Post Extraction Cellular Viability Of Pulpal Tissue

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Abstract

Aims & Objectives: The aim of this study was to assess the viability of dental pulp cells at different intervals so that the clinical viable time of pulpal cells can be determined before pulp extirpation after traumatic injuries. **Materials & Methods**: After ethical approval and with informed consent the pulp tissue samples were collected from 12 subjects meeting the inclusion and exclusion criteria. The sample was immediately transported to the laboratory for processing which included washing of the sample, mincing and enzymatic digestion followed by incubation and review for observation of growth. **Results & Conclusion**: A total of twelve samples were processed out of which two samples showed positive growth of cells. The cells appeared to be mesenchymal cells and morphologically resembled fibroblast indicating the possibility that they are derived from dental pulp and are different from microorganisms. It can be concluded that the tissue can be kept viable for a certain period of time, post extraction, but there are stringent requirements of a completely sterile environment.

Key words: Dental pulp, Fibroblasts, Sterilization, Viability

INTRODUCTION

Clinical practice is being improved by scientific research. The reproducibility of results and continuity in research and biomedical processes depends on genetically stabilizing living cells for which tissue culturing and engineering has become an important part. Limitations of tissue culture include the need for a high level of expertise, stringent environmental control, genetic and phenotypic instability, and differences in cell behavior *in vitro*. There are three main methods of initiating a culture: 1) organ culture, 2) primary explant culture, 3) and cell culture. Extracted human primary teeth represent an easily accessible, often discarded tissue that may be a valuable source of pulp tissue cells for future research and clinical applications. Several studies have demonstrated that intact periodontal ligament[1] or cells isolated from dental pulp[2, 3] can be successfully cryopreserved with good viability and function upon thawing, suggesting the possibility for banking dental pulp and other dental tissues for future utilization. To date, little has been published regarding practical aspects of obtaining and banking dental tissues, such as how quickly after tooth extraction cultures must be initiated, and for how long cells can be kept viable under laboratory conditions. The knowledge of pulp tissue viability will help us to know till what time the pulp tissue can be preserved and accordingly a treatment plan

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can be formulated. The aim of this study was to assess the viability of dental pulp cells at different intervals post extraction and to assess the possibility of culturing these cells in laboratory conditions which in turn will have implication on tissue engineering.

MATERIALS & METHODS

This study was conducted in the Department of Pedodontics & Preventive Dentistry in association with Department of Biotechnology, ITS-CDSR, Muradnagar. A total of 12 dental pulp tissue samples were obtained from children in the age range of 5-13yrs, visiting the department on day to day basis. The institutional ethical committee approval was obtained before beginning with the study. Written informed consent based on the ethical committee guideline was obtained from parent/guardian of each subject after complete explanation of the procedure. The subjects for the study were selected depending on the following criteria.

Inclusion criteria

- Tooth nearing physiologic exfoliation
- Individual free of any systemic illness
- Individual not on any drug therapy (antibiotics)

Exclusion criteria

- Tooth not indicated for extraction
- Child with systemic illness or on drugs therapy

Methodology

1. Isolation of Human dental pulp

Procedure of pulp extirpation

Patient was seated comfortably on the dental chair and advised to rinse with 0.2% chlorhexidine mouth rinse for 60 seconds prior to the sample collection to minimize the bacterial contamination. A topical anesthetic gel was applied and lignocaine local anesthetic with 1:200000 adrenaline was administered. Tooth was extracted following which pulp tissue was scooped out from the chamber using a sterile spoon excavator and from the root canals using a barbed broach.[4]

Transportation Of The Collected Sample

The tissue was collected in a conical base centrifuge tube, containing phosphate buffered saline (PBS) solution of 1X concentration containing no calcium or magnesium ions and was transported to the lab in an ice box.[4]

