Review

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Stem cell secretome as a new booster for regenerative medicine

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Summary **Stem cells are an undifferentiated cell population that has the ability to develop into many different cell types and also has the ability to repair damaged tissues in some cases. For a long time, the stem cell regenerative paradigm has been based on the assumption that progenitor cells play a critical role in tissue repair by means of their plasticity and differentiation potential. However, recent works suggest that the mechanism underlying the benefits of stem cell transplantation might relate to a paracrine modulatory effect rather than the replacement of affected cells at the site of injury. This paracrine modulatory effect derives from secretome which comprises a diverse host of growth factors, cytokines, chemokines, angiogenic factors, and exosomes which are extracellular vesicles that are produced in the endosomal compartment of most eukaryotic cells and are from about 30 to several hundred nanometers in diameter. The role of these factors is being increasingly recognized as key to the regulation of many physiological processes including leading endogenous and progenitor cells to sites of injury as well as mediating apoptosis, proliferation, migration, and angiogenesis. In reality, the immunomodulatory and paracrine role of these factors may mainly account for the therapeutic effects of stem cells and a number of *in vitro* and *in vivo* researches have proved limited stem cell engraftment at the site of injury. As a cell-free way for regenerative medicine therapies, stem cell secretome has shown great potential in a variety of clinical applications including prevention of cardiac disfunction, neurodegenerative disease, type 1 diabetes, hair loss, tumors, and joint osteoarthritis.**

Keywords: Secretions, stem cells, regenerative medicine, exosomes, conditioned medium

1. Introduction

Study results of the application of stem cells in various diseases are accumulating. Some researches showed beneficial effects of stem cell therapy in degenerative diseases such as myocardial infarction and revealed that stem cells cause tissue repair due to their ability to secrete molecules that perform a beneficial impact on the damaged tissue, rather than their capacity to differentiate into the necessary cells (*1*). Various studies on stem cell-derived molecules showed that the secreted factors alone without the stem cell itself may cause tissue repair in various diseases. The secreted factors are referred to as secretome, including growth factors, cytokines, chemokines, angiogenic factors, and exosome and can be found in the medium where the stem cells are cultured. The medium is called conditioned medium (*2*).

The application of secretions from medium has several merits compared to the application of stem cells, for medium can be manufactured, freeze-dried, packaged, and transported more easily. Moreover, as it is cell-free, there is no need to match the donor and the recipient to avoid a rejection reaction. Therefore, stem cell secretome has a promising role for regenerative medicine.

2. Brief overview of stem cell secretome

2.1. *What is the stem cell secretome*

Stem cell secretome is a collective term for the soluble factors produced by stem cells and utilized for their inter-cell communications (*3*). The secretome is thought to be encoded by approximately 10% of

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the human genome and includes a diverse array of serum proteins, growth factors, angiogenic factors, hormones, cytokines, extracellular matrix proteins, extracellular matrix proteases, hormones, and even, in low abundance, lipid mediators and genetic material (*4,5*). These secreted molecules are released by stem cells through classical and non-classical secretion mechanisms, including protein translocation, exocytosis, and vesicle or exosome encapsulation (*6,7*). The soluble factors and vesicles secreted by stem cells may act directly by mediating intracellular pathways in injured cells, or indirectly, by inducing the secretion of functionally active products from adjacent tissues.

2.2. *Why will we use secretome?*

The utility of cell-free therapies in regenerative medicine has more merits than conventional stem cell based therapies. The utility of secreted molecules could potentially avoid immune compatibility, tumorigenicity, and transmission of infections in stem cell therapies. Secretome could also largely reduce the cost and time associated with expansion and maintenance of cell lines because secretome could be prepared beforehand in large quantities and could be promptly available for treatment when needed. This makes their application suitable for emergencies such as myocardial infarction, cerebral ischemia, or trauma.

3. Various roles and mechanisms of secretome

3.1. *Tissue repair*

Stem cells showed advantageous therapeutic effects on tissue repair and wound healing without a significant degree of tissue engraftment at the site of injury (*8*). This research result in a number of animal models of multiple diseases led to the hypothesis that secretome of stem cells rather than direct tissue differentiation and engraftment may play a leading role in tissue repair. This assumption has been tested in a number of different clinical therapies *via* the utility of stem cell conditioned medium. The study of cell-specific secretions often begins from cell culture. However, *in vitro* researches can't totally simulate and test the full view of secretions in the microenvironment *in vivo*, studies quest to replicate the effects of the stem cell secretome *via* the utility of medium conditioned by stem cells (*9*). Study has demonstrated that the utility of stem cell conditioned media alone can replicate the therapeutic effects previously observed with the utility of stem cells directly (*1,10,11*).

