A novel personalized burn treatment: In-situ electrospun nanofibers 3D scaffold with cultured autologous keratinocytes

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Background

In cases of extensive burns, cultured epidermal autografts (CEA) is considered a lifesaving procedure, yet the technique is prone to several limitations, such as long culturing time, it requires multiple cell expansions, has uncertain take rate, sensitivity to infection, high production cost and scarring due to the lack of dermis. Therefore, clinical practice, for severe burn treatment, has evolved to incorporate combined treatments of skin substitutes with CEA that will serve as an adjunct to speed up re-epithelialisation for wound closure. Yet, no satisfactory standard-of-care coverage material exists to promote rapid and appropriate healing of burn wounds. Spincare (Nanomedic Technologies Ltd, Israel), a portable wound care system, uses electrospinning technology to create transient electrospun polymer nanofibrous matrix (EPNM). The portable system allows the generation of nanofibres at the bedside, directly on the exposed tissue, providing a substrate of an ECM-like structure to the damaged tissue.

Objectives

We propose an improved approach for treating extensive burn wounds by covering CEA grafts with EPNM, on-site. Moreover, we were able to demonstrate that spraying cultured autologous keratinocytes integrated with an EPNM, created directly on a wound bed, resulted in a larger expansion ratio and promoted wound healing. In vitro experiments support the cell's ability to proliferate within the EPNM .

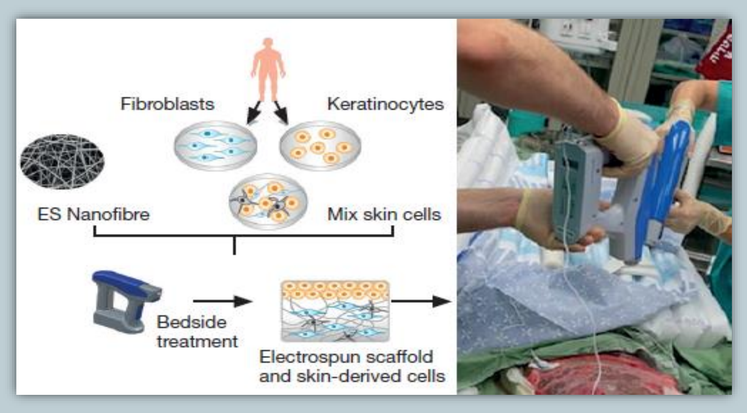

Fig 1. Proposed approach for combining an electrospun polymer nanofibrous matrix (EPNM) with autologous skin cells to be applied at the bedside over the wound. ES—electrospun.

Methods

Ethical approval and patient consent - All procedures were performed according to the Israeli law, after obtaining the legal guardian's informed consent.

Cell culture - Keratinocytes were isolated from a patient's biopsy, and cultured on irradiated 3T3-J2 feeder cells, for the production of CEA sheets or keratinocytes in suspension.

Grafting – 1. CEA sheets (~30cm^2) were grafted over meshed MEEK autografts (MEEK expanded to ratios ranging from 1:6 to 1:9) and wound areas without dermis, 7 days post MEEK grafting surgery. The grafted regions were directly overlaid with EPNM using the hand-held Spincare system on-site. 2. Dissociated keratinocytes in suspension were obtained by trypsinisation of cultured epithelium and suspended in DMEM at a concentration of 1×106 cells/ml. The suspension was loaded into a 3ml syringe with an Intranasal Mucosal Atomization Device (Pulmodyne, US). The syringe was held ~15cm from the sprayed surface and firm pressure was applied to the plunger, allowing a homogenous delivery of cells simultaneously applied with EPNM. CEA spraying was applied over debrided, deep dermal wounds that had previously failed with CEA or upon which other grafts had failed to take.

Gauze backings were removed on day 7 after CEA sheet placement or spraying.

In-vitro experiments- cell and culture viability, identity, purity and potency were determined using Flow cytometry, Colony-forming efficiency assay, XTT Cell proliferation and viability assay, and using fluorescent microscopy.

