[[Original Investigation]]

Closure of Long Surgical Incisions with A Novel Hemostatic Tissue Adhesive in Porcine Skin Model

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ABSTRACT

Objective: Skin adhesives offer many advantages over traditional wound closure devices. Recently, the current research group reported novel tissue adhesives composed of natural polymers (gelatin and alginate), which are biocompatible with mechanical properties suitable for tissue adhesion. The objective of the present study was to conduct clinical and histologic assessment of this hemostatic bioadhesive in the healing

of long skin incisions (≥4 cm) in comparison with traditional and commercially available methods.

Methods: Researchers created 24 long incisions on the ventral side of two domestic pigs to compare four different treatment modalities: two novel topical bioadhesives based on gelatin and alginate combined with hemostatic agent Kaolin, nylon sutures, and commercial tissue adhesive N-butyl-2-cyanoacrylate. The bioadhesive compounds were spread on the incision surface and then either mixed manually or by using a double-headed syringe. After 14 days, clinical and histologic measurements were performed to evaluate the healing phase of the wounds.

Results: The formulation that contained a relatively low crosslinker concentration demonstrated superior results to the formulation that contained a standard crosslinker concentration. However, no significant statistical differences were observed compared with the two control incisions (sutures and commercial adhesive N-butyl-2-cyanoacrylate). This was verified by immunohistochemical analysis for epithelial integrity and scar formation as well as by clinical assessment.

Conclusions: This newly developed bioadhesive demonstrated suitable properties for the closure of long incisions in a porcine skin model.

Keywords: bioadhesive, cyanocrylate, hydrogel, incision, kaolin, skin, wound closure, wound healing

INTRODUCTION

Every year, millions of people suffer traumatic wounds, such as skin lacerations, or surgical wounds that cause disruption of organs, connective tissue, muscles, and tendons.[1] Although small skin lacerations (<4 cm) can heal spontaneously without intervention, large skin lacerations (≥4 cm), especially those that are irregular shaped or deep are more complex and may exhibit impaired or delayed healing. In these cases, sutures are the traditional treatment for wound closure and bleeding control because of their high tensile strength and low dehiscence. However, inconvenience, pain, relatively slow handling, the need for removal in some cases, and concern over possible disease transmission through the use of needles are major disadvantages of suturing. Other techniques have been suggested to address these issues, including the use of clips, staples, tapes, hemostasis agents, and tissue bioadhesives.[2,3] Although tissue bioadhesives are an adequate solution for treating small lacerations, their use is limited in larger wounds mainly due to lower strength and flexibility and cytotoxicity issues[4].

Tissue bioadhesives represent a group of compounds that can be applied locally for a variety of indications, including bleeding control, wound closure, hemostasis, sealing air and body fluid leaks, repair of fistulas and aortic dissections, external fixation of devices, and drug delivery.[5,6] N-butyl-2-cyanoacrylate, fibrin, and

polyethylene glycol-based bioadhesives are among the most commonly used surgical bioadhesives. However, none of them satisfy the requirements of an ideal bioadhesive including both adequate mechanical properties particularly in wet environment, together with high biocompatibility.

Cyanoacrylates are the most widely used FDA-approved bioadhesives in clinical practice.[7] Cyanoacrylates have a high bonding strength to biological tissues and rapid curing time and are easy to use. However, they have been limited to external or temporary applications because of the toxicity of its degradation byproducts, its low viscosity, and its high stiffness. All of the above can cause adhesion failure, tissue irritation, and inflammatory responses.[1] N-butyl-2-cyanoacrylate is rarely used for long incisions, but only as a final touch on top of intracuticular suturing.