3.2. *Angiogenesis*

Stem cells and their secretome play an important part in regulation of angiogenesis that has been validated both *in vitro* and *in vivo*. Great interest emerges in the role of stem cells in angiogenesis for there are a lot of clinical diseases related to insufficient angiogenesis, including atherosclerotic diseases like coronary artery disease and peripheral vascular disease, and wound healing disorders, as well as a great number of diseases related to pro-angiogenic factors such as chronic kidney disease, tumor growth and metastasis, and proliferative retinopathy (*12*). Angiogenesis is defined as the physiological process through which new blood vessels form from pre-existing vessels, formed in the earlier stage of vasculogenesis. Angiogenesis continues the growth of the vasculature by processes of sprouting and splitting [wikipedia]. A great deal of secreted molecules such as growth factors, chemokines, enzymes, matrix metallopeptidases, and adhesion molecules strictly regulate this process (*13*).

A great number of angiogenesis related molecules have been identified in stem cell secretome by antibody-based assays like ELISA assays, and immunohistochemistry methods. The secretions include vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF-2), interleukin-6 (IL-6), and placental growth factor (PGF), angiopoietin-1, monocyte chemoattractant protein-1 (MCP-1), and cysteine-rich angiogenic inducer 61 (Cyr61) (*14-18*). Investigations have also found that the secretion of these angiogenic factors can be regulated by numerous chemokines and hypoxic conditions. For example, research by De Luca *et al*. showed that transforming growth factor alpha (TGF-α) had the ability to up-regulate the level of a few growth factors (VEGF, HGF, platelet-derived growth factor BB, IL6- and IL-8) in the stem cell secretome (*19*). Conditioned medium treated with TFG-α induced much more blood vessel growth compared to control medium *in vivo*. A few *in vitro* studies have validated the effects of stem cell secretome on each key step in angiogenesis. For instance, some mesenchymal stem cells like adipose-derived mesenchymal stem cells and bone marrow-derived mesenchymal stem cells have the ability to induce endothelial cell proliferation, migration, and tube formation, as well as prevent endothelial cell apoptosis *in vitro* (*20-23*). Successful application of stem cells to increase angiogenesis has been demonstrated in animal models for peripheral artery disease, myocardial infarction, cerebral ischemia/stroke, stress urinary incontinence, and neurogenic bladder disease among other diseases (*24-26*).

3.3. *Anti-apoptosis*

Apoptosis is a form of programmed cell death that occurs in multicellular organisms (*27*). Stem cells play a role of preventing cell death not only *via* the restoration of the local niche but also by specifically secreting molecules that have been identified as inhibitors

of apoptosis and by down-regulating expression of apoptotic proteins. Li *et al*. reported a study which demonstrated that stem cells decreased apoptosis of alveolar macrophages when co-cultured at particular ratios (*28*). Moreover, the stem cells decreased expression of the pro-apoptotic factors Bax and cleaved caspase-3 while increasing expression of anti-apoptotic protein Bcl-2. A study by Tang *et al*. similarly showed that Bax expression was decreased while expression of pro-angiogenic factors, including basic FGF and VEGF, and stem cell homing factor CXCL-12 were increased in stem cells-treated hearts compared to medium-treated hearts (*29*). Whereafter, stem cells-treated hearts demonstrated increased capillary density and improved left ventricular contractility two months after treatment, which presumed to indicate improved function. A study by Gnecchi *et al*. additionally found that Akt up-regulated stem cells prohibit ventricular remodeling and restore cardiac function in less than 72 hours, an effect that they hypothesized was most likely attributable to the secretion of paracrine factors rather than myocardial regeneration (*30,31*). They also found that the conditioned medium derived from stem cells distinctly inhibited hypoxia induced apoptosis in adult rat cardiomyocytes *in vitro*. Furthermore, the utility of conditioned medium *in vivo* led to an obvious decrease in infarct size and increased ventricular function compared to controls.

