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RESEARCH ARTICLE

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A systematic review and meta-analyses on animal models used in bone adhesive research

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Abstract

Currently, steel implants are used for osteosynthesis of (comminuted) fractures and intra-articular bone defects. These osteosyntheses can sometimes be complicated procedures and can have several drawbacks including stress shielding of the bone. A bone glue might be a safe and effective alternative to current materials. Despite numerous animal studies on bone adhesives, no such material is clinically applied yet. We have conducted a systematic review to summarize the evidence in experimental animal models used in research on bone adhesive materials for trauma and orthopedic surgery. Additionally, we analysed the efficacy of the different bone adhesives for different experimental designs. A heterogeneity in experimental parameters including animal species, defect types, and control measurements resulted in a wide variety in experimental models. In addition, no standard outcome measurements could be identified. Meta-analysis on bone regeneration between adhesive treatment and nonadhesive treatment showed a high heterogeneity and no statistically significant overall effect (*M*: −0.71, 95% confidence interval [CI]: −1.63–0.21, $p = 0.13$). Besides, currently there is not enough evidence to draw conclusions based on the effectiveness of the individual types of adhesives or experimental models. A positive statistically significant effect was found for the adhesive treatment in comparison with conventional osteosynthesis materials (*M*: 2.49, 95% CI: 1.20–3.79, $p = 0.0002$). To enhance progression in bone adhesive research and provide valuable evidence for clinical application, more standard experimental parameters and a higher reporting quality in animal studies are needed. Statement of Clinical Significance: Current materials restoring anatomical alignments of bones have several drawbacks. A (biodegradable) adhesive for fixating bone defects can be a treatment breakthrough. Although numerous bone adhesives have been researched, most seemed to fail at the preclinical stage. An overview in this field is missing. This systematic review highlights the relevant parameters for design of experimental bone adhesive studies. It demonstrates evidence regarding

benefit of bone adhesives but also that the quality of reporting and the risk of bias in studies need to be improved. The results will aid in designing better quality animal studies for bone adhesive research with higher translational value.

KEYWORDS
animal model, Bone adhesive, bone defect, meta-analysis, systematic review

1 | INTRODUCTION

Osteosyntheses materials including pins and plates are commonly implanted as treatment method for dislocated fracture care in trauma and orthopedic surgery. The aim of these osteosynthesis is to restore the anatomical alignment of the bones, to reduce the fracture gap, and to provide mechanical stability thus facilitate early mobilization and enhance patient recovery.[1–3] However, the stiff materials used in osteosynthesis devices can cause stress-shielding of the fractured bone, resulting in weakening of the bone and delayed bone healing.[4–7] In addition, application of screws for fixation of osteosynthesis devices creates new bone defects.[3] On occasion, patients complain about the steel implants and a new operation is needed to remove the nondegradable fixation devices.[8] These disadvantages have led researchers to search for alternative fixation options, including bone adhesive materials.

A bone glue or bone tape should form a sufficiently strong bond with bone tissue to stabilize the bone defect long enough to allow bone healing and even in compromised circumstances, for example, bleeding, exudate formation and inflammation.[9] These bond strengths are often first evaluated by experiments on a mechanical test bench with cadaveric bone. In addition, it is important that the adhesive does not form a barrier for tissue growth and causes no adverse biological effects. Ideally, the bone adhesive is biodegradable, easy to apply and has a fast fracture healing with a short immobilization time for the patient.[9,10] These (long term) safety and efficacy issues will have to be tested on in vivo animal models. When designing these studies, attention should be paid to the type of animal species (e.g., small or large animals), anatomical location (e.g., craniofacial or long bones) and type (e.g., burr hole or fracture) and size of the defect. In addition, outcome measurements and techniques can differ based on the type of research question and experimental model. Since no standard experimental design is advocated for animal studies on bone adhesive materials, the diversity in all these parameters causes a high variety in experimental studies with equivocal outcomes.

The aim of this systematic review is to summarize the evidence in experimental animal models used in research on bone adhesive materials for trauma and orthopedic surgery, identifying the most appropriate experimental models for bone adhesive research, and analysing the efficacy of the different bone adhesives on tissue regeneration for the different experimental designs. We focussed on bone adhesive materials that are used to bind bone tissue, bone graft or tendon and that specifically adhere to bone tissue rather than filling the bone defect. Outcomes of this systematic review can guide future bone adhesive research with evidence-based design and model selection.

2 | MATERIALS AND METHODS

The full review protocol was registered on June 18th, 2018 in PROSPERO[11] under registration number CRD42018091831.

