tissue ischemia [3,4]. The application of surgical adhesives is a convenient alternative method for wound closure because of their characteristics, such as simple implementation procedure, shorter time, less painful to patients, and no need for removal. Toward this goal, various types of surgical materials have been used for sealing, reconnecting tissues, or attaching devices to the tissues [5]. Recently, extensive research efforts have been made to engineer biocompatible, biodegradable, and flexible sealants for the formation of leak-free closures in soft tissues [6–8]. Surgical sealants are commonly used to prevent leakage of fluid and/or gas from an incision. The sealant materials are required to be elastic and compliant to allow normal function and movement of elastic native tissues such as lungs, skin, blood vessel, and heart tissues. Sealants or surgical adhesives can be divided as biological, synthetic, semi-synthetic and biomimetic [9–12] Natural polymers includes chitin, chitosan, gelatin, collagen, alginate, etc [13–19]. Among those natural polymers, alginate is a naturally occurring anionic and hydrophilic polysaccharide. It is one of the most abundant biosynthesized materials [20–23], and is derived primarily from brown seaweed and bacteria. Due to its outstanding properties in terms of biocompatibility, biodegradability, non-antigenicity and chelating ability alginate is one of particular interest for a broad range of applications as a biomaterial and especially as the supporting matrix or delivery system for tissue repair and regeneration, drug delivery and in wound dressing [24–27]. Sodium alginate hydrogels was also found to have the highest bio adhesive property in vitro and in drug delivery [28,29]. Till now in the field of polysaccharide-based surgical sealants, chitosan [12], dextran [30] and chondroitin sulphate [31] were successfully used. However, bio-adhesives properties of alginate as surgical sealants were not yet evaluated. In the present study, we try to evaluate the functional efficacy of alginate hydrogel as surgical sealants.

## 2. Materials and methods

### 2.1. Materials

Sodium Alginate was purchased from Wako Pure Chemical Industries (Osaka, Japan). The preparation of alginate hydrogel has been previously described [21]. Briefly, 0.3%–1% (w/w) sodium alginate solution was prepared by dissolving into autoclaved distilled

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water. These solutions either soaked in calcium chloride solution (5% w/w) (Nacalai Tesque, Kyoto, Japan) or keep them in solution form for animal experiments. The obtained hydrogel were washed several times for further use.

### 2.2. Mechanical property analysis

Young's modulus was measured using Autograph (AGS-J, Shimadzu, Kyoto, Japan). To fit the autograph instrument, the alginate hydrogel was made into thick cylindrical shape with dimensions of 35mm□ × □35mm□ × 15 mm. The experiments were performed using a crosshead speed of 2.0 mm $min^{-1}$. After adjusting the experimental parameters five samples of each kind of gel were used for compressive experiment. The elastic modulus, E, was determined from the slope of linear dependence.

### 2.3. Chemical property analysis

The chemical structure characterization of alginate solution was conducted by infrared spectroscopy. The infrared spectra of alginate solution were measured with an FTIR spectrophotometer (Fourier Transform Infrared Spectrophotometer, FT/IR-6000, Jasco, Tokyo, Japan).

### 2.4. Animal experiments

ICR mice were purchased from CLEA Japan inc., (Tokyo, Japan). Animal experiment procedures strictly adhered to the Guidelines for Animal Experiments of Yamagata University and were carried out with the approval of the Animal Use and Care Committee of Yamagata University.

Jcl: ICR mice, weighting 32–33 g were used in this study. The animals were given general anaesthesia with 3% sevofrane (Maruishi Pharmaceutical Co., Ltd., Osaka, Japan). Subsequently, each mouse was sedated with an intramuscular injection of 10 mg $kg^{1}1$ xylazine hydrochloride (Sedeluck, Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan). Dorsum hair was removed with hair removal cream. After disinfecting with iodine, 2% lidocaine hydrochloride containing 1:80,000 epinephrine (Xylocaine Poly Amp 2%, Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan) was administered locally. Two skin defects with 3 mm × 10 mm in diameter were made on each side of the body. The control wound groups were sealed with traditional nylon (No. 6.0) suture materials (Natsume Seisakusho Co., Ltd、Tokyo, Japan). Experimental group were sealed with sodium alginate solution. Finally all wound covered with OPSITE◊ quick guard (Smith & Nephew plc, London, UK). All the experiment repeated twice (n = 6). Evaluation was performed at 1 and 2 weeks interval.

