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Published in:
Biomaterials Advances

DOI:
10.1016/j.bioadv.2024.213986

Publication date:
2024

Biomaterials Advances 164 (2024) 213986

Contents lists available at ScienceDirect

Biomaterials Advances

journal homepage: www.journals.elsevier.com/materials-science-and-engineering-c

Review

The role of extracellular matrix hydrogels and adipose-derived stromal cells in soft tissue vascularization – A systematic review

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ARTICLE INFO

Keywords:
Adipose-derived stromal cells (ASC)
Conditioned medium (CM)
Extracellular matrix
Vascularization
Angiogenesis
Regenerative medicine

ABSTRACT

Decellularized extracellular matrix (dECM) hydrogels loaded with adipose-derived stromal cells (ASC) or their conditioned medium (ASC CM) present a promising and versatile treatment approach for tissue vascularization and regeneration. These hydrogels are easy to produce, store, personalize, manipulate, and deliver to the target tissue. This literature review aimed to investigate the applications of dECM hydrogels with ASC or ASC CM for *in vivo* tissue vascularization. Fourteen experimental studies have been reviewed using vessel density as the primary outcome parameter for *in vivo* vascularization. The studies consistently reported an increased efficacy in augmenting angiogenesis by the ASC or ASC CM-loaded hydrogels compared to untreated controls. However, this systematic review shows the need to standardize procedures and characterization, particularly of the final administered product(s). The findings from these experimental studies highlight the potential of dECM hydrogel with ASC or ASC CM in novel tissue regeneration and regenerative medicine applications.

1. Introduction

Tissue engineering aims to repair or regenerate sustained acute or chronic tissue and organ damage. Currently, organ transplantation is among the limited clinical approaches available, but this is challenging due to the limited supply of organs. Adequate perfusion is essential for tissue regeneration; therefore, augmented vascularization is a key to stimulating regeneration. Vasculogenesis refers to the formation of new blood vessels from mesodermal cells, whereas angiogenesis refers to the formation of blood vessels from already existing blood vessels (ref). Both terms result in the formation of a new vascular network, here referred to as vascularization. Combining technological and biological knowledge, regenerative medicine is developing and implementing diverse tissue 3D-engineered scaffolds as a novel treatment option for implementation in clinical practice.

Adipose tissue-derived stromal cells (ASC) belong to the multipotent mesenchymal stromal cells (MSC) family. With their extensive proliferative capacity, multilineage differentiation, angiogenic, and immunomodulatory properties [1–4], ASC have emerged as a promising option to treat diverse pathologies, including cardiovascular [5], metabolic [6], neurodegenerative diseases [7], among others. In the last decade, an alternative approach exploring ASC has been investigated: implementing cell-free therapy using the ASC-conditioned medium (CM), which contains all factors secreted by the cells under chosen conditions [8–11]. This rich mixture offers a variety of advantages: 1) it reduces the risk of unwanted immune reactions; 2) the protein content can be modified by pre-conditioning cells beforehand [11]; 3) easy to deliver to the target tissue (either injectable as liquid or implantable within a gel) [8,10,12]; and 4) easy to produce and store.

ASC promote proliferation and suppress apoptosis *in vitro*, but results from clinical trials have not met expectations [13–15]. The efficacy of ASC or the CM treatment *in vivo* is challenging due to the fast diffusion of soluble factors into surrounding tissue and the emigration of injected cells. In damaged tissue, the lymphatic system has increased activation; once ASC or CM are injected, those will naturally disperse and be cleared by the lymphatic system. Implementing a scaffold for delivering and retaining factors *in vivo* helps resolve those issues. Hydrogels derived from the decellularized human or animal extracellular matrix (dECM)

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https://doi.org/10.1016/j.bioadv.2024.213986
Received 27 November 2023; Received in revised form 12 July 2024; Accepted 2 August 2024
Available online 9 August 2024

offer several advantages: they are easy to produce on a large scale, promote induction of angiogenesis, can be seamlessly combined with CM or cells, and efficiently bind the proteins in the CM, releasing them gradually over time [16]. The dECM comprises proteins such as collagens, fibronectin, elastin, laminins, proteoglycans, and others [17,18]. The dECM hydrogel mimics the extracellular tissue microenvironment from which it was derived. Besides, dECM contains a host of glycosaminoglycans (GAGs), strongly negatively charged polysaccharides that efficiently bind growth factors and retain water. The efficiency with which GAGs are retained during decellularization depends on protocol and organ origin. An essential characteristic of dECM hydrogels is their viscoelasticity. They can resist and adapt to strain, and their natural cross-linking is temperature-dependent (solidifying at 37 °C). Therefore, the hydrogel is injectable and generally is thought not to suffer from shear-thinning. Upon injection, the pre-gel's temperature will quickly reach 37 °C, *i.e.*, body temperature, and gelate *in situ* [18]. Within limits, the stiffness of the hydrogel is tunable to the stiffness of the organ of choice by altering its concentration [19].

