Long-term *in vivo* assessment of bioengineered skin substitutes: a clinical study

Giovanni Nicoletti¹²³*, Federica Brenta⁴, Mariella Bleve⁵, Tommaso Pellegratta⁴, Alberto Malovini⁶⁷, Angela Faga¹²³ and Paola Perugini⁸

¹Department of Clinical Surgical Diagnostic and Paediatric Sciences, University of Pavia, Italy
²Advanced Technologies for Regenerative Medicine and Inductive Surgery Research Centre, University of Pavia, Italy
³Plastic and Reconstructive Surgery Unit, Salvatore Maugeri Research and Care Institute, Pavia, Italy
⁴Plastic Surgery Residency Programme, University of Pavia, Italy
⁵Etichub s.r.l., Department of Drug Sciences, University of Pavia, Italy
⁶Department of Computer Engineering and Systems Science, University of Pavia, Italy
⁷Laboratory of Informatics and Systems Engineering for Clinical Research, Salvatore Maugeri Research and Care Institute, Pavia, Italy
⁸Department of Drug Sciences, University of Pavia, Italy

Abstract

The aim of the study was an objective *in vivo* assessment of skin properties after reconstruction with two artificial dermal substitutes, Integra® and Hyalomatrix®. Twenty-seven patients underwent reconstruction of 36 skin-loss sites with full-thickness skin graft, split-thickness skin graft, Hyalomatrix® bioengineered skin substitute and sequential split-thickness skin graft and Integra® bioengineered skin substitute and sequential split-thickness skin graft. Objective assessments were carried out using three instrumental devices: Multi Probe Adapter System MPA; 22 MHz ultrasound skin scan; and Primos Pico for a three-dimensional (3D) skin scan. The skin parameters under study in our sample were: corneometry, transepidermal water loss, elastometry, colorimetry, skin thickness and 3D skin surface pattern. A skin reconstruction with Hyalomatrix seemed to most closely approach the hydration, transepidermal water loss and skin surface 3D pattern of normal skin. A skin reconstruction with Integra seemed to demonstrate the best skin colour feature and elastic properties. Although no statistically significant differences were observed, the descriptive analysis of the outcomes might suggest a better cell regulation, regenerated extracellular matrix and neoangiogenesis with the use of Hyalomatrix, and the formation of a more elastic regenerated dermis, with overall better physical, mechanical and optical properties, with the use of Integra. © 2014 The Authors. *Journal of Tissue Engineering and Regenerative Medicine* published by John Wiley & Sons Ltd.

Received 27 September 2013; Revised 28 April 2014; Accepted 27 May 2014

Keywords bioengineered skin; dermal substitute; skin graft; skin reconstruction; wound repair

1. Introduction

Bioengineered skin substitutes gained a growing consensus for tissue loss reconstruction in difficult cases where the need for autologous skin grafts exceeds the available donor sites. This encouraged the development of skin-replacement materials. The lack of dermal tissue in full-thickness wounds and the poor quality of the scars after treatment with split-thickness autologous skin grafts or cultured epithelial grafts, which contain little or no dermal component, respectively, further promoted the development of dermal substitutes.

In current clinical practice, artificial skin substitutes are generally used for the acute treatment of huge skin loss in patients in critical general conditions (Wolter et al., 2005; Weigert et al., 2011), for the treatment of burns (Heimbach et al., 1988; Gros et al., 2005; Gravante et al., 2007), to promote regeneration in non-healing wounds (Motolese et al., 2013) or for functional and aesthetic indications in secondary surgery, thus replacing the traditional indications for flap surgery in an increasing number of cases (Giovannini and Teot, 2002; Nicoletti et al., 2008, 2014; Faga et al., 2012). Nevertheless, there is a lack of biological background information on the design and use of different types of materials and their long-term anatomical and functional outcomes.

*Correspondence to: Giovanni Nicoletti, Plastic Surgery Unit, University of Pavia, Salvatore Maugeri Research and Care Institute, Via Salvatore Maugeri 30, 27100 Pavia, Italy. E-mail: giovanni.nicoletti@unipv.it; giovanni.nicoletti@fsm.it*
The aim of our study was an objective in vivo assessment of the skin anatomical and functional properties after reconstruction with two popular artificial dermal substitutes, Integra® (Integra LifeSciences, Plainsboro, NJ, USA) and Hyalomatrix® (Anika Therapeutics, Bedford, MA, USA).

2. Materials and methods

The study was carried out in cooperation between the Plastic Surgery Unit, University of Pavia, Salvatore Maugeri Research and Care Institute and the Department of Drug Sciences, University of Pavia, Pavia, Italy; 27 patients (10 females and 17 males) underwent reconstruction of skin loss with one of the following procedures:

1. Full-thickness skin graft.
2. Split-thickness skin graft.
3. Hyalomatrix bioengineered skin substitute and sequential split-thickness skin graft (Hyalomatrix SG).
4. Integra bioengineered skin substitute and sequential split-thickness skin graft (Integra SG).