3.4. *Immunoregulation effect*

The innate immune response plays an important role of being the body's frontline to infection or tissue damage, and severe immune and inflammatory responses to tissue injury can often have adverse effects. Stem cells possess immunomodulatory and immunological tolerance inducing effects that have been revealed to relieve and modulate possible damaging inflammatory reactions. Interestingly, these stem cells typically express MHC-I but have an absence of MHC-II, CD40, CD80, and CD86. On account of the absence of co-stimulatory cell surface molecules, stem cells often can't induce an immune response by the transplant host (*32*). Moreover, stem cells have been shown to work inhibiting immune responses by three main mechanisms: first, by mediating interaction between cells; second, through the action of soluble factors; and third, *via* induction of regulatory T cells. The first report of suppression of cell-mediated immune responses by stem cells was by Di Nicola *et al*., who found that stem cells inhibit proliferation of CD4+ and CD8+ T cells even in the absence of direct cell-cell contact (*33*). Immunomodulatory abilities of stem cells have been validated to work in each of the three main steps of the immune response: first, antigen recognition and presentation; second, T cell activation, proliferation, and differentiation; and third, the T-cell effector stage (*34*).

4. Effects of secretome on various diseases

4.1. *Cardiac*

Stem cells have been reported to promote cardiomyocyte recovery after myocardial ischemia. Originally this was supposed to be due to their capability to differentiate into cardiomyocytes to substitute for damaged tissue. Nevertheless, recent papers suggest that differentiation into tissue alone is not adequate to explain the beneficial effects seen after stem cell therapy. Moreover, a few researchers have reported that the stem cell secretome alone is sufficient to improve functional recovery. In a study by Uemura *et al*., bone marrow stem cells were injected into the left ventricle of mice following coronary artery ligation (*35*). Twenty eight days after myocardial infarction, stem cells were found in very low numbers in the mice cardiac tissue. But the good effects of treatment were still observed including induction of infarct area compared to control group and increased left ventricular ejection fraction. This result suggested that expansion and differentiation of stem cells were not adequate to explain the tissue functional improvements. The researchers also studied the role of preconditioning stem cells. A group of mice was treated with preconditioned bone marrow-derived stem cells that experienced hypoxia to up-regulate several secretions; these mice subsequently obtained the greatest functional recovery. These findings suggest possible future study in which secretome could be justified by cellular preconditioning to increase particular factors and to develop a more effective secretome therapy.

Other work by Kshitiz *et al*. reinforced the idea that secretome of bone marrow-derived stromal cells revealed a cardioprotective biochemical cocktail (*36*). In this study, researchers reconstructed dynamic secretory signatures of cells based on a very limited number of time points. By using this method, they demonstrated that the secretory signatures of CD133-positive bone marrow stem cells are uniquely defined by distinct biological contexts, including signals from injured cardiac cells undergoing oxidative stress, characteristic of cardiac infarction. Moreover, they showed that the mixture of recombinant factors reproducing the dynamics of bone marrow stem cell secretome can mediate a highly effective rescue of cells injured by oxidative stress and an improved cardiac output.

Besides adult stem cell secretome, umbilical cord stem cell secretome also has been researched by a few of laboratories. Han *et al*. reported that human umbilical cord stem cell derived exosomes encapsulated in functional peptide hydrogels promote cardiac repair (*37*). In a rat myocardial infarction model, these researchers demonstrated that the PA-GHRPS peptide protected H9C2 cells from H_2O_2-

