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Emergence of the Stem Cell Secretome in Regenerative Engineering

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Abstract

The secretome is defined as the set of molecules and biological factors that are secreted by cells into the extracellular space. In the past decade, secretome-based therapies have emerged as a promising approach to overcome the limitations associated with cell-based therapies for tissue and organ regeneration. Considering the growing number of recent publications related to secretome-based therapies, this review takes a step-by-step engineering approach to evaluate the role of the stem cell secretome in regenerative engineering. We discuss the functional benefits of the secretome, the techniques used to engineer the secretome and tailor its therapeutic effects, and the delivery systems and strategies that have been developed to use the secretome for tissue regeneration.

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The Stem Cell Secretome: a New Paradigm for Cell-Free Regenerative Engineering

As the population continues to age, there is a critical clinical need to develop therapeutic strategies to repair and regenerate damaged organs and tissues and to restore their normal functions [1]. Regenerative engineering is a transdisciplinary approach that is defined as the convergence of advanced materials sciences, stem cell sciences, physics, developmental biology, and clinical translation to regenerate complex tissues and organs [2–6]. The capacity to converge the latest advancements made in each of these respective fields can lead to highly translational technologies and solutions that overcome the need for organ transplantation. Thus far, regenerative engineering strategies have primarily focused on using materials science and engineering tools to develop biomaterials that can modulate cellular functions and harness the body's innate regenerative potential [7–12]. Within the regenerative engineering paradigm, however, the role of progenitor/stem cells and bioactive factors is becoming increasingly evident and of central importance [13].

Cell therapy relies on the delivery of cells, either autologous or allogeneic, to promote tissue repair and regeneration. Cell therapy using differentiated cells is constricted by the limited availability and low proliferative capacity of the cells [14]. Stem cells, however, are superior in that they can be isolated from adult tissues and expanded in large quantities for use. They are integral in cell-based therapies for a variety of tissues secondary to their multipotent differentiation capacity, immunomodulatory properties, and anti-inflammatory effects [15–17].

The therapeutic effects of stem cells are regulated by three main mechanisms: homing, that is, migration to the site of injury; differentiation into various cell types that can engraft to the damaged tissue for repair; and secretion of bioactive factors [18]. While promising, however, the use of stem cells in tissue repair faces several challenges, including immune compatibility, tumorigenicity, and transmission of infections [19,20]. The large quantity of cells that is required for cell therapy mandates continuous growth and passaging of the cells outside the body in *in vitro* culture conditions, which may result in spontaneous alterations in the behavior and properties of the stem cells [21]. What is more, diverging from earlier studies attesting that the therapeutic benefits of stem cells are due to their engraftment and differentiation at damaged tissue sites, recent studies now suggest that it is the paracrine factors secreted from these cells that are mainly responsible for their therapeutic effects [22–25]. These paracrine factors are collectively termed the secretome and can be individually isolated for therapeutic purposes.

The secretome is defined as the repertoire of molecules and biological factors that are secreted from cells into the extracellular space. These secretory factors play important roles in many biological functions, including **homeostasis** (see Glossary), development, signaling, immunomodulation, inflammation, angiogenesis, **apoptosis**, **proteolysis**, adhesion, and extracellular matrix (ECM) organization [26,27]. The secretome comprises various serum proteins, growth factors, angiogenic factors, hormones, **cytokines**, ECM proteins and **proteases**, and even in low abundance, lipid mediators, and genetic material. It is broadly categorized into soluble factors (growth factors, cytokines, **chemokines**, and enzymes) and

extracellular vesicles that transport lipids, proteins, and RNA and DNA subtypes (Figure 1) [28–31]. The composition of the secretome is dynamic, depending on the cell type and microenvironmental stimuli. However, generally, the stem cell secretome is known to have therapeutic benefits for tissue repair including proangiogenic, antiapoptotic, antifibrotic, anti-inflammatory, and immunomodulatory effects [32–34]. In addition to the biological benefits and obviating many of the safety concerns surrounding the direct use of cells, cell-free secretome-based therapies have several logistical advantages for clinical use, including scalability, availability, and longer shelf-lives [18,35].

Engineering the Secretome

The composition of the stem cell secretome can vary based on numerous factors including the species, tissue source, and isolation procedure, and the microenvironment and chemical and physical stimuli to which the cells are exposed to. Although this dynamic nature may seem challenging for progression toward large-scale clinical applications, a thorough systematic and comparative analysis of the influence of the aforementioned factors can pave the way to select the secretome most appropriate for the intended use. More notably, by amplifying or suppressing certain biomolecules, a wide range of possibilities is unraveled to engineer the secretome and customize its therapeutic effects (Box 1).

