



Composite Acellular Collagen Glue: A Novel Treatment Method for Aural Hematomas in Dogs

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ABSTRACT

Background: An aural (auricular) haematoma is a collection of blood within the cartilage plate of the ear and occurs mostly in dogs. This study aimed to assess the healing and cosmetic effectiveness of collagen glue in treating aural hematomas in dogs.

Methods: Eighteen clinical cases of dogs suffering from haematoma were divided into three groups. In group I (n=6), horizontal mattress sutures were applied, in group II (n=6), horizontal mattress sutures along with collagen glue was applied and in group III (n=6), sole application of collagen glue was done for the obliteration of dead space. Various parameters such as thickness of pinna, degree of exudation and inflammation, cosmetic appearance, healing time, surgery time and recurrence were recorded.

Result: Post-operative evaluation revealed that the thickness of ear pinna was reduced mostly in group III (5.43 ± 0.46 mm) along with reduced exudation and inflammation by day 14 and 21 respectively.

Key words: Aural haematoma, Collagen glue, Dog, Horizontal mattress suture.

INTRODUCTION

Aural haematoma is the accumulation of serosanguinous fluid within the pinna caused by rupture of auricular capillary (Lahiani and Niebauer, 2019). Conditions like otitis, ectoparasitism and allergic dermatitis are predisposing factors for aural haematoma resulting, blood-filled swelling on the pinna due to constant ear shaking and rubbing (Safwan *et al.*, 2018). If this condition is left untreated, it can lead to fibrosis and ear pinna deformity (Hewitt and Bajwa, 2020). Conservative treatment modality for aural haematoma includes fine-needle aspiration, corticosteroid injections and suturing. These treatments often face issues like recurrence and deteriorated cosmetic outcomes (Lahiani and Niebauer, 2019).

Recent studies have explored tissue adhesives like collagen glue and cyanoacrylates for their potential to bind cartilage and skin. However, cyanoacrylates and aldehyde-based adhesives can be cytotoxic (Yang *et al.*, 2021). Collagen, on the other hand, is a biocompatible and bioactive material that promotes structural integrity, faster healing and regeneration without causing any harmful local or systemic effects (Jain *et al.*, 2023). Previous research has demonstrated its effectiveness in wound healing in animals (Mishra, 2023).

Collagen derived from bubaline sources acts as an adhesive for treating aural haematoma. This method can be helpful in reducing invasiveness of surgery, shorten anaesthesia duration, improve cosmetic outcome and provides better results than conventional methods.

MATERIALS AND METHODS

The present study was conducted from 2022 to 2024 in eighteen clinical cases of dogs suffering from aural haematoma presented in Department of Veterinary Surgery

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and Radiology, VCC, MHOW. The dogs were selected irrespective of age, sex or breed and divided into three groups of six dogs each. Each dog underwent a clinical examination to assess the presence of ticks, lice, dermatitis, otitis and the extent of haematoma. The research proposal was approved by Institutional animal Ethics Committee (IAEC), according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA). A written consent was also taken from the owners before the surgery.

Collagen glue

The collagen glue is obtained from bubaline offal's and processed to be used as medical adhesives and sealants. Collagen being a biomimetic and bioactive material supports structural integrity, promotes quicker cosmetic healing, regeneration and fills in tissue deficits. Decellularized collagen is biocompatible, bio adhesive,

non-antigenic and non-toxic (Mishra, 2023). A significant component of the extracellular matrix, collagen has been demonstrated to play a number of critical roles in the control of cellular adhesion, division, migration and organ regeneration (Sekine *et al.*, 2000) (Fig 1a).

AQsilk suture

These sutures are non-absorbable, sterile and non-mutagenic surgical sutures which are composed of natural proteinaceous silk fibers known as fibroin. They are easy to handle, have excellent knot-tying properties and are highly unlikely to cause an allergic reaction in patients. Additionally, silk sutures are biocompatible, meaning they do not induce tissue reactions. These qualities make them ideal for surgeries that require fine suturing (Fig 1b).

