

Review

The Regenerative Medicine in Oral and Maxillofacial Surgery: The Most Important Innovations in the Clinical Application of Mesenchymal Stem Cells

Marco Tatullo^{1,2*}✉, Massimo Marrelli^{1,2*}, Francesco Paduano^{1*}

1. Tecnologica Research Institute, Biomedical Section, Crotona, Italy

2. Calabrodental clinic, Biomaterials test unit, Crotona, Italy

* All the Authors have equally contributed to this paper

✉ Corresponding author: Dr. Marco Tatullo, PhD, Scientific Director, Tecnologica Research Institute, Biomedical Section, Str. E. Fermi, Crotona, Italy, Email: marco.tatullo@tecnologicasrl.com

© Ivyspring International Publisher. This is an open-access article distributed under the terms of the Creative Commons License (<http://creativecommons.org/licenses/by-nc-nd/3.0/>). Reproduction is permitted for personal, noncommercial use, provided that the article is in whole, unmodified, and properly cited.

Received: 2014.10.02; Accepted: 2014.10.31; Published: 2015.01.01

Abstract

Regenerative medicine is an emerging field of biotechnology that combines various aspects of medicine, cell and molecular biology, materials science and bioengineering in order to regenerate, repair or replace tissues.

The oral surgery and maxillofacial surgery have a role in the treatment of traumatic or degenerative diseases that lead to a tissue loss: frequently, to rehabilitate these minuses, you should use techniques that have been improved over time. Since 1990, we started with the use of growth factors and platelet concentrates in oral and maxillofacial surgery; in the following period we start to use biomaterials, as well as several type of scaffolds and autologous tissues. The frontier of regenerative medicine nowadays is represented by the mesenchymal stem cells (MSCs): overcoming the ethical problems thanks to the use of mesenchymal stem cells from adult patient, and with the increasingly sophisticated technology to support their manipulation, MSCs are undoubtedly the future of medicine regenerative and they are showing perspectives unimaginable just a few years ago. Most recent studies are aimed to tissues regeneration using MSCs taken from sites that are even more accessible and rich in stem cells: the oral cavity turned out to be an important source of MSCs with the advantage to be easily accessible to the surgeon, thus avoiding to increase the morbidity of the patient.

The future is the regeneration of whole organs or biological systems consisting of many different tissues, starting from an initial stem cell line, perhaps using innovative scaffolds together with the nano-engineering of biological tissues.

Key words: Regenerative medicine; Mesenchymal Stem Cells; Bone regeneration; Dental Pulp Stem Cells; human Periapical Cysts Mesenchymal Stem Cells; hPCy-MSCs.

Introduction

Regenerative medicine is an emerging field of biotechnology that combines various aspects of medicine, cell and molecular biology, materials science and bioengineering in order to regenerate, repair or replace tissues.

The oral surgery and maxillofacial surgery have a role in the treatment of traumatic or degenerative

diseases that lead to a tissue loss: frequently, to rehabilitate these minuses, you should use techniques that have been improved over time. Since 1990, tissue engineering has developed protocols in which it has been proposed the use of platelet concentrates, which showed enormous benefits for the patient: they favored and accelerated the post-surgical and provided

a support for tissue regeneration due to growth factors contained in them. Several authors¹⁻⁴ have described the importance of growth factors in tissue repair processes, in fact, they are important elements for new tissue production, moreover, they perform feedback controls on inflammatory processes within the tissue graft, in cases of regenerative surgery.

Whitman⁵ and Marx⁶ published the first studies on the use of growth factors contained in platelet gel, called Platelet-Rich Plasma (PRP).

Thanks to Marx's studies, it was possible to verify that the platelet concentrate is a very effective tool for the modulation of wound healing and tissue regeneration. However, the PRP showed a number of disadvantages, such as the need of having to run a complex and expensive protocol for its production. To overcome some of these problems, the PRGF (Plasma Rich in Growth Factors) was introduced in the list of platelet concentrates. The PRGF is considered an evolution of the PRP^{7,8} and it allows a higher concentration of growth factors in platelet preparation. Among the advantages of the PRGF, we can cite the lesser amount of blood taken for the preparation and a procedure relatively faster, while, among the disadvantages we can mention the rapid clot formation, which require speed in its surgical use.

In 2001, Choukroun *et coll.* have instead proposed an alternative technique: the PRF (Platelet Rich Fibrin). PRF is derived from a simple preparation protocol that does not require alteration of the blood; it is a platelet concentrate rich in GFs that contains a three-dimensional matrix of autologous, elastic and flexible fibrin.

