

Isolation of stem cells from pets using good manufacturing proceedings

Vivian Gonzaga^[a], Jessica Castanheira^[b], Alexandre Kerkis^[a], Irina Kerkis^[a], Cristiane Wenceslau^[b]

^[a] Butantan Institute, São Paulo, SP – Brazil

^[b] Federal University of São Paulo (UNIFESP), São Paulo, SP – Brazil

Abstract

For nearly half a century, pets have played a key role in advancing stem cell therapies because they are recognized as critical translational models of human diseases. Nowadays, primary care veterinarians are using adult stem cell therapies, particularly for orthopedic conditions. However, veterinary stem cell therapies are still deficiency in good manufacturing proceedings to isolation of stem cells with substantial quantities and excellent quality. Thus, this study provides isolation of multipotent stem cell from dental pulp (DP) and umbilical cord (UC) that are noninvasive sources and have pluripotential niches according to requirements for human. In order to isolate multipotent stem cells from DP were used clinically healthy pets with ranged age from 6 months to 3 years and UC from new born kittens and puppies. Herein, DP and UC cultured generating was performed by explant also multiples mechanical transfers into a new culture dish. The mesenchymal stem cells (MSC) from DP and UC were cultivated in DMEM-F12, supplemented with 15% SFB-hyclone. These cells were characterized using following antibodies: canine anti-Oct3/4, canine anti-SOX2, canine anti-NANOG, canine anti-CD44 antibody and canine anti-CD146, CD105 vimentin, alfa-actnina and fibronectina by immunofluorescence assay. Firstly, fibroblast-like cells appeared after five or six days. A total of four mechanical transfers of DP and UC were performed each 5-6 days without using enzymatic treatment. No changes in both early transfer (T0) and later transfer (T4) were observed in morphology and expression of stem cells markers. DP and UC expressed filaments intermediary vimentin, fibronectin and alfa-actnin. The DP and UC cells expressed to CD44 and CD149 proteins. A few DP cells reacted positively to pluripotent stem cells markers, such as Oct3/4 and Sox2, while UC expressed Sox2 and nanog. The DP and UC are phenotypic similar and even isolated by mechanical transfers, and these cells expressed proteins associated to MSC. Furthermore, ours finding are important for the future of pet stem cell therapies, providing scaling-up of stem cells with minimum risk of losing their “stemness”.

