Influence of negative pressure wound therapy (NPWT) on fibroblasts in 3D-culture

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Introduction

Negative pressure wound therapy (NPWT) has been shown to be clinically effective in the treatment of chronic-stagnating wounds. However, the exact mechanism of action on wound healing still remains to be elucidated. It is thought that the decrease of the local and interstitial tissue edema, increased perfusion of the (peri-)wound area, reduction of bacteria, and mechanical stimulation of the wound bed account for the beneficial effects [1,2]. Hence, we have established an *in vitro* model for NPWT on chronic wounds using fibroblast in a 3D-culture system to investigate the influence of NPWT with different wound dressings on cell viability and migration.

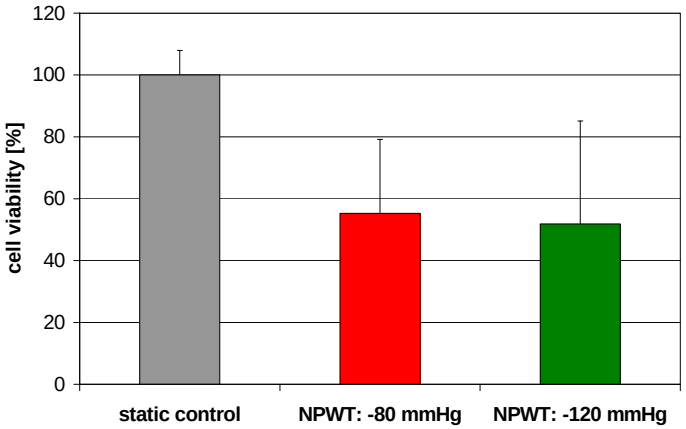

Fig. 1: *NPWT with -80 mmHg and -120 mmHg decreased fibroblast viability in 3D-culture compared to a static control.*

Material & Methods

Fibroblasts were seeded on collagen pellicles and cultured for 14d. The samples (KerlixAMD / Kendall and V.A.C. GranuFoam Dressing / KCl) were placed on the cultures; this assembly was positioned in a 6-well plate and sealed with a vacuum-applicator-lid (VAL). VALs were connected to medium supply and vacuum pump (PRO-I, Prospera). Experiments were carried out at -80 mmHg and -120 mmHg for 48h. Static controls were run at each assay. Histology specimens were stained with haematoxylin/eosin and anti-vimentin. Cell viability (CellTiter-Blue viability assay, Promega) and ingrowths of cells into samples (ATPlite M kit, Perkin Elmer) was determined.

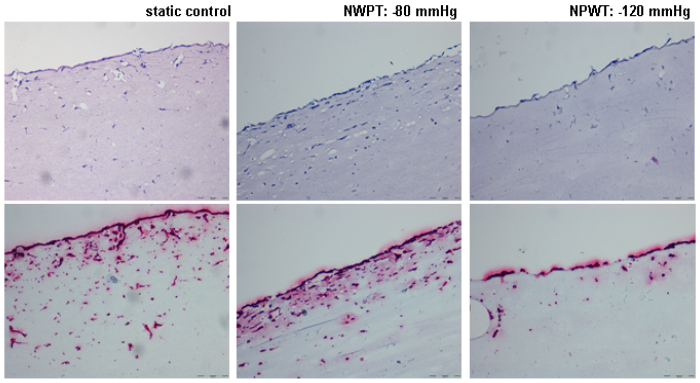

Fig. 2: *Fibroblasts in the 3D-cultures responded to NPWT by migrating in the direction of the applied vacuum (upper panel: staining with haematoxylin/eosin, lower panel: staining with anti-vimentin).*

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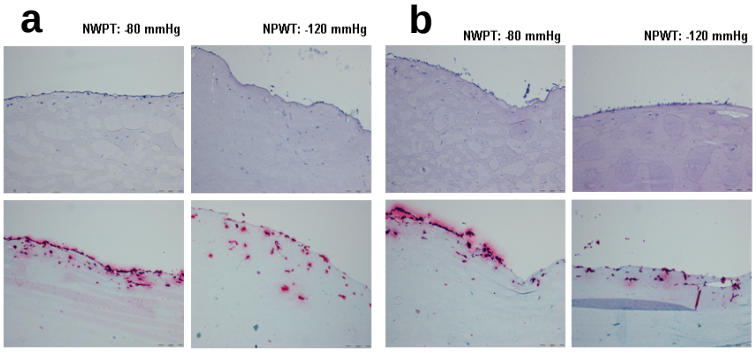

Fig. 3: *Kerlix AMD (a) and V.A.C. GranuFoam Dressing (b) affected cell migration differently (upper panel: staining with haematoxylin/eosin, lower panel: staining with anti-vimentin).*

Results

NPWT decreased fibroblast viability compared to static controls (fig. 1). No difference between cells treated with -80 mmHg and -120 mmHg was observed. The cells responded to the subatmospheric pressure by migrating in direction of the applied vacuum (fig. 2). Wound dressings affected cell migration differently; in cultures treated with KerlixAMD cells were localized at the pellicle edge (fig. 3a), while cells continued to migrate into the V.A.C. GranuFoam Dressing (fig. 3b). The high amount of cells in the GranuFoam sample could be confirmed by measurement of the cellular ATP content (fig. 4).

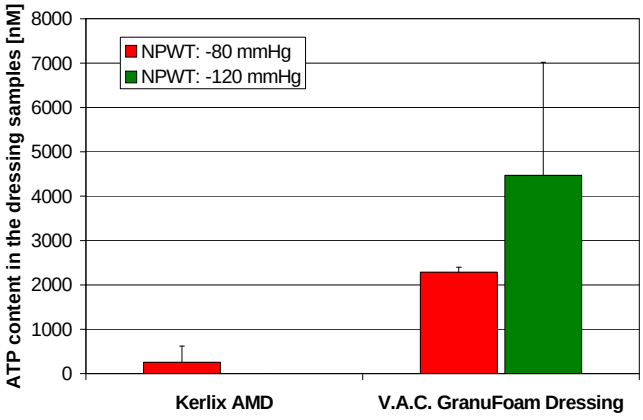

Fig. 4: *Measurement of ingrowth of cells into the wound dressing samples by determination of the ATP content (mean ± SE).*

Conclusions

The study outcome suggests that the positive effects observed clinically for NPWT may result from the recruitment of cells to the wound site, where they contribute to the formation of granulation tissue. It is important that during the early stages of wound healing fibroblasts migrate into the wound from surrounding tissue to synthesize new 'bricks' for tissue repair. However, the various wound dressings used for NPWT exhibit different effects. While fibroblasts did not migrate into the fine-grained KerlixAMD, the cells showed a significant tendency to grow into the V.A.C. GranuFoam Dressing. *In vivo* this may lead to the disruption of newly formed tissue during dressing changes.

References

1. *Moues CM et al. Wound Rep Reg 2008; 16:488-494*
2. *Borgquist O et al. Wounds 2009; 11*