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Review Article

Novel Progression from Cell-Based Therapies to Increasingly Refined, Cell-Free Approaches

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Abstract

In the late 20th century, stem cell research took off with the discovery of mesenchymal stem cells (MSCs) and embryonic stem cells (ESCs). MSCs demonstrated potential to regenerate musculoskeletal tissues, while ESCs offered broader differentiation capacities. Stem cells, particularly MSCs, can differentiate into chondrocytes (cartilage-producing cells), osteoblasts (bone-forming cells), or insulin-producing beta cells. This plasticity positioned them as therapeutic candidates for OA and T1D. Early stem cell therapies focused on cartilage repair. MSCs were directly injected into joints to regenerate cartilage and reduce inflammation. In T1D, researchers sought to replace destroyed beta cells. Early efforts included differentiating stem cells into insulin-producing cells to restore glycemic control. By the early 2000s, studies revealed that much of the therapeutic effect of stem cells was mediated by their secreted factors rather than direct engraftment. This led to a focus on stem cell-derived peptides, short chains of amino acids secreted by or derived from stem cells. These peptides play roles in modulating inflammation, promoting tissue repair, and enhancing cell signaling. They are components of the secretome of stem cells. Stem cell-derived peptides have been shown to promote extracellular matrix (ECM) synthesis and reduce catabolic processes in cartilage, delaying OA progression. Peptides derived from stem cells have demonstrated immunomodulatory effects, protecting residual beta cells from autoimmune destruction and enhancing the survival of transplanted insulin-producing cells. By the late 2000s and 2010s, exosomes-small extracellular vesicles secreted by stem cells-emerged as the central mediators of the therapeutic effects of stem cells. Exosomes are enriched with proteins, lipids, RNAs, and microRNAs that influence tissue repair, reduce inflammation, and enhance cellular communication. Unlike peptides, exosomes carry complex molecular cargo capable of targeting multiple pathways simultaneously. Exosomes derived from MSCs or induced pluripotent stem cells (iPSCs) can promote cartilage repair by stimulating chondrocyte proliferation and ECM synthesis while reducing inflammation. They offer significant advantages over cell-based therapies, including easier storage, reduced risk of tumorigenesis, and the ability to deliver precise molecular signals. The novel progression from cell-based therapies to increasingly refined, cell-free approaches has been driven by the desire to reduce the complexities and risks associated with stem cell transplantation while retaining (and enhancing) therapeutic efficacy.

Keywords: Peptides, Nano Organo Peptides (NOP), Mito Organelles (MO) Peptides, Cell Therapy, Regenerative Medicine, Exosomes

Regenerative Therapy

Significant interest in regenerative treatments aimed at repairing and replacing the injured cells and tissues with new ones continues to increase. Promising new bioregenerative technologies offer exciting therapeutic options, however, current widespread use of novel regenerative approaches has been somewhat limited.

Stem cells (SCs) have been proposed as regenerative cell therapy for various conditions, due to the potential for self-renewal and differentiation. Multipotent stem cells are distributed extensively in the bone marrow, trabecular bone, fat pad tissue, synovial membrane, and several other tissues. The differentiation capacity and maintaining immune modulating capabilities has been leveraged in several preclinical and clinical studies confirming the potential for mesenchymal SCs (MSCs) as a novel therapeutic strategy for the treatment of OA [1]. While a systematic review of 61 studies of OA and stem cell therapy in humans [2] concluded that MSC therapy has a positive effect on OA patients, the limited high-quality evidence available and that long-term follow-up as well as lack of consistency of MSC preparations presents significant challenges.

Indeed, different tissue sources of MSCs demonstrate different characteristics that confer advantages and disadvantages for therapeutic usage, including proliferative capacity, immunomodulatory capacity and cytokine secretion profiles [3]. For example, umbilical cord and amniotic-derived MSCs and adipose tissue-derived MSCs (AD-MSCs) have superior immunomodulatory capacity, including immune regulation, compared to bone marrow MSCs and placental MSCs. On the other hand, umbilical cord MSCs secrete more cell growth factors than bone marrow MSCs. Notwithstanding, several clinical trials have demonstrated the potential efficacy of MSCs derived from bone marrow, adipose tissue, and umbilical cord blood in the treatment of musculoskeletal and joint conditions [4-6].

