Mesenchymal Stromal Cells from the Maternal Segment of Human Umbilical Cord is Ideal for Bone Regeneration in Allogenic Setting

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Abstract Umbilical cord (UC) is a discarded product from the operating theatre and a ready source of mesenchymal stromal cells (MSCs). MSCs from UC express both embryonic and adult mesenchymal stem cell markers and are known to be hypoimmunogenic and non-tumorigenic and thus suitable for allogeneic cell transplantation. Our study aimed to determine the degree of immunotolerance and bone-forming capacity of osteodifferentiated human Wharton's jelly-derived mesenchymal stromal cells (hWJ-MSCs) from different segments of UC in an allogenic setting. UCs were obtained from healthy donors delivering a full-term infant by elective Caesarean section. hWJ-MSCs were isolated from 3 cm length segment from the maternal and foetal ends of UCs. Three-dimensional fibrin constructs were formed and implanted intramuscularly into immunocompetent mice. The mice were implanted with 1) fibrin construct with maternal hWJ-MSCs, 2) fibrin construct with foetal hWJ-MSCs, or 3) fibrin without cells; the control group received sham surgery. After 1 month, the lymphoid organs were analysed to determine the degree of immune rejection and bone constructs were analysed to determine the amount of bone formed. A pronounced immune reaction was noted in the fibrin group. The maternal segment constructs demonstrated greater osteogenesis than the foetal segment constructs. Both maternal and foetal segment constructs caused minimal immune reaction and thus appear to be safe for allogeneic bone transplant. The suppression of inflammation may be a result of increased anti-inflammatory cytokine production mediated by the hWJ-MSC. In summary, this study demonstrates the feasibility of using bone constructs derived from hWJ-MSCs in an allogenic setting.

Keywords Bone · Mesenchymal stromal cells · Tissue engineering · Wharton's jelly · Allogeneic

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Abbreviations

MSCs	Mesenchymal stromal cells
hWJ-MSCs	Human Wharton's jelly-derived mesenchymal stromal cells
hBM-MSCs	Human bone marrow-derived mesenchymal stromal cells
TE	Tissue engineering
UC	Umbilical cord

1 Introduction

The worldwide incidence of bone disorders has increased steeply over the years. There are many causes of bone defects, from hereditary diseases such as scoliosis and osteogenesis imperfecta to degenerative diseases such as osteoporosis and osteoarthritis, as well as other pathological states such as osteomyelitis and osteosarcoma. It has been estimated that five to twenty percent of fracture cases resulted in impaired healing and required therapeutic intervention [1].

Fractured bone has the ability to regenerate if there is space available and a scaffold is in place [2]. A bone graft can be autologous, allogeneic or synthetic. Autologous grafts can be harvested from the patient's own iliac crest, tibia or fibula. Allogeneic graft tissue is normally collected from cadaver. Synthetic grafts are usually made from ceramic containing calcium phosphate (e.g. hydroxyapatite and tricalcium phosphate).

Autologous bone grafts are the current gold standard for bone repair as the graft is osteoinductive, i.e. it recruits cells from the edge of the defect to regenerate the lost tissue. Furthermore, bone autografts promote regeneration without risk of disease transmission [3]. The disadvantages of bone autografts are the need for an additional surgery to harvest the graft, limited availability and high risk of donor site morbidity [4, 5]. Although bone allografts are readily available they carry the risks of disease transmission and immune rejection [6–8]. Synthetic bone grafts are a potential alternative to autografts and allografts as they can be prepared from readily available materials and are devoid of all the aforementioned disadvantages. Viable synthetic grafts for bone repair have been made possible by the emergence of regenerative medicine and tissue engineering. Viable synthetic grafts can be prepared by adding stem cells or growth factors to three-dimensional (3D) porous scaffolds, either individually or in combination [9, 10].

One of the most intriguing properties of mesenchymal stromal cells (MSCs) is their immunomodulatory properties [11]. MSCs suppress T- and B cell proliferation and modulation immune cells such as NK cells and macrophages, via the secretion of soluble factors [12]. MSCs secrete anti-inflammatory cytokines that suppress

inflammation mediated by activated T-cells that come into contact with allogeneic cells or mitogens [13–15]. The immunomodulatory properties of MSCs make them ideal candidates for allogeneic cell therapy [16]. Bone regeneration is regulated by the inflammation in that acute inflammation aids healing whereas chronic inflammation inhibits it. MSCs may be able to promote bone regeneration.

