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ORIGINAL ARTICLE

Microvascular Anastomosis Using Less Sutures and Fibrin Glue in Male Albino Rat: An Experimental Study

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ABSTRACT

Background: Traditional microvascular anastomosis can be time-consuming, which raises the risk of thrombosis. Damage to the endothelium during the process triggers a repair mechanism that involves platelet aggregation, smooth muscle cell proliferation, and thickening of the endothelium. Microvascular anastomosis plays a vital role in free flap transfers and replantation surgeries. Although standard suturing remains the preferred technique, it presents challenges due to its technical difficulty, extended duration, and potential for vessel wall trauma. Fibrin glue, derived from a combination of autologous cryoprecipitate and thrombin, was originally introduced for peripheral nerve repair in humans. The success of these early uses led to its expanded application in areas such as wound closure, skin grafting, and osteotomy healing. This study aims to evaluate the effectiveness of fibrin adhesive in addressing these challenges within the context of microvascular anastomosis.

Methods: This experimental study included 22 male Sprague-Dawley albino rats, the rats were divided into two groups Group (1): Experimental group, we used 4 core sutures with 9/0 polypropylene placed 90 degrees from each other and 0.1 ml of fibrin glue at site of anastomosis. Group (2): As a control group we used the standard repair technique of the transected artery by 5-8 primary sutures with 9/0 polypropylene.

Results: The use of fibrin adhesive significantly decreased both the number of sutures required and the time needed to complete the anastomosis. The immediate and long-term patency rates were not adversely affected by the application of fibrin glue. Histological analysis of the anastomosed vessels revealed no significant differences between the two techniques.

Conclusions: In conclusion, the application of fibrin adhesive did not produce any adverse effects in microvascular anastomosis. The authors advocate for using fibrin adhesive in clinical settings, particularly in more complex cases where multiple microvascular anastomoses are necessary.

Keywords: Microvascular anastomosis; Fibrin glue; Albino Rat.

INTRODUCTION

Before the early 1900s, ligation was the only method available for managing vascular injuries [1]. In 1761, Richard Lambert, from Newcastle upon Tyne, detailed in a letter his success in closing a small tear in the brachial artery using a steel pin to approximate the wound edges. Unfortunately, this technique could not be reproduced, stalling advancements in vascular anastomosis for over a century [1], [2]. Limited understanding of vessel physiology and the mechanisms of thrombosis likely contributed to this delay. It wasn't until progress in microscopy and

tissue histology occurred that vascular anastomosis became a viable option [3].

Achieving successful blood vessel anastomosis is crucial for many contemporary surgical procedures. Nowadays, it is commonly applied in organ transplants, vascular repair following trauma, and free tissue transfers under the microscope [4]. However, traditional vascular anastomosis techniques can be time-consuming, often exceeding 30 minutes, which increases the risk of thrombosis. Disruption of the endothelium during the procedure can trigger platelet aggregation, smooth muscle proliferation, and endothelial thickening [4].

Additionally, the intraluminal sutures used in these methods may provoke a foreign body response, leading to thrombocyte aggregation, intimal hyperplasia, and vessel stenosis. Despite these significant limitations, the needle-and-suture technique remains widely used in standard surgical practices for both large and small vessel anastomoses [5].

In 1974, fibrin glue, composed of autologous cryoprecipitate and thrombin solution, was first employed in human peripheral nerve repair. Its success in these early trials prompted its use in wound closure, skin graft fixation, and osteotomy repair [6]. Fibrin glue replicates the final stage of blood clotting, transforming fibrinogen into fibrin [7].

Despite its promising application in other fields, the role of fibrin glue in microvascular anastomosis remains insufficiently studied. While some evidence suggests it may reduce thrombosis and improve outcomes, its long-term effects on vascular integrity and patency are not well understood. There is also limited data on whether fibrin glue can shorten anastomosis time without compromising vascular repair quality [8,9]. This knowledge gap highlights the need for further investigation. The aim of this study is to assess the effect of fibrin glue on microvascular anastomosis, specifically evaluating its impact on anastomosis time, thrombosis rates, and vessel patency in a controlled experimental setting.

METHODS

Study Design and Animal Model

The study included 22 male Wistar albino rats weighing 150–300 gm, carried out at Zagazig University Hand and Microsurgery Center (ZUHMC). The animals were kept under controlled environmental conditions with a temperature of 22°C, a light/dark cycle of 12 hours, and free access to standard laboratory food and water. The Institutional Animal Care and Use Committee of Zagazig University approved all experimental procedures (Approval number: ZU-IACUC/3/F/67/2023).

