Matrix Flaps – New Approaches to Flap Prefabrication: Experimental Data and First Clinical Applications

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5.1 Introduction: Flaps, Cells, Matrices, and Modulation

Reconstruction of large soft tissue defects caused by tumors or trauma remains a surgical challenge. Flap prefabrication of customized tissue components has been one of the recent advances in reconstructive surgery [1, 2], and the technique has fulfilled the need for flaps with multiple surfaces or functional properties, such as long-term stability.

Major technical breakthroughs have been made with recently developed surgical and non-surgical tools, such as continuous vacuum therapy [3–7] and techniques learned from tissue engineering (i.e., cell cultures, matrix composition, cytokines, etc.) [8–10]. Combining traditional knowledge of flap creation with the insights from clinical and experimental findings from these new techniques has been the basis for the customized prefabrication of three-dimensional autologous flaps for functional tissue defect reconstruction.

It has been shown by our group that application of topical negative pressure during the time of prefabrication may help to reduce the incidence of infection and tissue necrosis [11–13]. However, while the blood flow in flaps may be improved, the prefabrication process may be less timely. The integration of autogenous and alloplastic materials, for example titanium or resorbable polylactide scaffolds, offers another prospect in this context [14, 15].

On the other hand, our experimental findings with the induction of axial vascularization in tissue-engineered three-dimensional constructs give rise to the hope that further advances toward an optimization of customized flaps and minimizing donor site effects can be achieved in the future [8, 16–18].

Clinical experience of the use of prefabricated free and pedicled flaps for partial laryngeal reconstruction in ten patients with various defects in the head and neck region has followed our experimental results with vascular axialization by means of arteriovenous loops in an animal model [2, 12, 16] (Figs. 5.1, 5.2).

Due to advances in cell culture techniques, virtually all types of single tissue elements can now be expanded in vitro with tissue specific cells [10, 19–21]. Furthermore, efforts have focussed on pluripotent cells able to produce tissues of different kinds. A particular subset of these progenitor cells has attracted special interest, i.e., the so-called mesenchymal stem cells (hMSCs). Although adult mesenchymal stem cells were first isolated in 1975, it was not until 1994 that a better understanding was achieved of their role as protagonists in homeostasis and regeneration of all tissues [22, 23].

During the past decade, there has also been an explosive growth in the field of biomaterials. It was recognized at an early stage that the key element of success for a scaffold was its interaction with the surrounding tissue at the cellular level. Scaffold technology has provided a vast variety of materials for all applications, with an emphasis on tissue engineering of bone [8, 15].

Surface chemistry as well as geometry and porosity was found to have a large influence on cellular differentiation and growth. The addition of growth factors enhanced the recruitment and differentiation of host cells, e.g., the use of bone morphogenetic proteins (BMPs) to promote osteogenesis. Surface properties and chemistry were altered to boost specific adhesion of cells or to hinder it. An example is bioartificial vessels with enhancement of endothelial growth and concomitant inhibition of platelet adhesion. Geometry and porosity were modified for optimal growth of cells, with optimal pore size found to be in the range 200–900 µm [15].

For the favorable modulation of these interactions, biomolecular research has focussed on tissue engineering for the advanced application of growth factors and

