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Review

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Stem cells and tissue engineering in plastic surgery: an update

Gregory R. D. Evans, Alan D. Widgerow

Department of Plastic Surgery, University of California, Irvine, Orange, CA 92868, USA.

Correspondence to: Prof. Gregory R. D. Evans, Department of Plastic Surgery, University of California, Irvine, 200 S Manchester Suite 650, Orange, CA 92868, USA. E-mail: gevans@hs.uci.edu

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Abstract

Stem cells and tissue engineering have made great strides in plastic surgery. This review of the literature evaluates some current background information and recent advances in our laboratory to bring these areas more into the clinical setting.

Keywords: Stem cells, plastic surgery, fat

INTRODUCTION

Plastic surgeons are innovative. The specialty has been founded on cutting technology, new ways to look at procedures and adaptive behavior. Plastic surgeons have embraced the field of regenerative medicine and have attempted to understand stem cell technology and fat grafting and how this might relate and apply to the clinical setting[1-5]. Unfortunately despite much progress, there remains significant unanswered questions on clinical applicability and our ability to regulate and adjust these cells for clinical use.

Embryonic stem cells were first isolated and described by Dr. James Thomson at the University of Wisconsin in 1998[5-13]. The pluripotent nature of these cells generated a great deal of excitement but with this excitement came concerns on potential embryonic sacrifice along with the potential experimental concern with the use of these cells. Consequently, the use of embryonic stems cells has been isolated to a few discrete clinical trials[14-20].

Induced pluripotent cells (iPS cells) are genetically modified cells that take on the characteristic of embryonic stem cells. The initial excitement over the use of these cells revolved around the opportunity

for researchers to utilize a cell with similar pluripotency but with the lack of embryonic moral concerns relating to embryonic stem cells[21-30]. However, concerns on genetic modification along with tumorigenic potential has led to caution for clinical indications[31-56].

Adult stem cells, although not as robust as embryonic stem cells, carry significant potential in regenerative medicine and, with their abundance in fat, carry great significance to plastic surgeons[18,19,37,39,54,56]. They are capable of transforming into a limited number of cellular phenotypes within a given family and there is some thought that they may induce further local transformation of cells in a microenvironment through paracrine influences. In 2001, Zuk *et al.*[13] reported a source of mesenchymal stem cells in abundance in one particular tissue - that being fat. During processing with collagenase, they found an abundance of cells known as the stromal vascular fraction (SVF) that includes red and white blood cells, immune cells, endothelial cells, and stem cell precursors[56-80]. Their process of isolation and determination of unique physical identifiers known as clusters of differentiation allowed the identification of a variety of cells that with multipotent potential. Various processing techniques have been utilized to isolate these cells. Collagen isolation is perhaps the most efficient but concerns with the FDA on the use of collagen in the clinical environment and regulations on minimal manipulation have led to the search for alternative options such as other processing techniques, filtering or drying, washing, or centrifugation.

While SVF may contribute to regeneration by its different components, it may also use a paracrine signaling as a means for regeneration based on cross-talk between different cell populations[11,12]. Co-culture of SVF with adipocytes has yielded induced progenitor preadipocyte formations. SVF can also promote angiogenesis and neovascularization. We believe that this has great opportunities in the clinical setting for promoting wound healing, particularly in patients with diabetes and other chronic diseases. Co-implantation of SVF with endothelial progenitor cells and adipose derived stem cells (ADSCs) resulted in improved neovascularization potential[11]. With lipotransfer there is often significant volume loss but combining with SVF has demonstrated enrichment of fat revascularization, probably through this mechanism of promoting secretion of a diverse array of cytokines. SVF can also be used to create highly vascularized tissue engineered human dermo-epidermal skin substitutes for burn wounds. Rigotti postulated a common sequence of events occurring when SVF is transplanted to radiation-damaged tissue that ultimately results in tissue reperfusion and recovery of some of the damaged skin[50,81-92].

Many of therapeutic studies have also described an initial decrease in inflammation and immune response at the site of SVF injection. When applied to certain disease models, it also tends to decrease inflammatory cytokines and growth factors[92-117].

SVF also has a regenerative capacity, probably through the release of cytokines. SVF increases proliferation of fibroblasts when injected in diabetic foot ulcers. There is also some evidence that it might promote nerve regeneration[21]. Diabetic foot disease is a multibillion dollar drain to the health care system, thus utilization of options that could prevent or improve wound healing would be monumental.