The regeneration-inducing and immunomodulatory capacity of the ASC or CM, combined with dECM hydrogels, brings exciting new perspectives for clinical applications. This systematic review presents *in vivo* implementations of dECM hydrogels loaded with ASC or ASC CM for tissue vascularization, demonstrating the potential of this innovative approach for future clinical implementation.

2. Materials and methods

The systematic review protocol was registered with PROSPERO (registration number CRD42023453435). Due to the heterogeneity of the data, animal models, and study designs of the 14 included studies, a meta-analysis was performed on three studies that reported similar parameters for vessel density assessment.

2.1. Search strategy

The literature search was conducted in five independent databases, clinicaltrials.gov, Cochrane, Embase, Medline, and Scopus, for studies up to August 2023. The search strategy combined search words/terms and Medical Subject Headings (MeSH) for extracellular matrix, hydrogel, adipose stromal/stem cells, conditioned medium, and vascularization (Supplementary files, Appendix 1).

2.2. Inclusion criteria

To our knowledge, no similar previous systematic review on this topic had been conducted; therefore, there was no date limit for our search. Our inclusion criteria focused only on studies with a scaffold from human or animal organ-derived ECM hydrogel, ASC, or ASC CM that measured angiogenesis/vascularization *in vivo* or clinical trials.

2.3. Exclusion criteria

Studies involving synthetic ECM or only individual ECM proteins (*e.g.*, collagen gels) were excluded. Additionally, any studies involving stem cells other than ASC were excluded. *In vitro* studies were also excluded. Articles without full text, only abstract papers, case reports/series, and systematic reviews were excluded. Furthermore, ongoing and unpublished trials were excluded.

2.4. Data extraction

Two independent researchers extracted data by reviewing the title and abstracts per the abovementioned protocol. Full-text reviews were conducted on articles that met the inclusion criteria. Both reviewers independently conducted data extraction.

2.4.1. Data extraction vascularization

Numerical data regarding vessel density, quantified through reported CD31 ($n = 6$) and vWF ($n = 4$), eNOS ($n = 1$) expression, was extracted from the studies. Only data from the endpoint measurements were collected. In two CD31 [20,21] and two vWF [22,23] studies, exact results were not provided, and data had to be approximated from the available graphs. As only one study looked at eNOS, no comparison was possible [24]. Considering the heterogeneity of the data and the different treatment groups in each study, we decided to compare only the untreated control and the dECM+ASC treated group. This selection was based on the groups that showed the most consistent overlap between the studies, allowing a more robust and meaningful comparison. Of the six CD31 studies, two were excluded from the meta-analysis; one quantified CD31 positive cells and not vessels [25], and one presented the vessel density in percentage; all other studies reported the vessel density as number of vessels [26]. One of the four CD31 studies was excluded due to the lack of an untreated control group [27]. Meta-analysis was performed with Jamovi software [28] for CD31 ($n = 3$) [20,21,29]. Of the four vWF, only two were quantified and provided sufficient data on vessel density [22,23]. Meta-analysis could not be performed on the two vWF studies, as the study by Bai et al. did not provide the number of subjects in the *in vivo* trial [23].

3. Results

3.1. Literature search results

The five datasets yielded 444 articles, which underwent title and abstract review. Of those, 22 articles underwent full-text review, and 14 covered all inclusion criteria and were included in this systematic review (Fig. 1). The studies' characteristics and methodologies are summarized in Table 1 [20–27,29–34].

3.2. Extracellular matrix and the tissue engineering scaffold

The included studies used human ($n = 6$) or animal ($n = 8$) organ-derived dECM (Fig. 2A). None of the studies utilized cell-derived ECM. The most used dECM was human adipose-derived (n = 6), followed by porcine adipose-derived dECM ($n = 2$). Among the selected studies, nine solely used dECM to compose the scaffolds/treatments, and five combined the dECM with another biomaterial in their scaffold: mussel adhesive protein mixed with poly(N-isopropylacrylamide) (MAP-PNIPAM) [26], methacrylated gelatin with methacrylated hyaluronic acid [21], poly(L-lactide-*co*-caprolactone) (PLCL) [31], methacrylated chondroitin sulfate (MCS) and methacrylated glycol chitosan (MGC) [33], or PEGylated platelet free plasma (PFP) [25].

3.2.1. Lack of dECM characterization before in vivo applications

Fundamental quality control needs to be conducted to ensure successful decellularization of the ECM before *in vivo* implementation. The process of obtaining dECM involves mechanical and/or chemical dissociation and organ digestion, followed by washing with various detergents to remove residual DNA, sterilization, and milling into a fine powder, after which mild proteolysis with pepsin will generate a hydrogel [35]. During the decellularization procedure and treating the dECM with diverse reagents, GAGs may be lost [36–38]. Hence, a balance must be found between sufficiently removing residual cellular materials and preserving the integrity of the ECM.