The patients were enrolled in the study following long-term stable reconstruction; mean 28 (minimum 18, maximum 60, median 24) months. The trial was carried out over a period of 24 weeks in May–October 2012. Mean age was 62.85 (minimum 15, maximum 88, median 71) years. We excluded from the study patients with poor individual and/or family compliance.

The reconstructed skin loss area was considered the experimental unit of the study, irrespective of the number of reconstructions/patient. An overall number of 36 skin loss reconstructions were included in the sample. Seven areas underwent reconstruction with full-thickness skin graft, 10 with split-thickness skin graft, eight with Hyalomatrix SG and 11 with Integra SG; 19 skin loss reconstructions involved the head, seven the trunk, five the upper limb and five the lower limb. In all cases the reconstructions were carried out on fresh wounds, following radical excisions of skin tumours (21 areas), excision of unstable scars (11 areas) and debridement in healthy tissue of difficult-to-heal wounds (4 areas). Twenty-two skin grafts were harvested from the thigh, seven from the arm, four from the supercavicular region, two from the groin and one from the retro-auricular region.

Skin grafts were sutured with stitches in the recipient site and a petrolatum gauze plus non-woven fabric dressing was tied over, in order to ensure contact between the graft and wound bed. At day 7 the tie-over dressing was removed to check that the skin graft had regained its former pink colour, which is the clinical sign of effective revascularization.

Hyalomatrix was applied to the recipient site and fixed with metallic staples and a petrolatum gauze plus non-woven fabric dressing was tied over. The patients were instructed to keep the operated areas as still as possible. At day 3, the tie-over dressings were removed to check the wounds. A simple inspection of the silicone layer was carried out at this stage and, if appropriate, serum draining from the margins and from the holes in the silicone sheet was gently removed with sterile saline solution. The tie-over dressing was replaced by a tight dressing until day 6, when the staples were removed. After an average of 19 (range 13–27) days, according to clinical evidence of regenerated tissue growth, a second surgical procedure provided stable wound repair with a split-thickness skin graft. At this stage, the silicone sheet was removed to expose the wound bed. Any clinically detectable granulation tissue was carefully removed in order to expose the underlying regenerated pink dermal-like tissue. The wound bed was eventually grafted with an autologous split-thickness skin graft.

Similarly, Integra was applied to the recipient site and fixed with metallic staples and a petrolatum gauze plus non-woven fabric dressing was tied over. The patients were instructed to keep the operated areas as still as possible. At day 3, the tie-over dressings were removed to check the wounds and replaced by a tight dressing. The staples were removed after an average of 25 (range 15–45) days, according to clinical evidence of regenerated tissue growth, and the silicone sheet was removed to allow immediate cover with an autologous split-thickness skin graft.

Perioperative antibiotic prophylaxis was routinely administered (amoxicillin 2 g + clavulanic acid 200 mg intravenously intra-operatively, and amoxicillin 875 mg + clavulanic acid 125 mg per os twice a day for 1 week after surgery), irrespective of the surgical reconstruction modality.

Objective assessments were carried out using three instrumental devices:

1. Multi Probe Adapter System MPA (Courage and Khazaka, Cologne, Germany), equipped with Cutometer MPA 580, Corneometer CM825, Tewameter TM300, Mexameter MX18, Colorimeter CI 400, allows assessment of skin corneometry, transepidermal water loss (TEWL), elastometry and colorimetry.
2. DUB system (Tpm Taberna pro medicum, Luneburg, Germany) allows a detailed ultrasound scan of the skin and subcutaneous tissue from epidermis to fascia, with excellent definition of all anatomical layers.
3. Primos Pico (GF Messtechnik GmbH, Teltow, Germany) allows a three-dimensional (3D) skin scan.

These diagnostic techniques already have proved their effectiveness in the objective anatomical functional assessment of the skin in several previous reports in the literature (Fisher et al., 1999; Piérard, 1999; Szymańska et al., 2000; Nguyen et al., 2010; Sin et al., 2010; Bloemen et al., 2011; Moiemen et al. 2011). All of the devices are CE certified and passed the safety tests before use.

Formal informed written consent was obtained from all the patients and the study conformed to the Declaration of Helsinki.

The anatomical functional parameters under study in our sample of skin reconstructions were: corneometry;
transdermal water loss; elastometry; colorimetry; ultrasound skin scan pattern; and 3D skin surface pattern.