Dohan *et al.* have shown that platelet cytokines (PDGF, TGFbeta1 and IGF-1) are present in three-dimensional fibrin matrix derived from these platelet concentrates; moreover, PRF matrix traps glycosaminoglycans such as heparin and hyaluronic acid, which have considerable affinity with some peptides present in the bloodstream and therefore show strong ability of chemotaxis and diapedesis, useful for the healing of tissue damaged, for example, by trauma⁹. Moreover, it was shown that this matrix can be a valuable support for the transplantation of bone morphogenetic proteins (BMP) issued in a progressive manner to induce osteogenic differentiation, as demonstrated by recent studies on muscle preparations^{10,11}; about this, the results of Wiltschko *et al.* are encouraging, in fact, they show an improvement of osteoblast proliferation in cases in which it was used the PRF compared to PRP¹².

Marrelli *et al.* described a case in which is documented the filling with PRF of a large osteolytic cavity and complete bone reformation¹³. Tatullo *et al.* have suggested that the osteoinductive potential of

PRF is related to its neoangiogenic ability and concentration of GFs, in relation to the fibrin content and platelet cytokines present, all suitable for the totipotent cell migration and activation of pre-osteoblastic cells present in the surgical site, fundamental aspects for bone regeneration¹⁴.

Platelets concentrates are, thus, versatile products in surgery, with regard to their biological properties and their easy manipulation in the form of gel or membranes; these features allow the use of PRF as well as other platelet concentrates in cases, for example, of maxillary surgical sites or in the surgery of maxillary sinus¹⁵.

The frontier of regenerative medicine nowadays is represented by the mesenchymal stem cells (MSCs): overcoming the ethical problems thanks to the use of mesenchymal stem cells from adult patient, and with the increasingly sophisticated technology to support their manipulation, MSCs are undoubtedly the future of medicine regenerative and they are showing perspectives unimaginable just a few years ago. Most recent studies are aimed to tissues regeneration using MSCs taken from sites that are even more accessible and rich in stem cells: the oral cavity turned out to be an important source of MSCs with the advantage to be easily accessible to the surgeon, thus avoiding to increase the morbidity of the patient.

Mesenchymal stem cells of oral origin

The aim of the regenerative medicine and tissue engineering is to regenerate and repair the damaged cells and tissues in order to establish the normal functions¹⁶. The regenerative medicine involves the use of biomaterials, growth factors and stem cells¹⁷. Regeneration of the tissues exists naturally due to the presence of stem cells with the potential to self-regenerate and differentiate into one of more specialized cell types. However, this regenerative potential decreases with age and regeneration is not sufficient to repair the damages produced by degenerative, inflammatory or tumor based diseases¹⁸. Stem cells are immature and unspecialized cells with the ability to renew and divide themselves indefinitely through "self-renewal" and able to differentiate into multiple cell lineages¹⁹. The stem cells use for regenerative medicine should fit the following criteria: they can be: *a*) found in abundant numbers and can be differentiated in multiple cell lineages in a reproducible and controllable manner; *b*) isolated by minimally invasive procedure with minimal morbidity for patients, *c*) produced in accordance with GMP (Good manufacture Practice) and *d*) transplanted safely^{20,21}. In the last decade, several improvements have been produced in the comprehension of stem cells properties in view of the fact that these cells have an important role in the repair of

every organ and tissue.

In general, the stem cells are divided into three main types that can be utilized for tissue repair and regeneration: *i*) the embryonic stem cells derived from embryos (ES) ^{22,23}; *ii*) the adult stem cells that are derived from adult tissue ²⁴; and *iii*) the induced pluripotent stem (iPS) cells that have been produced artificially via genetic manipulation of the somatic cells ²⁵. ES and iPS cells are considered pluripotent stem cells because they can develop into all types of cells from all three germinal layers. Both stem cells have technical and moral obstacles, in addition these cells are not easy to control and they can form tumors after injection²². On the contrary, adult stem cells are multipotent because they can only differentiate into a restricted number of cell types. Adult stem cells, also termed postnatal stem cells or somatic stem cells, are discovered in a particular area of each tissue named "stem cell niche."

Different type of postnatal stem cells resides in numerous mesenchymal tissues and these cells are at the same time referred to as mesenchymal stem cells (MSCs) ^{24,26}. MSCs were first isolated and characterized from bone marrow (BMSCs) by Friedenstein *et al.* in 1974 ²⁷. Subsequently, different studies have showed that MSCs can be isolated from other tissues, such as peripheral blood, umbilical cord blood, amniotic membrane, adult connective, adipose and dental tissues²⁸⁻³².

