15

Oral Tissues as Source for Bone Regeneration in Dental Implantology

Dilaware Khan[1], Claudia Kleinfeld[1], Martin Winter[2] and Edda Tobiasch[1]
[1]*University of Applied Sciences Bonn-Rhine-Sieg, Rheinbach*
[2]*Oralchirurgische Praxis, Rheinbach*
Germany

1. Introduction

One of the most common problems in Regenerative Medicine is the regeneration of damaged bone with the aim of repairing or replacing lost or damaged bone tissue by stimulating the natural regenerative process. Particularly in the fields of orthopedic, plastic, reconstructive, maxillofacial and craniofacial surgery there is need for successful methods to restore bone. From a regenerative point of view two different bone replacement problems can be distinguished: large bone defects and small bone defects. Currently, no perfect system exists for the treatment of large bone defects. Autologous bone material from the hip or the split calvarial graft is the gold standard to repair bone defects, as it has osteoinductive and osteoconductive properties (Tessier, 1982; Tessier et al., 2005a; Laurencin et al., 2006). Unfortunately this method is associated with an additional invasive intervention that leads to an increase risk of infection, pain during recovery, morbidity and frequent long periods of convalescence due to surgical trauma. Besides, only a limited amount of tissue can be obtained and harvested (Younger & Chapman, 1989; Tessier et al., 2005b). Also, the outcome is not always satisfactory after surgical treatment using bone splits (Baltzer et al., 2000; Lietman et al., 2000; Sorger et al., 2001). Heterologous transplants on the other hand, bear the risk of infection and rejection of the donor material. If the required amount of implant material cannot be obtained, another source is bovine-derived xenografts. There is, however, a potential risk for prion infection that cannot be totally avoided. Last not least large bone defect replacement needs nutrient and oxygen supply via blood vessels, so angiogenesis must be considered. This is very different in small bone defects: here angiogenesis is not an issue, but most of the other problems addressed above do play a role here too. This chapter will focus on small bone defects, especially those linked to dental implants.

2. Bone structure and regulation

The skeletal system is composed of bones that support the body, protect internal organs, and allow movement. Bone itself can be described as a natural composite material that consists of minerals and collagen that are merged in a complex amalgam. It consists mainly of two structures: an organic component as a matrix that contains collagen and a mineral component that is predominantly hydroxyapatite (Rho et al., 1997). The complex mineral substances give hardness to the bone and the softer organic collagen matrix causes visco-

elasticity and toughness (Hutmacher et al., 2007). Together with cartilage, connective tissue, nerves, blood vessels, and marrow, they constitute the bone.

In the mineralized organic bone matrix, living and dead cells are present. Three types are known to play a role in bone homeostasis: osteoblasts, osteocytes and osteoclasts.

Osteoblasts are derived from MSCs and are cuboidal in shape (Fig. 1). They contain prominent Golgi bodies with a well developed rough endoplasmic reticulum, which is a histological sign for prominent protein production. These cells are located on the endosteal and periosteal bone surfaces. They secrete collagen type I and the non-collagenous proteins of the organic bone matrix. These cells also synthesize the enzyme alkaline phosphatase (ALP) that regulates the mineralization of the bone matrix. Their lifetime is about three months, after which they become metabolically inactive, flattened bone lining cells (Fig. 1). Bone lining cells are found covering inactive bone surfaces where they serve as a barrier for certain ions. The osteocytes originate from metabolically inactive osteoblasts and become trapped within the newly formed bone matrix during bone formation. Osteocytes have reduced synthetic activity compared to osteoblasts but maintain their sensitivity to vitamin D while continuing to participate in calcium regulation. On the other hand osteoclasts are derived from the fusion of monocyte and macrophage lineages (Ash, 1980) (Fig. 1). They are multi-nucleated cells that resorb bone. Osteoblasts regulate the differentiation of osteoclasts and osteocytes, which secrete factors in a feedback loop that play a role in regulating the functions of osteoblasts (Hartmann, 2006) and osteoclasts (Seeman & Delmas, 2006). The formation and resorption of bone is a continuous process that is kept in balance by the regulation of these three types of cells, with emphasis on osteoblasts and osteoclasts.

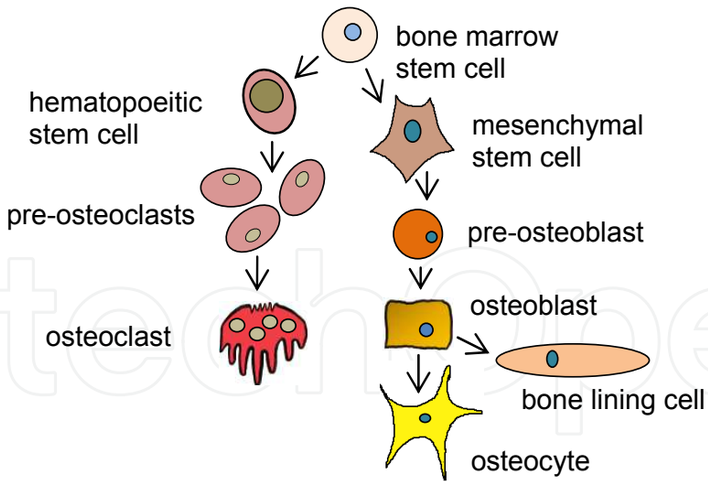


Fig. 1. Development of Bone Cells. Bone marrow stem cells give rise to hematopoietic stem cells and mesenchymal stem cells. Hematopoietic stem cells give rise to osteoclasts and mesenchymal stem cells are differentiated into osteoblasts together with other cell types. Osteoblasts further develop into bone lining cells and osteocytes.