Human bone marrow-derived mesenchymal stromal cells (hBM-MSCs) are commonly used as precursor cells to initiate bone regeneration. Collection of hBM-MSCs is an invasive procedure and may cause aspiration site morbidity. The number and proliferative potential of MSCs present in the bone marrow vary between individuals. The autologous MSCs of aging patients may have limited expansion capacity and thus be unsuitable for cell therapy, making allogeneic MSCs a better choice for this patient group [17]. Human Wharton's jelly-derived mesenchymal stromal cells (hWJ-MSCs) are plentiful and have excellent proliferative potential and are thus the ideal candidate to replace hBM-MSCs. hWJ-MSCs can be infused intravenously or administered at the site of injury, but their effects on lymphoid organs and the immune response to them remain unknown.

Our previous study divided human umbilical cord (UC) into three segments i.e. the maternal, middle, and foetal segments. There are biological differences between the hWJ-MSCs isolated from the different UC segments [18]. It was demonstrated that hWJ-MSCs isolated from the maternal and foetal segments were more suitable for tissue engineering purposes in terms of cell growth, cell viability and expression of pluripotent embryonic markers. MSCs from maternal and foetal segments also showed greater *in vitro* osteogenic potential than the middle segment making them the preferred choice for bone tissue engineering. A subsequent *in vitro* study assessed the immunomodulatory properties of hWJ-MSCs derived from the maternal, middle, and foetal segments of the human UC before and after osteogenic differentiation [19]. It showed that osteodifferentiated hWJ-MSCs were less immunogenic than undifferentiated cells, a find that suggested that osteodifferentiated hWJ-MSCs from maternal and foetal segments could be a reliable allogeneic cell source for bone tissue engineering. Nevertheless as all these data were from *in vitro* research the findings need to be verified *in vivo*.

The research reported here consisted of *in vivo* experiments designed to determine the bone-forming capacity and immunogenicity of osteodifferentiated hWJ-MSCs from different UC segments. We used a xenogeneic approach as it is impossible to conduct a human allogeneic transplant at this stage. The immunological response to implantation was assessed on the basis of changes in the concentrations of pro- and anti-inflammatory cytokines in

the plasma and histological changes in the lymphoid organs. The implanted constructs were harvested and analysed histologically to assess bone formation.

2 Materials and methods

2.1 hWJ-MSCs isolation and culture

All samples were collected from healthy donors who delivered by elective caesarean section after a full-term pregnancy of 38–40 weeks with informed consent and approval from UKM Research Ethics Committee (FF-2014-066). Three segments of human UC were identified (maternal, middle, and foetal). The procedures used to isolate and culture MSCs from each segment have been described elsewhere [18].

2.2 Osteogenic differentiation of hWJ-MSCs

Passage 3 hWJ-MSC were cultured for 21 days in osteogenic medium consisting of alpha-MEM (Sigma-Aldrich, USA) supplemented with 0.1 μ M dexamethasone (Sigma-Aldrich), 10 mM β -glycerol phosphate (Sigma-Aldrich) and 0.2 mM ascorbic acid (Sigma-Aldrich). The culture medium was changed twice weekly.

2.3 Fibrin preparation

Five millilitres of whole blood was withdrawn from volunteers who have given their written informed consent and placed in a vacutainer containing 3.2% sodium citrate (Greiner Bio-One, Austria). Soluble fibrin was extracted from whole blood by centrifugation at 1850 rpm for 5 min at 4 °C. The soluble fibrin was then sterile-filtered through a 0.22 μ m filter (Sartorius, Germany) and stored at –20 °C for later use.

2.4 Formation of 3D bone constructs

A total of 7.5×10^6 cells were suspended in 1 ml of fibrin to form a cell-fibrin construct. Next 50 μ l of 0.5 M calcium chloride was added to solidify the fibrin. The cell-fibrin constructs were cultured in osteogenic medium consisting of alpha-MEM supplemented with 10 mM β -glycerol phosphate, 0.1 μ M dexamethasone, and 0.2 mM ascorbic acid for 3 days. Bone constructs were fixed with 10% neutral buffered formalin for bone formation analysis via Alizarin red (AR) staining.

