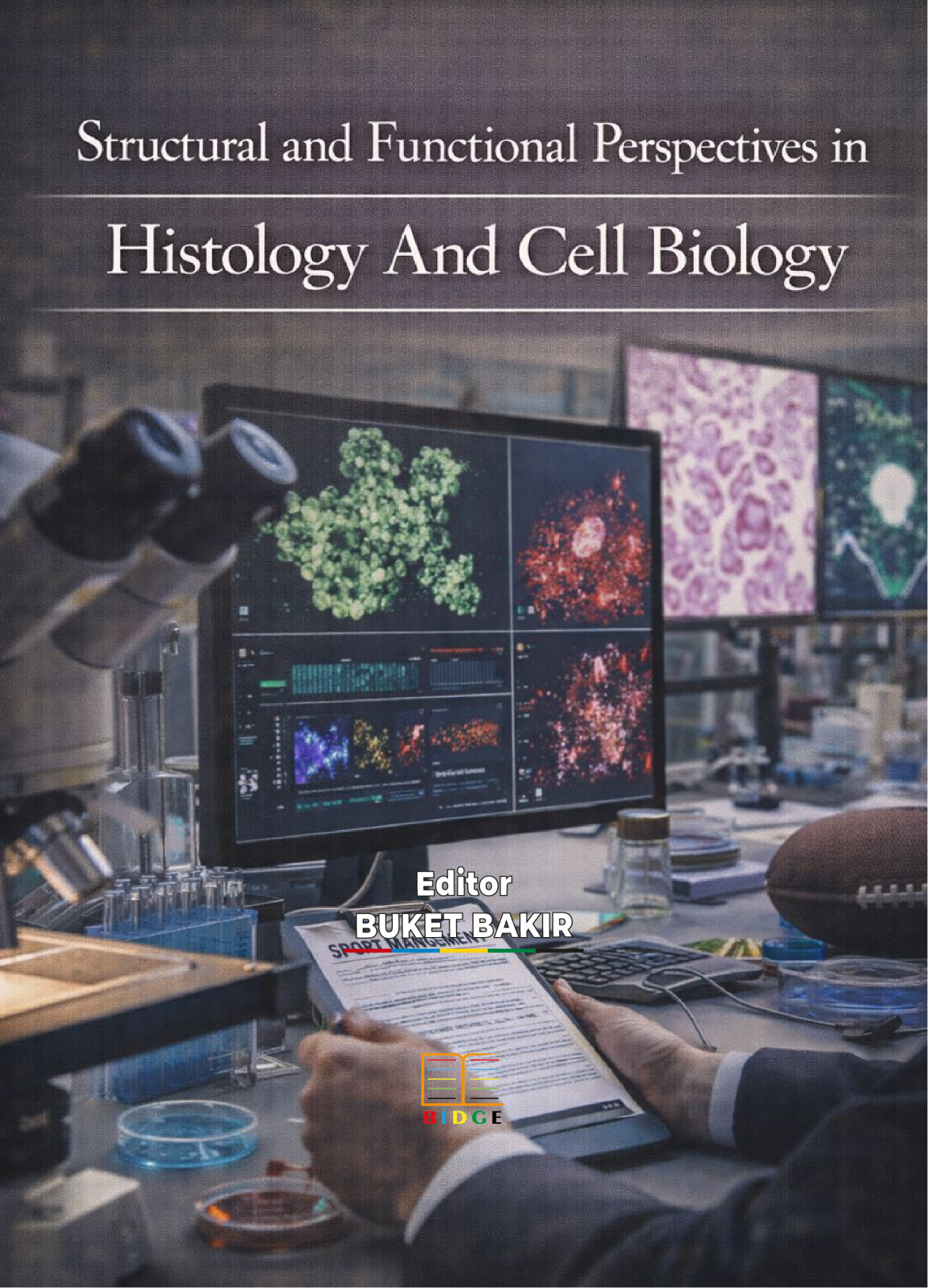
Structural and Functional Perspectives in Histology And Cell Biology



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İÇİNDEKİLER

MATURATION IN ORGANOID AND ORGAN-ON-CHIP MODELS: FROM HISTOLOGICAL CRITERIA TO PHYSIOLOGICAL PERFORMANCE
BÖLÜM 1

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Introduction

Human relevant in vitro organotypic models have moved quickly over the last decade, largely because the field has been looking for better ways to bridge the translational gap between conventional 2D culture and animal models. Within this broader effort, organoids and organ on a chip (OoC) platforms are often presented as complementary strategies. Organoids draw heavily on developmental biology and self organization, allowing stem or tissue derived cells to form miniaturized tissue like structures. Organ on a chip systems, by contrast, use microscale engineering to recreate organ level microenvironments and dynamic cues in a more controlled format (Z. Wang et al., 2020).

Despite the progress, both approaches continue to face a shared challenge that has become central for translation and benchmarking, namely maturation. In practical terms, maturation describes how far an in vitro model progresses beyond early developmental phenotypes toward adult like structural organization, cell type composition, and physiological function. The difficulty is that the field still lacks a universal agreement on what counts as “mature enough,” and that threshold shifts with the intended use, for example mechanistic biology, safety pharmacology, or efficacy screening. This ambiguity is made worse by protocol and laboratory level variability. A point emphasized in organoid on a chip discussions is that non standardized methods do not only increase variability, they can also destabilize organoid maturation and vascularization. In that situation, maturation becomes a moving target unless the culture environment is brought under tighter control (Suhito & Kim, 2022).

