

Tissue Augmentation with Fibrin Sealant and Cultured Fibroblasts: A Preliminary Study

Gwendolyn Hoben · Volker J. Schmidt ·
Holger Bannasch · Raymund E. Horch

Received: 30 December 2010 / Accepted: 25 March 2011 / Published online: 22 April 2011
© Springer Science+Business Media, LLC and International Society of Aesthetic Plastic Surgery 2011

Abstract

Background Nonoperative subdermal tissue augmentation is one of the most frequently performed procedures in plastic surgery and dermatological practice. Many products, from biological to synthetic filler substances, are currently available. However, none has achieved ideal clinical efficacy, especially regarding volume maintenance and longevity. We examined the use of fibrin sealant as a biological and fully degradable matrix for dermal augmentation in combination with precultured human fibroblasts and hyaluronic acid gel (HYAFF).

Methods Four implant preparations were studied: fibrin glue only (F); 1% HYAFF mixed in fibrin glue (FH); 1.8×10^6 cells/ml of fibrin glue (FC); and 1% HYAFF and 1.8×10^6 cells/ml of fibrin glue (FHC). Each mouse was given two separate subcutaneous injections of implant material. At 1, 3, and 6 weeks two mice from each group were sacrificed, such that there was an $n = 4$ for each

implant group at each time point. The mice were grossly examined for implant retention and the implants were evaluated by means of immunohistochemistry for fibrosis, integration into surrounding tissue, presence of elastin, and blood vessel infiltration.

Results Only the implants in the cell-containing groups, FC and FHC, remained after 6 weeks. Moreover, with the exception of a mild inflammatory response, no adverse affects of the cell-seeded implants were noted.

Conclusion Presence of fibroblasts increases implant durability. Further studies should evaluate the ideal hyaluronic acid and fibroblast concentration for long-term longevity.

Keywords Tissue augmentation · Fibroblasts · Fibrin · Filler

Introduction

The demand for dermal augmentation for the treatment of contour defects, scarring, and aging-related wrinkling has increased greatly in recent years. Likewise, there has been an increase in the number of different products available on the market. The ideal material for dermal augmentation would be nonimmunogenic, hypoallergenic, immobile, cost-effective, easily administered, readily available (i.e., little to no cultivation or preparation time), have a minimal risk for infection, and provide a permanent desired effect. However, the current materials in use do not meet all of these qualifications; most notably they do not provide a permanent treatment. The currently available materials have significant volume loss in under a year and many require repeat treatments, with the continual threat of adverse side effects.

G. Hoben (✉)
Division of Plastic and Reconstructive Surgery, Washington
University School of Medicine, 660 South Euclid Avenue, St.
Louis, MO 63110, USA
e-mail: gmbhoben@gmail.com

V. J. Schmidt · R. E. Horch
Department of Plastic and Hand Surgery,
Friedrich-Alexander-University of Erlangen-Nuernberg,
Krankenhausstr. 12, 91054 Erlangen, Germany
e-mail: volker.schmidt@uk-erlangen.de

R. E. Horch
e-mail: raymund.horch@uk-erlangen.de

H. Bannasch
Department of Plastic and Hand Surgery, University Hospital
Freiburg, Hugstetter Strasse 55, 79106 Freiburg, Germany
e-mail: holger.bannasch@uniklinik-freiburg.de

Current strategies for dermal augmentation include the use of synthetic materials such as silicone, PTFE (Gore-Tex®), poly(*n*-vinyl-2-pyrrolidone) (PVP), calcium hydroxylapatite, and poly(methyl methacrylate) beads (Artecoll). One of the materials in longest use, silicone, has been used to treat wrinkles and scarring but it is associated with a variety of unpredictable side effects such as adverse erythema, tissue induration, cellulitis, and granulomatous reaction [1]. Multiple-system organ failure was also reported after subcutaneous silicone injection [2]. Studies with PVP show a volume loss of 33% after 1 month and PTFE implants are not injectable; they require a surgical operation and the aesthetic effects are less malleable by the physician [3]. Poly(methyl methacrylate) treatments must be repeated at 1 to 3-month intervals to maintain the desired effect, and in a study by McClelland et al. [4], the implants tended to migrate from the implant site [5]. Calcium hydroxylapatite is proposed to induce a process that results in collagen synthesis [6, 7] and, therefore, has been suggested for tissue augmentation [8].