Extensive efforts are therefore underway to develop a safer, nontoxic, degradable, and hemostatic bioadhesive. Recently, the present research group developed a novel bioadhesive formulation based on a combination of gelatin and alginate crosslinked with carbodiimide (EDC) and loaded with the hemostatic agent kaolin.[8,9]

Gelatin is a biocompatible, biodegradable, water-soluble polypeptide that is obtained from collagen.[10] It is popular in medical applications such as tissue

bioadhesives, drug-delivery systems, and wound dressings.[11] Alginate is a naturally occurring polysaccharide, extracted from brown algae.[12] It is biodegradable under normal physiologic conditions and its high and controllable gel porosity makes it a good candidate for protein and cell delivery.[13] However, to maintain the mechanical properties of gelatin and alginate gels, they need to be crosslinked with an appropriate crosslinking agent.

The cross-linking reaction of this system is achieved using N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC). This is a zero-length crosslinker that creates the cross-linking reaction and leaves urea as a byproduct, which is much less toxic than formaldehyde and glutaraldehyde (as are formed with cyanoacrylate use). Previous studies by this research group[11-13] indicated that a formulation containing 400 mg/ml gelatin, 10 mg/ml alginate, and crosslinked EDC has high potential for wound-closure applications, because of its relatively high bonding strength, burst strength (sealing ability), and strength of the bulk material, and suitable gelation time and viscosity. This formulation was also found to be biocompatible with low cytotoxicity in vitro and in vivo.[14-16] Further studies also succeeded in lowering the concentration of the crosslinker agent, using N-hydroxysuccinimide (NHS), without decreasing the tissue-bonding strength.[17]

Based on the researchers' previous in vitro studies, in the present study they tested two bioadhesive formulations (A and B)[17,18] to evaluate the effectiveness of the novel gelatin-alginate-EDC-based bioadhesive in the healing process of skin incisions in a porcine skin model. Whereas most studies of bioadhesives have focused on skin closure of short wounds, this report focuses on the results of an in vivo study comparing the novel investigational tissue adhesive with sutures and other commercially available skin-closure devices for epidermal closure of long (≥4 cm) surgical incisions.

METHODS

Materials

Gelatin "type A" from porcine skin (90-110 g bloom), low viscosity alginic acid sodium salt, EDC, NHS, and kaolin (K1512) were purchased from Sigma-Aldrich, Rehovot, Israel.

Preparation of Tissue Bioadhesives

Preparation of the bioadhesives was based on dissolving 400 mg/mL gelatin and 10 mg/mL alginate (Gel-Al) together with the hemostatic agent powder (3% w/v kaolin) in distilled water, under heating up to 60 °C in order to create homogenous hydrogel. The crosslinking agents (EDC and NHS) were added to the Gel-Al solution containing the hemostatic

agent by two different methods. In the first method, the polymer solution and crosslinking agent solution were loaded in separate syringes and mixed in the incision site. In the second method, a double-headed syringe was used to mix the two solutions prior to application in the incision site.

Two formulations were used: Formulation A (standard) contained 20 mg/mL EDC, and formulation B (low EDC content) contained 10 mg/mL EDC and 1 mg/mL NHS (Table 1). Both formulations exhibited similar ex vivo bonding strengths of approximately 30 KPa.[18]

Animal Model and Surgical Procedures

Porcine skin is anatomically and physiologically similar to human skin; both have a thick epidermis and a similar dermis-epidermis thickness ratio.[19] They also both have well-developed epithelial extensions that project into the underlying connective tissue (rete pegs), papillary bodies, similar dermal collagen, and rich subdermal adipose tissue.[19] The size, orientation, and distribution of blood vessels in the pig dermis are similar to blood vessels in human skin. Functionally, porcine and human skin are similar in terms of epidermal turnover time, type of keratinous proteins, and lipid composition. In addition, human and porcine skin heal through similar physiologic processes. Asa result, the porcine is an excellent animal

model for the assessment of post-trauma wound healing agents destined for use in human wounds.

Animal handling was conducted in accordance with national guidelines and was approved by the Institutional Review Board and the Institutional Committee on Animal Use, Rappaport Faculty of Medicine, Technion, Israel Institute of Technology.

