Clinical Use of Cultured Epithelial Autograft Cellular Suspension for Clinical Use

SCAR Meeting 2006 – Montpellier, France
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Western Australian Burns Service
Tissue Regeneration
WHY LIZARDS SHOULD NEVER BUNGEE JUMP
'There is no magician’s mantle to compare with the skin in its diverse roles of waterproof, overcoat, sunshade, suit of armour and refrigerator. Sensitive to the touch of a feather, to temperature and pain, withstanding the wear and tear of three score years and ten and executing its own running repairs.'
Skin Replacement Criteria

- Rapidly available
- Autologous
- Site matched
- Reliable wound adherence
- Minimal donor site morbidity
- Clinically manageable
- Improved quality of scar
- Affordable

Horch, Munster & Achauer (2001) Cultured human keratinocytes and tissue engineered skin substitutes
Optimal Surgical Timing

10 days to heal                      Scar Risk 4%

>21 days to heal                     Scar Risk 78%

Early treatment decreases risk and level of scarring

Deitch EA, Wheelahan TM, Rose MP et al (1983) Hypertrophic burn scars: analysis of variables J Trauma 23(10); 895-8
Cultured epithelial autograft (CEA) sheets

- Cultured using a large, full-thickness biopsy
- Cultured as confluent sheets
- Generally 3 to 5 cell layers thick
- Available 3 to 5 weeks after initial biopsy

Horch (2001) Tissue engineering and the skin: Development of cultured skin substitutes from sheets to composites to suspensions and monolayers on biological carrier materials
Problems with CEA Sheets

- Lengthy culture process
- Extensive application time
- Variability in product quality (cell numbers and yield)
- Fragile and difficult to apply
- Poor “take” in contoured and high shear areas
- High costs, especially when associated with poor “take”
Advantages of C3’s CEA Suspensions

- Improved epithelialisation and scar outcomes
- Reduced culture time
- Improved “take”
- Small, site matched biopsy
- Ease of handling
- Reduced length of stay/treatment requirements
- Cost effective

J. Investigational Dermatology, 112(3): 391-391
Application of Cell Sheet vs. Cell Suspension to the Wound Bed

Porcine model – wound healing

- Yorkshire pig model to study the effects of sprayed keratinocytes delivered on wounds within a meshed autograft
- Full thickness wounds covered with 3:1 split thickness autograft, one group treated with sprayed keratinocytes, one group treated with culture medium alone

Navarro, Stoner, Park et. al. (2000) Sprayed keratinocyte suspensions accelerate epidermal coverage in a porcine microwound model, J. Burn Care & Rehabilitation, 21(6): 513-518
Conclusions:

• Day 5 and 8 histology - Increased epidermal thickness, confluence, keratin cysts and blood vessels in cell treated wounds
• Faster and better quality of epithelialisation in the wounds treated with cells
Yorkshire spotted pig model used to study methods of restoring melanocytes
• Paired, full-thickness wound covered with split thickness grafts. Both sprayed with an epidermal spray suspension, one group highly-pigmented (HPS), one group non-pigmented (NPS)

Navarro, Stoner, Lee et. al. (2001) Melanocyte repopulation in full-thickness wounds using a cell spray apparatus, J. Burn Care & Rehabilitation, 22(1): 41-46
Conclusions:

- Viable melanocytes successfully transferred and evenly distributed with C3 suspension
- C3 suspension could be a legitimate method to restore colour to skin
## Variability of Cell Yields and Viability

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>Epilepsy</th>
<th>Bali</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>52yrs</td>
<td>36yrs</td>
<td>25yrs</td>
</tr>
<tr>
<td><strong>BSA Injury</strong></td>
<td>12%</td>
<td>50%</td>
<td>25%</td>
</tr>
<tr>
<td><strong>Cell Yield</strong></td>
<td>$4.8 \times 10^6$</td>
<td>$1.8 \times 10^6$</td>
<td>$3.4 \times 10^6$</td>
</tr>
<tr>
<td><strong>Cell Viability</strong></td>
<td>94%</td>
<td>59%</td>
<td>60%</td>
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</table>

Level of Resuscitation and Co-Morbidities Greatly Impact Cell Yields and Viabilities
CellSpray® in deep dermal partial thickness burn (12% TBSA)

Day 5 (pre-treatment)  
7.5 months post injury
Type of graft by year of injury, n=94

The graph shows the distribution of different types of grafts used over the years. The x-axis represents the year of injury, with categories for 2000 and 1990. The y-axis represents the number of children. The graph categories include:
- No grafting
- Split skin grafts only
- SSG & CEA
- CEA only
- SSG & ReCell
- ReCell only

The data indicates a significant increase in grafting procedures over time, particularly between 2000 and 1990.
ReCell® Safety and Efficacy Study

- To establish the safety & efficacy of ReCell® in injuries and scars
- Total patients 80 (70 treated so far)

- Interim results:
  - No signs of significant infection or adverse events
  - Complete healing in 60% of patients by one week
  - 100% wound healing by six-weeks
  - No issues with donor sites
  - Scars described as matched or mildly mismatched
  - All patients (bar one) described outcome as exceptional or better

PSR-01 and PSR-02, Australia (ongoing)
CellSpray® in deep full thickness burn
Survival curve for patients admitted with major burn injuries, 1992-2002
Characteristics of all patients treated with cultured epithelial autografts, n = 62

