THE PREPARATION OF AN AUTOLOGOUS TRIPLE LAYERED PATCH CONTAINING PLATELETS, LEUKOCYTES AND FIBRIN AND ITS CLINICAL USE IN THE TREATMENT OF CHRONIC WOUNDS

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Study aim:

To setup a clinical pilot study to assess the clinical effect, safety and the feasibility of ambulant use of a fully autologous platelet and leukocyte rich fibrin product³.

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Process & Product

Process:

Venous blood is drawn into a vacuum device.

The filled device is centrifuged in a two-step automated process.

The device is opened and the formed patch is transferred to the cleaned wound.

Product:

The formed patch has a gelatinous appearance (A,B) and histology analyses (HE stain) reveals a triple layered construct (C). The top layer is composed of fibrin, the middle layer concentrated platelets whereas the lower layer is composed of leukocytes (E).
CLINICAL STUDY SETUP

**Inclusion Criteria:**
- Chronic cutaneous ulcers on the lower extremities, chronic diabetic foot ulcers (grade I-II according to the Wagner scale) or amputation wounds, that had been present for at least 2 months and had failed to heal by conventional means.
- Age > 18 years
- Written informed consent

**Exclusion criteria:**
- Clinical signs of infection or osteomyelitis
- Significant medical conditions likely to impede wound healing
- Wound necrosis
- Poor nutrition (Albumin < 2.5 g/dl)
- Ischemia demanding vascular reconstruction or amputation,
- Vascular reconstruction within the last 4 weeks
- Uncontrolled diabetes ([HbA1c] > 10% [13.7 mmol/L])
- 3 or more wounds

**Treatment:**
Eligible patients were included for treatment with LeucoPatch. The study procedure included weekly:
- Debridement (if needed)
- Planimetry
- Blood draw
- Patch generation
- Treatment with patch
- Primary dressing (Profore WCL, S&N)
- Secondary dressing
- Offloading.

CLINICAL STUDY RESULTS

The mean age of the 15 patients was 59 years (range 19 to 86 years). All but one patient was male. Wounds varied in their aetiologies (malleolar wound (2), diabetic foot ulcer (5), surgical heel wound (3), venous ulcer (5), amputation wound (1)) and initial size (mean 3.6 cm$^2$ (0.4 to 15.7 cm$^2$)). The mean wound duration was 38 months (2 to 108 months).

Safety: No device related adverse event were found during the 106 treatments in the study.

Percentage change in wound area during the treatment period (per-protocol analysis), mean ± 95% confidence interval, $r^2 = .919$ ($P = .0007$).

Percentage change in wound area

- 0% to 10%
- 10% to 20%
- 20% to 30%
- 30% to 40%
- 40% to 50%
- 50% to 60%
- 60% to 70%
- 70% to 80%
- 80% to 90%
- 90% to 100%

Time (weeks)

1 to 6 weeks
Patient 1, male, 58 years, Malleolar wound for 4 years

Debrided

3 weeks

3 months

Patient 10, male, 54 years, diabetic foot ulcer for 8 months

Debrided

6 weeks

3 months
Selected in Vitro Data

Method:
The effect of the patch on primary human dermal fibroblast (NHDF) cells was determined using cell culture systems. Briefly, NHDFs (Lonza) were expanded in fibroblast growth medium (FGM) with 10% fetal calf serum (FCS). For growth assays, cells were grown in fibroblast basal medium (FBM) added 2% FCS (Control) in 0.2 μm Anapore® membrane inserts (Nunc, Denmark) submerged in wells added FBM 2% FCS and a 2 mm biopsy of patch (LeucoPatch).

For collagen and VEGF secretion assays, cells were grown to confluence and kept in FBM2%FCS (control) and a 2 mm biopsy of patch (LeucoPatch).

Cell numbers in the inserts were determined by ATP detection. CICP and VEGF were detected by ELISA assays.

The autologous patch activates fibroblast cells to:
- Grow
- Secrete collagen
- Release VEGF
- Key elements in wound healing
CONCLUSIONS

The patch tested in the study was:

• Safe
• Effective on the tested wounds
• Applicable in the multidisciplinary treatment setting

A larger multi-center trial on 50 DFU patients is currently ongoing

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