The study was performed on two large white juvenile domestic swine (*Sus scrofa domestica*) weighing 60 and 55 kg. The study was conducted in two stages, involving one pig each time, allowing for a staged assessment of the effects of adhesive on porcine subjects. The animals were purchased from the Animal Research Institute, Kibbutz Lahav, Israel. They were housed in individual pens with an artificial 12-hour light/dark cycle and constant temperature. The animals were acclimated for 1 week prior to the study and were fed standard chow and water ad libitum.

During the 14-day follow-up period, the animals were examined daily for the following signs: food and water intake, urine/feces, general appearance, and behavior. The nutrition state, integument, eyes, nose and mucosa membrane, lymph nodes, respiratory tract, cardiovascular system, digestive system, mammary glands, and nervous system were monitored once a week.

On the day of the experiment, the animals were anesthetized with an intramuscular injection of ketamine (20 mg/kg) and ACP1 (1 mg/kg), followed by induction with propofol (5-7 mg/kg). After intubation, anesthesia was maintained with isoflurane 2% delivered by PPV (Pulse Pressure Variation) plus Fentanyl (5-10 mcg/kg/h). The ventral skin surface of the animals was shaved using an electric shaving machine. The skin was then disinfected using a septal scrub (chlorhexidine disinfectant) and 70% ethanol.

Twelve 10-cm-long incisions were made in each animal using a #10 blade.[20] The 24 incisions were divided into the following treatments: sutures, commercial N-butyl-2-cyanoacrylate, formulation A, formulation B, formulation A using a double-headed syringe, and formulation B using a double-headed syringe. Sutured incisions (10 sutures per incision using interrupted 3-0 nylon) and incisions treated with commercial N-butyl-2-cyanoacrylate served as positive controls. The 12 incisions performed on the first animal were divided equally, with two incisions for each of the above treatments. The 12 incisions performed on the second animal were divided as follows: two incisions were treated with sutures, two were treated with commercial N-butyl-2-cyanoacrylate, four were treated with formulation B and four were treated with formulation B using a double-headed syringe.

For all incisions treated with N-butyl-2-cyanoacrylate or the tested bioadhesive, the material was applied into the incisional gaps. Mechanical pressure was then applied for 30 seconds to hold the two adjacent edges of the incision. Following this, researchers applied skin closure strips on each wound and secured a large bandage with surgical staples. After the surgical procedures, the animals were treated with Tramadol (100 mg once a day) and Optalgin (500 mg twice a day) for three days.

The animals were anesthetized again at the endpoint, 14 days post-operation. The incisions were photographed documented, and 1 cm biopsies were taken from the center of each wound and immediately fixed in phosphate-buffered formalin for histological and immunohistochemical analysis. The animals were then sacrificed in the standard procedure by receiving an overdose of 5% isoflurane for 5 minutes followed by KCL (potassium chloride) IV.

Histologic Analysis

The skin biopsies that were fixed in phosphate-buffered formalin were dehydrated with an increasing alcohol gradient. The biopsies were then embedded in paraffin and 5-µm thick sections were made using a Leica microtome. The slides were deparaffinized and hydrated with a decreasing alcohol gradient. The sections were then taken for standard hematoxylin and eosin or trichrome stain (Gomori Kit,

Sigma-Aldrich), and were analyzed according to the manufacturer's instructions.

The sections were observed and photographed under ×200 and ×400 magnification using an Olympus upright light microscope. Healing analysis was conducted in a double-blind manner by four separate evaluators using a quantitative grading system. The sections were evaluated based on structure and content. The healing criteria examined included epithelial confluence, epithelialization, clinical collagen assessment, scar width, and mononuclear cell infiltrate. Criteria were graded on a scale of 0 to 5: 0 = absence, 1 = minimal presence, 2 = mild presence, 3 = moderate presence, 4 = high presence and 5 = extensive presence.The presented score is the average of the four evaluators.

