

Reconstruction of scalp defects with exposed bone using dermis template (Integra®) with or without autologous skin micrografts (Rigenera®) and Flowable wound matrix (Integra®)



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Background: The aim of this study was to investigate the use of autologous micrografts obtained by the Rigenera Micrografting Technology, dermal substitute and flowable wound matrix (Integra®) on injuries involving the scalp with exposed skull. For large scalp wounds with exposed calvarium, drilling of the exposed bone followed by skin grafting is usually not successful, requiring more appropriate options. **Methods:** The authors report their experience over 3 years of the reconstruction of extensive trauma of the head with loss of skin coverage, due to thermal, chemical and electrical burns. **Results:** The use of dermal template provides coverage of the defect, the application of the autologous micrografts suspension allow for faster tissue colonisation of the scaffold and neo-dermis formation, while the flowable wound matrix provide an optimal environment, resulting in a shorter interval until definitive cover by autografting. **Conclusions:** The authors' protocol proved to be a good option, out of 10 consecutive patients, results were considered good or excellent in nine patients and satisfactory in one, in terms of elasticity, pliability, sensation and sweating.

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Injuries involving the scalp with exposure of the skull present a surgical challenge (Shonka et al, 2011; Greenwood et al, 2016). Large defects are difficult to treat, excision of the necrotic outer cortex of the skull down to viable tissue, followed by primary or delayed skin grafting is recommended for most cases (Koenen et al, 2008), however, grafting over bone is often followed by poor graft take. Although rotational skin flap or more complex reconstructive surgery are recommended for better cosmetic results of the surrounding tissue is often not suitable, especially in patients with scalp burns. Due to the rare occurrence and the long and complex treatment required, there is limited literature on this type on injuries (Heimbach et al, 2003; Richardson et al, 2016).

Dermal substitutes have become popular in the reconstruction of exposed calvarium; after removing the outer table of the skull, the

dermal substitute is placed on the resulting wound-bed. The whole process allows the neo-dermis growth and its vascularisation, which can be grafted during a second procedure (Dingman and Argenta, 1982; Wilensky et al, 2005; Andreone and den Hollander, 2019). Several dermal templates have been used in the management of exposed calvarium due to burn and trauma.

The use of grafts to resurface the wound is the most common approach used by surgeon worldwide, however, in the last years was developed a new technology related to the use micron-sized-grafts (micrografts), named Rigenera Micrografting Technology. The objective of this technology is to mechanically disaggregate autologous tissue along with sterile saline generating a micrografts liquid suspension of a calibrated size of 80 microns. This technology was developed after several

Table 1. Distribution of patients in this series.

Case	Age	Sex	Type of injury	Risk factors	CD4 count	Wound size	DM	FM	AM	NPWT
1	2	M	Electrical burns	-	-	3x2cm (6cm ²)	✓	-	-	-
2	3	F	Food burns	-	-	4x6cm (24cm ²)	✓	-	-	-
3	17	F	Trauma	HIV +	334	5x2 cm (10cm ²)	✓	✓	✓	✓
4	43	F	Trauma	HIV +	456	24x6 cm (144cm ²)	✓	✓	✓	✓
5	34	M	Trauma	HIV +	237	19x7 cm (133cm ²)	✓	✓	✓	✓
6	37	M	Flame burns	HIV +	167	27x6 cm (162cm ²)	✓	✓	✓	✓
7	28	M	Chemical burns	HIV +	134	23x5 cm (115cm ²)	✓	✓	✓	✓
8	32	M	Trauma	HIV +	456	9x11cm (99cm ²)	✓	-	✓	-
9	44	F	Chemical burns	HIV +	231	8.5x7cm (59.5cm ²)	✓	-	✓	✓
10	21	M	Flame burns	HIV +	489	9.4x5cm (47cm ²)	✓	-	✓	✓

years of experimental research to create a medical device able to disaggregate the tissue mechanically (not enzymatically) while maintaining a high the tissue viability. The chosen 80 microns cut-off is designed to obtain micrografts positive for different MSCs markers such as CD73, CD90, CD115, CD146 but negative for hematopoietic markers such as CD34 and CD45 (Zanzottera et al, 2014; Purpura et al, 2016; Monti et al, 2017). Additionally, these studies have reported a percentage of cell viability inside the micrografts ranging in 70–90%. Finally, *in vitro* results also showed that when combined with collagen sponges, micrografts were able to form a viable and proliferative bio-complex, confirming their regenerative potential (De Francesco et al, 2017).

