VII

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

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General discussion
Discussing results of this thesis in the background of current literature.

Future perspectives
A personal view on times to come.
Chapter 7 - General discussion and future perspectives

General discussion

Volumetric reconstruction by lipofilling on a large scale has been around for over a quarter of a century, with treatment modalities in both aesthetic as well as reconstructive plastic surgery. Almost every aspect of the technique, however, is still subject to discussion in current literature. Unfortunately, several surgeons who pioneered in lipofilling made strong claims which lacked sound scientific support. This caused a vigorous debate on the efficacy of lipofilling. Fortunately, in the past decade, basic biological knowledge with regard to lipograft increased, which has resulted in variations of harvesting-processing-and injection techniques. Although knowledge of biological principals behind lipofilling has significantly increased during the past years, many questions remain, such as: ‘what is the optimal technique?’ ‘what is the percentage of graft survival?’ ‘does lipofilling rejuvenate overlaying skin?’ ‘what is the role of additives like PRP?’ ‘what is the influence of sex?’ and ‘what is the influence of age and underlying (patho)physiology?’ to mention a few. Increased knowledge of the underlying principles of the procedure may result in optimal – or at least improved – use of lipofilling and could clarify its efficacy in facial rejuvenation. This thesis does not provide answers to all these questions, but delivers a better understanding of the role of lipofilling and the addition of PRP in facial rejuvenation by lipofilling. Also, we present potential pitfalls in future ASC-based human therapies during our journey.

Traditionally, facial rejuvenation is achieved by vertically repositioning the sagged soft tissue, countering the effects of gravity: the facelift. With loss of facial volume due to bone and fat atrophy, identified as a major factor in the aging face, restoring volume by lipofilling could potentially enhance rejuvenation significantly. Despite uncertainties that surround lipofilling, it could offer a ‘natural’ method of volume restoration of the aging face. Furthermore, it has been suggested that lipofilling improves overlying skin quality, a process that is attributed to the ASC. Permanent fillers like Bioalcamed and silicon were readily used in the ‘80 and ‘90’s, but were abandoned due to a high complication rate. The outcomes of our study (Chapter II) support the fact that lipofilling combined with a lifting procedure significantly increases the overall rejuvenation effect compared to a lifting procedure alone. We corroborate the findings published by other authors. It is nowadays commonly accepted that maximal rejuvenation of the aging face can only be achieved by addressing both volume loss as well as lifting sagged soft tissues. In other words: by employing a combination therapy.

With mixed reported clinical results, various additions were explored in order to increase fat graft survival and the predictably of graft take. Because of promising results reported on the use of platelet rich plasma in chronic wound closure and orthopedics, the first experimental studies revealed an increase in fat graft survival when added to the lipograft. Growth factors in the platelet like PDGF and VEGF are known to play a crucial role in tissue inflammatory response and angiogenesis: PDGF promotes wound healing via activation of the local stroma, i.e., connective tissue, while, e.g., VEGF promotes local angiogenesis. Angiogenesis is essential to improve perfusion, i.e., oxygen delivery, but also delivery of immune cells that are essential in adequate wound healing such as alternatively activated macrophages. In a way, the augmentation of lipografts with PRP is thought to reiterate and augment physiological wound healing. Therefore, addition of PRP to the lipograft could increase the rejuvenation effect in facial lipofilling by increasing graft retention and thus gaining volume in a single procedure instead of two or three procedures. Besides the potential effect on surviving lipograft volume, several authors suggested that PRP could also speed up the healing process that would result in a faster recovery time. PRP could also enhance
the claimed rejuvenating skin effects of lipofilling, by influencing collagen deposition, turnover and re-modulation\textsuperscript{23,25}. In our retrospective study (Chapter III), we found that the addition of PRP indeed boosted the overall aesthetic outcome significantly with a reduction in recovering time after surgery. Combined with a MACS-lift, the rejuvenation effect appeared to be the most profound. Possible explanations of this observation can be found in increased graft retention and improvement of the overlying skin, albeit speculative. No comparable studies exist thus far that have focused on recovery time after facial lipofilling, but additional clinical evidence of reduction of recovery time in facial rejuvenation by PRP was also found in the study of Na et al. \textsuperscript{32}.

