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The Role of Menstrual Blood in Wound Healing

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Abstract: *Treatment for persistent wound are still elusive despite significant progress in understanding the cellular and molecular mechanism of wound healing. Here, we go over the genuine roles of mesenchymal steam cell (MenSCs) produce from menstrual blood. Mesenchymal steam cell have observable immunomodulatory , immunosuppressive and regenerative properties.*

To enhance MSCs migratory, angiogenic, immunodulatory and reparative capacities, it is necessary to identify the best sources for them. Menstrual fluid (MenSCs) is an alternate source that is appropriate for repeated , non-invasive MSc collection and offers a significant practical benefit over alternative sources.

While, chronic wounds are unable to end the inflammatory phase and cannot progress towards a regenerative state, the wound healing process is a complex of overlapping and coordinate processes that moves past the inflammatory phase towards wound resolution. Chronic wound immunopathology has been linked to tissue and cellular senescence, protracted inflammation, and dysregulation of microenvironments that cause an imbalance between pro- and anti-inflammatory states. Here, we address the mesenchymal steam cell (MenSCs) produced from menstrual blood and their genuine roles, particularly their migratory, angiogenic and immunosuppressive capabilities .

Keywords: Menstrul blood, Mesenchymal stem cells, wound healing, regeneration, Cell therapy, Tissue regeneration

I. INTRODUCTION

Treatment for chronic wounds is still difficult, despite significant progress in our understanding of the cellular and molecular reactions involved in wound healing.

Innovative techniques employing therapeutic cells have grown in popularity over the past few decades and shown promise in tissue regeneration. Mesenchymal stem cells (MenSCs) in particular have been demonstrated to exhibit several advantageous traits for wound healing. MSCs are tissue resident progenitor cells that can differentiate into a variety of cell types and have immunomodulatory, anti-inflammatory and self renewing properties. Given the promising result of using MSCc to repair wounds, research into the best sources of MSCs is still required to enhance their migratory, angiogenic, immunodulatory, and reparative properties. Menstrual blood derived mesenchymal stem cells (MenSCs) offer an alternate source for repeated, non-invasive collection that poses no ethical issue [1]

Effective healing of chronic wounds has a remaind a significant challenge in the Healthcare system, despite significant advancement in our understanding of the underlying cellular and molecular mechanism of wound healing. Regretfully, nearly 50% of chronic wounds do not achieve adequate healing and regeneration. Therefore, in order to improve patients quality of life, new therapeutic approaches must be created for successful wound healing management. Over the past 10 years, research based on stem cell and tissue engineering has produced innovative methods for Managing chronic wounds. Because stem cells have special qualities like the capacity to self-renew , to undergo transdifferentiation, and to secrete a variety of trophic factors that can control immune responses, using them for these therapeutic interventions is an appealing alternative for these therapeutic interventions. [2]

One of the main goals of clinical therapy is still to promote the healing of these intentional and inadvertent wounds, minimize the patients asthetic impact and maximize tissue function restoration. Larger injuries or the presence of several physiological and common disease state,such as age, infection, diabetes/ vascular disease and cancer can

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adversely affect the healing process in ways that are currently poorly understood, evenly through minor injuries in healthy people typically heal well Fig:-1 [3]

