Review Article

CELL CULTURE AND MICROSCOPY AS RESEARCH AIDS IN CONSERVATIVE DENTISTRY AND ENDODONTICS

Teena Sheethal Dsouza[1*]

[1]*Department of Conservative Dentistry & Endodontics, AB Shetty Memorial Institute of Dental Sciences, Nitte (Deemed to be University), Mangalore, India.*
Tinsha_7@hotmail.com

https://doi.org/10.51847/jFEn5AEnsF

ABSTRACT

A plethora of dental materials and types of equipment are being invented and manufactured in the sector of dentistry on a swift move. The onus is on the dental professionals before the implementation of these materials and devices for safer use. When compared to other dental fields, the sector of conservative dentistry and endodontics has overtaken the improvements in the subject due to the quick development of many materials and techniques. The field has progressed, although extrapolation of results and approaches for application have been questioned. Research aids in the form of microbiological aids, instrumental aids, and microscopy assist in the dissemination of knowledge, as well as innovative designs and approaches, to improve the profession and the professional. This makes high-quality research available to everyone through publications, resulting in changes in concepts and beliefs. The subject's foundation is formed by research aids, which will impact the eventual usage of any material, technique, or treatment.

Key words: Research, Conservative dentistry, Endodontics, Cell culture, Microscopy.

Introduction

The area of restorative dentistry and endodontics is rapidly evolving with the emergence of newer materials, instruments, and technology [1, 2]. However, there is very little information about their clinical utility. On the one hand, material and laboratory research are vital; nevertheless, clinical research and proper aids are insufficient to support their translation into clinical practice. The field has progressed, although extrapolation of results and approaches for application have been questioned. As a result, research aids assist in the dissemination of knowledge, as well as innovative designs and approaches, to improve the profession and the professional. This makes high-quality research available to everyone through publications, resulting in changes in concepts and beliefs. The subject's foundation is formed by research aids, which will impact the eventual usage of any material, technique, or treatment [3].

Cell culture

It's critical to determine whether a material is potentially dangerous to patients or dental staff, how this potential harm manifests, how it may be avoided, and what countermeasures are available. Laboratory and clinical research and observations can provide answers to these issues. When investigating the biological behavior of materials, the standard technique and idea are to perform basic in vitro experiments [4].

Cell culture is the process wherein cells are harvested directly from an animal or plant and disaggregated by enzymatic or mechanical techniques before cultivation, or they can be generated from a previously established cell line or cell strain and subsequently grown in a controlled environment. The cells can be directly extracted from the tissue. Primary cell culture is one that began with cells, tissues, or organs extracted directly from living creatures. Until it is effectively subcultured for the first time, a primary culture can be considered such. It's then referred to as a cell line'. At the time of the first successful subculture, a cell line emerges from a primary culture. The term "cell line" refers to the fact that cultures developed from it are made up of many lineages of cells found in the original culture [5-7].

The American Type Culture Collection collects available cell lines and provides a catalog with information on viability, growth medium, growth parameters, plating efficiency, age of culture since inception, morphology, karyology, sterility testing, and viral susceptibility for each cell type.

In dentistry

Many manufacturer assertions about product biocompatibility are based on evidence from microbiological experiments. Permanent cell lines are commonly utilized since they can be easily multiplied and their activity is familiar, relatively organized, and steady. Human epithelial cells (HeLa) or persistent mouse fibroblasts (L-929, 3T3) are frequently employed. Other cells, such as gingival or pulpal fibroblasts, are generated directly from biopsies of target tissues [8].

Cells can also be cultivated three-dimensionally in vitro, allowing for improved in vivo simulation. The ingredients or their extracts are used to incubate these cell cultures.