2.5 Bone constructs analysis

Bone constructs were fixed in 10% neutral buffered formalin overnight. Tissue sections of 5 μ m thickness were

dewaxed and rehydrated before being stained with AR, which stains calcified tissue red. Images for analysis were captured using a bright field microscope (Olympus CH30RF200 with Olympus E A 10X Microscope Objective Lens (numerical aperture 0.25) using QImaging MicroPublisher 3.3 RTV camera). Calculation of the surface area bone mineralization was based on three histological slides obtained from each construct. In each slide, the outline of calcification area was drawn manually on nine random views of the tissue section and the area calculated using the Image J software (National Institutes of Health, USA).

2.6 Animal handling

Animal experiments were carried out using protocols approved by the Animal Research Ethics Committee of Universiti Kebangsaan Malaysia (UKM 1.5.3.5/244/FF-2015-180). Six-week-old Balb/C mice (*InVivos*, Singapore) weighing 25–30 g were used. The animals were housed individually and given ad libitum access to food (Altromin#1324 Diet, Germany) and water. The animals were acclimatised to the laboratory environment for at least 2 weeks before experimentation started.

2.7 *In vivo* construct implantation

Animals were randomly divided into four groups: control group receiving sham surgery, maternal construct group receiving fibrin construct with osteodifferentiated hWJ-MSCs from maternal segment, fetal construct group receiving fibrin construct with osteodifferentiated hWJ-MSCs from fetal segment and fibrin group receiving acellular fibrin construct. Animals were anesthetized via intramuscular injection of a combination of tiletamine (2.8 mg/kg Zolatil 20; Virbac Laboratories, France), ketamine (5.6 mg/kg Ketamav 50; Mavlab, Australia), and xylazine (5.6 mg/kg Ilium xylazil-100; Troy Laboratories, Australia). The skin was disinfected with an alcohol swab and a 10 mm incision was made using a scalpel at the gluteal region. The gastrocnemius muscle fibers were split carefully and the implant was placed intramuscularly. Thirty days implantation was done to determine if there's any acute reaction and at the same time allowing sufficient time for bone formation. Thirty days after implantation, cardiac puncture was performed to collect whole blood under anesthesia. The animals were then euthanized. The spleen, thymus and lymph nodes were collected and fixed in 10% neutral buffered formalin overnight. Incision was made at the original site of implantation, muscle was split to reveal the construct. Construct was removed with a forcep and immediately weighed and measured, then fixed with 10% formalin.

2.8 Histopathological evaluation of the lymphoid organs

Samples of spleen, thymus and axillary lymph nodes fixed with 10% neutral buffered formalin were dewaxed and rehydrated before being cut into 10 µm sections and stained with haematoxylin and eosin (H&E), which stains cytoplasm pink or red, and nuclei purple. Images were captured using a bright field microscope (Olympus CH30RF200 with Olympus E A 10X Microscope Objective Lens (numerical aperture 0.25) using QImaging MicroPublisher 3.3 RTV camera). Measurements were made using Image Pro Plus software.

2.9 Protein array analysis

Whole blood collected via cardiac puncture was centrifuged to prepare serum samples for an antibody-based cytokine array that was performed according to the manufacturer's protocol. A 19-cytokine assay was customised using the Human G-Series Array (RayBiotech, USA). The array slide includes 12 subarrays, and each subarray consists of antibodies recognising 19 different cytokines in duplicate spots. Briefly, microarray glass slide wells were blocked with blocking buffer at room temperature for 30 min and incubated overnight with 100 µl of two-fold diluted serum at 4 °C. Washing buffer was used to rinse the slides, which were then incubated with biotin-conjugated anti-cytokine antibodies for 2 h. After another round of washing, samples were incubated for 2 h with fluorescent conjugated dye in the dark. Water droplets were removed by centrifuging at 1000 rpm for 3 min. Images were captured with a LuxScan10 K-A scanner (CapitalBio, China). Signal strength data were imported into the RayBio antibody array tool for analysis.

