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Comparative study of regenerative effects of mesenchymal stem cells derived from placental amnion, chorion and umbilical cord on dermal wounds

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ABSTRACT

Objective: Mesenchymal stem/stromal cells derived from human term placentas (PMSCs) are novel therapeutic agents and more topical than ever. Here we evaluated the effects of three types of PMSCs on wound healing in an *in vivo* mouse model: Amnion-derived MSCs (AMSCs), blood vessel-derived MSCs (BV-MSCs) from the chorionic plate and Wharton's jelly-derived MSCs (WJ-MSCs) from the umbilical cord.
Methods: We topically applied PMSCs onto skin wounds in mice using the dermal substitute Matriderm® as carrier and evaluated wound healing parameters. In addition, we investigated the effects of all PMSC types under co-application with placental endothelial cells (PLECs). After 8 days, we compared the percent of wound closure and the angiogenic potential between all groups.
Results: AMSCs, BV-MSCs and WJ-MSCs significantly induced a faster healing and a higher number of blood vessels in the wound when compared to controls (Matriderm®-alone). PLECs did not further improve the advantageous effects of PMSC-treatment. Quantitative data and 3D analysis by high resolution episcopic microscopy confirmed a lower density of vessels in Matriderm®/PMSCs/PLECs co-application compared to Matriderm®/PMSCs treatment.
Conclusion: Results indicate that all three PMSC types exert similar beneficial effects on wound closure and neovascularization in our mouse model.
Practice: Using Matriderm® as carrier for PMSCs propagates rapid cell migration towards the wound area that allows a fast and clinically practicable method for stem cell application.
Implications: These promising effects warrant further investigation in clinical trials.

1. Introduction

Wound healing is a complex process that involves the interaction of different blood cells, extracellular matrix components and soluble mediators such as growth factors and cytokines. If one of those factors does not work properly, chronic, non-healing wounds occur, which bear a high risk of infections leading to amputations and sepsis [1]. Thus, many research groups are searching for the right and effective wound treatment. Until now, most of the used items like topical drugs, dressings or skin grafts did not show the desired healing results. A new promising approach to stimulate wound healing might be the application of mesenchymal stem/stromal cells (MSCs), which are delivered to the wound as intradermal injection or in combination with appropriate scaffolds

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[1–3]. Especially MSCs from human term placenta and umbilical cord are attractive candidates for cell transplantation, due to their non-invasive isolation. They have anti-inflammatory, anti-cancer and anti-fibrotic characteristics and are immunologically tolerated *in vivo* [4,5]. Placental tissues and its derivatives have clinical benefits in a wide range of wound repair and surgical applications [6].

We could demonstrate that placental amnion-derived MSCs (AMSCs) support the survival of endothelial cells and stabilize endothelial networks *in vitro* [7] and that placental blood vessel-derived MSCs (BV-MSCs) have even higher pro-angiogenic properties [8]. We recently proved that AMSCs promote wound healing *in vivo* by enhancing angiogenesis and re-epithelialization [9]. Also, Wharton's jelly-derived MSCs of the umbilical cord (WJ-MSCs) were reported to enhance wound healing [10]. They have a higher proliferative capacity and are less senescent than adipose-derived MSCs (ADSCs) [11]. The efficacy of MSCs from different sources appears to vary [2], but proper studies comparing the wound-healing abilities of various placental MSC types are still lacking. Thus, the aim of this study was to test the therapeutic effects of three different types of placental tissue-derived MSCs (PMSCs), namely: AMSCs, BV-MSCs and WJ-MSCs, on wound healing in an *in vivo* mouse model. We topically applied PMSCs on full thickness skin wounds using Matriderm® [12], a dermal skin substitute, as carrier and evaluated skin regeneration with special focus on neovascularization and re-epithelialization. As subcutaneous co-implantation of AMSCs with placental endothelial cells (PLECs) induced a higher number of blood vessels [13], we additionally investigated whether PMSCs/PLECs co-application further enhances neovascularization.

2. Materials and methods

2.1. Cell culture

AMSCs, BV-MSCs, PLECs were isolated from human term placentas and characterized as published [7,8,14]. WJ-MSCs were isolated from human umbilical cords similar to the method of [15]. Briefly, the umbilical cord was cut into 1.5 cm length pieces. The amnion and umbilical vessels were removed. The remaining tissue was minced into 1–3 mm pieces, which were plated on 6-well plates. After a drying period of 5 min, 2 ml of EGM-2 (Lonza) was added. After 4 days, 1 ml EGM-2 was replaced by fresh medium. Then medium was changed every 2–3 days. After 10–14 days, the tissue explants were removed and the outgrown cells were further cultured in EGM-2. Approval of the Ethical Committee of the Medical University of Graz was granted (No. 21–070 ex 09/10). Flow cytometric analysis demonstrated that AMSCs, BV-MSCs, WJ-MSCs showed a similar surface marker profile of common MSC markers. They expressed CD90, CD73, CD105, and were negative for the immune and endothelial markers, CD45, HLA-DR, CD14, and CD34 (supplementary figure).

