The suppression of Type X Collagen Expression is Maintained when Mesenchymal Stem Cells from Osteoarthritis Patients are Pre-Cultured on Nitrogen Rich Polymers and then Transferred to Pellet Cultures

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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 connective tissues. Using MSCs from osteoarthritic (OA) patients for cartilage repair has been proposed in several studies because these patients will require a source of autologous stem cells if biological repair of cartilage lesions is to be a therapeutic option. Previous studies had shown that a major drawback of current cartilage and intervertebral disc tissue engineering and repair is that human MSCs from OA patients express high levels of type X collagen [1]. Type X collagen, a marker of late stage chondrocyte hypertrophy [2], is linked with endochondral ossification, which is a prelude to bone formation. However, these studies also revealed that a novel atmospheric-pressure plasma-polymerized thin film material, called 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 serum-free 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, non-adherent cells were discarded after 72 h of culture and the adherent ones were thoroughly washed twice with DMEM. 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. At the time of experiments, cells were cultured on polystyrene dishes and on two different PPE:N surfaces: high (H) and low (L) pressure deposition, each one differing in their concentration of primary amines [1-3]. Both PPE:N surfaces were prepared as previously described [4]. After 3 or 7 days of culture on the surfaces, cells were transferred for additional days in a chondrogenic serum-free media (DMEM high glucose supplemented with 2 mM L-glutamine, 20 mM HEPES, 45 mM NaHCO3, 100 U/ml penicillin, 100 µg/ml streptomycin, 1 mg/ml bovine serum albumin, 5 µg/ml insulin, 50 µg/ml ascorbic acid, 5 ng/ml sodium selenite, 5 µg/ml transferrin) in pellet culture [5].

RNA was extracted using a standard TRIzol RNA extraction protocol (Invitrogen). After quantification, 0.5µg of RNA was converted to cDNA, which was then subjected to PCR using 10µM of type I and X collagen primers (Invitrogen). GAPDH gene expression was used as a control to determine if the suppression of type X collagen by PPE:N is maintained.

RESULTS

As observed in previous studies [1, 3], type X collagen mRNA was expressed in MSCs from OA patients cultured on polystyrene but was suppressed when cultured on both H- and LPPE:N after 3 and 7 days (Fig 1). However, HPPE:N was more effective in decreasing type X collagen expression after 7 days in culture than LPPE:N, suggesting that type X collagen expression may be sensitive to the level of primary amines.

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 mRNA levels maintained not only when cells were removed from the PPE:N surfaces and transferred to new polystyrene culture dishes in the presence of chondrogenic media, but also when transferred to pellet cultures (Fig 2). Culturing MSCs from OA patients on PPE:N surfaces and in pellet culture had no effect on the levels of type I collagen mRNA (results not shown).

DISCUSSION AND CONCLUSION

The use of MSCs is promising for tissue engineering of cartilage and intervertebral disc, as autologous sources of stem cells for OA patients. However, the expression of type X collagen in these cells greatly limits their use for tissue engineering. The present study confirmed the potential of PPE:N surfaces in suppressing type X collagen expression. More importantly, when these cells are transferred to pellet cultures, type X collagen suppression is maintained. These results may lead us one step closer to the production of large amounts of reprogrammed MSCs for tissue engineering applications.

REFERENCES


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