Anaesthesia

All dogs were placed under general anaesthesia using the following protocol: Atropine sulfate @ 0.04 mg/kg b.wt. S/C, Xylazine @ 1 mg/kg b.wt. I/M as preanesthetics and Ketamine @ 10 mg/kg body weight for induction. Anaesthesia was maintained by administering ketamine and diazepam at 1:1 ratio as and when needed. Prior to the surgery the diameter of each haematoma was measured using vernier callipers to determine the size of haematoma (Fig 2 a,b).

Surgical technique

Under strict aseptic conditions, S-shaped incision was made along the entire length of haematoma on the ventral surface of the pinna (Fig 3a). With the help of curator, all blood clots and fibrous material was removed (Fig 3b). The dead space was obliterated using different surgical techniques in different groups. In group I horizontal mattress sutures were placed parallel to the incision line, covering the entire length of the pinna, with knots on the outer surface (Fig 3c). In group II, horizontal mattress sutures along with collagen glue were applied (Fig 3d). In group III, a thin layer of collagen glue was applied inside the inner surface of the damaged ear (Fig 3e).

Antiseptic dressing with 10% povidone-iodine ointment and compression bandaging was done every third day until suture removal and complete healing in groups I, II and until complete healing in group III (without sutures).

Parameters of the study

Various parameters were recorded postoperatively, including the thickness of ear pinna, degree of exudation and inflammation on 0, 3rd, 7th, 14th and 21st days. The time required for surgical intervention, healing time,



Fig 1: (a) Composite acellular bubaline collagen glue; (b) AQsilk non-absorbable surgical suture no.1.

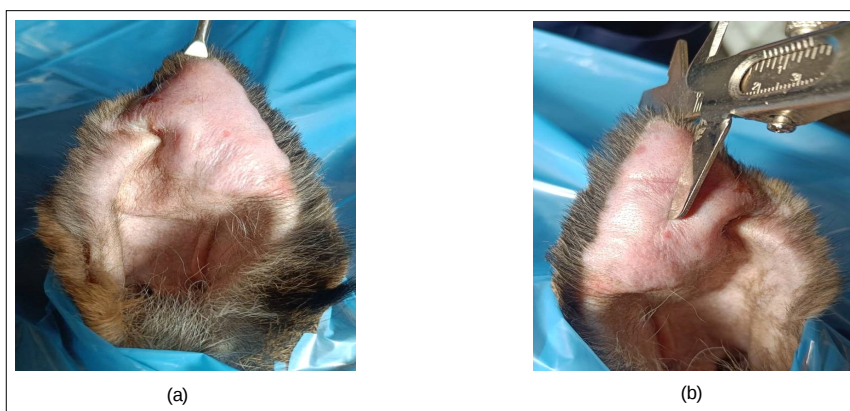


Fig 2: (a) Aural haematoma at tip of ear pinna; (b) Measurement of size of aural haematoma by vernier calipers.

cosmetic appearance of the pinna and any recurrence was also noted. Degree of exudation was graded as: nil (0), mild (1), moderate (2) and severe (3) (Natthuji, 2008). Degree of inflammation was scored as: nil (0), mild (1), moderate (2) and marked (3) (Nema *et al.*, 2016). The cosmetic appearance of ear flap was assessed on a visual analogue scale with some modifications, where 1 represented excellent, 2-good, 3-fair and 4-poor based on carriage of ear pinna, scar formation, embedding of sutures, fibrosis and distortion of the ear (Safwan *et al.*, 2018) with some modifications. The data were analysed by using complete randomized design (CRD) (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The thickness of the ear pinna decreased significantly across all groups over time. When comparing the groups, Group III showed the most notable reduction in ear pinna thickness by the third day and maintained a significantly lower thickness ($p < 0.05$) than the other groups until the 21st day.

In case of group II the ear pinna thickness was significantly lower than group I but higher than group III. The order of normalization of ear pinna thickness is group III > group II > group I (Table 1). The group III achieved

normal ear pinna thickness due to collagen infiltration, which provides a scaffold for new tissue growth and promotes healthy scar formation and early healing.