### 2.5. Macroscopic and histological evaluation

After 1 and 2 weeks, an overdose of anaesthetic (Pentobarbital sodium salt, Nacalai tesque, Inc., Kyoto, Japan) was delivered intraperitoneally to euthanize the mice Skin tissue. At first the optic wound dressing was remove from the wound area and digital photography of the wounds were taken and aesthetical evaluation was done. Finally wounds were collected for histological evaluation. The excised tissues were fixed with 20% phosphate-buffered paraformaldehyde (PFA) for 48 h. The fixed tissues were then embedded in paraffin wax to make a paraffin block. The specimens were sectioned into thicknesses of 6 μm using microtome. The sections were stained using hematoxylin eosin staining (HE staining) and masson's trichrome (MT) staining. All the samples were then observed with an optical microscope (BX53, OLYMPUS, Tokyo, Japan).

### 2.6. In vitro cell movement and wound scratch assay

For cell movement assay bone marrow derived MSCs were isolated from bone marrow of BALB/C1 mice (Charles River Laboratories, Japan)) as previously descried [32]. Briefly, MSCs were generated from tibia and femur bone marrow of 8 week-old mice. For wound scratch test mouse fibroblastic cell line L929 purchased from European Collection of Authenticated Cell Cultures (ECACC). Both type cells were cultured in basic medium alpha minimal essential medium (α-MEM, Wako pure chemical) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Invitrogen, CA) and 1% Penicillin/Streptomycin (PS, Nacalai Tesque). After seeding non-adherent cells were removed after 24 h, and adherent cells were harvested and re-cultured in 100 mm cell culture dish for 3–4 passages before using. Medium was replaced every 3 days. After 72 h culture scratch wounds were created and filled with alginate solution in the cell monolayer to evaluate the cell migration. Sample without adding alginate represent the control group. Scratch images were acquired after 30 min considered as 0 h. After 24 and 48 h the cells were fixed with 4% PFA followed by HE staining and took images. The scratch gap width at each time point in each group was measured at four different positions from three individual experiment and compared with the gap width at 0 h, which was arbitrarily set as 1.

### 2.7. Statistical analysis

Statistical evaluation of data was performed using the Microsoft Excel 2011. Tests were carried out in triplicate (n = 5) and all data are reported as mean ± standard deviation at a significance level of $*p \leq 0.05$, $**p \leq 0.01$ using one-way analysis of variance (ANOVA) and scheffe test.

## 3. Results and discussion

Bio-adhesives have been used in surgery as hemostatic and wound healing agents. It has been suggested that the bio adhesive used in surgical applications should contain a reasonable bonding strength to tissue in the presence of moisture [33]. Also, the flexibility of adhesive is important to permit it to conform to the adhered tissue. Additionally, prior to cure, the adhesive should be viscous enough to be easily applied to a specific tissue site without dissipating. Furthermore, the adhesive should be non-toxic and able to degrade in vivo. Finally, the presence of the adhesive should not interfere with normal progress of the natural repair process.

It is well known that human tissues possess high water content; e.g., skin is 45–50% water and muscles are 70–80% water [34]. Cell migration and proliferation are best facilitated in a suitable aqueous environment, not in dehydrated tissues [35]. To accelerate healing, ideal wound dressings should establish these optimal conditions for cell vitality; maintain adequate moisture and effective oxygen circulation [36]. Recent studies have shown that hydrogels can be used as wet dressings for skin wound healing and accelerate the healing process [23,35,37]. Hydrogels are cross-linked, hydrophilic materials made of natural or synthetic polymers. Due to their high water content and polymeric network structure comparable to native tissue matrix, hydrogels are widely used in tissue engineering applications. In the present study we focus on the alginate, a natural base biomimetic hydrogel materials for surgical tissue adhesion.

The elasticity/compliance of a sealant is critically important; particularly on dynamic structures or organs within the body. In this work we have prepared two different concentrated alginate solutions, 0.3% and 1% (w/w). Fig. 1. A showed the representative pictures of 0.3% (w/w) sodium alginate solution. To understand the mechanical strength of the alginate solution concentration, elastic modulus was measured. The elastic moduli of the prepared alginate gel altered according to the alginate concentration: they increased from around 3.7 to 21 kPa when

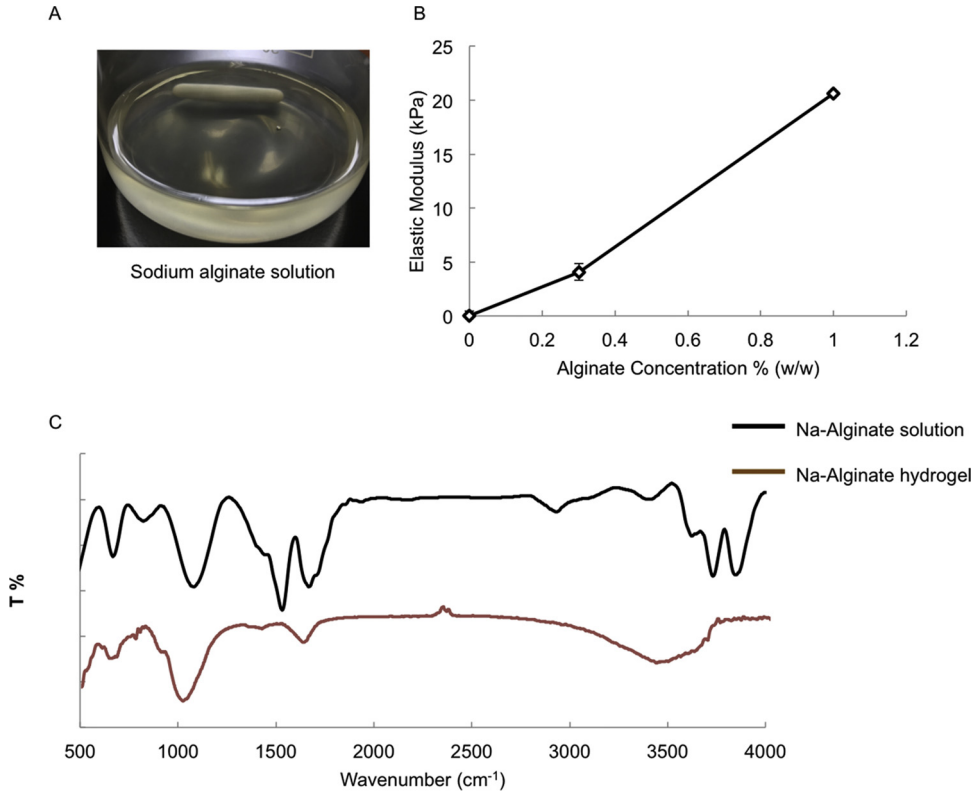


**Fig. 1.** A). Prepared alginate hydrogel solution. B) Elastic modulus (4–21 kPa) of gel increased as alginate concentration increased (0.3–1 wt%) (n = 4). C) FTIR image of sodium alginate hydrogel and solution.

the alginate concentration was increased from 0.3 to 1% (w/w) (Fig. 1B). This range was set on the basis of the Young's modulus of biological tissue (e.g., 1 kPa in embryonic tissue and brain, muscles are around 10 kPa and 60 kPa are immature bone tissue) [38]. Also a lower modulus is indicative of a sealant that is less stiff and more compliant, as compared to a sealant with high modulus. For that reason we choose 0.3% (w/w) alginate solutions with 3.7 kPa elastic moduli for further experiment.

To confirm the intermolecular bonding of the alginate, FTIR of the alginate solution as well as hydrogel were performed. The data showed that both solution and gel had the characteristic peak for carbonyl group at round 1018–1,037, 1384–1,519, and 1590–1639 $cm^{-1}$ and peak showed for OH- group at 3380–3718 $cm^{-1}$ (Fig. 1C).

The term bioadhesion can be generally defined as the adhesion or contact between two surfaces, with one being a biological substratum [39]. If one of the surfaces involved is a mucosal layer, the term mucoadhesion is then used [40]. Studies have shown that polymers with charge density can serve as good mucoadhesive agents [40,41]. Alginate, with its carboxyl end groups, is classified as an anionic mucoadhesive polymer. In alginate muco-adhesion studies, it has been found that alginate has the highest mucoadhesive strength when compared to polymers such as polystyrene, chitosan, carboxymethylcellulose and poly (lactic acid) [41]. Restoration of tissue integrity and homeostasis following the surgical procedure is of vital importance, as the integument provides the first barrier against invading microbes and pathogens as well as for the aesthetic appearance. To evaluate the wound healing efficiency, wound were closed with either nylon suture or 0.3% alginate solution for predetermined periods (Fig. 2A). Alginate hydrogel are commonly formed by adding of alginate solution into an aqueous solution of calcium ions. In the present experiments, to receive sufficient $Ca^{2+}$ ions to promote alginate gelation, we induced bleeding on wound surface (Fig. 2A), taking into account that in mouse serum mean values of calcium is 8.40 + /-1.09 (mg %) [42].

The general appearance and clinical course of all animals after surgery was good, with no apparent disease was detected. At the end of the surgical procedure when suture or adhesives were used, most of the incisions in the mice skin were closed in full by primary closure. However, after excised the tissue, it was observed that the wounds surface sealed with alginate heals much uniformly than those in the control group. On day 7, in alginate sealed group, no persistent wound could be observed. However in traditional suture group clear skin lesion were still visible. And also after stretching, it was found that the suture sealed wound was not closed completely (Fig. 2B). On day 14, no surgical scratch marks were visible in the wound sealed with alginate. However, wound sealed with nylon thread showed clear surgical scratch with thickening of the epidermis and dermis layer (Fig. 2C). The healing efficiency was further evaluated at the microscopic level using histological staining. MT staining, which can stain collagen blue, nuclei black, muscle and blood vessels red, was used to visualize the cells and analyze collagen deposition. After 1 week, microscopic observation of the wound exhibited deeper wound in suture sample compare to alginate sample (Fig. 3A). Even the epithelium layer was presence in both control and experimental group, surrounding tissue were markedly elevated and prominently roughs in suture sample compare to the alginate sample. In both cases abundant granulation tissue, composed of bundles of collagen, neovascularization along with infiltration of the inflammatory cells was observed (Fig. 3A). Microscopic images of the wounds on 2 weeks are shown in Fig. 3B. Thicker and uniform epidermis layer reconstructed by the keratinocytes and fibroblast resemble to normal tissue was detected in alginate group. Collagen underlying the epidermis was also better organized and more compact as higher collagen density can help reconstruct the extracellular matrix and further support skin tissue regeneration (Fig. 3B). However, the wound sealed with suture showed elevation of the epidermis surrounding the wound with thin collagen layer. More interestingly, the suture sample showed the remained residual wound gap with inflammatory cells infiltration (Fig. 3B). These results suggested that wound sealed with

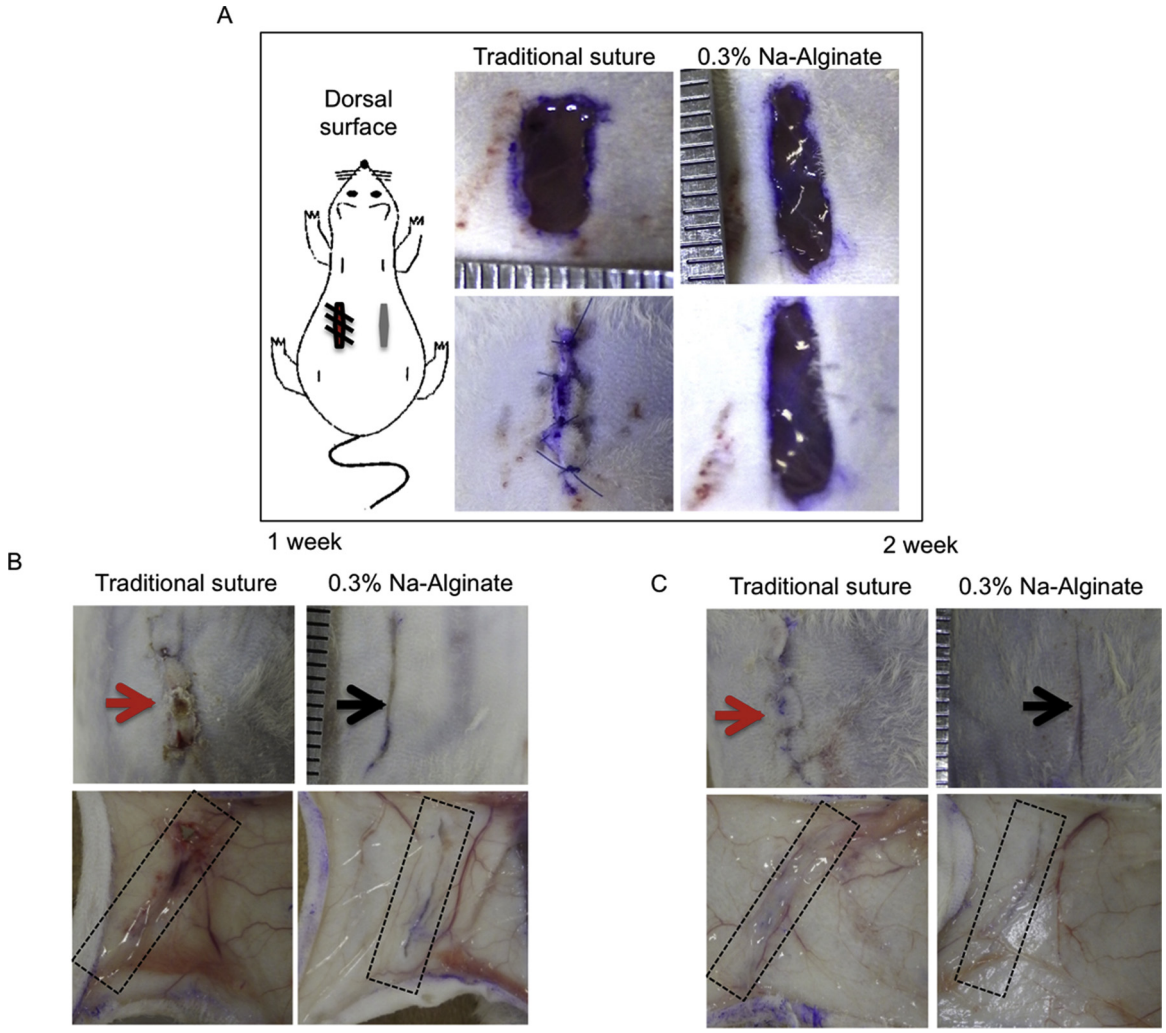


**Fig. 2.** A. Method and place of wound creation and sealing using alginate solution and traditional suture. Macroscopic photographs of wounds condition at each time point. A) 1 st week; B) 2nd week.

alginate promote faster wound healing process compared to the suture group.

