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A New Source of Stem Cells: Mesenchymal Stem Cells from Menstrual Blood

Sydney R. Slattery and Vincent S. Gallicchio*

Department of Biological Sciences, College of Science, Clemson University, Clemson, SC 29634, Clemson, SC, USA.

*Correspondence:
Dr. Vincent S. Gallicchio, Department of Biological Sciences, College of Science, Clemson University, Clemson, SC, USA.

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ABSTRACT

Menstrual blood is a bodily fluid that consists endometrial cells, and, therefore, contains mesenchymal stem cells (MSCs). Over the past few years, these cells have been catching the attention of researchers and medical professionals due to the advantages they hold over alternative stem cell sources, such as the frequent production, pain free collection process, and lack of complex, ethical concerns associated with collecting the fluid. Menstrual blood-derived mesenchymal stem cells (MenSCs), still demonstrate the typical characteristics of mesenchymal stem cells, but research has found that they have an even faster rate of replication, as well as an increased proliferation and differentiation potential compared to other stem cell sources. Though MenSCs have been gaining more attention and there is evidence showing that would work well to help slow the progression or cure many diseases, there are still several unanswered questions and the occasional concerning data, which creates a need for further preclinical and clinical research before it is a well-accepted tool in medicine.

Keywords

Stem cell, Menstrual blood, Endometrial, Trials.

Abbreviations

ASCs: Adult Stem Cells; CPD: Citrate Phosphate Dextrose solution; CPP: Chronic Pelvic Pain; CFU: Colony-Forming Unit; D&C: Dilation and Curettage, EDTA: Ethylenediaminetetraacetic Acid, ESCs: Embryonic Stem Cells; EndoSCs: Endometrial Stem Cells; MenSCs: Menstrual Blood-derived Stem Cells, MSCs: Mesenchymal Stem Cells, MLR: Mixed Lymphocyte Reaction, POF: Premature Ovarian Failure.

Introduction

Stem cells, independent of the source are capable of dividing and renewing over an extended period of time, unspecialized, and can become specialized cells. Adult stem cells (ASCs) are constantly growing as a topic of research due to continual new findings related to these undifferentiated cells and the potential the cells have in regenerative medicine. As research continues, more tissues are being found to be sources of ASCs. There are two different types of adult stem cells – hematopoietic stem cells and mesenchymal stem cells (also referred to as bone marrow stromal stem cells) [1].

Research is being pursued on the various types of ASCs from several areas of the body and tissues, and most of this is done in vitro. Scientists are continuing research and working to manipulate the cells in order to create cells types that are able to fight or treat diseases that currently have little to no treatment available [1].

MSCs are currently used frequently within clinical trials and research as a way to cure disease – from a variety of cancers to Alzheimer's [2]. These cells have been found to be effective and best at treating immune and inflammation-mediated diseases [2]. MSCs currently define non-blood stem cells from several different tissues, but there is no certainty in the fact that these stem cells are the same from all of the tissues [1]. The following criteria was generated in 2006 by the International Society for Cellular Therapy, to define MSCs [3]:

- plastic adherent in the environment of a typical culture
- express specific surface markers – CD105, CD73 and CD90 – but also lack expression of CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR,
- *in vitro*, ability to differentiation into osteoblasts, adipocytes, and chondrocytes.

These cells can differentiate into several types of cells within the human body such as cartilage, bone, fat, muscle, marrow, tendon, adipose, and connective tissues [4]. The multi-lineage potential

and ability to be genetically modified, is what makes MSCs a common topic of research.

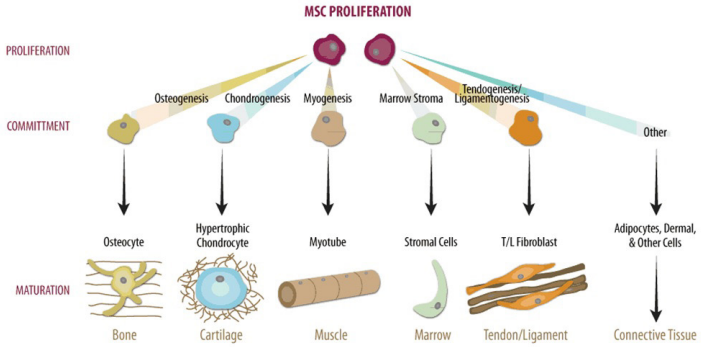

Figure 1: MSC have the potential to differentiate into a wide range of cell types [5].