From a histology perspective, maturation is often first argued through morphology and marker based criteria. Researchers look for expected tissue compartments, polarity, differentiated lineages, and canonical immunophenotypes. Intestinal organoid systems, for example, can reproduce crypt villus like architecture and strong apical basolateral polarity, and these features can be supported using lineage and differentiation markers such as proliferative markers and mature epithelial markers. At the same time, bioengineering oriented work repeatedly points out that classical organoid culture still depends heavily on poorly defined matrices and handling intensive formats. These features can increase heterogeneity and compromise reproducibility, which directly undermines any attempt to compare maturation levels across conditions or across laboratories (Kakni et al., 2020).

This is where microengineering begins to shift how maturation is framed. Rather than treating maturity as a purely descriptive histology problem, organoid on a chip approaches often treat it as a structure function alignment problem. Chips are positioned as tools that can improve both structural and physiological aspects of organoids by controlling environmental parameters such as nutrient and gas delivery, as well as physical and chemical stimulation. They can also support integrated monitoring, which may help reduce culture to culture variation (Suhito & Kim, 2022). In other words, the maturation question becomes measurable not only by “what the tissue looks like,” but also by “what the tissue does,” and whether function scales appropriately with the observed histological organization.

A concrete illustration comes from vascularization and endothelial maturation in kidney organoids. Kidney organoids often resemble early developmental stages and are constrained by limited culture longevity and a lack of functional vasculature, which in turn restricts size and maturation status. In a microfluidic organ on a chip context, endothelial populations can be interrogated using maturation related markers such as MCAM (CD146) and PECAM (CD31), where developmental transitions are reflected by shifts in marker expression patterns. Notably, when kidney organoids were cultured on chip, analyses suggested more directed endothelial maturation patterns. This included increased MCAM positive and PECAM positive areas and distinct colocalization

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profiles compared with transwell culture (Bas et al., 2022). For a histology to physiology framing, the point is that microenvironment control, including flow, can be linked to marker defined maturation trajectories that are easier to interpret in developmental terms, rather than producing a mixed phenotype that is hard to evaluate.

Epithelial systems provide a similar lesson, but through a different constraint. Maturation is frequently limited by diffusion and by closed architectures that trap debris and waste. An organoid chip hybrid mini colon model addresses this by combining organoid biology with luminal access and flow. In that system, flow removes shed cells and improves tissue longevity and differentiation. Growth factor gradients are used to sustain compartmentalization and differentiation, and single cell RNA sequencing is presented as evidence of emerging mature absorptive lineages, interpreted as a sign of more in vivo like maturation (Mitrofanova et al., 2023). Here, maturation is not treated as a label applied at the end. It is operationalized through environmental design, specifically flow and gradients, and then supported using both tissue organization and cell state readouts.

Beyond endothelium and epithelium, maturation also depends on whether a model can reproduce relevant multicellular interactions. Immune epithelial crosstalk is a good example where histology alone is not enough. Even if an organoid expresses appropriate epithelial markers, the model may still fail to support physiologic immune recruitment or contact formation. In a tissue chip co culture setting, Matrigel, which is commonly used for organoid culture, was shown to prevent dendritic cell migration toward organoids, limiting direct immune epithelial contacts. The same study highlights that migration through Matrigel can be reduced relative to uncoated transwell conditions and can be susceptible to batch to batch variation, which threatens reproducible validation claims. Replacing the matrix with a synthetic hydrogel, VitroGel ORGANOID 3, enabled improved chemotaxis and deeper immune cell penetration in the chip format while maintaining viability (Cherne et al., 2021). In the context of this chapter, this offers a clear bridge between histological maturity markers and physiological performance, because interaction capacity can itself be a defining dimension of maturity for certain organs and disease questions.

Finally, maturation is not only biochemical. It can also be influenced by biophysical and electrophysiological cues that are difficult to reproduce in static culture. A cochlea on a chip platform integrating cochlear organoids within a conductive hydrogel and providing electroacoustic stimulation illustrates this direction. The system supports organoid formation with structurally mature hair cells, while the chip architecture enables dynamic, higher throughput drug evaluation via concentration gradients. The microfluidic setting is also framed as enabling continuous nutrient and oxygen transport and waste removal, which are classic determinants of long term differentiation and maturation capacity. In addition, the stimulation is positioned as a regulator of proliferation, differentiation, and maturation related behaviors (Hu et al., 2024).

Taken together, these examples support a shared message. Maturation in organoid and organ on a chip systems is best treated as a multi axis construct that requires alignment between histological identity, physiological performance, and system level reproducibility. Histological identity includes architecture, polarity, lineage composition, and marker expression. Physiological performance includes transport and barrier function, mechanosensitivity or electrophysiology, secretion and absorption, and related outputs. Reproducibility depends on standardized matrices, controlled environmental cues, and measurable benchmarks. The literature increasingly suggests that microphysiological engineering, through flow, gradients, defined biomaterials, and integrated monitoring, does not merely "improve culture conditions." It can reshape maturation trajectories in ways that are more interpretable, more reproducible, and more tightly connected to functional endpoints (Kakni et al., 2020; Mitrofanova et al., 2023; Suhito & Kim, 2022).