In some diseases this balance is disrupted, as in osteoporosis, where increased osteoclast activity results in more resorption of bone than formation by osteoblasts. Along with osteoporosis, other medical conditions like bone cancer and osteogenesis imperfecta can

lead to weakness of bones that can result in fractures. Bone defects can also occur due to trauma after accidents (Schäffler & Büchler, 2007). In addition, changes in recreational behavior especially in young adults lead to more need for bone replacement. Also, improved conditions of public health, nutrition and medicine have increased the life expectancy that resulted in an enhanced need for dental replacement. Taken together there is a growing need for bone regeneration and replacement.

3. Bone regeneration and replacement

3.1 The need for bone regeneration in dental defects

Studies revealed that approximately 70 % of all adults between 35 and 44 years lost at least one permanent tooth and by the age of 74 around 26 % of the adults lost all their permanent teeth (National Institutes of Health, 2001). Additionally, 45 % of the adults between 35 and 44 years and 54 % of the seniors between 65 and 74 years suffered from a middle heavy periodontitis, which is connected with a higher risk of tooth-loss (Holtfreter et al., 2010). To overcome these problems dental implants are one of the most common features to realize oral prosthetic reconstruction.

In order to guarantee a long and successful osseointegration of dental implants, they should be circumferentially covered with bone. Furthermore, it seems advantageous that the intraosseous part of the fixture is longer than the extraosseous prosthetic part. At least, the length of the implant should not be shorter than the abutment. Nowadays correct implant placement is determined by esthetic and prosthetic aspects, which often cannot be realized when only the residually available bone (restoration-driven implant placement) is being used (Garber et al., 1995). (National Institutes of Health, 2001)

There are defects of the alveolar bone which occur as a result of trauma, inflammation, resective surgical intervention such as tumor resection, bone loss after periodontal disease or athrophia after tooth loss or agenesis. In the posterior maxilla the phenomenon of pneumatization of the sinus maxillaris increases after tooth loss, which results in a vertical compromized bone level (Fig. 2A). Thus, bone reconstruction before or simultaneously to implant placement is often necessary (Fig. 2B). To do so guided, bone regeneration with autologous material such as bone graft material or other autologous or artificial grafting

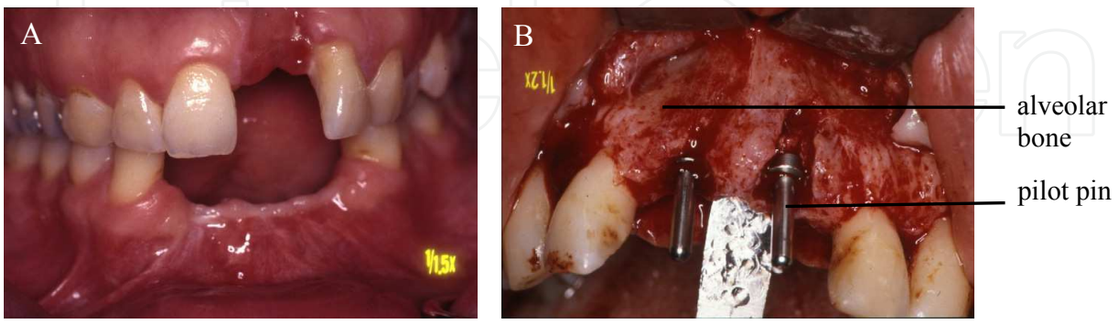


Fig. 2. A) Bone Degeneration. After tooth loss, reduced jawbone is as a result of trauma in a 24 years old male. B) Pilot pins *in situ* demonstrate the compromized bone-situation (male, 43 years old). Stable integration of implants is dependent on a thick jawbone. Stem cells could be used to fill the gaps and increase the thickness and induce osseointegration of implants.

procedures are methods of choice. Nevertheless, there exist many unsolved problems such as a e.g. higher morbidity in conjunction with the second wound of the donor site.

Therefore the use of stem cells (SCs) as source material for bone regeneration could represent an interesting approach for dental implantology.

3.2 Stem cells for bone regeneration

A modern strategy in Regenerative Medicine is the approach to combine living cells and scaffold material to establish a biological alternative for the diseased organ or tissue that can restore the functions. (Sittinger et al., 1996; Vacanti & Langer, 1999; Khademhosseini et al., 2009). Some degradable polymers, ceramics, or a combination of both can provide desirable mechanical and osteoconductive properties as basic scaffold material for bone replacement (Zippel et al., 2010b). Different factors should be considered for the use of such a biomaterial scaffold. It should imitate the three dimensional environment of the extracellular matrix, it should provide stability until replaced by regrown bone tissue and serve as an extended surface area for migration, adhesion, and differentiation of cells to encourage the growth of new tissue (Schultz et al., 2000; Ringe et al., 2002; Moroni et al., 2008).

The proliferating cells cover the scaffold and can grow into three dimensional tissue within. They are also an important factor for forming new tissue through extracellular matrix synthesis (Bonassar & Vacanti, 1998). Due to the development of new blood vessels towards, and to some extent onto, the new tissue, the scaffold begins to degenerate from the outside and is reconstituted by new natural bone tissue. As tissue related cell types cannot always be obtained in an adequate number or quality, SCs are a useful alternative for tissue regeneration.

Stem cells are the precursors of all cells and are involved in the repair system of the body. They are defined by three characteristics: self sustainability, self renewal and the potential of differentiation into different tissue types. For example adipocytes, astrocytes, chondroblasts, or osteoblasts come from mesenchymal stem cells (MSCs) (Pittenger et al., 1999; Pansky et al., 2007). In several publications, it has been suggested that MSCs can differentiate towards lineages that are naturally derived from the endoderm (Zuk et al., 2002; Tobiasch, 2009). Thus, increasing their potential because of these properties, the use of SCs to heal or rebuild damaged organs may provide an approach in future Regenerative Medicine (Zippel et al., 2010a).

