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Review Article

Dental Medicine Applications of Stem Cells

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Abstract

Investigation using stem cells started in 1960 with the discovery of the first viable source of these cells: bone marrow. Several studies detailed their role in tissue renewal and regeneration after damage, as well as its characterization as a heterogeneous group of undifferentiated cells called 'clonogenic' (defined by their self-renewal capacity and differentiation into mature cells).

Nowadays, these cells have gained popularity as a therapeutic alternative for many diseases such as diabetes, congenital abnormalities, nervous tissue injuries, Parkinson, pulpal exposure, periodontal defects, loss of teeth, Alzheimer and other degenerative disorders.

Studies have also helped to identify five populations of stem cells of dental origin (DPSCs, SHEDs, DFPCs, SCAPs and PDLCs). Additional research into stem cell therapy is still required, however, the use of these cells in tissue engineering is considered obligatory as it has the potential to provide new tools for regenerative medicine via the combined use of biomaterials and biological agents. It offers highly desirable biomimetic principles and, as stated by 'conservation practitioners', regenerative medicine is the most promising way for the development of personalized medicine.

For these reasons, results on stem cell research have triggered much debate and high expectations in the field of tissue engineering.

Keywords: "Stem Cells", "Dental Stem Cells", "Tissue Engineering using Dental Stem Cells".

Introduction

Stem cells studies started fifty years ago, when researchers found out that the bone marrow is a viable source of these cells. Nowadays, due to their properties, stem cell implementation in Tissue Engineering looks promising [1].

Tissue Engineering is an interdisciplinary field that combines engineering, materials and biological sciences backgrounds towards the development of therapeutic strategies that restore, keep, substitute or improve biological functions. Promoting this knowledge potentiates the development of new treatment possibilities, in most biomedical areas, including Dental Medicine [2].

Stem cells have the capacity of division, giving rise to an identical cell able to generate different tissue types [3]. The pluripotent nature of these cells is the basic concept of regenerative therapies, since they can differentiate into cells belonging to all types of tissues of the body [4]. The big hope is that stem cells produced in big amounts and later transferred, give rise to new cells that will be used to replace lost or damaged tissues. This tissue regeneration capacity is the focus of the emerging field of personalized medicine. The main disadvantages of this are ethical dilemmas (depending on the origin of the cells), the carcinogenic potential and the risk of immunological rejection [5].

Recent studies showed the presence of stem cells in dental tissues, with an expansive differentiation potential concerning mesodermal and ectodermal lines [6-8]. These cells can be collected from dental pulp, periodontal ligament, apical papilla and precursor cells of dental follicle [6-12].

All these exciting discoveries put Dental Medicine in the frontline of a potential revolution on therapeutic options. The dental doctor plays an important role in the recovery and use of dental stem cells (obtained from deciduous and permanent teeth) as well as in the execution of regenerative medical therapies.

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To benefit from therapies able to save lives through the use of stem cells, it is essential to know all the surroundings, advantages and limitations.

Dental Stem Cells

Post-natal stem cells can have different origins, including teeth [13]. The first dental stem cell described in the literature was isolated from dental pulp and was called Dental Pulp Stem Cell (DPSC) [6]. After that, three other groups of stem cells were isolated and characterized: Stem Cells from Human Exfoliated Deciduous Teeth (SHEDs) [7], Periodontal Ligament Stem Cells (PDLSCs) [8] and Stem Cells from the Apical Papilla (SCAPs) [12]. More recently, were identified four more groups: Dental Follicle Precursor Cells (DFPCs) [11], Alveolar Bone-Derived Mesenchymal Stem Cells (ABMSCs) [14], Tooth Germ Progenitor Cells (TGPCs) [15] and Gingival Mesenchymal Stem Cells (GMSCs) [16].

