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Validation of novel microsurgical vessel anastomosis techniques: A systematic review

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Abstract:
Introduction: Thorough validation of novel microsurgical techniques is deemed essential before their integration into clinical practice. To achieve proper validation, the design of randomized controlled trials (RCTs) should be undertaken, accompanied by the execution of comprehensive statistical analyses, including confounder adjustment and power analysis. This systematic review aims to provide an encompassing overview of the validation methodologies employed in microsurgical studies, with a specific focus on innovative vessel anastomosis techniques.
Methods: A literature search was conducted in PubMed for articles describing the validation of novel microsurgical vessel anastomosis techniques in animal or human subjects.
Results: The literature search yielded 6,658 articles. 6,564 articles were excluded based on title and abstract. Ninety-four articles were assessed for full-text eligibility. Forty-eight articles were included in this systematic review. Out of 30 comparative studies, nine studies validated novel modified interrupted suture techniques, six studies modified continuous techniques, six studies modified sleeve anastomosis techniques, one study a modified vesselotomy technique, seven studies sutureless techniques, and one study a modified lymphaticovenular anastomosis technique. Twenty-eight studies contained animals (n=1,998). Fifteen animal studies were RCTs. Two studies contained human/cadaveric subjects (n=29). Statistical power-analysis and confounder adjustment were performed in one animal study. Out of eighteen non-comparative studies, five studies validated novel modified interrupted suture techniques, one study a modified continuous technique, two studies modified sleeve anastomosis techniques, four studies modified vesselotomy techniques, four studies sutureless techniques, and two studies modified lymphaticovenular anastomosis techniques. Ten studies contained animal subjects (n=320), with two RCTs. Eight studies contained human subjects (n=173). Statistical power-analysis and confounder adjustment were performed in none of the animal or human studies.
Conclusion: The current methods of microsurgical technique validation should be reconsidered due to poor study design. Statistical analysis including confounder adjustment and power-analysis should be performed as a standard method of novel technique validation.

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Table 1. Studies validating novel microsurgical techniques: Comparative studies (30 studies)									
Technique type	**Study design (No. studies)**	**Type of subjects (No. studies)**	**Type of modification (No. studies)**	**Anastomosis type (No. studies)**	**Main outcome (No. studies)**	**Control group (No. studies)**	**Technique is clinically used (No. studies)**	**Power analysis performed (No. studies)**	**Confounder adjustment performed (No. studies)**
Modified interrupted technique (9 studies) [3,10,11,14,15,18,21,31,32]	Comparative (3) RCT (5) RO (1)	Animal (7) Human (2)	Posterior wall first (1) Posterior wall support (1) Fewer sutures + glue (1) Extra cut placement (1) Flow-through anastomosis (1) Antegrade anastomosis (1) Intravascular stent (1) Double stitch everting (2) Horizontal mattress sutures (1)	ETE (6) ETS (7)	Postoperative PR (4) Postoperative FR (3) Flap survival (1) Ischemic time (1) Suture symmetry score (1) Anastomotic leakage (1) Anastomotic time (1) Thrombosis rate (1)	Conventional interrupted suture technique (9)	Yes (2) No (2) N/A (5)	Yes (1) No (8)	Yes (1) No (8)
Modified continuous technique (6 studies) [9,13,19,25,29,30]	Experimental (2) RCT (4)	Animal (6)	Posterior wall first (1) Absorbable sutures (1) Horizontal mattress sutures (1) Knotless (2) Inside-to-outside suture (1)	ETE (4) ETS (2)	Postoperative PR (2) Postoperative FR (2) Anastomotic leakage (1) Aneurysm formation (1) Postoperative anastomosis bleeding and	Conventional continuous suture technique (4) Conventional interrupted suture technique (3)	Yes (1) N/A (6)	No (6)	No (6)

					stenosis (1) Graft and subject survival (1)				
Modified sleeve anastomosis technique (6 studies)[4-6,20,23,27]	Comparative (1) Experimental (3) RCT (2)	Animal (6)	Assymetrical sleeve (1) Symmetrical sleeve (1) Extra cut placement (2) Suture placement (3) Glue (1)	ETE (6)	Postoperative PR (5) Elongation, tensile strength, and elasticity of vessels (1)	Conventional interrupted suture technique (5) Conventional interrupted suture technique (1) Arteries with adventitia (1)	No (1) N/A (5)	No (6)	No (6)
Modified vesselotomy technique (1 study)[7]	RCT (1)	Animal (1)	Elliptic vesselotomy (1)	ETS (1)	Postoperative PR (1)	Slit anastomosis in arteries and veins (1)	Yes (1)	No (1)	No (1)
Sutureless technique (7 studies)[8,16,17,22,23,26,28]	Comparative (2) Experimental (3) RCT (2)	Animal (7)	Photochemical bonding (1) Absorbable cuff (1) Unabsorbable cuff (1) Glue + absorbable stent (1) Glue + venous cuff (1) Biodegradable laser (1) Glue (1)	ETE (7)	Postoperative PR (4) Postoperative FR (1) Cuff absorption rate (1) Surgical succes rate (1) Anastomotic time (2)	Conventional interrupted suture technique (6) Unabsorbable cuff technique (1)	Yes (1) No (1) N/A (5)	No (7)	No (7)
Modified lymphaticovenular anastomosis technique (1 study)[12]	RCT (1)	Animal (1)	Intima-to-intima coaptation (1)	ETE (1)	Postoperative PR (1)	Conventional lymphaticovenular implantation technique (1)	No (1)	No (1)	No (1)

