Dental Pulp Stem Cells for Regenerative Medicine

S. SAMSON [1], V. BURLACU [2], E. ZOTA[1], V. NACU [1] V.PĂLĂRIE[1]

[1]*Laboratory of Tissue Engineering and Cells Culture SMPhU "Nicolae Testemitanu"*
[2]*Dentistry Department SMPhU "Nicolae Testemitanu"*

***Abstract* — Stem cells can self-renew and produce different cell types, thus providing new strategies to regenerate missing tissues and treat diseases. In the field of dentistry, adult mesenchymal stem/stromal cells (MSCs) have been identified in several oral and maxillofacial tissues, which suggests that the oral tissues are a rich source of stem cells, and oral stem and mucosal cells are expected to provide an ideal source for genetically reprogrammed cells such as induced pluripotent stem cells. Furthermore, oral tissues are expected to be not only a source but also a therapeutic target for stem cells, as cellular and tissue engineering therapies in dentistry continue to attract increasing clinical interest. This review outlines various types of intra- and extra-oral tissue-derived stem cells with regard to clinical availability and applications in dentistry. Additionally, appropriate sources of stem cells for regenerative dentistry are discussed with regard to differentiation capacity, accessibility and possible immunomodulatory properties..**

***Index Terms* — Dental stem cells; Induced pluripotent stem cells; Mesenchymal stem cells; Regenerative dentistry; Stem cell sources.**

I. INTRODUCTION

Stem cells are immature, unspecialized cells that have the potential to develop into many different cell lineages via differentiation. By the conventional definition, these cells can renew themselves indefinitely through ''self-renewal'' [1], and they vary in terms of their location in the body and the type of cells that they can produce. Recent studies have revealed that the oral tissues, which are easily accessible for dentists, are a rich source of stem cells. Given their unique abilities, stem cells are particularly important for developing innovative technologies for tissue engineering strategies [2] to regenerate or replace damaged, diseased or missing tissues and even organs by *in vitro* cell manipulation and design of the extracellular environment. In dentistry, tissue engineering is also considered to be a new frontier in the regeneration of missing oral tissues/organs [3, 4]. For example, various degrees of alveolar bone resorption occur after tooth loss/extraction because of periodontal disease, severe caries, root fractures or accidental trauma [5]. In addition, the bone resorption in the residual ridge, particularly in the mandible, continues throughout life in many edentulous patients [6]. The severe bone resorption in edentulous areas makes it difficult to restore the missing teeth with dental implants or denture treatment [7–9]. Therefore, stem cell and tissue engineering therapies are expected to provide a novel capability to regenerate large defects in periodontal tissues [10] and alveolar bone [11–13], and to ultimately replace the lost tooth itself [14, 15]. The tissues and organs targeted for such regenerative medicine strategies in dentistry include the salivary gland [16], tongue [17] and craniofacial skeletal muscles [18], as well as the condylar cartilage of the temporomandibular joint [19, 20]. Many basic and translational studies with stem cells and the other key elements of tissue engineering, i.e., bioactive factors and extracellular matrix scaffolds [21, 22], have been conducted in animal models to develop the concept of oral tissue and organ regeneration for clinical application in dentistry. In addition, stem cell-based tissue engineering has already been applied to clinical trials with demonstrated efficacy in orofacial bone tissue regeneration [11–13]. Despite these promising successes, recent findings that various types of stem cells can be obtained from the oral and maxillofacial region may lead to confusion regarding the role of stem cells and regenerative biology in dentistry, particularly with regard to the optimal type of stem cells for oral tissue and organ regeneration. There are two primary sources of stem cells: adult stem cells and embryonic stem (ES) cells. In addition to these stem cells, which are naturally present in the human body, induced pluripotent stem cells have been recently generated artificially via genetic manipulation of somatic cells [23, 24]. Embryonic stem and induced pluripotent stem cells are collectively referred to as pluripotent stem cells because they can develop into all types of cells from all three germinal layers. In contrast, most adult stem cells are multipotent, i.e., they can only differentiate into a limited number of cell types. We herein outline the different types ofstem cells under consideration for applications in dentistry in terms of their clinical availability. Stem cells can be defined as self-replicate cells that are able to differentiate into at least two different cell types. Both conditions must be present for a cell to be considered a stem cell. For example, osteoblasts are not stem cells. Although osteoblasts differentiate into osteocytes, they typically do not differentiate into other cell types except osteocytes. Osteocytes are not stem cells; they are end-lineage cells that typically neither self-replicate and not differentiate in to another cells type . **Mesenchymal stem cells (**MSC) can be isolated from different sources. First described in bone marrow, MSC have been extensively characterized *in vitro* by the expression of markers such as STRO-1, CD146 or CD44. STRO-1 is a cell surface antigen used to identify osteogenic precursors in bone marrow, CD146 a pericyte marker, and CD44 a mesenchymal stem cell marker. MSC possess a high self-renewal capacity and the potential to differentiate into mesoderm lineages thus forming cartilage, bone, adipose tissue, skeletal muscle and the stroma of connective tissues. The potential of

