Encapsulated Mesenchymal Stem Cells for Dental Pulp Regeneration. (RanoKure)

Clinical trial register: NCT03102879.
Date: 01/05/2019.

PROTOCOL STUDY

Manufacturing and quality control of clinical grade UC-MSC

Umbilical cords were obtained from full-term human placentas delivered by cesarean section after informed consent, from healthy donors, and processed within 4 hours after birth. To elaborate the end product, UC-MSCs were cultured from the Master Cell Bank (MCB) of Cells for Cells S.A. and cryopreserved in aliquots of $1x10^6$ cells in FBS + 10% DMSO. For quality control of the MCB and end cellular product, UC-MSCs were evaluated for identity and purity for the following surface markers: CD105, CD90, CD73, CD34, CD45, CD19 and HLA-DR by Flow Cytometry in a FACS Canto II Flow Cytometer (BD Biosciences) and analyzed with FlowJo analysis software. Furthermore, sterility of the product was evaluated by Hemoculture, Fast Gram Test, Endotoxin (Endosafe PTS, Charles River, Wilmington, MA, USA) and Mycoplasma (MycoAlert, detection kit, Lonza, Basel, Switzerland). Finally, cell count and viability were determined by Trypan Blue exclusion assay. In addition, MCB was evaluated for tri-differentiation potency and tumorigenicity.

Platelet-Poor Plasma (PPP) storage and UC-MSCs encapsulation

PPP fractions were obtained from AB Rh-positive universal irradiated plasma from healthy donors collected from the Blood Bank Unit of the Clínica Universidad de los Andes, Chile. All the donors were screened for mandatory reportable diseases and stored at –80°C. At 48 hours before treatment, cells were thawed and seeded in a T150 flask with αMEM supplemented with 5% Plasma AB+. MSCs were then harvested using TrypLE Express 1X (Walthan, MA, USA) and washed twice with PBS 1X and resuspended in 175uL de saline solution. The resuspended cell was then mixed with 760uL of freshly thawed PPP, 15uL of Tranexamic acid and 50uL of a 2% solution CaCl2 in a 1,5ml microfuge tube. All the components were mixed by pipetting and the suspension was incubated at 37°C for 5 minutes. After checking the gelification, the end-product was stored and transported at 4°C for transplantation.

Clinical Procedure

The operators were two endodontic specialists who had been previously calibrated with a standard operating procedure. The first session was the same for every patient. Tooth sensitivity (cold, heat and electric) was assessed. Tooth vitality was tested using a Laser Doppler flowmetry (LDF) (Moor VMS-LDF; Moor Instruments Limited, Axminster, UK). Then intervention was started by the administration of local anesthesia (2% lidocaine hydrochloride with epinephrine 1:100,000; Septodont, Saint-Maur-des-Fosses, France). The access cavity was prepared using sterile high-

speed round burs, size 018 (Dentsply Sirona, Ballaigues, Switzerland). In all cases, rubber dam isolation was maintained (Hygienic; Coltene/Whaledent AG, Altstätten, Switzerland). A glide file was established with a size 10 hand K-file (Dentsply, Ballaigues, Switzerland). The working length to the apical constriction was confirmed using an electronic apex locator (Root ZX; J Morita, Tokyo, Japan) and verified radiographically. All root canals were enlarged using Reciproc® (VDW, GmbH, Munich, Germany) selected according to manufacturer's recommendation. All teeth were irrigated with 20 ml 2.5% sodium hypochlorite and Endoactivator system (Dentsply Tulsa Dental Specialties, Tulsa, OK) was used for final sonic activation. Finally, calcium hydroxide (Hertz Pharmaceutical, Santiago, Chile) was used as an intracanal medication and closed with glass ionomer (Vitrebond; 3M ESPE, St Paul, MN, USA). After three weeks in the second session, the medication was removed by irrigation with 20 ml 17% EDTA and canal dried with sterile paper points. Subsequently, each tooth was randomized and assigned to each group following a specific protocol. ENDO Group: canals were filled using gutta-percha cones (Reciproc® VDW, GmbH, Munich, Germany) and Topseal® sealer (Dentsply Sirona, Switzerland) through a continuous wave condensation technique with an Elements™ Obturation Unit (Sybronendo, Orange County, CA, USA). REP Group: apical bleeding was induced by using a size 8 stainless steel hand K-file (Dentsply Sirona) 3 mm beyond the apex. Once the apical portion of the root canal was filled with blood, PPP-UC-MSCs was implanted and absorbable gelatin sponge hemostats (Gelita-Spon® GmbH, Eberbach, Germany) was used to contain the Biodentine™ (Septodont, France) filling material. Final restorations in both groups were done with resin (Filtek™ Z350 XT Universal Restorative; 3M ESPE, St Paul, MN, USA). Patients were blinded to treatment group. All clinical procedures were performed under an operating microscope (OPMI® Pico; Carl Zeiss AG, Göttingen, Germany).

Statistical Analysis

The categorical variables were described by absolute frequencies and percentages by group of treatment and were evaluated by the Chi-square test of independence or Fisher's Exact test at each time point. The quantitative variables were described by the median and interquartile range and they were compared between treatment groups using the Mann Whitney test at each time point. Lesion size differences 6 months versus basal time point and 12 months versus 6 months were compared between treatment group through Mann Whitney test. The evolution over time in each treatment arm of perfusion, dimensions of lesion, and cortical compromise was evaluated through a generalized estimating equation (GEE) model assuming repeated measures over time. It was reported 95% confidence interval and p-values. For variables with zeros as a result, as is the pain to percussion and sensitivity test, differences between basal point and 12 months was used McNemar test and p-value was reported. It was considered significant a p-value<0.05. Effect size by

bootstrapping estimation was reported if it was >0.4. The analysis was performed using the software Stata® (StataCorp 2015-Stata Statistical software 14.2, College Station. TX: StataCorp LP).