DNA measurements of the dECM samples should be performed to confirm the success of the decellularization protocol. However, only six out of the 14 studies [21,25,29,31,32,34] reported DNA measurements, and in two of the six studies, only percentages [31] or general conclusions [25] were provided without exact readouts. Moreover, one of the remaining four studies reported >50 ng/mg of DNA [32], typically considered a standard cut-off point in the field [35,39] (Table 1). A DNA concentration of 50 ng/mg or less is necessary to conclude if the

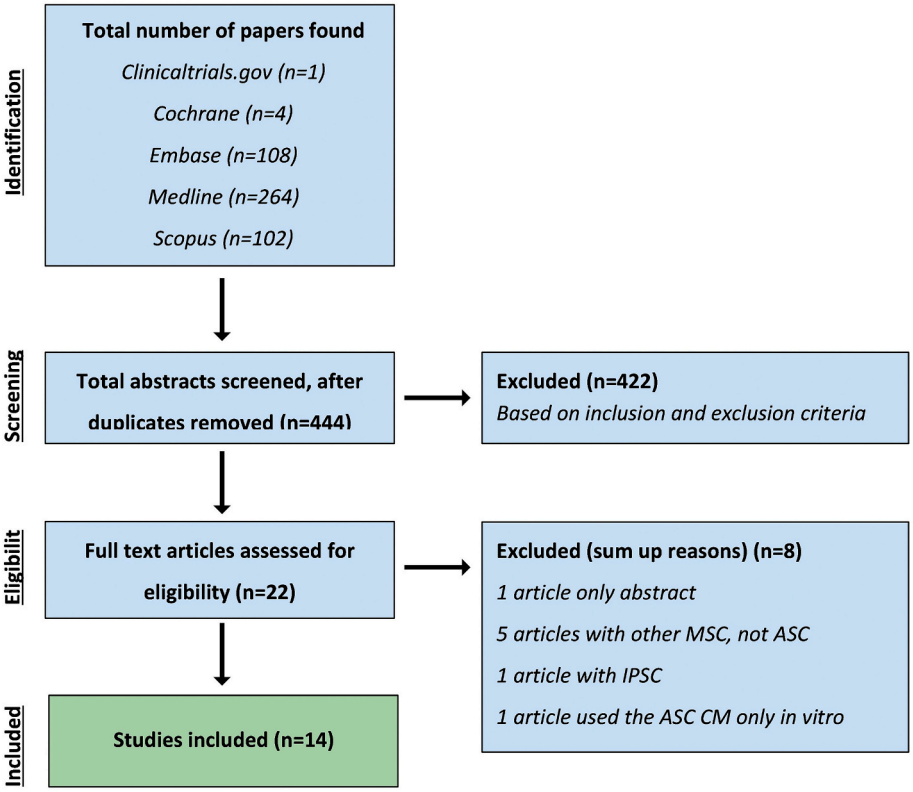

Fig. 1. Flow diagram of the studies' selection process. A comprehensive literature search in five databases yielded 444 articles. After screening titles and abstracts, 22 articles met the eligibility criteria. Following full article screening, 14 of the 22 were included in this review. Abbreviations: Adipose-derived stromal cells (ASC); Conditioned medium (CM); Induced pluripotent stem cells (iPSC); Mesenchymal stem cells (MSC).

decellularization protocol was effective and ensure the quality of the dECM before it is used *in vivo*.

The ECM comprises two major classes of molecules: GAGs and fibrous molecules such as collagens [40]. GAGs are a family of polysaccharides that are key for facilitating essential processes of the ECM, such as cell adhesion and proliferation, making them essential for tissue regeneration. Most importantly, they bind to growth factors within the hydrogel, resulting in a prolonged secretion time *in vivo*. Inevitably, the decellularization process leads to the loss of GAGs. Measuring GAGs post-decellularization is thus necessary to ensure viable dECM samples, considering possible organ-dependent GAGs content variability [41]. Only three of the 14 studies reported GAGs measurements (0.72–7.2μg/ml) (Table 1).

3.2.2. *The manipulability and tunability of dECM hydrogels result in versatile administration modalities and therapeutic applications*

A dECM hydrogel exhibits viscoelastic properties with a temperature-dependent consistency: liquid at room temperature and gel-like at 37 °C. This unique characteristic allows for easy molding and manipulation into different shapes. It is injectable and transforms into a gel within seconds to minutes once inside the body, remaining at the injection site. Therefore, dECM hydrogels can be introduced through diverse methods. The studies discussed in this review included injection ($n = 6$), skin dressing ($n = 4$), and implantation (n = 4). The versatile characteristics and regenerative capacity of dECM hydrogels enabled a wide range of therapeutic applications, including regeneration and inflammation modulation: adipose regeneration ($n = 5$), skin regeneration (n = 4), cardiac regeneration ($n = 2$), general vascularization (n = 2), and pancreatitis ($n = 1$) (Fig. 2B).

