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Dear readers,

Appropriate management of soft tissue has immense impact on final functional and aesthetic results. Not only devastating injury and extensive loss of soft tissue but also any kind of injury (including operative injury) with prolonged healing leads to excessive superficial and deep scaring and a significant aesthetic and functional deficit.

Over the past years, plastic surgeons developed and introduced many simple and complex reconstructive techniques to fasten and improve healing processes. Plastic surgery as a specialty is best equipped and competent to solve complex soft tissue problems.

Due to the involvement of plastic surgery, many patients with extensive trauma got the chance to save their extremities and even life.

Those who suffer from aesthetic and functional limitation receive reconstructions to restore function and improve outcomes.

Current research in plastic surgery is focussed on the development of new, more sophisticated techniques involving tissue engineering methods and tissue regenerative techniques.

Many originally plastic surgery methods became incorporated in the armamentarium of other specialties.

Despite this, we are witnessing many patients who did not receive the appropriate care they deserve. Poor treatment leads to their poor results. They have to undergo secondary procedures to get some, usually only partial improvements. What is omitted initially can never be completely restored. The role of a proper management of soft tissue never can be overestimated.

The current issue of *Acta chirurgiae plasticae* is dedicated to soft tissue management. It should motivate colleagues to share their experience and increase awareness about capability with other surgical specialties. The benefit to the patient is immense.

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DIFFUSION OF INJECTED COLLAGENASE CLOSTRIDIUM HISTOLYTICUM FOR DUPUYTREN'S DISEASE: AN IN-VIVO STUDY

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ABSTRACT

Introduction. While injecting Collagenase Clostridium Histolyticum (CCH) as a non--surgical treatment for Dupuytren's disease on the palmar side of the hand the recommended depth of the needle should be "around 2 to 3 mm in depth". The diffusion of CCH inside the soft tissues around the cord might explain the occurrence of common adverse events reported in the literature such as oedema, injection site swelling, blood blister, skin laceration, and pain in extremity. We hypothesized that the injected CCH does not only concentrate inside the cord but also dissipates both along the cord and into the adjacent tissues. This study investigated our hypothesis by visual intraoperative findings after injecting Povidone iodine into the cord.

Material and methods. Povidone iodine was injected into the cord on six patients with Dupuytren's contracture (DC) before an open surgical operation (partial fasciectomy). We marked three hypothetical CCH injection points at 2 mm intervals on the skin above the cord around the metacarpo-phalangeal joint and the depth of the injection (distance from the skin surface to the middle of the cord) was measured by ultrasonography. After dispensing 0.25 ml of PI into the three points at the measured depths, we performed careful dissection and investigated the extent of diffusion of Povidone iodine visually.

Results. The injection depth averaged 2.6 mm. In all cases, the cord was homogenously stained about 10 mm along its extent centrally to the injected sites and infiltration of Povidone iodine into the subcutaneous structure and fat tissue occurred. Three cases showed diffusion into the neurovascular bundles and two cases showed infiltration underneath the cord structure.

Conclusions. This study simulated the likely diffusion outcomes of injected CCH around the cord. This implies that even if CCH is injected into the centre of the cord, CCH does not concentrate inside the cord only but also dissipates along the cord and infiltrates into the adjacent tissues with potential secondary damages.

KEYWORDS

Collagenase Clostridium Histolyticum; Dupuytren´s disease; injection; diffusion

INTRODUCTION

Dupuytren´s disease is a thickening of the palmar, digital fascia and adjacent soft tissues mainly affecting the ring finger and the little finger.[1]

In 8 to 33% of cases the progressive nature of Dupuytren´s contracture (DC) may form progressive fixed flexion contracture (FFC), that inhibit normal function of the hand.[2-4] The mainstay treatment for DC is surgical fasciectomy or fasciotomy.[5] Collagenase Clostridium Histolyticum (CCH) was introduced in 2009 as an alternative nonsurgical, minimally invasive treatment for DC and the efficacy and safety of CCH has been confirmed in clinical studies subsequently. [6-10] It relies on enzymatic cleavage of the pathologic cords via local injection of collagenase, followed by delayed manual manipulation.[11] The most common adverse events of CCH injection are swelling, ecchymosis, blisters, lymphadenopathy, and skin tears that usually resolve without further surgical therapies,[5-9] on the other hand, severe complication is tendon rupture requiring technically demanding surgical procedures. [13]

The technical instruction leaflet for CCH injection (Auxilium Pharmaceuticals, Inc., Malvern, PA, USA) recommends a "2–3mm depth" of injection but gives no further guidance. [12] We hypothesized that the injected CCH does not only concentrate inside the cord but also dissipates both along the cord and into the adjacent tissues. Povidone-iodine (PI) is a widely used water-soluble antiseptic solution consisting of polyvinylpyrrolidone with water, iodide and 1% available iodine introduced by Shelanki in 1956.[14] It has bactericidal ability against a large array of pathogens.[15] This study investigated our hypothesis by visual intraoperative findings after injecting Povidone iodine (PI) into the cord.

MATERIALS AND METHODS

This study enrolled five male and one female patient with DC (four little fingers and two ring fingers) with a mean age of 71 (range; 60–78 years old) on whom we performed partial fasciectomies due to FFC of the metacarpophalangeal (MP) and proximal interphalangeal (PIP) joints (Table 1). Before the surgical procedure, we marked three hypothetical collagenase injection points at 2 mm intervals on the skin above the cord around of the MP joint and measured the distance from the skin surface to the middle of the cord as hypothetical injection depth by ultrasonography (US) with long axis images (SNiBLE; Konica Minolta, Tokyo, Japan). We then dispensed a total amount of 0.25 ml of PI, which is a commonly used antiseptic solution used to reduce the risk of surgical site infection before the skin incision or to irrigate infected tissues [16,17] (Mylan Inc., Tokyo, Japan), into the three points after adjusting the needle length to the hypothetical injection depth by our silicone tube method.[18] The silicone tube method involves placing a precut, measured and sterilized silicone tube (Phycon tube SH No. 1, inner diameter-outer diameter; 1.0-2.0mm, Fuji Systems, Tokyo, Japan) prepared for us by BEAR Medic Corporation's factory (Ibaraki, Japan) over the 13 mm non-removable needle of VA syringes (Nipro, Osaka, Japan) which effectively adjusts the needle length (Figure 1). The restricted depth provides not only precise injection into the middle of the cords but also avoids needle tip migration. Stabilizing the tube with sterilized forceps while depressing the plunger simplifies the procedure. Soon after the injection of PI, we performed a careful dissection and visually examined the extent of the PI diffusion checking A) the longitudinal extent in the cord central to the injected sites and B) the infiltration into the adjacent tissues located inside the subcutaneous structures i.e. fat tissue, neurovascular bundles and the space underneath the cord structure. After completing the partial fasciectomies, the remaining PI was irrigated with saline thoroughly and skin was closed.

RESULTS

The hypothetical injection depth averaged 2.6 mm. In detail it was equivalent to 2.1 mm (Case 1), 3 mm (Case 2), 2.5 mm (Case 3), 3 mm (Case 4), 2.8 mm (Case5) and 2.1 mm (Case 6).

Case	Age	Affected finger	FFC of MP	
joint (degree)	FFC of PIP			
joint (degree)				
1	78	little	10	25
2	60	little	15	10
3	76	little	50	85
4	69	ring	0	60
5	65	ring	0	20
6	60	little	50	0

Table 1. Baseline patient characteristics

Figure 1. 0.25 ml of PI prepared with the syringe. Note the needle length adjusted by a silicone tube sleeve

A) The longitudinal extent in the cord central to the injected sites

In all cases the cord was homogeneously stained with PI to the following extents; 10 mm (Case 1), 6 mm (Case 2), 10 mm (Case 3), 8 mm (Case 4), 15 mm (Case 5) and 13 mm (Case 6) with an average of 10 mm.

B) The infiltration into the adjacent tissues

The infiltration of PI into the subcutaneous structure underneath the injection sites and into the bilateral fat tissue was seen in all cases (Figure 2a). In addition, Case 1, 3 and 6 showed the infiltration into the neurovascular bundles (Figure 2b). Furthermore, Case 1 and 6 showed the infiltration of PI underneath the cord structure (Figure 2c).

DISCUSSION

Although CCH injection treatment is reported to have a high rate of both clinical success with FFC ≤5 degree and patients' satisfaction, there is a persistent rate of adverse events, such as oedema, injection site swelling, blood blister, skin laceration and pain in extremity. [6-10] The incidence of the severe complication of tendon rupture is low [6,7,10,13] and it is postulated that this should be prevented by avoiding injection too deeply. For this reason, physicians may be tempted to inject CCH shallowly. We suggest this likely exacerbates the occurrence of the adverse events. Therefore, we consider it is important to inject CCH in the centre of the cord in an effort to minimize those complications and maximize CCH efficacy. Verheyden also recommended a direct CCH injection into the centre of the cord by feeling the firm pressure at plunging of the syringe.[19] However, swelling (40%) and skin lacerations (35%) were noted which were comparable to previous reports. [6-10] This implies that even if CCH is injected into the centre of the cord, the liquid does not only concentrate inside the cord but also dissipates along the cord and infiltrates into the adjacent tissues. This

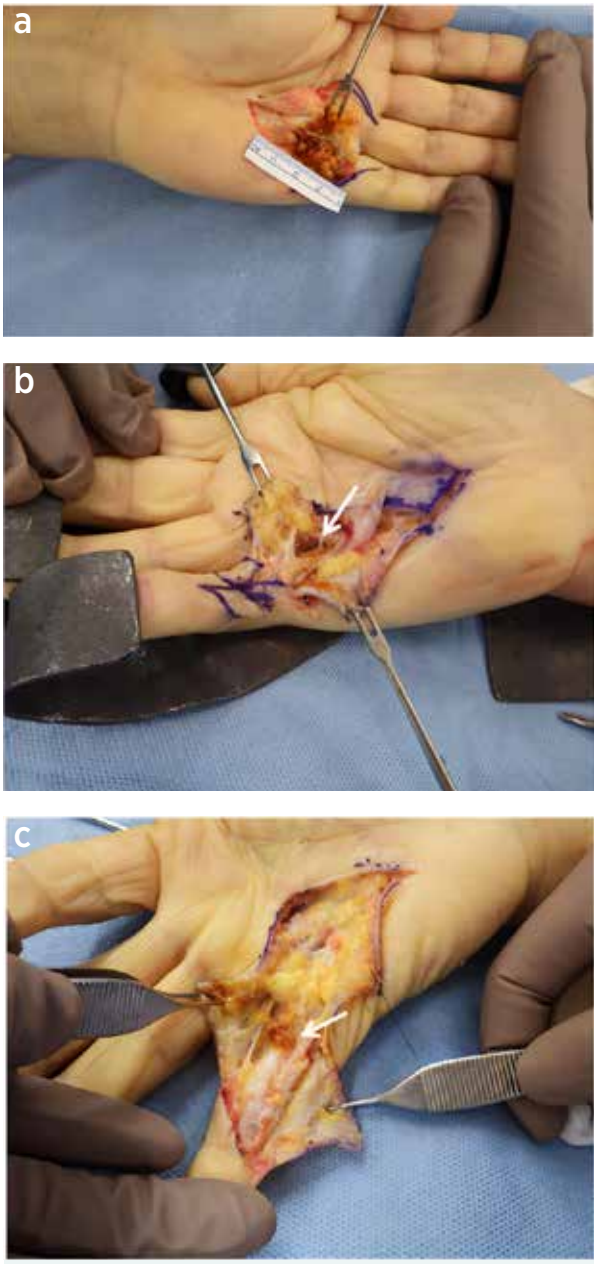

Figure 2a. The infiltrati on of PI into the subcutaneous structure underneath the injection sites and into the bilateral fat tissue. Note the medio-lateral spread of the solution
Figure 2b. The infiltration into the neurovascular bundles (arrow). Note the relative superficial allocation of the two digital nerves
Figure 2c. The infiltration of PI underneath the cord structure (arrow). Note the close position of the cord to the flexor A1 pulley and flexor tendons with bowstringing effect of the two neurovascular bundles

may explain why CCH injection at the MP joint improved the FFC of the PIP joints[9] but with the occurrence of adverse events. [6-10] In fact, as direct visual inspection of injected CCH is impossible, we designed this study to simulate an in vivo condition by injecting PI into the cord of the cases that were due to have partial fasciotomies. Although CCH and PI are different agents, they are soluble liquid substrate mixtures. The diffusion of PI inside and outside of the cord implies that CCH would behave similarly. Crivello et. al.[21] reported the inflammatory changes about the flexor tendons (60%) and around the cord (100%) by slightly increased signal intensity on the MRI one month after CCH injection. Those findings were attributed to a reaction to CCH because there was no sign of inflammation on the MRI taken before CCH injection. They commented about the importance of understanding the effect of CCH on the neighbouring soft tissues, for example, the possible spread of CCH to nearby flexor tendons and pulleys. Our study confirms their comments showing the infiltration of PI into the subcutaneous structure underneath the injection sites and the bilateral fat tissue in all six cases, into the neurovascular bundles in three cases, and underneath the cord structure in two cases.

The limitations of this study were firstly, PI and CCH are different agents and secondly, the case numbers are small. However, we believe this study demonstrates the likelihood of CCH dissipation inside the cord and infiltration into the adjacent tissues.

CONCLUSIONS

This study simulated the likely diffusion outcomes of injected CCH around the cord.

Even if CCH is injected into the centre of the cord, CCH may not concentrate inside the cord only but also may dissipate along the cord and infiltrates into the adjacent tissues with potential secondary damages.

Role of authors: All authors have been actively involved in the planning, preparation, analysis and interpretation of the findings, enactment and processing of the article with the same contribution.

Declaration: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for being included in the study. Some data from this paper were presented at the 73rd Annual Meeting of the American Society for Surgery of the Hand, September 15–18, 2018, Boston.

Conflict of interest: None.

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REFERENCES

1. Badalamente MA., Stern L., Hurst LC. The pathogenesis of Dupuytren's contracture: contractile mechanisms of the myofibroblasts. *J Hand Surg Am*. 1983, 8:235–43.
2. Diep GK., Agel J., Adams JE. Prevalence of palmar fibromatosis with and without contracture in asymptomatic patients. *J Plast Surg Hand Surg*. 2015. 49:247–50.
3. Degreef I., De Smet L. A high prevalence of Dupuytren´s disease in Flanders. *Acta Orthop Belg*. 2010. 76:316–20.
4. Lanting R., van den Heuvel ER., Westerink B., Werker PM. Prevalence

of Dupuytren disease in The Netherlands. *Plast Reconstr Surg*. 2013, 132:394–403.
5. Crean SM., Gerber RA., Le Graverand MP., Boyd DM., Cappelleri JC. The efficacy and safety of fasciectomy and fasciotomy for Dupuytren´s contracture in European patients: a structured review of published studies. *J Hand Surg Eur*. 2011, 36:396–407.
6. Badalamente MA., Hurst LC., Benhaim P., Cohen BM. Efficacy and safety of CCH in the treatment of proximal interphalangeal joints in Dupuytren contracture: combined analysis of 4 phase 3 clinical trials. *J Hand Surg Am*. 2015, 40:975–83.
7. Gaston RG., Larsen SE., Pess GM., et al. The Efficacy and Safety of Concurrent CCH Injections for 2 Dupuytren Contractures in the Same Hand: A Prospective, Multicenter Study. *J Hand Surg Am*. 2015, 40:1963–71.
8. Gilpin D., Coleman S., Hall S., Houston A., Karrasch J., Jones N. Injectable CCH: a new nonsurgical treatment for Dupuytren´s disease. *J Hand Surg Am*. 2010, 35:2027–38.e1
9. Hirata H., Tanaka K., Sakai A., Kakinoki R., Ikegami H., Tateishi N. Efficacy and safety of CCH injection for Dupuytren´s contracture in non-Caucasian Japanese patients (CORD-J Study): the first clinical trial in a non-Caucasian population. *J Hand Surg Eur*. 2017, 42:30–8.
10. Hurst LC., Badalamente MA., Hentz VR., et al. Injectable CCH for Dupuytren´s contracture. *N Engl J Med*. 2009, 361:968–79.
11. Desai SS., Hentz VR. The treatment of Dupuytren disease. *J Hand Surg Am*. 2011, 36:936–42.
12. Xiaflex (CCH) [package insert].Or Malvern, PA: Auxilium Pharmaceuticals, Inc.; 2016.
13. Zhang AY., Curtin CM., Hentz VR. Flexor tendon rupture after collagenase injection for Dupuytren contracture: case report. *J Hand Surg Am*. 2011, 36:1323–5.
14. Shelanski KA., Shelanski MV. PVP-iodine: history, toxicity and therapeutic uses. *J Int Coll Surg*. 1956, 25:727–34.
15. Zamora JL. Chemical and microbiologic characteristics and toxicity of povidone-iodine solutions. *Am J Surg*. 1986, 151:400–6.
16. Sindelar WF., Mason GR. Efficacy of povidone-iodine irrigation in prevention of surgical wound infections. *Surg Forum*. 1977, 28:48–51.
17. Chundamala J., Wright JG. The efficacy and risks of using povidone-iodine irrigation to prevent surgical site infection: an evidence-based review. *Can J Surg*. 2007, 50:473–81.
18. Kanatani T., Nagura I., Harada Y. CCH Injection with Precise Needle Length Adjusted by Silicone Tube Interposition for Dupuytren Contracture. *J Hand Surg Asian Pac Vol*. 2018, 23:437–9.
19. Verheyden JR. Early outcomes of a sequential series of 144 patients with Dupuytren´s contracture treated by collagenase injection using an increased dose, multi-cord technique. *J Hand Surg Eur*. 2015, 40:133–40.
20. Leclère FM., Mathys L., Vögelin E. Traitement de la maladie de Dupuytren par collagénase injectable, évaluation de l´échographie assistée [Collagenase injection in Dupuytren´s disease, evaluation of the ultrasound assisted technique]. *Chir Main*. 2014, 33:196–203.
21. Crivello KM., Potter HG., Moon ES., Rancy SK., Wolfe SW. Does collagenase injection disrupt or digest the Dupuytren´s cord: a magnetic resonance imaging study. *J Hand Surg Eur*. 2016, 41:614–20.

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OPTIMAL INJECTION DEPTH FOR COLLAGENASE CLOSTRIDIUM HISTOLYTICUM DETERMINED BY ULTRASONOGRAPHY IN THE TREATMENT OF DUPUYTREN'S DISEASE

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SUMMARY

Introduction. A non-surgical procedure for the treatment of Dupuytren's disease is a palmar injection of Collagenase Clostridium Histolyticum to the recommended depth of "around 2–3 mm". However, there is little supporting evidence from the literature to substantiate this. The aim of this study was to evaluate the "optimal depth" for injection of Collagenase Clostridium Histolyticum by ultrasonography for the treatment of Dupuytren's disease.

Material and methods. A total of 43 patients were enrolled in this study. We marked the collagenase injection point on the skin above the cord before injection. We then measured the distance from the surface of the skin to the middle of the cord by ultrasonography long axis imaging and defined this as the "optimal depth".

Results. The average depth from the skin to the centre of the cord was 2.4 mm. The average distance from the surface of the skin to the proximal surface of the cord was 1.0 mm and the average thickness of the cord was 2.7 mm.

Conclusion: By precise measurement of individual cases utilising ultrasonography we were able to confirm that the recommendations for injection depth as provided by the supplier of Collagenase Clostridium Histolyticum (2–3 mm) were in agreement with our findings. However no objective guide was supplied as with regards to interindividual variability between patients and we suggest that the use of preliminary ultrasonography will likely provide improved outcomes.

KEYWORDS

Clostridium Histolyticum Collagenase; Dupuytren's contracture; ultrasonography; injection; interventional ultrasound

INTRODUCTION

Dupuytren's disease (DD) is a common, benign, fibroproliferative disease with thickening of the palmar and digital fascia and adjacent soft tissues mainly in the ring finger and the little finger.[1] Normal palmar fascia affected by DD transforms into pathologic nodules and cords, which may form progressive flexion contractures and inhibit normal function of the hand. The mainstay treatment for DD is surgical fasciectomy or fasciotomy.[2,3] The surgical treatment is associated with significant morbidity, with a reported overall complication rate of 17%, including skin complications, hematoma, digital nerve injury, and complex regional pain syndrome.[4] Despite resection of the pathological lesion, subsequent secondary operations are sometimes required because of recurrence and revision surgery is even more challenging, with a higher rate of complications.[5] Due to the risks of primary surgery and the challenges of additional surgery on a previously scarred bed, hand surgeons have long been searching for nonsurgical treatments for DD.[6]

An alternative nonsurgical treatment for DD is Collagenase Clostridium Histolyticum (CCH) injection (Auxilium Pharmaceuticals, Inc., Malvern, PA, USA), which was introduced in 2009.[7,8] It relies on enzymatic cleavage of

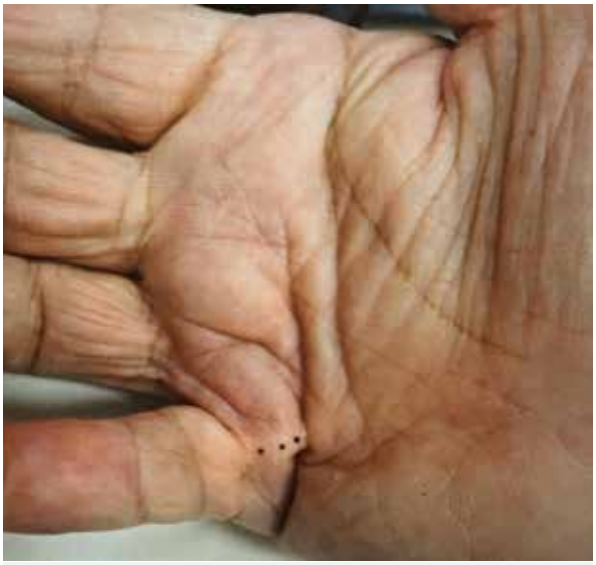

Figure 1. Marking points before the injection

the pathologic cords via local injection of collagenase, followed by delayed manual manipulation.[9] The efficacy and safety of CCH has been confirmed in clinical studies and CCH subsequently became a therapeutic drug for the minimally invasive treatment of DD. [7-11] The most common adverse outcomes reported for CCH injection are pain with injection and manipulation, edema, ecchymosis, lymphadenopathy, and skin tears that are usually resolved without further surgical therapy.[7-12] However, a serious complication is tendon rupture, requiring technically demanding surgical procedures to repair.[8-12] It is important to minimise the occurrence of such complications, therefore injection to the correct depth by a skilled physician is recommended. In Japan, only hand surgeons board certified by The Japanese Society for Surgery of the Hand (JSSH) are allowed to perform CCH injections.[11]

The technical instruction leaflet for CCH injection recommends a "2–3 mm depth" of injection,[13] however, there is little supporting evidence to rationalise the appropriateness of this assertion. We hypothesized that the optimal injection depth is the distance from the surface of the skin to the middle of the cord because we believe that it would produce maximum efficacy and minimise side effects. We note that Leclere et al proposed a US guided injection technique[14] as a way to achieve the same result. The aim of this study was to evaluate the use of US to determine the optimal injection depth in different patients with different cord thicknesses.

MATERIAL AND METHODS

A total of 43 patients affected by DD with fixed flexion contracture (FFC) of the metacarpophalangeal (MP) or proximal interphalangeal (PIP) joint were included in this study. There were 40 males and 3 females with a mean age of 71.4 years (range 53–87). Treatment was given to only one finger in each patient. Affected digits were the little (n = 24), the ring (n = 17) and the middle (n =2) fingers. The mean FFCs caused by the palpable cords were of 45 degrees at the MP joint (range 0-80) and 17 degrees at the PIP joint (range 0–65).

We marked three collagenase injection points at 2 mm intervals on the skin above the cord around the MP joint of the involved digit before injection. All points were located not more than 4 mm distal to the palmar crease (Figure 1). Then pulling each finger under tension, we measured the hypothesized "optimal depth", i.e. the distance from the skin surface to the centre of the cord, by high resolution ultrasonography with long axis images (SNiBLE; Konica Minolta, Tokyo, Japan) with an 18 MHz linear transducer. The linear transducer was applied over the cord with care to prevent compression of subcutaneous soft tissues. We also measured the distance from the skin surface to the superficial aspect of the cord, the width of the cord by long axis imaging and used this to calculate the distance from the skin surface to the centre of the cord (Fig 2). We analyzed the correlation between the FFC of the MP joint and the width of the cord additionally (Pearson's correlation analysis).

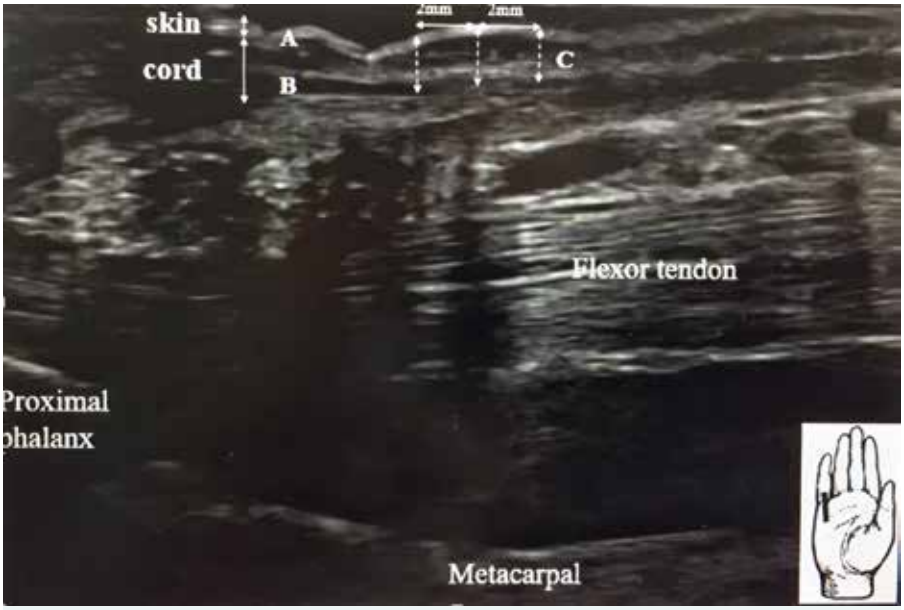

Figure 2. Long axis image of the US evaluation
(A) Distance between the skin and the cord
(B) Width of the cord
(C) Distance from the skin to the middle of the cord

RESULTS

All cords showed a mixture of isoechogennic or hypoechogennic lesions. The average distance from the skin surface to the cord was

Finger (Number)	A (mm)	B (mm)	C (mm)
Middle (N=2)	0.9±0	3.1±0.2	2.5±0.1
Ring (N=17)	1±0.4	2.7±0.6	2.4±0.4
Little (N=24)	1.1±0.4	2.7±0.6	2.4±0.4
Total (N=43)	1±0.4	2.7±0.6	2.4±0.4

Table 1. Patient list

1.0 mm (range: 0.4–2.0) and the average width of the cord was 2.7 mm (range: 1.5–3.8). The average optimal depth, i.e. distance from the skin to the middle of the cord, was 2.4 mm (range: 1.5–3.0) (Table 1). Interestingly, 4 cases were less than the 2 mm minimum recommendation. No correlation between the FFC of MP joint and the width of the cord was found (R2 = 0.183) (Table 2).

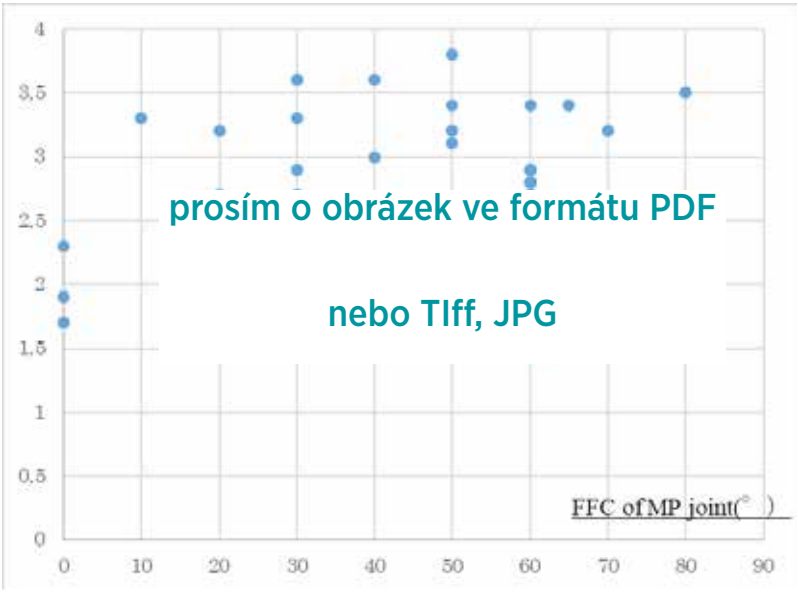

Table 2. Correlation between the FFC of MP joint and the width of the cord

DISCUSSION

CCH injection has been approved as a minimally invasive non-surgical procedure to treat DD for more than 10 years in various countries.[11, 13-15] Its efficacy and safety have been reported in clinical settings.[7, 11, 16, 17] Although the technical instruction leaflet for CCH injection recommends a "2–3 mm-in depth injection within 4 mm distal to the palmar digital crease"[14], there is still room for error owing to the different thicknesses and depths of the cords. Furthermore, there is little guidance with objective evidence in the literature regarding the optimal depth for the CCH injection. Physicians tend to inject CCH shallowly to avoid the serious complication of flexor tendon ruptures and this might exacerbate the occurrence of adverse events such as bruising, swelling and skin laceration. These adverse events are mild or moderate and resolved without intervention within a median duration of 10 days.[8,18] Patients' satisfaction is superior to that of needle fasciotomy[19] despite this disadvantage. Verheyden et al. suggested injecting CCH into the centre of the cord in an effort to maximize CCH efficacy and to avoid the greater potential for spread of the enzyme to nearby flexor tendons or pulleys.[20] We agree with the authors and believe that injection into the middle of the cord might reduce the rate of the aforementioned complications.

Meals et al. reported their intraoperative findings underlining that the superficial aspect of the cords is located 2 to 4 mm from the skin surface, and cord thickness is rarely more than 4 to 5 mm.[21] In our study, the average width of the cord was 2.7 mm (range; 1.5–3.8), which was smaller than their findings. We hypothesize that candidates for surgery may have thicker cords affected by more long-lasting conditions than those selected for non-surgical therapy or alternatively that surgical specimens are free from in-vivo anatomical pressure when measured.

Our US-findings are comparable to previous reports[22,23] but we further investigated the widths of the cord and skin thickness in DD patients. We were able to determine the injection depth by direct measurement-and demonstrated that FFC of the MP joint could not be used to determine the injection depth as it did not correlate with the width of the cord. However, the skin thickness may help in predicting the propensity for skin laceration after manipulation. Further research is necessary to define the exact usefulness of US in these procedures. Uehara et al.[24] reported US evaluation of the relationship between the cords and neurovascular bundles, utilizing a short axial image. We selected a long axial image because it was easier to detect and measure the anatomical characteristics of the cords and to elucidate the three injection points at the 2 mm intervals. Moreover, pragmatically, it is difficult to hold the probe on the convex surface of the cord for a short axial image because the contact point is smaller compared to the long axis image.

The limitations of this study are: (a) the small number of enrolled patients and (b) the lack of investigation of the spiral, lateral digital and retrovascular cords in the digital forms. This study is mainly focused on the palmar forms with the MP joint main involvement only. The improvement of FCC as a result of this procedure is to be reported elsewhere and is not included here.

CONCLUSION

In this study, we confirmed that "2–3mm in depth" for CCH injection was appropriate, in most circumstances,

however 4 cases (9.3%) presented as less than 2 mm in depth, which could have resulted in delivery of CCH underneath the cord. Experienced surgeons know the anatomy of DD and the nodules but we conclude that measuring the injection depth by US would be beneficial.

To inject the planned depth, the 1.2 mm bevel at the needle tip helps estimate the depth of insertion [21] or a silicone tube interposition to adjust needle length is practical for precise injection.[25]

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Declaration: All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients included in this study.

Role of authors: All authors have been actively involved in the planning, preparation, analysis and interpretation of the findings, enactment and processing of the article with the same contribution.

Conflict of interest: None.

REFERENCES

1. Badalamente MA., Stern L., Hurst LC . The pathogenesis of Dupuytren´s contracture: contractile mechanisms of the myofibroblasts. *J Hand Surg Am*. 1983, 8, 235–43.
2. Crean SM., Gerber RA., Le Graverand MP., Boyd DM., Cappelleri JC. The efficacy and safety of fasciectomy and fasciotomy for Dupuytren's contracture in European patients: a structured review of published studies. *J Hand Surg Eur*. 2011, 36:396–407.
3. Dias JJ., Braybrooke J. Dupuytren´s contracture: an audit of the outcomes of surgery. *J Hand Surg Br*. 2006, 31:514–21.
4. Watt AJ., Curtin CM., Hentz VR. Collagenase injection as nonsurgical treatment of Dupuytren's disease: 8-year follow-up. *J Hand Surg Am*. 2010, 35:534–9.
5. Rodrigues JN., Zhang W., Scammell BE., et al. Functional outcome and complications following surgery for Dupuytren's disease: a multi-centre cross-sectional study. *J Hand Surg Eur*. 2017, 42:7–17.
6. Coert JH., Nérin JP., Meek MF. Results of partial fasciectomy for Dupuytren disease in 261 consecutive patients. *Ann Plast Surg*. 2006, 57:13–7.
7. Gilpin D., Coleman S., Hall S., Houston A., Karrasch J., Jones N. Injectable collagenase Clostridium histolyticum: a new nonsurgical treatment for Dupuytren's disease. *J Hand Surg Am*. 2010, 35:2027–38.e1.
8. Hurst LC., Badalamente MA., Hentz VR., Hotchkiss RN., Kaplan FT., Meals RA., Smith TM., Rodzvilla J. CORD I Study Group. Injectable collagenase clostridium histolyticum for Dupuytren's contracture. *N Engl J Med*. 2009, 361:968–79.
9. Desai SS., Hentz VR. The treatment of Dupuytren disease. *J Hand Surg Am*. 2011, 36:936–42. doi: 10.1016/j.jhsa.2011.03.002.PMID: 21527148.
10. Badalamente MA., Hurst LC., Hentz VR. Collagen as a clinical target: nonoperative treatment of Dupuytren's disease. *J Hand Surg Am*. 2002, 27:788–98.
11. Sakai A., Zenke Y., Menuki K., Yamanaka Y., Tajima T., Tsukamoto M., Uchida S. Short-term efficacy and safety of collagenase injection for Dupuytren's contracture: Therapy protocol for successful outcomes in a clinical setting.*J Orthop Sci*. 2019, 24:434–40.
12. Zhang AY., Curtin CM., Hentz VR. Flexor tendon rupture after collagenase injection for Dupuytren contracture: case report. *J Hand Surg Am*. 2011, 36:1323–5.
13. Xiaflex_ (collagenase clostridium histolyticum) [prescribing information]. Chesterbrook, PA: Auxilium Pharmaceuticals, Inc.; October 2014.
14. Leclère FM., Mathys L., Vögelin E. [Collagenase injection in Dupuytren´s disease, evaluation of the ultrasound assisted technique]. *Chir Main*. 2014, 33:196–203.
15. Badalamente MA., Hurst LC., Benhaim P., Cohen BM. Efficacy and safety of collagenase clostridium histolyticum in the treatment of proximal interphalangeal joints in dupuytren contracture: combined analysis of 4 phase 3 clinical trials. *J Hand Surg Am*. 2015, 40:975–83.
16. Peimer CA., Blazar P., Coleman S., Kaplan FT., Smith T., Lindau T. Dupuytren Contracture Recurrence Following Treatment With Collagenase Clostridium histolyticum (CORDLESS [Collagenase Option for Reduction of Dupuytren Long-Term Evaluation of Safety Study]): 5-Year Data. *J Hand Surg Am*. 2015, 40:1597–1605.
17. Witthaut J., Jones G., Skrepnik N., Kushner H., Houston A., Lindau TR. Efficacy and safety of collagenase clostridium histolyticum injection for Dupuytren contracture: short-term results from 2 open-label studies. *J Hand Surg Am*. 2013, 38:2–11.
18. Gaston RG., Larsen SE., Pess GM., Coleman S., Dean B., Cohen BM., Kaufman GJ., Tursi JP., Hurst LC. The efficacy and safety of concurrent collagenase clostridium histolyticum injection for 2 Dupuytren contractures in the same hand; a prospective, multicentre study. *J Hand Surg Am*. 2015, 40, 2015, 1963–71.
19. Skov ST., Bisgaard T., Søndergaard P., Lange J. Injectable Collagenase Versus Percutaneous Needle Fasciotomy for Dupuytren Contracture in Proximal Interphalangeal Joints: A Randomized Controlled Trial. *J Hand Surg Am*. 2017, 42:321–8.e3.
20. Verheyden JR. Early outcomes of a sequential series of 144 patients with Dupuytren contracture treated by collagenase injection using an incresed dose, multi-cord tequnique. *J Hand Surg Eur*. 2015, 40:133–40.
21. Meals RA., Hentz VR. Technical tips for collagenase injection treatment for Dupuytren contracture. *J Hand Surg Am*. 2014, 39:1195–1200.
22. Creteur V., Madani A., Gosset N. Ultrasound imaging of Dupuytren's contracture. *J Radiol*. 2010, 91:687–91.
23. Morris G., Jacobson JA., Kalume Brigido M., Gaetke-Udager K., Yablon CM., Dong Q. Ultrasound features of palmar fibromatosis or Dupuytren contracture. *J Ultrasound Med*. 2019, 38:387–92.
24. Uehara K., Miura T., Morizaki Y., Miyamoto H., Ohe T., Tanaka S. Ultrasonographic evaluation of displaced neurovascular bundle in Dupuytren disease. *J Hand Surg Am*. 2013, 38:23–8.
25. Kanatani T., Nagura I., Harada Y. Collagenase Clostridium Histolyticum Injection with Precise Needle Length Adjusted by Silicone Tube Interposition for Dupuytren Contracture. *J Hand Surg Asian Pac*. 2018, 23:437–9.