2.1. Corneometry

Corneometry is a technique used to assess the hydration of the outer layer of the epidermis, the stratum corneum. As skin is a dielectric medium, all variations in hydration show up through corresponding changes in the skin capacity. The device used in our trial was equipped with a 49 mm² surface probe that allows precise measurement in 1 s within a 10–20 μm depth range in the stratum corneum.

2.2. Transepidermal water loss (TEWL)

Transepidermal water loss was assessed in terms of g/m²/h by a skin evaporimeter made of a small cylindrical open chamber (1 cm diameter × 2 cm height) with a couple of hygrometric sensors connecting to a microprocessor plugged into a computer workstation. The device allows recording of TEWL (range 0–90 g/m²/h), the relative humidity (range 0–100%) and the probe temperature. The latter parameters allow the system an extremely accurate measurement of TEWL by excluding any influence from the external environmental conditions.

2.3. Elastometry

The cutaneous elasticity was assessed using a cutometer, measuring the vertical deformation of the skin induced by vacuum aspiration. A negative pressure of 450 mbar was applied to the skin for 1–3 s through a 2 mm diameter probe. Each aspiration was followed by a release time, allowing the skin to return to its resting condition. The probe is provided with an optic sensor, assessing variation of light transmission due to the aspirated skin bulking inside the probe. The following parameters were considered reliable indicators of skin elasticity:

- **Skin compactness** (R0): the passive skin behaviour following application of negative pressure.
- **Skin resistance** (R2): the resistance against the return to the rest conditions at the end of suction.
- **Net skin elasticity** (R5): the ratio between the maximum skin extension and the residual skin deformity.

The parameters were expressed on an arbitrary score scale.

2.4. Colorimetry

Skin colorimetry was measured using two methods, mexameter and colorimeter.

In the *mexameter* method, a 5 mm diameter probe emits light at three different wavelengths (568, 660 and 870 nm); an optic sensor measures the light after reflection on the skin. The device measures the emitted light absorption rate by both melanin and haemoglobin, providing an arbitrary melanin index (MI) and an arbitrary haemoglobin index (HI), respectively, range 0–999.

In the *colorimeter* method, an 8 mm diameter probe emits white LED light; an optic sensor measures the light after reflection on the skin, using an arbitrary score scale for the following parameters: luminosity (L), range 0 (black)–100 (white); green and red (a), tolerance –120/+120; blue and yellow (b), tolerance –120/+120. The skin colour is calculated using the formula: \( L \times a \times b \).

2.5. Ultrasound skin scan

A 22 MHz probe ultrasound scan allowed a skin anatomical assessment up to 8 mm in depth. Dermal depth was measured in μm.

2.6. 3D skin scan

The phase-shift rapid *in vivo* measurement of the skin (PRIMOS) system allowed detailed 3D skin surface scanning. It provides high-resolution assessment of skin surfaces by using phase-shifted light stripes projected by micro-mirrors to generate a 3D profile of the measured skin surface (area 18 × 13 mm²). The reflected light is captured by a high-resolution camera and a software package converts the image into a colour-coded picture of the skin surface, with different colours for different heights. The device therefore allows direct measurement of the skin surface reliefs and hollows as follows:

- Maximum absolute height in μm of the skin profile, calculated from the maximum depth of the skin hollows to the top of the skin reliefs.
- Mean furrows depth (μm).
- Mean depth of the deepest furrow (μm).
- Maximum depth of the deepest furrow (μm).
- Furrow count.
- Furrows overall volume (mm³).
- Overall furrows surface (mm²).
- Furrow surface ratio: percentage of the skin area with furrows vs the area without furrows.
- Furrow overall length (mm): length sum of all furrows.

The 3D synthetic assessment of the skin surface was expressed by the function integral of the skin surface profile (Ra). Each reconstructed area was compared with the skin in the corresponding normal contralateral anatomical area used as the control.

2.7. Statistical analysis

The Shapiro test was used to evaluate whether quantitative variables deviated significantly from the normal distribution. The non-parametric Kruskal–Wallis test was employed to test for differences in terms of quantitative
distributions among subgroups. The two-sided non-parametric Wilcoxon rank-sum test was applied to perform pairwise comparisons between treatments within the same parameter. Fisher’s exact test was applied to compare categorical distributions among subgroups. The presence of statistically significant correlations between quantitative variables was tested by the Spearman test: the strength of the correlation is expressed by the coefficient \( \sigma \), range \(-1\) to \(+1\). Adjustments for multiple testing were performed by rescaling the significance threshold according to the Bonferroni correction, dividing \( \alpha = 0.05 \) by the number of tests evaluated for each analysis. Statistical calculations were performed using R statistical software (www.r-project.org/).

