Regeneration of tissues or organs using stem cells is an advancing treatment modality in the recent days. Stem cell therapy normally utilizes autologous or heterologous tissues, which is isolated, expanded, propagated, and characterized for the potential clinical application. It is gaining momentum due to lack of organ donors which makes it imperative and unavoidable recent days. Understanding the mechanism of pluripotency in embryonic stem cells (ESCs) was the inspiration for induced pluripotent stem cells (iPSCs) regeneration, which gave an impetus for this discovery to convert adult cells into iPSCs with known candidate transcriptional factors. Unlike adult stem cells (ASCs), iPSCs behave like ESCs, which makes it superior than ASCs in regenerating tissues or organs, thereby avoiding ethical concerns involved in ESCs. The biggest advantage of iPSCs is the patient-specific personalized tissue can be made, which revolutionized the regenerative medicine field. This brief review highlights the research and clinical implication of iPSCs with respect to dentistry.

Evolution

Stem cells, by definition, will have an ability for potency and self-renewal. They are broadly classified into ESCs, ASCs, and iPSCs, in which the first two types occur naturally whereas iPSCs are generated by reprogramming the differentiated cells. ESCs, though totipotent, can form all germ layers that have an ethical issue it needs for the destruction of inner mass of blastocyst, thereby killing the embryo. ASCs, which are widely used currently, were harvested from many tissues such as bone marrow, adipose tissue, umbilical cord, and dental pulp, and have less pluripotency than ESCs and iPSCs. The iPSCs have a tremendous advantage over ESCs that it does not have any ethical issues and still continues to behave like ESCs. To achieve pluripotency in matured cells, the underlying molecular mechanism and sequence of events leading to the change should be thoroughly studied. Numerous studies were carried out to know the exact mechanism of growth of an embryonic cell to form an organ. Reprograming of somatic cells was studied by researchers over decades, in which frog somatic cells were reprogramed after inoculating into an enucleated oocyte, which formed tadpole. Cloning of animals is also an excellent example of reprogramming.

Induced Pluripotent Stem Cell Generation

The first successful iPSCs generation was achieved using skin fibroblasts of mice with defined transcriptional factors such as...
c-Myc, Oct4, Sox2, and Klf4 using retrovirus. The first human iPSCs were developed using the similar approach. To generate iPSCs, 24 candidate genes were assessed, which include beta-catenin, c-Myc, Stat3, Grb2, Ecat1, Dppa5 (Esg1), Fbxo15, Nanog, Eras, Dmnt3l, Ecats, Gdf5, Sox15, Dppa4, Fib17, Sox2, Rex1 (Zfp42), UTF1, Tcl1, Dppa3 (Stella), Klf4, Sall4, and Oct3/4. Ten candidate genes were screened, out of which 24 genes and finally 4 genes were zeroed to generate iPSCs.

Oct3/4, Sox2, and c-Myc were commonly used to derive iPSCs using retrovirus by reprogramming. C-Myc can cause tumorigenesis by the activation of proto-oncogenes, which was confirmed by subsequent studies by researchers. The Myc family has three form of heterodimers, namely, C-Myc, N-Myc, and L-Myc. L-Myc with short N-terminal region showed less tumorigenesis than other heterodimers. Recent researchers prefer L-Myc for iPSCs generation than C-Myc. Apart from these candidate genes, other factors also involve in iPSCs' generation, among which, Lin28, a micro RNA regulator, has shown to accelerate the speed. It facilitates iPSCs' generation by cell cycle-dependent manner and overexpression of Oct3/4. In this cascading process, there will be an inhibition of p53 and p21 and overexpression of Lin28.

Araki et al. found that retroviral induction of transcriptional factors showed its division several times symmetrically maintaining its fibroblastic shape. Transformation in the morphology of cells happened within 48 h of retroviral induction of transcriptional factors, normally four factors (among which, C-Myc plays a major role) which convert them into ESC-like shape. Asymmetric division of one descendant of the cells will become iPSCs and the other descendant will undergo cell death. MEFs convert into the form of epithelial cells after losing its mesenchymal property, which is known as mesenchymal epithelial transition (MET). MET, which is normally found in organogenesis, involves a lot of transcriptional factors in tandem. On the other hand, epithelial mesenchymal transition (EMT) promoted by factor snail is downregulated by Sox2 and Oct3/4. Transforming growth factor beta which also promotes EMT is downregulated by C-Myc, thereby enhancing MET.

