—Review—

Adipose-derived Stem Cells for Tissue Repair and Regeneration: Ten Years of Research and a Literature Review

Hiroshi Mizuno

Department of Plastic, Reconstructive and Regenerative Surgery, Graduate School of Medicine, Nippon Medical School

Abstract

Stem cell based therapies for the repair and regeneration of various tissues and organs offer a paradigm shift that may provide alternative therapeutic solutions for a number of diseases. Although embryonic stem cells and induced pluripotent stem cells are theoretically highly beneficial, there are various limitations to their use imposed by cell regulations, ethical considerations, and genetic manipulation. Adult stem cells, on the other hand, are more easily available, with neither ethical nor immunoreactive considerations, as long as they are of autologous tissue origin. Much research has focused on mesenchymal stem cells isolated from bone marrow stroma which have been shown to possess adipogenic, osteogenic, chondrogenic, myogenic, and neurogenic potential *in vitro*. However bone marrow procurement is extremely painful for patients and yields low numbers of harvested cells.

When compared with bone marrow-derived mesenchymal stem cells, adipose-derived stem cells are equally capable of differentiating into cells and tissues of mesodermal origin. Because human adipose tissue is ubiquitous and easily obtainable in large quantities under local anesthesia with little patient discomfort, it may provide an alternative source of stem cells for mesenchymal tissue regeneration and engineering. Based on our previous experimental findings, this review highlights the molecular characteristics, the potential for differentiation, the potential for wound healing, and the future role of adipose-derived stem cells in cell-based therapies and tissue engineering.

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Key words: adipose-derived stem cells, regenerative medicine, tissue engineering, cell therapy, wound healing

Introduction

The emerging field of regenerative medicine requires a reliable source of stem cells in addition to biomaterial scaffolds and cytokine growth factors. By definition, a stem cell is characterized by its ability to self-renew and to differentiate along multiple lineage pathways.

Candidates for such strategies include embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs)[1] and postnatal adult stem cells. Although the therapeutic potential of ESCs and iPSCs is enormous due to their auto-reproducibility and

Correspondence to Hiroshi Mizuno, MD, Department of Plastic and Reconstructive Surgery, Nippon Medical School, 1–1–5 Sendagi, Bunkyo-ku, Tokyo 113–8603, Japan
E-mail: hmizuno@nms.ac.jp
Journal Website (http://www.nms.ac.jp/jnms/)

pluripotentiality, there are still some limitations to their practical use, including cell regulations, ethical considerations and genetic manipulation[1–3]. In contrast, postnatal adult stem cells are, by nature, immunocompatible, and there are no ethical issues related to their use. Cells obtained from bone marrow stroma, termed mesenchymal stem cells (MSCs), are the representative cells of this type, and possess adipogenic, osteogenic, chondrogenic, myogenic and neurogenic potential *in vitro*[4–6]. However, MSCs with similar characteristics to bone marrow-derived MSCs[7,8], have recently been isolated from several different tissue sources in addition to bone marrow stroma. Our laboratory has shown previously that cells obtained from human liposuction fat aspirates can also differentiate into adipogenic, osteogenic, chondrogenic, and myogenic cells in a lineage-specific culture medium, and we termed these cells adipose-derived stem cells (ASCs)[9,10]. Furthermore, we have successfully performed several studies of *in vivo* tissue regeneration and primary wound repair[11–15].

Adipose tissue represents an abundant and accessible source of adult stem cells that can differentiate along multiple lineage pathways. The aims of this review are: (1) to describe the availability of ASCs, (2) to describe the isolation procedures and molecular characterization of ASCs, (3) to describe both the *in vitro* and *in vivo* differentiation potential of ASCs, (4) to introduce current and on-going applications for clinical use and (5) to discuss the future clinical perspective of ASCs.

