Use of amniotic membrane for radial forearm free flap donor site coverage: clinical, functional and cosmetic outcomes

Stefan Hunger¹ · Lukas Postl¹ · Raphael Stehrer¹ · Lukas Hingsammer¹ · Stefan Krennmair² · Wolfgang Feistl¹ · Michael Malek¹ · Gerald Krennmair³

Received: 30 June 2017 / Accepted: 30 September 2019 / Published online: 15 November 2019
© The Author(s) 2019

Abstract

Objective To evaluate the clinical, functional and aesthetic outcomes for radial forearm free flap (RFFF) donor sites covered with amniotic membrane (AM).

Material and methods The healing process of patients with RFFF donor sites covered with AM was prospectively followed for 1 year. Additionally at the 12-month evaluation, objective scoring systems were used to assess the aesthetic (Vancouver scar scale, VSS: range 1–13) and functional outcome (skin sensibility, hand/wrist functionality [goniometer], grip strength [score 1 = excellent, 5 = poor]). By using a subjective rating system (score 1 = excellent, 5 = poor), the patient-reported aesthetic and functionality outcome was correlated with objective data analysis.

Results Twenty-one out of 23 patients were followed for 12 months (dropout: 2 patients at 3 months). In 17/23 (73.9%) patients RFFF defect covered with AM showed an uneventful healing period (< 3 months). Prolonged healing periods (> 3 months < 6) for 6 patients (26.1%) were attributed to wound infections (4×), seroma (1×) and inflammation (1×). At the 1-year evaluation, there was a significant ($p < 0.01$) correlation between subjective (2.0 ± 0.71) and objective aesthetic scores (VSS 3.74 ± 2.18), and a successful grip strength (score 1.67 ± 0.86); however, thumb hyposensibility in 76.2% was seen. A high body mass index (BMI) was in conjunction with a negative ($p = 0.012$) and the use of antihypertensive medications provided positive effects ($p = 0.041$) on the aesthetic outcome.

Conclusion RFFF donor site defects covered using AM show excellent clinical, aesthetic and functional outcome representing patient comorbidities (BMI, antihypertensive drugs) might affect the aesthetic outcome.

Clinical relevance In relation to the excellent outcomes found, the use of AM offers an alternative treatment procedure for RFFF defect covering.

Keywords Amniotic membrane · Radial forearm free flap · Aesthetic outcome · Functional outcome

Introduction

The radial forearm flap (RFFF) initially introduced in 1981 by Yang et al. [1] has become a standard free flap

procedure covering defects in head and neck tumour surgery and also represents one of the most commonly used flaps in plastic and reconstructive surgery [2–5]. The beneficial effects of the flap characteristics such as pliability, thinness, mostly hairless skin, large diameter, long and constant vascular pedicle, and low bulk allow repair of complex three-dimensional defects [6–9]. However, despite the excellent results for their primary use in defect reconstruction, radial donor site morbidity continues to represent a particular problem which needs to be further addressed for improving overall clinical patient-related outcome [10, 11].

Although several different skin closure methods for improving the functional and aesthetic outcome of the forearm donor site have been described in detail, the

✉ Stefan Hunger
Stefan.hunger@kepleruniklinikum.at

¹ Medical Faculty, Department of Oral and Maxillofacial Surgery, Johannes Kepler University Linz, Krankenausstraße 9, 4020 Linz, Austria

² Ludwig-Maximilian University (LMU), Munich, Germany

³ Sigmund Freud University Vienna, Head of Department of Prosthodontics, Sigmundfreudplatz 1, 1020 Vienna, Austria

split-thickness skin graft (STSG) continues to be the predominantly used one for covering the radial forearm defect [10, 12, 13]. However, wound healing defects and skin graft loss as well as tendon adhesion resulting in reduced motion and cosmetic discomfort have been reported with the use of split-thickness skin grafts [8, 10, 13]. In addition, radial forearm defects may also be repaired with a full-thickness skin graft (FTSG) and can also be closed primarily resulting in minimal morbidity [14–18]. Alternatively, the defect may be repaired using an acellular human dermal skin matrix, although prolonged healing periods must be anticipated in such cases [19–22].

Several publications discussing soft tissue and wound covering procedures have also described the use of an amniotic membrane associated with high clinical success rates for various indications [23–25]. Thus, separate studies of Rohleder et al. [26] and Kesting et al. [27] have described the clinical use of amniotic membrane for repairing oral defects and/or oroantral communications with excellent outcome. In addition, successful and effective use of amniotic membrane has also been reported as graft material in vestibuloplasty as well as for the treatment of burns [28–30]. A beneficial effect of amniotic membrane may be assumed as it provides for a reservoir of growth factors and cytokines and is known to modulate inflammation and enhance the healing process [31]. In addition, amniotic membrane is also described to show antimicrobial, antifibrotic and anti-scarring properties [31].

When considering coverage of the forearm defect following radial flap elevation, the use of amniotic membrane may represent an alternative tool for the various methods for donor site closure already described. Amniotic membrane shows almost unrestricted availability and permits donor site coverage regardless of wound size dimensions [25, 31, 32]. Moreover, a gliding surface between the underlying structures, e.g. paratenon or nerves, provides for an additional beneficial effect of amniotic membrane by avoiding complications of adhesion [31, 32]. However, although the beneficial properties of the amniotic membrane have been described and demonstrated in several studies in oral and maxillofacial surgery, there is a lack of information regarding its use in covering radial donor sites after radial forearm flap elevation in detail [27, 29].