Published data show that DPSCs, SHEDs, DFPCs, PDLCs, ABMSCs, TGPCs and GMSCs fulfil all the requirements to be considered stem cells: adhere to plastic; express the cellular markers CD73, CD90 and CD105; and have the capacity to differentiate into osteoblasts, adipocytes and chondrocytes both *in vitro* and *in vivo*. SCAPs, although they present mesenchymal markers, it was still not possible to show chondrogenic differentiation [9].

Comparative Odontogenic Capacity Between Bone Marrow And Dental Pulp Stem Cells

BMMSCs are in close proximity to hematopoietic stem cells and were initially called "bone marrow stromal cells" [17]. Their ability to form clonogenic colonies is similar to hematopoietic stem cells, and explains their self-renewal capacity. These cells can be isolated from suspensions obtained by aspiration of the bone marrow [18] and present migration capacity [19,20]. Morphologically, they present a considerable heterogeneity in terms of size, morphology, histochemistry, proliferation, cellular maturity and development potential [6,21-23]. They are able to differentiate into osteoblasts, chondrocytes or retinal cells, which means that they have are able to differentiate into cells of at least two different germ layers [18,24,25]. Some studies have reported the possibility of myogenic differentiation [26-29].

Due to certain difficulties in obtaining BMMSCs (including pain, morbidity and low numbers of cells in each crop), alternative sources of stem cells have been sought [9]. Recently, dental cell therapies have been discussed through the combination of non-dental mesenchymal stem cells and dental stem cells [30-32]. Following the accomplishment of numerous studies in this scope, it was concluded that BMMSCs can be reprogrammed in order to constitute a valid possibility for the development of dental tissue engineering [33]. These data are promising, but the use of dental stem cells - such as DPSCs - seems more feasible since these cells are closely related to mature dental tissues [34].

DPSCs and BMMSCs, due to their ability to differentiate into multiple mesenchymal cell lines, are excellent candidates for tissue engineering with the aim of reconstructing the entire dental element and bone tissue respectively [6,35-38]. Previous studies have shown that dentinogenesis and osteogenesis mediated by DPSCs and BMMSCs occur through distinct mechanisms, promoting the differentiated organization of different dental elements and bone [37]. Studies have shown that both DPSCs and BMMSCs can be used to generate dental structures under appropriate conditions [6,31,33,35,37,39]. Knowledge is limited concerning differences in odontogenesis when mediated by different cell groups, although DPSCs appear to be more competent than BMMSCs in this function [40].

Dental Medicine Applications Of Stem Cells

Losing teeth is a common disease that occurs frequently in elderly populations, affecting negatively the masticatory efficiency, speech, aesthetics and self-esteem. According with data from World Health Organization, dental caries is present in most countries all over the world (with 100% incidence in certain populations), serious periodontal diseases affect 5-20% of adult populations and incidence of total edentulousness has been estimated between 7% and 69% [41].

Therapeutic strategies used so far are mainly centered in artificial material or non-biological implants that might reduce life quality, due to the limited physiological functions and, in some cases, can even lead to immunological rejection [42]. The development of a biomimetic technique that allows the formation of substitution teeth, constitutes a highly desirable approach for permanent replacement of teeth *in situ* [43]. This strategy was used by Modino and Sharpe [43] where aggregates of stem cells of mice were inserted in the mandibular primordium. These researchers performed *in vitro* and *in vivo* assays in adult mice and observed normal sized teeth formation that were connected to the underlying bone. This study shows that developing tooth germs might be successfully implanted into the gingiva of patients.

In order to develop cellular therapies based on regenerative medicine, it is essential to understand: the self-renewal mechanisms that allow the *in vitro* regulation of adult stem cells growth, giving rise to sufficient number of cells for different applications [40,44,45]; the regulation of stem cells during differentiation and production of a specific tissue [46]; the interactions between stem cells and the immunological system [47]; how to perform monetarization of the transformed and expanded stem cells [48].