Availability of ASCs

Several literature reviews indicate that MSCs obtained from bone marrow, adipose tissue, and umbilical cord show no differences in fibroblast-like morphology, immune phenotype, success rate of isolation MSCs, colony frequency, and differentiation capacity[16,17]. However, Gimble et al. have suggested that a stem cell for regenerative medicinal applications should ideally meet the following criteria[18,19]:

1. Can be found in abundant quantities (millions to billions of cells)
2. Can be harvested with a minimally invasive procedure
3. Can be differentiated along multiple cell lineage pathways in a regulatable and reproducible manner
4. Can be safely and effectively transplanted to either an autologous or allogeneic host
5. Can be manufactured in accordance with current Good Manufacturing Practice guidelines

Adipose tissue could be considered to fulfill all these criteria. With the increased incidence of obesity in modern populations, subcutaneous adipose tissue is abundant and readily accessible. To harvest the adipose tissue, a liposuction procedure is less invasive than bone marrow aspiration. In general, well-trained plastic surgeons are familiar with liposuction procedures, and the technique produces less patient discomfort and donor site morbidity. Small amounts of adipose tissue (100 to 200 mL) can be obtained under local anesthesia. In addition, 1 g of adipose tissue yields approximately 5×10^3 stem cells[20], which is 500-fold greater than the number of MSCs in 1 g of bone marrow[21]. As such, adipose tissue can be considered as a rich source of stem cells.

Isolation, Proliferation and Molecular Characterization of ASCs

In previous studies, we established a standard protocol for the isolation of ASCs from adipose tissue using enzymatic digestion[9], which has been broadly applied by most scientists. Briefly, the raw liposuction aspirate or finely minced adipose tissue is washed extensively with sterile phosphate-buffered saline to remove blood cells, saline, and local anesthetics. The extracellular matrix is digested with 0.075% collagenase at 37℃ for 30 minutes to release the cellular fraction. Collagenase is inactivated with an equal volume of Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS). The infranatant is centrifuged at 250 g for 10 minutes to obtain a high-density cell pellet. The pellet is resuspended in DMEM and 10% FBS, and plated in 100-mm tissue culture dishes at a density of 1×10^6 cells per plate. These cells are maintained in control medium

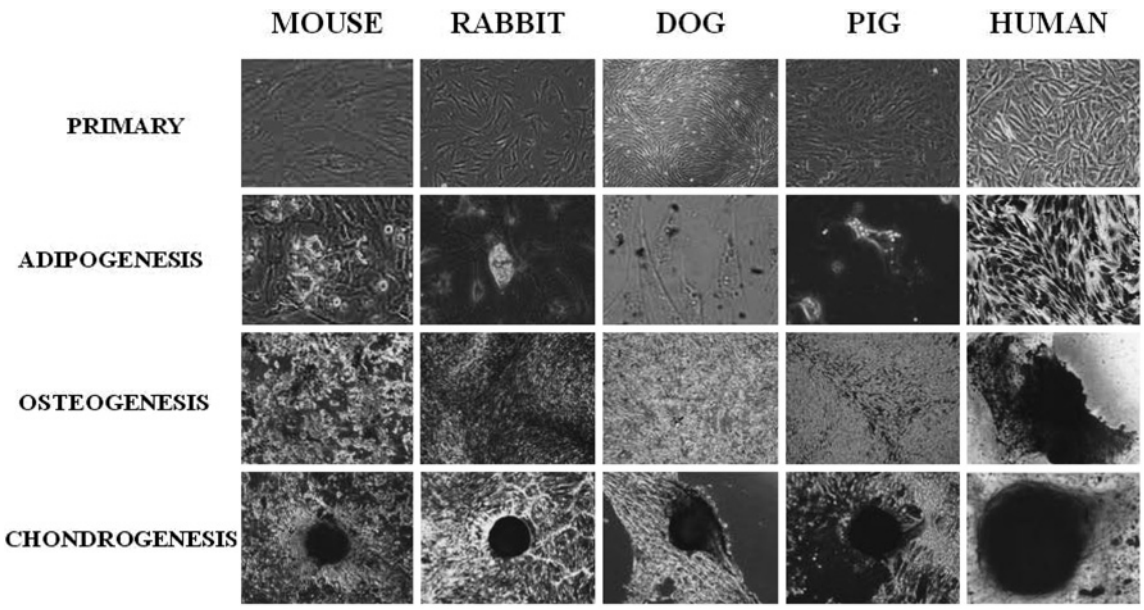

Fig. 1 Adipose derived stem cells (ASCs) isolated from humans and other species. Regardless of the species, ASCs exhibit fibroblast-like morphology and multipotency towards adipogenic, osteogenic and chondrogenic differentiation.