Immunohistochemical Analysis

Immunohistochemical analysis was performed on formalin-fixed paraffin sections. The slides were deparaffinized and hydrated with a decreasing alcohol gradient and immersed in distilled water. The following antibodies were used: anti-laminin antibody (Abcam, ab11575), Ki-67 antibody (Zymed Laboratories, 7B11), and anti-α smooth muscle actin (anti-αSMA) antibody (Abcam, ab5694).[21–23] For anti-laminin staining, antigen retrieval was performed using 1 mM Tris-EDTA buffer solution (pH 8) at 90 °C for 13 min, followed by

proteinase K digestion at 37 °C for 10 min. For anti-αSMA and Ki-67 staining, antigen retrieval was performed using 1 mM Tris-EDTA buffer solution (pH 8) at 90 °C for 20 minutes. The sections were then blocked with suitable serum for 30 minutes, followed by 14 hours of incubation at 4 °C with the primary antibody. This was followed by incubation with an appropriate biotinylated secondary antibody, streptavidin-peroxidase conjugate, and S-(2-aminoethyl)-l-cysteine as substrate (Histostain-SP kit; Zymed Laboratories). Counterstaining was performed with hematoxylin and the slides were examined under a light microscope.

The evaluated criteria were proliferation index, scar tissue formation, and basement membrane integrity. The integrity of the newly formed basement membrane was determined by evaluating the percentage of anti-laminin staining in the scar area.[22,23] The proliferation index of the epidermis was quantified in the scar area as the percentage of Ki-67-positive cells to measure keratinocyte activation. Scar formation was evaluated by counting anti-αSMA positive myofibroblasts in high-power fields (average of 5 fields). Anti-αSMA stain of hair follicles and in smooth muscle of vessels was not counted in the analysis.[22] All evaluations were performed by two observers in a single-blind trial under a light microscope.

Ex-Vivo Bonding Force of the Healed Skin Sections

After the 14-day follow-up period, the skin area containing closed incisions (sutured or attached using bioadhesive) was harvested under general anesthesia, using a sterile #10 scalpel blade, and was cut into 5 × 2 cm sections for the tensile force test using a 5500 Instron Universal Testing Machine with a 100 N load cell. The two parts of the skin samples were strained at a constant velocity of 10 mm per minute until separation was achieved. The bonding force was defined as the maximum strength in the force-displacement curve measured by the Instron Merlin Software. At least two repetitions were carried out for each formulation.

Statistical Analysis

Means and standard errors of the mean (SEMs) were calculated for the histologic scoring and the immunohistochemical analysis and SD was calculated for the bonding force analysis. Differences between means were analyzed for statistical significance using a one-way analysis of variance with the Tukey-Kramer multiple comparisons posttest (SPSS version 17.0, IBM Corp). *P* values ≤ .05 were considered significant.

RESULTS

Clinical Evaluation

Overall, the animals tolerated the experimental procedure well and did not show signs of distress or systemic or local inflammation. Fourteen days post-operation, macroscopic photographs were taken and clinical evaluation was performed.

Four 10-cm incisions (two in each animal) were sutured and served as a control. All four incisions demonstrated good clinical appearance with a satisfactory healing process and no signs of inflammation (Figure 1). Four additional incisions served as the N-butyl-2-cyanoacrylate control. In two of the four incisions a scab was formed and the overall healing process was delayed. However, the other two incisions demonstrated a good healing process. The two under-healed incisions may be explained by the cytotoxic nature of N-butyl-2-cyanoacrylate and its byproducts.

Formulation A and formulation A using a double-headed syringe were applied on two incisions each. The overall appearance demonstrated a poor healing process that did not progress into a stable adhesion of the two adjacent incision lips. Scab formation was observed in all four incisions (Figure 1).

Formulation B demonstrated a good healing process in the first animal. Therefore, the researchers elected to use it in four incisions in the second pig. Five of the six incisions treated with formulation B demonstrated a

satisfactory healing process, with good skin contact, minimal scabbing, and no inflammation process (Figure 1). One incision failed to heal properly, with an apparent scab formation. Formulation B was also applied using a double-headed syringe with a built-in stirrer in six incisions. In three of the six incisions, the clinical appearance was satisfactory with a good healing process. However, three incisions failed to heal properly and a scab formed. The relatively inferior results obtained in the three incisions treated using a double-headed syringe may be explained by a poor mixing process of the polymer solution and the crosslinker solution.