Musculoskeletal/Joint Conditions

Orthopedic diseases such as osteoarthritis (OA), ligament ruptures, and dysplasia are prevalent in pets, with OA affecting over 20% of dogs in the U.S. aged over one year. These conditions are exacerbated by factors such as age, obesity, trauma, and genetic predispositions [7,8]. Traditional treatments, including surgery and pain management, often provide limited relief and carry risks. Stem cell therapy, particularly with mesenchymal stem cells (MSCs) derived from adipose tissue or bone marrow, has emerged as a promising alternative due to their ability to promote cartilage regeneration, reduce inflammation, and improve joint function. Studies demonstrate that intra-articular injections of MSCs result in significant improvements in mobility, pain reduction, and quality of life in pets with OA and other joint issues, with effects lasting up to two years in some cases. Additionally, MSC therapy has shown efficacy in treating hip and elbow dysplasia, cranial cruciate ligament ruptures, and tendon injuries. As highlighted in our recent review, the emerging utility of stem cell therapy in veterinary medicine as innovative treatment has the potential to revolutionize the management of age-related disorders and orthopedic diseases in animal companion animals despite challenges and limitations [9-11].

In humans, joint degenerative disorders, OA, are a growing global burden driven by mechanical and inflammatory factors. Initial treatment strategies focus on non-pharmacological and pharmacological approaches to manage pain and slow disease progression, with surgical intervention as a subsequent option. Stem cell-based therapy is gaining significant attention due to its potential to regenerate cartilage, promote chondrocyte formation, and modulate inflammation. Several clinical trials have demonstrated safety and potential efficacy of bone marrow (BM-MSCs), adipose derived (AD-MSCs), and umbilical cord (UC-MSCs) in the treatment of OA [12].

To preserve joint structure and function in early stages, while addressing extensive cartilage loss and subchondral bone changes in later stages the basic goal of therapeutic strategies is to preserve tissue structure and function. Cartilage stem cells (CSCs) and chondrogenic progenitor cells (CPCs) play a vital role in joint repair by enhancing extracellular matrix (ECM) production, chondrocyte regeneration, and anti-inflammatory signaling. Migratory CPCs, identified in both early and late-stage OA, possess high chondrogenic potential and may either exacerbate or mitigate tissue damage depending on their role in ECM turnover. Studies show that cartilage injuries trigger CPC migration via chemotactic signals such as HMGB-1, aiding in cartilage repair. However, the efficacy of CPC-driven repair depends on their local microenvironment or niche, which influences their differentiation and regenerative potential. Effective OA therapies may require a combination of CPCs with niche-modifying strategies, including growth factors, scaffolds, and siRNAs, to optimize the repair response and address progressive cartilage degeneration [13].

Specific matrix molecules can also be used to regulate cell behavior and help provide an optimal microenvironment to promote chondrogenesis, while simultaneously inhibiting chondrocyte hypertrophy and terminal differentiation. Stem cell-based approaches for cartilage repair leverage natural ECM components, scaffolds, and growth factors to enhance stem cell attachment, growth, and differentiation. Decellularized ECMs (dECMs) serve as scaffolds that mimic the native cartilage environment, promoting effective cell function and tissue repair while exhibiting low cytotoxicity. Studies, including canine models, have shown that dECM-based scaffolds integrated with stem cells improve cartilage and subchondral bone repair compared to cell-free scaffolds. ECM-derived biphasic scaffolds demonstrate potential for addressing high-load-bearing osteochondral defects. Alternatively, individual ECM proteins, like collagen, can be used to create biocompatible scaffolds. Type I collagen sponges have successfully delivered growth factors such as insulin and FGF, enhancing proteoglycan production and generating repair tissue resembling native hyaline cartilage. These strategies underscore the importance of preserving and optimizing the tissue microenvironment for effective cartilage regeneration [14]. Both autologous and allogeneic MSCs are being explored, with cartilage stem cell-based therapies emerging as a particularly effective regenerative strategy. When combined with traditional treatments, stem cell therapy holds the potential for enhanced outcomes in managing degenerative joint disorders.