A recent finding has drawn attention to a new method of stem cell collection – menstrual blood [6]. Menstruation is a process that occurs each month for women from menarche (usually between ages 11 and 14) until menopause (around age 51) [6]. This regular, vaginal bleeding that occurs, happens due to the lack of pregnancy; the body prepares for embryo implantation, and then pregnancy, but the uterine lining is shed when this does not occur [7]. On average, every woman sheds the endometrial lining more than 400 times; this means there is a constant cycle of regeneration, differentiation, and shedding during the reproductive years of a woman's life [8]. When the endometrial lining is shed, a fluid referred to as menstrual blood is produced, and this is what is expulsed from the body each month. This fluid contains blood, vaginal secretions, and endometrial cells of the uterine wall [7].

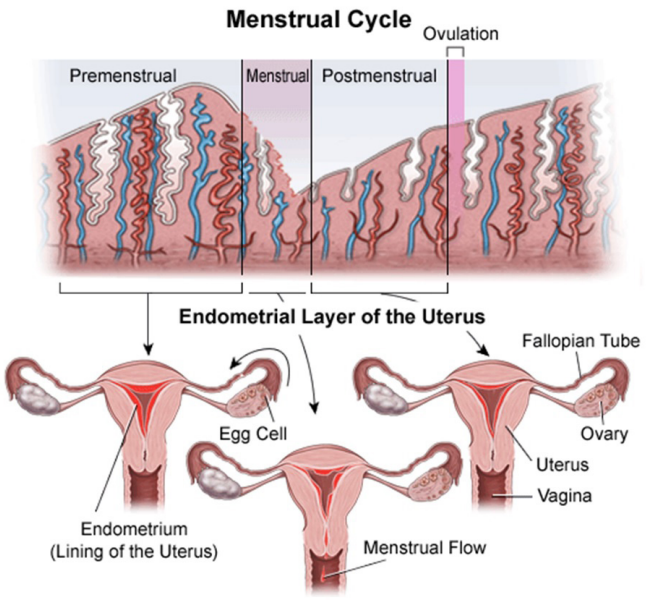

Figure 2: Menstrual cycle and the shedding of the endometrium [9].

It is evident that these cells, within the endometrium, play an important part in cyclic regeneration and repair – which could be vital in finding other common diseases these cells may be beneficial in curing or reducing the effects of [8]. As endometrial stem cells (EndoSCs) are released within the menstrual blood each month, they contain epithelial, stromal, and endothelial cells, all of which factor into that cyclic regeneration of the endometrium. Since these cells are released by the body via menstrual fluid, they are referred to as menstrual blood-derived stem cells (MenSCs) [8].

Discussion

Are Menstrual Blood-Derived Stem Cells the Better Option?

The question now is whether or not this source of stem cells could be a better candidate or more efficient method of stem cell collection. There are several reasons that indicate it does overcome a lot of the current issues (ethical, physical, and pain-related), that are associated with previous methods of stem cells such as embryonic, bone marrow (hematopoietic), cord blood, and adipose tissue. The most common method of gathering stem cells currently is through bone marrow collection – a highly invasive and painful procedure. The collection of menstrual blood however, is a pain free process. In terms of ethics, there is a lot of debate over whether collecting embryonic stem cells (ESCs) is an ethical procedure; so, this offers another positive aspect of MenSCs since it avoids that ethical debate [10].

Another source, stem cells from the endometrium, was a recent discovery in research. This lining was found to be a large source of stem cells, which could help to continue growing the field of regenerative medicine. However, the same issue arises with this as with bone marrow; it is an intrusive procedure. Yet, since the originally finding, researchers have been able to find ESCs within menstrual blood and have the same crucial characteristics of other stem cells – ability to replicate and differentiate into numerous cell types [11].