Histologic Evaluation

At the study endpoint, 1 cm biopsies were taken from each incision. Hematoxylin and eosin and Gomoris trichrome staining for collagen fibers were performed. Clinical photographs of representative incisions from the various treated groups are presented in Figure 2. The following criteria were independently evaluated by four observers: epithelial confluence, epithelialization, clinical collagen assessment, scar width, and mononuclear cell infiltrate.

Figure 3 presents a cumulative graph demonstrating the superiority of formulation B and formulation B using a double-headed syringe in comparison with formulation A. Formulation B and formulation B using a double-headed

syringe were superior in all tested parameters compared with formulation A, including improved organization of the epithelium, better epithelialization, less mononuclear cell infiltrate, less collagen deposition, and smaller scar width. Formulation B and formulation B using a double-headed syringe received general scores of 12.7 ± 2 and 10.1 ± 2.2, respectively, compared with formulation A and formulation A using a double-headed syringe which yielded general scores of 4.2 ± 0.9 and 4.9 ± 2, respectively. The scar width of the incisions treated with formulation B did not differ significantly from the control incisions that were sutured (12.7 ± 2 and 13.2 ± 3, respectively). Incisions treated with formulation B had a non significantly lower score compared with the incisions treated with N-butyl cyanoacrylate (12.7 ± 2 and 11.7 ± 1.3, respectively). Figure 4 presents the histologic scores for all criteria. No significant differences were found in the collagen organization between incisions treated with formulation B, sutures, and N-butyl cyanoacrylate. Both formulation A with and without the use of a double-headed syringe and formulation B with the use of a double-headed syringe demonstrated less-organized collagen fibers, probably as a consequence of poor wound healing.

Immunohistochemical Analysis

Immunohistochemical staining to laminin, αSMA, and Ki67 was performed to evaluate the healing process. For basement membrane integrity analysis, it demonstrated a nonsignificant superiority of treatment with formulation B (89%) compared with sutures (79%) and N-butyl cyanoacrylate (85%). Formulation A demonstrated a significant decrease in laminin expression compared with formulation B (55 vs 89, respectively, $P < .005$). Surprisingly, formulation A using a double-headed syringe demonstrated elevated laminin expression (Figures 5 and 6).

Scar formation was evaluated by counting anti-αSMA positive myofibroblasts. Myofibroblasts are key players in the reconstruction of connective tissue after injury and in generating scar fibrosis, which means a less favorable scar. Both formulation B and formulation B using a double-headed syringe demonstrated less αSMA expression compared with both A formulations. No differences were found between incisions treated with formulation B, sutured incisions, or incisions treated with N-butyl cyanoacrylate. Ki-67 staining, a marker for epidermal proliferative basal layer, was performed to determine the proliferation index of the epidermis and to measure keratinocyte activation. No differences were found between all incisions. Nevertheless, the sutured incisions (control) demonstrated a significantly higher proliferation index compared with

incisions treated with either N-butyl cyanoacrylate or bioadhesives.

In Vivo Bonding Force

Formulation B, which contained a relatively low EDC concentration (with or without the use of a double-headed syringe), demonstrated superior in vivo results compared with formulation A, which contained a standard EDC concentration. The bonding forces of the incision skin samples are presented in Table 2.

The in vivo bonding force of the skin samples 14 days post-operation was weakest for the sutured incisions (58 N), and strongest for the incisions treated with N-butyl cyanoacrylate (118 N). Formulation B showed a bonding force similar to that resulting from sutures (53 N); however, this formulation showed a much higher bonding force when applied using a double-headed syringe (80 N), although with a large SD. These results are consistent with the clinical, histologic, and immunohistochemical analyses.