Recently, orofacial and dental tissues have acquired interest as a further accessible source of mesenchymal stem cells ³³ due to the fact that the oral area is rich in MSCs (Table 1). Today, every cell population which has the following characteristics independently of its tissue source, is usually referred as MSCs: *i*) they adhere to plastic and have a fibroblast-like morphology; *ii*) they have the capacity of self-renewal and

could differentiate into cells of the mesenchymal lineage such as osteocytes, chondrocytes and adipocytes. In addition, MSCs also can also differentiate, under appropriate conditions, into cells of the endoderm and ectoderm lineages such as hepatocytes and neurons, respectively ^{34,35}. Phenotypically, MSCs express the CD13, CD29, CD44, CD59, CD73, CD90, CD105, CD146 and STRO-1 surface antigens, and they do not express CD45 (leukocyte marker), CD34 (the primitive hematopoietic progenitor and endothelial cell marker), CD14 and CD11 (the monocyte and macrophage markers), CD79 and CD19 (the B cell markers), or HLA class II ³⁶. Research related to MSC from oral origin began in 2000 ³⁷ and every year numerous investigations have demonstrated that oral tissues, which are simply available for dentists, are a rich source for mesenchymal stem cells ^{33,38}.

Today numerous types of MSCs have been isolated from teeth: in 2000 MSCs were first isolated by Gronthos *et al.* from dental pulp (DPSCs) ^{37,38}. These cells possess phenotypic characteristics similar to those of BMSCs ³⁹, and they have definitive stem cell properties such as self-renewal and multi-differentiation capacity, and can form the dentin-pulp structure when transplanted into immunocompromised mice ⁴⁰. Moreover, DPSCs participate in the regeneration of non-orofacial tissues, in fact, these cells have been differentiated into hair follicle-, hepatocyte-, neuron-, islet-, myocyte- and cardiomyocyte-like cells ⁴¹⁻⁴⁶. Subsequently, MSCs have been also isolated from dental pulp of human exfoliated deciduous teeth (SHEDs). These cells, like DPSCs, have the ability to differentiate *in vitro* in odontoblasts, osteoblasts, adipocytes and neuron-like cells. Also SHEDs were able to form dentin and bone when transplanted with HA/TCP *in vivo*⁴⁷.

Table 1: Mesenchymal Stem Cells from dental tissues

| Name | Site | Date of discover | Authors | Country | Institution |
|-----------|----------------------------------|------------------|----------------------------------------------------------------------------------------------------------------|------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|
| DPSCs | Dental Pulp | 2000 | S. Gronthos, M. Mankani, J. Brahim, P.G. Robey, S. Shi | USA. Bethesda, Maryland | National Institute on Dental Research, National Institutes of Health |
| SHED | human Exfoliated Deciduous Teeth | 2003 | M. Miura, S. Gronthos, M. Zhao, B. Lu, L.W. Fisher, P. G. Robey, S. Shi | USA. Bethesda, Maryland | National Institute on Dental Research, National Institutes of Health |
| PDLSs | Periodontal Ligament | 2004 | B. M. Seo, M. Miura, S. Gronthos, P.M. Bartold, S. Batouli, J. Brahim, M. Young, P.G. Robey, C.Y. Wang, S. Shi | USA. Bethesda, Maryland | National Institute on Dental Research, National Institutes of Health |
| SCAP | Apical Papilla | 2006 | W. Sonoyama, Y. Liu, D. Fang, T. Yamaza, B.M. Seo, C. Zhang, H. Liu, S. Gronthos, C.Y. Wang, S. Wang, S. Shi | USA. Los Angeles, California JAPAN. Okayama | University of Southern California School of Dentistry; Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences |
| DFSCs | Dental Follicle | 2005 | C. Morsczeck, W. Götz, J. Schierholz, F. Zeilhofer, U. Kühn, C. Möhl, C. Sippel, K.H. Hoffmann | GERMANY. Bonn | Stiftung Caesar, Center of Advanced European Studies and Research |
| hPCy-MSCs | human Periapical Cyst | 2013 | M. Marrelli, F. Paduano, M. Tatullo | ITALY. Crotone | Calabrodental, Unit of Maxillofacial Surgery; Tecnologica Research Institute, Biomedical Section |