Cardiology

As individuals age, the human heart experiences a variety of structural and functional changes. These age-related alterations are influenced by genetic predispositions, environmental factors, lifestyle choices, and the presence of other medical conditions. With the global population continuing to age, cardiovascular diseases

(CVDs) are becoming an increasingly significant concern, contributing substantially to disability, death, and rising healthcare costs. According to the World Health Organization, CVDs are expected to cause more than 23 million deaths annually by 2030, making up nearly half of all global deaths [15]. Thus, cardiovascular health is a critical factor in both the lifespan and quality of life for older adults.

Cardiac aging is a multifaceted process that involves numerous structural and functional changes, which are largely responsible for the higher incidence of heart disease in older individuals [16]. Age-related cardiomyopathies, such as dilated, hypertrophic, restrictive, and amyloid cardiomyopathies, present major challenges for healthcare providers. Understanding the underlying molecular and cellular mechanisms driving these changes is essential for the development of more effective treatments. Ongoing research into innovative therapeutic approaches, including gene editing, regenerative medicine, and the discovery of new drugs, will be pivotal in addressing the increasing burden of cardiovascular disease among the aging population [17].

As age-related cardiomyopathies continue to pose a significant challenge, research efforts are increasingly directed toward identifying effective strategies for preventing or reversing these conditions. To mitigate the rising prevalence of cardiovascular diseases, it is essential for healthcare systems to emphasize early detection, prevention, and the creation of personalized treatment plans tailored to individual patient needs. Peptides offer several benefits over traditional small-molecule drugs, such as enhanced specificity, reduced toxicity, and the potential to target multiple disease mechanisms at once. As a result, peptide-based therapies are increasingly being explored in cardiology, especially for addressing age-related heart conditions [18].

Peptides influence cardiac health by binding to specific receptors or interacting with signaling pathways that regulate heart function and repair. The main mechanisms through which peptides exert their effects on the heart include: modulation of inflammation, promotion of regeneration and repair, antioxidant activity, stimulation of angiogenesis and vasodilation, and regulation of cell signaling pathways [19,20]. Mitochondrial peptides, which are small signaling molecules derived from mitochondrial proteins, represent a promising new class of therapeutic agents in cardiology, particularly for the treatment of cardiovascular diseases (CVDs). These peptides play a crucial role in regulating mitochondrial function, cellular metabolism, and tissue regeneration, all of which are vital for maintaining heart health. In some of our previous works we have explored mitochondrial peptides (i.e. MitoOrganelles™) for their potential to promote cardiac regeneration, enhance mitochondrial function, and reduce the impact of heart diseases, including myocardial infarction, heart failure, and ischemia [21]. These peptides exert multiple effects on the heart by modulating mitochondrial biogenesis, reducing oxidative stress, promoting anti-apoptotic signaling, and enhancing cellular adaptability to ischemic injury [22]. With age, energy production reduces, oxidative stress increases and nuclear signaling pathways deteriorate due to mitochondrial dysfunction and decreased production of mitochondrial peptides. As a result, peptide therapies, such as MO and NOP, have emerged as promising anti-aging strategies. Those therapies aim to repair and replace damaged mitochondria and improve mitochondrial function by reducing apoptosis and ROS production [23-29]. Furthermore, it has also been reported that treatment with MO resulted in improved lipid profiles, increased insulin sensitivity [30]. Of note, peptides such as MO and NOP can be used not only as an anti-aging treatment but also because of their ability to repair and regenerate damaged tissues, making this approach promising in myocardial infarction treatment Moreover, those peptides have shown potential in decreasing inflammation and stimulating vessel formation, therefore they might be a part of heart failure management [31].