The enhanced healing process decreases the thickness of affected area, resulting in a smoother, more natural appearance. Infiltration of collagen also minimizes repeated skin punctures, reducing patient discomfort and stress compared to other treatments. Parte (2021) also concluded that thickness of ear pinna reduced very less in horizontal mattress sutures treated group over the time, when compared to other treatment modalities.

It was observed that most significant reduction in degree of exudation was present in group III by day 3rd and remained significantly lesser up to day 7th to become nil thereafter. The decreasing order of degree of exudation of ear is group I < group II < group III (Table 2). Balwada (2014) also concluded in similar trials that the exudation stopped in groups treated with bovine collagen sheet from day 6th onwards. The exudation was significantly less in group III because collagen reduces exudation by absorbing excess fluid (Ayello and Cuddigan, 2004), enhances haemostasis through platelet activation and modulates inflammation for a controlled healing process (Robson *et al.*, 2001) thus, decreasing inflammatory exudate production. The micro fibrillar collagen is a biomaterial that can be used to

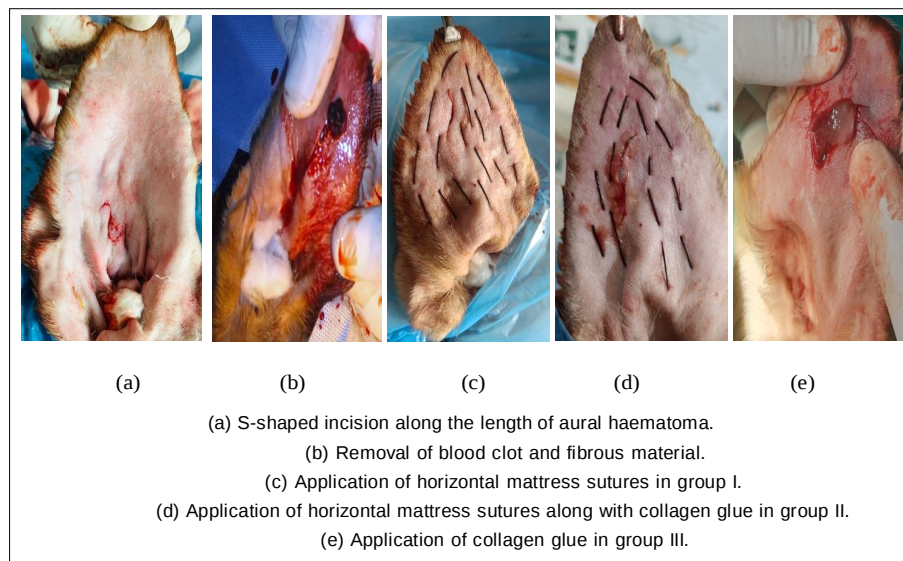


Fig 3: Steps of surgical technique.

Table 1: Mean $\pm$ S.E of thickness of ear pinna (mm) at different time intervals.

Groups	Days				
	0	3 rd	7 th	14 th	21 st
I	10.02 ^{Aa} $\pm$ 0.28	9.13 ^{Ab} $\pm$ 0.19	8.52 ^{Ab} $\pm$ 0.48	7.72 ^{Ab} $\pm$ 0.20	6.63 ^{Ac} $\pm$ 0.35
II	10.23 ^{Aa} $\pm$ 0.25	9.08 ^{Ab} $\pm$ 0.41	8.16 ^{Ab} $\pm$ 0.23	7.33 ^{Ab} $\pm$ 0.24	6.45 ^{Ac} $\pm$ 0.32
III	10.16 ^{Aa} $\pm$ 0.25	8.19 ^{Bb} $\pm$ 0.34	6.94 ^{Bc} $\pm$ 0.34	5.87 ^{Bc} $\pm$ 0.30	5.43 ^{Bc} $\pm$ 0.46

Mean bearing different superscript differ significantly ($p < 0.05$) (a,b,c = within groups and A,B,C=between groups).

manage post-operative exudation and bleeding during surgical procedures. It can be used as a skin adhesive or packing material.