DISCUSSION

Traditional wound-healing methods use surgical suturing techniques, but this approach increases the risk of infection because bacteria have an affinity for certain suture materials. Further, suturing requires the use of

anesthesia and later suture removal. An alternative to suturing that has been proposed to overcome these limitations is the use of bioadhesives for nonsurgical wound closure. An ideal bioadhesive should have rapid and strong bonding strength to the tissue, hemostatic properties, and tissue healing regeneration characteristics that do not interfere with the body's natural healing process. It should also be cost-effective, nontoxic, degradable, and absorbable within the healing period with minimal cytotoxic byproducts.

Cyanoacrylate-based skin adhesives are commonly utilized in wound closure because of their ease of use, rapid application, and ability to provide superficial protection.[24-26] Grimaldi et al[24] evaluated the incidence of infection and complications of patients treated with octyl-2-cyanoacrylate. They concluded that octyl-2-cyanoacrylate reduces not only the risk of surgical site infections, but also the timing and the number of postoperative checks, thus increasing patient satisfaction. Although widely used, the limitations associated with cyanoacrylates (eg, toxicity of degradation byproducts, low viscosity, high stiffness) make them unsuitable for long incisions and restricts their usage to external or temporary applications.

To address these limitations, the present study proposes novel adhesives based on natural polymers for the treatment of long surgical incisions (≥4 cm). The researchers compared this new adhesive formulation with well-established closure techniques, including surgical sutures and the commercial N-butyl cyanoacrylate tissue adhesive. The novel adhesive formulation is composed of natural biocompatible polymers and previously demonstrated high biocompatibility in both in vitro and in vivo studies.[9,14–16] In particular, the Gel-Al formulation with 20 mg/ml EDC exhibited excellent cell viability (above 90%) in the Alamar Blue assay. The Alamar Blue assay was performed on human fibroblasts that participate in the wound-healing process to evaluate cell viability in the presence of the hydrogels. Formulations that result in a decrease of more than 30% in viability are considered cytotoxic. In contrast, the commercially available adhesive tested in this study, N-butyl cyanoacrylate, exhibited high cytotoxicity, resulting in low cell viability of 5%.[9,14,15] These findings highlight the biocompatible nature of the proposed bioadhesives, which are based on natural polymers (gelatin and alginate) crosslinked by EDC and enriched with layered silicates such as kaolin.

Effect of the EDC Concentration

The results of the clinical and histologic analyses showed a superior efficacy of formulation B (low EDC content) compared with formulation A (standard) in the treatment of wounds. The assessment of epithelial confluence, epithelialization, clinical collagen, scar width, and mononuclear cell infiltrate all indicated better results for formulation B. Further, immunohistochemical analysis revealed higher levels of expression for laminin and Ki-67, markers of epithelial integrity and proliferation, respectively, in the healed tissue treated with formulation B compared with formulation A. In addition, αSMA, a marker for scar formation, was upregulated in formulation A compared with formulation B and the control incisions. This result highlights the potential advantage of formulation B in reducing scar formation, a common challenge in human wound healing. Based on these clinical, histologic, and mechanical results, the current findings suggest that formulations with lower EDC content, such as formulation B, may offer improved wound healing outcomes compared with formulations with higher EDC content.

The observed superiority of formulation B versus formulation A in the present study can be explained by the lower EDC (crosslinker) content in formulation B. The use of a crosslinker such as EDC to enhance mechanical properties and slow degradation can be advantageous. However, despite being a zero-length crosslinker, in high

concentrations EDC may negatively impact cell migration and tissue integration, therefore negatively affecting wound healing.[27] These findings are consistent with previous studies that have demonstrated the benefits of using low concentrations of EDC for improving biochemical stability and promoting stable wound closure. Powell et al[28] evaluated the use of collagen-glycosaminoglycan sponges as a substitute for the extracellular matrix of dermal tissue. They concluded that low concentrations of EDC can effectively improve the biochemical stability of the collagen-glycosaminoglycan component of cultured skin substitutes(CSS) and promote stable wound closure.[28]