Table 2. Studies validating novel microsurgical techniques: Non-comparative studies (16 studies)								
Technique type	**Study design (No. studies)**	**Type of subjects (No.**	**Type of modification (No. studies)**	**Anastomosis type (No. studies)**	**Main outcome (No. studies)**	**Technique is clinically used (No.**	**Power analysis performed (No.**	**Confounder adjustment performe**

		studies)				studies)	studies)	d (No. studies)
Modified interrupted technique (5 studies)[36,39,45,47,49]	Experimental (1) RCT (1) RO (1) CS (1) PO (1)	Animal (2) Human (3)	Posterior wall first (1) No turn-over (1) Fewer sutures (1) Single loop (1) Temporary assisting suspension suture (1)	ETE (2) ETS (3)	Initial success rate (1) Postoperative PR (2) Postoperative FR (1) Complication rate (1) Operative time (1) Postoperative venous thrombosis (1)	Yes (1) N/A (4)	No (5)	No (5)
Modified continuous technique (1 study)[37]	Experimental (1)	Animal (1)	Anterior and posterior wall sutures placed separately (1)	ETE (1)	Ischemic time (1)	N/A (1)	No (1)	No (1)
Modified sleeve anastomosis technique (2 studies)[34,38]	Experimental (2)	Animal (2)	Heat-induced tissue-welding (1) Suture placement (1)	ETE (2)	Postoperative PR (1) Postoperative FR (1)	N/A (2)	No (2)	No (2)
Modified vesselotomy technique (4 studies)[33,43,44,48]	Experimental (1) RO (2) CS (1)	Animal (1) Human (3)	Longitudinal vesselotomy (1) V-shaped flap (1) Oblique transection (1) Diamond-shaped vesselotomy (1)	ETE (3) ETS (1)	Postoperative FR (1) Operative time (1) Vessel discrepancy ratio (1) Flap survival rate (1) Postoperative complication rate (1)	Yes (2) N/A (2)	No (4)	No (4)
Sutureless technique (4 studies)[35,40-42]	Experimental (3) RCT (1)	Animal (4)	Heat-induced tissue-welding (2) Unabsorbable cuff (2)	ETE (4)	Operative time (2) Postoperative PR (1) Postoperative FR (1) Postoperative subject survival (1)	No (2) N/A (2)	No (4)	No (4)
Modified lymphaticovenu	PO (2)	Human (2)	Double barrel (1)	ETE (2)	Relief of lymphede	No (2)	No (2)	No (2)

lar anastomosis technique (2 studies)[46,50]			Multiple barrel (1)		ma symptoms (2)			

Validation of novel microsurgical vessel anastomosis techniques: A systematic review

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Abstract

Introduction: Thorough validation of novel microsurgical techniques is deemed essential before their integration into clinical practice. To achieve proper validation, the design of randomized controlled trials (RCTs) should be undertaken, accompanied by the execution of comprehensive statistical analyses, including confounder adjustment and power analysis. This systematic review aims to provide an encompassing overview of the validation methodologies employed in microsurgical studies, with a specific focus on innovative vessel anastomosis techniques.

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Keywords

Microsurgery – Surgical technique – Vessel anastomosis – Validation

Introduction

Microsurgery is a complex and precise field of surgery, which requires a high level of technical skills (1). Microsurgical techniques are used in a wide variety of surgical subspecialties for e.g., vessel and nerve anastomosis, which allows for repair of human tissue and regain of function after trauma, tumor resection, anatomical reconstructions, congenital abnormalities, and impending ischemia (Figure 1a & Figure 1b) (1). To construct patent microsurgical vessel anastomoses in patients needing complex vascular reconstructions using new and improved techniques, these techniques need to be validated. Proving the validity of a new microsurgical technique could predict their safe and effective use in the clinical practice. Designing randomized controlled trials (RCT), statistical analysis and confounder adjustment involving outcome measures such as anastomosis time and patency rate are necessary to be able to grant validation to new microsurgical techniques. In the past decades, over two thousand articles on microsurgical anastomosis techniques have been published (2). This systematic review aims to provide an overview of validation methods used by microsurgical studies, proposing new vessel anastomosis techniques.

Methods

A literature search was conducted in PubMed from database inception to January 6th, 2024. Combinations of search terms regarding "Microsurgical techniques". To be included, articles had to meet the following criteria: (1) microsurgical techniques had to be applied to blood vessel anastomosis (2) studies had to include human or animal subjects (3) articles had to contain more than 5 subjects, (4) articles were written in English. Reasons for exclusion of articles were (1) non-original articles, (2) articles reporting data on other types of anastomoses than blood vessels, (3) reviews, (4) training models.

The title and abstract of the articles were screened for eligibility by five of the authors (YS, IM, AJ, ATG, HS). If their abstracts met the inclusion criteria, full-text articles were obtained. Each full-text article was then assessed for final inclusion in this systematic review. All articles were rescreened and reassessed by one author (YS). Disagreements were resolved through discussion with the senior author (VV).

Relevant data from included articles were extracted by independent authors (YS, IM, AJ, ATG, HS) into an extraction template. Study information extracted included: study design (comparative, non-comparative, RCT, experimental, retrospective observational cohort, case series), country, number of study subjects and type of subjects (animal, human), type of novel modification, type of anastomosis (end-to-end, end-to-side, side-to-side), description of technique, the study's main outcome(s), control group, if technique is clinically used, whether power analysis was performed, and whether confounder adjustment was performed. All data was reextracted by one author (YS). The novel microsurgical techniques were divided into six groups: modified interrupted suture techniques, modified continuous techniques, modified sleeve anastomosis techniques, modified vesselotomy techniques, sutureless techniques, and modified lymphaticovenular anastomosis techniques.

Quality assessment of the included articles was performed by the senior author (VV) using Cochrane RCT-2 for RCTs and A Cochrane Risk Of Bias Assessment Tool: for Non-Randomized Studies of Interventions (ACROBAT-NRSI) for non-randomized studies.

