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# Human Mesenchymal Stem Cells Derived from Adipose Tissue and Umbilical Cord, in Combination with Acellular Human Amniotic Membranes, for Skin Healing Processes in Animal Models: a Systematic Review

## Células Troncales Mesenquimales Humanas Derivadas de Tejido Adiposo y Cordón Umbilical, Combinadas con Membranas Amnióticas Humanas Acelulares, para Procesos de Regeneración Cutánea en Modelos Animales: una Revisión Sistemática

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## ABSTRACT

This systematic review aims to document the available research evidence regarding using mesenchymal stem cells (MSCs) and acellular amniotic membranes (AAM) as scaffolds in the murine model for tissue regeneration. This research was developed by analyzing available information on databases like Google Scholar, Pubmed, Scopus, and Web of Science, using the following key terms ''Human Stem Cells'', ''Amniotic membrane'', ''Wound healing' ' and ''Animal model''. A total of 519 articles published from January 2013 to March 2024 were found, but only 8 studies were included in this review, the inclusion criteria were as follows the use of human-derived stem cells (UCMSCs and ADMSCs) seeded in decellularized hAM, in murine models with induced wounds (incisions or burns); exclusion criteria: stem cells obtained from non-human origin, combination of human stem cells from different tissues, use of a different biological scaffold, and studies that not assess efficacy in skin regeneration. The main outcomes were decreased wound closure time, increased angiogenesis, remodeling and increase in extracellular matrix deposition, increased synthesis of growth factors and anti-inflammatory cytokines, and optimization of biomechanical properties. Moreover, one of the main findings was that combining these methods can improve the healing process in chronic wounds. The main bias was related to the inclusion of more studies that used ADMSC (5 of 8); additionally, there were differences in the animal model used, the induced wound, and the comparison of different variables between the studies. In conclusion, we found that the combination of MSCs and AAM as a bio-scaffold improves general tissue healing and regeneration.

**KEYWORDS:** adipose-derived MSCs, biological scaffold, human amniotic membrane, mesenchymal stem cells, umbilical cord MSCs, wound healing

## RESUMEN

Esta revisión sistemática tiene como objetivo documentar la evidencia disponible sobre el uso de células madre mesenquimales (MSC) y membranas amnióticas acelulares (AAM) como andamios biológicos en modelos murinos para la regeneración de tejidos. Esta investigación se desarrolló buscando información disponible en bases de datos como *Google Scholar*, *Pubmed*, *Scopus* y *Web of Science*, utilizando los siguientes términos clave "Células madre humanas", "Membrana amniótica", "Curación de heridas" y " 'Modelo animal'. Fueron encontrados un total de 519 artículos publicados desde enero de 2013 hasta marzo de 2024, pero solo se incluyeron 8 estudios en esta revisión. El criterio de inclusión: uso de células madre derivadas de humanos (UCMSC y ADMSC) sembradas en hAM descelularizadas en modelos murinos con heridas inducidas (incisiones o quemaduras). Los criterios de exclusión fueron: células madre obtenidas de origen no humano, combinación de células madre humanas de diferentes tejidos, uso de un andamio biológico diferente y estudios que no evalúen la eficacia en la regeneración de la piel. Los principales resultados fueron una disminución del tiempo de cierre de la herida, aumento de la angiogénesis, remodelación, aumento del depósito de matriz extracelular, síntesis de factores de crecimiento y citocinas antiinflamatorias junto con la optimización de las propiedades biomecánicas, que en conjunto pueden mejorar el proceso de curación en heridas crónicas. El sesgo principal se relaciona con la inclusión de más estudios que emplearon ADMSC (5 de 8), adicionalmente hubo diferencias entre el modelo animal empleado, la herida inducida y la comparación de diferentes variables entre los estudios. En conclusión, encontramos que la combinación de MSC y AAM como bioestructura mejora la curación y regeneración general del tejido.


**PALABRAS CLAVE:** andamio biológico, cicatrización de heridas, células madre mesenquimales, CMM derivadas de tejido adiposo, CMM del cordón umbilical, membranas amnióticas humanas


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## INTRODUCTION

Given the complexity of its treatment, skin lesions represent a great challenge nowadays, in the United States alone, wound care costs reached $126.864 billion in 2019[1]. A huge portion of the investment is destined for chronic wounds, characterized by not following a normal healing course and failing to restore functional or anatomical integrity after 3 months[2]. Additionally, its prevalence is estimated to affect around 40 million people worldwide[3]. Similarly, other types of wounds, such as burns, have a worldwide incidence of 11 million cases per year[4], and depending on the depth and extension, they can progress towards chronic non-healing wounds.

Under normal circumstances, the healing process consists of 4 phases. Phase I: Hemostasis occurs immediately after the injury, aiming to restore the normal barrier function of the skin. It begins with vasoconstriction, finishing with the formation of a clot that covers the wound, acting as a provisional scaffold for cell migration[3]. The platelets within this clot produce a variety of pro-inflammatory agents, including thrombin, fibrinogen, angiogenic factors, growth factors, cytokines, and chemokines, which in conjunction promote migration of fibroblasts, monocytes, neutrophils, endothelial cells, along with bone marrow-derived mesenchymal stem cells (BMSCs). Phase 2: Inflammation begins with neutrophils producing bacterial lysis and removal of cellular debris. Likewise, monocytes help by differentiating into M1 macrophages aiming to amplify the inflammatory response. At the end of this stage, M2 macrophages and BMSCs initiate the healing process through their anti-inflammatory properties. Phase 3: Proliferation occurs when granulation tissue is formed by the action of fibroblasts, while the migration of keratinocytes initiates the re-epithelialization process[3]. Phase 4: Remodeling, a progressive decrease of cellularity and blood vessels occurs. In this transition process from granulation tissue to scar formation, metalloproteinases (MMPS) degrade collagen type III fibers previously arranged as temporary scaffolds. At the same time, fibroblasts increase the amount of collagen type I fibers to complete the healing and regeneration process[3].

