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Original Paper

Evaluation of Immunogenicity of Alginate Encapsulated Human Wharton's Jelly-Derived Mesenchymal Stem Cells in the Peritoneal Cavity of Rat

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Background: Human Wharton's Jelly-derived Mesenchymal Stem Cells (hWJ-MSCs) have immunosuppressive and anti-inflammatory properties. As such, they exhibit an attractive therapeutic option for autoimmune disorders. Microencapsulation provides adequate protection against immune destruction of transplanted cells. We aimed to investigate the cellular and humoral immune responses in the host rats transplanted with alginate microencapsulated hWJ-MSCs in their peritoneal cavities.

Methods: hWJ-MSCs were microencapsulated in alginate microspheres and were transplanted intraperitoneally to rats. After 30 days, for the evaluation of rats' cellular and humoral immunity against hWJ-MSCs, the spleen mononuclear cells of rats were stained with CFSE and co-cultured with hWJ-MSCs for 3 days. Then the proliferation of spleen cells against hWJ-MSC was evaluated by flow cytometry, and Interferon-gamma (IFN-γ) secretion was measured by ELISA. For evaluating humoral immunity, the serum of different rat groups was incubated with hWJ-MSCs, and serum-mediated cytotoxicity was measured by MTT assay and antibody binding by cell-based ELISA.

Results: There were no differences between the spleen mononuclear cells proliferation, secretion of IFN-γ, serum-mediated cytotoxicity, and anti-WJ-MSCs antibodies in rats transplanted with encapsulated and non-encapsulated hWJ-MSCs. Encapsulated and non-encapsulated hWJ-MSCs did not stimulate the immune system in rats at the same time.

Conclusion: We suggest using a 3D culture for proliferation and having a more favorable function in the transplantation of these hWJ-MSCs.

Keywords: Alginate, Immunogenicity, Human Wharton's jelly-derived mesenchymal stem cells, Microencapsulation

Introduction

In the last decade, the therapeutic use of Mesenchymal Stem Cells (MSCs) has increased dramatically. The weight of existing evidence backing that the short-term application of MSCs is safe and possible. Not only do concerns stay over the possibility of unwanted long-term effects in addition to unwanted interactions of MSCs with the host immune system, but MSCs may also promote tumor progression or even result in cancer themselves [1].

Because of the easy harvesting and the high quantity obtained from these sources (Wharton's jelly), these cells were used in this study. MSCs express adhesion molecules of CD44 and CD105, markers integrin of CD29, CD51, and mesenchymal stem cells markers of SH2, SH3, CD68, and CD73, but they do not express markers of CD34 and CD45 [2].

Human Wharton's Jelly-derived Mesenchymal Stem Cells (hWJ-MSCs) is a new source of MSCs that shows a high degree of self-renewal capacity and multi-differentiation potential. hWJ-MSCs have a wider range of collection sources than Bone Marrow Stem Cells (BMSCs) [3]. However, the immune rejection problems associated with their use need to be solved before they can be considered for successful transplantation. As previously demonstrated, MSCs can suppress lymphocyte

proliferation induced by Phytohemagglutinin (PHA), and that they are not restricted by Major Histocompatibility Complex (MHC) [4].

Mesenchymal Stem Cells (MSCs) derived from the umbilical cord's Wharton jelly can migrate to the damaged area and differentiate into different tissue cells and restore tissue. Besides, they are beneficial for tissue regeneration and function in clinical applications for low immunity [5].

MSCs express adhesion molecules (CD44 and CD105), markers integrin (CD29, CD51), and markers of mesenchymal stem cells (SH2, SH3, CD68, and CD73), but they do not express the markers (CD34 and CD45) [2].

These unique immunological properties of MSCs increase their potential for using in the organ transplantation, the prevention of rejection, and the treatment of autoimmune diseases [6].

MSCs have been identified as an agent that regulates the immune system. The immunomodulatory impact of these cells on a variety of immune cells, including lymphocytes, T, B, natural killer cells, neutrophils, macrophages as well as dendritic cells, resulted in using these cells in the treatment of inflammatory and especially those such as multiple sclerosis and rheumatoid arthritis are autoimmune.

Alginate is mainly isolated from marine algae and is one of a few substances that, in physiological conditions, causes the microencapsulation process to occur. Microcapsules are done at the body or room temperature and in physiologic pH to an isotonic solution. Microcapsules for small spherical and small size compared with large capsules provide a suitable level in proportion to the volume and capacity for a proper release. The other advantage is that they are not easily broken and mechanically steady, and they do not require complicated and costly construction techniques [7].

MSCs have an advantage of safety, and more importantly, they show immunosuppressive capacity, although the underlying mechanisms of MSCs in immunosuppression have not been determined. The properties of their immunosuppression have been used in previous clinical studies such as maintaining the tolerance of the transplantation, autoimmunity, and maternal-embryo tolerance [1].

Moreover, in multilineage differentiation, MSCs also have strong immunomodulatory effects comprising inhibition of proliferation and function of T cells, B cells, and natural killer cells [8-11].