Once isolated from adipose tissue, the stromal cell populations represent a diverse collection of cell types. The key concept however is that they are free of lipid and one can mark the cell types with numerous CD (cluster of differentiation) markers. Endothelial precursors are distinctive for bearing the CD 31 antigen and comprise approximately 25%-30% of the mixture of cells. It should be noted however that endothelial precursors bear more markers than just CD 31. Cells for CD 34 markers are considered early multipotent progenitor cells and are considered the true "pre-adipocyte". Further "pericytes" are thought to give rise to many of the stromal cell populations. Stem cells sense and respond through differentiation to various mechanical cues, growth factors adhesive ligand density, and other factors. It is not only cell differentiation but also cell proliferation, angiogenesis, and cell signaling that are affected by these factors. The rest of this manuscript considers the use of these cells in the clinical setting[11,12].

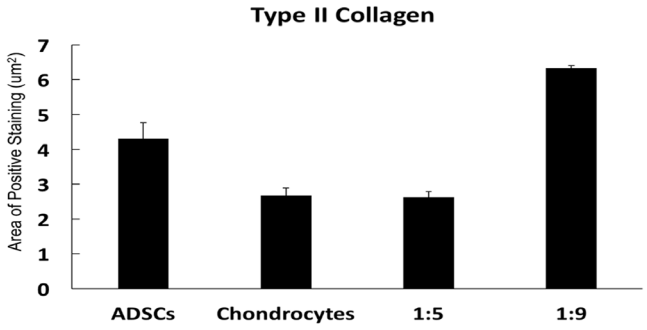

Figure 1. The paraffin embedded sections were also stained for type II collagen. The images were quantified, and the 1:9 condition showed the highest level of staining, which was significantly greater than the chondrocytes alone. ADSCs: adipose derived stem cells

It should be noted that, although clinically there does not appear to be an increased influence on carcinogenesis, several studies indicate that ADSC/ADSC secretomes significantly stimulate proliferation transmigration and 3D invasion of primary normal and tumor epithelial cells[118-120].

Soft Tissue Augmentation and Regeneration is often completed by fat grafting. Variable percentages of absorption occur with fat grafting and volume estimation and preservation may not be optimal[25]. The SVF can provide various growth factors such as vascular endothelial growth factor that can promote neovascularization as well as other growth factors through a technique coined as cell-assisted lipotransfer[62,63]. Utilization of ways to preserve and maintain fat moving forward under the FDA guidelines will help us utilize these options for future clinical studies.

Other studies in our laboratory involve the utilization of ADSCs as a source for chondrocyte progenitors. Porcine chondrocytes can be successfully isolated, expanded, frozen, and thawed, but past a certain point they lose their chondrogenic features. Co-culturing these chondrocytes with ADSCs on AAM (allograft adipose matrix) was hypothesized to enhance their chondrogenic features. Chondrocytes were co-cultured on disc with varying concentrations. After 10 weeks in culture, the discs were paraffin imbedded and stained with H&E. The 1:5 and 1:9 conditions (chondrocytes:ADSCs) demonstrated evidence of extracellular matrix deposition with the 1:9 condition showing a more compact tissue structure, indicating potential chondrogenesis. The paraffin imbedded sections were also stained for type II collagen [Figure 1] with the 1:9 condition showing the highest level of staining, which was significantly greater than the chondrocytes alone. Because the chondrocytes were from porcine and the ADSCs were from human, the contribution of each cell type to chondrogenesis was able to be determined. Hy-PYD (Hydroxylysyl Pyridinoline) assesses collagen crosslinking [Figure 2]. Here, the expression of Hy-PYD was assessed from the discs by enzyme-linked immunosorbent assay (ELISA) and the 1:9 condition increased the expression over the chondrocytes alone. The 1:9 condition was significantly greater than the 1:5 condition. This indicated that ADSCs were stimulating the chondrocytes to be more chondrogenic. The supernatants were collected from the co-culture wells for each condition. ELISA tests were conducted to assess the secretion of type II collagen using species specific antibodies. The human collagen II results were normalized to the chondrocytes because they were presumed to show no expression, and the porcine collagen II results were normalized to the ADSCs, which were presumed to show no expression. The ELISAs were conducted using the supernatants from the collections at Weeks 3, 5, 7, and 9 [Figure 3]. For the human collagen II expression, there was essentially no expression throughout the experiment. However, for the porcine expression, there was a time-dependent increase for the 1:9 co-culture condition, with the nine-week reading showing the highest expression of any of the supernatants tested. The other conditions did not reveal any distinct pattern, indicating that the 1:5 co-culture condition was not different from culturing the chondrocytes alone. In summary, porcine ADSCs can be successfully isolated, expanded, frozen, and