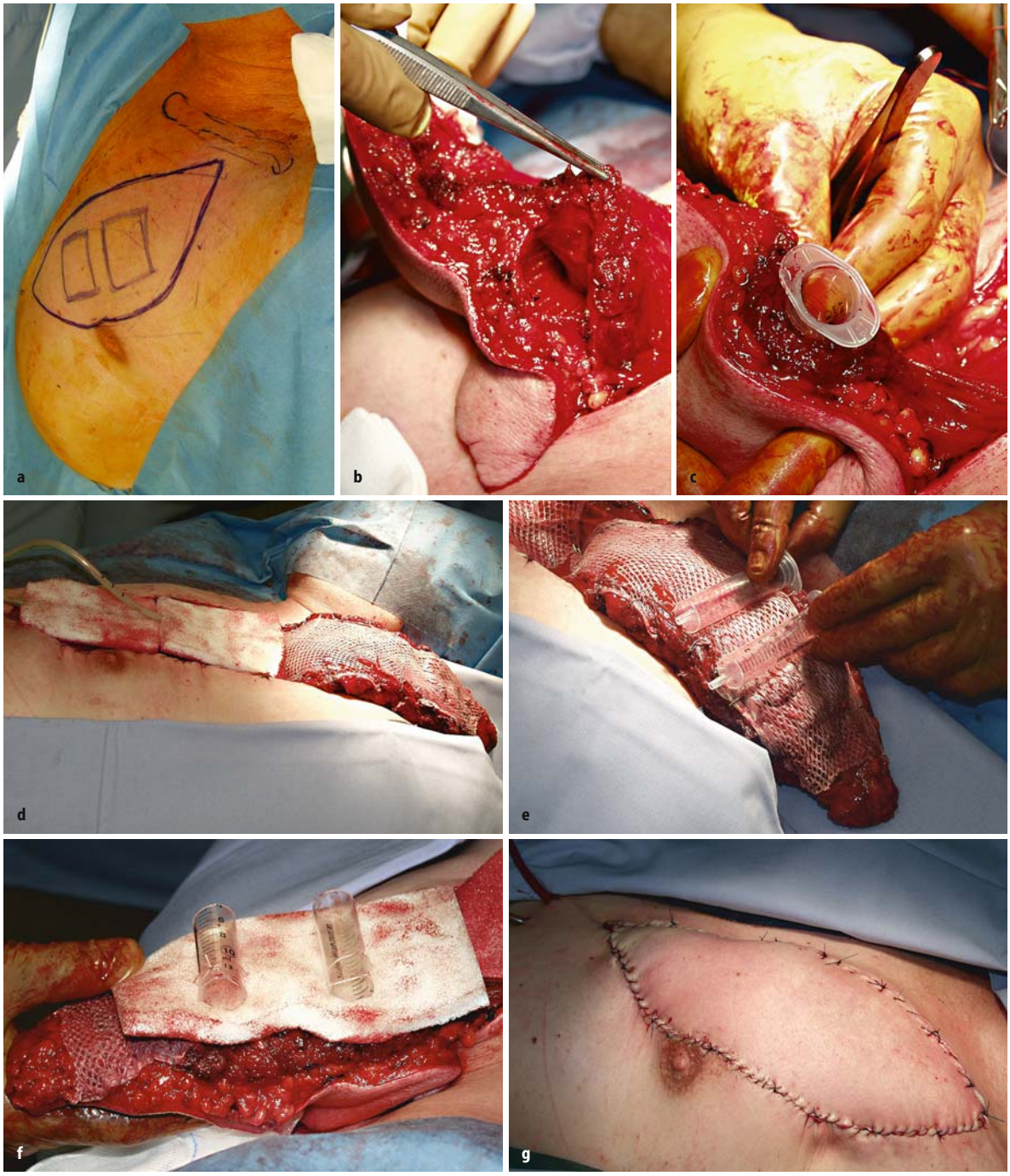

Fig. 5.1. a Schematic drawing of a planned double half pipe construct with integration of an alloplastic biomaterial scaffold into a pectoralis major muscle flap for tracheal reconstruction. **b** Implantation of a half pipe shaped bioresorbable scaffold into the pectoralis muscle. **c** Stabilization and shaping into the desired shape with a plastic syringe. **d** Closure of the donor site at the chest with a meshed split thickness skin graft and fixation of the graft with vacuum polyvinyl (PV) foam. **e** Demonstration of the creation of half pipe shapes of corresponding muscle parts plus creation of a new flap undersurface for inner lining of later tracheal replacement. **f** Placement of PV foam upon the split thickness skin graft on the undersurface of the flap. Syringes serve as spacers. **g** The prefabricated flap is left in situ utilizing vacuum closure for the securing of grafts and allografts until integration of tissue and materials allows for flap transfer