Thus, the aim of this prospective follow-up study was (1) to evaluate the clinical outcome of forearm flap coverage of the donor site using amniotic membrane and also (2) to evaluate the aesthetic appearance as well as aspects of hand/wrist functionality following of radial forearm flap elevation covered by amniotic membrane from the perspective of patients and clinicians.

Material and methods

Study design/patients

The study was designed as a prospective 12-month follow-up study for 23 patients with RFFF and subsequent forearm defect closure using an amniotic membrane. During the period between January 2011 and March 2012 patient included underwent primary tumour resection combined with unilateral or bilateral neck dissection and oral defect reconstruction using RFFF. Exclusion criteria comprised preoperative disabilities at the donor site extremity, minor age and patients with coagulation disorders. The charts of patients at the Department of Oral & Maxillofacial Surgery at the University Hospital of Linz, Austria, having undergone radial forearm free flap (RFFF) elevation were additionally reviewed for demographic patient data. Clinical details including age, gender, body mass index (BMI), tumour characteristics, flap size elevated, and presence of systemic comorbidities (diabetes, nicotine abuse, toxic nutritional disease or cardiovascular disease with concomitant antihypertensive therapy) can be found in Table 1.

Each patient included was given a detailed description of the procedure and signed an informed consent document prior to participation in the follow-up program. The anonymity of the patients' data was ensured by the use of decipherable

Table 1 Patients' ($n = 23$; results from the first 3 months) characteristics and comorbidities subjected to RFFF with several indications

Age: 56.0 ± 14.9 years (18.0–83.0)
Male/female: 19 (80.9%)/4 (19.1%)
Body mass index (BMI): 23.5 ± 3.4 (18.2–30.4)
Smoker: 14
CVD: 5
Diabetes mellitus (type II): 2
Alcohol abuse: 12
Hypothyroidism: 2
Immunotherapy: 1
RFFF indications:
SSC: 18
ME-Ca: 2
Melanoma: 1
BCC: 1
BRONJ: 1
RFFF-dimension:
Length (cm): 5.74 ± 0.98 (2.5–5.0)
Width (cm): 3.76 ± 0.64 (3.5–7.0)

CVD, cardiovascular disease (including hypertension: antihypertensive medication [diuretica, ca-antagonist; ACE inhibitors]); RFFF, radial free forearm flap; SSC, squamous cell carcinoma; ME-Ca, mucoepidermoid carcinoma; BCC, basal cell carcinoma; BRONJ, bisphosphonate-related osteonecrosis of the jaw

patient identification numbers. The study protocol had been approved by the local ethics committee (approval number 1045), and the study was self-funded by the authors and their institution.

Surgical technique

Radial forearm free flap elevation

Patients underwent RFFF elevation in a subfascial plane preferably from the non-dominant arm. The Allen test was performed preoperatively and the flap was outlined according to the size of the defect. Dissection was started from the ulnar side and deepened towards the radial artery. Special attention was given to leaving the paratenon of the flexor tendons intact. The donor site was then partly downsized by primary closure of the defect and on the residual defect site wound dressing was done with Melolin/Solvaline (Smith and Nephew; Bristol UK/Lohmann and Rauscher). The downsized wound was measured (width/length: cm) and dressing was changed daily until tendons were covered by granulation tissue. (Fig. 1).

Donor site coverage—processing of amniotic membrane

In an average of 31 days after radial forearm flap elevation, the granulation tissue was covered with amniotic membrane in a second surgical procedure (Fig. 2). Covering tissue was fixed with stitches and a tie-over dressing was applied. Change of the wound dressing was done for 3–4 weeks until complete healing had been achieved. Complete healing was assessed by subjective evaluation and defined as restauration of sustained functional and anatomic continuity similarly described in previous studies [33, 34].

The amniotic membrane had been obtained from healthy mothers who had provided their informed consent for further use. The AM was collected from the placenta in the course of a Caesarean section. Processing was done at the Linz Blood Bank. In compliance with the Austrian Tissue Safety Act, Ringer's solution at a temperature of 4 °C and BASE 128 (Fa. Alchimia). At the laboratory, blunt separation of the amniotic membrane from the placenta was done. The

Fig. 1 Granulation tissue before covering with amniotic membrane

Fig. 2 Covering the granulation tissue with amniotic membrane

seronegative AM was placed on a carrier (Sugi) and was available at different sizes (e.g. 3 × 3 cm, 3 × 2 cm and 8 × 8 cm). A cryomedium (RPMI, 10% human albumin; 10% DMSO) was used for preservation at a temperature of −80 °C. Prior to application the membrane was thawed in a water bath at 37 °C and then rinsed using isotonic saline.

Clinical analysis

Patients included were enrolled in a regular prospective follow-up program with monthly recalls during the first 3 months and scheduled appointments for the 6-, 9- and 12-month postoperative follow-up. Additional recall visits were scheduled, if required for the follow-up of clinical problems encountered by the patients included.

At all follow-up visits, the primary outcome measurement comprised evaluation of the clinical outcome for the forearm defect covered with AM. At the final evaluation (12-month follow-up), the secondary outcome measurements included assessment of functional and aesthetic results.

