

Investment In Healthcare Through Dental Stem Cell Banking in India.

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Abstract

Tooth Banking is a simple alternative to recover stem cells with the potential for differentiation into various cells of the body. Young and healthy pulp is a enriched source of stem cells. These cells can be preserved for a longer time to treat various ailments like heart disease, cancer, repairing or even growing damaged organs etc. Likewise, stem cells recovered can be used in regenerative procedures in dentistry. There are many companies offering tooth banking with simple protocols for stem cell banking and storage. This review is based on various dental stem cell banks and details of harvesting with the applications and limitations of procedures. Sources of information are various articles on stem cell banking from reputed journals. Likewise, informative survey of official websites of dental stem cell banks and verbal information extracted from their representatives.

Keywords: Dental Stem cells, Stem Cell Banking, Cryopreservation.

Introduction

It is an acclaimed fact that overall health has a strong connection with oral health. Researchers are working day and night to improve human life. Among all the subjects, stem cell therapy plays a significant role in the regenerative medicine field. The concept of stem cells has brought revolutionary changes in the therapeutic world. According to data collected, more than 40,000 stem cell transplants are performed yearly all over the world. [1] India has a record of 2000 transplants. [2] Considering the disease's prevalence, the need is increasing for stem cell collection and banking.

Stem cell sources are categorized as [3]

- Embryonic stem cell sources
- Adult stem cell sources like, Bone marrow, Peripheral blood, neuronal, muscle, Liver, Pancreas, cornea and Retina, Mammary gland, Salivary glands, Skin, Tendon, Synovial membrane, Heart, Cartilage, Thymic progenitors, Adipose tissue Umbilical cord blood, Amniotic stem cells, Blood vessels- mesangioblasts

Mesenchymal stem cells are cells with multilineage potential[4] as they are mesodermal and neuroectodermal in origin. These can easily differentiate into many cells, like adipocytes, Osteocytes and chondrocytes. The dental pulp is a rich source of Mesenchymal stem cells and acts as a sealed niche. These stem cells are different from bone marrow MSC as these cells are derived from the neural crest. Sources of stem cells from dental sources are: [5]

- Permanent teeth – DPSC (dental pulp stem cells): derived from the third molar.
Deciduous teeth -SHED: stem cells are present within the pulp of deciduous teeth.
- Periodontal ligament - Periodontal ligament stem cells
- Stem Cells from apical papilla
- Stem cells from the supernumerary tooth – Mesiodens.
- Stem cells from teeth extracted for orthodontic purposes.
- Dental follicle progenitor cells
- Stem cells from human natal dental pulp

Stem cells of dental origin have high plasticity, [6] an easy approach of banking, and better cryopreservation. Dental stem cells have a quality of good interaction with scaffold and growth factors. Also, transplantations need immunosuppression, so autologous stem cell sources like dental stem cells are the best option. Dental pulp stem cells can fit to criteria due to low morbidity of the anatomical site after the collection of the pulp.

Tooth eligibility criteria for collection. [7]

- Tooth should have an intact blood supply.
- It should be free of infection, deep caries, and other pathologies.

Dental pulp Stem cells are diffusely spread in the healthy pulp. [8] The best time to recover stem cells is when a patient is young and healthy and the stem cells are at their most proliferative stage. But Stem cells can also be recovered from the permanent teeth of middle-aged individuals.

Stem cell Banking and Stem cell storage

Banking is the process of collecting blood, extracting, storage and preservation of stem cells from different sources for its potential future medical use. [9]

Dental stem cell banking can be stored at various stages of life. The discovery of SHED (Stem cells from human exfoliated deciduous teeth), behaving like embryonic stem cells have opened a new gateway for the concept of dental stem cell banking. The protocols for harvesting and isolation of the stem cells were much simpler than cord stem cell banking. [10]

There are two methods of stem cell storage.

- Cryopreservation. [11]
- Magnetic freezing. [12]

Dental stem cells after cryopreservation or magnetic freezing will not lose their capability for differentiation. [13] These can be used later for future medical treatments and regenerative treatments.