On the contrary, the healing process in chronic wounds has been altered by the increase of IL-1, IL-6, TNFα, and the permanence of MMPS; along with a rise in the macrophages differentiation to M1 pro-inflammatory subtype, causing Fibroblasts to decrease their proliferation, with a consequent reduction in the synthesis of extracellular matrix and the making of granulation tissue[4]. Similarly, the formation of new blood vessels decreases, resulting in a pro-inflammatory, hypoxic, and ischemic environment[5]. Together, these conditions lead to the chronification of many diseases, such as diabetes, peripheral arterial disease, venous insufficiency, or ulcers derived from localized pressure. Consequently, this propitiates the development and progression of non-healing chronic wounds[6].

In addition to the complex phases of the healing process, the often-observed imbalance in diverse ailments further complicates the course of chronic wounds and their treatment when co-morbidity is observed. Therefore, therapeutic alternatives have been developed to optimize the outcomes of treating skin lesions, while decreasing the burden imposed on the health system. Among these therapeutic options, stem cell therapy has been widely studied for its role in the tissue regeneration process. It is currently a topic of ongoing research and evolution.

Regarding wound regeneration, the most analyzed human mesenchymal stem cell populations have been adipose tissue and umbilical cord-derived MSCs. Advantages of using human umbilical cord-derived mesenchymal stem cells (UCMSCs) include their accessibility, as they are obtained from waste tissue at the time of delivery; low immunogenicity due to the low expression of HLA-1 as well as greater potential for proliferation, compared to other sources of stem cells[7]. Similarly, adipose tissue-derived stem cells (ADMSCs) have demonstrated great abil-

ity to modulate the inflammatory response of dendritic cells and T lymphocytes; as well an important cellular plasticity, which gives them the ability to differentiate into various cell lineages such as fibroblasts, keratinocytes, endothelial and epithelial cells[8]. Furthermore, ADMSCs present multiple autocrine and paracrine effects, which promote tissue repair; and are easily obtained through minimally invasive liposuction procedures[8][9]. Remarkably, studies have been carried out utilizing exosomes derived from ADMSCs, which have shown similar effects, by decreasing the synthesis of proinflammatory cytokines, such as IL-1β and TNFα[10], (Figure 1).

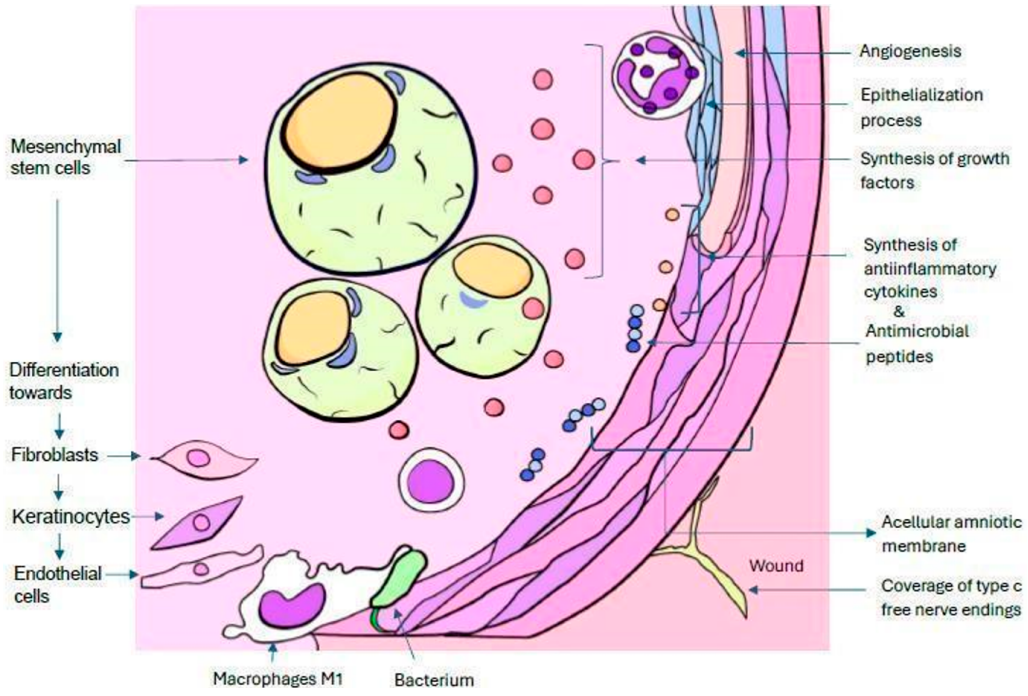


**FIGURE 1. Synergistic effects between stem cells and amniotic membrane in wound healing. Courtesy of Guillermo Mayorga. Elaborated using Adobe illustrator.**

Although subcutaneous local infiltration of stem cells has been tested in non-healing chronic wounds, the main drawbacks are the increased apoptosis susceptibility; secondary to the shear stress caused at the time of infiltration, accompanied by an excessive inflammatory process at the wound site, which perpetuates the non-optimal environment for cell survival[11]. Consequently, different research has been done using stem cells in association with biological scaffolds, many of them developed with tissue engineering, while others performed with natural human amniotic membranes (hAM). Overall, results evidence improvement in cell survival rate, overcoming the limitations of using MSCs applied directly into the wound without a scaffold, and exhibiting significant synergistic effects on tissue regeneration.

Biological polymer scaffolds can be used in the tissue regeneration process, these can be naturally based on proteins (collagen, fibrin) or polysaccharides (cellulose, hyaluronic acid). Likewise, synthetic polymers (polyethylene, polystyrene) have been developed, which are distinguished mainly by their mechanical properties[12].

In this case, hAM was chosen due to its numerous advantages, such as its easy procurement, good aesthetic results in the healing process, and its ability to synthesize a wide variety of molecules that promote tissue regen-

eration. Its use avoids resorting to the exploitation of natural resources, its cost is significantly lower compared to polymers, laborious manufacturing processes are avoided, and rejection by the recipient is an adverse reaction that occurs in few cases[13].

