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CASE REPORT

Novel isolation technology with convincing evidence in secretome clinical application: A Case Report

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ABSTRACT

A novel and natural mesenchymal stem cell isolation procedure without the incorporation of any chemical or biological agents from fresh umbilical cord through cultural expansion and harvest is described, the resulting cells can be maintained up to 20 passages and exhibited MSC morphology and characteristics. Proficient and straight forward secretome complex was harvested from these stem cell cultures after cells reached genetic stability. The process was straightforward, sustainable and scalable; the secretome complex depicted reproducible clinical end-point evident disease-modifying activity in various clinical applications. These stem cell isolation processes exemplified a proficient and sustainable cell expansion protocol and the harvested secretome component that depicted clinical efficacy warrants further investigation.

Abbreviations:

MSCs: Mesenchymal Stem Cells
nMS: Natural Multipotent MSC
UC-MSC: Umbilical Cord MSC
iPSC: Inducible Pluripotent Stem Cell
hPSC: Human Pluripotent Stem Cell
hUC-MSC: Human Umbilical Cord MSC
ISCT: International Society for Cellular Therapy

Introduction

The recent advent in frontier medicine involving stem cells is one of the most astonishing advancements in the medical field. The stem cells' self-renewing abilities and the capabilities to differentiate into specialized cells have been fully demonstrated. They serve as a reserved repair system for the body[1]. There are well known two main types of stem cells: embryonic stem cells and adult stem cells. Embryonic stem cells are pluripotent stem cells capable of differentiating into various tissues and organs in the body. Adult stem cells can be found in various tissues, they are multipotent cells, and there are five major types of adult stem cells: epithelial, hematopoietic (blood), mesenchymal, neural and skin stem cells[1,2].

Adult stem cell mesenchymal stem cells (MSCs) can modulate the immune system and have been used to successfully treat a variety of inflammatory diseases in numerous preclinical studies. MSCs show homing ability to sites of inflammation, injury and tumor. Recent evidence has implicated paracrine signaling as the predominant mechanism of MSC therapeutic activity[3].

MSCs appear to be an extremely powerful tools for tissue engineering and regenerative medicine, not just for their multilineage differentiation potential (MSC engraftment after *in vivo* delivery is low) but mostly for their paracrine activity, exerted through the release of soluble factors such as anti-inflammatory cytokines, anti-apoptotic and trophic molecules that stimulate tissue repair and counteract inflammation[3,4,5]. Stem cell technology and treatment methods can resolve many major, challenging diseases and injuries that individuals may encounter throughout their lives[5,6]. Nevertheless, based on current science, it is believed that the effect of stem cells in clinical trials conducted to date is not from the cells as such, but rather via the "secretome"[7,8].

The secretome is the totality of all messenger substances released to the outside of the cell, including the micro-vesicles and exosomes, but also hundreds of important factors that are dissolved in the fluid[7]. The secretome of cells encompass a diverse array of molecules beyond proteins involved in signaling pathways to have different functions, for instance, protein-like growth factors, hormones, and cytokines. However, which substances of the secretome trigger the actual effects is still largely unknown.

A stem cell's secretome is a bioactive molecule or substance composed of soluble proteins, immune cells, growth factors, and nucleic acids. It has five active components, namely pro-collagen, keratinocyte growth factor, vascular endothelial growth factor, fibroblast growth factor, and stromal cell-derived factor. The benefit of secretome therapy is that both the production and handling processes are readily efficient, convenient and achievable[6-8].

Techniques:

MSCs are cells with the capacity for self-renewal, and differentiate into the mesenchymal lineages, including skeletal myoblasts, chondrocytes, and adipose tissue. Recent reports have shown their ability to differentiate into neural (non-mesenchymal) tissues as well[1]. MSCs are the prime candidate for regenerative medicine and cell therapy. MSCs can be obtained and isolated from a variety of tissues such as the umbilical cord, the placenta, amniotic fluid, adipose tissue, etc. Their regenerative and immunomodulatory properties can promote recovery in damaged tissues, and their relative genetic stability makes them a promising choice of MSC isolation[2,3].

