CHITOSAN-COLLAGEN SCAFFOLDS CAN REGULATE THE BIOLOGICAL ACTIVITIES OF ADIPOSE MESENCHYMAL STEM CELLS FOR TISSUE ENGINEERING

Idiberto Jose Zotarelli Filho[1, 2, 3], Luiz Fernando Frascino[3], Elias Naim Kassis[2, 3] , Marinônio Lopes Cornélio [1]

[1] State University of Sao Paulo-IBILCE-UNESP, Rua Cristovao Colombo 2265, Sao Jose do Rio Preto SP Brazil.
[2] *Unipos - Post graduate and continuing education, Street Ipiranga, 3460, São José do Rio Preto SP Brazil 15020-040.*
[3] *Portuguese Beneficent Hospital, São Jose do Rio Preto SP-Brazil.*

BACKGROUND

The use of mesenchymal stem cells from human adipose tissue (ADSC - Adipose Derived Stem Cell), aimed at repairing injured cardiac tissue, appears as a promising treatment option for patients with cardiomyopathy. To facilitate the infusion of these cells in the heart, use of matrices for growing ADSC is an important strategy in the field of cardiac tissue engineering. Among the biomaterials used for this purpose chitosan and collagen type I (Blenda) because they are biocompatible, biodegradable and nontoxic. The use of chitosan as a biomaterial has several attractive properties for tissue regeneration because their cationic nature permits electrostatic interactions with the cell anionic species such as glicosaminoclicanos (GAG) and proteoglycans .

OBJECTIVE

The objective was to evaluate the physico-chemical ADSC with respect to growth kinetics, size, production of the enzyme alkaline phosphatase, inducing the production of nitric oxide by macrophages and examine the adhesion and proliferation of mesenchymal stem cells from adipose tissue (ADSC) in chitosan matrices (Ch), collagen (C) and the blend (B), within **petakas.**

METHODOLOGY

In the methodology, 07 female patients, aged between 20 and 60 years, underwent liposuction hospital in IMC, using techniques which involve manual aspiration, mechanical aspiration with vacuum lipoaspirador (Nevoni ® mod 3003). Digestion of the fat tissue was made with the enzyme collagenase for two hours. Then, the pellet obtained by centrifugation was resuspended with 6.0 x 10^8 (± 2) cell viability and 99.0 (± 3) % by volume final. All analyzes were performed after the third passage, and immunophenotyping the ADSC. We carried out physico-chemical studies of the kinetics of growth ADSC, measures of cell sizes in **Petakas (Celartia-USA)** in bottles and traditional activity of the enzyme alkaline phosphatase (AP)-mediated cytotoxicity and nitrogen oxide (NO) in the presence of activated macrophages and the presence of the chitosan matrix (Ch) and collagen (Co) for biocompatibility studies. Made up of simple randomization fat samples, Pearson correlation between the volumes and number of fat ADSC sampled by the method parametric analysis of variance (ANOVA).

DISCUSSION

The physicochemical properties of chitosan-collagen matrices induced adhesion, proliferation and differentiation of ADSC (figure 1). Thus, the reduction in alkaline phosphatase enzyme activity in the presence of scaffolds of Ch, and Ch-Co-Ge as compared with the increase of NO synthesis and decrease in cell proliferation may be indicative that the ADSC initiate cellular differentiation (figures 2 and 3). importance of the presence of chitosan, collagen, ADSC genipin and in the process of tissue regeneration, because these scaffolds strongly stimulated NO production that may be responsible, *in vivo*, by vasodilation present in physiological angiogenesis, in addition to function as a mediator in wound healing, and as an inhibitor of leukocyte adhesion in post capillary microvessels.

RESULTS

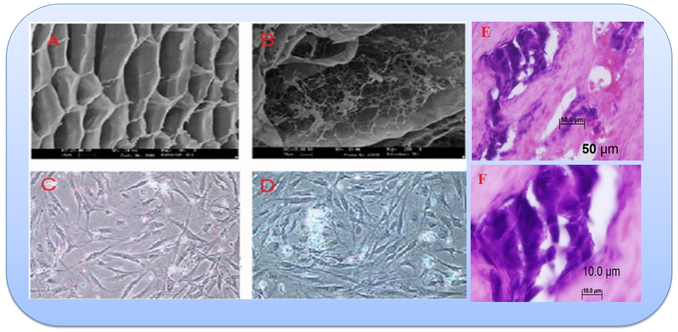

Figure 1 - Images of scanning electron microscopy obtained in previous studies from Zotarelli et.al., 2011 as an example of the morphological structure of scaffolds of chitosan (Ch) (**A**) and chitosan-collagen (Ch-C) (**B**) (blend) and optical microscopy images of ASC adhered and proliferated in Petakas (**C** and **D**). And scaffolds with ADSC adhered and proliferated in blends for a period of 48 hours, all stained with hematoxylin-eosin (**E** and **F**). The images of the three controls are 50.0µm and 10.0µm.