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FUNCTIONAL RECONSTRUCTION OF SOFT TISSUE OROFACIAL DEFECTS WITH MICROVASCULAR GRACILIS MUSCLE FLAP

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SUMMARY

Introduction. Free flap reconstructive surgery of middle size and large oral and facial defects enables aesthetic and functional rehabilitation. Microvascular flap reconstructive surgery, with flap survival success rate more than 90–98%, is a gold standard in head and neck extensive reconstructions. Currently, head and neck reconstructive surgery is focused not only on defect occlusion and adequate aesthetical result, but the same emphasize is aimed at functional result. Functional result post tongue and lip resection means defect occlusion with the possibility of movement restoration. Free gracilis muscle flap appears to be a choice for functional tongue and lip reconstruction.
Material and methods. We present 1-year experience with 5 microvascular flap functional reconstructions of middle size and large defects of tongue and lip with free gracilis muscle flap. Four patients post tongue resection and one post subtotal lower lip and cheek resection underwent immediate functional microvascular gracilis muscle flap reconstruction.
Results. All five patients were successfully reconstructed with functional free gracilis muscle flap, with no flap loss. We found gracilis muscle flap harvest is not technically demanding, provides adequate tissue volume for middle size orofacial defects reconstruction, with possibility for skin island harvest, and simple primary closure of donor site with very low morbidity. Patients after tongue reconstruction with free gracilis flap were swallowing spoon food 1 week post operation. Patient after total lip resection and immediate reconstruction with free gracilis flap presented with oral competence before the discharge. The functional result in the group of patients with free gracilis flap reconstruction for orofacial defect will have to be further evaluated again after 2 years post operation considering the ability to swallow and articulate during the speech for the tongue reconstructions and the oral competence and facial mimic for the lip reconstruction.
Conclusion. Microvascular gracilis muscle flap reconstruction compared to radial forearm flap reconstruction enables functional reconstruction of soft tissue defect. Functional reconstruction of soft tissue defects of tongue or lip with microvascular gracilis muscle flap appears to have advantage of adequate volume, very low donor side morbidity and expectancy of movement renewal compared to other microvascular flap reconstructive options such as anterolateral thigh flap, superficial circumflex iliac artery perforator flap, lateral arm free flap or deep inferior epigastric perforator free flap.

KEYWORDS

Orofacial defect; gracilis muscle; microvascular free flap; functional reconstruction

INTRODUCTION

Free flap reconstructive surgery of middle size orofacial defects enables aesthetic and functional rehabilitation in maxillofacial region. Microvascular flap reconstructive surgery with flap survival success rate more than 90–98% is a gold standard in head and neck reconstructions. The role of the surgeon is not only to close the post-ablative defect, but also to reestablish the lost function. The gracilis muscle free flap (Figure 1) provides adequate volume for middle size oral and facial defects and enables functional rehabilitation.

Reconstructive surgery in general and not only in maxillofacial region has two major rules. The first is to reconstruct the lost tissue with most alike type of tissue. The second is to choose the simplest reconstructive option, which fulfills the criteria of aesthetic and functional rehabilitation. For small and some middle size soft tissue orofacial defects local pedicled flap is the workhorse for adequate reconstruction. Most of the small and part of middle size orofacial defects can be adequately reconstructed with one of the following local pedicled flaps: submental flap, FAMM (facial artery musclemucosal flap), nasolabial perforator flap, buccal fat pad, paramedian forehead flap or supraclavicular flap. Distal pedicled flaps such as pectoralis major flap, deltopectoral flap or latissimus dorsi flap can provide enough tissue to close middle size and large soft tissue maxillofacial defects

and usually work very effectively as salvage option in case of complication or loss of the first choice flap.

For large and middle size soft tissue defects in maxillofacial region is usually one of the following microvascular flaps the reconstructive choice: anterolateral thigh (ALT) flap, forearm flap, superficial circumflex iliac artery perforator (SCIP) flap, lateral arm free flap (LAFF) or deep inferior epigastric perforator (DIEP) free flap. In defined middle size or large size soft tissue defects of the tongue, lip or cheek with ambition for functional reconstruction the gracilis free flap is the flap of choice with adequate soft tissue type and volume, but also with simple straightforward harvest and low donor side morbidity. The use of the gracilis muscle *(musculus gracilis)* in reconstructive surgery was first described by Pickrell in 1952 for rectal sphincter reconstruction.[1] With skin island was the gracilis pedicled myocutaneous flap described in 1972 by Orticochea.[2] It was one of the first myocutaneous flaps described in humans. In 1976, Harii published a series of patients reconstructed with free gracilis flaps for soft tissue cover[3] and a functional free gracilis flap for facial reanimation.[4] Gracilis flap has become one of the essential tools of the reconstructive surgeon, as a functional flap, for soft tissue coverage and for contour deformities such as in breast reconstruction. For reconstruction of head and neck defects the free gracilis muscle flap is not widely used, but has the potential to provide good results.[5] Use of the free gracilis muscle flap has gained popularity due to its predictable vascular anatomy and minimal donor-site morbidity.[6] The aim of this paper is to share our experiences with the gracilis muscle flap in reconstructions of orofacial defects.

Functional reconstruction of soft tissue defects of the tongue or lip with microvascular gracilis muscle flap appears to have advantage of adequate volume, straightforward flap harvest technique, acceptable pedicle length and satisfactory vessel diameter with very low donor site morbidity compared to other microvascular flap reconstructive options such as radial forearm flap, anterolateral thigh (ALT) flap, superficial circumflex iliac artery perforator (SCIP) flap, lateral arm free flap (LAFF) or deep inferior epigastric perforator (DIEP) free flap or vertical rectus musculocutaneous (VRAM) flap.

Gracilis muscle takes origin from the symphysis pubis and inserts distally into the medial surface of the proximal tibia. Gracilis is the most superficial muscle of the adductors group of the lower limb. The muscle belly length is approximately 30 cm.[7] Distal end of the muscle lies between sartorius anteriorly and semitendinosus muscle posteriorly. The function of the muscle is to adduct, flex and medially rotate the hip and to flex the knee. The muscle is most commonly raised on the dominant vascular pedicle only, and the entire muscle may be harvested this way. Dominant pedicle is a branch of the medial femoral circumflex artery or direct branch from the deep femoral artery, usually with a caliber of 1 to 2.5 mm. In adults, the main vascular supply enters the gracilis approximately 10 cm inferior to the pubic tubercle. The course of the pedicle runs deep between adductor magnus

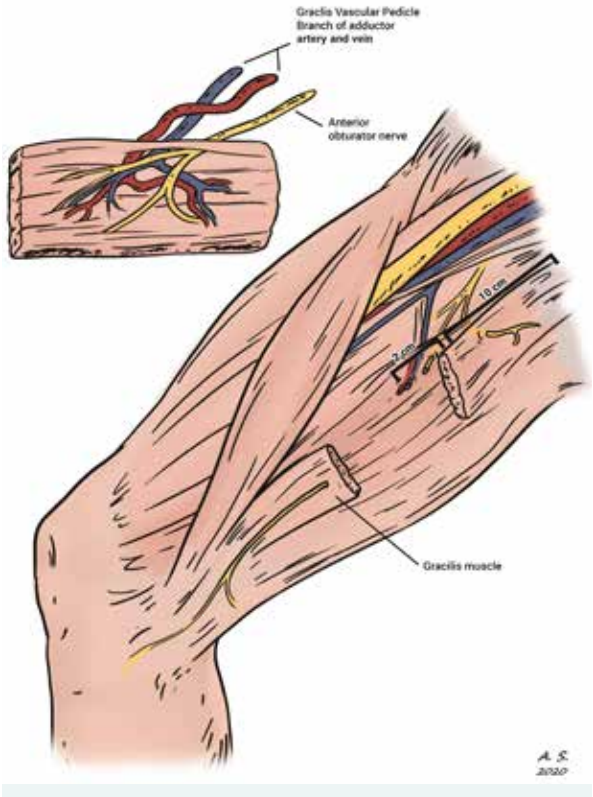

Figure 1. Gracilis flap anatomy (author´s photo archive)

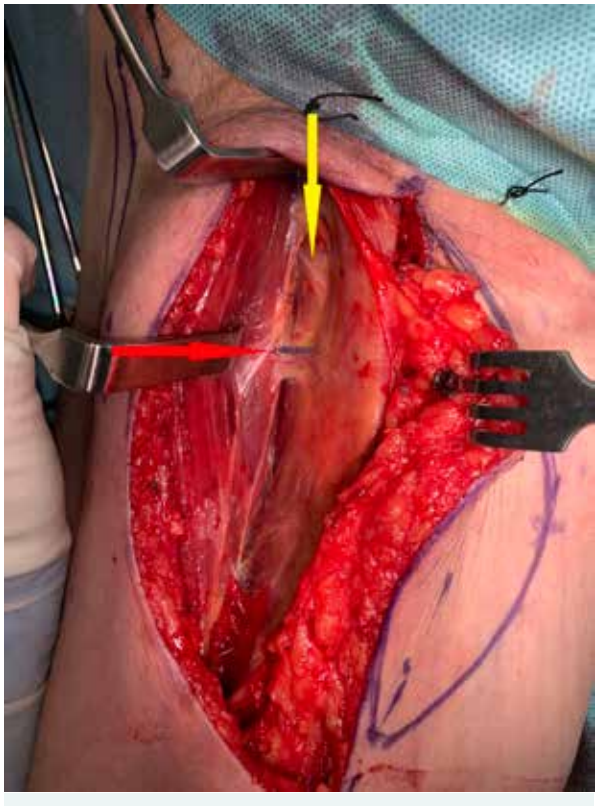

Figure 2. Free gracilis flap harvest with skin island marked, vascular pedicle (red arrow) and anterior branch of obturator nerve -yellow arrow (author´s photo archive)

and longus with median length of 6 cm (5–7 cm). Usually two commitant veins are found accompanying the artery. They drain into the deep femoral vein and may converge to form a larger common vein (3–4 mm diameter), a short distance before entering the deep one. There are usually two secondary segmental pedicles to the gracilis muscle that can be found distally to the main one. They are both ligated and divided when the muscle is harvested. Somatomotoric supply is provided by the anterior branch of the obturator nerve. The nerve originates from the lumbar plexus (L2–L4), runs through the obturator foramen and branches into anterior and posterior divisions beneath the pectineus muscle. The anterior division then travels between adductor longus and brevis (supplying it) before entering the gracilis muscle close to the location of the dominant pedicle. The nerve has a more oblique course proximally than the vascular pedicle and runs 2–3 cm cranially to the pedicle.[8]

Gracilis free flap (Figure 1, 2) is a versatile option for tongue reconstruction from middle size defects (hemiglossectomy) to total tongue defect after glossectomy. Advanced tongue cancer normally treated with total glossectomy with laryngeal preservation (TGLP) has a high risk of severe postoperative morbidity due to the loss of swallowing and articulation. Innervated functional gracilis musculocutaneous flap permits an appropriate dynamic reconstruction, and along with an adequate course of rehabilitation, can provide good swallowing and articulation outcomes, which permits a satisfactory long-term quality of life.[9] Gracilis free flap is the first-line treatment used in facial palsy reanimation, with numerous advantages including low morbidity, a strong motor impulse, high reliability, and fast reinnervation. In this case cross-face facial nerve or ipsilateral masseter nerve are used for neuroanastomosis and reinnervation with excellent results.[10] Proper neuroanastomosis and flap reinnervation are crucial to preserve flap volume and to achieve superior swallowing capacity.[11]

Reconstruction of the lower and upper lip or both lips should meet both aesthetic and functional requirements, which is a major challenge particularly in extensive lip defects requiring microvascular flap reconstruction. Free fasciocutaneous flaps such as composite radial forearm flap or anterolateral thigh flap in conjunction with static tendon slings provide satisfactory results. However, neurovascular gracilis muscle transfer in recent years has been introduced to overcome noncontractile properties of these flaps and to restore oral competence by muscle contractility.[5] The use of gracilis muscle to reconstruct lower lip defects has gained popularity since the study conducted by Ninkovic et al. in 2007; the authors used the gracilis flap arranged with the facial artery musculomucosal flap for the mucosa restoration and used a skin graft harvested from the scalp for external skin.[12] Raising the flap with the overlying skin island allows to avoid morbidity in other donor site, to avoid scar retraction of the skin graft on the gracilis that could limit its movement, and to plan aesthetical refinements such as hair transplantation or tattoo of the beard on the skin.[13] The innervated gracilis muscle transfer became a basic choice for functional lip reconstruction.[14]

MATERIAL AND METHODS

We present recent less than one-year experience with functional reconstructions of middle size orofacial defects (tongue or lip) with free gracilis muscle flap in a group of 5 patients. Four patients with squamous cell carcinoma (SCC) of tongue and one with SCC of lip and cheek underwent radical resection with subsequent immediate functional microvascular gracilis muscle flap reconstruction. All five patients were males, with an average age of 59 years. In the subgroup of 4 patients post tongue resection 2 underwent pure gracilis muscle flap reconstruction only, and the other 2 composite free gracilis flap with skin island reconstruction (myocutaneous flap). Gracilis flap with skin island attached was

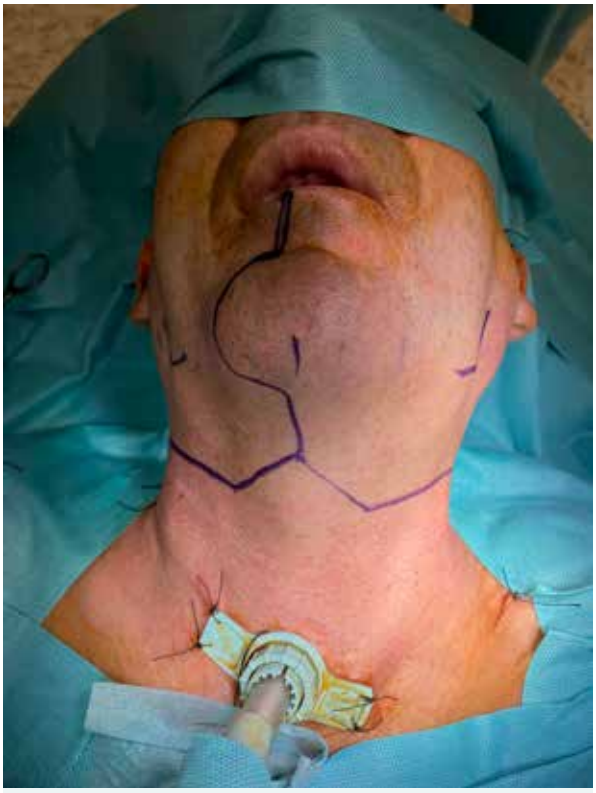

Figure 3. Case 1 – planned skin incision for mandibular split approach to glossectomy (author´s photo archive)

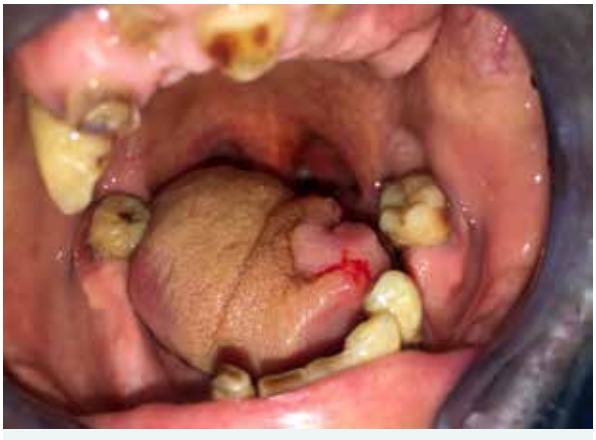

Figure 4. Case 1 – SCC of tongue T4aN2cM0 (author´s photo archive)

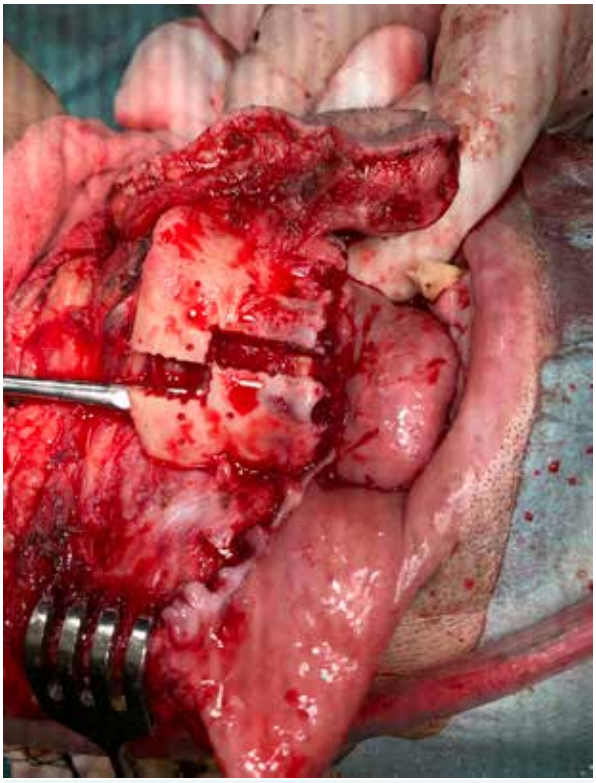

Figure 5. Case 1 – osteotomy for mandibular split approach (author´s photo archive)

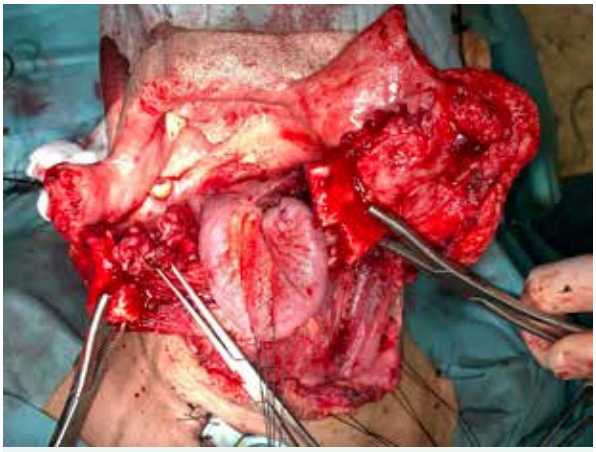

Figure 6. Case 1 – mandibular split approach to glossectomy (author´s photo archive)

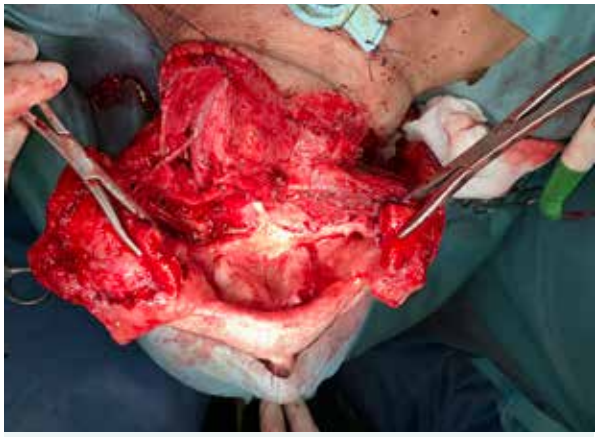

Figure 7. Case 1 – glossectomy defect (author´s photo archive)

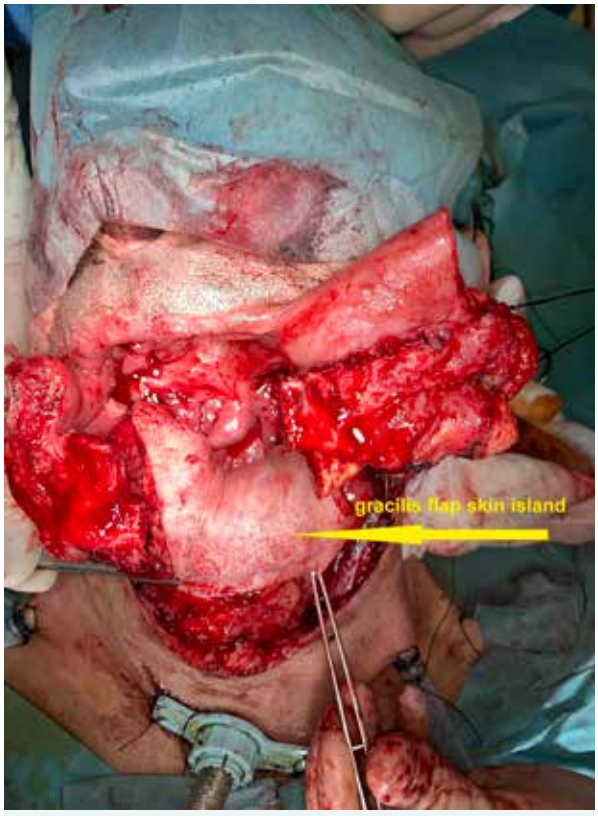

Figure 8. Case 1 – gracilis myocutaenous flap inserted into glossectomy defect, skin island marked with an arrow (author´s photo archive)

preferred for larger tongue defects glossectomy or extended hemiglossectomy. The skin island attached to the gracilis muscle was also used to cover extraoral defect in the single-stage functional reconstruction of subtotal lower lip and unilateral cheek defect with free myocutaneous gracilis flap.

All 5 patients had confirmed SCC in diagnostic biopsy, followed by standard preoperative studies: computed tomography (CT) or magnetic resonance imaging (MRI) of the head and neck, chest X-ray and abdomen ultrasonography (USG) or CT. Microvascular flap reconstruction was planned for all patients with the aim not only for aesthetic, but also for functional reconstruction, therefore radial forearm flap was not a choice. In all 5 patients were the expected post-ablative orofacial defects of middle size, therefore free gracilis muscle was the flap of choice over ALT flap, which would be the second choice in our department.

No special preoperative studies were obtained considering gracilis free flap reconstruction. The donor site was

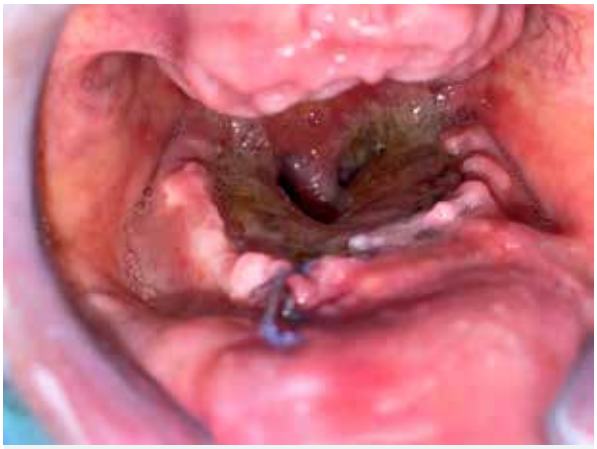

Figure 9. Case 1 – glossectomy defect reconstructed with myocutaneous gracilis flap on discharge (POD 12); (author´s photo archive)

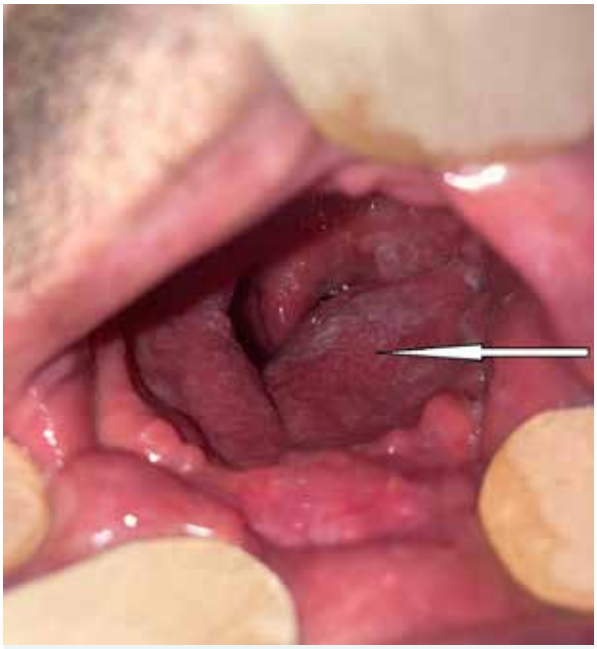

Figure 10. Case 1 – epithelized myocutaneous gracilis flap 6 months after surgery and 3 months after radiochemotherapy (author´s photo archive)

shaved and Doppler ultrasonography was used to mark the femoral artery, and then the branch of the medial femoral circumflex artery, main gracilis muscle vascular pedicle, which is located approximately 10 cm below the pubic tubercle. The obturator nerve is usually located 2 cm (1,5–3cm) above the vascular pedicle. Position of patient during surgery is supine with flexed leg (sole at the level of contralateral knee) – well known as unilateral frog leg position. Contralateral hip is elevated for better surgical fied exposure. The gracilis muscle flap was in 2 cases harvested as a pure muscle flap and in 3 cases as myocutaneous flap with skin island and in all 5 cases as a functional flap with the anterior branch of obturator nerve. The vascular pedicle was dissected to its origin from the medial circumflex femoral vessels or directly to the deep femoral vessel to gain the maximum length.

CASE REPORT 1

A 51-year-old man referred to our department with 6-week history of complaints with tongue pain, speech impairment and dysphagia. On physical examination a tumor of the right tongue margin was noticed and histologically verified as SCC; standard preoperative examinations were performed and tumor was classified as stage T4aN2cM0. The patient was indicated for primary surgical treatment as follows: tracheostomy and bilateral neck dissection regions I–V on the left side and I–III on the right side, radical tumor resection was performed through temporary mandibular split, total glossectomy was performed with tumor-free margin confirmed intraoperatively. Second team simultaneously harvested the gracilis flap with 15 cm muscle length and skin island sized 10 x 6 cm and the defect was immediately reconstructed. The vascular pedicle was anastomosed end-to-end to the recipient left-sided vessels: lingual artery, facial vein and external jugular vein for second venous anastomosis. The obturator nerve was neuroanastomosed to left hypoglossal nerve. During the reconstruction of the tongue the flap was oriented with long axis transversally and skin island intraorally. Patient was discharged home on postoperative day (POD) 12 and underwent adjuvant postoperative radiochemotherapy, due to bilateral neck nodal involvement. (Figure 3–10.)

CASE REPORT 2

A 64-year-old patient referred with primary advanced SCC of the right tongue, with chief complaints of pain, dysphagia and 15 kg weight loss in 7 weeks. Staging CT of the head and neck showed bilateral tumor of tongue staged T3N2 and following whole body (chest and abdomen) CT confirmed M0. Primary surgical treatment was indicated as follows: tracheotomy, bilateral neck dissection in regions I–V, extended hemiglossectomy 1.5 cm into the contralateral left half of tongue to gain negative resection margins in intraoperative histopathology. The second team simultaneously harvested myocutaneous gracilis flap with skin island sized 13 x 6 cm

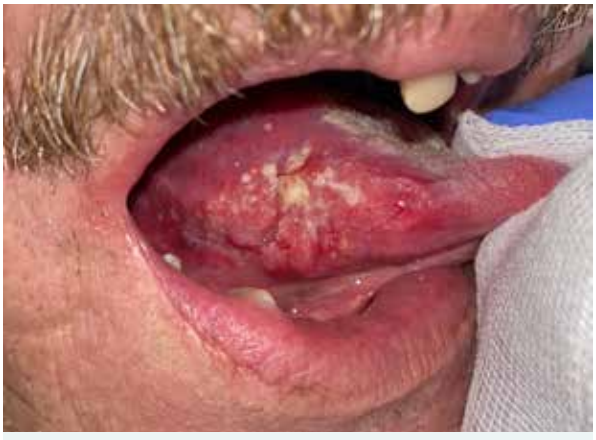

Figure 11. Case 2 – right-sided tongue SCC T3N2M0 (author´s photo archive)

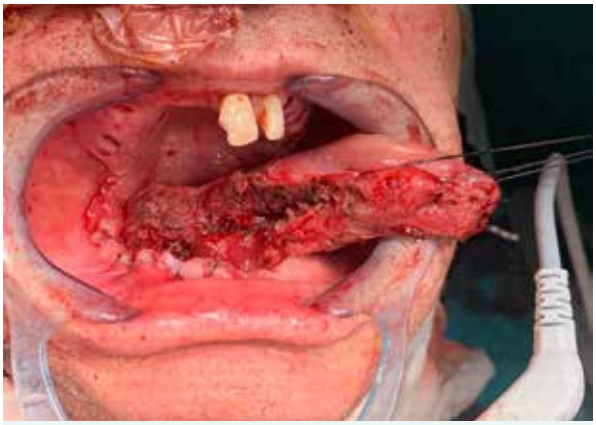

Figure 12. Case 2 – extended hemiglossectomy right side (author´s photo archive)

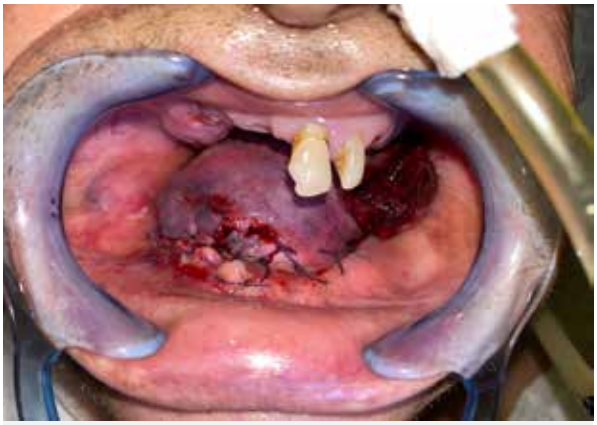

Figure 13. Case 2 – myocutaneous gracilis flap reconstruction with isles of venous congestion and positive pin prick test in medial anterior flap margin (POD 3); (author´s photo archive)

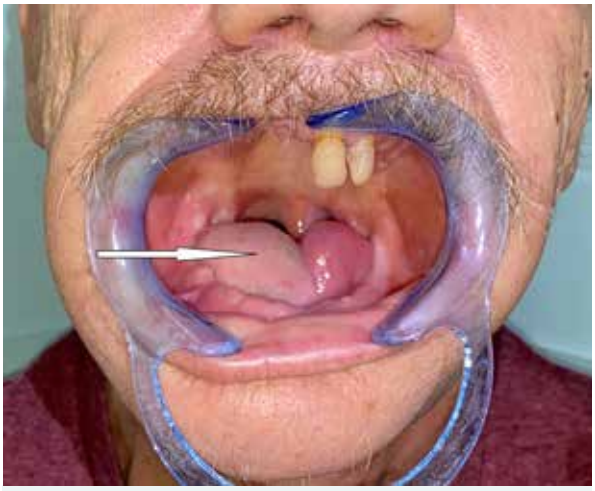

Figure 14. Case 2 – tongue at rest; 6 months post operation, arrow showing myocutaneous gracilis flap (author´s photo archive)

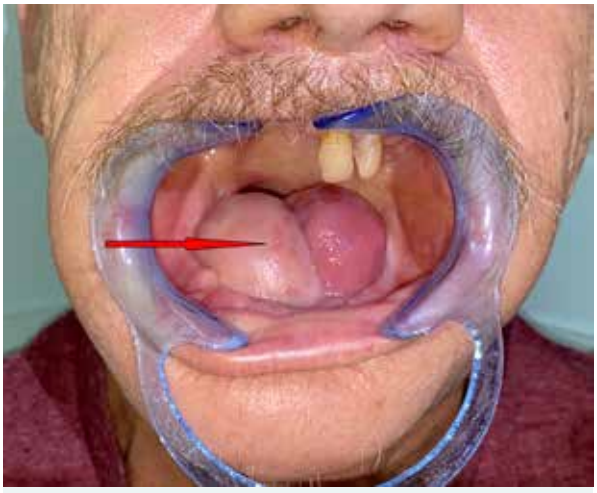

Figure 15. Case 2 – tongue at crawling; 6 months post operation, arrow showing myocutaneous gracilis flap (author´s photo archive)

and muscle length 12 cm. The vascular pedicle was anastomosed end-to-end to recipient right-sided vessels: facial artery, facial vein and superior thyroid vein for second vein anastomosis. The obturator nerve was neuroanastomosed to the right hypoglossal nerve. The gracilis flap was oriented in antero-posterior direction with long axis of muscle during the reconstruction. Partial isles of venous congestion were observed in the skin island of the gracilis flap during first week after surgery without any subsequent wound dehiscence or skin loss. The patient was discharged home POD 11 on oral feeding and despite extensive tumor resection was the patient able to speak and was clearly understood. The patient underwent subsequent adjuvant postoperative radiochemotherapy. (Figure 11–15.)

CASE REPORT 3

54-year-old male referred to our department with second cancer in the head and neck region, currently diagnosed SCC of the left margin of the tongue. The patient was 6 years in remission for oropharyngeal carcinoma (staged T3N2cM0) treated with primary radiochemotherapy, total dose (TD) 70Gy with concomitant cisplatin and fluorouracil (5-FU). Positron emission computed tomography (PET CT) showed a highly FDG-avid left-sided tumor of the tongue, with no local enhancement in oropharynx, neither regional neck nor whole body involvement staged T2N0M0. Salvage surgery was indicated by a tumor board, consisting of tracheostomy, unilateral neck dissection, resection of the left half of tongue (hemiglossectomy) and reconstruction with gracilis flap. The gracilis free muscle flap was harvested simultaneously with the ablative surgery, and the defect was reconstructed with muscle only flap of total length 13cm. The vascular pedicle was anastomosed end-to-end to recipient left-sided vessels: facial artery, facial vein and superior thyroid vein; and the obturator nerve to left hypoglossal nerve. Prolonged neck lymphorrhea was observed during the hospital stay. The patient was discharged POD 10, but came with a dehiscence

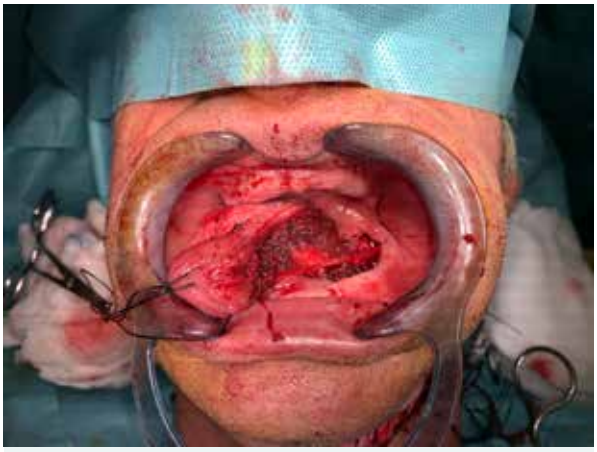

Figure 16. Case 3 – left-sided hemiglossectomy (author´s photo archive)

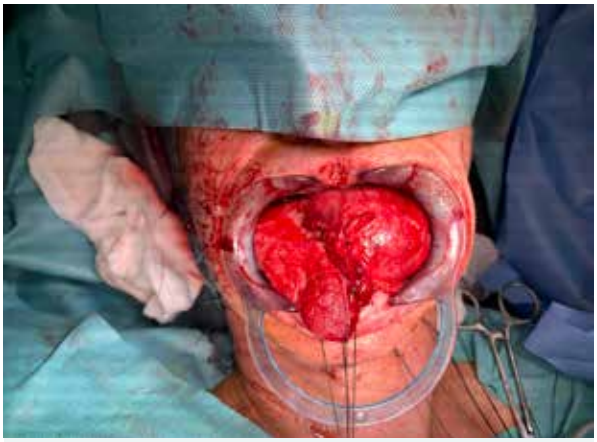

Figure 17. Case 3 – left-sided hemiglossectomy reconstructed with gracilis free flap (author´s photo archive)

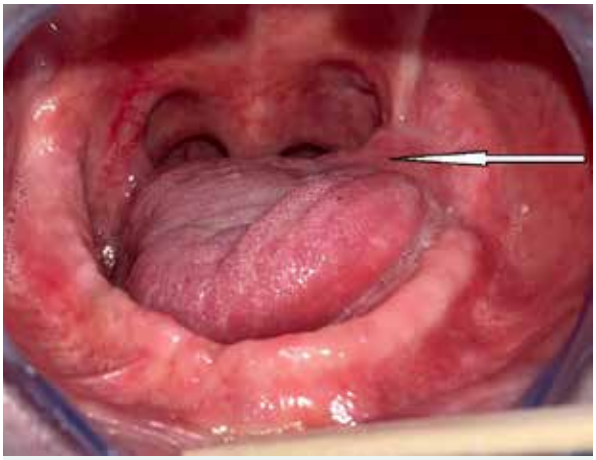

Figure 18. Case 3 – left-sided hemiglossectomy reconstructed with gracilis free flap, 4 months after surgery with significant gracilis flap atrophy, but no orocutaneous fistula (author´s photo archive)

of the wound after neck dissection two weeks post-op. The wound was revised and re-sutured primarily with successful subsequent healing despite previously irradiated neck. The patient was not indicated for adjuvant oncologic treatment and is under meticulous oncologic follow-up with PET/CT planned 3 months after surgery. (Figure 16–18.)