SCs have been isolated from embryonic sources and well developed tissues of adult organism such as bone marrow, skin, dental pulp and adipose tissue (Kern et al., 2006). In addition two other sources for SCs have been discovered: cancer stem cells and induced pluripotent stem cells (iPS) (Takahashi et al., 2007; Aoi et al., 2008). Since the higher potency of embryonic stem cells and iPS compared to adult stem cells goes together with a higher risk of tumor formation, and embryonic stem cells are ethically problematic. Therefore, adult stem cells present themselves as an interesting cell source for bone replacement.

Adult stem cells can be divided into two main subpopulations: hematopoietic and mesenchymal stem cells (MSCs). Hematopoietic stem cells derived from bone marrow have been investigated best and could be a source for osteoclasts (Ash, 1980) (see Fig. 1). MSCs have been found in umbilical cord blood, bone marrow, and adipose tissue among others (Zuk et al., 2002). Generally, the isolation of MSCs is accomplished by plastic adherence resulting in colonies that are heterogeneous in size and morphology might contain

contaminating non-mesenchymal cells such as macrophages or fibroblasts. The purity of isolated MSCs can be investigated by using the surface markers: CD73, CD90, and CD105 (should be expressed) and CD14, CD34 and CD45 (should not be expressed). These markers serve next to the adherence to plastic as a second feature for the identification and characterization of MSCs as suggested by the 'International Society for Cellular Therapy' (Dominici et al., 2006).

Another group of adult stem cells that has attracted attention are the ectomesenchymal stem cells derived from oral tissues. This stem cell group includes the dental pulp stem cells (DPSCs) and stem cells of human exfoliated deciduous teeth (SHEDs), both deriving from the pulpa, dental periodontal ligament stem cells (DPLSCs), dental follicle cells (DFCs), and stem cells from the apical papilla (SCAPs) (see Fig. 3). These cell types have the potential to differentiate into cells of all dental tissue types and bone as well. They share common phenotypic markers of MSCs (Alipur et al., 2010).

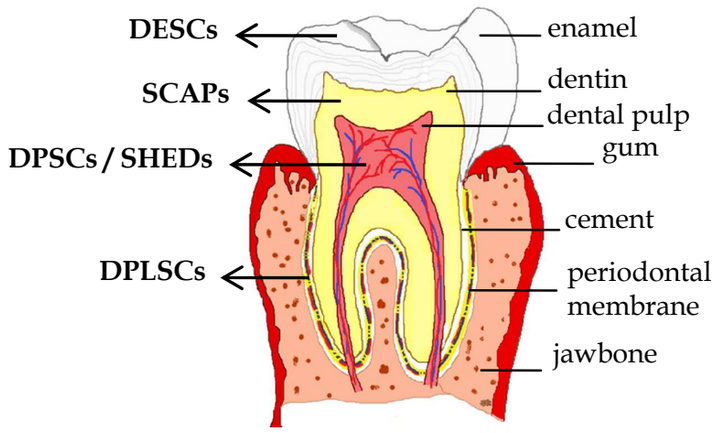


Fig. 3. Stem Cell Types in Tooth. From tooth different stem cell types namely, dental pulp stem cells (DPSCs), stem cells of human exfoliated deciduous teeth (SHEDs), dental periodontal ligament stem cells (DPLSCs), dental enamel derived stem cells (DESCs), stem cells from the apical papilla (SCAPs) and dental follicle cells (DFCs) can be obtained.

In comparison to other dental sources, dental follicle cells (DFCs) can be easily obtained in high amounts from young and healthy donors, since they are isolated from tooth extraction material collected during surgical removal of wisdom teeth. As these cells are derived from young donors, long telomeres extend their lifespan which makes them interesting cells for Regenerative Medicine (Shay & Wright, 2010). The dental follicle develops from ectomesenchyme. It surrounds the developing tooth germ before eruption (Ten Cate, 1997; Wise, 2002). During embryonic development, the ectomesenchyme is partly derived from migrating cells of the cranial neural crest. Therefore, the cells derivative from dental follicle differ from mesenchymal stem cells isolated from other sources (Chung et al., 2004; Slootweg, 2009). Due to having the more ectodermal character, these cells can have a differentiation potential diverse from MSCs. As expected these cells can differentiate into hard tissue such as the periodontal, cementoblastic, chondrocytic, and osteogenic lineages.

ATSCs and DFCs, both show osteogenic differentiation potential and are thus suitable candidates for the use in bone regeneration for stable osseointegration of dental implants. As these cells are obtained from healthy individuals, they might be used as an autograft in

the future. The transplantation not only of autologous but also of allogenic sources could provide benefits in comparison to other common procedures in bone regeneration. As MSCs have low immune characteristics, they appear to be suitable for allogenic therapeutic purposes, without activating the immune response in immunocompetent patients (Jung et al., 2009). In different studies the use of MSCs has been investigated to replace lost or damaged bone (Schaefer et al., 2000; Ringe et al., 2002). After tooth loss, jawbone degenerates and stable integration of dental implant needs a thick jawbone. To overcome this problem there are two different alternatives that can be considered for using SCs in dental implants. The reconstruction after bone defects with SCs to achieve a sufficient bone thickness to insert the implants and the loading of an implant or artificial tooth-root with SCs with the aim to realize a sufficient integration in the bone.

SCs have the capability to re-establish cell function, reverse cellular damage, and heal damaged tissue (Conrad and Huss, 2005). SCs could also be a source to regenerate human teeth in the future, as these cells have been successfully used to regenerate living teeth in rabbit extraction sockets (Hung et al., 2011). In some mammals like rodents, rabbits, prairie dogs, and pikas, the teeth can grow throughout life because in these mammals as the pulp cavity remains open permanently. While on the other hand in humans tooth cannot grow continuously as pulp cavity closes when the teeth are fully grown. Therefore this study cannot be adapted easily for the regeneration of teeth or teeth related tissues in humans but it at least provides interesting basic results that can be helpful for use of SCs in dental tissues.