induced oxidative stress and the gelatinization ability of PA-GHRPS can be enhanced by peptide NapFF. Therefore, these two peptides were mixed to form a PGN hydrogel, which was used to encapsulate exosomes. Their results showed that the PGN hydrogel was able to encapsulate exosomes effectively and ensured a stable and sustained release of exosomes. The exosome/PGN hydrogel mixture was injected into the infarcted border zone of rat hearts. Compared to the exosome treatment alone, the mixture improved myocardial function by reducing inflammation, fibrosis and apoptosis, and by promoting angiogenesis. A similar study was also published by Zhou *et al*. (*38*). In addition, Lazzarini *et al*. from another team reported that human amniotic fluid stem cell secretome effectively counteracts doxorubicin-induced cardiotoxicity (*39*). In this research, they showed that, following hypoxic preconditioning, amniotic fluid stem cell conditioned medium antagonizes senescence and apoptosis of cardiomyocytes and cardiac progenitor cells, two major features of doxorubicin, a conventional chemotherapeutic medicine, causing cardiotoxicity. Mechanistic studies with mouse neonatal ventricular cardiomyocytes reveal that medium inhibition of doxorubicin-elicited senescence and apoptosis is associated with decreased DNA damage, nuclear translocation of NF-kB, and upregulation of the NF-kB controlled genes, *Il6* and *Cxcl1*, promoting mouse neonatal ventricular cardiomyocyte survival. Furthermore, medium induces expression of the efflux transporter, *Abcb1b*, and doxorubicin extrusion from neonatal ventricular cardiomyocytes. The PI3K/Akt signaling cascade, upstream of NF-kB, is potently activated by medium and pre-treatment with a PI3K inhibitor, which abrogates NF-kB accumulation into the nucleus, modulation of *Il6*, *Cxcl1* and *Abcb1b*, and prevention of doxorubicn-initiated senescence and apoptosis in response to medium.

4.2. *Oncology*

There is a lot of interest in leading tissue regenerative strategies to restore function in the wake of cancer treatment and remission (*40*). Nevertheless, a serious concern is the oncologic potential surrounding stem cell therapy and the possibility of triggering a cancer recurrence. Unfortunately, many of the properties unique to stem cells such as tissue revascularization, multipotentiality, immunomodulatory effects, and cell homing and migration are also characteristics that exist in tumor progression and metastasis. However, utility of a cell-free therapy such as stem cell secretome could further avoid these risks.

A few models have been established for studying stem cell-cancer cell interactions. The effect of stem cells on tumor growth remains controversial and is a complex field of current study. Conflicting research results have been shown such as that stem cells have been shown to have both pro- and anti-tumorigenic effects, even in the same cancer model, and, sometimes, even using the same cancer cell lines (*41,42*). Some potentially concerning oncogenic effects of stem cells include modulation of paracrine activities resulting in local immunosuppression, angiogenesis, promotion of tumor growth and invasion *via* remodeling of the extracellular matrix, promotion of the epithelial to mesenchymal transition of tumor cells necessary for invasion and later metastasis, and inhibition of tumor necrosis/apoptosis. Secretions such as VEGF, TGF-β, and IL-6 which are normally secreted by stem cells are expressed in increased quantities by stem cells that have been recruited by tumor cells, supporting tumor growth and invasion (*3*).

4.3. *Diabetes*

Stem cells have gained attention due to their potential for providing a limitless source of glucose responsive insulin-producing β cells as well as their ability to enhance the survival and function of transplanted islets. This holds potential to solve the problem of limited availability of suitable donor islets, and can also enhance the therapeutic outcome of islet transplantation in T1D patients (*43*).

In a recent study, Mahdipour *et al*. demonstrated that stem cell-derived exosomes lead to regeneration of β islets through a Pdx-1 dependent mechanism in a rat model of type 1 diabetes (*44*). In their study, exosomes were intravenously injected into animals at different time points and in single or repeated therapeutic doses. After about 6 weeks, animals were euthanized and the pancreas was analyzed for the presence of regenerated β islets as well as insulin secretion. Non-fasting blood glucose and serum insulin level were also monitored during the study. The results represented that menstrual blood-derived mesenchymal stem cell-derived exosomes enhance the β cell mass and insulin production in the pancreas of diabetic animals that received repeated doses of exosomes. Immunohistochemistry analysis also confirmed the presence of insulin in the islets of treated animals. Further investigations proposed that exosomes induce islet regeneration through the pancreatic and duodenal homeobox 1 pathway. Exosome tracking also revealed homing of injected exosomes to the pancreas.

