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(REVIEW ARTICLE)

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The essentials of stem cell-derived secretome in wound healing

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Abstract

As the primary method for encouraging tissue regeneration, stem cells are known to exert paracrine effects. A collection of biocomponents produced by the activated stem cells as the paracrine effect, is called secretomes. It includes the extra-vesicular components and the soluble factors. Secretomes are being used in cell-free stem cell treatments that are currently being developed. The secretome includes cytokines and key growth factors such as VEGF, TGF-β, FGF, PDGF, EGF, bFGF, and HGF; which aid in coordinating cellular communication to stimulate tissue regeneration. Studies have shown the benefits of stem cell-derived secretome applications in the wound healing process. The content of various growth factors in secretomes is known to accelerate wound healing by increasing cellular chemotaxis, and fibroblast contraction, stimulating the proliferation of fibroblast and keratinocyte, and promoting neovascularization through angiogenesis stimulation. Moreover, the secretomes also demonstrated the ability to stimulate the proliferation and migration of skin cells. The use of stem cell-derived secretomes in the future has the potential as an alternative cell-free therapy in various wound healing processes. In this review, we will discuss the potential role of stem cell-derived secretomes for wound healing and how to acquire them. The benefit of secretomes is promising as an alternative cell-free treatment to improve wound healing.

Keywords: Stem cells; Secretomes; Growth factors; Wound healing; Paracrine effect

1. Introduction

Cell-based therapy is a treatment that uses stem cells, which are unspecialized cells that may grow into many types of cells and make up different types of tissues in the human body. Stem cells play an important role in the development, growth, maintenance, and repair of various organs of the body. Stem cells can be found in various tissues of the human body [1].

However, until now there are still various limitations possessed by stem cells, such as the potential risk of Graft versus Host Diseases (GvHD) which is a mismatch response between transplanted cells and body cells, as well as the potential for tumors and cancer due to excessive proliferation [2]. An alternative to cell-based therapy is the secretomes, which are a collection of proteins secreted by stem cells [3]. The secretomes present to be a solution to the various limitations of stem cells. It has advantages including overcoming the ethical barriers of cell transplantation, not causing complications of wrong differentiation of cells in recipient tissues and maintaining the paracrine action potential of cellular therapy [4]. Several studies have shown that growth factors and secreted vesicles (secretomes) can be better agents in cell differentiation when compared to stem cells themselves [5]. Secretome analysis revealed the presence of a large number of proteins known to be involved in skin inflammation, hemostasis, and wound repair [6,7].

The process of wound healing is dynamic and involves the role of various cells and structures. This process is separated into three overlapping phases: inflammatory response, proliferation, and remodeling. To achieve effective tissue repair

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and restoration of tissue function, it is necessary to involve the actin filament cytoskeleton. This cytoskeleton is essential in the formation of platelet plugs that halt bleeding at the time of injury and secreting extracellular matrix (ECM) proteins that regulate the inflammatory response, help stimulate the angiogenesis, and increase the formation of granulation tissue, as well as integrin receptors which play a role in the differentiation, proliferation, and adhesion of cells in the area of injury and assist in the attachment and signal transduction between cells and the extracellular matrix [6,7].

The use of secretomes is proven to accelerate wound healing with its ability to increase angiogenesis and proliferation of fibroblast cells in the skin. Secretomes also have the ability to provide protection to skin fibroblast cells so that they avoid apoptosis mediated by oxidative stress and accelerate wound closure with a stimulatory effect for fibroblast migration [8]. Various studies regarding the use of stem cell-derived secretomes for wound healing have been carried out with various sources. The results demonstrated that MSC-derived secretomes accelerated healing and increased cell proliferation, and cell migration which led to effective wound healing [7]. Even so, further studies are needed regarding the effect of secretome administration and its role in detail in the wound-healing process. In this review, we will discuss the potential role of stem cell-derived secretomes in enhancing the wound-healing process as well as the isolation methods.

2. Stem Cells

Stem cells are cells that still have not yet developed to be specified into a certain type of cell. As basal or basic cells, stem cells generally have totipotent traits that can develop into various types of cells and tissues in the body. On the other side, stem cells can continuously replicate by the process of mitosis and renew themselves, making it important in the development and growth of tissue and organs [9]. Stem cells are divided into two classifications, namely based on their ability to differentiate into other cells and based on their sources (Figure 1).

