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Experimental methodology used for testing *in vivo* adhesion of composite meshes for incisional hernia repair: a scoping review

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This review investigates the variability in the experimental methodology used in animal studies exploring the optimal composite mesh for incisional hernia (IH) repair. Eight databases were searched from inception to April 1, 2023. Animal studies conducted to evaluate the anti-adhesion effect of the composite mesh were included. Standardized forms were used to extract the experimental design characteristics. The extracted data were presented in tabular format and summarized using frequency analysis. The inherent risk of bias in the included studies was assessed using SYRCLE's risk of bias tool for animal studies. The results showed that 71 studies were included in the final analysis. Rats represented the most common animal (65%) used for studies. Conventional models (92%), high-adhesion models (4%), and abdominal cavity pollution models (4%) were reported in the included studies. The sample size of animals varied between studies (2–31/group). A variety of quantitative (calculation of adhesion area or testing of adhesion strength) and qualitative (45 assessment systems) adhesion assessment methods were reported. One month (41%) and 1 week (30%) were the most common time points used to evaluate the adhesion. The results of the risk of bias assessment showed that, of the 71 animal studies included, only one was a randomized controlled study, and only two studies reported that animal breeders and investigators were blinded. In conclusion, a large number of animal studies have been conducted to explore the ideal intraperitoneal anti-adhesive composite mesh for IH repair. However, these animal studies have significant differences in animal models, implantation procedures, control selection, and adhesion assessment. These differences directly affect the comparability between studies and the reproducibility of the studies.

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Introduction

Incisional hernia (IH) is defined as "Any abdominal wall gap with or without a bulge in the area of a postoperative scar perceptible or palpable by clinical examination or imaging",[1] which is one of the most common complications post-laparotomy. The IH rate estimated by pooling the published literature is 12.8% after about two years.[2] Laparoscopic intraperitoneal onlay mesh placement (IPOM) is clinically available for IH repair.[3] However, due to direct contact of mesh materials with abdominal viscera in IPOM, tissue-to-mesh adhesion is easily formed, leading to complications such as chronic pain,[4] intestinal obstruction,[5] difficulties at reoperation,[6] and even fistulation.[7,8] In 2006, the number of patients who underwent IH repair in the United States was estimated at 348 000.[9]

Although recent years have seen a growing number of review articles on incisional hernia management, most have concentrated on clinical treatment strategies and mesh applications in

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surgical practice. For example, Quiroga-Centeno *et al.* conducted a scoping review of emergency incisional hernia repair, outlining current clinical approaches including timing of intervention, surgical techniques, and postoperative management. They highlighted the lack of standardized terminology and high-quality evidence in this area.[10] Similarly, Najm *et al.* reviewed currently available abdominal wall meshes and emerging materials, focusing on their biological properties, postoperative complications, and performance in clinical trials.[11] However, neither review addressed the preclinical evaluation of anti-adhesion composite meshes in animal models. Given the critical role of animal studies in the preliminary assessment of mesh safety and efficacy, systematically examining experimental design variability, scoring systems, and observation durations is essential for facilitating clinical translation.

Identifying an ideal mesh for IH has been a hot topic of tremendous interest for general surgeons and medical device companies for decades. Composite meshes represent the ideal design for IPOM applications in recent years,[12] as these are comprised of a permanent synthetic mesh material on the parietal side and an adhesion barrier layer on the visceral side. The mesh side is intended to promote tissue ingrowth and anchor the prosthesis to the abdominal wall, whereas the barrier layer prevents adhesion of the abdominal viscera to the underlying mesh.[12] Composite meshes can be divided into two basic categories: meshes with permanent barrier layers, and meshes with absorbable (*i.e.*, temporary) barrier layers or coatings.

The anti-adhesion effect of composite mesh is a critical parameter reflecting product safety, while animal studies are usually performed to evaluate the anti-adhesion effects of the barrier layers applied to meshes before potential translation to clinics.[13] Adhesion formation to the mesh can only be researched using experimental models, since patients cannot be reoperated for evaluation of this key aspect.[14] Animal models for incisional hernia mesh repair can be broadly categorized into three types: the conventional model, the high-adhesion model, and the abdominal cavity pollution model. The conventional model typically involves creating an abdominal wall defect and repairing it with mesh under sterile conditions with intact peritoneum, leading to baseline levels of mesh-viscera adhesions. The high-adhesion model deliberately promotes extensive adhesions—for example, by abrading the peritoneal surface of the bowel to mimic severe injury (the Harris adhesion model)—resulting in markedly higher adhesion formation.[15] The abdominal cavity pollution model simulates infected or contaminated surgical fields by introducing bacterial inoculum or other contaminants into the abdominal cavity at the time of mesh implantation; for instance, inoculating methicillin-resistant *Staphylococcus aureus* (MRSA) after mesh placement produces an effective infection model.[16] The purpose of the adhesion testing is to provide evidence of a product's safety, and to demonstrate that a novel composite mesh is at least substantially equivalent, if not superior, to an established product.[17] Preclinical *in vivo* testing data has been commonly used in regulatory submission of mesh products.[18,19] However, there has been no recognized gold standard for the methods used to evaluate meshes *in vivo* for several pivotal characteristics, such as inflammation, shrinkage, ingrowth, remodeling, and adhesion formation to the mesh.