Natural materials tend to cause fewer adverse reactions in patients but the lack of permanence is still problematic. The use of aspirated fat for dermal augmentation and large soft-tissue defect treatment generally suffers from up to 50% volume and weight loss, and the results are very technique-dependent [5, 9–12]. In addition, this method requires a surgical intervention, is limited in slim patients, and may require frequent procedures [13]. Bovine collagen products such as Zyderm® and Zyplast® produce somewhat greater longevity but they also require multiple treatments since resorption typically occurs over 3–4 months, 2–3% of patients experience an allergic reaction, and there is some risk for disease transmission [3, 5, 14]. A novel porcine collagen-based dermal filler (Dermicol-P35) is proposed to be more persistent (up to 12 months) compared with bovine collagen-based dermal fillers [15, 16]. Human collagen products (Allogen) show greater longevity than the bovine products but they require a sample of patient skin to cultivate the implant: 3 in.² of skin will produce 1 ml of implant [17–21]. Dermalogen, an autologous collagen implant, removes the need for a donor site, but it still requires multiple treatments and lacks permanence [21]. Varying in degree of crosslinking, several hyaluronic acid-based fillers are also available [22]. But again, longevity is an issue, with only a 66% clinical correction seen after 1 year [5, 23].

Another possible matrix for dermal augmentation is fibrin sealant. Beneficial effects of fibrin in tissue generation have already been reported in the context of tissue engineering [24–29]. Fibrin sealant creates a natural matrix for cell infiltration and has been found to be angiogenic [30]. These characteristics make it a good candidate for creating permanent tissue at the site of implantation through fibroblast migration and infiltration in addition to the deposition of

autologous collagen as the fibrin matrix degrades. In this study, fibrin sealant is evaluated for its longevity in implantation in the nude mouse model at 1, 3, and 6 weeks post implantation. The addition of fibroblasts and hyaluronic acid gel to the fibrin sealant was also evaluated.

Materials and Methods

Cell Culture

Human dermal fibroblasts were isolated from a skin sample obtained from the Plastic and Hand Surgery Department of the University of Freiburg Hospital. The skin was washed in phosphate buffered saline (PBS), washed briefly in 70% ethanol, cut into strips, and treated overnight at 4°C with 0.5% dispase (Boehringer Mannheim). The epidermis was then removed and the samples were digested sequentially in collagenase (type III, Biochrom, 185 U/ml) prepared in PBS at 37°C for 2 h. The cells were then counted and plated with DMEM supplemented with 10% v/v NCS (PAA Laboratories), 1% v/v antibiotics (10 U/ml penicillin, 10 µg/ml streptomycin, Gibco) in 25-cm² vent cap cell culture flasks (Corning). After the first passage, they were transferred to 75-cm² vent cap cell culture flasks. Medium was changed every 3–4 days and cells were passaged weekly.

Implant Preparation

All implants were prepared using a two-component fibrin glue provided by Baxter (Tissucol® Duo S, 0.5 ml). The hyaluronic acid portion was prepared by first mixing a 20% solution of HYAFF (Hyalobarrier gel, Fidia Advanced Biomaterials) under sterile conditions. Immediately prior to implantation, 0.05 ml of the 20% solution was mixed with the 0.5-ml fibrinogen component of the fibrin glue. This component, when mixed with the 0.5-ml thrombin component, produced a 1% HYAFF solution. The cell component was prepared by trypsinizing (0.05% trypsin, 0.02% EDTA, Gibco) 16 confluent flasks of second-passage human fibroblasts and resuspending the cells in the thrombin component of the fibrin glue, producing cell implants at a concentration of 1.8×10^6 cells/ml of fibrin glue. The following experimental groups were prepared: fibrin glue alone (F), 1% HYAFF in fibrin glue (FH), 1.8×10^6 cells/ml of fibrin glue (FC), and 1% HYAFF and 1.8×10^6 cells/ml of fibrin glue (FHC).