Mesenchymal stem cells are immune-privileged because of their low immunogenicity. In other words, they express deficient levels of MHC class I, no MHC class II, and do not induce activation of allogeneic lymphocytes [12]. In vitro, MSCs effectively exclude the proliferation of syngeneic and allogeneic T cells equally, suggesting that their immunosuppressive properties do not depend on MHC expression by MSCs and lymphocytes [13, 14].

Hypothetically, their low immunogenicity would enable allogeneic MSCs to escape the allogeneic immune system and allow their usage across MHC barriers. Mesenchymal Stem Cells (MSCs) can become potently immunosuppressive via unknown mechanisms. It was found that the immunosuppressive function of MSCs is evoked by Interferon-gamma (IFN-γ) and the simultaneous presence of any of three other proinflammatory cytokines, Tumor Necrosis Factor-alpha (TNF-α), Interleukin (IL)-1a, or IL-1b. These cytokine combinations provoke the expression of high levels of several chemokines and inducible nitric oxide synthase by MSCs [15].

This immunoisolation by encapsulation not only allows successful transplantation of cells in the default of immunosuppression [16, 17] but also enables transplantation of cells from the nonhuman origin, i.e., xenografts, which could be a mean of overcoming the impediment of a limited supply of donor tissue till exogenous be applied in a wide range of treatments organ shortage [18, 19].

The immunoregulatory properties of hWJ-MSCs have not been fully defined. IFN-γ activates and develops lymphocyte function as a positive immune regulator in immune rejection. In this study, the immunological characteristics of hWJ-MSCs encapsulated in alginate and their effects on lymphocyte proliferation and the secretion of IFN-γ were investigated. Moreover, we investigated whether direct cell-to-cell interactions and soluble factors such as IFN-γ were essential for balancing hWJ-MSC-mediated immune regulation in the discrepancy-free cell (cells with no encapsulation) [6].

This study aimed to evaluate the immunogenicity of hWJ-MSCs in both capsulated and non-encapsulated states and their performance in these two situations.

Materials and Methods

Isolation and Culture of hWJ-MSCs

Umbilical cord collection was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (ethical code: IR.AJUMS.REC.1396.30).

Human umbilical cords were collected from healthy full-term infants born delivered by cesarean section. The infant's mother gave informed consent before collecting umbilical cords. Isolation of hWJ-MSCs was accomplished as previously described [2].

Isolation of MSCs from Wharton Jelly by Explant Method

The isolation and culture methods were explained briefly as described earlier [2]. After thorough washing with Phosphate-Buffered Saline (PBS), the cord was cut open, the arteries and veins were removed, and the extracellular matrix of the Wharton's jelly was cut into 1-2 mm pieces. Then, 6 to 9 pieces were seeded onto a 25-cm^2 T-flasks culture dish with Dulbecco's modified eagle medium containing nutrient mixture F-12 (DMEM-F12; Gibco), supplemented with 10% (v/v) Fetal Bovine Serum (FBS; Gibco), penicillin (100 U/mL), streptomycin (100 µg/mL), and amphotericin B (2.5 µg/mL) and cultured in 5% CO_2 at 37^0C in an incubator. The culture medium was changed twice a week. After two weeks, the pieces were removed, trypsinized with 0.25% trypsin containing 0.02% EDTA (Gibco). When the adherent cells reached 80% confluence, they were passaged into a new flask.

Cell Viability Assay

To compare the proliferation of cells in 2D and 3D media, the MTT test was used according to the following procedure.

The encapsulated and unencapsulated hWJ-MSCs viability was assessed by the MTT assay explained earlier by Son et al. Briefly, for MTT assay, the capsules were transferred into a 24-well plate [2].

After adding 1 mL of the culture medium to each of the wells, the steps were taken following the methodology recommended by the kit. The absorbance at 570 nm was measured on days 5,

10, and 15.

Immunophenotyping

Expression of the surface markers of hWJ-MSCs by flow cytometry technique for CD105, CD73, and CD45, CD34 antigens was investigated before encapsulating the cells in the alginate and when the cells were cultured in monolayer.

The expression of cell surface markers methods was explained briefly as described earlier [2].

The cells were analyzed using flow cytometry (Becton Dickinson, San Diego, CA, USA). At least 20000 events were recorded for each sample, and the data were analyzed using FlowJo™ software.

Differentiation of hWJ-MSCs

For osteogenic differentiation, hWJ-MSCs were incubated in DMEM with 10 nM dexamethasone (Sigma), 50 µg/mL ascorbic acid 2-phosphate (Sigma), and 10 mM b-glycerol phosphate (Sigma). For adipogenesis, the cells were treated with DMEM supplemented with 50 µg/mL indomethacin (Sigma) and 100 nM dexamethasone (Sigma) for 3 weeks. After 21 days, the cells were stained with Alizarin Red for osteogenic differentiation and Oil Red for adipogenic differentiation.