Preliminary results from our study (Chapter III) inspired us to design of a randomized controlled trial in order to eliminate confounding factors and for the first time investigate the claimed effects of improved skin quality after lipofilling combined with PRP as additive (Chapter IV). Results of our study reproduced the previously reported reduction in recovery time. We found effect, however, of the lipofilling procedure with or without PRP on skin elasticity, a parameter that is commonly accepted in determining skin quality and age\textsuperscript{36-40}. This finding raises questions about the claims made by Coleman\textsuperscript{11} and many others “that lipofilling is more than a permanent filler”. Results as presented in our study, however, might suffer from our limited sample size. During study design and power calculation (estimated minimal cohort size for a given effect that could result in significant differences), literature did not offer a potential clinical effect size. Because of this, the effect size was a rough estimate derived from fundamental studies and a minimal desired effect (5% gain in elasticity). There could, potentially, be an increase in skin elasticity, but this increase would be lower than 5% compared to a control group.

Thus far, most studies focus on lipofilling in damaged (e.g., radiation, thermal) skin\textsuperscript{41,43} in order to reduce excessive scarring, a process that seems to be influenced by ASC in the lipograft. Within the aesthetic domain, the study of Rigotti et al. \textsuperscript{38} shows minimal changes in collagen and elastin deposition after facial lipofilling. This finding, however, seems clinically insignificant, as shown by Amirkhani et al. \textsuperscript{36}. Recent studies\textsuperscript{44, 45}, however, show some effect with SVF/ASC boosted lipografts, again underlining the important role of the ASC. Future studies are definitely needed to focus on the relevancy of this effect in a clinical setting, and, if applicable, long term effects to surrounding tissue and for instance the influence of altering physical characteristics of a patient (e.g. aging, BMI-index, diseases). This knowledge seems paramount prior to widespread clinical application. Current literature on the role of SVF/ASC boosted lipografts is reviewed in Chapter VI.

With the clinical relevancy of small volume facial lipofilling still under debate\textsuperscript{4, 46}, we noted no effect of lipofilling nor lipofilling with PRP on the depth of the nasolabial fold (Chapter IV), despite the fact that previous research with the “Merz Scale” could determine small volume changes (e.g., filler injection) \textsuperscript{47}. The absence of changes in nasolabial fold depth probably can be explained by the fact that lipofilling has increased the overall facial volume of the different fat compartments and thereby did not alter the relative differences between facial compartments / zones. It is our opinion that lipofilling can only have an effect on the depth of the nasolabial fold in combination with a facelift. Moreover, in our study, changes in facial volume were minimal because of the limited amounts of lipografts that have been injected. Until now, only one study has clearly demonstrated facial graft retention after facial lipofilling as determined with external 3D photographic reconstruction\textsuperscript{46}. In this study, an overall retention of 32% was reported. The range and variation of the reported data in this publication, however, questions its real scientific merit. In addition, the vast number of patients in this study also received some form of facial lifting procedure that most likely also changed the facial volume distribution, and
thereby influenced facial volume that has been attributed to lipofilling. Even though lipograft survival in the face has been documented with MRI imaging\(^4^8\), the clinical relevance of facial lipofilling procedures without lifting procedures on facial fold depth remains to be elucidated. Our study raises further questions: is lipofilling really as good as claimed in the past? Apart from the limited level of evidence of Chapter III, one could also state that lipofilling alone is insufficient in facial rejuvenation, since only a vertical lift of soft tissue is able to correct the sagging of the midface and lower facial area (jowls). Increasing facial volume with small volume amounts of fat, as used in our RCT, will not correct the aspect of soft tissue sagging enough. This is in line with the conclusion as drawn in Chapter II: lipofilling will only increase the overall aesthetic outcome of the face when combined with a vertical lift of the sagged soft tissues.