3. Results

Our primary aim was to evaluate whether the score distribution observed in the treated skin tissue samples was similar to the distribution of scores observed in the normal tissue, and measured in the same patients. To this end, we estimated the absolute value of the difference in terms of score distributions between treated and matched control tissue samples (delta). Delta scores deviated significantly from normality (Shapiro \( p < 0.05 \)), therefore their distribution is described by median and interquartile range (IQR), instead of median \( \pm \) standard deviation (SD).

No statistically significant difference in terms of delta values distribution was observed among treatment groups for each parameter \( (p > 0.005, \text{ based on the Bonferroni correction for multiple testing, adjusting by 10 tests}) \). Pairwise comparisons showed no statistically significant difference in terms of delta values distribution between all possible treatment groups for each of the 10 parameters evaluated \( (p > 0.0008) \) (Table 1).

No statistically significant correlation between delta measurements and time lapse was observed for specific parameters (Spearman \( \sigma \), range \(-0.23 \) to \(+0.21; p > 0.20\)).

The median age distributions within split-thickness skin graft, full-thickness skin graft, Hyalomatrix SG and Integra SG were: 67 (IQR = 62–72), 78 (IQR = 64–81.5), 57 (IQR = 26.5–80.5) and 42 (IQR = 38–42), respectively. No statistically significant difference was observed among subgroups (Kruskal–Wallis test, \( p = 0.13 \)).

Age did not correlate significantly with the measured delta variations regarding any of the analysed parameters (Spearman \( \sigma \), range \(-0.28 \) to \(+0.35; p > 0.075\)).

Aetiology of skin loss was unbalanced across the treatment groups (Fisher’s exact test, \( p < 2 \times 10^{-10} \)).

We observed no statistically significant difference in terms of anatomical locations distribution among treatment groups (Fisher’s exact test, \( p = 0.31 \)). These results suggest that anatomical locations were balanced among treatments.

No statistically significant difference in terms of median delta variations was observed across different anatomical origins for any of the analysed parameters (Kruskal–Wallis test, \( p > 0.07 \)).

Post hoc analyses revealed that we had statistical power in the range \(<0.01 \) to \( 0.53 \), given the effect sizes observed for single comparisons, the sample size and the significance threshold (set at \( p < 0.0008, \text{ based on the Bonferroni correction for multiple testing} \)). These results show that our analyses were not sufficiently powered to state that there are no statistically significant differences between the analysed subgroups. Nevertheless, some interesting considerations are suggested by descriptive analysis of all of the assessed parameters (Fig. 1A–J):

A. Corneometry. A lesser degree of hydration of the stratum corneum was demonstrated in all of the reconstructed areas vs the controls. The best approximation to the corneal hydration of the normal skin was featured by Hyalomatrix SG, while STSG, FTSG and Integra SG displayed marked progressively lower degrees of epidermal hydration.

B. Transepidermal water loss. Integra SG demonstrated a higher water loss than the normal skin, while all the other reconstructions displayed a lower loss in comparison with the control sites. Within the latter group, Hyalomatrix SG featured the best approximation to the normal skin, while the skin grafts did significantly worse, FTSG being the worst.

C. Skin compactness. Hyalomatrix SG, STSG and Integra SG in decreasing order, appeared more deformable than the control skin, while FTSG displayed an increased stiffness vs the control sites.

D. Skin resistance. A lesser degree of skin resistance was demonstrated in all of the reconstructions vs the controls. The best approximation to the normal skin was featured by FTSG, while Integra SG, Hyalomatrix SG and STSG followed, in decreasing order.

E. Net skin elasticity. FTSG appeared stiffer than the normal skin, while the other reconstructions appeared more lax than the controls. Within the latter group, Hyalomatrix SG was the reconstruction modality that allowed the highest degree of skin laxity; Integra SG and STSG then followed in decreasing order.

F. Melanin index. Hyalomatrix SG showed a melanin index higher than the normal skin, while all of the other reconstructions had an index lower than the control sites. Within the latter group, STSG displayed the best approximation to the normal skin and FTSG and Integra SG followed, in decreasing order.

G. Haemoglobin index. Hyalomatrix SG displayed a haemoglobin index higher than the normal skin, while all the other reconstructions had an index lower than the controls. Within the latter group, STSG displayed the best approximation to the normal skin and FTSG and Integra SG followed, in decreasing order.

H. Colorimetry. None of the reconstructions could match the normal skin in colorimetry. The best approximation to the normal skin was featured by Integra SG, followed, in decreasing order, by STSG, FTSG and Hyalomatrix SG.

I. Dermal thickness. Hyalomatrix SG and STSG showed a thicker dermis than normal skin and Hyalomatrix SG had the thickest dermis of all reconstructions and
We used the bioengineered skin substitutes for the acute treatment of huge skin loss in patients in critical general conditions or for functional and aesthetic indications in secondary surgery, where a flap was not available (Nicoletti et al., 2008, 2014; Faga et al., 2012).