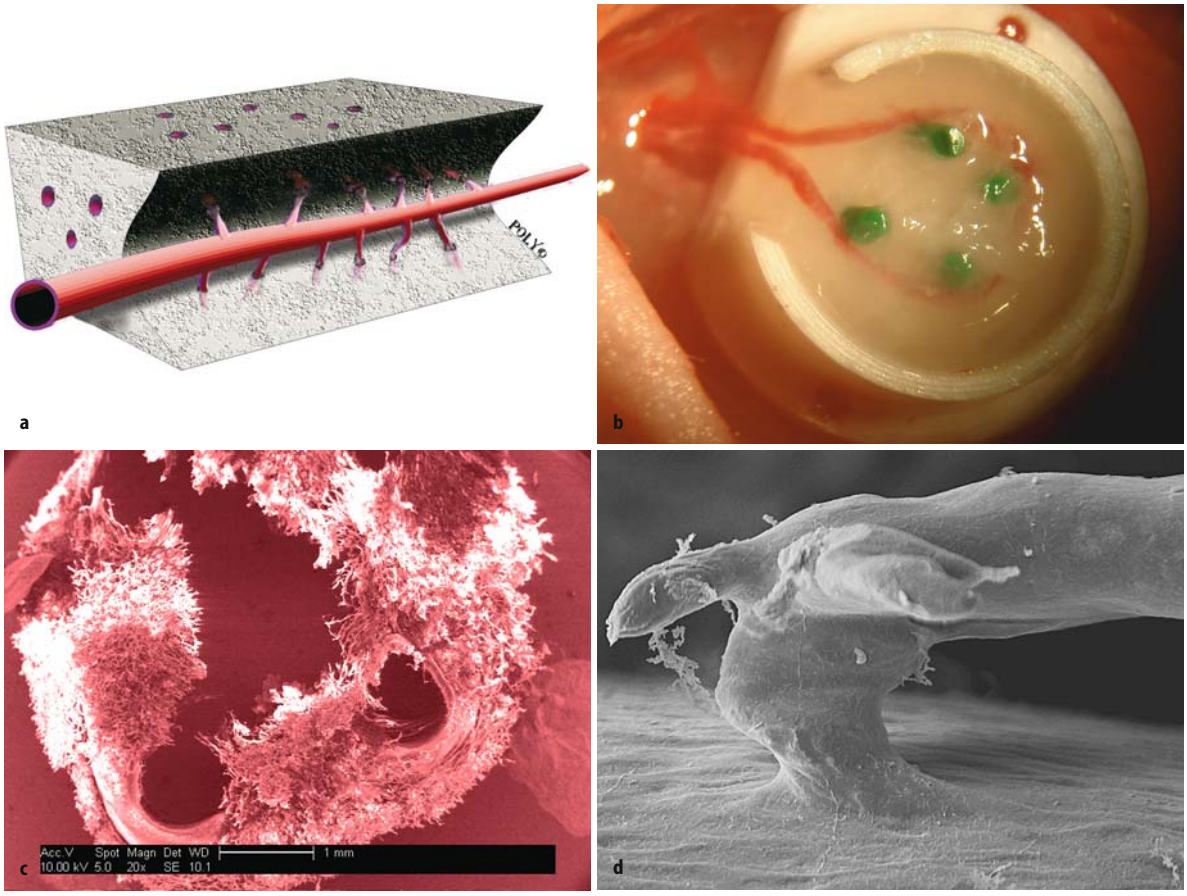

Fig. 5.2. a Schematic representation of the "matrix flap." An alloplastic or allogenous matrix is placed in the vicinity of a vascular axis. The organism serves as bioreactor and vascularization of the matrix is achieved prior to microsurgical or pedicled transfer. **b** The surgical field upon experimental implantation of an arteriovenous loop in a gel matrix. The construct is encased in an isolation chamber. The perforations at the base of the matrix are used for fixation of the assembly on the medial thigh of the rat. **c** The loop will induce a vivid angiogenic response in the fibrin matrix. Here a microvascular replica of the neocapillary network 14 days after implantation is shown. The vascular replica was constructed by means of corrosion casting with a low viscosity resin. Scanning electron microscopy was used, ×20. **d** The nuclei of the endothelial cells are imprinted on the vascular replica of the venous portion of the arteriovenous loop. A new vessel by means of direct luminal sprouting is visible. The impression on the basis of this new vessel might be signs of blood flow controlling structures such as a sphincter or a valve. The illustration was produced by means of corrosion casting and scanning electron microscopy, ×1,000

bioactive proteins. At the high end of these technologies, cells and scaffolds have been designed to release growth factors of their own [24–27].

These ex vivo technologies were transferred to the in vivo environment with promising results [28]. However, in most cell seeded biomaterial constructs, the volume-to-surface-ratio of the constructs was kept low and successful implantation took place in a site of rich perfusion; in other words, small constructs in healthy recipients. The matrices were rapidly invaded by capillaries from the local vascular network, which, in turn, ensured functional interaction and biointegration of the constructs [9, 29, 30].

5.2 Tissue Engineering in Plastic Surgery

The clinical situation requiring tissue substitution differs completely from the ideal laboratory setting, however. Tissue defects can be the result of radical tumor excision [31, 32], fulminant infection [33, 34] or compound tissue trauma [35, 36]. In such cases there is either ongoing inflammation with exhausted immune resources or marked fibrosis with virtually zero local angiogenic capacity [37]. The absence of sufficient perfusion represents a further high risk factor for bacterial inoculation. Any means of tissue substitution would have to introduce vascularization to the site so as to promote healing and restore functionality. The standard treatment nowadays is microsurgical plastic re-

construction by means of free flaps [36] or vascularized bone grafts [38].