Surgical Procedure

All procedures performed on the rats in this experiment were conducted with the highest standards of ethics and compassion. Out of 22, only 18 rats survived until the study's end, while four did not. Anesthesia was administered via an intraperitoneal injection of a Ketamine/Xylazine mixture (25 mg of Ketamine and 10 mg of Xylazine per mL) at 0.1 mL per 100 grams of body weight. Following anesthesia, the rats' lower limbs were shaved in preparation for the procedures. All the procedures were performed using surgical microscope and microsurgical tools to enable the proper dissection and repair of the femoral arteries. The rats were divided into two groups.

In group (1): Experimental group, we used four core sutures with 9/0 polypropylene placed 90 degrees from each other and 0.1 ml of fibrin glue at the site of anastomosis **(Figure 1)**.

In group (2): Control group, we used the standard repair technique of the transected artery by 5-8 primary sutures with 9/0 polypropylene **(Figure 2)**.

Postoperative Care and Assessment

The rats were closely monitored throughout the surgery and recovery phases. Each rat was housed individually in a cage equipped with food and water. During the first week, they were checked daily for feeding, cleanliness, antibiotic administration, and wound care. For the remaining duration of the experiment, which lasted up to 2 weeks, the rats were monitored every three days. Antibiotics were administered for a period of 7 days, with Tetracycline given in the drinking water at a concentration of 0.8 mg per 100 grams of body weight per 24 hours. Once the skin had fully healed and the sutures were removed, the rats were divided into two groups and placed in large communal cages approximately two weeks post-operatively.

Histological Evaluation

After two weeks, all rats were anesthetized, and the femoral artery was exposed in each animal. The flow and patency of the femoral artery were assessed, and a 10 mm segment containing the arterial anastomosis was harvested and placed in a sample tube with 10% formalin solution for histological examination.

The femoral arteries from the rats preserved in formol-saline were processed using an automated tissue processor. The process began with fixation and dehydration. Tissues were immersed in 10% buffered formalin for 48 hours for fixation, followed by a 30-

minute rinse in distilled water to eliminate the fixative. Dehydration involved passing the tissues through a series of alcohol solutions: 70% alcohol for 120 minutes, 90% alcohol for 90 minutes, and two cycles of absolute alcohol, each lasting one hour. After dehydration, the samples were cleared in xylene, first using a 50% alcohol and 50% xylene mixture for one hour, followed by pure xylene for one and a half hours. The tissues were then impregnated with molten paraffin wax, embedded, and blocked out. Paraffin sections, cut to 4–5 micrometers, were stained with hematoxylin and eosin. The stained sections were examined for circulatory disturbances, inflammation, degeneration, apoptosis, necrosis, and other pathological changes in the tissues.

Statistical analysis

The collected data were coded and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences software version 26.0 (IBM Corp., Armok, NY, USA). The quantitative data were presented as mean, standard deviations and ranges. Also, qualitative variables were presented as numbers and percentages. The one-sample Kolmogorov-Smirnov test can test whether a variable is normally distributed. The normality of the variables was checked with the Shapiro-Wilk test. The statistical analysis was considered significant at $p < 0.05$.

RESULTS

Shows that there was statistically significant decrease in the time used for anastomosis in experimental group [12.67±1.0] than control group [19.67 ± 1.8] with p-value <0.001 **(Table 1) (Figure 3).**

There was an increase in the percentage of patency in control group [9 (100.0%)] than experimental group [6 (66.7%)] but with no statistically significant difference between both groups with p-value = 0.058 may be due to lower number of samples **(Table 2) (Figure 4).**

Histopathological result

Histopathological assessment using routine H&E processing and staining, the results were comparable to each other as in:

Group (1): Examined sections from transected femoral arteries of (fibrin glue-treated rats), showed that (66.7%) of arterial anastomoses were completely patent with empty laminae and the arterial intima appeared free of any destructive changes or occluding structures The tunica media and the peri-arterial tissue are also free of any inflammatory cells infiltrates or tissue scars **(Figure 5)**.

While (33.3%) of examined arterial anastomoses revealed a partially patent arterial wall with intraluminal partially occluding thrombi, the thrombotic material and many inflammatory cells appeared focally damaged the arterial intima. Moderate peri-arterial tissue infiltration by inflammatory exudate could also be seen.

