Functionalised collagen-MnO_2 fibres inhibit oxidative-induced apoptosis in degenerated IVD

DR Pereira[1-3], C Tapeinos[1], JM Oliveira[2,3], RL Reis[2,3], A Pandit[1]

[1] *CÚRAM SFI Centre for Research in Medical Devices, National University of Ireland, Galway.*
[2] *3B's Research Group, Biomaterials, Biodegradables and Biomimetics, Univ. Minho, Portugal.*
[3] *ICVS/3B's- PT Government Associate Laboratory, Braga/Guimarães, Portugal.*

INTRODUCTION: Intervertebral disc cell apoptosis has been reported as the major factor responsible in promoting disc degeneration. In this study we hypothesize that collagen fibres with manganese dioxide (MnO_2) nanoparticles (NPs) can increase oxygen levels by scavenging ROS species and converting it into byproducts. The specific objective of this study is to fabricate collagen fibres incorporating NPs (Fig. 1), with controlled degradability that are able to scavenge ROS species and generate O_2 while inhibiting annulus fibrosus (AF) cell apoptosis under inflamed conditions.

METHODS: MnO_2 NPs were synthesized and the functionalised fibres were characterised structurally (XRD & IR) and morphologically (TEM) and their charge was measured by DLS. An oxygen sensor was used to measure the oxygen levels after the reaction of NPs with various H_2O_2 concentrations. The NPs were mixed with collagen type I (3.5 mg/ml) and fibres were synthesized by the fibre extrusion method (Fig. 1B). The degradation profile as well as the oxygen levels after reaction in presence of H_2O_2 was measured. Cytotoxicity of the functionalised fibres was carried out on Il-1β induced inflamed AF cells. Changes in the apoptosis rate and intracellular ROS levels in cultured inflamed AF cells were measured by flow cytometry and immunocytochemistry.

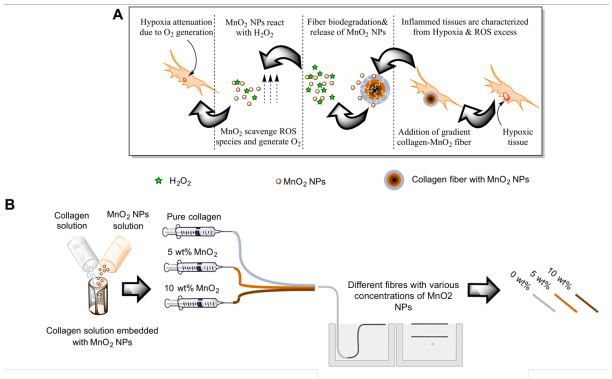

Fig. 1: (A) Potential mechanism of action in inflamed tissues and (B) Schematic representation of functionalised collagen-MnO_2 fibres.

RESULTS: MnO_2 NPs were successfully synthesized and incorporated within collagen fibres. Oxygen was generated following the reaction of the NPs with H_2O_2 (Fig. 2B) and the increasing oxygen levels are proportional to the nanoparticle concentration. *In vitro* studies on show good cellular metabolic activity indicating the scavenging effect of the NPs (Fig. 2C). Flow cytometry results showed that MnO_2 loaded collagen fibres exhibit increased cell viability, decreased ROS production and reduced apoptosis.

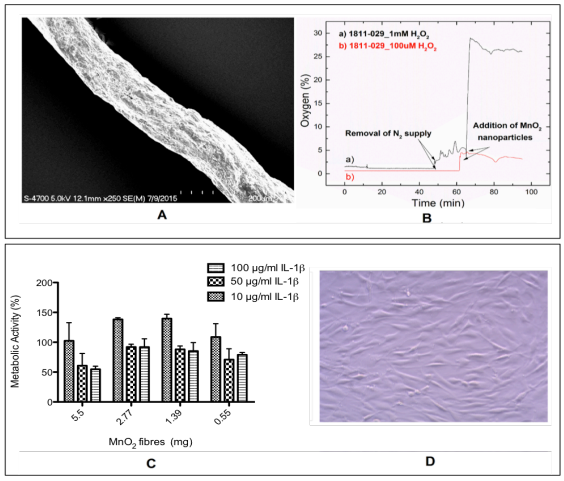

Fig. 2: (A) SEM picture of functionalised collagen-MnO_2 fibres; (B) Oxygen measurements of collagen-MnO_2 fibres after treatment with different concentrations of H_2O_2; (C) Metabolic activity of IL-1β inflamed AF cells after treatment with functionalised collagen-MnO_2 fibres and (D) IL-1β inflamed AF cells

DISCUSSION & CONCLUSION: The functionalised collagen-MnO_2 fibres were able to generate oxygen H_2O_2 by MnO_2 NPs scavenging reaction. Future studies will include measurements of altered mitochondrial membrane potential and molecular markers involved in ECM production of AF cells.

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