Steps involved in tooth banking. [14]

1: Tooth Collection

Exfoliated teeth should have red color pulp. The pulp with adequate blood flow at the time of removal, is indicative of cell viability

In the case of a Scheduled procedure

- *Inspection* of the freshly-extracted tooth. It is to confirm the healthy pulpal tissue.
- *Transferring* the extracted tooth into the vial (hypotonic phosphate buffered saline solution).
Hypotonic phosphate buffered saline solution
While transporting, this solution prevents drying and provides nutrients. The vial is stored at room temperature after sealing.
- *Transportation* -This vial is kept in a temperature phase change carrier and placed into a metallic vessel. Also known as **Sustentation**.

2: Stem Cell Isolation

- **Cleansing**
Wash the tooth structure with a buffer solution without Ca^{++} and Mg^{++} .
- **Disinfection**
Povidine Iodine is used as a disinfectant and again washed with a buffer solution.
- **Isolation**
Sterile small forceps or dental excavator is used for isolation of the pulpal tissue. These stem cells can be flushed with salt water from the pulp. Isolated pulp is placed in a petri dish and then Washed again with Buffer solution.
- **Addition of Digestive Enzymes like**
Dispase and Collagenase Type I are added at 37°C for 1 hour. Trypsin - EDTA can also be used. This obtained mass is filtered to have a single-cell suspension.
- **Culturing of Stem cells**
Mesenchymal Stem Cell Medium is the culture media for harvesting and colonies can be seen after 24 hours. Different cell lines are obtained.

3: Stem Cell Storage

- Cryopreservation. [11]
- Magnetic freezing. [12]

Guidelines

When parents decide to bank dental stem cells for future reference, the most important aspect is to choose a dental stem cell banking company. The important and crucial points for choosing the best dental stem cell bank are:

1. Knowledge about the company
2. Procedure of extracting pulpal tissue
3. Their laboratory and equipment
4. Their team of qualified people
5. Technology of isolation and storage of stem cells from pulpal tissue
6. Knowledge about the premises of a company

7. Availability of their sales team
8. Services provided by a company.

Here are few Commercial Tooth Banks. [15, 16]

- *BioEden (USA)*
- *National Dental Pulp Laboratory (USA)*
- *Oothy (USA)*
- *Future Health Biobank (UK)*
- *Mothercell (India)*
- *ReeLabs (India)*
- *Stemade (India)*
- *Dentcell (Mexico)*

Dental Stem cell banks in India

Mother cell Biosciences [17,18]

Mother cell is known to be the World's biggest Dental stem cell bank with a well-equipped laboratory. The company is established in Hyderabad meeting standards globally. They own a well-qualified technical team. Also, Mothercell has connected with 21 major medical institutes, like Max, Apollo, Manipal and Cloud9 etc. They approved more than 5927 dental clinics all over India for tooth collection. The company has availed the best transport facilities after tooth collection.

How the company works as a TOOTH BANK

Enlistment with company

Clients (parents interested) can initiate the process of tooth stem cell banking by enrolling with company. One of the company representatives will explain the process with all benefits. Payment plans will be explained along with the allotment of IBN (International Barcode number).

Screening and Evaluation with OPG

Screening for an ideal case is an important part of the procedure. The eruption and exfoliation phase is studied and evaluated with the help of OPG. The company employs a team of specialists who evaluate the right time for banking. Accordingly, the company connects with clients.

Tooth Collection

A highly skilled team collects the tooth in good sterile conditions. The whole process is supervised by the company's specialized authority for the protocol followed.

Transportation

It is a step critically followed with quality ensured to transport the tooth viable for stem cell isolation. The team is provided with a proprietary transportation kit for an appropriate temperature of 2-8 degrees for 72 hours.

Processing

A few tests are done before preserving the tooth.

- For testing the presence of microbes like bacteria and fungi, **Sterility tests** are performed.
- A Sample needs an ample number of stem cells for preservation. So, the **Quantity test** is done.
- Stem cells need testing for their **Proliferative** capacity in terms of P1, P2, and P3 (Passages). It is considered a stability measure.