Periodontal Regeneration

Periodont is a group of specialized tissues that surrounds and supports teeth in order to keep them in the jaw. Periodontitis is an inflammatory disease that affects periodont and results in irreversible loss of teeth insertion in the connective tissue and supporting alveolar bone. Procedures to achieve periodontal regeneration

have included root surface conditioning, bone graft placement, guided tissue regeneration and growth factor application. Sander and Karring [49] used the guided tissue regeneration procedure for healing of periodontal lesions in monkeys. However, current regenerative procedures have limitations in attaining complete and predicable regeneration, especially in advanced periodontal defects [49].

The challenge for tissue engineering, in this particular case, consists on the use of different populations of dental stem cells in order to replicate the key events of periodontal development and promote sequential regeneration of periodont [50].

Four factors must be respected in order to promote a successful periodontal regeneration: it is essential to check the epithelial sealing to avoid migration of epithelial cells to periodontal defects; new acellular cementum must be regenerated at the root surface; height of alveolar bone must be re-established and new Sharpey fibers must be inserted in the newly formed cementum [51]. A successful outcome of periodontal tissue engineering also requires the following essential factors: an adequate supply of appropriate progenitor cells with the capacity to differentiate into the required mature tissue-forming phenotypes, including osteoblasts, cementoblasts and fibroblasts; the appropriate signals to modulate cellular differentiation and tissue neogenesis; and a conductive three-dimensional extracellular matrix scaffold to support and facilitate these processes [52]. Additionally, angiogenic signals, promoting new vascular networks, are essential to provide the nutritional base for tissue growth and homeostasis. Furthermore, appropriate mechanical loading is required for the development of highly organized, functional periodontal ligament fibers. Finally, as periodontal defect sites are accompanied by a microbial load, strategies to control infection and host response are required for optimum periodontal regeneration [53].

Although research in this field usually involves the use of non-dental stem cells, regarding their differentiation potential, PDLSCs constitute an ideal candidate for periodontal tissue engineering [54]. One approach to periodontal regeneration involves incorporation of progenitor cells in a periodontal defect [55]. Published data indicate that these cells, when expanded *in vivo* and transplanted into mice (using hydroxyapatite phosphate and tricalcium phosphate) are able to regenerate fibers of periodontal ligament and a layer similar to acellular cementum [8, 56]. The main doubt related with this technique concerns the maintenance of integrity and function of regenerated periodont [57].

Although direct application of PDLSCs looks promising, it is still in its stage of infancy, and more work is needed in this area to validate results. Stem cell assistance in periodontal regeneration technique could overcome the limitations and concerns of *ex vivo* cell culture techniques as they are costly and sensitive and there is chance of loss of stem cells during cell passage, genetic alteration and tumorigenic potential [58]. The autologous stem cell assistance in periodontal regeneration technique has emerged as a constructive avenue in the treatment of periodontal osseous defects. In a recent study, Vandana et al. [59] harvested autologous PDLSCs for regeneration of intraosseous periodontal defects, bypassing *ex vivo* culture. These authors obtained successful clinical and radiographic parameters like gain of clinical attachment, decreased probing pocket depth and satisfactory defect fill of intraosseous defects when evaluated during a one-year period [59].

At present, the limitations are the uncertainty on the number and viability of cells transplanted immediately after scraping the tissues from the root surfaces of extracted teeth. For now, lack of histological evidence and *in vitro* analysis of stem cell characterization and osteogenic potential are some important shortcomings [59].

Pulp Regeneration

Dental pulp regeneration would change current practice of replacing infected pulp by inorganic materials (devitalization).

According with the characteristics of cell sources, if the goal is to regenerate dental pulp the best dental cellular sources are SHEDs and DPSCs [60]. The protocol established by Yan et al. [42] for this purpose consists on isolation of DPSCs and SCAPs from human third molars, cover these cells with a matrix of copolymer (poly-DL-lactide and glicolide) and insert this mixture into the canal space previously emptied [42]. A study performed in mice to evaluate this procedure allowed to see that, after 3-4 months, the root canal had been completely filled by pulp tissue with well-established vascularization and a continuous layer of tissue similar to mineralized dentine had been deposited over the existent dental walls in the canal [42].

