Textile-based Silk Scaffolds for Bone Tissue Engineering Applications

Viviana Ribeiro[1,2], Alain Morais[1,2], Vitor M. Correlo[1,2], Alexandra P. Marques[1,2], Ana Ribeiro[3], Carla Silva[3], Nuno Durães[3], Graça Bonifácio[4], Rui Sousa[1,2], Joaquim M. Oliveira[1,2], Rui L. Reis[1,2] and Ana L. Oliveira[1,2,5]

[1]3B's Research Group–Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Guimarães, Portugal; [2]ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal; [3]CeNTI, Centre for Nanotechnology and Smart Materials, V.N. Famalicão, Portugal; [4]CITEVE, Technological Center for Textile and Clothing Industry, V.N. Famalicão, Portugal; [5]CBQF–Center for Biotechnology and Fine Chemistry, Portuguese Catholic University, Porto, Portugal.

viviana.ribeiro@dep.uminho.pt

INTRODUCTION

Scaffolds developed for bone tissue engineering (TE) need to facilitate and promote cell adhesion, proliferation and neo-tissue formation. They must possess specific properties to allow the new tissue to integrate with the material, without inducing any inflammatory response[1]. The intra-architectural scaffold geometry, porosity, scaffold material and surface area play important roles in this process[2]. Several polymeric systems (natural and/or synthetic) and processing methods and have been proposed to develop the "ideal" scaffold for bone TE. However, so far the proposed strategies do not fulfil all the requirements for effective bone regeneration. Textile-based technologies constitute an innovative alternative for the production of 3D structures for bone TE applications, offering a superior control over scaffolds' design, manufacturing and reproducibility[3]. Silk fibroin (SF) derived from silkworm Bombyx mori has already proved to be a good biomaterial for bone TE applications[4]. SF-based structures offer impressive mechanical properties, biodegradability, biocompatibility and stability, which meets the basic requirements for the design of structures for bone regeneration applications[5].

EXPERIMENTAL METHODS

In this work we describe for the first time the processing of natural silk yarns into 3D scaffolds, combining standard knitting fabrics spaced by a monofilament of polyethylene terephthalate (PET). A comparative study is established using a stable polymeric system made entirely of PET. The obtained knitted spacer constructs were described in terms of morphology and mechanical properties. An *in vitro* biological assay was performed to evaluate the potential of the developed structures to support human Adipose-derived Stem Cells (hASCs) adhesion, proliferation and osteogenic differentiation. Cells were cultured over 28 days in standard basal and osteogenic conditions and evaluated through different quantitative (DNA, ALP, Calcium, RT-PCR) and qualitative (SEM, Alizarin Red, immunocytochemistry) assays. The *in vivo* biocompatibility of the textile materials was assessed by subcutaneous implantation in mice model. After 2 and 4weeks of implantation the explants were collected and the obtained slides were stained with hematoxylin and eosin (H&E).

RESULTS AND DISCUSSION

The cross-sections of the developed spacer textile constructs reveal a significant increase of the scaffolds three-dimensionality induced by the PET monofilament (Figure 1a). The hASCs seeded onto the spacer textile scaffolds were able to attach, spread, proliferate and differentiate, both in osteogenic and basal conditions (Figure 1b). Great evidences of ECM mineralization were observed, also penetrating and colonizing the PET monofilament (Figure 1a). The preliminary *in vivo* results revealed that the implanted scaffolds allowed tissue ingrowth, without inducing any acute inflammatory reaction (Figure 1c).

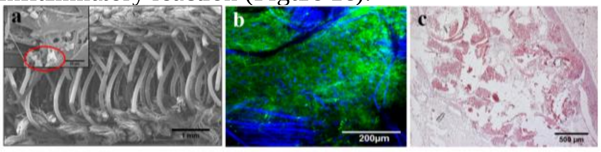

Figure 1- SF textile scaffolds seeded with hASCs and cultured for 28 days in osteogenic medium: (a) SEM micrographs from a cross-section and top view perspective. The red circle indicates mineralization nodules; (b) Confocal micrographs, nuclei stained in blue and Col I in green. (c) H&E staining of a SF textile scaffold in the explant, after implantation for 2 weeks.

CONCLUSION

In this study, innovative 3D biotextile scaffolds able to support cell adhesion, proliferation and osteogenesis were successfully developed. Furthermore, these scaffolds allowed the new tissue formation and integration within the material, when subcutaneously implanted in mice. Thus, the proposed textile-based scaffolds can be promising candidates for bone TE applications such as the craniomaxilofacial complex[6].

REFERENCES

1. Burg K. *et al.*, Biomaterials. 21(23):2347-2359, 2000
2. Salgado J. *et al.*, Macromol Biosci. 4(8):743-765, 2004
3. Sumanasinghe R. *et al.*, Research Journal of Textile and Apparel. 9(3):80-90, 2005
4. Kundu B. *et al.*, Adv Drug Deliver Rev. 65(4):457-470, 2013
5. Yan L-P. *et al.*, J Bioact Compat Pol. 28(5):439-452, 2013
6. Liu G. *et al.*, Biomaterials. 34(11):2655-2664, 2013

ACKNOWLEDGMENTS

Portuguese Foundation for Science and Technology (FCT) for the project TISSUE2TISSUE (PTDC/CTM/105703/2008). Investigator FCT program IF/00423/2012 and IF/00411/2013 are also greatly acknowledged.

Keywords:
3D biotextile, Silk fibroin, Knitting, Spacer, Human adipose-derived stem cells, Bone tissue engineering