Results

The search strategy yielded 6,658 articles, which were put through initial screening. 6,564 articles were excluded based on title and abstract. Ninety-four articles remained to be assessed for eligibility based on full-text sifting. Ultimately, 48 articles were included in this systematic review based on full-text eligibility (Figure 2). Quality assessment revealed a low risk of bias for RCTs (n=17), and for the non-randomized studies (n=31) a critical risk of bias.

Comparative studies

Thirty of the included studies were comparative studies (Table 1). In total, 28 studies contained animal subjects (n=1,998 animal subjects) and two studies contained human subjects (n=29) (Supplementary Appendix: Table 1).

Nine studies proposed a novel modified interrupted anastomosis technique, with modifications such as double stitch everting in two studies (Table 1). Seven studies were performed on animal subjects and two on human subjects (Table 1). Five studies were RCTs (Table 1). Conventional interrupted suture technique was used in the control group in all nine studies (Table 1). The immediate postoperative patency rate (n=4) and the postoperative flow rate (n=3) were the most investigated main outcomes (Table 1). Two techniques were clinically used, and for five techniques details on their clinical use was unavailable (Table 1). Statistical power-analysis and confounder adjustment were performed in one animal study by Dindelegan et al. (21) (Table 1).

Six studies proposed a novel modified continuous anastomosis technique, with modifications such as knotless continuous anastomosis in two studies (Table 1). All studies were performed on animal subjects (Table 1). Four studies were RCTs (Table 1). Conventional interrupted suture technique was used in the control group in three studies and conventional continuous suture technique in four studies (Table 1). The immediate postoperative patency rate (n=2) and the postoperative flow rate (n=2) were the most investigated main outcomes (Table 1). Two techniques were clinically used, and for five techniques details on their clinical use was unavailable (Table 1). Statistical power-analysis and confounder adjustment were performed in none of the studies (Table 1).

Six studies proposed a novel modified sleeve anastomosis technique, with modifications such as suture placement in three studies, and extra cut placement in two studies (Table 1). All studies were performed on animal subjects (Table 1). Two studies were RCTs (Table 1). Conventional interrupted suture technique was used in the control group in five studies and conventional continuous suture technique in one study (Table 1). The immediate postoperative patency rate (n=5) was the most investigated main outcomes (Table 1). For five techniques details on their clinical use was unavailable (Table 1). Statistical power-analysis and confounder adjustment were performed in none of the studies (Table 1).

Seven studies proposed a novel sutureless anastomosis technique, with modifications such as use of absorbable (n=1) and non-absorbable cuffs (n=1) in two studies. All studies were performed on animal subjects (Table 1). Two studies were RCTs (Table 1). Conventional interrupted suture technique was used in the control group in six studies and unabsorbable cuff technique in one study (Table 1). The immediate postoperative patency rate (n=4) and anastomotic time (n=2) were the most investigated main outcomes (Table 1). For five techniques details on their clinical use was unavailable and one technique was clinically used (Table 1). Statistical power-analysis and confounder adjustment were performed in none of the studies (Table 1).

One RCT proposed a novel elliptic vesselotomy technique (7) on animals, which was clinically used (Table 1). Statistical power-analysis and confounder adjustment were not performed (Table 1). One RCT proposed a modified lymphaticovenular anastomosis technique (12) on animals, with no statistical power-analysis and confounder adjustment (Table 1).

Non-comparative studies

Eighteen of the included studies were non-comparative studies (Table 2). In total, ten studies contained animal subjects (n=320) and eight studies contained human subjects (n=173) (Supplementary Appendix: Table 2).

Five studies proposed a novel modified interrupted anastomosis technique, with modifications such as fewer sutures and single loop sutures (Table 2). Two studies were performed on animal subjects and three on human subjects (Table 2). One RCT, one prospective observational cohort study, and one retrospective observational cohort study were conducted (Table 2). The immediate postoperative patency rate (n=2) was the most investigated main outcomes (Table 2). One technique was clinically used, and for four techniques details on their clinical use was unavailable (Table 2). Statistical power-analysis and confounder adjustment were performed in none of the studies (Table 2).

One experimental study proposed a novel modified continuous anastomosis technique (37) on animals (Table 2). Statistical power-analysis and confounder adjustment were not performed (Table 1). Two experimental studies proposed modified sleeve anastomosis techniques on animals, with modifications such as heat-induced tissue-welding (Table 2). Statistical power-analysis and confounder adjustment were not performed in both studies (Table 2). Two prospective observational studies proposed multiple barrel modified lymphaticovenular anastomosis techniques on human subjects (Table 2). As the main outcome, relief of lymphedema symptoms were measured (Table 2). Statistical power-analysis and confounder adjustment were not performed in both studies (Table 2).

Four studies proposed a novel modified vesselotomy technique, with modifications such as longitudinal vesselotomy (Table 2). One study was performed on animal subjects and three studies were performed on human subjects (Table 2). Two techniques were clinically used, and for two techniques details on their clinical use was unavailable (Table 2). Statistical power-analysis and confounder adjustment were performed in none of the studies (Table 2).

Four studies proposed a novel sutureless anastomosis technique, with modifications such as heat-induced tissue-welding (n=2) and non-absorbable cuffs (n=2) (Table 2). All studies were performed on animal subjects (Table 2). One study was an RCT (Table 2). Operative time (n=2) was the most investigated main outcomes (Table 2). For five techniques details on their clinical use was unavailable and one technique was clinically used (Table 1). Statistical power-analysis and confounder adjustment were performed in none of the studies (Table 1).