3.3. *ASC, in combination with dECM hydrogel, support tissue regeneration*

Across all studies, accelerated tissue regeneration was consistently observed, emphasizing enhanced vascularization when combining dECM with ASC or ASC-CM, compared to controls or standard treatments. Among the studies, nine quantified vascularization by accessing vessel density. This was done by quantitative immune staining either for CD31 ($n = 6$), vWF ($n = 3$), or eNOS ($n = 1$) [24], while four studies either provided insufficient data or did not report vessel density (Fig. 3A). All studies that translated CD31 and vWF expression into vessel density reported increased vessel density with treatment using dECM+ASC compared to the untreated control (Fig. 3B and C) [20–23,29].

3.4. *Meta-analysis: Vessel density measured by CD31 staining [28]*

Three studies were included in the meta-analysis of the vessel density determined with CD31 staining [20,21,29]. The observed standardized mean differences ranged from 4.3 to 6.9 [42]. The estimated average standardized mean difference based on the random-effects model was 4.7 (95 % CI: 3.5 to 5.8), visually represented in a Forest plot (Fig. 4). Therefore, the average outcome differed significantly from zero ($z = 8.0899$, $p < 0.0001$). According to the Q-test, there was no significant heterogeneity in the true outcomes ($Q(2) = 2.5$, $p = 0.2886$, $tau^2 = 0$, $I^2 = 0$ %). A 95 % prediction interval for the true outcomes is given by 3.5 to 5.8. Hence, even though there may be some heterogeneity, the true outcomes of the studies are generally in the same direction as the estimated average outcome. An examination of the studentized residuals revealed that none of the studies had a value larger than ±2.4. Hence, there was no indication of outliers in the context of this model. According to Cook's distances, none of the studies could be considered

Table 1
Experimental studies overview. A comprehensive summary of the 14 included papers. Details on study design, scaffold characterization application, cells or conditioned medium information, animal model, histological and immunohistochemistry analysis.

Study	Aim	Scaffold						Cells		Animal model		Angiogenesis assessment		
		Biomaterial	Administration	dECM characteristics				Cells or conditioned medium	Cell source	Animals; model	Duration of animal study	Histology staining	Immuno histochemistry	Vessel density
Chen, Z. et al., 2021 (29)	Cell delivery for wound healing acceleration	Human AT dECM hydrogel	Skin wound dressing	Collagen content 0.72 ± 0.04 mg/mg	sGAG 2.34 ± 0.47 μg/mg	Protein profile 142 proteins (LS-MS/MS)	DNA measurement 4.74 ± 0.71 ng/mg	Cells	Human subcutaneous AT	Mice; full-thickness wound model on diabetic model	2 weeks	H&E	CD31	Dermal microvessels density per high-power field
Qiao, L. et al., 2019 (22)	Cell delivery for acute MI	Porcine myocardium dECM hydrogel	Intramyocardial injection	ND	ND	ND	ND	Cells	Rat inguinal AT	Rats, myocardial infarction	4 weeks	Masson's Trichrome	vWF	Number of vessels: vWF-positive
Jeon, E. Y. et al., 2020 (30)	Tissue adhesive hydrogel for soft tissue restoration	Human AT or breast AT dECM hydrogel with MAP-PNIPAM	Subcutaneous injection	ND	ND	ND	ND	Cells	Rat AT (source undisclosed)	Rats; subcutaneous model	3 weeks	H&E	CD31, collagen IV	ND
Fu, H. et al., 2023 (21)	Skin substitute for wound healing	Human AT dECM pre-gel with GelMA and HAMA	Skin wound dressing	ND	ND	ND	24.5 ± 7.1 ng/mg	Cells	Human ASC cell line (HUXMD-01001)	Mice, full-thickness skin wound model	2 weeks	H&E, Masson's Trichrome	CD31	Number of capillaries
Liu, P. et al., 2021 (27)	Adipogenesis and angiogenesis promotion	Porcine AT dECM hydrogel	Subcutaneous injection	ND	ND	ND	ND	Cells	Human subcutaneous AT (from mastectomy samples of women with early breast cancer)	Nude mice, subcutaneous injection	4 weeks	H&E	CD31, Ki67	Number of vessels: CD31-positive
Bai, R. et al., 2019 (23)	Cardiac regeneration and repair	Rodent heart dECM hydrogel	Intra-myocardium injection	ND	ND	ND	ND	Cells	Rat brown AT	Rats, myocardial infarction	4 weeks	Masson's Trichrome	vWF, αSMA	Number of vessels: vWF- and α-SMA-positive vessels
Lin, S. et al., 2021 (20)	Wound healing	Porcine skin dECM hydrogel	Skin wound dressing	ND	ND	ND	ND	Cells	Rat inguinal AT	Rats, full-thickness skin wound model	2 weeks	H&E	CD31	Number of vessels: CD31-positive
Kim, S. H. et al., 2021 (31)	Adipogenesis and angiogenesis promotion	Human AT and porcine heart dECM hydrogel with PLCL	Subcutaneous implantation	88.13 % and 84.21 % of collagen remained	75.61 % and 86.63 % of GAGs were present	ND	97.26 % and 98.50 % of the DNA was present	Cells	Human subcutaneous AT	Nude mice	12 weeks	H&E	von Willebrand, αSMA	Number of vessels: vWF- and α-SMA-positive vessels
Kojima, H. et al., 2023 (32)	Cell delivery in pancreatitis	Porcine liver dECM hydrogel	Pancreatic injection	7.2 ± 0.7 μg/mg	1.1 ± 0.1 μg/mg	ND	65 ng/mg	Cells	Rat ASC cell line (MSA01C)	Rats, dibutyltin dichloride-induced pancreatitis model	4 weeks	H&E	Collagen I, αSMA, CD31	ND
Cheung, H.K. et al., 2014 (33)	Cell delivery for natural soft tissue regeneration	Human AT or breast AT dECM hydrogel with MGC and MCS	Subcutaneous implantation	ND	ND	ND	ND	Cells	Rat epididymal AT	Rats; subcutaneous model	12 weeks	Masson's Trichrome	ND	ND