To prepare rats for transplantation, the rats were categorized into 4 groups of 7 rats each. All 28 rats are present in the groups as follows:

Group 1: Rats without the alginate and the cell transplantation (control)

Group 2: Rats with a cell-free scaffold link

Group 3: Rats with hWJ-MSCs transplantation scaffold containing cells

Group 4: Rats with cell transplant without alginate scaffold

Preparation of Alginate 1/2 M

About 119 mg NaCl and 115 mg HEPES were solved in 25 mL Deionize Warm Water (DW) and heated until a temperature of 60°C. Then 312.5 mg alginate (PRONOVA UP MVG) was added to it with a stirring rod until the 1/2 M homogenous alginate solution was prepared, filtrate, and then given UV.

Ministration of $CaCl_2$ 102 mM Solution

First, 100 mL of DW, 1.5 g of calcium chloride powder was added to the total amount of 238 mg of HEPES solution, and the pH of 7.4 was set. The resulting solution was filtered and then autoclaved.

Polymerization of Alginate

In sterile conditions, using a syringe, 2 or 3 mL alginate was added to the solution $CaCl_2$ in the vicinity of the solution for 20 minutes. Then, the scaffolds were formed and washed with normal saline.

Encapsulation of hWJ-MSCs

For group 3 with scaffold containing cells hWJ-MSCs, a sterile solution alginate encapsulation process was carried out as follows:

Using trypsin 0.25%, MSCs were isolated from the bottom flask at a density of 90%-100%. DMEM was used to neutralize enzymes and then centrifuge the cell platform with added alginate solution, pipetting up the solution to provide a uniform cell solution. The cell solution polymerization process with alginate was obtained, as mentioned earlier, and finally made polymerized.

In this way, two kinds of scaffold-free (for group 2) and with (for group 3) hWJ-MSCs transplantation in peritoneal rats was ready.

Transplantation of Scaffolds or hWJ-MSCs

Female rats weighing approximately 250 g and aged 8-6 weeks were selected. The general anesthesia with ketamine at a dose of 80 to 100 mg/kg and xylazine at a dose of 10 mg/kg body weight in the form of intraperitoneal was induced. The vertical incision in the anterior abdominal wall and access to the peritoneal cavity scaffolds depending on the groups (3 or 2) cellular or a cellular were transplanted in the peritoneal cavity. In group 4, hWJ-MSCs cells in DMEM solution with a concentration of one million in 200 µL of DMEM low-glucose after cell counts were prepared and injected intraperitoneally.

Immunological Method

A) Humoral Immunity Assay

Measurement of Anti-hWJ-MSCs Antibodies

One month after transplantation of hWJ-MSCs in rat peritoneum, the rats' serum was isolated in different groups. A number of 1×10^4 hWJ-MSCs were cultured in 200 µL DMEM-F12 medium (Sigma) with 10% FBS on 96-well plates overnight. After that, the cells were incubated in PBS containing 20% of serum from rats in different groups for 120 min at room temperature. The cells were washed and then incubated with diluted HRP-conjugated goat anti-rat Immunoglobulin M (IgM), IgG, or IgA antibody (Sigma) for 60 mines at room temperature. hWJ-MSCs incubated with PBS containing 20% inactivated FBS were used as negative controls. The cells were washed three times, and Tetramethylbenzidine (TMB; Sigma) was added into each well (100 µL/well). The color reaction was stopped with 50 µL/well 2 N H_2SO_4 and measured in an ELISA assay at 450 nm.

Measurement of Serum-Mediated Cytotoxicity on WJ-MSCs

One month after transplantation of hWJ-MSCs in rats' peritoneal cavity, the rats' serum was isolated in different groups. hWJ-MSCs were seeded in a 96-well plate (1×10^4 cells/well) overnight and incubated in PBS containing 20% of serum from rats in different groups. Cells incubated with 20% normal rat serum or with 1% Triton-X-100 in PBS were used as negative or positive controls, respectively. After 2 or 4 hours of incubation, cytotoxicity experiments were assessed using 3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) (Sigma) reduction. At the end of the incubation period, 20 µL of MTT (0.5 mg/mL) solution was added to each well. Then, the cells were incubated for an additional 3 h at 37°C. The medium was removed, and 200 µL DMSO was added to each well.

The wells were shaken slowly and remained in darkness for 10 min to dissolve the dark blue Formosan crystals. The absorbance of each well solution was measured at 570 nm. Cells grown in the medium alone was used as reference value at 100% viability [20].

B) Cellular Immunity Measurement

IFN-γ Secretion from Spleen Cells of the Rats Treated with hWJ-MSCs

Thirty days after implantation, the rats were killed. Their

spleens were isolated and dissociated mechanically, and red blood cells were lysed by suspension in 1 mL of 8.3 g/L ammonium chloride red blood cell lysing buffer (Sigma). Splenocytes were subsequently washed with PBS for 2 times. For the IFNγ release assay, 1×10^4 WJ-MSCs were cultured in 200 µL DMEM-F12 medium with 10% FBS on 96-well plates overnight. Then, splenocytes were isolated from different groups and co-cultured at 2×10^5 cells/well, with WJ-MSCs in 200 µL RPMI 1640 media supplemented with 10% FBS. After 3 days, the supernatant was harvested and tested for the existence of rat IFNγ by ELISA according to the manufacturer's instructions.