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cultured sheets</th>
<th>Sheets and Suspension</th>
<th>Suspension (CellSpray)(\textsuperscript{\dagger})</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\textsuperscript{\dagger}) Patients treated, n</td>
<td>14</td>
<td>12</td>
<td>36</td>
<td>62</td>
</tr>
<tr>
<td>Days to first treatment (Mean ± SD)</td>
<td>24.7 ± 6.9</td>
<td>14.8 ± 7.7</td>
<td>10.6 ± 6.7</td>
<td>14.6 ± 8.9</td>
</tr>
<tr>
<td>%TBSA injury (Mean ± SD)</td>
<td>60.7 ± 16.0</td>
<td>61.0 ± 10.3</td>
<td>63.8 ± 15.4</td>
<td>62.3 ± 14.8</td>
</tr>
<tr>
<td>Number of cell applications (Mean ± SD)</td>
<td>2.9 ± 2.0</td>
<td>7.3 ± 5.5</td>
<td>6.1 ± 5.8</td>
<td>5.6 ± 5.3</td>
</tr>
<tr>
<td>Operative procedures (Mean ± SD)</td>
<td>5.9 ± 4.3</td>
<td>6.1 ± 3.1</td>
<td>4.3 ± 2.3</td>
<td>5.0 ± 3.1</td>
</tr>
<tr>
<td>Microbes isolated (Mean ± SD)</td>
<td>7.1 ± 3.4</td>
<td>6.2 ± 4.0</td>
<td>5.6 ± 3.2</td>
<td>6.1 ± 3.4</td>
</tr>
<tr>
<td>Re-grafts in theatre (number)</td>
<td>9</td>
<td>8</td>
<td>11</td>
<td>28</td>
</tr>
<tr>
<td>Patients who also had split skin grafts, n</td>
<td>11</td>
<td>12</td>
<td>35</td>
<td>58</td>
</tr>
<tr>
<td>Total length of stay (days) (Mean ± SD)</td>
<td>127.9 ± 95.2</td>
<td>111.9 ± 65.9</td>
<td>76.6 ± 96.9</td>
<td>95.0 ± 70.0</td>
</tr>
<tr>
<td>TLOS/%TBSA (Mean ± SD)</td>
<td>2.0 ± 1.2</td>
<td>1.8 ± 0.9</td>
<td>1.3 ± 0.7</td>
<td>1.5 ± 0.9</td>
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\(\textsuperscript{\dagger}\) Includes 6 patients who had cultured sheets applied on the ward to small patchy areas, and 3 patients who had limited cultured sheets at the time of secondary surgery, ie “patchy areas” in theatre

\(\textsuperscript{\dagger}\) Treatment may also includes the use of split skin grafting with/without artificial dermal replacement
Results: Continuous SEC
Dependent vs non-dependent

Magnusson M, Papini R, Rea S, Reed C, Wood FW
Cultured autologous keratinocytes in suspension accelerate maturation in an in-vivo wound model as measured by surface electrical capacitance. Plastic and Reconstructive Surgery
Accepted for Publication, 2005
ReCell® and Integra®

- The use of dermal substitutes have been limited by poor take, high infection rates and poor cosmetic outcomes

- Study Design:
  - 10 full-thickness wounds in two pig models (n=20)
    - 14 treated with ReCell® and Integra®
    - 3 treated with Integra® alone
    - 3 received ReCell® alone
  - Observation and wound histology at days 7, 14, 21, 28 and 35

Wood FW, Fowler BV, Rea SM. A prospective clinical study to evaluate the safety and efficacy of ReCell® Autologous Cell Harvesting Kit in Epidermal Reconstruction Journal Burn Care and Rehabilitation. Submitted for Publication
Results & Conclusions:

- No infection noted in any wound

- Improved healing in ReCell® and Integra® wounds at all stages of the study

- Healing by secondary intention only in wounds not treated with ReCell®
The Skin and its Repair

• The ability of epidermal cells to regenerate is highly dependent on the substrate on which they are grown;
  • Growth factors
  • Adhesion
  • Architecture

• Well established that alteration in structure of scaffolds at the micron-level affects wound healing;
  • Integra™
Tissue Engineering

• But what about at the nano-scale...

• As yet, there has been no study into the effects of nano-scale architecture on keratinocyte behaviour.

• If keratinocytes are sensitive to changes on the nano-scale then it may be possible to optimise behaviour and enhance the wound healing process.
Microscopy images of prepared AAO membranes. Pore size is ~ 160 nm.
AFM of skin cells deposited on AAO membrane
After 24 hour contact with skin cell suspension and washing

High resolution AFM images of skin cells on AAO membrane.
⇒ 3 and 2 micron scans.
Proliferation Assay (MTS) – to determine which pore size gives optimum proliferation.
Assessment is key in understanding the extent of injury. Debridement is focused on tissue salvage. Reconstruction balances repair with regeneration. Investigation of multimodality, multiscale characterisation, including confocal microscopy and synchrotron technology will quantify assessment. Debridement using autolytic inflammatory control techniques with image guided physical methods will ensure the vital tissue frameworks are retained. Tissue guided regeneration afforded by self-assembly nanoparticles will provide the framework to guide cells to express the appropriate phenotype in reconstruction. To solve the clinical problem a multi-disciplinary scientific approach is needed to ensure the quality of the scar is worth the pain of survival.
Acknowledgements

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Thank You