At Top IVF USA, we can deliver full-term infants, securing fresh umbilical cords from consenting donors: about 15–20 cm per umbilical cord. Nevertheless, the general health examination status of stem cell donors under evaluation would have included most of these medical tests: CBC, LFTs, RFTs, lipid profile, ECG (ECG), tumor markers, chest X-ray, spine X-ray, bone densitometry (DEXA), electrocardiogram (EKG), and cerebroamgiography, autoimmune antibody screening.

MSCs from umbilical cords were prepared by the tissue explant method as described with minor modification[8-10]. Briefly, fresh umbilical cord tissue from a healthy donor was minced into small pieces and were cultured in α-MEM (Thermo Fisher Scientific, Grand Island, NY, USA) supplemented with human platelet lysate (HPL, Fisher Scientific International, Pittsburg, Pennsylvania, USA). The medium was replaced three times a week, and adherent cells were allowed to reach 80 % - 85 % confluency before they were sub-cultured with trypsin-EDTA or preferably via biophysical technique to detach the cells.

It is not advisable to use tissue decomposing agents or digestive enzymes during the stem cell expansion process. Without adding any chemical agents, inducers, hormones, antibiotics, etc. during the isolation process and cultured in an incubator under a hypoxia condition (carbon dioxide, oxygen and balanced with nitrogen), the culture was observed under inverted microscope for cell attachment and any microbial contamination, and the medium was changed every three days. Reports suggest that culturing at physiological oxygen levels delays senescence and inhibits senescence-related genes, preventing cell cycle arrest[11]. The freshly isolated cells were considered P_0 and they should have saved the "most primitive, original stem cells" or the "progenitor" of umbilical cord mesenchymal stem cells. It is believed that these "ancestors" were the most effective MSC of clinical treatment. Cells can be propagated up to 20 passages and this naturally preparation MSC was called "nMS". We have validated the morphology, spindle shaped and fibroblast-like cells, and the expression of classical surface markers on these nMS cells, that were isolated from each umbilical cord donor; they were conformed to the International Society for Cellular Therapy (ISCT) cluster marker display characterization ($CD45^-$, $CD73^+$, $CD90^+$, and $CD105^+$).

MSCs have long been characterized with higher resistance to DNA damage and undergo apoptosis. Maintaining this stability involves various cellular mechanisms that safeguard genetic information from damage, mutations, or unwanted alterations[9-11]. The resulting nMS cells were harvested and expanded in vitro for 4-5 weeks and expanded when they reached ~80 % confluency in culture dishes[4,5]. Isolated cells were quantified, their morphology was assessed and cells in passage three (3) were characterized based on their immunophenotyping and differential potentials to ensure stem cell lineage characteristics.

In contrast to most published reports, nMS cells were subcultured for at least seven (7) passages, when cells will reach genetical stability and attach to the culture dish, only then was the "Secretome Complex" preparation harvested from the growth medium of 7-12 passage cells; no serum starvation, without centrifugation or any manipulation, basically they were culture media but free of stem cell debris nor contained any cells[9]. The cell-free bioactive secretome has been shown to consist of cytokines, proteins, growth factors, and various non-coding RNAs such as miR-21, miR-24, and miR-26 carried via exosomes secreted by actively culturing cells, no change in composition, in ready-to-use liquid format, and were provided to patient and orally administered[13,14].

The flavor was added to secretome complex preparations prior to treat patients with many various diseases' conditions, below are four examples:

Case 1: Patient Reference #1, Joseph R, a 64-year-old accountant, often felt uncomfortable on the left side of his chest because of stenosis of the blood vessels in his heart, left ventricle. He underwent a cardiovascular stent procedure. After the operation, he suffered severe pain in his chest, back and shoulders, and was prescribed and took painkillers pills, Chinese medicine, acupuncture, ointment, etc., all of which were ineffective. The patient was referred to our clinic and took the "Secretome Complex" prepared by Dr. Wang Funan, each dose was 70~75 mL three time daily, and after 2~3 days, all the pain had disappeared, and there was no visible side effect, and no recurrence of chest pain.