An ideal protocol for difficult to treat wounds/burns would require a faster neo-dermis formation to allow the grafting to resurface the lesion as soon as possible. To do so our in our department we have introduced the use of the Rigenera micrografts technology which allow the generation of a suspension on autologous micron-sized grafts (micrografts) at the bed site of the patient, without any manipulation of the tissue. Such micrografts suspension is then partially used to increase the cellularity of the dermal template by imbibition of the scaffold, creating a bio-complex, and for grafting onto the wounds. The management of scalp injuries with exposed bone is challenging. The authors report a series of 10 consecutive patients treated with dermal template or Flowable matrix with or without the use of the micrografts suspension over a 3-year period. This protocol proved to be a good option in the reconstructive ladder for coverage of medium to large defects presented after full-thickness burns of the skull and large degloving injuries with loss of the skin.

Experimental section

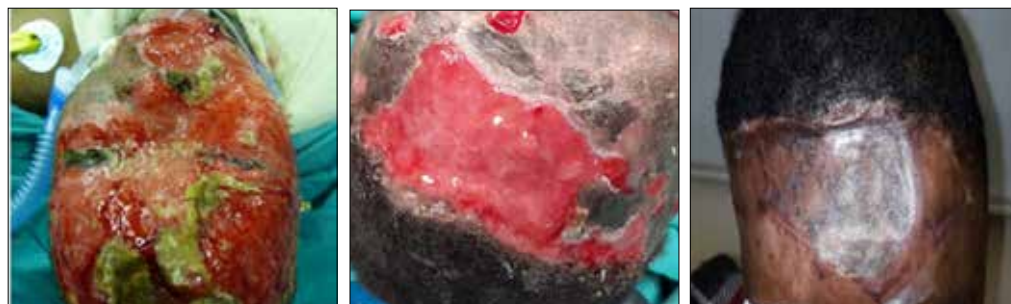
All patients with scalp wounds and exposed calvarium admitted to the Burn and Soft Tissue Injury Unit at Inkosi Albert Luthuli Central Hospital in Durban, South Africa, from January 2017 to January 2020 were included in this series. Three of the included patients were admitted to the Plastic and Reconstructive Surgery and Burns Unit at Robert Mangaliso Sobukwe Hospital in Kimberley, South Africa; those cases were performed during an outreach service provided by one of the authors. Ethical approval for retrospective studies using the Trauma/Burns database was granted by the UKZN-BREC ethics committee (Class Approval BE 207/09). All patients gave written consent to publish their photographs. *Table 1* depicts the summary of the patients reported in this series. After debridement of overlaying cutaneous necrotic tissue resulting from the initial trauma, the wound margins were excised, and the exposed necrotic outer table of the cranium was debrided until cancellous bone was reached. Debridement was performed either with bone driller or with osteotome. Bovine dermal replacement matrix (Integra®, Life Science, USA; Baroque SA) (Xin et al, 2010) was then applied directly to the cancellous, viable bone and also all the resulting wounds.

Among all, eight patients also received autologous micrografts (Rigenera®, Human Brain Wave, Italy) and five received Flowable Wound Matrix (Integra® Life Science, USA; Baroque Medical, SA) as well. The Rigenera Micrografts were applied both on the dermal template and partially injected on the wound margins. Flowable matrix was injected into the wound margins to promote faster healing and to prevent tissue fragmentation with creation of stable neo-dermis wound bed.

Figure 1. Case #2. 3-year-old child Flame burn (A) Day 5 post admission. (B) 6 months and (C) 1 follow-up post-Integra application.



Figure 2. Case #9. 34 years old female chemical assault, resulting in blindness. (A) on presentation to burns unit 12 days after injury. (B) after skin graft of the face 3 weeks after decortication of outer skull table and application of Integra Dermal template and Rigenera Micrografts. (C) result after 1 year.



