Canine adipose stem cells: the influence of the anatomy and passaging on the stemness and osteogenic differentiation potential

J. F. Requicha[1,2,3], C. A. Viegas[1,2,3], C. M. Albuquerque[2], J. M. Azevedo[4], R. L. Reis[1,3], M. E. Gomes[1,3]

[1] *3B's Research Group – Biomaterials, Biodegradables and Biomimetics, Department of Polymer Engineering, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Guimarães, Portugal*

[2] *Department of Veterinary Sciences, School of Agrarian and Veterinary Sciences, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal*

[3] *ICVS/3B's – PT Government Associated Laboratory, Braga/Guimarães, Portugal*

[4] *CECAV – Centre for Studies in Animal and Veterinary Sciences, Department of Animal Sciences, School of Agrarian and Veterinary Sciences, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal*

Abstract

Periodontal disease (PD) is an inflammatory pathology highly prevalent both in humans and in dogs, which is characterized by destruction of the periodontal ligament, cementum and alveolar bone. The development of new therapies, such as Tissue Engineering strategies, is mandatory due to the inefficacy of conventional therapies currently used.

Dog is a very relevant animal model to study several human diseases, such as PD and simultaneously an important subject in veterinary medicine. Adipose derived stem cells have a great potential for application in cell based therapies, such as tissue engineering.

The aim of this study was to assess the potential of the canine adipose tissue derived stem cells (cASCs) to differentiate into osteoblasts and chondroblasts and study the anatomical origin and cell passaging effect on the cASCs stemness and osteogenic potential. In addition, we aimed at assessing the behavior of cASCs when cultured onto a newly developed double layer scaffold, specifically designed for the regeneration of periodontal defects.

The adipose tissue was harvested from the abdominal subcutaneous layer and from the greater omentum from adult healthy dogs, according to the animal welfare Portuguese legislation. cASCs were isolated by an enzymatic method and expanded along 4 passages in basal medium. cASCs were cultured using either osteogenic medium or chondrogenic medium. The stemness and osteogenic differentiation was followed by real time RT-PCR analysis of typical markers of MSCs, namely CD73, CD90 and CD105, and osteoblasts, like COLIA1, RUNX2 and Osteocalcin.

The behavior of the cASCs was then evaluated on a previously developed double layer scaffold based on a starch and polycaprolactone (SPCL) blend that comprises a functionalized 3D fiber mesh to promote osteogenesis (an thus support alveolar bone regeneration) combined with a membrane aiming at acts as a physical barrier and promote periodontal ligament regeneration. The cellular proliferation was assessed by dsDNA quantification and SEM observation. The gene expression of MSCs/osteogenic typical markers was assessed by real time RT-PCR.

The obtained data revealed that cASCs exhibit a progressively decreased expression of the MSCs markers along passages and also a decreased osteogenic differentiation potential. Moreover, the results showed that the anatomical origin of the adipose tissue has an evident effect in the differentiation potential of the cASCs.

The resemblances with the human ASCs make the canine ASCs a suitable cell model for study new cell therapies for humans. Additionally, we reported the high potential of a newly developed scaffold combined with ASCs for periodontal tissue engineering.

Keywords: Adipose stem cells; Tissue engineering; Canine model; Periodontal regeneration