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Extracellular ATP Enhances Proliferation after in Vitro Shockwave Treatment by ERK Dependent Pathways

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Device and producing company: Dermagold100, MTS

Introduction:
Shockwave treatment, a mode of mechanical stress, accelerates wound healing *in vivo*. Yet the mechanisms underlying the beneficial effect of shockwave treatment still remain largely unknown. In this study, we investigated whether shockwave induced ATP release is essential for the proliferative effect of shockwave treatment and if the ERK1/2 signaling pathway is involved in this phenomenon.

Methods:
In our *in vitro* model C3H10T1/2 mouse mesenchymal stem cells were subjected to shockwave treatment and ATP release was assessed. Cell cycle phase distribution after application of shockwaves was evaluated by propidium iodide staining followed by flow cytometry. Proliferating cells were also quantified using a BrdU incorporation assay. Western blot analysis was performed to assess the activation of ERK1/2. Apyrase and suramin were used to evaluate the roles of ATP release and P2 purinergic receptors in the effect of shockwave treatment on proliferation.

Results:
Shockwave treatment released ATP in C3H10T1/2 cells dependent on applied energy and pulse number. Shockwave treatment significantly increased the amount of cells in S-phase in an energy dependent manner. Hydrolysis of released ATP with apyrase completely diminished the proliferative effect of shockwave treatment. Shockwaves induced significant pERK1/2 activation. Pretreatment of cells with the P2 receptor antagonist suramin as well as depletion of ATP prevented this activation.

Discussion:
We conclude that *in vitro* shockwave treatment releases cellular ATP that activates downstream signaling such as ERK1/2 via purinergic receptors, ultimately causing the proliferative effects of shockwave treatment.

Conclusion:
This signaling cascade could be one of the underlying principles of the beneficial effects of shockwave treatment in wound healing.