These therapies perform quite well, but there is always a cost for autologous microsurgical transplantation in terms of donor site morbidity and remaining functional. Furthermore there is limited availability in the size and form of tissue to be spared, this holding true for all tissues and bone grafts in particular. Therefore plastic reconstructive surgeons have been forced to come up with new ideas; modern approaches include perforator flaps and flap prefabrication.

Prefabricated free flaps represent a method of staged microvascular transfer where a graft is introduced in form and implanted ectopically into a site of rich vascularity and adjacent to a vascular axis. During a second step, the construct is harvested as a compound free flap including the vascularized graft and the surrounding tissue, with the vessel serving as pedicle. This method has been further refined in a hybrid strategy incorporating tissue engineering concepts into flap prefabrication [2, 12].

Experimentally we investigated the implantation of tissue specific cells in a suitable matrix which permitted interaction with the environment [9, 39]. In vivo experiments with various materials when implanted after loading with expanded hepatocytes [29] and osteoblasts [9] clearly demonstrated the problem of low transplantation efficiency. Histological findings were complemented by labeling studies to track down the fate of the cells in vivo and confirmed a high cell-death ratio especially in the central portions of the construct [17, 40]. The single most important factor leading to cell loss after in vivo transplantation was thought to be the lack of vascularization. Nutrient supply and metabolite evacuation were ensured in vitro by frequent changes of culture medium. After implantation of the cell-loaded matrices in vivo, local diffusion was insufficient, especially for cell populations residing in the central portions of the matrix. The importance of vascularity for cell survival therefore is considered to be a core issue in improving tissue engineering concepts clinically. The necessary step to linking the in vitro findings with in vivo application was rendering the matrix vascularized, prior to cell transplantation.

5.3 Materials and Methods

In a preliminary study we developed a device consisting of an isolation chamber and a matrix in the form of a disc. The cylindrical Teflon chamber was constructed by the Institute of Materials Research (Professor Dr. P. Greil, Division of Glass and Ceramics, University of Erlangen). The chamber comprised a base plate (diameter: 15 mm), under a cylindrical shell (height 6 mm × diameter 12 mm) and an upper cup (height: 2 mm × diameter: 14 mm). The basal plate had two peripheral perforations for stabilization on the fascia of the medial musculature of the thigh.

The matrix consisted of processed bovine cancellous bone (Tutogen Medical AG, Neunkirchen, Germany). The pore size of the matrix was 300 – 500 µm with a porosity of 65 – 80 % rendered acellular and non-antigenic by a standardized procedure. Canals for future injection of gel-immobilized osteoblasts were included in the matrix design. The matrix was a disc 9 mm in diameter with a groove (1.5 × 2.0 mm) around the periphery of the disc allowing for optimal accommodation of the arteriovenous loop.

The vascularization of the matrix itself was to be effected by implantation of a vascular axis. The femoral artery and vein were used as donor vessels. During the same study, microsurgical techniques were established for construction of the vascular configuration to drive angiogenesis within the construct [41].

Several patterns for the vascular axis were tested. The arteriovenous loop model performed best in terms of vascularization potential and rate of thrombosis.

This vascularization pattern was based on a model introduced by Erol and Spira in 1979 and augmented by Morrisson and coworkers in the 1990s [42, 43].

In this model an arteriovenous loop is created by interposition of a venous graft between the femoral artery and vein in the medial thigh of the rat in the following manner. The femoral neurovascular bundle is exposed through a 4-cm-long incision at the medial thigh. Dissection of the vessels extends from the pelvic artery in the groin to the popliteal artery in the knee. After dissection of the artery and vein, a femoral venous graft is harvested from the contralateral side and interposed between the femoral vessels by anastomoses using an 11-0 nylon suture (Ethilon, Ethicon, Norderstedt, Germany). The processed bovine cancellous bone (PBCB) disc is placed in the arteriovenous loop and the vascular axis is positioned in the peripheral groove. The construct is placed in the Teflon chamber with the artery and vein exiting through an opening at the proximal pole.

For control purposes, different configurations for the vascular carrier were also tested. An arteriovenous fistula between the femoral artery and vein without the use of a venous graft did not provide a sufficient loop radius to accommodate the matrix. An arteriovenous bundle constructed by en bloc dissection of the femoral vessels and distal ligation was also used. Furthermore, matrices void of vascular carrier encased in the isolation chamber as well as a processed bovine cancellous bone (PBCB) disc implanted subcutaneously without the use of an isolation chamber were evaluated.