On comparison between the groups, it was observed that, at all-time intervals, the degree of inflammation in group III animals remained at the least scores and most significant ($p < 0.05$) reduction was present in group III by day 7th itself which reduced almost completely significantly ($p < 0.05$) by day 14th and thereafter. In group II and group I reduction in degree of inflammation was similar. The order of inflammation is group I and group II > group III. The least inflammation was observed in group III due to anti-inflammatory properties of collagen glue, by switching cytokines from pro-inflammatory to anti-inflammatory phase (Voigt and Driver, 2011).

Collagen acts as a scaffold for tissue remodelling, repair and implant stabilization (Gokulakrishnan *et al.*, 2023). It promotes cell migration and proliferation, foster new blood vessel growth and extracellular matrix development (Park *et al.*, 2007), maintaining a moist wound environment (Schultz *et al.*, 2011) and possessing antimicrobial properties to prevent infection and inflammation (Kamble *et al.*, 2004) (Table 3) (Fig 4).

The time required for healing was recorded from day of surgery till complete healing. In group I, the total time required for healing was 24.5 ± 0.76 days. This was significantly ($p < 0.05$) more than group II where the complete healing was observed in 20.00 ± 0.96 days. In group III, healing occurred on an average time span of 13.83 ± 0.47 days which was significantly ($p < 0.05$) lesser than both group I and II. Ahirwar (2007) also concluded that the duration of the healing process was the longest among the conventional group. Horizontal mattress sutures alone, was found inadequate for proper healing (Natthuji, 2008). According to Preethi *et al.* (2021), the use of collagen membrane was effective in promoting early tissue healing

and accelerating the phases of tissue regeneration and early graft resorption.

The cosmetic appearance of ear was assessed after complete healing based on different parameters. In group I, the cosmetic outcome of ear pinna was scored to be poor with a cumulative score of 3 due to, poor ear pinna carriage, distortion of ear and scar formation where ever sutures were present in most of the cases. In group II, cosmetic appearance of ear was fair with a total score of 2. In most of the cases embedding, drooping of ear and scar formation was observed. However, in case of group III, the cumulative score was 0 indicating excellent cosmetic outcomes without any complications. The order of best cosmetic appearance among all the groups, based on parameters described above is group III > group II > group I (Table 4).

Coulthard *et al.* (2004) found a statistically significant difference in the surgeon's assessment of cosmetic appearance between the tissue adhesive group and the suture group. The study concluded that surgeons can consider using tissue adhesives as an alternative to sutures or adhesive tape for closing incisions. Ahirwar (2007) reported that the horizontal mattress suture method resulted in disfiguration and drooping of the ear in all animals studied. The needle bite injuries caused cartilage damage and subsequent pain and discomfort, which could be attributed to the drooping of the ear. Cosmetic alterations typically occur due to improper suture placement and excessive suture tension.

The recurrence of aural haematoma was noted down during the recovery period and after complete healing. On comparison among all groups, no recurrence was observed in any cases of group III. However, in group II, recurrence was observed after complete healing on day 21st whereas, in group I, it was observed on day 14th during recovery period. Recurrence of haematoma could also be due to negligence

Table 2: Mean $\pm$ S.E of degree of exudation at different time intervals.

Groups	Days				
	0	3 rd	7 th	14 th	21 st
I	0.00 $\pm$ 0.00	2.33 ^{Aa} $\pm$ 0.21	1.83 ^{Aa} $\pm$ 0.17	0.83 ^{Ab} $\pm$ 0.17	0.17 ^{Ac} $\pm$ 0.17
II	0.00 $\pm$ 0.00	2.50 ^{Aa} $\pm$ 0.22	1.67 ^{Ab} $\pm$ 0.22	0.50 ^{Ac} $\pm$ 0.22	0.17 ^{Ac} $\pm$ 0.17
III	0.00 $\pm$ 0.00	1.50 ^{Ba} $\pm$ 0.22	1.00 ^{Ba} $\pm$ 0.37	0.00 ^{Ab} $\pm$ 0.00	0.00 ^{Ab} $\pm$ 0.00

Mean bearing different superscript differ significantly ($p < 0.05$) (a,b,c= within groups and A,B between the groups).