The periodontal ligament is another adult MSCs source in dental tissue, and periodontal ligament stem cells (PDLSCs) were isolated from extracted teeth⁴⁸. PDLSCs have the ability to regenerate periodontal tissues such as the cementum, periodontal ligament and alveolar bone⁴⁹. Moreover, MSCs have been also isolated from developing dental tissues such as the dental follicle (DFPCs)⁵⁰ and apical papilla (SCAPs)⁵¹. DFPCs have the ability to regenerate periodontal tissues whereas SCAPs demonstrate better proliferation and better regeneration of the dentin matrix when transplanted in immunocompromised mice with compared to DPSCs^{50,52,53}. Zhang *et al.* have isolated mesenchymal stem cells from the gingiva, these MSCs exhibited higher clonogenicity, self-renewal and multipotent differentiation capacity similar to that of BMSCs⁵⁴. Moreover, the salivary glands derived MSCs could differentiate into the salivary gland duct cells as well as mucin and amylase producing acinar cells *in vitro*⁵⁵. In addition, De Bari *et al.* demonstrated that single-cell-derived clonal populations of adult human periosteal cells possess mesenchymal multipotency, as they differentiate to osteoblast, chondrocyte, adipocyte and skeletal myocyte lineages *in vitro* and *in vivo*. Therefore, expanded MSCs isolated from periosteum could be useful for functional tissue engineering, especially for bone regeneration⁵⁶.

The MSCs contained within the bone marrow aspiration from the iliac crest, and liposuction from extra-oral tissue are not easily-accessible stem cells. On the contrary, the orofacial bone marrow, periosteum, salivary glands and dental tissues are the most accessible stem cell sources. Moreover, the isolation of MSCs from these sources may still not be convenient because it requires surgical methods or tooth or pulp extraction. In addition, even if impacted wisdom teeth could be a mesenchymal stem cell source, these MSCs are present in a low percentage and can, therefore, be difficult to isolate, purify and expand. Furthermore, not all adults need the extraction of the wisdom teeth. To overcome these limitations, recently, Marrelli *et al.* demonstrated that MSCs derived from periapical cysts (hPCy-MSCs) have a mesenchymal stem cell immunophenotype and the ability to differentiate into osteogenic and adipogenic lineages⁵⁷. The periapical cyst, which is a tissue that is easily obtainable and whose cells can be simply expanded from patients with minimal discomfort, seems to be a promising source of adult stem cells in dentistry for regenerative medicine. In fact, a recent study of Marrelli *et al.* showed that hPCy-MSCs similarly to DPSCs have neural progenitor-like properties by expressing spontaneously neuron and astrocyte specific proteins and neural related genes before any differentiation. Furthermore, hPCy-MSCs, under appropriate neural

stimulation, acquire neural morphology and significantly over-express several neural markers at both protein and transcriptional level (in press, not yet published research by Marrelli *et al.*).

Mesenchymal stem cells in regenerative medicine

It was reported that MSCs isolated from whole bone marrow aspirates in combination with scaffolds and growth factors are able to repair cranial defects in several animal models⁵⁸⁻⁶⁰. These studies demonstrated that MSCs can alleviate the complications of craniofacial surgical procedures that required allogenic tissue grafts or extraction of autologous bone from secondary sites. This approach may alleviate donor site morbidity and allow a virtual unlimited source of cellular material derived from allogenic MSCs⁶¹.

The identification of MSC residing in the oral cavity tissues increases clinical interest in MSCs as a cell source for regeneration of other connective tissues such as cementum, dentin and periodontal ligament (PDL). Many research studies have been performed to assess the capacity of dental derived MSCs to enhance periodontal regeneration. Seo *et al.* have demonstrated that human PDLSCs were able to generate a cementum/PDL-like structures when transplanted into immunocompromised mice, and consequently transplantation of PDLSCs could be considered as a therapeutic approach for regeneration of tissues damaged by periodontal diseases⁴⁸. Moreover, Kim *et al.* compared the alveolar bone regeneration achieved from implantation of PDLSCs and BMSCs and identified no significant difference in regenerative potential *in vivo* between these MSCs⁶².

The three key elements in the field of tissue engineering are stem cells, scaffolds and growth factors⁶³. Recently, researchers are trying to identify the ideal scaffold that facilitate growth, cell spreading, adhesion, integration and differentiation of MSCs. This scaffold should be biocompatible and biodegradable, should have optimal physical features and mechanical properties⁶⁴. Different material have been designed and constructed for tissue engineering approaches, using natural or synthetic polymers or inorganic materials, which have been fabricated into porous scaffolds, nanofibrous material, hydrogels and microparticles. Natural materials include collagen, elastin, fibrin, silk, chitosan and glycosaminoglycans⁶⁵. Recently, hydrogels have been investigated for tissue engineering applications because they offer numerous properties including biocompatibility and mechanical characteristics similar to those of native tissue^{66,67}. Synthetic poly lactic-co-glycolic acid (PLGA) and titanium provide excellent chemical and mechanical