(DMEM supplemented with 10% FBS and 1% antibiotic/antimycotic) at 37℃ and 5% CO_2. Attached cells obtained from human and other species exhibit a fibroblast-like appearance and the potential to differentiate into adipogenic, osteogenic, chondrogenic, myogenic, and neurogenic lineages under the appropriate culture conditions: this potential indicates they are multipotent ASCs (**Fig. 1**).

In our previous report, the proliferation assay showed that ASCs obtained from 20 donors, and cultured under standard conditions, exhibited an average population doubling time of 60 hours[9]. Generally, ASCs display a cell doubling time of 2 to 4 days, depending on donor age, the type (white or brown adipose tissue), and location (subcutaneous or visceral) of the adipose tissue, the type of surgical procedure, culturing conditions, plating density, and media formulations[16,22].

The proliferation of ASCs can be stimulated by several exogenous supplements. These include fibroblast growth factor 2 (FGF-2) via the FGF receptor 2[23], sphingosylphosphorylcholine via the activation of c-jun N-terminal kinase (JNK)[24], platelet-derived growth factor via the activation of JNK[25], and oncostatin M via activation of the microtubule-associated protein kinase (MEK)/extracellular signal-regulated kinase (ERK) and the JAK3/STAT1 pathway[26].

On the other hand, Rubio et al. have shown that human ASCs undergo malignant transformation with prolonged passaging over more than 4 months[27]. These results indicated that care must be taken in the manipulation and culture of ASCs. Moreover, such a phenomenon implies that freshly isolated ASCs might be safer and more practical than cultured ASCs for clinical use.

Cell surface immunophenotypes, such as CD markers (determined with fluorescence-activated cell sorting), of ASCs isolated from humans and other species have been investigated by various independent groups[9,28–32]. Regardless of differences in isolation, culture procedures and time in passage, the reported immunophenotypes were relatively consistent between research groups. Indeed, such ASC surface marker expression profiles seem to be similar to those of bone marrow-derived MSCs[28,33].

Finally, ASCs secrete potent growth factors, such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), FGF-2, and insulin-like growth factor 1 (IGF-1)[34–36]. In addition, the levels of VEGF or HGF or both secreted by ASCs can be induced by exposure of the cells to hypoxia[34], growth factors, differentiation factor[37] or tumor necrosis factor-α[38].