Following that, many other characteristics will be assessed, such as the number of "surviving" cells, protein production, enzymatic activity, or inflammatory mediator synthesis. The dye "neutral red" was used as one of the earliest methods for assessing cellular damage caused by any products. This dye will stain vital cells, but will not stain cells with membrane damage. Another way, which is still used today, is to use a color change reaction to measure the activity of mitochondrial enzymes photometrically (MTT assay). Dentin-barrier experiments, which imitate tooth conditions by inserting a dentin disc between target cells and the sample specimen, are a more contemporary approach. As target cells, three-dimensional cultures of immortalized pulpal fibroblasts can be employed. Cultures are continuously perfused with growth media, keeping them alive for up to many weeks. As a consequence, animal tests will be unnecessary in some situations. Molecular toxicology methods have also been introduced. Fluorescence-activated cell sorting (FACS) and Western blotting (a method for detecting specific proteins by gel electrophoresis, transfer to a membrane such as nitrocellulose, and detection by antibodies) are used to detect the impact of a biomaterial on cell metabolism, such as signaling pathways within the cell [8].

Microscopy for chemical analysis

Three-dimensional (3D) structural information on a range of length scales is crucial in biological investigations. There are good ways for acquiring atomic resolution structures of molecules, organelles, and tissue at electron microscopic resolution and light microscopic resolution, respectively [9].

Scanning electron microscopy

For a long time, scanning electron microscopy (SEM) has been a helpful technique in the study. Images at high magnification (50x – 10,000x and more) can be seen with SEM.

Principle

An electron beam scans the surface of the sample to create a range of signals, the features of which rely on several parameters, including the energy of an electron beam and the nature of the sample, as explained by Saghiri *et al*. There is no usage of light, and the color of the sample has no influence on the picture, which is important in dentistry because dental tissues and materials are often white or light in color, making optical microscopes difficult to use [10].

Because teeth surfaces can be fixed and dried out, high-vacuum images are usually attained. Images with a higher magnification can be obtained using high-vacuum imaging, but materials must be conductive. Because neither teeth nor dental materials (for example, composites, ceramics, and cement) are conductive, sputtering of the samples is required, which can be accomplished using an Au or Au-Pd target. Depending on the research, carbon coating is also employed [11, 12].

Transmission electron microscopy

Ernst Ruska devised the transmission electron microscope (TEM) in 1931 with the help of Max Knolls. Transmission electron microscopy (TEM) is a form of microscopy in which a stream of electrons flows through an ultra-thin object and interacts with it. The interaction of electrons passing through the specimen creates an image that is amplified and focused onto an imaging device, such as a fluorescent screen, a layer of photographic film, or a sensor, such as a CCD camera, for detection [13].

Advantages

1. The maximum magnification power is found in TEMs, which may reach one million times or more.
2. Transmission electron microscopes (TEMs) reveal the structure of elements and compounds.
3. The images are detailed, high-resolution.
4. Surface properties, shape, size, and structure may all be determined using TEMs.
5. They are easy to operate when properly trained.

Disadvantages

1. TEMs are large and expensive.
2. Sample preparation takes a long time.
3. Artefacts of sample preparation that may occur
4. Operation and analysis require specialized skills.
5. Samples must be electron transparent, endure the vacuum chamber, and be small enough to fit inside.
6. TEMs require special housing and maintenance.
7. The photographs are in black and white [14].

Fluorescent microscopy

Fluorescing compounds have been used in dentistry research for almost 40 years to explore a myriad of contents, including 1. microleakage and/or adaptation of bonded restorations to preparation walls, 2. characteristics, and structure of bonded restoration hybrid layer, or 3. interfacial morphology between different types of restorative materials

Fluorescent dye microscopy is a powerful investigative tool. These compounds are useful as tracers to determine a compound's path or current location since they can be detected at low concentrations, are inexpensive, and are non-toxic, making them suitable for clinical and laboratory study. Fluorophores and fluorochromes are dyes that absorb light at one wavelength range (excitation) and then re-emit it at a longer, lower-energy wavelength range (emission). Fluorescence is defined as luminescence in which a substance produces light within the visible spectrum as a result of higher energy, shorter wavelength radiation stimulating or exciting it [15].

Confocal laser scanning microscope

The Confocal Laser Scanning Microscope (CLSM) is an optical microscope with a laser light source as well as an electronic system for image processing. It creates high-

resolution, ultra-thin optical image sections by eliminating interference caused by light arriving from distinct optical fields across the sample thickness and focussing on a single plane (confocal). As a result, because the images are digital, unanticipated magnifications for optical microscopy can be attained [16].

