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VARIABLE P53 RESPONSE IN A 3D MATRIX

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Detachment of an attached (stressed) fibroblast-populated collagen matrix (FPCM) induces fibroblast apoptosis; this may be secondary to increased p53 after detachment. We have noted, however, that the p53 response after detachment of an attached FPCM (i.e., stress-release) varies among cells strains. We hypothesized that modulation of p53 after FPCM stress-release may be related to the fibroblast population density at the time of release. Human foreskin fibroblasts (initially seeded at 0.5×10^6/ml in 0.2 ml) were cultured for 48 hr in attached collagen matrices prior to detachment for 0–6 hr; p53 levels and lysate DNA concentration (used as a measure of fibroblast population density) were determined with immunoblot densitometry and a spectrophotometer, respectively. The experiment was performed with 15 different strains of fibroblasts. The p53 level increased (defined as $\geq$100% increase in baseline p53 level) during the 6 hr detachment period in 6/15 (40%) of fibroblast strains (responders), and downregulated or unchanged in the remaining strains (nonresponders). The mean DNA concentration in the responders *vs.* nonresponders was 1.56 μg/ml (median 1.63, range 0.89–2.25) *vs.* 2.57 (median 1.94, range 1.38–5.09), respectively ($p < 0.05$, Wilcoxon rank sum test). Stress-release of the FPCM resulted in a variable p53 response which appeared to have a relationship with the fibroblast population density; a lower density was associated with p53 upregulation after detachment. This variable p53 response may in turn be related to varying proliferative capacity in the collagen matrix, as there was a broad range (nearly an order of magnitude) of DNA concentration among the 15 cell strains after 48 hr of attached FPCM cuture.

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MACROPHAGE SUSPENSIONS TREATMENT OF INFECTED STERNAL WOUNDS FOLLOWING CABG SURGERY

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Macrophages serve as the coordinators of the wound healing process. Since 1998 following the Israeli Ministry of health authorization, macrophage suspensions have been used for the treatment of ulcers, in more than 800 elderly and paraplegic patients suffering from decubital chronic ulcers.
As previously published, a significant number of genes showed increased levels of expression in hypo-osmotic shock activated cells, using DNA microarrays technique. The majority of these genes are considered to be directly involved in the macrophage function and in the wound healing process.
Macrophge suspensions are prepared from a whole blood unit of healthy, young volunteer blood donors in a closed, sterile system, as previously described. The activated cells are applied to the wounds either by local injection or by direct deposition to the wound.
Between January 2000 and October 2003, 112 patients with postoperative sternal wound infection were treated with macrophage suspension. Full closure of the wounds was achieved in 104 (93%) of the patients.

Original wound surface area	8–175 cm2 (mean 93)
Days until treatment	6–180 (mean 47)
Days until 50% closure	6–60 (mean 21)
Days until full closure	10–138 (mean 49)

No side effects were noted.
The use of macrophage suspension is a safe and effective therapeutic strategy that reduces risk of complications and morbidity and improves the quality of life for long -suffering patients. Length and cost of hospital stay may be reduced, as the treatment requires no hospitalization.

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IMPACT OF HYDRATION ON MMP-ACTIVITY

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Hydration of keratinocytes modifies the levels of cytokines they secrete, which in turn impacts the secretory behaviour of dermal fibroblasts. In an in vitro coculture model, conditioned media (CM) collagen content was decreased 44% when keratinocytes were hydrated. We hypothesized that this is partly due to increased MMP-activity. We used the same coculture model to study changes in MMP-activity and TIMP secreted by keratinocytes as well as by fibroblasts in monoculture and in coculture in relation to air-treatment or hydration of keratinocytes. Stratified human epidermal keratinocytes (HEK) and confluent human dermal fibroblasts (HDF) were cocultured for 72 h under serum-free conditions. HEK were either kept at the air-interface or hydrated. CM was assayed for MMP-1, -2, -9, TIMP-1 and –2 were assayed using zymograms, western blotting, and ELISA. MMP-1, secreted by both cell types, increased significantly in cocultures compared to monocultures (4-fold in the air-treated group, 26-fold in the hydrated group). MMP-2, secreted mainly by HDFs, was significantly increased by coculture (hydration: 2.4-fold, air: 2.8-fold). MMP-9, predominantly secreted by air-treated HEKs and was significantly decreased in hydrated monoculture (76%) and coculture. HEK-monoculture hydration also significant decreased MMP-1 (86%) and MMP-2 (81%) activity. HDF-secreted TIMP-1 expression was significantly increased by coculture and was unaffected by hydration. Our findings demonstrate that paracrine interactions between HEK and HDF modify MMP activity and that HEK hydration significantly effects on MMP activity. The findings provide insight into the role of hydration on HEK and HDF ctivity during the wound healing process.

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TNF-ALPHA MEDIATED INDUCTION OF MMP-9 IS MODULATED BY P21-ACTIVATED KINASE (PAK)

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Matrix metalloproteinase-9 (MMP-9) transiently expresses in acute wound. In non-healed wounds, MMP-9 together with other proteinases persistently elevate, which may lead excessive ECM degradation and failure of wound closure. To understand the molecular regulation of MMP-9 we investigated the signal transduction for TNF-alpha mediated induction of MMP-9 by dermal fibroblasts. TNF-alpha initiates three major signal pathways including NF-11B, JUN N-terminal kinase (JNK), and p38 MAPK. On the other hand, Rho-GTPase plays an important role in a variety of cellular functions including cell morphogenesis, motility, survival, angiogenesis, and mitosis. It remains unknown if the "cross talk" of these signals having a role in regulation of matrix metalloproteinases (MMPs). In this study we found that over expression of the p21-activated kinase (PAK) specifically attenuates TNF-alpha mediated induction of MMP-9. However, TNF-alpha mediated induction of MMP-3 and proMMP-2 activation was intact. NF-κB signal is regarded as a common pathway for many MMPs. Indeed, PAK did not affect TNF-alpha mediated degradation of Ikappa B, suggesting additional signal is targeted by PAK. In contrast, MMP-3 but not MMP-9 expression is specifically blocked by p38 MAK. Thus TNF-alpha induced expression of multiple MMPs in wound healing may utilize different intracellular signal pathways.