The Food and Drug Administration (FDA) has developed a guidance document outlining suitable testing methods for new surgical mesh products;[18] however, the guidance does not provide details on the technical aspects of *in vivo* studies that should be conducted and analyzed. In 2021, Whitehead-Clarke *et al.*[17] performed a scoping review of relevant studies to analyze the methodologies used for *in vivo* hernia mesh testing, and they found that standardization is absent from the current practice of *in vivo* mesh testing. There has been significant inconsistency in the methodology of every category of testing, encompassing mechanical testing, histology-structural analysis, and histology-inflammatory cellular analysis. Another systematic review conducted in 2020 (ref. 20) examined the experimental methodology behind *in vivo* testing of hiatus hernia and diaphragmatic hernia mesh and also found significant variation between existing studies.

A variety of composite meshes reducing adhesion have been developed and the number of animal studies exploring the optimal composite mesh for IH repair has been increasing. However, to our knowledge, no studies have investigated their experimental methodologies. Therefore, our scoping review analyzed the variability in this area of *in vivo* testing of IH composite meshes, contributing to identifying the need for standardization in the field and the areas in which standardization attracts clinical attention.

Materials and methods

The study design and reporting of data were compliant with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) extension for Scoping Reviews.[21] The methods of this scoping review have been specified in the predetermined protocol (Appendix I).

Search strategies

We conducted a systematic search of the following eight national and international databases from inception to April 1, 2021, including PubMed (1946–2021), Ovid-Embase (–2023), Web of Science (–2023), China National Knowledge Infrastructure Database (CNKI) (–2023), Chinese Scientific Journals Full-Text Database (–2023), Wanfang Database (–2023), and China Biological Medicine Database (CBM) (2023). The free words and medical subject heading (MeSH) in three groups of search terms, including "abdominal wall defect", "adhesion", and "search filters of animal studies"[22,23] are combined. The complete search strategy for the above databases is detailed in Appendix II. To identify the potential additional studies, we also searched the pertinent reviews[12,24] and the references cited in the included studies.

Eligibility criteria

The eligibility criteria related to study characteristics were as follows: (1) study design: controlled studies, with no restriction on randomization; (2) participants: animal models of

abdominal wall defect, with no limitations to the animal species nor modeling methods; (3) intervention/comparison: composite meshes (comprised of permanent synthetic mesh materials on the parietal side and an adhesion barrier layer on the visceral side) implanted in the abdominal wall. Studies that compare different fixation techniques, adjuncts that are not meshes, or new pharmacological products were excluded; (4) outcomes: tissue-to-mesh adhesions, regardless of measurements used.

The eligibility criteria related to the report characteristics were as follows: (1) language of publication: English or Chinese; and (2) status of publication: abstracts of studies were excluded.

Study selection

All search results from electronic databases were exported to EndNote X8 software and duplicates were removed. Two authors independently screened titles and abstracts based on the eligibility criteria. Before the formal selection of studies, a random sample of 10% of records was independently evaluated by the two reviewers, and the final selection process was not initiated until a satisfactory agreement (>90%) was achieved between them. Studies were subcategorized into three groups (included, excluded, and unsure) in this step. Afterwards, two authors independently examined the full text of potentially eligible and unclear studies to reach the final decision on inclusion or exclusion. Any disagreement between them was resolved through discussion or consultation with the third reviewer.

Charting the data

Two of three authors extracted the data from the included studies using a standardized, predefined data collection form prepared using Microsoft Excel 2016, and another author checked the extracted data. Any discrepancies were resolved by consensus or through consultations with the fourth author. The four authors from our group had previously collaborated on a similar project,[25] which created a good understanding of the process. Before the final extraction, a pretest using a random sample of ten included studies was carried out to revise the form, and its final version was consulted with the general surgeons.

The extracted information included the following: (1) general study characteristics (the first author and year of publication); (2) animal species, weight, age, and sex; (3) the number of animals used; (4) whether the sample size was calculated; (5) type of animal model; (6) whether the modeling method was reported; (7) whether published standard animal models were cited; (8) barrier layers and mesh layers of composite meshes; (9) product name of the marked composite meshes; (10) time points of meshes and tissues explanted; (11) methods for assessing adhesions.

Critical appraisal of studies

Two authors independently assessed the inherent risk of bias in the included studies using SYRCLE's risk of bias tool for animal studies,[26] which consisted of 10 questions grouped into the following six domains: selection bias, implementation bias, measurement bias, follow-up bias, report bias, and other biases. The answer to the assessment questions should be either "yes" for a low risk of bias, or "no" for a high risk of bias. For unclear items, an answer with "unclear" was assigned. The two authors cross-checked the evaluation results. Any disagreements were resolved through consultation with the third author.

Synthesis of results

The results from the data extraction tool were collated and summarized to provide a narrative review of how published literature reports on the experimental methodology for *in vivo* adhesion testing of the composite meshes used in IH repair. Tabular and graphical representations of the data were used to illustrate the identified results and were supported by narrative descriptions of the data.

Results

Search and selection results

Following the removal of duplicates, 3036 studies were identified from the search of electronic databases, and 13 additional potentially relevant studies were identified from a supplementary search conducted on April 1, 2023. After a detailed assessment based on eligibility criteria, 71 studies were included in the scoping review (Appendix III). The study selection process is presented in Fig. 1.