Clinical studies on prevention of diabetes-associated complications also are growing vigorously. Reports have indicated that T1DM patients have an approximately fivefold higher risk of hip fracture compared with individuals without diabetes, which is partly due to reduced bone mineral density and bone quality (*45*). In a rat calvarial defect model, Zhu *et al*. demonstrated that *in vitro*, bone marrow-derived stem cell exosomes enhanced osteogenic differentiation

of BMSCs and promoted the angiogenic activity of HUVECs (*46*). Similarly, *in vivo*, exosomes promoted bone regeneration and neovascularization in rat calvarial defects. In addition, diabetic nephropathy is another serious complication of diabetes mellitus and a common cause of end-stage renal disease. Also autophagy has a defensive role against kidney damage caused by hyperglycemia. Ebrahim *et al*. reported that stem cell-derived exosomes ameliorated diabetic nephropathy by autophagy induction through the mTOR signaling pathway (*47*). The research data shows that exosomes markedly improved renal function and showed histological restoration of renal tissues, with significant increase of LC3 and Beclin-1, and a significant decrease of mTOR and fibrotic marker expression in renal tissue. All previous effects were partially abolished by the autophagy inhibitors chloroquine and 3-MA.

4.4. *neurodegenerative disease*

Neurologic complications are commonly regarded as irreversible impairments that stem from limited potential of regeneration of the central nervous system (CNS). On the other side, the regenerative potential of stem cells has been evaluated in basic research, as well as in preclinical studies. Since exosomes can be obtained from different cell sources to mediate neuroprotective and neurotherapeutic functions, investigations have been focusing on the best cell source to generate and deliver exosomes to the CNS niche (*48*).

Exosomes have produced beneficial effects in a variety of models of neurodegenerative diseases, such as Parkinson's disease. 6-Hydroxydopamine (6-OHDA) is commonly used as an *in vivo* and *in vitro* model of Parkinson's disease because it triggers selective apoptosis of dopaminergic neurons. Jarmalavičiūtė *et al*. reported that exosomes obtained from human dental pulp stem cells were able to suppress apoptosis of dopaminergic neurons following treatment with 6-OHDA (*49*). However, if the same stem cells were cultured through normal settings, the exosomes failed to inhibit apoptosis, demonstrating that culture situation has an important impression on the properties of the exosomes. 6-OHDA induces apoptosis through the generation of reactive oxygen species (ROS) (*50*), suggesting that exosomes can decrease the sensitivity of dopaminergic neurons to oxidative stress. Future investigations are required to determine the specific proteins or miRNAs, which account for these neuroprotective characteristics of exosomes. In an experimental autoimmune encephalomyelitis mouse model, Riazifar *et al*. found that intravenous administration of exosomes produced by stem cells stimulated by IFNγ(IFNγ-Exo) *i*) reduced the mean clinical score of mice compared to PBS control, *ii*) reduced demyelination, *iii*) decreased neuroinflammation, and iv) up-regulated the number of CD4+CD25+FOXP3+ regulatory T cells (Tregs) within the spinal cords of mice (*51*) [Nuro].

4.5. *Anti-aging*

Aging is a biological process that induces changes to the structural integrity and physiological function of skin (*52*), such as the development of dyschromia, roughness, and fine rhytids followed by persistent deeper folds. Structural changes are a result of dermal atrophy, decreased collagen, the loss of subcutaneous fat, the loss of inherent elasticity, and increased melanogen (*53*). In the present study, researchers focused on fibroblasts because they are the main source of dermal extracellular matrix (ECM) proteins, mainly are collagens and elastins that are processed to assemble fibers conferring tensile strength and resilience to skin, and consequently maintain the homeostasis and juvenscence of skin (*54,55*).

In a skin recovery study, Wang *et al*. irradiated human dermal fibroblasts with ultraviolet radiation B at different senescent levels, and then treated them with stem cell conditioned medium (*56*). Then they found medium therapy can slightly or significantly improve cellular proliferative activity and restore functions both in irradiated and non-irradiated HDFs. Besides, medium therapy decreased cellular apoptosis and senescence induced by UVB. A similar finding was also reported by Kim *et al*. who found that medium stimulated both collagen synthesis and migration of dermal fibroblasts, which improved the wrinkling and accelerated wound healing in animal models, as well as protected dermal fibroblasts from oxidative stress induced by chemicals and UVB irradiation (*57*).

In a few studies, effective factors in the secretome were screened and analyzed. Proteomic analysis of human bone marrow stem cell secretome identifies nineteen secreted proteins, including extracellular matrix structural proteins, collagen processing enzymes, pigment epithelium-derived factor (PEDF) and cystatin C. Immunodepletion and reconstitution experiments show that PEDF is the predominant fibroblast chemoattractant in the conditioned medium (*58*) [Skin-D]. This stimulatory effect of PEDF on fibroblast chemotaxis is in contrast to the PEDF-mediated inhibition of endothelial cell migration, reported previously. These differential functional effects of PEDF toward fibroblasts and endothelial cells may serve to program an ordered temporal sequence of scaffold building followed by angiogenesis during wound healing.

