

### **ASC isolation and *ex vivo* expansion**

The harvested tissue was dissociated by digestion with 0.075% type IA collagenase (Sigma-Aldrich, Inc.) for 45 minutes. Enzyme activity was stopped and the cell suspension was centrifuged at 300g for 15 minutes. Pelleted cells were recovered and plated onto 10-cm culture plates (NUNC, Rochester, NY). At 24-hour intervals, cultures were washed with PBS to remove contaminating erythrocytes and other unattached cells, and then reseeded with fresh medium. The plating and expansion medium consisted of low glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and penicillin/streptomycin antibiotics (Invitrogen Corporation, Carlsbad, CA).

Cells were maintained at 37°C with 5% CO<sub>2</sub> in tissue culture dishes and fed twice a week until they reached 80% of confluence - usually within 5 to 7 days after the initial plating. Once 80% confluence was reached (passage 0), adherent cells were detached with 0.25% trypsin-EDTA (Vitrocel Embriolife, Campinas, SP, Brazil) and were either replated at  $1 \times 10^4$  cells/cm<sup>2</sup> or used for experiments. Cultures were passaged every 3 to 5 days and used for experimental procedures until passage 3.