First, mesenchymal stem cells (MSCs) isolated from different tissue origins present variations in their secretory profile that can be used to the advantage. For instance, the secretome of MSCs from adipose tissue (adipose-derived stem cells; ADSCs) consists of a broader range of angiogenic factors and thus may be preferred over the secretome of bone marrow MSCs (BMSCs) for angiogenesis-mediated tissue regeneration [36]. The ADSC-secretome may also be preferred for neuroregenerative applications due to its stronger capabilities in promoting neuronal axonal growth [37,38]. The secretome of MSCs from the Wharton’s jelly is similarly better suited for neurogenesis and angiogenesis compared to that of BMSCs [37,39]. The secretome of MSCs isolated from the placenta and bone marrow have different effects on the functional properties of endothelial progenitor cells (EPCs); one of the important cell populations in neovascularization. While the secretome of placental MSCs enhanced the migration of EPCs, the BMSC-secretome has more of an effect on the invasion and vessel-forming capacity of the cells [40]. These differences highlight the important considerations that are required when selecting a cell source for secretome isolation.

The stem cell secretome can be modulated by genetically modifying the stem cells. Through various techniques, the stem cells can be modified to overexpress or underexpress certain factors leading to a secretome that is tailored in its therapeutic potential. Both BMSCs and ADSCs have been genetically modified to produce greater amounts of angiogenic, antiapoptotic and cardioprotective factors such as vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF)-1, hepatocyte growth factor (HGF), and basic fibroblast growth factor (b-FGF) that have functional relevance for angiogenesis, cell survival, wound healing, and for preventing tissue fibrosis and inflammation [41–43]. Additionally, genetic modification can be avoided through engineering microparticles that target a central regulatory cellular pathway. This approach was particularly found effective

in inflamed MSCs and through targeting the nuclear factor (NF)-κB central regulatory pathway, which promotes the secretion of proinflammatory factors. Intracellular delivery of an NF-κB inhibitor loaded into microparticles of 1–2 μm, sustainably inhibited NF-κB activation and attenuated secretion of proinflammatory factors under inflammatory conditions [44].

One of the more common approaches in secretome engineering has been to expose the cells to biochemical stimuli during the **preconditioning** stage. Much of the work involving biochemical stimulation of MSCs has been through exposing the cells to various inflammatory mediators [e.g., tumor necrosis factor (TNF)-α), interferon (IFN)-γ, transforming growth factor (TGF)-β, interleukin (IL)-1, and lipopolysaccharides (LPS)] that elicit the cells to produce respondent biomolecules that are useful. These include various cytokines and chemokines (IL-1β, IL-6, and IL-8), proteases (matrix metalloproteases), protease inhibitors, and proangiogenic and prosurvival factors (VEGF, FGF-2, HGF, IGF-1, angiopoietin, and monocyte chemoattractant protein (MCP)-1] that play roles in immunomodulation, angiogenesis, and regeneration, and have anti-inflammatory, antifibrotic, and neuroprotective effects [45–52]. Modulating the secretion of cytokines, chemokines, and exosomes that are involved in immunomodulation, immunosuppression, and allergic responses could have significant implications for immune-associated disorders [53,54].

Physical stimuli can also be applied during the preconditioning stage to modulate the composition of the secretome. **Hypoxic** culture has been one of the most widely used methods to engineer the secretory profile of MSCs and enhance its therapeutic potential for tissue and organ regeneration. Hypoxic culture promotes the secretion of numerous prosurvival and proangiogenic factors, such as VEGF, angiogenin, b-FGF, FGF-2, HGF, IL-6, MCP-1, MCP-3, IGF-1, TGF-β, platelet-derived growth factor (PDGF)-BB, and epidermal growth factor (EGF), that have shown beneficial effects in promoting angiogenesis and survival in ischemic conditions, and in promoting neurorestoration, cardioprotection, **chemotaxis**, wound healing, skin regeneration, and hair regrowth [51,55–63]. Of note, the conditions of hypoxic exposure during preconditioning should be chosen with care. Different oxygen levels - typically from 0.1% to 5% O_2 - have different effects on the properties of the secretome and can influence the secretome's functional potential [64–66]. In addition, the duration of exposure is important. For instance, prolonged hypoxic exposure can act in the negative and lead to a secretome that is detrimental for tissue repair [66,67].