In the remainder of this chapter, we therefore treat maturation as a bridge concept between histology and physiology. We first outline commonly used histological criteria and their limitations, then map these criteria onto functional assays and performance metrics, and finally discuss how organ on a chip integration can support validation and benchmarking by making maturation measurable, comparable, and fit for purpose across organ systems and use cases.

Defining "Maturation" as a Structure–Function Construct

In organoid and organ-on-chip work, *maturation* is often treated as a shorthand for "becoming closer to the in vivo organ." A more useful definition for this chapter is operational: maturation is the

co-evolution of tissue structure and tissue function under controllable conditions, such that structural markers and architecture align with measurable physiological outputs. This framing matters because a model can look convincing under the microscope yet still behave in ways that are not predictive for the intended application, and because microenvironment control is frequently the lever that pushes both structure and function forward (Suhito & Kim, 2022; Z. Wang et al., 2020).

Why Maturation Is A Central Bottleneck For Translation

Maturation becomes a translational bottleneck when organoids remain in partially developed states or when their phenotype is unstable across batches. Reviews of organoid-on-a-chip technology emphasize that progress is often constrained not by the ability to generate organoid-like tissue per se, but by limitations in culture environments and by the lack of standardized, comparable benchmarks that would support confident interpretation across laboratories (Suhito & Kim, 2022).

A recurrent biological reason is that static 3D culture does not naturally reproduce key in vivo drivers of maturation, particularly perfusion-like transport and vascular support. Work focusing on vascularization highlights that sustaining long-term organogenesis and maturation requires overcoming oxygen and nutrient transport constraints—limitations that become more pronounced as tissues increase in size and complexity (Wang, Bijonowski, & Kurniawan, 2023). In kidney organoid settings, the rationale for creating an organoid–vasculature interaction model on-chip is closely tied to the same bottleneck: insufficient nutrient and oxygen delivery without vascular interaction can limit maturation trajectories and functional relevance (Bas et al., 2022).

From an engineering perspective, microphysiological systems are explicitly designed to mimic essential microenvironment features so that primary organ-level functions can be supported in vitro. That design logic implies that maturation is not merely "time in culture," but the outcome of deliberately reconstructed cues—transport, forces, gradients, and materials—selected to support organ-relevant function (Z. Wang et al., 2020).

Dimensions of maturation: structural identity, functional performance, and reproducibility

A practical way to keep maturation claims disciplined is to treat maturity as having three linked dimensions:

Structural identity (histological fidelity) includes architecture, compartmentalization, polarity, and cell-type composition. However, structural identity is only meaningful if it can be assessed consistently. Methods that reduce heterogeneity and control starting conditions are therefore part of "maturation," not just convenience. For example, microwell-array strategies for intestinal organoids are presented as ways to reduce culture heterogeneity and improve controllability—conditions that make any structural maturity claim more interpretable (Kakni et al., 2020).

Maturation claims are most convincing when they are supported by **what the tissue does**, not only by what it expresses. In our source set, several functional axes are difficult to infer from histology alone: longevity and waste clearance under luminal flow and gradients in engineered colon systems (Mitrofanova et al., 2023), immune–epithelial interaction capacity that depends strongly on matrix choice in chip co-culture (Cherne et al., 2021), and sensory-system performance contexts enabled by cochlear organoid integration into a drug-evaluation chip platform (Hu et al., 2024). Taken together, these studies point to the same practical conclusion: maturation becomes credible when structural features and functional performance improve in tandem and can be measured.

Reproducibility is the third maturity axis: even when structural identity and functional performance look strong, a model is hard to translate if results do not hold across batches, chips, or operators. Recent organoid and organoid-on-chip discussions therefore tie platform value to tighter environmental control and repeatable workflows, because these determine whether maturation claims are comparable and whether readouts are stable enough to support downstream decisions (Suhito & Kim, 2022; Z. Wang et al., 2020).

"Fit-for-purpose" maturity: what "mature enough" means depends on use case

"Mature enough" is not a single universal threshold. In practice, the maturity bar shifts with the question you are trying to answer.

For screening and drug testing, the most pragmatic definition is often a stable, interpretable stimulus–response under controlled exposure, even if the model is not fully adult-like in every structural detail; cochlea-on-a-chip work designed around performance-oriented drug evaluation reflects that logic (Hu et al., 2024).

For mechanistic biology, by contrast, an intentionally developmental or transitional state may be the right target if the goal is to understand how structure and function emerge over time; engineered colon systems that use controlled environments and gradients to study spatial organization and differentiation fit this use case (Mitrofanova et al., 2023).

In cancer and personalized applications, "mature enough" often means the platform supports clinically meaningful behaviors and robust testing workflows, with microfluidic organoids-on-chip positioned as enabling more efficient and potentially more reproducible pipelines while standardization remains an open challenge (Duzagac et al., 2021).