Hyalomatrix or Integra were used without specific indications, as the long-term outcome of each product was not known at the time of our early experience. Actually, after several years of random use of both bioengineered skin substitutes, we designed this study to objectively assess the particular long-term outcomes of each product in comparison with the traditional skin-grafting procedures. Although the potential sample of patients with a bioengineered skin reconstruction could have been much larger, the rigorous compliance requirements limited the number of patients who eventually participated to the study. All the measurements were non-invasive in nature, but patients’ attendance was limited by two main factors: (a) place of residence far from the hospital; and (b) heavy emotional burden.

J. Skin surface profile. FTSG, STSG and Hylomatrix SG, in decreasing order, displayed a more wrinkled surface than normal skin, while Integra SG showed the smoothest surface of all reconstructions and controls.

4. Discussion

Our results demonstrated that none of the skin reconstruction modalities could reproduce the anatomical and functional features of healthy skin. In our practice, a split-thickness skin graft is best indicated in large soft-tissue loss where a better cosmetic result is a priority. In general, we used the bioengineered skin substitutes for the acute treatment of huge skin loss in patients in critical general conditions or for functional and aesthetic indications in secondary surgery, where a flap was not available (Nicoletti et al., 2008, 2014; Faga et al., 2012).

Table 1. Statistical analysis of Δ scores distribution among treatment groups for each parameter

| Parameter                          | Treatment          | n    | Median Δ (IQR) | +/- | Global p |
|------------------------------------|--------------------|------|----------------|-----|----------|
| Corneometry                        | HSTSG              | 8    | 6.55 (1.93–9.38) | −   | 0.595    |
|                                    | STSG               | 10   | 9.3 (5.58–20.85) | −   |          |
|                                    | FTSG               | 7    | 14.2 (4.7–19.95) | −   |          |
|                                    | ISTSG              | 11   | 16.1 (6.85–25.7) | −   |          |
|                                    | HSTSG              | 8    | 6.2 (2.88–10.55) | −   | 0.803    |
|                                    | ISTSG              | 11   | 6.7 (3–22.5)     | +   |          |
|                                    | STSG               | 9    | 7.4 (1.9–9.9)    | −   |          |
|                                    | FTSG               | 7    | 8 (5.75–9.35)    | −   |          |
| TEWL                               | STSG               | 9    | 0.01 (0.01–0.04) | +   | 0.486    |
|                                    | ISTSG              | 7    | 0.02 (0.02–0.04) | +   |          |
|                                    | FTSG               | 6    | 0.025 (0.01–0.06) | −   |          |
|                                    | HSTSG              | 6    | 0.055 (0.02–0.08) | +   |          |
| Skin compactness (R0)              | STSG               | 9    | 0.26 (0.1–0.55)  | −   |          |
|                                    | ISTSG              | 7    | 0.17 (0.08–0.35) | −   | 0.568    |
|                                    | FTSG               | 6    | 0.08 (0.04–0.16) | −   |          |
|                                    | HSTSG              | 6    | 0.195 (0.13–0.26) | −   |          |
|                                    | STSG               | 9    | 0.26 (0.1–0.55)  | −   |          |
|                                    | FTSG               | 7    | 40 (17.5–62)     | −   |          |
| Net skin elasticity (R5)           | STSG               | 8    | 53.5 (35–102.5)  | +   |          |
|                                    | ISTSG              | 11   | 81 (51–131.5)    | +   |          |
| Melanin index                      | STSG               | 10   | 28.5 (20.25–89.25) | −   | 0.167    |
|                                    | FTSG               | 7    | 40 (17.5–62)     | −   |          |
|                                    | HSTSG              | 8    | 53.5 (35–102.5)  | +   |          |
| Haemoglobin index                  | STSG               | 10   | 49 (43.75–70.5)  | −   | 0.037    |
|                                    | FTSG               | 7    | 59 (38.5–72.5)   | −   |          |
|                                    | HSTSG              | 8    | 70.5 (39.75–128.5) | +   |          |
| Colorimetry (colorimeter method)   | ISTSG              | 11   | 1894.5 (704.1–3972.1) | −   | 0.252    |
|                                    | STSG               | 10   | 2117.45 (1137.38–3062.38) | −   |          |
|                                    | FTSG               | 7    | 2698.7 (2254.7–4660.45) | −   |          |
|                                    | HSTSG              | 8    | 4448.6 (1353.25–6029.29) | −   |          |
| Dermal thickness                   | FTSG               | 4    | 290 (155.75–425.25) | −   | 0.079    |
|                                    | STSG               | 10   | 519 (238.75–1278.25) | +   |          |
|                                    | HSTSG              | 6    | 731 (329–935.75)  | +   |          |
|                                    | ISTSG              | 10   | 1836 (811.5–2453.75) | −   |          |
| Skin surface profile (Ra)          | HSTSG              | 7    | 2 (2–3.5)       | +   | 0.076    |
|                                    | STSG               | 9    | 6 (2–17)        | +   |          |
|                                    | FTSG               | 6    | 8 (2.75–12.5)   | +   |          |
|                                    | ISTSG              | 10   | 10 (6.25–11)    | −   |          |