4.6. *Hair loss*

Hair loss, also known as alopecia or baldness, refers to

a loss of hair from part of the head or body (*59*). Hair loss in some people causes psychological distress (*60*). Pattern hair loss by age 50 affects about half of males and a quarter of females (*61*). Interventions that can be tried include the medications minoxidil (or finasteride) and hair transplant surgery (*62,63*).

In a number of researches, several growth factors from stem cells and fibroblasts conditioned medium were demonstrated to have a beneficial effect on hair growth. VEGF has been proven to affect hair growth and follicular size by angiogenesis (*64*). Hepatocyte growth factor and IGF also activate hair growth through various pathways (*65,66*). Platelet-derived growth factor induces and maintains anagen hair in murine hair follicles (*67*). In a recent study, Shin *et al.* (*68*) found that after treatment with stem cell conditioned medium for 12 weeks hair density increased from 105.4 to 122.7 hairs/cm^2 ($p < 0.001$). Hair thickness increased from 57.5 μm to 64.0 μm ($p < 0.001$). None of the patients reported severe adverse reactions. Another research performed by Yan *et al.* (*69*) suggested that exosomes from dermal papilla cells (DPCs) have ability to mediate hair follicle stem cells (HFSCs) proliferation and differentiation [hair-b]. They cultured hair follicle stem cells with DPCs and found exosomes from DPCs attached to the surface of HFSCs. Using micro RNA (miRNA) high-throughput sequencing, they identified miR-22-5p-LEF1 was a novel axis regulating HFSCs proliferation.

5. Outlooks

Although the utility of secretome as a cell-free way could be a promising substitute for stem cell therapies, numerous challenges remain to be dealt with before clinical translation. One of the most serious challenges is to determine a therapeutic schedule in consideration of a great number of complex interactions of secreted molecules during tissue damage (*70*). To illuminate how cytokines are expressed during tissue damage and wound healing and how they impair therapeutic effects will be helpful for developing more effective therapeutic methods (*11*). Further research is required to exactly analyze the secretome and to identify which molecules are responsible for its therapeutic effects. Better understanding of both signaling pathways that regulate secretome expression and of secretome constitution itself will be beneficial for improving regulation of its secretion, a major barrier for translating secretome into clinical application.

The utility of conditioned medium as a form of application of stem cell secretome also exists with a few deficiencies. First, stem cells possess a dynamic expression profile that is difficult to capture with the utility of conditioned medium, which only has a static composition. Second, conditioned medium can't reflect all stages that stem cells experience and thus some secretions will be absent in a given medium. Therefore, the production of conditioned medium requires careful collection for each cell type to avoid omitting intracellular secretions from dead cells or from cells undergoing apoptosis (*71*). Finally, the preparation and concentration of secretome in quantities sufficient for clinical administration are also a problem.

6. Conclusions

Since the discovery of stem cells in the 1960s, many kinds of stem cells have been identified and used for various diseases such as cardiovascular, neurodegenerative, autoimmune, and diabetes. One of the most important functions of stem cells is the paracrine/autocrine action of stem cell secretome including its imunomodulatory effects and ability to promote tissue regeneration. Nevertheless, exploration of the stem cell secretome is only in its nascent stages for most diseases. Advances in high-throughput detective technologies and bioinformatics have already benefited analysis of the complex secreted factors that constitute the secretome and will continue to be beneficial for validation of secretome ingredients of various stem cell kinds under different conditions. So far, stem cell secretome has been proven to play a helpful role in a number of cellular processes including angiogenesis, autoimmune and inflammatory modulation, and tissue repair. Stem cell secretome has been studied mainly in the form of conditioned medium and has shown effectiveness in cell and animal experiments for various pathologic processes including those in cardiac, neurologic, diabetic, dermal, and hair fields. However, up to now a lot of barriers remain to be surmounted before making secretome a clinically useful method for regenerative therapies. More studies on secretome component analysis, composition dynamic change, molecular mechanisms, and production of conditioned medium are still needed.

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