The role of PRP on graft survival in a clinical setting also remains unclear: current literature is inconclusive\(^4^9\). Variations in methodology probably create significant bias and obstacles in interpreting and comparing the results\(^5^0-5^2\). The presence of ASC’s is most likely an important factor enhancing lipograft retention\(^2^0\) and improving skin quality\(^3^6\), but understanding the role of PRP on ASC is also of paramount importance. As stated in our general introduction, ASCs could attribute to graft survival by several mechanisms: 

1. Direct support of adipocytes during the first days after transplantation
2. Inhibit apoptosis pathways of adipocytes
3. Support vascular ingrowth by direct or indirect effects on endothelial cells
4. Differentiate into adipocytes.

It has been shown that the concentration of produced PRP is rather variable, depending on the methods of creation and donor platelet count\(^5^2\). Fundamental studies in vitro have shown a clear concentration dependent effect of PRP on various cell-lines\(^5^3-5^6\). With findings, as presented in Chapter V, we unveiled the importance of PRP concentration in the lipograft: a higher concentration of PRP is not always better. Although high concentrations of PRP proved to be to most powerful mitogen for ASC expansion, it inadvertently seems to change the genotype ASC into a fibroblast like form: apparently, gene expression of ECM related genes are decreased and pro-angiogenic gene expression are also depressed when exposed to high PRP concentrations in the culture medium. Elaborating on this finding, ASC altered genotype translated into its secretome: with inhibition of endothelial cell sprouting capabilities (Chapter V). Both of these changes negatively influence the outcome of facial lipofilling.

The results from our study (Chapter V) will give us hints in how we have to use PRP in lipofilling and ASC cell culture. Care must be taken for the right dose of PRP: its concentration, as well as the end concentration within the lipograft or cell culture, has significant influence on graft / cell survival and differentiation. This concentration aspect may explain the variety of results observed thus far in “PRP-lipofilling literature”. This conclusion was made after we already had designed and started our RCT (Chapter IV). Besides reducing recovery time, absence of PRP effects on skin elasticity and lipograft volume in this study could partially be explained by findings as described in Chapter V, and questions the value of its use as described in our RCT. In our opinion, adding PRP to the lipograft can only work synergetic within a very small therapeutic window that cannot be achieved with the current available disposable PRP kits. Moreover, transdermal delivery of PRP into the lipofilled tissue planes, as in our RCT, is also probably not precise enough. Future ‘tailor made’ lipografts, with known ASC-adipocyte composition, might leave a role for PRP addition.

Besides PRP being an interesting additive to lipofilling in a clinical setting because of faster recovery time, PRP could also play an import role in ASC-therapies. The study of Kolle et al.
clearly demonstrated that ASCs exert a dose-dependent influence in the lipograft\textsuperscript{20,57}, and also showed that ASCs might expand in vivo in lipografts\textsuperscript{57}. Expansion of ASC’s in vitro can only be achieved by means of adding growth factors to the culture medium. In an experimental setting, most studies use bovine serum components. Interestingly, many positive effects attributed to ASC (Chapter VI) are based on the use of culture media that are supplemented with bovine serum, whereas ASCs cultured on human serum (in our case PRP) differ significantly from bovine cultures as demonstrated in Chapter V. Results of our study are supported by the Bieback et al.\textsuperscript{58} and Blande et al.\textsuperscript{59}. Future ASC’s based clinical therapies preferably should use human platelet derivatives in order to minimize inflammatory responses towards bovine proteins and the potential of animal pathogen transmission that may be harmful to humans (e.g. Creutzfeldt-Jakob disease\textsuperscript{59}). Despite the promising results observed in anti-fibrotic ASC therapies in animal and limited human studies (Chapter VI), reliable achievement of these effects in large clinical series is only possible with a better understanding of platelet lysate based ASC cultures and the effects of the lysate concentrate.

