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Banking of stem cells from deciduous teeth: from culture to clinics

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Abstract

Until recently, stem cells harvested from umbilical cord blood were the only storage option to guard against future illness or disease. Unfortunately, this opportunity comes only once and many parents regret missing it. Stem cells from human exfoliated deciduous teeth [SHED] are able to differentiate into a variety of cell types to a greater extent than many of the other post natal mesenchymal stem cells. SHED can be collected every time a milk tooth falls out. Banking SHED cells costs less than one third of the cost of cord blood storage. In addition, the proven facts that SHED can be directed to become pluripotential cells and generate solid tissue types, which cord blood cannot; has made them a simple, reasonable and convenient replacement to the umbilical cord blood. This review attempts to discuss the protocol, therapeutic applications and the present scenario of tooth banking.

Key Words: Stem cells, regeneration, deciduous teeth.

INTRODUCTION

Cells in the human body have a remarkable capacity to regenerate. The regenerative capability of a living creature was recorded as early as 330 BC, when Aristotle observed that a lizard could grow back the lost tip of its tail. Since then, there have been numerous attempts at understanding the regenerative capabilities of human being.[1] The discovery of the powerful cells that allow us to regenerate some tissues like blood and skin was first revealed when experiments with bone marrow in the 1950s established the existence of stem cells. For the first time in history, it became possible for physicians to regenerate a damaged tissue with a new supply of healthy cells by drawing on the unique ability of stem cells to create many of the body's specialized cell types. One cell type stems from the other and hence these were named 'stem cells'.[2]

Stem cells can be defined as a group of unique, clonogenic cells capable of self renewal with an intrinsic ability to transform into specialized cell types. The possibility of offering therapy for a number of incurable diseases has made stem cell research more fascinating.[3] Use of stem cells, either of embryonic or post natal derivation, to fabricate new replacement body tissues commonly involves expanding cells in culture, seeding them at various stages of differentiation with in scaffolds, which can then be implanted. Several "loci" or "niches" within the adult human body are colonized by a significant number of stem cells. However, access to these potential collection sites often is a limiting point. The interaction with biomaterials (scaffolds) is a further point that limits the therapeutic usage of stem cells.[4] Stem cells from human exfoliated deciduous teeth (SHED) have been demonstrated to answer all of these issues: easy access to the collection site, efficient extraction of stem cells, extensive differentiation ability; and the demonstrated interactivity with biomaterials makes them ideal for tissue reconstruction.[5]

SHED were isolated for the first time in 2003 by Miura et al, who confirmed that they were able to differentiate into a variety of cell types to a greater extent than many of the other post natal mesenchymal stem cells. The ethical constraints associated with the use of embryonic stem cells, together with the limitations of readily accessible sources of autologous postnatal stem cells with multipotentiality, have made SHED an attractive alternative for tissue engineering.[6,7]

Recent studies have shown that SHED has the ability to develop into more types of body tissues than other stem cells. Storing SHED holds enormous potential in future in the treatment of neuronal degenerative diseases like Alzheimer's, Parkinson's, Amylotropic lateral sclerosis; chronic heart conditions such as congestive heart failure and chronic ischemic heart disease; periodontal disease and facial bone regeneration.[8-14] SHED are able to generate solid tissue types that cord blood cannot- such as potentially repairing connective tissues, dental tissues, neuronal tissue and bone.[15,16] The application of stem cell therapy using SHED to treat these diseases is currently being pursued many researches around the world.

Many parents around the world are now considering the option of storing stem cells from their children in **stem cell banks**. The most common method is storing blood from babies' umbilical cords soon after birth. This opportunity comes only once and hence unfortunately is beyond the reach of many people. In wake of the recent advances discovering the multipotentiality of SHED, this review attempts to discuss the protocol, therapeutic applications and the present scenario of tooth banking.

Protocol of SHED cell banking

The technique is simple and non- invasive; involves collection, isolation and storage of SHED.

Step 1: Tooth Collection

The teeth especially primary incisors and canines with no pathology and at least one third of root left contain these unique

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types of cells in sufficient number. If the tooth is exfoliated at home, it can be stored in sterile saline solution in a refrigerator and can be brought to tooth bank for stem cell retrieval. The tooth exfoliated should have pulp red in color, indicating that the pulp received blood flow till the last moment, which is indicative of cell viability. Teeth that become very mobile due to disease or trauma, often have a compromised blood supply, and hence are not considered for stem cell retrieval. That is why recovery of SHED is preferred after an extraction rather than an exfoliation. The viability of stem cells is both time and temperature sensitive.[17]

Step 2: Stem Cell Isolation

When the tooth bank receives the vial, the following protocol is followed.[18]

1.The tooth surface is washed with phosphate buffer saline (PBSA) without calcium and magnesium ions.