Outcome measures

The primary outcome measures during the first year of evaluation assessed the clinical outcome of the defect closure process and included the following:

- Total healing time (days) for achieving complete AM integration
- Distribution of different duration of healing time: < 3 months; > 3–< 6 months; > 6 months
- Prevalence of wound infection/dehiscence, graft rejection, demarcation, tendon exposure

The secondary outcome measures at the 12-month follow-up included assessments of the subjective patient-related and the objective clinician-based aesthetic and functional outcome parameters. The objective clinician-based outcome measures included evaluation of the aesthetic outcome using the Vancouver scar scale (VSS), assessment of hand/wrist sensibility and functionality as well as determination of the grip

strength [35, 36]. The subjective patient-based outcomes evaluated the subjective aesthetic and functional outcome using appropriate questionnaires. In addition, patient-related risk factors such as age, gender, body mass index, smoking status, diabetes mellitus, alcohol consumption, antihypertensive therapy (AHT), immunotherapy, and hypothyroidism were assessed in a multivariate regression model for affecting the aesthetic outcome (VSS).

Clinician-based aesthetic and functional outcome

(1) For the clinician-based aesthetic evaluations, scar scale quality was determined using the modified Vancouver scar scale (VSS). This version of the modified VSS assesses the four different subgroups of pigmentation, vascularity, pliability, and height as described in previous studies [35, 36]. All scars were photographically documented, and the VSS rating of the defect area was provided by 2 independent, blinded assessors (H.S.; K.G.) not involved in the surgical procedure. The subgroups rated comprise the following items:

- Vascularity was rated as normal (score 0), pink (score 1), red (score 2) or purple (score 3).
- Pigmentation was rated normal, hypo- or hyperpigmentation (scores 0, 1, 2).
- Scar pliability was measured using the pinch test for a skin fold located 1 cm from the lateral and 1 cm from the medial border being lifted and its height measured. The value obtained was divided by two. A value of 5 mm or greater represented good tissue laxity including the ability to separate the skin from underlying structures and border and measuring its height. A value of > 7 mm or greater represented good tissue laxity including the ability to separate the skin from underlying structures (score 1); a tissue laxity of > 5–7 mm height was rated as good (score 2), a height of > 3–5 mm was rated as moderate tissue laxity (score 3), > 1–3 mm was rated as acceptable (score 4), and 0 mm as non-acceptable (score 5)
- Scar height was defined as flat-normal = score 0; < 2 mm = score 1; > 2 mm to < 5 mm = score 2; or > 5 mm = score 3.

The scores in the modified VSS for the defect area rated ranged from 1 to 13. The lowest possible score on this scale indicating the best scar quality, i.e. the quality of healthy skin, is 1; the highest score is 13 (worst rating). All VSS data evaluated at the 12-month follow-up by the two observers were compared for interobserver variability. Each sub-score of the VSS scale was analysed individually and were summarised to a total score.

- (2) Sensibility was determined by two-point discrimination (2PD) at the center of the scar [33, 35]. A 2PD of 30 mm or less was rated as positive (score 0) and the sensibility determined to be intact. 2PD > 30 mm was rated as negative (score 1).
- (3) Active range of motion (AROM) of the wrist with respect to palmar and dorsal extension/flexion was determined, and the non-operated side was used as a control. Active range of motion was measured with a goniometer, and when a difference of more than 10° was noticed between operated side and control, this was classified as pathologic [35, 37]. The different ranges of motion measurements were as follows:

Flexion/extension: The forearm was placed on a flat underlay with free mobility of the wrist. One wing of the goniometer was placed to the ulnar the other was placed to the metacarpus.

Pronation/supination: The forearm was placed on a flat underlay with 90° in the cubital joint and 0° in the wrist. Wrist, cubital joint and shoulder joint are positioned on one straight line. For supination, the goniometer was placed just medial to the ulnar styloid process. For pronation the goniometer was placed just lateral to the radial styloid process.

- (4) Grip strength of hand/thumb was assessed by having the patient squeeze the examiner's hands while applying the maximum possible force. Pinch or grip strength of the healthy collateral side was defined as possible maximum power to be achieved. Results were measured by establishing a scale from 0 to 5 according to the Medical Research Council (modified) scale for muscle strength in which the patient's effort is classified as follows: score 1 (normal muscle contraction against full resistance); score 2 (reduced muscle strength against resistance); score 3 (hardly any resistance detected against observer's contraction); score 4 (fasciculation observed in the muscle); score 5 (no movement observed).
- (5) Sensibility of the thumb was assessed by two-point discrimination (2PD) at the dorsal part of the thumb. A 2PD of 30 mm or less was rated as normal aesthesia. 2PD > 30 mm was rated as hypaesthesia.

Patient-based aesthetic/functional outcome—patient questionnaires

Using a patient questionnaire with a visual analogue scale (VAS), the donor site was assessed with regard to patient's

satisfaction with the aesthetic outcome and with their wrist/hand functionality [35, 38].

- The aesthetic and functional result was rated by the patients at the time of the evaluation on a scale ranging from one to five based on following categories: 1, very satisfied; 2, rather satisfied; 3, neither satisfied nor dissatisfied; 4, somewhat dissatisfied; 5, very dissatisfied.
- Functionality of the hand, i.e. movement, was assessed using a rating score ranging from 1 to 5 (1 = excellent, 2 = good; 3 = average, 4 = reduced, 5 = severely impaired).