Results of the risk of bias assessment

The results of the risk of bias assessment are shown in Fig. 2. Of the 71 animal studies included, only one was a randomized controlled study, which reported the method for random allocations but did not apply allocation concealment. The baseline features of 18 studies were balanced. Only two studies reported that animal breeders and investigators were blinded. Twenty-six studies randomized the placement of laboratory animals. In two studies, animals were randomly selected for results evaluation. In 19 studies, the evaluators were blinded to the results. Experimental animals from 47 studies were included in the final analysis. We only obtained one study protocol, and all the pre-identified outcomes were reported in the study. For the remaining 70 studies that we did not obtain study protocols for, all the expected results were reported in the studies. For other sources of bias, 31 studies did not report funding or conflict of interest statements, and 15 studies only analyzed the surviving animals.

Experimental technique

Animal species. In all 71 studies, rats represented the most common animals used for studies (65%, 46/71), followed by rabbits (28%, 20/71). Pigs (7%, 5/71) and dogs (1%, 1/71) were also used in several studies (Fig. 3). Before the experiments, 87% (62/71) provided details on animal weights; 27% (19/71) provided details on animal ages; 75% (53/71) provided details on animal sex, and most studies (35/53) only used male animals.

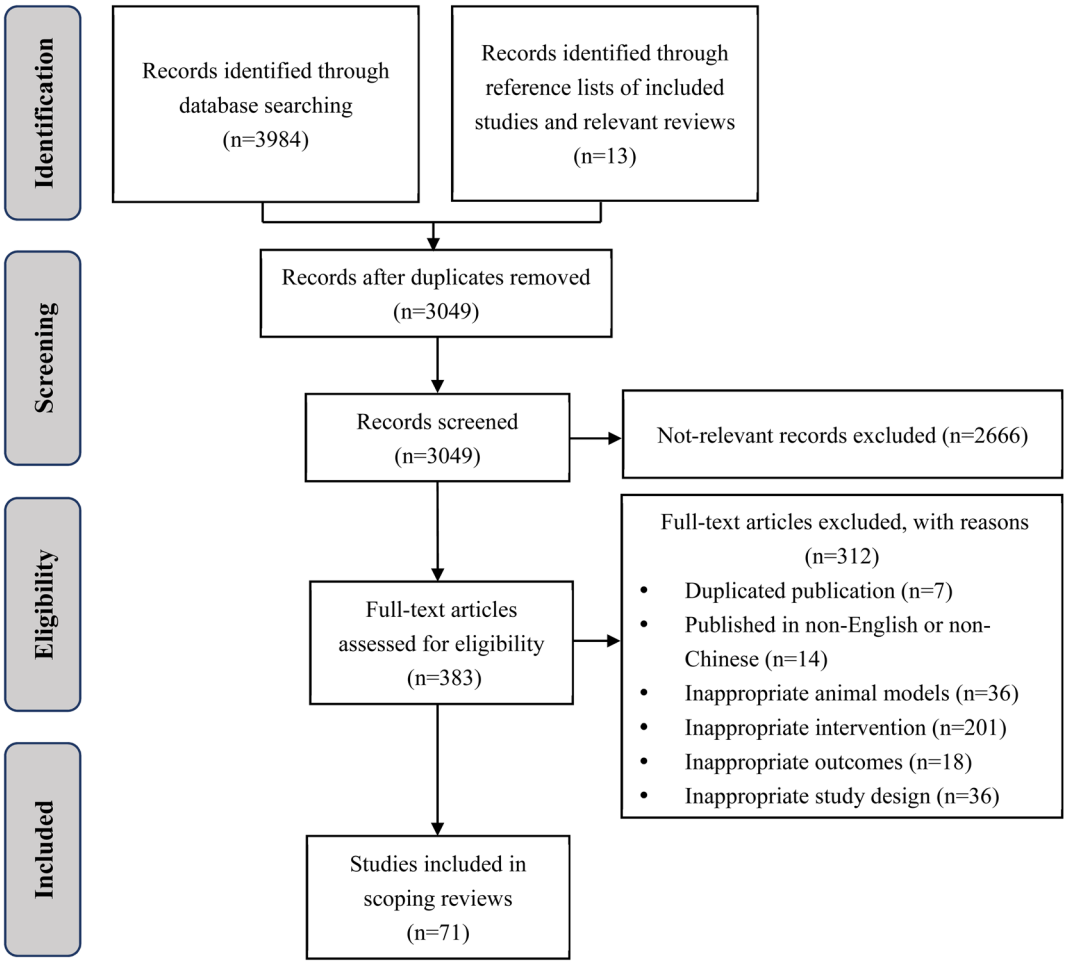

Fig. 1 The study screening and selection process.