properties for bone tissue regeneration *in vivo* using DPSCs⁶⁸. Furthermore, recent studies demonstrated that DPSCs loaded onto scaffolds of chitosan formed a dentine-pulp complex *in vivo*⁶⁹ whereas DPSCs cultured on hydroxyapatite (HA) and placed subcutaneously in nude mice formed bone⁷⁰. A great number of investigations for evaluating the *in vivo* application of MSCs isolated from the oral cavity were carried out on animal models. A clinical study conducted by Pappaccio's group gave evidence of the possibility to utilize DPSCs to repair bone defect in humans. In fact, they showed that DPSCs/collagen biocomplex completely restored human mandible bone defects subsequent to DPSCs transplantation⁷¹.

Conclusions

The future is the regeneration of whole organs and complex biological systems consisting of many different tissues, starting from an initial stem cell line, probably using innovative scaffolds together with the nano-engineering of biological tissues: this approach is already a research topic in several international research institutes, and the best way to merge the numerous skills needed to get a so ambitious result is the multicenter collaboration. The authors are closely collaborating together with high-level international Universities, to develop protocols aimed to control and lead the tissues regeneration. This goal could make born a new generation of stem-cells based therapies, so to open the door to a new high-performing regenerative medicine.

Starting from 2000, in only fifteen years, researchers have changed the face of the tissues engineering and the expectation of quality of life in more than 2 billions of patients undergone to a regenerative surgery: the challenge is to continue to make the patient's life better, to make the surgery more predictable and to simply replace damaged or degenerated tissues with MSCs from dental and oral sources.

Acknowledgements

The present study was supported by "PROMETEO Project - Progettazione e Sviluppo di piattaforme tecnologiche innovative ed ottimizzazione di processi per applicazioni in Medicina rigenerativa in ambito oromaxillofaciale, ematologico, neurologico e cardiologico". PON01_02834.

Competing Interests

The authors have declared that no competing interest exists.

