ADVANCES IN STEM CELL THERAPY FOR REPRODUCTIVE MEDICINE: APPLICATIONS, TECHNIQUES, AND POTENTIAL OUTCOMES

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SUMMARY

Stem cell therapy, a revolutionary approach in reproductive medicine, is making significant strides, offering new avenues for addressing infertility and related reproductive challenges. This article delves into the impact of stem cells, particularly mesenchymal stem cells (MSCs), and their pivotal role in regenerative medicine. We explore various methods for obtaining stromal cells from adipose tissue, including direct approaches such as adinizing and enzymatic techniques and indirect methods like emulsification and microfragmentation/micronization. Direct methods, such as adinizing, preserve the fat tissue for graft use, while enzymatic methods discard the fat due to potential contamination. Indirect methods aim to increase the relative proportion of stromal cells by removing parenchymal cells. Additionally, we discuss the application of MSCs in treating conditions such as premature ovarian failure and Asherman's syndrome, highlighting their role in tissue repair and fertility restoration. Our findings underscore the transformative potential of stem cell therapy in reproductive health, offering promising solutions for individuals facing infertility and other reproductive challenges. The continued research and refinement of these techniques, along with the exploration of new methods and technologies, hold promise for advancing reproductive medicine, providing a wealth of knowledge for professionals and individuals interested in this field.
Keywords: stem cells, mesenchymal stem cells, adipose tissue, regenerative medicine, infertility, PRP, reproductive health

Stem cell therapy is an emerging and transformative field in reproductive medicine. With its potential to regenerate and repair tissues, it offers a new paradigm in fertility treatments, providing hope for individuals and couples struggling with infertility.

The study explores the roles of parenchymal and stromal cells, adipose-derived stem cells (ADSCs), and Platelet-Rich Plasma (PRP) in regenerative medicine. Methods for obtaining stro-

mal cells from adipose tissue are classified as direct (adinizing, enzymatic techniques) and indirect (emulsification, microfragmentation). PRP separation and activation processes are also detailed.

Parenchymal Cells:

Definition: Parenchymal cells are the functional cells of an organ or tissue. They carry out the organ's specific functions.

These cells are the workhorses of the organ responsible for its primary physiological functions. For instance, in the liver, parenchymal cells, known as hepatocytes, perform tasks like detoxification and protein synthesis.

Stromal Cells:

Definition: Stromal cells are the supporting connective tissue cells that provide structure and support for the parenchymal cells. They constitute the framework or matrix of an organ or tissue.
Function: Stromal cells are crucial in maintaining the structural integrity of tissues and organs. They also participate in various physiological processes, such as immune responses, tissue repair, and communication with parenchymal cells.

Adipose cells and adipocytes are terms used to describe the cells that make up adipose tissue, a type of connective tissue that specializes in storing energy in the form of fat. Adipose cells refer to the cells that make up adipose tissue. These cells are specialized for the storage of fat.
Function: The primary function of adipose cells is to store energy as triglycerides (fat). They also play a role in insulation, cushioning organs, and serving as an energy reserve.

Adipocytes are the specific cells within adipose tissue responsible for storing and releasing fat.
Structure: Adipocytes have a unique structure characterized by a large, centrally located lipid droplet (vacuole) that occupies most of the cell volume. The nucleus and other organelles are pushed to the periphery of the cell. The primary function of adipocytes is to store excess energy in the form of triglycerides when the body has more power than it needs. These stored fats can be released when the body requires additional energy.

Adipose tissue is essential for normal physiological function and plays a critical role in energy balance, insulation, and protection of organs. However, excess adipose tissue, especially visceral fat (fat around internal organs), is associated with various health risks, including cardiovascular diseases and metabolic disorders.

White adipose tissue (WAT) is the body's most common type of adipose tissue and is crucial in energy storage. It is mainly composed of adipocytes (fat cells) that store triglycerides. Two major types of white adipose tissue are subcutaneous and visceral.

Subcutaneous Adipose Tissue:

Subcutaneous adipose tissue is found beneath the skin (subcutaneous layer).