Proliferation Assay

Splenocytes were isolated from different groups one month after implantation, as explained above. Proliferation assay was evaluated using CellTrace™ Carboxyfluorescein succinimidyl ester Cell Proliferation Kit (CFSE; Invitrogen) as described previously [20]. Briefly, splenocytes were labeled with 5 mM CFSE for 5 min and washed 4 times with PBS to remove excess CFSE. hWJ-MSCs were co-cultured with 2×105 CFSE-labeled splenocytes at 1:10 ratio (hWJ-MSCs: splenocytes) in 200 µL RPMI-1640 medium (Invitrogen) with 10% FBS on 96-well plates for 4 days. On the fourth day, the proliferation was evaluated by flow cytometry, analyzed using FlowJo™ software.

Scanning Electron Microscopy

The alginate beads were fixed in 2.5% glutaraldehyde and then dried on filter paper.

The beads' morphology and the encapsulated cells were assessed by scanning electron microscopy.

Statistical Analysis

GraphPad Prism (version: 5.04) was used for data analysis and drawing figures. Then, the Kolmogorov-Smirnov test was used to investigate the normal distribution of the parameters. The statistical tests of ANOVA and the Kruskal-Wallis test were used for the intergroup comparison after investigating data normality. The confidence level was considered 95% in all experiments, and $P<0.05$ was considered statistically significant.

Results

The expression of cell surface markers after passage 3-4 was investigated.

The flow cytometry results revealed high levels of positive markers for the hWJ-MSCs of CD73, CD105, but CD45, and CD34, which indicates that the markers of hematopoietic cells were not expressed (Figure 1).

After about 10-14 days, the explants were removed from the tissue culture flask, the cells adherent to the floor of the flask after 2-3 passages were usually uniform and had peudocyte (fibroblast-like shape) (Figure 2A).

These cells were round-shaped and uniform after transferring into the beads of alginate (3D cultured) (Figure 2B).

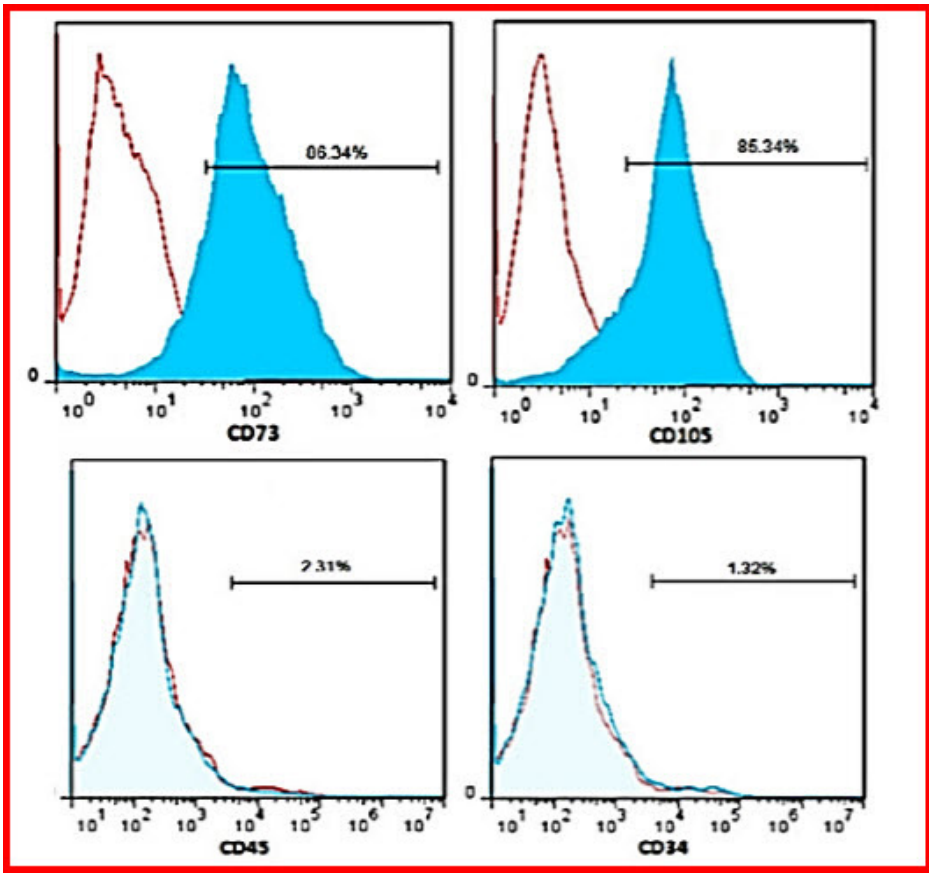

Figure 1: Flow cytometry analysis of surface markers of WJ-MSC

Flow cytometry analysis of surface markers showing that WJ-MSC were positive for CD73 and CD105 but negative for CD34 and CD45. Isotype control was used to obtain the autofluorescence levels during flow cytometry (red curve).