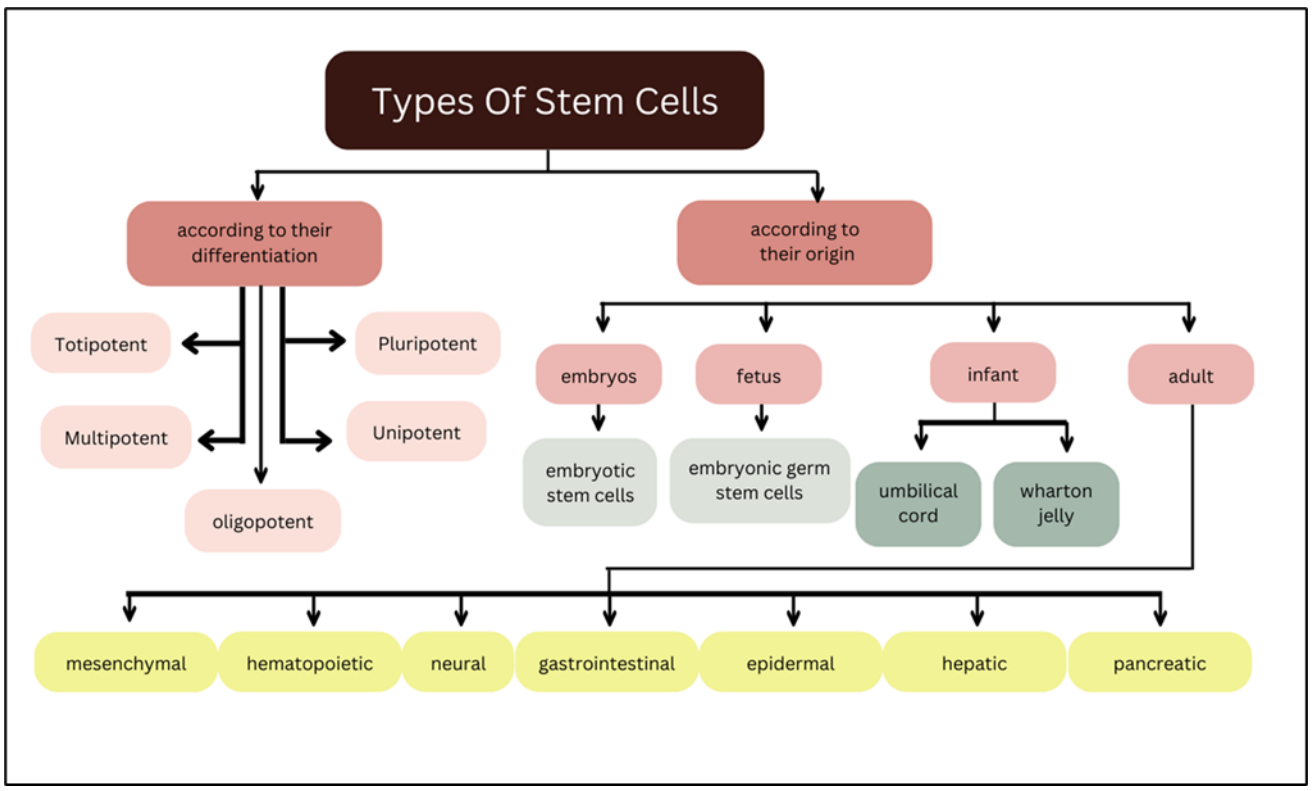

Figure 1 Stem Cell Classification

Based on their ability to differentiate into other cells, stem cells are divided into 1) *totipotent* stem cells, which can differentiate into all cell types, 2) *pluripotent*, which has the ability to differentiate into almost all cell types, 3) *multipotent*, which can differentiate into several types of related cells or closest to the cell, 4) *oligopotent* is the ability to differentiate into only certain cells, and 5) *unipotent* is the ability to be able to differentiate into only one particular cell. As for the classification based on its source, stem cells are classified into two types, namely those from embryonic cells and adult cells. Generally, embryonic cells can be found in the blastocyst while adult cells can generally be found

in adult body tissues, which include mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), neural stem cells, and other stem cells from organs [1].

3. Secretomes as an alternative to stem cell-based therapy

The provision of renewable drug therapy with cells currently offers a good cure rate and success. However, it has disadvantages including requiring high costs, special storage areas, the potential for tumorigenicity, the possibility of infection, and rejection reactions occurring. Therefore, tends to be difficult to use effectively in the community [10]. In addition, direct cell application by injection via a syringe and needle can also reduce cell viability until only 1-32% remains and can cause fatal damage to the cell membranes involved which is irreversible [11]. This should be a further consideration because non-viable cells with large populations of necrotic and apoptotic cells can stimulate the body's immune response which will impair the healing process.