Spheroid culture of stem cells provides a more physiologically relevant environment that allows the cells to exhibit improved biological properties. In secretome engineering, spheroid culture can elevate the secretion of proangiogenic factors (VEGF, bFGF, HGF, angiogenin, IL-11), anti-inflammatory markers (IL-1ra, granulocyte-colony stimulating factor, prostaglandin E_2 (PGE_2)], and antifibrotic molecules that have mostly shown therapeutic implications for angiogenesis and cardiac tissue repair [68–71]. In addition to standard spheroid culture methods, spheroid culture in 3D spinner flasks is particularly shown to produce a secretome enriched in angiogenic and antiapoptotic factors that is

clinically relevant and efficacious for improving angiogenesis, blood perfusion, and limb salvage in ischemic limbs [72].

Cells in the body are constantly experiencing mechanical forces. These forces are generally converted into biochemical signals through **mechanotransduction** that can stimulate different signaling pathways and influence cell behavior [73]. While the majority of studies using mechanical forces have been to regulate stem cell differentiation, it is evident that mechanical stimulation can also be used as a means to modulate the secretome. For bone applications and in bone cells, mechanical loading increases the secretion of various paracrine factors, including nitric oxide and PGE_2, two important signaling molecules that mediate the response of bone cells to mechanical forces [74]. However, the secretome produced from the various bone cells or MSCs is distinct, and has different effects on the proliferation, migration, and differentiation of other MSCs or bone cells [75–77]. Of note, mechanical loading can be applied to MSCs undergoing differentiation to produce a secretome that is customized in its regenerative and therapeutic potential. For instance, mechanical stimulation of MSCs during chondrogenic induction amplified the secretion of various proteins and cytokines involved in cartilage development, regeneration, and disease, such as type II collagen, aggrecan, keratan sulfate, TGF-β1, matrix metalloproteinase-13, PDGF-AA, VEGF, and angiogenin [78,79].

Secretome Applications and Delivery Strategies

Numerous delivery systems and strategies have been developed to harness the therapeutic potential of the stem cell secretome for organ/tissue repair and regeneration. Depending on the target site, the secretome or conditioned medium (CM) can be delivered either through direct injections, systemic injections, or through using delivery vehicles such as scaffolds, hydrogels, and microparticles (Table 1).

Systemic/Local Direct Administration

Systemic administration is a convenient approach that relies on rapidly increasing the concentration of the agent in the systemic circulation for distribution to sites of action. In a mouse model of rheumatoid arthritis, intravenous injections of CM from human deciduous dental pulp stem cells markedly improved arthritis symptoms and joint destruction [80]. Intravenous (IV) injections of exosomes derived from the CM of cardiac progenitor cells exerted cardioprotective effects in a rat model of drug-induced cardiotoxicity [81]. In a mouse model of traumatic brain injury, IV injecions of the exosomes collected from MSCs suppressed neuroinflammation and rescued the cognitive impairments [82]. A recent study evaluated the efficacy of IL-1α-primed MSC-derived CM on brain injury and recovery after cerebral ischemia in mice. The composition of the secretome was engineered by priming the MSCs using IL-1α toward a more anti-inflammatory and proreparative phenotype. Subcutaneous injections of the CM were performed to produce systemic effects against stroke, which included significant neuroprotective effects and improved functional recovery [83]. While there have not been many studies comparing local versus systemic injections for secretome delivery, the protective effects of the MSC secretome in reducing fatty

degeneration and atrophy of the rotator cuff muscles was shown through both delivery routes in a rat massive rotator cuff tear model [84].

The more common approach taken for delivering the secretome to a target organ has been through direct injections at the site of injury. For example, the efficacy of direct injections of MSC-derived exosomes in promoting cartilage repair has been shown in a few studies. In an immunocompetent rat model of **osteochondral** defects, weekly intra-articular injections of exosomes from human embryonic stem cell-derived MSCs induced the regeneration of the cartilage and subchondral bone tissue in as early as 2 weeks and led to orderly regeneration of both tissues by 12 weeks [85]. The exosomes from human embryonic stem cell-derived MSCs can also impede cartilage destruction in osteoarthritic mice through balancing the synthesis and degradation of the ECM [86]. Interestingly, exosomes from induced pluripotent stem cell-derived MSCs were shown to have greater therapeutic efficiency against osteoarthritis than those from synovial membrane-derived MSCs, possibly due to their greater stimulatory effects on chondrocyte migration and proliferation [87].