One of the most fascinating properties of hAM described in the literature is the ability to regulate the production of proinflammatory cytokines, while promoting the release if IL-4, IL-10, TGF-β, hepatocyte growth factor (HGF), prostaglandin E2 (PGE-2), histocompatibility antigen HLA- G and indoleamine 2,3- dioxygenase (IDO), all of them exhibiting anti-inflammatory properties[14].

Additionally, hAM displays antimicrobial functions through the release of elafin and beta-defensins, both of which are antimicrobial peptides. Moreover, it can contribute to analgesia acting as a physical barrier when used to cover the wound and nerves, while stimulating angiogenesis via the release of vascular endothelial growth factor (VEGF-A), angiopoietin 1, and fibroblast growth factor (FGF-2). Studies have also reported that the use of hAM promotes cell differentiation and adhesion through structural proteins such as type I collagen, laminin, and fibronectin, which also support epithelialization. Finally, this biocompatible scaffold can be easily obtained and exhibits outstanding regenerative properties, making it a feasible option for treating non-healing chronic wounds[15].

To optimize the use of hAM, several research has been developed to decellularized the membrane scaffold and colonize it with stem cells. Aiming to prevent graft rejection, thus allowing a more synergic treatment of non-healing chronic wounds. This decellularization process removes the epithelium while preserving the stroma of the hAM (Figure 2), maintaining the components of the extracellular matrix, and the presence of tissue regenerating-associated growth factors. These associated biomolecules include nerve growth factor (NGF), epidermal growth factor EGF, Keratinocyte Growth Factor KGF, Basic fibroblast growth factor bFGF, and Transforming growth factor beta α and β (TGFα and β)[16], resulting in the AAM. Human amniotic membranes offer a combination of biological and structural benefits that make them ideal for applications in regenerative medicine, especially for skin regeneration.

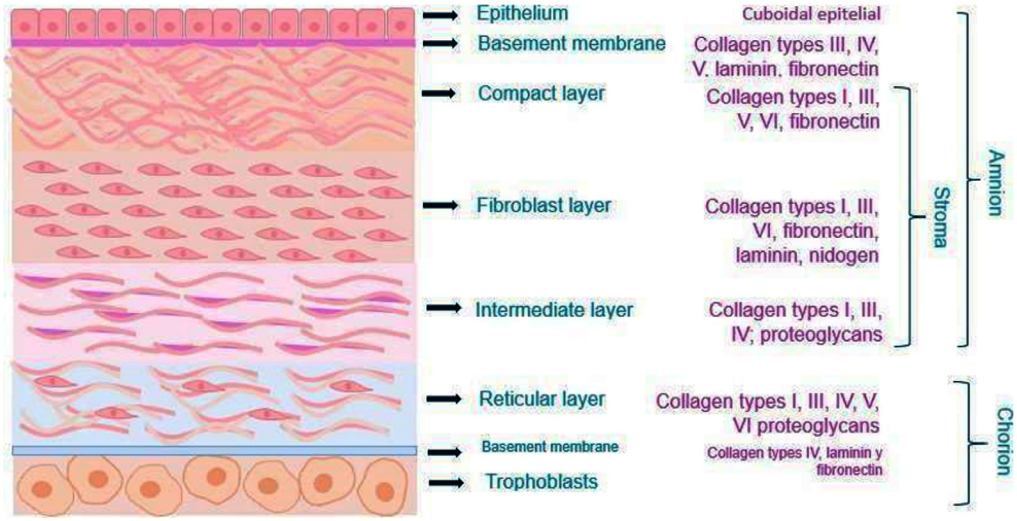


FIGURE 2. **Structure of the amniotic membrane, prepared using Microsoft Word, adapted from[14].**

Interestingly, available evidence regarding the use of stem cells in combination with human amniotic membranes has been documented with great outcomes, in in vivo experiments carried out in mice and rat models. Hence, this systematic review aimed to analyze the studies using human stem cells derived from adipose tissue and/or umbilical cord in combination with acellular human amniotic membrane in the healing process of skin wounds in murine models and provide solid bases for defining the type of stem cells to be used in future human trials.

## MATERIALS AND METHODS

### Literature research

Between May 8 and 12 2024, an electronic search was carried out in the Google Scholar, Pubmed, Scopus, and Web of Science databases, grouping the terms that constituted the PICO question: ''Human Stem Cells'', ''Amniotic mechanisms'', ''Wound healing'', and ''Animal model'', articulated to fulfill the objective stated in the previous section. The final structure of each search strategy was adjusted based on the thesaurus vocabulary and the Boolean operators specific to each database.

### Inclusion and exclusion criteria

Studies published from January 2013 to March 2024 were considered eligible, in which the effects on skin regeneration were evaluated, using human mesenchymal stem cells (UCMSC and ADMSC) seeded in decellularized hAM, in murine models with induced incisions or burns, with prior approval from the associated animal care and use committees. The reason for only considering murine models is that a gap and discrepancy were identified in the literature regarding the most studied stem cell (ADMSCs) and the stem cell used in the few studies identified in humans (UC-MSCs), thus, this systematic review arises to provide solid bases for future trials in humans. Exclusion criteria were considered as follows: non-primary studies, obtaining stem cells of non-human origin, a combination of human stem cells from different tissues, use of a biological scaffold different from the one stated, studies not designed to assess efficacy in skin regeneration, and articles written in languages other than English or Spanish. These exclusion criteria were considered to enable a comparison with the studies finally selected.

### Study selection

Just the authors VG and GM participated in the selection of potentially relevant studies to reduce the risk of selection bias and this process was carried out in three stages. The first stage focused on eliminating duplicate records. The second stage centered on exclusion of articles according to their title and abstract. In these two stages, the collaborative web application named Rayyan was used. Finally, the third stage consisted of a full-text analysis. The last two stages were guided by inclusion and exclusion criteria. This process was based on the statements established in the PRISMA 2020 protocol.

### Data extraction

The studies were required to contain this information: population sample, type of injury, methodology in terms of origin and processing of stem cells and amniotic membranes, information on the interventions, and outcome measures. Data were independently extracted and organized in a detailed manner on a worksheet for further analysis.