2.Disinfection is done with povidone iodine and the tooth is again washed with PBSA.

3.The pulp tissue is isolated from the pulp chamber with a sterile endodontic file or a small forceps and placed in a sterile petri dish which was washed with PBSA.

4.The tissue is then digested with type I collagenase and diaspase for one hour at 37^{o}C. Trypsin- EDTA can also be used.

5.Isolated cells are passed through a 70-µm strainer to obtain single cell suspensions.

6.Then the cells are cultured in an α- Minimal Essential Medium, which consists of 2Mm glutamine, 15% fetal bovine serum (FBS), 0.1 Mm L-Ascorbic acid phosphate, 100U/ml Pencillin and 100 µg/ml streptomycin at 37 $^{\circ}$C and 5% CO_2 in air.

7.Isolated colonies are visible after 24 hours. Different cell lines can be obtained such as odontogenic, adipogenic and neural by making changes in the culture medium.

8.If the sample is contaminated, colonies of cells with morphology resembling epithelial cells or endothelial cells can be established, which disappear during course of successive cell passages.

9.The most reliable method of isolating stem cells in a culture is by using Fluorescent activated cell sorting (FACS) with STRO -1 or CD146.

Confirmation of current health and viability of these cells is given to the donor's parents.

Step 3: Stem cell Storage

At present, Cryopreservation and Magnetic freezing are the two approaches used in stem cell storage.

Cryopreservation –

The cells or whole tissues are preserved by cooling them to sub zero temperatures. At these freezing temperatures, the cell processes leading to cell death are stopped. SHED can be successfully cryopreserved over an extended period of time and when needed, carefully thawed to maintain their viability. The sample is divided into four cryo tubes and each part is stored in a separate location in cryogenic system so that even in the unlikely event of a problem with one of storage units, there will be another sample available for use. The cells are preserved in liquid nitrogen vapor at a temperature of less than -150ºC. This preserves the cells and maintains their latency and potency.[19,20]

Magnetic freezing

This technology is called CAS and exploits the little known phenomena that applying even a weak magnetic field to water or cell tissue will lower the freezing point of that body by up to 6-7 degrees Celsius. The idea of CAS is to completely chill an object below freezing point without freezing it, thus ensuring distributed low temperature without the cell wall damage caused by ice expansion and nutrient drainage due to capillary action, as normally caused by conventional freezing methods. Then, once the object is uniformly chilled, the magnetic field is turned off and the objects snap freezes. Using CAS, Hiroshima University claims that it can increase the cell survival rate in teeth as high as 83%.This compares to 63% for liquid nitrogen (-196 ^{0} C), 45% for ultra-cold freezing (-80 ^{0} C), and just 21.5% for a household freezer (-20 ^{0} C). Maintaining a CAS system is a lot cheaper than cryogenics and more reliable as well.[17]

Therapeutic applications of SHED

Orofacial Bone regeneration

SHED were able to induce bone formation and repair critical sized parietal defects in immunocompromised mice when implanted subcutaneously using hydroxyapetite/tricalcium phosphate as a carrier vehicle.[19] Although the stem cells from bone marrow (BMMSC) and adipose tissues,[20,21] which are of mesodermal origin, could repair critical size- bone defects in animal models, Seo et al hypothesized that neural crest derived SHED offer optimal orofacial bone repairing with a matched neural crest origin. The osteogenic differentiation potential of cultured SHED in vitro was demonstrated by positive expression of osteogenic growth factors like TGF β, FGF, VEGF and CC9 / MUC 18/ CD146 (early mesenchymal stem cell marker).[19]

There is a great demand for regeneration of orofacial defects caused by trauma, cancer, genetic malformation and periodontal diseases. Clinically, autologous grafts from long bones used to heal orofacial defects often result in an unfavorable outcome, which may be, due to the fact that the orofacial bones and long bones originate from the neural crest and mesoderm respectively.[22] Therefore, SHED, originating from neural crest cells, could be a superior accessible tissue resource for autologous transplantation from a practical perspective. Furthermore, SHED maintains a high proliferation rate than BMMSC and has the capacity to provide sufficient number of cells for clinical therapies.[19] It has a significant potential in the orofacial complex for fracture healing, bone augmentation, Temporomandibular Joint cartilage repair or regeneration, pulpal repair, periodontal ligament regenerationand osseointegration for implants.[7]

Induced pluripotential stem cells

Induced pluripotent stem cells (iPSCs) are adult cells that have been genetically reprogrammed to an embryonic stem cell–like state .They are a type of pluripotent stem cells artificially derived from a non-pluripotent cell, typically an adult somatic cell, by inducing a "forced" expression of specific genes and factors and are very similar to natural pluripotent stem cells, such as embryonic stem (ES) cells. iPSCs were first produced in 2006 from mouse cells and in 2007 from human adult fibroblasts by retroviral transduction of four transcription factors, namely Oct3/4, Sox2, Klf4, and c-Myc.