Dentin Regeneration

Regenerative property of the pulp-dentin complex mainly depends on the formation of tertiary dentin (reparative dentin).

DPSCs are the better type of stem cells to be used on tertiary dentinogenesis [6,61-63]. They migrate, proliferate and differentiate into odontoblasts, which then synthesize matrix to form the tertiary dentin at the damaged sites [64].

There are two different approaches implemented in dentin regeneration by the use of tissue engineering techniques. The first approach includes a device which can be used as a filling material into a deep cavity of tooth with partial layer of dentin on top of the pulp. In this process, they used some growth factors or molecules that can form reparative dentin [65,66]. The second approach is to put scaffold on open pulp along with odontoblast-like cells to grow on it, and these cells will synthesize reparative dentin [67].

DPSCs have been cultured on a variety of scaffolds to engineer dentin tissues. There are several examples in literature on the use of these strategies as is the case of Wang et al. [63] that performed *in vitro* and *in vivo* (in rat models) experiments with DPSCs cells

overexpressing CCN3. This study showed that CCN3 can promote dentinogenesis by coordinating proliferation and odontoblastic differentiation of DPSCs. This is a promising therapeutic strategy in dentin regeneration.

Another example is work of Chiang et al. [65] who used an inorganic degradable biomaterial that acts as a growth factor reservoir to promote reparative dentinogenesis in HDPC cells and in rat animal models. In this study, scientists observed the formation of dentin-pulp tissue, concluding that this strategy has a high potential to form the reparative dentinogenesis *in vivo* [65].

Moreover, Zhang et al. [66] demonstrated that the vascular endothelial growth factor promotes reparative dentin formation both *in vitro* and *in vivo*. Data from the *in vivo* assays (rat models) indicated that this growth factor enhanced pulp cell proliferation and neovascularization, and markedly increased formation of reparative dentin in dental pulp.

However, the signalling pathways underlying the regulation of DPSCs in dentin regeneration remains largely unknown, limiting their effective application in dentin tissue engineering [68].

Apicogenesis and Apicification

Closing of the root apice of a permanent tooth occurs until 3 years after tooth eruption. The presence of irreversible pulp damage in an immature permanent tooth represents a clinical challenge and this justifies the appearance of the concepts of Apicogenesis and Apicification [69].

Apicogenesis is a root complementation therapy performed in teeth with pulp vitality and consists in the removal of infected coronal pulp, maintenance of vital root pulp and its protection with biocompatible material. Calcium hydroxide and mineral trioxide aggregate have been the chosen materials, but, so far, there is no ideal material since none of them is able to stimulate pulp tissue regeneration [70,71]. Apicification is a therapy based on closure induction of apical foramen in teeth having pulp necrosis and consists in deposition of a hard tissue barrier in the apice. This last technique is more desirable and ideally uses stem cells [69].

To perform apicification with stem cells, these cells have to be grown and expanded *in vitro* or *in vivo* to be used later on. If the expansion is done *in vitro*, cells are implanted and must adhere to the walls of the root canal (previously disinfected). The implanted tissue lacks the needed vascular supply and previous data describe that it is technically difficult to introduce the regenerated pulp tissue without damaging cells. The *in vivo* approach overcomes some problems concerning the re-introduction of cells and one of the possible techniques consists in growing SHEDs and endothelial cells in biodegradable matrixes and apply the substrate in human teeth implanted in immune-compromised mice. This was performed by Cordeiro et al. [72] and the obtained data showed that SHEDs differentiated *in vivo* (rat model) into odontoblast-like cells as well as into endothelial-like cells.