Operation and Implantation Procedures

The use of 26 athymic nude mice (BALB/c-Nu/Nu, 6–8 weeks old, Harlan-Winkelmann) was approved by the

Animal Ethics Committee of the University of Freiburg Hospital. The animals were kept in single boxes in a DuoFlo laminar airflow system at 37°C with 12-h light exposure.

Mice were anesthetized and 0.25-ml subcutaneous injections were made in the backs of the mice using the duploject syringe, included in the Tissucol kit, with an Abbocath-T needle (Venisystems). Two injections were made in each mouse; each composed of the same material and located on either side of the spine. Six mice were prepared for each of the four experimental groups, and the one remaining mouse was prepared within the FHC group. This last mouse was included in the third-time-point implant evaluation.

After 1, 3, and 6 weeks, two mice from each experimental group were killed. The implant was excised with a portion of the skin and underlying muscle. One half of the sample was preserved in 4% formalin PBS solution (Merck) for paraffin embedding and the other half was immediately placed in liquid nitrogen for cryopreservation.

Implant Evaluation

Sustained presence of the implants at each time point was qualitatively recorded with digital photography and palpation, followed by full dissection of the site. Implant retention was regarded as being able to ascertain any remaining implant component via palpation and histological exam of the implant site. The implant samples were prepared in 5- μ m sections for histological and immunohistochemical staining. Paraffin sections were used for H&E evaluation; two sections from each implant were evaluated for implant presence, fibrosis, integration into surrounding tissue, and blood vessel infiltration. Paraffin sections from the last time point were also stained for elastin (Accustain, Sigma) and compared with normal human skin and mouse skin. Frozen samples from the fibroblast experimental groups were evaluated for the presence of human fibroblasts using the Mouse on Mouse Immunodetection Kit (Linaris) with a mouse anti-human fibroblast primary antibody (1:100, AS02, Dianova). The sections were incubated for 40 min with the primary antibody, stained with AEC chromagen (Dako) for 10 min, and counterstained with Mayer's hematoxylin (Chroma). Cell-free implants and normal mouse skin were run with each staining to check for background staining, and human skin was used as positive control. No background staining or nonspecific binding was noted. A semiquantitative evaluation of human fibroblast retention within the implant was performed by counting the antibody-positive fibroblasts in 40 $\times$ magnification fields on a Zeiss Axioplan 2 microscope: five fields within the implant were counted and averaged for each implant. Histological images were photographed with a Matrox Meteor digital camera.

Results

Macroscopic Evaluation of Implants

The implants were clearly visualized as they were injected (Fig. 1) and no redness, swelling, or scarring was noted at the injection sites at any subsequent evaluations. Injection volume was kept as uniform as possible; however, some inconsistency was inevitable as the fibrin glue tended to spread out amorphously from the injection site. Thus, a quantifiable evaluation of volume retention was not possible. At each time point, two mice from each group were randomly chosen to be killed and the implant area was excised. The implants were solid, smooth, and white-clear-appearing masses that moved with the skin and were easily separated from the skin. Graft retention, defined as both palpable and evident on histology, at each time point is given in Table 1. Some amount of each type of implant was found after 1 and 3 weeks, but after 6 weeks, only cell-seeded implants remained (FC and FHC).

Histological Observations

Staining with H&E revealed the fibrin implants as smooth-appearing eosinophilic masses at the first time point. A layer of granulocytes and lymphocytes denoting moderate inflammation surrounded each implant. Little change was seen in this layer over time nor was the inflammation any different among the experimental groups.

The cell-free implants were smooth, eosinophilic masses at the first time point, while at the third week it was observed that the cells around the periphery began to infiltrate the implant. At that time point, the implant itself had a more striated and broken-up appearance and was spotted with cells and tendrils of invading cell masses from the periphery. No difference was seen between the F and FH groups (Fig. 2).