The major concern with the potential clinical application of iPSCs is their propensity to form tumors. A non-genetic method of producing iPSCs has been demonstrated using recombinant proteins, but its efficiency was quite low. Other approaches such

as using adenovirus or plasmids are generally thought to be safer than retroviral methods. During the culturing of human iPSCs, it was observed that the retroviruses used as vectors were strongly silenced in human iPS cells, indicating that these cells are efficiently reprogrammed and do not depend on continuous expression of the transgenes for self renewal. However, refinements to the existing methodology in near future can make them suitable for therapeutic use.[23]

Tamaoki et al tried to establish iPSCs from SHED and were successful in establishing five cell lines. They concluded SHED as an optimal source of iPS cells, since they are easily obtained from extracted teeth and can be expanded under simple culture conditions and suggested that the resulting iPSCs could be stored in a bank. They can be used for drug development, modeling of disease, treating spinal cord injuries, cancer, Alzheimer's disease, Parkinson's disease, diabetes, auto-immune disorders etc.[24]

SHED banking – Present Scenario

The key to successful stem cell therapy lies in being able to harvest the cells at the right point of development and to safely store them until accident or disease requires their usage. Needless to say, 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. To make it feasible, the researchers working with these cells are strongly lobbying for consideration of banking of SHED as 'Biological Insurance' and if this is done, could be a ray of hope for the treatment of various ailments already discussed in the paper. Till date, tooth banking is not very popular but the trend is catching up mainly in the developed countries.[17]

In the USA, Bio Eden (Austin, Texas), has international laboratories in the UK (serving Europe) and Thailand (serving South East Asia) with further expansion plans for Russia, Australia, India and the Middle East. Stem Save (USA) and Store –A- Tooth (USA) are also companies involved in banking tooth stem cells and expanding their horizon in other countries. In Japan, the first tooth bank was established in Hiroshima University in 2005. Nagoya University (Kyodo, Japan) also came up with a tooth bank in 2007.Taipei Medical University (TMU) in collaboration with Hiroshima University opened the nation's first tooth bank in September, 2008 with the goal of storing teeth for natural implants and providing a potential alternative source for harvesting and freezing stem cells including SHED. The Norwegian Tooth Bank set up in 2008 is collecting exfoliated primary teeth from 100,000 children in Norway.[17]

CONCLUSION

Multipotent stem cells from bone marrow and umbilical cord blood are currently used to treat some diseases, such as leukemia and lymphoma. Other potential treatments may be available in the future for conditions like type 1 diabetes, stroke, and cardiovascular injuries. SHED are also multipotent, which means they could be used for generating a wide range of cellular tissues like cartilage for the treatment of arthritis, adipocytes, skeletal muscle cells, bone cells, tendons, cardiac tissue and even neural cells for the treatment of brain injuries (Figure.1).Apart from the wide range of diseases and injuries that could be treated by storing milk teeth stem cells, this type of biological material could be a convenient replacement for umbilical cord blood for a couple of reasons. First of all, cord blood has to be collected soon after birth, while SHED can be collected every time a milk tooth falls out. This means that parents have more time to make a decision whether to store their child's biological sample or not. Also, banking SHED cells costs less than one third of the cost of cord blood storage. It's too early to say how much impact SHED can have on future medical breakthroughs. The research is extremely encouraging, and it doesn't hurt to bank these cells if cost is not a problem.

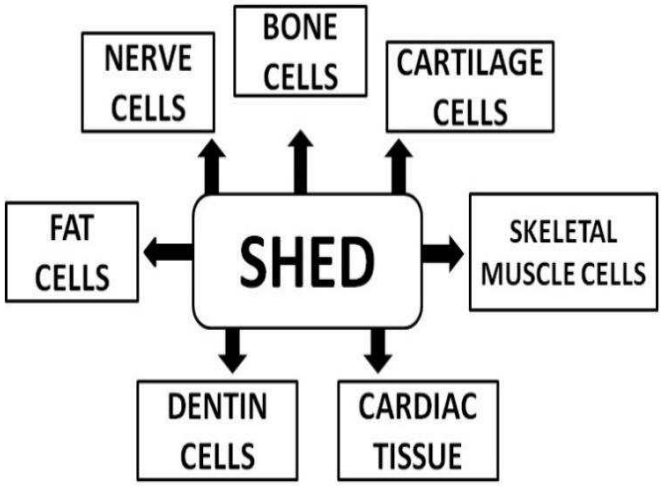

Figure.1 -Therapeutic applications of SHED

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