Group (2): examined serial sections from the transected femoral artery of the control group (Standard microsurgery repair technique), showed partially organized thrombus in (22.2%), partially occluded the anastomosed end of the artery. The arterial intima was damaged with an attached thrombus from the approximated ends. The fibrin clot was partially replaced by fibro and angioblasts with focal canalization. While examining serial sections of the control group (standard microsurgery repair technique), it was revealed that arteries were completely patent with clear lumens in (77.8%). The arterial intima was free of any destructive changes or obstructive structures. The tunica media and the surrounding peri-arterial tissue also showed no signs of inflammatory cell infiltration or tissue scarring.

Table 1: Difference in the time used for anastomosis in the studied groups.

		Control group No. = 9	Experimental group No. = 9	Test value	P-value	Sig.
Time (mins)	**Mean ± SD**	19.67 ± 1.8	12.67 ± 1	10.186***	0.000	HS
	Range	18 – 22	12 – 14			

Table 2: Difference in the percentage of patency in the studied groups.

		Control group No. = 9	Experimental group No. = 9	Test value	P-value	Sig.
Patency	**Patent**	9 (100.0%)	6 (66.7%)	3.600*	0.058	NS
	Partially patent	0 (0.0%)	3 (33.3%)			

Figure 1: repair of femoral artery using 4 core sutures and 0.1 ml of fibrin glue.

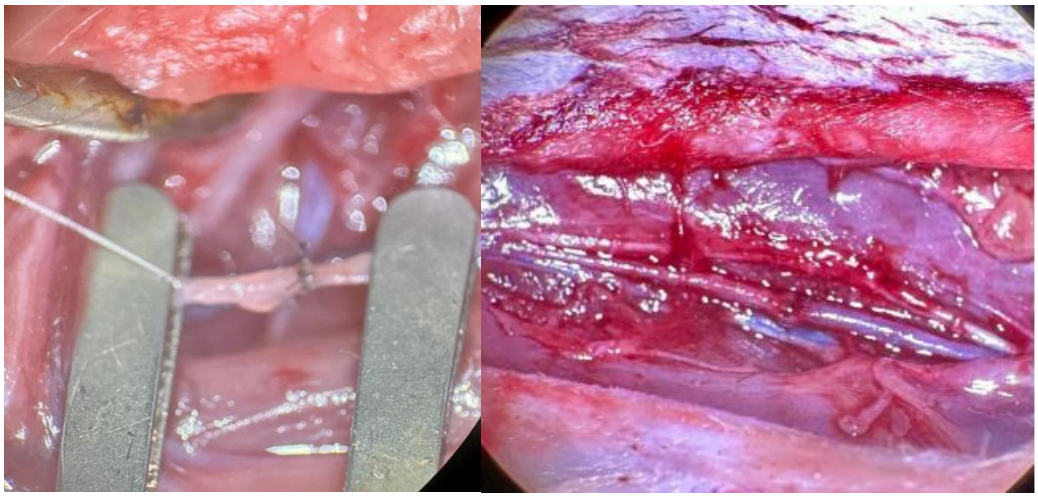

Figure 2: standard repair technique of the transected artery by primary sutures.

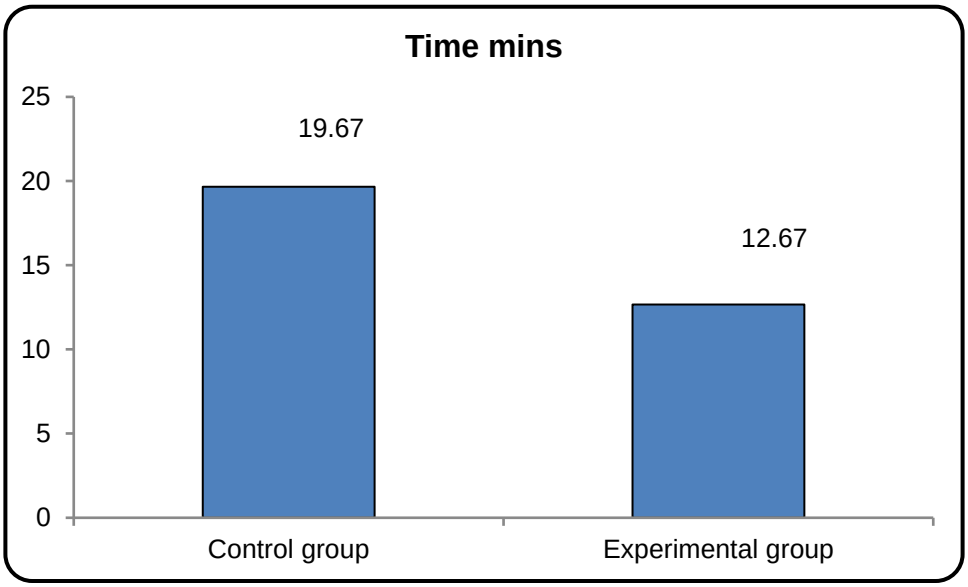

Figure 3: Comparison between control and experimental groups regarding time used for anastomosis of the studied samples.