Case report

We present the case of a 26-year-old male patient with 98% total body surface area (TBSA) deep-dermal to full-thickness burns. We used a combined treatment approach of widely expanded Meek grafts taken from his secondarily healed scalp and over 500 CEA grafts, produced from two 2cm^2 biopsies taken from his foot and applied over the burn wounds in 17 sequential transplantations. After 376 days of hospitalisation in intensive care, the patient reached 95% wound closure and was discharged to rehabilitation.

Table 1. Grafting aspects and procedures presented by grafting method

Anatomical site	Integra	Meek	CEA	CEA + EPNM	Spray + EPNM
Abdomen		1	4	1	1
Chest	1	4	5		1
Back		4	4	1	2
Right scapula		2	3		2
Left scapula		2	2		
Buttocks		2	7	1	3
Right arm		3	3		1
Left arm		4	4		
Right leg		3	6	3	2
Left leg		4	7		
Total of 8 meek surgeries over several body areas					
Total of 17 transplantations from cultured cells (CEA/spray)					

Results

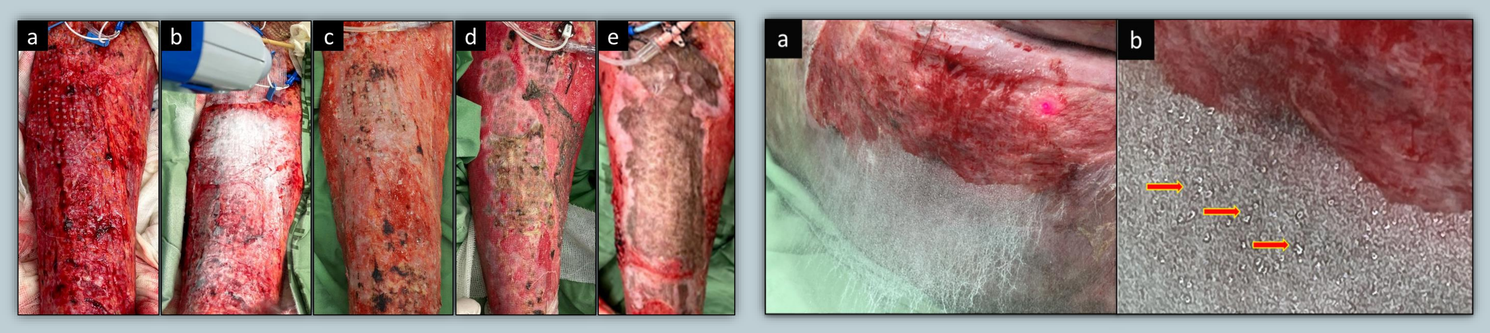

Fig 2. Re-epithelialisation process of a CEA grafted area, applied on top of Meek, covered by EPNM. (a). Electrospinning technology was used to cover the CEA grafts with EPNM **(b)**. 7 days post grafting, showing a large re-epithelialisation area over the combined transplanted area **(c)**. 3 weeks post grafting, showing an almost complete wound closure **(d)**. 3 months post grafting, showing re-epithelialisation and a complete wound closure of the front thigh **(e).**