2.1 | Search strategy

To identify as many in vivo animal studies as possible on bone adhesive materials, a comprehensive and systematic search strategy was performed in three databases; Pubmed, EMBASE (via Ovid) and Web of Science, in December 2020. The search strategy combined an "adhesive" search component containing synonyms for adhesive-related terms, with a component for "bone defect" or "bone-tendon rupture" (complete search strings in Supplements). Thereafter, the search terms were combined with the SYRCLE animal filters designed by Hooijmans et al.[12] (PubMed) and De Vries et al.[13] (EMBASE). An adapted version of the EMBASE animal filter was used in the Web of Science database. In the EMBASE search, conference abstracts and conference reviews were omitted. No restriction on language or publication data were applied.

2.2 | Study selection and exclusion criteria

All articles were collected in Endnote (version X9.2; Thomson Reuters) and duplicates were manually removed. Title and abstracts were screened for relevance in the online tool Rayyan QCRI (Doha, Qatar, http://rayyan.qcri.org) by two independent researchers (MvE and FY, RFL or MI), using the following exclusion criteria: (1) no bone or bone-tendon defect; (2) no bone-binding adhesive; (3) no in vivo animal model; (4) no healthy animal model; (5) not an original study (i.e., reviews or opinion letters). Studies were included where the bone adhesive material (i) had (tissue-specific) chemical and/or physical bonding properties, (ii) was used to hold at least two pieces of (artificial) bone or bone and tendon together, (iii) was applied on the defect or recipient site and (iv) was not primarily used as a bone

substitute or filling material. Selected studies were independently screened based on full articles by at least two researchers (MvE, and RFL or FY) with the additional exclusion criteria: (6) adhesive is used as a scaffold or carrier for cells or biomolecules; (7) no outcome measures regarding bone regeneration; (8) not used for trauma or orthopedic surgery; (9) a case study. In all stages of the selection procedure discrepancies between two reviewers were resolved by discussion until consensus was reached. From all included articles, references were screened for cross-references and citations. Articles not available or not translatable were excluded from this review.

2.3 | Study characteristics

General information from all included studies (author, year, country) were registered. Animal model information (number of animals, species, strain, age, sex, weight, location, and type of the defect), intervention characteristics (type of adhesive, location defect, treatment group and control intervention) and outcome characteristics (time of evaluation, tissue regeneration and drop-outs) were extracted and listed in Table S1. Google Translate and/or native speakers were used to retrieve study characteristics from articles in languages other than English (e.g., Chinese, Japanese, and Russian).

2.4 | Quality assessment

To gain insight into the methodological quality of the included studies, the risk of bias was assessed according to an adapted version of the Risk of Bias (RoB) tool for animal studies.[14] The RoB of Russian and Japanese articles was not assessed due to translating difficulties. Two additional questions on reporting quality were added to the tool: (1) Is it mentioned that the experiment was randomized?, and (2) Is it mentioned that the experiment was blinded? All items about internal validity (items 1–8 of the RoB tool) were scored. Two independent researchers (MvE and RFL) assessed the risk of bias by using "high," "low," or "unclear" for all items. Discrepancies were discussed until unanimity was reached. Considering the focus of this review, we included the results of (bone) tissue regeneration for the assessment.

2.5 | Meta-analyses

Experiments with quantitative data on neo-bone formation or bone strength were included in meta-analyses to visualize the effects of the different animal models and bone adhesives. Distinction was made between studies comparing adhesive with nonadhesive treatment and with conventional methods as control treatment. Only bone-to-bone fixation was included in the meta-analysis. The standard error of the mean, the most conservative assumption, was chosen when the presentation of data was not clearly stated in the article. The outcome "reduced defect size" was converted to "increased bone volume" to be used in the analysis. In studies where outcomes at different time points were measured, the time of highest effect was used in the analysis. Statistical differences between subgroups were only assessed when subgroups contained at least three independent experiments per subgroup.

2.6 | Statistical analysis

The inverse variance method was used to pool continuous data from independent measurements. The random-effect model was used in all analyses. Results were presented as standardized mean difference with 95% confidence intervals. All analyses were performed with Review Manager (version 5.3).

3 | RESULTS

3.1 | Description of included studies

The comprehensive search strategy yielded a total number of 6856 articles (2411 articles from PubMed, 2676 from EMBASE and 1769 articles from Web of Science), which where de-duplicated and screened on title and abstract for possible inclusion using predefined criteria. 378 preclinical studies were screened in full text. Screening reference lists revealed one extra study. In total, 65 articles were included in this systematic review (Figure 1) of which 61 studies were assessed for risk of bias. Seven articles described more than one separate animal experiments, in the end 81 separate animal experiments were reviewed.