Discussion

Summary of findings

This systematic review summarized evidence from 48 articles, including data on both animal (n=2,318) and human (n=202) subjects. Thirty of the included studies were comparative studies, with fourteen RCTs containing animal subjects and one RCT containing human subjects. Nine comparative studies validated novel modified interrupted suture techniques, six studies modified continuous techniques, six studies modified sleeve anastomosis techniques, one study a modified vesselotomy technique, seven studies sutureless techniques, and one study a modified lymphaticovenular anastomosis technique. Statistical power-analysis and confounder adjustment were performed in one comparative animal study. Eighteen of the included studies were non-comparative studies, with two RCTs containing animal subjects. Five non-comparative studies validated novel modified interrupted suture techniques, one study a modified continuous technique, two studies modified sleeve anastomosis techniques, four studies modified vesselotomy techniques, four studies sutureless techniques, and two studies modified lymphaticovenular anastomosis techniques. Statistical power-analysis and confounder adjustment were performed in none of the non-comparative animal or human studies.

Current methods of technique validation

Many types of validity are used in scientific literature (51). Face validity, construct validity, and predictive validity are important aspects on which validity of microsurgical techniques should be based. Firstly, face validity indicates if the new technique is as effective as the standard technique or not. Secondly, construct validity compares and correlates the current anastomosis outcomes with other outcomes, to reveal possible associations. At last, when these associations have been revealed, predictive validity can determine how the studied techniques will perform in clinical practice based on the used method of measurement. It remains abundantly clear from the studies assessed that while a plethora (hundreds) of reports is available detailing either a new trick, technique, or anastomosis modification, researchers and clinicians are completely unaware of the possibilities and responsibilities regarding proper validation. Even when techniques are validated, the exact facet of validity is not specifically mentioned, despite some articles being of high methodological rigor. Reviewers and editors should be wary of these practices and demand exact validation methodologies for the different facets of validity. Non-comparative studies should not be accepted as proper proof of validation. Best of all, predictive validity should be encouraged, i.e. that a new trick of technique actually leads to better overall results in the clinical setting.

The non-comparative studies (n=16) included in this systematic review failed to properly validate their newly proposed techniques by including no control group, which makes their outcomes less reliable. No studies evaluated construct validity, except for Dindelegan et al. (21), which performed confounder adjustment. Besides, predictive validity has not been proven in any of the studies included, except for Dindelegan et al. (21), which is the most important and relevant type of validity in case of microsurgical techniques. Out of over 6,000 published studies about microsurgical techniques, only forty-seven proposed new techniques, of which only one study succeeded to properly validate their technique. The optimal situation of technique validation is when a technique is first validated in an RCT comparison in animals, and then for predictive validity in an RCT in a clinical setting. No study in our systematic review succeeded in reaching this standard. Therefore, the second-best strategy is to validate a technique in a prospective study/RCT clinically using a control group, worst case scenario historical matched controls, which is also not met by the included studies. At last, a fair method of validation is performing RCT comparison with conventional suture techniques in animals, which has been conducted in fourteen studies included in this systematic review (5-7, 9-12, 17-19, 21, 22, 29, 30). However, only one study amongst these RCTs succeeded in proper predictive validation, which has been mentioned earlier (21). The validated techniques in these studies are merely modifications to existing anastomosis techniques or efforts to minimize steps in suturing anastomosis, aiming to reach a faster and more efficient anastomosis time, with the same or improved patency rate as conventional methods. Additional adhesive tools such as fibrin glue and cyanoacrylate were added to the anastomosis suturing steps to speed up the process of suturing and tricks, such as open guide suturing, were introduced to provide a better exposure during suturing and improve eversion of the vessel wall.

Recommendations

Our results indicate that only very few studies, and even fewer RCTs have been conducted to validate new microsurgical techniques, the least of which in the patient population of interest. Although, these techniques are being routinely used in surgeries without proper evidence of their effectiveness or proof that they achieve their goal when used clinically. The current scientific methods employed in the validation of new microsurgical techniques is rudimentary and scientifically barren and needs to be refined. Confounder adjustment with predefined confounders should be part of any data analysis plan, as well as adequate power analysis before commencing any study.

Strengths and limitations

To our knowledge, this article is the first systematic review discussing the current methods of validation of microsurgical techniques. Some limitations should be noted such as the fact that three studies did not define their main outcomes, which they simply address as e.g., 'success rate'. Besides, the main outcomes measured in the studies appeared to be quite heterogenous, which prevented us from pooling results or performing statistical inferences on the data collected. Lastly, thirty-one (67%) of the articles failed to mention whether their novel technique is being used in clinical practice. Articles should at least mention if their technique has been clinically used in their own institute, and if not, this information should also be reported.

Conclusion

The current scientific methods employed in the validation of microsurgical techniques are rudimentary and should be reconsidered. Statistical analysis plans, including confounder adjustment and power analysis should be employed routinely in each predefined statistical analysis plan.

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Supplementary Appendix: Table 1. Studies validating novel microsurgical techniques: Comparative studies (30 studies)											
Study (First author, year)	**Study design**	**Country**	**Number of subjects**	**Name of technique validated**	**Type of technique**	**Description of technique**	**Main outcome(s)**	**Control group**	**Technique is clinically used**	**Power analysis performed**	**Confounder adjustment performed**
Animal subjects											
Hou, 1987[3]	Comparative	USA	40 rats	Alternative interrupted technique	ETE	The first stitch is placed on the posterior wall of the vessel	Postoperative PR	Conventional interrupted suture technique	N/A	N	N
Siemionow, 1987[4]	Comparative	USA	160 rabbits	Asymmetrical vs. symmetrical adventitia sleeve technique	ETE	Asymmetrical sleeving of adventitia vs. Symmetrical trimming of the adventitia with collagen cuff wrapping vs. Symmetrical trimming of adventita vs. Sleeve anastomosis technique	Postoperative PR	Arteries with adventitia and vessel preparation prior to anastomosis	N/A	N	N
Zhang, 1991[5]	RCT	China	40 rats	Sleeve anastomosis	ETE	One sidecut and the placement of two sutures in	Postoperative PR	Conventional interrupted suture technique	N/A	N	N