Advantages

1. Higher resolution: As the numerical aperture of the objective rises, resolution increases with shorter wavelengths.
2. Increased contrast: The veil that creates out-of-focus parts is removed.
3. The ability to obtain optical slices: By varying the pinhole aperture and focus plane, different plane slices can be obtained.
4. Three-dimensional reconstruction: A three-dimensional image of the material under study can be created using slices collected in different focal planes.
5. Image analysis: The image can be digitalized and morphometric measurements were taken using imaging techniques [16]

Applications

In dentistry, the Confocal Laser Scanning Microscope has been used to evaluate new restorative materials in dental therapy and to determine the bone-implant contact.

CLSM's application in dental therapy

1. CLSM has been utilized to study the resin-dentin contact in various repair materials [17].
2. CLSM allows the width of space to be measured without destroying the samples. In contrast to the normal drying artifacts seen with SEM specimen preparation processes, CLSM keeps specimens under constant humid conditions.
3. CLSM has been utilized to assess the impact of various dental operations on both healthy and diseased dental tissues. As a result, the effects of bleaching agents on normal enamel and enamel with early artificial caries lesions, surface analysis of enamel and dentin after Nd: YAG laser, Er: YAG laser, and CO2 laser irradiation, dentin tubule occlusion with a desensitizing dentifrice, improvement of the remineralization effect of topical fluoride using iontophoresis, caries removal effectiveness of dentin excavation methods
4. CLSM has been utilized to evaluate the cariostatic effect of various restorative materials and fluoride compounds [18, 19].

Atomic force microscopy

Atomic Force Microscopy (AFM) is a sort of scanning probe microscopy that uses a pointed probe or tip to map the contours of a material. Its resolution is not restricted by diffraction effects because it is a near-field microscope.

In a conventional AFM, a silicon nitride tip is microfabricated on the apex of a flexible cantilever. In its normal mode of operation, the tip sweeps in a raster pattern over the sample surface, with a very low repulsive force between tip and sample. Because of the undulations in the surface topography, the cantilever is deflected. A laser bounces off the rear of the cantilever and is detected by a split photodetector. Following that, a feedback signal is given to the piezo scanner, which continuously changes the sample height such that the cantilever's deflection stays constant. The voltages delivered to the piezo are converted into a false-color picture that shows the surface topography at constant deflection during scanning [20].

Applications

1. The AFM can be used to examine the topography of gutta-percha cones and is a strong new technique for assessing the properties of gutta-percha cone surfaces. Caroline *et al*. (2004) investigated the topography of the apical portion of four different gutta-percha kinds using the lateral force mode of the AFM [21].
2. The AFM is a powerful microscope that allows for a high-resolution examination of the surface structure of the salivary pellicle in its natural (hydrated) state. Fixing and dehydration artifacts, which are frequent in scanning electron microscopy, are eliminated. Hannig *et al*. (2001) utilized AFM to assess the surface of the salivary pellicle (adsorbed layer of salivary proteins) formed in vivo on tooth enamel and glass surfaces [22].
3. Sanches *et al*. (2009) used the AFM to image the characteristics of bovine enamel and dentin following acid etching [23].
4. The AFM may be used to investigate the adhesion behavior of osteoblast cells in vitro, which can be used to verify the biocompatibility of implant materials. On a nanoscale, this technology allows researchers to investigate the cytomorphology and cytomechanical aspects of living cells [24].

Conclusion

In recent years, the field of dentistry research has grown at an exponential rate. The basic goal of the research is to generate new knowledge or to find innovative ways to make existing knowledge more accessible to people who require it. And with the proper use of research aids, this can be accomplished.

When compared to other dental fields, the sector of Conservative dentistry and Endodontics has overtaken the improvements in the subject due to the quick development of many materials and techniques. However, the use of microbiological analysis, instrumental methods of chemical analysis, and microscopy studies are essential before applying them to clinical practice.