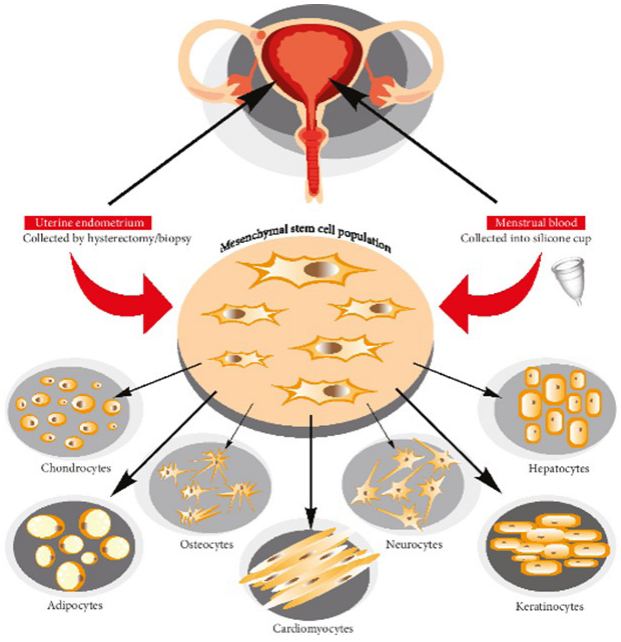

Figure 3: Mesenchymal stem cell proliferation from two sources – uterine endometrium and menstrual blood [12].

Additional research has found that this source of stem cells, has an even faster rate of replication than stem cells drawn from umbilical cord blood and bone marrow, another benefit of this new source. This is due to a unique growth factor that is created by this cell due a fundamental part of the uterine phase of the menstrual cycle: the growth of new blood vessels from previously existing vessels [13].

MenSCs, which do have the necessary characteristics of typical adult stem cells (ASCs), have also been discovered to have low immune responses [15]. This is due to the lack of expression of the HLA-DR surface markers [3]. HLA is the human leukocyte antigen, which is also referred to as the "immune privilege status" of MSCs. The HLA incompatibility provides very little issues, making these cells ideal for transplantation since the subjects being tested are less likely to reject these cells in comparison to other types of stem cells [16]. And, based on existent research, transplanting MenSCs into animal models has not only never resulted in any immune response, but has not produced teratoma or ectopic formation [17].

Though MenSCs have been studied and seem to outdo the negative effects previously encountered by other stem cell sources, there is still limited research on the topic and a lot of issues that need to be overcome. There are very few stem cell treatments that are completely safe and proven effective today, and these consist mainly of hematopoietic stem cell treatments. But, since the same treatment is likely to be ineffective for different diseases, it is important that the topic continues to be researched and better understood because this will allow for what is currently just science to grow and change into medical treatment [18].

Endometriosis

Endometriosis has been an interesting topic of stem cell research. Treatment for this disease currently consists of hormone therapy to prevent growth and a laparoscopic surgery to remove the lesions caused by the disease [19]. This disease currently effects more than 7 million women in the U.S. and has serious effects such as infertility, chronic pelvic pain (CPP), and the need for surgery, as mentioned [20].

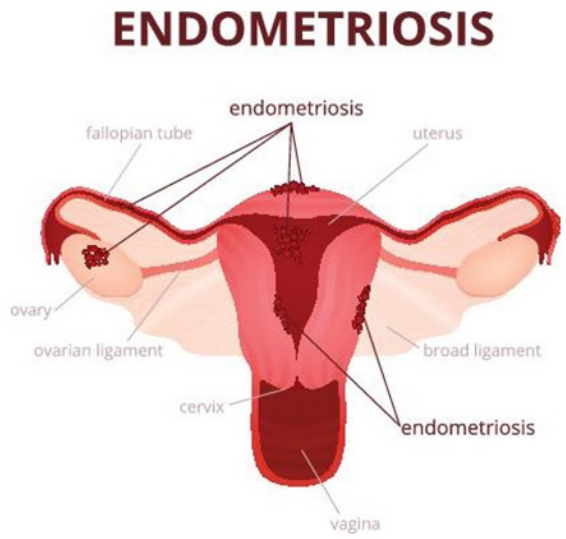

Figure 4: Endometrial lining from the uterus attaches to areas and organs outside of the uterus with no way to leave the body once attached [20].