References

- Gibble JW, Ness PM. Fibrin glue: the perfect operative sealant? *Transfusion*. 1990;30:741-7.
- Saltz R, Sierra D, Feldman D, Saltz MB, Dimick A, Vasconez LO. Experimental and clinical applications of fibrin glue. *Plast Reconstr Surg*. 1991;88:1005-15, discussion 1016-7.
- Hotz G. Alveolar ridge augmentation with hydroxylapatite using fibrin sealant for fixation. Part I: An experimental study. *Int J Oral Maxillofac Surg*. 1991; 20:204-7.
- Hotz G. Alveolar ridge augmentation with hydroxylapatite using fibrin sealant for fixation. Part II: Clinical application. *Int J Oral Maxillofac Surg*. 1991; 20:208-13.
- Whitman DH, Berry R, Green D. Platelet gel: an autologous alternative to fibrine glue with applications in oral and maxillofacial surgery. *J Oral Maxillofac Surg*. 1997; 55: 1294-9.
- Marx RE, Carlson ER, Eichstaedt RM, et al. Platelet-rich plasma: growth factor enhancement for bone grafts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 1998; 85: 638-46.
- Weibrich G, Kleis WKG, Hitzler WE, Hafner G. Comparison of the Platelet Concentrate Collection System with the Plasma-Rich-in-Growth-Factors Kit to Produce Platelet-Rich Plasma: A Technical Report. *Int J Oral Maxillofac Implants*. 2005;20:118-123.
- Weibrich G, Kleis WKG, Hafner G, Hitzler WE, Wagner W. Comparison of platelet, leukocyte and growth factor levels in point of care platelet-enriched plasma, prepared using a modified Curasan kit, with preparations received from a local blood bank. *Clin Oral Implants Res*. 2005; 14: 357-362.
- Gibble JW, Ness PM. Fibrin glue: the perfect operative sealant? *Transfusion* 1990;30:741-7.
- Mosesson MW, Siebenlist KR, Meh DA. The structure and biological features of fibrinogen and fibrin. *Ann N Y Acad Sci*. 2001;936:11-30.
- Kawamura M, Urist MR. Human fibrin is a physiologic delivery system for bone morphogenic protein. *Clin Orthop*. 1988; 235: 302-10.
- Wiltfang J, Kloss FR, Kessler P, Nkenke E, Zimmerman R, Schlegel KA. Effect of prp on bone healing in combination with autogenous bone end bone substitutes in critical-size defects. An animal experiment. *Clin Oral Implants Res*. 2004; 15: 187-93
- Marrelli M, Pacifici A, Di Giorgio G, Cassetta M, Stefanelli LV, Gargari M, Promenzio L, Annibaldi S, Cristalli MP, Chiaravalloti E, Pacifici L, Tatullo M. Diagnosis and treatment of a rare case of adenomatoid odontogenic tumor in a young patient affected by attenuated familial adenomatosis polyposis (aFAP): case report and 5 year follow-up. *Eur Rev Med Pharmacol Sci*. 2014;18(2):265-9.
- Tatullo M, Marrelli M, Cassetta M, Pacifici A, Stefanelli LV, Scacco S, Dipalma G, Pacifici L, Inchigolo F. Platelet Rich Fibrin (P.R.F.) in reconstructive surgery of atrophied maxillary bones: clinical and histological evaluations. *Int J Med Sci*. 2012;9(10):872-80. doi: 10.7150/ijms.5119. Epub 2012 Nov 7.
- Marrelli M, Tatullo M. Influence of PRF in the healing of bone and gingival tissues. Clinical and histological evaluations. *Eur Rev Med Pharmacol Sci*. 2013 Jul;17(14):1958-62.
- Mason C, Dunnill P. A brief definition of regenerative medicine. *Regenerative medicine* 2008; 3: 1-5
- Sundelacruz S, Kaplan DL. Stem cell- and scaffold-based tissue engineering approaches to osteochondral regenerative medicine. *Seminars in cell & developmental biology* 2009; 20: 646-655
- Rodriguez-Lozano FJ, Bueno C, Insausti CL, Meseguer L, Ramirez MC, Blanquer M, Marin N, Martinez S, Moraleda JM. Mesenchymal stem cells derived from dental tissues. *International endodontic journal* 2011; 44: 800-806
- Weissman IL. Stem cells: Units of development, units of regeneration, and units in evolution. *Cell* 2000; 100: 157-168.
- Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. *Circulation research* 2007; 100: 1249-1260
- Gimble JM. Adipose tissue-derived therapeutics. *Expert opinion on biological therapy* 2003; 3: 705-713
- Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature* 1981; 292: 154-156
- Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proceedings of the National Academy of Sciences of the United States of America* 1981; 78: 7634-7638
- Korbling M, Estrov Z. Adult stem cells for tissue repair - a new therapeutic concept? *The New England journal of medicine* 2003; 349: 570-582
- Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 2007; 318: 1917-1920
- Keating A. Mesenchymal stromal cells: New directions. *Cell stem cell* 2012; 10: 709-716
- Friedenstein AJ, Chailakhyan RK, Latsinik NV, Panasyuk AF, Keiliss-Borok IV. Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo. *Transplantation* 1974; 17: 331-340
- Zhang Y, Huang B. Peripheral blood stem cells: Phenotypic diversity and potential clinical applications. *Stem cell reviews* 2012; 8: 917-925
- Zarrabi M, Mousavi SH, Abroun S, Sadeghi B. Potential uses for cord blood mesenchymal stem cells. *Cell journal* 2014; 15: 274-281