Based on various obstacles to cell-based therapy mentioned above, the current interest is using the cellular products of the stem cell namely secretomes. Secretomes are a collection of proteins secreted by certain cells or tissues which consist of various protein molecules such as cytokines, chemokines, growth factors, anti-inflammatory factors, and extravascular proteins. The secretomes can be isolated from MSCs. According to recent research, MSCs' therapeutic function is mostly mediated through the paracrine impact of the secretome [3]. This new concept of cell secretome-based therapy can overcome several ethical-related barriers to cell transplantation, does not cause complications of wrong differentiation of cells in recipient tissues, and can still maintain the paracrine action potential of cellular therapy [4]. Secretomes are known to influence neighboring cells and influence some of their biological processes through their, paracrine or trophic properties [12]. Of the components it has, the secretome can contribute positively to the wound-healing process. Analysis of the secretome revealed the presence of large amounts of proteins known to be involved in skin inflammation, hemostasis, and wound repair. The benefits of secretomes include convenience in the application process and tend to be easier to produce in large quantities, easier to store, and easier to administer [13].

4. Characteristics of Stem Cell-derived Secretome

The secretome functions as both a component of secretor proteins and a component of secreted native proteins [14]. The secretome is secreted by cells, tissues, or organisms into the extracellular space under certain environmental conditions [15]. The secretome consists of various active bio components and can be divided into two sub-sections: the first is the soluble part such as essential proteins and solvents, and cytokines. Apart from soluble factors, it also has lipids and extracellular vesicles (EVs) which carry important molecules [16]. The second is the vesicular part which consists of exosomes, micro vesicles, and apoptotic bodies [17].

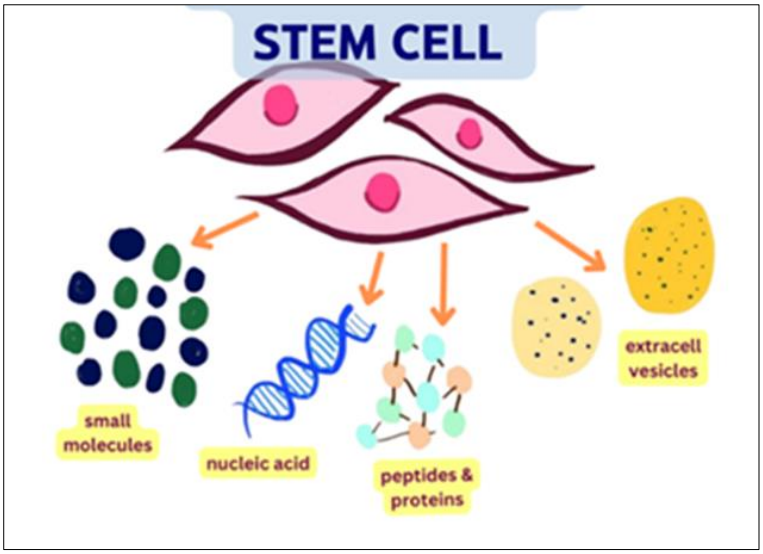

Figure 2 Bioactive components in stem cell secretome

The secretomes derived from stem cells can be defined as various paracrine factors secreted by stem cells. As a response to environmental conditions at the injury site, stem cell will be activated and secrete many cytoprotective factors (paracrine) to increase the regeneration process in damaged/injured tissues [18]. This paracrine effect is mediated by the secretome which provides soluble bio-components that include cytokines, growth factors, hormones, neurotransmitters, salt ions, exosomes or micro vesicles containing lipids, DNA, and microRNA (miRNA) (Figure 2) [19].

Meanwhile, extracellular vesicles, (EVs), are released by cells into the extracellular space. EVs are known as membrane-bound, have nanoparticle sizes (30-1000 nm), have a crucial role in transporting important biomolecules between cells [20], and maintain physiological homeostasis [21]. EVs can be classified into different subtypes based on their physical features such as size or density. Small EVs (sEV) have a typical size lower than 200 nm, whereas medium/large vesicles are characterized by sizes greater than 200 nm. The classification of EVs is also based on their biochemical composition, such as the presence of transmembrane or glycosyl phosphatidyl inositol (GPI) anchored proteins (eg, CD63, CD81, and MHC class I), cytosolic or periplasmic proteins (eg, TSG101, Flotillin-1, Alix, and HSP-70), and proteins associated with non-EV structures (eg, albumin and ApoA1/2) [20,21].