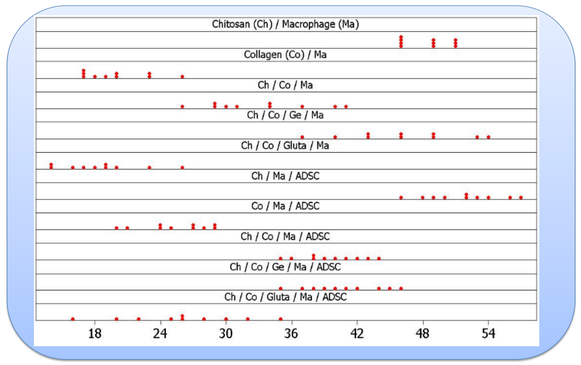

Figure 2. DotPlot graphic model (Minitab 15) showing NO production, whose values are in micromol ml-1 by stimulated macrophages chitosan, collagen, chitosan, collagen, chitosancollagen-genipin to 0.50% collagen-chitosan-glutaraldehyde : 50 %, with and without the presence of ASC, with n = 10.

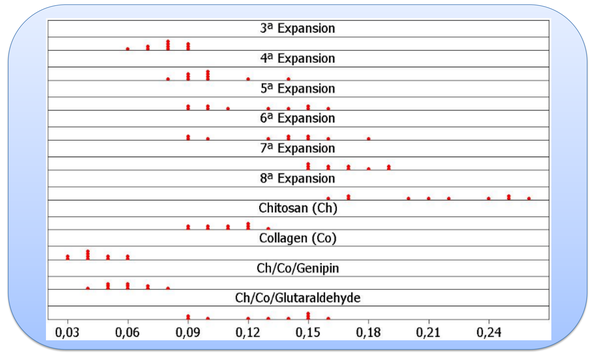

Figure 3. Graph model DotPlot (Minitab 15 showing the values of the activity of the enzyme alkaline phosphatase secreted by ASC in U mL-1 in the case of the third expansion of ASC and eighth in the presence of scaffolds of chitosan, collagen, collagenchitosan-genipin and chitosan-collagen with glutaraldehyde ADSC, with n =10.

CONCLUSION

The physicochemical properties of chitosan-collagen matrices induced adhesion, proliferation and differentiation of ADSC possible because there was a significant relationship between the production of the enzyme alkaline phosphatase by ADSC and NO production by macrophages. This relationship of AF showed that the enzyme activity decreased as increased NO production and this occurs when the cells are beginning to differentiate. Added to this, the matrix of chitosan-collagen - genipin stimulates this process, which can result in a major course of neovascularization or angiogenesis tissue.

REFERENCES

BALDWIN, A. D.; KIICK, K. L**. Polysaccharide-modified synthetic polymeric biomaterials**. Biopolymers ,94, 128–140, 2010.

CHAN, Y. L.; NGAN, A. H. W.; KING, N. M. **Use of focused íon bean milling for investigating the mechanical properties of biological tissues: A study of human primary molars**. Journal of the Mechanical Behavior of Biomedical Materials, **2** (4), 375-383, 2009.

BODO-ECKEHARD STRAUER, MUHAMMAD YOUSEF, AND CHRISTIANA M. SCHANNWELL. **The acute and long-term effects of intracoronary Stem cell Transplantation in 191 patients with chronic heart failure: the STAR-heart study.** European journal of Heart Failure (2010) 12, 721-729.

ACKNOWLEDGMENTS

The work was financially supported by the Unipós – Graduate School of São José do Rio Preto – Brazil and also appreciate the support of the Life Group (Brazil) and Celartia (Ohio, USA). We appreciate the support of Duke University (Durham, NC, USA) in the field of research and statistical studies also and Universidade Estadual Paulista - UNESP, São José do Rio Preto.