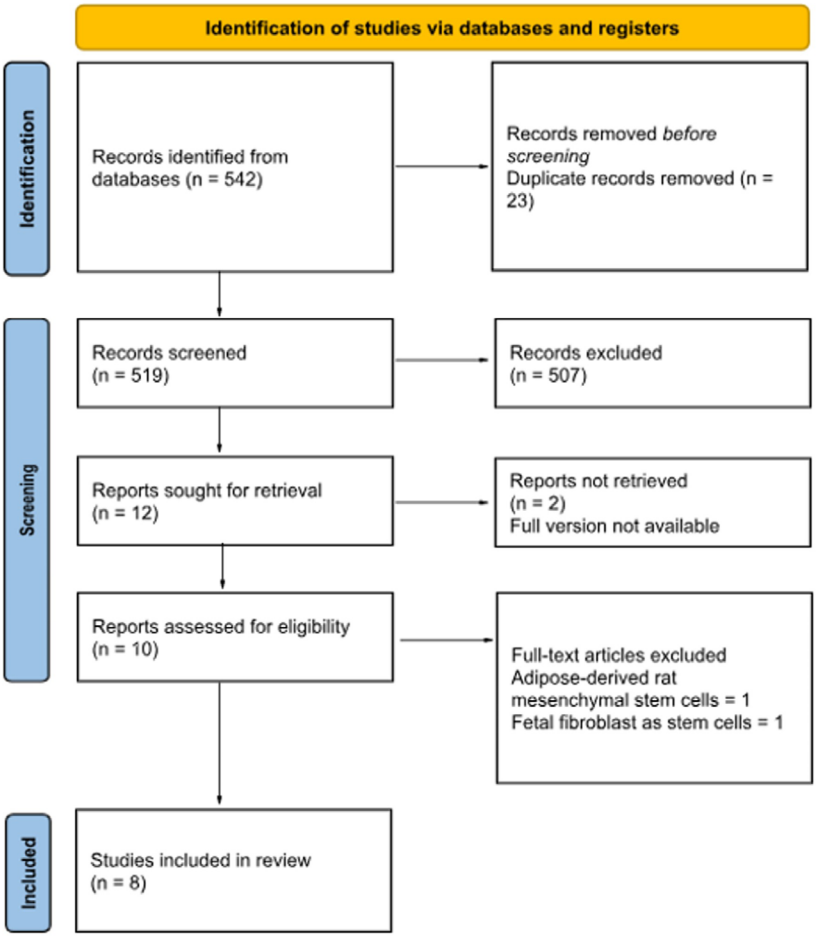


FIGURE 3. **Prisma flowchart.**

## RESULTS AND DISCUSSION

### Search results

After removing duplicated studies, according to the PRISMA 2020 flowchart, the search performed yielded a total of 519 studies (Figure 3). Considering the information found on the titles and abstracts, 12 studies were fully assessed, out of which 4 studies were excluded. Finally, 8 primary studies were included.

### Characteristics of the studies

The main findings of the analyzed studies are presented in Table 1. All studies used murine models, with the most used species being BALB/c mice and Wistar rats. All studies included signed informed consent for aesthetic liposuction procedures and elective cesarean sections to obtain ADMSCs, UCMSCs, and hAMs.

Isolation and culture procedures for human mesenchymal stem cells were similar, along with their characterization to meet the criteria established by the International Society of Cellular Therapy (ISCT) for MSCs. As per the de-epithelization of the human amniotic membrane, the methods used were mechanical and enzymatic. Of the included studies, 6 described macroscopic characteristics, 8 referred histological features, 4 made immunohistochemical analyses, 2 presented biomechanical characteristics, and 1 performed molecular biology studies.

**TABLE 1. Analysis of the characteristics of the studies included in the systematic review. (Continue in the next page).**

| | Animal model | Type of induced wound | Methodology | Analysis/studies carried out | Outcomes |
|---|---|---|---|---|---|
| Khalatbary, *et al.*, (2023)[10] | 60 experimental Wistar rats | Excisional wound on the back and two sagittal incisions on the sides of the wound | Stem cell process: human origin, obtained by liposuction, subsequently washed, isolated, centrifuged, seeded, incubated and finally characterized<br><br>AAM process: human origin, obtained during cesarean sections, subsequently washed, decellularized with trypsin solution, triton, sodium deoxycholic acid, washed again, and lyophilized. Decellularization was confirmed by H&E<br><br>Diabetes mellitus was induced in all rats by intraperitoneal injection of streptozotocin<br><br>They were divided into four groups: control, injection of ADMSCs-derived exosomes, AMS, AMS + ADMSCs- derived exosomes | Samples were taken from five mice from each subgroup at the end of the 1, 2, 3 weeks for analysis:<br><br>-Histological<br>-H&E: nuclei and cytoplasm<br>-Toluidine blue: mast cells<br>-Masson's trichrome: collagen<br>-Immunohistochemical<br>-Anti-ki67 antibodies: cell proliferation<br>-Anti-CD86 antibodies: M1 macrophages<br>-Molecular: TGF-β, bFGF, VEGF, TNF-α and IL-1β by qRT-PCR<br>-Tensiometric: maximum force and energy absorption were determined by a material testing machine | -Wound area Wound closure rate<br>-Total volumes of the new epidermis and dermis<br>-Numerical density of fibroblasts, neutrophils, mast cells, blood vessels in the dermis<br>-Cell proliferation and macrophages M1<br>-Collagen deposit Levels of TGF-β, bFGF,<br>-VEGF, TNF-α, IL-β Tensile strength test |
| Zhou, *et al.*, (2023)[13] | 48 experimental C57BL/6J mice | Second- degree burns of 1 cm in diameter at dorsal level | Stem cell process: obtained from healthy donors by cesarean section, subsequently washed and incubated<br><br>AAM process: obtained from healthy donors by cesarean section, stored, frozen, thawed, decellularized by enzymatic digestion and confirmed by DAPI<br><br>They were divided into four groups: control, mAM + UC-MSCs, UC-MSCs, sham | Five mice from each subgroup were sacrificed on days 7 and 11 for analysis:<br>-RNA<br>-Seq transcriptomic analysis to compare the gene expression profile<br>-Biological effects of mAM<br>-MSC on HUVECs using transwell cell migration and tube formation assays<br>-Survival of mAM-MSC using bioluminescence imaging<br>-Histological: H&E<br>-Immunohistochemical: anti-CD31, antibodies, angiogenesis | -Effects of mAM on MSC<br>-Survival of mAM-MSC<br>-Wound area<br>-Wound closure rate<br>-Vascularization |
| Moghimi, *et al.*, (2023)[17] | 60 experimental Wistar rats | Two excisional wounds of total thickness of 1 cm in diameter at the dorsal level | Stem cell process: obtained from liposuction material from healthy patients undergoing cosmetic surgery, washed, isolated, centrifuged, incubated and characterized<br>AAM process: obtained after cesarean deliveries of healthy women, washed, frozen, thawed, decellularized by swabbing with NaOH and washed<br>They were divided into three groups: control, dAM + ADMSCs, sAM + ADMSCs | The rats were sacrificed on days 3,7,16,21 for analysis:<br><br>-Molecular: VEGF and Col-I by qRT-PCR<br>-Histological<br>-H&E<br>-Masson's trichrome | -Real-Time PCR results<br>-Wound area<br>-Vascularization<br>- Collagen deposit |