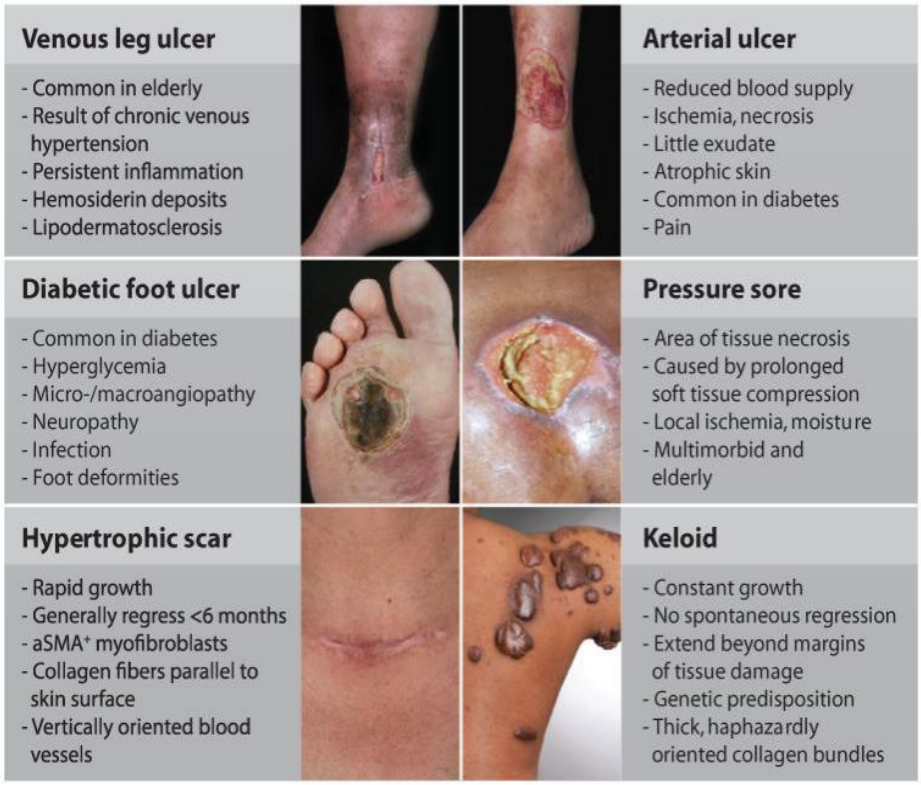

Fig:- (1) clinical / features of most common wound healing pathologies.

Numerous systemic and local variables can disrupt the repair process, resulting in a variety of wound healing disorders (A) venous leg ulcer (VLU) on the medial portion of the lower leg (B) DFU or a diabetic foot ulcer (C) an arterial ulcer on the lowerlegs arterial surface (D) soreness from pressure (E) after thyroid surgery,hypertrophic scar (F) keloid. [3]

Mesenchymal stem cells (MSCs) are a promising therapeutic approach and a special tool in a tissue engineering and regenerative medicine. MSCs appear to be the preferred modality technique for these individuals because of their distinct biological characteristics. Numerous preclinical and clinical

investigation point to the potential applications of these cells in scar remodeling, Acute and chronic wound healing and tissue regeneration. The goal of these studies is to provide an overview of the preclinical and current knowledge on MSCs, their biological properties and mechanism of action during healing and regenerative processes and their clinical use in the treatment of chronic wounds [4]

Like MSCs, MenSCs' immunomodulatory qualities and paracrine function, which promotes endogenous cellular repair or regeneration without significant cell-tissue integration or differentiation, have been the basis for research into their therapeutic potential.[5]

Graphical abstract of MenSCs:-

MENSES is the first step in the procedure, which yields a "Cell Mix" that isolates MenSCs. MenSCs' therapeutic potential is highlighted in the diagram, as evidenced by their demonstrated: The capacity to alter the immune system is known as immunomodulatory properties. Cell Reparative/Regenerative Properties: The ability to differentiate into multiple cell types and repair and regenerate tissue through secreted chemicals. **(Fig:-2)** [5]

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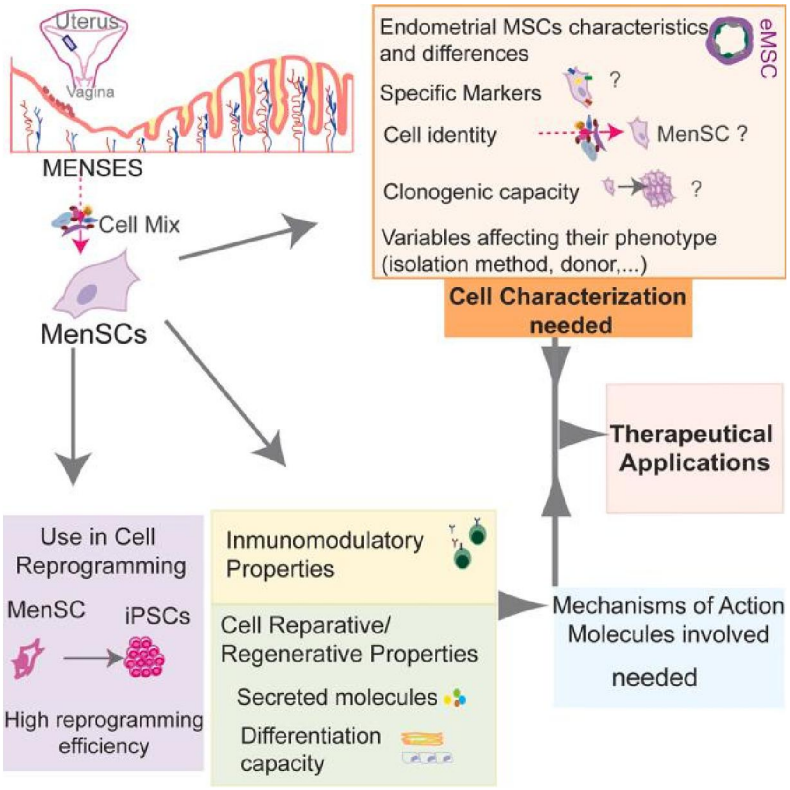

Fig 2:- Graphical abstract

Therapeutic mechanism Of MenSCs:-

MSCs' therapeutic potential for tissue repair has been thoroughly investigated. According to recent research, MenSCs primarily produce therapeutic effects through following mechanisms.