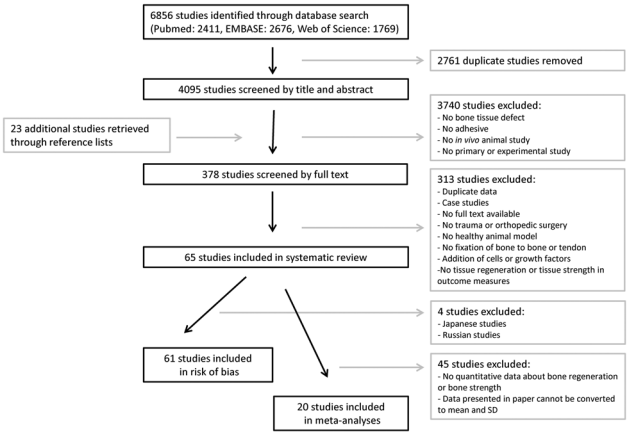

FIGURE 1 Flow chart of search and screening process

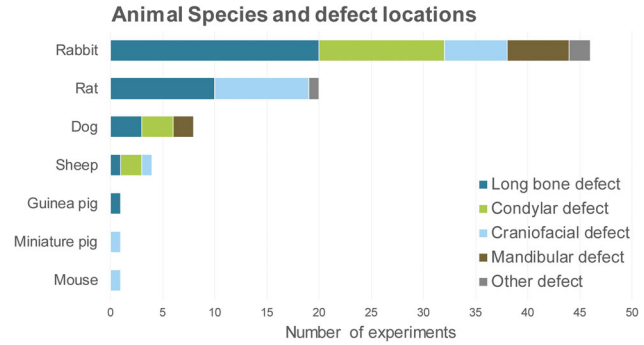

FIGURE 2 Bar chart of animal species used in all included experiments, split into different defect models. Other defect types included scapula, ilium, and rotator cuff. Some experiment used more than one animal species [Color figure can be viewed at wileyonlinelibrary.com]

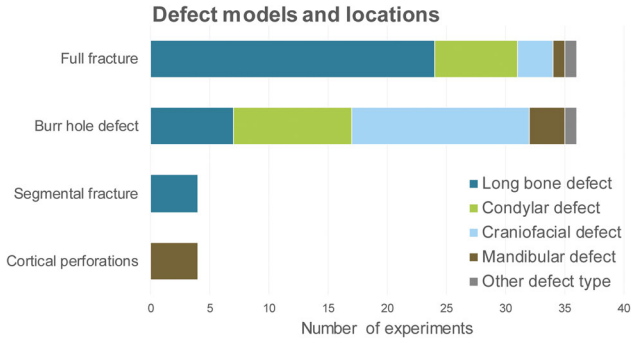

FIGURE 3 Bar chart of anatomical locations of created defects, split into different types of defect. Other defect types included scapula and ilium. Some experiment used more than one defect model [Color figure can be viewed at wileyonlinelibrary.com]

3.2 | Experimental models

3.2.1 | Animal models

Seven different animal species were studied of which rabbits were most often used (56.8%), followed by rats (24.7%), dogs (9.9%), sheep (4.9%), guinea pigs, miniature pigs, and mice (all three 1.2%) (Figure 2). Strain, sex, and weight of the animals were mentioned in 63.0%, 56.2%, and 56.2% of studies, respectively. In most articles, animal age was described using terms as "adult" or "grown up," only 18 experiments (24.7%) reported exact age in weeks, months, or years.

3.2.2 | Defect models

Defect models were categorized based on the anatomical location of the created defects in: long bones (43.2%) (including femur, humerus, radius, tibia, and ulna), craniofacial sites (22.2%, calvaria and zygomatic bones), condyles (21.0%), mandibula (9.9%), scapula (1.2%), rotator cuff (1.2%), and ilium (1.2%) (Figure 2). Altogether, sixteen different locations of bone defects were used in the studies (Table S1). The different defect types and treatment options depending on the anatomical location are shown in Figure 3. For example burr hole defects (44.4%) where a hole was created with a (trephine) burr, the bone fragment was removed and the defect filled with the harvested bone tissue or with autologous bone harvested elsewhere in the body or with (artificial) bone particles. These defects were created in long bones, condyles, mandible and in the craniofacial region. Cortical perforations (4.9%) were only created in combination with autologous graft fixation and always in the mandibula. The most frequently used bone defects were osteotomies including complete fractures (44.4%) and segmental fractures (4.9%), mainly created in long bones. In addition to the bone-bone defect, one bone-tendon rupture (1.2%) was included.

Over time, the long bone fracture model was the most common experimental model used in bone adhesive research. In recent years researchers opt more often for more challenging interventions including non-weight-bearing craniofacial defects (Figure S2). Recommendations for the use of animal models are described in Section 6.

3.2.3 | Defect reinforcement strategies

In seventeen experiments (21.0%), additional fixation or reinforcement materials were used in the experimental groups, including external (1.2%) or internal metal fixation (12.3%), casts (3.7%), and sutures (3.7%). The use of these materials was mostly dependent on the location and type of defect; weight-bearing defects were more often reinforced than non-weight-bearing defects. In several long bone fractures and defects, the adjacent long bone, for example, radius/ulna was supporting the fractured bone or bone defect in addition to the extra fixation and reinforcement materials.

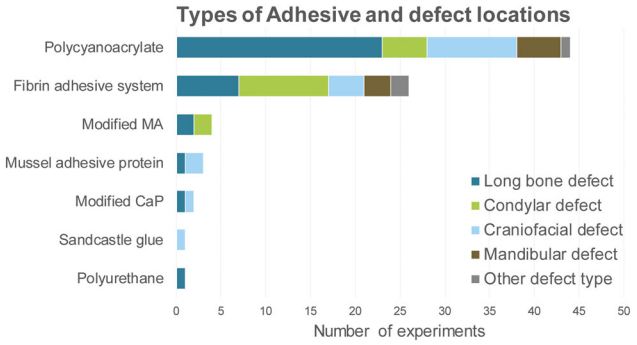

FIGURE 4 Bar chart of types of adhesive used in the included studies, split into different types of defect. Some experiment used more than one type of adhesive [Color figure can be viewed at wileyonlinelibrary.com]

3.2.4 | Effects of bone adhesives

Adhesives

Seven different categories of bone adhesive materials were evaluated (Figure 4). The most used and earliest studied adhesives were poly-cyanoacrylate (CA, 44 experiments, 54.3%) and fibrin glue (32.1%). Another category was the modified cements (7.4%), including modified methacrylate (four studies) and calcium phosphate cement (two studies) which modification resulted in an enhanced adhesion to bone tissue. Other adhesives were polyurethane (1.2%), and adhesives of biological origin including mussel adhesive protein glue (3.7%) and sandcastle glue (1.2%). Poly-cyanoacrylates were tested more frequently in long bone defect models, whereas fibrin adhesives were mainly tested in non-weight-bearing defects including condylar defects.

Control groups

In 59.3% of experiments, a control group was present in which the model, defect type and intervention were similar to the experimental group except for the application of an adhesive. In most publications without the use of a latter described control groups, an active control group was included (in 16% of all experiments) in which no adhesive but a conventional fixation technique was used such as a K-wire or plates and screws. In studies without a control or active control group, the experimental group was compared with no other group (4.8%), with a group with both an adhesive and a metal implant (1.2%), with another adhesive group (2.4%) or an empty defect (1.2%).

Quality assessment

The quality of the included papers was assessed for all studies except for two Japanese and two Russian articles. Figure 5 shows the results of the risk of bias. Due to the poor reporting quality of the majority of included papers, we also assessed two additional reporting criteria (items 9 and 10, randomization and blinding); 31.1% of studies mentioned randomization and 13.1% mentioned blinding. Randomization was reported more frequently in the last decades as were blinding of the investigators and/or outcome assessors (Figure 6A,B). Overall, the quality of reporting details in bone adhesive research was poor and there seemed to be a serious risk of bias.

Outcome measurements and follow-up period

Several different, however not standardized, measurements were used, of which qualitative descriptive histology, evaluating new tissue formation and biological response in bone adhesive research, was the most common technique in 67 experiments (82.2%). This technique was used in combination with a quantitative technique including histo(morpho)metry in 18 (21.9%), with biomechanical tests in 11 (13.4%) or bone density measurements in five experiments 4.9%) (Figure S4). There was no consistency in follow-up period reported, not even in experiments with similar animal models and anatomical location of defects. Recommendations for outcome assessments are described in Section 6.

Effectiveness of bone adhesives compared to nonadhesive treatment

Meta-analyses were performed assessing the effect of the bone adhesive on bone regeneration. Two different treatments were compared: (1) adhesive treatment fixating the bone fracture, fragment or granules in the bone defect with an adhesive; (2) nonadhesive treament fixating the bone fracture, fragment or granules in the bone defect without an adhesive or reinforcement material. The studies were grouped per type of adhesive and the overall effect per subgroup is showen in case of three or more independent comparisons. Ten studies were included in the meta-analysis on bone area and bone volume outcomes (Figure 7A); three cyanoacrylate, four fibrin glue and three studies with modified methacrylate. No significant effect (*M*: −0.71, 95% confidence interval [CI]: −1.63–0.21, $p = 0.13$) in bone area and volume was demonstrated comparing the adhesive and nonadhesive treatment groups.

Six articles were included assessing the outcome bone strength and density; one cyanoacrylate, two fibrin glue and three modified methacrylate studies (Figure 7B). No statistically significant overall effect in favor of one of the treatments was present (*M*: 0.25, 95% CI: −0.49–0.98, $p = 0.36$).

Effectiveness of bone adhesives compared to conventional metal fixation

Six experiments described conventional metal fixation treatment compared to an adhesive treatment. These are visualized in two separate forest plots (Figure 8). Three experiments, of which two in one study, regarded cyanoacrylates with bone growth as primary outcome. There was more bone regeneration in the adhesive groups compared to the metal fixation groups with a significant overall effect in favor of the adhesive intervention (*M*: 2.49, 95% CI: 1.20–3.79, $p = 0.0002$, Figure 8A).

In the other three experiments, polycyanoacrylate, fibrin glue and modified calciumphosphate cement fixation were compared with conventional metal fixation treatment with bone strength as outcome, showing also a significant effect in favor of the adhesive fixation (*M*: 1.13, 95% CI: 0.26–2.01, $p = 0.01$, Figure 8B).

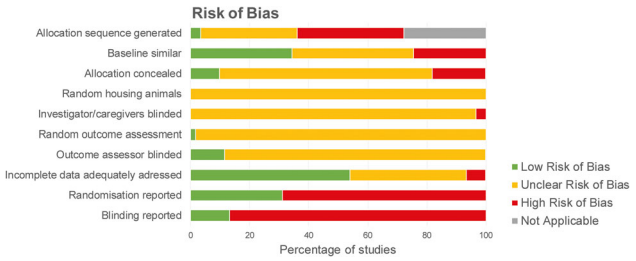

FIGURE 5 Risk of bias analysis. The risk of bias was analysed using several signaling questions, using the SYRCLE's Risk of Bias Tool. Depicted results are the answers for all studies per question [Color figure can be viewed at wileyonlinelibrary.com]

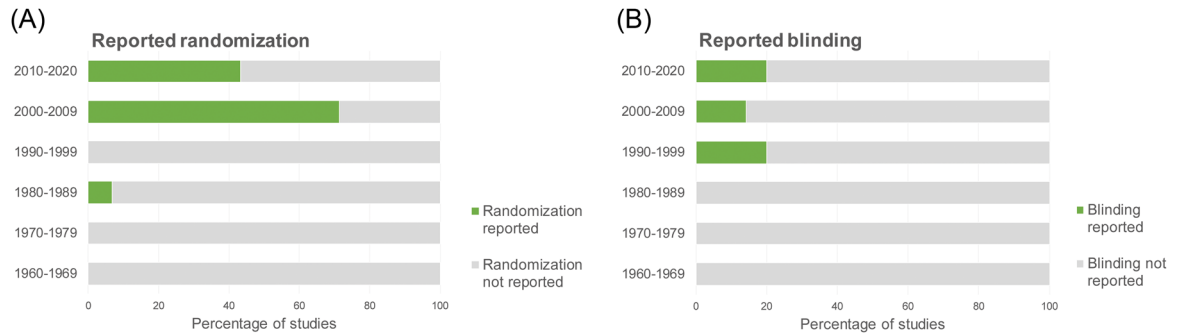

FIGURE 6 (A) Percentage of studies in which randomization was reported in the article. (B) Percentage of studies in which blinding was reported in the article [Color figure can be viewed at wileyonlinelibrary.com]

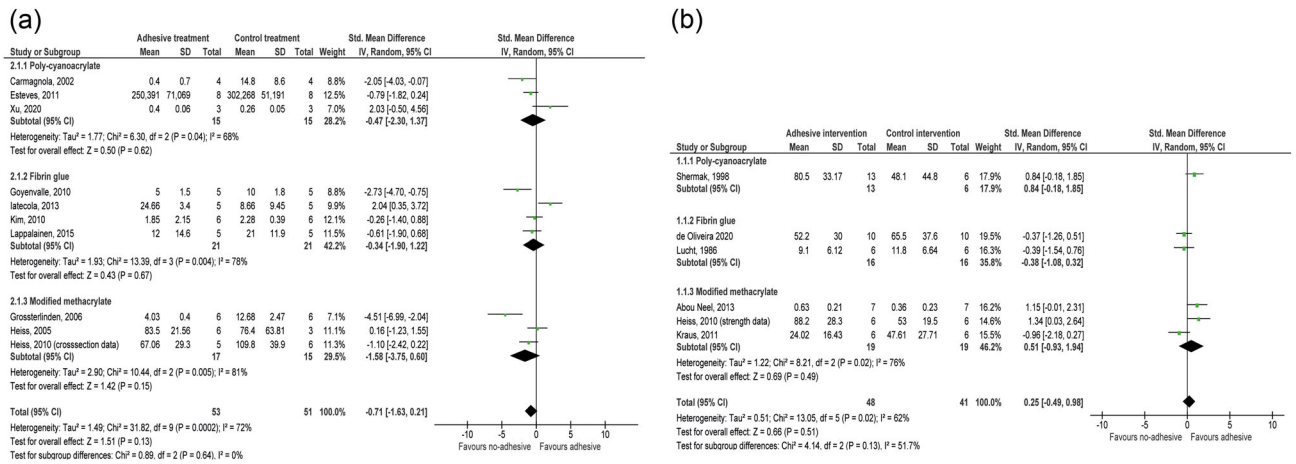

FIGURE 7 (A) Forest plot of the results of bone area or volume after adhesive treatment in comparison with nonadhesive treatment. Random effect model was applied. (B) Forest plot of the results of bone strength after adhesive treatment in comparison with nonadhesive treatment. Random effect model was applied

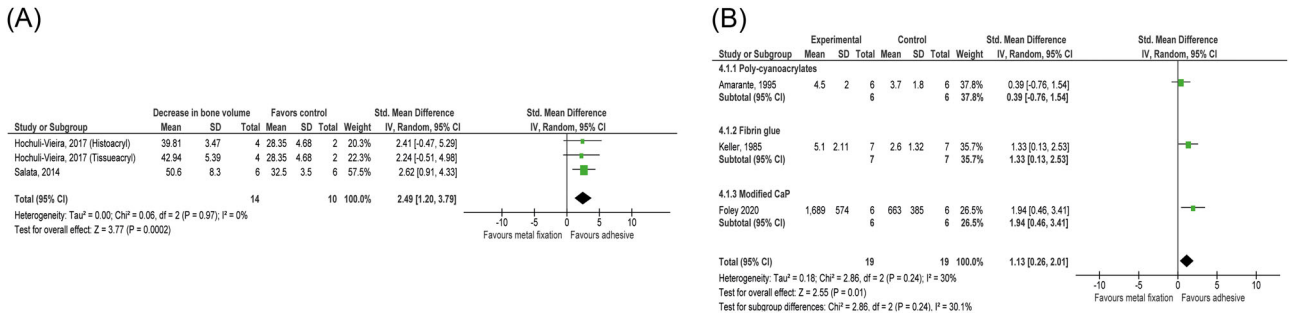

FIGURE 8 (A) Forest plot of the results of bone area or volume after adhesive treatment in comparison with metal fixation materials. Random effect model was applied. Studies are subdivided per type of adhesive. (B) Forest plot of the results of bone strength after adhesive treatment in comparison with metal fixation materials. Random effect model was applied. Studies are subdivided per type of adhesive [Color figure can be viewed at wileyonlinelibrary.com]

Adverse effects

Drop-outs of animals were only reported in seven studies. Severe adverse effects reported were severe inflammation, often linked to the presence of adhesive material, tissue necrosis in the bone graft, refractures and nonunions. Slight inflammatory reaction was reported in experimental and control animals of almost all studies. In several articles, a barrier effect was noticed caused by the adhesive layer blocking effective bone regeneration.

4 | DISCUSSION

In this systematic review all experimental studies on bone adhesives until December 2020 were analysed to summarize the available literature in bone adhesive research for trauma and orthopedic surgery. The lack of standard model parameters including animal species, strain, defect type, and intervention characteristics resulted in a large heterogeneity in experimental models (Table S1). Additionally, the efficacy of the different bone-binding materials on tissue regeneration was presented in Forest plots to visualize the available evidence. Few studies included quantitative results on tissue regeneration eligible for meta-analyses, complicating cross-study comparisons for effectiveness of adhesives and experimental models. Bone adhesive treatment was superior to conventional metal osteosynthesis, while no significant difference (*M*: −0.71, 95% CI: −1.63–0.21, $p = 0.13$) regarding bone regeneration was found in the comparison between adhesive and nonadhesive treatments.

The lack of standard parameters is a general problem in bone research previously described.[15–17] Not one single animal model is suitable for all research purposes and in all research phases.[15] However, standard measurements in a selected number of appropriate and validated models could have improved the comparability and prevented unnecessary repetition of animal studies, for example, by making use of clinically relevant and previously used in vivo models and interventions. In addition, a relevant and accurate timepoint for the assessment of bone tissue at which differences between the groups is expected, must be chosen as well as quantitative outcome measurements and the presence of a control and/or active control group. In Section 6, recommendations are further explained.

Seven different animal species and an unknown number of strains were used in the experiments. Due to anatomic, biochemical, and gene expression differences, results in fracture healing studies among different animal species are difficult to compare and results may even conflict when using the same defect model.[18] Small animal species including rabbits have dominated the bone adhesive research, similar to other research on musculoskeletal and hard tissue regeneration,[19] even though these animal models are less predictive to the human clinical situation than larger models including goat and horses.[20] Advantages of using rabbits are their early skeletal maturity and the high rate of secondary bone remodeling compared to larger animal models and rodents.[19,21] Rats are increasingly used replacing rabbits for the last two decades both in bone adhesive research and in other research fields.[22] Rodent species including the rat have reduced maintenance costs, less handling difficulties and a short reproductive cycle compared to large animals,[19] but the bone structure differs in that it does not include Haversian systems.[18] Remarkably, mice models are rarely used in bone adhesive research while they are widely used in biomedical research and even in studies on fracture repair.[23] This might be explained by the small skeletal size of the animal which can hinder the application of a bone adhesive.[17]