The benefits of local secretome administration have also been investigated for wound healing applications. Weekly injections of the secretome from both human fetal skin-derived stem cells and human umbilical cord MSCs effectively enhanced wound healing and angiogenesis in radiation-induced skin injuries in rats [88]. In diabetic rats, BMSC-CM effectively improved wound closure rates and the quality of healed skin in chronic diabetic wounds, through modulating the behavior of fibroblasts, angiogenesis, and the inflammatory and immune responses [89]. In a larger diabetic swine model, topical application of ADSC-CM or human umbilical vein endothelial cell-CM accelerated wound closure, possibly through the secretome's angiogenic and immunomodulatory effects [90]. The paracrine effects of human adipose tissue for wound healing applications was evaluated by preparing a cell-free liquid extract from the tissue and directly injecting it into the wound bed of mice. The extract, which was collected from the liquid portion of lipoaspirates after centrifugation, contained as many as 1975 human proteins (including bFGF, EGF, TGF-β1, VEGF, HGF, and PDGF) that were mainly from the cytoplasm, followed by the nucleus, extracellular space, and plasma membrane. Direct injections of this minimally processed tissue extract increased wound healing rates, angiogenesis and adipogenesis in full-thickness wounds [91].

Delivery Vehicles

Direct injections often result in a rapid **clearance** rate from the delivery site; therefore, it is important to develop carriers that prolong secretome retention for more efficacious therapeutic effects [92]. The use of the stem cell secretome in combination with biomaterials for bone regeneration has been extensively investigated by Katagiri and colleagues. The addition of MSC-CM to atelocollagen (Terudermis) scaffolds implanted into the calvarial defect of rats increased new bone regeneration, which was shown to be due to the secretome's proangiogenic properties and ability to induce early migration of endogenous stem cells to the defect site [93,94]. A following study showed that a cytokine cocktail of only IGF-1, VEGF-A, and TGF-β1 at concentrations similar to that of the MSC-CM was sufficient to induce bone regeneration at comparable levels to the MSC-CM [95]. β-Tricalcium phosphate (β-TCP) scaffolds soaked in MSC-CM for 5 min effectively delivered

the secretome to promote early bone regeneration in a rabbit maxillary sinus floor elevation model [96]. Katagiri and colleagues have further evaluated the safety and regenerative capacity of the MSC-CM in two small human studies. Patients requiring maxillary sinus floor elevation and bone grafts received β-TCP implants that were either soaked with MSC-CM (four patients) or as is (control, two patients). There was greater new bone formation in patients receiving the MSC-CM, particularly in the center of the augmented area [97]. In patients needing bone augmentation prior to dental implants also β-TCP- or atelocollagen-based scaffolds soaked with MSC-CM demonstrated great osteogenic potential and were found safe, causing no systemic or local complications [98].

Synthetic polymer-based scaffolds have also been used for secretome delivery. To increase hydrophilicity and improve CM immobilization, poly(lactic-co-glycolic acid) membranes were treated with NaOH before CM loading. NaOH treatment increased the immobilization of CM on the membranes which in turn led to greater BMSC proliferation and alkaline phosphatase activity on the membranes *in vitro*, and more new bone formation in rat calvarial defects [99]. Another study used polyethyleneimine to incorporate the extracellular vesicles of human gingival MSCs onto 3D-printed polylactic acid (PLA) scaffolds. Implantation of the PLA+polyethyleneimine extracellular vesicles scaffolds into rat calvarial defects led to complete bridging and repair of the defects, and greater new bone formation and vascularization (Box 2) [100].

Hydrogels are another class of biomaterials that can be used to retain the secretome for a controlled release profile. Injectable hydrogels, specifically, provide a minimally invasive means for localized delivery to target organs. One of the earliest works on secretome delivery was based on developing an injectable peptide-based hydrogel sponge to soak up the secretome *in situ*, and subsequently release it in the therapeutic environment [101]. Silk fibroin-based injectable hydrogels were used to deliver the secretome of human umbilical cord MSCs into the bone marrow of osteoporotic rats. These hydrogels were shown to provide a slow and sustained release for a period of 30 days *in vitro*. Intratibial injections localized the secretome at sites of osteoporosis within the bone, enabling the secretome to exert its antiaging effects and attenuate the drastic loss of bone [102]. To treat myocardial infarction (MI) in rats, an injectable hydrogel composed of gelatin and Laponite was developed. Laponite was chosen due to its high protein adsorption capacity, and contribution to the shear-thinning behavior of the hydrogels. The secretome from spheroid culture of human ADSCs was loaded and delivered to the peri-infarct regions of the heart through intramyocardial injections. Secretome delivery improved overall cardiac function and vascularization, and reduced fibrosis and scar tissue formation [69].