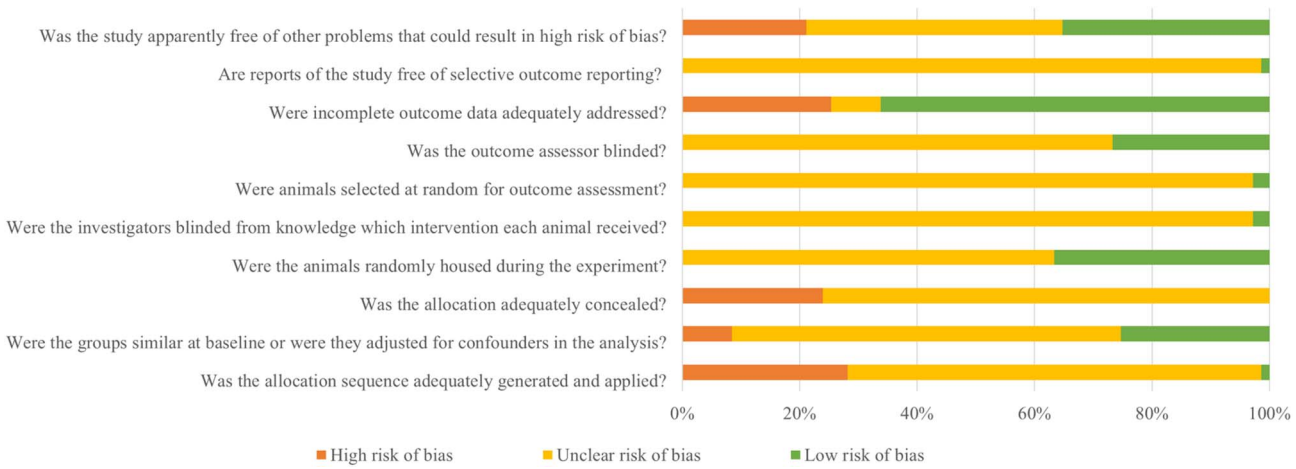

Fig. 2 Results of the risk of bias assessment.

Modeling methods. In these studies, 93% (66/71) reported the modeling methods for the experimental animals, but 15% of them (10/66) cited standard published animal models. Ninety-two percent (92%) (65/71) used the conventional animal models of IH. Drawing on the conventional model, 4% (3/71) performed mechanical abrasion of the bowel to create a high-adhesion model, and 4% (3/71) used effective pollution sources to create a model of abdominal cavity pollution (Fig. 4).

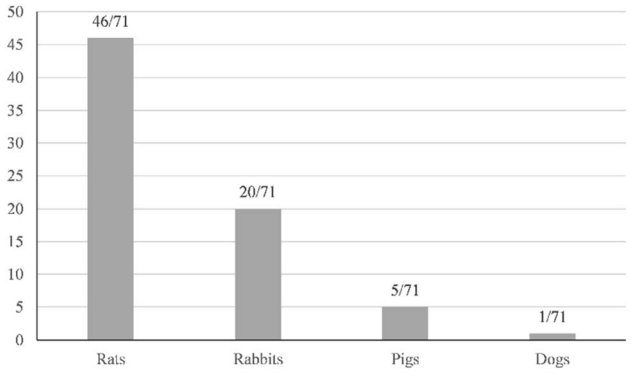

Fig. 3 Animal species.

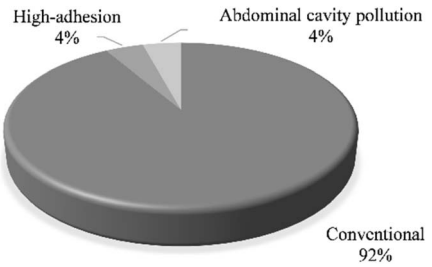

Fig. 4 The type of animal model.

Sample size. The sample size of animals varied between studies (2–31/group). Forty-five percent (45%) (32/71) had less than 10 animals per group, 42% (30/71) had 10–20 animals per group, and 13% (9/71) had more than 20 animals per group (Fig. 5). None of the studies reported how the sample size was calculated.

Explant time. The meshes and tissues were explanted from animals at different time points. Fifty-eight percent (58%) (41/71) explanted the meshes and tissues from animals at one fixed time point, 30% (21/71) involved two explant time points, and 14% (10/71) involved three to five explant time points (Fig. 6). The reported time points included one week (30%, 21/71), two weeks (20%, 14/71), one month (41%, 29/71), and three months (21%, 15/71).

Composite mesh. A total of 44 types of composite meshes were involved in the included studies, and 15 of them were marketed products. For the involved composite meshes, the permanent synthetic meshes on the parietal side involved nine kinds of materials. The adhesion barrier layer on the visceral side involved four types of materials, including seven non-absorbable synthetic materials, four absorbable synthetic materials, 22 natural materials, and six composite materials. The components of the composite meshes are described in Appendix IV. In all 71 included studies, 39% (28/71) compared the anti-adhesion effects of marketed and non-marketed products, 45% (32/71) compared the anti-adhesion effects of marketed and marketed products, and 16% (11/71) compared the anti-adhesion effects of non-marketed and non-marketed products (Fig. 7).

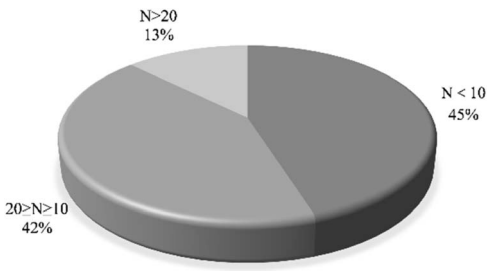

Fig. 5 The number of animals in each group.

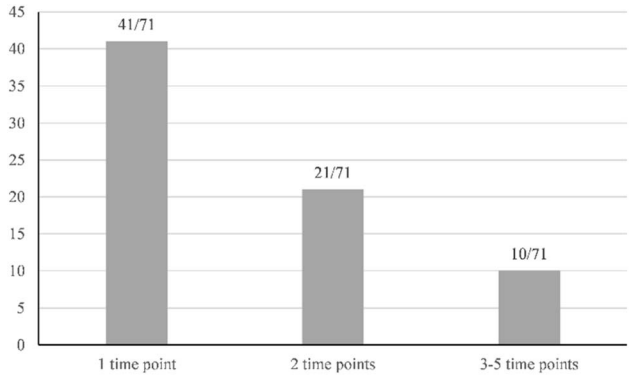

Fig. 6 The number of explant time points.

