Engineering Cell-adhesive Gellan Gum Spongy-like Hydrogels

Lucélia da Silva1,2, Mariana T. Cerqueira1,2, Rui A. Sousa1,2, Rui L. Reis1,2, Vitor M. Correlo1,2, Alexandra P. Marques1,2
13B’s Research Group - Biomaterials, Biodegradables and Biomimetics, University of Minho, Guimarães, Portugal; 2ICVS/3B’s - PT Government Associate Laboratory, Braga/Guimarães, Portugal

Introduction
The resemblances between soft tissues extracellular matrix (ECM), characterized by a viscoelastic polymeric network with high water content, and hydrogels has been sustaining its advance for Regenerative Medicine purposes1. However, hydrogels limitations, such as mechanical instability and elasticity, as well as lack of cell-adhesive properties have been hampering major successes. The most followed but laborious and expensive strategy to overcome the absence of cell adhesion sites within hydrogels relies on binding cell adhesive sequences, such as, RGD, or blending ECM proteins with other polymers. Considering these constrains, here we propose a simple method of processing Gellan Gum (GG) hydrogels with non-adhesive features, into GG spongy-like hydrogels that depict physical features of both sponges and hydrogels, with high water content and improved microstructure and mechanical performance, but more importantly, cell adhesive character.

Materials and Methods
Spongy-like hydrogels were obtained, following a patented methodology (Provisional patent 20131000027163), from GG hydrogels and upon rehydration of a freeze dried polymeric network by dropwise addition of PBS or a cellular suspension prepared in adequate culture medium. Freezing thermodynamics under different freezing temperature and time, as well as after varying the solute amount and type, was analyzed. The onset of freezing temperature was determined by Differential Scanning Calorimetry (DSC) and the microarchitecture of the dried polymeric networks was characterized by micro-computed tomography (µ-CT). The precursor hydrogels and spongy-like hydrogels were characterized in terms of morphology by cryo-scanning electron microscopy (cryo-SEM), and regarding their water content and mechanical strength. Human adipose stem cells (hASCs), keratinocytes (hKCs) and umbilical cord vein endothelial cells (HUVECS) were respectively isolated from lipoaspirates, skin samples and umbilical cord vein, following standard procedures and entrapped/encapsulated within the spongy-like and precursor hydrogels, respectively. After 3, 7 and 14 days of culture, cell adhesion was evaluated after phalloidin/DAPI staining, cell viability by calcein/PI, and cell proliferation by ki-67 staining.

Results
The most critical step during spongy-like hydrogels preparation was the freezing stage, whose thermodynamics was highly influenced by the solutes existing in the hydrogel prepared at previous processing steps as well as by freezing duration and temperature.

The microarchitecture of spongy-like hydrogels was dissimilar from hydrogels. The average pore size of spongy-like hydrogels was significantly higher than hydrogels (Fig. 1A), while its water retention capability was lower (~200%) nevertheless, presented equivalent compressive modulus. Moreover, spongy-like hydrogels, in contrast to hydrogels presented a great capacity of shape adaptability, a high degree of flexibility and a fast and complete recover applying a compressive force (up to 60% strain) (Fig. 1B). hASCs entrapped within the spongy-like hydrogels adhered to the pore walls and organized their cytoskeleton, exhibiting their typical adherent morphology, not observed in hydrogels (Fig.1C). Furthermore, spongy-like hydrogels were able to support hASCs proliferation (Fig. 1D) presenting a significantly low amount of non-viable cells after 7 days of culture (Fig. 1E). Cell adhesion was similarly observed with hKC (Fig. 1F), although hDMEC were only able to adhere in fibronectin-soaked spongy-like hydrogels (Fig. 1G).

Discussion and Conclusions
In this work, we propose a simple and cost-effective processing methodology to obtain GG spongy-like hydrogels that present significant advantages over traditional and precursor hydrogels. Those structures permit to overpass limitations such as the reduced physical stability and flexibility with limited handling possibility as well as off-the-shelf availability. Moreover, the reduced temperature window for viable cell encapsulation and homogeneous cell dispersion within the hydrogel structure are overcome with spongy-like hydrogels that result from a prompt hydration of a dried polymeric network available off-the-shelf, under physiological conditions. Most importantly, when re-hydrated with a cell suspension, cells became entrapped and are able to adhere and proliferate, without using time-consuming and expensive cell-adhesive peptide sequences incorporation used for hydrogels biofunctionalization.

References

Acknowledgments
We acknowledge FCT for the grant SFRH/BD/87025/2011 and Project RL1 - ABMR - NORTE-01-0124-FEDER-000016 co-financed by North Portugal Regional Operational Programme (ON.2 – O Novo Norte), under the National Strategic Reference Framework (NSRF), through the European Regional Development Fund (ERDF).