Type X Collagen Expression in Mesenchymal Stem Cells from Osteoarthritis Patients in Pellet Culture

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INTRODUCTION

Mesenchymal stem cells (MSCs) are multipotent stem cells that can differentiate into chondrocytes, osteoblasts, myocytes, adipocytes, and a variety of other cell types. Several studies have been directed toward using MSCs from osteoarthritic (OA) patients for cartilage repair not only because these patients are the ones that will require a source of autologous stem cells if biological repair of cartilage lesions is to be a therapeutic option, but also to further an understanding of stem cell differentiation. Previous studies had shown that a major drawback of current cartilage and intervertebral disc tissue engineering repair is that human MSCs from OA patients rapidly express type X collagen [1]. Type X collagen, a marker of late stage chondrocyte hyperthrophy [2], is implicated in endochondral ossification. However, these studies also revealed that a novel atmospheric-pressure plasma-polymerized thin film material, named nitrogen-rich plasma-polymerized ethylene (PPE:N), is able to inhibit type X collagen expression in committed MSCs [3]. The specific aim of this study was to determine if the suppression of type X collagen by PPE:N is maintained when MSCs are transferred to pellet cultures in chondrogenic defined media.

MATERIALS AND METHODS

Human MSCs were obtained from aspirates from the intramedullary canal of donors undergoing total hip replacement for OA using a protocol approved by the Research Ethics Committee of our institution. Bone marrow aspirates were processed essentially as previously described [3]. Briefly, after 72 h, non-adherent cells were discarded and the adherent ones were thoroughly washed twice with DMEM. Thereafter, the cells were then expanded for 2-3 passages in DMEM high glucose supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin on polystyrene cell culture dishes (Control) or PPE:N surfaces (2-D culture) [1-3]. PPE:N surfaces were prepared as previously described [4]. After 3 days of culture, cells were transferred for 3 additional days in a chondrogenic serum free media (DMEM high glucose supplemented with 2 mM L-glutamine, 20 mM HEPES, 45 mM $NaHCO_3$, 100 U/ml penicillin, 100 µg/ml streptomycin, 1 mg/ml bovine serum albumin, 5µg/ml insulin, 50 µg/ml ascorbic acid, 5 ng/ml sodium selenite, 5 µg/ml transferrin) in pellet culture [5].

Cells were then lysed and proteins were separated on 4-20% acrylamide gels (SDS-PAGE) and transferred to nitrocellulose membranes. Type X collagen was detected by Western blot using specific antibodies directed against type X collagen (Sigma-Aldrich, Oakville, ON). GAPDH (Cedarlane, Hornby, ON) expression was used as an internal control for protein loading. HRP-conjugated goat anti-mouse antibody (Zymed, South San Francisco, CA) was used as secondary antibody. Proteins were detected using *NEN Renaissance* chemiluminescence reagents (Perkin Elmer, Woodbridge, ON) and analyzed using a Bio-Rad VersaDoc instrument equipped with a cooled CCD 12 bit camera.

RESULTS

As expected from previous studies [1,3], type X collagen protein is expressed in MSCs from OA patients cultured on polystyrene but suppressed when cultured on PPE:N (Figure 1, 2D culture). Since defined chondrogenic medium are commonly used in pellet culture to promote *in vitro* chondrogenesis, we then investigated the effect of transferring cells pre-cultured on PPE:N into pellet culture on type X collagen expression. Results showed that the decreased type X collagen expression was not maintained and that the expression returned to control values (Figure 1, Pellet culture).

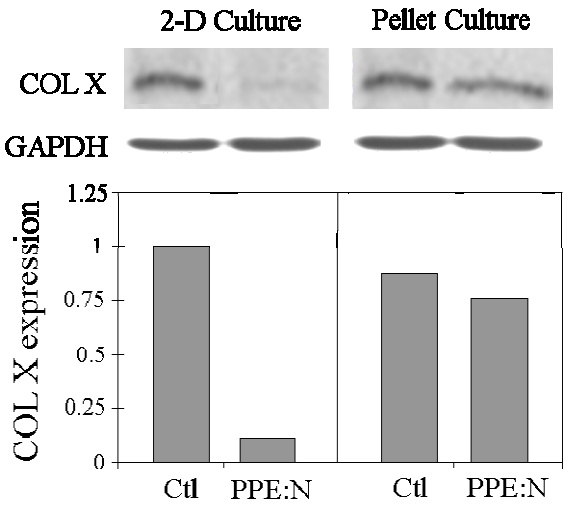

Figure 1: Expression of type X collagen (COL X) in MSCs cultured either on polysterene (control) or pre-cultured on PPE:N (PPE:N) (2D culture) and transferred to pellet cultures after pre-culturing for 3 days on PPE:N (pellet culture).

DISCUSSION AND CONCLUSION

The use of MSCs is promising for tissue engineering of cartilage and intervertebral disc. However, the expression of type X collagen in these cells from OA patients greatly limits their use for tissue engineering. The present study confirmed the potential of PPE:N surfaces in suppressing type X collagen expression. However, when MSCs stem cells are transferred to pellet cultures, type X collagen is rapidly re-expressed suggesting that pellet cultures may not be suitable for chondrogenesis of MSCs from OA patients. It is also possible that the pre-culture time (3 days) may not be enough to really completely re-program the stem cells into a chondrocyte-like phenotype. Thus, longer culture times on PPE:N surfaces may then be necessary to achieve chondrogenesis, in which hypertrophy is suppressed.

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