						separate vertical planes					
Saitoh, 1993[6]	RCT	Japan	29 rats	Loop sutures in sleeve anastomosis	ETE	Two loop sutures placed on the proximal stump of the vessel are passed through the wall of the distal vessel	Postoperative PR	Conventional interrupted suture technique	N/A	N	N
Adams Jr, 2000[7]	RCT	USA	104 rats	Elliptic hole, slit anastomosis	ETS	Elliptic vesselotomy with anastomosis in arteries and veins	Postoperative PR	Slit anastomosis in arteries and veins	Y	N	N
O'Neill, 2007[8]	Comparative	USA	60 rats	Photochemical tissue bonding technique (PTB)	ETE	PTB uses a combination of visible light and a photoreactive dye to create immediate bonds and a tight seal between tissue surfaces	Postoperative PR	Conventional interrupted suture technique	N	N	N
Cigna, 2008[9]	RCT	Italy	40 rats	Posterior wall first -	ETE	The first stitch	Postoperative PR	Continuous-Interrupte	N/A	N	N

				Continuous interrupted - Airborne (PCA) technique		is placed on the posterior wall of the vessel, The second and third sutures are placed very close to the first stitch, one above and one below it. The anastomosis is completed by using the Continuous-Interrupted technique		d technique			
Zhang, 2010[10]	RCT	China	40 rats	Modified interrupted suture technique	ETE	The modified technique entailed using fewer sutures (5–6 sutures) and fibrin glue	Postoperative PR	Conventional interrupted suture technique	Y	N	N
Huang, 2011[11]	RCT	Germany	18 rats	Modified interrupted suture technique	ETS	The modified technique allowed for the	Postoperative PR	Conventional interrupted suture technique	N/A	N	N

						compensation of size mismatches between donor and recipient vessels by placing a small incision from the edge of the recipient vessel					
Ishiura, 2017[12]	RCT	Japan	12 rats	Supermicrosurgical lymphaticovenular anastomosis	ETE	The largest selected lymphatic vessel was anastomosed to the recipient vein in an intima-to-intima coaptation manner	Postoperative PR	Lymphaticovenular implantation technique	N	N	N
Firsching, 1984[13]	Experimental	Germany	20 rats	Continuous-suture technique	ETE	Continuous-suture technique with resorbable suture materia	Postoperative FR	Conventional interrupted suture technique	Y	N	N
Miyamoto, 2008[14]	Comparative	Japan	120 rats	Flow-through arterial anastomo	ETE, ETS	ETE anastomoses were	Postoperative FR	Conventional interrupted suture	N	N	N

				sis		performed between the proximal and distal stumps of the axillary artery and the stumps of the common carotid artery		technique			
Miyamoto, 2008(15)	Comparative	Japan	60 rats	Retrograde arterial anastomosis	ETE, ETS	The anastomosis is performed in an antegrade fashion	Postoperative FR, flap survival	Conventional interrupted suture technique	N	N	N
Euler, 1989(16)	Experimental	Germany	11 rats	Cuff technique	ETE	Nonsuture microsurgical vessel anastomosis using an absorbable cuff	Absorption rate	Unabsorbable cuff technique	N/A	N	N
Zhou, 2010(17)	RCT	Multi-country	80 mice	Cuff technique	ETE	Nonsuture microsurgical vessel anastomosis using a cuff	Surgical success rate	Conventional interrupted suture technique	N/A	N	N
Miyamoto, 2010(18)	RCT	Japan	45 rats	Intravascular stenting technique vs. open guide suture technique	ETE	Intravascular stenting technique: The first stay	Ischemia time, Postoperative PR	Conventional interrupted suture technique	N/A	N	N

						suture was placed on the side opposite the surgeon and tied. The stent was then inserted into both stumps, thereafter, anastomosis was completed as with the conventional technique Open guide suture technique: Two interrupted sutures were placed on the anterior wall with untied method and tied in order.					
Başar, 2012[19]	RCT	Turkey	36 rats	Continuous horizontal mattress suture technique	ETS	Between the two knots, on the front side first	Anastomotic leakage, aneurysm format	Conventional interrupted suture technique, Conventional	N/A	N	N

						and then on the back side, four sutures were passed as horizontal mattress, then two sutures were tied with each other.	ion, Postoperative FR	continuous suture technique			
Szabo, 2020[20]	Experimental	Hungary	60 chicken thighs 12 rats	Modified Lauritzen's sleeve-technique	ETE	Four sutures, including corner and pulling stitches, were added to the conventional sleeve anastomosis	Elongation, tensile strength, and elasticity of vessels	Conventional interrupted suture technique, Conventional continuous suture technique	N/A	N	N
Dindelegan, 2021[21]	RCT	Romania, The Netherlands	177 chicken thighs 43 rats	The double stitch everting technique	ETS	Leaving the needle inside the vessel wall in-between stitching	Suture symmetry score, Postoperative PR	Conventional interrupted suture technique	Y	Y	Y
Orădan, 2022[22]	RCT	Romania	24 rats	The use of cyanoacrylate in anastomosis	ETE	Anastomosis with three interrupted sutures with tissue	Total operative time, anastomotic time, Postoperative	Conventional interrupted suture technique	Y	N	N