Statistical analysis

The parameters were recorded in descriptive statistical manner, tabulated and evaluated. The relationship between metric variables was estimated by the Bravais-Pearson correlation coefficient and by the Spearman correlation coefficient, respectively, in case of ordinal or non-normal-distributed metric variables (verification with the Kolmogorov-Smirnov test with Lilliefors correction). As test for the correlation coefficient against the reference value zero (“no correlation”), a test based on the *t* test distribution was used. A multivariate linear regression model was used to estimate factors affecting VSS scoring. The inter-rater reliability between the two raters (H.S., K.G.) was assessed with the intraclass correlation coefficient (ICC). The ICC showed an excellent agreement between the two raters (ICC = 0.977). The sub-scores of the VSS (vascularity: Cohen’s Kappa = 0.609; pigmentation: Cohen’s Kappa = 0.568; pliability: Cohen’s Kappa = 0.516; height: Cohen’s Kappa = 1.000) showed also a perfect agreement (Cohen’s Kappa 0.516–1.000). For the comparison of the active range of wrist/hand motion between the operated and non-operated site, the paired *t* test in case of normality (verification with the Kolmogorov-Smirnov test with Lilliefors correction) and the exact Wilcoxon-test in case of non-normality, respectively, was used. $p < 0.05$ was taken as the statistical significance level and represented with “*”. No adjustment for the type I error was made. For evaluating risk factors affecting aesthetic outcome, a multivariate regression model was used.

For statistical analysis, the statistical computing software R Version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org>) was used. Graphical presentation of the data was also performed in the same software (R Version 3.3.2) like the statistical analysis. Continuous data is presented as mean and standard deviation (mean $\pm$ standard deviation).

Results

Clinical findings—dropouts

Twenty-three patients could be followed for 6 months postoperatively, and 21 (91.3%) patients had continuous follow-up for 12 months. Two patients were lost to follow-up during the period of 6 to 12 months due to death and patient immobility (12-month dropout rate, 8.7%).

For all patients included ($n = 23$) after amniotic membrane application, the radial donor-site defect ranging from 5 cm (width) to 7 cm (length) healed without problems in 17 (73.9%) patients (Table 2) within 3 months. During the healing period, epithelial migration was noticed over the amniotic membrane graft from the adjacent normal skin towards the center of the donor site defect (Figs. 3 and 4). For all patients included, complete defect healing was noted after 62.5 ± 9.3 days. Donor site complications including graft rejection, graft failure, tendon exposure or surgical revision were not seen in any of the patients. At the 12-month evaluation, the covered defects for the patients followed ($n = 21$) showed a healing outcome without signs of complication (Fig. 5).

However, six patients (26.1%) reported an extended wound therapy over a time period of 8 weeks including regular (weekly) presentation at the clinic (Table 2). Four (17.4%) of these six patients showed a putrid infection at the donor site and were treated with local antiseptics and oral antibiotics (amoxicillin/clavulanic acid 2 g/day for 7 days). One patient (4.3%) developed a seroma (Fig. 6), which was punctured, and one (4.3%) patient showed moderate inflammation at the wound edges. Defect closure could be finally achieved by conservative treatment. No graft loss was noted.

Aesthetic/functional outcome (clinician- and patient-based)

Table 3 shows the subjective aesthetic and functional findings evaluated at the 12-month evaluation in detail. There was an excellent inter-rater reliability (ICC 0.977) allowing to summarise the data of both observers with one overall number [33,

Table 2 Clinical outcome of RFFF defect covered using amniotic membrane

RFFF—covered with amniotic membrane: $n = 23$	
Healing uneventful	17 (74%)
Prolonged healing	6 (26%)
Infection: 4 (17.4%)	
Seroma: 1 (4.3%)	
Border Inflammation: 1 (4.3%)	
Duration of healing:	62.5 ± 9.3 days

Fig. 3 Epithelial migration over the amniotic membrane graft from the normal skin towards the center of the donor site defect—3 weeks after application

34]. For patients followed ($n = 21$), the modified Vancouver scar scale (VSS) evaluated at the 12-month follow-up showed scores of 3.74 ± 2.18 for the defect area. Figure 7 shows the overall as well as detailed data for the 4 subgroups evaluated for VSS.

Considering the individual criteria, scar pliability of the tissue showed the least favourable results (area 1.65 ± 0.64 , [range 1–3]. Next to this, scar vascularity showed average results of 1.13 ± 0.86 (range 0–3) for VSS on defect area. However, all scars were of flat height (VSS: mean 0.29 ± 0.56 ; range 0–2) being in line with the absence of any hypertrophic scarring and keloid formation (Fig. 7).

The covered radial defect did not show any signs of sensibility within a two-point discrimination test for a distance of < 30 mm evaluated.

At the 12-month evaluation, hand/wrist functionality of any of the patients evaluated showed a limitation in excess of 10° compared with the healthy (non-operated) control side, and patients did not complain about any problems using the respective hand in daily activities. Differences between pre-operative and postoperative values for palmar, dorsal flexion, as well as for pro- and supination were within the 10° range. Table 3 shows the detailed differences between pre- and post-operative goniometer measurements for palmar and dorsal flexion as well as for pronation and supination for the operated and non-operated arm at the 12-month follow-up.

Fig. 4 Epithelial migration over the amniotic membrane graft from the normal skin towards the center of the donor site defect—6 weeks after application

Fig. 5 Final evaluation (12-month follow-up) including secondary outcome measurements of functional and aesthetic results

Grip strength measured on the operated arm reached the maximum value of 1.67 ± 0.86 (range 1.0–4.0) in all patients and showed no limitations as compared with the non-operated side. Measurement (2PD) of thumb sensibility showing significantly more hypaesthesia (16/21 [76.2%]) than normaesthesia (5/21[23.8%]) was noted for the thumb (Table 3).

Patients' subjective evaluation of the aesthetic and functional outcome as assessed by patients' self-estimation at the 12-month follow up can be seen in Table 4. There were significant correlations between the objective clinician-assessed VSS (Spearman rank correlation 0.446; $p = 0.043$) and the patients' subjective aesthetic score (Fig. 8).