Efficacy and Bonding Strength of the Novel Bioadhesives

The results of the current clinical and histologic analyses showed a slightly superior efficacy of formulation B in comparison with the control, commercial N-butyl cyanoacrylate. More specifically, nonsignificant elevations in the histologic scoring of the epithelial confluence and clinical collagen assessment were observed. Moreover, results obtained from immunohistochemical analysis demonstrated better (although not significant) epithelial integrity, fewer αSMA positive cells, and more proliferating basal epithelial cells in the incisions treated with formulation B compared with those treated with N-butyl cyanoacrylate.

N-butyl cyanoacrylate has several disadvantages in comparison with the newly developed formulation B. The main components of formulation B, gelatin and alginate, are both natural polymers and, unlike N-butyl cyanoacrylate, do not cause a foreign body reaction which may lead to local ischemia, necrosis, and tissue damage. In addition, the degradation of N-butyl cyanoacrylate in the tissue can release certain byproducts, including formaldehyde and lipid hydroperoxide; this does not occur when using the biocompatible formulation B. Taken together, the slightly better efficacy of formulation B, its nontoxic reactions, and its cost effectiveness compared with N-butyl cyanoacrylate led the researchers to conclude that formulation B may serve as a potentially better alternative to the FDA-approved n-butyl cyanoacrylate.

In comparison with sutures, most tested histologic parameters for the efficacy of wound closure demonstrated equal efficacy between the incisions treated with the bioadhesives. Immunohistochemical analysis demonstrated a slight, nonsignificant superiority in epithelial integrity and fewer αSMA positive cells for formulation B. However, the sutured incisions demonstrated more proliferating epithelial basal cells. Suturing has several drawbacks: It requires technical expertise, is time consuming for large wounds, may cause injury to the physician and possible transfer of infectious diseases, is painful if a local

anesthetic drug is not used, and results in stitch marks. In contrast, the novel bioadhesives studied do not require follow-up visits for removal, are less time consuming to apply, and offer a potentially valuable and economical approach for treating skin lacerations.

The superiority of formulation B was further confirmed in the in vitro bonding force measurements of the skin samples. The results indicated that the maximal forces in tension were comparable to sutured incisions, which are considered the conventional treatment method. Moreover, when formulation B applied using a double-headed syringe, it demonstrated even higher results, approaching the maximum values (obtained for N-butyl cyanoacrylate). N-butyl cyanoacrylate is known to have high bonding strength to biological tissues due to its synthetic composition. However, as a result of the toxicity of its degradation byproducts, its low viscosity, and its high stiffness, it is limited to external or temporary applications, and poses a greater risk when used for larger incisions. It should be noted, however, that the skin samples cut from the animals varied in size and thickness. Thus, the in vivo bonding force method can be considered only as a partially quantitative method, which affords a rough estimate of the strength of the healed tissue.

Previous studies have compared the clinical outcomes of skin closure with octyl cyanoacrylate skin adhesive and subcuticular suture closure and found no significant difference in scar cosmesis and patient outcomes between the two methods, although skin closure time was faster with skin adhesive. These findings suggest that formulation B may offer a promising alternative for wound treatment.[29]

Effect of Application Methods

In this study, researchers evaluated the use of a double-headed syringe as a more convenient method for future clinical use. The results demonstrated that use of the syringe impaired the good healing process achieved when formulation B was applied to the incisions manually. The relatively high SD indicates that better fitting of the syringe system should be considered. Therefore, an optimal syringe that is fitted especially to the bioadhesives may lead to better results.

Limitations

The limitations of this study are inherent to this kind of study design and use of large animals for in vivo study and include a small sample size and brief follow-up period. Therefore, the results of this study may serve as a preliminary experimental model for further investigation of the newly proposed hydrogels when applied to human skin closure. Further research with larger sample sizes, longer

follow-up periods, and broader comparisons to various closure techniques is warranted to fully understand the limitations and potential benefits of the bioadhesive in clinical practice.