(*continued on next page*)

Table 1 (*continued*)

Study	Aim	Scaffold						Cells		Animal model		Angiogenesis assessment		
		Biomaterial	Administration	dECM characteristics				Cells or conditioned medium	Cell source	Animals; model	Duration of animal study	Histology staining	Immuno histochemistry	Vessel density
Aurora, A. et al., 2017 (25)	Cell delivery system and *in vivo* vascularization	Porcine muscle dECM with PEGylated PFP hydrogel	Placed during surgery	ND	ND	ND	No evidence of DNA fragments	Cells	Human subcutaneous AT	Rats, volumetric muscle loss	2 weeks	H&E or Masson's Trichrome	CD31	Number of vessels: CD31-positive
Matsuda, K. et al., 2013 (24)	Tissue growth and angiogenesis	Rat cardiac dECM hydrogel based on alginate	Implantation into chamber with AV loop	ND	ND	ND	ND	Cells	Human subcutaneous AT	Rats, AV loop in the groin	1 week	H&E	Mouse anti-eNOS	Counted mouse anti-eNOS/ NOS type III staining area
Vriend, L. et al., 2022 (34)	Skin flap viability and wound repair	Porcine skin dECM hydrogel	Placed in the skin wound	ND	ND	ND	11.6–0.6 ng/ mg	CM	Human subcutaneous AT	Rats, skin flap model	4 weeks	H&E, Picrosirius, Masson's Trichrome	Ki67	Number of vessels per microscopic picture
Young, D. A. et al., 2014 (26)	Adipogenesis and angiogenesis promotion	Human AT dECM hydrogel	Subcutaneous injection	ND	ND	ND	ND	Cells	Human subcutaneous AT	Nude mice	4 weeks	H&E	CD31, αSMA	Number of vessels: CD31-positive

Abbreviations: Not described (ND); conditioned medium (CM); adipose-derived stromal cells (ASC); adipose tissue (AT); decellularized extracellular matrix (dECM); Methacrylate chondroitin sulfate (MCS); Methacrylate glycol chitosan (MGC); mussel adhesive protein (MAP); poly(N-isopropyl acrylamide) (PNIPAM); Poly(l-lactide-co-caprolactone) (PLCL); Sulphated Glycosaminoglycan (sGAG); hematoxylin & eosin (H&E); alpha-smooth muscle actin (αSMA); cluster of differentiation 31 (CD31); von Willebrand (vWF); endothelial nitric oxide synthase (eNOS).

overly influential. Neither the rank correlation nor the regression test indicated any funnel plot asymmetry ($p = 0.3333$ and $p = 0.2002$, respectively). In summary, all studies showed increased vessel density in the treated group compared to the control.

3.5. Cell-free approach: using the conditioned medium

In our review, only one study used the ASC CM in combination with dECM [34] (Table 1) (Fig. 2C). This study reported similar results in dermal wound healing among all groups. However, the group treated with ASC CM alone showed increased vessel density on day 7 compared to the untreated control group, which was the first measuring point; the study endpoint was 28 days [34]. Further comparison between ASC and ASC CM was not possible due to the lack of published *in vivo* studies using ASC CM.

4. Discussion

This systematic literature review reported 14 available articles investigating the implementation of a dECM hydrogel with ASC or ASC CM to augment tissue vascularization. All studies showed that the ASC or ASC CM containing hydrogels improved vascularization *in vivo*. Vascularization is a crucial process in tissue regeneration, vital for wound healing, providing oxygen and nutrients, and facilitating the influx of cells to the damaged area. Insufficient blood supply is the main pitfall of tissue regeneration scaffolds, resulting in potential necrosis and implant failure [43,44]. All main findings of our literature analysis are graphically represented in Fig. 5.