At the field level, the rapid growth and increasing coupling of organoids and organ-on-chip technologies has made these distinctions more pressing, because maturity definitions need to travel across studies, labs, and applications (Z. Wang et al., 2020).

For the remainder of this chapter, we therefore treat maturation as **fit-for-purpose structure–function alignment**: histological benchmarks are necessary but not sufficient, physiological readouts provide the decisive test, and reproducibility determines whether "mature" is a meaningful claim beyond a single experiment (Suhito & Kim, 2022; Z. Wang et al., 2020).

Histological Benchmarks

Histological evidence is usually the first thing researchers reach for when they argue that an organoid, or an organoid-on-chip construct, has moved toward a more organ-like state. It is also where maturation language can become slippery, because a tissue can look convincing under the microscope while still behaving in an incomplete or unstable way. For that reason, it helps to treat histology as necessary but not sufficient, and to focus on structural benchmarks that appear repeatedly across the literature.

A consistent starting point is **tissue architecture and spatial organization**. A mature phenotype is rarely just a matter of having the right cell types. Many organs are defined by compartmentalization, polarity, and an ordered arrangement of lineages, and organoid culture often struggles precisely with controlling these features. In intestinal systems, Kakni and colleagues note that conventional culture typically embeds organoids in an excess amount of a poorly defined, tumor-derived extracellular matrix, most notably Matrigel. They describe how this complicates handling and downstream processing and contributes to organoid-to-organoid variation, and they present improved controllability and uniformity as a motivation for alternative culture formats (Kakni et al., 2020).

In kidney organoids, Menéndez and colleagues describe organoids as resembling aspects of the human fetal kidney. They emphasize that nephron structures such as glomeruli and tubules can be validated histologically using established markers, while also linking limited maturation to constrained culture conditions and lack of vascularization. In their framing, necrotic core formation becomes an architectural sign that organoid growth and developmental progression are hitting a practical ceiling (Bas et al., 2022).

Once the architectural "blueprint" is established, most studies rely on **cell-type identity and differentiation markers** to support structural claims. Across the included papers, immunostaining-based marker panels are the dominant approach, and the stronger examples are the ones that go beyond "one marker, one claim." Kakni et al. provide a clear intestinal organoid example: their confocal microscopy descriptions include Ki67 for proliferative cells, lysozyme for Paneth cells, chromogranin A for enteroendocrine cells, and villin as an apical enterocyte marker, with E-cadherin indicating basolateral localization (Kakni et al., 2020). The inclusion of polarity-related markers matters because it supports a structural claim about organization, not merely lineage presence.

Menéndez et al. take a similar approach in kidney organoids, describing nephron structures and using marker-based validation for glomerular or podocyte-associated compartments and tubular segments, including WT1 and PODXL for glomerular or podocyte-related structures, villin for proximal tubuli, and E-cadherin for distal tubuli (Bas et al., 2022). Review-level summaries in organoid-on-chip research show how commonly this comparison logic is used.

Suhito and Kim, for example, describe studies where brain organoids grown on-chip show higher proportions of SOX2-positive neural progenitors and TUJ1-positive neurons than organoids grown in conventional dish culture, with immunohistochemistry used as the core evidence of a state shift (Suhito & Kim, 2022). Even when presented as an overview example, it captures how

histological benchmarks are often used not only to confirm "organoid identity," but also to argue that specific environments bias the balance of cell states and organization.

A third recurring benchmark is **vascularization**, which is discussed as both a structural feature and an enabling condition for continued maturation. Wang and colleagues make the underlying logic explicit in their review: vascularization is crucial during in vivo organ maturation for oxygen and nutrient supply and waste removal as organs increase in size; similarly, diffusion constraints in growing organoids motivate strategies to vascularize organoids to promote long-term organogenesis on chip (X. Wang et al., 2023).

Menéndez et al. add an important nuance, namely that vascular maturation is not only about the presence of endothelial cells, but also about the state of those endothelial populations. They analyze MCAM and PECAM staining patterns and report larger MCAM-positive and PECAM-positive areas on-chip compared with transwell culture, along with distinct colocalization profiles that they interpret as a more directed maturation trajectory (Bas et al., 2022).

Wang et al. further highlight that endothelial vessel formation and maturation in vitro depends on supportive cues and co-culture with vascular support cells such as pericytes or MSCs, reinforcing that vascular histology is inherently tied to microenvironment design (X. Wang et al., 2023).

Finally, histological benchmarking has a practical side that is easy to underestimate: it is also about **controllability and heterogeneity**. If organoids vary widely in size, shape, and cell-type ratios, then "more mature" can quietly become a statement about distribution shifts rather than a reproducible biological change. Suhito and Kim emphasize that methodological standardization is difficult and that organoid generation can show high variability in morphology and cell-type ratios, which undermines uniform interpretation of maturation status (Suhito & Kim, 2022).

Kakni et al. respond with an engineering intervention, moving intestinal organoid culture into polymer film-based microwell arrays. They present this as improving stability and extending culture compared with Matrigel domes, while also reducing handling constraints associated with viscous gels (Kakni et al., 2020). In practice, formats that fix organoid positions and reduce heterogeneity also make histological comparisons more meaningful, because the model can be imaged and quantified consistently over time.