Table 3: Mean $\pm$ S.E of degree of inflammation at different time intervals.

Groups	Days				
	0	3 rd	7 th	14 th	21 st
I	2.00 ^{Aa} $\pm$ 0.37	1.50 ^{Aa} $\pm$ 0.22	1.33 ^{Aa} $\pm$ 0.21	0.33 ^{Ab} $\pm$ 0.21	0.33 ^{Ab} $\pm$ 0.21
II	2.17 ^{Aa} $\pm$ 0.31	1.50 ^{Aa} $\pm$ 0.22	1.33 ^{Aa} $\pm$ 0.21	0.56 ^{Ab} $\pm$ 0.33	0.33 ^{Ab} $\pm$ 0.21
III	2.04 ^{Aa} $\pm$ 0.22	1.33 ^{Aa} $\pm$ 0.21	0.50 ^{Bb} $\pm$ 0.34	0.17 ^{Ab} $\pm$ 0.17	0.00 ^{Ab} $\pm$ 0.00

Mean bearing different superscript differ significantly ($p < 0.05$) (a,b = within groups and A,B = between groups).

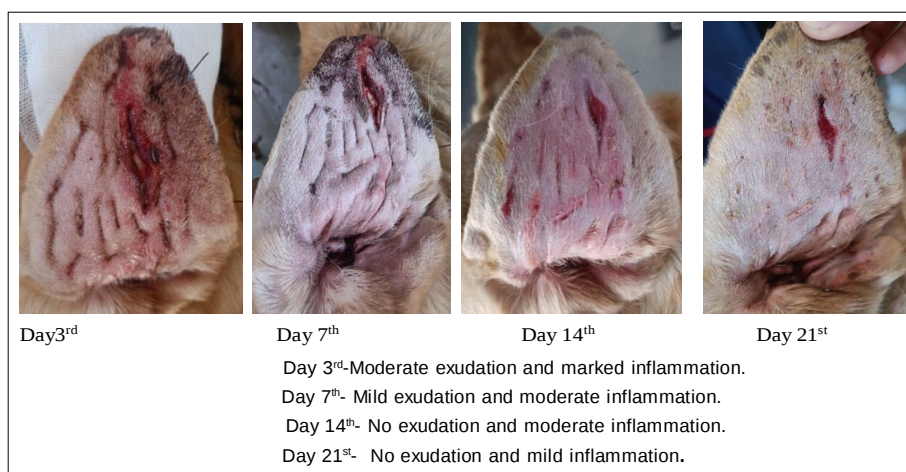


Fig 4(a): Degree of exudation and inflammation in group I at different time intervals.

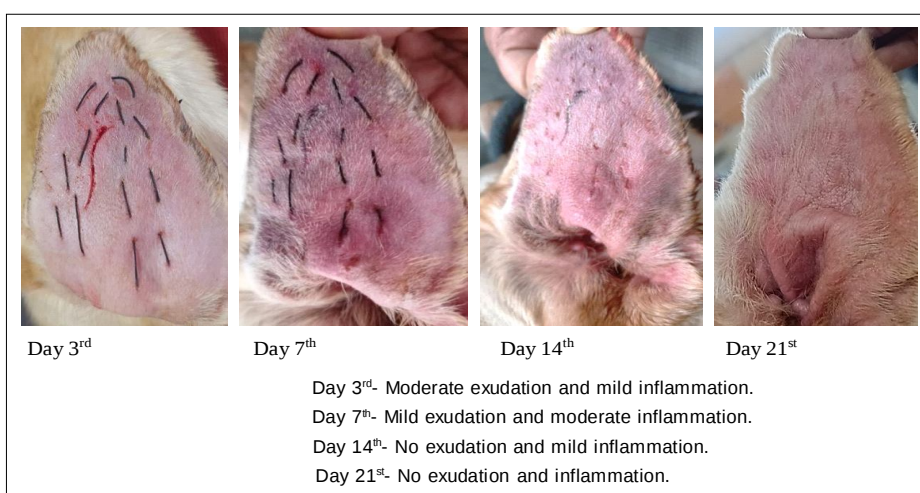


Fig 4 (b): Degree of exudation and inflammation in group II at different time intervals.