An outline of potential MenSC treatment pathways is shown.

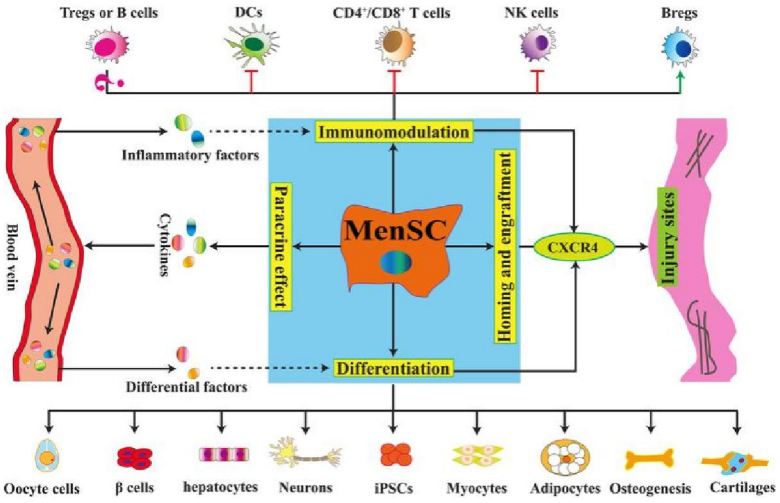

Fig 3:- MenSCs therapeutic effects through following mechanisms

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(1) MenSCs primarily work by differentiating into targeting cells, such as cartilaginous, adipocytic, osteogenic, cardiogenic, muscle, neurogenic, oocyte-like, iPSCs, myocytic, granulosa, and hepatic tissues;

(2) immunomodulation interacting with different immune cells, such as promoting the production of regulatory B (Breg) cells and inhibiting the proliferation of T cells, NK cells, and dendritic cells.

(3) A range of cytokines, including VEGF, BDNF, NT-3, IL-4, TGF-β 2, EGF, PDGF, NO, HIF-1α, MMP-3, MMP-10, IL-6, MCP-1, HGF, IL-8, GRO, OPG, angiopoietin, elastin, thrombospondin-1, SDF-1, and IGF-1. MenSCs engage with immunomodulation and produce inflammatory factors by secreting these cytokines through the blood vein. Similar to this, some differential factors are also produced by the paracrine action through the blood vein to play the role of differentiation; and

(4) certain chemokine receptors, such CXCR4, target wounded regions by homing and engraftment. Red T-shapes indicate a negative role, whereas green arrows indicate a positive role. **(Fig:-3)** [6]

Explanation of mechanism:-

1) Differentiation:-In order to replace damaged tissues, MenSCs can differentiate into a variety of cell types. They can differentiate into neurones, hepatocytes, myocytes, adipocytes, osteogenesis cells, and cartilage, according to the diagram.[7]

2) immunomodulation:-Immune responses, especially those involving cytokines, chemokines, and the interaction of different immune cells, play a crucial role in the intricate process of wound healing. Haemostasis, inflammation, proliferation, and remodelling are the phases of wound healing that overlap. The immune system, which coordinates a sophisticated reaction to harm, has a significant impact on each stage. In order to eradicate infections and promote the ensuing healing processes, the inflammatory phase is a crucial reaction to tissue damage. [8]

MenSCs regulate the immune system by interacting with various immune cells. This entails encouraging the development of regulatory B (Breg) cells while inhibiting the growth of T lymphocytes, natural killer (NK) cells, and dendritic cells (DC). [9]

3) Paracrine effect:- MenSCs have a paracrine function by secreting a range of cytokines and other substances. The graphic shows a number of cytokines that are released into the bloodstream to affect inflammatory factors and support differentiation, including VEGF and BDNF [10]

4) homing and engraftment:- Migration via the interstitium to the site of injury: MSCs detect chemotactic gradients in the tissue, such as the SDF-1/CXCR4 axis, and move in the direction of the injured region.

enhancing adhesion and migration by upregulating integrins, ligands, CXCR4, and CXCR7 (for the SDF-1 gradient). [11]

Evidence from wound healing model;-

1)Excisional wound model in rats using MenSCs-seeded decellularized amniotic membrane:-

Rats with full-thickness ~1.5 × 1.5 cm wounds were used in the study "Promotion of excisional wound repair by a menstrual blood-derived stem cell-seeded decellularized human amniotic membrane (DAM)." MenSCs were administered to the wounds after being seeded onto a human amniotic membrane scaffold that had been decellularized.