2.10 Statistical analysis

Data are presented as mean $\pm$ standard error of the mean (SEM). Descriptive analysis was performed and one-way ANOVA (two-tailed) followed by Tukey's post hoc tests was used to compare group means. The statistical significance level was set at $p < 0.05$.

3 Results

3.1 Gross pathology

No mortality was recorded during the experiments. Animals in all the groups looked healthy and gained weight gradually throughout the experiment. No abnormal growth was noticed at the implantation site. Lymph node size was

normal in all groups, but mice in the fibrin group showed two-fold enlargement of the thymus relative to controls. Slight splenomegaly was also noted in the fibrin group (Fig. 1). No abnormalities were observed in the lymphoid organs of the maternal and foetal construct groups.

3.2 Bone construct analysis

Most of the constructs retrieved 30 days post-implantation had a smooth surface and appeared to be semi-translucent. The size of the construct was determined by the weight and length as shown in the pictures. The construct appears to be whitish to semi-translucent and can be easily distinguished from the surrounding muscle bulk. The construct can be easily removed without any attachment to the surrounding, as shown in Fig. 2. The fibrin group underwent the most construct shrinkage and reduction in length after implantation (Table 1). Mineralisation of constructs was demonstrated by AR staining. Red precipitates indicate calcium deposition by the cells (Fig. 3A–C). The total surface area of the bone nodules formed was determined using Image J software. The maternal segment construct had significantly ($p = 0.043$) more bone nodules than the foetal segment construct (Fig. 3D).

3.3 Histopathology

Histopathological analysis was based on previous study [17] in which necrosis, lymphocyte apoptosis and lymphoblastic activities were noted. The samples were assessed by two blinded histopathologists to avoid inter-observation bias with $n = 3$.

3.3.1 Spleen

The gross histology of the spleen is shown in Fig. 4. The average size of the lymphoid follicles was less than 1.22 mm in the control, maternal segment and foetal segment groups, but greater than 1.22 mm in the fibrin group. The fibrin group showed the highest number of lymphoblasts within lymphatic follicles, followed by the foetal segment group and then the maternal segment group. Mild angiogenesis was noted in all four groups. Lymphocyte apoptosis was only present in the fibrin group (Table 2).

3.3.2 Lymph nodes

The gross histology of the lymph nodes is shown in Fig. 5. Relative to the control group, the fibrin group had the highest number of lymphocytes, followed by the foetal segment and maternal segment groups. The only group showing lymphocyte apoptosis and necrosis was the fibrin group. The fibrin group had a more macrophages and a

Fig. 1 Representative lymphoid organs isolated from the **A** Control group, **B** Maternal segment group, **C** Foetal segment group and **D** Fibrin group. Lymph nodes, spleen and thymus are indicated by the bold, dashed and normal arrows, respectively

larger vacuolar area in the interfollicular region than the control, maternal segment and foetal segment groups (Table 3).

3.3.3 Thymus

The gross histology of the thymus is shown in Fig. 6. The ratio of the cortical and medullary areas remained unchanged in all groups. Severe lymphocytic apoptosis and histiocytosis were noted in the fibrin group, whilst the maternal segment and foetal segment groups showed moderate apoptosis (Table 4).

3.4 Protein array analysis

Thirteen of the 19 measured cytokines are pro-inflammatory cytokines. Relative to the control group only IL-3 was significantly ($p = 0.0043$) upregulated in the maternal and foetal segment groups, MMP-2 was significantly ($p = 0.00002$) downregulated in all experimental groups and fractalkine was significantly ($p = 0.023$) downregulated in the foetal segment group. The concentrations of other measured pro-inflammatory cytokines did not change significantly relative to the control group.

Six anti-inflammatory cytokines were analysed. Three, galectin-1, IL-13, and IL-10, were upregulated in the foetal and maternal segment groups relative to the fibrin group. Only interleukin-13 was significantly ($p = 0.0125$) upregulated in the foetal segment group relative to the fibrin group. IL-10 was significantly ($p = 0.0027$) upregulated in the maternal segment group relative to the control, fibrin and maternal segment groups. TIMP-2 was significantly ($p = 0.0007$) downregulated in all experimental groups relative to the control group (Fig. 7).