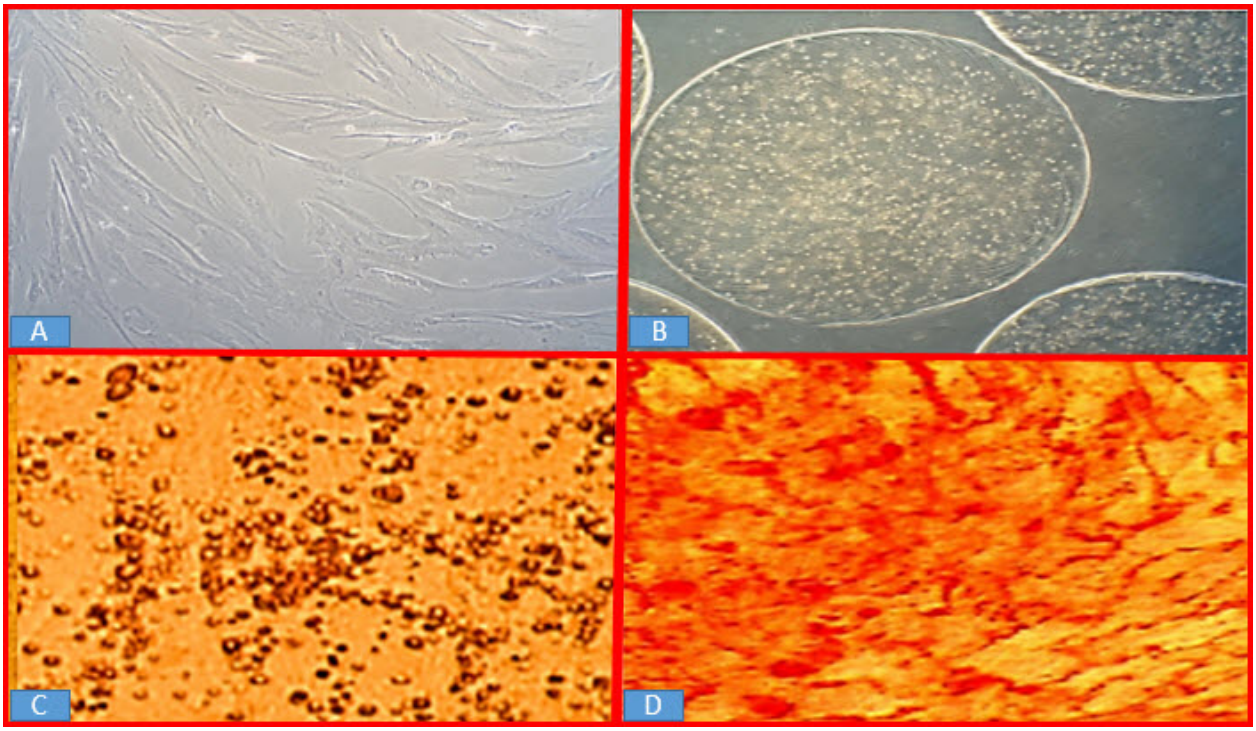

Figure 2: A) MSCs in 2D culture noticed the peudocyte and fibroblast-like cell MSCs; B) Cells encapsulated with a scaffold of alginate containing cells (bead); C) Differentiation potential assay of hWJ-MSCs, lipid vacuoles inside the cytoplasm; D) Calcium deposition inside the cytoplasm

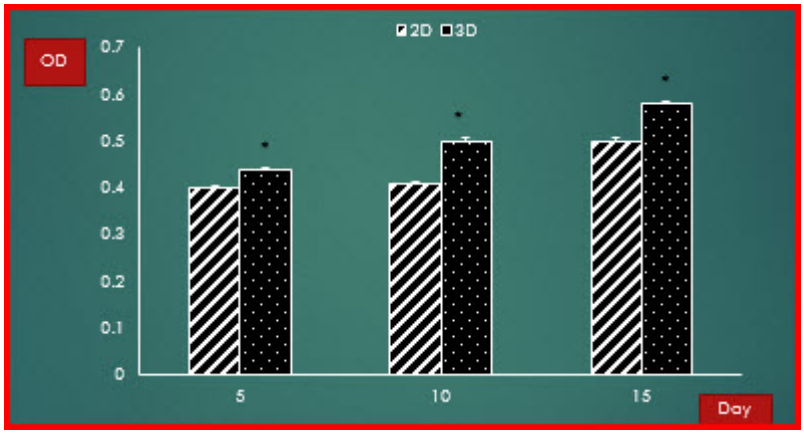

Figure 3: Comparison of cell proliferation on days 5, 10, and 15 in 2D and 3D culture media

The comparisons between cell proliferation in 2D and 3D culture by MTT assay exhibited that the proliferation of hWJ-MSCs in 3D culture in alginate beads on days 5, 10, and 15 was higher than that 2D culture ($P<0.05$) (Figure 3).

IFN-γ Secretion

For this purpose, 1 month after transplantation of hWJ-MSCs, the rats' spleen was first isolated in different groups, and splenocytes were co-cultured with hWJ-MSCs for three days. Three days later, the supernatant was collected, and an ELISA kit was used to measure IFN-γ in different groups. The results showed that in none of the groups, the splenocytes stimulated against hWJ-MSCs; moreover, they did not produce IFN-γ, which was not detectable in any group.