5.3.1 Methods of Assessment

5.3.1.1 Experimental Design and Groups

Four different groups of animals were incorporated in the studies. Animals with a PBCB matrix containing an arteriovenous loop were included in group A. Animals bearing a construct with an arteriovenous bundle as a vascular carrier were incorporated in group B. Group C included animals with constructs encased in an isolation chamber and implanted without a vascular carrier at all, whereas group D comprised animals with a construct implanted subcutaneously without the use of an isolation chamber. Each group contained 15 animals. The explantation intervals were 2, 4 and 8 weeks after the initial operation for all groups.

Explantations with India ink injection, corrosion casting, and histology were performed as described by previous publications, and morphometric analysis was performed according to our established protocols [8, 16, 18].

Magnetic resonance angiography was performed using a 4.7-T Bruker Biospec scanner, and MRIan software (www.biocom-online.de) was used for evaluation of volume datasets. Amira software (www.mc.com/tgs) was used for visualization.

5.4 Results and Discussion

The clinical results of flap prefabrication have been published elsewhere [2, 12]. The principle of three-dimensional flap generation is demonstrated in Fig. 5.1.

Experimental data of the microsurgical prevascularization of constructs showed that all animals tolerated the implantation operations well. There was no loss of weight or cyanosis of the extremity, indicating that there was no circulatory compromise of either the animal or the isolated extremity as a result of the artificial arteriovenous shunt. The capacity of the AV loop to fully vascularize the matrices was evident upon explantation; well vascularized constructs displayed a distinct dark coloration due to perfusion with India ink. In those constructs perfused with methylmethacrylate, patency of the arteriovenous loop was confirmed by the rigid pedicle exiting the chamber due to polymerization of the resin. The incidence of abscess formation in the chamber was significantly lower in the AV loop group. Histology and morphology confirmed the macroscopic findings. Quantitative evaluation of vascularization displayed a clear advantage of the arteriovenous loop as a means of vascularizing the PBCB disc. However, there were also several morphological landmarks in favor of the method. In subcutaneous implantation, the angiogenetic front propagated from the periphery toward the center, representing a fibrovascular "ingrowth." In this setting perfusion derives per se from the outer side of the construct; hence the term "extrinsic vascularization" coined by Cassell [44].

In contrast, in the AV loop constructs vascularization radiated from the center toward the periphery: representing an "outgrowth" from within; hence the term "intrinsic vascularization." Furthermore, in the subcutaneously implanted matrices there was a significant amount of inflammatory reaction and scarring.

That was not the case in the AV loop group. The dominating generated tissue here was loose vascularized connective tissue as opposed to a marked fibrosis with abundant polymorphonuclear infiltration in the subcutaneously implanted discs [17].

Finally, although from a quantitative point of view results between subcutaneous and axial vascularization were comparable, the capillary tree in the latter displayed a higher degree of variability in luminal diameter, implying a higher degree of differentiation and more advanced arborization.

In the constructs harboring an arteriovenous bundle, there was frequently a thrombosis of the vessels, mostly the artery. Neovascularization propagated even distally to the point of thrombosis. The level of thrombosis was variable, ranging from complete thrombosis of both the vein and the artery to distal thrombosis of the artery alone. However, the more proximal the occlusion the more inflammatory the character of the neovascularization and the less the extent and volume of the newly formed fibrovascular tissue. At none of the constructs could any osteogenesis be witnessed.

Scanning electron microscopy of the vascular replicas, after corrosion of the matrix and the fibrovascular tissue, confirmed these results. In the proximal-arterial part of the loop the neovascular beds were oriented parallel to the vascular axis, as an adaptation to higher pulsatile pressure. On the venous side, the new vessels assumed a rather cavernous form.

New vessels emerged from the remaining perivascular tissue and the vasa vasorum as well as the main loop elements themselves (femoral artery, venous graft, femoral vein). Luminal sprouting was a sign of a very vivid angiogenic response. Not only the venous segment participated in this phenomenon but the arterial and the graft parts too. Direct luminal sprouting from the arterial and especially the graft portions of a vascular axis have not previously been documented in the literature.

Propagating angiogenesis seemed to occur at "hot spots." In these areas, clusters of neovascular sprouts revealed a higher occurrence of angiogenetic activity.

