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# Human chorionic villous mesenchymal stem/stromal cells modify the effects of oxidative stress on endothelial cell functions

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Mesenchymal stem/stromal cells derived from chorionic villi of human term placentae (pMSCs) produce a unique combination of molecules, which modulate important cellular functions of their target cells while concurrently suppressing their immune responses. These properties make MSCs advantageous candidates for cell-based therapy. Our first aim was to examine the effect of high levels of oxidative stress on pMSC functions. pMSCs were exposed to hydrogen peroxide ($H_2O_2$) and their ability to proliferate and adhere to an endothelial cell monolayer was determined. Oxidatively stressed pMSCs maintained their proliferation and adhesion potentials. The second aim was to measure the ability of pMSCs to prevent oxidative stress-related damage to endothelial cells. Endothelial cells were exposed to $H_2O_2$, then co-cultured with pMSCs, and the effect on endothelial cell adhesion, proliferation and migration was determined. pMSCs were able to reverse the damaging effects of oxidative stress on the proliferation and migration but not on the adhesion of endothelial cells. These data indicate that pMSCs are not only inherently resistant to oxidative stress, but also protect endothelial cell functions from oxidative stress-associated damage. Therefore, pMSCs could be used as a therapeutic tool in inflammatory diseases by reducing the effects of oxidative stress on endothelial cells.

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## 1. Introduction

Mesenchymal stem/stromal cells (MSCs) (also called multipotent stromal cells depending on the source) are isolated from adult and fetal tissues, such as bone marrow, liver, dental pulp, adipose tissue, endometrium, muscle, amniotic fluid, placenta and umbilical cord blood [1–3]. MSCs have self-renewal ability and multipotent differentiation potential that includes cells of multiple organs and systems such as bone, fat, cartilage, muscle, neurons and hepatocytes [3,4]. Previously, we isolated and characterized MSCs from chorionic villi of human term placentae (pMSCs) [3]. pMSCs produce a unique combination of molecules that influence important functions of target cells including proliferation, differentiation, migration and angiogenesis, while concurrently suppressing their immune responses [3]. We showed that pMSCs are capable of self-renewal and differentiation into the three mesenchymal lineages of adipocytes, osteocytes and chondrocytes [3]. Moreover, we demonstrated the immunosuppressive properties of pMSCs [3,5,6]. We showed that pMSCs can induce an anti-inflammatory phenotype in human macrophages by shifting the differentiation of human monocytes from M1 inflammatory into

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M2 anti-inflammatory macrophages [6]. In addition, we provided evidence for their inhibitory effects on the differentiation, maturation and function of human dendritic cells, and their ability to inhibit T cell proliferation. Together, these data show the ability of pMSCs to control the immune responses at multiple levels [7]. These characteristics of pMSCs make them an attractive source for cell-based therapies.

Cell-based therapies rely on MSC migration to sites of inflammation and injured tissue in response to various stimuli including cytokines, chemokines and growth factors. At these sites, MSCs repair the damaged tissues in a hostile and toxic inflammatory environment, either by engrafting and differentiating into tissue-specific cell types, or more likely through paracrine mechanisms. Paracrine effects of MSCs include various combinations of the following; stimulating endogenous stem cells, preventing cell apoptosis, increasing cell proliferation and modulating the functions of the innate and adaptive immune cells such as antigen presenting cells and lymphocytes [3,5–8].

Oxidative stress is characterized by the imbalance between pro-oxidant molecules including reactive oxygen and nitrogen species, and antioxidant defenses [9,10]. Oxidative stress plays a key role in the pathogenesis of many diseases [9]. Accumulating evidence supports the notion that acute and chronic uncontrolled over-production of oxidative stress-related factors, including reactive oxygen species (ROS), are causative agents in cardiovascular diseases, such as atherosclerosis and diabetes [11]. Furthermore, ROS mediate various signaling pathways that underly vascular inflammation in ischemic tissues [11]. pMSCs are derived from the fetal part of the placenta and exposed to the fetal circulation, and thus experience lower levels of inflammation and oxidative stress [12,13]. The exposure of pMSCs to high levels of inflammation and oxidative stress may negatively impact their therapeutic potential, as is the case for MSCs derived from other sources [14].