Other bioregenerative biological therapeutic agents used in cardiology are exosomes. Exosomes are small nanovesicles ranging in size from 30 to 120 nm that exist in various types of cells, including stem cells. They exert their biological effects and, as extracellular microvesicles, facilitate the communication of organelles within cells (as endosomes), and when excreted outside the cell, can transport biologically active RNA, proteins, lipids and other signaling molecules depending on physiological purposes (as exosomes). Thus, exosomes act as communicators [32]. As a kind of important paracrine signaling vehicles, these biomolecules containing important information in various cells, exosomes are able to influence gene transcription and cell proliferation [33]. Typical biomarkers of exosomes include proteins such as CD9, CD81, CD63, ceramide, tumor susceptibility gene 101 (TSG101), as well as apoptosis-linked gene 2-interacting protein X (ALIX) [34].

Thus, exosomes can perform two main functions: diagnostic (as biomarkers of cell damage) and therapeutic (stimulating regeneration processes) [35]. The advantages of the diagnostic potential of exosomes over known cardiac markers (such as NTproBNP, troponin, etc.) are the prediction of the further course of the disease and the choice of targeted therapy [36]. The bioregenerative potential of exosomes lies in the ability to regulate transcription and translation of genes, promote cell survival and proliferation, enhance angiogenesis and healing of damage, waste management, balance of immune response, apoptosis, cellular differentiation and neoplasia, metabolic regulation [37].

The positive effect of using exosomes has been proven in the treatment of myocardial infarction [38], cardiomyopathy [39,40], ischemic heart diseases [41], endothelial dysfunction in Hypertension [42], Heart Failure [43]. The regenerative abilities of mesenchymal stem cells derived exosomes have been proved in preclinical trials [44]. The immunomodulatory effect of MSC exosomes via the PP2A/p-Akt/Foxo3 signaling pathway was used in an experimental model of myocardial infarction [45]. Also, MSC-derived exosomes suppress apoptotic cell injury under hypoxic conditions by delivering miR-144 into cells where it targets the PTEN/AKT pathway, which may be a promising therapeutic tool to facilitate the delivery of miRNA therapy in ischemic cardiomyocyte injury [46]. In research [47]. MSC-derived exosomes enhanced cardiomyocyte survival to hypoxia. So, his targeted microRNA delivery presents an

effective and safe strategy as a stem cell and exosomal therapy in ischemic/ reperfusion cardiac repair. Results of a systematic review showed that the use of exo-microRNAs in heart failure may be a promising direction in cardiology [48].

Dermatology

Skin aging is characterized by a gradual decline in skin density, elasticity, and the overall ability to maintain a youthful appearance. This slow, multifactorial process involves various biological mechanisms that result in significant changes to the skin's structure and function. In the epidermis, the aging process causes alterations in the shape and texture of the skin, while in the dermis, it leads to a reduction in extracellular matrix (ECM) proteins, increased breakdown of collagen, and a decline in key cellular components such as melanocytes, keratinocytes, and fibroblasts [23].

Photodamage in the epidermis often presents as variations in skin thickness, marked by alternating areas of thinning (atrophy) and thickening (hypertrophy), as well as nuclear atypia in keratinocytes (KCs), reflecting the dysregulation of KC growth due to ultraviolet radiation (UVR) exposure. Several cytokines derived from fibroblasts, including keratinocyte growth factor (KGF), epidermal growth factor (EGF), and hepatocyte growth factor (HGF), play a role in promoting KC proliferation. Additionally, mast cells and other inflammatory cells in the skin release cytokines that contribute to the pathological changes observed in UV-exposed, sun-damaged skin. After UVB irradiation, the levels of KGF and chemokine receptor-2 (CXCR-2) in human keratinocytes are significantly reduced, impairing the epidermis's ability to regenerate and respond to increased dermal KGF synthesis and epidermal overproduction of IL-8 and Gro-α [24].

Melanocytes, the pigment-producing cells of the skin, are also affected by signals from keratinocytes and dermal cells, including fibroblasts and inflammatory cells. The activity of melanocyte growth factors, such as β-FGF, endothelin-1 (ET-1), HGF, and stem cell growth factor (SCF), as well as melanocyte tyrosinase, plays a role in the pigmentation changes associated with photoaging. In sun-damaged skin, blood vessels are often damaged, leading to a loss of the normal horizontal plexus architecture in the upper dermis. The number and size of blood vessels are significantly reduced, particularly in the papillary dermis of photoaged skin, which can contribute to poor skin health and function.