It is an energy reserve that provides insulation to regulate body temperature and a cushion that protects organs and tissues from physical trauma.

Subcutaneous fat is the fat that you can pinch between your fingers. It is distributed throughout the body but is commonly found in areas like the thighs, buttocks, and abdomen.

Visceral Adipose Tissue:

Visceral adipose tissue is located around internal organs in the abdominal cavity, such as the liver, pancreas, and intestines. While it also serves as an energy store, visceral fat is metabolically active

and associated with increased health risks. It produces hormones and cytokines that can affect metabolic processes, and excess visceral fat is linked to conditions like insulin resistance, cardiovascular diseases, and metabolic syndrome.

Adipose tissue has gained attention in regenerative medicine due to its potential as a source of regenerative cells and therapeutic applications. The process involving adipose tissue for regeneration is often called "liporegeneration." Here are some critical aspects of adipose tissue in the context of regeneration:

Adipose tissue is rich in a type of stem cell called adipose-derived stem cells (ADSCs). These stem cells can differentiate into various cell types, including adipocytes, myocytes, chondrocytes, and osteocytes.

The abundance of ADSCs in adipose tissue makes it a valuable source for regenerative purposes.

Adipose-derived stem cells, rich in regenerative properties, are believed to contribute significantly to tissue repair and regeneration. Their potential applications in various regenerative medicine fields, including wound healing, tissue engineering, and treatment of degenerative diseases, provide a wealth of knowledge for professionals and individuals interested in this field.

Receiving stem cells from Adipose Tissue

However, there is no accepted classification in terms of methods. In this presentation, a new classification is proposed for the first time. Accordingly, stromal cells can be obtained from adipose tissue by two approaches: direct methods for the bonds between parenchymal and stromal cells and indirect methods, which target parenchymal cells rather than strong bonds and increase the stromal cell ratio relatively. These methods can also be subclassified as fat (+), fat (–), and fat (±) in terms of using the remaining fat in the final product as a graft. Direct methods include adinizing and enzymatic techniques; indirect methods include emulsification and micro-fragmentation/ micronization techniques. In the enzymatic method, the fat tissue in the final product is considered dirty because it contains enzymes and must be discarded. That is why it is a fat (–) method. The adinizing method using ultra-sharp blades is fat (+) because the adipose tissue can be used after the procedure. Because the fat tissue is exposed to blunt pressure in emulsification techniques, it cannot be used as a graft. Thus, these are fat (–) methods. There may still be intact adipocytes in micronization techniques using filter systems; therefore, it should be classified as fat (±). Adinizing provides the highest efficiency and the full use of the end product. This detailed classification will guide clinicians in choosing the right product, making them feel more informed and knowledgeable.

DIRECT METHODS

Direct methods separate the stromal cells without killing the directly affected parenchymal cells by the bonds and bridges between parenchymal and stromal cells.

Fat (+) methods: Adinizing is a method where adipocytes and stromal cells are separated with ultra-sharp blades. The fat tissue can be used after the procedure, and the ECM is preserved. Copcu and Oztan named the final product obtained using TOST (total stromal cell).

Fat (–) methods: These involve obtaining stromal cells via enzymatic methods. The final product is called SVF (stromal vascular fraction); the fat obtained during this process is not used. It is

considered waste because of the potential risk of enzymes being in it. The loss is about 90% and almost ten times as many cells can be obtained by mechanical methods.

INDIRECT METHODS

These methods aim to remove the parenchymal cells entirely or partially and ensure that relatively more stromal cells remain in the final product.

Emulsification: Adipocytes, which are extremely sensitive to trauma, are emulsified by passing fat tissue between two syringes with the help of a nanofat connector stromal cells, which are more resistant to trauma, remain in the environment.

Microfragmentation/micronization: The aim is to fragment and eliminate the parenchymal cells, decrease their efficiency, and increase the relative stromal cell rate. For this purpose, filter, bead, centrifuge, membrane systems, and blunt pressure systems are used. There is little or no amount of adipocyte spring in the final product. Although these can be fat (±) depending on the extent of the pressure applied in the procedure, there will be some adipocyte death; so, most adipocytes will never survive, as in adinizing and Supplemental Digital Contents.