Endothelium comprises a squamous epithelium that lines the lumen of all blood vessels that is highly metabolically active. Endothelium plays a major role in vascular homeostasis and acts as an endocrine organ by producing variety of molecules including hormones, growth factors, coagulation factors, and adhesion molecules [15]. Moreover, the endothelium is the active biologic interface between blood and tissues that regulates the sensitive balance between vasoconstriction/vasodilatation, coagulation/fibrinolysis and proliferation/apoptosis. Finally, the endothelium is the interface for the transient adhesion, and subsequent diapedesis, of blood-borne leukocytes [15]. Inflammatory diseases, such as atherosclerosis, are characterized by endothelial cell activation, which results from oxidative stress and increased inflammation [16]. Intense and sustained endothelial cell activation culminates in a damaged endothelium [15]. This damage manifests in major phenotypic changes, such as increased inflammatory marker expression in the endothelium, and endothelial dysfunction as a result of increased proliferation, which is a characteristic of atherosclerosis [17].

Therefore, an essential prerequisite to the use of pMSCs in a cell-based therapy is to determine their effects on the functional characteristics of endothelial cells in a hostile and toxic inflammatory environment. Here, we examined the potential of pMSCs to interact with endothelial cells in a hostile, toxic and inflammatory environment.

Our results showed that pMSCs exposed to hydrogen peroxide ($H_2O_2$) were able to proliferate and adhere to an endothelial cell monolayer. Furthermore, pMSCs prevent oxidative stress-related damage to endothelial cells. pMSCs were able to reverse the damaging effects of oxidative stress on the proliferation and migration but not on the adhesion of endothelial cells. Together, these data show pMSCs are inherently resistant to oxidative stress and they can also protect endothelial cell functions from oxidative stress-associated damage. Therefore, pMSCs could be used as a therapeutic tool in inflammatory diseases because of their ability to reduce the effects of oxidative stress on endothelial cells.

## 2. Materials and methods

### 2.1. Ethics of experimentation

This study was approved by the institutional research board (Reference # IRBC/021/14) at King Abdulla International Medical Research Centre/King Abdulaziz Medical City, Riyadh, Saudi Arabia. All term placentae and umbilical cords were obtained with informed patient consent.

### 2.2. Placentae

Human placentae were obtained from uncomplicated pregnancies following normal vaginal delivery (38–40 weeks of gestation). The gestational age and fetal viability of all pregnancies were confirmed by early ultrasound examination before 20 weeks of gestation. The placentae were used within 2 h of delivery.

### 2.3. Isolation of pMSCs from chorionic villi of human term placenta in vitro

pMSCs were isolated from chorionic villi of human term placenta using our published method [3]. Briefly, placental tissues were dissected and then washed thoroughly with sterile phosphate buffered saline (PBS), pH 7.4. After removing the superficial layer of maternal decidua on the maternal side of the placenta, the underlying fetal chorionic villi were cut into small pieces of approximately 40 mg total wet weight. The tissue was washed with PBS and then incubated with 2.5% trypsin (Life Technologies, Grand Island, USA), which was diluted in DMEM-F12 medium (Invitrogen, Saudi Arabia) containing (271 unit/ml) DNase (Life Technologies, Grand Island, USA), 100 μg/ml streptomycin and 100 U/l penicillin, with gentle rotation overnight at 4 °C. Tissues were then washed thoroughly with PBS and allowed to adhere to the plastic in 6 well plates for 1 h at 37 °C in a humidified atmosphere containing 5% $CO_2$ (a cell culture incubator). Subsequently, DMEM-F12 medium containing 10% Mesenchymal Stem Cell Certified fetal bovine serum (MSC-FBS) (Life Technologies, Grand Island, USA), 100 μg/ml of L-glutamate, 100 μg/ml streptomycin and 100 U/l penicillin was gently added to the tissues and cultured at 37 °C in a cell culture incubator. Every two days, the medium was replaced with fresh medium. On day 14, the tissue pieces were removed and cells that had migrated out from the cut ends of the tissues were then harvested with TrypLE™ Express detachment solution (Life Technologies, Grand Island, USA) and characterized by flow cytometry using MSC positive markers (CD44, CD90, CD146, CD166 and CD105) and hematopoietic negative markers (CD14, CD19, CD45, HLA-DR, CD80, CD83, CD86, and CD40), as described previously [3]. Cells at a density of $1 \times 10^5$ cells in 75 $cm^2$ flasks (Becton Dickinson, New Jersey, USA) were re-cultured until they reached 75% confluency and then used in subsequent experiments. Prior to use in experiments, pMSCs at passage 2 were assessed for differentiation into adipocytes, chondrocytes and osteocytes as previously described [3]. Adipogenic, osteogenic and chondrogenic differentiation was performed by incubating pMSCs in adipogenic (#390415), osteogenic (#390416) and chondrogenic (#390417) media respectively, and were purchased from R&D Systems (Abingdon, UK). Each differentiation medium was supplemented with 10% MSC-FBS, 100 μg/ml of L-glutamate, 100 μg/ml streptomycin and 100 U/l penicillin. Adipocytes, osteocytes and

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