**TABLE 1. Analysis of the characteristics of the studies included in the systematic review. (Continue in the next page).**

| | Animal model | Type of induced wound | Methodology | Analysis/studies carried out | Outcomes |
|---|---|---|---|---|---|
| Aghayan, *et al.*, (2022)[18] | 24 experimental Wistar rats | Excisional wound of total thickness of 1 cm at dorsal level | Stem cell process: obtained from liposuction surgery waste (<40 years) and from full-term cesarean births (>38 weeks), washed, isolated, centrifuged, incubated and characterized<br><br>AAM process: obtained from full-term cesarean deliveries, washed, frozen, thawed, decellularized by cell scraper and multiple washing and incubated. Decellularization was confirmed by H&E<br>They were divided into four groups: control, AAM, ADMSCs + AAM, PLMSCs + AAM | Three rats from each subgroup were sacrificed on days 7 and 14 for analysis:<br>-Histological<br>-H&E<br>-Masson's trichrome | -Wound closure rate<br>-Epithelialization<br>-Angiogenesis<br>-Collagen deposit<br>-Epidermal and dermal thickness<br>-Number of inflammatory cells<br>-Cutaneous annexes |
| Xiao, *et al.*, (2021)[19] | 40 experimental BABL/C mice | Total thickness defect of 1 cm in diameter at dorsal level | Stem cell process: obtained during liposuction procedures, isolated, centrifuged, incubated and characterized<br><br>AAM process: obtained from healthy women undergoing cesarean section, frozen, thawed, washed, incubated, decellularized by cell scraping and multiple washing, confirmed by H&E. Finally lyophilized<br><br>Diabetes mellitus was induced in all mice by intraperitoneal injection of streptozotocin. They were divided into four groups: control, ADMSCs-derived exosomes, hAAM, hAAM + ADMSCs-derived exosomes | The healing of the wounds was evaluated by macroscopic observation on days 1,3,7 and 14. Three analyses were performed:<br><br>-Biological effects ADMSC-exos on HDFs and HUVECs using tube formation assays and scratch assay<br><br>-Histological:<br>-H&E<br>-Masson's trichrome<br>-Immunohistochemical<br>-Anti-CD206 antibodies: M2 macrophages<br>-Anti-collagen III antibodies: extracellular matrix<br>-Anti-CD31 antibodies | -Effects ADMSC-exos on HDFs and HUVECs<br>-Appearance and rate of closure of the wound<br>Infiltration of inflammatory cells<br>-Macrophage M2<br>-Vascularization<br>-Extracellular matrix reservoir<br>-Type III collagen<br>-Cutaneous annexes |
| Hashemi, *et al.*, (2020)[20] | 32 experimental albino mice | Third-degree burns (20% SCT) in the cervical region | Stem cell process: obtained from mothers who are candidates for cesarean section, washed, incubated and characterized<br>AAM process: obtained from mothers who are candidates for cesarean section, decellularized using a mechanical method and cold peeling<br>They were divided into four groups: DHAM + HWJMSCs, HWJMSCs injection, HWJMSCs application and DHAM. The corresponding treatment was administered 24 hours of the burn | Half of the rats in each subgroup (4) were sacrificed on the 7th and 14th for analysis:<br><br>-Molecular: gene cGFP and MTT assay<br>-Histological: H&E | -Effects of HWJMSCs on acellular hAM, the viability of cells after labeling and perching on DHAM<br>-Histological findings: re-epithelialization, granulation tissue, hemorrhage and inflammation |

**TABLE 1. Analysis of the characteristics of the studies included in the systematic review. (Continue from previous page).**

| | Animal model | Type of induced wound | Methodology | Analysis/studies carried out | Outcomes |
|---|---|---|---|---|---|
| Wu, *et al.*, (2016)[21] | 32 experimental albino mice | Defects of total thickness of 0.8 x 0.8 cm on the back | Stem cell process: not specified<br><br>AAM process: obtaining not specified, decellularized with sodium dodecyl sulfate, hypotonic tris buffer and treatment with protease and nuclease inhibitors. Confirmation by H&E<br><br>They were divided into four groups: hAM, AAM, ADMSC + AAM | The wound healing rate at 7.14 and 28 days and two analysis at 28 days:<br><br>-Immunohistochemical<br>-Anti-CK19 and human derived mitochondria antibodies<br><br>-Histological: H&E | -Wound healing rate<br>-Histological characteristics: epidermal layers, cutaneous annexes |
| Sabapathy, *et al.*, (2014)[7] | 9 SCID experimental mice | Excisional wound with an area of 1 $cm^2$ on the back | Stem cell process: obtained from patients undergoing elective cesarean section with full-term pregnancy, washed, isolated, incubated and characterized<br><br>AAM process: obtained from patients undergoing elective cesarean section with full-term pregnancy, washed, decellularized with Tris buffer and sodium dodecyl sulfate, washed, immersed in a buffer reaction and sterilized<br><br>They were divided into three groups: HWJMSCs injection, AAM + HWJMSCs,<br>control | The analyses were carried out after 14 days:<br><br>-Multilineage differentiation analysis by stains and flow cytometry<br>-Establishing the safety of isolated and expanded MSCs by flow cytometry and qRT-PCR<br>-Histological: Masson's trichrome<br>-Tracking the transplanted cells in vivo by ICG and using IVIS imaging system<br>-Tensiometric: characterize the mechanical properties by a tensile testing machine | -Differentiation Plasticity<br>-Safety<br>-Singer classification<br>-Cell survival<br>-Biomechanical properties |