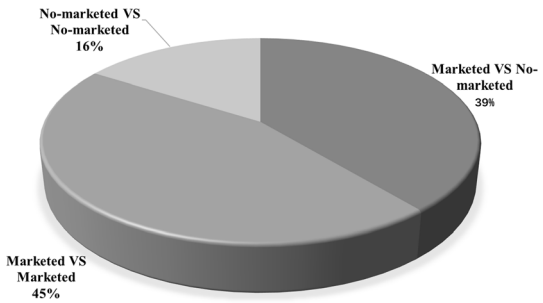

Fig. 7 Types of reference products.

Methods for adhesion assessment

A variety of adhesion assessment methods were reported in the included studies. Adhesions were assessed quantitatively in 58% of studies (41/71), qualitatively in 8% (6/71), or both quantitatively and qualitatively in 34% of studies (24/71) (Fig. 8). The quantitative assessment methods encompassed the calculation/estimation of adhesion area and testing of adhesion strength. Four adhesion indicators were involved in the included studies, including adhesion area, adhesion scope, adhesion strength, and adhesion appearance. Adhesion area was assessed in 73% of studies (52/71); adhesion scope was assessed in 15% (11/71); adhesion strength was assessed in 54% (38/71); and adhesion appearance was assessed in 34% (24/71) (Fig. 8 and 9).

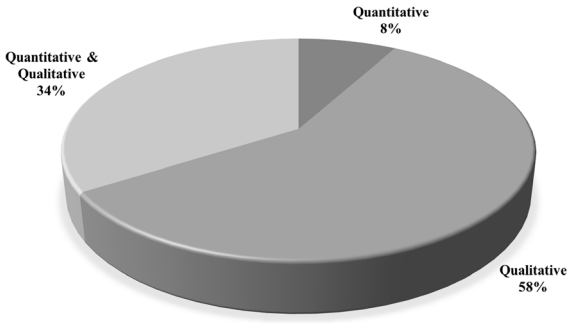

Fig. 8 Methods for adhesion assessment.

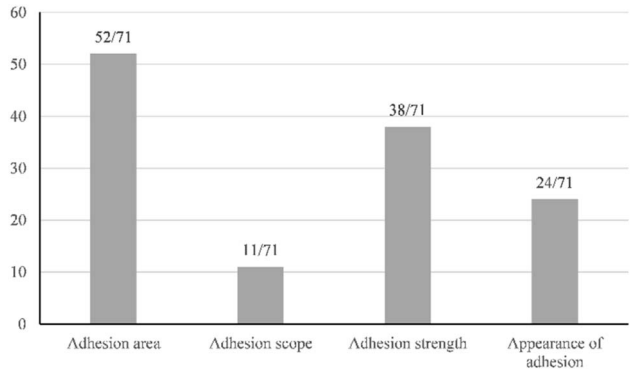

Fig. 9 Adhesion indicators involved in the included studies.

Thirty-nine percent (39%) (28/71) reported the quantitative estimation/calculation of adhesion area. The calculation/estimation tools involved in these studies are image analysis software, planimetry by digital caliper equipped with a liquid crystal display screen, and macroscopic evaluation, and 2 cm × 2 cm side length grid estimation. Among the 28 studies, nine did not report which tool was used for quantitative calculation/estimation of adhesion area. In all 71 included studies, four reported quantitative testing of adhesion strength with tensile testing equipment.

Among the studies, a qualitative adhesion assessment by macroscopic observation was reported in 92% (65/71). Departing from the assessment systems used to evaluate the existence and severity of adhesions in 61 studies, no assessment systems were used in four studies. A total of 45 assessment systems were involved, and 40 of them used severity level to grade adhesions.

In 65 studies, 37 used a single indicator system for adhesion assessment, involving 24 systems, as outlined in Appendix V. There were four assessment systems to grade adhesion based on the percentage area of the mesh surface covered, and four different severity levels of adhesions were used in these assessment systems. The frequency of adhesion scores appearing in different evaluation systems is shown in Fig. 10. Four systems were assessed based on the adhesion scope, involving the abdominal organs, and three of them used severity level. Thirteen systems were based on the adhesion strength between meshes and tissues, and 12 of them used

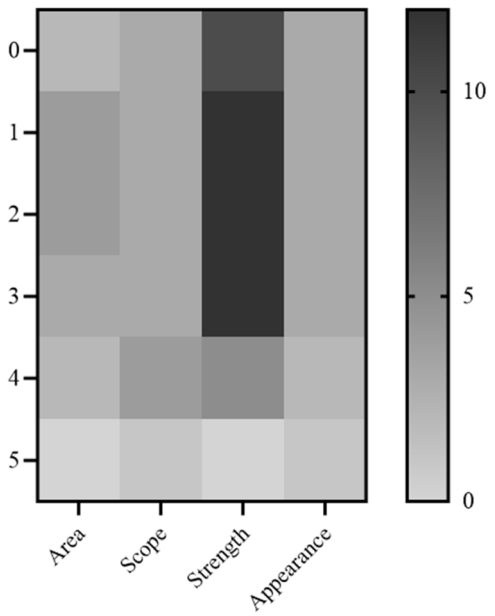

Fig. 10 Adhesion scores.

severity level. Three systems were based on the adhesion appearance, and all of them used severity level.