Regeneration of Dental Element

Nowadays clinical practice indicates tooth implants as the most advanced technique for tooth substitution. It is known that this approach presents several disadvantages, like requiring a minimal amount of existing bone and the absence of periodontal ligament that sustains masticatory forces. Due to these problems, scientists started to think about bio-tooth. It consists of a biological tooth that can be re-integrated in the jaw and execute normal functions of a natural tooth (including the regenerative capacity when there is damage). Several studies have shown that the bio-tooth can be rebuild from dental cells involved or not in matrixes, from dental pre/postnatal cells and even from non-dental cells [42].

The minimal requirement for an adequate biological substitution is the formation of minimal components necessary for the development of a functional tooth (that includes roots, periodontal ligament, nervous and blood supply) [57]. If the goal is to regenerate tooth root it is better to use a combination of SCAPs and PDLSCs [12]. Coronal replacement constitutes the lower source of problems because, although essential, synthetic tooth coronas are aesthetically and functionally compatible [57].

The main challenges are: to identify non-embryonic cell sources that show the same properties of dental germinative cells and to develop culture conditions that allow expansion and maintenance of the potential of these cells. All these becomes more challenging when tooth development requires two types of cells, epithelial and mesenchymal cells [73-75]. By fulfilling these criteria, it becomes possible to obtain a three-dimensional structure that is functional and differentiated, capable of avoiding transplant rejection [76].

As teeth are formed by the cooperation of two different tissues, tooth reconstruction requires the interaction between them [77]. The odontogenic potential present in the tooth epithelium, when combined with mesenchymal cells is responsible for morphogenesis and cyto-differentiation – leading to tooth formation [78-81]. This is only possible due to the plasticity characteristic of some adult tissues. Molecular mechanisms involved in the interaction between epithelium and mesenchyme involve diffusible proteins like bone morphogenic proteins, fibroblast growth factors and tumor necrosis factor [76].

After epithelial induction of mesenchyme, this one becomes an inductive tissue and retrieves the induction to the non-inductive epithelium. Tooth regeneration can then occur through the use of epithelial or mesenchymal cells that induce tooth development into the other type of cells [57]. That cellular interaction leads to the formation of a tooth primordium that is chirurgically transplanted. The formed tooth must show certain characteristics: functional odontoblasts, ameloblasts, pulp, cuspid formation and also roots and periodontal ligament [82].

Although *in vitro* cell culture techniques are being progressively developed and improved, it is still needed to find stem cell

populations that can replace embryonic tooth epithelium and tooth mesenchyme. DPSCs have been used on regeneration of partial tooth structures, however, due to their *in vitro* limited expansion, it was still not possible to promote regeneration of a tooth that is morphologically and functionally competent [6].

Complete tooth regeneration still faces several obstacles: abnormal establishment of tooth size, failure in root formation and absence of functional occlusion [9]. In order to overcome these problems, Sonoyama et al. [12] showed that the best would be to create a bio-root and complete the tooth by using a tooth corona. Still, literature reports that frequently there is a change in the bio-root dentin that leads to two thirds decrease in mechanical resistance [9].

Replacement of missing teeth with "natural" tissue engineered teeth in humans is some way off and indeed may prove too difficult, irreproducible or expensive. However, there is a real possibility that this will be achievable and, although there are no guarantees, what we learn along the way will be invaluable in our understanding of tooth development and stem cell biology [16].

Clearly, recent recognitions of dental stem cells and their role in making a bio-tooth provide a substantial basis upon which we can begin to explore their therapeutic potential at the preclinical level. However, making a bio-tooth with masticatory function and supportive tissues from dental stem cells may be much more complicated than expected. Key techniques must be developed to reproduce the highly specialized arrangements of dental stem cells that constitute a bio-tooth. Several issues involving in the making of a stem cell-mediated bio-tooth must be solved, including identification and 'stemness' maintenance of stem cells, dental morphogenesis, tooth type determination, odontogenic signal cascades, odontogenic epithelium availability, controllable bio-tooth growth and eruption, pulp revascularization and neural regeneration, and host-graft immune rejection in the jaws [70, 83-85]. Although there are many technical barriers to overcome, stem cell-based approaches to tooth reconstruction will, of necessity, provide an unpredictable applicative opportunity to treat tooth loss and other dental diseases [86].