Results: When compared to DAM alone, the MenSC-seeded DAM greatly enhanced wound closure. Improved tissue architecture was seen in histopathology. The authors came to the conclusion that MenSCs could be a possible source for skin regeneration through this scaffold.[12]

2) MenSCs in diabetic wound models:-

(a) "Streptozotocin-induced diabetic rats were used in an experimental study on the effects of human amniotic membrane in combination with menstrual blood-derived stem cells on wound healing in a diabetic rat model." Groups: MenSCs alone, HAM + MenSCs, control, and HAM alone.

Results: The HAM+MenSC group had a markedly higher rate of wound healing, more collagen deposition, thicker new dermis and epidermis, and enhanced mechanical and tensiometric characteristics. TGF-β, bFGF, and VEGF gene expression was elevated in treatment groups, particularly HAM+MenSCs. Concurrently, HAM+MenSCs showed a greater downregulation of pro-inflammatory gene[13]

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(b) Mice with full-thickness excisional wounds were employed in the study "Application of Menstrual Blood Derived Stromal (stem) Cells Exert Greater Regenerative Potency Than Fibroblasts/Keratinocytes in Chronic Wounds of Diabetic Mice." MenSC-seeded bilayer scaffolds were contrasted with controls and scaffolds seeded with keratinocytes or fibroblasts.

Results: From days 7–14 after wounding, the MenSCs group showed improved wound contraction, re-epithelialization, more mature dermal/epidermal regeneration, higher vascular markers (CD31, vWF), higher M2/M1 macrophage ratio (indicating more reparative macrophages), and lower type III collagen (less scar-prone).[14]

3)use of MenSCs derived exosomes :-

produced full-thickness dorsal skin lesions in streptozotocin-induced diabetic mice; PBS, exosomes, and MenSCs were the therapy groups.

Key findings: Treatment with exosomes led to: Quicker re-epithelialization, Increased angiogenesis (VEGF-A overexpression),M1→M2 macrophage shift that favours the repair/anti-inflammatory phenotype, decreased collagen I:III ratio, or less scar formation [15]

4) mechanistic/in vitro supportive work:-

MenSCs release a range of growth factors and cytokines that aid in wound healing, such as angiopoietin, PDGF, elastin (ELN), MMP3, and MMP10. MenSCs enhanced vascularity and wound closure in a mouse excisional wound model.[16]

Limitations & caveats:-

1)predominantly preclinical evidence:-

Menstrual blood-derived mesenchymal stem cells (MenSCs) are frequently studied in vitro or in rodent models for wound repair. For instance, in a rat excisional model, a decellularized amniotic membrane seeded with MenSCs enhanced wound closure. [12]

2) heterogeneity of source:-

Although some studies show good consistency up to a specific age, different donors may produce MenSCs with varying proliferative capacities.[17]

3) model wound types vs real world complexity:-

Acute excisional wounds in young, healthy animals are used in several research. For instance, young, healthy skin models with minor wounds were employed in a study on menstrual fluid plasma. They pointed out a drawback: the incisions in non-compromised skin were only 1 mm deep.[18]

4) mechanistic understanding still limited:-

MenSCs and menstrual fluid exhibit encouraging paracrine/secretome effects (angiogenesis, immunomodulation, migration), but it is unclear exactly how they affect wound extracellular matrix, cell recruitment, and immune regulation in vivo. For instance, the MenSC study found that in pro-inflammatory conditions, factors such as PDGFB and MMP3 were upregulated [18]

5) safety, immunogenicity, long term outcomes:-

MenSCs offer favourable characteristics (low immunogenicity, non-invasive source), and there is some safety information (such as in tumorigenicity/toxicity studies). [17]

Key features of MenSCs beneficial for wound healing:-

1)Non invasive & renewable source

Unlike bone-marrow MSCs and adipose MSCs, MenSCs may be extracted from menstrual blood, which is shed once a month and does not require an intrusive surgery. [19]

