Development of Chitosan/Hyaluronic Acid Scaffolds for Cartilage Tissue Engineering
Clara Correia1, Liliana S. Moreira Teixeira2, Lorenzo Moroni2, Clemens A. Van Blitterswijk2, Marcel Karperien2, João F. Mano1*

1 3B’s Research Group – Biomaterials, Biodegradables and Biomimetics, Department of Polymer Engineering, University of Minho, AvePark, 4806-909 Taipas, Guimarães, Portugal; IBB – Institute for Biotechnology and Bioengineering, Braga, Portugal.
2 MIRA - Institute for BioMedical Technology and Technical Medicine, Twente University. Department of Tissue Regeneration, P.O. Box 217, Enschede 7500 AE, The Netherlands.

* Corresponding Author: jmano@dep.uminho.pt

Introduction
Cartilage tissue engineering demands the use of adequate materials which mimic mechanical properties and functionality native cartilage. Freeze dried Chitosan (CHT) scaffolds are promising materials for cartilage tissue engineering but biocompatibility appears low. Chitosan is a linear polysaccharide based on repeated glucosamine units. Hyaluronic acid (HA) is a glycosaminoglycan composed of repeating disaccharide units of D-glucuronic acid and N-acetylglucosamine. It is an important component of the cartilaginous matrix and has a high water-holding capacity [1,2,3]. We reasoned that incorporation of a natural component of the ECM will improve the performance of freeze dried CHT scaffolds for cartilage tissue engineering. For this purpose, we have prepared novel freeze dried composite scaffolds of CHT and HA in different weight ratios. We have tested material characteristics and have performed cytocompatibility studies.

Materials and Methods
Scaffolds preparation: CHT and HA were first separately dissolved (1% w/v) in Acetic Acid (1% v/v) and subsequently mixed in different ratios (w/v) in a final polymer concentration of 1% (w/v). Six types of CHT scaffolds were obtained by freeze-drying, containing 0% (control), 0.5%, 1%, 2%, 5% or 10% of HA.

Microstructure observation: the morphologies of the scaffolds were imaged using scanning electron microscopy (SEM).

Cell viability and proliferation: bovine chondrocytes (BC) were seeded on CHT/HA scaffolds (5x10^5 cells/scaffold) to evaluate their in vitro cytocompatibility. MTT and live-dead assays were performed at 1, 3, 7, 14 and 21 days. Cell morphology was addressed by SEM.

Results
The incorporation of HA proved to have an important effect on pore formation as different porous structure could be obtained by varying its content (Fig.1 (S)). With the highest amount of HA incorporated, the pores seemed to lose their circular shape and become more compact (Fig. 1 (S10)). The scaffolds were able to support chondrocytes adhesion, culture viability and metabolic activity (Fig.1 (L), (M)).

Discussion and Conclusions
In this study, incorporation of HA in freeze dried CHT scaffolds improved pore formation and cytocompatibility. The scaffolds with intermediate HA concentrations (1%, 2% and 5%) seemed to have better porous interconectivity and cytocompatibility compared to CHT scaffolds containing 0 or 10% HA. Thus, it can be conclude that CHT scaffolds with low amounts of HA may be appealing instructive matrices for cartilage tissue engineering.

References