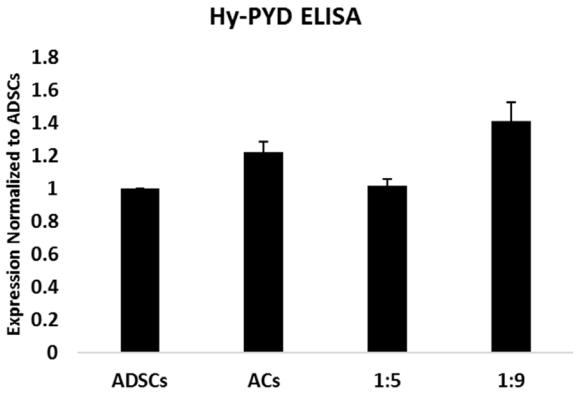

Figure 2. Because the chondrocytes were from porcine and the ADSCs were from human, we determined the contribution of each cell type to chondrogenesis. Hy-PYD assesses collagen crosslinking. Here, the expression of Hy-PYD was assessed from the discs by ELISA, and the 1:9 condition increased the expression over the chondrocytes alone. The 1:9 condition was significantly greater than the 1:5 condition. This indicated that ADSCs were stimulating the chondrocytes to be more chondrogenic. ADSCs: adipose derived stem cells; ELISA: enzyme-linked immunosorbent assay

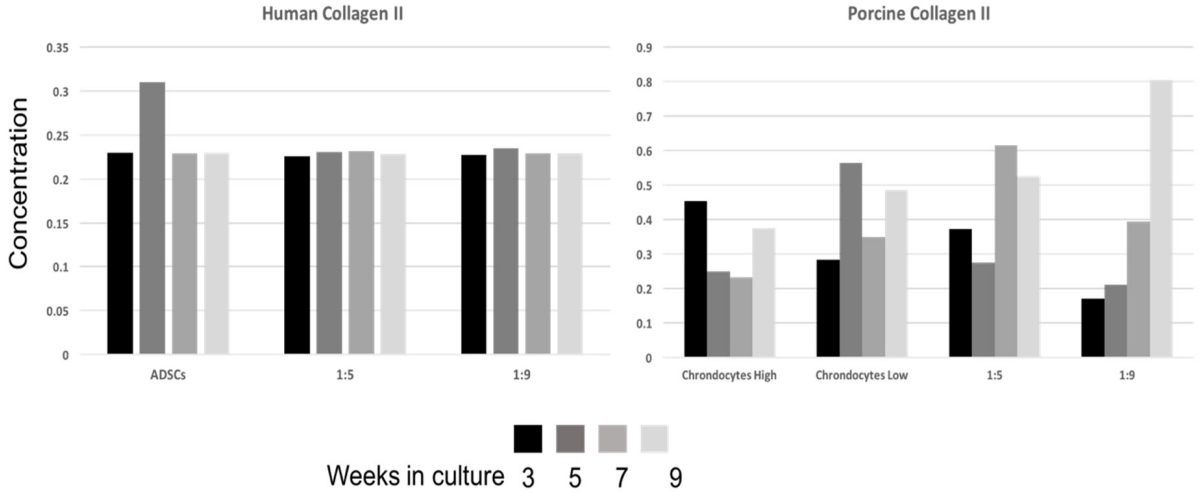

Figure 3. The supernatants were collected from the co-culture wells for each condition. Then, ELISA tests were conducted to assess the secretion of type II collagen using species specific antibodies. The human collagen II results were normalized to the chondrocytes because they were presumed to show no expression, and the porcine collagen II results were normalized to the ADSCs, which were presumed to show no expression. The ELISAs were conducted using the supernatants from the collections at Weeks 3, 5, 7, and 9. For the human collagen II expression, there was essentially no expression throughout the experiment. However, for the porcine expression, there was a time-dependent increase for the 1:9 co-culture condition, with the nine-week reading showing the highest expression of any of the supernatants tested. The other conditions did not reveal any distinct pattern, indicating that the 1:5 co-culture was not different from culturing the chondrocytes alone. All quantitative data are expressed as the mean ± SD. A Student's *t*-test was performed to assess the differences between the groups. A *P*-value < 0.05 was considered statistically significant. ADSCs: adipose derived stem cells; ELISA: enzyme-linked immunosorbent assay

thawed, but past a certain point they lose their chondrogenic features. Co-culturing these chondrocytes with ADSCs on AAM enhanced their chondrogenic features, revealing more structural changes, an increase in Hy-PYD and type II collagen staining, and secretion in the 1:9 (Chondrocytes:ADSCs) co-culture condition. Chondrocytes contribute to the production of collagen, and the presence of the ADSCs increases this production when these cells were cultured on AAM[118-125].

