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Review Article

Advanced Microscopy and Cell Culture Techniques in Regenerative Endodontics

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ABSTRACT

The dental industry is fast in inventing and producing a wide range of dental materials and equipment. Before these materials and technologies can be used for safer purposes, it is the responsibility of dentists to use them. This study aimed to examine cell culture and microscopy as research aids in conservative dentistry and endodontics. Compared to other dental professions, conservative dentistry, and endodontics have outpaced advancements in this area due to the rapid growth of several materials and techniques. The field has progressed, yet the extrapolation of outcomes and methodologies for application has been contested. Microbiological aids, instrumental aids, and microscopy all contribute to the diffusion of information, in addition to creative designs and techniques, for the betterment of the profession and professionals. This makes excellent studies accessible to all through publications and leads to changes in notions and attitudes. The basis of the subject is built by study aids, which will influence the future use of any substance, method, or therapy.

Keywords: Research, Conservative dentistry, Endodontics, Cell culture, Microscopy

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Introduction

With the introduction of improved tools, materials, and technology, the fields of endodontics and restorative dentistry are developing quickly [1, 2]. On the other hand, not much is known regarding their clinical usefulness. While laboratory and material studies are important, clinical research and appropriate tools are not enough to assist their translation into clinical practice. Although there have been concerns about extrapolating results and application strategies, the discipline has advanced. For this reason, research helps spread information and new ideas and methods to advance the field and the profession. This leads to shifts in ideas and perspectives by making excellent research accessible to everybody through publishing. The basis of the topic is established by research aids, which will influence the final use of any substance, method, or therapy [3].

This study aimed to investigate cell culture and microscopy as research aids in conservative dentistry and endodontics.

Results and Discussion

Cell culture

It's crucial to ascertain whether a substance poses a risk to patients or dental personnel, how this risk materializes, how to prevent it, and what safeguards are in place. These questions can be addressed by laboratory and clinical research as well as observations. Basic in vitro tests are the standard method and concept for examining the biological behavior of materials [4]. Cell culture can be produced from a pre-existing cell line or cell strain and then cultivated in a controlled environment, or it might include directly harvesting cells from an animal or plant and disaggregating them using mechanical or enzymatic methods before cultivation. It is possible to remove the cells straight

from the tissue. Cells, tissues, or organs taken straight from live things are the starting point for primary cell cultivation. Until it is satisfactorily subcultured for the initial moment, a basic culture can be considered such; from that point on, it is referred to as a cell line; the term “cell line” refers to the reality that the cultures obtained from an initial culture are made up of multiple cell lineages from the original culture [5-7].

Cell lines are gathered by the American Type Culture Collection, which also offers a catalog that details each cell type's viability, growth medium, growth parameters, plating efficiency, age of culture from inception, morphology, karyology, sterility testing, and virus susceptibility.

In dentistry

Microbiological investigations provide proof for many of the manufacturer's claims regarding the biocompatibility of their products. Because they are easily replicated and exhibit familiar, somewhat ordered, and constant activity, permanent cell lines are frequently used. Commonly used are persistent murine fibroblasts (L-929, 3T3) or human epithelial cells (HeLa). Other cells, such as pulpal or gingival fibroblasts, are produced straight from target tissue biopsies [8].

Furthermore, three-dimensional cell culture in vitro improves in vivo modeling. The elements or their derivatives are utilized to incubate these cell cultures. The number of “surviving” cells, protein synthesis, enzymatic activity, and the production of inflammatory mediators are among the many other characteristics that will subsequently be assessed. The “neutral red” dye was one of the first ways to measure the amount of cellular harm that any substance may produce. Vital cells will be stained by this dye; however, cells with damaged membranes won't be stained. An additional approach that is still in use today is the photometric measurement of mitochondrial enzyme activity using a color change reaction (MTT test). A more recent method is dentin-barrier tests, which substitute a dentin disc between the sample specimen and the target cells to mimic tooth circumstances. Cultures of immortalized pulpal fibroblasts in three dimensions are able employed as target cells. Cultures may survive for several weeks because growth medium is regularly infused into them. Therefore, there will be some circumstances in which animal testing is not required. Additionally, molecular toxicological techniques have been introduced. Two methods are used to determine how a biomaterial impacts cell metabolism, including signaling pathways within the cell: Western blotting, which uses gel electrophoresis, transfer to a membrane such as nitrocellulose, and antibody detection to identify particular proteins, and fluorescence-activated cell sorting (FACS) [8].

Microscopy for chemical analysis

In biological study, three-dimensional (3D) structural data, on a range of length scales, are essential. Atomic resolution structures of molecules, organelles, and tissues may be obtained at electron microscopic and light microscopic resolution, respectively, using efficient techniques [9].

Scanning electron microscopy

Scanning electron microscopy, or SEM, has been for years a useful research tool. SEM enables the viewing of images at great magnification (50x to 10,000x and beyond).

Principle

An electron beam scans a sample's surface to produce a range of signals, the properties of which depend on several factors, such as the material's type and the electron beam's energy, based on Saghiri *et al*. [10]. Dental tissues and materials are often white or light in color, making optical microscopes challenging to utilize. This is important in dentistry because there is no need for light, and the color of the sample has no effect on the image [10].

Because tooth surfaces may be repaired and dried off, high-vacuum images are frequently acquired. Higher magnification pictures may be obtained using high-vacuum imaging, although conductive materials are required. The samples have to be sputter-deposited, which can be accomplished by employing an Au or Au-Pd target because teeth and dental materials (like cement, composites, and ceramics) are not conductive. According to the study's findings, carbon coating is also utilized [11, 12].