CASE REPORT 4

65-year-old patient presented to our department with rapid growing tumor of the right margin of the tongue, excessive pain, halitosis and impaired swallowing lasting for more than 4 weeks. Histopathology confirmed SCC, the patient subsequently underwent CT of the head and neck and standard preoperative examination and was staged T3N2M0. Primary surgical treatment was indicated by tumor board as follows: tracheotomy, unilateral right sided extended cervical dissection regions I–V, tumor resection

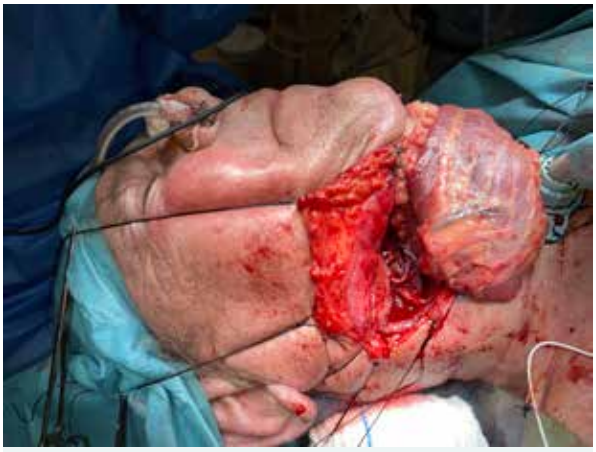

Figure 19. Case 4 – gracilis free flap anastomosed to facial vessels, shortly before relocating flap intraorally for reconstruction (author´s photo archive)

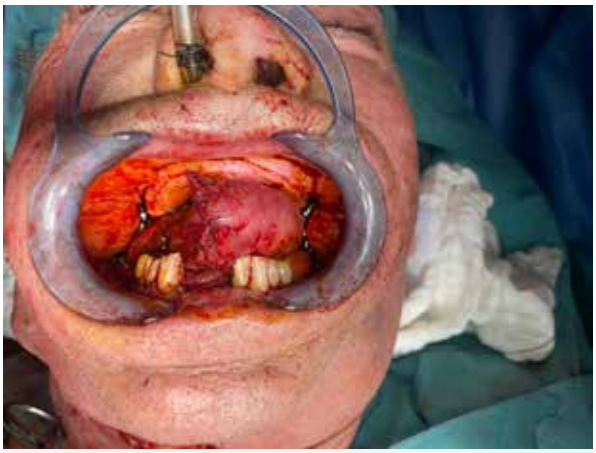

Figure 20. Case 4 – hemiglossectomy defect reconstructed with muscle only gracilis flap (author´s photo archive)

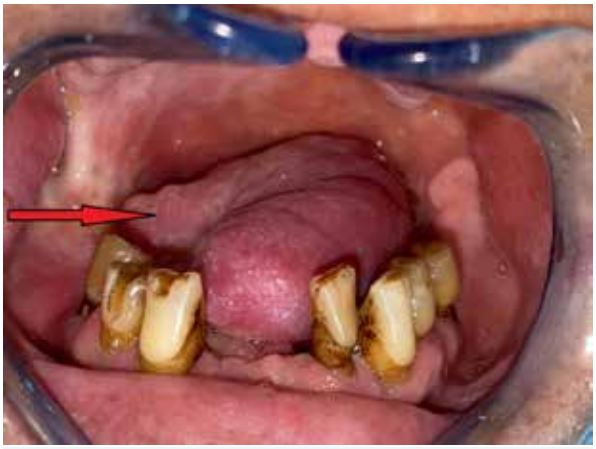

Figure 21. Case 4 – hemiglossectomy defect reconstructed with muscle only gracilis flap 3 months after surgery, the patient finished adjuvant radiochemotherapy, arrow showing mild gracilis flap atrophy (author´s photo archive)

(extended hemiglossectomy) and immediate reconstruction with microvascular gracilis flap without skin island. The vascular pedicle was anastomosed end-to-end to recipient ipsilateral vessels: lingual artery, facial vein and superior thyroid vein. The anterior branch of obturator nerve was neuroanastomosed to the right hypoglossal nerve. Even though the flap showed good vascular perfusion immediately after completing microvascular anastomoses and release of the pedicle, unfortunately soon afterwards the arterial flow decreased and during 20 minutes total arterial occlusion was noticed. The arterial anastomosis was revised with no flow in pedicle artery or in recipient lingual artery. Afterwards the flap artery was reanastomosed successfully to the facial artery with normal perfusion to the flap. After less than 24 hours was the patient transferred from intensive care unit to our ward and subsequent hospital stay was without any other complications. The patient was discharged POD 9 without nasogastric tube, with acceptable speech articulation. The patient was scheduled for adjuvant radiochemotherapy. (Figure 19–21.)

CASE REPORT 5

The last case report concerns a 63-year-old patient with newly diagnosed invasive SCC of the lower lip and left cheek, with clinical staging T4N2 and M0, confirmed by whole body CT staging scan. Standard preoperative exams were performed and primary surgical treatment was indicated. Surgical treatment consisted of tracheostomy, bilateral neck dissection region I-V, subtotal resection of lower lip with partial left cheek resection. All intraoperative frozen sections' resection margins were clear and the reconstruction with simultaneously harvested myocutaneous gracilis flap followed. The gracilis free flap was harvested with 18cm muscle length and skin island size of 15 x 8 cm. The whole skin island was used for extraoral defect coverage and intraorally was left uncovered gracilis muscle for secondary epithelization. No partial split thickness skin graft was used. The anterior branch of obturator nerve was anastomosed to marginal branch of left facial nerve. The vascular pedicle was anastomosed end-to-end to left sided recipient vessels: facial artery, facial vein and submental vein for second vein anastomosis. During second postoperative day a partial venostasis in skin island was observed and hirudotherapy was indicated with a temporary effect. Marginal right sided skin island necrosis was treated with debridement and primary resuture under local anesthesia. The patient was discharged on POD 15 and scheduled for adjuvant radiochemotherapy due to advanced cancer stage pT4N2cM0. (Figure 22–25.)

RESULTS

All five patients with oral or facial defects were successfully reconstructed with functional gracilis muscle flap, with no flap loss. We found free gracilis muscle flap harvest not to be technically demanding, with the possibility for skin island harvest if needed, and simple primary closure of donor site with very low morbidity. Hand held Doppler USG was always used preoperatively to mark the gracilis vascular pedicle position, which corresponded

Figure 22. Case 5 – lower lip and left cheek SCC staged T4N2M0 (author´s photo archive)

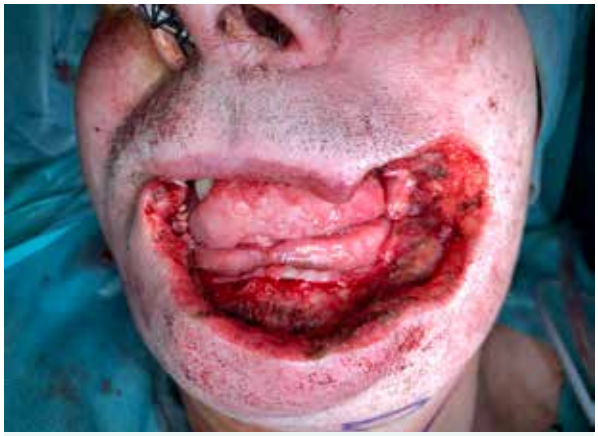

Figure 23. Case 5 – defect after subtotal lower lip and partial left cheek resection (author´s photo archive)

Figure 24. Case 5 – lower lip and cheek defect reconstruction with free gracilis myocutaneous flap (author´s photo archive)

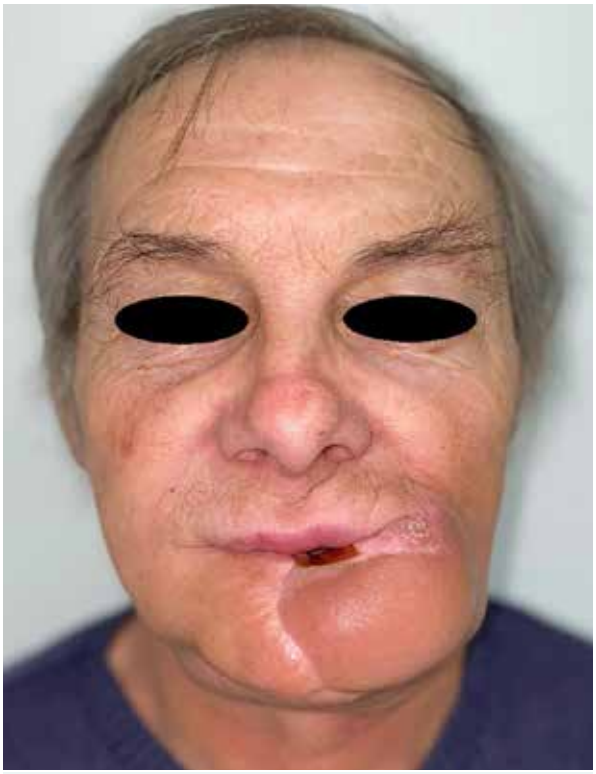

Figure 25. Case 5 – lower lip and cheek defect reconstruction with free gracilis myocutaneous flap 5 months after surgery and 3 months after radiochemotherapy with mild flap atrophy (author´s photo archive)

accurately with the real position during the surgery in all five cases. The donor site was always closed primarily with one redon drain and no major donor site morbidity was observed. In two flaps we observed minor partial skin island venous congestion and hirudotherapy was used successfully in one flap. The second skin island needed surgical debridement with primary re-suture. No wound dehiscence nor orofacial fistula was observed.

DISCUSSION

Radical surgery should be the primary treatment modality for most resectable oral cavity cancers and for T4a laryngeal/hypopharyngeal cancers and preferred modality of treatment for most early (T1–T2, N0) laryngeal and hypo/oropharyngeal carcinomas, because this strategy offers an opportunity to reserve radiotherapy for a potential recurrence or second primary tumor.[15] Primary surgical treatment consists of radical resection and adequate reconstruction, both equally important. With locally advanced cancer (T3, T4) reconstruction is the prerequisite for successful radical resection. Flaps in general, both pedicled and microvascular, are the gold standard for middle size and large maxillofacial defects reconstruction, which allows to treat radically even locally advanced cancer stages considered in the past as inoperable. In general, successful radical primary surgery for head and neck cancer provides the patients with higher 5-year survival rates and better quality of life.

The potential complications of microvascular flap reconstruction in general are free flap loss, scar formation in donor or recipient site, donor site morbidity, sensorial loss. Special consideration for lip reconstruction include microstomia, loss of oral competence and loss of gingivobuccal sulcus and oral mucosa.[15]

According to literature, overall complication rate for free gracilis flap may be underreported and be even 9.6% with the two most commonly occurring complications: postoperative hematoma (3.6%) and infection (3.5%).[16]

Ablative orofacial defects incorporating mimic facial musculature/nerve cause expressive dysfunction and considerable morbidity. The disrupted branches of facial nerve provided a source for functional free muscle flap reinnervation. Reconstruction of oncologic defects including expressive facial musculature/nerve with gracilis free functioning muscle transfer can restore oral continence and facial expression primarily.[17]

Objective assessment of smile outcome after microvascular free gracilis transfer is challenging, and quantification of smile outcomes in the literature is inconsistent; however oral commissure excursion and facial symmetry both at rest and when smiling are evaluated.[18] Terzis score for facial mimic movements´ assessment can be used. Standardizing follow-up schedule, assessing spontaneity in an objective and reproducible fashion, and use of consistent outcome measures would allow for future meta-analyses and better understanding of the options for functional reconstruction.[19]

Extensive defects of both lower and upper lip and commissure may require reconstruction with a free gracilis muscle combined with a forearm flap transfer for better functional result and good oral sphincter function for eating, speaking and air inspiration.[20] Prefabricated gracilis flap can be used for extensive lip and commissure defects. Ueda et al. reported total lower lip reconstruction in an 18-month-old boy following a dog bite. In order to obtain an optimal functional result and to avoid any additional facial scarring, a prefabricated gracilis muscle free flap was used in a two-stage procedure. This allowed both lip occlusion and normal speech development to be restored without any impairment of mandibular growth during a 4-year follow-up.[21]

Functional reconstruction of soft tissue defects of tongue or lip with microvascular gracilis muscle flap appears to have advantage of adequate volume and very low donor site morbidity compared to other microvascular flap reconstructive options, such as anterolateral thigh (ALT) flap, superficial circumflex iliac artery perforator (SCIP) flap, lateral arm free flap (LAFF) or deep inferior epigastric perforator (DIEP) free flap. Reinervation of microvascular gracilis flap should restore the movement also in the reconstructed part of the tongue or lip. In contrast, if the tongue or lip defect is reconstructed without innervated soft tissue flap with muscle component (either pedicled or microvacular), the function will be carried only by surrounding innervated healthy tissue.

CONCLUSION

Currently, head and neck reconstructive surgery is focused not only on defect occlusion and adequate aesthetic result, but the same emphasize is aimed at functional result. Functional result post tongue and lip resection means defect occlusion with the possibility of movement restoration. Microvascular gracilis muscle flap reconstruction compared to radial forearm flap reconstruction enables functional reconstruction of orofacial soft tissue defect. In all 5 patients the free gracilis flap healed primarily with adequate volume of tissue for the defect occlusion. Patients after tongue reconstruction were swallowing spoon food and oral competence was present in the patient after total lip resection before the discharge. Free gracilis muscle flap appears to be a choice for functional tongue and lip reconstruction. The functional result for the group of our patients will be evaluated each 6 months after surgery and finally evaluated after 2 years post operation considering the ability to swallow and articulate during the speech for the tongue reconstruction and the oral competence and facial mimic movements´ assessment (Terzis score) for the lip reconstruction. Post-operative rating of the gracilis flap function and the range and rate of atrophy will be the subject of further scientific evaluation.

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Disclosure: The authors declare that there is no conflict of interest.

This statement is to certify that all Authors have seen and approved the manuscript being submitted. We warrant that the article is the Authors´ original work. We warrant that the article has not received prior publication and is not under consideration for publication elsewhere. On behalf of all Co-Authors, the corresponding Author shall bear full responsibility for the submission.

We declare that this study has received no financial support. All procedures performed in this study involving human participants were in accordance with ethical standards of the institutional and/or national research committee and with the Helsinki declaration and its later amendments or comparable ethical standards

REFERENCES

1. Pickrell KL., Broadbent TR., Masters FW., Metzger JT**.** Construction of a rectal sphincter and restoration of anal continence by transplanting the gracilis muscle; a report of four cases in children. *Ann Surg.* 1952, 135:853–62.
2. Orticochea M. The musculo-cutaneous flap method: an immediate and heroic substitute for the method of delay. *Br J Plast Surg.* 1972, 25:106–10.
3. Harii K., Ohmori K., Sekiguchi J. The free musculocutaneous flap. *Plast Reconstr Surg.* 1976, 57:294–303.
4. Harii K., Ohmori K., Torii S**.** Free gracilis muscle transplantation, with microneurovascular anastomoses for the treatment of facial paralysis. A preliminary report. *Plast Reconstr Surg.* 1976, 57:133–143.
5. Del Frari B., Schoeller T., Wechselberger G. Reconstruction of large head and neck deformities: experience with free gracilis muscle and myocutaneous flaps. *Microsurgery.* 2010, 30:192–8.
6. Kropf N., Cordeiro CN., McCarthy CM., Hu QY., Cordeiro PG**.** The vertically oriented free myocutaneous gracilis flap in head and neck reconstruction. *Ann Plast Surg.* 2008, 61:632–6.
7. Macchi V., Vigato E., Porzionato A., Tiengo C., Stecco C., Parenti A., Morra A., Bassetto F., Mazzoleni F., De Caro R. The gracilis muscle and its use in clinical reconstruction: an anatomical, embryological, and radiological study. *Clin Anat.* 2008, 21:696–704.
8. Wei FC, Mardini S**.** *Flaps and reconstructive surgery.* Taipei: Elsevier, 2017. 872 p. ISBN 978-0-323-24322-3.
9. Righini S., Festa BM., Bonanno MC., Colombo V., Luca N. Dynamic Tongue Reconstruction With Innervated Gracilis Musculocutaneos Flap After Total Glossectomy. *Laryngoscope.* 2019, 129:76–81.
10. Oh TS., Kim HB., Choi JW., Jeong WS**.** Facial reanimation with masseter nerve-innervated free gracilis muscle transfer in established facial palsy patients. *Arch Plast Surg.* 2019, 46:122–8.
11. Jeong WH., Lee WJ., Roh TS., Lew DH., Yun IS**.** Long-term Functional Outcomes After Total Tongue Reconstruction: Consideration of Flap Types, Volume, and Functional Results. *Microsurgery.* 2017, 37:190–6.
12. Ninkovic M., Spanio di Spilimbergo S., Ninkovic M. Lower lip reconstruction: introduction of a new procedure using a functioning gracilis muscle free flap. *Plast Reconstr Surg.* 2007, 119:1472–80.
13. Spaggiari A., Benanti E., Starnoni M., Sala P., Baccarani A., Santis G**.** Lower Lip and Chin Reconstruction with Functional Myocutaneous Gracilis Flap. *Indian J Plast Surg.* 2019, 52:242–5.
14. Sacak B., Gurunluoglu R**.** The Innervated Gracilis Muscle for Microsurgical Functional Lip Reconstruction: Review of the Literature. *Ann Plast Surg.* 2015, 74:204–9.
15. Bozec A., Culié D., Poissonnet G., Dassonville O. Current role of primary surgical treatment in patients with head and neck squamous cell carcinoma. *Curr Opin Oncol.* 2019, 31:138–45.
16. Kuriakose MA. *Contemporary Oral Oncology.* Buffalo, USA: Springer, 2017. 380 p. ISBN 978-3-319-43852-8.
17. Garcia RM., Gosain AK., Zenn MR., Marcus JR**.** Early Postoperative

Complications following Gracilis Free Muscle Transfer for Facial Reanimation: A Systematic Review and Pooled Data Analysis. *J Reconstr Microsurg.* 2015, 31:558–64.
18. Lin CH., Wallace C., Liao CT**.** Functioning free gracilis myocutaneous flap transfer provides a reliable single-stage facial reconstruction and reanimation following tumor ablation. *Plast Reconstr Surg.* 2011, 128:687–96.
19. Bhama PK., Weinberg JS., Lindsay RW., Hohman MH., Cheney ML., Hadlock TA**.** Objective outcomes analysis following microvascular gracilis transfer for facial reanimation: a review of 10 years' experience. *JAMA Facial Plast Surg.* 2014, 16 :85–92.
20**.** Vila PM., Kallogjeri D., Yaeger LH., Chi JJ. Powering the Gracilis for Facial Reanimation: A Systematic Review and Meta-analysis of Outcomes Based on Donor Nerve. *JAMA Otolaryngol Head Neck Surg.* 2020, 146:429–36. 26:e200065. doi: 10.1001/jamaoto.2020.0065. Online ahead of print, 2020.
21. Ueda K., Oba S., Nakai K., Okada M., Kurokawa N., Nuri T. Functional Reconstruction of the Upper and Lower Lips and Commissure With a Forearm Flap Combined With a Free Gracilis Muscle Transfer. *J Plast Reconstr Aesthet Surg.* 2009, 62:337–40.
22. Lengelé BG., Testelin S., Bayet B., Devauchelle B**.** Total lower lip functional reconstruction with a prefabricated gracilis muscle free flap. *Int J Oral Maxillofac Surg.* 2004, 33:396–401.

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DERMAL REPLACEMENT WITH MATRIDERM ® – FIRST EXPERIENCE AT THE PRAGUE BURN CENTRE

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ABSTRACT

Introduction. The quality of resulting scar tissue plays an important role in patients' return to normal life and full functioning in society. The use of artificial skin substitutes in clinical practice improves functional and cosmetic outcomes. This is true for any patient, and not only those suffering from burns.
Material and methods. The collagen elastin dermal substitute Matriderm® allows for immediate application of a dermal substitute together with a skin graft. The authors present a group of 10 patients representing their first experience in utilizing Matriderm® as a dermal substitute in the treatment of skin losses due to various etiologies.
Results. The average healing time in the group was 19.6 days. Healing took place without serious infectious complications and with good functional results.
Conclusion. Matriderm® can be utilized as an alternative to the most commonly used dermal substitute so far, Integra®, in the treatment of acute skin loss due to various etiologies and in reconstructive surgery.

KEYWORDS

Skin substitutes; artificial dermis; split--thickness skin graft

INTRODUCTION

In recent decades, we have witnessed declining mortality and morbidity in burn patients[1]. Already in 2012, for example, Kraft et al. published a paper on changing mortality in children treated in burn centers relative to the extent of total body surface area (TBSA) burned. Whereas 40% TBSA burned was formerly associated with a high risk of mortality, today that figure is more like 60%[2]. Improved survival of burn patients is attributed not only to the centralization of patients and to the advances in intensive and pre-hospital care, but also to considerable developments in surgical therapy. One of the pillars of modern surgical intervention is the use of artificial skin grafts that can provide quick compensation for skin defects and lead to excellent functional and aesthetic characteristics of the resulting scars. At present, there exists an extensive selection of products on the market. The most widely used product in the world is the dermal substitute Integra® (Integra LifeSciences, Plainsboro, NJ, USA), with which our unit has the most practical experience[3]. Since the year 2003, we have performed a total of 59 applications utilizing Integra®. The greatest disadvantages of its use include the maturation time, which is approximately 3–4 weeks, and the necessity for subsequent application of an ultrathin dermoepidermal skin graft in a second operation[4]. The development of new and especially thinner materials gradually enabled using a prosthesis in a single operation along with a dermoepidermal graft. The primary representative of this trend in the development of skin substitutes is the Matriderm® dermal template (MedSkin Solutions Dr. Suwelack, Billerbeck, Germany).

The aim of the presented paper is to present the first experience with the application of Matriderm® at our centre during the period 2017–2020.

MATERIAL AND METHODS

Patients

At our centre, we used Matriderm® from January 2018 to June 2020 in a total of 10 patients; 90% of whom were children. The average age in the group of children was 4 years, and 60% of the patients were males, while 40% were females. One patient was electively operated due to scar contracture. Seven patients suffered from a thermal injury, one child suffered from a rare congenital Volkmann´s ischemic contracture, and one child sustained a loss of a finger due to an injury of non-burn etiology. Matriderm® was used in one patient during the treatment of a skin defect after a meningococcal sepsis. In nine cases was the material used on the limbs, and in one patient we used Matriderm® on the face (see Figure 3). Seven patients were treated at our unit, the child with a loss of a finger in cooperation with the Departmend

of Plastic Surgery3 FM CU and UHKV, and two children (a patient after meningococcal sepsis and a newborn with congenital skin ischemia) at the Department of Pediatric Surgery and Traumatology, 3rd Medical Faculty, Charles University and Thomayer Hospital in Prague.

Technique of application

Matriderm® is a dermal prosthesis consisting in a three-dimensional highly porous matrix composed of collagen I, III and V (originating from bovine dermis) and elastin (obtained by hydrolysis from bovine nuchal cartilage). A dermal matrix with a thickness of 1 mm serves as a scaffold for fibroblast proliferation and rapid angiogenesis. Elastin promotes rapid capillary growth and formation of elastic fibers. The highly porous matrix enables diffusion of nutritional factors and subsequent rapid vascularization as a condition for the survival of a thin skin graft applied to the wound bed at the same time as a dermal substitute[5]. By its design, Matriderm® has also a hemostatic potential. It is always supplied in a sterile form (sterilization by gamma irradiation). It is easy to handle and does not require special storage or preparation[6]. Once removed from the package, Matriderm® is applied to the wound and moistened with sterile physiological saline. This changes the material's consistency and provides better adherence to the base of the wound. A thin dermoepidermal graft of 0.15–0.3 mm and harvested with a Zimmer® dermatome (Zimmer Germany, Freiburg, Germany) is immediately applied to its surface. In order to achieve the best possible cosmetic result, it is important not to expand the grafts by meshing. The transplant often needs to be fixed with sutures at the edge and at the bottom of the wound. Tulle gras, antiseptic dressing, and other sterile non-adhesive dressing are applied on the graft to facilitate its efficacy. The same conditions apply to the successful application of Matriderm® as in the cases of other skin substitutes or skin transplants. The wound must be treated after complete excision and be without infection and hematomas. The greatest danger is the presence of bacteria that cause hydrolysis of collagen matrix of the prosthesis, blocking angiogenesis and preventing its engraftment[7]. The main indications are acute skin losses at predilection sites with high risk of contractures, such as hand dorsum and defects extending to deep structures[8]. Matriderm® is advantageously used in reconstructive surgery to treat scarring contractures[9]. It can also be used, however, for skin losses due to other etiologies, such as chronic wounds or defects after tumor excision[10]. The main disadvantages include its high cost and risk of infectious complications, especially when used in the acute phase of trauma[11]. (Figure 1.)

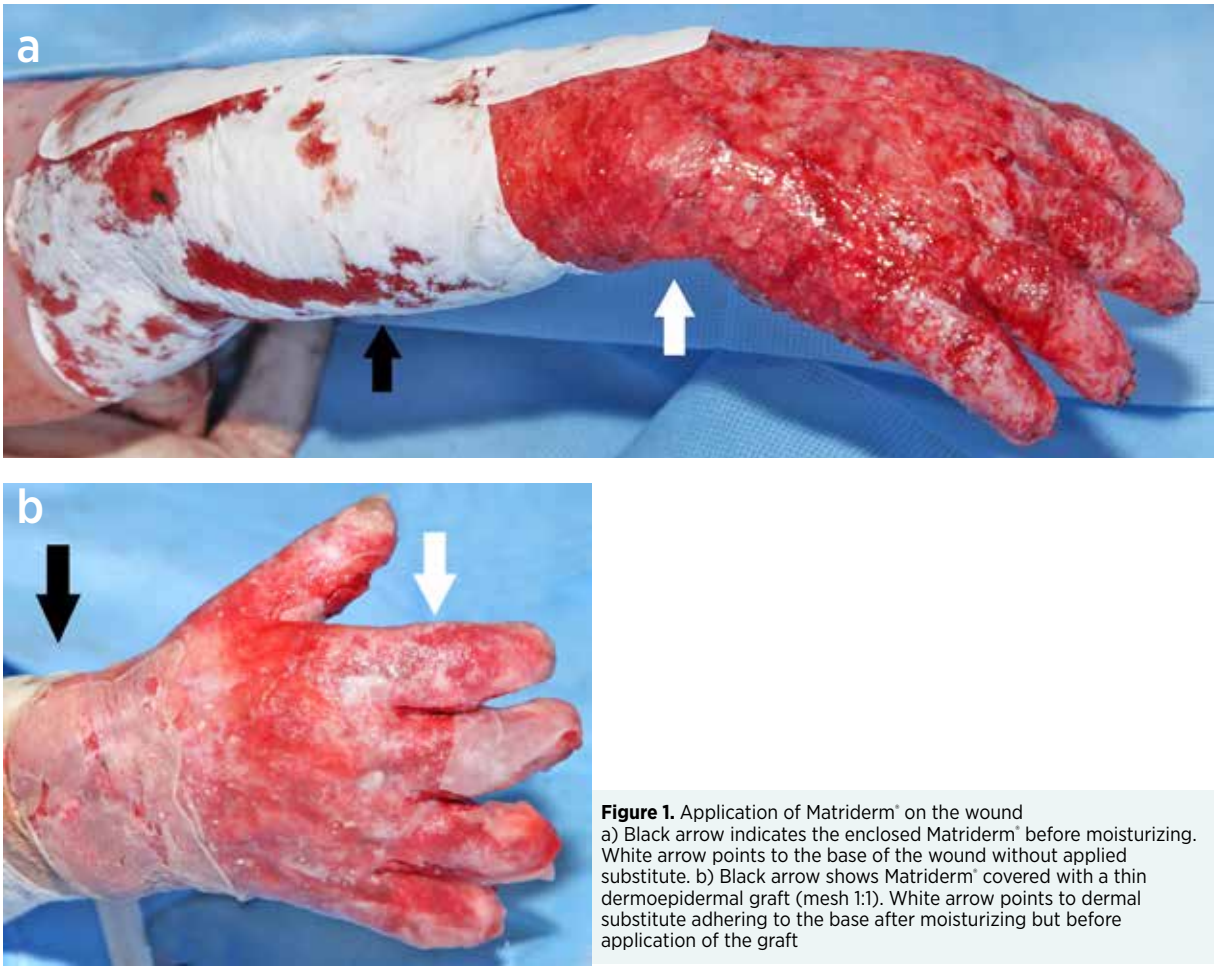

Figure 1. Application of Matriderm® on the wound
a) Black arrow indicates the enclosed Matriderm® before moisturizing. White arrow points to the base of the wound without applied substitute. b) Black arrow shows Matriderm® covered with a thin dermoepidermal graft (mesh 1:1). White arrow points to dermal substitute adhering to the base after moisturizing but before application of the graft

Patient No.	Age (years)	Sex	Indication	Localisation	ATB	Healing time (days post-op)	Complications
1	8	F	Scar contracture	Dorsum of foot	+	24	Mechanical defects
2	14	M	Skin defect within scar	Cubitus	+	15	-
3	24	M	Contact burn	Dorsum of hand	+	20	-
4	8	F	Electric burn	Face, hands	+	18	-
5	2,5	M	Electric burn	Thumb	-	18	Local infection
6	1,5	F	Contact burn	Dorsum of hand, fingers	+	20	-
7	2	M	Fire burn	Hands, forearms	+	19	Mechanical defects
8	0	M	Congenital ischemic contracture	Forearm	+	24	-
9	2	F	Meningococcal sepsis	Achilles tendon region	+	22	-
10	2	M	Amputation	Finger	-	16	-

Table 1. Patients treated with dermal substitute Matriderm®, 2018–2020

RESULTS

The average time for complete attachment of the dermoepidermal graft to the skin matrix was 19.6 days in our group. We defined the time of complete healing as ≥95% healing of a skin graft as assessed by a specialist. Perioperative antibiotics were administered in 80% of our patients. Complications occurred in 3 patients. In 2 cases there was mechanical damage to the artificial prosthesis or transplant, while in 1 case the area was locally infected (*Staphylococcus aureus*) without the need for additional transplantation. (Table 1, Figure 2, Figure 3.)

DISCUSSION

The treatment of burns is based on a comprehensive approach, and the goals include not only to avoid mortality but also to the improvement of long-term quality of life of burn patients. The quality of scar tissue, in particular, plays a key role in the return of patients to normal life and full functioning in society[12]. The introduction of artificial skin prostheses into clinical practice is improving the functional and cosmetic outcomes in burn patients[13]. The most widely used prosthesis worldwide is the bilaminar dermal substitute Integra®, which is supplied with a silicone layer (functioning as a temporary epidermal cover) and its use has saved the lives of many, especially extensively burned, patients[14]. After successful vascularization is Integra® providing temporary fully-sealed occlusion until the size of the harvest area allows repeated harvest of dermoepidermal grafts so that complete coverage of all areas was achieved.

Moreover, new materials, such as the single-layer dermal substitute Matriderm®, which enables immediate coverage with a dermoepidermal graft in a single operation, are gradually being introduced to our clinical practice. In our opinion, the so-called "one-stage procedure" has indisputable advantages: it reduces the number of operations while diminishing the risk of infection, costs of treatment, and length of hospital stay. A disadvantage of this strategy is the need for an immediate application of the graft when we treat a large burn area with a limited donor site for skin harvest. For burn patients, we prefer Matriderm® in risk areas of limited extent, the treatment of which would otherwise be associated with greatest functional and cosmetic consequences. This corresponds to the operated sites in our patients' group. We have successfully applied Matriderm® to areas with exposed deep structures such as the tendons. The main indication for the use of Matriderm®, however, is in reconstructive procedures, and not only in cases of burn trauma[15,16]. Published studies have shown mainly high elasticity of the resulting scars after the use of the dermal substitute Matriderm®[17]. At present, there are alternative products available on the market, such as the Integra Dermal Regeneration Template Single Layer® (Integra LifeSciences), which consists of 1.3 mm thick porous collagen and chondroitin-6-sulphate. Experimental work on an animal model, however, shows comparable biological properties during graft adhesion between Matriderm® and the Integra® single layer. Matriderm®, however, has a higher degree of biodegradability, probably due to its lower density at a thickness of 1 mm[18]. Clinical data comparing long-term results with different substitutes are not yet available.