While there is evidence that supports stem cells could potentially help or be a method of curing endometriosis, there is also opposing evidence – that stem cells are the cause of this disease. The theory that stem cells are the cause of the disease is known as the Stem Cell Theory of Endometriosis, which attributes endometriosis to the regenerative properties of stem cells [21]. The regenerative characteristic of cells within the uterus walls is crucial due to the constant shedding and regrowth that occurs each month [8]. However, this theory suggests that when these cells are shed each month (through the shedding of the endometrial or uterine lining), there is the chance that the cells end up outside of the uterus where they continue to naturally regenerate. Then, since they are capable or differentiating, if they happen to differentiate into endometrial cells, it could lead to endometriosis. Though the continued regeneration and spreading of these cells within the pelvic cavity, the severity of endometriosis increases [21].

This theory is often combined with Sampson's theory of Retrograde Menstruation, which is simply the idea that during a menstrual cycle, the blood flows back into the body. When this occurs, the tissue that has been shed from the endometrial lining is carried back into the body by the blood. Thought retrograde menstruation does in fact occur in every woman, the relationship directly with endometriosis is still unknown and does need to be studied more [22].

An additional theory suggests that the endometrial stem cells within bone marrow may link bone marrow to endometriosis. The thought behind this is that since the bone marrow circulates throughout the body, and consists of so many different cell forms, it could explain why the disease causes lesions to reoccur despite surgical treatments [21].

Contrary to these theories, however, are the studies that have shown stem cells to be effective against inflammatory diseases [2]. Because these cells do exhibit those regenerative abilities, there have been effective at repairing and curing so many diseases, and research has shown that using MSCs as cell-based therapy can significantly reduce inflammation. Other researchers have started to study the effect of MSCs as drug carriers since they do not evoke a response from the immune system [21].

Donating & Banking Cells

Most women, who are within the reproductive age, are able to retrieve stem cells from their menstrual fluid since all it takes a sample from one day during their period. As long as the women have normal menstrual cycles, ovulation, and endocrine functions, there is nothing to prevent them from collecting their sample when everything seems biologically normal and healthy [23].

One procedure that disrupts the uterine lining is a Dilation and Curettage (D&C), which is a quick process, usually only lasting about 20 minutes, and the patient receives anesthesia. A D&C

is used following a miscarriage that occurs prior to the 20-week mark of the gestation period. At this stage, when a miscarriage occurs, part of the fetus and placenta remain within the uterus. Because they are not naturally expelled, this procedure removes the contents of the uterus along with the uterine lining. However, there is evidence indicating that this procedure does not have negative or long-lasting effects. Following the procedure, within about 4-6 weeks, a woman's normal menstrual cycle should return; and, within 3 cycles, the uterine lining should be completely restored [24]. Additionally, studies have shown that within a year of having a D&C because of a failed pregnancy, 87% of women can get pregnant [25].

Collection Method

The process of collecting menstrual fluid is a non-invasive process. Prior to the process, and for the most sterile results, it is important that the participant showers, and empties their bladder completely – this is just to prevent the need to during the time of collection. A silicone menstrual cup (frequently referred to as a diva cup), is placed within the vagina in order to collect the menstrual fluid [26]. The placement of the cup should be somewhat low – about one centimeter from the vaginal opening and it is important that it is not too high and not over the opening of the cervix. This is important to prevent any leaks or an uncomfortable experience, which can occur when it is in too high or against the cervix [27].