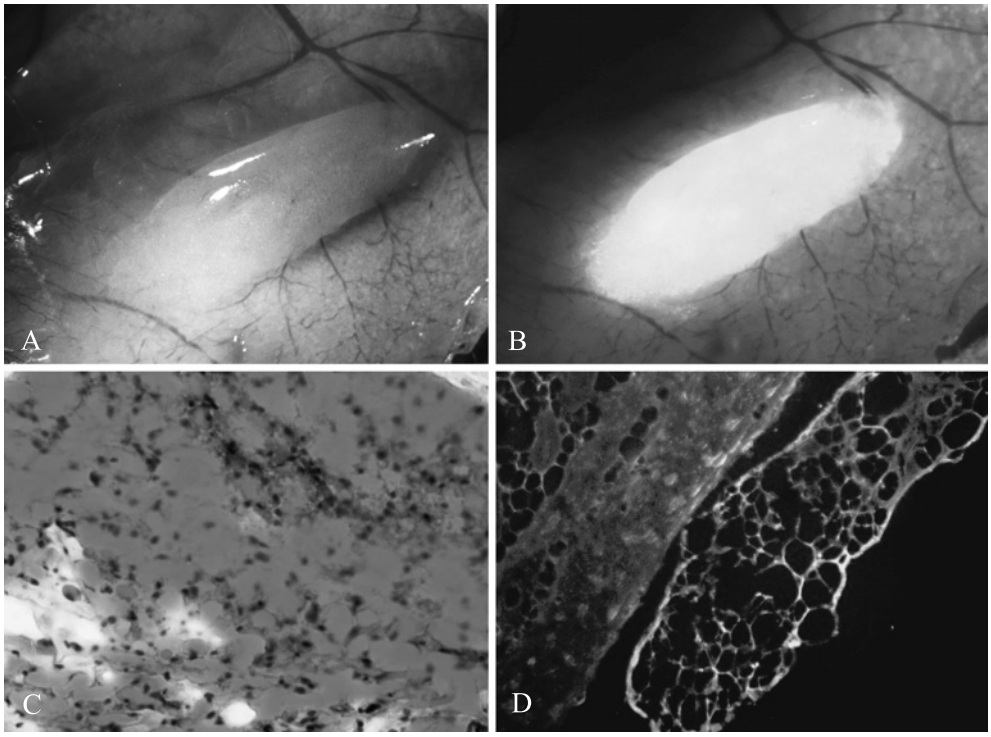

Fig. 2 In vivo regeneration of adipose tissue by ASCs. **A**: A yellowish and more three-dimensional structure was regenerated after 8 weeks of ASC implantation with fibrin glue. **B**: Fluorescence-microscopic observation demonstrated green fluorescent protein more strongly. **C**: At 8 weeks, increased lipid accumulation was confirmed by ORO staining. **D**: Fluorescence microscopic image shows green fluorescence along the cell surface membranes of the newly formed tissue. (Reprinted from Mizuno et al. *Cells Tissues Organs*. 187: 177, 2008)

In vitro Differential Potential of ASCs

There are numerous examples from the literature demonstrating the multipotency of ASCs *in vitro*. Because ASCs are of mesodermal origin, potential lineages obviously include the adipogenic[9,10,39], osteogenic[9,10,40], and chondrogenic[9,10,41] lineages, and the myogenic lineage leading to skeletal muscle[9,10,42,43], smooth muscle[44,45], and cardiomyocytes[46]. Interestingly, however, ASCs have also been shown to possess the potential to differentiate into non-mesodermal lineages including neuron-like cells[47–50], endothelial cells[35,51], epithelial cells[52], hepatocytes[53,54], pancreatic cells[55], and hematopoietic supporting cells[56,57].

Potential of *In vivo* Tissue Regeneration

In addition to *in vitro* studies of differentiation assays in ASCs, tissue/organ regeneration and repair experiments using ASCs with or without appropriate scaffolds have been performed *in vivo*.

With regard to tissue of mesodermal origin, ASCs pre-induced in adipogenic differentiation medium and seeded onto a scaffold of such materials as poly lactic-co-glycolic acid, type I collagen sponge, and fibrin glue, have been successfully induced to differentiate into adipose tissue. This differentiation has been confirmed with macroscopic morphology, histology, and immunohistochemistry[13,58,59]. In our experiments, ASCs isolated from green fluorescent protein (GFP) transgenic mice were successfully induced to differentiate into three-dimensional adipose tissue after 8 weeks of implantation. Moreover, immunohistochemical analysis showed that the newly formed adipose tissue originated mostly from the transplanted ASCs derived from the GFP transgenic mice. These results indicate that newly formed tissue is composed mainly of