Transmission electron microscopy

In 1931, Ernst Ruska and Max Knolls collaborated to create the transmission electron microscope (TEM). A form of microscopy known as transmission electron microscopy (TEM) involves electrons interacting with and passing through extremely thin objects. A picture created by the interaction of electrons passing through the specimen is magnified and focused onto a sensor, including a CCD camera, a layer of photographic film, or a fluorescent screen for detection [13].

Advantages

1. TEMs have the highest magnification power that can exceed up to a million times.

2. Elements and compounds' structures can be seen using transmission electron microscopes (TEMs).
3. The images are detailed and high-resolution.
4. TEMs have the highest magnification power, which may be up to a million times.
5. Compound and elemental structures are revealed using transmission electron microscopes (TEMs).

Disadvantages

1. TEMs are large and expensive.
2. Sample preparation takes a long time.
3. Artifacts 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

Research in dentistry has been using fluorescing compounds for nearly 40 years to investigate a wide range of topics, such as 1. bonded restorations' microleakage and/or adaptation to preparation walls, 2. the properties and structure of the bonded restoration hybrid layer, or 3. the interfacial morphology between various restorative material types.

Fluorescent dye microscopy is a potent research instrument. Because these compounds are non-toxic, affordable, and detectable at low concentrations, they may be used as tracers to ascertain a compound's route or present position. This makes them appropriate for both laboratory and clinical research. One wavelength of light is absorbed by fluorophores and fluorochromes, which are dyes that subsequently produce light at a less energetic wavelength. Fluorescence is defined as luminescence, which is the production of visible light in a substance when it is stimulated or excited by higher energy, shorter wavelength radiation [15].

Confocal laser scanning microscope

One kind of optical microscope that makes use of an electronic computational imaging system and a laser light source is the confocal laser scanning microscope (CLSM). It produces high-resolution, ultra-thin optical image components by focusing on a single plane (confocal) and removing interference from light entering from many optical fields via the sample thickness. Because the pictures are digital, it is possible to acquire unexpected magnifications for optical microscopy [16].

Advantages

1. Greater resolution: At shorter wavelengths, resolution climbs as the objective's numerical aperture increases.
2. More contrast: The veil that produces out-of-focus areas is eliminated.
3. The capability to produce optical slices: You may produce various plane slices by altering the focus plane and pinhole aperture.
4. Three-dimensional reconstruction: By assembling slices from several focal planes, a three-dimensional representation of the material being studied can be produced.
5. Picture analysis: Using imaging methods, morphometric measures were made once the picture was digitalized [16].

Applications

The CLSM is utilized in dentistry to assess novel restorative materials in dental treatment and to assess the interface between implants and bone.

CLSM's application in dental therapy

1. CLSM was used to investigate the resin-dentin contact in a variety of repair materials [17].
2. CLSM permits the measurement of the width of space without annihilating the samples. Unlike SEM specimen preparation procedures, which typically result in drying artifacts, CLSM maintains specimens in a constant, humid environment.
3. CLSM has been used to evaluate how different dental procedures affect the tissues of both healthy and damaged teeth. Following Nd: YAG laser, Er: YAG laser, and CO2 laser irradiation, the effects of bleaching agents on normal enamel and enamel with early artificial caries lesions, surface analysis of enamel and dentin, dentin tubule occlusion with a desensitizing dentifrice, enhancement of the remineralization impact of topical fluoride employing iontophoresis, and the efficacy of dentin excavation techniques for removing caries.
4. A variety of restorative materials and fluoride substances have had their cariostatic effects assessed using CLSM [18, 19].

Atomic force microscopy

AFM is a type of scanning probe microscopy in which a pointed probe or tip is used to map the contours of a substance. Because it is a near-field microscope, its resolution is unaffected by diffraction effects.

On the apex of a flexible cantilever, a silicon nitride tip is microfabricated in a traditional AFM. Under typical operating conditions, the tip moves over the sample surface in a raster pattern while the force of repulsive force between the tip and the sample is relatively low.

This deflection is caused by the surface topography's undulations. After bouncing off the back of the cantilever, a split photodetector detects the laser. After that, a feedback signal is sent to the piezo scanner, which keeps adjusting the sample height to maintain a consistent cantilever deflection. A false-color image of the surface topography at constant deflection is created during scanning from the voltages applied to the piezo [20].

Applications

1. The AFM is a powerful new method for evaluating the characteristics of gutta-percha cone surfaces and may be utilized for investigating the topography of gutta-percha cones. Valois *et al*. [21] used the AFM's lateral force mode to examine the topography of the apical section of four distinct gutta-percha types.
2. The AFM is a strong microscope that enables a high-resolution analysis of the salivary pellicle's surface structure in its unaltered (hydrated) condition. Repairing and dehydration artifacts are removed, which are common in scanning electron microscopy. The surface of the salivary pellicle, or adsorbed layer of salivary proteins, that forms in vivo on glass and tooth enamel surfaces was evaluated by Hannig *et al*. [22] using AFM.
3. Sanches *et al*. [23] imaged the properties of bovine dentin and enamel after acid etching using the AFM.
4. The adhesion behavior of osteoblast cells in vitro may be evaluated using the AFM, which might be used to gauge the implant materials' biocompatibility. This method enables scientists to investigate the cytomorphology and cytomechanical characteristics of living cells at the nanoscale [24].

Conclusion

The area of dental research has expanded at an exponential rate in the last few years. The main objective of the research is to either produce new information or come up with creative ways to make already existing knowledge more accessible to those who need it. This may be achieved by using research tools appropriately.

The field of endodontics and conservative dentistry has advanced more quickly than other dental specialties due to the quick growth of various tools and techniques. However, before using microbiological analysis, instrumental chemical analysis techniques, and microscopy studies in clinical practice, they must be used.

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