AAM, acellular amniotic membrane; H&E, hematoxylin and eosin; ADMSCs, adipose tissue-derived stem cells; AMS, bioengineered three-dimensional microporous amniotic membrane scaffold; qRT-PCR, quantitative Real Time-Polymerase Chain Reaction; mAM, micronized amniotic membrane; UC-MSCs, umbilical cord-derived mesenchymal stem cells; HUVECs, Human Umbilical Vein Endothelial Cells; dAM, decellularized amniotic membrane; sAM, stromal amniotic membrane; PLMSCs, placenta-derived mesenchymal stem cells; hAAM, human acellular amniotic membrane; HDFs, primary human dermal fibroblasts; DHAM, decellularized human amniotic membrane; HWJMSCs, Wharton's jelly-derived mesenchymal stem cells; hAM; human amniotic membrane, ICG, Indocyanine green; IVIS, In Vivo Imaging System.

## Results found in the analyzed studies

### *Macroscopic characteristics*

Macroscopic observation through photographic recording of the wound area at transverse moments showed that the intervention groups (AMS + ADSCs-derived exosomes, mAM + UC-MSCs, dAM + ADMSCs, hAAM + ADSCs-derived exosomes and ADMSCs + AAM) had a greater and faster effect in reducing the wound area, compared to other interventions, including the control group[10][13][17][19][21].

### *Immunological and immunohistochemical characteristics*

In one study, a mathematical equation was used to calculate the newly formed epidermal and dermal volumes in the treated groups. Interestingly, the AMS + ADSCs-derived exosome groups showed greater volumes compared to the other groups on days 7, 14, and 21[10]. Additionally, via the histological study, authors reported that the AMS + ADSCs-derived exosomes group had, comparatively, higher vascular density on days 7, 14, and 21[10]. Although several studies compare AAM + HWJMSCs to other intervention groups, one of them evaluated hyperkeratosis and epidermal hyperplasia, finding higher values in comparison[7]. Similarly, in two studies staining with CD31 was performed and a greater pro-angiogenic effect was observed in the intervention groups (mAM + UC-MSCs and hAAM + ADSCs-derived exosomes), compared to the other groups[13].

Regarding collagen deposition, Masson's trichrome staining was used to stain collagen fibers, revealing that in the AMS + ADSCs-derived exosomes and dAM + ADMSCs groups, the wound bed had a greater amount of collagen with a better arrangement of fibers; therefore, constituting a more organized regenerated structure[10][17]. In another study, collagen expression was determined by immunohistochemical staining of collagen III and it was evident that the hAAM + ADSCs-derived exosomes group showed the highest expression compared to the other groups[19]. Likewise, the group HWJMSCs + AAM was evaluated through the orientation of collagen fibers, which was superior to the other intervention groups[7].

In terms of cell proliferation, immunohistochemical staining of ki67 and CD86 demonstrated that cell proliferation in the AMS + ADSCs-derived exosomes group was significantly higher compared to the other groups on days 7, 14 and 21; while the density of M1 macrophages was considerably lower in this group[10]. Immunohistochemical staining of CD206 demonstrated increased recruitment of M2 macrophages to the wound bed[19]. Furthermore, three of the studies performing immunohistochemical analyses evaluated the regeneration of skin annexes. One of the studies performed immunohistochemical staining of cytokeratin 19 and mitochondria and reported the observation of hair follicle structure on day 28 (ADMSCs + AAM group). Though a second study aiming to improve wound healing in diabetic mice by applying hAAM + ADSCs-derived exosomes, hair follicles and sebaceous glands

failed to regenerate[19], the third experiment used the Singer classification to quantify the regeneration of skin wounds via Masson's trichrome staining, with great outcomes. Authors report the presence of hair follicles and apocrine glands in the AAM + HWJMSCs group[7].

*Biomechanical characteristics*. Two of the studies included tensile strength tests. In the first one, the AMS + ADSCs-derived exosomes group exhibited greater maximum strength and energy absorption on day 21, compared to the other groups[10]. While on the second study, the analysis of all biomechanical parameters (stress, strain, Young's modulus, stiffness, tenacity modulus and tensile strength) indicated that the AAM + HWJMSCs group had better scores when compared to other groups (HWJMSCs and control)[7].

*Molecular biology studies*. In one interesting study, the level of gene transcription of various representative factors was evaluated through real-time polymerase chain reaction. Factors included in this study were associated with proliferation and regeneration (TGF-β and bFGF), angiogenesis (VEGF), and inflammation (TNFα and IL-1β) on day 7. In comparison to other groups, the AMS + ADSCs-derived exosomes group had greater expression of the factors associated with proliferation, regeneration, and angiogenesis and lower expression of proinflammatory factors[10].

## Discussion

The function of the human amniotic membrane as an acellular scaffold for mesenchymal stem cells derived from different tissues and its role in tissue regeneration has been extensively studied over time[22][23], this systematic review aims to consolidate the available evidence on the combined use of human stem cells derived from adipose tissue and umbilical cord seeded in acellular human amniotic membranes and their role in the skin wound healing process in mice and rats.