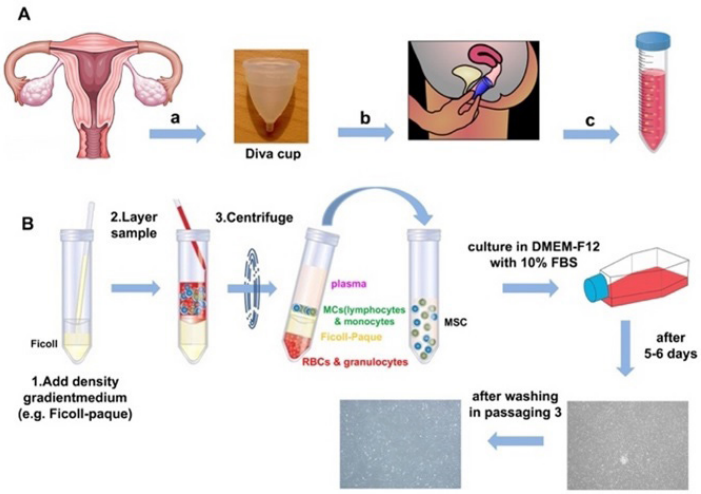

Figure 5: Collection method for menstrual fluid and procurement of stem cells [26].

Anticoagulants

The body naturally creates blood clots when blood leave the body, which means that anticoagulants must be added during the stem cell collection process to prevent the blood from clotting. In general, heparin, is the most commonly used anticoagulant, and, when collecting stem cells, is most likely to be used; however, different coagulants can be used to collect blood and include ethylenediaminetetraacetic acid (EDTA) and citrate phosphate dextrose solution (CPD), in addition to heparin [28].

Since the use and type of anticoagulant can have a serious impact on the ability to use the stem cells that have been collected, studies have used different anticoagulants to test one against another. A study published in 2009, used both CPD and heparin with cord blood to see which produced better results, and found that CPD produced more nucleated cells than heparin – despite previous research and cord blood banks often recommending heparin [29]. However, in 2017, a study looked at bone marrow stem cells cytotherapy and notes the benefits of heparin [30]. During the infusion process, the use of heparin reduced coagulation and the rejection of the stem cells, which increased the efficiency of the therapy and decreased many of the potential side-effects [30].

As far as menstrual blood collection and anticoagulants, there is a lot of missing information and many studies do not discuss the necessity of anticoagulants in the process. One study from 2010, examined the benefits of having "individually tailored donor cells" and the potential benefits these cells could have for cell therapy, specifically in stroke victims [31]. During the collection process, which took place in an approved facility, about 8-10 milliliters of menstrual fluid was collected. Once collected, at least a million cells were required for the culture, and throughout the isolation process DPBS (a buffered saline media) was used, which contained heparin [31]. However, another study completed in 2015, collected menstrual fluid from five donors by use of a menstrual cup, and then transferred the contents into a collection tube [33]. At this point, phosphate buffered saline (PBS) was added, along with antibiotics and EDTA. The use of EDTA allowed for adherent cells to be detached [32].

Since it is not clear which anticoagulant is the most effective, and various studies have used either heparin or EDTA, it is evident that there is a lack of data and information regarding anticoagulants and stem cell collections – even more so, specifically for the collection of MenSCs.

Introductory Studies

A study published in the Journal of Women's Health in 2019, looked at women's attitudes, thoughts, and opinions regarding MenSCs and their willingness to donate menstrual fluid for medical purposes by surveying 100 women. This study found that, in general, women are willing to donate and have relatively positive thoughts on the process, which reflected a relatively positive future for this process. One interesting finding of this study showed that as women become more educated on MenSCs and the potential use of the cells, many women have a better outlook on periods in general – this is true for about 40% of the women that were surveyed in this study [33]. The table below is also pulled from this study and demonstrates different categories of participants depending on their most commonly used menstrual hygiene product and the percentage of people within that group who would be willing to donate menstrual fluid. Based on the data within this table, no significant association was found between the women in each group; however, it is interesting to note that those who used menstrual cups were willing to donate 100% of the time, yet compiled a much smaller percentage of the surveyed population [33].

Participants' Willingness to Donate Menstrual Blood Depending on Preferred Menstrual Hygiene Product (Internal and External)

Study	*Percentage of participants surveyed*	*Percentage that would donate menstrual blood*	p
Preferred menstrual hygiene product			0.819
Sanitary towel	36	77	
Tampon	30	75	
Menstrual cup	3	100	
Combination[a]: Tampons and sanitary towels	22	69	
Combination: Menstrual cups and sanitary towels	5	100	
Combination: Tampons and menstrual cups	4	100	
Type of menstrual hygiene product used:			0.914
Internal (*i.e.*, tampon, menstrual cup)	64	78	
External (*i.e.*, sanitary towel)	36	77	

[a]Participants that regularly use more than one type of menstrual hygiene product.