In contrast, the cell-seeded groups appeared more striated and broken-up from the first time point onward. The seeded cells were generally dispersed throughout the implant, though clusters of cells within the implant were also observed. Tendrils of invading cell masses were also seen on the periphery of the implants. No gross differences were observed between the FC and FHC groups (Fig. 3).

The elastin staining revealed no obvious elastin in any of the implants. Blood vessels were observed within at least two of the remaining implants at 6 weeks.

Fibroblast Retention in Cell-seeded Implants

The antibody staining revealed that in almost all of the cell-seeded implants there was a layer of human fibroblasts around the periphery of the implant in addition to cells



Fig. 1 Injections of 0.25 ml of the fibrin glue solutions were made on either side of the spine of each mouse

Table 1 Implant persistence at each time point

Group	1 week	3 weeks	6 weeks
F	4/4	1/4	0/4
FH	4/4	2/4	0/4
FC	4/4	4/4	4/4
FHC	3/4	4/4	3/6

Implant persistence was gauged by gross palpation and presence on histological exam

dispersed throughout the implant. Significant numbers of mouse fibroblasts were not found within the implants. No statistically significant difference was found between the different time points, regardless of HA presence, in terms of the average number of cells counted in a field by the semiquantitative method (Fig. 4).

Discussion

Fibrin sealant mimics the natural fibrin clot formation found in wound healing and has both hemostatic and

adhesive properties. Fibrin sealant used in wound repair has been found to result in the development of increased cellularity and blood vessel formation in the area of the implanted material [30]. Our group has shown that fibrin sealant works well as a transfer matrix for dermal cells [26, 29, 31], and Schoeller et al. [32] showed that preadipocytes suspended in fibrin sealant were able to maintain their volume for up to 1 year and neovascularization was also noted.

The addition of a cellular component to the fibrin sealant is also promising in terms of enhancing the implant for longevity. The addition of fibroblasts to collagen sponges increased their tensile strength and slowed degradation [33, 34]. Likewise, the addition of cells to calcium alginate hydrogels increased their volume retention from 58 to 88% over 8 weeks [35]. In addition to greater longevity, the use of fibroblasts in dermal substitutes has been found to improve cosmetic outcome and reduce scarring [33, 36–38].

The properties of hyaluronic acid gel and its current use in cosmetic surgery suggested that it too would be a valuable component in a material for dermal augmentation. Hyaluronic acid is linked to reduced scarring, enhanced tissue regeneration, and cell proliferation through decreased cell differentiation [39–42]. At a concentration of 150 $\mu\text{g/ml}$, hyaluronic acid was found to have a significant effect on cultured adult fibroblasts: greater cell proliferation, higher protein production, and the production of finer collagen fibers [39].

Our results indicate that fibrin sealant has significant potential for use in dermal augmentation. The implants appeared to produce minimal inflammation and no adverse reactions at the implantation site. Mixing the sealant with fibroblasts produced greater longevity: 70% retention of implant material after 6 weeks in comparison to the cell-free implants. The semiquantitative counts of fibroblasts in the implants confirmed that cells lasted in the implant material for at least 6 weeks. The longevity of the fibroblasts suggests that they will be able to play a significant role in building new tissue.

However, there was decreased implant retention in the FHC group compared to the FC group, suggesting a possible deleterious effect of the hyaluronic acid. Nor did the semiquantitative counts reveal any increase in cell quantity in the presence of hyaluronic acid. These were surprising findings that suggest that the mixture concentration of the hyaluronic acid and even the formulation of the hyaluronic acid component merit greater investigation.

Since this was a preliminary study, this work was limited by sample size and the necessity of choosing single concentrations of the materials tested (i.e., the concentration of HA, the concentration of cells in the implants). In addition, in looking toward aesthetic applications, there are two main issues in translating this study: the animal model

Fig. 2 H&E-stained ($\times 40$) paraffin cuts of the F and FH implants at the 1- and 3-week time points. The images show both implant material and mouse epidermis for orientation. **a** A smooth pink subcutaneous implant was seen in group F 1 week after implantation. **b** By 3 weeks after implantation, the implant appeared more broken up, but there was evidence of cell infiltration. **c** Similarly, the FH implant appeared homogeneous at 1 week. **d** By 3 weeks, the FH implant showed cell infiltration