Independently of these processes and concomitant to them, the so-called "non-sprouting" or "intussusceptive" form of angiogenesis has been observed as

well. However, the incidence of this form of new vessel formation was significantly higher in the constructs implanted subcutaneously and bearing an AV bundle.

The axial character of the newly formed vascular tree in the AV loop constructs was conserved even in the 8-week groups. By this time the venous graft was showing signs of arterialization as indicated by spindle like impressions of the nuclei of the endothelial cells on the cast, oriented along the long axis of the vessel.

The axial pattern of perfusion even at advanced stages of vascularization was confirmed by micro-MRI angiography. Inflow through the artery and outflow through the vein could be visualized at the entrance of the vascular axis into the chamber. However, the shunt pattern changed over the weeks from a strictly serial mode exclusively through the venous bypass during the first weeks, to a more diffuse mode throughout the experiment, indicating ever increasing participation of the newly formed capillary network in the arteriovenous exchange. In other words, the construct assumed with time the perfusion pattern of a distinct organoid with a rise in intrinsic impedance. This is an example of self-regulation as in the embryonic organogenesis.

Numerous studies with implications regarding the importance of vascularization for cell survival have been conducted since the beginning of the twentieth century [45, 46].

As early as 1961, Greene showed that tiny tumors implanted for more than a year in the anterior chamber of the guinea pig eye would not exceed 1 mm in diameter because they could not become vascularized. When these tumors were reimplanted in the muscle of a rabbit where they could become vascularized, they grew to a large size [47].

However, even in the case when a cell loaded construct is slim enough to allow for rapid vascularization and the site of implantation offers conditions favorable for angiogenesis, the cells are still in peril. An intense inflammatory response at the site of implantation of a cell loaded matrix hinders cellular growth of the tissue specific cells immobilized in the biomaterial. The cells were overrun by the inflammatory cells and the subsequent fibrosis [48].

5.5 The Future

There are preliminary results indicating that prevascularization of a matrix by means of the arteriovenous loop largely increases survival of secondary transplanted cells. Further, angiogenetic growth factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) greatly enhance and accelerate angiogenic response. Transfer of the model into large animals and upscaling the size of tissue defects to be replaced by prevascularized constructs will provide further insight into how well prevascularization strategies are applicable in the clinical setting. The combination of our clinical experience with flap prefabrication using the vacuum technique and the initial results from our promising axially vascularized tissue engineered constructs will offer new perspectives for customized tissue replacement.

References

1. Khouri RK, Upton J, Shaw WW (1991) Prefabrication of composite free flaps through staged microvascular transfer: an experimental and clinical study. Plast Reconstr Surg 87:108 – 115
2. Schipper J, Leffler M, Maier W, Kopp J, Bach AD, Horch RE (2006) Reconstruction of tumor induced defects in head and neck surgery with individualized prefabricated three dimensional flaps with the use of continuous vacuum therapy. Zentralbl Chir 131 [Suppl 1]:S141–S145
3. Horch RE (2004) Basics foundation and results of the vacuum therapy in the reconstructive surgery. Zentralbl Chir 129 [Suppl 1]:S2–S5
4. Kopp J, Strnad V, Bach AD, Sauer R, Horch RE (2005) Vacuum application increases therapeutic safety and allows intensified local radiation treatment of malignant soft-tissue tumors. Strahlenther Onkol 181:124 – 130
5. Kneser U, Leffler M, Bach AD, Kopp J, Horch RE (2006) Vacuum assisted closure (V.A.C.) therapy is an essential tool for treatment of complex defect injuries of the upper extremity. Zentralbl Chir 131 [Suppl 1]:S7–S12
6. Horch RE (2006) Changing paradigms in reconstructive surgery by vacuum therapy? Zentralbl Chir 131 [Suppl 1]:S44–S49
7. Argenta LC, Morykwas MJ (1997) Vacuum-assisted closure: a new method for wound control and treatment: clinical experience. Ann Plast Surg 38:563 – 576; discussion 577
8. Kneser U, Schaefer DJ, Polykandriotis E, Horch RE (2006) Tissue engineering of bone: the reconstructive surgeon's point of view. J Cell Mol Med 10:7 – 19
9. Kneser U, Voogd A, Ohnolz J, Buettner O, Stangenberg L, Zhang YH, Stark GB, Schaefer DJ (2005) Fibrin gel-immobilized primary osteoblasts in calcium phosphate bone cement: in vivo evaluation with regard to application as injectable biological bone substitute. Cells Tissues Organs 179:158 – 169
10. Horch RE, Kopp J, Kneser U, Beier J, Bach AD (2005) Tissue engineering of cultured skin substitutes. J Cell Mol Med 9:592 – 608
11. Von Gossler CM, Horch RE (2000) Rapid aggressive soft-tissue necrosis after beetle bite can be treated by radical necrectomy and vacuum suction-assisted closure. J Cutan Med Surg 4:219 – 222
12. Schipper J, Ridder GJ, Maier W, Horch RE (2003) The preconditioning and prelamination of pedicled and free microvascular anastomised flaps with the technique of vacuum assisted closure. Laryngorhinootologie 82:421 – 427
13. Horch RE, Stark GB (2000) The contralateral bilobed trapezius myocutaneous flap for closure of large defects of the dorsal neck permitting primary donor site closure. Head Neck 22:513 – 519
14. Warnke PH, Wiltfang J, Springer I, Acil Y, Bolte H, Kosmahl M, Russo PA, Sherry E, Lutzen U, Wolfart S, Terhey-