For clinical application, selection of appropriate donor cell type is mandatory which should be easy to obtain from the patients. Most commonly, fibroblasts and peripheral blood cells were used. It can also be generated from the fibroblasts of gingiva, buccal mucosa, and also from dental pulp. It involves forcible conversion of adult cells into pluripotent cells by inducing genes via vectors, normally retroviruses. Since it involves external factors and viruses, extreme care should be taken to avoid viral integration into the host, as well as tumor formation. Numerous viruses were used for integration, among which, Sendai virus-mediated iPSCs were a very efficient integration method in the conversion of fibroblasts and peripheral blood cells, but it is expensive and also has a risk of carrying residual viral matter. Lesser risk-integrating techniques are available in the recent days which include single polycistronic vector, PiggyBac transposon system, episomal vectors, and minicircle DNA free of transgenes and vectors. Nonintegrating vectors such as protein-induced iPSCs which do not require vectors are gaining popularity in the recent days, and also synthetic mRNA which has a higher conversion efficiency and lesser side effects is widely used by researchers.

Characterization of Induced Pluripotent Stem Cells

Ideally, iPSCs should be subjected to markers for characterization to understand its pluripotency, efficacy, and purity. Apart from cell surface markers, flow cytometry analysis was done to quantify the cells for their expression. In addition, alkaline phosphatase staining, polymerase chain reaction, and karyotyping for chromosome stability analysis of iPSCs should be done. Compulsory embryoid body formation and teratoma assay should be carried out on the subcutaneous tissue of nude mice to confirm the formation of three germinal layers. Chan et al. in their live cell imaging of iPSCS found three types of human iPSCs, depending on the expression of these cells for cell surface markers such as SSEA-4 and TRA-1-60 and their negativity for fibroblast marker. They also categorized iPSCs based on the retroviral silencing of the cells, thereby ideally programed cells can be isolated and used for therapeutic purposes.

General Applications of Induced Pluripotent Stem Cells

Numerous animal models were researched with relation to human-specific diseases, which necessarily need not cause the same symptoms and effect in humans. Cell cultures from human tissues are considered suitable for the disease-specific iPSC line models. In comparison with ESCs and ASCs, iPSCs can be generated for disease-specific, human-specific, and also different tissues of the same individual. Patient-specific iPSCs will have an advantage of retaining genetic and epigenetic memories and also reduce the chance of immune rejection and also host versus graft reaction. Parkinson’s disease models were effectively made using reprogramed fibroblasts of rats. With gene editing, it can also be extended to other diseases such as sickle cell anemia. Animal studies carried out for drug discovery can be substituted with iPSCs, which will be a cheaper and better alternative. It also has an advantage of real simulation of human disease and large-scale production.

The Induced Pluripotent Stem Cells from Oral and Maxillofacial Tissues

The iPSC generation was achieved by various tissues in the body starting from the skin fibroblasts to an array of varying tissues such as red blood cells, white blood cells, platelets, and neural tissue. It was also generated from dental tissue such as pulp, gingival fibroblasts, periodontal ligament, and buccal mucosa. Dental pulp can be easily accessed and retrieved without much morbidity from the discarded teeth. Dental pulp is found to be an excellent candidate for iPSC generation because it requires less factors than other tissues and also has better reprogramming capacity. The colony-forming frequency is higher with dental pulp tissue when comparing with other
mesenchymal stem cells (MSCs) of bone marrow. Dental pulp stem cells proliferated rapidly in culture and also showed population doubling up to 100 times and beyond. Stem cells from exfoliated deciduous teeth have an ESCs-like quality and also have superior reprogramming ability as it readily expresses OCT4, Nanog, C-Myc, SSEA-3, SSEA-4, TRA-1-60, and TRA-1-8. The iPSCs were also generated from the third molars using Lentivirus and three candidate transcriptional factors KLF4, SOX2, and OCT3/4. It also showed the properties of ESCs which include gene expression, morphology, surface markers, in vitro differentiation, teratoma formation, and DNA methylation. Dental stem cells differ from other MSCs, having high colony-forming ability and more population doubling before it attains senescence.

Clinical Applications of Induced Pluripotent Stem Cells in Dentistry

The clinical translation of iPSC technology is gaining momentum in the recent days. The first clinical application of iPSCs has got approval in Japan for the human application of age-related macular degeneration.

This great technology has reached pinnacle to treat genetic diseases and other systemic diseases. Application of iPSCs for treating oral and maxillofacial diseases is in its early stages, which needs more research and trials for clinical translation. The clinical application of iPSCs in dentistry is in its formative stage, which will get a huge potential in future. The biggest edge of iPSCs over ESCs and MSCs is that it can be tailored and generated for the patients with different genetic and epigenetic backgrounds. Application of iPSCs in dentistry mainly involves regeneration of teeth, its parts, and its allied structures such as periodontium and alveolar bone. The complex structures in the oral and maxillofacial region include skeletal muscles, cranio-facial bones, skin, tempo-mandibular joint, nerves, and salivary glands. Various investigators were able to regenerate all these structures in some human models and in most of the animal models using MSCs and ESCs. It can be conveniently extended to iPSC, which has a huge potential because of the patient-specific property.