Peptides are versatile molecules that have gained attention for their potential to address various skin conditions in cosmeceuticals. They play a significant role in combating skin aging by modulating collagen turnover and influencing specific neurotransmitters to reduce age-related wrinkles. The primary mechanism of peptides in preventing photoaging involves stimulating collagen production and restoring lost ECM components, helping to reduce wrinkles. Fibroblasts, the key cells in the dermis responsible for synthesizing and secreting collagen and elastin, become less abundant with age, leading to a decline in collagen production and a loss of skin elasticity. Peptides can help regulate fibroblast activity and promote the synthesis of ECM components, particularly through the action of signaling peptides [25]. For example, the elastin peptide Val-Gly-Val-Ala-Pro-Gly (VGVAPG) stimulates fibroblast proliferation by binding to elastin-binding proteins. Bioactive peptides can also counteract the negative effects of photoaging, such as oxidative stress, inflammation, the abnormal expression of matrix metalloproteinases (MMPs), hyaluronidase, elastase, and excessive melanin production. Anti-aging peptides improve skin cell viability, enhance skin proliferation, reduce pigmentation, alleviate tissue inflammation, strengthen the skin barrier, and provide overall support to the skin [26].

Anti-aging peptides are classified based on their mechanisms of action, which include signal peptides, carrier peptides, neurotransmitter inhibitor peptides, and enzyme inhibitor peptides. As biologically active compounds, peptides offer significant potential for combating the early signs of skin aging, fortifying the skin barrier, and protecting against UV radiation [27]. A deeper understanding of the functions and interactions of peptides has led to their use in both therapeutic and supplemental applications, supporting the skin's health and addressing the effects of aging and environmental damage [27].

Peptides

Peptides can act as signaling molecules, interacting with receptors on other cells to stimulate specific cellular behaviors like migration, proliferation, and differentiation, depending on the peptide sequence. Peptides can be incorporated into scaffolds to enhance stem cell adhesion and promote tissue growth in areas where regeneration is needed. "Stem cell-derived peptides" refer to short protein fragments (peptides) that are extracted or produced from stem cells, which can mimic the functions of the original stem cell proteins and have potential applications in regenerative medicine by promoting cell adhesion, proliferation, and differentiation, particularly in tissue repair and regeneration processes. These peptides are derived from various types of stem cells, including MSCs, which are known for their ability to regenerate tissues. By stimulating cell migration and proliferation, stem cell derived peptides can accelerate wound closure. In addition, peptides derived from stem cells may have anti-inflammatory properties, potentially useful in treating inflammatory diseases. Further, peptides mimicking components of the ECM can promote cell adhesion and migration.

Despite similar content, unique cellular function and morphology dictates the ultrastructure and intracellular biologically active substances, with certain biologically active substances predominantly synthesized or accumulated in a tissue-specific manner. Further, cell type determines the signaling activity and function of intracellular peptides. Capitalizing tissue and organ-specificity, peptide therapy has the potential to renew the strength of the signals received by cells to either induce peptide production or trigger normal signaling processes, thereby rejuvenating and revitalizing tissues as well as the organism. The short lengths of peptides and their low molecular weights, allow large-scale biosynthesis and extraction processes and distribution for use in therapeutic treatments [3]. Nano Organo Peptides (NOPs) and MitoOrganellesTM (MO) Peptides have been extensively used in humans and animals.

NOPs are 3nm in size and have a molecular weight of less than 10kDA [3], procured from mammalian stem cells and are processed through a proprietary parallel-extraction process that includes multiple ultrafiltrate steps through specialized Millipore's to obtain the cellular material within the cell, known as the molecular-level ultrafiltrates. Owing to the extraction process, these ultrafiltrates are specific to the cell type that they are derived from. NOP contents are extracted from organ specific cells with an initially high molecular mass and subsequently separated through various ultrafiltration steps through micro-Millipore filters. This selective filtration process only allows substances with a molecular mass of less than 10kDa to pass, thereby ensuring peptide specificity. Moreover, the small molecular weights and high solubility of NOPs permits their delivery via both sublingual and injectable routes (either subcutaneous or intramuscular) [3]. NOPs have been investigated and utilized for a variety of applications including cosmetics [8] and regenerative organ repair [9].