One of the more interesting approaches for secretome delivery has been to fabricate secretome-loaded microparticles the size of stem cells and to coat them with fragments of the stem cell membrane to create ‘synthetic MSCs’ or ‘cell-mimicking particles’. These microparticles provide a controlled and sustained release for up to 7 days *in vitro*. In a mouse model of acute MI, intramyocardial injection of the microparticles that were loaded with the secretome of BMSCs significantly reduced the infarct area and promoted endogenous heart repair [103]. The injection of similar microparticles loaded with the secretome of cardiac stem cells (CSCs), rather than BMSCs, in a similar model of acute MI

also improved cardiac function. It is important to note that the cardioprotective effects of the secretome-loaded particles were similar to that of CSC injections, however, CSC injections caused severe immune rejection and substantial T cell infiltration, which were found to be negligible in the secretome group [104].

Concluding Remarks and Future Prospects

Secretome-based therapies have emerged as a promising alternative to cell-based therapies. The ability to be manufactured, stored, and used as off-the-shelf ready-to-go products while maintaining the therapeutic benefits of stem cells but with fewer safety concerns have situated the secretome at the forefront of next-generation tissue and organ-regenerative engineering applications.

There are many challenges, however, that need to be overcome to translate this promise to the clinic (see Outstanding Questions). There is a need to develop guidelines and standardize the methods and procedures used in cell isolation and culture, and the techniques used to extract and purify the secretome. As discussed, the secretome is dynamic and responsive to microenvironmental changes and its composition can vary depending on its source of origin and culture conditions. Thus, it is imperative to consider the implications of these factors as variations in each may influence outcomes and obscure findings. This threat can, however, be used to advantage by modulating the composition of the secretome and tailoring its therapeutic effects for target applications. The methods described in secretome engineering can be used in combination to synergistically produce a secretome more fitting and efficient in its therapeutic effects. With this, it is important to note that the dynamic concentrations and combinations of proteins in the secretome respond differently to microenvironmental cues and that the effects of biochemical and physical stimuli should be considered on the entirety of the secretome, and not just a few biomolecules. As an example, there are components in the secretome such as some inflammatory mediators that are not necessarily beneficial for tissue regeneration and that through secretome engineering may become to adversely impact the secretome's therapeutic potential, and thus must be considered in secretome manipulation. The technological advancements and improvements in the methodologies and tools that are used in proteomics and **secretomics** to decipher the secretome and have a global understanding of the secreted factors will be integral for further progress and to further define the safety and efficacy of secretome-based therapeutics.

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Glossary

Apoptosis
a regulated and programmed process of cell death and self destruction.

Chemotaxis
directed movement and migration of cells in response to a chemical signal.

Chemokines (chemoattractant cytokines)
a subgroup of cytokines that induce chemotaxis in other cells.

Clearance
drug elimination from an organ.

Cytokines
a large family of small signaling protein molecules that are secreted by cells for cell signaling and cell-cell communication.

Homeostasis
the ability of the body or a cell to regulate and maintain a steady and stable physiological condition.

Hypoxia
the reduction or lack of oxygen in organs, tissues, and cells. Hypoxic culture refers to performing the cell culture at O_2 levels lower than ambient air (21% O_2) and typically at 0.1–5% O_2.

Mechanotransduction
the process in which cells sense and respond to mechanical stimuli by converting them into biochemical signals.

Osteochondral
pertaining to bone and cartilage.

Preconditioning
exposing the cells to certain physical or chemical stimuli to manipulate cell behavior for a desired response.

Protease
an enzyme that helps break down proteins or peptides

Proteolysis
the process in which proteins are degraded, partially or completely, by the activity of proteases.

Proteome
the entire set of proteins expressed by an organism.

Proteomics
the large-scale study of the proteome to analyze the structure, function, and interactions of all the proteins of a cell for a global and integrated biological view.

Secretomics
is a subfield of proteomics that involves the global study of the secretome - proteins that are secreted by organisms.

Spheroid

3D structures of cells grown in aggregates that recreate the natural *in vivo* environment of the cells more closely than 2D culture conditions.

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Highlights

The stem cell secretome has emerged as a promising cell-free alternative to cell-based therapies.

Secretome-based therapies have many of the therapeutic benefits of cell-based therapies, while obviating many of the safety and logistical concerns associated with directly using stem cells.

The secretome is highly dynamic and its therapeutic effects can be engineered and customized according to its intended application.

Delivery systems and strategies have been developed to harness the therapeutic benefits of the secretome for the repair and regeneration of various tissues and organs.

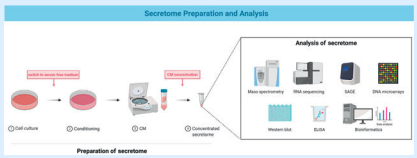
Box 1.

