Supplementary information of Development and characterization of an immunomodulatory and injectable system composed by collagen modified with trifunctional oligourethanes and silica

Pedro U. Muñoz-González, Jorge Delgado, Laura E. Castellano*, and Birzabith Mendoza-Novelo*

Science and Engineering Division, University of Guanajuato. Loma del bosque # 103, Col. Lomas del campestre, C.P. 37150, León, GTO, México.

Mauricio Flores-Moreno

The Research Center in Optics, Loma del bosque # 115, Col. Lomas del campestre, C.P. 37150, León, GTO, México.

Peadar Rooney, Isma Liza Mohd Isa, Abhay Pandit

CÚRAM, Centre for Research in Medical Devices, Biomedical Sciences, National University of Ireland, Galway, Ireland.

*Corresponding authors, E-mail: laedcato@ugto.mx, b.mendozanovelo@ugto.mx

THP-1 monocyte differentiation into macrophages control

Monocytes were cultured on polystyrene wells and stimulated with PMA for 48h, after the first 6h of stimulation control monocytes started to attach to the bottom of the well, this suggest that the monocytes started to differentiate into macrophages (Fig. S1).

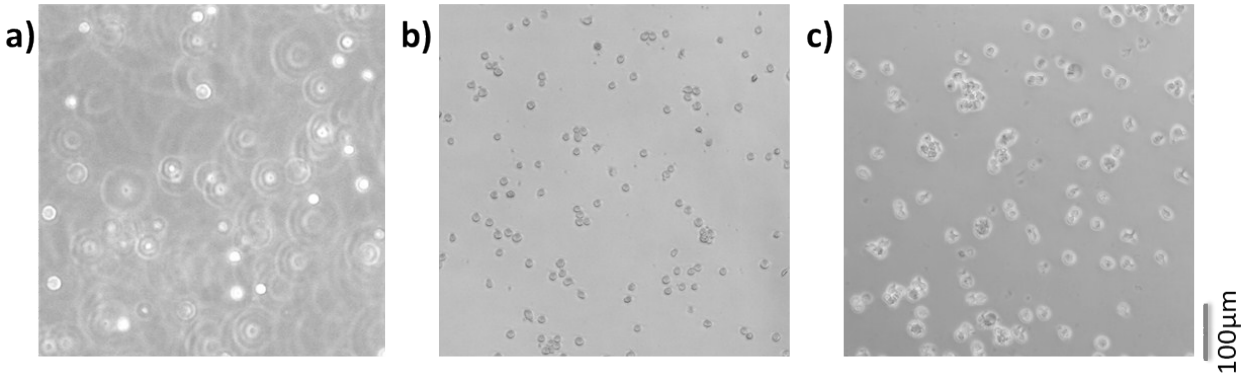

Figure S1. Micrographs where is shown the THP-1 cells attachment as an effect of the differentiation of PMA stimulated monocytes into macrophages after **a)** 0h, **b)** 6h and **c)** 48h.