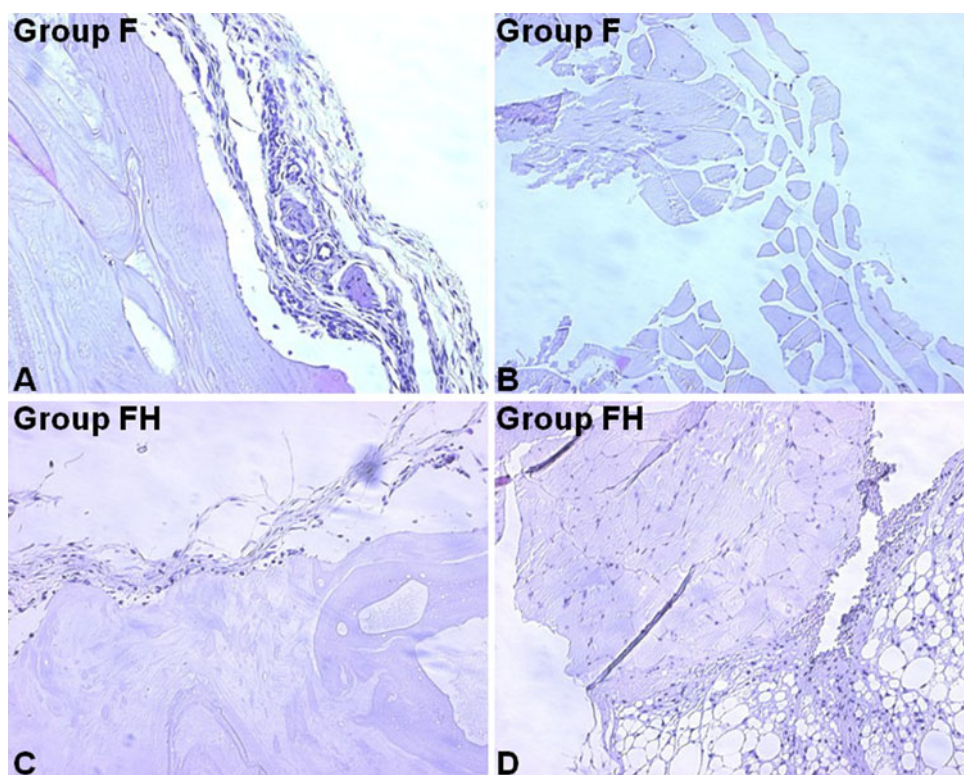
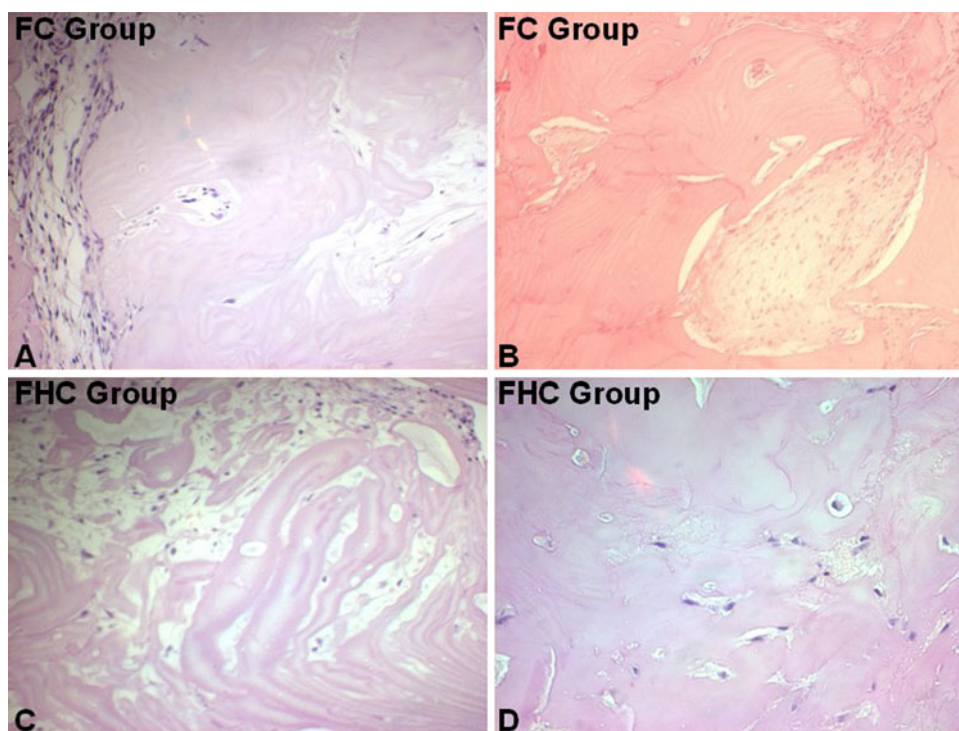