Four broad main categories according to the anatomical location of the defect could be distinguished in the included studies, for example, mandible, condyle, craniofacial and long bones. The latter category has been used since the 1960s for its translational value, however has the drawback that the defects are weight-bearing and often need additional reinforcement materials such as pins and screws. These (stiff) reinforcement materials can cause differences in loading patterns, affecting the quantity and quality of the newly formed bone thus skewing study outcomes.[24] We therefore suggest starting exploring the bone adhesive capacities of new material in non-weight-bearing defects, such as cranial burr hole defects, before taking the next step toward more complicated weight-bearing defects in a larger animal model such as sheep.

Although comminuted fracture repair is a main application for a bone adhesive, less than half of the publications used a model representing comminuted fracture. One of such models was the burr hole defect model filled with grinded bone graft or granules which was used both at load bearing and nonload bearing locations and was used to evaluate the capacity to hold small bone fragments together. Another model, however barely described in bone adhesive literature, is when the guillotine is used for fracturing with dislocation of bone parts. In almost all included publications, the defect was created with an electrical burr/saw, resulting in minimal fracture dislocations,[23] or the technique was not mentioned. Since we expect that the repair of small bone fragment and nonload bearing comminuted fractures benefit most of an adhesive, we recommend to focus on these clinically relevant animal models in bone adhesive studies.

We included only one tendon-bone study in this review. Most tendon-bone studies were excluded due to the use of cements for anchoring rather than gluing the tendon tissue to bone.[25–27] Apparently, fixation of tendon-bone defects requires different materials than those used for bone adhesion.

The limited evidence that is currently available shows high heterogeneity and allows only small meta-analyses and barely any subgroup analysis. For example, the effectiveness of individual adhesives could not be determined in subgroup analyses. All adhesives included, the meta-analyses demonstrated more bone regeneration in the adhesive compared to the metallic fixation treated animals. However, these conclusions must be interpreted with caution as only three independent experiments were eligible for inclusion in this analysis. Hochuli et al. and Salata et al. reported less resorption of grafts fixed with adhesives compared to grafts fixed with metal devices.[28,29] The reduction in bone resorption might be due to the application of the adhesive material; hen the adhesive is applied on the complete defect area and thus on a large surface, a better graft stability is created in contrast to the application of a single screw or pin. More stability and smaller gap sizes may lead to less micromovements and more angiogenesis, and subsequently less graft resorption and an increase in bone healing.[28,30] In addition, it is suggested that a negative internal pressure exerted by screw fixation on the bone graft can also contribute to resorption of the graft.[28,31] These findings indicate potential for adhesive treatment as an alternative to current metallic osteosynthesis materials.

Various techniques were applied for measuring outcomes, making it challenging to compare studies in the meta-analyses.

Despite the qualitative nature, histology seems an important technique, used in most studies on bone regeneration assessment. In contrast, biomechanical analysis was only performed in eleven (13.4%) in vivo studies included in this review (Figure S4). This is surprising given the fact that biomechanical measurement is frequently used in ex vivo and in vitro experiments and is a required test by regulatory authorities for a new bone fixation material or agent.[9,32] The development of new and more advanced techniques such as computed tomography scans, magnetic resonance imaging, and histo(morpho)metrical measurements has contributed to the diversity in reported diagnostic techniques in this study field. These quantitative outcome measurements are highly recommended for bone regeneration assessment and better allows cross-study comparisons than qualitative measurements. The high variety of techniques that are used for the measurement of regenerated bone and its advantages and disadvantages has been described in more detail by Guda et al.[33]

Seven different main bone adhesives with a large number of subtypes were included in this systematic review. Since the definition of an adhesive is rather vague,[34,35] we chose to limit our review to products with a "real" binding capacity to bone tissue rather than mechanical interlockings, that are applied on the bone defect surface. Consequently, studies on products with minimal adhesive properties to bone tissue and products that are used to fill bone defects or as bone substitutes were excluded. This approach is different from other reviews on bone adhesives [9,10,36–39] and is a more focussed and more specific review on the beneficial and adverse characteristics of animal models and bone-bonding materials in general.

