

Suppression of Hypertrophy and Osteogenesis in Committed Human Mesenchymal Stem Cells Cultured on Novel Nitrogen-Rich Plasma Polymer Coatings

F.Mwale^a, P-L.Girard-Lauriault^b, H.T. Wang^a, S. Lerouge^c, J. Antoniou^a, M.R. Wertheimer^b.

^aMcGill University, and Lady Davis Institute for Medical Research. ^bDepartment of Engineering Physics, École Polytechnique. ^cResearch Center, Notre-Dame Hospital, Montréal, Québec Canada.

INTRODUCTION: Mesenchymal stem cells (MSCs) are pluripotent progenitor cells with the ability to generate cartilage, bone, muscle, tendon, ligament and fat. However, recent evidence indicates that a major drawback of current cartilage- and intervertebral disc-tissue engineering is that human MSCs isolated from some arthritic patients (a clinically relevant source of stem cells) express type X collagen (a marker of chondrocyte hypertrophy associated with endochondral ossification) and osteogenic markers [1]. Some studies have attempted to use growth factors to inhibit type X collagen expression, but none has addressed the possible effect of the chemical composition of the substratum on chondrocyte hypertrophy and osteogenesis. Here, we examine the growth and differentiation potential of human MSCs cultured on extremely N-rich plasma polymer layers, which we call "PPE:N" (N-doped plasma-polymerised ethylene, containing up to 36% [N]).

METHODS: *Deposition of PPE:N-* PPE:N films were deposited on biaxially oriented polypropylene (BOPP 50 μm thick isotactic polymer film, graciously provided by 3M Company. Films containing 29.5% nitrogen, [N], were deposited from the precursor gas mixture composed of nitrogen (N_2 , 10 standard liters per minute, slm) and ethylene (C_2H_4 , 10 standard cubic centimeters per minute, sccm). Other PPE:N films with different nitrogen concentrations, [N], were also studied, for comparison. Changing the flow rate of the ethylene gas precursor, $F_{\text{C}_2\text{H}_4}$, from the lowest value of 5 sccm to higher values gradually reduces [N]. Thus, for $F_{\text{C}_2\text{H}_4} = 5$ sccm, [N] \approx 36%, while for the highest $F_{\text{C}_2\text{H}_4}$ used here, 20 sccm, [N] \approx 25%.

Source and preparation of mesenchymal stem cells (MSCs). MSCs were obtained from aspirates from the intramedullary canal of donors (60-80 years of age) undergoing total hip replacement for osteoarthritis, using a protocol approved by the Research Ethics Committee of the Jewish General Hospital. Bone marrow aspirates were processed essentially as previously described [1].

Cell culture

One million of 3 or 4 passage MSCs were cultured on each of the three different PPE:N coatings on BOPP, in DMEM + 10%FBS. Commercial polystyrene (PS) tissue culture dishes (Sarstedt, Inc. Canada) were used as controls. The medium was changed every 2 days for up to 14 days, after which cells were harvested at different times or at the endpoint for gene expression studies.

Polymerase Chain Reaction (PCR)

PCR was performed as previously described [1].

RESULTS: We show that PPE:N almost completely suppresses the expression not only of type X collagen but also of osteogenic marker genes such as alkaline phosphatase (ALP), bone sialoprotein (BSP) and osteocalcine (OC). In contrast neither aggrecan nor type I collagen expression were significantly affected.

DISCUSSION & CONCLUSIONS: These results indicate that PPE:N coatings may be suitable surfaces for inducing MSCs to a chondrocyte or disc like phenotype for tissue engineering of cartilage or intervertebral disc in which hypertrophy and osteogenesis are suppressed.

REFERENCES:

- 1 Mwale, F., Wang, H. T., Nelea, V., Luo, L., Antoniou, J. and Wertheimer, M. R. (2006) The effect of glow discharge plasma surface modification of polymers on the osteogenic differentiation of committed human mesenchymal stem cells. *Biomaterials* **27**, 2258-2264.

ACKNOWLEDGEMENTS: This work is supported by the Canadian Institutes for Health Research (CIHR), AO Foundation Switzerland, and the Natural Sciences and Engineering Research Council of Canada (NSERC).