Extracorporal shock waves induce healing of chronic leg ulcers via activation of cell-cycle regulatory proteins and pro-inflammatory cytokines

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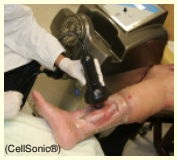
Introduction

Chronic leg ulcer is a tissue disorder with high and increasing incidence. Its treatment is multidisciplinary and challenging, and over the years many conservative (e.g. wound dressings), interventional (e.g. vacuum therapy) or surgical therapeutic approaches (e.g. vein operations, skin transplantation) are conducted.

Until now, clinically efficient therapy for chronic leg ulcers has not yet been described.

Here, we report that >80% of chronic leg ulcers (n=80) with various pathophysiologies show an induction of wound healing after repetitive **Extracorporeal Shock Wave Therapy (ESWT).** Based on this suprising clinical observation, we analyzed the underlying molecular processes that induce wound healing *in vitro* (in keratinocytes, fibroblasts and endothelial cells) after ESWT **"from bedside-to-bench"**.

Conclusions

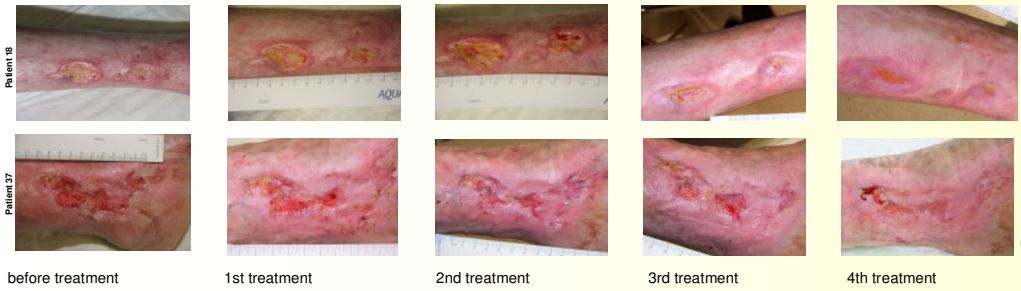


bedside ⟷ bench

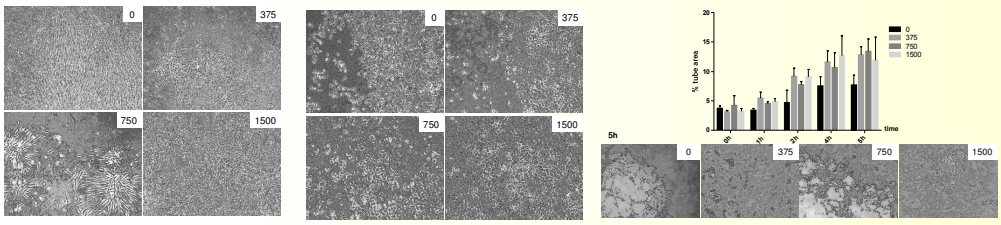
- Morphological switch: „wound" fibroblasts, keratinocytes with cobblestone pattern
- Endothelial cells increase rate of angiogenesis
- ESWT induce cell migration
- Induction of cell-cycle regulatory genes involved in mitosis (fibroblasts)
- Inductions of immune response genes (keratinocytes)

Results

1 Clinical documentation of ulcers treated with ESWT

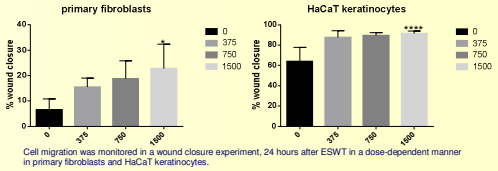


2 Morphological transformation of skin cells and increased angiogenic activity of endothelial cells



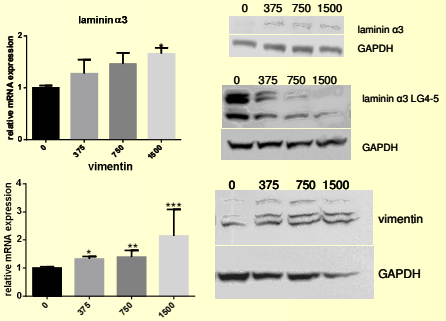
Fibroblasts, keratinocytes, or endothelial were treated with increasing shocks rates (0, 375, 750, 1500). After 24 hours fibroblasts or keratinocytes were monitored and visualized microscopically. Capillary network formation of human dermal microvascular endothelial cells (HMEC) increased over time (0 -5 hours) measured as tube area in percent compared to untreated cells (0) as can be seen for time point 5 hours.