EVs are also characterized by a specific charge consisting of mRNA, microRNA (miRs), protein, or DNA. This genetic material is protected from the oxidative extracellular environment and can be carried to distant cells to modulate the repair of damaged tissues [20,21]. Messenger RNA and micro-RNA are nucleic acids that make up the vesicular section, including small proteins that are secreted through the membrane layer to the extra-vesicular area [8].

5. Growth Factors within Secretome

The secretome is known to contain various cellular growth factors, that are essential for biological processes since it is involved in the control of many cellular activities [22]. One of the most important growth factors is VEGF, known as an activated mitogen protein, that aids in the development of new blood vessels and cellular activities, such as proliferation, growth, and cell defense [23]. Another growth factor is FGF, a multi-functional growth factor that is crucial in the process of angiogenesis as well as in the development and function of central nervous system (CNS) cells [4]. The TGF-β is engaged in several wound healing processes such as inflammation, angiogenesis stimulation, fibroblast proliferation, collagen synthesis, and deposition and remodeling of the new extracellular matrix. The various molecules and proteins contained in the secretome can be seen in Table 1.

Table 1 Molecules and proteins within secretome

Growth Factors	Inflammatory Proteins	Extracellular Matrix Proteins
PDGF	IL-1	MMP-1
IGF-1	IL-8	MMP-2
EGF	IL-10	MMP-3
FGF	IL-6	MMP-7
G-CSF	TNF-α	TIMP-1
GM-CSF	ILF	TIMP-2
HGF	IL-11	ICAM
PGE-2	MCP-1	Elastin
TGF-β	PGE2	Collagens
VEGF	IL-9	Decorin
KGF	IL-13	Laminin

6. Methods for secretomes isolation

Because secretomes are extremely dynamic, selecting the isolation procedure is a key aspect that must be considered in the creation of bioprocess components. The type and amount of biocomponents released by a cell are also affected by the treatment performed on the cells, such as the duration of culture, culture growth phase, physical/chemical conditions that affect the culture, and cell sources [24].

Several factors need to be considered related to cell culture when aiming to obtain its secretomes. *First* is the source of cells, because it will affect the character of the secretome. The *second* is culture medium, whether to be conditioned with supplements or even with non-supplementation/ starvation methods. The *third* is culture's oxygen levels, whether normoxia or even less oxygen (hypoxia). In some circumstances, hypoxic conditions are said to increase the quantity of biocomponents involved in cell proliferation, differentiation, angiogenesis, and immunomodulation.

The *fourth* is the culture conditions, whether static or dynamic, and using 2D or 3D (spheroid). This condition plays a role in changing cell morphology and effectiveness in expressing micro vesicles and suppressing anti-inflammatory responses which affect its immunomodulatory characteristics. The *fifth* is the duration of cell culture, whether 24 hours, 48 hours, or 72 hours. It matters because the secretome is a very dynamic component of biomolecules, therefore the length of the intended culture must be properly estimated in order to attain the desired conditions [25].

The idea of secretome isolation is to non-invasively isolate particles from their complex biological environment while preserving the structure and composition of the biocomponents intact. The secretome can be isolated in order to understand its composition and possible therapeutic uses. We can obtain secretome from the expanded media where the stem cell is cultivated, also called a conditioned medium (CM). This CM comprises both fractions of the stem cell-derived secretome, the soluble components, and the extracellular vesicles (EVs); after being centrifuged to remove cell debris.

Several principles are mainly used to isolate the secretome components: [25–27]

- *Ultracentrifugation*. This method is the gold standard for EVs isolation. Exposure of a heterogeneous mixture (suspension) to centrifugal force centrifugation will cause sedimentation of particle elements in the suspension based on their density, size, and shape. This method is achieved by combining low-speed and high-speed centrifugation and requires repeating the centrifugation process or ultracentrifugation phases.
- *Size-based isolation*. Secretome components such as exosomes can be isolated using size-based isolation techniques. These approaches rely on particle size variations to differentiate secreted molecules from other components. This method can be done by membrane filters.
- *Commercial Kits*. It is available, providing easy and standardized processes. For optimal results, these kits frequently combine various isolation approaches.
- *Others*. These include immunoaffinity, microfluidics, and field flow fractionation.

The International Society for Cellular Therapy (ISCT) conducted a survey in 2019 to determine the procedures utilized for EVs isolation. The most often utilized procedures for EVs separation include ultracentrifugation and a mixture of size-based isolation approaches. Although ultracentrifugation is still the most widely utilized EVs separation method, the number of responders who employ size exclusion chromatography (SEC) has climbed significantly since 2016 (26).