Proliferation Assay

Thirty days after transplantation, the splenocytes were first labeled with CFSE and co-cultured with hWJ-MSCs for 4 days to examine the proliferation of splenocytes of rats against hWJ-MSCs. Flow cytometry was used to investigate proliferation. Results showed that splenocytes did not proliferate in different groups and did not stimulate against hWJ-MSCs (Figure 4).

Cell Viability Assay

To compare the proliferation of cells in 2D and 3D media, the MTT test was used according to the following procedure.

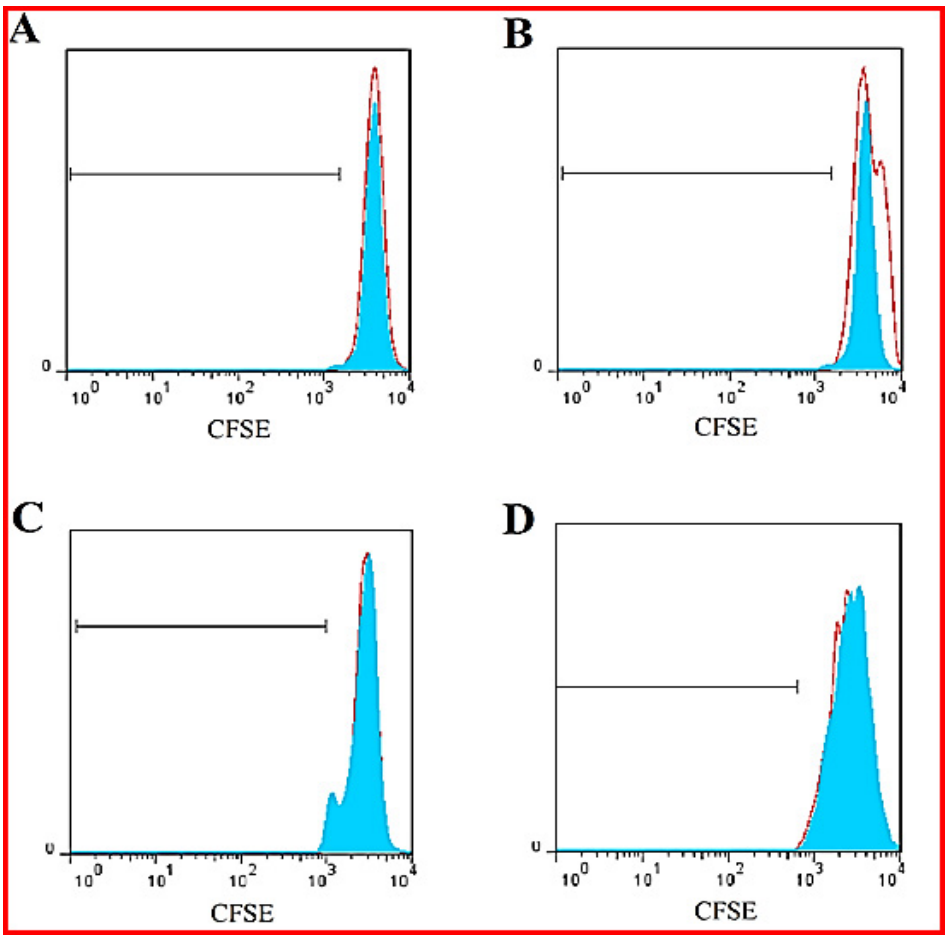

Figure 4: Proliferation assay by co-culture of splenocytes with hWJ-MSCs assessed by CFSE method. Splenocytes proliferation was analyzed by flow cytometry after 4 days. Isotype control (red curve) overlays to splenocytes proliferation (blue curve); A) Rats without the alginate and the cell transplantation; B) Rats with a cell-free scaffold link; C) Rats with MSCs transplantation scaffold containing cells; D) Rats with cell transplant without alginate scaffold. Splenocytes did not proliferate against WJ-MSCs in different groups (P<0.05).

The encapsulated and unencapsulated hWJ-MSCs' viability was assessed by the MTT assay explained earlier by Son et al. Briefly, for MTT assay, the capsules were transferred into a 24-well plate.

After adding 1 mL of the culture medium to each well, steps were taken following the methodology recommended by the kit, and the absorbance at 570 nm was measured on days 5, 10, and 15 (Figure 5).

Measurement of Anti-hWJ-MSCs Antibodies

One month after transplantation, the serum of mice was isolated in different groups and incubated with hWJ-MSCs. The presence of IgM, IgG, IgA antibodies against hWJ-MSCs was measured by the ELISA method. The results showed that IgM, IgG, and IgA antibody classes against hWJ-MSCs had not been made in rat serum in different groups, and hWJ-MSCs transplantation did not stimulate the immune system of rats to stimulate humoral immunity. Antibodies were not detectable in any group.

Measurement of Serum-Mediated Cytotoxicity on hWJ-MSCs

One month after the transplantation, the rats' serum in different groups was co-cultured with hWJ-MSCs for 2 and 4 hours. MTT was used to show antibody cytotoxicity. There was no cytotoxicity in the serum of rats in different groups. Transplantation of hWJ-MSCs did not stimulate humoral immunity in rats (Figure 5).

The expression of cell surface markers after passage 3-4 was investigated.