3.3 Bone chips for the stabilization of dental implants

Another approach next to scaffold loaded with stem cells to overcome the problem of unstable dental implants is the use of particulated non-vascularized bone autografts. The particles can be collected during the implant-bed preparation in the process of drilling the hole for the implant into the bone. An advantage of the use of these bone chips is that this material can be expected to facilitate bone regeneration. However, contradictory statements were made about the quality of this material such as if it contains living cells. In addition, it is not clear how to disinfect the bone chips, which are contaminated with bacteria of the oral cavity due to the sampling process. To address these questions bone chips were collected from two different regions of bone: carticular bone and spongy bone (see Fig. 4).

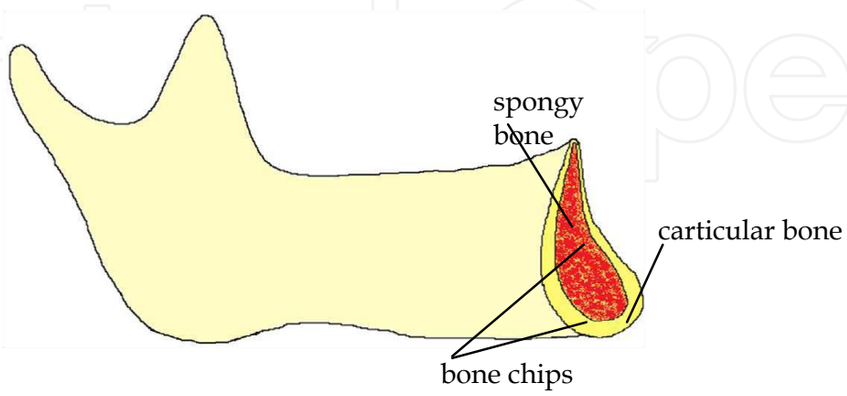


Fig. 4. Schematic Structure of Lower Jaw. Bone is composed of two tissue types mainly: spongious and carticular bone. Bone chips obtained during dental surgery for implant-bed preparation is derived from both bone tissue types.

4. Research methods

4.1 Isolation of primary cells for osteo-differentiation

4.1.1 Isolation of cells from tooth extraction material

For the isolation of ectomesenchymal stem cells, dental follicles were collected from human third molars before tooth eruption after surgical removal. The dental follicles were washed three times with 1 x PBS. Afterwards, the dental follicles were separated from the mineralized tooth and minced with a scalpel under sterile conditions. The tissue was digested in Collagenase (0.1 U / mL) and Dispase (0.8 U / mL) for 2 h at 37 °C in humidified atmosphere with 5 % CO_2. The cells were passed through a 100 µm strainer to obtain single-cell suspensions and seeded in 10 cm dishes in stem cell medium (SCM) that consisted of DMEM supplemented with 10 % FCS, 2 mM L-glutamine, 100 units / mL penicillin, 100 mg / mL streptomycin and 1 % amphotericin and cultured at 37 °C in a humidified atmosphere with 5 % CO_2. After 24 hours, non-adherent cells were removed by washing with 1 x PBS. The medium was changed and the plastic adherent cell fraction was cultured until 80 % confluent for further use.

The bone chip particles were collected with a bone filter integrated into a surgical suction pipe during the implant-bed preparation to isolate primary cells. For the isolation of bone chip derived cells (BCDCs), the same procedure as described above for DFCs was followed.

4.1.2 Isolation of adipose tissue derived stem cells

Human adipose tissue derived stem cells (ATSCs) were isolated from lipoaspirate obtained from plastic surgery. The isolation technique used during surgery was the tumescent liposuction technique. Using this particular technique, diluted epinephrine and lidocaine is infiltrated into the body fat to be removed, which leads to swelling and firmness of the targeted region, providing more accuracy during the liposuction procedure. The protocol was adjusted and modified to the procedure described by Zuk and colleagues (Zuk et al., 2001). The obtained lipoaspirate was augmented with PBS in a 1:2 ratio. After incubation for 30 minutes at room temperature (RT), two phases, a lower aqueous and upper fat phase of the lipoaspirate were obtained.

The lower phase was centrifuged at 200 x g for 10 minutes at RT. The resulting pellets, comprising the cells, were pooled and washed with 1 x PBS. Remaining erythrocytes were removed by applying 10 mL erythrolysis buffer for 10 minutes at RT. After another centrifugation step, under the same conditions as mentioned before, the cells were cultured in 60 cm^2 culture plates in SCM medium.

The upper phase comprising the fat tissue was augmented with 10 mg / mL type I collagenase in 1 x PBS and incubated for 45 minutes at 37 °C with agitation. The following steps for the treatment of the upper phase were according to the treatment of the lower phase. Cells of both phases were incubated at 37 °C with 5 % CO_2 in a humidified atmosphere. ATSCs were isolated due to their adherence to plastic and purified by washing with 1 x PBS after 24 hours, to remove undesired non-adherent cells.

4.2 Fluorescence activated cell sorting

The percentages of ATSCs or DFCs positive for the mesenchymal stem cell markers CD44, CD90 and CD105 and negative for CD14, CD45 and CD34 were measured using FACS analysis. The stem cells were trypsinized, centrifuged at 200 x g for 5 min and counted. 1 x 10^6 cells were resuspended in 1 mL 0.1 % PBSB and passed through a 100 µM cell strainer to obtain a single cell solution. 100 µL of the cell solution (100.000 cells) were incubated for 20 min in the dark with either the isotype control or the antibodies. Cells were washed with 2 mL 0.1 % (w / v) PBSB, centrifuged at 200 x g for 5 min and resuspended in 1 mL 0.1 % (w / v) PBSB. The cytometer settings and cell gates were adjusted to the isotype control, followed by measurement of the stem cell markers using the same conditions.