Ultra-sharp blades not only allow adipocytes to reach the desired diameter but also reveal their regenerative properties.

Enzymatic methods, such as collagenase or trypsin, aim to release stromal cells by dissolving the bonds between parenchymal and stromal cells.

Enzymatic methods are much more expensive, time-consuming, and complicated, requiring more equipment and staff than mechanical methods.

Enzymatic methods are considered dirty; they must be discarded after obtaining the stromal cell.

In mechanical methods, the aim is to cut the ligaments directly with ultra-sharp blades, defined as “adinizing” by Copcu et al. Mechanical methods are classified as “emulsification” and “micronization/microfragmentation.”

Copcu and Oztan have published a rather detailed review of mechanical methods. This study showed the evolution of mechanical processes and presented them in four steps.

These are:

1. Nanofat, defined by Tonnard,
2. “beads” system defined by Tremolada,
3. a “connector and filter system” defined by Cohen
4, and, finally, ultra-sharp blade systems defined by Copcu and Öztan, whose extensive literature review showed that the highest efficiency in terms of cell number and viability is in the exact blade systems.

They called cutting fat tissues with ultra-sharp blade systems “adinizing.” This method is not microfragmentation because it directly targets the stromal and parenchymal intercellular bonds, and while obtaining stromal cells, the parenchymal cells are not damaged.

Sources of Regenerative Cells: Regenerative cells refer to cells that have the potential to repair or replace damaged tissues in the body. These can include various types of stem cells, which can differentiate into different cell types.

Blood PPP and PRP: PPP stands for Platelet-Poor Plasma, and PRP stands for Platelet-Rich Plasma. Both are components of blood.

PRP is derived from a patient's own blood and contains a higher concentration of platelets than normal blood. Platelets contain growth factors that can potentially stimulate tissue repair and regeneration. PRP is sometimes used in regenerative medicine, particularly orthopedics and sports medicine, to promote healing in injured tissues.

Adipose Tissue: Adipose tissue is fat tissue. It is a rich source of mesenchymal stem cells (MSCs). MSCs have the potential to differentiate into various cell types, and they are being explored in regenerative medicine for their ability to promote tissue repair.TOST (total stromal-cell).

PRP + Mesenchymal stem cells + PPP is called Supercharged MEST

Stem Cells Hierarchy: Totipotent cells are the only cells that can form all the cell types in a body. They have the extraordinary ability to give rise to all cell types in an organism, including both embryonic and extraembryonic tissues. The term "totipotent" is derived from the Latin words "toti" meaning all and "potens" meaning potential.

During the early stages of embryonic development, totipotent cells emerge from the fusion of sperm and egg to form a zygote. The zygote undergoes successive cell divisions, and each resulting cell remains totipotent, meaning it has the potential to develop into a complete organism.

Pluripotent cells are a pivotal stage in cellular differentiation, possessing the ability to develop into many, but not all, cell types in the body. Unlike totipotent cells, which can give rise to both embryonic and extraembryonic tissues, pluripotent cells are primarily capable of forming the three germ layers: ectoderm, mesoderm, and endoderm.

Embryonic stem cells (ESCs) are a classic example of pluripotent cells. They are derived from the inner cell mass of the blastocyst during early embryonic development. Pluripotent cells have the remarkable potential to differentiate into various cell types, including neurons, muscle cells, and blood cells, among others.

Multipotent mesenchymal cells are a fascinating category of cells with a notable capacity for differentiation. These cells, often called mesenchymal stem cells (MSCs), exhibit the ability to differentiate into multiple, but more limited, cell types within a specific lineage.

Derived from various tissues such as bone marrow, adipose tissue, and umbilical cord, multipotent mesenchymal cells can give rise to cells like osteoblasts (bone cells), chondrocytes (cartilage cells), and adipocytes (fat cells). This differentiation potential makes them particularly valuable for regenerative medicine applications.