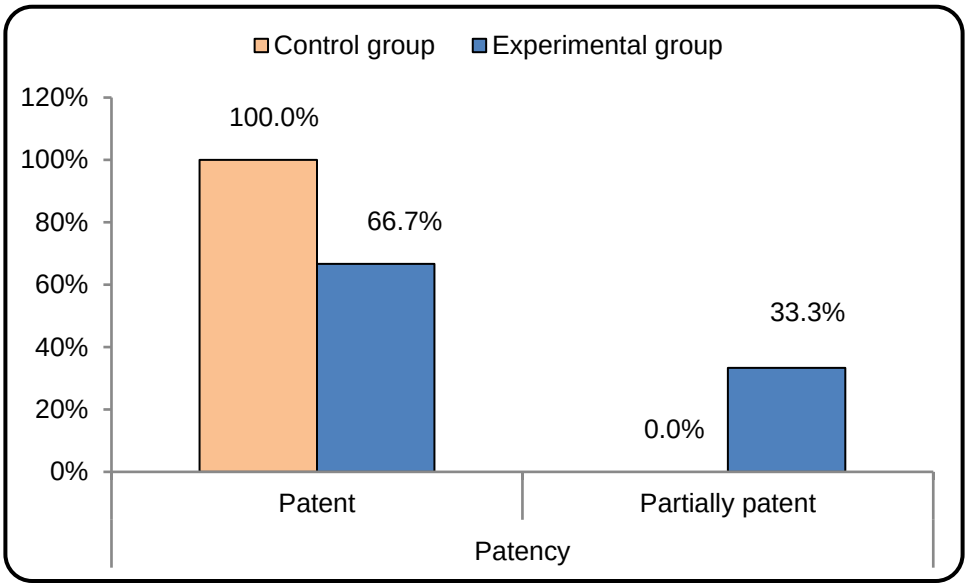

Figure 4: Comparison between control and experimental groups regarding percentage of patency among the studied samples.

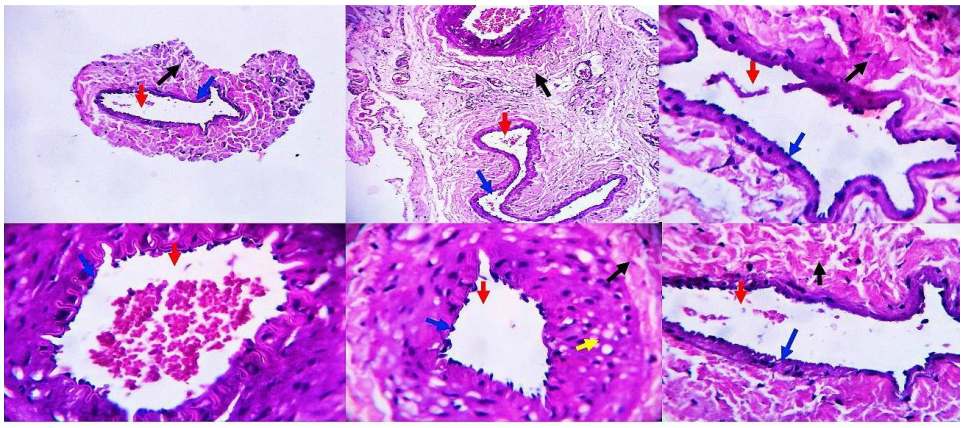

Figure 5: Photomicrographs from rat's femoral artery of (fibrin glue treated rats) demonstrating the recorded histopathologic changes. **(Red arrows)** show completely patent arteries, the arterial intima appeared free of any destructive changes **(dark blue arrows)**, the periarterial tissue are also free from any inflammatory infiltrate **(black arrows),** occasionally the tunica media shows vacuolar degenerative changes **(yellow arrows).**

DISCUSSION

Microvascular anastomosis is a crucial technique in reconstructive surgery. Several approaches have been developed to enhance vessel patency, reduce the time required for anastomosis, and improve hemostasis. There is no single, universally accepted method for performing microvascular anastomosis, as practices can vary depending on the surgeon [10]. Nevertheless, it is essential to avoid common issues, such as constriction of the vascular lumen, inconsistencies in vessel diameters leading to folds and irregularities, excessive suture material inside the vessel lumen, and, notably, transmural sutures that may obstruct the lumen by penetrating the posterior wall [11].

