Adipose tissue therapeutics for scar rehabilitation after thermal injury

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Introduction

THE PROBLEM

- Burn injuries are common and always lead to scarring, especially pathological skin tissue.
- Most basic research focuses on acute phase.
- N = 50 CD1 Nu/Nu (Athymic, nude) mice received standardized 70°C 10s burn (under anesthesia and analgesia) with a brass rod to dorsal skin and monitored for six weeks while chronic scars formed (Fig 1-3).
- At six weeks animals were randomized to five groups (Table 1): non-injected controls received no injection, other groups received subcutaneous injection of 0.6mL human liposarpirate, human ADSCs in matrigel hydrogel suspension, or matrigel control. Adipose tissue from discarded human pannus. ADSCs from SVF ex-vivo culture.
- Skin perfusion measured with Hyperspectral Imaging (HSI) and digital photos were taken at 4 time points.
- Mice were sacrificed at 10 weeks post-burn (PB) (4 weeks after engrafment) for skin histology.
- Scar wound area and oxy and deoxy hemoglobin (Hb) measurements were determined at all time points.
- Skin tissue samples were stained for vascularity (CD31) and collagen composition (Picrosirius red, Masson’s Trichrome). Matrigel explants were H&E stained.

MATERIALS & METHODS

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RESULTS

- Scar wound area: Liposarpirate treated mice had significantly reduced perceived scar area compared to controls at 10 weeks (Fig 4).
- Histology: CD31 IHC trouble. Dermal vessels visualized on Masson’s Trichrome counted in 3 hps/slide show increase in G3 compared to G1 (Fig 5).
- Collagen picrosirius photomorphometry ongoing. H&E stain of matrigel explants show living cells within hydrogel matrix.
- Hyperspectral imaging: Changes in oxy, deoxy, and total hemoglobin (Hb) consistent all mice until week 6 prior to treatment (not shown).
- CD3 significantly increased oxy Hb from week 6 to 10 compared to other groups (Fig 6). G3-5 significantly lower deoxy Hb compared G1 10.6 wks (~0.9, not shown).
- Total Hb reduced (~0.9) in G4-5 compared to G1 at 10.6 wks (not shown).
- ADSC FACS analysis: CD34+, CD45-, CD24+, CD144+, CD90+, CD44+, CD29+, CD73 and CD105 mostly (~fits MSC phenotype).

A POTENTIAL SOLUTION

- Autologous adipose tissue grafting (“Fat Grafting”) and adipose-derived stem cell (ADSC) therapy may improve wound healing and scar outcomes in acute burn, excisional, and radiation skin injury models.
- Clinical case reports suggest adipose therapeutics may improve the remodeling of chronically scarred skin tissue by improving skin color, texture, pliability, and patient symptoms. At least one clinical trial is ongoing.
- Most basic research focuses on acute phase intervention, few if any studies examine adipose derived therapeutics for improved remodeling of chronic scars.

PROJECT GOALS

- Determine if adipose tissue can improve scar remodeling subacutely after acute wound healing phases have concluded in a mouse model of thermal injury.
- Compare the effects of processed liposarpirate to adipose-derived stem cells.

TABLE 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Non-injected Control</td>
<td>Matrigel Control</td>
<td>Human high density Liposarpirate</td>
<td>Low Dose (10⁶) ADSCs in Matrigel</td>
<td>High Dose (10⁶) ADSCs in Matrigel</td>
</tr>
<tr>
<td>N=10</td>
<td>N=10</td>
<td>N=10</td>
<td>N=10</td>
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Conclusions

- Liposarpirate may improve scar remodeling, possibly mediated by increased blood vessel density improving oxygenation, resulting in smaller perceived scar area.
- Liposarpirate may retain a native scaffold allowing improved cell survival and angiogenesis preferentially to de novo vasculogenesis from direct ADSC differentiation.
- ADSCs, although promising in other studies, did not improve scar area, perfusion, or BV density in this model. Matrigel may have contributed to this finding as growth factor penetration to healing site may be limited.
- Limitations: Variability in mouse skin phenotype may have contributed error. Burn models are difficult to standardize: burn injury may not have been equal across all mice. Time and resources limited extent of analysis.

Future Directions

- Molecular analysis of scar remodeling targets such as TGF-β1/3, α-SMA, col1/3, VEGF, MMP9, SMAD-3.
- Experiment other hydrogels or no hydrogel in this case this was a factor in reducing ADSC efficacy.
- Continue attempts at improving photomorphometric analysis of picrosirius red collagen staining.
- Include a “supercharged” liposarpirate group for comparison (Lipo + ADSCa).
- Consider porcine studies, a higher fidelity human skin model and better model of hypertrophic scarring.
- Consider consultation with dermatopathology experts for new clues for histological analysis.

References


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Examples of burn scarring. Hypertrophic scar areas are raised and erythematous. Note also changes in texture, pigmentation, and contraction of skin limiting mobility.


Figure 1. Project timeline and outcomes.

Figure 2. Scar micro and macroscopic progression from pilot study. Fig. 3 (below) shows scar area progression through 6 weeks post-burn due to scar contraction.

Figure 3. Wound area all groups to 6 weeks (% of original, mean +/- SD)

Figure 4. Percent change in scar area from 0 to 10 weeks (mean %, +/- SD)

Figure 5. Vessels per hps on Masson’s Trichrome (mean +/- SD)

Figure 6. Vessels per hps on Masson’s Trichrome (mean +/- SD)

Table 1. Group 1 vs. Group 2, ** p < 0.01 vs. group 1, + p <0.05 vs. group 2, ++ p < 0.01 vs. group 2.