Taken together, the histological benchmarks in this literature converge on a pragmatic core: architecture and compartmentalization, marker-defined lineage and polarity, vascular organization and endothelial state, and the controllability needed to make those readouts comparable (Kakni et al., 2020; Suhito & Kim, 2022). These benchmarks establish structural identity, but they do not by themselves guarantee physiological performance. That is why the next step is functional readouts, where maturation claims ultimately have to be tested.

Physiological Readouts: Linking Histology to Functional Performance

Physiological readouts are where maturation becomes testable. A model can look organ-like, but the maturity claim only becomes convincing when the tissue performs in ways that match the intended organ function and when those outputs can be reproduced across runs. In practice, the most useful readouts are the ones that map onto a known function, can be repeated across batches, and are sensitive enough to detect incremental gains that histology alone may not resolve. Organoid-on-chip approaches help because they introduce controlled perfusion and gradients, and they also provide a clearer route for integrated sensing and real-time monitoring than standard static organoid formats (Suhito & Kim, 2022).

A common entry point is **transport and barrier behavior**, because these are quantifiable endpoints that are difficult to "argue around." Microfluidic intestinal models can be probed through uptake or absorption outputs that reflect epithelial transport performance. In one gut-on-a-chip setup described in the microphysiological systems literature, glucose absorption was higher than in a standard Transwell configuration, suggesting that the chip environment can shift the system toward more in vivo-like function (K. Wang et al., 2020). This is mechanistically plausible because chips allow dynamic cues, including flow and mechanical stimulation, that are difficult to reproduce in static wells. More broadly, external mechanical inputs such as cyclic strain and fluid flow are repeatedly treated as functionally meaningful drivers, precisely because they connect architecture to performance rather than simply changing marker expression (K. Wang et al., 2020).

A related point is that organoid-on-chip designs can reduce diffusion limitations and create more standardized microenvironments. When the culture format improves mass transport, downstream functional assays, including secretion-linked or metabolite-linked endpoints, become less confounded by local depletion and accumulation effects (Kakni et al., 2020). In other words, improved transport conditions do not just make culture easier; they change what functional measurements mean.

Secretory physiology provides another strong maturity signal, especially for epithelial systems, because secretion is organ-specific and dynamically regulated. A useful example is the bioengineered "mini-colon" platform that integrates organoid and organ-on-chip logic. Here, luminal access and flow remove shed cells and waste, supporting longer-lived tissue and more advanced differentiation than physically closed organoids (Mitrofanova et al., 2023).

In this system, maturity is framed as a shift toward more adult-like organization and output, supported by growth factor gradients that sustain in vivo-like patterning and concurrent differentiation. The mucus layer becomes a particularly concrete functional endpoint. The authors report continuous mucus release and note that without perfusion the lumen fills with mucus, an observation that is physiologically relevant because it shows how function depends on fluid handling and clearance, not only on the presence of goblet cells. This is a broader lesson for interpretation: a secretion phenotype is not only about which cells are present, but whether the system supports secretion, distribution, and removal under controlled boundary conditions (Mitrofanova et al., 2023).

Perfusion-linked readouts and endothelial behavior are similarly central, especially in models where vascular interaction is expected to shape maturation. Perfusion matters because oxygen and nutrient delivery can limit organoid size and bias outputs toward stress responses rather than organ-typical function. Vascularization-focused work therefore frames diffusion constraints as a major barrier to long-term organogenesis and maturation, motivating strategies that support vascularization on chip (X. Wang et al., 2023).

In a kidney organoid–vasculature interaction model, Menéndez and colleagues treat endothelial behavior as a functional component rather than decorative detail. They describe endothelial migration into kidney organoids and the formation of lumen-like structures, and they position the model as useful for studying vascular contributions to organoid maturation as well as for drug testing (Bas et al., 2022). From a physiological readout perspective, the key point is that vascular integration creates measurable targets, including endothelial organization and lumen formation, that sit between histology and whole-organ physiology.

Some organs demand readouts that are explicitly function-forward. In the inner ear, morphology alone is rarely persuasive unless it is paired with performance proxies tied to the sensory task. Hu and colleagues present a cochlea-on-a-chip platform that integrates cochlear organoids with electroacoustic stimulation and uses endpoints related to hair-cell differentiation and hair-cell or bundle gene expression to probe the effects of the engineered electrical microenvironment (Hu et al., 2024).

For translation, their design also incorporates a microfluidic concentration-gradient generator to support dynamic, higher-throughput drug evaluation. They demonstrate sensitivity to cisplatin across different concentrations using Live/Dead staining and then evaluate a protective intervention, alpha-lipoic acid, under gradient conditions, positioning the platform as a drug screening and inner-ear drug evaluation system rather than only an organoid culture showcase (Hu et al., 2024). This is a clean example of maturation being operationalized as a measurable performance shift under a clinically relevant perturbation, not simply an increase in markers.