The greatest risk of dermal substitutes consists of infectious complications. In clinical practice and when using Integra®, we often encounter complications in the late stages of maturation at the edges of the silicone foil. With Matriderm®, this problem is eliminated by a single application. We did not notice any more serious infectious complications in our small patients' group. Only one patient developed a local wound infection (*S. aureus*), which we managed by topical means and without the need for additional transplantation. Eighty percent of the patients in the cohort were perioperatively treated with antibiotics according to the culture from the area. Prophylactic antibiotics admin-

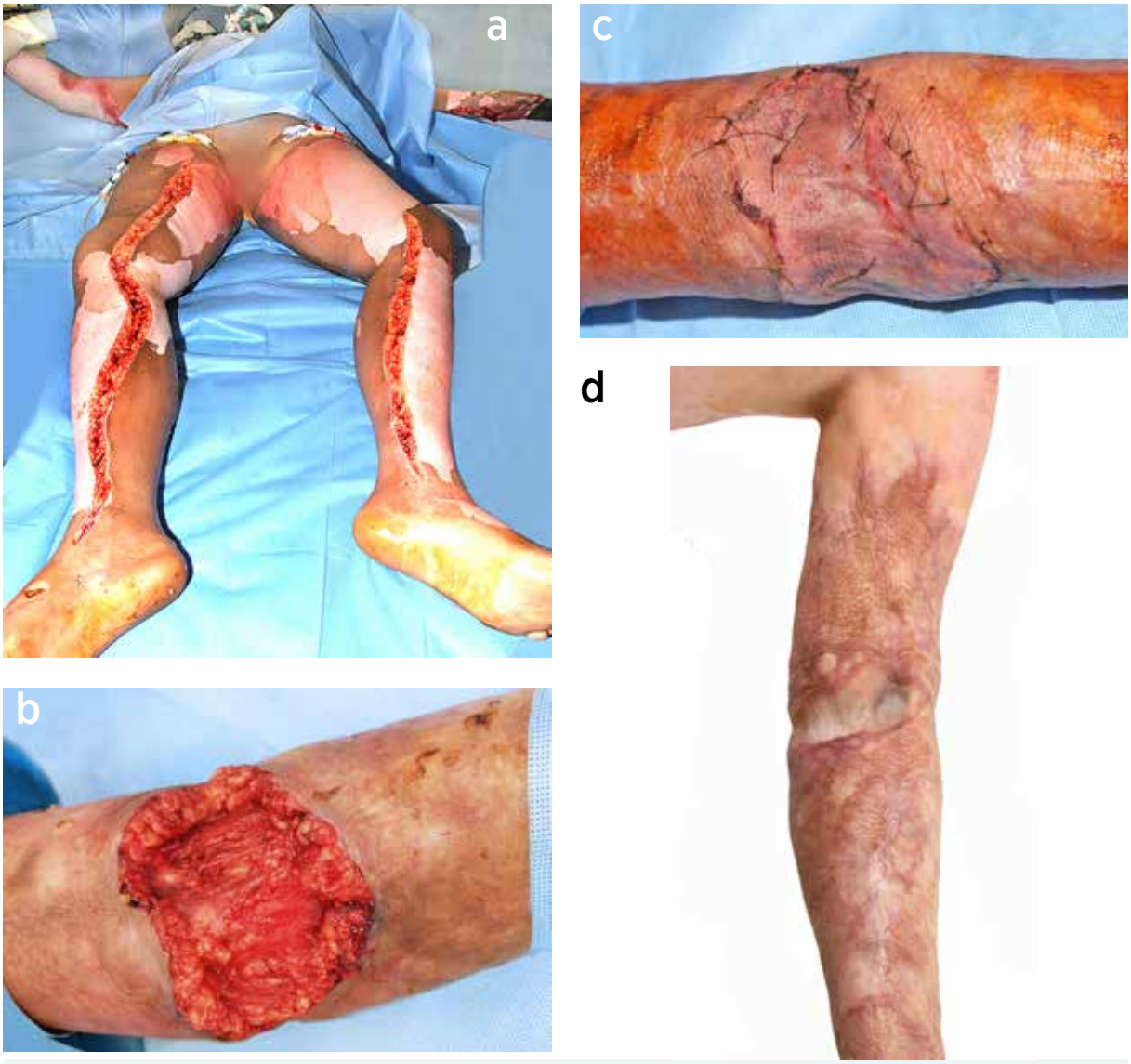

Figure 2. Application of Matriderm® in critical burn trauma of a child
a) A 13-year-old patient with 80% TBSA involvement with 3rd degree burns, as a result of clothing ignition. b) Despite intensive rehabilitation, a contracture formed during hospitalization in the area of the left elbow, and this impaired movements. During mobilization in the operating room, the area in the elbow was reopened. The resulting defect of approximately 9 × 7 cm was treated using Matriderm® and a skin graft. c) 15 days after transplantation; the area is completely healed. d)Condition 3 months after discharge. Since that time, no contracture has developed in the elbow and the patient is capable of fully extending the elbow joint

istration is generally not recommended in burn patients[19]. Indications for systemic antibiotic treatment in our group of patients were other infectious complications in the context of their underlying diseases. A widely debated issue relates to the adhesion of a thin dermoepidermal graft to the neodermis (after application of the dermal matrix). Graft take depends on the ingrowth of blood vessels from the base and interposition material (such as dermal matrix) could interfere with vascularization of the graft. The porous structure and presence of elastin enable rapid vascularization without affecting the success of the dermoepidermal graft healing. Published data agree with our interim results[20], whereby we observed no serious problems with graft vascularization to the neodermis. Our earliest experience has shown that it is necessary to allow longer period for graft healing. The average time of complete graft healing in our group was 19.6 days, which is longer than reported in the literature[8]. We explain the difference by the fact that we used the substitute for complicated injuries or diseases (e.g., meningococcal sepsis) in places with limited perfusion, such as a skin defect based on ischemia in utero. Another problem related to fragility of the healed area several weeks after complete healing. This corresponds to prolonged vascularization of the graft until there is a firm connection between the graft and the neodermis. In one patient out of 10, we had to supplement a minor part of the graft with a second transplant due to

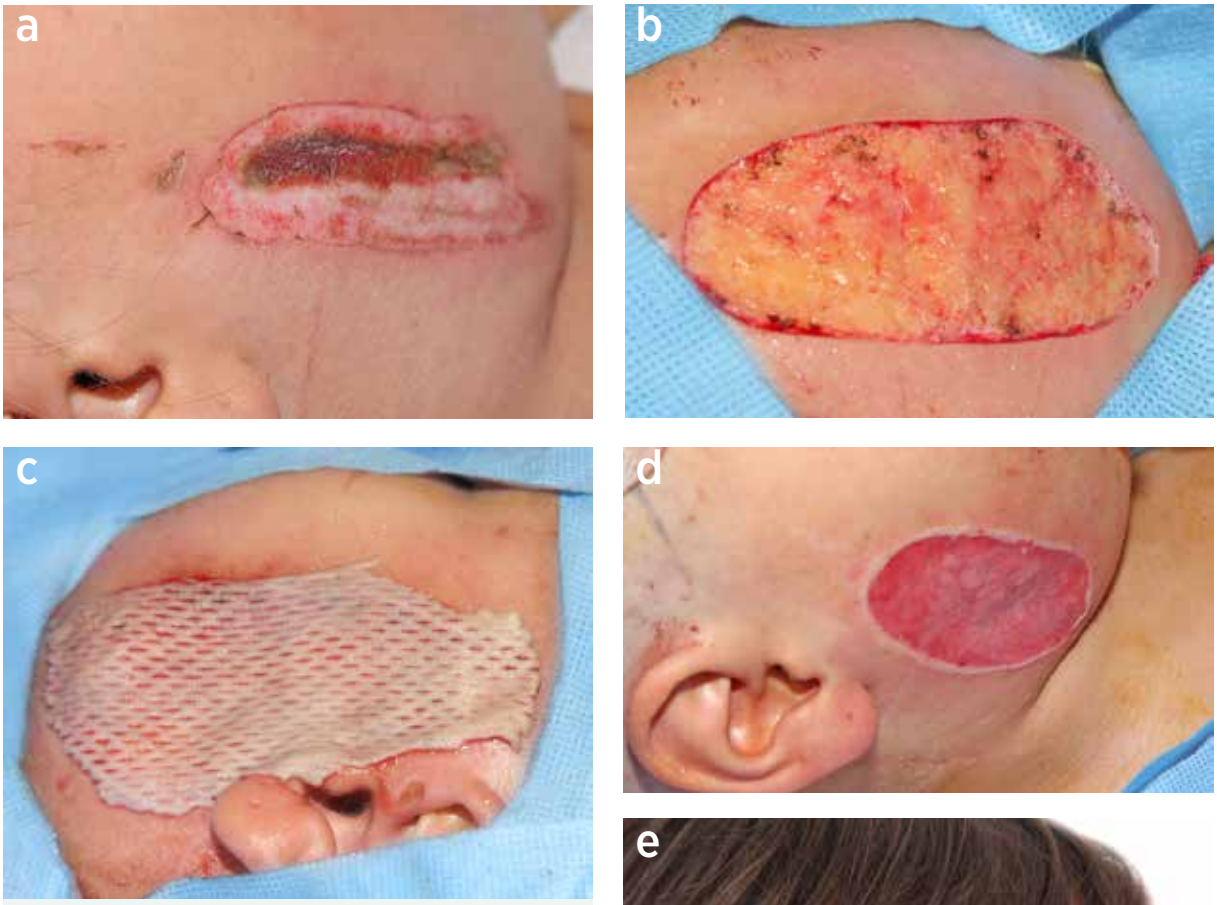

Figure 3. Use of Matriderm® in an electric shock injury in an 8-year-old child
a) Full thickness skin loss of the face after electric injury. b) Wound after excision, 3rd day post trauma. c) Temporary coverage of the defect with a glycerolized allograft. d) After placement of the allograft (day 19) was Matriderm® applied to the defined and well-vital base in a single procedure using a dermoepidermal graft harvested from the hairy part of the head. e) The resulting condition one year after the accident

anatomical complexity and the extent of damage. A child with a history of hyperkinetic disorder (ADHD) mechanically damaged the transplanted area (Figure 4). Therefore, in patients with low compliance, the indication for the use of dermal substitute must be considered on a strictly individual basis.

CONCLUSION

Based on our preliminary clinical experience, we are convinced that the Matriderm® skin substitute represents an alternative variant to the most frequently used dermal substitute, Integra®. The possibility for immediately covering the material with a skin graft is advantageous, as it shortens the treatment time and eliminates the two-stage operation. We see the main indication in reconstructive surgery or its use in acute treatment of thermal trauma in areas at risk of developing contractures. To assess long-term results, it will be necessary to increase the number of operated patients and objectively evaluate the nature of the resulting scar over an extended period of time.

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Declaration of acquaintance with the manuscript and consent to publication: All the stated coauthors are acquainted with the manuscript and agree with its publication.

Figure 4. Complications of healing – dislocation of the graft
In an 8-year-old child with a history of hyperkinetic disorder was Matriderm® substitute used to reconstruct scar contracture of both legs. After releasing the scar and transfixing the toes with K wires, Matriderm® and a thin dermoepidermal graft were placed on the defect. Failure of the child to cooperate was the cause for partial dislocation of the attached graft 2 days later

Statement on ethical procedures used in the publication: The procedures used in preparing the publication were in accordance with the Declaration of Helsinki 1975, as revised in 2000. The procedures used were approved by decision of the Ethics Committee of the Royal Vinohrady University Hospital EK-R-06-20 from 22 June 2020.

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REFERENCES

1. Jeschke MG., Pinto R., Kraft R., et al. Morbidity and survival probability in burn patients in modern burn care. *Crit Care Med*. 2015, 43:808–15.
2. Kraft R., Herndon DN., Al-Mousawi AM. Burn size and survival probability in paediatric patients in modern burn care: a prospective observational cohort study. *Lancet*. 2012, 379:1013–21.
3. Zajíček R., Grossová I., Šuca H. Experience with Integra at the Prague Burn Centre 2002–2016. *Acta Chir Plast*. 2017, 59:18–26.
4. Zajíček R., Sticová E., Šuca H., Brož L. Kožní náhrada Integra® v klinické praxi. *Rozhl Chir*. 2013, 92:283–7.
5. Min JH., Yun IS., Lew DH., Roh TS., Lee WJ. The use of matriderm and autologous skin graft in the treatment of full thickness skin defects. *Arch Plast Surg*. 2014, 41:330–6.
6. Choi JY., Kim SH., Oh GJ., Roh SG., Lee NH., Yang KM. Management of defects on lower extremities with the use of Matriderm and skin graft. *Arch Plast Surg*. 2014, 41:337–43.
7. Heitland A., Piatkowski A., Noah EM., Pallua N. Update on the use of collagen/glycosaminoglycate skin substitute-six years of experiences with artificial skin in 15 German burn centers. *Burns*. 2004, 30:471–5.
8. Haslik W., Kamolz LP., Nathschläger G., Andel H., Meissl G., Frey M. First experiences with the collagen-elastin matrix Matriderm as a dermal substitute in severe burn injuries of the hand. *Burns*. 2007, 33:364–8.
9. Rehim SA., Singhal M., Chung KC. Dermal skin substitutes for upper limb reconstruction: current status, indications, and contraindications. *Hand Clin*. 2014, 30:239–52.
10. Bertolli E., Campagnari M., Molina AS., Macedo MP., Pinto CA., Cunha IW., et al. Artificial dermis (Matriderm®) followed by skin graft as an option in dermatofibrosarcoma protuberans with complete circumferential and peripheral deep margin assessment. *Int Wound J*. 2015, 12:545–7.
11. Heimbach D., Luterman A., Burke J., et al. Artificial dermis for major burns. A multi-center randomized clinical trial. *Ann Surg*. 1988, 208:313–20.
12. Anzarut A., Chen M., Shankowsky H., Tredget EE. Quality-of-life and outcome predictors following massive burn injury. *Plast Reconstr Surg*. 2005, 116:791–7.
13. Jones I., Currie L., Martin R. A guide to biological skin substitutes. *Br J Plast Surg*. 2002, 55:185–93.
14. Zajíček R. et al. Complex approach to skin repair in an extensively burned child: a case study. *Journal of Wound Care*, 2020, 29(8):458–63.
15. Jeon H., Kim J., Yeo H., Jeong H., Son D., Han K. Treatment of diabetic foot ulcer using matriderm in comparison with a skin graft. *Arch Plast Surg*. 2013, 40:403–8.
16. Min JH., Yun IS., Lew DH., Roh TS., Lee WJ. The use of matriderm and autologous skin graft in the treatment of full thickness skin defects. *Arch Plast Surg*. 2014, 41:330–6.
17. Hamuy R., Kinoshita N., Yoshimoto H., Hayashida K., Houbara S., Nakashima M, et al. One-stage, simultaneous skin grafting with artificial dermis and basic fibroblast growth factor successfully improves elasticity with maturation of scar formation. *Wound Repair Regen*. 2013, 21:141–54.
18. Böttcher-Haberzeth S., Biedermann T., Schiestl C., et al. Matriderm® 1 mm versus Integra® Single Layer 1.3 mm for one-step closure of full thickness skin defects: a comparative experimental study in rats. *Pediatr Surg Int*. 2012, 28:171–7.
19. Ramos G., Cornistein W., Cerino GT., Nacif G. Systemic antimicrobial prophylaxis in burn patients: systematic review. *J Hosp Infect*. 2017, 97:105–14.
20. van Zuijlen PP., van Trier AJ., Vloemans JF., Groenevelt F., Kreis RW., Middelkoop E. Graft survival and effectiveness of dermal substitution in burns and reconstructive surgery in a one-stage grafting model. *Plast Reconstr Surg*. 2000, 106:615–23.

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COMPLEX FACIAL RECONSTRUCTION BASED ON 3D MODELS: PRELAMINATION CASES AND LITERATURE REVIEW

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SUMMARY

3D models allowed a huge advance of reconstructive surgery. A literature review was performed in order to ascertain the current applicability of 3D modeling combined with free flaps, the most commonly used free flaps, and whether the use of 3D models alongside prelaminated free flaps has already been described. Also, two patients with significant facial disfiguration scheduled for surgeries that included 3D modeling and prelamination techniques were selected.

3D modeling is mostly used for the correction of mandibular defects with fibular free flaps. No literature reports the combined use of 3D models and prelamination, but we have demonstrated its applicability: two costume-made prelaminated free flaps were obtained and the patients achieved an important aesthetic and morphological improvement.

When no local grafts or flaps are available, and a three-dimensional facial reconstruction is intended, prelamination is a complex option to be considered. This complexity can be reduced by the use of 3D models, being a personalized flap obtained in a decreased operating time. Prelamination used alongside 3D modeling can be a powerful tool in the correction of complex facial deformities.

KEYWORDS

Facial reconstruction; prelamination; 3D modeling

INTRODUCTION

The face is one of the most important regions of the human body, which contains complicated and delicate features that help to define the person's identity[1]. The deformities in this region can have multiple etiologies: oral cancer, burns, infections, osteoradionecrosis, vascular lesions, traumatic injuries and congenital anomalies. There are various methods of reconstruction and it is important to perform a morphologic and dynamic evaluation of the patients in order to find the best one[2].

Advances in 3D technology have given surgeons the answers to complex reconstructive surgery questions. With the introduction of 3D models, it was possible to stop using two-dimensional images or photos for preoperative planning and to start using three-dimensional ones, resulting in a significant decrease of intraoperative time. 3D models can be virtual or physical: the latter ones usually involve the conversion of computerized tomography or magnetic resonance imaging data – 3D printing[3] – and can be used in an intraoperative context being a useful reference, since they provide not only visual, but also tactile information. On the other hand, these models have improved the doctor-patient relationship since they enable better understanding of both the craniofacial problem and the desired surgical result, diminishing a lot of anxiety that the patients feel about the possible outcome. The 3D models not only enable a more accurate surgical planning, but also allow a more customized and specific reconstruction for each case. Mandible defects have been successfully fixed with the use of 3D modeling, being its use described in fracture repair surgeries[4-8] and aesthetic surgeries regarding mandibular features[9-14]. The processes of orbital reconstruction[15-18], ear reconstruction[19], zygomatic bone repair[20,21], rhinoplasty[22,23] and nose reconstruction[24,25] have also achieved successful aesthetic results due to 3D modeling. To emphasize its wide range of applications, it can also be used in female feminization surgeries, where an individual approach is the key[26].

The term prelamination means a process in which a 3D structure – which can be composed of tissues, or tissue engineering products – is engrafted or implanted into a reliable vascular territory without interfering with its blood supply, being transferred to the recipient site 2 to 3 weeks later[27,28]. This surgical delay is a period of neovascularization and

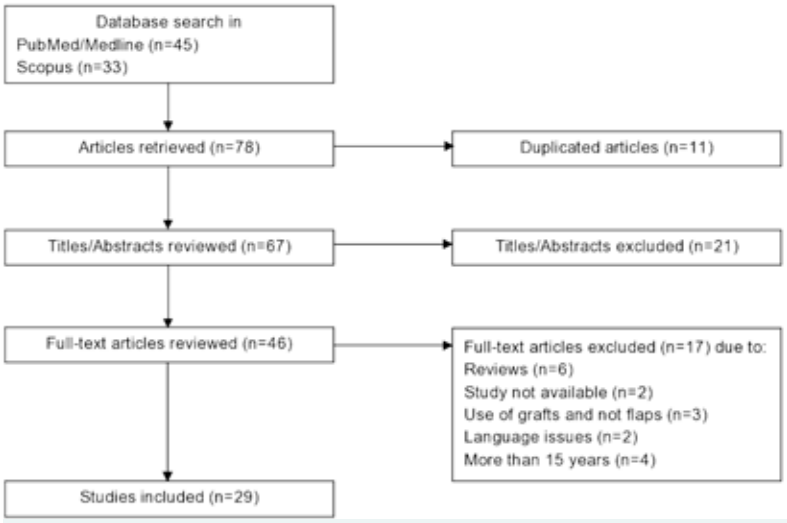

Figure 1. Flowchart of the literature review

integration, in which there is an increase in blood flow, an increase in angiogenesis and a reduction of edema so that when the flap is transferred, it is completely vascularized and with defined contours.

The purposes of the present study were to investigate the main applications of 3D modeling used alongside free flaps in facial reconstruction, which free flaps were the most commonly used in these cases, to ascertain if the combined use of 3D models and prelaminated free flaps has already been described and to emphasize the advantages that can come from the use of these last two methods together.

EVALUATION OF THE TOPIC

We conducted a literature review in order to ascertain the current applicability of 3D modeling combined with free flaps in reconstructive surgery.

PubMed and Scopus electronic databases were searched for articles using the following query: ("Reconstructive Surgical Procedures" [Mesh] OR "Surgery, Plastic" [Mesh] OR reconstructive [Text Word] OR reconstruction [Text Word]) AND ("Imaging, Three-Dimensional" [Mesh] OR 3D [Text Word] OR three-dimensional [Text Word]) AND ("Models, Anatomic" [Mesh] OR model [Text Word] OR modeling [Text Word]) AND ("Free Tissue Flaps" [Mesh] OR "free flaps" [Text Word]).

We identified 45 articles from PubMed and 33 from Scopus, amounting to a total of 78 articles (Figure 1). After removing duplicates, we scanned 67 titles and abstracts, selecting 46 for full-analysis. After accessing the full text of the included articles, 17 were excluded. The reasons for exclusion were: reviews (n=6), study not available (n=2), use of grafts and not flaps (n=3) and language issues (n=2). Also, studies with more than 15 years were excluded considering the great technical advances recognized in reconstructive surgery (n=4). Finally, 29 articles were included in the literature review.

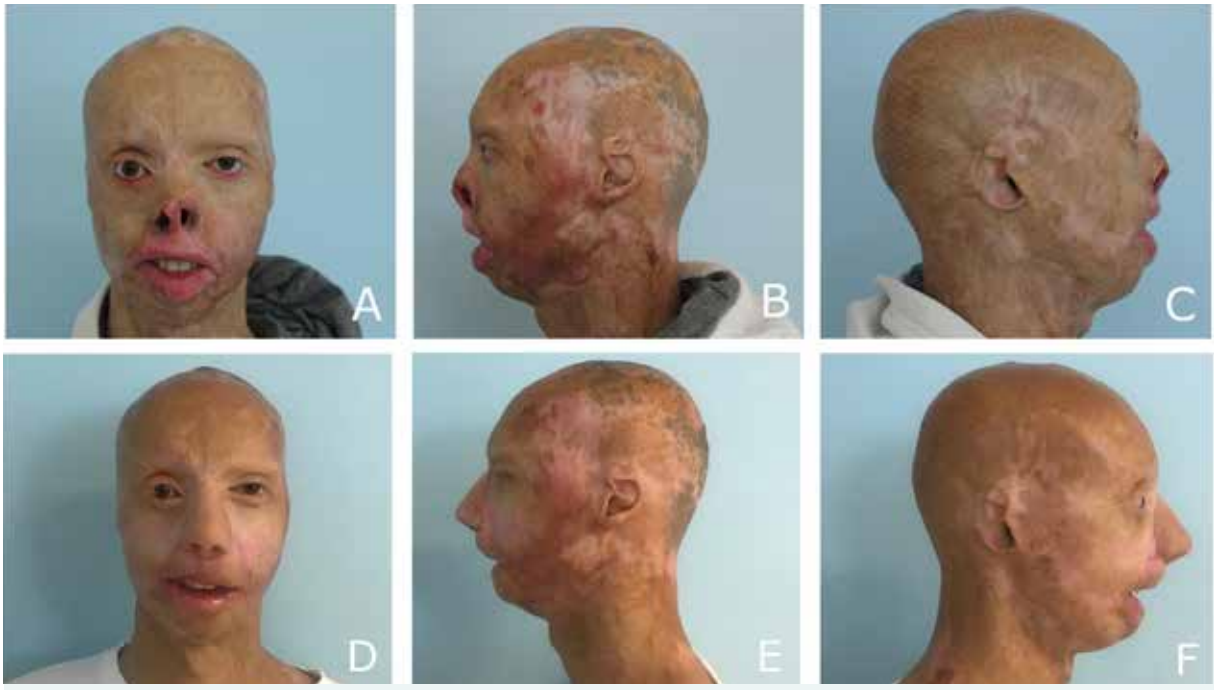

Figure 2. A 27-year-old patient with severe sequelae of thermal burns including ectropium, upper and lower lip retraction and partial nasal loss. Preoperative views: (A) Frontal view. (B and C) Lateral views. Postoperative views: (D) Frontal view with the patient using a silicone prothesis for the nose. (E and F) Lateral views with the patient using a silicone prothesis for the nose

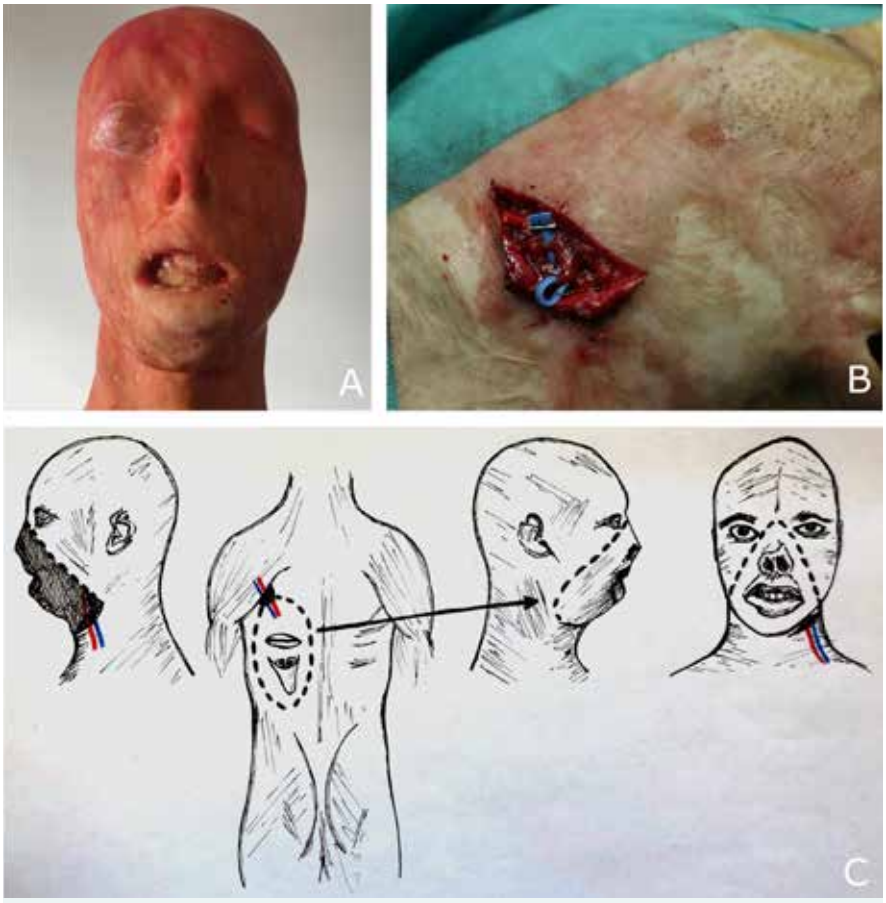

Figure 3. (A) 3D latex model. (B) Facial artery and vein identified on the left side of the neck. (C) Drawn scheme of the prelamination steps

We also selected two patients who were treated at our institution who represent suitable examples of innovative prelamination techniques that involved 3D modeling procedures.

CASE 1

A 27-year-old patient with severe sequelae of thermal burns (55 percent total burn surface area), including ectropium, upper and lower lip retraction and partial nasal loss (Figure 2), was a candidate for facial transplantation, but as he did not want this procedure, other options were investigated.

Prelamination was the selected method. The only unburned area in the body of the patient was the left side of the back, where there is a constant pedicle (parascapular artery and vein). In the first stage, an elective surgery was performed to identify and tag the recipient vessels on the neck (Figure 3). The prelamination process was then initiated with a drawing of a facial model on the back of the patient. This was based on a 3D latex model created at the Faculty of Arts as a print of the patient's face (see Figure 3). At this stage, a partial delay of the flap was performed and the nose and the lips were drawn and open inside the flap, with subsequent placement of biomaterials (porous polyethylene implant) and grafts (Figure 4). After three months was the flap transferred and microvascular anastomosis between the subscapular vessels and the recipient vessels on the neck were created (see Figure 4). Although the perioral reconstruction was achieved successfully, revision surgeries were needed due to the different skin texture and bulk. Reconstruction of the eyelids and correction of the ectropium were performed at the same time through scar release, skin grafting and canthopexy. Later on, due to columellar and alar retraction resulting from a lack of cartilage support and secondary fibrosis of a rather thick flap, and in order to improve nasal shape, a costal cartilage graft was placed. However, this did not provide enough support either and as other local or distant flap options were not available, an anaplastologist fabricated a silicone model for alar rim and columella support to avoid airway collapse and nostril occlusion.

From 2012 (the year of the incident) to 2020, the patient has gained better feeding and mouth opening capacity, more effective breathing with more permeable areas and more robust eye protection, which translated into an extremely important functional gain. The aesthetic result has also experienced some improvement (see Figure 2).

CASE 2

A 27-year-old man suffered a motor vehicle accident and presented an almost complete amputation of the left auricle and traumatized temporal skin and fascia (Figure 5)[29]. He was not a candidate for traditional repair techniques because of an abrasive injury that reached preauricular and retroauricular skin. A porous polyethylene implant was considered, however local flap coverage such as temporoparietal fascia flap was not available due to initial damage to the surrounding tissues. Consequently, a prelaminated radial forearm free flap was selected for reconstruction with prolonged prelamination time and surgical delay (two months). Firstly, a 3D-printed ear made of silicone was created based on the patient's CT-scan of the contralateral ear and used for intraoperative molding of the polyethylene implant (Medpor®). The implant consists of two components – extended ear base and helical rim – and both were adjusted into a desirable shape, having the contralateral silicone printed ear as a guide. A subdermal pocket was then dissected on the anterior aspect

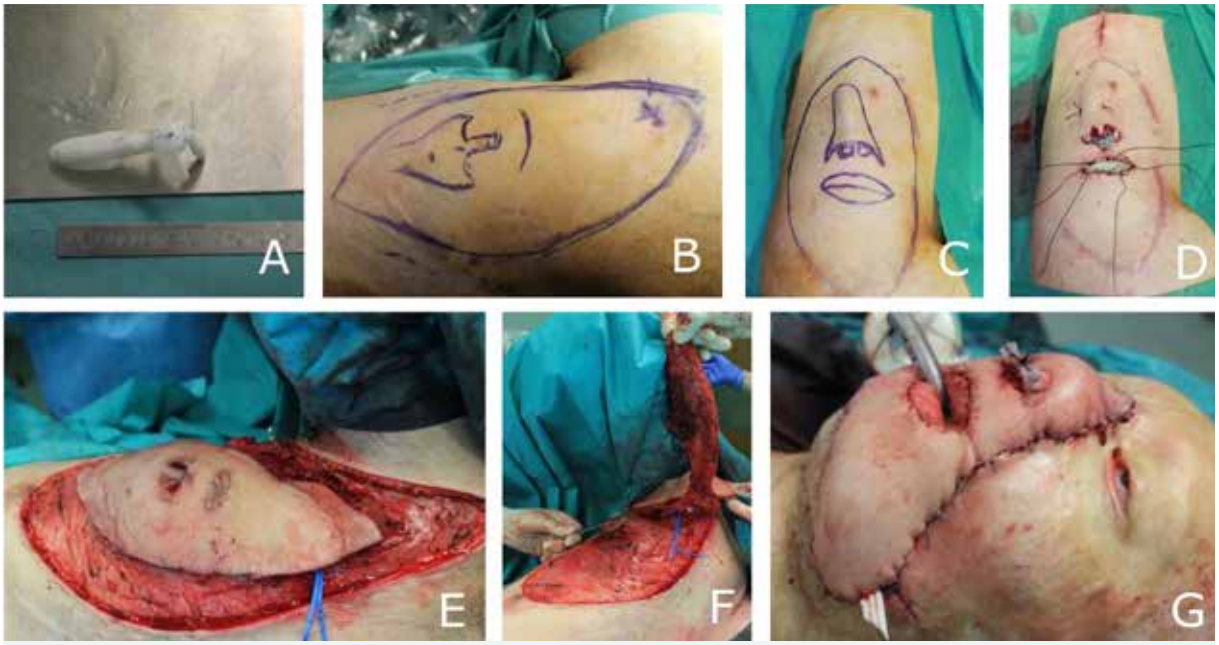

Figure 4. (A) Porous polyethylene implant for the nose reconstruction. (B and C) Drawing of facial model on the back of the patient. (D) Opened nose and lips inside the flap, with placement of biomaterials and grafts. (E, F and G) Flap transfer

of the left forearm along the projection of the radial artery for placement of the porous polyethylene implant that was subsequently inserted subdermally (Figure 6). After a two-month period of integration and neovascularization of the added tissue, the prelaminated flap was transferred (Figure 7). Flap reinnervation was performed by direct coaptation of the great auricular nerve to the lateral antebrachial cutaneous nerve. The flap fully survived and there were no complications in the early postoperative period. Revision surgeries were performed. In three to six months, the patient returned to normal state in terms of warmth and cold perception and recovered the discriminative facial sensibility. After one year, the auricular reconstruction was intact and satisfactory aesthetic results were achieved. Four years after the intervention, the patient already wears short hair and is extremely satisfied with his new ear (see Figure 5).

3D MODELING AND FREE FLAPS

The combination of 3D modeling and free flaps has been described in reconstructive surgeries, being more commonly used in maxillary[30-38] and hemimaxillary reconstruction[39], orbital defects reconstruction[32,33] and mandibular reconstruction[35,38,40-47,48-56,40-47]. Moreover, reconstructive surgeries regarding Parry-Romberg syndrome and Treacher Collins syndrome have also made use of this combined tool[57,58]. Fibular free flaps were the most commonly used for the correction of bone defects, but the use of a thoracodorsal scapula composite free flap[30], vascularized iliac bone combined with a superficial inferior epigastric artery flap[37], iliac bone free flap[48], scapular osteocutaneous free flap[54], anterolateral thigh dermal adipofascial flap[57] and temporoparietal galeal flap[58] were also described (Table 1).

Figure 5. A 27-year-old patient with a posttraumatic left ear amputation. Preoperative views (after healing): (A) Lateral view. (B) Oblique view. Postoperative views (result after 4 years): (C) Lateral view. (D) Oblique view

Author, year	Application	Type of free flap
Modest, 2017	Maxillary reconstruction	Thoracodorsal scapula composite free flap
Jędrzejewski, 2012	Maxillary reconstruction	Fibular free flap
Fu, 2017	Orbitomaxillary reconstruction	Fibular free flap
Zhu, 2020	Orbitomaxillary reconstruction	Fibular free flap
Rohner, 2012	Maxillary reconstruction	Fibular free flap
Modabber, 2012	Maxillary and mandibular reconstruction	Fibular free flap
He, 2009	Maxillary reconstruction	Fibular free flap
Hu, 2007	Palatomaxillary reconstruction	Vascularized iliac bone combined with a superficial inferior epigastric artery flap
Kääriäinen, 2016	Maxillary and mandibular reconstruction	Fibular free flap
Nkenke, 2014	Hemimaxillary reconstruction	Fibular free flap
Mazzoni, 2013	Mandibular reconstruction	Fibular free flap and Iliac bone free flap
Ciocca, 2012	Mandibular reconstruction	Fibular free flap
Katsuragi, 2011	Mandibular reconstruction	Fibular free flap
Roser, 2010	Mandibular reconstruction	Fibular free flap
Cornelius, 2015	Mandibular reconstruction	Fibular free flap
Ren, 2018	Mandibular reconstruction	Fibular free flap
Nuri, 2019	Mandibular reconstruction	Fibular free flap and scapular free flap
Vakharia, 2012	Mandibular reconstruction	Fibular free flap
Valentini, 2005	Mandibular reconstruction	Fibular free flap
Mottini, 2016	Mandibular reconstruction	Fibular free flap
Kadowaki, 2017	Mandibular reconstruction	Fibular free flap
Jacek, 2018	Mandibular reconstruction	Fibular free flap
Iglesias-Martín, 2018	Mandibular reconstruction	Fibular free flap
Arce, 2018	Mandibular reconstruction	Fibular free flap
Lee, 2018	Mandibular reconstruction	Fibular free flap
Wang, 2013	Mandibular reconstruction	Fibular free flap
Weitz, 2018	Mandibular reconstruction	Fibular free flap
Chai, 2015	Parry-Romberg syndrome treatment	Anterolateral thigh dermal adipofascial flap
Herlin, 2013	Treacher Collins syndrome midface reconstruction	Temporoparietal galeal flap

Table 1. Literature review findings: the applicability of 3D modeling in reconstructive surgeries using free flaps and the type of free flaps chosen

Although the use of 3D modeling for free flap surgery in facial reconstruction is common, the use of 3D modeling to design prelamination for free flaps in facial reconstruction has never been described, being a novel approach. We present two prelaminated free flaps obtained with the use of 3D models: a prelaminated parascapular free flap and a prelaminated radial forearm free flap. They were used in reconstructive surgeries related with burn injuries and ear reconstruction, respectively.