2)high proliferative/clonogenic capacity

When compared to certain other MSCs, MenSCs exhibit quick proliferation and a quicker doubling time (about 20 hours in one report).[19]

They preserve chromosomal integrity over a long period of time—up to 68 generations, according to one review—which is crucial for growth.[1]

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3) multipotent differentiation potential

MenSCs have the ability to develop into a variety of lineages, including keratinocytes (skin epidermal cells). For example, a study stimulated MenSCs to express the keratinocyte markers K14, p63, and involucrin. [20]

4) Strong paracrine/secretory profile (growth factors, cytokines, MMPs etc)

MenSCs' gene expression for wound-repair-related components was much higher in the study comparing them to umbilical-cord MSCs, such as PDGFB ~791-fold, MMP3 ~21.6-fold, and ELN ~13.4-fold under pro-inflammatory stimulation.[1]

The paracrine secretome also contains elements that support angiogenesis, such as ANGPT1, VEGF, matrix remodelling (MMPs), elastin/ELN, etc.**(16)**

5) Angiogenic potential

For wound healing, effective neovascularisation is essential. MenSC-treated in vivo wound models demonstrated improved vascular networks and increased VEGF expression.[1]

The exosome analysis found that wounds in diabetic mice had increased angiogenesis due to VEGF overexpression.[15]

6) immunomodulatory and anti-inflammatory effects

In the comparison investigation, MenSCs suppressed T-cell proliferation in co-culture, indicating immunosuppressive potential.[1]

In wound models, they reduced pro-inflammatory cytokines (TNF-α, IL-1β) and encouraged M1 → M2 macrophage polarisation, which is beneficial for healing.[21]

7) favourable safety/ immunogenic profile

MenSCs have little tumorigenicity in several preclinical contexts and express low HLA-DR, which reduces immunogenicity (however clinical data is lacking).[22]

8) suitability for chronic/ difficult wound microenvironment

Numerous research focus on diabetic wounds, which are a common example of poor healing. MenSCs are especially well-suited for such difficult environments because of their angio-, immunomodulatory-, and matrix-remodeling characteristics.[14]

Advantages:-

1) Non-invasive & easily accessible source

Unlike bone marrow or adipose tissue, menstrual blood is obtained non-invasively from healthy women of reproductive age without the need for discomfort or surgery. offers a source of stem cells that is autologous (or allogeneic) and renewable with little ethical issues.[16]

2) High proliferation and clonogenic potential

When compared to other mesenchymal stem cells (MSCs), such as bone marrow-derived MSCs (BM-MSCs), MenSCs have a higher rate of proliferation and a shorter population doubling time.[17]

3) Strong paracrine (secretory)and immunomodulatory effects

Release growth factors such as VEGF, TGF-β, PDGF, HGF, and cytokines that support: Angiogenesis, or the creation of new blood vessels Re-epithelialization

and collagen production Macrophage polarisation (anti-inflammatory phenotype, M1 → M2)

Lower pro-inflammatory cytokines (TNF-α, IL-1β) to aid in the healing of chronic wounds.[15]

4) Effective in Chronic & diabetic wound models

MenSCs or their exosomes in mice with diabetes: hasten the healing of wounds Boost collagen organisation and vascularization Decrease the production of scars and fibrosis Boost epidermal and dermal renewal [23]

5) Compatibility with biomaterials

MenSCs enhance structural support and regulate the release of restorative factors by integrating effectively with scaffolds such hyaluronic acid, silk fibroin nanofibers, and decellularized amniotic membrane.[24]

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Disadvantages:-

1) Poor cell survival / engraftment in wound microenvironment

MenSCs have good migratory and proliferative characteristics, but their survival in the hostile wound microenvironment—which includes hypoxia, inflammation, and oxidative stress—after transplantation is restricted.[17]

2) Lack of robust human clinical trial data

The majority of the evidence supporting MenSCs in wound healing comes from in vitro research or animal models; human clinical data are few. For instance, the translation into human wound healing (particularly chronic wounds and concomitant patients) has not yet been established, despite the fact that numerous preclinical studies demonstrate promise.[2]

3) Donor variability & heterogeneity

MenSCs may vary in yield, quality, and function depending on donor factors (age, hormonal status, menstrual cycle phase, health status) or collection/processing methods.[16]

4) Microenvironmental challenges reduce efficacy

The unfavourable microenvironment and extremely low oxygen (<1% O_2) seen in wounds, particularly chronic wounds, can hinder the survival and function of stem cells.[17]