Craniofacial Bone Regeneration

Congenital craniofacial deformities or caused by trauma are big challenges for clinicians, since commonly used surgeries present limited therapeutic outcomes. For this reason, new strategies based on the use of stem cells have been developed with the purpose of bone repair.

Several studies have been done where different types of stem cells were used for cranium-facial bone regeneration. A good example is a study of Alhadlaq and Mao [87] where a human-shaped mandibular condyle was tissue-engineered from rat mesenchymal stem cells encapsulated in a biocompatible polymer. These authors observed *de novo* formation of mandibular condyles, eight weeks after *in vivo* implantation [87].

Adipose derived stem cells are an alternative cell source and have been successfully applied to scaffolds, leading to *de novo* formation of bone in subcutaneous tissue, calvarial defects, and critical sized bone defects in murine models [88-90]. This strategy has been applied to human calvarial bone defects [83,91].

Other studies have used dental pulp stem cells for oromaxillofacial bone repair. d'Aquino et al. [92] applied this type of stem cells on collagen sponge scaffolds and showed that it can be an effective therapeutic strategy for bone defects restoration [92]. In another study, d'Aquino et al. [93] have shown that several cytotypes can be obtained from dental pulp stem cells owing to their multipotency and that transplantation of new-formed bone tissue obtained from dental pulp stem cells leads to the formation of vascularized adult bone and integration between the graft and the surrounding host blood supply [93]. This study has demonstrated that: DPSCs can be used for human mandible bone defect repair, the use of DPSCs on appropriate absorbable scaffolds produces and efficient biocomplex and collagen sponges can be considered an optimal support for stem cells in cell-guided regeneration [93]. That have given evidence that DPSCs can be used in a low-risk and effective therapeutic strategy for the repair of bone defects. Despite the optimal results, the flaws of this study reside mainly in the small number of patients enrolled. Longer patient follow-up would ascertain the lifespan of the regenerated bone [94].

Recent work of Chamieh et al. [95] focused on therapies using mesenchymal dental pulp stem cell seeded scaffolds for several areas of regenerative medicine, like cranio-maxillofacial surgery. The collagen scaffolds with these stem cells were able to increase osteogenic differentiation of these stem cells in a rat model having a calvarial defect contributing to the bone healing process [95].

Moreover, Maruyama et al. [96] showed that suture mesenchymal stem cells can be used for injury repair and skeletal regeneration. This work has identified those cells as skeletal stem cells with innate capacities to replace the damaged skeleton by the use of a cell-based therapy [96].

These studies suggest that optimal cell types and local factors are essential to make possible human application of these strategies.

Standardization Of SHEDs Collection, Isolation And Conservation

Success in collecting, isolating and conserving SHEDs is achieved when harvesting is done at the optimum developmental stage and stored safely until needed. Needless to say, this often means storing the cells for decades, which significantly increases the cost and difficulty of the technique [97]. To this end, among all sources of existing dental stem cells, SHEDs are the best alternative. Huang et al. [98] *in vitro* study concludes that: different isolation methods give rise to different populations or lineages of pulp cells during *in vitro* passage, direct contact of pulp cells with mechanically and chemically treated dentin may

promote pulp cells to differentiate into odontoblasts with processes extending into dentinal tubules and collagen matrix alone may not be a suitable scaffold for pulp tissue engineering [98]. The protocol for the isolation and conservation of these cells is presented below [97].