Immune co-culture provides another kind of functional stress test. For many applications, maturity becomes more convincing when the model can respond to a controlled challenge that probes tissue permissiveness, matrix constraints, and signaling competence. In a GOFlowChip-based approach, Cherne et al. assess dendritic cell viability and maturation status after chip culture, and they quantify chemotactic migration using a Transwell chemotaxis assay with a luminescence-based readout (CellTiterGlo). In their framing, motility and recruitment are functional endpoints that help define whether the system supports meaningful immune-epithelial interaction dynamics (Cherne et al., 2021).

Their study also highlights that extracellular matrix selection changes the meaning of "immune interaction." They discuss limitations of Matrigel for efficient recruitment in earlier work and compare matrices in terms of their ability to permit migration while preserving viability (Cherne et al., 2021). In the context of maturation, this is more than a methods detail. It reinforces that physiological readouts are conditional on the engineered microenvironment, and that reproducibility depends on controlling those conditions.

Finally, many "mature enough" arguments in organoid-on-chip work converge on **drug response**, particularly in oncology. Microfluidic devices are described as enabling precise control of drug distribution and supporting dynamic drug-screening regimens, with continuous monitoring and biochemical analysis options that are difficult to achieve in static formats. This aligns with the fit-for-purpose logic from earlier sections. For screening, what matters is not only whether a tissue looks adult-like, but whether it produces stable, interpretable response curves under controlled exposure conditions (Duzagac et al., 2021).

How Chips Support Maturation

Organoid-on-chip systems do not automatically make tissues "more mature." Their value is that they let researchers shape the microenvironment in a more deliberate way, so maturation can be guided and, just as importantly, evaluated in comparable terms. The microphysiological systems literature frames this around recreating physical, mechanical, and biochemical features that influence organ development and function, rather than relying on static culture conditions alone (Z. Wang et al., 2020). Reviews of organoid-on-a-chip technology take a similar position: chips can improve organoid structure and physiology under more controlled conditions, while validation and standardization remain ongoing challenges (Suhito & Kim, 2022).

A major contribution of chips is that they turn diffusion-limited culture into a more controlled transport problem through **perfusion and flow**. As organoids grow and diversify, oxygen and nutrient delivery, along with waste removal, can become limiting, which can stall development or increase heterogeneity. This is one reason vascularization and perfusion are repeatedly discussed as enabling conditions for sustained organogenesis and maturation (X. Wang et al., 2023). Chips address this by enabling **controlled flow** and defined exchange conditions.

In kidney organoid work, an organoid-vasculature interaction model is developed in an organ-on-chip context precisely to study and support vascular contributions to organoid development, with flow and perfusion as central design elements (Bas et al., 2022). In gut models, the same principle appears in a different form: luminal access and flow can remove shed cells and waste, supporting longer-lived tissue and promoting differentiation in engineered colon organoid systems (Mitrofanova et al., 2023). In both cases, the maturation argument depends on changing the boundary conditions of the culture so that transport becomes controllable rather than incidental.

Chips also support maturation by stabilizing **spatial organization** through gradients and compartmentalization. Many maturation claims ultimately depend on whether a system can maintain niche-like patterning instead of drifting toward a mixed or unstable state over time. Compared with static formats, chip-based approaches can impose and maintain gradients more reliably. In the mini-colon platform, gradients are used to sustain in vivo-like patterning and differentiation trajectories (Mitrofanova et al., 2023). In a sensory-organ context, the cochlea-on-a-chip platform incorporates microfluidic gradient logic in a way that is directly tied to performance-oriented drug evaluation, which shows how spatial control often becomes part of the functional testing workflow rather than an isolated design choice (Hu et al., 2024).

A third, practical lever is the choice of **matrices and materials**. Many organoid protocols depend on poorly defined ECM preparations, which can amplify variability and complicate integration with devices and co-cultures. Engineering strategies can reduce that dependence, or at least make it more controlled. Kakni et al. describe microwell-array culture as a way to reduce heterogeneity and improve controllability for intestinal organoids, which is an important precondition for interpretable maturation comparisons (Kakni et al., 2020).

Matrix choice also determines what functions are even observable. In a tissue-chip co-culture model, Cherne et al. show that a synthetic hydrogel, VitroGel ORGANOID-3, improves immune cell-epithelial interaction readouts such as chemotaxis through the matrix while maintaining viability,

illustrating how biomaterials can unlock physiologically relevant behaviors that may otherwise be suppressed by the culture environment (Cherne et al., 2021).

Finally, chips matter because they support **standardization and workflow robustness**. Fixed geometry, controlled exposure, and repeatable handling strengthen the credibility of both histological and functional maturity claims by reducing uncontrolled variability. This theme is explicit in organoid-on-chip reviews that emphasize controlled improvement of organoid structure and physiology while also noting that benchmarking and standardization remain essential for translation (Suhito & Kim, 2022). In oncology-oriented organoids-on-chip discussions, the rationale is similar: microfluidics is positioned as enabling more structured and potentially more reproducible testing pipelines, while limitations related to consistent device and organoid production still need to be addressed (Duzagac et al., 2021).

Short Case Examples: When Maturation Becomes Measurable Structure-Function Performance

A useful way to keep "maturation" concrete is to look at systems where the claim is supported by a clear pairing of structural features and functional outputs. The following examples are intentionally brief. Each one illustrates a slightly different route by which organoid or organoid-on-chip models make maturity visible as something that can be measured rather than inferred.