dental MSC for tooth regeneration and repair has been extensively studied in the last years. Below, we will present the mesenchymal progenitors that have been assessed for tooth engineering purposes, such as progenitors derived from teeth (adult dental pulp, apical part of papilla, dental follicle, periodontal ligament) and bone marrow .**Stem cells from human exfoliated deciduous teeth (SHED).** The isolation of post-natal stem cells from an easily accessible source is indispensable for tissue engineering and clinical applications. Recent findings demonstrated the isolation of mesenchymal progenitors from the pulp of human deciduous incisors. These cells were named SHED (Stem cells from Human Exfoliated Deciduous teeth) and exhibited a high plasticity since they could differentiate into neurons, adipocytes, osteoblasts and odontoblasts. *In vivo* SHED cells can induce bone or dentin formation but, in contrast to dental pulp, DPSC failed to produce a dentin-pulp complex .**Adult dental pulp stem cells (DPSC).** The possibility that tooth pulp might contain mesenchymal stem cells was first suggested by the observation that severe tooth damage that penetrates both enamel and dentine into the pulp stimulates a limited natural repair process, by which new odontoblasts are formed, which produce new dentine to repair the lesion (Smith A. J., Lesot H., 2001). Putative stem cells from the tooth pulp and several other dental tissues have now been identified. The first stem cells isolated from adult human dental pulp were termed dental pulp stem cells (DPSCs) . They were isolated from permanent third molars, and exhibited high proliferation and high frequency of colony formation that produced sporadic, but densely calcified nodules. Additionally, *in vivo* transplantation into immunocompromised mice demonstrated the ability of DPSCs to generate functional dental tissue in the form of dentine/pulp-like complexes [2]. Further characterization revealed that DPSCs were also capable of differentiating into other mesenchymal cell derivatives *in vitro* such as odontoblasts, adipoctyes, chondrocytes and osteoblasts (Koyama N., et al., 2009). DPSCs differentiate into functionally active neurons, and implanted DPSCs induce endogenous axon guidance, suggesting their potential as cellular therapy for neuronal disorders (Arthur A. et al., 2009).**Stem cells from the apical part of the papilla (SCAP).** Recently was discovered another type of MSCs in the apical papilla of human immature permanent teeth termed stem cells from apical papilla (SCAP) (Wataru Sonoyama, Yi Liu, Takayoshi Yamaza, 2008). Was found that apical papilla is distinctive to pulp in terms of containing less cellular and vascular components than those in pulp. Cells in apical papilla proliferated 2- to 3-fold greater than those in pulp in organ cultures. Both SCAP and DPSCs were as potent in osteo/dentinogenic differentiation as MSCs from bone marrows while weaker in adipogenic potential. The immunophenotype of SCAP is similar to that of DPSCs on the osteo/dentinogenic and growth factor receptor gene profiles. Double staining experiments showed that STRO-1 co-expressed with dentinogenic markers such as bone sialophosphoprotein (BSP), osteocalcin (OCN) and growth factors FGFR1 and TGFβRI in cultured SCAP. Stem cells from the apical part of the human dental papilla (SCAP) have been isolated and their potential to differentiate into odontoblasts was compared to that of the periodontal ligament stem cells (PDLSC). SCAP exhibit a higher proliferative rate and appears more effective than PDLSC for tooth formation. Importantly, SCAP are easily accessible since they can be isolated from human third molars.**Stem cells from the dental follicle (DFSC).** DFSC have been isolated from follicle of human third molars and express the stem cell markers Notch1, STRO-1 and nestin. The dental follicle is a loose of ectomesenchyme-derived connective tissue sac surrounding the enamel organ and the dental papilla of the developing tooth germ before eruption (Ten Cate, 1998). It is believed to contain progenitors for cementoblasts, PDL and osteoblasts. Dental follicle cells (DFC) form the PDL by differentiating into PDL fibroblasts that secrete collagen and interact with fibres on the surfaces of adjacent bone and cementum. DFC can form cementoblast-like cells after transplantation into SCID mice (Handa K. et al., 2002). Dental follicle progenitor cells isolated from human third molars are characterized by their rapid attachment in culture, expression of the putative stem cell markers Nestin and Notch-1, and ability to form compact calcifiednodules *in vitro* (Lin N. H. et al., 2008). DFC were transplanted into immunocompromised mice, however, there was little indication of cementum or bone formation (Lin N. H. et al., 2008). DFC, in common with SCAP, represent cells from a developing tissue and might thus exhibit a greater plasticity than other dental stem cells. However, also similar to SCAP, further research needs to be carried out on the properties and potential uses of these cells. **Periodontal ligament stem cells (PDLSC).** The PDL is a specialized tissue located between the cementum and the alveolar bone and has as a role the maintenance and support of the teeth. Its continuous regeneration is thought to involve mesenchymal progenitors arising from the dental follicle. PDL contains STRO-1 positive cells that maintain certain plasticity since they can adopt adipogenic, osteogenic and chondrogenic phenotypes *in vitro*. It is thus obvious that PDL itself contains progenitors, which can be activated to self-renew and regenerate other tissues such as cementum and alveolar bone. It was shown that cultured PDLSCs proliferate in higher rate on the rough surface especially at the 75μm Al_2O_3 particle treated surface than other surfaces. Also, osteocalcin was highly expressed on the rough surfaces treated with 75μm and 125μm Al_2O_3 particles (Heo Y. Y., Um S., Kim S. K., Park J. M., 2011). **Bone marrow derived mesenchymal stem cells (BMSC).** BMSC have been tested for their ability to recreate periodontal tissue. These cells are able to form *in vivo* cementum, PDL and alveolar bone after implantation into defective periodontal tissues. Thus, bone marrow provides an alternative source of MSC for the treatment of periodontal diseases (Kawaguchi H., 2004). BMSC share numerous characteristics with DPSC and are both able to form bone-like or tooth-like structures. However, BMSC display a lower odontogenic potential than DPSC (Yu J. et al., 2007), indicating that MSC from different embryonic origins are not equivalent. Indeed, DPSC derive from neural crest cells, whereas BMSC originate from the mesoderm. Furthermore, the comparison of the osteogenic and adipogenic potential of MSC from