Burn injuries demand challenging 3D reconstructions. When it comes to reconstructive options that can be performed in patients with severe burns, skin grafts are often not used because of poor color matching and secondary contracture and local flaps are rarely obtainable in large burns[59]. Therefore, when analyzing reconstructive options in burn patients, it may be necessary to resort to techniques that optimize the resources. Facial transplant surgery is an option to be considered in these cases since it presents better aesthetic results and is usually a one-time surgery. However, the need of lifelong immunosuppressive therapy, which can bring a lot of nefarious complications, such as increased incidence of malignancies, infections and end-organ toxicity[60], made the patient described in case 1 to refuse this method. Prelamination is one of the most effective mechanisms available for approximating aesthetic reconstructive outcomes using autologous tissue[61], not requiring immunosuppressive therapy, and at the end the patient was scheduled for this technique. When selecting the free-tissue transfer donor

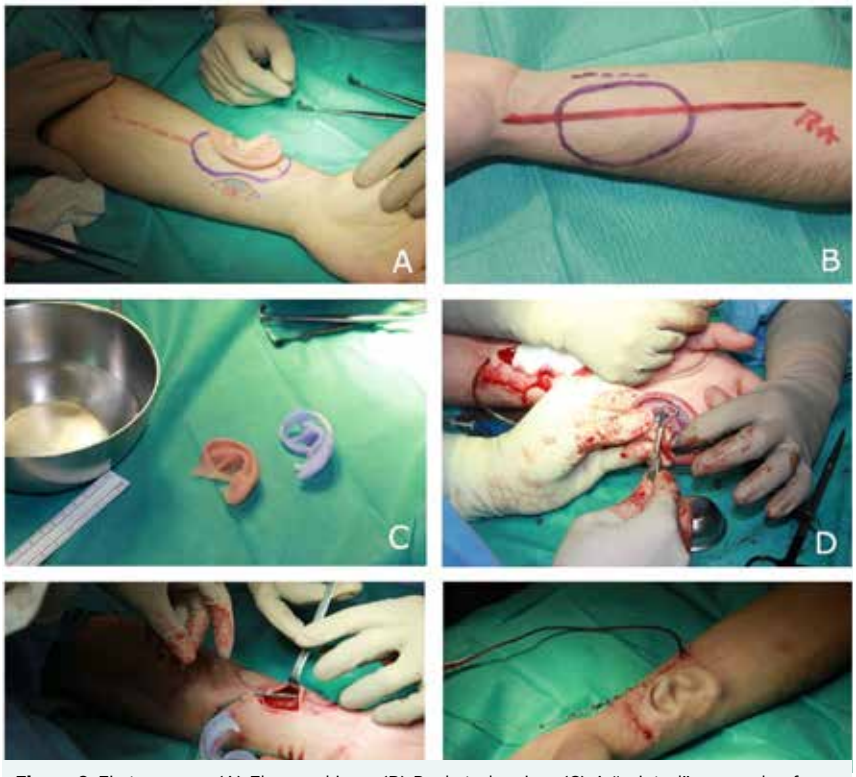

Figure 6. First surgery: (A) Flap markings. (B) Pocket planning. (C) A "printed" ear made of silicone based on the **patient's CT**-scan of the contralateral ear was used for intraoperative comparation and molding of the future reconstruction. (D and E) The implant was trimmed to a desired shape and inserted into the pocket. (F) Immediate aspect after closure and placement of two suction drains

icle is relatively short in length. Moreover, the 3D model enabled that, by direct comparison, the design of the lower half of the face (which included the mouth and nose) drawn in the parascapular region had the appropriate proportions and dimensions, therefore enabled programming a more efficient and personalized surgery. Furthermore, in cases like this, where more than half of the surface area is burned and there are few local or distant flaps available, the 3D model is of great importance since it allows surgical planning in order to take the maximum advantage of the little tissue available. Nevertheless, since a rise in facial transplantation cases mortality had been noticed and some apprehension has emerged among transplant candidates, here we have described a method that is also safe in the long-term – in fact, this method offers several advantages when compared to facial transplantation, especially when it comes to long-term risks for the patient (Table 2). Despite the number of revision surgeries required and the modest aesthetic results because of a discrepancy of color and texture between the dorsal and facial skin, the patient still reported a remarkable functional gain. In fact, the improvements in the capacity of feeding and opening the mouth were mainly due to the prelamination technique that was essential in the perioral rehabilitation.

site, since the two most commonly ones used to replace facial subunits – the radial forearm and temporoparietal fascia flaps – were compromised, other sites had to be explored[62]. The parascapular tissue on the left dorsal region was intact, and since the parascapular tissue has a constant pedicle, it was the area selected for the prelamination. This optimization of resources was also possible thanks to the 3D model that brought advantages at both the pre- and the intraoperative level. Effectively, the model assumed an important role in the calculation of distances to perform anastomoses, allowing us to obtain tension-free vascular anastomoses. This was particularly important since the parascapular pedicle is relatively short in length. Moreover, the 3D model enabled that, by direct comparison, the design of the lower half of the face (which included the mouth and nose) drawn in the parascapular region had the appropriate proportions and dimensions, therefore enabled programming a more efficient and personalized surgery. Furthermore, in cases like this, where more than half of the surface area is burned and there are few local or distant flaps available, the 3D model is of great importance since it allows surgical planning in order to take the maximum advantage of the little tissue available. Nevertheless, since a rise in facial transplantation cases mortality had been noticed and some apprehension has emerged among transplant candidates, here we have described a method that is also safe in the long-term – in fact, this method offers several advantages when compared to facial transplantation, especially when it comes to long-term risks for the patient (Table 2). Despite the number of revision surgeries required and the modest aesthetic results because of a discrepancy of color and texture between the dorsal and facial skin, the patient still reported a remarkable functional gain. In fact, the improvements in the capacity of feeding and opening the mouth were mainly due to the prelamination technique that was essential in the perioral rehabilitation.

When it comes to ear reconstruction, creating an ear shape from the costal cartilage is the gold standard technique[63]; however it is quite demanding, it does not have the same elastic properties as ear cartilage and it results in donor site morbidity. Therefore, an alloplastic material – high-density porous polyethylene – which is highly biocompatible, stable, durable and easily moldable was selected.

Figure 7. Second surgery: (A) Flap markings. (B and C) Intraoperative images of the flap before and after insertion

	Advantages	Limitations
Facial Transplantation	One-stage surgery Better aesthetic outcomes Restoration of the appearance	Requires life-long immunosuppression Increases the incidence of infections (opportunistic infections), cancer and nephrotoxicity High incidence of acute rejection Difficult matching process (tissue antigen, skin color, texture, age, and gender) Identity issues
Reconstruction with prelaminated free flaps based on 3D models	No need for immunosuppressive therapy Allows the maturation of the primary flap before its transfer Allows en-bloc transfer of 3D composite flaps 3D Modelling allows a more accurate surgical planning 3D Models decrease the intraoperative time and make prelamination less complex No long-term morbidity	Sub-optimal aesthetic results Requires at least two surgeries Post-transfer scar contracture may require revision surgeries Disparity of color and texture between local and donor sites

Table 2. Advantages and limitations of facial transplantation vs. reconstruction with prelaminated free flaps based on 3D models

Regarding the importance of the 3D model used here, it is important to note that Medpor® implants used in auricular reconstruction are often shaped based on a radiographic film with markings of the contralateral ear[64]. However, this method offers only two-dimensional information. The 3D model makes the molding process easier because it provides visual and tactile information and the surgeon manages to make the implant configuration as identical as possible to that of the mold. In addition, using the 3D silicone model as a means of direct comparison in the operating room reduces the intraoperative time. Effectively, a 3D-printed ear mold makes the trimming of the implant an easier and more time-saving procedure, being the final result more similar and symmetrical to the contralateral ear. Finally, the use of this model leads to a reduction in costs: a 3D-printed ear of porous polyethylene may also be ordered from the manufacturer, but it is much more expensive than the conventional universal alloplasts that were used in this case – they are composed of two pieces and are available in three sizes. In fact, here we showed that if a silicone 3D model, based on the contralateral ear, is used as a guide, a more low-cost but yet effective procedure is reachable – ultimately a personalized prelaminated flap was achieved and a symmetrical aesthetic result was obtained.

Nowadays, reconstructive surgery can make use of the techniques that allow us to manage complex cases, achieving very good aesthetic and funcional results. Since the facial anatomy is crucial for the patient's identity and 3D modeling allows an individual approach, it is easy to understand its extreme importance when it comes to reconstruction of craniofacial defects. 3D modeling has been used for a more efficient planning in surgeries using free flaps and is more often used in mandibular reconstruction with fibular flee flaps. Although its use in the planning of prelamination surgeries hasn't been described in the literature, we have demonstrated its applicability: the first phase of the prelamination technique was simpler, with achievement of a customized free flap in a significantly decreased intraoperative time, and with both patients, who were complex cases of different etiologies (burn injuries and trauma), achieving good aesthetic and morphologic results.

CONCLUSIONS

The craniofacial region is one of the most complex in the human body and is of great functional and aesthetic importance, making its reconstruction challenging.

3D models allow to obtain a personalized facial reconstruction and this custom-made approach is extremely important for the conservation of the aesthetic appearance and, consequently, individual well-being. On the other hand, prelamination is a complex technique used when we intend to achieve a three-dimensional reconstruction and there are no local grafts or flaps available. This complexity can be reduced with the use of 3D models, which play an important role in the first surgical phase of the technique, allowing achievement of a customized flap designed in the most appropriate way.

Therefore, prelamination and 3D models can be used together as a powerful tool to achieve customized 3D reconstructions in complex cases where the existing reconstructive options are limited.

Role of authors: Francisca Frias acquired, analyzed and interpreted the data and created the manuscript. Ricardo Horta originated the concept of the submission and performed a critical revision of the manuscript.

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REFERENCES

1. Li QF., Zan T., Li H., Gu B., Liu K., Xie F., et al. Reconstruction of postburn full facial deformities with an integrated method. *J Craniofac Surg*. 2016, 27:1175–80.
2. Horta R., Nascimento R., Vilas-Boas J., Sousa F., Orvalho V., Silva A., et al. Thermographic analysis of facially burned patients. *Burns*. 2016, 42:236–8.
3. Hsieh TY., Dedhia R., Cervenka B., Tollefson TT. 3D Printing: Current use in facial plastic and reconstructive surgery. *Curr Opin Otolaryngol Head Neck Surg*. 2017, 25:291–9.
4. King BJ., Park EP., Christensen BJ., Danrad R. On-Site 3-Dimensional Printing and Preoperative Adaptation Decrease Operative Time for Mandibular Fracture Repair. *J Oral Maxillofac Surg*. [Internet]. 2018, 76:1950.e1–1950.e8. Available from: https://doi.org/10.1016/j.joms.2018.05.009
5. Yuan X., Xuan M., Tian W., Long J. Application of digital surgical guides in mandibular resection and reconstruction with fibula flaps. *Int J Oral Maxillofac Surg* [Internet]. 2016, 45:1406–9. Available from: http://dx.doi.org/10.1016/j.ijom.2016.06.022
6. Sinha P., Skolnick G., Patel KB., Branham GH., Chi JJ. A 3-dimensional–printed short-segment template prototype for mandibular fracture repair. *JAMA Facial Plast Surg*. 2018, 20:373–80.
7. Qassemyar Q., Assouly N., Temam S., Kolb F. Use of a three-dimensional custom-made porous titanium prosthesis for mandibular body reconstruction. *Int J Oral Maxillofac Surg*. 2017, 46:1248–51.
8. Abbasi AJ., Azari A., Mohebbi SZ., Javani A. Mandibular Rami Implant: A New Approach in Mandibular Reconstruction. *J Oral Maxillofac Surg*. 2017, 75:2550–8.
9. Yi CR., Choi JW. Three-Dimension-Printed Surgical Guide for Accurate and Safe Mandibuloplasty in Patients With Prominent Mandibular Angles. *J Craniofac Surg*. 2019, 30:1979–81.
10. Wang L., Tian D., Sun X., Xiao Y., Chen L., Wu G. The Precise Repositioning Instrument for Genioplasty and a Three-Dimensional Printing Technique for Treatment of Complex Facial Asymmetry. *Aesthetic Plast Surg*. 2017, 41:919–29.
11. Chang PC. Computer-assisted planning and 3D printing-assisted modeling for chin augmentation. *Aesthetic Surg J*. 2018, 38:1–10.
12. Zhang YL., Song JL., Xu XC., Zheng LL., Wang QY., Fan YB, et al. Morphologic analysis of the temporomandibular joint between patients with facial asymmetry and asymptomatic subjects by 2D and 3D evaluation: A preliminary study. Med (United States). 2016, 95:3052.
13. Ryu J., Cho J., Kim HM. Bilateral temporomandibular joint replacement using computer-assisted surgical simulation and three-dimensional printing. *J Craniofac Surg*. 2016, 27:450–2.
14. Hatamleh M., Turner C., Bhamrah G., Mack G., Osher J. Improved virtual planning for bimaxillary orthognathic surgery. *J Craniofac Surg*. 2016, 27:568–73.
15. Dave T., Tiple S, Vempati S., Palo M., Ali M., Kaliki S. et al. Low-cost three-dimensional printed orbital template-assisted patient-specific implants for the correction of spherical orbital implant migration. *Indian J Ophthalmol*. 2018, 66:1600–7.
16. Kim YC., Jeong WS., Park T kyung., Choi JW., Koh KS., Oh TS. The accuracy of patient specific implant prebented with 3D-printed rapid prototype model for orbital wall reconstruction. *J Cranio-Maxillofacial Surg* [Internet]. 2017, 45:928–36. Available from: http://dx.doi.org/10.1016/j.jcms.2017.03.010
17. Nekooei S., Sardabi M., Razavi M., Nekooei A., Kiarudi M. Implantation of customized, preshaped implant for orbital fractures with the aid of three-dimensional printing. *Middle East Afr J Ophthalmol*. 2018, 25:56–8.
18. Moura LB., de Azambuja Carvalho PH., Gabrielli MAC., Pereira-Filho VA. Three-dimensional printed model and transantral endoscopy to orbital fracture repair. *J Craniofac Surg*. 2018, 29:594–5.
19. Weissler JM., Sosin M., Dorafshar AH., Garcia JR. Combining Virtual Surgical Planning, Intraoperative Navigation, and 3-Dimensional Printing in Prosthetic-Based Bilateral Microtia Reconstruction. *J Oral Maxillofac Surg* [Internet]. 2017, 75:1491–7. Available from: http://dx.doi.org/10.1016/j.joms.2016.12.037
20. Gibelli D., Cellina M., Gibelli S., Oliva AG., Termine G., Pucciarelli V., et al. Assessing symmetry of zygomatic bone through three-dimensional segmentation on computed tomography scan and "mirroring" procedure: A contribution for reconstructive maxillofacial surgery. *J Cranio-Maxillofacial Surg* [Internet]. 2018, 46:600–4. Available from: https://doi.org/10.1016/j.jcms.2018.02.012
21. Roy AA., Efanov JI., Mercier-Couture G., Chollet A., Borsuk DE. Zygomaticomaxillary Reconstruction with Computer-aided Manufacturing of a Free DCIA Osseous Flap and Intraoral Anastomoses. *Plast Reconstr Surg – Glob Open*. 2017, 5:1–4.
22. Khan G., Choi YS., Park ES., Choi YD. The application of three-dimensional simulation program and three-dimensional printing in secondary rhinoplasty. *J Craniofac Surg*. 2018, 29:774–7.
23. Klosterman T., Romo T. Three-Dimensional Printed Facial Models in Rhinoplasty. *Facial Plast Surg*. 2018, 34:201–4.
24. Jung JW., Ha DH., Kim BY., Seo BF., Han HH., Kim DH., et al. Nasal Reconstruction Using a Customized Three-Dimensional–Printed Stent for Congenital Arhinia: Three-Year Follow-up. *Laryngoscope*. 2019, 129:582–5.
25. Zeng W., Chen G., Ju R., Yin H., Tian W., Tang W. The Combined Application of Database and Three-Dimensional Image Registration Technology in the Restoration of Total Nose Defect. *J Craniofac Surg*. 2018, 29:484–7.
26. Padula S La., Hersant B., Chatel H., Aguilar P., Bosc R., Roccaro G., et al. One-step facial feminization surgery: The importance of a custom-made preoperative planning and patient satisfaction assessment. *J Plast Reconstr Aesthetic Surg* [Internet]. 2019, 72:1694–9. Available from: https://doi.org/10.1016/j.bjps.2019.06.014
27. Pribaz JJ., Caterson EJ. Evolution and limitations of conventional autologous reconstruction of the head and neck. *J Craniofac Surg*. 2013, 24:99–107.
28. Guo L., Pribaz JJ. Clinical flap prefabrication. *Plast Reconstr Surg*. 2009, 124:340–50.
29. Horta R., Valença-Filipe R., Carvalho J., Nascimento R., Silva A., Amarante J. Reconstruction of a near total ear amputation with a neurosensorial radial forearm free flap prelaminated with porous polyethylene implant and delay procedure. *Microsurgery*. 2017, 38:203–8.
30. Modest MC., Moore EJ., Abel KMV., Janus JR., Sims JR., Price DL., et al. Scapular flap for maxillectomy defect reconstruction and preliminary results using three-dimensional modeling. *Laryngoscope*. 2017, 127:8–14.
31. Jędrzejewski P., Maclejewski A., Szymczyk C., Wierzgoń J. Maxillary reconstruction using a multi-element free fibula flap based on a three-dimensional polyacrylic resin model. Pol Prz Chir *Polish J Surg*. 2012, 84:49–55.
32. Fu K., Liu Y., Gao N., Cai J., He W., Qiu W. Reconstruction of Maxillary and Orbital Floor Defect With Free Fibula Flap and Whole Individualized Titanium Mesh Assisted by Computer Techniques. *J Oral Maxillofac Surg* [Internet]. 2017, 75:1791e1–1791e9. Available from: http://dx.doi.org/10.1016/j.joms.2017.03.054
33. Zhu B., Han M., Heaton C., Park AM., Seth R., Knott PD. Assessing Free Flap

Reconstruction Accuracy of the Midface and Orbit Using Computer-Aided Modeling Software. 2020, 22:93–9.
34. Rohner D., Guijarro-Martínez R., Bucher P., Hammer B. Importance of patient-specific intraoperative guides in complex maxillofacial reconstruction. *J Cranio-Maxillofacial Surg* [Internet]. 2013, 41:382–90. Available from: http://dx.doi.org/10.1016/j.jcms.2012.10.021
35. Modabber A., Legros C., Rana M., Gerressen M., Riediger D., Ghassemi A. Evaluation of computer-assisted jaw reconstruction with free vascularized fibular flap compared to conventional surgery: a clinical pilot study. *Int J Med Robot Comput Assist Surg*. 2012, 8:215–20.
36. He Y., Zhu H guang., Zhang Z yuan., He J., Sader R. Three-dimensional model simulation and reconstruction of composite total maxillectomy defects with fibula osteomyocutaneous flap flow-through from radial forearm flap. *Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology* [Internet]. 2009, 108:e6–12. Available from: http://dx.doi.org/10.1016/j.tripleo.2009.07.027
37. Hu YJ., Hardianto A., Li SY., Zhang ZY., Zhang CP. Reconstruction of a palatomaxillary defect with vascularized iliac bone combined with a superficial inferior epigastric artery flap and zygomatic implants as anchorage. *Int J Oral Maxillofac Surg*. 2007, 36:854–7.
38. Kääriäinen M., Kuuskeri M., Gremoutis G., Kuokkanen H., Miettinen A., Laranne J. Utilization of Three-Dimensional Computer-Aided Preoperative Virtual Planning and Manufacturing in Maxillary and Mandibular Reconstruction with a Microvascular Fibula Flap. *J Reconstr Microsurg*. 2016, 32:137–41.
39. Nkenke E., Eitner S. Complex hemimaxillary rehabilitation with a prefabricated fibula flap and cast-based vacuum-formed surgical template. *J Prosthet Dent* [Internet]. 2014, 111:521–4. Available from: http://dx.doi.org/10.1016/j.prosdent.2013.07.028
40. Mottini M., Seyed Jafari SM., Shafighi M., Schaller B. New approach for virtual surgical planning and mandibular reconstruction using a fibula free flap. *Oral Oncol* [Internet]. 2016, 59:6–9. Available from: http://dx.doi.org/10.1016/j.oraloncology.2016.06.001
41. Kadowaki M., Kubo T., Fujikawa M., Tashima H., Nagayama H., Ishihara O., et al. A two-tiered structure device based on stereolithography for residual mandible repositioning in mandibular reconstruction with fibular flap. *Microsurgery*. 2017, 37:509–15.
42. Jacek B., Maciej P., Tomasz P., Agata B., Wiesław K., Radosław W., et al. 3D printed models in mandibular reconstruction with bony free flaps. *J Mater Sci Mater Med*. 29, 2018, p. 10–15.
43. Iglesias-Martín F., Oliveros-López LG., Fernández-Olavarría A., Serrera-Figallo MÁ., Gutiérrez-Corrales A., Torres-Lagares D., et al. Advantages of surgical simulation in the surgical reconstruction of oncological patients. *Med Oral Patol Oral y Cir Bucal*. 2018, 23:e596–601.
44. Arce K., Waris S., Alexander AE., Ettinger KS. Novel Patient-Specific 3-Dimensional Printed Fixation Tray for Mandibular Reconstruction With Fibular Free Flaps. *J Oral Maxillofac Surg*. 2018, 76:2211–9.
45. Jędrzejewski P., Maclejewski A., Szymczyk C., Wierzgoń J. Maxillary reconstruction using a multi-element free fibula flap based on a three-dimensional polyacrylic resin model. *Pol Prz Chir Polish J Surg*. 2012, 84:49–55.
46. Wang WH., Zhu J., Deng JY., Xia B., Xu B. Three-dimensional virtual technology in reconstruction of mandibular defect including condyle using double-barrel vascularized fibula flap. *J Cranio-Maxillofacial Surg*. 2013, 41:417–22.
47. Weitz J., Wolff KD., Kesting MR., Nobis CP. Development of a novel resection and cutting guide for mandibular reconstruction using free fibula flap. *J Cranio-Maxillofacial Surg* [Internet]. 2018, 46:1975–8. Available from: https://doi.org/10.1016/j.jcms.2018.09.007
48. Mazzoni S., Marchetti C., Sgarzani R., Cipriani R., Scotti R., Ciocca L. Prosthetically guided maxillofacial surgery: Evaluation of the accuracy of a surgical guide and custom-made bone plate in oncology patients after mandibular reconstruction. *Plast Reconstr Surg*. 2013, 131:1376–85.
49. Ciocca L., Mazzoni S., Fantini M., Persiani F., Marchetti C., Scotti R. CAD/CAM guided secondary mandibular reconstruction of a discontinuity defect after ablative cancer surgery. *J Cranio-Maxillofacial Surg* [Internet]. 2012, 40:e511–5. Available from: http://dx.doi.org/10.1016/j.jcms.2012.03.015
50. Katsuragi Y., Kayano S., Akazawa S., Nagamatsu S., Koizumi T., Matsui T., et al. Mandible reconstruction using the calcium-sulphate three-dimensional model and rubber stick: A new method, "mould technique", for more accurate, efficient and simplified fabrication. *J Plast Reconstr Aesthetic Surg* [Internet]. 2011, 64:614–22. Available from: http://dx.doi.org/10.1016/j.bjps.2010.08.010
51. Roser SM., Ramachandra S., Blair H., Grist W., Carlson GW., Christensen AM., et al. The accuracy of virtual surgical planning in free fibula mandibular reconstruction: Comparison of planned and final results. *J Oral Maxillofac Surg* [Internet]. 2010, 68:2824–32. Available from: http://dx.doi.org/10.1016/j.joms.2010.06.177
52. Cornelius CP., Smolka W., Giessler GA., Wilde F., Probst FA. Patient-specific reconstruction plates are the missing link in computer-assisted mandibular reconstruction: A showcase for technical description. *J Cranio-Maxillofacial Surg* [Internet]. 2015, 43:624–9. Available from: http://dx.doi.org/10.1016/j.jcms.2015.02.016
53. Ren W., Gao L., Li S., Chen C., Li F., Wang Q., et al. Virtual planning and 3D printing modeling for mandibular reconstruction with fibula free flap. *Med Oral Patol Oral y Cir Bucal*. 2018, 23:e359–66.
54. Nuri T., Ueda K., Iwanaga H., Otsuki Y., Nakajima Y., Ueno T., et al. Microsurgical mandibular reconstruction using a resin surgical guide combined with a metal reconstructive plate. *Microsurgery*. 2019, 39:696–703.
55. Vakharia KT., Natoli NB., Johnson TS. Stereolithography-aided reconstruction of the mandible. *Plast Reconstr Surg*. 2012, 129:194–5.
56. Valentini V., Agrillo A., Battisti A., Gennaro P., Calabrese L., Iannetti G. Surgical planning in reconstruction of mandibular defect with fibula free flap: 15 Patients. *J Craniofac Surg*. 2005, 16:601–7.
57. Chai G., Tan A., Yao CA., Magee WP., Junjun P., Zhu M., et al. Treating Parry-Romberg Syndrome Using Three-Dimensional Scanning and Printing and the Anterolateral Thigh Dermal Adipofascial Flap. *J Craniofac Surg*. 2015, 26:1826–9.
58. Herlin C., Doucet JC., Bigorre M., Khelifa HC., Captier G. Computer-assisted midface reconstruction in Treacher Collins syndrome part 1: Skeletal reconstruction. *J Cranio-Maxillofacial Surg* [Internet]. 2013, 41:670–5. Available from: http://dx.doi.org/10.1016/j.jcms.2013.01.007
59. Daugherty THF., Pribaz JJ., Neumeister MW. The Use of Prefabricated Flaps in Burn Reconstruction. *Clin Plast Surg*. 2017, 44:813–21.
60. Pomahac B., Aflaki P., Chandraker A., Pribaz JJ. Facial transplantation and immunosuppressed patients: A new frontier in reconstructive surgery. *Transplantation*. 2008, 85:1693–7.
61. Mathy JA., Pribaz JJ. Prefabrication and Prelamination Applications in Current Aesthetic Facial Reconstruction. *Clin Plast Surg*. 2009, 36:493–505.
62. Weber SM., Wang TD. Options for Internal Lining in Nasal Reconstruction. *Facial Plast Surg Clin North Am*. 2011, 19:163–73.
63. Reinisch JF., Lewin S. Ear reconstruction using a porous polyethylene framework and temporoparietal fascia flap. *Facial Plast Surg*. 2009, 25:181–9.
64. Ali K., Trost JG., Truong TA., Harshbarger RJ. Total Ear Reconstruction Using Porous Polyethylene. *Semin Plast Surg*. 2017, 31:161–72.

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HIRUDOTHERAPY IN RECONSTRUCTIVE SURGERY: CASE-REPORTS AND REVIEW

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SUMMARY

Medicinal leeches *(Hirudo medicinalis, Hirudo verbana)* have been used in the field of medicine to treat various diseases for thousands of years. Popularity of their use changed over time and in Europe, it peaked at the beginning of the 19th century. In modern medicine, application of leeches on flaps with venous congestion was first used and described by Deganc and Zdravic in 1960. A certain renaissance of leech use is currently taking place, especially in the field of reconstructive surgery. In general, use of leeches is indicated during critical post-operative period, in which the microcirculation and veins are incapable of sufficient drainage of venous blood, which can lead to stagnation of circulation in tissues at all levels, clinically manifested as a change in color and turgor of the flap. If this venostasis is not recognized in time and treated adequately, tissue necrosis can develop. Medicinal leeches can be used in venous drainage disorders after a replantation of fingers, auricles, lips and parts of the nose. In head and neck reconstructive surgery, there are many studies that confirm the success rate of hirudotherapy in hematoma evacuation or in dealing with complications after scalp replantation and transfers of free and pedicled flaps. Leech application therapy can also be indicated as a part of non-surgical methods that improve conditions of the venous system.

KEYWORDS

European medicinal leech; hirudotherapy; venostasis; reconstruction; complication; flap

INTRODUCTION

Reconstructive surgical procedures are most often indicated in case of injuries resulting in a loss of tissues, severe burns, radical oncosurgical procedures and in the prevention of cicatricial contractures after secondary healing of wounds[1]. In accordance with classic reconstructive diagram, the spectrum of reconstructive procedures includes skin grafts, local tissue transfers, distant flaps on vascular pedicles or flap surgeries using microsurgical techniques. Currently, the gold standard in head and neck reconstruction lies in free microvascular procedures, healing of which can be complicated by flap ischemia as a result of impairing the vascular pedicle before performance of a microanastomosis[2].

Restitution of blood supply is the primary requirement for proper healing of the flap to the surrounding tissues. Nevertheless, changes in tissue tension, or, on the other hand, compressions of the pedicle and the subsequent changes in blood supply can lead to a sluggish perfusion in all types of flaps. The main complication then lies in the arterial or venous insufficiency caused by the aforementioned vein compression or an arterial spasm. Despite the high success rate of free flaps – up to 7–10% of cases require a revision surgery due to endangered tissue viability[3].

Timely diagnostics and intervention are the most important measures in a failing flap, and a surgical revision should be the method of first choice[4]. The success rate of the revision procedure is between 28% and 90% [5,6]. The success rate of a salvage surgery is higher in case of a flap failing on the basis of a venous thrombosis.

While a partial obstruction of the venous component can be physiologically compensated by an incipient neovascularization within 3–4 days[7], total obstruction caused by a vessel wall collapse or a venous thrombosis lead to a significant involvement of microcirculation within 3 hours, and within 8–12 hours usually results in irreversible changes and in "no-reflow" phenomena in deep tissues, which in turn leads to a flap necrosis[8]. Even though a timely surgical revision is the most frequent solution of the complication, the physiological circulation is not always restored in all layers of the damaged tissue. A subcutaneous application of heparin or an application of a heparin-soaked gauze into the area of venostasis after a minor incision can represent an alternative conservative solution[9]. Hyperbaric oxygenation therapy (HBOT), which can have an excellent effect not only

in case of venostasis, but also in case of tissue ischemia, can represent another option. A combination of hirudotherapy and hyperbaric oxygenation seems to be the ideal solution[10]. In accordance with our first experiences, hirudotherapy – a method using local application of medicinal leeches – can also be a useful solution of venous insufficiency.

OVERVIEW OF PROBLEMS OF HIRUDOTHERAPY: HISTORY AND CURRENT UTILIZATION OPTIONS

European medicinal leech (*Hirudo medicinalis*) is a representative of the phyllum Annelida, endemic in Southeast Asia and Europe and it is the most commonly used species in medicine[11]. Leeches live in clean fresh water and they are poikilothermic animals, capable of surviving temperatures from 0–35°C. Their body can be up to 15 cm long and it has a sucker on each end (Figure 1). The top part of the body is brownish-green with pronounced red and brown stripes. The bottom part of the body is light with darker spots. The body of a European medicinal leech is composed of approximately 100 outer annuli, covered with a thin cuticle. This non-cellular protective layer is very flexible and pliable and it allows the leech to increase its volume several times after feeding[12]. Its body muscles allow crossing rather large distances in water, breathing through body wall and also an attachment to a host. Leech's head contains a small anterior sucker, which surrounds three chitin jaws located in a Y position, each of which is equipped with 100 tiny

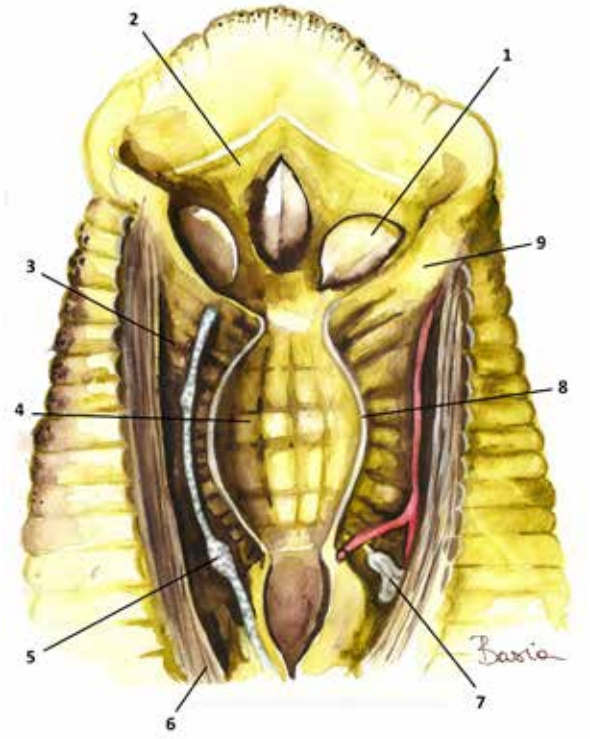

Figure 1. Lateral section of the oral part of a leech: 1. jaw, 2. velum, 3. radial muscles, 4. pharynx, 5. nervous system, 6. longitudinal muscles, 7. salivary gland, 8. annular muscles, 9. lacunas (Barbara Heinzová, watercolor paining, picture printed with author's permission, redrawn according to Pfurtscheller)

Figure 2. Oral part of a leech, attached to a glass (photo by Peter Heinz)

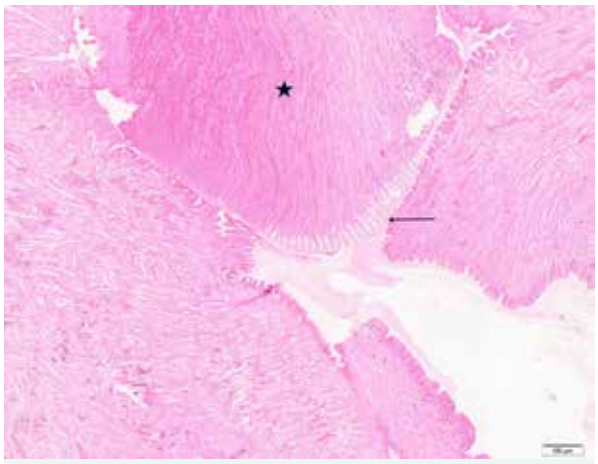

Figure 3. Detail of a leech's jaw (asterisk), which is equipped with a number of tiny teeth (the thin arrow). HE staining, magnification stated on the figure (Department of Clinical and Molecular Pathology, Faculty of Medicine and Dentistry, Palacky University Olomouc and University Hospital Olomouc, Tichý T.)

teeth (Figure 2, 3). The jaws can extend and thus penetrate skin or a mucous membrane. Subsequently, the jaws dilate rhythmically, thus creating vacuum effect. The body of a leech can absorb up to a tenfold of its original volume in single feeding and it can survive up to 2 years without the need to feed. Salivary glands in the laryngeal area produce an anticoagulant substance (hirudin). Therefore, a bite of a leech is accompanied by a prolonged bleeding from the wound, which can bleed for up to 12 hours[13,14]. The leech uses highly sensitive receptors located near the upper lip to find the right spot to attach. If it registers blood, glucose, sweat or pulsation with tissue temperature between 35–40°C, the leech will attach[15]. The rear sucker only serves for stabilization of the leech's position during blood sucking.

On the basis of experiences of large reconstructive centers (France, Germany, USA) using hirudotherapy[16,17,18], it is recommended to keep leeches for medicinal purposes in a sterile glass container with small perforations on the lid with a sufficient amount of non-saturated mineral or sterile water with an addition of hirudin salts, diluted in the concentration of 2g per 4.5 L. This enables to keep up to 50 leeches in 2.5 L of water at a temperature of 5–8°C for

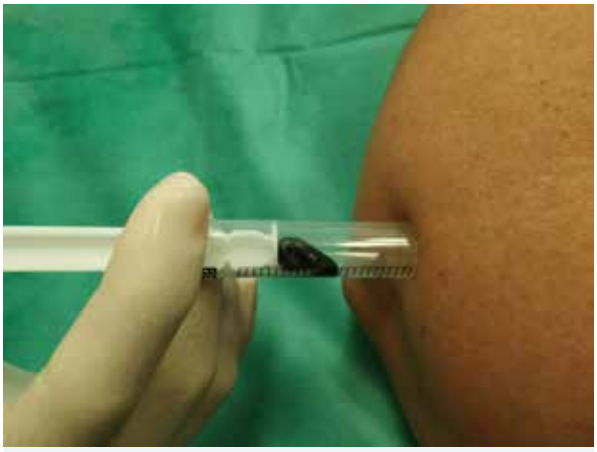

Figure 4. Manipulation with a leech (photo by Peter Heinz)

Figure 5. Side view of a leech's body, actively searching "sucker" end is raised (photo by Peter Heinz)

several weeks or even months. Replacement of the medium should be performed every week with minimum exposure to daylight. Manipulation should be done using wide anatomic forceps. It is recommended to provide patients with an antibiotic protection against Aeromonas hydrophila, a symbiotic gram-negative bacteria occurring in leeches' digestive tract, which is of significant importance for their growth. However, at the same time, it is a pathogen, which can cause muscle necrosis or formation of an abscess or cellulitis with possible development of sepsis. According to the available literature, the risk of manifestation of this complication is 7-20%; and that is why it is necessary to prevent it by administering Ciprofloxacin/Gentamicin for the period of hirudotherapy, but maximally for 5 days[19]. The actual application of a leech is performed with a 5 ml syringe to the affected location (Figure 4), which is delimited with a perforated sterile cloth to prevent unwanted migration of the leech. Its head can be identified by its fast, "searching" movements (Figure 5). If the leech does not attach on the desired location, the surface of the location can either be broken with a syringe, or a small incision can be made. After the leech attaches to a location, and when it is clear that it is starting to suck blood, the leech does not change its position any more. After the end of feeding, which usually takes from 15 to 45 minutes, the leech detaches from the tissue and it starts to move using peristaltic movements[20]. However, if the congestion area does not provide even minimum amount of blood, the leech can change its position or even move to a different body part[5]. Because of the possibility of its movement, careful monitoring of the leech's position is important, especially for example near a tracheostomy cannula[17]. Some authors recommend wrapping the body of the leech with a silk stitch and securing its ends with a clamp. A response to an adequate hirudotherapy will manifest itself with a visible improvement of perfusion in the order of minutes. After use, the leech has to be killed by an insertion into a 70% ethanl solution for 5 minutes and subsequently disposed of as a biological waste[16,18].