Secretome Preparation and Analysis

The secretome is isolated through a series of steps. (1) The stem cells are cultured in serum-containing culture medium, usually until 70–80% confluency. (2) To remove any remnants of serum proteins that may contaminate and interfere with the detection of minuscule amounts of cell-produced proteins, the cells are washed extensively and cultured in serum-free medium, usually for 12–48 h. This step may also include conditioning the cells using chemical or physical stimuli to obtain specific attributes from the secretome. (3) The conditioned medium (CM) consisting of the soluble components of the secretome and extracellular vesicles is collected by centrifugation to remove any dead cells or cell debris. (4) To increase the analytic resolution, the CM proteins are concentrated using centrifugation or lyophilization, and/or precipitated by ultrafiltration or trichloroacetic acid [33,105–107].

The secreted proteins of the secretome can be characterized using a targeted **proteomic** approach or a shotgun-based proteomic approach. The targeted approach involves protein microarrays such as antibody-based arrays (ELISA and western blotting) or bead-based arrays. The shotgun approach includes the following: gel-based (e.g., 2D gel electrophoresis) or gel-free (liquid chromatography) methods to separate the proteins in the secretome followed by mass spectroscopy to identify the proteins based on database knowledge; serial analysis of gene expression (SAGE), a short sequence-based method that quantitatively measures global gene expression patterns of tagged sites; RNA sequencing, a high throughput method that analyzes the transcriptome and can provide larger sequence information than SAGE; DNA microarray, a high-throughput method that measures differential gene expression based on known cDNA or oligonucleotide sequences; and secretion traps, a functional method that provides the sequence information of proteins. Bioinformatics tools (software and databases) can be used in combination with these approaches to manage and analyze the secretome/**proteome** results [33,105,108,109].

Figure I. Preparation and Analysis of the Secretome.



Box 2.

Extracellular Vesicle Based-Therapeutics

Extracellular vesicles (EVs) are nanosized membrane-enclosed vesicles that are released by the cells into the extracellular space. Such vesicles include exosomes (40–150 nm), formed by the fusion of intracellular multivesicular bodies with the plasma membrane, and microvesicles (50–1000 nm), which are shed directly from the plasma membrane. EVs contain various bioactive components such as nucleic acids (RNA, mRNA, miRNA, and DNA), proteins, cytokines, and lipids and are a significant means of communication between not only adjacent cells but also distant cells, to which they travel to through blood and bodily fluids. EVs have been shown to play important roles in regulating physiological and pathological processes including homeostasis, immunomodulation, regenerative processes, and tumorigenesis [110–113].

For many years considered as inert cellular debris, EVs are now receiving increasing attention as therapeutic delivery systems due to their natural ability to robustly transport biologically active components and genetic material to target cells, near or far. The EV delivery systems are used through loading the cargo either endogenously, that is, engineering the secretome for desired biomolecules and subsequently isolating and purifying the produced EVs, or exogenously, that is, encapsulating other drugs into already isolated EVs [114–116]. As a rapidly evolving and expanding field, EV-based therapies have so far been used toward the preclinical treatment or regeneration of various diseases and conditions including those of the respiratory, renal, hepatic, neurological, cardiovascular, and musculoskeletal systems. There are also clinical trials and applications involving the use of EVs particularly for cancer therapy and immunotherapy [117,118].

Outstanding Questions

How can we develop guidelines and standardize the procedures for secretome production?

How can we develop high-throughput systems for the large-scale production of the secretome?

How can we best engineer and customize the secretome for targeted therapeutic benefits?

What are the technical advances necessary to propel this technology to its next stage and progress into clinical practice?

What are the safety and regulatory concerns that need to be addressed?

Will secretome-based therapies provide long-term function and safety in patients?