different origins shows that, even if cells carry common genetic markers, they are not equivalent and are already committed toward a specific differentiation pathway (Musina R. A. et al., 2006). Commitment could arise from conditioning of stem cells by their specific microenvironment or stem cell niche.

II. MATERIAL AND METHODS

The study was effectuated on 25 extracted Vietnamese pigs teeth aged between 2-3 months. The cells were obtained from dental pulp by digestion in 0,25% dispase I (SIGMA) for 10 min at 37 ^{0}C. The cells were cultivated in 24 well in triplicate, in DMEM (HiMedia), 10% FBS (SIGMA), 5%CO_2, 96% humidity and temperature 37^{0}C.

III. RESULTS AND DISSCUTION

The cells were cultivated in 0,5x10^6 cells per well, in 3,0cm diameters culture dish during seven days. The half media was changed by fresh one every two days. At the end of this period cells were colored by Romanovski and counted under the light microscope. The number of the cells after seven days cultivation were: 4,5±0,3x10^6 per well.

With regard to accessibility, bone marrow aspiration from the iliac crest and liposuction from extra-oral tissue is not an easy operation for dentists because of the limitations of the dental license and the dental specialization. In contrast, orofacial bone marrow, periosteum, salivary glands and dental tissues are accessible stem cell sources for dentists; however, the isolation of stem cells from these locations may still not be convenient because it requires surgical procedures or tooth or pulp extraction. Additionally, even if impacted wisdom teeth could be a cell source, not all adults require the extraction of the wisdom teeth. Furthermore, these adult stem cells are present in small quantities and can therefore be difficult to isolate, purify and expand homogeneously. In contrast, the gingiva, which is a tissue that is easily obtainable by dentists and whose cells can be easily expanded from patients with minimal discomfort, seems to be a promising source of adult stem cells [98–100] and iPS cells [101] in dentistry. More studies are necessary to determine the regenerative abilities of gingival-derived stem cells in oral tissues. Research on all available stem cells in dentistry should be continued to permit their manipulation for the regeneration of oral tissues. Based on the accumulated knowledge, the type of stem cell to be used for a given application will be decided by considering a balance of the differentiation capacity with accessibility/availability, which may vary on a case-by-case basis.

Conclusions

The teeth's could be a rich source of adult stem cells. Many intra-oral tissues, such as deciduous teeth, wisdom teeth and the gingiva, are not only easily accessible from the oral cavity but can also often be obtained as a discarded biological sample. Therefore, dental professionals should recognize the promise of the emerging field of regenerative dentistry and the possibility of obtaining stem cells during conventional dental treatments that can be banked for autologous therapeutic use in the future. The discarded oral tissues can also be used to generate induced pluripotent stem cells that can be used not only for the autologous cell-based regeneration of complex oral tissues but also for the patientspecific modeling of oral diseases and the development of tailor-made diagnostic and drug screening tools for alveolar bone augmentation and oral cancer treatment.