30. Bongso A, Fong CY. The therapeutic potential, challenges and future clinical directions of stem cells from the wharton's jelly of the human umbilical cord. *Stem cell reviews* 2013; 9: 226-240
31. Romagnoli C, Brandi ML. Adipose mesenchymal stem cells in the field of bone tissue engineering. *World journal of stem cells* 2014; 6: 144-152
32. Egusa H, Sonoyama W, Nishimura M, Atsuta I, Akiyama K. Stem cells in dentistry--part i: Stem cell sources. *Journal of prosthodontic research* 2012; 56: 151-165
33. Mao JJ, Prockop DJ. Stem cells in the face: Tooth regeneration and beyond. *Cell stem cell* 2012; 11: 291-301
34. Wu XB, Tao R. Hepatocyte differentiation of mesenchymal stem cells. *Hepato-biliary & pancreatic diseases international: HBPD INT* 2012; 11: 360-371
35. Ferroni L, Gardin C, Tocco I, Epis R, Casadei A, Vindigni V, Mucci G, Zavan B. Potential for neural differentiation of mesenchymal stem cells. *Advances in biochemical engineering/biotechnology* 2013; 129: 89-115
36. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The international society for cellular therapy position statement. *Cytotherapy* 2006; 8: 315-317
37. Gronthos S, Mankani M, Brahimi J, Robey PG, Shi S. Postnatal human dental pulp stem cells (dpSCs) in vitro and in vivo. *Proceedings of the National Academy of Sciences of the United States of America* 2000; 97: 13625-13630
38. Tatullo M, Marrelli M, Shakesheff KM, White LJ. Dental pulp stem cells: function, isolation and applications in regenerative medicine. *J Tissue Eng Regen Med.* 2014; doi: 10.1002/term.1899
39. Shi S, Robey PG, Gronthos S. Comparison of human dental pulp and bone marrow stromal stem cells by cDNA microarray analysis. *Bone* 2001; 29: 532-539
40. Gronthos S, Brahimi J, Li W, Fisher LW, Cherman N, Boyde A, DenBesten P, Robey PG, Shi S. Stem cell properties of human dental pulp stem cells. *Journal of dental research* 2002; 81: 531-535
41. Reynolds AJ, Jahoda CA. Cultured human and rat tooth papilla cells induce hair follicle regeneration and fiber growth. *Differentiation; research in biological diversity* 2004; 72: 566-575
42. Iohara K, Zheng L, Ito M, Tomokiyo A, Matsushita K, Nakashima M. Side population cells isolated from porcine dental pulp tissue with self-renewal and multipotency for dentinogenesis, chondrogenesis, adipogenesis, and neurogenesis. *Stem cells (Dayton, Ohio)* 2006; 24: 2493-2503
43. Ishkitiev N, Yaegaki K, Calenic B, Nakahara T, Ishikawa H, Mitiev V, Haapasalo M. Deciduous and permanent dental pulp mesenchymal cells acquire hepatic morphologic and functional features in vitro. *Journal of endodontics* 2010; 36: 469-474
44. Yang R, Chen M, Lee CH, Yoon R, Lal S, Mao JJ. Clones of ectopic stem cells in the regeneration of muscle defects in vivo. *PLoS one* 2010; 5: e13547
45. Sugiyama M, Iohara K, Wakita H, Hattori H, Ueda M, Matsushita K, Nakashima M. Dental pulp-derived cd31(-)/cd146(-) side population stem/progenitor cells enhance recovery of focal cerebral ischemia in rats. *Tissue engineering Part A* 2011; 17: 1303-1311
46. Govindasamy V, Ronald VS, Abdullah AN, Ganesan Nathan KR, Aziz ZA, Abdullah M, Zain RB, Kasim NH, Musa S, Bhone RR. Human platelet lysate permits scale-up of dental pulp stromal cells for clinical applications. *Cytotherapy* 2011; 13: 1221-1233
47. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S. Shed: Stem cells from human exfoliated deciduous teeth. *Proceedings of the National Academy of Sciences of the United States of America* 2003; 100: 5807-5812
48. Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahimi J, Young M, Robey PG, Wang CY, Shi S. Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* 2004; 364: 149-155
49. Seo BM, Miura M, Sonoyama W, Coppe C, Stanyon R, Shi S. Recovery of stem cells from cryopreserved periodontal ligament. *Journal of dental research* 2005; 84: 907-912
50. Morszczek C, Gotz W, Schierholz J, Zeilhofer F, Kuhn U, Mohl C, Sippel C, Hoffmann KH. Isolation of precursor cells (pcs) from human dental follicle of wisdom teeth. *Matrix biology: journal of the International Society for Matrix Biology* 2005; 24: 155-165
51. Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, Huang GT. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: A pilot study. *Journal of endodontics* 2008; 34: 166-171
52. Yao S, Pan F, Prcic V, Wise GE. Differentiation of stem cells in the dental follicle. *Journal of dental research* 2008; 87: 767-771