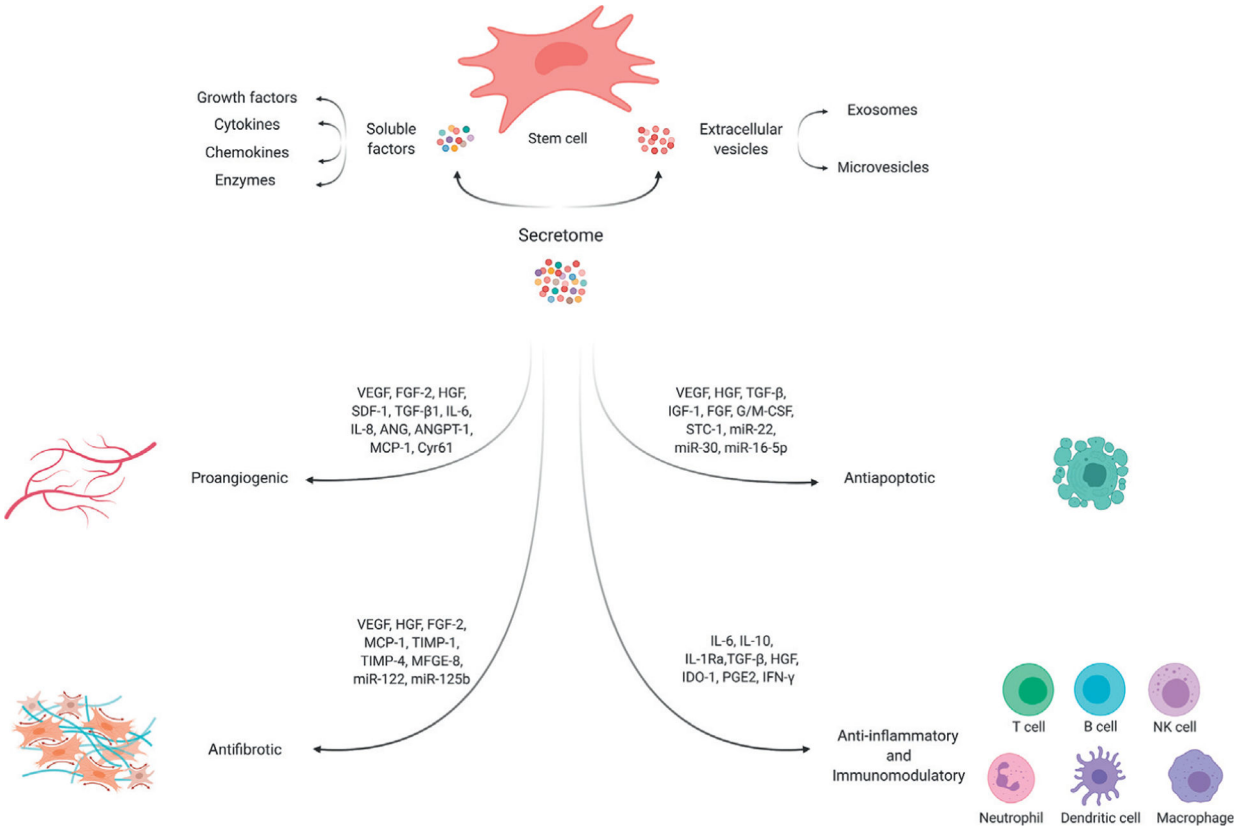


Figure 1. Schematic Representation of the Secretome and Its Therapeutic Effects.

Stem cells secrete various soluble factors and extracellular vehicles (including exosomes and microvesicles) that are collectively termed the secretome. These biologically active factors exert therapeutic effects through their proangiogenic, antifibrotic, antiapoptotic, anti-inflammatory, and immunomodulatory properties. Abbreviations: ANG, angiogenin; ANGPT-1, angiopoietin-1; Cyr61, cysteine-rich protein 61; FGF-2, fibroblast growth factor 2; G/M-CSF, granulocyte/macrophage colony stimulating factor; HGF, hepatocyte growth factor; IDO-1, indolamine 2,3-dioxygenase-1; IFN-γ, interferon-γ; IGF-1, insulin-like growth factor-1; IL-1Ra, IL-1 receptor antagonist; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; MFGE-8, milk fat globule-EGF factor 8; miR-, miRNAs found within the extracellular vesicles; PGE2, prostaglandin E2; SDF-1, stromal-derived factor-1; STC-1, stanniocalcin-1; TGF-β1, transforming growth factor-β1; TIMP-1, tissue inhibitors of metalloproteinase-1; VEGF, vascular endothelial growth factor.

Table 1.

Strategies to Deliver the Secretome for Regenerative Engineering Applications.