REFERENCES

[1] Slack JM. Origin of stem cells in organogenesis. Science 2008;322: 1498–501.

[2] Langer R, Vacanti JP. Tissue engineering. Science 1993;260:920–6.

[3] Kaigler D, Mooney D. Tissue engineering's impact on dentistry. J Dent Educ 2001;65:456–62.

[4] Koyano K. Toward a new era in prosthodontic medicine. J Prosthodont Res 2012;56:1–2.

[5] Kirkwood KL. Periodontal diseases and oral bone loss. In: Rosen CJ, editor. Primer on the metabolic bone diseases and disorders of mineral metabolism. 7th ed., Washington, DC: American Society for Bone and Mineral Research; 2008. p. 510–3.

[6] Atwood DA. Reduction of residual ridges: a major oral disease entity. J Prosthet Dent 1971;26:266–79.

[7] Darby I, Chen S, De Poi R. Ridge preservation: what is it and when should it be considered. Aust Dent J 2008;53:11–21.

[8] Egusa H, Saeki M, Doi M, Fukuyasu S, Matsumoto T, Kamisaki Y, et al. A small-molecule approach to bone regenerative medicine in dentistry. J Oral Biosci 2010;52:107–18.

[9] Egusa H. iPS cells in dentistry. Clin Calcium 2012;22:67–73.

[10] Izumi Y, Aoki A, Yamada Y, Kobayashi H, Iwata T, Akizuki T, et al. Current and future periodontal tissue engineering. Periodontology 2000 2011;56:166–87.

[11] Yamada Y, Ueda M, Hibi H, Baba S. A novel approach to periodontal tissue regeneration with mesenchymal stem cells and platelet-rich plasma using tissue engineering technology: a clinical case report. Int J Periodontics Restorative Dent 2006;26:363–9.

[12] Ueda M, Yamada Y, Kagami H, Hibi H. Injectable bone applied for ridge augmentation and dental implant placement: human progress study Implant Dent 2008;17:82–90.

[13] Yamada Y, Nakamura S, Ito K, Kohgo T, Hibi H, Nagasaka T, et al. Injectable tissue-engineered bone using autogenous bone marrow-derived stromal cells for maxillary sinus augmentation: clinical application report from a 2–6-year follow-up. Tissue Eng Part A 2008;14:1699–707.

[14] Ikeda E, Morita R, Nakao K, Ishida K, Nakamura T, Takano-Yamamoto T, et al. Fully functional bioengineered tooth replacement as an organ replacement therapy. Proc Natl Acad Sci U S A 2009;106:13475–80.

[15] Oshima M, Mizuno M, Imamura A, Ogawa M, Yasukawa M, Yamazaki H, et al. Functional tooth regeneration using a bioengineered tooth unit as a mature organ replacement regenerative therapy. PLoS One 2011;6:e21531.

[16] Lombaert IM, Knox SM, Hoffman MP. Salivary gland progenitor cell biology provides a rationale for therapeutic salivary gland regeneration. Oral Dis 2011;17:445–9.

[17] Luxameechanporn T, Hadlock T, Shyu J, Cowan D, Faquin W, Varvares M. Successful myoblast transplantation in rat tongue reconstruction. Head Neck 2006;28:517–24.

[18] Shah R, Sinanan AC, Knowles JC, Hunt NP, Lewis MP. Craniofacial muscle engineering using a 3-dimensional phosphate glass fibre construct. Biomaterials 2005;26:1497–505.

[19] Dormer NH, Busaidy K, Berkland CJ, Detamore MS. Osteochondral interface regeneration of rabbit mandibular condyle with bioactive signal gradients. J Oral Maxillofac Surg 2011;69:50–7.

[20] Yu H, Yang X, Cheng J, Wang X, Shen SG. Distraction osteogenesis combined with tissue-engineered cartilage in the reconstruction of condylar osteochondral defect. J Oral Maxillofac Surg 2011;69:558–64

[21] Akagawa Y, Kubo T, Koretake K, Hayashi K, Doi K, Matsuura A, et al. Initial bone regeneration around fenestrated implants in Beagle dogs using basic fibroblast growth factor-gelatin hydrogel complex with varying biodegradation rates. J Prosthodont Res 2009;53:41–7.

[22] Kubo T, Doi K, Hayashi K, Morita K, Matsuura A, Teixeira ER, et al. Comparative evaluation of bone regeneration using spherical and irregularly shaped granules of interconnected porous hydroxylapatite. A beagle dog study. J Prosthodont Res 2011;55:104–9.

[23] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663–76.

[24] Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al.