Histological analysis using Masson's trichrome staining confirmed our microscopic observations that alginate could better facilitate wound closure, skin epithelialization, hemostasis and collagen distribution (Fig. 3A, B). These effects may be associated with the unique properties of alginate, including its high water content, nonfouling properties, and biocompatibility and biomimetic nature [21–23]. Finally, to confirm

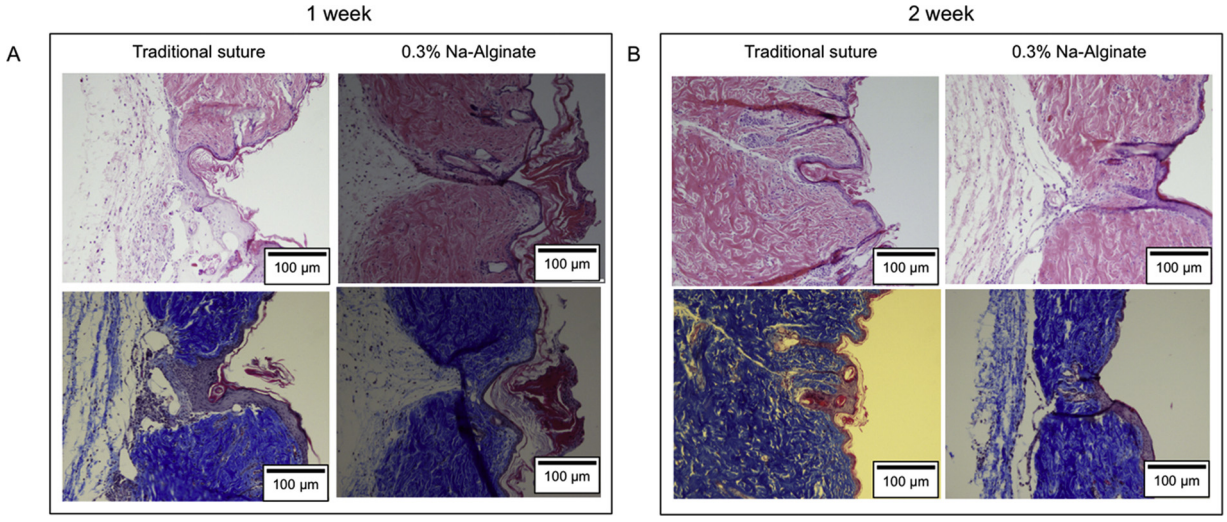


**Fig. 3.** HE and MT staining images of suture and alginate group wound at 1 week, A) and 2 weeks B). (Scale bar 100 µm).