Epidermis with all layers and cornification were formed using iPSCs, which formed keratinocytes that were layered on type I collagen matrix. Furthermore, in animal models with iPSCs, researchers were able to form skin with all appendages. Skeletal muscle regeneration was achieved by iPSCs on animal models which showed myogenic lineage differentiation and also increased isometric tetanic force of tibialis anterior muscle in mice. Human dermal fibroblasts were reprogrammed to cells which have electrophysiological character similar to neurons. These in vitro studies on animal and human iPSCs prove it to be a fitting tool for the generation of skin, muscles, and nerves of the facio-maxillary region.

The iPSCs were able to form ameloblast- and odontoblast-like cells with bone morphogenetic protein-4 (BMP-4) induction, confirming that BMP-4 plays a major role in differentiating iPSCs into odontogenic differentiation. Enamel matrix derivatives with iPSCs have shown to regenerate periodontal tissues, which include cementum, periodontal ligament, and alveolar bone. MSCs were able to generate tooth and tooth-like structures by various researchers on laboratory animals. Tooth regeneration requires three factors, namely, stem cells, scaffold, and morphogenetic signals. The iPSC technology will be the best source for stem cells used for tooth regeneration and can be procured from the same individual. Dental pulp will be an ideal choice for iPSCs' generation and it is also available from vestigial teeth-like third molars. The complete human tooth regeneration will take few more years to be a reality and replacement for dental implants.

Regeneration of craniofacial defects using MSCs was studied by various researchers, which rendered promising results. Tang et al. in their study were able to regenerate bone using iPSCs derived from MSC seeded on calcium phosphate scaffold. CD34+ cells of the bone marrow were taken for the study, which were exogenously maneuvered by vector pEB-C5 to generate iPSCs. It will be a better technique for osteogenic differentiation as it can be generated throughout life. Autologous chondrocyte implantation (ACI), a novel technique, is widely practiced by orthopedists for cartilage repair, which is a major boon for sports injuries. ACI is a successful regenerative procedure approved by the European Medicines Agency and the U.S Food and Drug Administration. It is a labor-intensive procedure which can be simplified by differentiating autologous chondrocytes from iPSCs. Boreström et al. were able to regenerate cartilage using human autologous chondrocyte by RNA-based technology and were able to generate foot print-free tissue, which is free of viral or reprogramed molecules. This has given an impetus for clinicians for the regeneration of cartilage, especially for tempo-mandibular joint defects and arthritis.

Management of oral cancer is one of the challenging areas which needs multidonal approach with average treatment outcome and substantial morbidity and mortality. Cancer immunotherapy utilizes autologous immune cells such as natural killer (NK) cells, anti-tumor monoclonal antibodies, and adoptive transfer for the regression of solid tumors. Among which, NK cells play a major role in immunotherapy, while adoptive transfer involves isolation and expansion of T-cells in vitro and putting back into the patients. The role of iPSC in immunotherapy is surmountable, and the generation of large-scale NK cells with this technology has a great future ahead. The research in the field of iPSC will be perpetual to achieve better treatment modality for oncology, especially oral cancer, and the biggest advantage is of its patient-specific nature.

Conclusions

Since the discovery of iPSCs, it has grown leaps and bounds and its contribution to dentistry is ostentatious. Clinical translation of it is in its infancy, which needs many experimental disease models for varying array of dental diseases ranging from developmental to acquired diseases. The clinical application of iPSC is hampered by patient safety issues such as teratoma
formation and viral integration, which needs to be addressed by strict guidelines for efficient clinical translation and outcome. The lacunae between iPSC research and its clinical translation have to be shortened by numerous clinical trials. The escalation of expenses involved to encompass the standard operating procedures and good manufacturing practice for safe clinical outcome has to be addressed. With all shortcomings, iPSCs have a surmountable capability to take regenerative medicine to the next level because of its patient-specific nature. The iPSC technology will be invaluable in the area of dentistry in future for its applications such as regeneration of maxillofacial, dental tissues, and also genetic disorders involved in the field. It is noteworthy that this technology may be the key in treating the malignant disease of oral and maxillofacial region in the future.

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Conflicts of interest

There are no conflicts of interest.

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