4.3 Adipogenic differentiation

For adipogenic induction, the isolated cells were seeded in a density of 2.8 x 10^3 cells / cm^2 in SCM. After one day, the medium was changed to adipogenic differentiation medium (AM), containing 1 µM dexamethasone, 1 µM insulin and 200 µM indomethacin. The cells were grown in AM for four weeks at 37 °C with 5 % CO_2 under humidified conditions. The AM was changed once a week. After four weeks, adipogenic differentiation was visualized with Oil Red O after fixing cells for 90 min with formalin (4 %) at 37 °C.

4.4 Osteogenic differentiation

The isolated cells were seeded in a density of 1.3 x 10^3 cells / cm^2 in 6 cm^2 and 12 well plates for osteogenic differentiation. After one day SCM was replaced with osteogenic medium (OM) containing dexamethasone, ascorbic acid and β-glycerophosphate. ATSCs were grown in OM for 4 weeks at 37 °C with 5 % CO_2 under humidified conditions. The OM medium was changed once a week. After four weeks, osteogenic differentiation was visualized by staining with Alizarin Red S after fixing cells for 5 min with formalin (4 %) at 37 °C.

4.5 Microbiological testing

Directly after surgery the obtained dental follicles were transferred into cold sodium chloride (0.9 % w / v) for determining possible microbial contaminations. The samples were kept cold until processing.

The samples were rolled over the surface of Columbia blood agar (CBA) and fastidious anaerobe agar (FAA) plates to isolate microorganisms. In addition the transport solution was put onto CBA and FAA plates. Under aerobic and anaerobic conditions the incubations were conducted over night at 37 °C. The Gas PakTM100-system was used for the incubation under anaerobic conditions. Single colonies were picked and isolated with respect to their morphological differences. Gram stainings, catalase- and oxidase-tests were used for the first characterization. API test strips were used to determine the exact bacteria species.

5. Comparison of stem cell sources

5.1 The characterization of ATSCs, DFCs and BCDCs for bone regeneration

The high plasticity of mesenchymal stem cells has resulted in an increased interest for their use in a variety of cellular therapies. However, different laboratories working with

these cells isolate them from various tissue sources by following different protocols and characterizing these cells by different markers. Therefore, to set a standard, the minimal criteria for the definition of human MSCs were suggested by the 'Mesenchymal and Tissue Stem Cell Committee of The International Society for Cellular Therapy' (Dominici et al., 2006). The multipotent character of the isolated adipose tissue derived stem cells, ectomesenchymal dental follicle cells and bone chip derived cells was tested according to these criteria. MSCs were isolated from human adult adipose tissue of different aged female donors. DFCs were isolated from dental follicles and BCDCs from the bone chips collected during implant-bed preparation of male and female donors. The enrichment of specific stem cells was achieved due to their property of plastic adherence that is the first criterion for the testing of aMSCs character (Dominici et al., 2006). Isolated mesenchymal and ectomesenchymal cells of all donors showed a morphology similar to fibroblasts, which is typical for these stem cells (Yoshimura et al., 2006).

According to the above mentioned criteria the isolated cells should express the stem cell specific surface markers CD73, CD90, and CD105, and should not express CD14, CD34, and CD45. All isolated SC types expressed the expected markers (CD73, CD90 and CD105) as assessed by RT-PCR. The mesenchymal character of ATSCs and DFCs was also confirmed using FACS analysis for the presence of CD90, CD105, and in addition CD44. Furthermore, the cell types ATSCs and DFCs did not show the expression of leukocyte marker CD45 and macrophage marker CD14. ATSCs were positive and DFCs were negative for CD34. The presence of the expression of CD34 on ATSCs is controversial discussed. Some studies confirm the absence of CD34 expression on ATSCs (Zuk et al., 2002; Lee et al., 2004; Wagner et al., 2005) while other investigations showed ATSCs expressing CD34 (Mitchell et al., 2006; Yoshimura et al., 2006; De Francesco et al., 2009). These differences could be due to different stem cell isolation protocols, passage number or a different gating strategy during FACS analysis. In this study a subpopulation of ATSCs was stained positive for CD34.

Another typical MSCs character is the multilineage differentiation potential towards various lineages such as adipocytes, chondroblasts and osteoblasts. ATSCs showed a strong adipogenic differentiation potential whereas DFCs and BCDCs could not differentiate towards adipocytes. However, Kémoun and colleagues reported DFCs to differentiate towards the adipogenic lineage (Kémoun et al., 2007). The differences during isolation and precipitation in cell population might be possible reasons for this discrepancy. Also, DFCs can be different in their potency because these cells are derived from ectomesenchyme that is more committed toward hard tissue as tooth enamel.

According to all the findings mentioned above, the isolated ATSCs can be considered to belong to the population of multipotent MSCs, whereas the DFCs and BCDCs have a limited differentiation potential. Haddouti and colleagues showed that DFCs have a strong commitment towards the osteogenic lineage and show a more quantitative osteogenic differentiation (Haddouti et al., 2009). Thus, DFCs and BCDCs seem to be more committed towards osteogenic lineage.

Taken together all these stem cell types are good candidates for bone regeneration. But material from the oral cavity for isolation of primary cells such as DFCs and BCDCs cannot be obtained without microbial contamination. The question arises if this is a draw back on the use of these stem cells.

5.2 Microbial load of the oral tissue material

In order to evaluate the quality of the cells derived from oral tissues, microbiological investigations were performed. Our results revealed that all samples contained microbial species. Pre-treatment of patients with the antibiotics chlorhexidine (0.2 %), which is done anyway to decrease the chances of inflammation after surgery, reduced the number of microorganisms to less than 5 % but did not suffice to eliminate all bacteria. On the other hand pre-surgical, antibiotic treatment seemed to be negative for cell-outgrowth. To reduce contamination of the harvested cell-material, an optimized surgical procedure is more important than pre-surgical irrigation with chlorhexidine (0.2 %), and the use of a stringent dual suction pipe procedure. The predominantly found species were gram-positive cocci being either catalase-positive and oxidase-negative or catalase- and oxidase-negative. Most microorganisms belonged to the families of *Streptococcaceae* and *Staphylococcaceae*. The detected microorganisms did not interfere with cell growth and differentiation. They can be easily suppressed with standard antibiotics, applied routinely in patient treatment during the implantation procedure. Thus, these stem cells can be used for bone regeneration in dental implants.