- The cells need to survive at such low temperatures during preservation, like -172°C to -196°C and while thawing. Hence, it needs a **Viability test**.
- **Characterization tests** are mandatory for each sample. Mesenchymal Stem Cells (MSC) must show positive for surface markers.
- Sample cells are examined for growth properties of MSC. If cells pass all the tests, the sample is selected for **Cryopreservation**. This is done in liquid nitrogen tanks at temperatures from -172 to -196 degrees Celsius.
- **Certification** by company.
- **Pricing (Table I)**

Eligibility Criteria by company

- Ideal child age -5 to 11 years, has healthy milk teeth with an adequate quantity of pulp.
- No caries, one or two Deciduous teeth without cavities.
- 1/3 root must be intact.
- No ill-health conditions on the day of tooth collection.

ReeLab [16]

Reelab is concerned with the ISRS-Indian society of Regenerative Science. They have a team of scientists and dentists working in stem cell research. The company has a large equipped laboratory to isolate and store stem cells for future use. These have expertise in banking from many human sources. The team is skilled at collecting stem cells from dental pulp and bone marrow along with other sources. The company has 10 international patents. Also, they have 15 proprietary technologies.

Steps of banking with Reelab

1. Enrollment

Tooth banking starts with a filling of a form and after signing, one gets enrolled with the company. The Price plan is explained to clients, and accordingly, payment is made.

2. Provision of the collection kit

A box containing a collection kit of samples is provided for the client and instructions are given to freeze the gel packs from the kit if the team takes time to arrive.

3. Call to Team

It is a recommendation by the company to keep the paramedic team updated and informed. Frozen packs are put back into collection kits for transportation.

4. Certification

The company issues a certificate of analysis and sends the client by post or email.

5. Sample testing

Tests are done for transmissible diseases like HIV, Hepatitis B&C, VDRL, etc.

6. Processing

Sample Processing is done with advanced technology.

7. Cryopreservation

Before storage, the sample undergoes quality control of sterility, viability, purity and potency. After the quality control, at the rate of 1 degree Celsius/minute till -150 degree Celsius, the sample is stored in liquid nitrogen.

Pricing Plan (Table II)

Stemade Biotech Pvt. Ltd. [19]

Indian Terrain has contributed a lot to regeneration by Stemade Biotech. Primary teeth and wisdom teeth have become good sources of stem cell banking. Thus, companies help to the bank smile of family. Utilizing the potential capabilities of stem cells, one can shield the family from critical health issues.

Steps to banking

1. Registration with the company

2. Extraction of tooth

The procedure is done at Stemade Smile Clinic and collection of samples is done after extraction.

3. Transportation

The dental sampling kit has given by Stemade (contains necessary components for safe transport) is sent to a laboratory in Chennai by courier.

4. Isolation of stem cells

Stem cells are isolated from the dental pulp.

5. Testing of sample

The Sample is tested for sterility, viability, purity, and quality control.

6. Certification

Stemade issues a certificate of banking to the harvester.

7. Processing

The process begins with the freezing and storage of stem cells at -196 degrees Celsius in liquid nitrogen.

Pricing Plan (Table III)

Applications of stem cells

Scientists are exploring varied applications of stem cells in treatments for illnesses like heart disease, cancer, repairing or even growing damaged organs, type 1 diabetes, and Alzheimer’s disease, potentially saving your child’s life. [20]