Treatment, treatment group; n, non-missing measurements; median Δ (interquartile range, IQR), median value of the difference between treated and matched control tissue; global p, p value testing the difference in terms of Δ distribution among subgroups within each parameter (significance level, p < 0.005); +, values higher than the values in normal skin; −, values lower than the values in normal skin; STSG, split-thickness skin graft; FTSG, full-thickness skin graft; HSTSG, Hylomatrix® bioengineered skin substitute and sequential split-thickness skin graft; ISTSG, Integra® bioengineered skin substitute and sequential split-thickness skin graft.
related to a past history of physical trauma, with subsequent refusal to undergo the perceived hospital admission experience again. Therefore, our sample could not be homogeneous for age, sex, pathologies requiring surgical treatment or indication for reconstruction modality. Nevertheless, the statistical analysis failed to demonstrate any influence of these factors on the eventual outcomes. However, some interesting considerations on the anatomical functional parameters under study follow on descriptive statistics.

Regarding the water regulation-related skin features, a skin reconstruction with Hyalomatrix SG appeared to most closely approach the hydration and transepidermal water loss (TEWL) of normal skin. These results might be related to both a hyaluronan-induced stimulation and regulation of the sweat gland remnants and a better epidermis–matrix interaction. TEWL mirrors the integrity of the skin barrier, which is directly related to both the production of a superficial hydrolipidic layer and the epidermal cells’ mutual cohesion. The superficial hydrolipidic

Figure 1. (a–j) Graphic representation of the differences between the healthy skin and the four skin reconstruction modalities under study, in terms of anatomical functional skin parameters: STSG, split-thickness skin graft; FTSG, full-thickness skin graft; HSTSG, Hyalomatrix® bioengineered skin substitute and sequential split-thickness skin graft; ISTSG, Integra® bioengineered skin substitute and sequential split-thickness skin graft
layer might be related to a hyaluronan-induced stimulation and regulation of the sweat and sebaceous gland remnants (Wang et al., 1992).

With regard to the functional parameters comprehensively investigating the elastic properties of the skin, FTSG, although showing some higher degree of stiffness, proved to be the best approximation to normal skin. All the other reconstructions displayed an increased overall laxity compared to the controls. Within the latter group, Integra SG demonstrated elastic properties that best approximated to normal skin.

In our sample, when considering the overall colour features, the reconstructions with Integra SG provided the best approximation to normal skin. The skin colour depends on multiple biological and physical factors: vascular network, number and functional activity of melanocytes, light absorption, refraction and reflection. The favourable outcome demonstrated by Integra SG is likely to be related to its dermal structural organization, which more closely resembled that of normal skin in terms of optic properties. In contrast, the poor result provided by Hyalomatrix SG might be explained by the increased red and black components of the skin colour that follow the lively hyaluronan-induced neoangiogenesis (Sattar et al., 1994; Lees et al., 1995) and melanocyte activation (Pianigiani et al., 1999).

Regarding dermal thickness, FTSG, although demonstrating a slightly thinner dermis than the controls, proved to be the best approximation to the normal skin. Integra SG demonstrated a thinner dermis than the controls and FTSG reconstructions. In contrast, a thicker dermis was demonstrated by STSG and Hyalomatrix SG, the latter being the thickest of all reconstructions and controls. This might be explained by two different hypotheses: STSG would promote a thick scar layer at the interface with the recipient wound bed, while the highly hydrophilic hyaluronan would attract larger amounts of water in the Hyalomatrix SG newly regenerated dermis.

Although the superficial skin wrinkledness might be influenced by many factors, the underlying scar retraction would play a relevant role for such an anatomical parameter. Our experience would therefore suggest some useful considerations for clinical use.

In our study, STSG proved to be the poorest soft tissue loss reconstruction modality, which is common knowledge in clinical practice. FTSG should be considered the best option when a reconstruction is expected to provide dermal thickness and elastic properties similar to normal skin, as in mobile and/or weight-bearing anatomical sites. On the other hand, Hyalomatrix SG would be the most appropriate solution when the skin surface quality, the water regulation-related skin features and the neoangiogenetic boost are relevant issues. Hyalomatrix (Anika Therapeutics) is a bilayered device: the bottom layer is a non-woven pad of HYAFF (Anika Therapeutics), a benzyl ester of hyaluronic acid;

---

Figure 1. Continued
the top layer is a semi-permeable membrane made of synthetic polysiloxane (silicone).