53. Huang GT, Sonoyama W, Liu Y, Liu H, Wang S, Shi S. The hidden treasure in apical papilla: The potential role in pulp/dentin regeneration and bioroot engineering. *Journal of endodontics* 2008; 34: 645-651
54. Zhang Q, Shi S, Liu Y, Uyanne J, Shi Y, Shi S, Le AD. Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. *Journal of immunology (Baltimore, Md : 1950)* 2009; 183: 7787-7798
55. Kishi T, Takao T, Fujita K, Taniguchi H. Clonal proliferation of multipotent stem/progenitor cells in the neonatal and adult salivary glands. *Biochemical and biophysical research communications* 2006; 340: 544-552
56. De Bari C, Dell'Accio F, Vanlauwe J, Eyckmans J, Khan IM, Archer CW, Jones EA, McGonagle D, Mitsiadis TA, Pitzalis C, Luyten FP. Mesenchymal multipotency of adult human periosteal cells demonstrated by single-cell lineage analysis. *Arthritis and rheumatism* 2006; 54: 1209-1221
57. Marrelli M, Paduano F, Tatullo M. Cells isolated from human periapical cysts express mesenchymal stem cell-like properties. *International journal of biological sciences* 2013; 9: 1070-1078
58. van den Dolder J, Farber E, Spauwen PH, Jansen JA. Bone tissue reconstruction using titanium fiber mesh combined with rat bone marrow stromal cells. *Biomaterials* 2003; 24: 1745-1750
59. Krebsbach PH, Mankani MH, Satomura K, Kuznetsov SA, Robey PG. Repair of craniotomy defects using bone marrow stromal cells. *Transplantation* 1998; 66: 1272-1278
60. Schantz JT, Huttmacher DW, Lam CX, Brinkmann M, Wong KM, Lim TC, Chou N, Guldberg RE, Teoh SH. Repair of calvarial defects with customised tissue-engineered bone grafts ii. Evaluation of cellular efficiency and efficacy in vivo. *Tissue engineering* 2003; 9 Suppl 1: S127-139
61. Shi S, Bartold PM, Miura M, Seo BM, Robey PG, Gronthos S. The efficacy of mesenchymal stem cells to regenerate and repair dental structures. *Orthodontics & craniofacial research* 2005; 8: 191-199
62. Kim SH, Kim KH, Seo BM, Koo KT, Kim TI, Seol YJ, Ku Y, Rhyu IC, Chung CP, Lee YM. Alveolar bone regeneration by transplantation of periodontal ligament stem cells and bone marrow stem cells in a canine peri-implant defect model: A pilot study. *Journal of periodontology* 2009; 80: 1815-1823
63. Rodriguez-Lozano FJ, Insausti CL, Iniesta F, Blanquer M, Ramirez MD, Meseguer L, Meseguer-Henarejos AB, Marin N, Martinez S, Moraleda JM. Mesenchymal dental stem cells in regenerative dentistry. *Medicina oral, patologia oral y cirugia bucal* 2012; 17: e1062-1067
64. La Noce M, Paino F, Spina A, Naddeo P, Montella R, Desiderio V, De Rosa A, Papaccio G, Tirino V, Laino L. Dental pulp stem cells: State of the art and suggestions for a true translation of research into therapy. *Journal of dentistry* 2014; 42: 761-768
65. Galler KM, D'Souza RN. Tissue engineering approaches for regenerative dentistry. *Regenerative medicine* 2011; 6: 111-124
66. Smith EL, Kanczler JM, Gothard D, Roberts CA, Wells JA, White LJ, Qutachi O, Sawkins MJ, Peto H, Rashidi H, Rojo L, Stevens MM, El Haj AJ, Rose FR, Shakesheff KM, Oreffo RO. Evaluation of skeletal tissue repair, part 1: Assessment of novel growth-factor-releasing hydrogels in an ex vivo chick femur defect model. *Acta biomaterialia* 2014; doi: 10.1016/j.actbio.2014.06.011
67. Smith EL, Kanczler JM, Gothard D, Roberts CA, Wells JA, White LJ, Qutachi O, Sawkins MJ, Peto H, Rashidi H, Rojo L, Stevens MM, El Haj AJ, Rose FR, Shakesheff KM, Oreffo RO. Evaluation of skeletal tissue repair, part 2: Enhancement of skeletal tissue repair through dual-growth-factor-releasing hydrogels within an ex vivo chick femur defect model. *Acta biomaterialia* 2014; doi: 10.1016/j.actbio.2014.05.025
68. Graziano A, d'Aquino R, Cusella-De Angelis MG, De Francesco F, Giordano A, Laino G, Piattelli A, Traini T, De Rosa A, Papaccio G. Scaffold's surface geometry significantly affects human stem cell bone tissue engineering. *Journal of cellular physiology* 2008; 214: 166-172
69. Yang X, Han G, Pang X, Fan M. Chitosan/collagen scaffold containing bone morphogenetic protein-7 DNA supports dental pulp stem cell differentiation in vitro and in vivo. *Journal of biomedical materials research Part A* 2012; doi: 10.1002/jbma.34064
70. Yang X, van der Kraan PM, Bian Z, Fan M, Walboomers XF, Jansen JA. Mineralized tissue formation by bmp2-transfected pulp stem cells. *Journal of dental research* 2009; 88: 1020-1025
71. d'Aquino R, De Rosa A, Lanza V, Tirino V, Laino L, Graziano A, Desiderio V, Laino G, Papaccio G. Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes. *European cells & materials* 2009; 18: 75-83