ASC have been clinically tested as a regenerative treatment in diverse fields, such as cardiac regeneration [13–15,45], dating back to 2012 when ASC were administered intracoronary to patients with acute myocardial infarction (MI) [45]. Ensuring effective delivery methods, cell retention, and survival are crucial for improving cell-based clinical therapy as it is a fluid substance that, when introduced *in vivo,* will diffuse into the tissue and bloodstream, potentially leading to a material loss in the target area. Therefore, the delivery method is of crucial importance to achieve optimal results. Most desired applications in regenerative medicine require long-term effects lasting weeks to months, making a slowly degrading 3D dECM scaffold an appropriate choice as a versatile delivery system. Vessel density measurement is a quantification of *in vivo* vascularization. All five studies, which reported sufficient data for comparison, showed increased vessel density in scaffold-treated groups; this was also the conclusion of the meta-analysis, underlining the regeneration ability of the versatile scaffold.

Our literature search yielded only one *in vivo* study implementing ASC CM and a dECM scaffold [34], reporting the limited efficacy of the scaffold (skin dECM with ASC CM) in wound healing. In the initial assessment stage of the trial, week one, the scaffold group showed increased vessel density, but the difference was minimized at the endpoint, the third week. More investigation into the regeneration efficacy of ASC CM in combination with dECM hydrogel is needed to draw further conclusions and to compare it to ASC.

Limitations of stem cell therapies have also been reported, such as availability and challenges with the *in vivo* administration approach to be maintained in the target area [46,47]. Safety aspects have been widely explored for MSC, in particular bone marrow-derived MSC. No data exists that these or other MSC transform into tumor cells. While the abbreviation MSC is often used to denote mesenchymal stem cells, these actually are mesenchymal stromal cells, very akin to *e.g.* fibroblasts. Stromal cells, in contrast to genuine stem cells, have a limited life span. In fact, *in vitro*, the MSC generally become senescent after several passages, depending on organ of origin and age of the donor. Regarding tumorigenicity, MSC are as prone to oncogenic stimuli as other stromal and parenchymal cells of the tissue to which these are administered.

To prevent the drawbacks of cell therapy, an alternative approach is to use the conditioned medium of these cells. Our literature search found

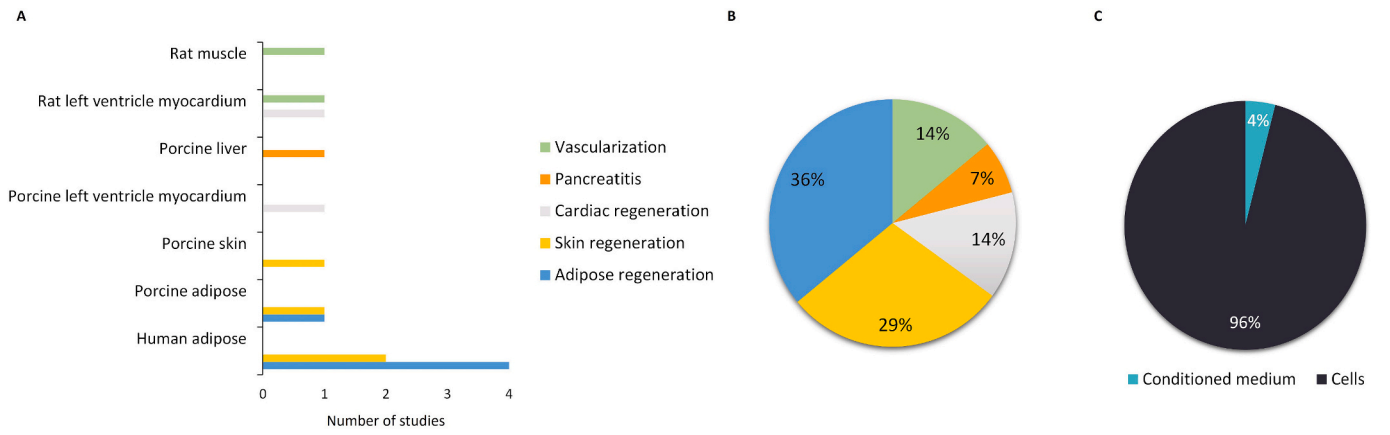

Fig. 2. Studies' heterogeneity. All data represents the 14 studies included in this review. A) Human and animal organ-derived dECM. Representation of the dECM source utilized in each reviewed study; B) Applications of dECM scaffold with ASC or ASC CM for tissue revascularization in diverse pathologies; C) Use of Adipose-derived stromal cells *vs.* Adipose-derived stromal cells' conditioned medium in tissue regeneration.