MO peptides are biologically extracted mixtures of cellular peptides that have predominantly mitochondria-specific functions [10]. Although cells of different organ systems have similar functions, variations in cellular functions between organs create the differential expression of peptides, which can be utilized for therapeutic purposes. As part of the aging process, the volume and strength of signals to the mitochondria declines, causing signals to be sent back to the nucleus to arrest cell proliferation and initiate apoptosis and cell death. MO peptides are organ-specific extracts that are aimed at revitalizing and rejuvenating mitochondrial activity, thereby regenerating cells and organisms. Unlike NOPs, MOs are larger in size and have predominantly mitochondria specific functions that allow for a more pronounced revitalization of mitochondrial function [11,12].

Peptides can be designed to mimic the activity of specific growth factors, promoting cell proliferation and differentiation. In the context of diabetes, peptides generated from stem cells have the potential to regulate blood sugar levels by mimicking the functions of naturally occurring insulin-producing beta cells, offering a potential therapeutic avenue for treating diabetes, particularly type 1 diabetes (T1D), where the body's own beta cells are destroyed by the immune system.

Our group investigated the ability of peptide therapy products to stimulate insulin production, protect beta cells from damage, and potentially modulate the immune response involved in diabetes development. European Wellness (EW) and the BioPep Research Group developed two distinct peptide therapy products made of organ-specific cellular extracts and peptide molecules, Mito Organelles (MO) peptides and Nano Organo Peptides (NOP), which are produced through a proprietary parallel-extraction process from mammalian precursor stem cells and rabbits bred in closed colonies under good manufacturing practices conditions. Given the wide distribution of Mitochondria-Derived Peptides (MDPs) through various tissues and their role in cryoprotection roles through maintaining cell viability and mitochondrial function under both pathological and normal conditions, our group conducted a study s to determine whether the administration of stem-cell derived MO peptides twice-weekly to NOD mice through intramuscular injections over 17 weeks delays or prevents the onset of the destruction of the insulin-secreting beta cells in pancreatic islets of Langerhans. Specifically, the stem-cell derived MO peptides were obtained from thymus and pancreatic extracts to target the regions of the beta cells and T-cell maturation [1,28].

A cytokine panel consisting of 45 cytokine assays was obtained using serum samples of each mouse. A significant difference in the concentration of Erythropoietin (EPO) and Chemokine Ligand 5 (CCL5), otherwise known as RANTES, was found between MO peptide mice and control mice. NOD MO peptide mice had an average EPO concentration of 374.88pg/mL while the NOD saline mice had an average EPO concentration of 203.68pg/mL ($p = 0.0062$) in the MO peptide treated group compared to sham controls. NOD MO peptide mice had an average CCL5 concentration of 14.37pg/mL while the NOD saline mice had an average EPO concentration of 8.08pg/mL ($p = 0.031$). The study provides important preliminary data that suggests that MO peptides may assist in delaying the onset or preventing T1D and represents an exciting therapeutic option to further investigate.

Despite the numerous studies highlighting the therapeutic effectiveness of NOP and MO peptides and the established procedures documented on obtaining them, little is known of the exact makeup of these formulations. Mass spectrometry has been shown to identify and quantify analytes in complex solution [13] and therefore is thought to be able to identify the population of peptides derived from peptide cocktail formulations. The mass spectrometer produces a readout of peaks plotted in relative abundance against the mass-to-charge ratios. By searching the experimentally derived peaks against a database of known proteins, it is possible to identify the peptides. The following report outlines the experimental methods and the results from performing mass spectrometry on various EW peptides.