The potential bias in the included studies in the review may have introduced an overestimation of the results of the meta-analyses. The quality of included studies is one of the main factors affecting the reliability of the results of this review. The risk of bias showed that many articles lacked (proper) reporting of important details of the experimental set-up. It is possible that these studies were correctly performed and only the reporting of details was not adequate. Although the quality of reporting improved over time, only a small number of papers mentioned randomization and/or blinding details (Figure 6A,B). Randomization of the animals and blinding of the researchers and outcome assessors proved to be important in experimental studies since nonrandomized studies can overestimate the effect of the treatment.[40,41] The poor reporting quality is a structural problem in preclinical studies not only regarding bone adhesive research. Several systematic reviews on animal studies have found low proportions of reporting randomization, allocation concealment and blinded outcome assessment.[40–42] Improvement of reporting transparency of in vivo studies can for instance be achieved by using the ARRIVE Guidelines Checklist.[43]

To our knowledge, this is the first systematic review on bone adhesives and the first review on animal models for bone adhesive materials. Previously reviews were narrative and focussing on the bone adhesives, not on in vivo studies and different animal models.[9,10,36,38,39,44,45] We also summarized previous literature and assessed the reporting quality of the papers. This makes this review more complete and valuable for the design of future animal studies having translational impact. The review has also limitations. Only few studies presented quantitative data suitable for inclusion in the meta-analyses precluding sub-analyses. Analyses of similar experimental models would have provided stronger evidence supporting recommendations for a bone adhesive. Instead, we had to pool all results independent of animal species, defect types, and interventions, despite the knowledge that bone healing is dependent on the animal species,[46] location of the defect (intramembranous bone healing for skull and mandible, enchondral bone healing in long bone fractures) [47] and the use of osteosynthesis materials.[3] Due to the lack of consistency in evaluation times and the variety in bone growth rate in different animal species, we could not conclude on the optimal follow-up time for assessing the efficacy of a bone adhesive. This is aggravated by the decision we had to make analysing only the time with the highest measured effect per experiment, since no consistency in time points was present. Because of the heterogeneity, the meta-analyses were only used to explore potential effects of bone adhesive treatment in comparison with nonadhesive treatment, rather than confirming the effects of the different adhesives.

5 | CONCLUSIONS

This systematic review highlights the absence of standard models and measurements for bone-binding materials and the poor to moderate quality of animal research in this field. This complicates comparing study outcomes across literature and obtaining evidence that can be translated to the clinic. Additionally, the body of currently available evidence is too small to draw strong conclusions from meta-analyses on the effectiveness of the individual adhesives or experimental models. This systematic review did however, provided an objective overview of the availability and quality of evidence, and identifying knowledge gaps. It succeeded in identifying the main parameters for designing future animal studies in bone adhesive research and for testing new products. It is recommended to improve standardization in experimental models and outcome measurements to better evaluate the safety and efficacy of the materials and to achieve more clinical impact.

6 | RECOMMENDATIONS

6.1 | Animal model

The choice of the size of the animal species is dependent on the study aim and outcomes defined; small animal species are recommended for "mechanism" studies and studies on safety and biological response with respect to the applied adhesive. Large animal species are recommended for efficacy studies before clinical testing. In addition, it is recommended to choose skeletally mature animals and ensure that the number of animals is properly powered.

6.2 | Defect

The type and location of the defect are strongly dependent on the characteristics and the purpose of use of the tested product. The location of the defect influences the need for additional fixation materials to reinforce the bone defect for example an oblique fracture in a weight bearing bone. Moreover, it also determines the outcome measures since biomechanical testing on cranial defects is difficult to perform.

6.4 | Control group

Relevant control group(s) (e.g., preferably a nonadhesive group and/or the gold standard fixation material in clinical use) is/are mandatory for proper conclusions on the efficacy of the tested material.

6.5 | Follow-up period

The right period chosen is dependent on the rate of bone growth that differs per animal species.[48] The optimal time of measurement (s) should be halfway the healing process, since differences between the experimental and control groups at the beginning and end of the bone regeneration process may not be detected (yet). Sometimes, a long term study is recommended to allow for valid conclusions regarding the biocompatibility of a biomaterial.[49]

6.6 | Outcome measurements

Relevant outcome measures should be considered such as listed in the article from Guda et al.[33] Preferably choose at least one quantitative outcome measure, and a combination of an imaging technique, a biomechanical analysis and a histological assessment.

6.7 | Blinding and randomization

Proper randomization and blinding are essential because nonrandomized and nonblinded studies can largely overestimate the effect of the treatment.[40,41]

6.8 | Reporting

All relevant baseline characteristics, procedure characteristics and results should be described in detail in the experimental paper. The ARRIVE Guidelines Checklist is very useful when writing the article.

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AUTHOR CONTRIBUTIONS

Machteld van Erk designed the study, analyzed the data and wrote the manuscript. Judith van Luijk, Fang Yang, and Rosa P. Félix Lanao contributed to the research design, acquisition, analysis, and interpretation of the data and critically revised the manuscript. Sander C. G. Leeuwenburgh, María J. Sánchez-Fernández, and Erik Hermans wrote and critically revised the manuscript. Harry van Goor designed the study, was involved in data interpretation and discussion of the research progress, wrote the manuscript and approved the final version. All authors were involved in drafting the paper, and have read and approved the final submitted manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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