One practical example is the intestinal mini-colon platform, where the engineering goal is not only to keep an epithelial tube alive but to make the lumen experimentally accessible. In this system, perfusion pulses through the luminal compartment are used to remove shed or dead cells and waste products, which stabilizes the tissue for longer-term work and makes longitudinal assessment more feasible. Functional maturation is then treated as a barrier property. The authors directly assess a leak-tight epithelial barrier using a fluorescent 40 kDa dextran permeability readout (Mitrofanova et al., 2023).

A second example comes from kidney organoids, where Menéndez and colleagues frame a central limitation as insufficient oxygen and nutrient supply due to limited vascularization. They address this through a human-cell-derived vascularization strategy in an organ-on-chip context. The maturation claim is supported at two levels. Histologically, nephron structures are described, while endothelial marker colocalization analysis (MCAM and PECAM) is used as a quantitative proxy for endothelial state. Functionally, the chip supports a hallmark behavior of vascular integration: HUVEC migration from endothelialized channels into organoid tissue and formation of integrated vascular structures with open lumens, reported as continuous with endogenous endothelial cells (Bas et al., 2022).

A third example treats maturity as performance under interaction, rather than a static endpoint. Cherne et al. focus on whether a gastric organoid system can support immune cell movement and epithelial interface behavior, which are central to many mucosal biology questions but are often difficult to reproduce in static culture. They show that VitroGel ORGANOID-3 can support gastric organoid growth in tissue-chip settings while enabling dendritic cell chemotaxis, and they evaluate immune-epithelial interaction behavior using barrier formation and interface-related readouts. In this framing, maturation is reflected in the ability to maintain a structured epithelium while permitting controlled immune dynamics that would otherwise be suppressed by the culture environment (Cherne et al., 2021).

Hu et al. provide a fourth example in an organ system where maturity is inseparable from stimulus responsiveness. They build an electroacoustic-responsive cochlea-on-a-chip and explicitly couple sensory-cell maturation goals, including abundant mature hair cells, to a platform designed for drug evaluation. A notable design element is a microfluidic gradient generator that enables dynamic, higher-throughput pharmacologic testing within the same device context. They demonstrate how this setup can be used in ototoxicity-related workflows, including cisplatin exposure with dose-dependent viability assessment and testing of a protective intervention such as alpha-lipoic acid. In practical terms, maturity is treated as both histologic or cellular differentiation and measurable vulnerability or protection under defined perturbations (Hu et al., 2024).

Finally, tumor organoids-on-chip illustrate fit-for-purpose maturity particularly clearly. Düzagac et al. argue that organoids are valuable in oncology because they can capture aspects of tumor architecture and microenvironment, and they emphasize that microfluidic integration offers

more controlled conditions for studying and manipulating those environments. In this use case, “mature enough” does not necessarily mean developmental maturity. Instead, it often means phenotypic stability under flow or gradient constraints, compatibility with treatment-response assays, and the ability to incorporate microenvironmental factors that can modulate drug sensitivity. The maturity target is therefore defined by the decision the model is meant to inform, such as mechanism, response prediction, or comparative screening, rather than by a universal endpoint (Duzagac et al., 2021).

Outlook and Remaining Challenges

Despite rapid progress, maturation still blocks translation in fairly predictable ways. Across organoid workflows, recurring barriers include low reproducibility, limited vascularization, restricted nutrient uptake and distribution, insufficient standardization, and intra-clonal variability (Suhito & Kim, 2022). These are not minor technical details. When protocols are not standardized, morphology, cell-type ratios, and functional outputs can drift, which changes what different groups end up calling “mature” in practice (Suhito & Kim, 2022).

A second constraint is mass transport. As organoids enlarge, diffusion alone becomes insufficient for oxygen and nutrient delivery and for waste removal, with survival biased toward cells closer to fresh medium (Duzagac et al., 2021). This diffusion-limited biology aligns with the broader vascularization argument: larger organoids risk hypoxia and necrotic cores unless perfusion or vascular strategies are introduced (X. Wang et al., 2023). Microenvironment fidelity is another persistent gap. Many systems still lack immune, stromal, or blood components and do not reproduce key biomechanical cues such as shear stress (Duzagac et al., 2021). The implication is straightforward: because maturation is a structure-function outcome, missing forces and missing cell populations can yield tissue that looks plausible histologically but behaves incompletely physiologically.

Materials and workflow variability are often the quiet sources of immature phenotypes. ECM surrogates are a central example. Matrigel-based culture is widely used, yet it is described as a poorly defined, tumor-derived matrix in organoid contexts. Beyond composition, the practical handling of viscous gels can complicate automation and downstream processing, which becomes a scaling problem when maturation assessment needs to be reproducible across batches and sites (Kakni et al., 2020). Experimental outcomes can also shift simply because the matrix shifts. For example, migration through Matrigel is reported to be affected by batch-to-batch variation (Cherne et al., 2021). Taken together, this means maturity cannot be treated as an intrinsic property of the tissue alone. It also reflects the broader materials ecosystem, including ECM and media, and the process ecosystem, including handling, imaging access, and operating discipline.