Table 1: Survey of women from the Journal of Women's Health, 2019, testing the relationship between hygiene product and willingness to donate menstrual fluid [28].

Preclinical Studies

Menstrual blood-derived mesenchymal stem cells are becoming a common subject amongst research as the concept becomes more well-known. A study on premature ovarian failure (POF) due to chemotherapy, used MenSCs and Bushen Tiaochong to improve POF within mice, which was done by inhibiting expressing GADD45b. Studies have shown that epirubicin induces POF, so this research used mice injected with epirubicin to study the effect of the MenSCs on the disease. The result was that the MenSCs reversed the effect that the epirubicin treatments had on the mice and were capable of improving the ovarian function. Though the MenSCs were effective, the most effective treatment was the combination with Bushen Tiaochong [34].

Another study was able to use MenSCs that had differentiated in vitro into nerve cells on rats [35]. These rats were animal models for stroke patients, which is simply characterized by neural cell death due to an interruption in blood supply to the brain. The stem cells that were introduced into the rats led to a reduction in behavioral impairments when compared to the control rats. However, although tissue repair is witnessed within laboratory settings, the significance of its contribution within this study is not completely clear. The conclusion of this study did find that MenSCs have therapeutic functions and in the future may be a great benefit in decreasing the disabilities caused by strokes in patients [35].

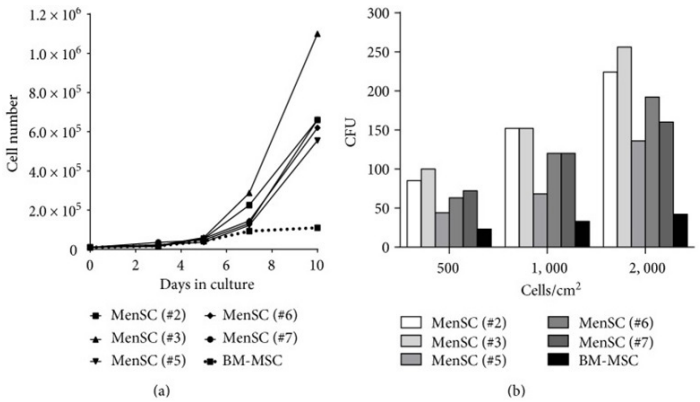

Figure 6: Growth of MenSCs from different donors over several days and the results of a colony-forming unit (CFU) assay [36].

An additional study tested the idea of using menstrual blood stem cells as cell carriers for oncolytic adenovirus. Within this study, the measured the growth potential of MenSCs (Figure 6), which was then compared to the potential of bone marrow stem cells. The morphology of the two stem cell types was found to be very similar, but when comparing the proliferation of the stem cells, MenSCs was found to have a higher growth potential than those from bone marrow. In the end, the conclusion was reached that MenSCs could be a strong alternative as oncolytic adenovirus carriers [36].

MenSCs abilities to inhibit the division of HeLa cells were studied within another experiment. This experiment tested the MenSCs against NIH 3T3 cells, when working against HeLa cells (an immortal cell line, which stems from cervical cancer cells). The study found that the stem cells had strong properties that worked against the cervical cancer cells. The MenSCs were found to secrete high levels of TGF-β, which cause cell cycle arrest and, therefore, prevent the proliferation of HeLa cells. In the end, this study supported the use of MenSCs in antitumor therapy for cervical cancer [37].

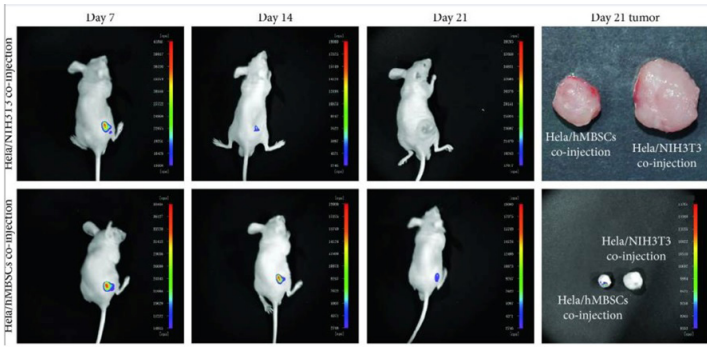

Figure 7: Fluorescent imaging with side-by-side comparison of tumor from mice injected with MenSCs versus NIH3T3 [37].