The flow cytometry results revealed high levels of positive markers for the HUMSCs of CD90 and CD105.

But CD45, which indicates the marker of hematopoietic cells, was not expressed.

After about 10-14 days, the explants were removed from the tissue culture flask, the cells adherent to the floor of the flask after 2-3 passages were usually uniform and had peudocyte (fibroblast-like shape) (Figure 2A).

These cells were round-shaped and uniform after transferring into the beads of alginate (3D cultured) (Figure 2B).

Scanning Electron Microscopy

Electron microscopy studies showed that the alginate of microbeads allows survival and proliferation to MSCs, and can be used to transmit a large number of cells and their transplantation in a specific place of the body (Figure 6).

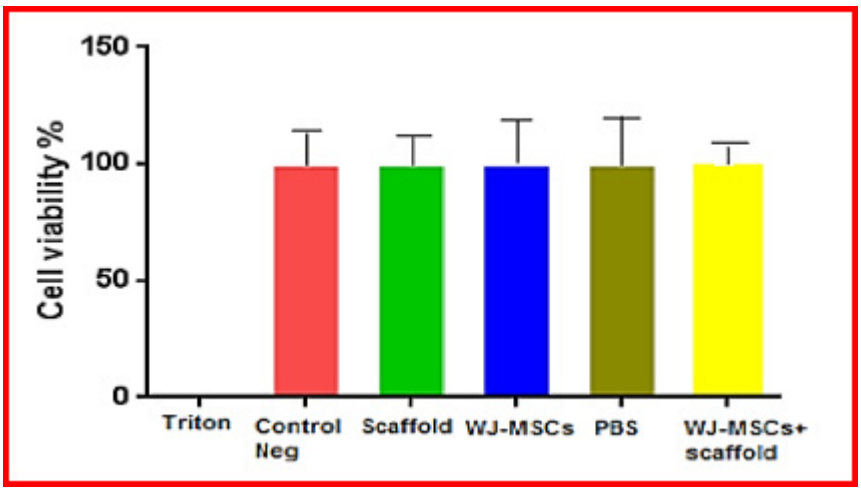

Figure 5: Comparison of serum mediated cytotoxicity on hWJ-MSCs in different groups.
After 2 and 4 h incubation with serum from different groups of rats with WJ-MSCS, there was no cytotoxicity in the serum of rats in different groups (P<0.05). The results were expressed as mean±SD in the three experiments.

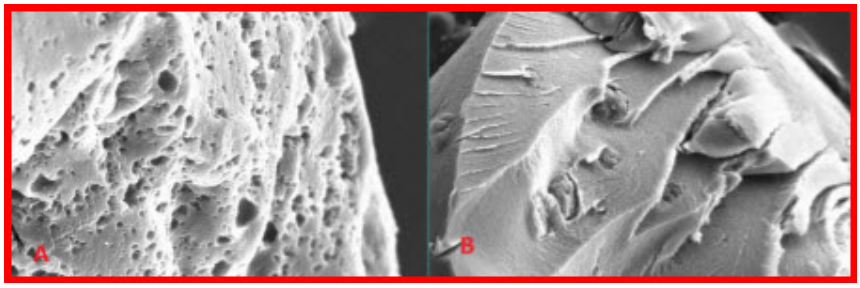

Figure 6: Images of an SEM from alginate beads. A) The outer surface of a bead; B) the cells' position in the alginate beads.

Statistical analysis

Statistical analysis was performed using GraphPad Prism (version: 5.04). One-way ANOVA and t tests were used to evaluate differences between groups, and P values less than 0.05 were considered statistically significant.

Discussion

MSCs can not only be derived from bone marrow but also blood, spleen, amniotic fluid, placenta, tendon, adipose tissue, synovial fluid, thymus cancellous bone, umbilical cord blood, skin, pulp, lungs, and umbilical cord [13, 14, 20, 21]. The umbilical cord is considered medical waste and can be easily obtained without adversely affecting the donor or provoking any ethical issues. Thus, it is an ideal source of cells for cell replacement therapy. MSCs derived from human umbilical cords possess self-renewal and multipotent differentiation potential [6].

Challenges with immune rejection need to be first addressed before hWJ-MSCs transplantation for the treatment of various degenerative diseases [6].

MHC-I functions to protect MSCs from destruction by Natural Killer (NK) cells [22]. The HLA-I antigen includes HLA-DR, HLA-DP, and HLA-DQ, with HLA-DR considered the most critical factors for allogeneic graft rejection. MHC-II can aid MSCs in escaping immune recognition by CD4+ T cells [23].

Stumpf et al. found that hWJ-MSCs produce an immunosuppressive isoform of HLA-I and do not express HLA-DR (MHC-II (.

This finding indicates that hWJ-MSCs are a type of low immunogenic cells. Allogeneic transplant rejection is mainly mediated by recipient T cells. Recent studies have demonstrated that the excessive activation and proliferation of T lymphocytes is one of the main reasons for graft-versus-host disease [24].