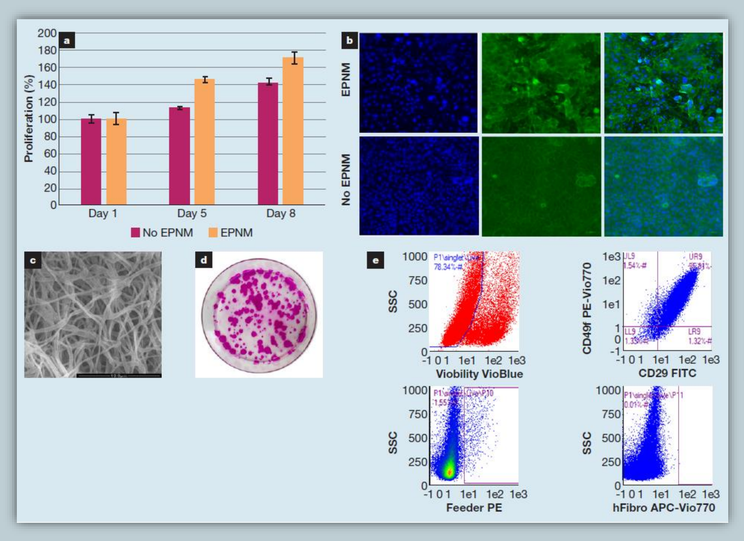

Fig 3. Skin-derived cell viability. Keratinocytes (7×10^4) and fibroblasts (3×10^4, derived from a skin biopsy, were seeded in a 24-well plate on glass coverslips covered with and without EPNM. XTT was measured daily on the indicated days. Proliferation was calculated relative to day 1. Representative experiment is presented, n=3 **(a)**. Cells were fixed on day 7 and fluorescence staining was performed, phalloidin for actin fibres (green) and DAPI for nuclear staining (blue). Scale bar represents 20µm **(b)**. EPNM structure under electron microscope simulates ECM. Scale bar represents 10µm **(c)**. CFE of extracted keratinocytes. 1×10^3 keratinocytes were seeded on irradiated murine feeder cells and grown for 12 days. Colonies were fixed and stained with Rhodamine B. Proliferative keratinocytes grown into keratinocyte colonies **(d)**. Keratinocytes were grown on irradiated murine feeder cells till confluence, trypsinised, stained and subjected to flow cytometry to determine cell viability (upper left), identity (CD29 and CD49f)(upper right), and purity (staining of PE-murine feeder cells) (lower left) and staining of fibroblast-APC-Vio 770 antibody (lower right)). **(e).**

Fig 4. Combined treatment of autologous cells sprayed with EPNM dressing on the buttocks. A total of 2.5×107 keratinocytes (1×106 cells/ml) were sprayed on a wound bed and covered with EPNM. EPNM forms a net, as an ECM structure, that captures the sprayed cell droplets underneath **(a)**. Enlarged area from **(a)** that depicts the captured cell droplets (marked with arrows) by the EPNM **(b)**.

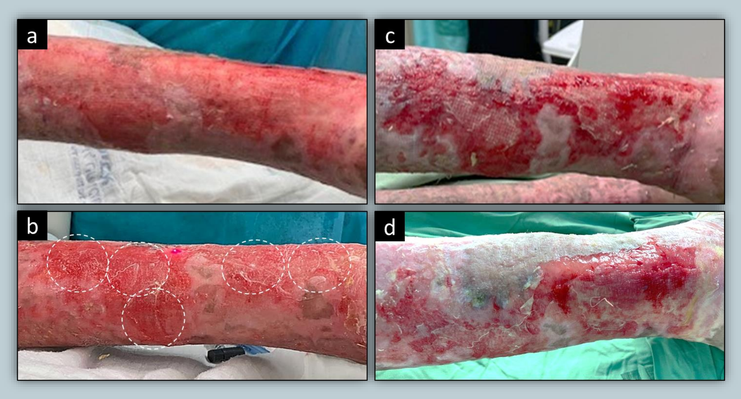

Fig 5. Combined treatment of autologous cells with EPNM dressing on the posterior calf. A total of 2.5×10^7 keratinocytes (1×10^6 cells/ml) were sprayed on a wound bed with no dermis and covered with EPNM **(a)**. 7 days post spraying, CEA grafts were placed over sprayed grafted area **(b)**. 7 days post CEA grafting, re-epithelialisation is seen in regions with no dermis underneath **(c)**. 14 days post CEA grafting, extensive re-epithelialisation of the same area is present **(d)**.

In this case study, under compassionate regulation treatment, by using an EPNM as a personalised graft cover at the bedside, we improved our treatment approach. In addition, we treated the full-thickness wound areas, which were poorly engrafted, with a combination of sprayed autografted cells integrated in the EPNM scaffold applied at the bedside. In all cases, there was no evidence of infection derived from CEA grafting nor from the EPNM dressing.

Conclusions

We have shown a promising new approach for the treatment of deep dermal wound areas with poor engraftment and delayed wound healing, based on a personalised wound dressing, created directly on the wound bed. We believe that these preliminary results support the development of a novel personalised, engineered skin equivalent created on-site. The engineered skin will be composed of a biologically relevant nanofibre scaffold (e.g., collagen, fibrin, elastin) and autologous skin cells. This future development will enable skin regeneration and functionality, which eventually has the capacity to improve patients' quality of life