Practice Implications and Recommendations for Further Study

In terms of clinical implications, the current study provides valuable insights into the comparative effectiveness of skin adhesives versus sutures and commercial adhesive N-butyl-2-cyanoacrylate for wound closure applications. The results suggest that the formulation B bioadhesive may be a viable alternative to current treatments with comparable healing and cosmetic outcomes and demonstrated no adverse effects on the skin structures. This information can guide clinicians in choosing the most appropriate method for wound closure, considering factors such as patient comfort, wound size, and potential complications.

Despite the promising results from the current study, further research is needed with larger sample sizes. This would help to assess the effectiveness, safety, and cost implications of using different skin adhesives compared with sutures and N-butyl-2-cyanoacrylate. Such studies would provide more robust evidence and facilitate the implementation of these methods in clinical practice.

The potential time-saving aspect of using skin adhesives rather than sutures is promising. Eliminating the need for suture removal and reducing the complexity of the closure process may lead to time savings, decreased healthcare costs, and increased efficiency in wound management. In terms of practice implications, the current research suggests that skin adhesives may offer a valuable alternative to traditional sutures for wound closure. This could lead to enhanced patient experience, reduced pain, and improved healing outcomes. However, further research is needed to explore specific clinical guidelines, training requirements, and regulatory considerations to ensure the safe and effective implementation of these methods in different healthcare settings.

CONCLUSIONS

This study demonstrated the efficacy of a novel bioadhesive for the closure of large incisions in a porcine skin model. This newly developed bioadhesive may serve as a less toxic and more tolerable alternative to FDA-approved bioadhesives commonly used in clinical practice and may also replace the need for suturing large incisions. Eliminating the need for suture removal and reducing the complexity of the closure process may lead to time savings, decreased healthcare costs, and increased efficiency in wound management. The

outcome of this study can be seen as a preliminary experimental model for further exploration of the application of the current bioadhesive in human skin closure. However, further research is needed to ensure the safe and effective implementation of these methods in different healthcare settings.

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Figure legends

Figure 1.

REPRESENTATIVE INCISIONS FROM EACH GROUP, 14 DAYS POST-OPERATION

Control groups were sutures and N-butyl-2-cyanoacrylate. The bioadhesive formulations were Formulation A, Formulation A using a double-headed syringe, Formulation B, and Formulation B using a double-headed syringe.

Figure 2.

REPRESENTATIVE HISTOLOGIC SECTIONS OF INCISIONS FROM EACH GROUP, 14 DAYS POST-OPERATION

Hematoxylin and eosin staining (Upper), Trichrome staining for collagen fibers (lower).

Figure 3.

HISTOLOGIC SCORING

Cumulative graph presenting the scoring of four independent observers for the following criteria: epithelial confluence, epithelialization, clinical collagen assessment, scar width, and mononuclear cell infiltrate. Grading was on a scale from 0 to 5: 0 = absence, 1 = minimal presence, 2 = mild presence, 3 = moderate presence, 4 = high presence, and 5 = extensive presence.

Figure 4.

HISTOLOGIC SCORING OF BIOPSIES TAKEN FROM THE INCISIONS 14 DAYS POST-OPERATION

The following healing criteria were investigated: epithelial confluence, epithelialization, clinical collagen assessment, scar width, and mononuclear infiltrate.

Figure 5.

REPRESENTATIVE IMMUNOHISTOCHEMISTRY SECTIONS OF INCISIONS FROM EACH GROUP, 14 DAYS POST-OPERATION.

Abbreviation: αSMA, smooth muscle actin.

Figure 6.

QUANTIFICATION OF THE IMMUNOHISTOCHEMICAL STAINING: (A) Basement membrane integrity (laminin expression), (B) SMA expression, (C) Ki-67 expression.

Abbreviation: αSMA, smooth muscle actin.