5) Sterility, scalability & manufacturing barriers

Issues with donor screening, GMP production, lot-to-lot uniformity, sterility, immunogenicity, cost, and regulation are included in general stem-cell therapy evaluations, albeit they are not usually specifically mentioned in every MenSC wound-healing study. [25]

6) Unclear long term safety & functional integration

Long-term human follow-up is inadequate, despite early research demonstrating safety (e.g., no overt tumorigenicity in short-term animal models).[25]

Consideration and caution:-

1) Translation from animal to human and to complex wound scenario

The majority of research on wound healing is conducted in animal models (rats, mice) with reasonably controlled wounds (excisional, full-thickness) or models with created diabetes. The microenvironment is more complicated for human wounds that occur in the real world, such as chronic diabetic foot ulcers, venous leg ulcers, wounds with infection or biofilm, and wounds with inadequate perfusion. For instance, the assessment of MSC-based cell-free therapy for wound healing highlights the fact that chronic human wound pathophysiology is not adequately captured by many preclinical models.[26]

Persistent inflammation, senescent cells, hypoxia, inadequate vascularization, and microbiological issues are common in chronic wounds. MenSCs' migratory traits might not be enough for them to home to chronic wound sites, according to a study that combined MenSCs with bilayer scaffold.[2]

2) Standardisation, manufacturing, dosing &delivery

The reviews highlight the continued variability of MenSC isolation, expansion, and characterisation techniques. MenSCs, for instance, "are attracting more and more attention... currently people are increasingly interested in their clinical potential," according to the evaluation of "multi-functional roles." but also suggests that translation is in its early stages. [16]

In wound healing applications, the cell dose, delivery timing in relation to wound stage, and application method (topical scaffold, injection around wound margins, whether with biomaterial) are still not standardised. The hypoxia study highlights how culture circumstances (normoxia vs. hypoxia) affect MenSC secretomes and, consequently, the therapy's potential efficacy. [17]

3) mechanistic clarity & fate of cells

The precise secreted molecules (growth factors, miRNAs in exosomes) responsible for the advantages are not entirely understood, even though paracrine pathways are probably predominant. This makes treatments less predictable and

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repeatable. There is still much to learn about the long-term fate of implanted MenSCs in wound-healing applications. Do they integrate, differentiate, endure over time, or are they cleared? Migration but little differentiation were seen in the liver fibrosis mode [27]

4) safety (tumorigenicity, immunogenicity and infection risk)

MenSCs may be different in wound-healing scenarios, even though they seem to be somewhat safe in early research (for instance, in non-wound applications). Cell behaviour in a wound bed may be influenced by immune and inflammatory reactions. Although it is still early, the cell-free exosome method may lower immunologic risk. For many applications, "the study... is still in its initial stages," according to the assessment on MenSC-derived tiny EVs.[28]

Future perspective:-

1) clinical translation &human trials

The majority of research to date on MenSCs' improved angiogenesis, epithelialisation, and anti-inflammatory properties has been done in small animal models. Clinical validation using Phase I/II human studies to evaluate safety is the next crucial stage.[29]

2) cell-free therapeutics (exosomes &secretome)

Future studies should concentrate on cell-free methods that can provide important bioactive molecules (including VEGF, miR-21, and TGF-β) without the dangers of live-cell transplantation, such as MenSC-derived exosomes or conditioned media. [30]

3) optimisation of delivery system

Cell survival, local retention, and tissue integration may be improved by combining MenSCs or their exosomes with biocompatible scaffolds such silk fibroin, amniotic membrane, or hyaluronic acid. [31]

II. CONCLUSION

According to available data, menstrual blood-derived mesenchymal stem cells (MenSCs) and the extracellular vesicles they release have a high potential for wound healing. MenSC-based treatments consistently improve tissue quality, promote angiogenesis, reduce inflammation, and speed up wound closure when compared to controls in pre-clinical animal and cell investigations. Pro-angiogenic, anti-inflammatory, and tissue-regenerative pathways appear to work together to provide these effects.

But the field is still mostly experimental. Thus yet, the evidence is restricted to small-animal and laboratory models; there is a dearth of solid human clinical data. Standardising cell isolation and processing, guaranteeing safety, and negotiating regulatory approval for therapeutic usage continue to be significant obstacles. Prior to clinical translation, logistical and ethical issues, as well as the biological diversity of materials produced from menstruation, must be taken into account.

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