3 Increased cell migration in fibroblasts and keratinocytes after ESWT



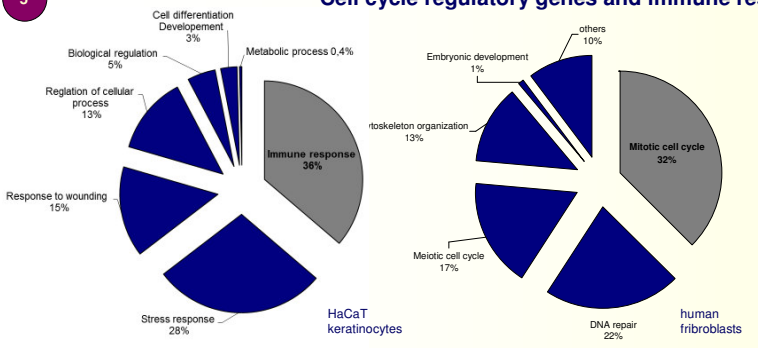
Cell migration was monitored in a wound closure experiment, 24 hours after ESWT in a dose-dependent manner in primary fibroblasts and HaCaT keratinocytes.

4 ESWT changes adhesion to ECM components



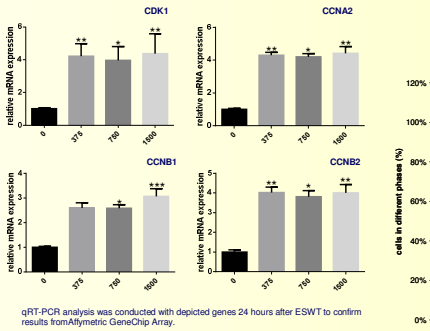
qRT-PCR analysis of induction of RNA expression (laminin α3) by ESWT in HaCaT keratinocytes. Western Blot analysis confirm slight increase of laminin α3 and decrease of its processed form in cell lysates. In fibroblasts vimentin is increased dose-denpendently also revealed by Western Blot Analysis.

5 Cell cycle regulatory genes and immune response genes influence cell regeneration process

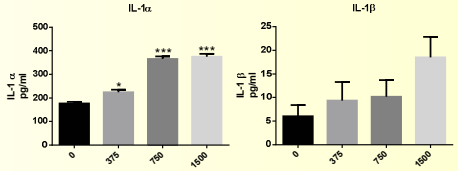


Differentially up-regulated genes in:	*HaCaT keratinocytes*	*primary fibroblasts*
1.	IL1α	CDK1
2.	IL7R	CCNB1, CCNB2
3.	IRF7	CDCA2, CDCA3
4.	IFI6	CCNE2
5.	IFI44	CDC6,7
6.	IFI44L	KIFs
7.	IFI27	POLQ
8.	C3	E2F (1,7,8)
9.	VEGFA	PLK1,4
10.	CCL5	CDK2

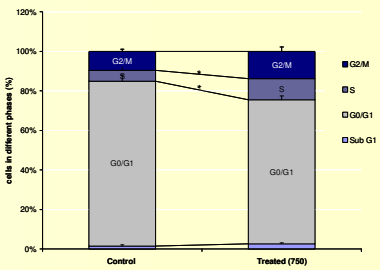
To investigate how shock waves affect gene expression, we used the Affymetrix GeneChip® Human Gene 2.0 ST Array to compare the transcriptomes of shock wave treated HaCaT keratinocytes and primary fibroblasts with a single dose of 750 shocks compared to untreated cells.



qRT-PCR analysis was conducted with depicted genes 24 hours after ESWT to confirm results fromAffymetric GeneChip Array.



ELISA was performed with supernatant of primary keratinocytes which 24 hours after ESWT



Cell cycle distributions were detected by flow cytometry analysis in primary fibroblasts 24 hours after EWST, which influenced significantly cell entrance to S phase (10.8 %) and increase in G2/M phase (13.7 %) compared to the untreated cells after 24 hours (5.4 % and 9.6 % , respectively).