Cho et al. conducted a study involving 30 anastomoses in the femoral artery and 30 in the carotid artery. For each artery, they divided the anastomoses into two groups: one group used interrupted sutures without fibrin adhesive (control group), while the other used fibrin adhesive along with fewer sutures (experimental group) [12].

In a similar vein, Orădan et al. performed an experimental study on rats, specifically comparing two anastomosis techniques on femoral arteries. One group received simple interrupted sutures, while the other group had a combination of fewer sutures and tissue adhesive [13].

We used in this study 18 Sprague Dawley rats divided into two groups equally the experimental

group we used 4 interrupted stitches placed 90 degrees apart and in control group we used simple sutures ranging from 5 to 8 interrupted sutures according to the size of the artery.

One of the key advantages of fibrin glue is its ability to shorten the time required for microvascular anastomosis. Research has demonstrated that fibrin glue, especially when used with minimal suturing, significantly reduces anastomosis time compared to traditional suturing methods [14]. This time reduction is due to the adhesive properties of fibrin glue, which quickly seals the vessel ends and minimizes the need for extensive suturing. Conversely, traditional suturing techniques can be labor-intensive and require a high level of skill and precision, often resulting in longer procedure durations [15].

In this study, we found that using fibrin glue and fewer sutures significantly reduced the time required to complete arterial anastomosis compared to the standard technique of simple interrupted sutures. The time for anastomosis was significantly shorter in the experimental group (12.67 ± 1.0 minutes) compared to the control group (19.67 ± 1.8 minutes), which aligns with the findings reported by previous studies [13,16,17].

Patency rates, which reflect the success of vessel connections, are critical for evaluating the effectiveness of anastomosis techniques. Recent studies indicate that patency rates for microvascular anastomoses using fibrin glue with minimal sutures are comparable to, or even exceed, those achieved with traditional suturing methods. For example, Lan et al. [17] reported no significant difference in patency rates between fibrin glue-assisted anastomoses and conventional sutures in a large animal model. This finding is further supported by a meta-analysis conducted by Koopman et al. 2022 [18], which confirmed that fibrin glue is an effective alternative for maintaining vessel patency.

In our study, the patency rate in the control group was 100%, with 22.2% experiencing thrombus formation followed by recanalization. In contrast, the experimental group had a patency rate of 66.7%. However, the difference between the two groups was not statistically significant.

The incidence of complications such as thrombosis, leakage, and infection is a critical factor in evaluating anastomosis techniques. Fibrin glue has been shown to reduce the risk of leakage and hematoma formation due to its superior sealing properties [19]. Additionally, the use of fibrin glue with fewer sutures may lower the risk of suture-related complications. However, some studies have indicated that improper application of fibrin glue could potentially increase the risk of thrombus formation. Thus, while fibrin glue can mitigate certain risks, it also presents its own set of challenges that must be carefully managed [20].

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The incidence of complications such as thrombosis, leakage, and infection are crucial when evaluating anastomosis techniques. Fibrin glue is known for its excellent sealing properties, which can reduce the risk of leakage and hematoma formation [19]. Additionally, the use of fewer sutures may decrease the likelihood of suture-related complications. However, some studies suggest that improper application of fibrin glue might increase the risk of thrombus formation. Thus, while fibrin glue can mitigate certain risks, it also presents challenges [18].

In our study, the experimental group experienced a (33.3%) incidence of intraluminal partially occluding thrombi, whereas the control group had a (22.2%), complication rate, primarily involving thrombus followed by recanalization. Both groups demonstrated effective hemostasis, with no statistically significant difference between them.

CONCLUSIONS

Employing fibrin glue with minimal sutures in microvascular anastomosis presents a viable alternative to traditional suturing methods. This approach offers advantages such as reduced procedure time, comparable patency rates, and simplified technical demands, making it a compelling option. Nonetheless, potential drawbacks, including a higher risk of thrombosis, must be carefully considered. Ongoing research and clinical trials will be crucial for validating these findings and optimizing the application of fibrin glue in microvascular surgery.

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