Fig. 3 H&E-stained ($\times 40$) paraffin cuts of the cell-containing implants. **a** FC implants 1 week after implantation show clusters of cells in the fibrin. **b** At the 3-week time point the FC implant showed enlarged clusters of cells. **c** The FHC implant appeared heterogeneous with dispersed cells. **d** At 3 weeks the implant showed cells dispersed in a more homogeneous-looking fibrin matrix



and the challenges of cell culture. The nude mouse model was chosen for this study so that human fibroblasts could be utilized, making the results more applicable to the clinical situation. One caveat to using nude mice, however, was that injections of the material could not be made

intradermally due to the thin nature of mice skin, so subcutaneous injections were evaluated. While the fibrin sealant is off-the-shelf, the advantage of the addition of cultured cells must be weighed with the additional preparation time and additional cost. Easy availability is a valued

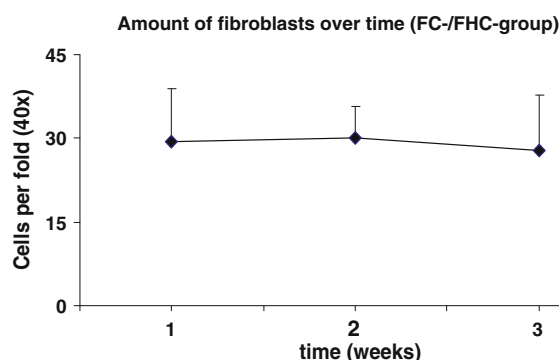


Fig. 4 Cell counts of human fibroblasts at distinct time points. Depicted is the merged data from the FH and FHC groups. No significant changes in the total amount of cells were observed over a time period of 6 weeks ($n = 4-7$). Data are presented as mean $\pm$ standard deviation

characteristic in aesthetic applications; however, as this study suggests, it may be at the cost of greater clinical efficacy.

The promise shown by these implants indicates that long-term studies should be done to evaluate fibrin sealant over a greater time period for longevity, implant migration, progression or regression of inflammation, and long-term clinical tissue reaction. Additionally, the effect of different concentrations of fibroblasts and of hyaluronic acid gel should be examined; perhaps a mix that preserves longevity and enhances tissue formation could be established. Our study demonstrated that off-the-shelf standard preparation fibrin sealant lasts 6 weeks with implanted fibroblasts. By changing the concentration of aprotinin in the fibrinogen component of the sealant it may be possible to enhance the longevity of the implant. Thus, with the optimization of these factors—HA concentration, aprotinin concentration, and cell concentration—fibrin sealant-based implants for dermal augmentation could become a useful tool in cosmetic and reconstructive surgery.

Acknowledgments We thank Dr. Techanu for her valuable assistance in examining the histology. We gratefully acknowledge the funding for this research provided by Baxter GmbH and the Fulbright Commission.

Disclosure None.