It is important to highlight that MSCs are easily obtained and have been successfully isolated from various human tissues[24]. When using stem cells to accelerate the skin regeneration process, it is evident that the viability of this type of cells is low, due to different friction forces generated during processing and application, ultimately compromising the survival of the stem cells[11]. Likewise, it has been shown that not all stem cells have the same capacity to migrate towards the site of injury, which prevents the recruitment of an adequate number of MSCs[25].

Various biological and synthetic scaffolds provide a favorable environment for growth, proliferation, and maintenance of stem cells destined for a specific tissue space[26]. AAM is a biological scaffold with excellent results in skin healing, and the evidence analyzed in this manuscript supports a synergistic effect with stem cells. Both present anti-inflammatory effects, through the release of growth factors and similar properties[27]. Of special interest in hAM, is the mechanical characteristics of the basement membrane conferred by its proteins[28].

This systematic review allowed the comparison and analysis of a variety of studies regarding the use of bioscaffolds (MSCs + dHAM) in skin wounds. Eight (8) articles were reviewed, and all of them evaluated one or more of the following criteria: macroscopic, histological, and immunohistochemical characteristics, collagen deposits, cell proliferation, and biomechanical properties.

This confirms that both ADMSCs and UC-MSCs, seeded in dHAM, show positive outcomes in terms of wound area reduction compared to the control groups[10][13][17][19][21]. Regarding immunohistochemical characteristics, it was determined that on days 7, 14, and 21, the epidermal volume and the rate of angiogenesis and cellular proliferation were

higher in the dHAM + ADSCs- derived exosomes group[10], suggesting a favorable and less inflammatory environment conducive to healing. Only one study measured the rate of hyperkeratosis and epidermal hyperplasia, which was higher in the dHAM + HWJMSCs group[7]. On the other hand, an important factor to consider in the skin healing process is the accumulation of collagen deposits and the organization of its fibers, exposing that in the dHAM + ADSCs-derived exosomes, dHAM + ADMSCs, and HWJMSCs + dHAM groups, better collagen fiber organization was observed[7][10][17]. Additionally, the dHAM + ADSCs-derived exosomes group reported a higher concentration of type III collagen[19]. It should be noted that only two studies evaluated the regeneration of skin appendages; of these, only two studies successfully regenerated skin appendages such as hair follicles by day 28[7][19] and apocrine glands[7]. The variability in these results may be due to the differences in the animal models used, the application methodology, or the source of the cells provided. When evaluating biomechanical properties, the study groups were subjected to traction, tension, deformation, and stiffness tests, where the dHAM + HWJMSCs group had the best outcomes[7]. This aspect is crucial to ensure not only rapid healing but also the provision of functional and resilient tissue. At the molecular level, the dHAM + ADSCs-derived exosomes group showed greater expression of factors associated with cellular proliferation and lower expression of pro-inflammatory factors[10]. This suggests that these combinations not only accelerate the healing process but also favorably modulate the cellular environment to promote more efficient healing with fewer inflammatory complications. Nevertheless, the use of human amniotic membranes combined with mesenchymal stem cells (MSCs) could have several limitations that are important to consider:

Immunological Rejection: Although hAM has immunomodulatory properties, there is a risk of immunological rejection when combined with MSCs. Quality Variability: The quality of amniotic membranes can vary depending on the source and processing method, which can affect the efficacy of the treatment. Risk of Infection: Being a biological tissue, there is a risk of transmission of infections if not handled properly[29]. Costs and Availability: Obtaining and processing amniotic membranes and MSCs can be expensive and are not always available in all regions. Limited Efficacy: In some cases, the observed benefits cannot be fully attributed to the cellular plasticity of MSCs, as the number of grafted cells may be low.

Overcoming the limitations of using human amniotic membranes combined with mesenchymal stem cells (MSCs) requires a multifaceted approach:

Improving Immunological Compatibility: Genetic engineering techniques can be used to modify MSCs and reduce the risk of immunological rejection. Process Standardization: Developing standardized protocols for obtaining and processing amniotic membranes can help reduce variability in quality. Quality Control and Safety: Implement strict quality control and safety testing to minimize the risk of infections. Research and Development: Invest in research to improve the efficacy of combination therapies and better understand the mechanisms of action of MSCs. Cost Reduction: Encourage mass production and process optimization to reduce costs and improve availability. Education and Training: Train healthcare professionals in the proper management of these therapies to maximize their benefits and minimize risks[30].

It is important to mention that the information obtained from ADMSCs exceeds that found about HWJMSCs, which explains the inequality when presenting the results found in the PRISMA search, since we found differences regarding the animal model and wound etiology. Additionally, it should be considered that not all articles compare the same parameters, which generates a bias when objectively evaluating the results. We consider that the fundamental parameters to evaluate similar investigations should be related to the effects on the wound area, the capacity for angiogenesis and collagen synthesis, the biomechanical properties, and the induction of molecular factors that favor cell proliferation.

Specifically in future clinical trials in humans, parameters such as improvement in pain and systemic effects of the use of this method for tissue regeneration should also be reported.

Moreover, some studies evaluated experimental results in up to four-time intervals, while other authors considered a two-time interval. Although evaluation periods may vary depending on the researchers' projections, resources, materials, and the disposition of the experimental and control groups, these determinants must be methodically evaluated before initiating the experiment to obtain reproducible results.

Another relevant consideration is that experimental studies carried out in humans are scarce and only studies of UC-MSCs + dHAM in skin regeneration were found. Hashemi and collaborators described the use of stem cells derived from the umbilical cord seeded in acellular human amniotic membrane, obtaining positive outcomes in terms of reducing wound size and healing time[31]. Overall, the analysis presented throughout this systematic review indicates that stem cells derived from adipose tissue and umbilical cord seeded in decellularized human amniotic membranes show positive results in clinical trials with murine animals. It is expected that in the future, these studies will pave the way for clinical trials in humans to be able to carry out evidence-based guided tissue regeneration therapies.

