

Evaluation of the molecular mechanism underlying the regenerating effects of MyCells®.

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Introduction

Activated platelets release numerous proteins, among them adhesive glycoproteins and growth factors. Following subcutaneous injection, these proteins and growth factors interact with cells residing in the subcutaneous tissues, e.g. skin fibroblasts, endothelial cells, osteoblasts. Upon binding to their cellular receptors, glycoproteins and growth factors activate intracellular signaling events, mediating cell proliferation, migration, survival, as well as production of extracellular matrix proteins.

In view of the crucial role of angiogenesis in wound healing and tissue regeneration, we examined the effect of activated platelets on the expression of 84 genes involved in positive and negative regulation of angiogenesis in endothelial cells, using a specialized real-time PCR array. Platelet activation was performed by thrombin- receptor activating peptide (TRAP).

Results

Proangiogenic growth factors

Upregulation (x fold)

Transforming growth factor, alpha	2.67
Transforming growth factor, beta 1	2.59
Vascular endothelial growth factor A	3.04
Hypoxia-inducible factor 1, alpha subunit - regulator of VEGF expression	6.29
Fibroblast growth factor 2 (basic)	13.02

Cell Adhesion Receptors

Upregulation (x fold)

Integrin, alpha V	3.16
Integrin, beta 3	3.17
Laminin receptor, alpha 5	7.56
Endoglin	4.05

Conclusions

Our preliminary results indicate that:

1. Exposure of endothelial cells to activated platelets induces enhanced expression of TGF –alpha, TGF- beta, Vascular endothelial growth factor (VEGF) (as well as HIF, a transcription factor regulating VEGF expression at the promoter level), and basic FGF, all of which are growth factors involved in regulation of angiogenesis, wound healing and tissue regeneration. In addition, endoglin, a cell membrane glycoprotein mediating TGF-beta induced signaling, was also found to be upregulated by exposure of endothelial cells to activated platelets.

2. Cell adhesion to the extracellular matrix proteins is a crucial step mediating numerous vital cellular processes. Exposure of endothelial cells to activated platelets induces increased expression of the integrin alpha v beta 3 and laminin receptor, which are involved in cell adhesion, survival, migration and regulation of growth factor receptor activity. All these processes play important roles in angiogenesis, as well as in and tissue regeneration and remodeling.