4 Discussion

Our previous study had shown that hWJ-MSCs from the maternal and foetal segments of UC had greater osteogenic differentiation potential [18], but as the experiments were conducted *in vitro* using a 2-D model the findings needed to be verified *in vivo*. Ectopic bone formation in rodent models is commonly used to evaluate the osteogenic potential of tissue-engineered bone constructs. Ectopic bone formation eliminates the bone cytokine stimulation effect of the host and the cell-to-cell interactions with host bone-forming cells, which will affect the results [20].

Fig. 2 Change in the size of bone construct over the 30-day implantation period. Gross morphology of bone construct before and after implantation. (S1-sample 1, S2-sample 2, S3-sample 3)

Table 1 Percentage of construct shrinkage after implantation. ($n = 3$)

Segments	Percentage of construct shrinkage	
	Weight	Length
Maternal	$48 \pm 1.03\%$	$67 \pm 1.01\%$
Fetal	$42 \pm 2.36\%$	$51 \pm 2.10\%$
Fibrin	$85 \pm 0.75\%$	$67 \pm 32.00\%$

Common ectopic sites for implantation are subcutaneous sites, intramuscular sites and the kidney capsule [21].

Numerous studies have demonstrated that MSCs exert immunosuppressive properties *in vitro* [22–24] and *in vivo* [17, 25]. It is thought that MSCs exert their immune modulatory effect in transplant tolerance, autoimmunity and peripheral tolerance through interactions with various types of immune cells that are mediated by cytokines and soluble factors [26]. These mediators act by inhibiting the production of pro-inflammatory cytokines or counteracting the effects of inflammation through various pathways. Thus MSCs are ideal for allogeneic cell-based therapies. We had shown previously that osteodifferentiated hWJ-MSCs have the ability to suppress proliferation of peripheral blood mononuclear cells *in vitro* and that osteodifferentiated hWJ-MSCs had a greater immunosuppressive effect than undifferentiated hWJ-MSCs [19].

In this study, none of the implanted animals showed any sign of toxicity, abnormal behaviour or restricted mobility, indicating good transplant tolerance. hWJ-MSCs from the maternal and foetal segments demonstrated a certain degree of immunotolerance and immune evasion as the constructs were not fully resorbed. In contrast, the fibrin construct was fully resorbed within 30 days. There was no observable angiogenesis in the constructs, probably due to the short implantation period. The maternal segment construct elicited greater bone formation: the surface area of bone nodule formation was larger *in vitro* and *in vivo* than in the foetal segment construct. These observations suggest that hWJ-MSCs from the maternal segment of UC possess greater osteogenic potential than those from the foetal segment. This could be due to higher number of MSC present at the maternal segment and plausibly higher plasticity, hence propensity to be differentiated into osteogenic lineage.

The lymphoid organs are the sites of immune cell production, so we carried out a histological study of the lymphoid organs (thymus, lymph nodes, and spleen) to evaluate immune modulation by osteodifferentiated MSCs. Currently there is no concrete evidence on how administration of allogeneic cells affects lymphoid organs, so this *in vivo* study was extended to assess changes in the lymphoid organs and the immunomodulatory properties of hWJ-MSCs in healthy immunocompetent Balb/C mice. Changes in the histopathology of the lymph nodes, spleen, and thymus are good indices of the severity of a host's

Fig. 3 Alizarin red staining of calcium deposition in **A** Maternal bone construct, **B** Foetal bone construct and **C** Fibrin construct. The apparent stained area was an artefact due to tissue folding. **D** Surface area of bone nodules formed on the maternal and foetal segment

immune response. We found apoptotic bodies in the lymphoid follicles of lymph nodes and in the white pulp of the spleen of the fibrin group, but not in the other groups. Apoptotic bodies are the result of lymphoid depletion or atrophy and are reflect a reduction in the number and size of lymphoid follicles and an absence or reduction germinal centres and/or depletion of paracortical lymphocytes due to immune reactions [27]. The fibrin group also had a high number of macrophages, widespread lymphoblast proliferation and large numbers of histiocytes and apoptotic bodies in the follicular region of the thymus. These observations are indicative of an ongoing acute immune response in the fibrin group. There was no evidence of a similar immune response in the maternal and foetal segment groups. It is important to note that fibrin was present in both the maternal and foetal segment constructs. The fact that the immune response evoked by fibrin was not seen in these two groups suggests that the immune response was suppressed by the presence of osteodifferentiated hWJ-MSCs. With the presence of osteo-differentiated hWJ-MSCs, the immune reaction towards fibrin (as seen in the fibrin only group) was suppressed. This could