## CONCLUSIONS

This systematic review of the literature evidence the synergy reported in the use of stem cells from adipose tissue and umbilical cord tissue of human origin in combination with the acellular human amniotic membrane for the treatment of skin lesions, such as ulcers and burns in rodents, promises great outcomes. Groups treated with MSCs reported better outcomes in terms of the reduction of the wounded area, better healing time, greater vascular density, and collagen formation. Interestingly, when evaluating biomechanical characteristics, better results were found when stem cells were combined with amniotic membranes. Based on these, we infer that stem cells derived from adipose and umbilical cord tissues of human origin, combined with acellular amniotic membranes, are a promising option in the treatment of wounds and burns. Although further clinical trials are needed, these investigations showed promising results that may promote the use of these biological scaffolds in humans, as part of a regenerative therapy.

## ACKNOWLEDGEMENT

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## AUTHOR CONTRIBUTIONS

V. G. conceptualization, data curation, formal analysis, investigation, methodology, project administration, and writing original draft; G. M. data curation, formal analysis, investigation, software, visualization, and writing original draft; K. S. data curation, formal analysis, investigation, and writing original draft; D. E. funding data curation, supervision, validation, and writing-review and editing; S. T. funding data curation, supervision, validation, and writing-review and editing; L. A. G. conceptualization, funding data curation, resources, supervision, validation, and writing-review and editing.

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## ANNEXE

PRISMA 2020 for Abstracts Checklist

| Section and Topic | Item # | Checklist item | Reported (Yes/No) |
|---|---|---|---|
| **TITLE** | | | |
| Title | 1 | Identify the report as a systematic review. | Yes |
| **BACKGROUND** | | | |
| Objectives | 2 | Provide an explicit statement of the main objective(s) or question(s) the review addresses. | Yes |
| **METHODS** | | | |
| Eligibility criteria | 3 | Specify the inclusion and exclusion criteria for the review. | Yes |
| Information sources | 4 | Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched. | Yes |
| Risk of bias | 5 | Specify the methods used to assess risk of bias in the included studies. | No |
| Synthesis of results | 6 | Specify the methods used to present and synthesise results. | Yes |
| **RESULTS** | | | |
| Included studies | 7 | Give the total number of included studies and participants and summarise relevant characteristics of studies. | Yes |
| Synthesis of results | 8 | Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured). | Yes |
| **DISCUSSION** | | | |
| Limitations of evidence | 9 | Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision). | Yes |
| Interpretation | 10 | Provide a general interpretation of the results and important implications. | Yes |
| **OTHER** | | | |
| Funding | 11 | Specify the primary source of funding for the review. | Yes |
| Registration | 12 | Provide the register name and registration number. | No |

*From:* Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

## PRISMA 2020 Checklist

| Section and Topic | Item # | Checklist item | Location where item is reported |
|---|---|---|---|
| **TITLE** | | | |
| Title | 1 | Identify the report as a systematic review. | Page 1 |
| **ABSTRACT** | | | |
| Abstract | 2 | See the PRISMA 2020 for Abstracts checklist. | Page 12 |
| **INTRODUCTION** | | | |
| Rationale | 3 | Describe the rationale for the review in the context of existing knowledge. | Page 2 |
| Objectives | 4 | Provide an explicit statement of the objective(s) or question(s) the review addresses. | Page 4 |
| **METHODS** | | | |
| Eligibility criteria | 5 | Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses. | Page 5 |
| Information sources | 6 | Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted. | Page 5 |
| Search strategy | 7 | Present the full search strategies for all databases, registers and websites, including any filters and limits used. | Pages 5 |
| Selection process | 8 | Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process. | Page 5 |
| Data collection process | 9 | Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process. | Page 5 |
| Data items | 10a | List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect. | Page 5 |
| | 10b | List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information. | Page 5 |
| Study risk of bias assessment | 11 | Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process. | Page 5 |
| Effect measures | 12 | Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results. | NA |
| Synthesis methods | 13a | Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)). | Pages 5 |
| | 13b | Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions. | Page 5 |
| | 13c | Describe any methods used to tabulate or visually display results of individual studies and syntheses. | Pages 5 |
| | 13d | Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used. | NA |
| | 13e | Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression). | NA |
| | 13f | Describe any sensitivity analyses conducted to assess robustness of the synthesized results. | NA |
| Reporting bias assessment | 14 | Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases). | NA |
| Certainty assessment | 15 | Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome. | NA |

## PRISMA 2020 Checklist

| Section and Topic | Item # | Checklist item | Location where item is reported |
|---|---|---|---|
| **RESULTS** | | | |
| Study selection | 16a | Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram. | Pages 5-6 |
| | 16b | Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded. | Pages 5-6 |
| Study characteristics | 17 | Cite each included study and present its characteristics. | Pages 7-10 |
| Risk of bias in studies | 18 | Present assessments of risk of bias for each included study. | - |
| Results of individual studies | 19 | For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots. | NA |
| Results of syntheses | 20a | For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies. | NA |
| | 20b | Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect. | NA |
| | 20c | Present results of all investigations of possible causes of heterogeneity among study results. | NA |
| | 20d | Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results. | NA |
| Reporting biases | 21 | Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed. | NA |
| Certainty of evidence | 22 | Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed. | NA |
| **DISCUSSION** | | | |
| Discussion | 23a | Provide a general interpretation of the results in the context of other evidence. | Pages 10-11 |
| | 23b | Discuss any limitations of the evidence included in the review. | Pages 10-11 |
| | 23c | Discuss any limitations of the review processes used. | Pages 10-11 |
| | 23d | Discuss implications of the results for practice, policy, and future research. | Page 11 |
| **OTHER INFORMATION** | | | |
| Registration and protocol | 24a | Provide registration information for the review, including register name and registration number, or state that the review was not registered. | - |
| | 24b | Indicate where the review protocol can be accessed, or state that a protocol was not prepared. | - |
| | 24c | Describe and explain any amendments to information provided at registration or in the protocol. | - |
| Support | 25 | Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review. | Page 12 |
| Competing interests | 26 | Declare any competing interests of review authors. | Page 12 |
| Availability of data, code and other materials | 27 | Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review. | Pages 14-17 |

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