Case 2: Patient #2, A 68-year-old man with pain in his left shoulder, unable to raise his left arm vertically or backwards, there was no history of injury, nor any unknown cause, it was inferred that it was due to degenerative arthritis of the left shoulder, known as adhesive capsulitis, also known as frozen shoulder, which meant the ligaments of the shoulder have hardened, causing pain and limited movement. Anti-inflammatory painkillers and muscle relaxants were prescribed by the attending physician, in the Department of Internal Medicine of a renowned medical hospital. Western medicine painkillers and surgery were recommended to relax the effected tendon. He took Tylenol and Motrin, but all were ineffective; he also didn't want to have surgery. He was referred to our clinic and drank the "Secretome Complex" prepared by Dr. Wang Funan for three days, three times a day, and was cured. As of now (the end of April 2025) his left shoulder is completely healed, and he is free to move about his arm, with normal range of motion, and no recurrence of pain.

Case 3: Patient #3, A 36-year-old woman had regular menstruating cycle and endured pain for a long time, but her experience during the period

was so painful that she couldn't get out of bed. After the obstetrician and gynecologist carefully examined her, she was told that she did not have endometriosis, uterine fibroids, or ovarian cysts, which was "primary menstrual pain", and she often took menstrual pills (or birth control pills) to relieve the pain, but the effect was very limited. She also often took painkillers and acupuncture treatments, but she still experienced a lot of pain. She was referred to our clinic and took the "Secretome Complex" prepared by Dr. Wang Fu Nan for a while, and found the pain subsided and gradually disappeared in a week. Currently, she experiences no more "menstrual pain", and in a very happy note, she successfully conceived and gave birth to a healthy baby naturally.

Case 4, Patient #4, A 72-year-old male suffered from Osteoarthritis in both knees and often experienced pain during and after movement. One day he was walking on a stairway and felt a sudden excruciating pain from his knee joints. Dr. Wang provided two doses of "Secretome complex" per day and which the patient drank, and he felt no pain at all the very next day.

All patients described above were scheduled for follow-up visits to ensure satisfactory results, and complete and comprehensive treatment procedures and proper filing for good medical record keeping purposes were completed.

Discussion

It is an undeniable reality that umbilical cord and cord blood remain the world's largest source of stem cell rich tissue, and when handled appropriately, there would be with no ethical and legal concern. There are key characteristics needed for the UC-MSC cells to effectively participate in tissue regeneration for a particular or a series of disease conditions[1-3].

The stem cell preparation is of paramount importance, umbilical cords, which are largely composed of MSC, extracellular matrix components: collagen, chondroitin sulfate, hyaluronic acid and sulfate and osteoglycan. Their derivation from fetal tissues solves ethical challenges represented by using other cell types such as embryonic stem cells.

Our team has pioneered, discovered and invented the preparation of natural multipotent stem cells (nMS) and isolated nMS via a "natural way" excluding exogenous inducers to avoid the potential problems that iPSC encountered[15-19]. Our institution has successfully identified, cultivated, and demonstrated the therapeutic potential of different tissue-resident adult stem cell types for treating specific pathological disorders, for thousands of patients, including genetic and degenerative diseases, neurologic, and vital organs failure or malfunction[21,24].

The technology of nMS preparation is unique and has been used for clinical treatment and demonstrated the successful safe treatment of thousands of diseases with an outstanding efficacy record[20-24]. It has not exhibited any noticeable adverse side effects. The efficacy ratio of measurable clinical endpoint was greater than 90 % of natural culture "adaptation" isolation processes, nMS isolation, compared to induced stem cells. The lab has found and cataloged different types of stem cells preparation and has applied them to clinical treatment with great success[21]. The successful and conclusive clinical treatment of stem cells, and examples of relevant case reports have been published in peer-reviewed medical journals[20-23].

Reports indicated that chromosome stability after seven (7) generations is maintained by cellular mechanisms that counteract excessive variation and contribute to gene and genome stability[9]. It's evidenced that these nMS can be propagated up to 20 passages under proper handling, especially without using the digestive enzymes, and it is anticipated faithful transmission of genetic material from generation to generation. Both the morphology and CD marker expression of nMS were consistent with ISCT definition. Maintaining genome integrity throughout these processes is crucial for cell and tissue homeostasis, and failure to do so is associated with accumulation of mutations and chromosomal aberrations, which contribute to the aging process and can lead to diseases[9].