Dental Collection

Since the best alternative is the collection of stem cells from deciduous teeth and, in this case, the donor still does not have decision-making power, it must come from the parents. It is therefore essential to inform them that they should store their teeth in a sterile saline solution when removing the tooth and inform the "tooth bank". The tooth must have reddish dental pulp (which demonstrates that it has received blood flow until removal), which is indicative of cell viability. If the pulp is greyish, it is likely that the blood flow to the pulp has been compromised and therefore stem cells are in necrosis and therefore no longer viable. Teeth exhibiting class III or IV mobility often have decreased blood supply and are not candidates for collection of stem cells. For these reasons, it is preferable to extract deciduous teeth than to expect them to fall naturally. Moreover, stem cells should not be collected from apical abscesses, tumors or cysts. The dentist should inspect the extracted tooth to confirm the presence of healthy pulp tissue and transfer it into a hypotonic phosphate buffered saline flask, which provides nutrients and helps preventing tissue dehydration during transport. Viability of stem cells is dependent on time and transport temperature. The period of time from collection to arrival to the storage and processing unit should not exceed 40 hours. The tooth should be placed in the vial at room temperature in order to induce hypothermia which is desirable for transport - this procedure is described as support.

Isolation of Stem Cells

When the stem cell bank receives the tooth, the following protocol is initiated:

- Cleaning the tooth surface by repeated washing (three times) with Ca^{2+} and Mg^{2+} (PBSA) Dulbecco's phosphate buffered saline.
- Disinfection with Povidone Iodine and re-washing with PBSA.
- Separation of pulp tissue from the pulp chamber using small forceps or sterilized dental excavator.
- The tissue collected from the dental pulp is placed in a sterile petri dish, which has been washed at least three times with PBSA. The tissue is then digested with collagenase type I and dispenses for 1 hour at 37°C. Trypsin-EDTA may also be used.
- Isolated cells are passed through a 70μm filter to obtain single cell suspensions.
- Cells are cultured in a culture medium suitable for mesenchymal stem cells consisting of 2mM glutamine supplemented with 15% fetal bovine serum (FBS), 0.1mM L-ascorbic acid phosphate, 100 U/mL Penicillin, 100 μg/mL streptomycin at 37°C and 5% CO_2 gas. Usually, isolated colonies are visible after 24 hours.
- Different cell lines, such as odontogenic, adipogenic and neural, can be obtained according to the changes made to the culture medium. After verification of the success of the process, cell viability is confirmed to the donor's parents.

Storage of Stem Cells

To store dental stem cells, it is possible to use Cryopreservation (process of preserving complete cells or tissues by lowering the temperature to below zero) or Magnetic Freezing (this technology uses a weak magnetic field that reduces 6-7ºC the solidification point of the body). Storage is proceeded in Tooth Baking. Needless to say that this means potentially storing for decades, and the cost and technical difficulty of doing this properly makes stem cell therapy using one's own cells a still uncertain bet. Nowadays, tooth banking is still not very popular but the trend is catching up mainly in developed countries, like USA, UK, Thailand, Japan and Norway).

Potential Limitations And Challenges

Stem cell therapy represents a fascinating new approach for the repair of defective tissues or functions through the transplantation of live cells. However, multiple key parameters need to be optimized through clinical research such as the required stem cell density and availability, as well as appropriate strategies, for their use. Nowadays, biotechnology companies working on stem cell therapy are focused on developing and commercializing human stem cell technology in the emerging field of regenerative medicine to treat degeneration of major organ systems. There are dozens of companies that are trying to develop cell therapy, however there are lots of questions… Can companies produce all the required cells under current good manufacturing practices? How much does it cost to produce these cells and how high are the profit margins? Will anybody be able to afford the therapy [50]?

Another issue is the availability of the cells over time, that is, DPSCs or SHEDs are not available throughout a patient's lifetime. Even though DSC banking may constitute a potential solution by cryopreserving them for future use, such a possibility is not only time-consuming and costly but limits their use in clinical applications [99].