One of the first documented records on the use of leeches in medicine was found in an Ancient Egyptian tomb from 1567–1308 B.C. [21]. The first written record of medicinal use of leeches is attributed to Nicander of Colophon (200–130 B.C.) in his medical poem *Alexipharmaca*[22]. Therapeutic use of leeches was widespread in the Ancient Greece, China, India and pre-Columbian America. These procedures are very well documented in works by Pliny the Elder, Gales or Avicenna[23]. In the Middle-Ages, leeches were used by barber-surgeons. Probably the largest use of leeches in Europe occurred during the Napoleonic wars in France thanks to a military surgeon François-Joseph-Victor Broussais (1772–1832), who is being referred to as "the bloodiest doctor of all times", because he applied hirudotherapy to all patients regardless of their problems. However, in this period, leeches have also been used rationally by one of the founders of maxillofacial surgery, Dieffenbach (1792–1847), in 17 cases of plastic and reconstructive surgeries of the face[25]. Year 1884 was a milestone in the history of hirudotherapy, because Haycraft has proven that the throat and the mouth of leeches contain a substance with an anticoagulant effect. This compound was later isolated, denoted "hirudin" by Jacob and used in a blood transfusion in 1915 [26]. Use of leeches in modern reconstructive surgery is dated back to 1960, when they were used by Slovenian plastic surgeons Derganc and Zdravic to restore flap microcirculation [27]. The same success was registered by Henderson in case of a scalp replantation in 1983 and by Upton after an avulsion of the auricle in a five-year-old boy in 1985 [28]. Since 2004, on the basis of an FDA decision, hirudotherapy has been recognized as an official treatment of complications in plastic and reconstructive surgery[29]. Among others, hirudotherapy is being used in the treatment of diseases affecting vessels, such as thromboembolia, limb varices[30], hemorrhoids[31], but also arthritis, tendinitis, hypertension diseases or in evacuation of voluminous soft tissue hematomas. Its role in reconstructive surgery is completely unique and unsubstitutable, because it can contribute significantly to saving pedicled or free flaps[32,33,34]. Despite the benefits of hirudotherapy, no unambiguous recommended indications are available regarding its use in replantation of fingers[35], auricles[36], lips[37], nose[38], penis[39] or other organs.

The effect of active substances from the salivary glands of leeches persists for 6–8 hours (Table 1), then the leeches can be replaced[40]. Under in vitro conditions, leeches are able to affect coagulation in up to 100 ml of blood[41], but there is no unified opinion regarding the amount that should be used in patients with free or pedicled flaps. You have to take into consideration the composition and volume of tissues, the condition and characteristics of the recipient location, degree of congestion and the time from the surgery. Literary

Component name	Effect
Hirudin	Inhibition of coagulation by binding to thrombin, vasodilatation, analgesic effect
Calin (Saratin)	Inhibition of coagulation using collagen-mediated aggregation of thrombocytes by blocking the binding site of von Willebrand's factor on the collagen
Destabilase	Thrombolytic effect
Hirustasin	Inhibition of kallikrein, trypsin, chymotrypsin, cathepsin G contained in neutrophils – significant anti-inflammatory effect
Hyaluronidase	Decreases interstitial viscosity, antibiotic effect
Bdelin	Anti-inflammatory effect, inhibition of trypsin, plasmin, acrosin
Factor Xa inhibitor	Inhibition of activity of the factor Xa by creating complexes
Eglin	Anti-inflammatory effect, inhibition of the activity of alpha-chymotrypsin, chymase, subtilisin, elastase and cathepsin G
Complement inhibitors	Can replace naturally occurring complement inhibitors in case of their deficiency
Carboxypeptidase A inhibitors	Increase blood influx at the location of attachment of leech's jaws
Histamine-like substances	Vasodilatation, they increase blood influx at the location of attachment of leech's jaws
Acetylcholine	Vasodilator
Chloramphenicol	Antibiotic effect

Table 1. Composition of the leech saliva

resources contain significant differences in protocols of individual sites (for similar situations, the available literature states usage of less than 10 up to 350 leeches per flap.[34,42]).

In a study performed by Nguyen et al.[43], the authors have described larger usage of leeches in a partially affected flap than in a completely affected flap. This difference can be explained with a different degree of venous insufficiency at the beginning. In cases of a distal insufficiency of the flap, an application of one to two leeches per every cycle in dependence on the flap size is generally recommended. If a severe congestion of the entire flap is present, then one leech can effectively improve microcirculation approximately in the area of 10 cm2 of tissue. Nevertheless, it seems logical to adjust the number of leeches based on flap thickness. More specifically, twice the number of leeches per every additional 2 cm of thickness is rational and supported by numerous studies[43,44,45].

Blood losses in the course of hirudotherapy are constant. On average, a medicinal leech will actively draw about 2.5 ml of blood in 20–70 minutes. The period of bleeding after the bite is described to last from 1.5 to 4 hours, up to 90 % of passive bleeding occurs within 5 hours after detachment of the leech, when the average blood loss cannot be estimated[48]. Patient's blood count should be known before to the surgery and monitoring of its possible drop after surgery each 24–48 hours in accordance with the number of leeches used is a matter of course. A general assumption is that in case of an application of 1 leech every 2 hours, the hemoglobin will drop by 10 g/L in 24 h.

Complications of hirudotherapy are rarely mentioned in the available literature. Whitaker et al.[42] calculated their frequency to 21.8%. Half of the complications are attributed to infection caused by the microbial flora inhabiting the digestive tract of leeches (Aeromonas hydrophila, Aeromonas, Serrata, Vibrio). The above-mentioned complication is described in 14% of cases in the metaanalysis performed by Whitaker et al.[42]. The infection manifested itself as lymphangitis, necrotizing fasciitis with formation of abscesses or even sepsis[47]. The infection can manifest itself from within 24 hours after leech application up to 4 weeks since treatment initiation[48]. Infectious complications significantly decrease the survival rate of the flap (from 88% to 37%, resp. 30% [42]). That is why a prophylactic administration of antibiotics is recommended by most authors, and it should be terminated no earlier than 24 hours after completion of hirudotherapy[49]. Cephalosporins of 2nd and 3rd generation are recommended most often[41].

Venous insufficiency develops most often during the first several hours after surgery as a continuously worsening of blood congestion. In flaps with skin islands, accelerated capillary refill time (less than 2s) is visible and in the following couple of hours, dark pink color of the skin transforms to pinkish-purple color. Very often, small spots ("mottled skin") start to appear on tissue surface, and flap margins will spontaneously start bleeding dark red blood.

In local flaps with random vascularization, distal venous insufficiency occurs quite often and it is usually very difficult to resolve with surgical procedures. Before we decide to apply leeches, we should decrease tension of the flap in several places by removing stitches, and, after the symptoms disappear, we should perform a resuture.

In case of pedicled flaps, venous congestion can be predicted already during the procedure or in a short period of time after harvesting[50]. It appears within several hours after the surgery as a distally delimited purple area. If you cannot perform a surgical correction of this condition, you have to initiate hirudotherapy as soon as possible[33] (Figure 6). However, if the congestion affects the entire flap, it is possible that the pedicle twisted. In such case, revision surgery is the method of choice. It consists of a decompression of the pedicle and a fixation of the flap in a different location or from its reversal into the place of harvest. After 48 hours, the

Figure 6. A leech on a submental flap, capillary drains introduced into the surrounding area, 4th day after surgery (photo by Peter Heinz)

flap can once again be moved into the desired position. This procedure should always be considered in large or voluminous flaps, in which the benefit of hirudotherapy is limited.

Apart from replantation of fingers or small organs such as parts of auricles, leeches cannot replace venous microanastomosis[43]. The use of leeches is more suitable in cases of repeated failures of the microsurgery for local or general causes. In free skin flaps or perforator flaps, you can use leeches in case of a distal perfusion insufficiency, which develops later than in more voluminous flaps. Hirudotherapy should be initiated as soon as possible (Figure 7). Leeches are to be applied on the affected area in higher numbers than in case of pedicled flaps. Hirudotherapy can be initiated at any time during the period between the discovery of congestion and the beginning of the surgical procedure. A prevention of spreading of thrombus is the clear advantage of this approach[51].

Contraindications of hirudotherapy include immunosuppression, severe arterial insufficiency, hemophilia or hemocoagulation disorders, sepsis, hepatic insufficiency, cachexia, religious reasons, or possibly mental disorders[52].

CASE REPORTS

In our department, we used hirudotherapy in three patients in 2019. These were two women (65 and 67 years old) and a man (41 years old). All patients have undergone surgery because of an oncological indication, the first case concerned a pedicled submental flap for a defect replacement after a resection of a tongue edge, in the second patient, a free forearm flap was used to reconstruct the inner side of a cheek. The last case concerned a replacement of a defect on the left side after a subtotal maxillectomy with an osteomyocutaneous free fibular flap.

Case Report 1

Male, 41 years old, minor hepatopathy caused by toxonutritive etiology, otherwise without other monitored internal disorders. Resection of the right edge and apex of the tongue was performed because of a moderately differentiated (G 2-3) squamous cell carcinoma (pT2, pN0, MX) with an elective neck dissection: IA, IB, IIA, IIB, III, IV, VA on the right; IB, IIA on the left. The defect was subsequently reconstructed with a submental flap on a left-sided pedicle. The patient only received low-molecular weight heparin 0.4 ml s.c. within thromboembolic disorder prevention. Within 12 hours after the surgery, venostasis developed centrally and the flap swelled up. In the course of a wound revision, we have performed hematoma evacuation, enlargement of the channel for vascular pedicle and a subsequent application of two leeches, which were later applied according to the patient's response in the amount of 1-2 every 8 hours. Over the total period of 4 days, patient's overall condition improved gradually, a total of 10 leeches was applied and no blood transfusion was needed. In this case, the flap healed fully to the tongue tissue and an adequate function of the flap was secured.

Case Report 2

Female, 65 years old, non-smoker. Patient's medical history includes hypertension, atrial fibrillation and ischemic heart disease. The patient has undergone a resection of the right cheek because of a moderately differentiated squamous cell carcinoma (G2) and an elective neck surgery reg. IA, IB, IIA, III. (pT2, N1, MX). Using the Dieffenbach-Weber approach, the defect was reconstructed with a free radial forearm flap. Symptoms of bleeding under the flap started to manifest after 72 hours in the form of an increased tension/swelling and a change in color of the flap. The patient received 5,000 international units (IU) of heparin per

Figure 7. A leech applied to a forearm fasciocutaneous flap, 2nd day after surgery (photo by Peter Heinz)

day continuously. After a subsequent surgical revision and hematoma evacuation without the need for an intervention in the area of microanastomosis, we applied 2 leeches every 8 hours; after 2 days, this frequency was changed to 1 leech every 8 hours because of an improvement in the flap color. Hirudotherapy was performed for a total of 5 days; a total of 18 leeches were used and two blood transfusions had to be administered. There were no other subsequent complications and the flap healed to the base and the surrounding tissue without any loss.

Case Report 3

Female, 67 years old, non-smoker. The patient is followed by her general practitioner for hypertension, bronchial asthma and hepatopathy caused by medication. In 2017, this patient has undergone a subtotal maxillectomy on the left side because of a plexiform sarcoma with a block neck dissection reg. IA, IB, IIA, IIB, III. (pT4a, pN0, pMX). Subsequently, the patient has undergone a dose of radiotherapy of 66 Gy. Two years later, we performed a reconstructive procedure – a replacement of the maxilla using 3D planning and an individually manufactured splint with an osteomyocutanneous free fibula flap. The patient was administered heparin in the amount of 10,000 IU/24 hrs and Fraxiparine 0,3ml s.c. per 12 hours. Mottled skin occurred on the flap 48 hours after the procedure and venostatic changes appeared on the skin island of the flap, and that is why we immediately performed a revision surgery of the microanastomosis and confirmed an extensive thrombosis of the venous component. Venous grafts have been harvested and reattached in order to improve venous drainage. As a supportive therapy, we have subsequently used 2 leeches applied on the center of the skin island every 6 hours, but despite a 24-hour improvement, the flap necrotized gradually and it had to be removed on the 3rd day after the revision surgery. A total of 16 leeches and four blood transfusions have been administered.

All the patients were secured with heparin after the surgery. We kept their hemoglobin level above 90 g/L. Despite these measures, tissues manifested typical symptoms of venostasis already several hours to days after the procedure. We have started the initial treatment after surgery by loosening the stitches around the edges of the flap, by an evacuation of hematomas and an introduction of capillary drainage on the base of the post-operative defect. If these measures failed to improve the perfusion of the tissues, we proceeded with surgical revision, i.e. loosening of the entire flap and an examination of the pedicle. In two cases, the color and texture of the tissues improved. We have promptly responded to the persisting symptoms of congestion by applying 1–2 leeches every 8 hours for the period of 4–5 days. In the first two cases (forearm and submental flap), which represented a combination of hirudotherapy and a surgical treatment, the flaps healed completely to the surrounding tissues.

In the last case (free fibula flap), surgically satisfactory result has not been achieved despite more than a 6-hour long revision surgery of the venous anastomosis. With regards to the initiation of hirudotherapy, the antibiotic therapy has always included a combination of broad-spectrum penicillins/lincosamides with gentaminicin[53]. Blood transfusions were necessary in both patients treated with free flaps.

DISCUSSION

Therapeutic use of leeches is a method that has been known since the times of Ancient Egypt[26]. A certain renaissance of this method currently takes place especially in the field of reconstructive surgery[13,14]. Medicinal leeches can be used very well to save venostatic tissues after a replantation of fingers, scalp, auricles, lips and parts of the nose until angiogenesis takes place and the conditions of venous drainage improve[15,38,54,55]. In specialized literature, there are number of studies confirming the success rate of hirudotherapy in free and pedicled flaps[15,54,55,56,57,58].

The simplest option with a high success rate of diagnostic recognition of a "failing flap" is a clinical observation of its color and temperature, capillary refill, turgor, monitoring of temporal latency of the discharge and blood color after a puncture with a needle, or monitoring with a Doppler flowmetry[5].

Kubo et al.[59] evaluated the surgical revision of a flap with an affected venous component as a first-choice method with the highest success rate. At the same time, he believes that non-surgical methods should be used if the revision surgery cannot be performed or if it fails repeatedly. Vascular endothelial growth factor (VGRF) in combination with blood drawing using leeches and a hyperbaric oxygenation can reportedly increase the flap survival chances. Nevertheless, controlled clinical studies performed on larger sets of patients are still missing and therefore, standardized indications of therapy of post-surgery venostasis remain limited. Apart from the replantation of fingers or small organs such as parts of auricles, leeches definitely cannot replace venous microanastomosis[43]. Foucher at al.[60] determined that in necrotic flaps, the attachment of leeches did not take place in all cases, which could be a good test to determine tissue quality. Delayed attachment of a leech to tissue together with small amount of blood drawn is considered a negative prognostic factor of flap viability despite its favorable color[8]. Several experimental studies have confirmed an improved perfusion after leech application in comparison to other, alternative methods[17,18].

The number of applied leeches varies significantly from one per day[54] up to several in the course of each hour[59], which points out the absence of a therapeutic scheme we could follow. The replacement interval is not unequivocally recommended as well. A study on brown rats has shown both the inefficiency of the use of large numbers of leeches and the lack of a positive effect on flap survival[49]. It is important to note that tissues should be carefully clinically examined prior to the leech application and they should not be used, if they show signs of a mixed arterial venous disease[61].

In hirudotherapy, blood losses cannot be predicted reliably. Whitaker et al.[42] described the need for transfusion in 50% of cases. The more voluminous flap and more advanced venostasis, the more aggressive hirudotherapy should be. Because of this, the hemoglobin level may drop faster, which will in turn worsen the alteration of the affected tissues even more[41]. Blood loss is the most visible and also the most important effect of hirudotherapy, but the effects of the discharge from salivary glands of the leeches are much more complex; they influence vasodilatation and they have anesthetic and anti-inflammatory properties[13,26].

In the available literature, the percentage of inflammatory complications associated with hirudotherapy is between

4% and 20% [19,52,56]. Aeromonas hydrophila was reported to be the most frequent cause of this complication [47], it constitutes 9% of all infections. Recent clinical studies confirm a higher frequency of infectious complications in patients, who were not administered the antibiotics prophylactically[52]. Recently, evidence of a resistance of the aforementioned pathogen to first generation cephalosporines, penicilines and tetracycline is being reported. In comparison, fluoroquinolones seem to be consistently able to deal with the infection[32].

CONCLUSION

Hirudotherapy can successfully be used in patients in cases where venostasis occurs in free or pedicled flaps and a surgical revision is either impossible or it cannot provide adequate restoration of circulation. Despite ambiguous recommendations in the available literature, it is possible to consider procedures concerning indications, dosage, application frequency and the adequate antibiotic treatment. The clinical and the instrumental monitoring and the related adjustments of leech dosage all play a fundamental role in the success of hirudotherapy. The anticoagulant and decongestive effect of this method in treatment of distal perfusion insufficiency in local or pedicled flaps is very well known, but this article also points out the options of a successful use of this treatment method in free or pedicled flaps in the intraoral location as well.

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The author of all photographs: Petr Heinz.

REFERENCES

1. Hidalgo DA., Jones CS. The role of emergent exploration in free-tissue transfer: a review of 150 consecutive cases. *Plast Reconstr Surg*. 1990, 86:492-8.
2. Hyodo I., Nakayama B., Kato H., Hasegawa Y., Ogawa T., Terada A., Torii S. Analysis of salvage operation in head and neck microsurgical reconstruction. *Laryngoscope*. 2007, 117:357-60.
3. Khouri RK., Cooley BC., Kunselman AR., Landis JR., et al: A prospective study of microvascular free-flap surgery and outcome. *Plast Reconstr Surg*. 1998, 102:711-21.
4. Bui DT., Cordeiro PG., Hu QY., et al. Free flap reexploration: indications, treatment, and outcomes in 1193 free flaps. *Plast Reconstr Surg*. 2007, 119:2092-100.
5. Okazaki M., Asato H., Takushima A., Sarukawa S., Nakatsuka T., Yamada A., Harii K. Analysis of salvage treatments following the failure of free flap transfer caused by vascular thrombosis in reconstruction for head and neck cancer. *Plast Reconstr Surg*. 2007, 119:1223-32.
6. Kroll SS., Schusterman MA., Reece GP., Miller MJ., Evans GR., Robb GL., Baldwin BJ. Choice of flap and incidence of free flap success *Plast Reconstr Surg*. 1996, 98:459-63.
7. Capla JM., Ceradini DJ., Tepper OM., et al. Skin graft vascularization involves precisely regulated regression and replacement of endothelial cells through both angiogenesis and vasculogenesis. *Plast Reconstr Surg*. 2006, 117:836-44.
8. May Jr JW., Chait LA., O'Brien BM., Hurley JV. The no-reflow phenomenon in experimental free flaps. *Plast Reconstruct Surg*. 1978, 61:256-67.
9. Sawada Y., Hatayama I., Sone K. The effect of continuous topical application of heparin on flap survival. *Br J Plast Surg*. 1992, 45:515-18.
10. Shah J. Hyperbaric oxygen therapy. J *Am Col Certif Wound Spec*. 2010, 24:9-13.
11. Utley DS., Koch RJ., Goode RL. The failing flap in facial plastic and reconstructive surgery: Role of the medicinal leech. *Laryngoscope*. 1998, 108:1129-35.
12. Mann KH. *Leeches (Hirudinea): their structure, physiology, ecology and embryology:* Elsevier. 2013, p. 36-43.
13. Michalsen A., Roth M., Dobos G. *Medicinal LeechTherapy*, Georg Thieme, New York, NY, USA, 2007, p. 13-17.
14. Grassberger M., Sherman R. A., Gileva O., Kim Ch. M. H., Mumcuoglu K .Y. Eds., *Biotherapy—History, Principles and Practice: A Practical Guide to the Diagnosis and Treatment of Disease Using Living Organisms,* Springer, Heidelberg, Germany, 2013.
15. Soucacos P. N., Beris A. E., Malizos K. N., Kabani C. T., Pakos S. "The use of medicinal leeches, Hirudo medicinalis, to restore venous circulation in trauma and reconstructive microsurgery," *International Angiology*. 1994, 13:251-58.
16. Guideline of the University of Toledo Medical Center (USA). [Internet]. Toledo: THE UNIVERSITY OF TOLEDO, 2801 W. Bancroft, St. Toledo, OH 43606 c2020 [cited 2020 Nov 11]. Available at: http://www.utoledo.edu/policies/utmc/nursing/guidelines/general. 2017
17. Guideline of the Mid Essex Hospital (United Kingdom) [Internet]. NHS Foundation Trust Mid Essex Hospital 2020 [cited 2020 Nov 11]. Available from: http://www.meht.nhs.uk/search/?q=leech.
18. Guideline of the BfArM (Germany). [Internet]. Bundesinstitut fur Arzneimittel und Medizinprodukte c 2013. [cited 2020 Nov 11]. http://www.bfarm.de/SharedDocs/Downloads/DE/Arzneimittel/Pharmakovigilanz/Service/mitteil/mittl_blutegel.pdf?__blob=publicationFile&v=3.
19. *Mercer NS., Beere DM., Bornemisza AJ., Thomas P. Medicinal leeches as sources of wound infections. Br Med J*. 1987, 294:937.
20. Tuncali D., Terzioglu A., Cigsar B., Aslan G. The value of medical leeches in the treatment of class IIC ring avulsion injuries: report of 2 cases. *J Hand Surg*. 2004, 29:943-6.
21. Wells MD., Manktelow RT., Boyd JB., Bowen V. The medical leech: an old treatment revisited. *Microsurgery*. 1993, 14:183-6.
22. Haycox CL., Odland PB., Coltrera MD., Raugi GJ. Indications and complications of medicinal leech therapy. *J Am Acad Dermatol*. 1995, 33:1053-5.
23. Grunner OCA. Treatise on the Canon of Medicine of Avicenna incorporating a translation of the first book. London: *Luzac and Co*. 1930, p. 513-4.
24. Rolleston JD. François-Joseph-Victor Broussais 1772 to 1832:his life and doctrines. *Proc R Soc Med*. 1939, 32:405-13.
25. Sawyer RT., Johann Friedrich Dieffenbach. Successful use of leeches in plastic surgery in the 1820s. *Br J Plast Surg*. 2000, 53:245-7.
26. Fields WS. The history of leeching and hirudin. *Haemostasis*. 1991, 21:3-10.
27. Deganc M., Zdravic F. Venous congestion of flaps treated by application of leeches," . *Br J Plast Surg*. 1960, 13:187-92
28. Henderson HP., Matti B., Laing AG., Morelli S., Sully L. Avulsion of the scalp treated by microvascular repair: the use of leeches for post-operative decongestion. *Br J Plast Surg*. 1983, 36:235-9.
29. Whitaker IS., Izadi D., Oliver DW., Monteath G., Butler PE. Hirudo medicinalis and the plastic surgeon. *Br J Plast Surg*. 2004, 57:348-53.
30. Niqar Z., Alam MA. Effect of taleeq (leech therapy) in dawali (varicose veins). *Anc Sci Life*. 2011, 30:84-91.
31. Fort CW. Leech Therapy: Current Uses for an Old Treatment. *Delaware Nurses Association* (DNA) *Reporter*. 2001, 26:16-7.

32. Azzopardi EA., Whitaker IS., Rozen WM., Naderi N., Kon M. Chemical and mechanical alternatives to leech therapy: a systematic review and critical appraisal. *J Reconstruct Microsurg*. 2011, 27:481-6.
33. Gideroglu K., Yildirim S., Akan M., Akoz T. Immediate use of medicinal leeches to salvage venous congested reverse pedi-cled neurocutaneous flaps. *Scand J Plast Reconstruct Surg Hand Surg*. 2003, 37:277-82.
34. Chepeha DB., Nussenbaum B., Bradford CR., Teknos TN. Leech therapy for patients with surgically unsalvageable venous ob-struction after revascularized free tissue transfer. *Arch Otolar-yngol Head Neck Surg*. 2002, 128:955-60.
35. Brody GA., Maloney WJ., Hentz VR. Digit replantation applying the leech Hirudo medicinalis. *Clin Orthop Relat Res*. 1989, 245:133-7.
36. Cho BH., Ahn HB. Microsurgical replantation of a partial ear, with leech therapy. *Ann Plast Surg*. 1999, 43:427-9.
37. Walton RL., Beahm EK., Brown RE., et al. Microsurgical replantation of the lip: a multi-institutional experience. *Plast Recon-struct Surg*. 1998, 102:358-68.
38. Frodel JL. Jr., Barth P., Wagner J. Salvage of partial facial soft tissue avulsions with medicinal leeches. *Otolaryngology*. 2004, 131:934-9.
39. Pantuck AJ., Lobis MR., Ciocca R., Weiss RE. Penile replantation using the leech Hirudo medicinalis. *Urology*. 1996, 48:953-6.
40. Eldor A., Orevi M., Rigbi MJBR. The role of the leech in medical therapeutics. *Blood Rev*. 1996, 10:201-9.
41. Houschyar KS., Momeni A., Maan ZN., et al. Medical leech therapy in plastic reconstructive surgery. *Wien Med Wochenschr*. 2015, 165:419-25.
42. Whitaker IS., Oboumarzouk O., Rozen WM., et al. The efficacy of medicinal leeches in plastic and reconstructive surgery: a systematic review of 277 reported clinical cases. *Microsurgery*. 2012, 32:240-50.
43. Nguyen MQ., Crosby MA., Skoracki RJ., Hanasono MM. Outcomes of flap salvage with medicinal leech therapy. *Microsurgery*. 2012, 32:351-7.
44. Elyassi AR., Terres J., Rowshan HH. Medicinal leech therapy on head and neck patients: a review of literature and proposed protocol. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2013, 116:167-72.
45. Kashiwagi K., Hashimoto I., Abe Y., Kotsu K., Yamano M., Nakanishi H. Quantitative analysis of hemodynamics of congested island flaps under leech therapy. *J Med Invest*. 2013, 60:213-20.
46. Conforti ML., Connor NP., Heisey DM., et al: Evaluation of performance characteristics of the medicinal leech (Hirudo medicinalis) for the treatment of venous congestion. *Plast Reconstr Surg*. 2002, 109:228-35.
47. Mackay DR., Manders EK., Saggers GC., Banducci DR., Prinsloo J., Klugman K. Aeromonas species isolated from medicinal leeches. *Ann Plast Surg*. 1999, 42:275-9.
48. Mumcuoglu KY. Recommendations for the use of leeches in reconstructive plastic surgery. *Evid Based Complement Alternat Med*. 2014, ID 205929. https://doi.org/10.1155/2014/205929
49. Schlaudraff KU., Bezzola T., Montandon D., Pepper MS., Pittet B. Mixed arterio-venous insufficiency in random skin flaps in the rat: is the application of medicinal leeches beneficial? *J Surg Res*. 2008, 150:85-91.
50. Herlin C., Sinna R., Hamoui M., Canovas F., Captier G., Chaput B. Distal lower extremity coverage by distally based sural flaps: Methods to increase their vascular reliability. *Ann Chir Plast Esthet*. 2017, 62:45-54.
51. Anavekar NS., Lim E., Johnston A., Findlay M., Hunter-Smith DJ. Minimally invasive late free flap salvage: indications, efficacy and implications for reconstructive microsurgeons. *J Plast Reconstr Aesthet Surg*. 2011, 64:1517-20.
52. Whitaker IS., Josty IC., Hawkins S., et al. Medicinal leeches and the microsurgeon: a four-year study, clinical series and risk benefit review. *Microsurgery*. 2011, 31:281-7.
53. Cattoir V., Poirel L., Aubert C., Soussy C-J., Nordmann P. Unexpected occurrence of plasmid-mediated quinolone resistance determinants in environmental Aeromonas spp. *Emerging Infect Dis*. 2008, 14:231-7.
54. Lim CL. Successful transfer of "free" microvascular superficial temporal artery flap with no obvious venous drainage and use of leeches for reducing venous congestion: Case report. *Microsurgery*. 1986, 7:87-8.
55. Stupka I., Dvorak Z., Vesely J., Novak P., Perrotta RE., Lombardo GA. Reconstruction of a Nose Bitten and Eaten by a Dog: A Ten-Year Follow-up of a Bitten-Off Nose Replantation Performed on an Eleven-Year-Old Boy. *Ann Plast Surg*. 2015, 75:585-7.
56. de Chalain TM. Exploring the use of the medicinal leech: a clinical risk-benefit analysis. *J. Reconstr. Microsurg*. 1996, 12:165-72.
57. Trovato MJ., Agarwal JP. Successful replantation of the ear as a venous flap. *Ann. Plast. Surg*. 2008, 61:164-8.
58. Riede F., Koenen W., Goerdt S., et al. Medicinal leeches for the treatment of venous congestion and hematoma after plastic reconstructive surgery. *J Dtsch Dermatol Ges*. 2010, 8: 881-8.
59. Kubo T., Yano K., Hosokawa K. Management of flaps with compromised venous outflow in head and neck microsurgical reconstruction. *Microsurgery*. 2002, 22:391-5.
60. Foucher G., Henderson MR., Maneau M. Distal digital replantation: One of the best indications for microsurgery. *Int J Microsurg*. 1981, 3:263-70.
61. Hullett JS., Spinnato GG., Ziccardi V. Treatment of an ear laceration with adjunctive leech therapy: A case report. *J Oral Maxillofac Surg*. 2007, 65:2112-14.

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TISSUE ENGINEERING IN PLASTIC SURGERY – WHAT HAS BEEN DONE

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SUMMARY

The field of tissue engineering applies principles of engineering and life sciences for the development of functional biologic substitutes. The increasing need of tissue for challenging reconstructive surgeries places plastic surgeons' involvement as vital in the research and development of engineered constructs and subsequent use. This narrative review aims to summarize tissue engineering principles, to update on its current uses and breakthroughs, to approach its current limitations and possible future directions for this exciting new field of medicine.
This revision addressed tissue engineering utilisation in skin lesions, craniocervical defects, musculoskeletal defects, peripheral nerves lesions, vascular tissue defects and adipose tissue uses.
Research in tissue engineering is increasing exponentially, however, and although there are already several engineered constructs available, its widespread clinical application is still a hope. More long-term studies that answer outstanding issues are needed in order for that to become reality.

KEYWORDS

Tissue engineering; bioengineering; plastic surgery; reconstructive surgery; stem cells; regeneration

INTRODUCTION

Tissue engineering (TE) was firstly defined as "the application of the principles and methods of engineering and the life sciences towards the fundamental understanding of structure–function relationships in normal and pathologic mammalian tissues and the development of biologic substitutes that restore, maintain, or improve tissue function" [1,2]. This relies on interdisciplinary collaboration between cell biologists, material engineers, biotechnologists, various medical specialties and industry manufacturers[3].

TE and Plastic surgery share the common goal of restoring structure and function[4]. TE has the potential to reduce morbidity of the current approaches by eradicating donor site lesions, reducing hospital stay and the associated risks and costs[3]. Classic TE approaches encompass three components for regeneration: cells to form a matrix, a scaffold for transplantation and temporary support, and environmental factors[2,3,5–8].

Possible *cell sources* include embryonic stem cells, adult stem cells and differentiated cells[3,5,6,8,9]. Embryonic stem cells are totipotent; however, they are difficult to obtain and raise ethical objections and concerns of tumorigenicity[3]. Mesenchymal stem cells (MSC) can be pluripotent or multipotent, they can be tissue derived, are available in large quantities and are increasingly recognized as the preferable cell source[3,8]. Induced pluripotent stem cells discovered by Takahashi and Yamanaka[10] made possible the use of somatic cells in tissue engineering by reversing their differentiation state into pluripotent cells, but their use is still at its infancy[3,8,9].

Scaffolds are designed to guide and support cells, allowing a physiologic three-dimensional proliferation and differentiation[5–9]. They can be biological or synthetic, depending on the fabrication materials. Furthermore, they can be solid, which requires surgical implantation, or can exhibit gel form, which – when injected – acquire the appropriated form of the defect in situ[3,6].

Environmental factors consist of bioactive molecules (growth factors and cytokines), oxygen tension and mechanical or electrical stimulation, that alter cell's response, improving wound healing and regeneration[3,5,7,9,11].

This review will present an overview of published literature regarding tissue engineering in plastic surgery in clinical and pre-clinical studies, its current cells' sources and applications, its disadvantages and future perspectives.

The aim of this work is to create awareness regarding one of the medicine's greatest evolutions and the importance of implementation into practice of these new methods in reconstructive and aesthetic plastic surgery.

USES OF TISSUE ENGINEERING IN PLASTIC AND RECONSTRUCTIVE SURGERY

Tissue engineering is the promising alternative to the current plastic and reconstructive surgery approaches and has yielded small successes so far.

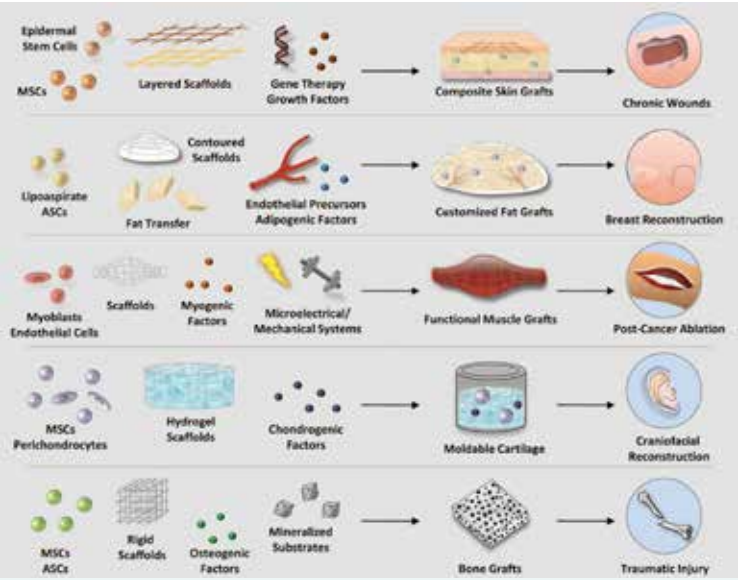

Figure 1. Potential strategies for tissue-specific engineering. (Above) Skin: seeding of epidermal stem cells and mesenchymal stem cells (MSC) onto epidermal and dermal scaffolds, respectively, with tissue-inducing factors to fabricate composite skin grafts for chronic wounds, burn injury, and skin diseases. (Second row) Fat: aspirated adipose-derived stem cells (ADSC) and fat transfer specimens incorporated into custom-shaped scaffolds with vascularization factors to engineer fat grafts for soft-tissue reconstruction. (Third row) Muscle: myoblasts and endothelial cells seeded onto flexible scaffolds in microelectrical/mechanical bioreactor systems to generate functionalized muscle grafts for myopathies, traumatic injury, and post-cancer ablation reconstruction. (Fourth row) Cartilage: mesenchymal stem cells and perichondrocytes seeded onto hydrogel scaffolds with chondrogenic factors to fabricate moldable and injectable cartilage for craniofacial and skeletal reconstruction. (Below) Bone: mesenchymal stem cells/ adipose-derived stem cells seeded onto rigid preformed scaffolds with mineralized substrates and osteogenic factors to engineer bone grafts for cancer, trauma, and congenital reconstruction. Image from "Tissue engineering in Plastic Surgery: A Review" used with author's permission[8]

Below, a brief outline of the various current and potential therapeutic applications of tissue engineering, the multiple tissue sources and their uses (Figure 1), are presented.

SKIN DEFECTS

The traditional autologous and allogeneic epidermal sheets have been proven successful in wound repair. However, they are limited in treating extensive wounds and fail to prevent wound contraction[12].

There are multiple bioengineered products used in *skin defects* (Table 1). These derivatives, besides their indications, vary in administration, preservation, shelf life (acellular derivatives have longer shelf life, lasting years) and host immune response (present with porcine or bovine derivatives)[9,13,14].