Acknowledgments: None

Conflict of interest: None

Financial support: None

Ethics statement: None

References

1. Kharalampos M, Put VA, Tarasenko SV, Reshetov IV. Comprehensive patient rehabilitation while performing immediate dental implant placement with the use of information-wave therapy (literature overview). J Adv Pharm Educ Res. 2020;10(2):11-4.
2. Yamany IA. The Employment of CBCT in Assessing Bone Loss around Dental Implants in Patients Receiving Mandibular Implant-Supported overdentures. Int J Pharm Res Allied Sci. 2019;8(3):9-16.
3. Dsouza TS, Srinivasan R, Pais KP. Research Aids in Conservative Dentistry and Endodontics- Instrumental Methods. Int J Eng Res Technol. 2019;8(6):473-6.
4. Singh S, Khanna VK, Pant AB. Development of in vitro toxicology: a historic story. InIn Vitro Toxicology. 2018 (pp. 1-19). Academic Press.
5. Culling CFA, Allison RT, Barr WT. Cellular pathology technique. Elsevier. 2014.
6. Ekwall B, Silano V, Stammati AP, Zucco F. Toxicity tests with mammalian cell cultures. 2009.
7. Bancroft JD, Gamble M. Theory and practice of histological techniques. Elsevier health sciences. 2008.
8. Schmalz G, Arenholt-Bindslev D. Biocompatibility of dental materials. Berlin: Springer; 2009.
9. Denk W, Horstmann H. Serial block-face Scanning Electron Microscopy to reconstruct three-dimensional tissue nanostructure. PLoS Biol. 2004;2(11):1900-9.
10. Saghiri MA, Asgar K, Lotfi M, Karamifar K, Saghiri AM, Neelakantan P, et al. Back-scattered and secondary electron images of scanning electron microscopy in dentistry: a new method for surface analysis. Acta Odontol Scand. 2012;70(6):603-9.
11. de Assumpção Pereira-da-Silva M, Ferri FA. Scanning Electron Microscopy. In Nanocharacterization Techniques. 2017;1-35.
12. Paradella TC, Bottino MA. Scanning electron microscopy in modern dentistry research. Braz Dent Sci. 2012;15(2):43-8.
13. Franken LE, Grünewald K, Boekema EJ, Stuart MC. A Technical Introduction to Transmission Electron Microscopy for Soft-Matter: Imaging, Possibilities, Choices, and Technical Developments. Small. 2020;16(14);1906198. doi:10.1002/smll.201906198
14. Watt IM. The principles and practice of electron microscopy. Cambridge University Press; 1997.
15. Paulo HP, Pereira JC, Svizero NR. Use of fluorescent compounds in assessing bonded resin-based restorations: A literature review. J Dent. 2006;34(9):623-34.
16. Watson TF, Petroll WM, Cavanagh HD, Jester JV. In vivo confocal microscopy in clinical dental research: an initial appraisal. J Dent. 1992;20(6):352-8.
17. Pioch T, Stotz S, Staehle HJ, Duschner H. Applications of confocal laser scanning microscopy to dental bonding. Adv Dent Res. 1997;11(4):453-61. doi:10.1177/08959374970110041201.
18. Watson TF. Applications of confocal scanning optical microscopy to dentistry. Br Dent J. 1991;171(9):287-91.
19. García-Herraiz A. Applications of Confocal Laser Scanning Microscopy in Dentistry. Study of the changes of the post-extraction sites. Curr Microsc Contrib Adv Sci Technol. 2012:569-81.
20. Vahabi S, Salman BN, Javanmard A. Atomic force microscopy application in biological research: A review study. Iran J Med Sci. 2013;38(2):76-83.
21. Valois CR, Silva LP, Azevedo RB, Costa ED Jr. Atomic force microscopy study of gutta-percha cone topography. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004;98(2):250-5.
22. Hannig M, Herzog S, Willigeroth SF, Zimehl R. Atomic force microscopy study of salivary pellicles formed on enamel and glass in vivo. Colloid Polym Sci. 2001;279(5):479-83.
23. Sanches RP, Otani C, Damião AJ, Miyakawa W. AFM characterization of bovine enamel and dentine after acid-etching. Micron. 2009;40(4):502-6.
24. Domke J, Dannöhl S, Parak WJ, Müller O, Aicher WK, Radmacher M. Substrate-dependent differences in morphology and elasticity of living osteoblasts investigated by atomic force microscopy. Colloids Surf B Biointerfaces. 2000;19(4):367-79.