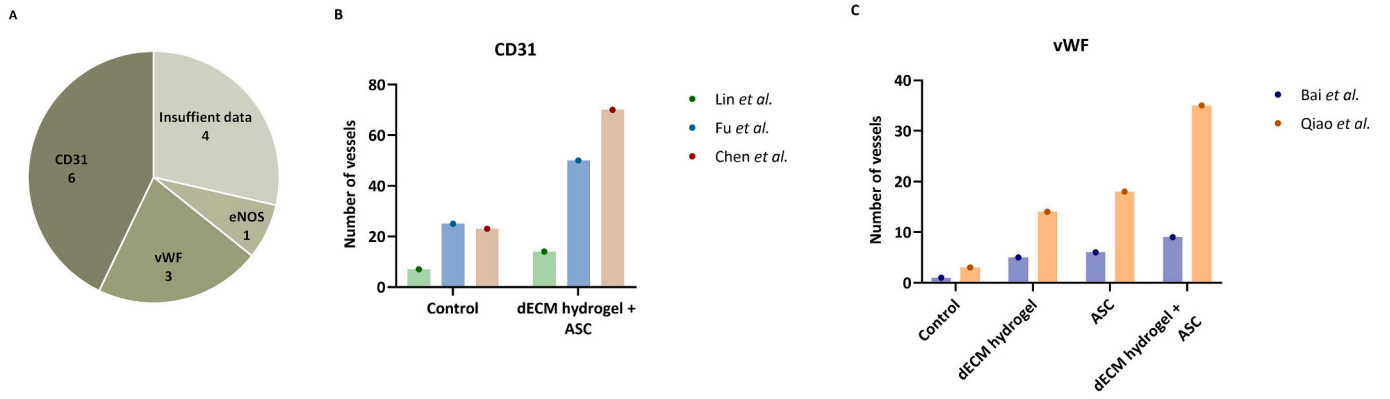

Fig. 3. Vessel density. Comparison was carried out among studies with heterogeneous vessel density analysis. A) Pie chart illustrating the distribution of vessels' density assessment methods among reviewed studies ($n = 14$); CD31 ($n = 6$), vWF ($n = 3$), eNOS (n = 1); insufficient information ($n = 4$).

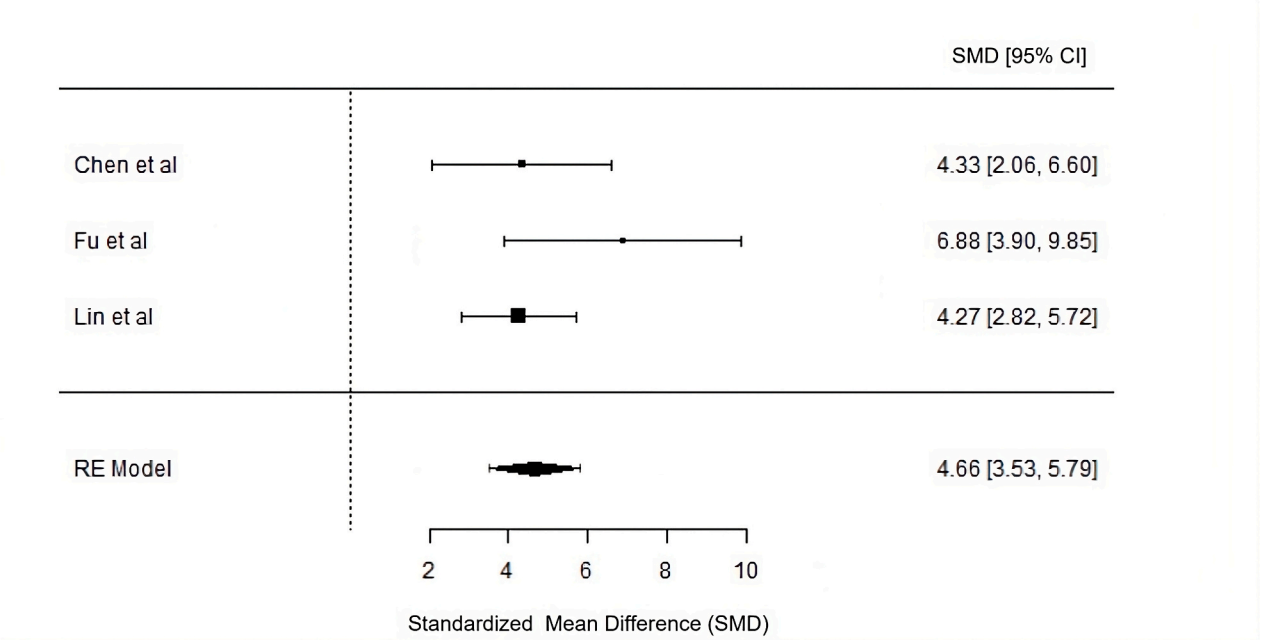

Fig. 4. Forest plot for CD31. Graphical representation of the standardized mean difference (SMD), including the 95 % confidence interval (CI) per study, as part of the meta-analysis. All studies are on the right side of the line of no effect (dotted vertical line).