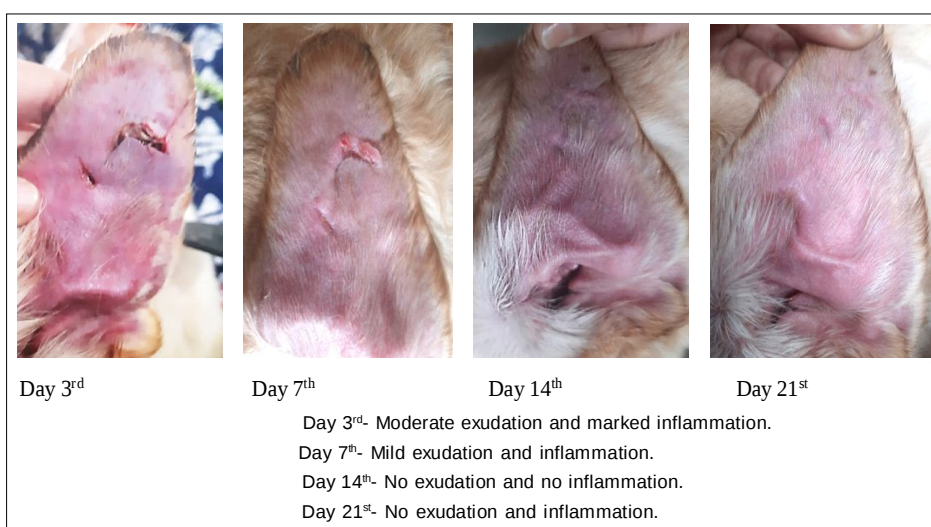


Fig 4(c): Degree of exudation and inflammation in group III at different time intervals.

Table 4: Cosmetic appearance graded on the basis of visual analogue scale.

Parameters	Groups		
	I	II	III
Ear pinna carriage	+	+	-
Embedding of suture	-	-	-
Scar formation	+	-	-
Fibrosis and distortion of ear	+	-	-
Total score	+++	++	-

(Parameters: Ear pinna carriage, Scar formation, Embedding of suture, Fibrosis and Distortion of ear. Scoring: Excellent (-), Good (+), Fair (++), Poor (+++).

in after care, uncooperative behaviour of animal, tissue response leading to discomfort. Moreover, the hypersensitivity reaction to the suturing leads to pawing, scratching and removal of bandage (Vapaavuori, 2017). If underlying causes are not identified or treated, it can result into recurrence (Ahirwar *et al.*, 2007). This recurrence could also be due to location of haematoma at base of ear, as pressure is not duly applied there with slippage of bandage (Parte, 2021).

The time required for surgery was calculated from giving incision on haematoma till placement of last suture in case of group I, II and collagen glue application in group III. In group I, group II and group III the surgery time was 32.16 ± 0.99 minutes, 27 ± 0.56 minutes and 19.16 ± 0.32 minutes respectively. On comparison among all the groups, group III required least time for performing surgery. However, group I and II, required almost similar time for surgical intervention. The time taken for surgery in group II was less than group I because the number of sutures placed in group II was less due to application of collagen glue in group II. The reduced time required for surgery can lead to a decrease in the amount of anaesthesia required, ultimately reducing the cost of the operation.

CONCLUSION

A post-surgical evaluation revealed that using collagen glue instead of sutures led to a faster reduction in ear pinna thickness, with minimal inflammation and exudation. The surgical duration was shorter, the healing period was quicker and the cosmetic outcomes were excellent. Future studies on aural hematomas should consider instilling collagen glue after fine needle aspiration to assess its effectiveness in promoting healing.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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