Culture conditions, dose of cell infusion, number of infusions, and route of cell delivery need to be optimized. Appropriate double-blind randomized clinical trials still need to be performed to confirm the true regenerative power of these stem cells. The immune rejection is a major risk for cell transplantation, so biosecurity is a crucial point for cell therapy, requiring control of cell transformation and a protocol for cellular biobanking. The risk

of transmission of bacterial, viral, and fungal or prion pathogens may lead to life-threatening reactions [100]. Manufacturing of cell-based medicinal products inevitably does not include terminal sterilization, purification, viral removal, and inactivation. Hence, viral and microbial safety is a pivotal risk factor associated with the use of non-autologous cells including stem cells. Further research and understanding of the stem cells physiology may enhance development of novel and more competent therapeutic approaches and will help in fulfilling the huge impact that stem cell therapy will have for future healthcare [68].

Finally, it is important to say that most of studies are made in animal species. Cellular characteristics between species (for instance, human vs rodent) appear to be significantly different when they are cultured *in vitro*. Rodent cells show high viability, stable growth and proliferation, and toughness to stimulation during culture (artificial conditions). Thus, it is easier to isolate and establish primary cells or new cell lines from harvested rodent tissue than from human samples. However, stem cell research, especially in regenerative medicine, may require culture experiments using human sources. Hence, data on human stem cell characteristics is necessary for safe and reliable clinical application. Further studies using human cells and the resulting accumulation of knowledge will lead us to the development of innovative cell-based therapy for dental and systemic diseases. Given the immense amount of funding provided for stem cell research, research and development will undoubtedly advance in the future, and patents and research reports will continue to appear [101].

Conclusion

These last years a growing enthusiasm by the biomedical community is felt concerning stem cell therapies. These are based on the theory that when healthy stem cells are injected into patients, they automatically go to damaged tissues, stimulating their regeneration [84].

It is possible to conclude that stem cells of dental origin constitute a viable alternative to regeneration. Cellular Therapy can benefit from this new source of cells, since they are self-renewal, multipotent, with simple isolation and able to give rise to different cell lines [85,102].

Advances in adult stem cell biology show up like an impulse for the clinical applications. Developments in isolation and understanding of tooth stem cells together with the development of biomaterial sciences, led to consider these cells a powerful tool for Regenerative Dental Medicine (capacity of tooth cells to replace other tooth cells) and, later on, for general medicine (capacity of tooth cells to differentiate into non-dental cells). In terms of applications in Dental Medicine, it is noticeable the differentiation capacity of these cells into diverse tooth components, allowing individual or generalized regeneration of the tooth [103].

The minimal intervention required to obtain these cells, absence of autoimmune rejection and repair efficacy (according with an analysis cost-benefit), constitute the main advantages to support therapies in the near future [60]. Although the advantages are numerous, certain negative aspects must be considered. It is required to evaluate the probability of development of a carcinogenic process. Cost associated with the therapeutic use of these cells is still very high, however history has shown that most revolutionary technologies become accessible when they start to be popular [104].

Nowadays, it is difficult to predict the impact that Tissue Engineering using tooth stem cells will have in the future. However, independently of this doubt, it is possible to conclude the great potential of these cells and the wish to use it efficiently. This requires better understanding of the mechanisms of self-renewal in order to sufficiently regulate adult stem cell growth *in vitro* (to generate required cell numbers needed for different applications); the regulation of stem cells during differentiation and maturation into tissue-specific cell types, as well as during wound healing; the interactions between stem cells and the immune system, in particular, regarding use of allogeneic cell populations; and mechanisms needed to control and prevent *ex vivo*-expanded mesenchymal stem cells from transformation [105].

Clinical trials previously performed suggest that additional information about tooth stem cells and their medical applications is essential to understand the therapeutic real efficacy, cell survival, integration, functionality and safety in long terms.

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