**Future perspectives**

Lipofilling and ASC therapies seem to harness the future of medicine: with lipofilling reconstruction and / or rejuvenation can be achieved on a cellular level. We probably stand at the beginning of a spectacular journey that may take several decades but finally will result in effective human stem cell therapies at its end. Evolutionary, this could be the next step for human species: fast (scarring) wound healing could be regulated by lipofilling and ASC therapies. In theory, this could result in scarless healing without functional loss, a healing very similar to that what occurs in early fetal stages.

We believe that future stem cell therapies will offer scarless fetal wound healing, an ability that has been lost in mature mankind wound healing. PRP, or platelet lysates, will definitely also have a role in these perspectives, probably for in vitro for cell expansion and potentially clinically if we gain further understanding of its working and interactions. However, it is currently unlikely that we can control in vivo PRP concentrations when used as an additive to the lipograft in the near future.

Currently, the scientific evidence for the “clinical observed” rejuvenation effects procedures with lipofilling needs to be further substantiated. No consensus exists about most aspects of the lipofilling procedure itself, and therefore no universal standard method has yet been established. With only case reports, claims like skin improvement have been accepted and are nowadays regularly used in patient consultations. Although the volume effect of lipofilling is established and definitely needed in optimal facial rejuvenation procedures, it is surprising that terms as skin improvement are still used so frequently in clinical consultation without sufficient scientific evidence. Because of spectacular clinical results observed in the early ‘90, extensive clinical application has spread over the world without real proof of the underlying idea or mechanism. Several factors may count for this course: the commercial circuit demanding speed and widespread application of new spectacular therapies and the spectacular results as observed in the early nineties. In order to get a better and sound scientific base for widespread clinical application of lipofilling and ASC related therapies, we must change our mentality towards ‘look before leaping’. Further elaboration with the research field of fundamental biology would be therefore most advantageous. Basic knowledge of what happens on a cellular level will finally lead to evidence-based clinical therapies. Furthermore, the gap between in vitro research and the clinician should be bridged, resulting in fundamental research that focuses on relevant clinical topics. As such, this thesis is an example of such a valuable collaboration.
In addition to a lack of basic understanding and science, there is a shortage of reproducible controlled clinical trials in aesthetic surgery. Literature thus far mainly consists of small, non-blinded non-randomized studies, often with various methodology. Lipofilling also is a perfect example for this aspect: variations in the technique itself have resulted in limited scientific evidence. The formation of an international scientific committee or consortium that dictates methodical guidelines for lipofilling could reduce the number of variables involved, and may subsequently result in reliable data for meta-analysis. Only well designed randomized controlled trials, with minimal variations in methodology, can answer the questions stated in the first paragraph of the general discussion in this thesis.

Publishing negative results, i.e., results that fail to confirm the hypothesis after well controlled clinical trials, should also be published and not be pushed away. As in this thesis, for example, the observation of the lack of increase in skin elasticity in our RCT (Chapter IV) has shown to be an unwelcome message for reviewers, probably because this observation is “not wanted to be found for whatever reason”. In my opinion, any result from an RCT should be of value, since it could form reference and baseline during designing and power calculations of future RCTs within the subject. Also, publication of RCT with a faulty design could be of great educational value when its fault is revealed and discussed.

Therefore, in order to eliminate any form of conflict of interest by reviewers and journals in publishing negative results, submission of the study in an earlier phase should be done. For example, with respect to an RCT, before the inclusion starts, submission of all the details, purpose and methods to journal should be undertaken to let them judge and decide whether or not the methodology is sound, and if the topic is within the journals’ scope. If accepted, the journal should be committed in publishing the results and whether they are to be expected (positive) or not (negative). Also, a critical review by independent reviewers of a suggested study’s methodology could result in better study quality. Fortunately, the rise of many open access journal greatly expands the reach of ‘negative result studies’ compared to recent history. In my opinion, open access journals are the way forward: data must be available for everyone at every time. Sharing could lead to increased efficiency, potentially lower costs and collaboration/synergy within research in general.

The future looks promising, but also is challenging and demanding: let’s learn from the past and join all our forces to finally discover the clues for scarless healing and rejuvenation.

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References

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