References

- Bigatà X, Ribera M, Bielsa I et al (2001) Adverse granulomatous reaction after cosmetic dermal silicone injection. *Dermatol Surg* 27:198–200
- Clark RF, Cantrell FL, Pacal A et al (2008) Subcutaneous silicone injection leading to multi-system organ failure. *Clin Toxicol (Phila)* 46:834–837
- Arpey CJ, Chang LK, Whitaker DC (2000) Injectability and tissue compatibility of poly-(N-vinyl-2-pyrrolidone) in the skin of rats: a pilot study. *Dermatol Surg* 26:441–445 discussion 445–446
- McClelland M, Egbert B, Hanks V et al (1997) Evaluation of Artecoll polymethylmethacrylate implant for soft-tissue augmentation: biocompatibility and chemical characterization. *Plast Reconstr Surg* 100:1466–1474
- Alster TS, West TB (2000) Human-derived and new synthetic injectable materials for soft-tissue augmentation: current status and role in cosmetic surgery. *Plast Reconstr Surg* 105:2515–2525 discussion 2526–2528
- Marmur ES, Phelps R, Goldberg DJ (2004) Clinical, histologic and electron microscopic findings after injection of a calcium hydroxylapatite filler. *J Cosmet Laser Ther* 6:223–226
- Lemperle G, Morhenn V, Charrier U (2003) Human histology and persistence of various injectable filler substances for soft tissue augmentation. *Aesthet Plast Surg* 27:354–366 discussion 367
- Mandy SH (2009) Satisfying patient expectations with soft-tissue augmentation. *Dermatol Online J* 15:1
- Kononas TC, Bucky LP, Hurley C et al (1993) The fate of suctioned and surgically removed fat after reimplantation for soft-tissue augmentation: a volumetric and histologic study in the rabbit. *Plast Reconstr Surg* 91:763–768
- Ullmann Y, Hyams M, Ramon Y et al (1998) Enhancing the survival of aspirated human fat injected into nude mice. *Plast Reconstr Surg* 101:1940–1944
- Cooper JS, Lee BT (2009) Treatment of facial scarring: lasers, filler, and nonoperative techniques. *Fac Plast Surg* 25:311–315
- Giugliano C, Benitez S, Wisnia P et al (2009) Liposuction and lipoinjection treatment for congenital and acquired lipodystrophies in children. *Plast Reconstr Surg* 124:134–143
- Kaufman MR, Miller TA, Huang C et al (2007) Autologous fat transfer for facial recontouring: is there science behind the art? *Plast Reconstr Surg* 119:2287–2296
- Fagien S (2000) Facial soft-tissue augmentation with injectable autologous and allogeneic human tissue collagen matrix (autologen and dermalogen). *Plast Reconstr Surg* 105:362–373 discussion 374–375
- Narins RS, Brandt FS, Lorenc ZP et al (2008) Twelve-month persistency of a novel ribose-cross-linked collagen dermal filler. *Dermatol Surg* 34(Suppl 1):S31–S39
- Goldberg DJ (2009) Correction of tear trough deformity with novel porcine collagen dermal filler (Dermicol-P35). *Aesthet Surg J* 29:S9–S11
- Sclafani AP, Romo T 3rd, Parker A et al (2000) Autologous collagen dispersion (Autologen) as a dermal filler: clinical observations and histologic findings. *Arch Faci Plast Surg* 2:48–52
- Sclafani AP, Romo T 3rd, Jacono AA et al (2000) Evaluation of acellular dermal graft in sheet (AlloDerm) and injectable (micronized AlloDerm) forms for soft tissue augmentation. Clinical observations and histological analysis. *Arch Faci Plast Surg* 2:130–136
- Sclafani AP, Romo T 3rd, Parker A et al (2002) Homologous collagen dispersion (Dermalogen) as a dermal filler: persistence and histology compared with bovine collagen. *Ann Plast Surg* 49:181–188
- Sclafani AP, Romo T 3rd, Jacono AA et al (2001) Evaluation of acellular dermal graft (AlloDerm) sheet for soft tissue augmentation: a 1-year follow-up of clinical observations and histological findings. *Arch Faci Plast Surg* 3:101–103
- Fagien S, Elson ML (2001) Facial soft-tissue augmentation with allogeneic human tissue collagen matrix (Dermalogen and Dermalogen). *Clin Plast Surg* 28:63–81
- Narins RS, Bowman PH (2005) Injectable skin fillers. *Clin Plast Surg* 32:151–162