den H (2006) Man as living bioreactor: fate of an exogenously prepared customized tissue-engineered mandible. Biomaterials 27:3163 – 3167
15. Salgado AJ, Coutinho OP, Reis RL (2004) Bone tissue engineering: state of the art and future trends. Macromol Biosci 4:743 – 765
16. Kneser U, Polykandriotis E, Ohnolz J, Heidner K, Grabinger L, Euler S, Amann KU, Hess A, Brune K, Greil P, Sturzl M, Horch RE (2006) Engineering of vascularized transplantable bone tissues: induction of axial vascularization in an osteoconductive matrix using an arteriovenous loop. Tissue Eng 12:1721 – 1731
17. Polykandriotis E, Horch RE, Arkudas A, Labanaris A, Brune K, Greil P, Bach AD, Kopp J, Hess A, Kneser U (2006) Intrinsic versus extrinsic vascularization in tissue engineering. Adv Exp Med Biol 585:311 – 326
18. Polykandriotis E, Arkudas A, Beier J, Hess A, Greil P, Papadopoulos T, Kopp J, Bach A, Horch RE, Kneser U (2007) Intrinsic axial vascularization of an osteoconductive bone matrix by means of an arteriovenous vascular bundle. Plast Reconstr Surg (in press)
19. Bach AD, Beier JP, Stern-Staeter J, Horch RE (2004) Skeletal muscle tissue engineering. J Cell Mol Med 8:413 – 422
20. Kneser U, Kaufmann PM, Fiegel HC, Pollok JM, Rogiers X, Kluth D, Herbst H (1999) Interaction of hepatocytes and pancreatic islets cotransplanted in polymeric matrices. Virchows Arch 435:125 – 132
21. Bach AD, Bannasch H, Galla TJ, Bittner KM, Stark GB (2001) Fibrin glue as matrix for cultured autologous urothelial cells in urethral reconstruction. Tissue Eng 7:45 – 53
22. Caplan AI (1994) The mesengenic process. Clin Plast Surg 21:429 – 435
23. Friedenstein AJ, Gorskaja JF, Kulagina NN (1976) Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Exp Hematol 4:267 – 274
24. Perets A, Baruch Y, Weisbuch F, Shoshany G, Neufeld G, Cohen S (2003) Enhancing the vascularization of three-dimensional porous alginate scaffolds by incorporating controlled release basic fibroblast growth factor microspheres. J Biomed Mater Res A 65:489 – 497
25. Murphy WL, Peters MC, Kohn DH, Mooney DJ (2000) Sustained release of vascular endothelial growth factor from mineralized poly(lactide-co-glycolide) scaffolds for tissue engineering. Biomaterials 21:2521 – 2527
26. Totsugawa T, Kobayashi N, Okitsu T, Noguchi H, Watanabe T, Matsumura T, Maruyama M, Fujiwara T, Sakaguchi M, Tanaka N (2002) Lentiviral transfer of the LacZ gene into human endothelial cells and human bone marrow mesenchymal stem cells. Cell Transplant 11:481 – 488
27. Blum HE, Hopt UT (2003) Hepatocellular carcinoma. Pathogenesis and multicentricity. Chirurg 74:709 – 716
28. Vacanti JP, Langer R, Upton J, Marler JJ (1998) Transplantation of cells in matrices for tissue regeneration. Adv Drug Deliv Rev 33:165 – 182
29. Kneser U, Kaufmann PM, Fiegel HC, Pollok JM, Kluth D, Herbst H, Rogiers X (1999) Long-term differentiated function of heterotopically transplanted hepatocytes on three-dimensional polymer matrices. J Biomed Mater Res 47: 494 – 503
30. Warnke PH, Springer IN, Wiltfang J, Acil Y, Eufinger H, Wehmoller M, Russo PA, Bolte H, Sherry E, Behrens E, Terheyden H (2004) Growth and transplantation of a custom vascularised bone graft in a man. Lancet 364:766 – 770
31. Horch RE, Stark GB, Beier JP (2005) Unusual explosive growth of a squamous cell carcinoma of the scalp after electrical burn injury and subsequent coverage by sequential free flap vascular connection – a case report. BMC Cancer 5:150