2. Culturing of cells

In the laboratory the sample was processed inside the culture room in a laminar flow chamber. The tissue sample was washed thrice with phosphate buffered saline solution with no calcium and magnesium. After this 0.05% trypsin was added and tissue minced to smaller pieces to increase the surface area for action of trypsin, using number 21 surgical scalpel. The tissue was incubated in the incubator for 10-20 minutes after mincing. After incubating the culture medium; Dulbeco's modified Eagle's Medium- F12 (DMEM-F12) with 10% Fetal Bovine Serum, 100µM ascorbic acid and 2mM L-Glutamax supplemented with 100U/ml penicillin and 100µ/ml streptomycin, was added to the incubated sample to minimize trypsin action. The sample was centrifuged at 1200 rpm for 7 min. The supernatant was discarded and the tissue pellet collected was plated in 35 mm^2 tissue culture flask marked appropriately containing 2 ml of the culture medium**.** The cells were incubated in a humidified atmosphere of 95% air and 5% CO_2 at 37^0C. The plates were reviewed after 48 hours to observe the growth and association of cells by using compound microscope.[5]

RESULTS

A total of twelve samples were processed out of which two samples showed the growth of cells. The cell growth in the culture plates was observed under the compound microscope and photomicrographs were taken. Initial observation showed few cells in each observation area. The number of cells increased from being few to approximately 10^3 to 10^4 cells per cm. sq. which indicated growth and multiplication of the cells in the medium over a period of 4 weeks. The cells appeared to be mesenchymal cells and morphologically resembled fibroblast indicating the possibility that they are derived from dental pulp and are different from microorganisms. Microscopic views of the cell colonies as seen under the microscope are shown in Figure 1(magnification 2X), 2(magnification 4X), 3 (magnification 4X) & 4(magnification 10X). Few dead cells were also observed by the end of 4 weeks which were visible as brownish discoloured areas (Figure 5; magnification 4X).