Results of the multivariate analysis evaluating factors affecting VSS scoring are shown in Table 5. There was a positive correlation between body mass index (BMI) and the VSS values evaluated (beta 0.946; $p = 0.012$) representing a deterioration of the aesthetic outcome (higher VSS values) in obese patients. However, patient age, gender, smoking habits, diabetes, and consumption of toxic nutrients did not affect VSS in the region. In contrast, for patients with cardiovascular disease using an antihypertensive therapy, the VSS obtained showed a significantly inverse correlation (beta -0.710 ; $p = 0.041$) and better clinical findings (reduced VSS values).

Fig. 6 Extended wound therapy by a patient with a seroma under the amniotic membrane

Table 3 Clinician objective 12-month evaluation of functional and aesthetic outcome of radial forearm donor site covered using amniotic membrane ($n = 21$ patients)

Donor site:				
VSS (modified)	3.74 ± 2.18	(0.75–7.75)		
Scar Sensibility: (2-point):				
< 30 mm:	0			
> 30 mm:	23 (100%)			
Hand/wrist functionality				
Goniometer: (postoperative–preoperative)				
Palmar flexion	Dorsal flexion	Pronation	Supination	
Operated site:	– 3.6° ± 5.5°	– 3.0° ± 3.7°	– 3.5° ± 4.5°	4.3° ± 5.2°
Non-Operated site:	– 0.4° ± 1.7°	– 0.8° ± 4.0°	0.5° ± 2.3°	– 0.3° ± 1.8
Force (grip):	Range: 1–5	Mean: 1.67 ± 0.86	(1.0–4.0)	
1	11	(52.4%)		
2	7	(33.3%)		
3	2	(9.52%)		
4	1	(4.8%)		
5	0			
Sensibility (thumb)				
Hypaesthesia:	16 (76.2%)			
Normaesthesia:	5 (23.8%)			

Discussion

The radial forearm free flap (RFFF) represents a reliable flap for plastic and reconstructive surgery as well as for soft tissue reconstruction following ablative maxillofacial tumour resection [1–3]. Although the primary postoperative concerns focus on reconstruction site repair, the associated forearm tissue defect has gained intensive interest after RFFF harvesting [4–6, 8, 10]. When considering coverage of the radial forearm defect, several attempts have been made to minimize functional and aesthetic morbidity and the complication rate and, consequently, optimize clinical outcome [8, 10, 13, 14]. Thus, direct closure, local flaps and different skin grafts (split or full thickness) as well as allogenic grafts have been described for defect closure with varying success rates including minor and major disadvantages and drawbacks [8, 10, 11, 13, 16, 18].

In addition to the currently recognized methods, the present study reports on the clinical, aesthetic and functional outcomes when using an amniotic membrane for covering the radial donor defect. Although the successful use of amniotic

membrane, i.e. for vestibuloplasty or for covering oral defects, has been as described in separate studies by Kesting et al. [27] and Keerthi et al. [29], detailed reports of the use of amniotic membrane for radial forearm defect closure are still lacking in the scientific literature.

Considering the clinical results for tissue regeneration after amniotic membrane application seen for the present study, this effect may be explained as a result of the immunological characteristics of the amniotic membrane [31, 38–43]. Stromal and epithelial amnion regions serve as a reservoir of epidermal and fibroblast growth factors and cytokines modulating inflammation and enhancing the healing process [38, 43]. According to Reilly et al. [41], the amniotic membrane also shows antimicrobial, antifibrotic and antiscarring properties for ensuring a successful tissue regeneration and wound healing process [31, 41]. AM has been reported to be a promoter of epithelialisation and represents a non-tumourigenic tissue without ethical problems or concerns for its use [43]. In the present study, no graft rejection and inflammation-free healing was seen, which may be attributed to the fact that the amniotic

Fig. 7 Box-whisker plot showing examiners rating due to the different VSS subgroups

Table 4 Patients' ($n = 21$) subjective 12-month evaluation of functional and aesthetic outcome of donor site covered with amniotic membrane

Aesthetic evaluation (score 1–5):	
Mean: 2.0 ± 0.71 (range: 1.0–3.0)	
Score 1:	5 (23.8%)
Score 2:	11 (52.4%)
Score 3:	5 (23.8%)
Score 4:	0
Score 5:	0
Functionality (score 1–5):	
Mean: 1.71 ± 0.85 (range: 1.0–4.0)	
Score 1:	10 (47.6%)
Score 2:	8 (38.1%)
Score 3:	2 (9.5%)
Score 4:	1 (4.8%)
Score 5:	0

membrane also includes antigenic structures and immune modulators like HLA-G und Fas ligands reflecting no immunological reaction of the host [28, 39, 42, 43].

The application of amniotic membrane on a wound bed prevents desiccation and excessive fluid loss and also provides for an analgesic effect by protecting exposed nerve endings from the environment [44]. Additionally, amniotic membrane also disposes of antiphlogistic properties and has been shown to produce human-beta-3-defensin [45]. These peptides are implicated in the resistance of epithelial surfaces to microbial colonization and have been shown to be upregulated in the inflamed amnion [46]. In obvious contrast, downregulation of certain tissue growth factors by mesenchymal hyaluronic acid leads to reduced cicatrization which may explain the beneficial effect of amnion on scar formation and

may be the reason why foetal wound healing is essentially scarless [32, 39].

However, in contrast to data reported on defect epithelialisation with the use of (full or partial) skin grafts as coverage materials, the present findings for the use of AM showed a significantly longer healing period for achieving full defect epithelialisation [25, 47]. This prolonged healing time is consistent with the solitary use of allografts (AlloDerm) as reported by Ho et al. [12] and Wax et al. [22] also demonstrating a time-dependent influence on defect epithelialisation with the beginning of migration from the graft borders to the central area [12, 22].

Interestingly, in the present study a statistically significant correlation between the objective clinician-based aesthetic VSS and the subjective patient-based aesthetic scoring variables could be found. Although the subjectively evaluated VSS scores were always within the range of rating of previous findings [35], generally, more beneficial values were seen in detail for all subgroups evaluated for the use of AM in the present study. The significant correlation found between subjective and objective variables evaluated is in contrast to previous findings of de Witt et al. [48] and Wirthmann et al. [35] in separate studies. The findings of the studies of de Witt et al. [48] and Wirthmann et al. [35] show a higher dissatisfaction score for female than for male patients regardless of the type of defect coverage used. This gender effect on the aesthetic outcomes evaluated could not be confirmed in the present study which showed no differences between males and females for the VSS results evaluated. The predominantly male population of the study cohort and the high satisfaction scoring of male patients may be attributed to the high subjective and objective success and satisfaction scoring and consequently also for the significant correlation found.

The overall high success and satisfaction score may preferably be attributed to the complication-free clinical wound healing process and to achievement of unrestricted functionality and aesthetic parameters such as vascularity and pigmentation with high evaluation score rating [45, 46]. As regards functionality, the present study showed favourable functional outcomes with no limitations in grip strength or wrist movement at the donor site. These findings may be attributed to both the suprafascial dissection technique used for flap harvesting and the tissue properties of the amniotic membrane coverage used [42, 49]. The beneficial effects of using the suprafascial dissection technique as compared with the infra fascial one with regard to tendon adhesion and sensory nerve damage at the donor site had already been reported for the study of Kamal et al. [49]. In addition, the amniotic membrane coverage used provides for a beneficial effect creating a gliding surface between the underlying structures, e.g. paratenon or nerves, with no resultant adhesion between tendons and covering tissue. The beneficial effect of the amniotic membrane epithelium is also responsible

Fig. 8 Correlations between the objective clinical-assessed VSS and the patients' subjective aesthetic score

Table 5 Patients' risk factors affecting aesthetic outcome of radial defect covered with amniotic membrane

	Unstandardized coefficients <i>B</i>	Standardized coefficients			
		Std. Error	Beta	<i>t</i>	<i>p</i>
Constant	−6.424	−6.424	4.243	1.514	0.161
Age	−0.019	0.038	−0.128	−0.509	0.622
Gender	−0.499	1.375	−0.090	−0.363	0.724
<i>BMI</i>	0.618	0.201	0.946	3.076	0.012*
Smoker	−3.157	1.642	−0.718	−1.923	0.083
Alc.	−1.422	1.738	−0.317	−0.818	0.432
DM	−1.717	1.461	−0.276	−1.175	0.267
<i>AHT</i>	−3.627	1.547	−0.710	−2.344	0.041*
Immun-TH	−2.720	2.605	−0.266	−1.044	0.321
Hypothyroidism	3.284	2.036	0.321	1.613	0.138

p < 0.05 was taken as the statistical significance level and represented with “*”

for other positive results seen with none of the patients included reporting tendon adhesions, nerve irritations, pain, or describing a significant loss in the range of motion of the wrist and hand [42, 43, 49, 50].

Interestingly, evaluation of the factors affecting the objective aesthetic outcome revealed a significant impact of the body mass index and of the use of antihypertensive medications in patients with cardiovascular disease. The influence of body mass index is consistent with previous findings of Butzelaar et al. [51] and Kim et al. [52] reporting a negative effect on the wound and scar healing process in obese patients [51, 52]. This may be explained by the fact that overweight is associated with a prolonged inflammatory phase during wound healing [53]. Prolongation of the inflammatory phase in overweight patients could cause prolonged secretion of proinflammatory and profibrotic cytokines allowing for more time and providing additional stimuli for depositing collagen into the wound bed.

In addition, the use of antihypertensive agents as ACE inhibitors and angiotensin II inhibitors has already been reported to reduce hypertrophic scar formation. According to Roderger et al. [54, 55], angiotensin II exerts proinflammatory effects by increasing influx of leucocytes into the wound and is also considered a profibrotic agent, since it stimulates collagen synthesis, angiogenesis and keratocyte proliferation. Therefore, the use of antihypertensive medication such as ACE inhibitors—as in the present study—might be attributed a beneficial effect on the aesthetic outcome measured.

In conclusion and for clinical relevance—within the limitations of the present study such as the small sample size and the need for using second-stage surgery—the data reported clearly show that defect coverage of the donor site after RFFF elevation can be successfully achieved with the use of amniotic membrane providing for stable and satisfactory long-term aesthetic and functional results.

Funding information Open access funding provided by Johannes Kepler University Linz. The work was supported by the Department of Oral & Maxillofacial Surgery, Med Campus III, Kepleruniversity Linz, Austria.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

1. Yang G, Chen B, Gao Y (1981) Forearm free skin flap transplantation. Natl Med J China
2. Yang G, Chen P, Gao Y, Liu X, Li J, Jiang S, He S, Boo-Chai K (1997) Forearm free skin flap transplantation: a report of 56 cases. Br J Plast Surg 50:162–165
3. Evans GR, Schusterman MA, Kroll SS, Miller MJ, Reece GP, Robb GL, Ainslie N (1994) The radial forearm free flap for head and neck reconstruction: a review. Am J Surg 168:446–450
4. Gurtner GC, Evans GR (2000) Advances in head and neck reconstruction. Plast Reconstr Surg 106:672–682
5. Soutar D, Tanner N (1984) The radial forearm flap in the management of soft tissue injuries of the hand. Br J Plast Surg 37:18–26

6. Soutar DS, McGregor I (1986) The radial forearm flap in intraoral reconstruction: the experience of 60 consecutive cases. *Plast Reconstr Surg* 78:1–8
7. Song R, Gao Y, Song Y, Yu Y, Song Y (1982) The forearm flap. *Clin Plat Surg* 9:6
8. Karimi A, Mahy P, Reyckler H (2007) Closure of radial forearm free flap donor site defect with a local meshed full-thickness skin graft: a retrospective study of an original technique. *J Craniomaxillofac Surg* 35:369–373
9. Avery C (2010) Review of the radial free flap: is it still evolving, or is it facing extinction? Part one: soft-tissue radial flap. *Br J Oral and Maxillofac Surg* 48:245–252
10. Richardson D, Fisher SE, Vaughan DE, Brown JS (1997) Radial forearm flap donor-site complications and morbidity: a prospective study. *Plast Reconstr Surg* 99:109–115
11. Lutz BS, Wei F, Chang SC, Yang K, Chen I (1999) Donor site morbidity after suprafascial elevation of the radial forearm flap: a prospective study in 95 consecutive cases. *Plast Reconstr Surg* 103: 132–137
12. Ho T, Couch M, Carson K, Schimberg A, Manley K, Byrne PJ (2006) Radial forearm free flap donor site outcomes comparison by closure methods. *Otolaryngology Head Neck Surg* 134:309–315
13. Sidebottom AJ, Stevens L, Moore M, Magennis P, Devine JC, Brown JS, Vaughan ED (2000) Repair of the radial free flap donor site with full or partial thickness skin grafts. A prospective randomised controlled trial. *Int J Oral Maxillofac Surg* 29:194–197
14. Zuidam JM, Coert JH, Hofer SO (2005) Closure of the donor site of the free radial forearm flap: a comparison of full-thickness graft and split-thickness skin graft. *Ann Plast Surg* 55:612–616
15. Loeffelbein DJ, Al-Benna S, Steinsträßer L, Satanovskij RM, Rohleder NH, Mücke T, Wolff K, Kesting MR (2012) Reduction of donor site morbidity of free radial forearm flaps: what level of evidence is available. *Eplasty* 12
16. Avery C, Iqbal M, Orr R, Hayter J (2005) Repair of radial free flap donor site by full-thickness skin graft from inner arm. *Br J Oral Maxillofac Surg* 43:161–165
17. Giordano L, Bondi S, Ferrario F, Fabiano B, Bussi M (2012) Radial forearm free flap surgery: a modified skin-closure technique improving donor-site aesthetic appearance. *Acta Otorhinolaryngol Ital* 32:158–163
18. Shiba K, Iida Y, Numata T (2003) Ipsilateral full-thickness forearm skin graft for covering the radial forearm flap donor site. *Laryngoscope* 113:1043–1046
19. Andrews BT, Smith RB, Chang KE, Scharpf J, Goldstein DP, Funk GF (2006) Management of the radial forearm free flap donor site with the vacuum-assisted closure (VAC) system. *Laryngoscope* 116:1918–1922
20. Murray RC, Gordin EA, Saigal K, Leventhal D, Krein H, Heffelfinger RN (2011) Reconstruction of the radial forearm free flap donor site using integra artificial dermis. *Microsurgery* 31: 104–108
21. Sinha UK, Shih C, Chang K, Rice DH (2002) Use of AlloDerm for coverage of radial forearm free flap donor site. *Laryngoscope* 112: 230–234
22. Wax MK, Winslow CP, Andersen PE (2002) Use of allogenic dermis for radial forearm free flap donor site coverage. *J Otolaryngol* 31
23. Zelen CM, Serena TE, Fetterolf DE (2014) Dehydrated human amnion/chorion membrane allografts in patients with chronic diabetic foot ulcers: a long-term follow-up study. *Wound Medicine* 4: 1–4
24. Garwood CS, Steinberg JS (2016) What's new in wound treatment: a critical appraisal. *Diabetes Metab Res* 32:268–274
25. Lullove EJ (2017) Use of a dehydrated amniotic membrane allograft in the treatment of lower extremity wounds: a retrospective cohort study. *Wounds*
26. Rohleder NH, Loeffelbein DJ, Feistl W, Eddicks M, Wolff KD, Gulati A, Steintraesser L, Kesting MR (2013) Repair of oronasal fistulae by interposition of multilayered amniotic membrane allograft. *Plast Reconstr Surg* 132:172–181
27. Kesting MR, Wolff K, Nobis CP, Rohleder NH (2014) Amniotic membrane in oral and maxillofacial surgery. *Oral Maxillofac Surg* 18:153–164
28. Sharma Y, Maria A, Kaur P (2011) Effectiveness of human amnion as a graft material in lower anterior ridge vestibuloplasty: a clinical study. *J Maxillofac Oral Surg* 10:283–287
29. Keerthi R, Vaibhav N, Raut R (2015) Amniotic membrane as a biological scaffold after vestibuloplasty. *J Maxillofac Oral Surg* 14:383–387
30. Kesting MR, Wolff KD, Hohlweg-Majert B, Steintraesser L (2008) The role of allogenic amniotic membrane in burn treatment. *J Burn Care Res* 29:907–916
31. Niknejad H, Peirovi H, Jorjani M, Ahmadiani A, Ghanavi J, Seifalian AM (2008) Properties of the amniotic membrane for potential use in tissue engineering. *Eur Cells Mater* 15:88–99
32. Fairbairn N, Randolph M, Redmond R (2014) The clinical applications of human amnion in plastic surgery. *J Plast Reconstr Aesthet Surg* 67:662–675
33. Lazarus GS, Cooper DM, Knighton DR, Margolis DJ, Percoraro RE, Rodeheaver G, Robson MC (1994) Definitions and guidelines for assessment of wounds and evaluation of healing. *Wound Repair Regen* 2:165–170
34. Takeo M, Lee W, Ito M (2015) Wound healing and skin regeneration. *Cold Spring Harb Perspect Med* 5:a023267
35. Wirthmann A, Finke JC, Giovanoli P, Lindenblatt N (2014) Long-term follow-up of donor site morbidity after defect coverage with Integra following radial forearm flap elevation. *Eur J Plast Surg* 37: 159–166
36. Finlay V, Burrows S, Kendell R, Berghuber A, Chong V, Tan J, Edgar DW, Wood F (2016) Modified Vancouver scar scale score is linked with quality of life after burn. *Burns*
37. Lee J, Alrashdan MS, Kim S, Rim J, Jabaiti S, Kim M, Kim S (2011) Functional and esthetic assessment of radial forearm flap donor site repaired with split thickness skin graft. *Eur Arch Otorhinolaryngol* 268:109–115
38. Riecke B, Kohlmeier C, Assaf AT, Wikner J, Drabik A, Catalá-Lehnen P, Heiland M, Rendenbach C (2016) Prospective biomechanical evaluation of donor site morbidity after radial forearm free flap. *Br J Oral Maxillofac Surg* 54:181–186
39. Koizumi N, Inatomi T, Sotozono C, Fullwood NJ, Quantock AJ, Kinoshita S (2000) Growth factor mRNA and protein in preserved human amniotic membrane. *Curr Eye Res* 20:173–177
40. Shimazaki J, Yang H, Tsubota K (1997) Amniotic membrane transplantation for ocular surface reconstruction in patients with chemical and thermal burns. *Ophthalmology* 104:2068–2076
41. Reilly DA, Hickey S, Glat P, Lineaweaver WC, Goverman J (2017) Clinical experience: using dehydrated human amnion/chorion membrane allografts for acute and reconstructive burn care. *Ann Plast Surg* 78:S19–S26
42. Anam K, Lazdun Y, Davis PM, Banas RA, Elster EA, Davis TA (2013) Amnion-derived multipotent progenitor cells support allograft tolerance induction. *Am J Transplant* 13:1416–1428
43. Mamede A, Carvalho M, Abrantes A, Laranjo M, Maia C, Botelho M (2012) Amniotic membrane: from structure and functions to clinical applications. *Cell Tissue Res* 349:447–458
44. Kim JD ABM, Joo BS, Kwon JY, Chung HJ, Yu SB (2014) Effect of propoal on prostaglandin E2 production and cyclooxygenase-2 expression in amniotic membrane cells. *J Anesth* 28:911–918
45. Hao Y, Ma DH, Hwang DG, Kim W, Zhang F (2000) Identification of antiangiogenic and antiinflammatory proteins in human amniotic membrane. *Cornea* 19:348–352