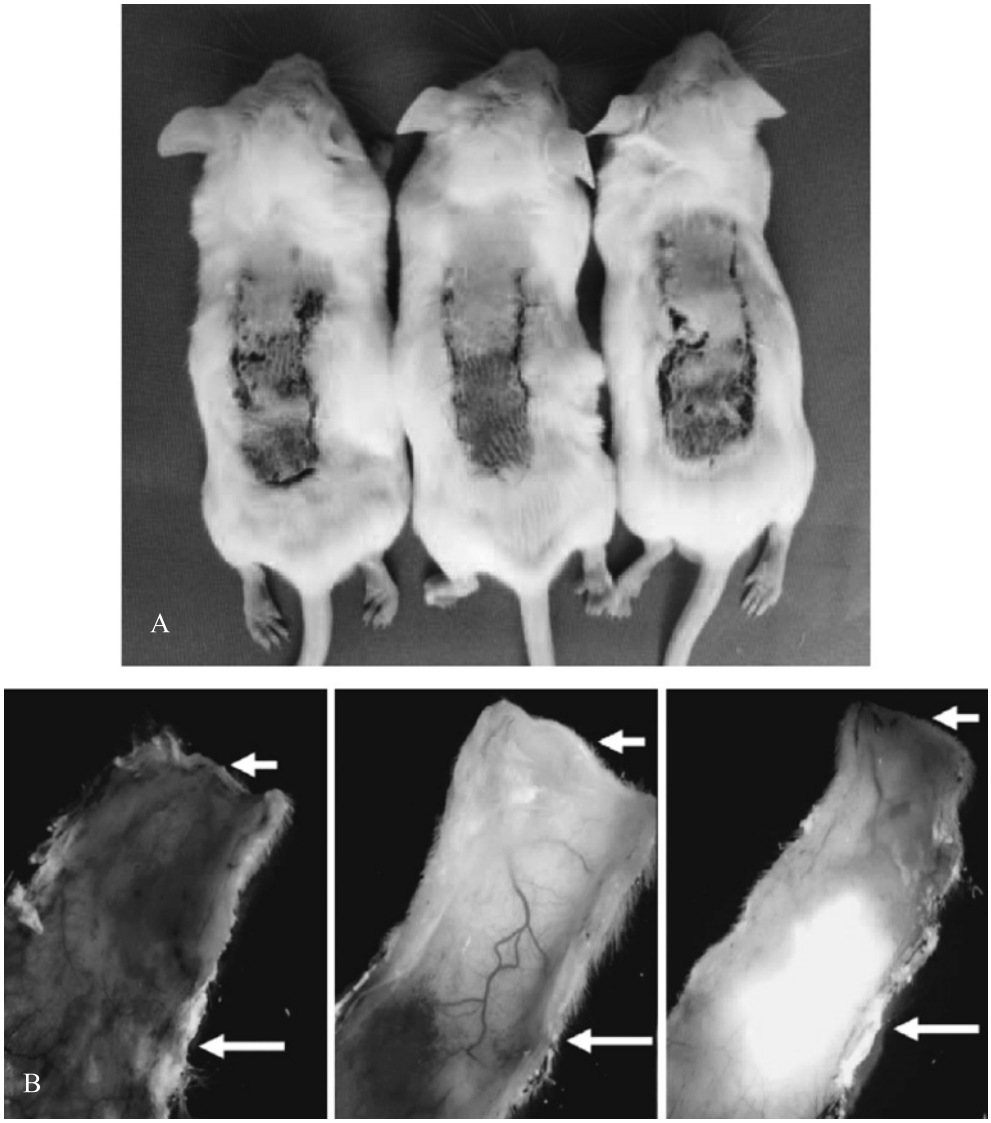

Fig. 3 Local implantation of ASCs into the flap decreased ischemia and lengthened subsequent survival. **A**: By postoperative day 7, the regions of survival and necrosis were clearly demarcated in the flaps and were easily distinguished by blinded observers. (*left*) Control (Dulbecco's modified Eagle medium); (*center*) injection of ASCs at the flap base; and (*right*) injection of ASCs at the center of the flap. **B**: Fluorescence distribution of DiI-labeled ASCs in the surviving areas of the flap. No fluorescent cells were identifiable in the control group (*left*). When ASCs were injected in the base of the flap, fluorescent density increased in the pedicle area (*center*). The fluorescence was evident in the area around the injection site, ranging from 0.5 to 2.0 cm distal to the pedicle (*right*). (**Small arrow** shows the base of the flap and **large arrow** shows 1.5 cm distal to the base of the flap). (Reprinted from Lu et al. *Plast Reconstr Surg*. 121: 50, 2008)

transplanted ASCs[13] **(Fig. 2)**.

In a rat ischemic hindlimb model, the intravenous or intramuscular administration of ASCs, which are negative for CD31, dramatically improved the vascular supply[34,35,51,60,61]. This mechanism accounts for both the direct differentiation of ASCs into endothelial cells and the indirect effect of ASCs, which secrete angiogenic growth factors. Consistent with the results of the ischemic hindlimb study, the survival area of an ischemic skin flap can also be increased by local injection of autologous ASCs into the skin flap[12] **(Fig. 3)**.

Topical administration of ASCs onto skin ulcers can accelerate the healing process of the skin wound[11]. In our such study, chemically-induced intractable ulcers were covered with ASCs in

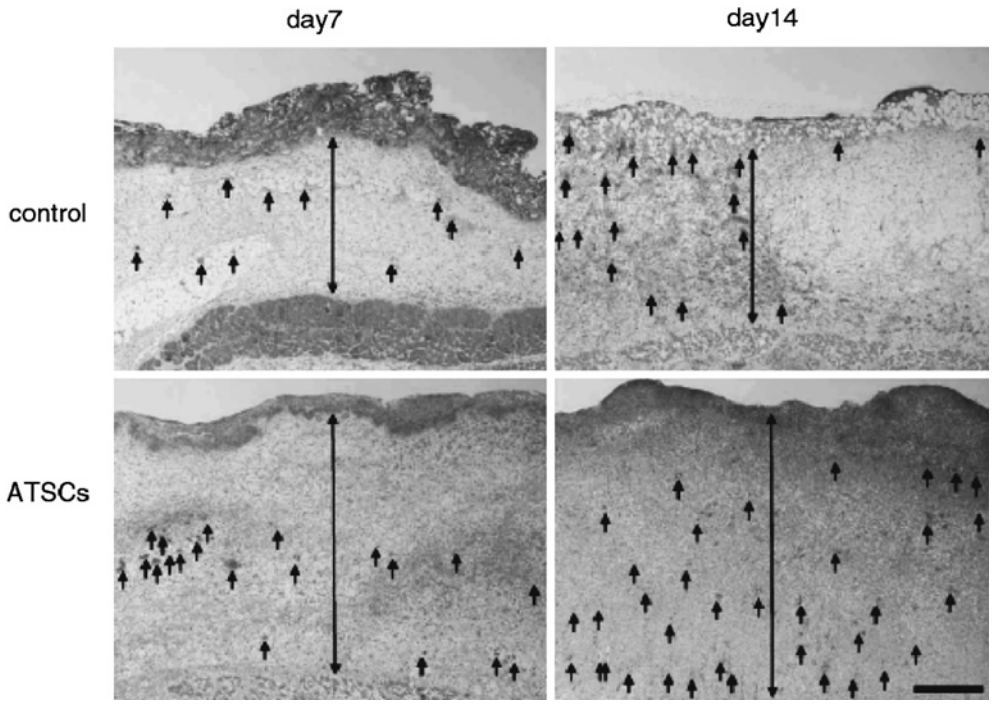

Fig. 4 Histological assessment of chemically mediated intractable ulcers treated with ASCs in conjunction with type I collagen sponge on days 7 and 14 after initial wounding. As compared with the control, ASC-treated groups showed greater numbers of mature vessels containing erythrocytes **(arrows)** and thickened granulation tissue. (Scale bar; 500 mm) (Reprinted from Nambu et al. *Would Repair Regen*. 15: 505, 2007)

conjunction with a type I collagen sponge, which increase both granulation thickness and capillary density when compared with a collagen sponge without ASCs **(Fig. 4)**. Further studies performed by the same research group showed that skin ulcers in diabetic mice were also repaired with autologous ASCs[62], indicating that ASCs can be used to treat skin ulcers, even in patients with diabetes.

Composite tissue regeneration by non-lineage-committed ASCs has also been confirmed. Periodontal tissue is composed of cementum (outer surface of the dentin), connected to the alveolar bone by periodontal ligaments, which lie perpendicular to the bone. Tobita et al. have clearly demonstrated that ASCs can promote periodontal tissue regeneration in a rat model[14]. Isolated ASCs, together with platelet-rich plasma obtained from inbred rats, were implanted into a periodontal tissue defect. The group observed that the regeneration of alveolar bone and cementum, and of a periodontal ligament-like structures 8 weeks after implantation and confirmed their results by histological and immunohistochemical analysis **(Fig. 5)**.

A variety of tissues and organs engineered with ASCs have been described in addition to those we have described above. These include cranial bone regeneration[63], articular chondrocyte regeneration[64], cardiac wall regeneration[65], the functional repair of myocardial infarction[66], and functional improvement of stroke[67].

Current Clinical Applications

On the basis of both *in vitro* experiments and pre-clinical studies, ASCs have been applied to various clinical fields. In the first clinical case, autologous ASCs were used for the regenerative treatment of widespread traumatic calvarial bone defects[68]. A 7-year-old girl with post-traumatic calvarial defects was treated with autologous cancellous iliac bone combined with autologous ASCs, fibrin glue and a biodegradable scaffold. Post-operative computed tomography showed new bone formation, and almost complete calvarial continuity was obtained.

The transfer of ASCs combined with free fat has been reported to play an important role in

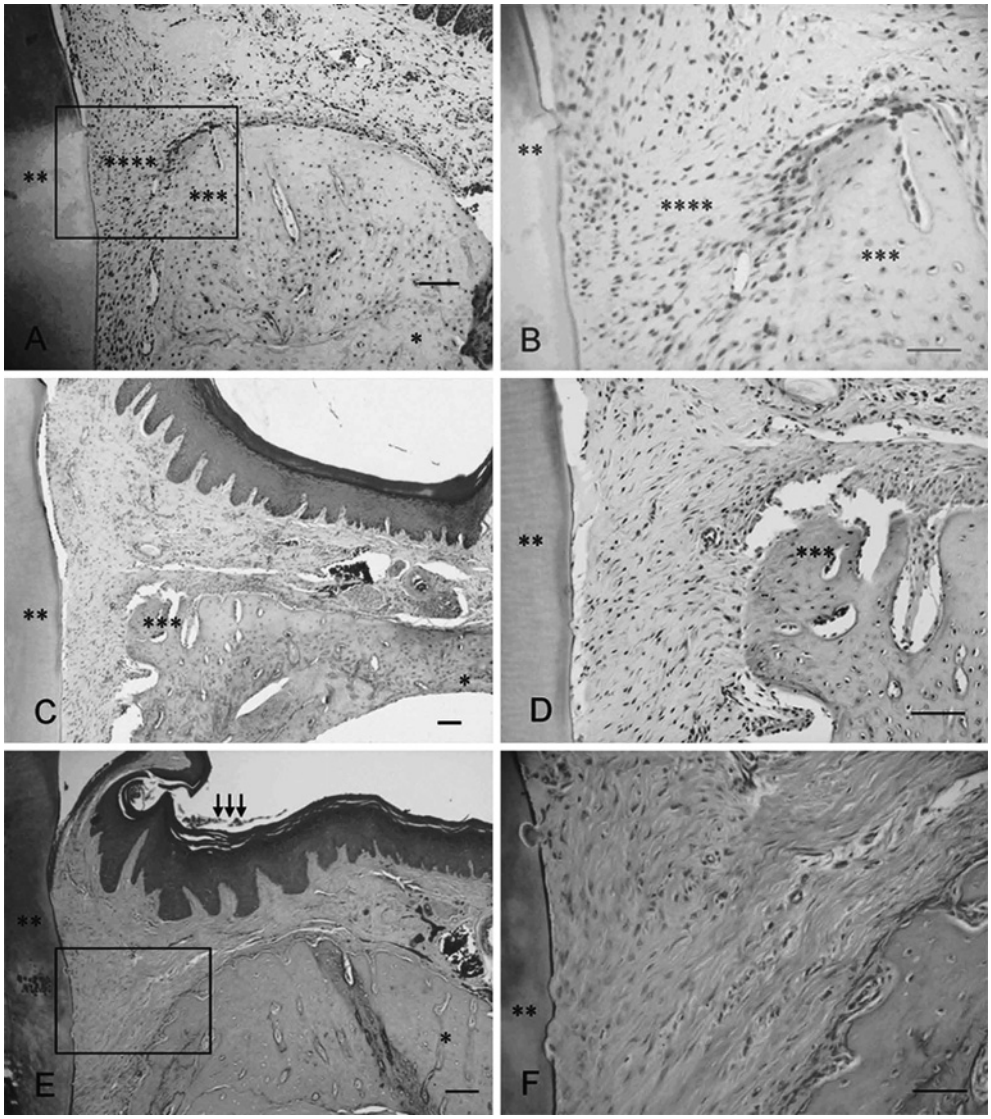

Fig. 5 Histological cross-section of the periodontal tissue defects 8 weeks after implantation of ASCs. **A**: In the ASC/platelet rich plasma (PRP) group, a cementum-like structure and alveolar bone with alveolar cristae had regenerated (scale bar: 100 mm). **B**: High magnification of the defects in the ASC/PRP group showed a periodontal ligament-like structure located perpendicularly between the cementum-like structure and the alveolar bone (scale bar: 50 mm). **C**: Alveolar bone regeneration was observed at the lowest level, although alveolar cristae were not seen in the PRP group (scale bar: 100 mm). **D**: A periodontal ligament-like structure was not evident between the alveolar bone and the dentin surface in the PRP group (scale bar: 100 mm). **E**: Little bone regeneration or alveolar cristae were noted in the no-implantation group. In addition, the volume of the gingiva was decreased **(arrow)** (scale bar: 100 mm). **F**: In the no-implantation group, dense collagen fibers and granulation tissue occupied the space between the dentin surface and alveolar bone (scale bar: 50 mm). * alveolar bone, ** dental root, *** regenerated bone, **** periodontal tissue-like structure. (Reprinted from Tobita et al. *Tissue Eng*. 14: 945, 2008)