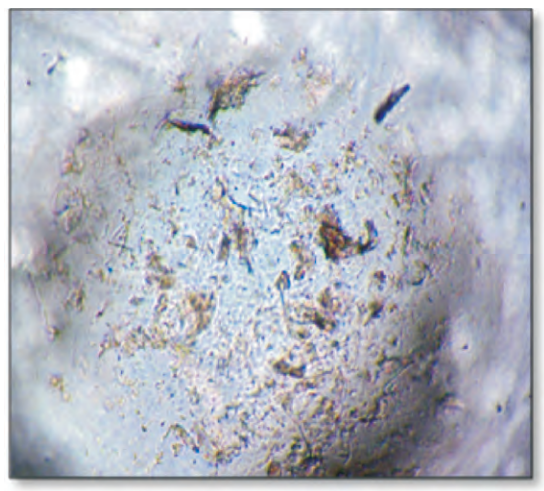

Fig. 1 The colony of cells along with darkened areas of cell debris

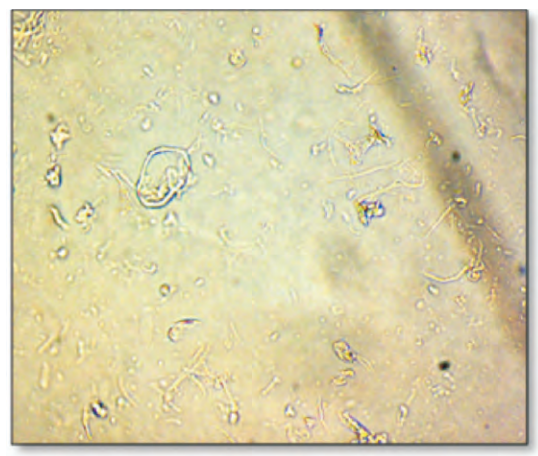

Fig . 2. Part of the colony showing scattered cells in the field of view

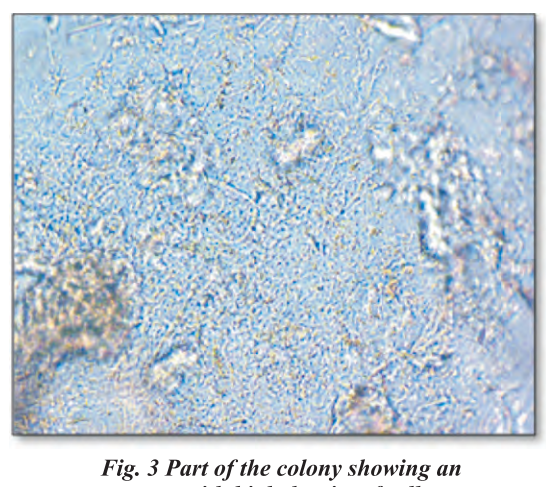

Fig. 3 Part of the colony showing an area with high density of cells

Fig. 4. A single cell magnified

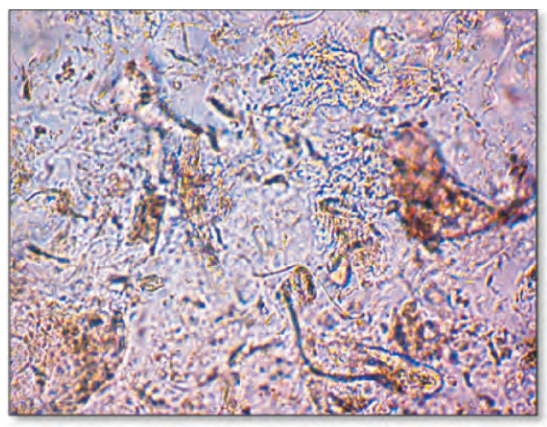

Fig. 5 Dead cells visible as brownish discoloured areas

DISCUSSION

The objective of this study was to establish cell cultures from pulp of extracted human primary teeth and to determine how long after tooth extraction pulp tissue viability can be sustained under in vitro conditions. It was found that cell cultures could be established from two out of twelve samples studied. The cells could be kept viable up to 4 weeks under culture conditions and could grow up to a density of approximately 10^3 to 10^4 cells per cm. sq.

There were a number of reasons for no evidence of cell growth in the culture media in the first ten samples. Most significant of them was the contamination in the environment. First few samples were processed under regular conditions of tissue culture, but with no results. A second attempt was made with ultraviolet ray sterilization of the laminar flow chamber where the samples were being processed, but no cell growth was observed in lieu of growth of micro organisms in the culture plate with no nutrients left for the cells to grow. Another attempt was made with complete fumigation of the tissue culture area and complete protection from contamination of the processing region. Cell growth could be observed only under absolute sterile environment. This is a major observation indicating the importance of a sterile atmosphere in all procedures of tissue culturing and engineering. The other factors for e.g. collection of sample, mode of transportation, method of processing and the composition of the culture medium containing antibiotics were standardised for all the samples except for the environment. In addition to the working environment, the complete sterility of the clinical as well as transporting conditions also play a crucial role for the growth of cells *in vitro*. In a study by Perry et al, 2008[6], it was observed that cultures from teeth stored for several days prior to processing compared to those of teeth processed shortly after extraction, suggested that donor related factors, such as oral health and oral flora variability, may play a role in culture contamination.

This observation proves to be a breakthrough for the success of various clinical procedures dealing with tissue regeneration and engineering. It shows that in cases requiring root end repair or regeneration after traumatic injuries the absolute sterility of the oral environment plays a pivotal role. Irrespective of the methods and the materials used, the absence of any kind of micro organisms itself can give a successful outcome in trials dealing with tissue repair and regeneration

It has also been observed that brief exposure to topical antiseptic agents like chlorhexidine and Listerine also efficiently kill oral pathogens, which helps to reduce the chances of sample contamination. The same procedure has been followed in the present study to cut down on the microbial blemish.

The tissue samples in our study were transported in phosphate buffered saline solution which in agreement with other studies

is an acceptable, inexpensive, and widely available collection/ transport medium, if the extracted sample has to be processed immediately.

CONCLUSION

In summary we observed that dental pulp tissue cells can be kept viable post extraction up to four weeks. It can also be commented through this study the importance of a completely sterile environment not only for regeneration of tissues but also for success in the clinical aspect dealing with repair and regeneration. We as dental professionals have difficulties in understanding biological and biotechnological intricacies. Our perception of the biological processes may serve as a foundation for the future design and fabrication of regenerated teeth. Research continues with the goal of being able to exploit natural processes in order to generate new therapies. Future work will continue to explore the possibility of tooth tissue restoration *in vivo* and the regeneration of whole teeth, both *in vivo* and *in vitro*.

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