be due to immunosuppressive properties of hWJ-MSCs that are maintained even after osteodifferentiation. Upregulation of anti-inflammatory cytokines (galectin-1, IL-10, and IL-13) was observed in this study. The level of IL-13 in the foetal segment group was three times higher than in fibrin group, whilst the level of IL-10 in the maternal segment group was 1.7 times higher than in the control group. IL-13 indirectly modulates T-cell function and T-helper type 1 cell differentiation via downregulation of the production of pro-inflammatory cytokines [28]. IL-10 is a cytokine synthesis inhibitory factor that suppresses the differentiation and activity of T-helper type 1 cells and limits the expansion and proliferation of T-cells [29, 30]. The level of galectin-1 was 1.5 times higher in the foetal segment group. This mediator induces differentiation of dendritic cells and reduces T-helper cell proliferation via IL-10 and IL-27 [31]. Galectin-1 also plays an important role in immune tolerance in pregnancy [32].

The level of pro-inflammatory galectin-3 was down-regulated in the maternal and foetal segment groups. Galectin-3 is expressed in monocytes, macrophages and epithelial cells [33, 34] and was shown to play an important

Fig. 4 H&E staining of longitudinal sections of the spleen. **A** Control specimen with follicle size < 1.22 mm. **B** Maternal segment construct specimen showing mild lymphocytic proliferation. **C** Foetal segment construct specimen showing moderate lymphocytic proliferation. **D** Fibrin construct specimen showing severe lymphocyte proliferation. **E** Higher magnification of a fibrin construct specimen with arrows indicating multinucleated giant cells. **F** Higher magnification of a fibrin construct specimen with arrows showing apoptotic bodies in the white pulp of the spleen

Table 2 Histopathological findings in the spleen

Histology	Control	Maternal	Fetal	Fibrin
Lymphoid follicles sizes	< 1.22 mm	< 1.22 mm	< 1.22 mm	> 1.22 mm
Number of lymphoblasts	+	++	+++	++++
Angiogenesis/small capillaries	+	+	+	+
Vacuolar degeneration	–	–	–	–
Fatty degeneration	–	–	–	–
Lymphocyte apoptosis	–	–	–	+

– represents absent, + represents present, ++ represents mild, +++ represents moderate, ++++ represents severe

role in the phagocytic clearance of microorganism and apoptotic cells in both innate and adaptive immunity [35, 36]. The concentration of fractalkine was significantly lower in the FT group than in maternal group. Fractalkine

is a pro-inflammatory cytokine involved in the recruitment of immune cells in various inflammatory disorders, including arteriosclerosis [37]. IL-3 was the only significantly upregulated pro-inflammatory cytokine; levels were

Fig. 5 H&E staining of longitudinal sections of lymph nodes. **A** Control specimen without lymphoblasts. **B** Maternal segment construct specimen with mild lymphocyte proliferation. **C** Foetal segment construct specimen with moderate lymphocyte proliferation. **D** Fibrin construct specimen with severe lymphocyte proliferation. In addition, fibrin construct specimens also showed **E** Vacuolar degeneration and **F** Sinusoids

Table 3 Histopathological findings in lymph nodes

Histology	Control	Maternal	Fetal	Fibrin
Lymphoid follicles				
Number of lymphoblasts	++	++	+++	++++
Angiogenesis/small capillaries	+	+	+	+
Vacuolar degeneration	–	–	–	+
Fatty degeneration	–	–	–	–
Lymphocyte apoptosis	–	–	–	+
Necrotic area	–	–	–	–
Medullary area				
Fat cells and necrotic debris	–	–	–	–
Interfollicular region				
Macrophages	++	++	++	+++
Fat cells	–	–	–	+
Vacuolar area	–	–	–	+

– represents absent, + represents present, ++ represents mild, +++ represents moderate, ++++ represents severe