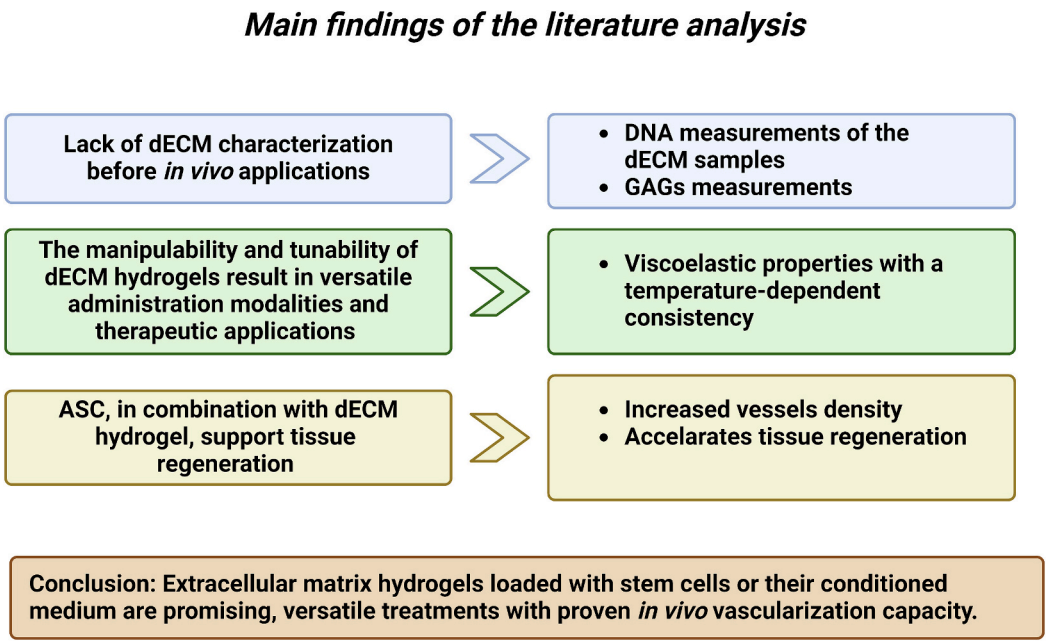

Fig. 5. Visual representations of the main finding of the systematic literature analysis.

one paper where ASC-CM was used for vascularization therapy, indicating that more research on this cell-free therapy is needed to evaluate its efficiency.

Our review underlined the need for essential dECM characterization to ensure its quality before its utilization *in vivo*. First, minimal DNA in the dECM amount must be ensured. The decellularization process leads to an inevitable loss of functional ECM proteins and GAGs; measuring remaining proteins and GAGs ensures the processed ECM has retained sufficient components to yield therapeutic relevance. Therefore, this review recommends essential quality control by DNA, proteins, and GAGs measurements of the dECM to ensure the successful decellularization and the preservation of ECM molecules.

In 2019, Traverse et al. reported the first human trial testing the safety of a dECM hydrogel for post-MI regeneration in 15 patients, confirming its safe application [48]. Although clinical trials implementing a dECM with ASC are yet to be conducted, our review summarizes 14 *in vivo* studies that tested a dECM hydrogel loaded with ASC or their CM for *in vivo* vascularization and showed increased vascularization in the treatment group compared to untreated controls. Despite the heterogeneity in therapeutic applications and administration, these experimental studies provide insight into translating this tissue engineering treatment into clinical practice. Given that both dECM hydrogel and ASC have separately been investigated in clinical trials, it is an exciting next step to combine them into one treatment for supporting tissue regeneration and improving vascularization.

5. Conclusion

This systematic review has identified and reported findings from 14 articles investigating the implementation of dECM hydrogels combined with ASC or ASC CM to augment tissue vascularization. The reviewed studies consistently demonstrated that these approaches significantly improve vascularization *in vivo*. Extracellular matrix hydrogels loaded with stromal cells or their conditioned medium show promising potential as versatile treatments for enhancing *in vivo* vascularization capacity.

Future research should focus on optimizing the design and composition of dECM-based scaffolds to tailor them for specific organ regeneration applications. This includes exploring advanced biomaterial combinations, refining delivery methods, and enhancing the bioactivity and biocompatibility of these constructs. Further investigations are needed to comprehensively assess long-term safety profiles and potential immunological responses associated with these therapies. Moreover, translating these promising preclinical findings into clinical applications remains a critical challenge. Overcoming regulatory hurdles and developing standardized protocols for manufacturing and application will be essential for ensuring the efficacy, reproducibility, and safety of dECM and ASC-based therapies in clinical settings.

In conclusion, dECM hydrogels integrated with ASC or ASC CM represent a cutting-edge approach with demonstrated *in vivo* vascularization capacity and broad potential for organ regeneration. Continued interdisciplinary efforts in research and development are pivotal to harnessing the full therapeutic potential of these innovative bioengineering strategies in regenerative medicine.

CRediT authorship contribution statement

Vasilena E. Getova: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Erika Pinheiro-Machado:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Martin C. Harmsen:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Janette K. Burgess:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Alexandra M. Smink:** Writing – review & editing, Project administration, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest. The funders had no role in the study's design, in the collection, analyses, interpretation of data, or the writing of the manuscript.

Data availability

Data will be made available on request.

Acknowledgements

We acknowledge the Central Medical Library, UMCG, for advising us on constructing the literature search strategy.

Funding

This project was supported by the University Medical Center Groningen (UMCG), the University of Groningen, and the Dutch Diabetes Research Foundation [2019.81.001]. JKB also acknowledges support from the NWO (Aspasia 015.013.010).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioadv.2024.213986.

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