6. Conclusion

The stability of dental implants is associated with a successful osseointegration into thick jawbone. Due to bone defects, bone regeneration is often needed before an implant can be inserted. For this stem cells can be a suitable candidates.

The stem cells isolated from adipose tissue, dental follicle and bone chips share mainly the multipotent character of mesenchymal stem cells. ATSCs can be successfully differentiated towards adipogenic and osteogenic lineages while DFCs and BCDCs did not show adipogenic differentiation. However, these cell types showed stronger commitment and differentiation towards osteogenic lineage. Therefore all three cell types are promising candidates for the treatment of various bone defects, and therefore also for the incorporation of tooth implants. They can be used to reconstruct jawbone defects to achieve enough bone thickness for the insertion of dental implants. It might be possible to load these cells on a dental implant or an artificial tooth root to increase its integration stability with the bone.

DFCs might be an ideal option if there will be a bank of donor material for these cells in the future, similar to those banks already existing as umbilical cord blood stem cells. If DFCs and BCDCs are not available for a specific patient, ATSCs are a reasonable option as they can differentiate towards the osteogenic lineage and be obtained from the patient itself as well, reducing the risk for rejection. Taken together all these tested stem cell types are suitable to improve the conditions for dental implants. Patients could preserve their dental follicle cells for later use in the future or their stem cells could be isolated from fat tissue directly before use. If a stem cell bank is arranged in the future, stem cells from other stem cell donors for dental follicle and fat tissue derived SCs could be used.

7. Acknowledgements

This work was supported by the BMBF-AIF, AdiPaD; FKZ: 1720X06 for ET and the Higher Education Commission of Pakistan, DAAD Germany for DK.

8. List of abbreviation

ALP	Alkaline phosphatase
AM	Adipogenic medium
ATSCs	Human adipose tissue derived mesenchymal stem cells
BCDCs	Bone chip derived cells
BMP2	Bone morphogenetic protien 2
°C	Degree centigrade
CBA	Columbia blood agar
CD14	Cluster of differentiation 14
CD34	Cluster of differentiation 34
CD45	Cluster of differentiation 45
CD73	Cluster of differentiation 73
CD90	Cluster of differentiation 90
CD105	Cluster of differentiation 105
cm	Centimeter
CO_2	Carbon dioxide
DESCs	Dental enamel derived stem cells
DFCs	Dental follicle cells
DMEM	Dulbecco's modified Eagle medium
DPLSCs	Dental periodontal ligament stem cells
DPSCs	Dental pulp stem cells
ECSs	Embryonic stem cells
FAA	Fastidious anaerobe agar
FACs	Fluorescence activated cell sorting
FCS	Fetal calf serum
iPS	Induced pluripotent stem cells
IGF-1	Insulin-like growth factor 1
LPL	Lipoprotein lipase
mL	Milliliter
mM	Millimolar
µL	Microliter
OM	Osteogenic medium
PBS	Phosphate buffer saline
PPARγ	Peroxisome proliferator-activated receptor gamma
RT	Room temperature
RT-PCR	Reverse transcriptase polymerase chain reaction
Runx2	Runt-related transcription factor 2
SCAPs	Stem cells from the apical papilla
SCM	Stem cell medium
SCs	Stem cells
SHEDs	Stem cells of human exfoliated deciduous teeth
w / v	Weight per volume
x g	Relative centrifugal force

9. References

Alipur, R., Sadeghi, F., Hashemi-Beni, B., Zarkesh-Esfahani, S.H., Heydari, F., Mousavi, S.B., Adib, M., Narimani, M., & Esmaeili, N. (2010). Phenotypic characterizations and

comparison of adult dental stem cells with adipose-derived stem cells. Int. J. Prev. Med, 1, 164-171

Aoi, T., Yae, K., Nakagawa, M., Ichisaka, T., Okita, K., Takahashi, K., Chiba, T. & Yamanaka, S. (2008). Generation of pluripotent stem cells from adult mouse liver and stomach cells. Science, 321, 699-702

Ash, P., Loutit, J.F. & Townsend, K.M. (1980). Osteoclasts derived from haematopoietic stem cells. Nature, 283, 669-670

Baltzer, A.W., Lattermann, C., Whalen, J.D., Wooley, P., Weiss, K., Grimm, M., Ghivizzani, S.C., Robbins, P.D., & Evans, C.H. (2000). Genetic enhancement of fracture repair: healing of an experimental segmental defect by adenoviral transfer of the BMP-2 gene. Gene Ther, 7, 734-739

Bonassar, L.J. & Vacanti, C.A. (1998). Tissue engineering: The first decade and beyond. J. Cell. Biochem, 72, 297-303

Chung, U.I., Kawaguchi, H., Takato, T. & Nakamura, K. (2004). Distinct osteogenic mechanisms of bones of distinct origins. J. Orthop. Sci, 9, 410-414

Conrad, C. & Huss, R. (2005). Adult stem cell lines in regenerative medicine and reconstructive surgery. J. Surg. Res, 124, 201-208

De Francesco, F., Tirino, V., Desiderio, V., Ferraro, G., D'Andrea, F., Giuliano, M., Libondi, G., Pirozzi, G., De Rosa, A. & Papaccio, G. (2009). Human CD34+/CD90+ ASCs are capable of growing as sphere clusters, producing high levels of VEGF and forming capillaries. PLoS One, 4, e6537

Diagnosis and management of dental caries throughout Life. Consensus development conference statement, national institutes of health 2001

Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F., Krause, D., Deans, R., Keating, A., Prockop, D. & Horwitz, E. (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy, 8, 315-317

Garber, D.A. & Belser, U. (1995). Restoration-driven implant placement with restoration-generated site development. Compend. Contin. Educ. Dent, 16, 796-804

Haddouti, E.-M., Skroch, M., Zippel, N., Müller, C., Birova, B., Pansky, A., Kleinfeld, C., Winter, M. & Tobiasch, E. (2009). Human Dental Follicle Precursor Cells of Wisdom Teeth: Isolation and Differentiation towards Osteoblasts for Implants with and without Scaffolds. Mater. Sci. Engin. Technol, 40, 732-737

Hartmann, C. (2006). A Wnt canon orchestrating osteoblastogenesis. Trends. Cell. Biol, 16, 151-158

Holtfreter, B., Kocher, T., Hoffmann, T., Desvarieux, M., & Michaelis, W. (2010). Prevalence of periodontal disease and treatment demands based on a German dental survey (DMS IV). J. Clin. Periodontol, 37, 211-219

Hung, C.N., Mar, K., Chang, H.C., Chiang, Y.L., Hu, H.Y., Lai, C.C., Chu, R.M. & Ma, C.M. (2011). A comparison between adipose tissue and dental pulp as sources of MSCs for tooth regeneration. Biomaterial, 32, 6995-7005

Hutmacher, D.W., Schantz, J.T., Lam, C.X., Tan, K.C. & Lim, T.C. (2007). State of the art and future directions of scaffold-based bone engineering from a biomaterials perspective. J. Tissue. Eng. Regen. Med, 1, 245-260

Jung, D.I., Ha, J., Kang, B.T., Kim, J.W., Quan, F.S., Lee, J.H., Woo, E.J. & Park, H.M. (2009). A comparison of autologous and allogenic bone marrowderived mesenchymal stem cell transplantation in canine spinal cord injury. J. Neurol. Sci, 285, 67-77

Kémoun, P., Laurencin-Dalicieux, S., Rue, J., Farges, J.C., Gennero, I., Conte-Auriol, F., Briand-Mesange, F., Gadelorge, M., Arzate, H., Narayanan, A.S., Brunel, G. & Salles, J.P. (2007). Human dental follicle cells acquire cementoblast features under stimulation by BMP-2/-7 and enamel matrix derivatives (EMD) in vitro. Cell. Tissue. Res, 329, 283-294

Kern, S., Eichler, H., Stoeve, J., Kluter, H. & Bieback, K. (2006). Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells, 24, 1294-1301

Khademhosseini, A., Vacanti, J.P. & Langer, R. (2009). Progress in tissue engineering. Sci. Am, 300, 64-71

Laurencin, C., Khan, Y. & El-Amin, S.F. (2006). Bone graft substitutes. Expert. Rev. Med. Devices, 3, 49-57

Lee, R.H., Kim, B., Choi, I., Kim, H., Choi, H.S., Suh, K., Bae, Y.C. & Jung, J.S. (2004). Characterization and expression analysis of mesenchymal stem cells from human bone marrow and adipose tissue. Cell. Physiol. Biochem, 14, 311-324

Lietman, S.A., Tomford, W.W., Gebhardt, M.C., Springfield, D.S. & Mankin, H.J. (2000). Complications of irradiated allografts in orthopaedic tumor surgery. Clin. Orthop. Relat. Res, 375, 214-217

Mitchell, J.B., McIntosh, K., Zvonic, S., Garrett, S., Floyd, Z.E., Kloster, A., Di Halvorsen, Y., Storms, R.W., Goh, B., Kilroy, G., Wu, X. & Gimble, J.M. (2006). Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers. Stem Cells, 24, 376-385

Moroni, L., Wijn, J. & Van Blitterswijk, A. (2008). Integrating novel technologies to fabricate smart scaffolds. J. Biomater. Sci. Polymer Edn, 19, 543-572

Pansky, A., Roitzheim, B & Tobiasch, E. (2007). Differentiation potential of adult human mesenchymal stem cells. Clin Lab, 53, 81-84

Pittenger, M., Mackay, A., Beck, S., Jaiswal, R., Douglas, R., Mosca, J., Moorman, M., Simonetti, D., Craig, S. & Marshak, D. (1999). Multilineage potential of adult human mesenchymal stem cells. Science, 284, 143-147

Rho, J., Tsui, T. & Pharr, G. (1997). Elastic properties of human cortical and trabecular lamellar bone measured by nanoindentation. Biomaterials, 18, 1325- 1330

Ringe, J., Kaps, C., Burmester, G.-R. & Sittinger, M. (2002). Stem cells for regenerative medicine: advances in the engineering of tissues and organs. Naturwissenschaften, 89, 338-351

Seeman, E & Delmas, P.D. (2006). Bone quality—the material and structural basis of bone strength and fragility. N. Engl. J. Med, 354, 2250-2261

Schaefer, D., Klemt, C., Zhang, X., & Stark, G. (2000). Tissue engineering with mesenchymal stem cells for cartilage and bone regeneration. Chirurg, 71, 1001-1008

Schäffler, A. & Büchler, C. (2007). Concise review: adipose tissue-derived stromal cells--basic and clinical implications for novel cell-based therapies. Stem Cells, 25, 818-827

Schultz, O., Sittinger, M., Haeupl, T. & Burmester, G.R. (2000). Emerging strategies of bone and joint repair. Arthritis. Res, 2, 433 – 436

Sengupta, A. & Cancelas, J.A. (2010). Cancer stem cells: A stride towards cancer cure? J. Cell. Physiol, 225, 7-14

Shay, J.W. & Wright, W.E. (2010). Telomeres and telomerase in normal and cancer stem cells. FEBS Lett, 584, 3819-3825