Topical therapies have the ability to promote wound vascularization promotion, reduce wound contraction and induce keratinization[9,15]. For example, recombinant human granulocyte/macrophage colony-stimulating factor (GM-CSF) topically applied had positive effects on venous and diabetic ulcers of the lower limb[16]. Also, *Regranex*, recombinant human PDGF, decreases wound closure time and increases complete wound healing[9]; this topical gel is used on diabetic neuropathic ulcers and advanced stage pressure ulcers, with great results, but as a growth factor, its application caries risk of malignancy[9,16].

Adipose tissue is a popular source of stem cells for wound healing[15–19]. The paracrine effect of adipose-derived stem cells (ADSC) stimulates the secretion of cytokines with anti-inflammatory properties and induces skin neovascularization. Furthermore, they recruit endogenous stem cells and have secretory effects over dermal fibroblasts and keratinocytes[15,19]. ADSC suspension in saline and platelet rich plasma (PRP) injected showed to be particularly successful in cases with small areas of skin necrosis after hyaluronic acid filler injection[18,20]. In Korea, this is widely used over hyaluronidase, and is very effective in the relief of local ischemia and pain[18,20].

Autologous ADSC administered by repeated hypoinvasive computer-assisted injections showed systematic improvement or remission in all 20 patients treated for chronic ulcers caused by radiotherapy[17].

Immersion of scaffolds in an ADSC cell suspension has been used for the treatment of recurrent or chronic cutaneous lesions, such as diabetic or chronic radiation ulcers[18]. As an example, *Terudermis* is used as an artificial dermis that must be sutured to adjacent skin as a protective surface. This approach may be an option for small traumatic defects or skin cancers to avoid the use of local flaps[18].

Cell sheet engineering, is a promising alternative that consists in a scaffold-free tissue-engineered product obtained through the culture of ADSC or human keratinocytes, dermal fibroblasts and dermal microvascular endothelial cells on a thermoresponsive surface or a standard cell culture surface[12,15]. Studies conducted on mice showed a short culture time period, great stability, high cell survival, high cell residence time and a stable neovascularization, which impacts on full-thickness skin regeneration[12,15].

The use of ADSC combined with *Platelet-rich plasma* (PRP) potentiate adipogenesis and graft maintenance, especially in association with insulin[19]. PRP is a small volume of plasma concentrated with autologous human platelets and growth factors, it is currently used in regenerative medicine due to its capacity to stimulate tissue regeneration[16,17,21].

PRP is indicated in a mixture with fat for chronic or post-traumatic ulcers and loss of substance of the lower limbs[16,17,21]. PRP used independently in a gel form or as an injection demonstrated great results in chronic/non-healing cutaneous ulcers, acute limb soft tissue wounds, trauma wounds and to stop bleeding in surgical flaps[16,21,22]. Possible associated risks include recurrence and growth of a pre-existing tumour[16].

As mentioned above, keratinocytes and fibroblasts are used in several tissue-engineered constructs. These constructs have several indications as aforementioned, but it is still unclear when they should be indicated over a skin graft[9].

Deformities associated with *burn injury* are frequently treated by plastic surgery procedures. Their incidence is in-

creasing and they can lead to deadly consequences like burn shock and sepsis[23,24]. Currently, burn wounds' treatment is based on the removal of devitalized tissue and coverage with an autologous split-thickness skin graft, but if the burned surface area is large this technique loses its value[23]. Temporary skin substitutes consist of biologically active patches that provide protection from infection and trauma, and provide pain control, while re-epithelialization occurs[23]. For example, *Integra Dermal Regeneration Template* is commonly used as a temporary approach in burn reconstruction, consisting of a layer of collagen and glycosaminoglycan covered by a semipermeable silicone[9]. Nowadays, as mentioned, there are already several bioengineered permanent skin substitutes in burn healing, including the FDA approved substitute, for treatment of severely burned patients – *Epicel* [9,13]. *Apligraf* and *Dermagraft* need to undergo more clinical trials to evaluate their potential in burn injuries[7,13].

Scarring is incomplete healing that leaves some deformation and/or defect[18]. Scars can affect negatively the appearance, can engender psychological illness, low self-esteem and isolation. ADSC therapy can be used to modulate scar formation. These cells injected repeatedly into the scarred tissue bed cause the scar to become soft, altering its remodelling process, which may avoid secondary surgery[18]. Although there is no scientific data published, ADSC-based cell therapy is used for prevention of scaring in severe facial trauma[18]. In clinical settings, if the healing process of an extensive wound will predictively take more than 2 weeks, skin grafting is advisable to minimize scarring[25]. There are two skin substitutes constructs indicated for scar reconstruction: *SureDerm* (acellular human cadaveric dermis) and *Integra*, used in burn scar contractures and keloid scars reconstructions[25].

Researchers believe that, by solving how to rapidly revascularize grafts/substitutes, the acceleration of the wound healing process will limit scar formation[25]. The goal for TE is therefore the generation of prevascularized constructs able to be transplantable with standard microsurgical anastomotic techniques. Klar et al. [26], for the first time, used adipose tissue's stromal vascular fraction (SVF) for assembling a capillary plexus, for anastomosis with the recipient's circulation, and transplanted it onto immune-deficient rats. This was beneficial in achieving an increased graft dimension and absence of shrinkage[26].

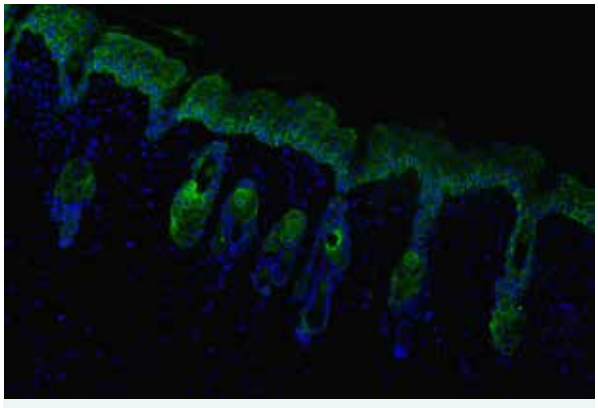

Figure 2. Rete ridges-like structures identified in the neoepidermis of mice treated with cell sheet engineered constructs of human adipose stem cells, at day 21, after immunohistochemical identification of keratin 14 (green). DAPI (blue) was used as nuclear staining for the immunostaining. 20x magnification. Image from "Human adipose stem cells cell sheet constructs impact epidermal morphogenesis in full-thickness excisional wounds" used with author's permission[15]

In the lab: At the 3B's Research Group, alternative methodologies for wound healing are being explored. Innovative scaffolds such as spongy-like hydrogels were shown to improve healing outcomes in animal[27–30], also by promoting neovascularization. The same was verified using cell sheet engineering, a scaffold-free strategy that uses sheets of cells, being a completely biologic approach (Figure 2)[12,15].

ADIPOSE TISSUE

Adipose derived stem cells have similar differentiation potential as bone marrow-derived MSC. They are easily harvested from lipoaspirates through enzymatic dissociation (with e.g. collagenase) and isolation from the SVF[4,7,8,13,31]. ADSC can then be used as a cell suspension, mixed with aspirated fat or seeded onto scaffolds[8]. Minonzio et al.[31] demonstrates that these cells can be preserved frozen without losing their ability to differentiate. This justifies the existence of a new business of ADSC banking[18].

Fat grafting is a frequent procedure used for soft tissue filling, but it has the disadvantage of significant absorption (40% to 80%) of the transplanted fat[8,13,32]. *Contour deformities* can cause both aesthetic and functional problems[32]. Many permanent (e.g. silicone, polymethyl-methacrylate) and temporary (e.g. HA, collagen) fillers are used, however, they often have poor results and complications[33]. ADSC in combination with fat grafting are indicated for volume restoration, because of their capacity to increase fat tissue survival rates, promote higher connective tissue formation, decrease necrotic tissue and improve patient satisfaction[32,34]. Bashir et al.[32] was the first to report clinically the use of ex vivo expanded ADSC-enriched fat graft for facial lipodystrophy[13,35,36]. This combination use was also reported in lower limb atrophy from critical limb ischemia[13,35] and chronic ulcers caused by radiation therapy[16,17].

Fat grafts enriched with ADSC were also used for *breast augmentation*, with cosmetic or cancer reconstruction purposes[8,13,17,36–38]. Their application resulted in breast's circumference enlargement, with minimal absorption and reduced complications[39,40]. However, these cells secrete proangiogenic growth factors, that may result in an increased breast cancer's metastatic risk[37]. ADSC were found to increase growth of active tumour cells but not resting tumour cells[41], while other study demonstrated that the risk depended on the delivery methods[42]. In contrast, there are also reports of a decrease in tumour growth and metastasis index[37,43].

Inoculation of Crohn's disease fistulas external openings with autologous ADSC was successful in 6 of 20 patients in a pilot study[16,17]. Since then, this technique was used to repair tracheomediastinal fistulas caused by cancer ablation[16,17,44].

ADSC, due to their immunomodulatory and anti-inflammatory effects, were used in other autoimmune diseases such as systemic lupus erythematosus, autoimmune arthritis, rheumatoid arthritis and acute graft-versus-host disease[16]. They also have applications for neural regeneration in Parkinson's and Alzheimer's disease[16,19]; hepatic regeneration[16]; and mixed with PRP for age-related macular degeneration and corneal epithelium repair[13,16,17,19].

CRANIOCERVICAL DEFECTS

Head and neck structures control several senses, vital functions, the swallowing process, communication, facial animation and aesthetics[45]. Craniofacial defects can be congenital (e.g. cleft lip, calvarial defects or Romberg syndrome) or acquired (e.g. trauma or oncological resection)[45–47]. The functional, aesthetic and social effects resulting from these anomalies can severally affect the patient's quality-of-life[45,46].

Soft tissue defects causing facial contour deformities require soft tissue augmentation therapies that usually consist of autologous fat grafting[32]. Case reports and prospective studies showed that ADSC enriched fat is more beneficial for facial contour deformities repair, reducing the need to repeat the grafting[32,34]. This combination is indicated for loss of substance on the face, volume loss in aging, hemifacial microsomia, scleroderma, Parry-Romberg syndrome and in facial skin necrosis[16–18]. PRP therapies have shown benefits in deep-plane rhytidectomy (for reducing tissue inflammation, oedema and ecchymosis)[48–50], Romberg syndrome and facial tissue atrophy type 1 and 2 [51–54].

Cranial bone defects are extremely common in children[55]. In craniofacial bone's replacement higher porosity is essential for vascularization, osteoblast proliferation and migration, but this leads to lower mechanical properties[46]. For bone replacement, it is thought to be essential to incorporate mineral phases onto the scaffolds for osteoinductivity[46,56]. Calvarial defects are usually treated with cranioplasty using autologous bone grafting, titanium mesh implants or polymethylmethacrylate[45]. Several animal and human studies showed promising results with the use of ADSC in tissue engineered constructs for these defects[9,13,16,17,45,57]. Case reports demonstrate new bone formation using ADSC in combination with an autologous cancellous bone from the iliac crest and fibrin glue for calvarial traumatic defects, without using exogenous growth factors[57,58]. The benefits of ADSC use were also reported in mandibular, maxilla, frontal sinus and nasal septum defects[59].

Bone marrow-derived MSC were beneficial on the improvement of bone defects healing[60] and have been used for a functional neomandible creation[61].

There is also a FDA approved product for alveolar ridge augmentation, including in alveolar clefts, *INFUSE* bone graft, that consists of a collagen sponge soaked with recombinant human BMP-2[62].

Cartilage tissue engineering could provide adequate amounts of tissue to overcome the cartilage donor site shortage and morbidity[45,47]. *Nasal lesions* often occur after oncologic resection of skin cancers, this resection is usually very mutilating requiring multiple local skin flaps[47]. Injected gelatinous chondroid matrix with chondrocytes harvested from the auricular cartilage, was used for nasal augmentation, with formation of hard neocartilage and satisfactory long-lasting effects[63]. In other human trial, resected alar cartilage was replaced with a tissue engineered mesh; however, the authors were incapable of proving the cartilaginous and not scar nature of the neotissue[64].

One of the biggest challenges in plastic surgery is *auricle reconstruction*, due to acquired or congenital deformities (e.g. microtia and prominent ears) [4,65]. The current treatments for these defects include an auricular shaped autologous rib cartilage or artificial implants[4,45,47,65]. One human trial, including 4 patients with microtia, showed, with great results, the possibility of injecting cultured chondrocytes into the subcutaneous pocket on the fascia of the lower abdomen to form a neocartilage block that was surgically harvested and carved into the shape of the auricle and implanted[66]. TE in nasal and auricle reconstruction is far advanced in animal studies, but still lacks human trials; there are high hopes for cartilage culture methods of chondrocytes or pluripotent stem cells on 3D scaffolds[4,45,47,65].

Tracheal defects can be life-threatening. The current synthetic materials that have been used as tracheal substitutes present a risk of granulation formation or stenosis, and allografts imply the need for lifelong immunosuppression[47]. There are several tissue- engineered substitutes, such as decellularized tracheal allografts[9,47,67,68], a rigid prosthetic tube lined by a free radial forearm flap[9,68,69], a decellularized tracheal autograft with bone marrow MSC and bronchial stem cells[11,70], and a tracheal allograft combined with a buccal mucosal graft[9,68,71]. A case report of the use of a decellularized tracheal autograft repopulated with recipient's respiratory epithelium and mesenchymal stromal cells on a 10-year-old child showed that the graft was completely integrated, but there was no proof of neocartilage formation and it didn't grow like the adjacent native trachea[47,70].

The *laryngeal* structures require a complex neural control and integration into reflex movement patterns. Therefore, tissue engineered laryngeal substitutes are still in a preclinical stage, since vocal fold motion has not yet been achieved[47].

MUSCULOSKELETAL TISSUES

The actual gold standards for *bone defects* are autologous bone grafting and cadaveric/decellularized bone allografts[5,9]. These methods are limited by variable graft resorption[72,73], risk of graft infection[72] and the potential of disease transmission[9,73].

Bone TE has been applied for years, in simpler forms such as corticotomy for osteoinduction[9]. Nowadays, the tissue engineered methods include transplantation of in vitro cultivated cells and guided tissue regeneration with bone mass or artificial material[4]. Osteoblasts, MSC, embryonic and skeletal muscle stem cells, all present osteogenic potential[8]. Scaffolds with calcium phosphate, hydroxyapatite, silica and collagen as the matrix are seen as necessary[8].

The first reported tissue engineered bone repair was in 2001. Bone marrow MSC were used in 3 patients to treat large bone defects, from the tibia, ulna and humerus[74]. The INFUSE bone graft, used for alveolar clefts repair, can also be used for open tibial shaft fractures[62]. A similar product, OP-1 Implant, consisting of recombinant human BMP-2 with a bovine collagen carrier, was FDA approved for long bones non-union refractory lesions[9,75]. MSC transplantations improved hematopoietic stem cell engraftment for healing critical sized bone defects and for children with *osteogenesis imperfecta*[11,61].

The current treatments for *articular cartilage* injury are essentially for symptoms' control[19]. Bone marrow MSC, cultured in a collagen gel scaffold, are useful in TE for intervertebral disk replacement, knee joint resurfacing and digital-joint engineering[5,11,76,77]. These cells were also used to repair articular defects with intra-articular injections[78]. These treatments showed efficiency in reducing pain and improving walking ability[13]. Other treatment alternative

is expanded autologous chondrocytes re-injected into the defect[79]. With engineered cartilage, it is difficult to obtain a secure healing of the cartilage to the underlying bone[7] and there is a danger of dedifferentiation of cells in chondrogenic grafts into fibroblastoid cells[5].

Tendon and muscle repair with tissue engineering techniques is still at a pre-clinical phase, with promising results in animal trials[8,19]. Bone marrow MSC and ADSC were described in several studies as useful in increasing tendon repair and tensile strength[19]. Recently was identified Growth Differentiation Factor – 5 as possibly having a significant role in tenogenic differentiation, but further studies are needed[80].

Engineering of skeletal muscle is challenging due to its complex microelectrical and mechanical networks[8]. Mechanical stimulation, electrical stimulation and vascularization of constructs, with myoblasts or myogenic stem cells in vitro, showed great potential for future approaches[8,81].

PERIPHERAL NERVE DEFECTS

The current replacement options for peripheral nerve defects include autologous, allogeneic and acellular nerve grafts, that are limited by neuroma formation, immune reaction and delayed regeneration, respectively[8,82]. Schwann cell transplantation showed capacity to enhance peripheral nerve repair and are considered the most suitable cell type for neural regeneration. However, there is considerable donor-site morbidity, these cells have a slow proliferation rate and cell transplantation alone is limited by suboptimal spatial arrangement[4,8,19]. Bone marrow MSC and ADSC have also shown success in enhancing neural regeneration by releasing neurotrophic factors, in vitro and in animal models[8,19,83,84].

VASCULAR TISSUES

When vascular grafts are needed in reconstructive surgery, the actual options are autologous vessels, which can be useless due to a disease, or synthetic grafts, which are prothrombotic[8]. The first bioengineered vessel was clinically used in 1999, on a four-year-old girl, to repair a total occlusion of the right intermediate pulmonary artery[85]. The construct was created by seeding cells extracted from the wall of a peripheral vessel into a biodegradable tube[85]. L'Heureux et al.[86] created a vessel with cultured human vascular smooth muscle cells without any synthetic material. The vessel displayed similar strength to a human vessel and demonstrated to be functional in vivo in animal models. A clinical trial examined the use of these vessel grafts as arteriovenous shunt in end-stage renal disease patients[87]. Expanded findings from this study showed that the majority of the patients enrolled had successful implantations[87].

Niklason et al. [88] reported a production of arbitrary lengths of bovine vascular conducts from smooth muscle and endothelial cells culture under pulsatile stimuli in vitro; these constructs demonstrated contractile responses including responses to pharmacological agents.

Smooth muscle cells (SMC) have limited proliferation and cultural senescence[89]. One study attempted to derive SMC from human hair follicle stem cells culture. These expressed similar markers to the SMC from human umbilical artery and also showed contractile function[89]. This could be a future reliable source of SMC for blood vessel engineering.

MSCs were also used in vascularization strategies, showing their potential to differentiate into endothelial cells, to promote endothelial repair by secretion of paracrine factors and prevent neointimal formation[19].

Clinical testing on human bone marrow MSCs, ADSCs, fetal stem cells, hematopoietic stem cells and others, showed their vascularization benefits in myocardial post-ischemic neovascularization, neovascularization in systemic sclerosis, myogenic regeneration and neovascularization in erectile dysfunction, angiogenesis in nonrevascularizable limb ischemia, wound neovascularization and cerebral injury revascularization[16].

The cell sheet technique was used as a vascularization strategy, without the use of extrinsic growth factors, with capillary-like structures organization when under hypoxic conditions[90].

In the lab: To tackle the current limitations related with the vascularization of constructs, the 3B's Research Group proposed the use of the Stromal Vascular Fraction of Adipose Tissue as a vascularization tool[91]. Cell sheets of SVF were shown to restore blood flux in a hind-limb ischemia mouse model[90]. SVF can also be used in combination with scaffolds, allowing their prevascularization without the use of growth factors (Figure 3).

CONCLUSION

The potential for tissue engineering is very big, however there are problems still to solve. There is the challenge of meeting the scientific needs while creating a durable and functional tissue and, at the same time, facing the complex regulatory processes until approval for human use[3]. Researchers need to better understand the role, interactions and fate of stem cells. Large constructs will still be limited by poor blood supply until significantly improved tissue engineering vascularization strategies arise. And the possible side effects (including malignancies) of growth factors

Figure 3. CD31 (green) and DAPI (blue) cell staining in a spongy-like hydrogel seeded with SVF cells. Extensive prevascular network formation is visible, in the absence of angiogenic growth factors. 63x magnification. Unpublished results

recurrent use in tissue engineering is still unknown[3,8,16]. Besides these biological problems, there is an increase in health care costs, regulatory restrictions and ethical concerns, that contribute to the delay of tissue engineering widespread application[8].

To allow this integration of tissue engineered constructs into clinical practice, there is the need for a multidisciplinary team, a clear understanding of manufacturing workflow and to understand the possibility/need of storage into specialized banks[3,8,23].

Tissue engineering has the potential to revolutionize clinical practice as we know it. Small successes so far were achieved, especially in plastic and reconstructive surgery, but there is still a great need for further investigations and clinical trials before this becomes reality.

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LITERATURE

1. Skalak R, Fox CF. Tissue engineering: proceedings of a workshop, held at Granlibakken, Lake Tahoe, California, February 26-29, 1988. Liss; 1988, 343 pages. ISBN-10: 0845147064
2. Sterodimas A., De Faria J., Correa WE., Pitanguy I. Tissue engineering in plastic surgery: an up-to-date review of the current literature. *Ann Plast Surg*. 2009, 62:97-103.
3. Al-Himdani S., Jessop Z.M., Al-Sabah A. et al. Tissue-Engineered Solutions in Plastic and Reconstructive Surgery: Principles and Practice. *Frontiers in Surgery*. 2017, 4(4):1-13.
4. Kratz G., Huss F. Tissue engineering--body parts from the Petri dish. *Scand J Surg*. 2003, 92:241-7.
5. Goessler UR., Hormann K., Riedel F. Tissue engineering with adult stem cells in reconstructive surgery (review). *Int J Mol Med*. 2005, 15:899-905.
6. Hillel AT., Elisseeff JH. Embryonic progenitor cells in adipose tissue engineering. *Facial Plast Surg*. 2010, 26:405-12.
7. Miller MJ., Patrick CW., Jr. Tissue engineering. *Clinics in Plastic Surgery*. 2003, 30:91-103.
8. Wong VW., Rustad KC., Longaker MT., Gurtner GC. Tissue engineering in plastic surgery: a review. *Plast Reconstr Surg*. 2010, 126:858-68.
9. Golas AR., Hernandez KA., Spector JA. Tissue engineering for plastic surgeons: a primer. *Aesthetic Plast Surg*. 2014, 38:207-21.
10. Takahashi K., Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006, 126:663-76.
11. Cetrulo C.L., Jr. Cord-blood mesenchymal stem cells and tissue engineering. *Stem Cell Rev*. 2006, 2:163-8.
12. Cerqueira MT., Pirraco RP., Martins AR., Santos TC., Reis RL., Marques AP. Cell sheet technology-driven re-epithelialization and neovascularization of skin wounds. *Acta Biomater*.2014, 10:3145-55.
13. Zarei F., Negahdari B. Recent progresses in plastic surgery using adipose-derived stem cells, biomaterials and growth factors. *J Microencapsul*. 2017, 34:699-706.
14. Kim JJ., Evans GR. Applications of biomaterials in plastic surgery. *Clin Plast Surg*. 2012, 39:359-76.
15. Cerqueira MT., Pirraco RP., Santos TC., Rodrigues DB., Frias AM., Martins AR., et al. Human adipose stem cells cell sheet constructs impact epidermal morphogenesis in full-thickness excisional wounds. *Biomacromolecules*. 2013, 14:3997-4008.
16. Gentile P., Scioli MG., Bielli A., Orlandi A., Cervelli V. Concise Review: The Use of Adipose-Derived Stromal Vascular Fraction Cells and Platelet Rich Plasma in Regenerative Plastic Surgery. *Stem Cells*. 2017, 35:117-34.
17. Gentile P., Orlandi A., Scioli MG., Di Pasquali C., Bocchini I., Cervelli V. Concise review: adipose-derived stromal vascular fraction cells and platelet-rich plasma: basic and clinical implications for tissue engineering therapies in regenerative surgery. *Stem Cells Transl Med*. 2012, 1:230-6.
18. Kim Y-J., Jeong J-H. Clinical application of adipose stem cells in plastic surgery. *J Korean Med Sci*. 2014, 29:462-7.
19. Naderi N., Combellack EJ., Griffin M., Sedaghati T., Javed M., Findlay MW. et al. The regenerative role of adipose-derived stem cells (ADSC) in plastic and reconstructive surgery. *Int Wound J*. 2017, 14:112-24.
20. Sung HM., Suh IS, Lee HB, Tak KS, Moon KM, Jung MS. Case Reports of Adipose-derived Stem Cell Therapy for Nasal Skin Necrosis after Filler Injection. *Arch Plast Surg*. 2012, 39:51-4.
21. Gentile P., Cervelli V. Adipose-Derived Stromal Vascular Fraction Cells and Platelet-Rich Plasma: Basic and Clinical Implications for Tissue Engineering Therapies in Regenerative Surgery. *Methods Mol Biol*. 2018, 1773:107-22.
22. Gentile P., Orlandi A., Scioli MG., Di Pasquali C., Bocchini I., Cervelli V. Concise review: adipose-derived stromal vascular fraction cells and platelet-rich plasma: basic and clinical implications for tissue engineering therapies in regenerative surgery. *Stem cells translational medicine*. 2012, 1:230-6.
23. Caterson EJ., Caterson SA. Regeneration in medicine: a plastic surgeons "tail" of disease, stem cells, and a possible future. *Birth Defects Res C Embryo Today*. 2018, 84:322-34.
24. Sheridan RL. Comprehensive treatment of burns. *Curr Probl Surg*. 2001, 38:657-756.
25. Markeson D., Pleat JM., Sharpe JR., Harris AL., Seifalian AM., Watt SM. Scarring, stem cells, scaffolds and skin repair. *Journal of Tissue Engineering and Regenerative Medicine*. 2015, 9:649-68.
26. Klar AS., Guven S., Biedermann T., Luginbuhl J., Bottcher-Haberzeth S., Meuli-Simmen C., et al. Tissue-engineered dermo-epidermal skin grafts prevascularized with adipose-derived cells. *Biomaterials*. 2014, 35:5065-78.
27. Cerqueira MT, da Silva LP, Santos TC, Pirraco RP., Correlo VM., Marques AP., et al. Human skin cell fractions fail to self-organize within a gellan gum/hyaluronic acid matrix but positively influence early wound healing. *Tissue Eng Part A*. 2014, 20:1369-78.
28. Cerqueira MT., da Silva LP., Santos TC., Pirraco RP., Correlo VM., Reis RL., et al. Gellan Gum-Hyaluronic Acid Spongy-like Hydrogels and Cells from Adipose Tissue Synergize Promoting Neoskin Vascularization. *ACS Applied Materials & Interfaces*. 2014, 6:19668-79.
29. da Silva LP., Santos TC., Rodrigues DB., Pirraco RP., Cerqueira MT., Reis RL., et al. Stem Cell-Containing Hyaluronic Acid-Based Spongy Hydrogels for Integrated Diabetic Wound Healing. *Journal of Investigative Dermatology*. 2017, 137:1541-51.
30. Silva LP., Pirraco R.P, Santos TC., Novoa-Carballal R., Cerqueira MT., Reis RL., et al. Neovascularization Induced by the Hyaluronic Acid-

Based Spongy-Like Hydrogels Degradation Products. *ACS Appl Mater Interfaces*. 2016, 8:33464-74.
31. Minonzio G., Corazza M., Mariotta L., Gola M., Zanzi M., Gandolfi E., et al. Frozen adipose-derived mesenchymal stem cells maintain high capability to grow and differentiate. *Cryobiology*. 2014, 69:211-16.
32. Bashir MM., Sohail M., Bashir A., Khan FA., Jan SN., Imran M., et al. Outcome of Conventional Adipose Tissue Grafting for Contour Deformities of Face and Role of Ex Vivo Expanded Adipose Tissue-Derived Stem Cells in Treatment of Such Deformities. *J Craniofac Surg*. 2018, 29:1143-7.
33. Lequeux C., Rodriguez J., Boucher F., Rouyer O., Damour O., Mojallal A., et al. In vitro and in vivo biocompatibility, bioavailability and tolerance of an injectable vehicle for adipose-derived stem/stromal cells for plastic surgery indications. *J Plast Reconstr Aesthet Surg*. 2015, 68:1491-7.
34. Kolle SF., Fischer-Nielsen A., Mathiasen AB., Elberg JJ., Oliveri RS., Glovinski PV., et al. Enrichment of autologous fat grafts with ex-vivo expanded adipose tissue-derived stem cells for graft survival: a randomised placebo-controlled trial. *Lancet*. 2013, 382:1113-20.
35. Lee HC., An SG., Lee HW., Park JS., Cha KS., Hong TJ., et al. Safety and effect of adipose tissue-derived stem cell implantation in patients with critical limb ischemia: a pilot study. *Circ J*. 2012, 76:1750-60.
36. Tiryaki T., Findikli N., Tiryaki D. Staged stem cell-enriched tissue (SET) injections for soft tissue augmentation in hostile recipient areas: a preliminary report. *Aesthetic Plast Surg*. 2011, 35:965-71.
37. Alperovich M., Lee ZH., Friedlander PL., Rowan BG., Gimble JM., Chiu ES. Adipose stem cell therapy in cancer reconstruction: a critical review. *Ann Plast Surg*. 2014, 73:104-7.
38. Yoshimura K., Asano Y., Aoi N., Kurita M., Oshima Y., Sato K., et al. Progenitor-enriched adipose tissue transplantation as rescue for breast implant complications. *Breast J*. 2010, 16:169-75.
39. Wang L., Lu Y., Luo X., Fu MG., Hu X., Dong H., et al. „Cell-assisted lipotransfer for breast augmentation: a report of 18 patients". *Zhonghua zheng xing wai ke za zhi= Zhonghua zhengxing waike zazhi= Chinese journal of plastic surgery*, 2012, 28.1:1-6.
40. Yoshimura K., Sato K., Aoi N., Kurita M., Hirohi T., Harii K. Cell-assisted lipotransfer for cosmetic breast augmentation: supportive use of adipose-derived stem/stromal cells. *Aesthetic Plast Surg*. 2008, 32:48-55.
41. Zimmerlin L., Donnenberg AD., Rubin JP., Basse P., Landreneau RJ., Donnenberg VS. Regenerative therapy and cancer: in vitro and in vivo studies of the interaction between adipose-derived stem cells and breast cancer cells from clinical isolates. *Tissue Eng Part A*. 2011, 17:93-106.
42. Altman AM., Prantl L., Muehlberg FL., Song YH., Seidensticker M., Butler CE., et al. Wound microenvironment sequesters adipose-derived stem cells in a murine model of reconstructive surgery in the setting of concurrent distant malignancy. *Plast Reconstr Surg*. 2011, 127:1467-77.
43. Sun B., Roh KH., Park JR., Lee SR., Park SB., Jung JW., et al. Therapeutic potential of mesenchymal stromal cells in a mouse breast cancer metastasis model. *Cytotherapy*. 2009, 11:289-98.
44. Alvarez PD., Garcia-Arranz M.., Georgiev-Hristov T, Garcia-Olmo D. A new bronchoscopic treatment of tracheomediastinal fistula using autologous adipose-derived stem cells. *Thorax*. 2008, 63:374-6.
45. Lott DG., Janus JR.. Tissue engineering for otorhinolaryngology-head and neck surgery. *Mayo Clin Proc*. 2014, 89:1722-33.
46. Nyberg EL., Farris AL., Hung BP., Dias M., Garcia JR., Dorafshar AH., et al. 3D-Printing Technologies for Craniofacial Rehabilitation, Reconstruction, and Regeneration. *Ann Biomed Eng*. 2017, 45:45-57.
47. Wiggenhauser PS., Schantz JT., Rotter N. Cartilage engineering in reconstructive surgery: auricular, nasal and tracheal engineering from a surgical perspective. *Regen Med*. 2017, 12:303-14.
48. Man D., Plosker H., Winland-Brown JE. The use of autologous platelet-rich plasma (platelet gel) and autologous platelet-poor plasma (fibrin glue) in cosmetic surgery. *Plast Reconstr Surg*. 2001, 107:229-37.
49. Powell DM., Chang E., Farrior EH. Recovery from deep-plane rhytidectomy following unilateral wound treatment with autologous platelet gel: a pilot study. *Arch Facial Plast Surg*. 2001, 3:245-50.
50. Guerrerosantos J., Guerrerosantos F., Orozco J. Classification and treatment of facial tissue atrophy in Parry-Romberg disease. *Aesthetic Plast Surg*. 2007, 31:424-34.
51. Guerrerosantos J. Evolution of Technique: Face and Neck Lifting and Fat Injections. *Clinics in Plastic Surgery*. 2008, 35:663-76.
52. Cervelli V., Gentile P. Use of cell fat mixed with platelet gel in progressive hemifacial atrophy. *Aesthetic Plast Surg*. 2009, 33:22-7.
53. Coleman SR. Long-term survival of fat transplants: controlled demonstrations. *Aesthetic Plast Surg*. 1995, 19:421-5.
54. Grimaldi M , Gentile P , Labardi L , Silvi E , Trimarco A , Cervelli V. Lipostructure technique in Romberg syndrome. *J Craniofac Surg*. 2008, 19:1089-91.
55. Hixon KR., Melvin AM., Lin AY., Hall AF., Sell SA. Cryogel scaffolds from patient-specific 3D-printed molds for personalized tissue-engineered bone regeneration in pediatric cleft-craniofacial defects. *J Biomater Appl*. 2017, 32:598-611.
56. Azami M., Samadikuchaksaraei A., Poursamar SA. Synthesis and characterization of a laminated hydroxyapatite/gelatin nanocomposite scaffold with controlled pore structure for bone tissue engineering. *Int J Artif Organs*. 2010, 33:86-95.
57. Thesleff T., Lehtimaki K., Niskakangas T., Mannerstrom B., Miettinen S., Suuronen R., et al. Cranioplasty with adipose-derived stem cells and biomaterial: a novel method for cranial reconstruction. *Neurosurgery*. 2011, 68:1535-40.
58. Lendeckel S., Jodicke A., Christophis P., Heidinger K., Wolff J., Fraser JK., et al. Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: case report. *J Craniomaxillofac Surg*. 2004, 32:370-3.
59. Sandor GK., Numminen J., Wolff J., Thesleff T., Miettinen A., Tuovinen VJ., et al. Adipose stem cells used to reconstruct 13 cases with cranio-maxillofacial hard-tissue defects. *Stem Cells Transl Med*. 2014, 3:530-40.
60. Petite H., Viateau V., Bensaid W., Meunier A., de Pollak C., Bourguignon M., et al. Tissue-engineered bone regeneration. *Nat Biotechnol*. 2000, 18:959-63.
61. Warnke PH., Springer IN., Wiltfang J., Acil Y., Eufinger H., Wehmoller M., et al. Growth and transplantation of a custom vascularised bone graft in a man. *Lancet*. 2004, 364:766-70.
62. Chin M., Ng T., Tom WK., Carstens M. Repair of alveolar clefts with recombinant human bone morphogenetic protein (rhBMP-2) in patients with clefts. *J Craniofac Surg*. 2005, 16:778-89.
63. Yanaga H., Imai K., Yanaga K. Generative surgery of cultured autologous auricular chondrocytes for nasal augmentation. *Aesthetic Plast Surg*. 2009, 33:795-802.
64. Fulco I., Miot S., Haug MD., Barbero A., Wixmerten A., Feliciano S., et al. Engineered autologous cartilage tissue for nasal reconstruction after tumour resection: an observational first-in-human trial. *Lancet*. 2014, 384:337-46.
65. Storck K., Staudenmaier R., Buchberger M., Strenger T., Kreutzer K., von Bomhard A., et al. Total reconstruction of the auricle: our experiences on indications and recent techniques. *Biomed Res Int*. 2014;2014: ID 373286.1-15. Doi: 10.1155/2014/373286.
66. Yanaga H., Imai K., Fujimoto T., Yanaga K. Generating ears from cultured autologous auricular chondrocytes by using two-stage implantation in treatment of microtia. *Plast Reconstr Surg*. 2009, 124:817-25.
67. Propst EJ., Prager JD., Meinzen-Derr J., Clark SL., Cotton RT., Rutter MJ. Pediatric tracheal reconstruction using cadaveric homograft. *Arch Otolaryngol Head Neck Surg*. 2011, 137:583-90.
68. Rich JT., Gullane PJ. Current concepts in tracheal reconstruction. *Curr Opin Otolaryngol Head Neck Surg*. 2012, 20:246-53.