32. Schipper J, Klenzner T, Arapakis I, Maier W, Horch R (2006) The transverse rectus abdominis muscle (TRAM) flap. A “second defensive line” in microvascular reconstructions of defects in the head and neck area. HNO 54: 20 – 24
33. Loos B, Kopp J, Bach A, Kneser U, Polykandriotis E, Hohenberger W, Horch RE (2004) Salvage of exposed alloplastic materials in irradiated wounds – a case report. Zentralbl Chir 129 [Suppl 1]:S133–S136
34. Loos B, Kopp J, Kneser U, Weyand M, Horch RE (2006) The importance of vacuum therapy in the treatment of sternal osteomyelitis from the plastic surgeons point of view. Zentralbl Chir 131 [Suppl 1]:S124–S128
35. Polykandriotis E, Stangl R, Hennig HH, Lennerz JK, Frank WM, Loos MD, Horch RE (2005) The composite vastus medialis-patellar complex osseomuscular flap as a salvage procedure after complex trauma of the knee–an anatomical study and clinical application. Br J Plast Surg 58:646 – 651
36. Horch RE, Stark GB (1999) The rectus abdominis free flap as an emergency procedure in extensive upper extremity soft-tissue defects. Plast Reconstr Surg 103:1421 – 1427
37. Horch RE, Gitsch G, Schultze-Seemann W (2002) Bilateral pedicled myocutaneous vertical rectus abdominus muscle flaps to close vesicovaginal and pouch-vaginal fistulas with simultaneous vaginal and perineal reconstruction in irradiated pelvic wounds. Urology 60:502 – 507
38. Bach AD, Kopp J, Stark GB, Horch RE (2004) The versatility of the free osteocutaneous fibula flap in the reconstruction of extremities after sarcoma resection. World J Surg Oncol 2:22
39. Stangenberg L, Schaefer DJ, Buettner O, Ohnolz J, Mobest D, Horch RE, Stark GB, Kneser U (2005) Differentiation of osteoblasts in three-dimensional culture in processed cancellous bone matrix: quantitative analysis of gene expression based on real-time reverse transcription-polymerase chain reaction. Tissue Eng 11:855 – 864
40. Kneser U, Stangenberg L, Ohnolz J, Buettner O, Stern-Straeter J, Möbest D, Horch RE, Stark GB, Schaefer DJ (2006) Evaluation of processed bovine cancellous bone matrix seeded with syngenic osteoblasts in a critical size calvarial defect rat model. J Cell Mol Med 10(3):695 – 707
41. Kneser U, Polykandriotis E, Ohnolz J, Heidner K, Bach AD, Kopp J, Horch RE (2004) Vascularized bone replacement for the treatment of chronic bone defects initial results of microsurgical hard tissues vascularization. Z Wundheilung 9:102 – 108
42. Erol OO, Sira M (1980) New capillary bed formation with a surgically constructed arteriovenous fistula. Plast Reconstr Surg 66:109 – 115
43. Mian R, Morrison WA, Hurley JV, Penington AJ, Romeo R, Tanaka Y, Knight KR (2000) Formation of new tissue from an arteriovenous loop in the absence of added extracellular matrix. Tissue Eng 6:595 – 603
44. Cassell OC, Hofer SO, Morrison WA, Knight KR (2002) Vascularisation of tissue-engineered grafts: the regulation of angiogenesis in reconstructive surgery and in disease states. Br J Plast Surg 55:603 – 610
45. Goldman E (1907) The growth of malignant disease in man and the lower animals with special reference to the vascular system. Lancet 2:1236
46. Folkman J (1971) Tumor angiogenesis: therapeutic implications. N Engl J Med 285:1182 – 1186
47. Greene HSN (1961) Heterologous transplantation of mammalian tumors. Exp Med 73:461
48. Wake MC, Patrick CW Jr, Mikos AG (1994) Pore morphology effects on the fibrovascular tissue growth in porous polymer substrates. Cell Transplant 3:339 – 343