The primary functions of hyaluronic in epidermis are the maintenance of the extracellular space and provision of an open, hydrated structure. Through its complex interactions with matrix components and cells, hyaluronic has multifaceted roles, ranging from a purely structural function to developmental regulation of cellular behaviour. Hyaluronic is also thought to have important biological roles in skin wound healing, as comprehensively stated by Chen and Abatangelo (1999). Because hyaluronic is a hygroscopic macromolecule, it creates an environment permissive for migration of cells to new tissue sites, while its free-radical scavenging, protein-exclusion properties and antioxidant effect offer protection to cells and extracellular matrix molecules against free-radical and proteolytic damage, as demonstrated by Presti and Scott (1994), Kvam et al. (1995) and Fukuda et al. (1997).

Integra SG should be the most appropriate solution when the best possible skin colour match is required. Should a FTSG not available, Integra SG is better than Hyalomatrix SG in reproducing the elastic properties of the normal skin. Integra Dermal Regeneration Template (Integra LifeSciences) is also a bilayered device; the bottom layer is made of a 3D porous matrix of fibres of crosslinked bovine tendon collagen and a glycosaminoglycan (GAG; chondroitin 6-sulphate) from shark cartilage; the top layer is a semi-permeable membrane made of synthetic polysiloxane (silicone). The bovine collagen and chondroitin 6-sulphate serve as a template for the infiltration of fibroblasts, macrophages, lymphocytes and capillaries, which form the neovascular network. As healing progresses, collagen is deposited by the host fibroblasts, which replaces the bovine collagen and chondroitin 6-sulphate, while the silicone layer provides adequate moisture control. Histological structure, physical properties and clinical features of the induced neodermis closely resemble those of normal skin. The majority of histological animal and human studies report good tissue compatibility and integrity, as well as controlled biodegradation and no adverse immunological reactions (Michaeli and McPherson, 1990). Integra therefore might provide a good substrate and act as an appropriate scaffold for elastic fibres regeneration, as suggested by Wang et al. (2005).

5. Conclusions

Bioengineered skin substitutes are conventionally indicated for reconstruction of soft-tissue loss where the recipient site is unfit for a standard skin graft and flap surgery is not possible, because of lack of adequate flap donor sites, or poor general clinical conditions where major surgery is contraindicated. Consensus on the latter indications for skin substitutes is not unanimous, as some authors consider their actual effectiveness questionable (Philandrianos et al., 2012).

Clinical indications for the use of such skin substitutes have been progressively extending and currently include cover of a huge variety of soft tissue defects, for both functional and cosmetic purposes. According to our experience, the two bioengineered skin substitutes under study in our trial could be recognized as first-choice surgical treatment for both functional and cosmetic soft tissue loss reconstruction in selected cases, and no longer a simple salvage second-line therapy.

Hyalomatrix seemed to demonstrate better cell regulation and stimulation activity, with subsequent production of a better regenerated extracellular matrix. On the other hand, Integra seemed to allow the formation of a more elastic regenerated dermis, with overall better physical, mechanical and optical properties. The ideal bioengineered skin substitute of the future would preferably share the best features of both: the Integra dermal scaffold structural organization and the Hyalomatrix cell and extracellular matrix regulation and regeneration properties.

Our study did not aim to provide new clinical guidelines, but rather to convey new information that might help the clinician to choose the most appropriate treatment, considering the long-term clinical outcome of the traditional vs two popular bioengineered skin grafts. The identification of the gold standard for skin grafting tissue transfer of the future would benefit from further developments of bioengineered tissue research, and this experience might be considered a small effort to reach that goal.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors thank Alan Serge McGhee MSc, Glasgow City Council Education Department, for his contribution to the submission of this paper. The authors also wish to thank Floriana Cazzola and Gian Mario Pelizzoli for their much-appreciated technical support.

References

Bloemen MC, van Gerven MS, van der Wal MB, et al. 2011; An objective device for measuring surface roughness of skin and scars. J Am Acad Dermatol 64(4): 706–715.
Chen WY, Abatangelo G. 1999; Functions of hyaluronan in wound repair. Wound Repair Regen 7(2): 79–89.
Faga A, Nicoletti G, Brenta F, et al. 2012; Hyaluronic acid three-dimensional scaffold for surgical revision of retracting scars: a human experimental study. Int Wound J 28: 1–8.
Fisher TW, Wigger-Alberti W, Elsner P. 1999; Direct and non-direct measurement techniques for analysis of skin surface topography. Skin Pharmacol Appl Skin Physiol 12: 1–11.
Fukuda K, Takayama M, Ueno M, et al. 1997; Hyaluronic acid inhibits interleukin-1-induced superoxide anion in bovine chondrocytes. Inflamm Res 46(3): 114–117.
Giovannini UM, Teot L. 2002; Aesthetic complex reconstruction of the lower leg: application of a dermal substitute (Integra) to an adipofascial flap. Br J Plast Surg 55: 171–172.

