Review Article

EXPLORING MICROBIOLOGICAL, MICROSCOPIC, AND INSTRUMENTAL RESEARCH AIDS IN ENDODONTICS: INSIGHTS FROM A NARRATIVE REVIEW

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ABSTRACT

From the dawn of civilization, humans have sought ways to enhance health, comfort, and quality of life. This pursuit has progressed from the discovery of fire and the invention of the wheel to the development of vaccines and, more recently, the integration of physical, chemical, and biological sciences into healthcare. As Albert Einstein remarked, "If we knew what it was we were doing, it would not be called research." Indeed, research drives survival, innovation, and advancement, transforming existing knowledge into new technologies. Endodontics, like many dental specialties, is rapidly evolving through the continuous development of novel materials and techniques. Ensuring their safe and effective application places a vital responsibility on clinicians. However, translating research outcomes into everyday clinical practice continues to present key challenges and considerations. Research aids, including microbiological tools, laboratory instruments, and advanced microscopy, are indispensable to expanding scientific understanding and fostering innovation. They support the design, validation, and dissemination of high-quality research, promoting evidence-based practice that challenges existing paradigms and refines clinical judgment. As integral components of scientific inquiry, research aids underpin the evolution of dentistry, guiding the selection and application of materials, techniques, and treatment modalities that shape the future of oral healthcare. This review highlights the pivotal role of research aids in endodontics, tracing their evolution and underscoring their ongoing impact on scientific progress and clinical excellence.

Key words: Microbiological, Microscopic, Instrumental, Research, Endodontics.

Introduction

Research in endodontics begins with the investigator's own initiative, encouraging independence and critical thinking while fostering a deeper understanding of the discipline [1]. Through systematic inquiry and careful evaluation of materials and sources, research validates, explains, and expands existing knowledge while uncovering new insights [2]. Dentistry as a whole, and endodontics in particular, continues to evolve rapidly due to the development of innovative materials, advanced instruments, and cutting-edge technologies [3]. Despite these advances, the clinical evidence supporting many newly introduced endodontic materials and techniques remains limited, and the reliable translation of experimental data into predictable clinical outcomes is still a major challenge [4]. In this context, research aids are indispensable: they facilitate the generation of reproducible data, stimulate innovation, and support the dissemination of high-quality findings. Microscopic methods such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), confocal laser scanning microscopy (CLSM), and atomic force microscopy (AFM) provide detailed structural and surface analyses; instrumental approaches—including the Gilmore needle apparatus, universal testing machine, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and atomic absorption spectroscopy (AAS) offer precise evaluation of physical and chemical properties; and cell culture techniques allow exploration of biological responses and biocompatibility [5]. This narrative review presents a comprehensive overview of these microscopic, instrumental, and cell culture methodologies **(Figure 1)**, underscoring their vital role in assessing the physical, mechanical, and biological characteristics of endodontic materials and in advancing evidence-based clinical practice.

Materials and Methods

Search strategy

An online search was conducted in the peer-reviewed journals indexed in PubMed to retrieve research studies related to the use of cell culture, microscopy, and instrumental aids in endodontic research, using the following search query: ("endodontic"[All Fields] OR "endodontics"[MeSH Terms] OR "endodontal"[All Fields] OR "endodontical"[All Fields] OR "endodontically"[All Fields]) AND ("cell culture"[All Fields] OR "cell line"[All Fields] OR "molecular microbiology"[All Fields] OR

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microscopy[All Fields] OR "scanning electron microscope"[All Fields] OR SEM[All Fields] OR "transmission electron microscope"[All Fields] OR TEM[All Fields] OR "confocal laser scanning microscope"[All Fields] OR CLSM[All Fields] OR "atomic force microscope"[All Fields] OR AFM[All Fields] OR "Gilmore needle"[All Fields] OR "universal testing machine"[All Fields] OR UTM[All Fields] OR "X-ray diffraction"[All Fields] OR XRD[All Fields] OR "Fourier transform infrared spectroscopy"[All Fields] OR FTIR[All Fields] OR "atomic absorption spectroscopy"[All Fields] OR AAS[All Fields]). Subsequently, a targeted search was performed for each methodological aid using individual queries: for cell culture techniques ("cell culture" OR "cell line" OR "molecular microbiology"), for microscopy including scanning electron microscope (SEM), transmission electron microscope (TEM), confocal laser scanning microscope (CLSM), and atomic force microscope (AFM), and for instrumental methods such as the Gilmore needle apparatus, universal testing machine (UTM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and atomic absorption spectroscopy (AAS), each combined with the terms ("endodontic" OR "endodontics") and limited to 2015/01/01:3000/12/31[Date—Publication]. All retrieved records were screened for relevance to endodontic laboratory research employing these specific aids, and reference lists of included studies were further examined to identify additional publications. A total of 85 articles were ultimately selected for inclusion in this narrative review.

Table 1. Search Strategy and Selection Criteria

Category	Inclusion Criteria	Exclusion Criteria
Type of Paper	Systematic reviews, meta-analyses, and original research studies	Abstracts only
Publication Form	Complete articles or book chapters	–
Language	English	Non-English languages
Content Focus	Topics relevant to Restorative dentistry or dental research involving tissues/materials	Content unrelated to dental tissues or restorative dentistry

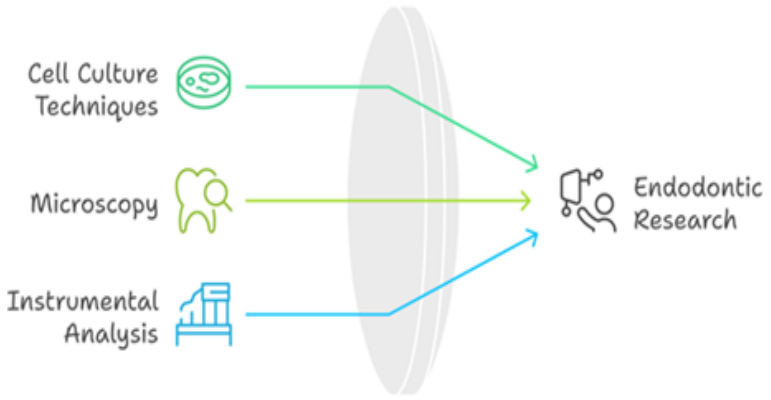

Figure 1. Aids in Endodontic Research (generated by Napkin AI)

Results and Discussion

Tools for microbial detection and characterization

Microbiology for dental applications has evolved through five successive generations **(Figure 2)**, reflecting chronological and technological advancements [6]. The investigative approaches used can be broadly classified into either open-ended or closed-ended methods. Open-ended methods aim to identify a wide spectrum of microorganisms present in a sample—although they typically highlight the dominant species within the detection capacity of the technique—and provide insights into microbial richness and relative abundance. Closed-ended methods, on the other hand, are designed to detect specific target organisms, generally in a presence/absence format, with the possibility of semi-quantitative or absolute quantification [7].

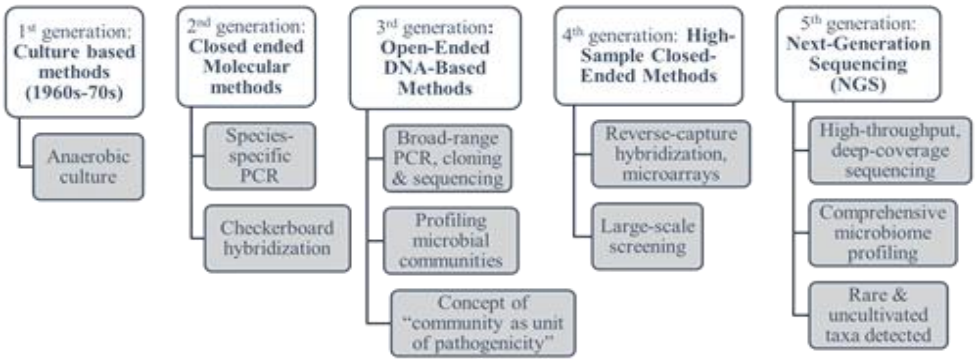

Figure 2. Flowchart depicting the Five Generations of Microbiology Studies in Dentistry [7]

Cell culture

Assessing whether a material poses risks to patients or dental personnel, understanding the nature of these risks, identifying ways to minimize them, and determining appropriate safety measures are all essential. Laboratory studies, clinical research, and careful observation can help address these concerns. In studying the biological properties of materials, the conventional approach involves conducting fundamental laboratory experiments [8, 9].

For decades, dental microbiology has primarily relied on culture techniques to detect, identify, and quantify bacteria. Successful cultivation requires that bacterial samples remain viable from collection through transport and laboratory processing, and that they are grown under controlled conditions with appropriate nutrients, temperature, moisture, atmosphere, salt concentration, and pH [10]. The process involves sample collection in a viability-preserving, non-supportive, anaerobic transport medium to avoid overgrowth of fast-growing species, followed by dispersion, dilution, plating, incubation under anaerobic (and sometimes aerobic/microaerophilic) conditions, and isolation of colonies for identification through morphological, biochemical, and molecular tests [11, 12]. Culture studies played a crucial role in establishing the infectious basis of the disease process, identifying the dominant cultivable species, and evaluating susceptibility to medications and systemic antibiotics [7]. However, their low sensitivity, particularly for fastidious anaerobes, and reliance on phenotypic traits often result in false negatives or misidentification, overlooking many species present in mixed communities [13, 14]. The inability to replicate specific physiological and nutritional requirements limits the cultivation of many bacteria [7]. New strategies such as culturomics, reverse genomics, and environmentally tailored media are being developed to expand cultivable ranges but are not yet applied in dental microbiology research [15, 16]. For more reliable identification, 16S rRNA gene sequencing remains the reference method [17].

Molecular microbiology methods

Molecular microbiology has both confirmed and extended traditional culture findings in dentistry, particularly endodontics [7]. Molecular methods have strengthened the association of several cultivable bacteria with apical periodontitis and revealed new, difficult-to-culture species. Most bacterial detection and quantification approaches target the 16S rRNA gene—the gold standard for taxonomy [17], though it can lack species-level resolution [18]. Polymerase Chain Reaction (PCR) and its variants are widely used: single, nested, and multiplex PCR employ species-specific primers, while real-time PCR (qPCR) provides sensitive quantitative data [19].

DNA–DNA hybridization techniques such as checkerboard (macroarray), microarrays, and fluorescence in situ hybridization (FISH) are commonly applied to identify bacteria in oral infections. These assays rely on labeled single-stranded DNA probes binding to complementary target sequences [20]. Checkerboard and microarray formats can simultaneously detect dozens to hundreds of taxa. Conventional checkerboard assays use whole-genomic probes prepared from cultured species but risk cross-reactivity. In contrast, reverse-capture checkerboard and microarray methods employ oligonucleotide probes, offering higher specificity and enabling detection of both cultivable and uncultivated bacteria [7].

Next-generation sequencing (NGS), also known as high-throughput sequencing, enables the simultaneous sequencing of hundreds of genes, providing deeper microbial coverage [21]. NGS can target either the 16S rRNA gene or entire genomes (metagenomics). Because 16S rRNA is present in all bacteria and archaea, it serves as a phylogenetic marker for assessing oral microbial composition [22]. Amplicon sequencing typically amplifies

short fragments spanning one or two hypervariable regions (e.g., V1–V3, V4, V4–V5) of the 16S rRNA gene, and the resulting sequences are compared with reference databases for taxonomic and abundance data [23]. While 16S sequencing usually resolves genera or species, whole-genome (shotgun) sequencing can reach species or strain level and reveal metabolic functions [22]. Shotgun NGS randomly fragments DNA (25–500 bp) and uses computational assembly to reconstruct genomes [24].

Microscopic techniques in research

Microscopy remains a core method in dental laboratory studies, but modern advancements extend well beyond traditional light and lens systems. The invention of electron and scanning probe microscopy has surpassed the limits of standard optical imaging, enabling today's instruments to use diverse energy sources for far higher magnification and resolution. These technologies enable researchers to examine the interface between dental tissues and materials at the nanoscale [25].

Researchers must address several factors when selecting the most suitable microscopic methods. Key considerations include the specimen's type and composition, whether it needs to be preserved for repeated observations, and the specific details required—such as overall morphology, surface features, internal structure, or micromechanical properties. Appropriate magnification, resolution, contrast enhancement, and awareness of specimen size and thickness are all essential for accurate imaging and meaningful results [26, 27].

Scanning electron microscope (SEM)

The concept of electron microscopy originated with Max Knoll and Ernst Ruska at the Technical University of Berlin, who in 1931 captured the first images using an electron-beam device. Since then, improvements in electron sources and detectors for secondary electrons have greatly enhanced the technique. More recent advances in the 21st century include digital image processing with computer systems and approaches that enable imaging in more natural settings, reducing the need for vacuum conditions or specimen fixation [25].

In dentistry, a scanning electron microscope (SEM) is an advanced instrument used to examine the microstructure of dental materials [28]. In this method, a focused electron beam scans across the specimen's surface, generating various signals whose properties depend on factors such as the beam's energy and the material's composition.

Among the signals produced, three are especially informative: secondary electrons (SE), backscattered electrons (BSE), and x-rays [29]. Secondary electrons originate from surface atoms and create images that are easy to interpret, with contrast influenced by the sample's surface topography. Because the primary electron beam is extremely fine, SEM can deliver images with very high resolution [30].

Back-scattered electrons are the primary beam electrons that rebound from the atoms of the specimen. The contrast in a back-scattered electron image is mainly governed by the atomic number of the elements present [31]. As a result, this imaging mode reveals the spatial distribution of various chemical phases within the material. However, because these electrons originate from different depths of the sample, the resulting image typically offers lower resolution compared with images formed by secondary electrons [32].

SEM in endodontic research

Scanning electron microscopy (SEM) is a high-resolution imaging technique that has become indispensable in endodontic-materials research because it uniquely reveals surface topography, microstructure, and failure modes at the micrometre–nanometre scale, allowing investigators to link visible morphology to function and handling characteristics [33]. Researchers routinely use SEM to examine sealer–dentine interfaces and quantify tubular penetration and interfacial adaptation (often paired with push-out or bond-strength tests) to compare epoxy-, bioceramic-, and resin-based sealers under clinically relevant conditions [34]. SEM remains the gold standard for evaluating smear-layer and debris removal along the coronal, middle, and apical thirds when assessing new irrigation regimens (including continuous vs. sequential chelation and novel etidronate-based protocols), because it directly visualises residual debris, open tubules, and smear-layer thickness [35]. When combined with energy-dispersive X-ray spectroscopy (EDS/EDX) or elemental mapping, SEM also identifies surface chemistry changes, mineral deposition, and early apatite-layer formation on bioactive sealers and hydraulic cements—information that guides formulation tweaks and predicts bioactivity [36]. Taken together, studies and reviews published across PubMed, Scopus, and Web of Science in the 2020–2025 window show SEM (±EDS) informing both hypothesis-driven research (for example, on sealing ability and washout resistance) and routine quality-control assessments during development of new bioceramics and resin-based root-canal materials [37].

Transmission electron microscopy (TEM)

Ernst Ruska, together with Max Knoll, developed the transmission electron microscope (TEM) in 1931. TEM is a microscopy technique in which a beam of electrons passes through an ultra-thin specimen and interacts with it, producing an image that is magnified and focused onto a detection device such as a fluorescent screen, photographic film, or a CCD camera [38]. Often regarded as a predecessor of the scanning electron microscope (SEM), TEM has evolved alongside SEM to serve distinct purposes in dental research [8]. Major advancements in the 1970s, particularly the introduction of high-resolution objective lenses and improvements in the field-emission electron gun, enhanced spatial resolution and improved the signal-to-noise ratio [25, 39]. Like SEM, TEM employs an electron beam as its

energy source, but the imaging mechanism differs: in TEM, the electrons pass through the specimen, where their interactions generate secondary signals—diffracted and transmitted electrons—that are collected by a detector [39]. To prevent electrical discharge and ensure proper beam transmission, the electron source operates under vacuum. A TEM system typically comprises an electron beam generator, various electromagnetic lenses, and a detector [39].

TEM in endodontic research

Transmission electron microscopy (TEM) has emerged as a key analytical tool in endodontic research from 2020 to 2025, offering nanometer-scale resolution for both biological and material investigations. Recent work highlights its role in characterizing antimicrobial or bioactive nanoparticles, revealing particle size, aggregation state, and core–shell architecture essential for intratubular penetration and controlled release [40]. TEM provides high-resolution visualization of dentinal collagen fibrils, intratubular deposits, and hybrid layers formed by sealers and remineralizing agents, clarifying the effects of irrigants and chelators on smear-layer removal [41]. It also enables ultrastructural analysis of calcium-silicate cements, documenting hydration products and nanocrystal morphology that influence setting reactions and bioactivity [42]. In regenerative endodontics, TEM is the gold standard for confirming the size and integrity of extracellular vesicles or exosomes derived from dental pulp stem cells or platelet-rich plasma [43]. Frequently combined with scanning electron microscopy and confocal laser scanning microscopy, TEM thus provides indispensable ultrastructural and crystallographic insights that advance the understanding of dentin–material interfaces, novel biomaterials, and cellular or microbial mechanisms central to successful endodontic therapy.

Confocal laser scanning microscopy (CLSM)

The advent of confocal microscopy represented a major milestone in fluorescence imaging. The concept was first proposed by Minsky in the 1950s and further developed about ten years later by Egger and Petran. The primary goal was to minimize distortions caused by out-of-focus light, thereby improving image clarity. With advancements in laser and computer technology, the first commercial confocal laser scanning microscope was introduced in 1987, quickly becoming widely adopted, especially in microbiological studies [25, 44].

Confocal laser scanning microscopy (CLSM) is an advanced fluorescence microscopy method that provides higher image resolution and contrast than conventional light microscopy. In CLSM, the excitation light passes through a small pinhole at the confocal plane **(Figure 1)**, producing focused illumination that reduces scattered out-of-focus signals. The specimen absorbs this light and emits fluorescence at longer wavelengths, which is detected and converted into an image. CLSM also allows optical sectioning, enabling imaging of thin specimen layers free from out-of-focus light, which can be compiled into three-dimensional reconstructions of the sample [44, 45].

CLSM in endodontic research

Confocal laser scanning microscopy (CLSM) has become an indispensable tool in endodontic research, offering high-resolution imaging capabilities that are crucial for evaluating various aspects of root canal therapy. Between 2020 and 2025, several studies have utilized CLSM to assess the effectiveness of different irrigation protocols and obturation techniques in enhancing sealer penetration and bacterial disinfection within dentinal tubules. For instance, a study employed CLSM to visualize the penetration of endodontic sealers into dentinal tubules, revealing that certain techniques resulted in deeper and more uniform sealer distribution [46]. Similarly, research by Tsesis *et al*. (2022) utilized CLSM to examine bacterial colonization patterns in root-end resected teeth, providing insights into the efficacy of various sealing materials in preventing microbial ingress [47]. Furthermore, CLSM has been instrumental in evaluating the impact of different irrigation solutions on smear layer removal and sealer penetration, as demonstrated in a study by Alghamdi *et al*. (2023), which found that specific irrigation protocols significantly improved sealer penetration compared to others [48]. These applications underscore the pivotal role of CLSM in advancing our understanding of endodontic procedures and materials, facilitating the development of more effective treatment strategies.

Atomic force microscopy (AFM)

Atomic force microscopy (AFM), developed by Binning and Rohrer in 1986 [49, 50] from scanning tunnelling microscopy, uses a sharp cantilever tip to map surfaces at the atomic level. The first nanoprobe appeared in 1991, and commercial AFM systems became available in 1998 [51]. AFM allows high-resolution imaging of non-conductive specimens under ambient or liquid conditions without surface coating, minimizing dehydration artifacts, which is particularly valuable in endodontic research [49, 50]. Tip–sample interactions, including van der Waals, chemical, and electrostatic forces, deflect the cantilever; these deflections are detected by a laser-photodiode system and converted into topographic images, providing detailed nanometer-scale information on the surface topography and mechanical properties of endodontic tissues and materials [52].

AFM in endodontic research

Atomic force microscopy (AFM) has emerged as a crucial technique in endodontic research, providing nanometer-scale visualisation and precise surface characterisation. From 2020 to 2025, numerous studies indexed in PubMed, Scopus, and Web of Science highlight its broad and evolving range of applications. AFM is widely used to evaluate the surface topography and roughness of root canal dentin after various irrigation regimens or activation methods, enabling researchers to monitor the removal of

smear layers and collagen exposure [53, 54]. Tsenova-Ilieva *et al*. (2022) quantified the roughness of human root canal dentin following different irrigation protocols [53]. Ibrahim *et al*. (2023) employed AFM to assess dentin morphology after Er, Cr: YSGG laser agitation of irrigants [54]. In addition, AFM detects nanoscale wear and surface defects on nickel–titanium rotary instruments after clinical use, chemical exposure, or sterilization cycles, which is critical for predicting cyclic fatigue and fracture resistance [55, 56]. Swathika *et al*. (2024) evaluated rotary NiTi instruments, correlating AFM-measured roughness with cyclic fatigue resistance and sterilization effects [55]. Shaik *et al*. (2024) likewise documented increased surface roughness of NiTi files after irrigant exposure and autoclaving [56]. Force-spectroscopy modes further enable measurement of bacterial adhesion forces to dentin, gutta-percha, and sealers, supporting investigations of biofilm formation and antimicrobial strategies [57]. Together, these investigations show that AFM provides three-dimensional nanoscale imaging and quantitative mechanical data, making it a powerful technique for analyzing dentin surfaces, endodontic instruments, and microbe–material interactions in modern endodontic research.

Table 2. The table below summarises the advantages and limitations of the microscopy techniques most frequently applied in endodontic research [25].

Technique	Advantages	Disadvantages
CLSM (Confocal Laser Scanning Microscopy)	- Reduces out-of-focus signal- High contrast due to fluorescence- Allows optical sectioning for 3D reconstruction	- Limited magnification (max ~1000×)- Resolution restricted by optical diffraction limit
SEM (Scanning Electron Microscopy)	- High-quality imaging of surface topography and adhesive interfaces- Higher resolution and magnification compared to CLSM- Can visualize larger specimen areas (millimetres)- Faster image acquisition than AFM- Can combine with spectroscopic methods and profilometry	- Non-conductive specimens require coating- Biological samples may require dehydration/fixation, potentially causing artefacts- Lower contrast compared to CLSM
TEM (Transmission Electron Microscopy)	- High-quality imaging of internal structures- Very high resolution and magnification (10^4× higher than CLSM)	- Requires ultrathin specimen sections- Preparation may be time-consuming for dental tissues
AFM (Atomic Force Microscopy)	- Provides the highest magnification and resolution- No vacuum required- Specimen coating, fixation, or dehydration not needed- Does not require fluorescent dyes- Specimen can be re-observed	- Requires a flat specimen surface for proper tip access- Limited scanning area- Imaging of dental tissues may take several minutes

Instrumental Methods for Endodontic Research

Gilmore needle apparatus

Indentation testing is a standard approach for evaluating how a material hardens over time [58, 59]. It determines the point at which the material can withstand indentation from a specified load. For water-based cements, such as mineral trioxide aggregate (MTA), the setting time is typically assessed using ISO 6876 (for endodontic sealing materials) or ISO 9917-1 (for restorative materials) [60, 61]. Both protocols measure the resistance of a material to a controlled indentation, which indicates when the final set is achieved. The ASTM C266 (Gillmore needle) method uses comparable pressures, defining the initial set in line with ISO 6876 and the final set similar to ISO 9917-1 [62]. The instrument assesses the physical development of the cement's hardness over time.

Figure 3. Flowchart illustrating the methodology for determining the setting time of cement using the Gilmore needle apparatus.

In endodontic research, the Gillmore needle method is frequently employed to evaluate the setting characteristics

of calcium-silicate–based cements and newer bioceramic sealers **(Figure 3)**. Accurate setting-time measurements are critical because they influence the material's sealing ability, dimensional stability, and clinical handling. Several studies have used this apparatus to compare traditional MTA with newer formulations and to assess how variables such as particle size, mixing liquids, or additives affect hardening. For example, Camilleri (2010) highlighted that the Gillmore test provides reliable final-set data for MTA when compared with ISO 9917-1 protocols [63]. Lovato and Sedgley (2011) similarly employed the Gillmore needles to evaluate the influence of different mixing techniques on hydraulic cements' setting time [64]. More recent investigations continue to apply ASTM C266 to novel bioceramic sealers, demonstrating its relevance for contemporary endodontic materials [65].

Universal testing machine

The Universal Testing Machine (UTM) is a versatile instrument widely used in dental and endodontic research to evaluate the mechanical properties of teeth, endodontic instruments, and restorative cements. Its primary function is to apply controlled loads or displacements to specimens and record their response, allowing precise quantification of properties such as fracture resistance, torsional strength, bending resistance, and cyclic fatigue [66, 67].

Principle: The UTM operates on the principle of controlled application of mechanical force. Specimens are placed between two fixtures, and either a compressive, tensile, or torsional load is applied at a constant rate. The machine measures the corresponding deformation, force, or stress until failure occurs. Modern UTMs are equipped with load cells, displacement sensors, and digital interfaces, allowing precise measurement of mechanical parameters such as maximum load, elongation, stiffness, and energy absorption [68]. In endodontic research, these measurements provide insights into the strength and resilience of teeth, instruments, and materials under simulated functional stresses [69].

In endodontic studies, UTMs are commonly used to assess the fracture resistance of endodontically treated teeth. For example, Hancerliogullari *et al*. (2025) evaluated the effect of different apical preparation sizes and root canal sealers on the fracture resistance of mandibular premolars using a UTM [70]. Similarly, UTMs are employed to test the mechanical performance of nickel-titanium (NiTi) rotary instruments, including torsional strength and cyclic fatigue, providing insights into instrument safety and longevity during clinical use [69].

UTMs are also instrumental in evaluating the mechanical properties of endodontic materials, such as posts and sealers. Ahmad *et al*. (2023) used a UTM to compare the mechanical behavior of different polyetheretherketone endodontic post systems, providing data that can guide material selection for clinical practice [71]. Overall, the Universal Testing Machine is a critical tool in endodontic research, enabling systematic assessment of materials and instruments under standardized conditions, thereby helping optimize treatment outcomes and ensure the safety and reliability of endodontic procedures [72-76].

X-ray diffractometer

In 1912, Max von Laue first demonstrated the diffraction of X-rays, a milestone that transformed the understanding of atomic structure and crystallography. Since that discovery, X-ray diffraction (XRD) has become an essential technique for elucidating the ordered arrangements of atoms in a broad spectrum of materials encountered in daily life [77].

Approximately 95 % of naturally occurring solids exhibit crystalline character. When a crystalline specimen is exposed to an incident X-ray beam, it produces a characteristic diffraction pattern. Because the spacing of crystallographic planes varies with direction and chemical composition, each crystalline substance yields a unique diffraction signature [78].

Diffraction Apparatus: The fundamental design of an XRD instrument is analogous to that of an optical grating spectrometer [4]. For single-plane collimation, an X-ray tube generates a beam that passes through either a bundle of metal capillaries or a sequence of precision slits [4]. Detection of the diffracted radiation can be accomplished via photographic methods or by electronic means such as gas-ionization detectors, scintillation counters, or semiconductor sensors (e.g., germanium or silicon) that utilize the photoelectric effect [78].

XRD in endodontic research

In recent years, X-ray diffraction (XRD) has become an indispensable tool for phase identification and quality control of endodontic sealers and hydraulic cements. For example, studies comparing commercially available calcium-silicate sealers with experimental formulations have used XRD in conjunction with Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) to delineate the crystalline phases present, detect impurities or secondary phases, and correlate phase assemblage with setting behavior and bioactivity [79]. Such analyses enable researchers to ensure consistency in manufacturing and to understand how changes in formulation (e.g., addition of radiopacifiers or alternative mixing media) affect the mineral phases, which in turn influence physical and biological performance [80].

Another important application has been tracking biomineralization at the sealer–dentin interface. XRD is routinely used to identify apatite or other calcium-phosphate phases that form after applying bioactive sealers onto root dentin or immersing them in simulated body fluid or phosphate-buffered saline. By comparing the diffraction patterns before and after immersion, researchers have shown the appearance or growth of characteristic apatite peaks, indicating mineral deposition that could improve the sealing

ability and biocompatibility of the sealer materials [81]. These findings help in evaluating the long-term performance of endodontic materials under physiologically relevant conditions.

XRD is also heavily employed in the characterization of novel biomaterials aimed at regenerative endodontics. For instance, when hydroxyapatite is synthesized from biological wastes such as eggshells or when scaffolds are produced that contain hydroxyapatite composites, XRD confirms the presence of the intended crystalline phase (usually hexagonal hydroxyapatite), measures crystallinity, and estimates crystallite size using methods such as the Scherrer equation. These parameters are critical because they influence important material properties such as solubility, ion release, surface reactivity, and mechanical stability—features that are vital in regeneration, repair, and sealing contexts [82].

In studies exploring antimicrobial or additive doping in endodontic materials, XRD has helped verify that nanoparticle additives (e.g., silver, zinc oxide, other metal oxides) are incorporated successfully and retain their intended crystalline structure. Such verification is important because the crystallinity and phase purity of these additives can strongly influence therapeutic performance (e.g., ion release kinetics, antimicrobial efficacy) and material stability [83]. Additionally, XRD has been used to examine compositional changes in dentin and other dental hard tissues—whether due to aging, disease, or treatment procedures. For example, in forensic and diagnostic contexts, researchers have employed XRD to analyze mineral phase shifts or altered crystallinity in dentin, which may be useful for age estimation or for understanding pathological changes [84].

Overall, XRD has found wide utility in endodontic research—not only for basic material characterization, but as a bridge toward clinically relevant performance metrics. When combined with complementary techniques like FTIR, SEM/EDS, and mechanical testing, XRD data enable a fuller understanding of how endodontic materials behave in situ, how they interact with biological tissues, and how they might be optimized for better sealing, biocompatibility, or regenerative capacity [85-89].

Fourier transform infrared spectroscopy (FTIR)

Spectroscopy has become an important tool in biomedical research and clinical diagnostics, showing notable progress in recent years. Various materials have been examined using spectroscopic methods such as FTIR, which are simple, reproducible, and non-destructive, requiring only minimal sample preparation and small sample volumes. These vibrational techniques provide detailed molecular information, enabling analysis of functional groups, bonding patterns, and molecular conformations. Their spectral bands are molecule-specific, narrow, and sensitive to structural and environmental changes [90].

FTIR spectroscopy, in particular, can detect molecular alterations within biological or other samples. It supports precise characterization of functional groups and bonding types, as many FTIR peaks can be assigned to the vibration of specific chemical bonds or individual functional groups [91].

To obtain a sample spectrum, a background scan is first recorded and then divided into the sample scan to remove atmospheric absorptions. Measurements can be made by transmission—detecting light that passes through the sample—or by various reflectance techniques (ATR, diffuse, or specular). Sample prep depends on the phase: solids are often mixed with IR-transparent KBr, while liquids use fixed-path cells with IR-transparent windows [92].

FTIR in endodontic research

FTIR spectroscopy has been increasingly applied in endodontic research to characterize materials and biological tissues with high specificity. Although FTIR spectroscopy has been used in endodontic material research for many years, the studies summarized here focus on the most recent five-year period. Recent work demonstrates the value of FTIR in analysing the chemical structure, bioactivity, and interactions with dentin of modern sealers. For example, Padmakumar *et al*. (2022) employed FTIR, SEM, and EDS to evaluate how common irrigants modify the mineral–organic composition of root canal dentin [93]. An experimental bioactive-glass–calcium-silicate sealer (Bio-G) was characterized by FTIR to confirm functional groups linked to hydroxyapatite formation [94]. Assiry *et al*. (2023) compared multiple commercial sealers, using FTIR to identify distinctive absorption bands and correlate them with chemical constituents [95]. FTIR has also been applied to a novel bioactive glass bioceramic sealer to detect hydroxyl, carbonate, and silicate groups indicative of bioactivity [96]. Also, detailed assessments of commercial sealers via FTIR with SEM/EDX have been made to identify the presence of functional bands that correspond to specific chemical constituents (e.g., C–H bending, stretching, and carbonate/apatite-type features), which helps in understanding their surface chemistry and potential implications for adhesion, stability, and cytotoxicity.

Atomic absorption spectroscopy (AAS)

The phenomenon of atomic absorption was first noted in 1802, when Wollaston observed dark lines in the Sun's emission spectrum. In 1859, Kirchhoff and Bunsen correctly attributed these lines to absorption of solar radiation by ground-state gas-phase atoms. Nearly a century later, Alan Walsh developed the first analytical atomic absorption spectrophotometer (1953), establishing atomic absorption spectroscopy (AAS) as a standard method for detecting both metallic and non-metallic elements. AAS is valued for its element specificity and ability to measure concentrations at the parts-per-million level or lower (1 ppm = 0.0001%). Routine applications include metal analysis in industry,

geology, medicine, and agriculture.

AAS involves two main steps: atomization, which converts analyte molecules into free gas-phase atoms, and measurement of the radiation absorbed by these atoms at an element-specific wavelength. Quantification follows the Beer–Lambert law, where absorbance (inverse of transmittance) varies linearly with the concentration of absorbing atoms. Instrumentation resembles other high-resolution spectroscopic systems but requires a narrow line radiation source and sufficient heat to create the atomic vapour. Hollow cathode lamps are typically used to provide the sharp spectral lines needed for accurate measurements [97].

AAS in endodontic research

Atomic Absorption Spectroscopy (AAS) has seen limited but meaningful application in endodontic materials research, particularly in quantifying ion release from root canal sealing materials. For instance, in a study, AAS was used to determine the release of Ca^{2+}, K^{+}, and Na^{+} ions from several silicone- and epoxy resin-based sealers after solubility testing [98]. Dsouza *et al*. (2021) analyzed calcium ion release from an experimental silver nanoparticle–incorporated calcium-silicate root-end cement using an atomic absorption spectrophotometer, demonstrating enhanced calcium ion release compared with ProRoot MTA and confirming its potential for hard-tissue formation [99]. While other contemporary investigations often rely on complementary techniques such as EDX, SEM, or ICP-MS for assessing elemental composition, specific applications of AAS in endodontics have remained sparse in the last 5 years. This suggests an opportunity for more recent studies to employ AAS for sensitive detection of trace metals or ion release, which could be critical for assessing biocompatibility and material performance.

Conclusion

The rapid evolution of newer materials and techniques in dentistry has brought remarkable opportunities for improved patient care, but it has also created challenges for clinicians in determining which tests to perform and how to accurately evaluate materials before clinical use. This paper has explored the critical role of research aids in guiding these decisions, highlighting essential tools and methodologies that support evidence-based practice. However, it is important to recognize that not all research aids and advanced evaluation tools can be covered, and emerging technologies continue to expand the possibilities for material testing and clinical validation. Additionally, limitations such as accessibility, cost, and the learning curve associated with sophisticated equipment must be considered. Despite these challenges, the judicious use of research aids remains central to safely integrating new materials and techniques, ensuring that innovation in dentistry translates into effective and reliable patient care.

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