Recent studies have utilized newer techniques for soft tissue augmentation. Rigotti evaluated a noninvasive technique, which he calls biological morphogenetic surgery, that can enlarge or reduce the shape and

volume of soft tissues by utilizing cannulas and augmentation with fat cells. He also noted an increase in adipose tissue under tissue expanders placed in rats below the latissimus muscle. The thought is that the tensile pressure associated with the tissue expander leads to adipose deposits[121,122].

Wound healing issues are a multibillion dollars business and the use of ADSCs offer potential therapeutic options. ADSCs have been promoted as favorable candidates for wound therapies and they secrete numerous growth factors and cytokines critical in repair. Recent studies have indicated that ADSCs may reverse or improve radiation-induced lesions as well as atrophy and scarring[94]. Animal studies suggest that the release of keratinocyte growth factor and the differentiation of ADSCs toward endothelial and epithelial cell line phenotypes may be the mechanisms of action. Further, the angiogenic properties of ADSCs may also benefit complications secondary to ischemia. Autogenous transplantation has demonstrated some promise in improving ankle-brachial index and transcutaneous oxygen pressure. ADSCs may also be useful for the treatment of pathological wound healing in the context of hypertrophic scar formation[20].

In addition, SVF and ADSCs have been used to promote extracellular matrix (ECM) synthesis, the groundwork for wound healing. The extracellular matrix acts as a potent scaffold in many tissue types, accelerating the regenerative functions of nearby cells. It is comprised of structural proteins such as collagen, laminin, fibronectin, and elastin, which are commonly secreted by the fibroblast[12]. Furthermore, the ECM contributes to the growth of vascular networks by mediating morphogenesis and migration speeds during angiogenesis. Since the SVF contains matrix-secreting fibroblasts and other stromal cells, the application of SVF is potentially advantageous for laying down the foundations for wound healing[11].

Finally, skin rejuvenation may have promise with ADSC use. It may be possible that these cells could reverse atrophic and photo-damaged cells. Animals studies have demonstrated that subcutaneous ADSC injections increase dermal thickness and collagen density in aged mice and perhaps reduced wrinkles induced by UVB-irradiation[11,12].

Our laboratory has also studied the mechanism of fat formation using acellularized adipose matrix by deconstructing adipo-induction in this acellularized adipose matrix (AAM). It has been observed that AAM injected subcutaneously in an area relatively devoid of fat may initiate lipogenesis. Because of this observation, our global aim was to delineate the sequence of events occurring following implantation of AAM to the final process of adipogenesis. We wanted to compare adipoinduction of adipose/fascia complex to that of adipose fraction alone - analyzing proteomics, bioinformatics, early and late cellular infiltrates, cytokines, adipokines, and enzymes (related to macrophage phenotype and lipoproteins). In addition, we examined the genesis of the adipocytes required to achieve a lipofilling effect, and detailed the involvement of stromal and stem cells in recruiting host tissue to fill the void via adipogenesis, neovascularization and fibrosis[120].

Recently, several groups have started to examine the use of human AAM as a scaffold for tissue engineering, which shows great promise as a vehicle for adipose stem cell delivery as well as a construct that promotes soft tissue regeneration though acellular mechanisms[119]. AAM secretes growth factors with adipo- and angio-inductive characteristics [vascular endothelial growth factor, bFGF (fibroblast growth factors), platelet-derived growth factor, and TGF-β (transforming growth factor)] and recruits preadipocytes, pericytes, and other cells responsible for local tissue regeneration. Degradation products of the ECM trigger a change in macrophage phenotype that can bring about progenitor cell recruitment[120].

Micronanobubbles (MNBs) technology is another area of exploration in fat graft survival. The bubbles afford the opportunity oxygenate tissues without the expense of utilizing hyperbaric chambers or other nonmobile options. What are the properties that make MNBs unique? MNBs are very small bubbles

in solution that slowly release their oxygen gas into the solution in which they are formed, providing a revolutionary new modality for tissue oxygenation. Strong data have demonstrated improved wound oxygenation, transplant survival and preservation times. This may have major implications for plastic surgery: oxygenation of lipoaspirate and improved fat graft survival[117].

CONCLUSION

Fat and stem cell technology offer tremendous clinical applications in plastic surgery. We still need to continue to work on the science of this technology. Only by understanding the molecular and tissue interactions will we be able to modify and utilize this technology to its full advantage.

DECLARATIONS

Authors' contributions

Dr Evans and Widgerow contribution was equal

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

Both authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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