Supplementary Materials

Supplementary Figure 1: Construction of DED-HSR. Skin was obtained as surgical discard material from subjects undergoing breast reduction or abdominoplasty surgery. Skin was trimmed to a thickness of ~1mm using a dermatome and cut into i) 1.5cm² pieces for preparation of decellularized dermis; ii) smaller misshaped pieces for keratinocyte preparation. 1) 1.5cm² pieces were immersed in 1M NaCl overnight (minimum of 16 hours) at 37°C with the epidermis peeled off and the de-cellularized dermis retained and washed the following day. 2) Keratinocyte preparation was performed by incubating skin in 0.125% trypsin overnight at 4°C. The following day the epidermis was peeled off the dermis and the keratinocytes scraped off the papillary side. Keratinocytes were re-suspended/colllected in full Green’s media and transferred to a 50ml centrifuge tube followed by centrifugation at 1000rpm for 5 minutes to pellet the cells. The supernatant was poured off, cells resuspended and counted prior to seeding onto irradiated 3T3 mouse fibroblast feeder cells until ~80% confluent when they were used to construct the DED-HSR. 3) Pieces of decellularized dermis (DED) were incubated in full Green’s media overnight prior to being placed, papillary side up, into the bottom of 24 well plates. A migration ring, consisting of a stainless steel cylinder with a hollow centre and a silicone ring at the bottom, was placed onto each DED and firmly pressed down to form a tight seal. To make the DED-HSR, Keratinocytes were trypsinnized and counted prior to seeding 2x10⁶ cells into the centre of each migration ring. 4) The keratinocytes were allowed to adhere and grow for 24 hours following which the migration rings were removed, the DED-HSR carefully removed from the wells and placed onto a porous metal grid before adding media until it was level with the bottom of the DED-HSR/below the seeded keratinocytes (i.e. feeding the sides and underneath) creating an air liquid interface. All treatments were added into the media and keratinocytes migrated laterally across/underwent proliferation on the DED. 5) At specific time points DED-HSRS were removed from culture and immersed in MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) with purple staining indicative of metabolically active viable cells. The MTT stained DED-HSRS were imaged using a dissecting microscope with camera, with area of MTT stain quantified using Image J. DED-HSR were then fixed in formalin for 24 hours, cut through the middle with a scalpel (dashed red line) to provide the best cellular representation of the cells present within the DED-HSR (away from the edges) and embedded in paraffin wax. These blocks were sectioned, stained with haematoxylin and eosin and the stratum corneum and cellular layer thickness quantified.

Supplementary Figure 2: Post-Mortem analysis of porcine wounds. Porcine wounds treated with MF (A) and PB (B) were imaged post mortem. Wounds demonstrated no differences in the cosmetic score (C), histological score (D), skin thickness (E) or % organizing granulation tissue (F) at post mortem.

Supplementary Figure 3: Immunohistochemistry for menstrual fluid proteins in native skin, role of FABPS in MF mediated skin repair and assessment of VEGF in MF versus PB. Immunohistochemical analysis of native skin obtained from breast reduction surgery and abdominoplasties localized MIF to the cellular layer (arrowheads, A & B) with highly variable levels of MIF immunoreactivity noted between subjects (A versus B). Faint NGAL immunoreactivity was noted localized to basal cells of the cellular layer (arrowhead, C) with some immunostaining occasionally noted within the stratum corneum (open arrowhead, D). Very faint immunostaining for FSTL1 was identified within the basal cells of the cellular layer (arrowheads, E & F) with no immunostaining evident for WFDC2 (G & H). Insets in all figures are negative control sectional. Scale bar = 100um. N=6 native skin samples examined for each protein, representative images from 2 different subjects presented for each. FABPS was depleted from MF samples by immunocapture using a specific FABP5 antibody. Depletion was confirmed by Western immunoblot analysis of non-depleted MF (A, lane 1), FABPS depleted MF (I, lane 2) and eluted immunocaptured MF (I, lane 3), demonstrating the presence of FABPS in non-depleted (lane 1) and immunocaptured (I, lane 3) samples, but not in FABPS-depleted MF (I, lane 2). FABPS-depleted MF (J, ), significantly reduced extent of keratinocyte repair versus non-depleted MF (J, ). Data presented as mean ± SEM of n=3 separate menstrual fluid samples. *p<0.05. Assessment of VEGF level in MF (K, ) versus matched PB ( ) demonstrates significantly elevated levels of VEGF in MF, *p<0.05.

Supplementary Figure 4: MF repair factors enhance proliferation of endometrial epithelial cells but have no effect on keratinocyte proliferation. Treatment of endometrial epithelial cells with MIF (A, ) and NGAL (B, ) mediated a trend towards increased proliferation. Treatment with FSTL1 (C, ) increased proliferation at 5, 24 and 48 hours. Treatment with CCL20 (D, ) increased adhesion/proliferation at 5 hours. Treatment with SLPI (E, ) had no effect on proliferation. Treatment with MIF (F), NGAL (G), FSTL1 (H), CCL20 (I) or SLPI (J) had no effect on proliferation of keratinocytes. Data presented as mean ± SEM of ≥ 5 separate experiments. *p<0.05.

Supplementary Table 1: Proteins elevated in total MF or PB, expressed as fold change versus corresponding fluid
Supplementary Table 2: Proteins exclusively present in total MF or PB
Supplementary Table 3: Heparin binding proteins exclusively present in MF or PB
Supplementary Table 4: Fibronectin binding proteins exclusively present in MF or PB
Supplementary Table 5: Combinatorial peptide ligand library enriched proteins exclusively present in MF or PB