In 28/65 studies, a combined indicator system was used for adhesion assessment. Twenty-one systems were involved in these studies and are outlined in Appendix VI. Among these systems, 12 considered two indicators (Scope and Appearance, 3; Strength and Appearance, 7; Area and Appearance, 1; Strength and Area, 1); 9 considered three indicators (Area and Appearance and Strength, 7; Strength and Area and Scope, 1; Strength and Area and Scope, 1), and 18 used severity level to grade adhesions, covering ten different grading levels.

Discussion

A large number of animal studies have been conducted to evaluate the anti-adhesive effects of composite meshes for intraperitoneal IHs; however, the results of our scoping review found significant methodological differences and deficiencies in these animal studies, leading to a lack of comparability between studies, as well as limiting the reproducibility of studies.

Animal models are central elements in the design, implementation, and evaluation of animal studies. The selection of animal models should be evaluated comprehensively. In terms of animal models of human disease, the higher the degree to which human disease characteristics can be reproduced or simulated, the better the model.[14,27] The similarity of disease characteristics is generally the first consideration in the selection and design of a particular animal model for study consideration.[27] This relates to the correlation between animal studies and clinical applications, and is important for maximizing the use of the evidence obtained from future animal studies and minimizing the risks of clinical trials.[27] Our scoping review found that the animal models of hernia used to study the anti-

adhesive effects of composite meshes involved small animals in rats (65%) and rabbits (28%) and large animals in pigs (7%) and dogs (1%). The rat model is the most commonly used animal model for IHs. Over 50% of all experimental hernia research focused on rat models.[28,29] Rats are economical, simple to manipulate, and their genetic variation can be easily controlled through inbred lines,[27] but they are less anatomically and physiologically compatible with humans.[30] On the one hand, they differ significantly from humans in terms of abdominal wall defect size and fascial thickness. On the other hand, rats have superior tissue healing ability to humans. Experts in the field have pointed out that the porcine model is physiologically the most appropriate choice to better mimic the structure of the human abdominal wall, particularly in terms of abdominal wall strength.[31] Recently, van den Hil *et al.*[32] found similar histological findings in rats and humans in terms of adhesion formation and foreign body response to meshes. This means that rats may not be the best animal model for evaluating the effect of hernia mesh repair, but may be one of the more desirable options for evaluating the anti-adhesive effects of composite meshes.

During one study, the growth of the animal had a significant impact on the evaluation of the efficacy of mesh repair for IHs. In 1975, Cerise *et al.*,[33] in an animal study of meshes, found that the rats doubled in size during the study. On the one hand, the increase in volume reflects the increase in the rupture strength of the abdominal wall, and this change will directly affect the evaluation of the mechanical strength of meshes. On the other hand, changes in animal volume have an impact on the interaction between the meshes and tissues, which in turn may affect the formation of adhesions. Of the included animal studies, the majority of studies (87%) reported only the animal weight at the start of the study and only 27% reported the animal age at the start of the study. The animals used for the study were reported to be quite young. To exclude the influence of animal growth and development on the experimental results, adult animals with stable body weight (size) should be used for future studies, and changes in animal weight before and after the experiment should be reported. Our scoping review found that animal sex was not reported in 25% of studies. Of the studies that reported the animal sex used, the majority (66%) used only male animals. Although there is no relevant evidence that the efficacy of mesh repair of IHs differs between male and female patients, to avoid potential physiological differences between the sexes from influencing experimental results,[34] we recommend that future studies balance and report animal sex in experimental studies of mesh repair of IHs in accordance with the Animal Research Reporting of *In Vivo* Experiments (ARRIVE) guidelines[35] and the recently published National Institutes of Health (NIH) policy.[36]

The majority of studies (92%) used a conventional IH model, and only a few studies simulated postoperative pathological abdominal adhesions (4%) and infections (4%) based on the IH model. The preservation of the animal's intact peritoneum in the former IH model, without any intestinal abrasion, is closer to the clinical reality of IH. A more complex clinical application scenario is created in the latter IH model, where the friction of the intestinal canal and peritoneum, and surgical infection in the modeling modality are all associated with higher probability of adhesions in the animal model, and the results obtained are more favorable for the evaluation of complex clinical application scenarios. Most of the studies (93%) reported the modeling method of the IH model, but we found that only 10 of these studies cited an IH model from previous studies. The current method of IH modeling varies significantly between studies.[14] Differences in animal modeling make it difficult to replicate study results and to directly compare results across the literature,[12,37] preventing direct access to information about the advantages and disadvantages of multiple commercially available products or developed biomaterials, and resulting in difficulties translating study results into clinical practice .[28,29,38] Therefore, we believe that it is appropriate to clarify the primary study objectives and the main evaluation measures when designing animal studies, limit the available modeling animals to a small scope focused on the primary study objectives and the main evaluation measures, and establish standardized models and cite them clearly, which may increase the impact of future publications and thus facilitate the clinical translation of the study results.[39–42]