						adhesive being applied between the sutures to complete the anastomosis	FR				
Lemaire, 2000[23]	Experimental	Canada	33 rats	Anastomosis with histoacryl glue and an intravascular soluble stent	ETE	The artery was prepared with histoacryl glue and an intravascular soluble stent was placed	Postoperative PR	Conventional interrupted suture technique	N/A	N	N
Le Hanneur, 2022[24]	Experimental	France	7 rats	Fibrin-glue-augmented sleeve anastomosis	ETE	Fibrin-based glue sleeve was added to conventional interruptured sutured anastomosis	Postoperative PR	Conventional interrupted suture technique	N	N	N
Mao, 2009[25]	Experimental	China	400 mice	Knotless technique	ETE	Knotless continuous suture	Postoperative anastomosis bleeding and stenosis	Conventional continuous suture technique	N/A	N	N
Maitz, 1999[26]	Comparative	Austria	90 rats	Biodegradable laser-activated solid protein solder	ETE	Sutureless microvascular anastomoses by a biodeg	Postoperative PR	Conventional interrupted suture technique	N/A	N	N

						radable laser-activated solid protein solder					
Riggio, 1999[27]	Experimental	Italy	81 rats	Modified sleeve technique	ETE	A vertical cut was placed in the sleeve (invagination)	Postoperative PR	Conventional interrupted suture technique	N/A	N	N
Sacak, 2013[28]	Experimental	Turkey	64 rats	Fibrin glue and venous cuff technique	ETE	Anastomosis was created with fibrin glue, a venous cuff was added to the anastomosis site	Postoperative PR	Conventional interrupted suture technique	N/A	N	N
Marni, 1996[29]	RCT	Italy	20 rats	Modified continuous technique	ETS	Last stitches are placed from the inside to the outside	Postoperative PR	Conventional interrupted suture technique	N/A	N	N
Ariyakhagorn, 2009[30]	RCT	Germany	72 rats	Knotless technique	ETE	The last stitch is made as close as possible to the starting stitch. The two starting and finishing	Anhepatic time, graft and subject survival	Conventional interrupted suture technique	N/A	N	N

						filaments of the suture are divided without making a knot.					
Human subjects											
Odobescu, 2014[31]	RCT	Canada	20 cadavers	Interrupted horizontal mattress sutures	ETE	The anastomosis started with a backhand pass of the horizontal matteress suture and returned with the forehand pass approximately 1 mm apart, such that the centre of the suture would coincide with the zenith of the vessel	Anastomotic leakage and time	Conventional interrupted suture technique	N/A	N	N
Miyagi, 2008[32]	RO	Japan	9 patients	Back wall support suture technique	ETE	Two sutures were placed at the deepes	Rate of hepatic artery throm	Conventional interrupted suture technique	N/A	N	N

						t, most difficult points in the hepatic artery for backside support. Each stitch was placed from the inner side of the arterial wall to the outer side with double needle sutures. The subsequent sutures were placed forward on either side adjacent to the previous suture	bosis				

RCT: Randomized Controlled Trial, RO: Retrospective Observational, Y: Yes, N: No, ETE: End-to-End, ETS: End-to-Side, Postoperative PR: Postoperative patency rate, Postoperative FR: Postoperative flow rate, N/A: Not Available.

Supplementary Appendix: Table 2. Studies validating novel microsurgical techniques: Non-comparative studies (16 studies)										
Study (First author, year)	**Study design**	**Country**	**Number of subjects**	**Name of technique validated**	**Type of technique**	**Description technique**	**Main outcome(s)**	**Technique is clinically used**	**Power analysis performed**	**Confounder adjustment performed**

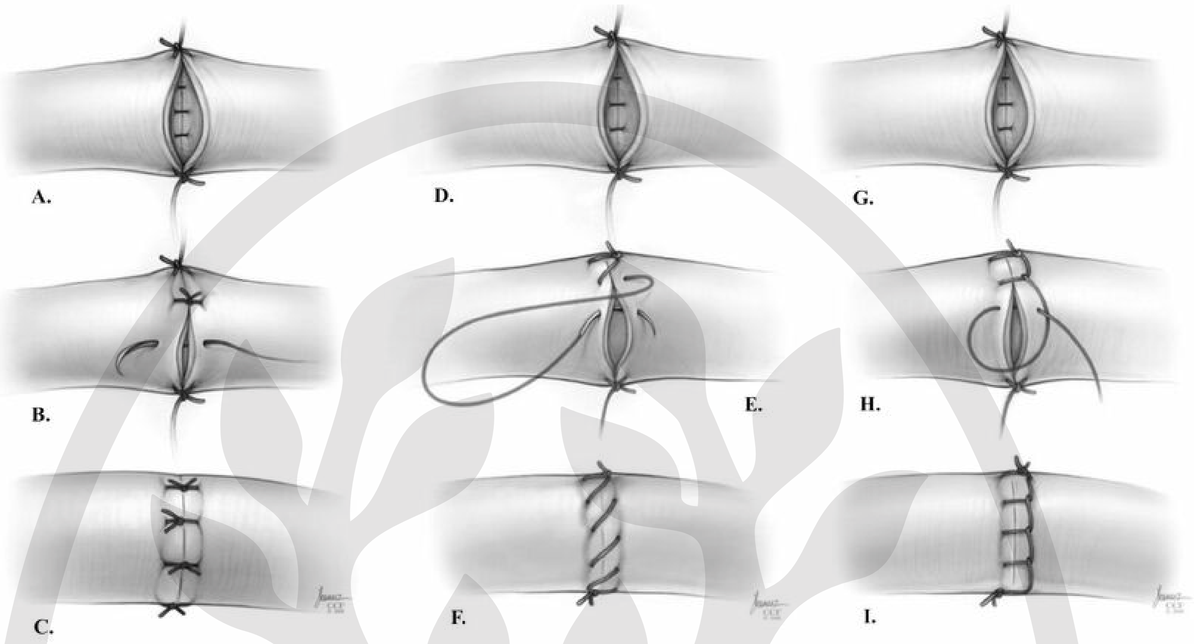
Animal subjects										
Orbay, 1985 [33]	Experimental	Switzerland	10 rats	Elongated and slitlike anastomosis	ETE	The anastomosis is facilitated by a longitudinally cut vein	Postoperative FR	N/A	N	N
Duarte, 1987[34]	Experimental	USA	72 rats	Thermic sleeve anastomosis	ETE	Proximal and distal stump of the vessel have been intussuscepted and are welded on two spots	Postoperative PR	N/A	N	N
Schubert, 2004[35]	RCT	Austria	42 rats	Bipolar anastomosis technique	ETE	Heat-induced tissue-welding is used to create the anastomosis	Operative time, Postoperative PR	N/A	N	N
Ulusal, 2005[36]	RCT	Taiwan	20 mice	Temporary assisting suspension suture technique	ETE	Placement of suspension loose sutures	Initial success rate	N/A	N	N
Hudson, 1998[37]	Experimental	South Africa	48 rats	Modified continuous anastomosis technique	ETE	The posterior wall and anterior wall continuous sutures are placed separately after two separate stay sutures. A closing suture is placed when flow through the anastomosis is confirmed	Ischemic time	N/A	N	N
Lauritzen, 1978[38]	Experimental	Sweden	20 rats	Sleeve anastomosis technique	ETE	Two starting sutures are placed and	Postoperative FR, postoperative PR	N/A	N	N