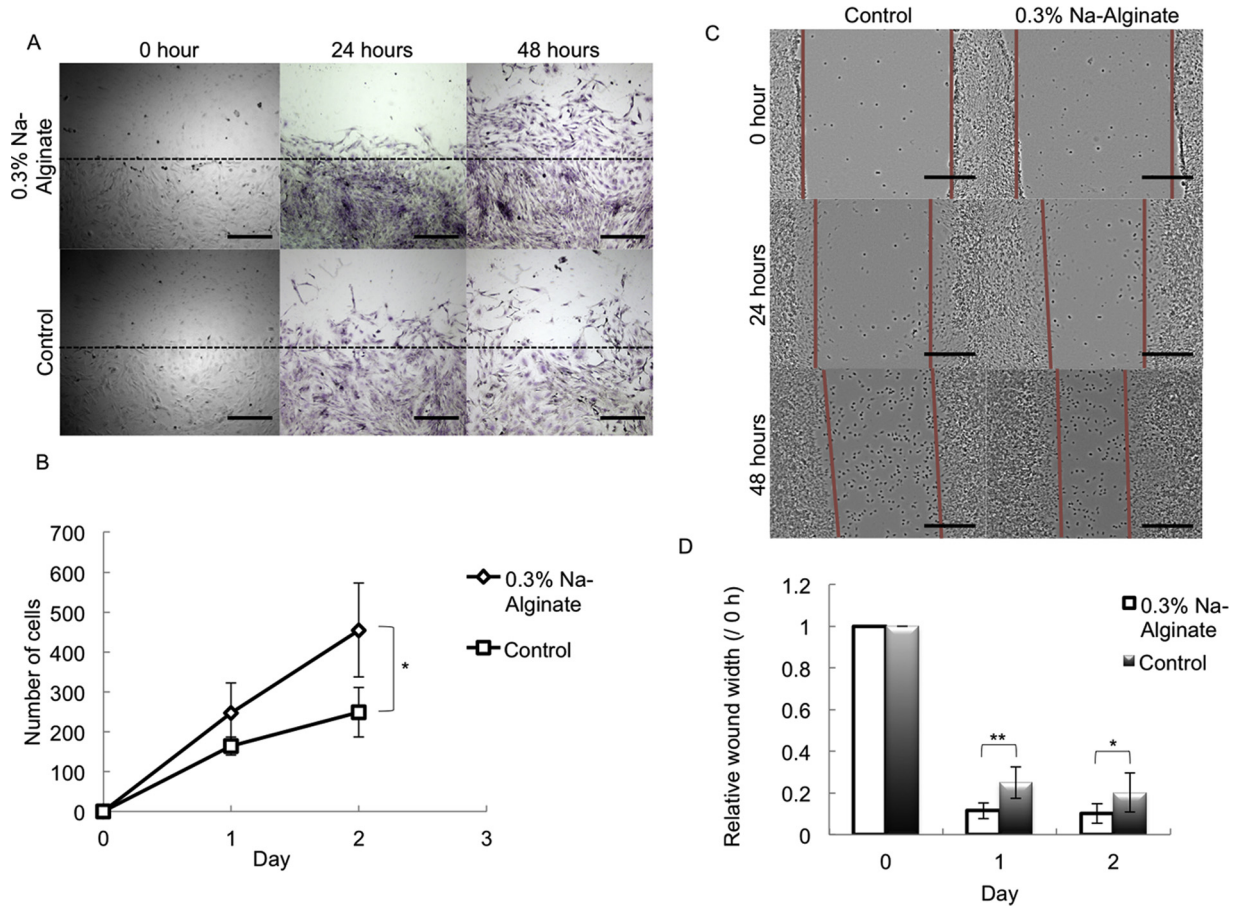


**Fig. 4.** Histological images (A) and graph depicting cellular migration and the number of migrated cells in vitro cell movement assay (B). (Scale bar 100 μm; * $p \leq 0.5$). Phase contrast images of wound scratch test (C) and the graph indicating the relative wound size at different time point (D). (Scale bar 1 mm; * $p \leq 0.5$; ** $p \leq 0.001$).

the animal results we evaluate the cell migration towards the alginate using simple in vitro cell movement and wound scratch test (Fig. 4A–D). Time series (0, 24 and 48 h after wounding) showing that the cellular migration and cell numbers were higher in the scratch filled with 0.3% alginate solution (Fig. 4A, B) compare to the control group. Similarly in wound healing assay, 0.3% Na-alginate solution promoted wound closure compare to the control (Fig. 4C, D). This result suggests that alginate can facilitate cell movement. And also alginate provides a better surrounding microenvironment so the cell proliferation also increased.

Basing on the above results, we hypothesized that alginate hydrogel can also used as surgical sealant. In vivo studies confirmed the effectiveness and convenience of using alginate in wound closure and bleeding control and in vitro studies disclosed the role of alginate in cell movement and proliferation.

To the best our knowledge, this is the first report about alginate as tissue adhesives that could effectively seal a bleeding wound, stop bleeding, and promote tissue regeneration without the aid of other surgical tools, such as suture and staples, or other adhesive mechanisms. This suture-free wound closure may be particularly very useful for those wounds on which sutures are hard to be placed and where aesthetic appearance are concerned. The convenient handling procedures, tissue adhesion, and aesthetic view of the wound surface, make alginate a promising materials for topical applications of surgical practice.

## 4. Conclusions

In summary the experimental results presented above demonstrate the effectiveness of sodium alginate solution as a new wet surgical sealants, that is safe and inexpensive constituents and via a one-step synthesis technique without involvement of any toxic reagents, and their applications in hemostasis and scar free wound closure.

## Conflict of interest

All the authors have approved that there are no conflicts of interest to declare.

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