For the regulatory clearance of new meshes, the FDA suggests the use of marketed products as standard controls for animal studies.[18] The use of standard controls helps to ensure the reproducibility of animal studies, and is beneficial for correctly determining the equivalence of the new composite meshes in the experimental group to their commercially available controls in terms of safety and efficacy. A total of 44 composite meshes or materials were covered in our scoping review, 15 of which were marketed products. 39% of animal studies used marketed products as a control. These products were used to validate the anti-adhesive effect of new composite meshes. In contrast, 16% of animal studies did not use any marketed products as controls. The rationale for choosing controls in these studies was relevant to the study's purpose. In the early stage of material design, it is necessary to select material combinations with different ratios as controls to determine the best material formulation for reducing adhesion. We suggest that animal studies aiming at obtaining evidence of safety and efficacy should use marketed meshes whose clinical benefits have been validated as controls, allowing researchers to make reliable judgments on whether the new composite meshes have the value of clinical translation, improve the value of animal studies, and reduce the waste of resources. Meanwhile, accumulating evidence suggests that chemical modifications can markedly influence the adhesion behavior of surgical meshes. One review highlighted that chemical alterations to the surface or internal structure of biomaterials, particularly through the incorporation of functionalized nanofillers such as poly(methyl methacrylate) grafted carbon nano-onions (PMPMA CNOs), can significantly enhance both the biological performance and mechanical integrity of composite materials.[43] Such strategies provide a strong foundation for the design and development of advanced implantable biomaterials, including anti-adhesion surgical meshes.

For adhesion evaluation measures, our scoping review found that a range of reported adhesion assessment methods were used in the pertinent literature, including quantitative assessment methods (adhesion area calculation and adhesion strength testing) and qualitative assessment methods (45 qualitative assessment systems). Qualitative assessment of adhesions (92%) was used in most studies. In the current systems for qualitative adhesion assessment, the severity of adhesions is classified according to one or more of four classification indicators: adhesion area, adhesion strength, adhesion extent, and observation of adhesion appearance. Some of the qualitative assessment systems examined the same adhesion classification indicators, but there were multiple ways of grading the severity of adhesions between studies.

No standard adhesion scoring system is widely accepted and used internationally.[24,44,45] In 2019, the National Medical Products Administration (NMPA) issued a guidance document titled Technical Review of Animal Study of Intraperitoneal Internal Hernia Mesh,[46] which suggested relevant requirements for the animal studies of composite hernia meshes under application for medical device registration in China. In this document, the relevance of animal studies to clinical practice was considered, incorporating the clinical applicability when establishing criteria for adhesion evaluation. Considering that mild adhesions without serious adverse events are clinically acceptable, in this guidance document, the rate of excellent adhesions was considered as a criterion to determine the acceptability of adhesions between meshes and tissues in animal studies. Moreover, since adhesion intensity and adhesion area show different effects on adhesion-related complications, such as intestinal fistula and intestinal obstruction, the "excellence" rate of adhesion is set as a composite indicator and there is an accompanying comprehensive evaluation table. In the evaluation table biased toward adhesion strength, adhesion area level 2 and adhesion strength level 3 are considered unacceptable, while adhesion area level 3 and adhesion strength level 2 are acceptable. Accordingly, investigators need to reach a consensus on a standard evaluation system for the anti-adhesion effects of meshes in animal studies. To better achieve clinical translation of animal study results, future animal studies on adhesion evaluation criteria need to be linked to those of clinical adhesions.

In the present study, the follow-up duration for adhesion evaluation varied widely among the included literature, ranging from 1 day to 1 year, most commonly at 1 month (41%) and 1 week (30%) time points. In animal studies, whether or not the barrier/coating of the composite mesh is degraded and the variability in degradation period may be one of the main reasons for the difference in follow-up time.[47] After implantation of the composite mesh in animals, an extensive inflammatory response may be provoked by contact between materials and abdominal tissues, which is one of the factors contributing to the development of adhesions.[48] Barrier materials such as polycaprolactone (PCL) and collagen exhibit markedly distinct degradation behaviors. PCL, a slow-degrading synthetic polymer, remains *in situ* for several months to years,[49] offering prolonged separation between viscera and mesh throughout various stages of tissue healing. In contrast, collagen-based barriers are natural biomaterials that are enzymatically broken down within weeks after implantation.[50] One study demonstrated that collagen-coated mesh significantly reduced adhesions at 7 days, but by 30 days post-op the collagen layer had been phagocytosed and adhesions had markedly increased.[48] Conversely, PCL barriers maintain physical isolation over a longer period, thereby minimizing adhesion formation until peritoneal regeneration is complete. Accordingly, the degradation timeline of the barrier layer should be carefully aligned with the biological window of peritoneal healing to maximize anti-adhesion efficacy. Reaction associated with surgical trauma is another factor contributing to adhesions. In accordance with ISO 10993-6:2007,[51] it may be difficult to distinguish between implant-induced or surgically-induced local tissue reactions in the first two weeks post-implantation procedure. According to the consensus of experts in this field,[24] a follow-up of 4 weeks or less is appropriate to assess short-term inflammatory responses in animal studies. In addition, reparation and peritonealization of the peritoneal mesothelial cell layer are critical factors in preventing adhesions in the abdominal cavity. Previous studies[52,53] noted that it typically takes 8 days for complete healing of the mural peritoneum. In our work, it is concluded that for the follow-up time of animal studies in this field, the observation time point and follow-up time should be determined by combining the barrier/coating degradation cycle of composite meshes and the duration of chronic inflammatory regression to ensure the scientific validity of outcome observations.