						the proximal vessel is placed inside the lumen of the distal vessel				
Holmin, 1983[39]	Experimental	Sweden	20 rats	Portacaval shunt	ETS	A simplified method of ETS anastomosis with three sutures	Postoperative PR	N/A	N	N
Savas, 1985[40]	Experimental	UK	40 rats	Sutureless cuff technique	ETE	A cuff was created from vessel lumps and connected together without sutures	Postoperative PR, postoperative subject survival	N/A	N	N
Fensterer, 2014[41]	Experimental	USA	8 rats	Cuff placement technique	ETE	Facilitating vessel eversion by the creation of a wedge-shaped gap to generate an adjustable cuff diameter at one end of the cuff and the creation of a barb to facilitate the fixing of draped vessel edges to the cuff.	Operative time	N	N	N
Schubert, 2006[42]	Experimental	Austria	40 chickens	Bipolar anastomosis technique (BAT)	ETE	Anastomosis is created by heat-induced tissue-welding	Postoperative PR and FR	N	N	N
Human subjects										
Bakhach,	CS	Lebanon	14 patien	V-Plasty technique	ETE	Creating a V-shaped	Vessel discrepan	Y	N	N

2015[43]			ts			flap in the vessel of larger diameter	cy ratio			
Inbal, 2019[44]	RO	USA	100 patients	Modified Kunlin's technique	ETE	An oblique transection of the donor and recipient vessels is performed with greater angle of transection for the smaller vessel to approximate circumferences	Flap survival rate, Postoperative complication rate	Y	N	N
Yamamoto, 1999[45]	CS	Japan	17 patients	Back Wall Technique	ETE	The back wall is sutured first	Postoperative FR and complication rate	N/A	N	N
Masoodi, 2022[46]	PO	Austria	12 patients	Double Barrel Lymphaticovenous Technique	ETE	Two lymphatic vessels have been intussuscepted into the vein	Relief of lymphedema symptoms	N	N	N
Nakagawa, 2008[47]	RO	Japan	9 patients	Modified technique for anastomosis of the posterior wall	ETS	Short pedicle without turn-over to the posterior wall	Postoperative venous thrombosis	N/A	N	N
Sen, 2006[48]	RO	Turkey	5 patients	Diamond arteriotomy technique	ETS	Diamond-shaped arteriotomy is performed	Operative time	N/A	N	N
Matsuo, 2020[49]	PO	Japan	7 patients	Single loop interrupted suture technique	ETS	Knots were placed after loop stitch placement	Postoperative PR, operative time	Y	N	N
Chen, 2015[50]	PO	Japan	9 patients	Octopus Lymphaticovenular Technique	ETE	Multiple lymphatic vessels have been intussusce	Relief of lymphedema symptoms	N	N	N