Our results indicated that there were no statistically significant differences in protein concentration (ug/ mL) between batches but were statistically significant differences between sample types ($p<0.05$). These results are in line with our experimental expectations as both the NOP and MO samples are cellularly derived solutions that contain a heterogenous mixture of molecules and peptides that will vary depending on the tissue that they are recovered from. Between batches, however, relative protein concentrations were expected to remain consistent as they were procured from the same tissue samples and processed in the same manner. In addition to the consistency between batches found in the protein concentration assay, consistency was also demonstrated between batches during our preliminary MS experiment. Following our preliminary MS experiment and protein concentration assay, LBS MO was chosen to progress and to perform an in-depth analysis due to its relatively high protein concentration and large number of peaks that indicate potential peptide candidates. Additionally, due to the differences in extraction and preparation procedures between the MO and NOP sample, the MO sample was deemed to be a more desirable

candidate due to the likelihood of finding peptides of larger size and weight. Although our preliminary data relied upon LC-MS/MS based peptide sequencing techniques to produce chromatograms and deconvolute our data, we utilized MALDI-TOF MS identification techniques for our in-depth analysis due to several calculated benefits [15]. First, MALDI-TOF utilizes a protein fingerprinting method in which the sample is digested by a proteolytic enzyme such as trypsin and used to generate an MS spectrum that can be searched against a database. Matched hits are ranked according to a scoring method in which the candidate protein that contains more proteolytic peptides has a higher score and generally represents the protein/peptide that is most probable. The desirability of MALDITOF also includes the speed at which each run is performed-often less than one minute to obtain-and the speed at which analysis can be performed against a database. In contrast, data acquired through LC-MS/MS typically requires multiple hours to obtain due to the length of runs-sometimes requiring over thirty minutes for each run-and requires several hours to parse through the data to identify a potential candidate. Unlike MALDI-TOF, LC-MS/MS requires data to be manually sorted and compared against a database. This can be time consuming and won't necessarily guarantee the most accurate result.

Our group has invested significant time and resources to evaluate and manufacture MO peptides intended for use in peptide therapy across species [18]. The demonstration of therapeutic application has been limited by the gaps in knowledge regarding the key peptides in these solutions. To address the challenge, we have leveraged MS for the direct identification of molecules based on their mass-to-charge ratios and fragmentation patterns. Comparisons of experimental MS data with that of well-established open-source databases provided the capacity to identity of the molecules, peptides, or proteins found within a solution, at lower cost and rapid efficiency. In brief, our use of the technique of using Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF), a protein fingerprinting method in which the sample is digested by a proteolytic enzyme was used to generate an MS spectrum that can be searched against existing databases, which can subsequently ranked according to a scoring method in which the candidate protein that contains more proteolytic peptides has a higher score. In general, this represents the most probable protein/peptide. Using MALDI-TOF, eleven major peptide products of interest were identified (five in batch one [14,969 Da, 15,300 Da, 8,449 Da, 8,294 Da and 4,618 Da], six in batch two [four of the same peptides – 14,969 Da, 15,301 Da, 8,294 Da, 8,449 Da in size– and two additional peptides of 5,436 Da, and 6,214 Da in size). Slight differences in peptide products between batches are likely due to the heterogeneous nature of cellularly-derived solutions and differences that occurred during the extraction process. We further evaluated the peptides to identify the stability and significance in peptide therapy.

The group also conducted analyses to assess the impact of temperature storage and duration of storage on peptide stability. We tested the acidic peptide N-06 D, seven acidic peptide combination (N-18A), a 7-peptide combination (N-18A) stored at 4°C, N-06D peptides in liver samples stored at 4°C over one- three-and six months. extracted from liver samples stored at 22°C for one-, three-, and six- months. Showing complex spectra and intensity at one month, with significant declines by six months for each. Overall, the collected data suggested that acidic peptides stored at 22°C experience degradation over time, with a clear reduction in both peak intensities and overall peptide stability by six months of storage. The results of our study suggests that while some peptides remain stable, there are notable changes in the peptide profiles over time. Slight differences in peptide products between batches are likely due to the heterogeneous nature of cellularly-derived solutions and differences that occurred during the extraction process. This approach lays the groundwork to identifying potential adverse effects on stability, and therefore therapeutic potential to ensure the safe application of stem cell technologies in clinical and research settings. Understanding these risks is essential for advancing stem cell science while safeguarding public health and promoting the responsible development of stem cell-based therapies.