There were large differences in the animal sample size between studies, and none of the studies reported the calculation of sample size. In animal studies, it is crucial to determine the sample size, *i.e.*, the number of animals per group. The sample size should meet the requirements of scientific research validity, but also comply with the ethical guidelines, national laws and regulations, and the 3rs principles of limits on the number of animals used.[27] There are two erroneous tendencies in determining the number of experimental animals in animal studies, too small or too large, and too small occupies a large proportion of these two erroneous tendencies.[27] Too small a sample size of animals leads to too little test efficacy in detecting meaningful or biologically significant results. Regarding how to scientifically determine the number of experimental animals when designing animal studies, the website of the Center for Biomathematics, Department of Pediatrics at Columbia University Medical Center has provided relevant calculation methods and formulas for different effect sizes,[54] future studies should incorporate power analysis during the design phase to calculate the minimum number of animals required. We recommend utilizing online sample size calculators, such as those provided by the Columbia University Biostatistics Center, which allow for calculation based on parameters including anticipated effect size, significance level (α), and statistical power ($1 - \beta$).[55] Researchers should also clearly report the methods and parameters used for sample size estimation in their publications to ensure transparency and reproducibility.

The studies included in this scoping review have major limitations in terms of experimental design and implementation, especially in terms of randomization, blinding, and allocation concealment, resulting in a high risk of various biases that affect the internal veracity of animal studies.[56–58] In the 71 studies, only one reported a randomized approach, where no concealed grouping was implemented, and 53 studies had uneven baseline characteristics and might have been subject to selective bias. Additionally, as an interventional animal study in surgery, it is crucial that the study executor and outcome assessors are blinded during the surgical performance and observation of subjective measures of adhesions, which could reduce implementation bias and measurement bias and improve the reliability and authenticity of the experimental results. Unfortunately, the executers and outcome assessors were unblinded in 52 studies. We recommend that future animal studies in this field be designed and conducted according to the risk of bias tool SYRCLE,[26] with an attempt to improve the internal veracity of the studies and ensure that the results are based on high-quality and unbiased data.

We further recommend standardizing several key parameters of animal experiments, in particular the type of animal model, follow-up time points, and adhesion assessment scoring system. First, researchers should select and clearly define the type of animal model (conventional, high-adhesion, or abdominal cavity pollution) appropriate for their study objectives, and explicitly state this in publications, to ensure results are contextually relevant and comparable.[15,16] Second, follow-up observation time points should be standardized, encompassing at a minimum an early phase (*e.g.*, 7–14 days post-surgery) and a longer-term phase (*e.g.*, 4–12 weeks post-surgery), to evaluate initial adhesion formation as well as its progression over time. Consistent time-point scheduling will facilitate direct comparisons of outcomes across different studies. Finally, drawing on the multiple adhesion scoring criteria compiled in this review, we advocate a unified multi-dimensional scoring framework for evaluating mesh adhesions. This scoring system includes metrics such as adhesion area (the percentage of mesh surface involved), adhesion extent (the number of adhesion bands and organs attached), adhesion tenacity (the force required to separate adhesions, reflecting their firmness), and adhesion appearance (*e.g.*, fibrous tissue thickness and degree of vascularization). Each metric can be graded on a scale (*e.g.*, 0 = no adhesion up to 3 or 4 = most severe adhesion) to quantitatively capture the overall severity of mesh adhesions. Employing such a standardized multi-parameter scoring system will reduce biases arising from disparate assessment methods and improve the objectivity and comparability of adhesion outcomes between studies.

To improve the comparability and reliability of animal study results, it is recommended that future researchers adhere to the ARRIVE guidelines, the core principles of ISO 10993-6, and the key elements outlined by Cheng *et al.*[59–61] These include a clear definition of study objectives, appropriate selection of test devices and controls, careful choice of animal models, adequate sample size, rational follow-up periods, robust outcome measures, comprehensive reporting, and implementation of an effective quality management system. By embracing the "3R + DQ" principle—replacement, reduction, refinement, combined with design and quality—researchers can enhance study quality, support inter-study standardization and reproducibility, and ultimately accelerate the clinical translation of anti-adhesion meshes for incisional hernia repair.

Conclusion

Existing animal studies exploring the ideal intraperitoneal anti-adhesive composite mesh for IH repair have significant differences in terms of animal models, implantation procedures, control selection, and adhesion assessment. There are serious deficiencies in the implementation of experimental design in terms of sample size determination, randomization, blinded experiments, and allocation concealment. These differences and deficiencies directly affect the comparability between studies and the reproducibility of the studies, severely limit the clinical translation efficiency of the composite hernia mesh under investigation, and lead to the waste of substantial animal research resources. Consequently, the necessary and more reliable data depend on the establishment of standardized methods for animal studies in this field. The standardized approach also enables direct comparison of data on various types of composite hernia meshes or newly developed materials among different research teams, facilitating the efficiency of clinical translation across the hernia mesh industry.

Author contributions

Xu Zhang and Kaiyan Hu are responsible for conceptualization and writing – original draft, Zhe Wang, Yanbiao Jiang, Zhenyu Zou, Jinwei Yang, Mingyue Jiao, Shuo Yang, Yingmo Shen, Yusha Liu, Xingzhi Li are responsible for visualization and formal analysis, Jing Xue is responsible for Writing – review & editing.

Conflicts of interest

The authors declare that they have no conflict of interest.

Data availability

No primary research results, software or code have been included and no new data were generated or analyzed as part of this review.

Supplementary information: Appendix I (Study protocol), Appendix II (Search strategy), Appendix III (List of included studies), Appendix IV (Description of the components of composite meshes), Appendix V (Rating system with the single indicator for adhesion assessment), and Appendix VI (Rating system with combination indicators for adhesion assessment). See DOI: **https://doi.org/10.1039/d5ra02062j**.

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