Harvesting, isolation and characterization of *Saimiri sciureus* adipose-derived mesenchymal stem cells

Luane L. Pinheiro[a], Ana T. Tobelem[a], Ana R. Lima[a], Edna C. S. Franco[b], Klena S. M. Silva[b], Érika R. Branco[a]

[a] Federal Rural University of Amazonia, Belém, PA – Brazil
[b] Evandro Chagas Institute, Ananindeua, PA – Brazil

Abstract

The *Saimiri sciureus* is one of the most used species of neotropical primates as a biological model due to its relatively small size and ease containment. The aim of this study was to establish a technique of harvest, isolation and differentiation of adipose-derived mesenchymal stem cells (ASCs) of *S. sciureus*, targeting future applications in regenerative medicine and cell therapy in future experimental studies. Upon approval of the Ethics Committee on Animal Use (CEUA) of the Evandro Chagas Institute (IEC), protocol N031/2013, it was used two adult male animals belonging to the National Primate Center (CENP/IEC/SVS/MS) – Ananindeua – PA. The specimens were physically restrained, tranquilized with Tiletamine and Zolazepam Hydrochloride (6mg/kg/IM), and the local anesthetic used was Lidocaine Hydrochloride. With the skin incision in the linea alba, the subcutaneous space was dissected to extract adipose tissue fragment. The animals were given ketoprofen (2mg/kg), IM every 8h for 3 days and the dressing was made with healing ointment. The process of enzyme digestion was performed with collagenase type IV (4.5mg/mL), at 37°C for 1h, and then it was inactivated by adding 5 mL of complete medium (DMEM:F12 with 20% FBS) and centrifugated for 10 min at 1000 rpm; then the pellet resuspended and centrifuged again under the same conditions. The cell stroma was cultured at 37 °C/5% CO_2 and 56% of relative humidity, with a medium change every 48h and cell viability analized through exclusion test with trypan blue dye. Using cytogenetic techniques for obtaining of chromosomes and G-banding, the cells were analyzed in P4, 6 and 8, in order to verify chromosomal abnormalities. P8 cells were cultured with specific commercial means (StemPro, Gibco) for differentiation osteogenic, adipogenic and chondrogenic, following manufacturer's recommendations, and proven with tissue-specific dyes. Immunocytochemical characterization with overnigth incubation in primary antibodies was performed (CD105 - 1:100, CD90 - 1:100, CD73 - 1:50, CD34 - 1:50 e CD79 - 1:100) and secondary antibodies bound to biotin by 30 min/37°C then to streptavidin and revealed with diaminobenzidine. In 24h there were small amounts of cells adhered to the plastic surface, but they had obtained confluence of approximately 90% within 8 days of cultivation. After P1, the ASCs have spread rapidly, forming a homogeneous population of cells with fibroblastic morphology, keeping karyotype stable 2n 44 chromosomes, an average of viability above 80% until P8 and exponential growth. The *S. sciureus* ASCs differentiated into osteogenic, adipogenic and chondrogenic lineages, and had positive markers for CD105, CD90, CD73 and negative for CD34 and CD79. *S. sciureus*

ASCs may be isolated by a simple procedure and expanded in vitro without loss of their potential for proliferation, multilineage differentiation and expression of specific cell surface markers, keeping karyotype stable and enabling their use in therapeutic trials.