Oriented growth of transdifferentiated mesenchymal stem cells on micropatterned polymeric substrates for neuroregeneration strategies

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Statement of Purpose: Nerve regeneration is a biological process required for regrowth of nervous cells and tissues. Various surgical methods such as coaptation, autologous nerve grafts (ANG) etc. are used for treatment of damaged peripheral nerves. For ANG, a segment of healthy nerve is used to bridge the gap at the injured nerve site. Even though ANG is considered the gold standard for treating nerve injuries, there are certain disadvantages such as donor site morbidity, limited length of graft material etc. Because of such limitations of conventional treatment procedures, researchers have started exploring alternative strategies including development of nerve regeneration conduits, transplantation of various cell types and other combinatorial treatment approaches. Previously, micropatterning was used for aligned growth of Schwann cells (SCs) [1]. In our group, we have also shown that a nerve regeneration conduit with a micropatterned inner lumen in combination with transplantation of SCs improved nerve regeneration in a rat model of peripheral nerve injury [2]. However, the only source of SCs is to isolate them after sacrificing healthy nerve tissue (similar to ANG) and despite that SCs do not propagate well in cell culture conditions, which makes this option nonviable. Recent studies have shown that multipotent mesenchymal stem cells (MSCs), which can easily be isolated from bone marrow, can be transdifferentiated to a SC-like phenotypic cells [3]. These SC-like cells express various SC marker proteins such as S100β, $p75^{NTR}$ etc. Here in this study we have transdifferentiated bone marrow derived mesenchymal stem cells(tMSCs) isolated from femoral bone of Brown Norway rats on micropatterned polystyrene (PS) and poly(lactic acid)(PLA) films to see if micropatterning affects the transdifferentiation of MSCs or not.

Methods: Reactive ion etching was used for fabricating micropatterned silicon wafers that were used as a negative template for fabrication of micropatterned PS and PLA films by means of solvent casting. 6% PS solution in toluene and 10% PLA solution in chloroform was used for preparation of the polymeric films. MSCs were cultured and transdifferentiated on poly-L-lysine coated polymeric substrates. Immunocytochemistry was used to identify SC marker proteins on the tMSCs followed by image acquisition using a florescent microscope. Morphometric analysis module of the MetaXpress software was used for quantifying the orientation, aspect ratio (width/height of cells) and cellular area of these tMSCs.

Results: MSCs transdifferentiated successfully (>70% of the cells express S100β & $p75^{NTR}$, Fig.1) on patterned and smooth substrates. No significant changes were observed in degree of transdifferentiation across patterned and smooth substrates. More than 80% of the transdifferentiated MSCs were oriented in the direction of microgrooves (0^o-10^o) on patterned substrates while tMSCs on smooth substrates were randomly oriented (Fig.2). Aspect ratio of tMSCs on patterned substrates (~5) was found to be significantly higher than tMSCs on smooth substrates (~1). However, no significant differences were observed in cellular area of tMSCs growing on patterned versus smooth substrates. A higher degree of orientation and elongation of MSCs was observed without effecting their transdifferentiation potential using patterned films.

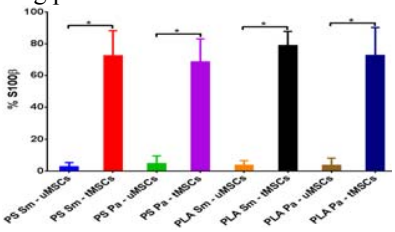

Figure 1. Comparison of percent S100β staining of undifferentiated MSCs (uMSCs) and transdifferentiated MSCs (tMSCs) on various films. Sm:Smooth, Pa:Pattern.

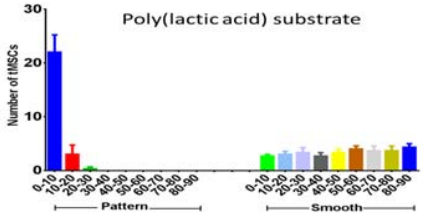

Figure 2. Alignment of tMSCs on pattern vs smooth PLA films. X-axis shows angle with patterns in degrees. N=3 independent experiments (30 cells per experiment).

Conclusions: This work can lead to potentially effective Schwann-cell replacements coupled with micropatterned films to facilitate and accelerate peripheral nerve regeneration.

References:

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