Ex vivo inflammation model of bovine intervertebral discs

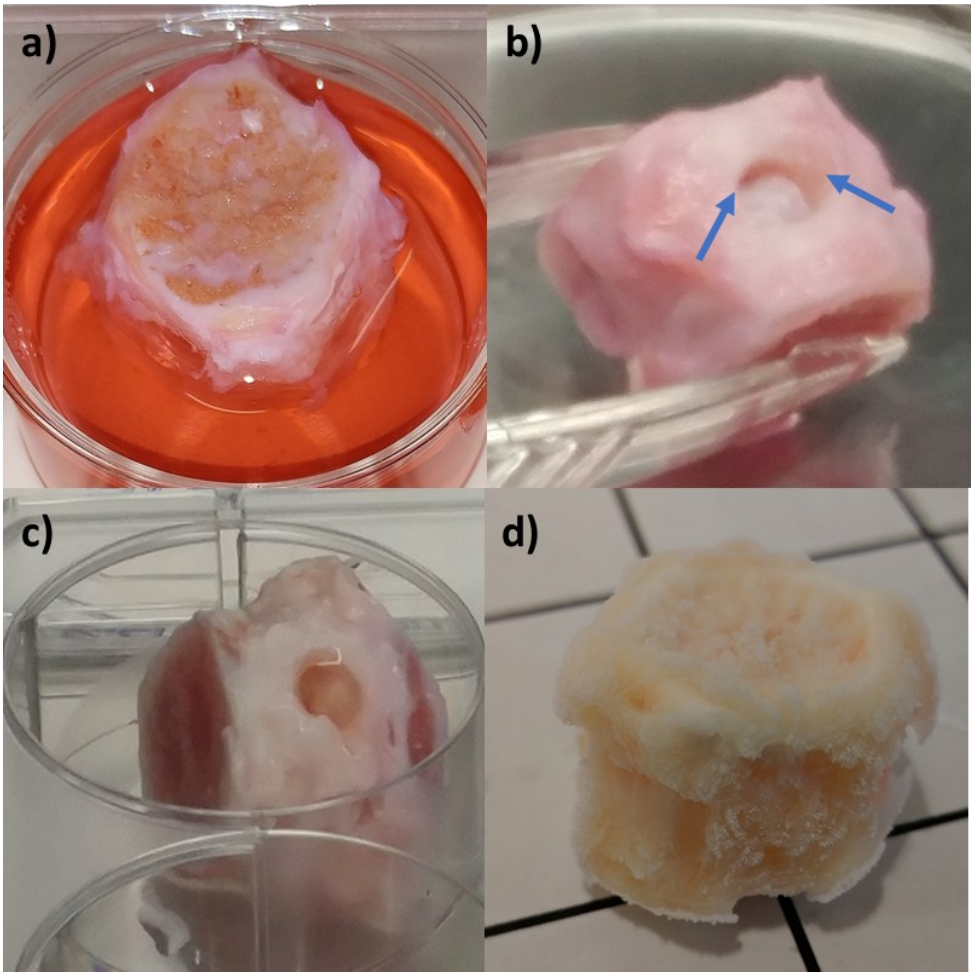

Figure S2. Representative images of bovine intervertebral disc *ex vivo* model progress, where is shown **a)** the culture of the intervertebral disc, **b)** the perforation of the intervertebral discs and the injection of the proinflammatory cytokine IL1β (blue arrows), **c)** the injection of the CXSi material in the perforation zone and **d)** the frozen intervertebral disc.

Intervertebral discs RNA isolation protocol

Tissue sections were explanted from the intervertebral discs inflammatory injection zone ($0.5cm^3$), then homogenized in 1mL of TRIzol reagent, 200 µL of chloroform were added for each 1 mL of trizol, the solution was mixed, and centrifuged (13,300 rpm, 15 min, 4°C). 600µL of the colorless phase containing the RNA were transferred into a new tube, then 600 µL of 70% molecular grade ethanol was added, 600 µL of the mixture was transferred into a mini/micro column RNeasy (Qiagen) and the solution was centrifuged (10,000 rpm, 15s, 23°C). RNA bound to the tube column was washed with 700 µL of RW1 buffer, and the solution was again centrifuged (10,000 rpm, 15s, 23°C). 500 µL of RPE buffer was added, and the solution was centrifuged (10,000 rpm, 15s, 23°C), following for another wash with 500µL de RPE buffer, the solution was centrifuged (10,000 rpm, 2min, 23°C). Then, 30 µL of RNAse free water was added, and the solution was centrifuged (10,000 rpm, 1m, 23°C) to collect RNA in a new collection tube. The RNA concentration was quantified from the solution in the bottom part of the tube using a spectrophotometer NanoDrop (Thermo Fishe Scientific) from ratio of absorbance 260 and 280 nm.

cDNA synthesis protocol

For reverse transcription, each 1 µL of RNA (20ng/µL), was mixed with 1µL of random primers and 3µL of RNAse free water in a PCR tube, the solution RNA and primers were

denatured (5 min, 70°C), then the solution was cooled (5 min, 4°C) and mixed with 15 μL of an ImPRom-II RM reaction buffer, $MgCl_2$, dNTP mix, Recombinant RNasin Ribonuclease inhibitor and ImPROM-II TM reverse transcriptase solution. The resulting solution was submitted to different thermal treatments, 5min 25°C, 60min 42°C, 15min 70°C, using a thermocycler DNA engine MJ Research PTC-200, getting the cDNA.