Fig. 6 H&E staining of longitudinal sections of the thymus. **A** Control specimen with minimal histiocytosis. **B** Maternal segment construct specimen showing minimal lymphocyte proliferation. **C** Foetal segment construct specimen showing moderate lymphocyte proliferation. **D** Fibrin construct specimen showing severe lymphocyte proliferation. **E** Higher magnification of a fibrin segment with an arrow indicating lymphocytic apoptosis

Table 4 Histopathological findings in the thymus for all four groups

Histology	Control	Maternal	Fetal	Fibrin
Lymphoid follicles				
Angiogenesis/small capillaries	+	+	+	+
Histiocytes	+	+	++	+++
Apoptosis	+	+	+	++
Ratio of cortical and medullary areas	C > M	C > M	C > M	C > M

— represents absent, + represents present, ++ represents mild, +++ represents moderate, ++++ represents severe

significantly higher in the maternal and foetal construct groups than the control group. IL-3, also known as multi-colony stimulating factor, is a cytokine produced by

activated T-cells and mast cells [38]. IL-3 can induce the growth and differentiation of haematopoietic progenitor cells, neutrophils, eosinophils and macrophages [39].

Fig. 7 Measured cytokines normalised against control group levels. **A** Changes in pro-inflammatory and **B** Anti-inflammatory cytokines detected in the blood are presented relative to control group levels. *Represents a difference ($p < 0.05$) from the control group,

#represents a difference ($p < 0.05$) relative to the fibrin group, \$represents a difference ($p < 0.05$) relative to the maternal segment group. (MT maternal segment group, FT foetal segment group)

Overall, there was a trend towards downregulation of pro-inflammatory cytokines in the maternal and foetal construct groups.

Matrix metalloproteinases (MMPs) are expressed by stromal cells in mature tissue and are responsible for breaking down the extracellular matrix during normal physiological processes such as tissue remodelling [40, 41]. MMP activity is regulated by tissue inhibitors of metalloproteinase proteins (TIMPs). Both MMPs and TIMPs are responsible for tissue remodelling [42]. TIMPs participate in bone formation by inhibiting bone resorption, which is important for the bone maintenance and remodelling [43]. In this study secretion of TIMP-2 and MMP-2 was significantly lower in the foetal segment group than the maternal segment group and the fibrin group. This may indicate reduced osteoclastic activity (bone resorption) and bone remodelling activity in the foetal segment group.

In summary, *in vivo* the hWJ-MSCs from maternal segment UC displayed greater osteogenic capacity than hWJ-MSCs from foetal segment UC. The xenogeneic hWJ-MSCs from foetal and maternal segments induced *in vivo* production of certain anti-inflammatory cytokines and suppressed *in vivo* production of selected pro-inflammatory cytokines. Nonetheless, histological evaluation of lymphoid organs demonstrated that hWJ-MSCs from maternal

segment were less immunogenic than hWJ-MSCs from foetal segment. Surprisingly, the fibrin group showed signs of immune rejection, but this immune response was suppressed or evaded in the presence of osteodifferentiated hWJ-MSCs.

In conclusion, allogeneic hWJ-MSCs are a potential alternative to autologous BM-MSCs in cell therapy especially when it comes to aged patients with poor cell yields. This study clearly shows that there are differences between hWJ-MSCs from foetal and maternal segments of UC with respect to osteogenic potential and immunomodulatory properties. hWJ-MSCs from maternal segment showed greater osteogenic potential and result in less host immune activation than hWJ-MSCs from foetal segment. These results suggest that maternal segment of UC is a safe source of MSCs for allogeneic bone transplants, however the implantation period in this study may have been too short to allow us to observe delayed responses such as implant rejection or a chronic reaction.

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article. Ruszymah Binti Haji Idrus revised it critically for the important intellectual content. All authors contributed substantially to the conception and design of the study and give final approval of the version to be published.

Compliance with ethical standards

Conflict of interest The authors have no financial conflicts of interest.

Ethical approval All umbilical cord samples were collected at the UKM Medical Centre with informed consent from patients and approval from UKM Research Ethics Committee (FF-2014-066). Animal experiments were carried out using protocols approved by the UKM Animal Research Ethics Committee (UKM 1.5.3.5/244/FF-2015-180).

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