Gravante G, Delogu D, Giordan N, et al. 2007; The use of Hyalomatrix PA in the treatment of deep partial-thickness burns. J Burn Care Res 28: 269–274.

Gros N, Guillot M, Zilliox R, et al. 2005; Use of an artificial dermis (Integra) for the reconstruction of extensive burn scars in children; about 22 grafts. Eur J Pediatr Surg 15(3): 187–192.

Heimbach D, Luterman A, Burke J, et al. 1995; Accelerated by angiogenic oligosaccharides of an artifical dermis for major burns. A multicenter randomized clinical trial. Ann Surg 208(3): 513–520.

Kvam BJ, Frgonas E, Degrassi A, et al. 1995; Oxygen-derived free radical (ODFR) action on hyaluronan (HA), on two HA ester derivatives, and on the metabolism of articular chondrocytes. Exp Cell Res 218(1): 79–86.

Lees VC, Fan TP, West DC. 1995; Angiogenesis in a delayed revascularization model is accelerated by angiogenic oligosaccharides of hyaluronan. Lab Invest 73(2): 259–266.

Michaeli D, McPherson M. 1990; Immunologic study of artificial skin used in the treatment of thermal injuries. J Burn Care Rehabil 11: 21–26.

Moiemen N, Yarrow J, Hodgson E, et al. 2011; Long-term clinical and histological analysis of Integra® dermal regeneration template. Plast Reconstr Surg 127(3): 1149–1154.

Motolese A, Vignati F, Brambilla R, et al. 2013; Interaction between a regenerative matrix and wound bed in nonhealing ulcers: results with 16 cases. Biomed Res Int 2013: 849321.

Nguyen DQ, Potokar TS, Price P. 2010; An objective long-term evaluation of Integra® (a dermal skin substitute) and split thickness skin grafts, in acute burns and reconstructive surgery. Burns 36: 23–28.

Nicoletti G, Ghilardi CG, Scevola S, et al. 2014; Hyaluronan induced cosmetic reconstruction of the nostril. Facial Plast Surg 30: 1–3.

Scevola S, Faga A. 2008; Bio-engineered skin for aesthetic reconstruction of the tip of the nose: a case report. Dermatol Surg 34(9): 1283–1287.

Philandrianos C, Andrac-Meyer L, Mordon S, et al. 2012; Comparison of five dermal substitutes in full-thickness skin wound healing in a porcine model. Burns 38(6): 820–829.

Pianigiani E, Andreassi A, Taddeucci P, et al. 1999; A new model for studying differentiation and growth of epithelial cultures on hyaluronan-based carrier. Biomaterials 20(18): 1689–1694.

Pierard GE. 1999; EEMCO guidance to in vivo assessment of tensile functional properties of the skin. Part 1: relevance to the structures and ageing of the skin and subcutaneous tissues. Skin Pharmacol Appl Skin Physiol 12: 352–362.

Presti D, Scott JE. 1994; Hyaluronan-mediated protective effect against cell damage caused by enzymatically produced hydroxyl (OH) radicals is dependent on hyaluronan molecular mass. Cell Biochem Funct 12(4): 281–288.

Sattar A, Rooney P, Kumar S, et al. 1994; Application of angiogenic oligosaccharides of hyaluronan increases blood vessel numbers in rat skin. J Invest Dermatol 103(4): 576–579.

Sin P, Stupka I, Brychta P. 2010; Evaluation and comparison of composite and split-thickness skin grafts using cutometer mpa 580. Ann Burns Fire Disast 23(4): 208–213.

Szymanska E, Nowicki A, Mlosek K, et al. 2000; Skin imaging with high frequency ultrasound – preliminary results. Eur J Ultrasound 12(1): 9–16.

Wang C, Tammi M, Tammi R. 1992; Distribution of hyaluronan and its CD44 receptor in the epithelia of human skin appendages. Histochemistry 98(2): 105–112.

Wang H, Pieper J, Péters F, et al. 2005; Synthetic scaffold morphology controls human dermal connective tissue formation. J Biomed Mater Res A 74(4): 523–532.

Weigert R, Choughri H, Casoli V. 2011; Management of severe hand wounds with Integra dermal regeneration template. J Hand Surg Eur Vol 36(3): 185–193.

Wolfer TP, Noah EM, Pallua N. 2005; The use of Integra in an upper extremity avulsion injury. Br J Plast Surg 58: 416–418.