69. Yu P, Clayman GL, Walsh GL. Long-term outcomes of microsurgical reconstruction for large tracheal defects. *Cancer*. 2011, 117:802-8.
70. Hamilton NJ., Kanani M., Roebuck DJ., Hewitt RJ., Cetto R., Culme-Seymour EJ., et al. Tissue-Engineered Tracheal Replacement in a Child: A 4-Year Follow-Up Study. *Am J Transplant*. 2015, 15:2750-7.
71. Delaere P., Vranckx J., Verleden G., De Leyn .P, Van Raemdonck D. Tracheal allotransplantation after withdrawal of immunosuppressive therapy. *N Engl J Med*. 2010, 362:138-45.
72. Neovius E., Engstrand T. Craniofacial reconstruction with bone and biomaterials: review over the last 11 years. *J Plast Reconstr Aesthet Surg*. 2010, 63:1615-23.
73. Oppenheimer AJ,, Mesa J,, Buchman SR. Current and emerging basic science concepts in bone biology: implications in craniofacial surgery. *J Craniofac Surg*. 2012, 23:30-6.
74. Quarto R., Mastrogiacomo M., Cancedda R., Kutepov SM., Mukhachev V., Lavroukov A., et al. Repair of large bone defects with the use of autologous bone marrow stromal cells. *N Engl J Med*. 344, 2001, p. 385-386.
75. Lissenberg-Thunnissen SN., de Gorter DJ., Sier CF., Schipper IB. Use and efficacy of bone morphogenetic proteins in fracture healing. *Int Orthop*. 35, 2011, p. 1271-1280.
76. Nejadnik H., Hui JH., Feng Choong EP., Tai BC., Lee EH. Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: an observational cohort study. *Am J Sports Med*. 2010, 38:1110-6.
77. Wakitani S., Imoto K., Yamamoto T., Saito M., Murata N., Yoneda M. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. *Osteoarthritis Cartilage*. 2002, 10:199-206.
78. Orozco L., Munar A., Soler R., Alberca M., Soler F., Huguet M., et al. Treatment of knee osteoarthritis with autologous mesenchymal stem cells: a pilot study. *Transplantation*. 2013, 95:1535-41.
79. Brittberg M., Lindahl A., Nilsson A., Ohlsson C., Isaksson O., Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med*. 1994, 331:889-95.
80. James R., Kumbar SG., Laurencin CT., Balian G., Chhabra AB. Tendon tissue engineering: adipose-derived stem cell and GDF-5 mediated regeneration using electrospun matrix systems. *Biomed Mater*. 2011, 6.2:025011. Doi: 10.1088/1748-6041/6/2/025011
81. Kwee BJ., Mooney DJ. Biomaterials for skeletal muscle tissue engineering. *Curr Opin Biotechnol*. 2017, 47:16-22.
82. Li GN., Hoffman-Kim D. Tissue-engineered platforms of axon guidance. *Tissue Eng Part B Rev*. 2008, 14:33-51.
83. Sowa Y., Imura T., Numajiri T., Nishino K., Fushiki S. Adipose-derived stem cells produce factors enhancing peripheral nerve regeneration: influence of age and anatomic site of origin. *Stem Cells Dev*. 2012, 21:1852-62.
84. Terenghi G., Wiberg M., Kingham PJ. Chapter 21: Use of stem cells for improving nerve regeneration. *Int Rev Neurobiol*. 2009, 87:393-403.
85. Shin'oka T, Imai Y, Ikada Y. Transplantation of a tissue-engineered pulmonary artery. *N Engl J Med*. 2001, 344(7):532-3.
86. L'Heureux N., Paquet S., Labbe R., Germain L., Auger FA. A completely biological tissue-engineered human blood vessel. *Faseb j*. 1998, 12:47-56.
87. Peck M., Gebhart D., Dusserre N., McAllister TN., L'Heureux N. The evolution of vascular tissue engineering and current state of the art. *Cells Tissues Organs*. 2012, 195:144-58.
88. Niklason LE., Gao J., Abbott WM., Hirschi KK., Houser S., Marini R., et al. Functional arteries grown in vitro. *Science*. 1999, 284:489-93.
89. Xu ZC., Zhang Q., Li H. Human hair follicle stem cell differentiation into contractile smooth muscle cells is induced by transforming growth factor-beta1 and platelet-derived growth factor BB. *Mol Med Rep*. 2013, 8:1715-21.
90. Costa M., Cerqueira MT., Santos TC.., Sampaio-Marques B, Ludovico P., Marques AP., et al. Cell sheet engineering using the stromal vascular fraction of adipose tissue as a vascularization strategy. *Acta Biomater*. 2017, 55:131-43.
91. Costa M., Pirraco RP., Cerqueira MT., Reis RL., Marques AP. Growth Factor-Free Pre-vascularization of Cell Sheets for Tissue Engineering. *Methods Mol Biol*. 2016, 1516:219-26.

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EXTRAMAMMARY PAGET'S DISEASE: A CASE REPORT OF VULVAR RECONSTRUCTION WITH GRACILIS MYOCUTANEOUS FLAP AFTER TOTAL VULVECTOMY

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SUMMARY

Extramammary vulvar Paget's disease is a very rare presentation of this disease, with few symptoms, whose initial complaint is the appearance of a slow-growing erythematous plaque in the anogenital region associated with pruritus. The evolution is chronic and the diagnosis is often late. Surgical excision is the main treatment and should be performed with wide margins, due to the high rate of local recurrence. Reconstruction is often complex, requiring the use of local or remote flaps. This manuscript presents the case report of a 65-year-old female patient, who had been suffering from pruritus in the vulvar region for 2 years and had a well-defined erythematous lesion, which showed no improvement with topical treatments and that was diagnosed as Paget's disease after biopsy. After surgical excision, reconstruction was performed using bilateral myofasciocutaneous flap of the gracilis muscle, with excellent aesthetic and functional results..

KEYWORDS

Extramammary Paget's disease; surgery; vulva; vulvectomy; cutaneous adenocarcinoma; Paget's carcinoma

INTRODUCTION

Extramammary Paget's disease is a rare tumor, often associated with multiple recurrences after extensive excisions.[1] It usually affects elderly patients, developing commonly in the anogenital region (vulva, penis, scrotum, perineum and perianal region) and less commonly in the axillary region.[1,2]

Paget's disease was first described by Sir James Paget in 1874, who described 15 patients with eczematous periareolar lesions, who later developed breast cancer.[3] The first description of extramammary Paget's disease was made by Crocker in 1889, who identified skin lesions histologically similar to Paget's disease, on the skin of the penis and scrotum.[4]

Vulvar reconstruction in Paget's disease is usually a challenge for the plastic surgeon, depending on the size and stage of the lesion, which can compromise the vulva both unilaterally and bilaterally, and can also compromise the vaginal or anorectal mucosa. Local or regional flaps are generally used for vulvar reconstruction, ranging from random to axial, both fasciocutaneous and myocutaneous.[5]

The ideal flap should (1) bring a good amount of well vascularized tissue with similar thickness to the defect, (2) with minimal morbidity to the donor areas, (3) without tension on the edges, (4) that is able to reestablish the functionally, (5) minimizing damage in gait and in the sitting position, (6) providing a good aesthetic result, (7) with sensitivity if possible, and (8) requiring only one surgical time.[6]

CASE REPORT

65-year-old female patient, hysterectomized for 38 years, with a family history of breast cancer and gastric neoplasia, complaining of 2-year history of pruritus in the vulvar region, without improvement with topical treatments,

Figure 1. Preoperative appearance of the lesion

Figure 2. Preoperative marking

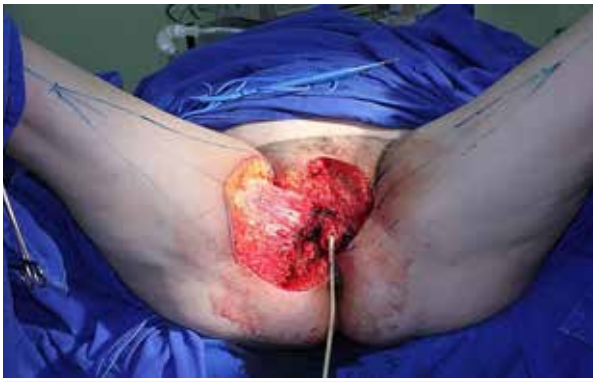

Figure 3. Aspect of the defect after excision and flap marking

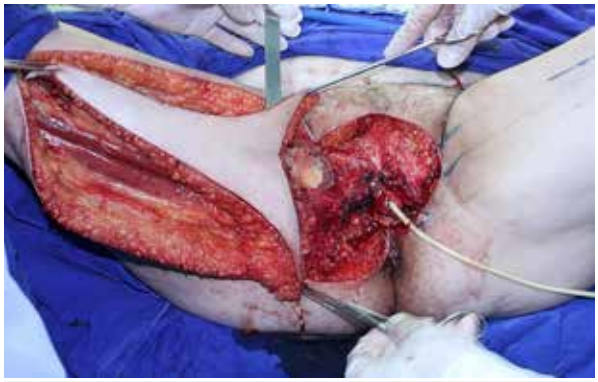

Figure 4. Attempt to advance the flap towards the defect

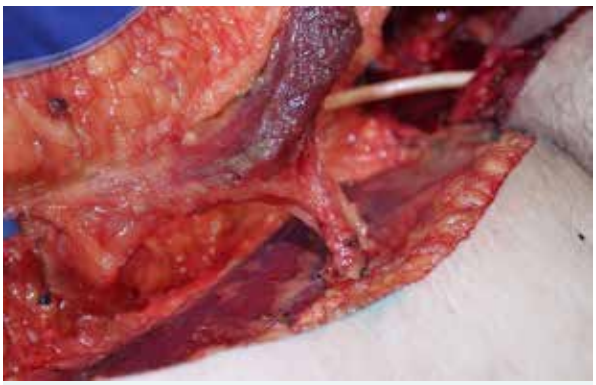

Figure 5. Identification of the flap feeding pedicle

presenting to the physical examination with an erythematous macula, slightly scaly, without erosion (Figure 1). After biopsy at the site, the presence of Paget cells was confirmed. It was initially treated with topical imiquimod, with a 20% reduction in the size of the lesion. No radiation therapy was performed at the site. Subsequently, she underwent total vulvectomy without lymphadenectomy by the gynecology team, according to the preoperative marking (Figures 2 and 3). For reconstruction, the fasciocutaneous V-Y flap of the medial region of the bilateral thigh was chosen, but after trying to advance the flap towards the defect, it was noted that it would arrive with difficulty, under tension, and its vascularization could be compromised (Figure 4). It was then decided to reconstruct the defect with a fascio-myocutaneous flap of the bilateral gracilis muscle, with perfusion being guaranteed through the branches of the medial circumflex arteries of the thigh (Figure 5). Then, the branches of the obturator nerve to the gracilis muscle were separated. At the end of the procedure, two continuous suction drains were placed below the flaps, bilaterally exiting through the inguinal crease. It was recommended, after the surgery, to use low molecular weight heparin (LMWH) subcutaneously, 40 mg daily for 10 days, in addition to antibiotic prophylaxis with ciprofloxacin 500 mg twice daily for 7 days. Gait was restricted for 1 week, but the patient could sit normally. After 1 week, the drains were removed and the patient was able to walk a few steps.

The patient returned again to the outpatient clinic, within 30 days, walking, without any functional deficit and a good aesthetic result was achieved, with adequate defect coverage, as seen both in the immediate postoperative period and 30 days after the surgery (Figure 6). She continued her follow-up with the team of gynecologists.

DISCUSSION

The lesions of extramammary Paget's disease usually appear as erythema plaques or spots that can later become erosive and infiltrative.[2,7] These tumors can present clinically in two ways, differing in prognosis. A primary form, as in the presented case, represented by an in situ epithelial car-

Figure 6. Immediate postoperative and after 30 days.

cinoma, usually originated from the proliferation of cells of the apocrine glands, such as the cells called Paget cells, and which has an excellent prognosis after extensive excision. The secondary form is usually represented by a primary, invasive cutaneous adenocarcinoma, underlying an injury, occurring synchronously, leading to a worse prognosis and having a greater variety of immunological markers.[8-10] There is an association with extravulvar adenocarcinoma in 30% of cases .[10] Cytokeratin 7 (CK7) is a Paget cell marker that has high sensitivity in detecting extramammary Paget's disease.[11] Other immunohistochemical marker numbers, such as Her2, p53, Ki-67, cyclin D1, CK20 and p-FAK, are recommended for disease detection and differentiation from primary and secondary forms.[7,12-15] The identification of the combined expression of high levels of Ki-67 and cyclin D1 is significantly associated with invasive lesions.[7] The patient in question had high levels of CK7 and p53; other markers were negative. Three biomarkers have been shown to be promissing, including serum cell-free (cf)DNA and a combination of serum cytokeratin 19 fragment 21-1 (CYFRA) and serum carcinoembryonic antigen (CEA), because they present a significant increase in patients with evidence of distant metastases, suggesting that they are useful as prognostic markers for therapeutic response.[16-17]

The most common differential diagnoses of extramammary Paget's disease include contact eczema, seborrheic dermatitis, psoriasis, ringworm and intertrigo.[8] It should also be differentiated from squamous cell carcinoma in situ, Pagetoid variant, which would be the vulvar equivalent of Bowen's disease.[18]

Surgical excision is the treatment of choice, being generally a complex procedure, involving extensive excisions and requiring major reconstructions. Excision generally requires safety margins of at least 2 cm[19], with a recurrence rate that can vary from 8-50% .[8,19] Other treatments have been recommended such as radiotherapy, CO2 laser and topical treatment with 5-fluoracil or imiquimod, however with results inferior to surgery.[8]Topical treatment with imiquimod 5% cream monotherapy or combined with 5-fluorouracil, can be useful for non-invasive extramammary Paget's disease, but these chemotherapeutic agents can induce cytological abnormalities in benign epithelial cells around the lesion that simulate a recurrent malignancy.

CONCLUSION

Extramammary Paget's disease is a disease with a high rate of recurrence when not resected with wide margins, which generally leads to great mutilation, making the reconstruction process difficult. Fortunately, this disease comprise only 1% of neoplasms of the vulva. Vulvar reconstruction with a myocutaneous flap of the gracilis muscle is a safe procedure, and although fasciocutaneous flaps are preferred in this type of reconstruction because they are easier, have good versatility and result in less morbidity, myocutaneous flaps may be necessary in the presence of major defects or to ensure better vascularization.

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Ethical compliance: The study conforms to the World Medical Association Declaration of Helsinki (June 1964) and subsequent amendments. The patient signed the informed consent for the procedure and for use of clinical data for scientific purposes and publication. Patient anonymity was ensured.

REFERENCES

1. Hendi A., Perdikis G., Snow JL. Unifocality of extramammary Paget disease. *J Am Acad Dermatol.* 2008, 59:811–13.
2. Shiomi T., Yoshida Y., Shomori K., Yamamoto O., Ito H. Extramammary Paget's disease: Evaluation of the histopathological patterns of Paget cell proliferation in the epidermis. *Journal of Dermatology*. 2011, 38:1054–7.
3. Paget J. One disease of the mammary areola proceeding cancer of the mammary gland. *St Barth Hosp Rep.* 1874, 10:87–9.
4. Crocker HR. Paget's disease affecting the scrotum und penis. *Trans Pathol Soc Lond*. 1889, 40:187–91.
5. Zeng A., Qiao Q., Zhao R., Song K., Long X. Anterolateral Thigh Flap–Based Reconstruction for Oncologic Vulvar Defects. *Plast. Reconstr. Surg*. 2011, 127:1939–45.

6. Salgarello M., Farallo E., Barone-Adesi L., Cervelli D., Scambia G., Salerno G., Margariti PA. Flap Algorithm in Vulvar Reconstruction after Radical, Extensive Vulvectomy. *Ann Plast Surg*. 2005, 54:184–90.
7. Aoyagi S., Akiyama M., Shimizu H. High expression of Ki-67 and cyclin D1 in invasive extramammary Paget's disease. *J Dermatol Sci*. 2008, 50:177–84.
8. Wagner G., Sachse MM. Extramammary Paget disease – clinical appearance, pathogenesis, management. *JDDG*. 2011, 9:448–54.
9. Grelck KW., Nowak MA., Doval M. Signet Ring Cell Perianal Paget Disease: Loss of MUC2 Expression and Loss of Signet Ring Cell Morphology Associated With Invasive Disease. *Am J Dermatopathol*. 2011, 33:616–20.
10. Fanning J., Lambert HC., Hale TM., Morris PC., Schuerch C. Paget's disease of the vulva: prevalence of associated vulvar adenocarcinoma, invasive Paget's disease, and recurrence after surgical excision. *Am J Obstet Gynecol*. 1999, 180:24–7.
11. Smith KJ., Tuur S., Corvette D., Lupton GP., Skelton HG. Cytokeratin 7 staining in mammary and extramammary Paget's disease. *Mod Pathol*. 1997, 10:1069–74.
12. Ellis PE., Fong LF., Rolfe KJ. et al. The role of p53 and Ki-67 in Paget's disease of the vulva and the breast. *Gynecol Oncol*. 2002, 86:150–6.
13. Wu ML., Guitart J. Low specificity of cytokeratin 20 in the diagnosis of extramammary Paget's disease. *Br J Dermatol*. 2000, 142:569–91.
14. Perrotto J., Abbott JJ., Ceilley RI., et al. The role of immunohistochemistry in discriminating primary from secondary extramammary Paget disease. *Am J Dermatopathol*. 2010, 32:137–43.
15. Chen S., Moroi Y., Urabe K., Takeuchi S. Concordant overexpression of p-FAK and p-ERK1/2 in extramammary Paget's disease. *Arch. Dermatol. Res*. 2008, 300:195–201.
16. Mijiddorj T., Kajihara I., Tasaki Y., et al. Serum cell-free DNA levels are a useful marker for extramammary Paget disease. *Br J Dermatol*. 2019, 181:505–11.
17. Nakamura Y., Tanese K., Hirai I., et al. Serum cytokeratin 19 fragment 21-1 and carcinoembryonic antigen combination assay as a biomarker of tumour progression and treatment response in extramammary Paget disease. *Br J Dermatol*. 2019, 181:535–43.
18. Armes JE., Lourie R., Bowlay G., Tabrizi S. Pagetoid Squamous Cell Carcinoma In Situ of the Vulva: Comparison With Extramammary Paget Disease and Nonpagetoid Squamous Cell Neoplasia. *Int J Gynecol Pathol*. 2008, 27:118–24.
19. Hatta N., Yamada M., Hirano T., Fujimoto A, Morita R. Extramammary Paget's disease: treatment, prognostic factors and outcome in 76 patients. *Br J Dermatol*. 2008, 158:313–18.

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Professor Ladislav Bařinka, MD, DSc
(July 1, 1927–July 28, 2020)

Professor Bařinka passed away at his home, accompanied by his wife Jarmila and other family members.

Professor Bařinka graduated from the Medical School of Masaryk University in Brno in 1955 and started working in the Plastic Surgery Institute (founded in 1949 in the building of previously nationalized Navratil GYN Private Sanatorium, Berková 34) at the same year. He was board certified from general surgery in 1958 and from plastic surgery under professor Karfík in 1961. Professor Bařinka´s research focused on – at that time – mysterious and difficult diseases like camptodactyly (PhD thesis in 1966) or treatment of lymphedema (Dr.Sc. in 1979). Later, together with the radiologist Professor Benda, they founded a Center for surgical treatment of lymphedema. This included both diagnostic processes and surgical treatment. Still today, we can meet patients after successful "radical operation" of low extremity lymphedema performed by professor Bařinka. The invention of "superdermatoma" allowed harvesting and processing large split skin grafts used in primary or delayed reconstructions. In the late 80s, professor Bařinka started his attempts to treat lymphedema by microsurgical anastomoses of lymphatic vessels into the veins using end-to-side L-V technique. This complex treatment of lymphedema was the only and unique in the whole country.

Prof. Bařinka earned most of his international fame by his innovative method of ear reconstruction using curved and sutured frame from costal cartilage. Artistic thinking and extremely skilled hands (both in speed and elegant movements to observe) allowed him to achieve better results than his contemporaries using older techniques. He operated more than 200 patients with congenital or acquired absence of the ear, unilateral or bilateral. He wrote a book "Rekonstrukce boltce ušního [Reconstruction of the auricle]" (1987, LF UJEP Brno), admirable witness even for today's generation of plastic surgeons who use modern alloplastic materials. Professor Bařinka´s reputation on ear reconstruction was well recognized in Europe and we, as young doctors, could benefit from numerous well-known plastic surgeons, like the members of "Mortier de Club", Prof. Mazola from Milan, Prof. Rafael from Grenoble or Prof. Van der Meulen from Rotterdam visiting and observing Prof. Bařinka in surgery. Prof. Bařinka´s presentation on this topic during the Centennial Symposium in Manhattan EET Hospital, New York, in 1968 was highly appreciated by the audience. Dr. Blair Rogers, one of the giants in ear reconstruction commented the presentation by the following words: "Listening and seeing Dr. Bařinka´s results today is like living through a veritable epoch in the history of advances in ear reconstruction".

Although this happened back in 1968, the American Society of Plastic Surgery honored Prof. Bařinka 24 years later by an invitation to give Honorary Maliniac Lecture during the Annual Meeting of ASPS in 1992. He became the first plastic surgeon from former communist countries who was privileged to give this lecture. Unfortunately, he was not able to travel to that meeting and invitation was given to Prof. Fára one year later.

Prof. Bařinka significantly contributed to establishing microsurgery as a new revolutionary technique in reconstructive procedures. He started enthusiastic attempts to replant amputated parts and very soon understood that microsurgery

needs new conditions for surgery and postoperative care. Nearly semiprivately he and the team of his friends constructed a new building – replantation and audiovisual centre in 1987. This centre serves to microsurgery very well until today and was actually the last new construction in Berkova 34 for the next 34 years till today.

At age of 65, at time for retirement, Prof. Bařinka started a private practice in Damascus where he founded a Department of Plastic Surgery in Teshreen Hospital and later he established a private clinic K.E.I. in Brno where he was operating till his age of 85 years.

Thanks to his inventive spirit, everyday hard work, and enthusiasm for plastic surgery, Prof. Bařinka´s life was fruitful in rare extent. His generosity and noble behaviour was inspiring the students and young doctors for 57 years of his active practice. His last academic publication was a majestic 800-page book "Plastická a rekonstrukční chirurgie" (2016) collecting professor´s whole life personal experience in the field of plastic surgery.

Prof. Bařinka was awarded many times, among the most important ones were state honours given by the president of the Czechoslovak Socialist Republic – "Vyznamenání za vynikající práci" in 1981 and by the president of the Czech Republic "Medaile za zásluhy" in 2012.

Professor Bařinka by his contribution to medical science has the right to be called "The Legend of Plastic Surgery".

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DIFÚZE INJEKTOVANÉ KOLAGENÁZY CLOSTRIDIUM HISTOLYTICUM U DUPUYTRENOVY NEMOCI: STUDIE IN VIVO

Kanatani, T., Nagura, I., Harada, Y., Lucchina, S.

Úvod. Při injektování kolagenázy Clostridium Histolyticum (CCH) jako nechirurgické léčbě Dupuytrenovy nemoci v dlani ruky by měla být doporučená hloubka vpichu „2 až 3 mm“. Difúze CCH uvnitř měkkých tkání kolem pruhu může vysvětlit výskyt běžných nežádoucích účinků uváděných v literatuře, jako jsou otoky, otoky v místě vpichu, krvavý puchýř, ruptury kůže a bolest v končetině. Předpokládali jsme, že injikovaná CCH se nejen koncentruje uvnitř pruhu, ale také rozptyluje jak podél pruhu, tak do sousedních tkání. Tato studie zkoumala naši hypotézu vizuálními intraoperačními nálezy po injekci jodopovidonu (PI) do pruhu.

Materiály a metody. Jodopovidon (PI) byl injikován do pruhu šesti pacientům s Dupuytrenovou kontrakturou (DC) před provedením otevřené chirurgické techniky (částečná fasciectomie). Vyznačili jsme tři hypotetické injekce kolagenázy Clostridium Histolyticum (CCH) ve 2mm intervalech na kůži nad pruhem kolem metakarpofalangeálního kloubu (MP) a hloubka injekce (vzdálenost od povrchu kůže ke středu pruhu) byla měřena ultrasonografií (US). Po dávkování 0,25 ml PI do tří bodů v měřených hloubkách jsme provedli pečlivou disekci a zkoumali jsme rozsah difúze PI vizuálně.

Výsledek. Průměrná hloubka injekce byla 2,6 mm. Ve všech případech byl pruhu homogenně obarven z injikovaných míst asi 10 mm ve svém rozsahu centrálně a došlo k infiltraci PI do subkutánních struktur a do okolní tukové tkáně. Ve třech případech došlo k difúzi kolem neurovaskulárních svazků a ve dvou případech k infiltraci struktur pod pruhem.

Závěr. Tato studie simulovala pravděpodobné difúzní rozšíření injektované CCH kolem pruhu. To znamená, že i když je CCH vstřikována do středu pruhu, nekoncentruje se jen uvnitř pruhu, ale také se rozptyluje podél pruhu a infiltruje do sousedních tkání s potenciálem sekundárního poškození.

OPTIMÁLNÍ HLOUBKA PRO APLIKOVÁNÍ INJEKCE KOLAGENÁZY CLOSTRIDIUM HISTOLYTICUM URČENÁ SONOGRAFIÍ PŘI LÉČBĚ DUPUYTRENOVY KONTRAKTURY

Nagura I., Kanatani T., Harada Y., Inui A., Mifune Y., Kuroda R., Lucchina S.

Úvod. Při injekci kolagenázy Clostridium Histolyticum jako nechirurgické léčbě Dupuytrenovy kontraktury v dlani je doporučená hloubka vpichu „2 až 3 mm“. V literatuře je však uvedeno jen málo důkazů podporujících toto doporučení. Cílem této studie bylo vyhodnotit optimální hloubku pro injekce kolagenázy Clostridium Histolyticum pro léčbu Dupuytrenovy kontraktury za pomoci ultrasonografie.

Materiály a metoda. Do studie bylo zařazeno 43 pacientů. Bod vpichu kolagenázy jsme označili na kůži nad pruhem před aplikací injekce. Pak jsme změřili vzdálenost od kůže k prostředku pruhu jako optimální hloubku za pomoci ultrazvukového zobrazení v podélné ose.

Výsledky. Průměrná vzdálenost od kůže ke středu pruhu byla 2,4 mm. Dále, průměrná vzdálenost od kůže k pruhu byla 1,0 mm a průměrná šířka pruhu byla 2,7 mm.

Závěr. Přesným měřením jednotlivých případů pomocí ultrasonografie se nám podařilo potvrdit, že doporučení pro hloubku injekce, které poskytl dodavatel kolagenázy Clostridium Histolyticum (2–3 mm), jsou v souladu s našimi nálezy. Nebyl však poskytnut žádný objektivní návod co se týče variability individuálních pacientů, a proto navrhujeme k zajištění lepších výsledků používat před výkonem ultrasonografii.

FUNKČNÍ REKONSTRUKCE MĚKKOTKÁŇOVÝCH OROFACIÁLNÍCH DEFEKTŮ MIKROVASKULÁRNÍM LALOKEM MUSCULUS GRACILIS

Stebel A., Hocková B., Abelovský J., Štorcelová D., Poruban D., Slávik R.

Úvod. Rekonstrukční mikrovaskulární chirurgie s použitím volných laloků umožňuje estetickou a funkční rehabilitaci středních a rozsáhlých měkkotkáňových defektů orofaciální oblasti. Mikrovaskulární laloková chirurgie s mírou úspěšnosti více než 90–98 % je zlatým standardem rozsáhlejších rekonstrukčních operací hlavy a krku. Rekonstrukční chirurgie je v současnosti zaměřena nejen na uzávěr defektu a přiměřený estetický efekt, ale stejný důraz se klade i na funkční výsledek. Funkční rekonstrukce po resekci jazyka či rtu znamená uzávěr defektu s možností obnovení pohybu. Volný lalok z gracilního svalu (musculus gracilis) je jedna z možností funkční rekonstrukce jazyka a rtů.

Metoda. Práce prezentuje roční zkušenost s pěti funkčními rekonstrukcemi středních a velkých defektů jazyka a defektu dolního rtu pomocí volného laloku musculus gracilis. Čtyři pacienti po resekci jazyka a jeden po subtotální resekci dolního rtu a části tváře podstoupili okamžitou funkční rekonstrukci volným lalokem musculus gracilis.

Výsledky. U všech pěti pacientů byly operační ablativní defekty primárně rekonstruované pomocí funkčního laloku musculus gracilis, bez ztráty laloku. Odběr laloku musculus gracilis považujeme za technicky nenáročný, přičemž lalok poskytuje dostatečný tkáňový objem na uzávěr středních orofaciálních defektů. Současně je možný odběr laloku s kožním ostrovem a následným jednoduchým uzávěrem odběrového místa primární suturou s nízkou morbiditou.

Pacienti po rekonstrukci jazyka mikrovaskulárním lalokem musculus gracilis byli před propuštěním domů schopni polykat kašovitou stravu. Pacient po totální resekci dolního rtu a rekonstrukci mikrovaskulárním lalokem musculus gracilis byl schopen před propuštěním domů přijímat potravu perorálně, ale i zavřít ústa. Funkční výsledek ve skupině pěti pacientů po rekonstrukci orofaciálního defektu mikrovaskulárním lalokem musculus gracilis bude třeba zhodnotit opakovaně v průběhu dvou let po operaci, a to vyhodnocením

schopnosti polykat a artikulovat po rekonstrukci jazyka a hodnocením uzávěru dutiny ústní a mimiky obličeje po rekonstrukci rtu.

Závěr. Rekonstrukce pomocí volného laloku musculus gracilis oproti čínskému laloku z předloktí umožňuje funkční rekonstrukci měkkotkáňových defektů. Funkční rekonstrukce defektů po resekci jazyka či rtu lalokem musculus gracilis garantuje dostatečný objem tkáně, velmi nízkou morbiditu odběrového místa a možnost obnovení pohyblivosti ve srovnání s použitím jiných mikrovaskulárních laloků, jako jsou anterolaterální stehenní lalok, perforátorový lalok a. circumflexa ilium superficialis, laterální pažní lalok nebo perforátorový lalok dolní hluboké epigastrické artérie.

DERMÁLNÍ NÁHRADA MATRIDERM® – PRVNÍ ZKUŠENOSTI NA KLINICE POPÁLENINOVÉ MEDICÍNY 3. LF UK A FNKV

Zajíček R., Šuca H., Grossová I., Fetissov V., Pafčuga I.

Úvod. Výsledná kvalita jizevnaté tkáně hraje důležitou roli v návratu pacientů do normálního života a společnosti. Používání umělých kožních náhrad v klinické praxi zlepšuje funkční i kosmetické výsledky nejen u popálených pacientů.

Materiál a metody. Kolagen-elastinová dermální náhrada Matriderm® umožňuje okamžitou aplikaci spolu s kožním štěpem. Autoři prezentují na souboru 10 pacientů první zkušenosti s použitím dermální náhrady Matridem® v léčbě kožních ztrát různé etiologie.

Výsledky. Průměrná doba hojení v soboru byla 19,6 dne, hojení probíhalo bez závažných infekčních komplikací s dobrým kosmetickým a funkčním výsledkem.

Závěr. Matriderm® představuje alternativní variantu dosud nejčastěji používané dermální náhrady Integra® v léčbě akutních kožních ztrát různé etiologie i v rekonstrukční chirurgii.

KOMPLEXNÍ REKONSTRUKCE OBLIČEJE NA ZÁKLADĚ 3D MODELŮ: PŘÍPADY S UŽITÍM PRELAMINACE A PŘEHLED LITERATURY

Frias F., Horta R.

3D modely umožnily obrovský pokrok v rekonstrukční chirurgii.

Byl proveden průzkum literatury s cílem zjistit současnou použitelnost 3D modelování kombinovaného s volnými laloky, které volné laloky se v těchto případech nejčastěji používají a bylo-li již popsáno použití 3D modelování u prelaminovaných volných laloků. Byli také vybráni dva pacienti s výrazným defektem obličeje, kteří podstoupili operační výkony zahrnující 3D modelování a prelaminační techniky.

3D modelování se nejčastěji používá při korekci mandibulárních defektů volnými fibulárními laloky. Žádný literární odkaz nevzpomíná kombinované použití 3D modelování a prelaminace, ale zde bylo demonstrováno použití těchto dvou technik společně. Byly získány dva custome-made a trojrozměrné prelaminované volné laloky, díky kterým dva vybraní pacienti dosáhli významného estetického a morfologického zlepšení.

Nejsou-li k dispozici žádné místní štěpy nebo laloky a je zamýšlena trojrozměrná rekonstrukce obličeje, je třeba zvážit využití prelaminační techniky. Složitost rekonstrukce může být usnadněna použitím 3D modelů, které umožňují získat personalizovaný lalok při zkrácení operačního času.

Prelaminace dohromady s 3D modelováním může být výkonným nástrojem pro korekci složitých deformit obličeje.

HIRUDOTERAPIE V REKONSTRUKČNÍ CHIRURGII: KAZUISTIKY A REVIEW

Heinz P., Tvrdý P., Pink R., Dvořák Z., Michl P.:

Pijavka lékařská *(Hirudo medicinalis, Hirudo verbana)* se v medicíně používána k léčbě různých onemocnění tisíce let. Popularita jejího použití se měnila v průběhu dějin a začátkem 19. století v Evropě dosáhla vrcholu. V moderní medicíně využili a popsali aplikaci pijavek poprvé u laloku s venózní kongescí v roce 1960 Deganc a Zdravic. V současnosti, zvláště v oblasti rekonstrukční chirurgie, dochází k určité renesanci hirudoterapie. Obecně je indikováno použití pijavek v průběhu kritického pooperačního období, kdy dochází k ustálení mikrocirkulace a vény nejsou schopny dostatečně odvádět přítok arteriální krve, což může vést k stagnaci oběhu v tkáních na všech úrovních, klinicky se projevující jako změna barvy a turgoru laloku. Není-li tato komplikace včas rozpoznána a adekvátně léčena, může dojít k nekróze tkáně. Medicinální pijavky lze použít při poruchách venózní drenáže po replantaci prstů, ušních boltců, rtu a části nosu. V rekonstrukční chirurgii hlavy a krku existuje řada studií potvrzujících úspěšnost hirudoterapie při evakuaci hematomu nebo řešení komplikací po replantaci skalpu a přenosech volných a stopkovaných laloků. Terapie přikládáním pijavek může být indikována i jako součást nechirurgických metod zlepšujících kondici venózního řečiště.

TKÁŇOVÉ INŽENÝRSTVÍ V PLASTICKÉ CHIRURGII – CO SE PODAŘILO

Ribeiro J., Pirraco R. P., Horta R.

V oblasti tkáňového inženýrství se při vývoji funkčních biologických náhrad uplatňují principy inženýrství a biologických věd. Rostoucí potřeba tkáně pro náročné rekonstrukční operace zapojuje plastické chirurgy do života a výzkumu inženýrských konstrukcí a do jejich následného použití.

Cílem tohoto narativního přehledu je shrnout principy tkáňového inženýrství, aktualizovat jeho současné použití a jeho průlomy, pochopit současná omezení a možné budoucí směry pro tuto vzrušující oblast léčby.

Tento přehled se zaměřil na použití tkáňového inženýrství u kožních lézí, kraniocervikálních defektů, muskuloskeletálních defektů, lézí periferních nervů, vaskulárních tkání a tukové tkáně.

Výzkum v tkáňovém inženýrství však roste exponenciálně, a ačkoli již existuje několik výstupů vytvořených s jeho pomocí, rozšířená klinická aplikace se stále teprve očekává. K tomu, aby se to stalo skutečností, je zapotřebí dlouhodobějších studií, které by zodpověděly nevyřešené problémy.

EXTRAMAMÁRNÍ PAGETOVA CHOROBA: ZPRÁVA O REKONSTRUKCI VULVY MYOKUTÁNNÍM LALOKEM M. GRACILIS PO ÚPLNÉ VULVEKTOMII

Simao T. S.

Extramamární vulvární Pagetova choroba je velmi vzácnou formou tohoto onemocnění, s několika příznaky, jejichž počátečním projevem je výskyt pomalu rostoucího erytematózního plaku v anogenitální oblasti spojeného s pruritem. Vývoj onemocnění je chronický a diagnóza je stanovena často pozdě. Chirurgická excize je hlavní léčebnou metodou a měla by být prováděna se širokým okrajem, vzhledem k vysoké míře lokálních recidiv. Rekonstrukce je často složitá a vyžaduje použití místních nebo vzdálených laloků. Tento text představuje případ 65leté pacientky, která po dobu 2 let trpěla svrběním v oblasti vulvy, měla jasně ohraničenou erytematózní lézi, která nevykazovala žádné zlepšení při lokální léčbě a ze které byla po biopsii potvrzena diagnóza Pagetovy choroby. Po chirurgické excizi byla provedena rekonstrukce pomocí oboustranného myofasciokutánního laloku m. gracilis s vynikajícím estetickým a funkčním výsledkem.

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