Exosomes

Since the discovery of exosomes as the fourth key player in cell-to-cell signaling, much has been discovered regarding the role they play in health and in diseases. Exosomes, small extracellular vesicles secreted by cells, including stem cells, facilitate intercellular communication by carrying proteins, lipids, RNA, and other bioactive molecules. In regenerative medicine, exosomes from stem cells act as key mediators of the therapeutic effects of stem cell therapy. They deliver regenerative signals to damaged or diseased tissues, influencing cell behavior, promoting repair, and reducing inflammation. Exosomes influence cell-to-cell communication locally and systematically, thus exhibiting both a paracrine and endocrine effect as they are carried by the environment into which they are secreted enabling action as protective shuttles to target cells, which is protected from DNAses and RNAses by the exosomal membrane. The protective exosomal membrane turn influence/change the behavior of the target cell, either promoting health or activating pathogenesis following fusion. Exosome "specificity" makes them suitable as biomarkers or predictors, and their "mobility" and "content" lend credence to drug delivery and therapeutic suitability as well as potential novel contributors within various pathways in the onset and progression of T1D.

As these advances continue to emerge through the medical technologies enhancing transplantation successes, there are several pieces of evidence that indicate that exosomes have utility in autoimmune diseases such as type 1 diabetes (T1D). Most notably, exosomes have been shown to provide preservation and survival of pancreatic islet cells mitigating complete destruction [28].

The pathogenesis of T1D involves a complex crosstalk between insulin-secreting pancreatic β-cells and immune cells, which is partially mediated by exosomes. β-cells secrete several exosomal miRNAs that stimulate monocytes and macrophages. In turn, signals from antigen-presenting cells activate T cells, leading to the synthesis and release of several miRNAs that induce apoptosis in β-cells. This vicious cycle is terminated only after the destruction of most of the β-cell mass. As such, the miRNA component represents a poten-

tial key contributor to the dialogue between pancreatic endocrine cells and the immune system. Crosstalk (i.e., dialogue between pancreatic endocrine and immune cells via RNA cargo of exosomes that is also functionally transferred to β-cells) between immune cells, especially macrophages, pancreatic endocrine cells, and insulin-target tissues occurring in T1D underlies the emerging link between exosomes and T1D. The enrichment of RNAi in exosomes and the underlying determination of the function of exosomes in T1D and T2D represent an auspicious area of further investigation. New technologies for the association of a specific marker with an exosome subtype and the exosome subtype with a particular function and/or group of functions warrant further investigation.

Discussion and Conclusion

The evolution from stem cells to stem cell-derived peptides and exosomes represents a scientific lineage that mirrors the desire for targeted, effective, and minimally invasive regenerative therapies. In musculoskeletal conditions like OA, this lineage has transitioned from direct cartilage repair to modulation of the joint microenvironment. Exosomes represent progression from cell-based therapies to increasingly refined, cell-free approaches. This evolution has been driven by the desire to reduce the complexities and risks associated with stem cell transplantation while retaining (and enhancing) therapeutic efficacy. In T1D, exosomes have shown promise in modulating immune responses, reducing islet inflammation, and enhancing beta-cell survival. Exosome-based therapies also aim to deliver beta-cell regeneration signals or insulin-mimetic peptides. Exosomes and peptides are now being integrated into biomaterials and scaffolds for enhanced delivery and localized action in cartilage repair. Exosomes loaded with specific growth factors or immune-modulatory molecules are being developed to complement beta-cell replacement strategies or even eliminate the need for transplantation. Similarly, in T1D, it has evolved from beta-cell replacement to immunomodulation and beta-cell regeneration. This progression not only highlights advancements in understanding cellular mechanisms but also opens the door for personalized, cell-free regenerative therapies.

Data Availability Statement

The data presented in this study are available in the study outlined.

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Conflicts of Interest

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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