7. The Role of Stem Cell-derived Secretome in Wound Healing

Wound healing is a process that often necessitates a well-coordinated integration of cellular and molecular activities that occur following the commencement of a tissue lesion to restore the damaged tissue. The involvement of cells and different biochemical substances distinguishes the four stages of wound healing, which are hemostasis, inflammatory reactions, cell proliferation, synthesis of extracellular matrix, and remodeling tissue formation. Unfortunately, skin wound healing is typically retarded and impaired, especially in the elderly or diabetic individuals, resulting in increased morbidity [27]. In wound therapy, cell-based treatments and products are not new. They have been available for decades, including skin substitutes/grafts, recombinant growth factors, cytokines, and platelet-rich plasmas. However, there is still a need for better cell-based therapies, as evidenced by the increase in the number of chronic wounds around the world [27].

The development of stem cell-derived secretome raises a new hope to address a high-quality therapy for chronic wounds. The main mechanisms underlying the regenerative effect of the secretome on wound healing are through the acceleration of the re-epithelialization process, stimulation of migration and recruitment of several inflammatory cells, increased neovascularization, increased production of extracellular matrix, and remodeling of granulation tissue (Figure 3) [28].

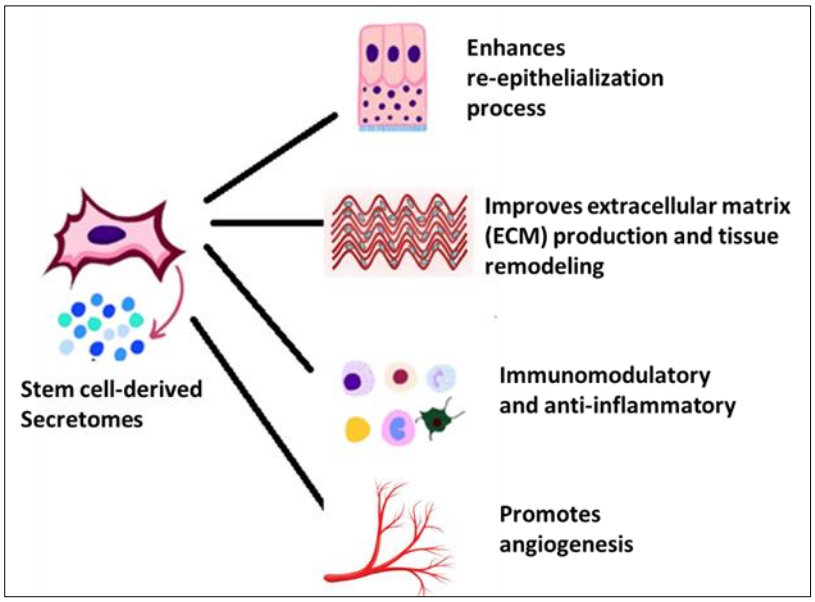

Figure 3 The role of the stem cell-derived secretome in the wound healing process

Ankrum et al, 2014 demonstrated that topical administration of secretomes in the burns wound area resulted in an increase in the number of fibroblast formation and improved vascularization through angiogenesis. This process in turn led to an acceleration of wound healing and wound closure [29]. In vivo, animal models to study the role of the secretome in wound healing have also been carried out. Mice, rats, and pigs are experimental animals of choice that are widely used to study the function of the secretome for wound healing [30]. The stem cell-derived secretomes with their bioactive content fulfill the demand of stimulating the healing process in wound models.

Various wound conditions have been studied, such as the use of secretomes for the treatment of radiation-induced wounds, burns, diabetic foot ulcers, skin ulcers, and chronic wounds [27]. These research findings suggest that the use of a secretome in wound care can hasten wound healing by enhancing the effects of cell migration and proliferation as well as inducing the wound healing processes of angiogenesis, re-epithelialization, neovascularization, and collagen deposition [24].

8. Conclusion

Secretome is a "waste" substance secreted by stem cells during its culture and response to the environment. The stem cell-derived secretome is composed of several bioactive components that are crucial for stimulating cellular processes to repair the damaged tissue in a wound. The application of secretomes to the site of damaged tissue will hasten the recovery process. The cytokines and growth factors within the secretome will promote angiogenesis, synthesis of a new granulation tissue, and stimulate keratinocyte migration which is crucial for wound closure and healing. With the limitations of stem cells in cell-based therapy, the development of secretome-based therapy has the potential to become an alternative therapy, particularly in wound treatment.

Compliance with ethical standards

Disclosure of conflict of interest

All of the authors stated there is no conflict of interest.

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