						pted into the vein				

RCT: Randomized Controlled Trial, RO: Retrospective Observational, CS: Case Series, PO: Prospective Observational N: No, ETE: End-to-End, ETS: End-to-Side, STS: Side-to-Side, Postoperative PR: Postoperative patency rate, Postoperative FR: Postoperative flow rate, N/A: Not Available.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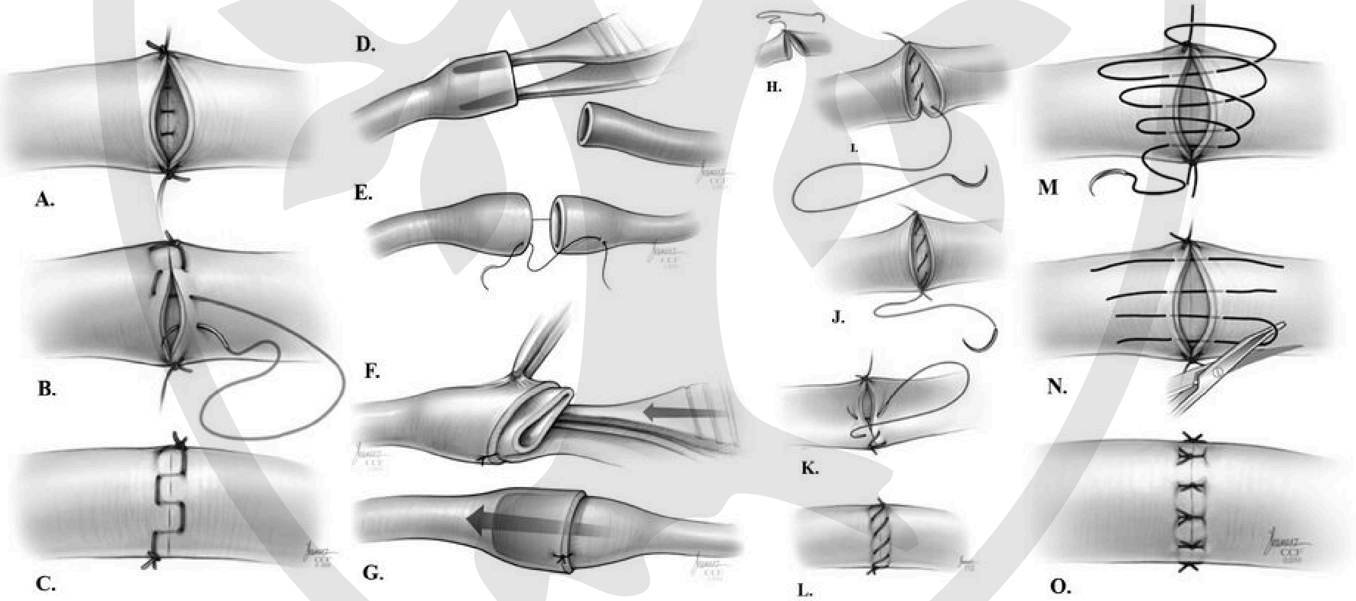
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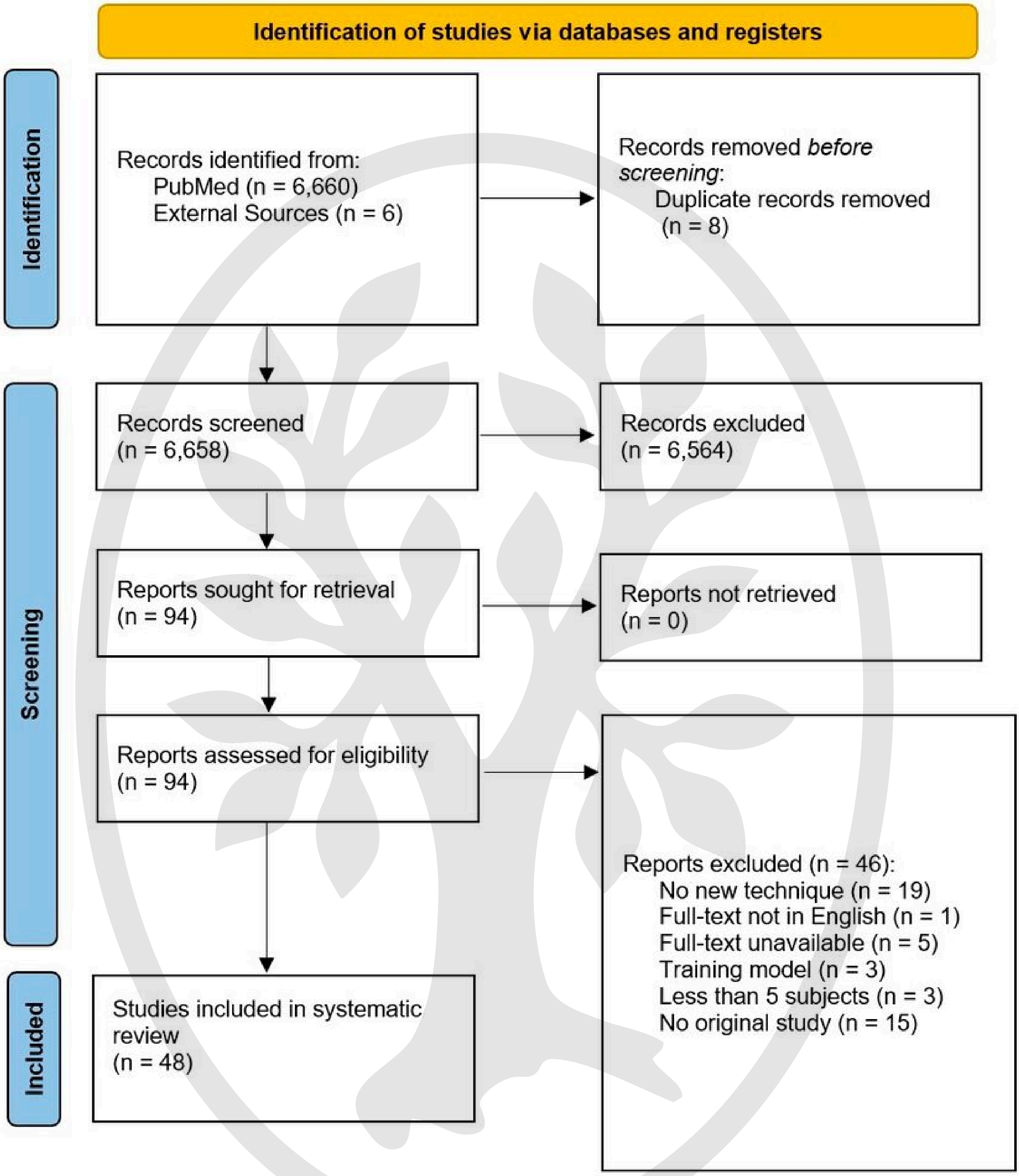
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External Sources (n = 6)
Records removed *before screening*:
Duplicate records removed (n = 8)
Screening
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Records excluded (n = 6,564)
Reports sought for retrieval (n = 94)
Reports not retrieved (n = 0)
Reports assessed for eligibility (n = 94)
Reports excluded (n = 46):
No new technique (n = 19)
Full-text not in English (n = 1)
Full-text unavailable (n = 5)
Training model (n = 3)
Less than 5 subjects (n = 3)
No original study (n = 15)
Included
Studies included in systematic review (n = 48)