The activation of naïve T cells needs the synergy of the 2 types of activation signals. When the first and second signals are bound by the corresponding ligands, T cells proliferate to form functional cell subsets. If T cells lack co-stimulatory signals, the first signal of antigen recognition cannot effectively activate specific T cells, leading to the loss of T cell function. Thus, the synergistic activation of the stimulatory molecules is essential for normal T cell activation [25].

In recent years, many studies have focused on the influence of hWJ-MSCs-regulated immune cells, particularly T cells. hWJ-MSCs can suppress the proliferation of lymphocytes [26, 27]. It has been suggested that the immunomodulatory effects of MSCs are mainly exerted through the following 2 mechanisms: direct contact between hWJ-MSCs and T lymphocytes or soluble cytokines secreted by hWJ-MSCs, indirectly inhibiting T lymphocytes [26-28]. IFN, the first discovered

soluble cytokine, is mainly produced by the activated T cells and NK cells. In a previous study, following hWJ-MSCs stimulation, IFN-γ secretion by T helper (Th)1 cells decreased by 50%, while Interleukin (IL)-4 secretion from Th2 cells increased significantly. This event indicates that hWJ-MSCs may induce T lymphocyte differentiation into Th2 cells [29].

Hong Wu Wang et al. suggested that hWJ-MSCs can inhibit T cells' proliferation activated by PHA. T cells' IFN-γ secretion is decreased, indicating that HUMSCs may exert immunosuppressive effects on T cells.

Adult stem cells, which include hWJ-MSCs, exhibit two important biological characteristics: first, injected adult stem cells can migrate to the target affected areas in the body after intravenous injection, and second, the stem cells can be induced to differentiate into appropriate cells required for the repair of damaged tissue. This phenomenon is termed site-specific differentiation [30].

Hong Wu Wang et al. found that hWJ-MSCs can colonize in the liver and pancreas after transplantation via caudal veins for 4 weeks without immune rejection. These results offered that hWJ-MSCs can survive in different parts of the body without immune rejection.

hWJ-MSCs have a great potential for treating various degenerative diseases and immune disorders. It is safe to expect that the first clinical application of stem cells will be the use of MSCs to correct improper immune reactions. Previous studies by various groups have had variable results in demonstrating immunosuppression by MSCs [13, 14]. Our studies show that the immunosuppressive ability of MSCs is not substantial but is instead induced by the proinflammatory cytokines IFN-γ in combination with TNFα, IL-1a, or IL-1b. Therefore, ironically proinflammatory cytokines can lead to immunosuppression under specific circumstances [15].

hWJ-MSCs express only low levels of MHCI and MHCII. Hoare et al. [31] and Potian et al. [32] demonstrated that third-party MSCs could reduce lysis of allogeneic target cells in a cytotoxicity assay with alloantigen-specific activated Cytotoxic T cells (CTLs) [20].

Krampera et al. showed that MSCs only reduced the proliferation of B cells in the presence of IFN-γ. The suppressive effect of IFN-γ was possibly related to its ability to stimulate the production of IDO by MSCs, which in turn suppresses the proliferative response of effector cells through the tryptophan pathway [33]. Zhang et al. have displayed the presence of "effector memory" CTL specific for viral antigens with potent recall function in the BM, which could be re-stimulated and clonally expanded [34]. Djouad et al. indicated that the growth of an allogeneic melanoma was significantly raised when the latter cells were co-transplanted together with the murine C3H10T1/2 MSC line [35].

In the present study, alginate increases the viability of the human MSCs. In agreement with our results, X et al. showed that alginate could enhance survival rates of the mesenchymal stem cells. Because of the effect of the encapsulation on maintaining the shape and function of the hWJ-MSCs, our group considers the 3D culture more suitable for bonding.

Our team acknowledges that based on the tests we used in this study, we found no difference between the two groups of encapsulated cells and unencapsulated ones in response to the humoral and cellular immune systems.

Conclusion

It was found that hWJ-MSCs neither stimulate the proliferation of lymphocytes nor induce allogeneic or xenogeneic immune cell responses.

The encapsulated or unencapsulated hWJ-MSCs, and free alginate microspheres beads (without cell) did not stimulate the rats' immune system.

According to the results of this research, a specific response of the immune system (humoral and cellular response) was evaluated for one month at the stimulation of xenografts (none specific transplant) in the rats. The results of four parameters examined were in favor of mass escape safety.

It is suggested to evaluate the responses against the second and third xenograft stimulation while investigating future responses to the second and third xenografts stimulation, which relates to the response of the memory B and T cell.

Future Perspectives

Encapsulation has a promising capacity regarding the treatment through cell replacement and biotechnology. Furthermore, this process has therapeutic and recoverable uses, although there are still many challenges against the encapsulation technique. For example, the proportion of alginate, substance density, and concentration of cells in the microbead should be carefully determined because of the preservation viability of cells in scaffold and cell differentiation capacity.

Like alginate scaffold, biohybrid organs have a desirable perspective for treating a wide variety of endocrine disorders.

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Conflict of Interest

The author declared no conflict of interest.

Authors' Contribution

Azandeh S. Bijan Nejad D, and Amari A developed the manuscript and revised it. Saki G revised it. Moghimi M wrote the manuscript and prepared the tables and the figures. Amari A performed the statistical analyses.

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