2.2. Animal experiments

The Animal Care and Use Committee (Veterinary University Vienna, Austrian Ministry of Science and Research) approved animal experiments. We chose 7 week old female NMRI-Foxn1nu/Foxn1nu mice (Janvier Labs), because their hairless appearance makes them perfectly suited for wound healing experiments. Mice were anesthetized by isoflurane inhalation and intraperitoneal injection of Fentanyl (0.005 mg/kg), Midazolam (2 mg/kg) and Medetomidin (0.15 mg/kg). We created two circular punch biopsy full-thickness wounds (8 mm diameter) on the dorsal side of the mice. Wounds were treated with Matriderm® (Medskin Solutions) soaked with 50 μl EGM-2 ± cells (Fig. 1A) using following settings (n = at least 5 mice, 5 wounds per treatment): Matriderm® + 3×10^5 cells (AMSCs alone, BV-MSCs alone, WJ-MSCs alone; PLECs mixed with the respective PMSC type in an established 80:20 ratio [13]) or Matriderm® without cells (negative control). Wounds were covered with 3M™ Tegaderm™ dressing (3M Corporate Headquarters). Directly after the surgical procedure, the mice received a single shot antibiotic to prevent infections. The mice received postoperative analgesia and were housed in groups until they were sacrificed by the 8th day at the latest.

For our *in vitro* study, we seeded AMSCs (2.5×10^5 cells) on Matriderm membranes (12 mm, diameter) placed on the bottom of 24-well culture plates or placed in Transwell inserts (0.4 μm pore size, Corning) to study the cell ingrowth of PMSCs into Matriderm® for 1, 2, 3, and 8 days *in vitro*.

2.3. Analysis of wound healing and immunohistochemistry

After sacrificing the mice, wounds were photographed to quantify the reduction of the wound areas. The wound areas were measured by tracing the wound margin and calculated (as area of original wound − area of actual wound)/area of original wound x 100) using Axiovision SE64 Re.4.9.1 software.

The wound areas were excised, fixed in 4% formalin, embedded in paraffin and sectioned. Every 100–200 μm intervals, 5 serial sections (5 μm) were collected up to the maximal wound area to perform H&E staining. Sections of interest were further processed for immunohistochemistry. Antigen retrieval was performed at pH9 (for Vimentin and smooth muscle actin (SMA) or pH6 (for CD 31). After blocking in H2O2 (10 min; ThermoScientific), Ultra V Block (5 min; ThermoScientific), M.O.M™ block (1 h; VectorLab), protein block (30 min; Dako), slides were exposed to anti-Vimentin (IgG1, 0.08 μg/ml; Dako) and anti-SMA (IgG2a, 0.07 μg/ml; Dako) for 30 min and developed with the UltraVision LP Detection System (ThermoScientific) to detect PMSCs and myofibroblasts, respectively. Rat-anti-mouse CD31 (2 μg/mL, 1 h, Dianova) was used to identify murine blood vessels. Staining was visualized using a biotinylated rabbit-anti-rat antibody (10 μg/mL, VectorLab) and Streptavidin-Peroxidase Reagent (10 min; ThermoScientific) after blocking with H2O2 (10min), Avidin and Biotin Block (both 15 min; VectorLab) and protein block (30 min).

2.4. Quantification of blood vessels

A section of the maximal wound area and 2 adjacent sections in a distance of 100 μm per treatment and mouse were stained with anti-CD31 to quantify murine blood vessels. Stained slides were photographed at fivefold magnification. The circumference of the whole wound area except healthy skin was marked and the number of blood vessels/mm^2 was calculated using Axiovision SE64 Re.4.9.1 software.

2.5. High resolution episcopic microscopy (HREM)

Samples devoted to three-dimensional analysis using HREM [16,17] were fixed in 2% PFA/PBS containing 4% carbolic acid for 3 days and dehydrated in a series of increasing ethanol concentrations (70% for 24 h; 80%, 90%, 100% for 2–3 h each). Samples were then transferred into JB-4 infiltration solution (Polysciences Europe) containing 0.4 g eosine spritlöslich (Waldeck GmbH) per 100 mL. After 28–32 h of infiltration at 4 °C (rocking, 3 changes) the samples were embedded in JB-4 resin, dyed with eosine (0.4 g per 100 mL). The resin blocks polymerised for 3 days at room temperature and were then baked for 48 h at 75 °C and subjected to HREM as previously described [18] to generate digital volume data. Briefly,