The suspended micrografts were obtained using the Rigeneracons medical device (Human Brain Wave, Italy). The technique consists in harvesting an autologous, full-thickness skin biopsy from the patient, generally the ratio between the wound size and the autologous sample to collect is 1:80. The tissue collected is inserted in the Rigeneracons device along with 4 ml of sterile saline solution then the device is activated for disaggregation for (time ranging from 2 to 10 minutes) to generate the ready to use micrografts.

Lastly, The dermal templates were covered with Nanocrystalline Silver dressing (Acticoat®, Smith and Nephew) and Negative Pressure Wound Therapy (NPWT) (Renesys EZ®, Smith and Nephew) was applied for seven days, using the continuous mode at -100 mmHg. After vascularisation of the dermal template took place at day 15 on average for the patient that received the Autologous Micrografts, the outer silicone layer was removed and meshed, or un-meshed thin split autograft was placed onto the neodermis.

Results

From a total of 339 patients with burns and soft tissue injuries admitted at our unit during the period of the study, six presented with scalp defects and exposed bone, four more patients were operated during outreach visits to other hospitals [Table 1]. The medium age was 26 years (range 2–44 years); six patients were male, and 4 females. The mechanism of injury was physical trauma in four patients, two chemical and two flame burns, one electrical injury and one food

burn; the two latter injuries were in children. Two patients were admitted to intensive care unit due to inhalation injury. Both required ventilation and for one of them a tracheostomy through burned tissue was necessary. Eight adults were HIV positive with an average CD4 count on admission of 313 (range 134–489). All patients continued receiving antiretroviral during the admission. The medium size of the scalp defect in adults was 96.2 cm² (range 10–162 cm²). The two children were managed only with dermal matrix (DM) only, followed by split thickness skin grafting (STSG) [Figure 1].

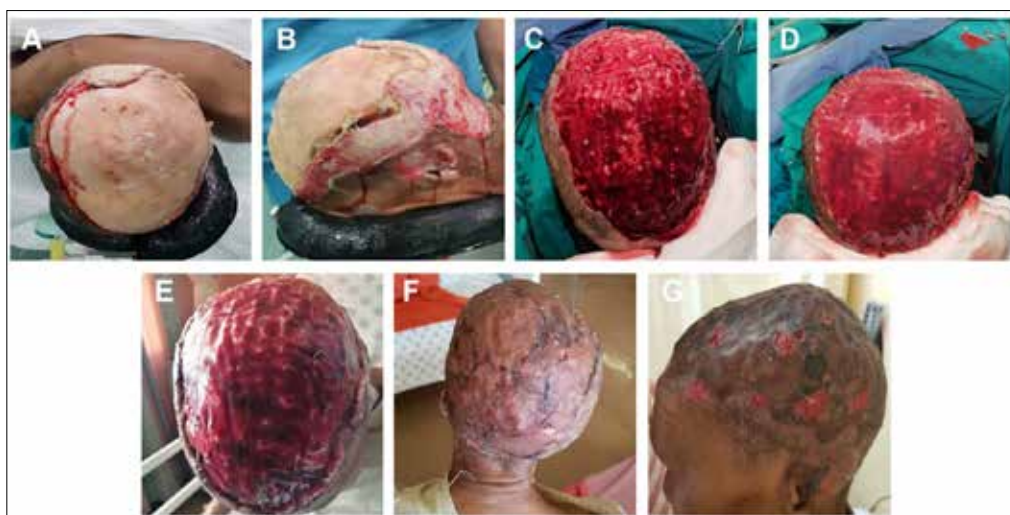
Three patients were managed with DM and Autologous Micrografts (AM) [Figure 2] and five with DM, AM and flowable collagen matrix (FCM) [Figures 3–5]. NPWT was applied on seven adult patients, over the dermal matrix encourages the growth of granulation tissue, reduces seroma and hematoma formation, decreases bacterial load and secures the fixation of the dermal substitute by conforming it to the contours of the wound bed (Andreone and den Hollander, 2019). A pressure exceeding the recommended -100 mmHg may cause though earlier lifting of the silicone layer present on top of the dermal matrix, leaving the immature neo-dermis exposed (Molnar et al, 2004).

In all patients after 14 to 16 days the outer silicone layer could be removed and meshed/un-meshed STSG was placed onto the neo-dermis. Table 2 depicts patients' outcomes. Three patients developed pneumonia postoperative and received 5 days of IV antibiotics, non-related to the wound; one had a hematoma that was drained with a small incision in the silicon layer

Figure 3. Case #5. 34-year-old adult victim of assault, sustaining degloving injury of the scalp. He was initially managed with suture of the degloved skin followed by necrosis of the sutured tissue. (A) after debridement and trepanation of exposed bone, prior to definitive surgery (B) after decortication of outer table of skull (C) after application of Integra® Dermal template/ flowable matrix and Rigenera Micrografts (D) day 10 post skin grating (E-F) three months post grafting.



Figure 4. Case #4. 43-year-old female sustaining degloving injury of the scalp and left arm caused by attack by a dog (A-B) after initial debridement of the vicious attack (C) after decortication of outer table of skull (D) after application of Integra® Dermal Template / Flowable matrix and Rigenera Micrografts (E) maturation of neodermis 2 weeks after initial surgery (F-G) 4 weeks after grafting.



with no loss in the dermal matrix. Time to wound closure from date of first surgery was 28 days (range 23–31 days) and in-hospital stay was 45 days (range 34–67 days).

In all patients the exposed bone was successfully covered with dermal matrix and autografts. Seven of the 10 have been followed for over 12 months. Results were considered good or excellent in nine patients and satisfactory in one, in terms of elasticity, pliability, sensation and sweating (modify Vancouver scale). In comparison with other burn scars treated with conventional methods, itching was significantly less in sites treated with dermal matrix and Autologous Micrografts. Hypertrophic scarring was also reduced in areas grafted with dermal matrix and autologous micrografts in all our patients (Fitzpatrick skin

types 4–6). No graft breakdown was found among the patients with late follow up post-skin graft.

Discussion

Scalp defects with exposed skull are a rare but challenging complication associated with full thickness burns to the head and extensive degloving injuries following a traumatic accident (Yannas and Burke, 1980; Koga et al, 2007). The management of such scalp defects has been the source of controversy, and a few approaches have been advocated (Yeong et al, 2006; Wood et al, 2007; Wain et al, 2010). The first stage involves surgical debridement of the defect and the exposed bone. Exposure of the skull for extended period of time can cause desiccation, osteomyelitis and epidural abscess

Figure 5. Case #7. 28-year-old adult sustaining chemical burns after an assault. (A) on presentation 3 days after the injury (B) Pre-debridement and application of Integra® Dermal Template / Flowable matrix and Rigenera Micrografts (C–D) results after 18 months.

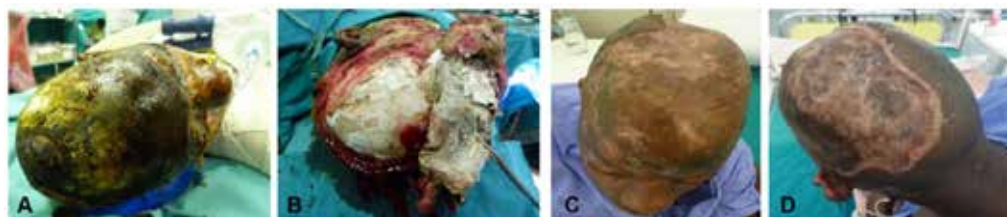


Table 2. Distribution of patients in this series.

Case no.	Length of stay (days)	Wound closure (days)	Outcome	Mortality	Morbidity	Tracheostomy
1	34	26	Good	II	–	–
2	42	29	Good	–	–	–
3	67	23	Good	–	–	–
4	58	27	Satisfactory	–	Pneumonia	–
5	61	30	Good	–	–	–
6	38	28	Good	–	Haematoma formation	–
7	36	31	Good	–	Pneumonia/septicemia	✓
8	40	30	Good	–	Septicemia episode	–
9	36	28	Good	–	–	–
10	41	27	Excellent	–	–	–

formation. To avoid further complications, the outer table of the exposed bone must be removed until viable tissue (cancellous bone) and immediately covered to prevent secondary desiccation, protein and fluid loss and infection (Koenen et al, 2008).

The coverage of the scalp defects after the bone debridement is cosmetically a better option using local flaps, which provide continuity of hair and better contouring of the defect. Particularly in burn patients with scalp burns the surrounding skin is often unsuitable to provide local flaps. Concomitant injuries, such as inhalational burns or lung contusion requiring ventilation may preclude the use of complex flaps such as free flaps in the acute setting when managing such injuries (Greenwood et al, 2016). However, simple drilling of the exposed bone followed by skin grafting is usually not successful, requiring better options to deal with.

The use of dermal template provides durable cover of the defect, especially in patients with concomitant injuries with an expected extended hospital stay. After initial excision of all devitalised and/or infected tissue the silicone layer provides temporary wound closure as an epidermal substitute, while the collagen matrix is populated with fibroblasts and neo-vascularised (Andreone and den Hollander, 2019).

Here, the authors report their 3 years' experience with a novel successful protocol for which we have injected the Autologous Micrografts inside the dermal template create a living bio-complex which results in a better neo-dermis formation until definitive cover by autografting is possible. Moreover, the combination of the dermal matrix with micrografts decreased the formation of hypertrophic scar tissue in this series, lastly the use of the Flowable collagen also 'filled out' any irregularities in the wound, resulting in scar with a better contour.

The Rigenera® technology produce within a few minutes a micrografts suspension from any kind of tissue composed by viable cells surrounded of Extra Cellular-Matrix (ECM), fragments of ECM and Growth Factors, opportunely selected by filtration (Balli et al, 2019). Moreover, with this tissue micrografts suspension it is possible to soak any kind of scaffolds (i.e. collagen, PLGA, bone substitute) creating in few minutes a biomaterial, without impacting on significant levels of postoperative morbidity. To date, the Rigenera® micrografting technology has demonstrated its efficacy in different clinical setting. It is widely used with dermal tissue sample for the treatment of complex wounds such as dehiscences, chronic ulcers and burns (Giaccone et al, 2014; Baglioni et al, 2016; Marcarelli et al, 2016; De Francesco

et al, 2017; Andreone and den Hollander, 2019; Riccio et al, 2019), for dermatological applications such as hypertrophic scars (Svolacchia et al, 2016) and for the management of androgenetic alopecia (Alvarez et al, 2018; Ruiz et al, 2020); with cartilage samples for the management of chondropathy (Dorta Fernandez and Baroni Luengo, 2018; Viganò et al, 2018); with bone samples for the treatment of the osteonecrosis of the femoral head (Marcarelli et al, 2020).

In a more recent study, a clinical record of 15 patients reported a wound reduction of $37.33\% \pm 19.35\%$ after 2 weeks, nine patients were healed after 8 weeks, 13 patients were healed after 16 weeks. The quality of scars was good and did not deteriorate at the six-month follow-up (Miranda et al, 2018). Considering all the above we have selected this technology with the aim of shortening the maturation time from the dermal template (from 3 weeks to 2 weeks) so that can be grafted earlier but also to increase the graft intake, which for the patient here reported was 100%.

Complications in this our protocol include hematoma formation (where was no possible to used NWTP), early detachment of the silicon layer if the NWPT is kept with a pressure higher than -100 mmHg. Integra dermal template is always at risk of infection, but due to the transparency of the silicon layer this is easy to diagnose (Lutz et al, 1998; Lee, 2012). Our complete protocol, with Integra dermal template, Flowable collagen and Rigenera Micrografting technology have many advantages, such as availability of the products off the shelf, all of them are easy to apply and report better cosmetic results (Svolacchia et al, 2016).

Conclusions

Our protocol which required extensive debridement of exposed bone, application of bovine dermal matrix with Autologous Micrografts suspension and flowable collagen, along with negative wound pressure therapy and split thickness skin graft was successfully used for the reconstruction of medium to large scalp injuries with minimal morbidity, shorter hospital stays, durable coverage and great cosmetic results. The outcomes suggest the recommendation of the technique as a safe option for these type of injuries within the reconstructive ladder.

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Conflict of interest

This research received no external funding. However, Dr. A Andreone received financial

assistance to travel and perform the cases in the outreach to other provinces.

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