Studies have examined the potential for MenSCs to be used as therapeutic treatment in cardiac disease, mainly because it is still the leading cause of death worldwide. The conclusion reached within these is that due to the easy and high collection rate, MenSCs offer a great alternative to the stem cells currently being used. Also, because of the cells ability to differentiate and cause little to no immune response, they have been effective in helping damaged myocardial tissue regenerate and improve overall cardiac function within preclinical and clinical studies [38].

Additionally, a study tested the ability of MenSCs to differential into functional hepatocyte-like cells by isolating the cells from donors and then separating out the mononuclear cells. The cells were then transferred into mice with liver damage. This study is working to help with the shortage of liver transplants needed that do not have a match. So, instead the goal is to create these cells that will be able to treat or slow the progression of liver disease. Following the study, and then checking again two months later, the MenSCs did not lead to the development of tumors within the mice. Therefore, it is concluded that the hepatocytes generated from MenSCs are safe for in vitro transplantation and could lead

to an alternative treatment plan [39].

One study does note that there are some research findings (a very small portion however), that do show immune cells may be negatively affected by MenSCs. This study shows that an allogenic mixed lymphocyte reaction (MLR), which is a test used to show the safety of a drug by pharmaceutical organization, may be inhibited or stimulated based on the concentration of MenSCs, but also that the cells may prevent monocyte-derived dendritic cells from reaching optimal maturation [40,41]. In order to further look into the potential pitfalls of MenSCs, this study tested the effects of MenSCs on natural killer (NK) cells. This study found that these cells encourage the proliferation of NK cells, which is contrary to the typical action of stem cells from other body sources, which inhibit NK function. This could be an issue because limiting NK cell function often can be beneficial since their presence or overabundance can quickly become a bad thing within the human body [40].

Clinical Studies

Few studies have actually been performed using menstrual blood-derived stem cells as there is still so much unknown about the cells. Two studies have been done in China that use MenSCs as a method in treating two different diseases and were completed in 2014 and 2015.

The first study looked at the transplantation of MenSCs as a method of treating patients with Type I Diabetes Mellitus [42]. The stem cells were infused into the patients via the pancreatic artery or intravenously. During the study, the usual control, exogenous insulin injection, was still administered daily. This study's primary measure was glycosylated hemoglobin (HbA1c) over the course of a year [42].

The other study collected data on the use of MenSCs and their effectiveness against liver cirrhosis. To date, the best way of treating this disease is with an orthotopic liver transplant; however, the high rate of rejection amongst other issues limits the ability to actually practice this method of treatment. Two groups were measured and monitored: one with conventional therapy plus intravenous injections of MenSCs, and the other with conventional therapy and a placebo treatment. This study will examine whether or not MenSCs are effective in improving the conditions of the patients with liver cirrhosis [43].

Conclusion

In summary, menstrual blood-derived mesenchymal stem cells have the potential to treat many diseases. Though there are no current clinical studies demonstrating the effect of these stem cells on patients, there are many preclinical trials that show the ability of these cells to reverse or slow the progression of diseases (in vitro and within mice used for the studies).

One of the biggest issues with this type of study is the stereotype that surrounds menstrual fluid, but also, the lack of knowledge about stem cells. Simple education, as seen within a study, can change the opinions and thoughts related to menstrual cycles. Another thing that could impact the future use of MenSCs, is the availability of collecting and banking these cells. Unlike bone marrow-derived stem cells, there is no current payment for donations or large companies that offer sterile collection and banking procedures. More research and review of current data is needed as there are still a lot of gaps within the data. One specific gap in the data is information regarding the collection process, what anticoagulant is used in each study, and why it is used. There also is still a need to ensure the effectiveness of MenSCs, especially for the diseases that have no real cure or treatment available prior to the start and completion of more clinical trials.

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