26 – Mesenchymal stem cells (MSCs) hold promise in regenerative medicine, particularly in addressing premature ovarian failure (POF). POF, characterized by the loss of ovarian function before the age of 40, poses significant challenges for affected individuals.

In the context of POF, MSCs can play a crucial role in regenerating ovarian tissues and restoring normal ovarian function. Studies suggest that MSCs, when administered either systemically or directly into the ovaries, may contribute to follicular development, enhance vascularization, and modulate the inflammatory microenvironment associated with POF.

The immunomodulatory and regenerative properties of MSCs make them potential candidates for promoting the repair of damaged ovarian tissue. Additionally, MSCs may stimulate the activation of dormant follicles, leading to the release of mature eggs. This offers hope for fertility restoration in women facing premature ovarian failure.

While research in this field is ongoing, the therapeutic potential of MSCs in addressing premature ovarian failure represents a promising avenue for the development of innovative and effective treatments. As we continue to delve into the intricacies of regenerative medicine, MSCs stand out as a beacon of hope for individuals grappling with the challenges of premature ovarian failure.

27- Mesenchymal stem cells (MSCs) present a compelling avenue for addressing the challenges posed by Asherman's syndrome. This condition, characterized by the formation of intrauterine adhesions or scar tissue, can lead to menstrual abnormalities, infertility, and recurrent pregnancy loss.

The regenerative properties of MSCs make them a potential therapeutic option for restoring the damaged uterine lining in Asherman's syndrome. By promoting tissue repair and modulating the inflammatory response, MSCs may contribute to the regeneration of healthy endometrial tissue, reducing adhesions and restoring the normal function of the uterus.

Research and clinical studies exploring the use of MSCs in Asherman's syndrome have shown promising results. Whether administered locally or systemically, MSCs have demonstrated their ability to differentiate into various cell types, including endometrial cells, fostering tissue regeneration and promoting a more conducive environment for pregnancy.

As we delve deeper into the potential applications of regenerative medicine, MSCs stand out as a promising tool in the quest to address the challenges of Asherman's syndrome. They offer hope for improved reproductive outcomes and quality of life for affected individuals.

31 - Mesenchymal stem cells (MSCs) exert their therapeutic effects through various mechanisms, making them a versatile tool in regenerative medicine. Here are critical aspects of their mechanisms of action:

1. Differentiation: MSCs can differentiate into various cell types, including bone, cartilage, adipose tissue, and more. This ability allows them to replace damaged cells and contribute to tissue repair.

2. Immunomodulation: MSCs possess immunomodulatory properties. They influence the immune system by suppressing inflammation and regulating immune responses, making them valuable in treating conditions where excessive inflammation plays a role.

3. Tissue Homing: MSCs have the capacity to migrate to sites of injury or inflammation in the body. This homing ability enhances their effectiveness in targeting and repairing specific tissues.

4. Paracrine Effects: MSCs secrete various bioactive molecules, such as growth factors, cytokines, and chemokines. These paracrine signals can stimulate tissue regeneration, reduce inflammation, and promote a healing environment.

5. Angiogenesis: MSCs can induce the formation of new blood vessels (angiogenesis), facilitating improved blood supply to damaged tissues. This is crucial for tissue repair and regeneration.

6. Anti-Apoptotic Effects: MSCs have anti-apoptotic (anti-cell death) properties, which can protect existing cells from undergoing programmed cell death in a damaged or inflamed environment.

7. Exosome Release: MSCs release extracellular vesicles, including exosomes containing proteins, nucleic acids, and other bioactive molecules. These exosomes contribute to MSCs' therapeutic effects, influencing neighboring cells and promoting regeneration.

40 - COMPONENTS OF PLATELET-RICH PLASMA (PRP)

Platelet-rich plasma (PRP) is a novel biomaterial that has garnered attention across diverse fields, including sports medicine and dermatology. This substance primarily comprises three key components, each playing a significant role in its therapeutic efficacy.