23. Duranti F, Salti G, Bovani B et al (1998) Injectable hyaluronic acid gel for soft tissue augmentation. A clinical and histological study. *Dermatol Surg* 24:1317–1325
24. Bleiziffer O, Horch RE, Hammon M et al (2009) T17b murine embryonal endothelial progenitor cells can be induced towards both proliferation and differentiation in a fibrin matrix. *J Cell Mol Med* 13:926–935
25. Beier JP, Horch RE, Hess A et al (2010) Axial vascularization of a large volume calcium phosphate ceramic bone substitute in the sheep AV loop model. *J Tissue Eng Regen Med* 4:216–223
26. Kopp J, Jeschke MG, Bach AD et al (2004) Applied tissue engineering in the closure of severe burns and chronic wounds using cultured human autologous keratinocytes in a natural fibrin matrix. *Cell Tissue Bank* 5:89–96
27. Horch RE (2006) Future perspectives in tissue engineering. *J Cell Mol Med* 10:4–6
28. Horch RE, Kopp J, Kneser U et al (2005) Tissue engineering of cultured skin substitutes. *J Cell Mol Med* 9:592–608
29. Horch RE, Bannasch H, Kopp J et al (1998) Single-cell suspensions of cultured human keratinocytes in fibrin-glue reconstitute the epidermis. *Cell Transpl* 7:309–317
30. Romanos GE, Strub JR (1998) Effect of Tissucol on connective tissue matrix during wound healing: an immunohistochemical study in rat skin. *J Biomed Mater Res* 39:462–468
31. Horch RE, Bannasch H, Stark GB (2001) Transplantation of cultured autologous keratinocytes in fibrin sealant biomatrix to resurface chronic wounds. *Transpl Proc* 33:642–644
32. Schoeller T, Lille S, Wechselberger G et al (2001) Histomorphologic and volumetric analysis of implanted autologous preadipocyte cultures suspended in fibrin glue: a potential new source for tissue augmentation. *Aesthet Plast Surg* 25:57–63
33. Lamme EN, Van Leeuwen RT, Brandsma K et al (2000) Higher numbers of autologous fibroblasts in an artificial dermal substitute improve tissue regeneration and modulate scar tissue formation. *J Pathol* 190:595–603
34. Marks MG, Doillon C, Silver FH (1991) Effects of fibroblasts and basic fibroblast growth factor on facilitation of dermal wound healing by type I collagen matrices. *J Biomed Mater Res* 25: 683–696
35. Marler JJ, Guha A, Rowley J et al (2000) Soft-tissue augmentation with injectable alginate and syngeneic fibroblasts. *Plast Reconstr Surg* 105:2049–2058
36. Zhang X, Deng Z, Wang H et al (2009) Expansion and delivery of human fibroblasts on micronized acellular dermal matrix for skin regeneration. *Biomaterials* 30:2666–2674
37. Murphy GF, Orgill DP, Yannas IV (1990) Partial dermal regeneration is induced by biodegradable collagen-glycosaminoglycan grafts. *Lab Invest* 62:305–313
38. Kuroyanagi Y, Yamada N, Yamashita R et al (2001) Tissue-engineered product: allogeneic cultured dermal substitute composed of spongy collagen with fibroblasts. *Artif Organs* 25:180–186
39. Deveci M, Ozturk S, Kopal C et al (2001) Effect of hyaluronan on adult and fetal fibroblast proliferation and collagen synthesis: in vitro study. *Eur J Plast Surg* 24:222–227
40. Meyer LJ, Russell SB, Russell JD et al (2000) Reduced hyaluronan in keloid tissue and cultured keloid fibroblasts. *J Invest Dermatol* 114:953–959
41. Iocono JA, Krummel TM, Keefer KA et al (1998) Repeated additions of hyaluronan alters granulation tissue deposition in sponge implants in mice. *Wound Repair Regen* 6:442–448
42. Mack JA, Abramson SR, Ben Y et al (2003) *Hoxb13* knockout adult skin exhibits high levels of hyaluronan and enhanced wound healing. *FASEB J* 17:1352–1354