Delivery system[a]	Animal model	*In vivo* effect	Refs
IV injection of human deciduous dental pulp stem cell-CM	Mouse rheumatoid arthritis model	• Improved clinical symptoms of arthritis • Improved histological scores of synovial inflammation, bone erosion, and cartilage damage • Reduced osteoclastogenesis in the joints • Induced M2 macrophages	[80]
IV injection of human cardiac progenitor cell-exosomes	Rat model of drug-induced cardiotoxicity	• Improved cardiac function • Attenuated myocardial fibrosis, inflammation, and iNOS expression	[81]
IV injection of MSC-EVs	Mouse model of traumatic brain injury	• Decreased IL-1β levels in brain • Persevered the pattern separation function and spatial learning ability of the mice	[82]
Direct injection of human UC-MSCs into the soleus muscle	Rat model of muscle atrophy	• Improved muscle mass, muscle fiber size, and metabolic activity • Improved muscle regeneration by activating satellite cells, stimulating myoblast proliferation and modulating the PI3K/Akt pathway	[119]
Weekly intra-articular injections of exosomes derived from human embryonic stem cell-derived MSCs	Rat critical-sized osteochondral defect	• Improved neotissue formation, and cartilage and subchondral bone regeneration • Increased cartilage ECM deposition of s-GAG and type II collagen • Enhanced cellular proliferation and attenuated apoptosis in the cartilage lesion and overlying synovium • Increased M2 macrophage infiltration and decreased M1 macrophages in the cartilage and synovium • Reduced levels of inflammatory IL-1 p and TNF-a cytokines in synovial fluids	[85]
Weekly subcutaneous secretome injections of human fetal skin-derived stem cells and UC-MSCs	Rat radiation-induced skin injury	• Improved rate and quality of wound healing • Enhanced angiogenesis	[88]
Topical administration of human ADSC-CM and HUVEC-CM	Diabetic swine full-thickness wound healing model	• Accelerated wound closure • Attenuated acute inflammation • Significantly decreased the production of TNF-α in the treated wounds	[90]
Atelocollagen-based sponge soaked with hMSC-CM	Rat calvarial bone defect	• Increased new bone formation • Enhanced early migration of endogenous MSCs to the lesion • Depletion of VEGF singly, or depletion of IGF-1, VEGF, and TGF-β1 collectively from the CM diminished its bone regenerative capabilities	[93–95]
β-TCP scaffold soaked with hMSC-CM	Rabbit maxillary sinus floor elevation model	• Increased early bone formation • Increased angiogenesis and cell proliferation during the early phase of bone regeneration	[96]

Delivery system[a]	Animal model	*In vivo* effect	Refs
β-TCP scaffold soaked with hMSC-CM	Human maxillary sinus floor elevation model	• Enhanced new bone formation in the center of the augmented area	[97]
NaOH-treated PLGA membrane soaked with rat BMSC-CM	Rat calvarial bone defect	• Increased new bone formation	[99]
3D-printed PLA scaffold loaded with PEI-engineered EVs	Rat calvarial bone defect	• Enhanced new bone formation, deposition of bone nodules, and integration with the host • Indications of a new vascular network formation	[100]
Silk fibroin-based hydrogel loaded with human UC-MSC-CM	Rat model of age-related osteoporosis	• Reduced attenuation in bone loss • Improved bone mineral density, and trabecular bone volume, thickness, and number	[102]
Gelatin and Laponite-based hydrogel loaded with hADSC-CM from spheroid culture	Rat myocardial infarction	• Improved cardiac function parameters including ejection fraction, fractional shortening, and cardiac output • Increased blood vessel density • Decreased infarct size	[69]
Hydroxyethyl cellulose hydrogel loaded with hADSC-exosomes	Rat excisional wound-splinting model	• Accelerated wound healing	[120]
PLGA microparticles coated with hMSC membrane fragments and loaded with hMSC-secretome	Mouse myocardial infarction	• Increased infarct wall thickness • Reduced infarct size • Increased vessel density	[103]
PLGA microparticles coated with hCSC membrane fragments and loaded with hCSC-secretome	Mouse myocardial infarction	• Ameliorated ventricular dysfunction • Increased blood vessel density and flow • Improved remuscularization and infarct thickness reduced scar size • Reduced apoptosis • Increased proliferation of endogenous cardiomyocytes	[104]

[a]ADSC, adipose-derived stem cell; β-TCP, β-tricalcium phosphate; CM, conditioned media; CSC, cardiac stem cell; ECM, extracellular matrix; EVs, extracellular vesicles; IL-1β, interleukin-1β; HUVEC, human umbilical vein endothelial cell; IGF-1, insulin-like growth factor; iNOS, inducible NO synthase; MSC, mesenchymal stem cell; PEI, polyethyleneimine; PI3K, phosphatidylinositol 3-kinase; PLA, polylactic acid; PLGA, poly(lactic-co-glycolic acid); s-GAG, s-glycosaminoglycan; TGF-β1, transforming growth factor-β1; TNF-α, tumor necrosis factor-α; UC-MSC, umbilical cord mesenchymal stem cell; VEGF, vascular endothelial growth factor.