1. Platelets: Platelets are tiny, disc-shaped cell fragments that play a pivotal role in blood clotting and wound healing. In PRP, platelets are concentrated to levels higher than those found in normal blood. These platelets are rich in growth factors, which stimulate cell proliferation, tissue repair, and regeneration.

2. Plasma: Plasma is the liquid component of blood that carries blood cells and platelets throughout the body. In the context of PRP, plasma serves as a vehicle for delivering concentrated platelets to targeted tissues. It contains proteins, nutrients, hormones, and electrolytes that support cellular functions and healing.

3. White Blood Cells: While some PRP preparations focus solely on platelets, others include white blood cells. White blood cells are integral to the immune system's response and contribute to the body's defense against infections. In PRP, they may enhance the immune-modulating properties of the treatment.

These three components work synergistically in PRP to promote tissue repair, reduce inflammation, and accelerate healing. Whether used in orthopedics for joint injuries, dermatology for skin rejuvenation, or other medical fields, PRP is a testament to the potential of harnessing the body's own healing mechanisms for therapeutic purposes.

As we delve deeper into the applications of PRP, we witness a paradigm shift in how we approach healing and regeneration. This innovative approach has the potential to reshape the landscape of medical treatments, offering patients new avenues for recovery and well-being.

41 - PRP Separation: The journey begins with a small sample of the patient's own blood, usually extracted from the arm. This blood undergoes a meticulous separation process, often employing a centrifuge. The centrifuge spins the blood at high speeds, causing its components to separate based on their densities. Through this process, the platelet-rich portion is isolated, creating PRP.

Platelet Activation: However, the true magic lies in activating these concentrated platelets. Once separated, the PRP contains inactive platelets ready to unleash their healing power. Activation is often achieved by introducing substances like calcium chloride and thrombin or physical means like laser light exposure. This activation prompts the release of growth factors and other bioactive molecules from the platelets.

Growth Factors Unleashed: Activated platelets release a cascade of growth factors, including platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), insulin-like growth factor (IGF), and many others. These growth factors play a pivotal role in tissue repair, angiogenesis (formation of new blood vessels), and modulation of the inflammatory response.

In essence, PRP separation and platelet activation transform a small blood sample into a potent elixir rich in growth factors, ready to be strategically applied in various medical fields. From orthopedics for tissue regeneration to dermatology for skin rejuvenation, the applications of activated PRP are diverse, offering a glimpse into the future of personalized and regenerative medicine.

As we explore the realm of PRP, we witness not just a separation of components but a convergence of science and healing, unlocking the body's innate capacity to regenerate and repair.

Transvaginal PRP Intraovarian Injection: In this approach, a minimally invasive procedure is employed, where a thin needle is introduced through the vaginal wall to access the ovaries. The targeted delivery of PRP directly into the ovaries aims to stimulate follicular development, enhance vascularization, and create a more conducive environment for healthy egg maturation.

Laparoscopic PRP Intraovarian Injection: For those cases requiring a more detailed intervention, laparoscopic injection of PRP offers a surgical option. A laparoscope, a thin tube with a camera, is inserted through small incisions in the abdomen to precisely guide the injection of PRP into the ovaries. This method allows for a more comprehensive assessment of the reproductive organs while delivering the regenerative benefits of PRP.

Mechanism of Action: The regenerative properties of PRP, with its rich source of growth factors, may contribute to repairing damaged ovarian tissue, promoting follicular development, and improving overall ovarian function. This approach holds potential for women facing challenges such as diminished ovarian reserve or premature ovarian failure.

While this field is still evolving and further research is underway, the prospect of PRP intraovarian injection signifies a paradigm shift in fertility treatments. The personalized and regenerative nature of this approach aligns with the growing trend of tailored interventions in reproductive medicine, offering hope to those navigating the intricate journey of fertility challenges.

As we delve into this frontier, let us anticipate the continued progress and the transformative impact that PRP intraovarian injection might bring to the lives of individuals and couples aspiring to build their families.

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