maintaining the volume of the injected fat tissue[69]. Free fat injection, together with ASCs isolated from the equivalent liposuction aspirates, termed cell-assisted lipotransfer (CAL), could become an alternative to soft tissue augmentation surgery, including cosmetic breast augmentation[70].

Autologous ASC therapy could also be used to treat fistulas in patients with Crohn's disease. In a

pilot study of 5 patients with Crohn's disease, the external opening in 6 out of 8 fistulas could be closed by inoculation of the fistulas with autologous ASCs[71]. Since this report was published, ASCs have been also used to repair tracheomediastinal fistulas caused by cancer ablation[72].

The therapeutic potential of ASCs for wound healing, which has also been shown in our pre-clinical studies[11,62], can be anticipated for the treatment of chronic ulcers caused by radiation therapy[73]. Twenty patients being treated for the side effects of radiotherapy, and with severe symptoms or irreversible functional damage, received autologous ASCs delivered via repeated hypo-invasive computer-assisted injections. The clinical outcome was systematic improvement or remission of symptoms in all patients evaluated. Although the mechanism of therapy at the molecular level is unknown, this therapeutic approach may play a pivotal role in the treatment of intractable ulcer.

In addition, ASCs have *in vivo* immunosuppressive properties that can be used to control graft-versus-host disease (GVHD)[74]. On the basis of this findings, ASCs were administered intravenously to patients with steroid-refractory acute GVHD[75]. In this clinical trial, acute GVHD resolved completely in 5 out of 6 patients, 4 of whom were alive, without side effects, after a median follow-up period of 40 months.

Finally, clinical trials of ASCs for the treatment of both chronic heart failure and acute myocardial infarction have begun in Europe. Although results of these studies have not been published, ASCs as well as bone marrow-derived MSCs are a promising source of cell-based therapies for the treatment of cardiovascular diseases.

Future Directions

We have recently researched the alternative application of ASCs for tissue repair and healing. Our preliminary data indicate that: (1) local injection of ASCs into skin ulcers induces rapid healing with less scarring in a rat model; (2) topical application of ASCs with fibrin glue around the site of primary Achilles tendon repair in rabbits dramatically increases the tensile strength of the tendon by both direct differentiation of ASCs into tenocytes and by the indirect effect of the release of growth factors, such as VEGF; and (3) topical administration of ASCs around the site of primary sciatic nerve repair in rats improves the functional restoration of the sciatic nerve, a result that has been confirmed by gait analysis, electroneurography and histology. These therapeutic models may be applicable to clinical situations in which the local environment for wound healing is compromised by inadequate blood supply and severely scarred tissue.

Gimble et al. have shown that ASCs delivered into an injured or diseased tissue may secrete cytokines and growth factors that stimulate recovery in a paracrine manner[19]. ASCs modulate the "stem cell niche" of the host by stimulating the recruitment of endogenous stem cells to the site and promoting their differentiation along the required lineage pathway. In a similar way, ASCs could provide antioxidants, free radical scavengers, and chaperone/heat shock proteins at an ischemic site. As a result, toxic substances released into the local environment would be removed, thereby promoting recovery of the surviving cells[19].

The easily repeatable access to subcutaneous adipose tissue provides a clear advantage for the isolation of MSCs, and both the isolation and culture techniques are easier to perform than bone marrow isolation. For the present generation, excess subcutaneous fat around the "waist" is generally considered to be a "waste". However, as stated above, adipose tissue is now regarded to be a rich source of stem cells. Further pre-clinical and clinical studies need to be performed so that ASC-based therapies fulfill expectations and can be successfully used to treat disorders for which the present medical and surgical therapies are either ineffective or impractical.

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