Sittinger, M., Bujia, J., Rotter, N., Reitzel, D., Minuth, W.W. & Burmester, G.R. (1996). Tissue engineering and autologous transplant formation: practical approaches with resorbable biomaterials and new cell culture techniques. Biomaterials, 17, 237-242

Slootweg, P.J. (2009). Lesions of the jaws. Histopathology, 54, 401-418

Sorger, J.I., Hornicek, F.J., Zavatta, M., Menzner, J.P., Gebhardt, M.C., Tomford, W.W. & Mankin, H.J. (2001). Allograft fractures revisited. Clin. Orthop. Relat. Res, 382, 66-74

Takahashi, K., Okita, K., Nakagawa, M. & Yamanaka, S. (2007). Induction of pluripotent stem cells from fibroblast cultures. Nat. Protoc, 2, 3081-3089

Ten Cate, A.R. (1997). The development of the periodontium - a largely ectomesenchymally derived unit. Periodontology, 13, 9-19

Tessier, P. (1982). Autogenous bone grafts taken from the calvarium for facial and cranial applications. Clin. Plast. Surg, 9, 531-538

Tessier, P., Kawamoto, H., Matthews, D., Posnick, J., Raulo, Y., Tulasne, J.F. & Wolfe, S.A. (2005a). Autogenous bone grafts and bone substitutes--tools and techniques: I. A 20,000-case experience in maxillofacial and craniofacial surgery. Plast. Reconstr. Surg, 116, 6-24

Tessier, P., Kawamoto, H., Posnick, J., Raulo, Y., Tulasne, J.F. & Wolfe, S.A. (2005b). Complications of harvesting autogenous bone grafts: a group experience of 20,000 cases. Plast. Reconstr. Surg, 116, 72-73

Tobiasch, E. (2009) Adult human mesenchymal stem cells as source for future tissue engineering, Forschungsspitzen und Spitzenforschung. Zacharias, C., Horst, KW Ter., Witt, K.-U., Sommer, V., Ant, M. Essmann, U and Mülheim, L. 329-338, Spring Verlag, ISBN: 978-3-7908-2126-0

Vacanti, J. & Langer, R. (1999). Tissue engineering: the design and fabrication of living replacement devices for surgical reconstruction and transplantation. Lancet, 354, I32-34

Wagner, W., Wein, F., Seckinger, A., Frankhauser, M., Wirkner, U., Krause, U., Blake, J., Schwager, C., Eckstein, V., Ansorge, W. & Ho, A.D. (2005). Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. Exp. Hematol, 33, 1402-1416

Wise, G.E., Frazier-Bowers, S. & D'Souza, R.N. (2002). Cellular, molecular, and genetic determinants of tooth eruption. Crit. Rev. Oral. Biol. Med, 13, 323-334

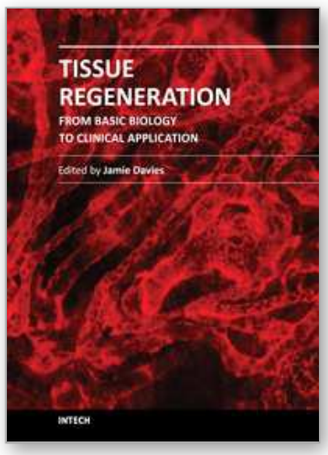
Yoshimura, K., Shigeura, T., Matsumoto, D., Sato, T., Takaki, Y., Aiba-Kojima, E., Sato, K., Inoue, K., Nagase, T., Koshima, I. & Gonda, K. (2006). Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates. J. Cell. Physiol, 208, 64-76

Younger, E.M. & Chapman, M.W. (1989). Morbidity at bone graft donor sites. J. Orthop. Trauma, 3, 192-195

Zippel, N., Limbach C.A., Ratajski, N., Urban, C., Luparello, C., Pansky, A., Kassack, M.U., & Tobiasch, E. (2010a) Purinergic Receptors Influence the Differentiation of Human Mesenchymal Stem Cells," Stem cells & Development. Epub ahead of print doi:10.1089/scd.2010.0576.

Zippel, N., Schulze, M. & Tobiasch, E. (2010b). Biomaterials and mesenchymal stem cells for regenerative medicine. Recent. Pat. Biotechnol, 4, 1-22

Zuk, P., Zhu, M., Ashjian, P., De Ugarte, D., Huang, J., Mizuno, H., Alfonso, Z., Fraser, J., Benhaim, P. & Hedrick, M. (2002). Human adipose tissue is a source of multipotent stem cells. Mol. Biol. Cell, 13, 4279-4295



Tissue Regeneration - From Basic Biology to Clinical Application
Edited by Prof. Jamie Davies

ISBN 978-953-51-0387-5
Hard cover, 512 pages
Publisher InTech
Published online 30, March, 2012
Published in print edition March, 2012

When most types of human tissue are damaged, they repair themselves by forming a scar - a mechanically strong 'patch' that restores structural integrity to the tissue without restoring physiological function. Much better, for a patient, would be like-for-like replacement of damaged tissue with something functionally equivalent: there is currently an intense international research effort focused on this goal. This timely book addresses key topics in tissue regeneration in a sequence of linked chapters, each written by world experts; understanding normal healing; sources of, and methods of using, stem cells; construction and use of scaffolds; and modelling and assessment of regeneration. The book is intended for an audience consisting of advanced students, and research and medical professionals.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Dilaware Khan, Claudia Kleinfeld, Martin Winter and Edda Tobiasch (2012). Oral Tissues as Source for Bone Regeneration in Dental Implantology, Tissue Regeneration - From Basic Biology to Clinical Application, Prof. Jamie Davies (Ed.), ISBN: 978-953-51-0387-5, InTech, Available from: http://www.intechopen.com/books/tissue-regeneration-from-basic-biology-to-clinical-application/oral-tissues-as-source-for-bone-regeneration-in-dental-implants

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen