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Technique and Applications of tissue microarray in pathology study: A review

Omar M. Ameen [1a*] Ghada A. Taqa [2 b]

[1a] Department of Oral Diagnosis, College of Dentistry, University of Mosul, Mosul, Iraq
ORCID: https://orcid.org/0009-0003-8013-5617
Email address: Omar.mazin@uomosul.edu.iq
[1b] Department of Dental Basic Sciences, College of Dentistry, University of Mosul, Mosul, Iraq
ORCID: https://orcid.org/0000-0001-9683-7881 Email address: ghadataqa@uomosul.edu.iq

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* Corresponding Author: Ghada A. Taqa.
Email: ghadataqa@uomosul.edu.iq

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Abstract

Tissue microarray (TMA) technology is a high-throughput method that enables the simultaneous analysis of multiple tissue samples within a single paraffin block. By extracting small core biopsies from donor blocks and arranging them systematically into a recipient block, TMAs allow uniform experimental conditions, efficient biomarker evaluation, and preservation of valuable tissue resources. This review discusses the historical development of TMA technology, construction techniques, major applications in predictive, prognostic, validation, and progression studies, and its role in oncology and oral pathology research. The advantages, limitations—particularly tumor heterogeneity—and recent technological advancements in automation and digital pathology are critically evaluated. Special emphasis is placed on the application of TMAs in oral cavity diseases and bioimaging. TMA remains a powerful and evolving tool in molecular pathology and translational research.

Keywords: Biobanks, Tissue microarray, pathology, TMA, tumor heterogeneity.

Introduction

Tissue microarray (TMA) is a novel method that can be used to evaluate multiple tissue samples simultaneously. This cost-effective method has been used extensively in various fields including diagnosis, treatment, and research, demonstrating both its efficiency and versatility (1).

Clinical and biological research relies heavily on tissue samples to detect proteins, RNA, and DNA in situ, facilitating biomarker discovery and improving understanding of disease mechanisms within the tissue microenvironment. However, biobanked samples are often limited, and conventional biomarker analysis is costly, time-consuming, and

labor-intensive. To address these limitations, array-based technologies were developed in the late 1990s to enable high-throughput in situ examination of archived tissues (2).
TMA technology involves assembling hundreds to thousands of tissue cores in a standardized array format, which are then sectioned and analyzed on microscope slides for molecular studies (3). It is possible to evaluate hundreds of consecutive slides using various antibodies and probes, which makes it possible to quickly analyze numerous tissues at once as well as numerous biomarkers or targets from successive sections (2).
Fascinatingly, National Human Genome Research Institute's (NIH's) Cancer Genetics Branch is where what is today regarded as a standard research tool in molecular pathology had its start. This occurred at a time when genomics research was beginning to change due to technology like DNA microarrays. Given that setting, it made sense for us to think about using automated, high-throughput, highly parallel methods for molecular pathology. Together with an engineer named Steve Leighton, we constructed the initial prototype of a manual tissue arrayed. As later reported in numerous collaborative publications that fell between researchers at NIH and Basel, contributed valuable insights and enthusiasm for applying the technology in tissue banks made up of tens of thousands of samples in addition to feedback from the pathology laboratory. (4, 5).
We chose to utilize 0.6 mm- (600 µm) diameter cores from every tissue (3). In comparison to earlier "multitissue block"-based techniques, this enabled us to fit about One microscope slide held 1000 samples, which was nearly an order of magnitude greater (4). The little size of the tissue sample was also criticized, raising the question of how it could be indicative of a heterogeneous tumour. TMA publications still address this issue today (5). It goes without saying that TMAs are designed for throughput and cannot simultaneously offer a comprehensive analysis of every sample. TMAs are usually used to examine cohorts of samples or entire biobanks rather than individual malignancies. TMAs allow estimation of biomarker prevalence across a population, but to characterize expression patterns across an entire sample it is necessary to use multiple punches per tumour and use whole tissue sections. From genome sequencing We are currently learning about the coexistence and clonal evolution of many tumour sub clones in various tumour regions. Therefore, no single tumour component, regardless of its size, can fully capture the diversity of , of the different Clones and sub clones of cancer cells in any patient (5).

The Creation and History of a Tissue Microarray

Small cores of paraffin- embedded biological material are arranged onto a block using a tissue microarray, allowing for the simultaneous examination of hundreds or even thousands of samples at once. In the 1980s, Hector Battifora developed the TMA technique around a central core or rod, which was then embedded in paraffin. One of the numerous drawbacks of his "sausage" block method was that it was hard to tell between particular tissues and wrap the rod with tissue. However, after presenting the concept, Battifora and Mehta developed a checkerboard arranging method that put the components in a block pattern. (3 , 5).
To further refine the method, Wan et al. created the "punching" technique for removal and transfer into exact circular parts. A solid tissue sample is taken from the body during tissue biopsies, which produce the final array. Medical professionals typically need a significant amount of tissue for traditional tissue biopsies, which are essential for diagnosis and therapy. Less tissue is required when using the TMA technique. A circular needle that ranges in size from 0.6 mm to 4.0 mm, depending on the quantity of tissue

available, is used to punch into solid tissue in order to remove the tissue specimen. As illustrated in Figure. 1, smaller core diameters allow for higher sample density within the array (4) . After being divided into tiny slides, the tissue is either directly added to the final array or formalin-fixed and implanted in a donor paraffin block for longevity (3).

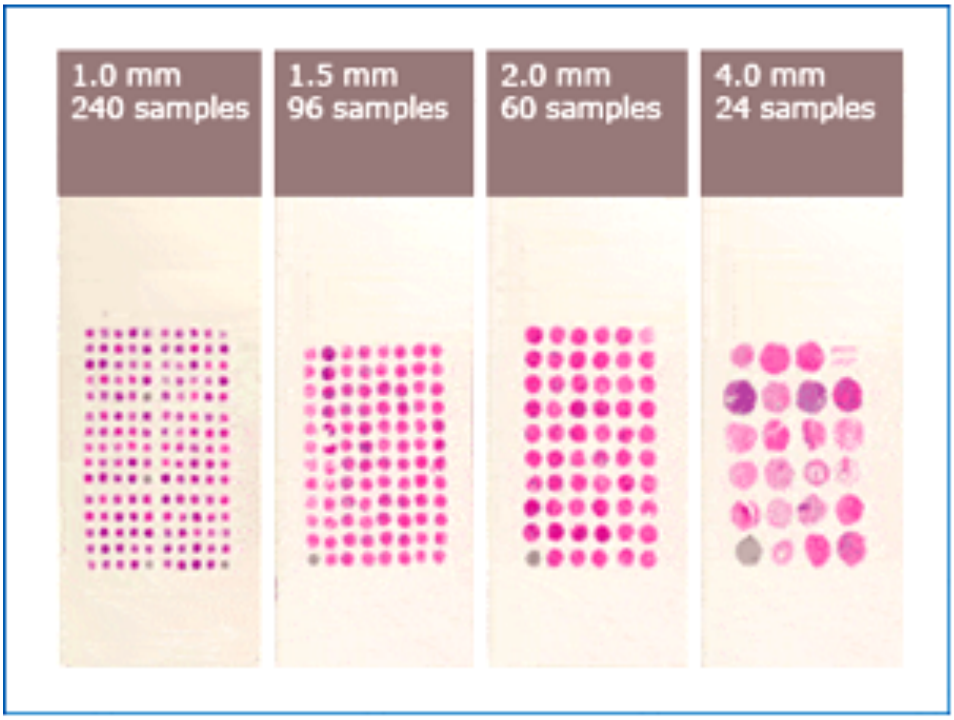

Figure. 1: Sections with Dimensions Between 1.0 and 4.0 Millimeters (3)

Once prepared, tissue cores are transferred into a separate block known as the recipient block. These cores may originate from multiple donor blocks. Paraffin with a low melting point must be present in the recipient block, and throughout the embedding procedure, To prevent disruptions in the array, it is crucial to avoid forming air bubbles within the block. Moreover, recipient block holes should be created immediately prior to the transfer, as paraffin's flexibility can cause them to lose their shape if made too early (6). The array needs to be allowed to set by cooling and incubation after the parts are moved within the block. This will make the sections firm and controllable. For optimal visualization, the array is typically stained using methods such as hematoxylin and eosin (H&E) or immunohistochemical/immunofluorescent techniques, depending on the research purpose (7). The array is set up such that the tissues can be tracked using X-Y coordinates. Individual tissue segment demographic and outcome data are frequently entered into a spreadsheet. to maintain orientation, recipient blocks typically marked at the upper left (8). In Figure. 2 depicts a variety of TMA production TMAs method (4).

Historical Development and Technique

The conceptual foundation of TMA technology was established in the 1980s with early multi-tissue block techniques. Subsequent refinement led to the development of the punching method, which allows precise extraction and transfer of cylindrical cores from donor blocks into a recipient block.(5).

Core diameters typically range from 0.6 mm to 4.0 mm, depending on tissue availability and study design. Smaller core diameters permit higher sample density within a single array. After transfer, the recipient block is cooled to ensure structural stability before sectioning. Sections are then cut using a microtome and mounted onto glass slides for staining (6).

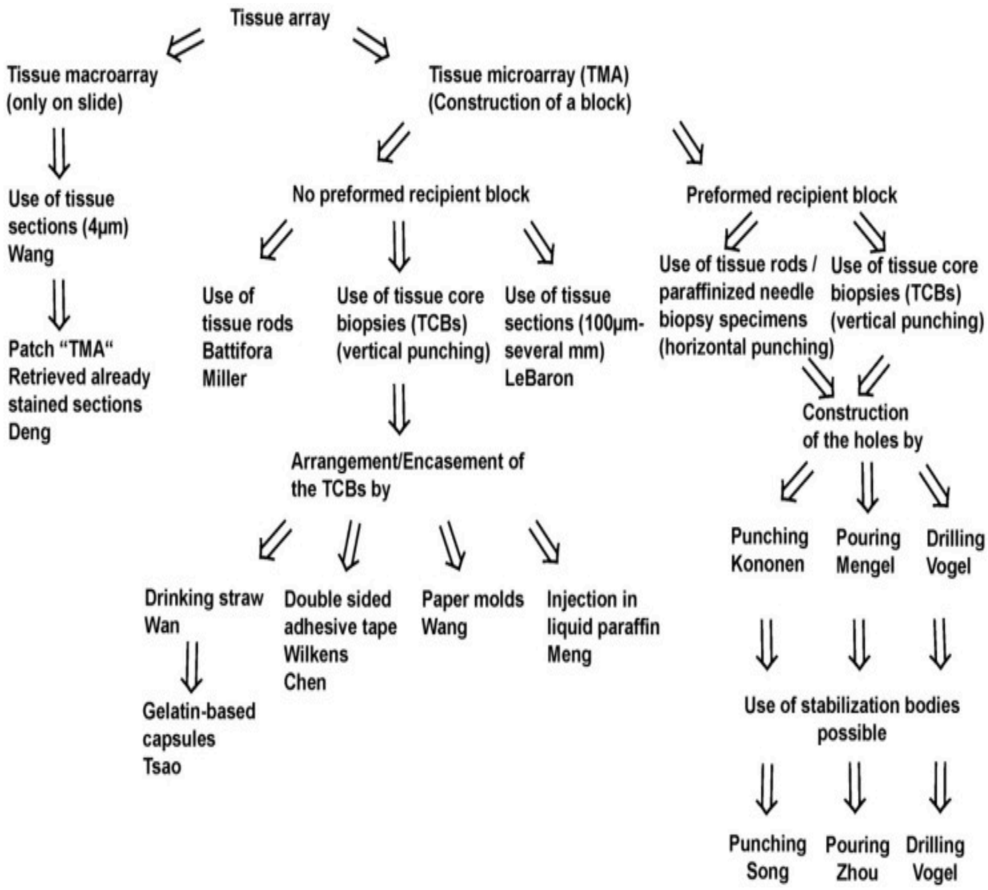

Figure 2: Techniques for Creating TMAs (4)

Uniform processing ensures that all samples are subjected to identical staining conditions, reducing experimental variability. Common analytical methods include hematoxylin and eosin staining, immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), and molecular assays (7). Proper array orientation is maintained using coordinate mapping systems. Each tissue core is linked to corresponding clinical and demographic data, enabling efficient data management and analysis.(8)

Rare tissue augmentation is one of the most important applications for TMAs. Because of the round, accurate sections with limited thickness, 50–500 sections can be employed in an array from a single standard histology sample. The number of sections can be doubled or tripled by further cutting the microarray into 2-3 millimeter pieces after it has been placed in the recipient block (8). As a result, TMAs can create tissue disks totaling tens of thousands from a single sample (7). A TMA's main advantage is that it allows for the full utilization of limited tissue material, hence it is feasible to remove portions for an array while preserving the original tissue block (9). This method is shown in Figure. 3. This guarantees that the content can be used simultaneously in several sectors, including study and diagnostics (10).

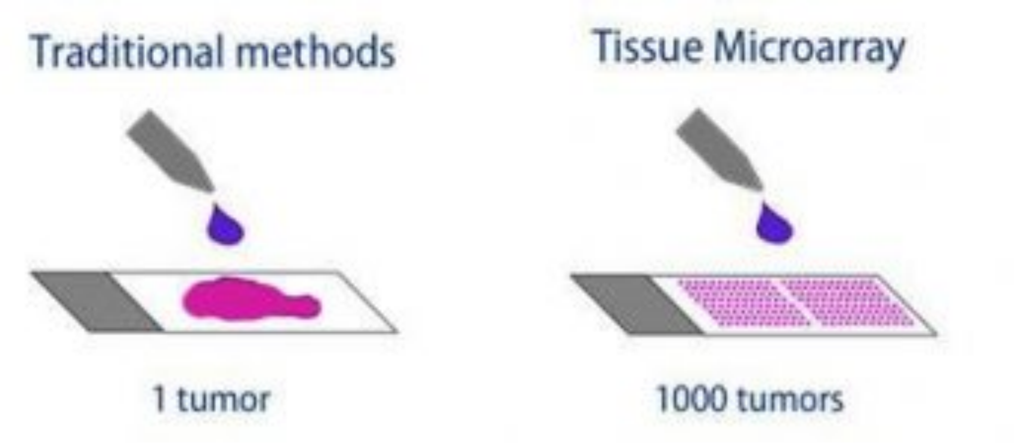

Figure. 3: Traditional Biopsy VS Tissue Microarray (10)

Applications of Tissue Microarrays

Predictive Applications

Predictive TMAs are widely used in therapeutic response studies and drug development. They facilitate evaluation of treatment-related biomarkers and identification of drug-resistant gene expression patterns (7).

Control Microarrays

Control TMAs provide internal quality assurance by including standardized reference tissues. This approach reduces inter-laboratory variation and enhances reproducibility.(8)(11)

Validation Studies

Validation TMAs are used to confirm findings from genomic or transcriptomic analyses at the protein level. They play a crucial role in biomarker validation before clinical implementation (11,12).

Prognostic and Progression Studies

Prognostic TMAs evaluate biomarker expression in relation to patient survival and disease outcome. Progression arrays monitor molecular changes across different stages of tumor development.(9,13). TMA applications extend beyond oncology to include cardiac, placental, neurological, and inflammatory diseases (14).

The drawbacks of tissue microarray technology

Although several studies report high concordance rates between TMAs and whole-section analysis, tumor heterogeneity remains a significant concern, particularly in highly heterogeneous malignancies such as hepatocellular carcinoma. Therefore, the use of multiple cores per tumor is recommended to improve representativeness. (15). A major limitation is that the small size of tissue cores may not accurately represent the entire specimen. Tumours are often histologically heterogeneous, with different regions exhibiting variable protein expression patterns (14). Not all of the tumours genetic markers may be visible in a TMA made up of sections from that tumour (15). In contrast, A new study by Parker and colleagues. revealed that 96% of the markers found in the whole tissue analysis and the tissue microarray analysis were the same for certain estrogen receptors in breast cancer (16). This trend has recurred in several trials with comparable outcomes, suggesting that this drawback is not a statistical barrier (13). One serious condition that tissue microarrays might not detect is hepatocellular carcinoma, a liver cancer that frequently manifests as a tumour that is diverse (16).

Tissue Microarrays' Prospects

There are ongoing advancements in the sector as a result of the many essential applications of TMAs, including improved both amount and quality, in addition to a reduction in the time, effort, and financial requirements (14). The expansion of the amount of tissue that can be used is one such development (17). Current standard TMA platforms typically accommodate 200–600 cores per array in routine laboratory settings. Higher-density experimental platforms may exceed 1000 cores; however, such formats require specialized equipment and may present technical limitations related to core integrity and tissue representativeness. (18). To do this, recipient blocks that are micrometers thick are sliced, and even finer needles are used to retrieve smaller cores (17).

The method for developing a TMA is also quickly approaching a completely automated procedure, which will save time and human labor (20). Several tissue blocks can be handled concurrently by automated tissue arrayer equipment (21). The information in each area must be corroborated, transferred, and recorded by them (22). There have also been advancements in the conversion of glass transforms into digital pictures that are saved until needed and analyzed by algorithms that monitor certain staining patterns and tissue physical characteristics (18).

A number of academic institutions, including Stanford, are developing software applications that would facilitate the acquisition of high-quality analysis and the monitoring of high-density microarrays using automated digital imaging (19). These simulated slides are expected to provide significant biomarkers (21). Through the use of computers, they can be distributed virtually everywhere on earth, creating a collection of specimens that can be utilized for long-term educational objectives (22). Figure. 4 provides examples of automated imaging (20).

Advancements in Non-Traditional Tissue Microarray Platforms: An overview has advanced. These include the use, frozen tissue which has been shown to affect specimen extraction, RNA purity, and tissue morphology (21). Cell line microarrays are another example of this type of technology, this can be used to contrast suspension cells with organically grown cells (22). grown cells to identify physical features in a cell (21).

It is evident from both superior molecular analysis and practicality that TMAs play a key role in their function(22). Although they are still in their infancy, tissue microarrays will eventually play a crucial role in both clinical practice and future research (21). TMAs must continue to advance in order to keep up with the rate of automation that drives biotechnological research, even though they can evaluate multi-origin cell blocks quickly (22). In addition to general oncological and molecular pathology applications, TMA technology has been increasingly utilized in oral pathology research, where standardized biomarker assessment is essential for understanding disease progression and therapeutic response.(21).

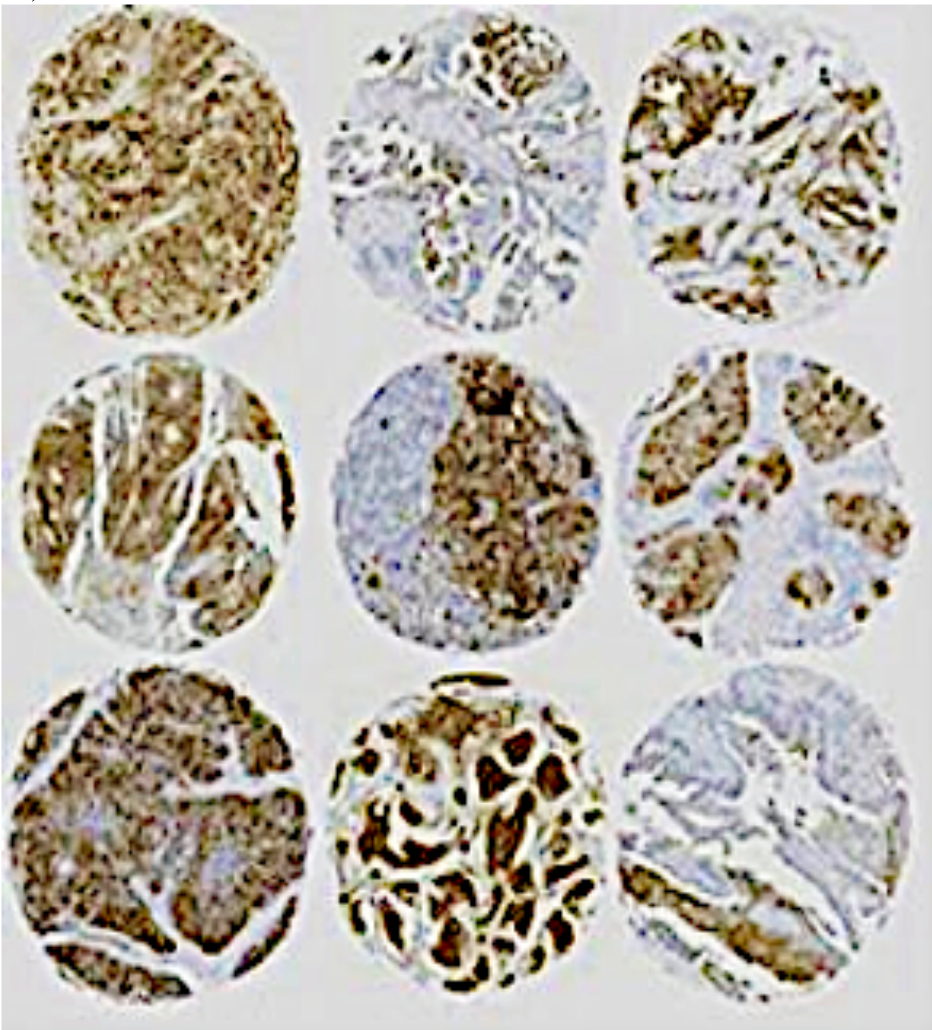

Figure. 4: Sections of Colon Cancer from Automatic Imaging (19)

Oral Cavity Tissue Microarrays in Bioimaging

The oral cavity, encompassing structures such as the lips, tongue, gums, and inner lining of the cheeks, plays a pivotal role in the overall health and well-being of individuals (14) (Figure.5). Diseases affecting the oral cavity, including various cancers, infections, and inflammatory conditions, can significantly impact quality of life (15). The advent of bioimaging techniques and tools, such as tissue microarrays (TMAs), has revolutionized the study and diagnosis of these diseases (16). This article explores the use of oral cavity tissue microarrays in bioimaging, highlighting their significance, applications, and future potential (17).

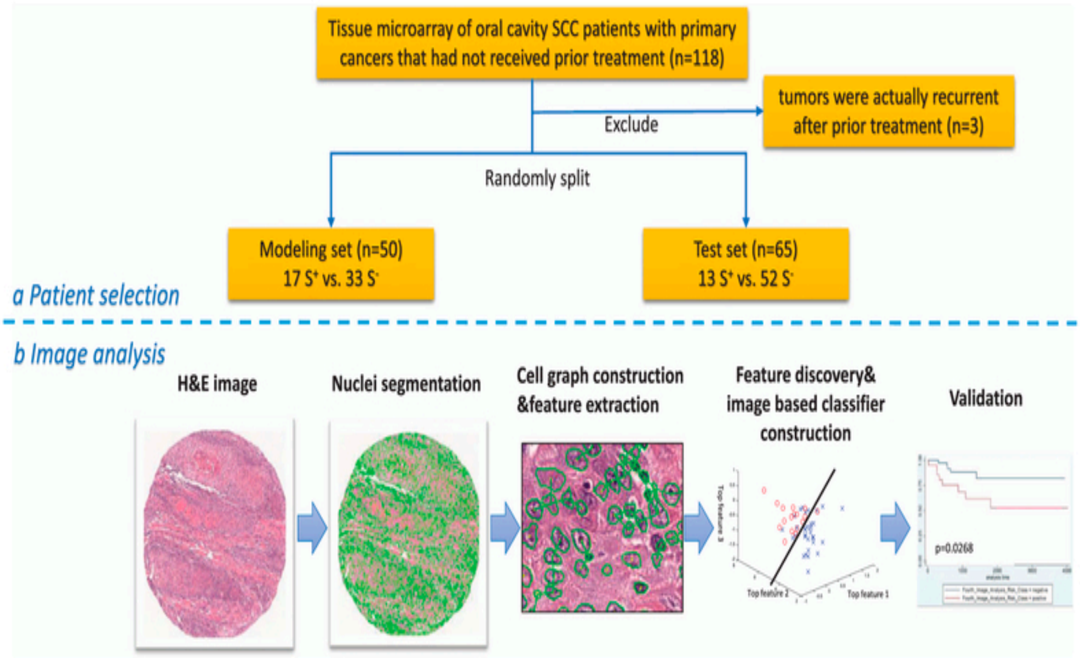

Figure. 5: Image analysis flowchart of the tissue microarray spots. (22)

Applications of Oral Cavity Tissue Microarrays in Bioimaging

Cancer Research and Diagnostics

The identification and validation of biomarkers linked to various stages and types of oral malignancies is made possible using oral cavity TMAs. Simultaneous analysis of several samples allows researchers to link particular biomarkers to patient outcomes and the course of the disease This facilitates early identification and individualized treatment strategies (24).

Drug Development and Testing

TMAs are very useful for testing new drugs to see how well they work and how safe they are. Researchers can look at how drugs affect different tissue samples, which speeds up the drug development process and gives people with oral cavity tumors more treatment options (25).

Histopathological Analysis

Morphological Studies: TMAs make it possible to examine oral tissues in great detail. On a single slide, pathologists can look at tissue architecture, cellular characteristics, and the existence of anomalies in several samples. This thorough study helps with precise diagnosis and comprehension of disease mechanisms (26). Oral cavity TMAs allow the simultaneous evaluation of protein expression patterns in multiple tissue samples, facilitating comparative studies and the identification of possible therapeutic targets. Immunohistochemistry (IHC) is a commonly used technique in which antibodies are used to detect specific antigens in tissue sections (27).

Infectious Disease Research

Pathogen Detection: By analyzing the distribution and localization of pathogens within tissues, researchers can learn more about the pathogenesis and progression of oral infections. Oral cavity TMAs can be used to identify and investigate the bacteria, viruses, and fungi that cause infections in the oral cavity (28).

Inflammatory and Autoimmune Diseases

Cytokine Profiling: TMAs make it possible to identify inflammatory mediators, including cytokines, in oral tissues, researchers can uncover important participants in autoimmune and inflammatory disorders by analyzing the patterns of cytokine expression in various samples, opening the door for targeted treatments (29).

Advantages of Using Oral Cavity TMAs in Bioimaging

High Throughput and Efficiency

TMAs greatly cut down on the time and expense involved with conventional histology techniques by enabling the simultaneous study of hundreds of tissue samples. Large-scale research and clinical trials benefit greatly from this high-throughput capability (25).

Standardization and Reproducibility

TMAs guarantee that all tissue samples are processed and stained identically by arranging them in an array on a single slide. This standardization reduces technical variances and improves the reproducibility of results, which facilitates cross-study comparison of findings (30).

Conclusion

A popular technique for visualizing tissue sections, ESI-MS has been used extensively to describe various disease states in a variety of tissue types. Here, we show how to create and examine high-density TMAs straight from frozen, Tissue microarray technology represents a robust and efficient platform for high-throughput biomarker analysis in pathology. Its ability to standardize experimental conditions and conserve tissue resources has significantly advanced translational research. Although limitations related to tumor heterogeneity persist, methodological refinements and the use of multiple cores per case improve reliability. Ongoing advances in automation and digital pathology are expected to further expand the clinical and research applications of TMAs. ,We prove this novel method using brain cancer samples, demonstrating that the spectral data acquired is of excellent quality and consistent with results that have been previously published, even though the sample size was small and the analysis duration was brief. Specifically, Additionally In dentistry, tissue microarrays provide a valuable research tool for investigating oral squamous cell carcinoma, odontogenic lesions, salivary gland tumors, and inflammatory oral disorders. By enabling simultaneous biomarker assessment across large patient cohorts, TMAs enhance diagnostic accuracy, prognostic evaluation, and therapeutic target identification. Their integration into oral pathology research will likely contribute to more precise, personalized, and evidence-based dental care, identifying biomarkers through in-depth investigation, and testing for drug distributions or biochemical activity in tissue due to its low sample preparation, small sample volume needed, and high throughput. In bioimaging, oral cavity tissue microarrays are a strong and adaptable technology

Declarations

Acknowledgment

None

Ethics statement

Ethics statement: The authors declare that this study was conducted in accordance with the ethical standards and guidelines outlined in the journal's "Ethics Approval" section of the author guidelines. As this work is built on electronic published article, formal ethical approval was not required.

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Competing interest's statement

The authors declare no conflicts of interest.

Author contributions

OMA: Data collection, preparation of study materials, and contribution to the scientific content of the manuscript; GAT: Study concept and design, selection of the study title, and writing and critical revision of the discussion section. All authors reviewed and approved the final version of the manuscript.

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