46. Buhimschi IA, Jabr M, Buhimschi CS, Petkova AP, Weiner CP, Saed GM (2004) The novel antimicrobial peptide β 3-defensin is produced by the amnion: a possible role of the fetal membranes in innate immunity of the amniotic cavity. *Obstet Gynecol* 191:1678–1687
47. Davis WJ, Wu C, Sieber D, Vandevender DK (2011) A comparison of full and split thickness skin grafts in radial forearm donor sites. *J Hand Microsurg* 3:18–24
48. de Witt CA, de Bree R, Verdonck-de Leeuw IM, Quak JJ, Leemans CR (2007) Donor site morbidity of the fasciocutaneous radial forearm flap: what does the patient really bother? *Eur Arch Otorhinolaryngol* 264:929–934
49. Kamal D, Fassio E, Goudot P, Yachouh J (2008) Suprafascial dissection of the forearm flap. *Ann Chir Plast Esthet* 55:14–18
50. Shores JT, Hiersche M, Gabriel A, Gupta S (2012) Tendon coverage using an artificial skin substitute. *J Plast Reconstr Aesthet Surg* 65:1544–1550
51. Butzelaar L, Soykan EA, Galindo Garre F, Beelen RH, Ulrich MM, Niessen FB, van der Molen M, Aebele B (2015) Going into surgery: risk factors for hypertrophic scarring. *Wound Repair Regen* 23:531–537
52. Kim JH, Sung JY, Kim YH, Lee YS, Chang H, Park CS, Roh MR (2012) Risk factors for hypertrophic surgical scar development after thyroidectomy. *Wound Repair Regen* 20:304–310
53. do Nascimento AP, Monte-Alto-Costa A (2011) Both obesity-prone and obesity-resistant rats present delayed cutaneous wound healing. *Br J Nutr* 106:603–611
54. Rodgers K, Xiong S, Felix J, Roda N, Espinoza T, Maldonado S, Dizerega G (2001) Development of angiotensin (1-7) as an agent to accelerate dermal repair. *Wound Repair Regen* 9:238–247
55. Rodgers KE, Ellefson DD, Espinoza T, Roda N, Maldonado S, Dizerega GS (2005) Effect of NorLeu3-A (1-7) on scar formation over time after full-thickness incision injury in the rat. *Wound Repair Regen* 13:309–317

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.