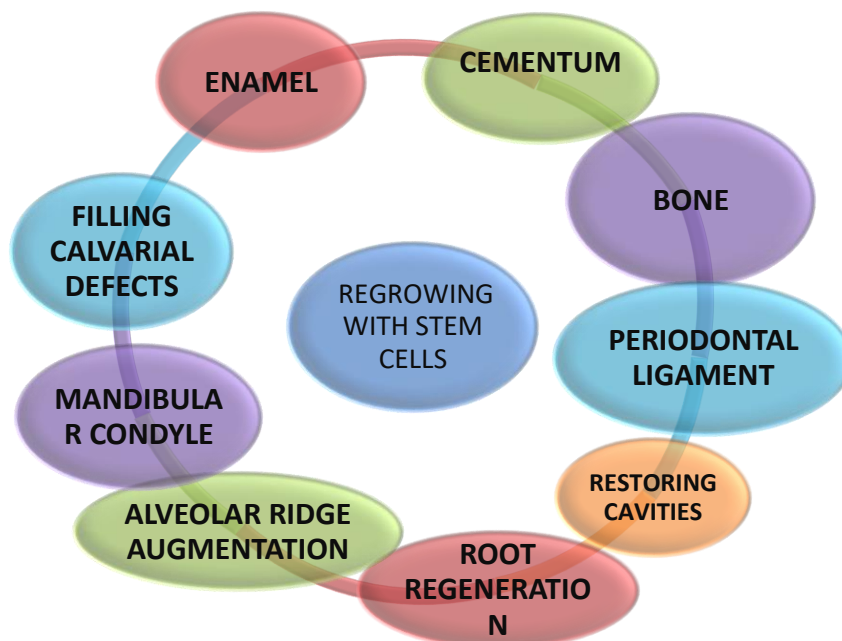


Figure 1

Other applications- Dental tissue regeneration (Figure 1)

- Craniofacial Regeneration. [21]
Dental stem cells help in bone regeneration and the correction of large craniofacial defects. The craniofacial defects can be due to trauma, tumor resection, and cyst enucleation. Likewise, a *temporomandibular joint* was created by having a natural bone building process as inspiration (Tissue Engineering). [22] This technique can be a future approach for regenerating other *oromaxillofacial bones*.
- Regeneration in Tooth structure. [23]
The day will soon come when in spite of dental implants, they will be replaced by tooth regeneration. Similarly, using the scaffold, stem cells and inductive morphogens, regeneration of the Dentin pulp complex is possible.

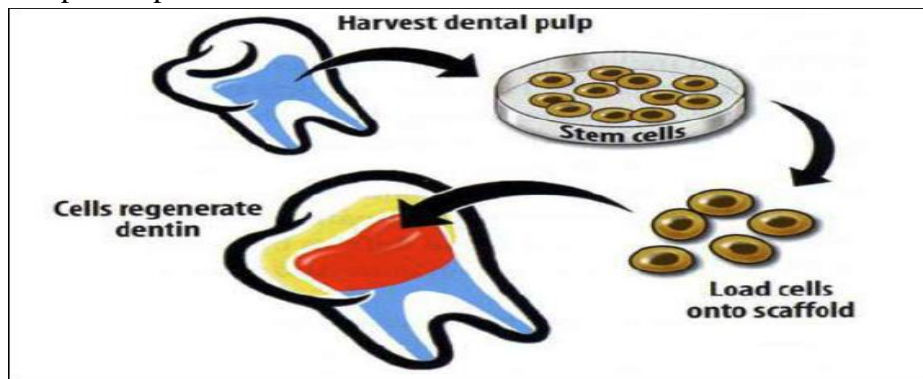


Figure2

In the same manner, other structures like enamel, periodontal ligament regeneration and dentin (figure 2) are also possible.

- Role of stem cells in Operative Dentistry and Endodontics. [24,25]
Research has shown that stem cells coaxed with drugs show regrowth of tissue to fill cavities in teeth.

Challenges In Dental Stem Cell Banking. [26]

- The tissue volume obtained from pulp and its microbial contamination may hinder the potential clinical application.
- The cost of banking is very expensive and needs to be renewed depending on the contract with the firm for storing the tooth.
- Stem cells stored for longer years might not show good results warranted at the time of usage.
- Following up on the samples is a difficult task.

Conclusion

From the last few decades, continuous research and awareness about stem cells, have brought society to a new platform of the possibility of the healthy future. Dental stem cell banking focus on the preservation of stem cells collected from dental tissues with easier and simple approach. When decision is for the family, one has to be assured about every aspect.

Considering the concept, health opportunities, pros & cons, one has to decide about Stem cell Banking. Likewise, one should learn about various companies offering Stem cell Banking with pricing and services provided by them.

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Table I: Price plan by Mothercell for Cryopreservation

Time span	Processing, Testing and Storage fees
30 years	INR 97,000 (20 million mesenchymal stem cells)
Life time storage	INR 1,27,000 (20 million mesenchymal stem cells)

Table II: Price plan by Reelab

Time span	Processing, Testing and Storage fees
21 years	INR 90,000
Life time storage	INR 1,10,000

Table III: Price plan by Stemade

Time span	Processing, Testing and Storage fees
21 years	INR 89,000 onetime payment or yearly payment of INR5000
Life time storage	INR 1,00,000

Legends

Figure 1: Dental tissue regeneration with dental stem cells

Figure 2: Dentin regeneration harvesting dental pulp using scaffold.