Human pluripotent stem cells (hPSC) are known to acquire chromosomal abnormalities, which range from point mutations to large copy number changes, including full chromosome aneuploidy. These aberrations have a wide-ranging influence on the state of cells, in both the undifferentiated and differentiated state[9,25]. On the contrary, multipotent mesenchymal stem cells have been

characterized with higher resistance to DNA damage and undergo apoptosis[9,10]. It's documented that cell cycle analysis of the third-generation hUC-MSCs cultured by modified enzyme digestion method indicated that most cells were quiescent. Immunofluorescence staining showed that these cells expressed MSC markers CD44 and CD90[16]. Taken together, this would argue the natural stem cell preparation technique, such as nMS, would be safer and superior to the conventional methodology available.

Many review articles indicate the main biochemical and physiological mechanisms of action of MSCs involve the following: Anti-inflammatory and immune-mediated action, angiogenic factor secretion, antioxidant factors production and anti-apoptotic reaction[11,18,25].

Minimal manipulation of the MSC, nMS together with cell-free secretome complex preparation was conducted; no filtration, no centrifugation, and no concentration steps were performed. Additional condition medium manipulation process might result in losing valuable secretome components compromising natural healing power and efficacy[8]. The ideal MSC isolation protocol might be the best. This novel yet naturally nMS preparation, without introducing any external chemical agents, inducers, hormones, digestive enzymes, catabolic enzymes, etc., might result in preserving the "most primitive stem cells" or the "progenitor" of stem cells and could enable these cells to be propagated up to 20 passages and maintain the intrinsic stem cell efficacy[24].

Secretome complex can be harvested starting with passage seven (7), and thereafter, that could ensure the genetic stability of stem cells as well as the preservation of secretome components during the harvesting processing; aliquot and natural isolation could be translated into large scale production using standard operating procedures.

The secretome of cells encompasses a diverse array of molecules beyond proteins. It includes not only soluble proteins such as cytokines, chemokines, and growth factors but also extracellular vesicles (EVs) like exosomes and microvesicles. These EVs also contain various biological molecules, including microRNAs (miRNAs), messenger RNAs (mRNAs), and other nucleic acids, which play significant roles in intercellular communication and modulation of physiological processes. However, the entity that impacts the successful cell and secretome therapy industry remains to be elucidated.

The great advantage of the secretome is that it can be frozen and, in this case, consists of minimal cell handling skill and equipment involvement. If stem cells are frozen, their properties change, and this can lead to a loss of efficacy. In the case of many chronic diseases, however, continuous therapy is needed, i.e. stem cells would have to be taken again and again[13]. In the case of secretome, equipped with quality by design and detailed planning, one can easily obtain 10-30 doses from one batch collection and thus perform repeated treatment over a period of 2 years.

Literature reveals there are five major components of secretomes: procollagen, keratinocyte growth factor, vascular endothelial growth factors, fibroblast growth factor and stromal cell derived growth factors; anti-inflammatory cytokines: IL-4 (15 kDa), IL-10 (18.6 kDa), IL-11 (19 kDa), IL-13 (12.6 kDa) and TGF-β (25 kDa)[26]. It's believed that these anti-inflammation cytokines, restoring immune homeostasis and preventing excessive inflammation, help combating the above three inflammation conditions, however which cytokine or growth factor is the major contributor remains to be investigated[26].

Furthermore, investigating the cell-secreted proteins not only might lead to creating specific treatments for a variety of diseases, but also could be beneficial to diagnose disorders in patients.

Conclusion

It is well understood that prior to harnessing their reparative nature for degenerative diseases, concerns regarding the genetic integrity and mutation acquisition of stem cells need to be addressed. Ensuring genomic stability in stem cell lines is required to achieve the quality control standards for safe clinical application[19].

The simplest MSC isolation and secretome complex harvest protocol might be the best scalable process procedure. This novel, straightforward yet natural approach, might not only preserve the "most primitive and effective stem cells", or the "progenitor" of stem cells, but also enable these cells to be propagated up to 20

passages and maintain the intrinsic stem cell regenerative and repair efficacy[24].

We have had many cases of acute and chronic trunk pain that have been treated with oral Secretome complex with excellent results, and we will not repeat them here. In the future, we will attempt using Secretome complex in treating other kinds of diseases, and hope to bring effective and alternative treatment options to patients, and will report in the near future.

To conclude, the secretome represents a frontier in biomedical research that will allow us to gain insight into the intricacies of disease mechanisms, cellular communication, and treatments.

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