Photo-immobilized Epidermal Growth Factor for Directed Keratinocyte Migration and Accelerated Wound Closure

Tracy Jane Stefonek, Kristyn S. Masters
Department of Biomedical Engineering, University of Wisconsin-Madison

Statement of Purpose:
Despite the creation of numerous wound healing technologies, there remains a lack of appropriate treatments for chronic skin wounds. Current bioactive wound dressings and gels have had limited success, mostly due to inappropriate delivery of the bioactive agents (i.e. rapid degradation and little control over spatial distribution). It is well known that migration of cells is critically important for effective wound repair, and that one of the most important factors in epidermal cell growth and migration is epidermal growth factor (EGF) (Martin P. Science 1997;276:75-81; Jost M. et al., Eur J Dermatol 2000;10: 505-510). Prior work has shown that EGF retains its biological activity following covalent immobilization to surfaces (Chen G, Ito Y. Biomaterials 2001;22:2453-2457). Based on this, we propose that a gradient of surface-immobilized EGF, and perhaps other factors and ECM components working synergistically, will induce directed migration of keratinocytes, thus promoting accelerated dermal wound healing.

Methods:
The photo-reactive heterobifunctional cross-linker Sulfo-Sanpah (SS) was conjugated to EGF using a 50-fold molar excess of SS to EGF in HEPES buffer. The SS-EGF was then photo-immobilized onto tissue culture polystyrene (TCPS) using film photomasks with gradients of 35% or 65%. Negative controls included gradient patterns of Tris-quenched SS and unpatterned TCPS. All samples, including TCPS controls with no SS-EGF, were exposed to ultraviolet light (Novacure; 365nm 90 mW/cm2) for 120 seconds. Immortalized keratinocytes (HaCaTs, courtesy of N. Fusenig, DKFZ, Germany) were seeded at $5x10^5$ cells/ml in $1cm^2$ temporary wells at the 'start' of each gradient pattern. The cells were allowed to attach for 24 hours, then the wells were removed and each dish was cultured in serum-free medium. Photomicro-graphs were taken of the leading edge at 12.5X every 24 hours for 16 days to track and measure migration. To prove that migration was due to specific interactions with tethered EGF, the experiment was repeated adding an irreversible EGF receptor blocker (PD168393; Calbiochem) to the media on days 0, 5, and 10.

Results/Discussion:
As shown in Figure 1, migration of HaCaTs on immobilized gradients of SS-EGF was significantly greater than the TCPS and SS controls, and preferentially in the direction of increasing EGF concentrations on the gradient. However, there appears to be no significant difference between the 35% and 65% EGF gradients. The EGFR blocking experiment confirmed that the observed increase in migration on experimental surfaces is directly attributable to HaCaT recognition of immobilized EGF. As the studies are carried out in serum-free medium, the only growth factor to which the cells are exposed is the tethered EGF. As seen in Fig. 1, cell migration in the absence of tethered EGF (TCPS and SS controls) is extremely slow. Addition of the EGFR inhibitor on Day 0 did not affect cell adhesion, but the cells on TCPS and 35% SS-EGF did not migrate more than a cell's width when seeded with PD168393, and stopped migrating within four hours of adding PD168393 on days 5 and 10 (Fig. 2). The endpoint for all studies was when HaCaTs reached the end of the SS-EGF gradient condition. These results indicate that our photo-immobilized SS-EGF induced accelerated and directed migration of HaCaTs, and remained active for the duration of the experiment. Further studies are needed to more accurately determine if this migration is directed or a proliferation-based response, and if a particular gradient pattern is more effective for migration induction.

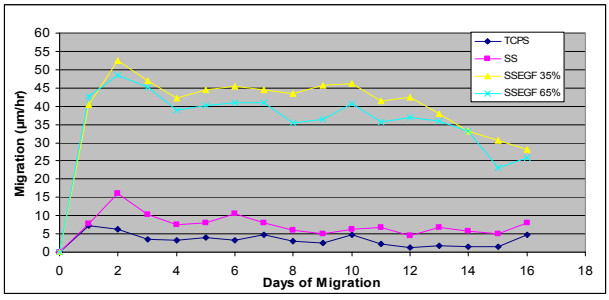

Figure 1. Average daily migration of HaCaTs in µm/hr on TCPS, SS, 35% and 65% SS-EGF gradients.

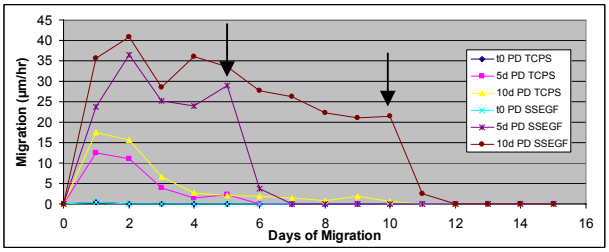

Figure 2. Average daily migration of HaCaTs in µm/hr on TCPS and 35% SS-EGF gradient. Arrows indicate addition of PD168393 to experimental samples.

Conclusions:
Acceleration of wound closure not only results in decreased patient suffering and cost of wound treatment, but may also minimize scarring and lead to formation of a more stable closed wound. The patterned, directed migration system used here could allow precise control over wound repair and have a significant impact on the development of novel biomaterials. Our method of gradient formation differs from existing techniques and allows precise control over gradient slope and density. Moreover, it can be adapted to form gradient patterns in 3-D.