The outlook is still optimistic, partly because organoid and organ-on-chip research continues to expand and the overlap between the two areas is growing (Mitrofanova et al., 2023). On the technical side, the logic of chips matches several maturation needs: microfluidics can serve as a circulatory analogue, transporting nutrients, gases, and metabolites while enabling controlled mechanical stimulation and gradient generation (K. Wang et al., 2020).

At the same time, “more chips” is not automatically the same as “more mature models.” The next step is methodological rather than purely technological. Operational standardization remains essential because poor reproducibility and protocol variability destabilize both morphology and maturity claims. Instrumented readouts can also reduce ambiguity, since sensors can be integrated to monitor responses and behaviors and help minimize culture variation (Suhito & Kim, 2022).

Finally, automation and throughput are not just convenience features. They reduce operator-driven drift and can support continuous monitoring and biochemical analyses in more structured formats, including multi-well approaches (Duzagac et al., 2021).

Conceptually, the field is moving toward a fit-for-purpose maturity philosophy. The most defensible approach is to define minimum structural criteria and minimum functional criteria that are explicitly tied to the intended application, whether that is toxicity screening, disease mechanism, or regenerative aims. This avoids claiming a single universal “mature state,” while still demanding measurable maturity thresholds grounded in reproducibility, transport sufficiency, and physiological performanc (Duzagac et al., 2021; Suhito & Kim, 2022).

Conclusion

In this chapter, we treated maturation in organoid and organ-on-chip models as a structure-function construct. In other words, a maturity claim becomes convincing when histological identity, including architecture, cell-type composition, and marker-defined organization, advances alongside measurable physiological performance, and when both can be reproduced with enough stability for the intended use (Suhito & Kim, 2022; Z. Wang et al., 2020). Across the studies we included, the most persuasive arguments tend to rely on practical control of the microenvironment. Transport conditions such as flow, perfusion, and waste removal matter, as do spatial cues such as gradients and compartmentalization. Matrix and material choices also repeatedly show up as decisive, because they can either enable key behaviors or quietly constrain them (Cherne et al., 2021; Kakni et al., 2020; Mitrofanova et al., 2023).

The case examples point to a simple pattern. Chip integration is most valuable when it turns a biological limitation into a controllable variable. Luminal access and flow can stabilize gut-like systems and make barrier function measurable in ways that are difficult to achieve in closed organoids (Mitrofanova et al., 2023). Perfusable architectures can support vascular interaction and make endothelial phenotypes quantifiable as part of the maturation story, rather than treating vasculature as a missing background feature (Bas et al., 2022; X. Wang et al., 2023). In sensory and translational drug-testing contexts, platforms built around stimulus-response and controlled exposure help move "maturity" away from a descriptive label and toward a testable performance property (Hu et al., 2024). In oncology-focused organoids-on-chip, "mature enough" is often defined more pragmatically, namely by whether the system supports robust and interpretable treatment-response workflows under microfluidic control, while standardization remains an important frontier (Duzagac et al., 2021).

Looking ahead, the rapid expansion of both organoid and organ-on-chip research makes fit-for-purpose maturity benchmarks increasingly important. These benchmarks need to be explicit, measurable, and comparable across studies if the field is going to translate reliably (Z. Wang et al., 2020). A practical takeaway for readers is to avoid treating maturation as a single universal endpoint. Instead, it is more defensible to report maturity as a combined package: structural benchmarks, functional readouts that match the application, and reproducibility controls that make the evidence transferable beyond a single experiment (Suhito & Kim, 2022; Z. Wang et al., 2020).

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BÖLÜM 2

Implantation and Its Mechanism

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Implantation

Implantation is the process by which an embryo (blastocyst) attaches to and implants within the endometrium. Implantation is a multistage process that occurs when the blastocyst interacts harmoniously with the developing endometrium. This interaction between the two components can only occur within a specific timeframe known as the "implantation window." The implantation window is the timeframe during which the uterus is ready to accept a fertilized egg. In a normal menstrual cycle, this period covers approximately day 16 to day 22. The chances of implantation outside this timeframe are significantly reduced, and if implantation occurs, it often results in miscarriage.

Prerequisites for Implantation

The remodeling of the uterine endometrium, which is shed during the menstrual cycle, is crucial for successful implantation. Estrogen and progesterone have decisive effects on the remodeling of the uterus's structure and function (1). These hormones perform their functions by interacting only with their specific receptors to initiate intracellular signaling pathways. Progesterone has PR-A and PR-B receptors, while estrogen has ERα and ERβ receptors. Genetically modified mouse models created in recent years have provided important information about the roles of ovarian steroid hormones in the implantation process. Receptors are crucial for implantation. PR-A receptor, in particular, is crucial for implantation. Similarly, the primary requirement for estrogen's effectiveness is the presence of ERα. It has been observed that animals lacking the ERα gene have hypoplastic uteruses and are infertile, while animals lacking the ERβ gene are fertile.

Estrogen levels rise due to developing ovarian follicles. Increased estrogen stimulates the proliferation of endometrial epithelial, stromal, and vascular endothelial cells, contributing significantly to tissue remodeling in the proliferative phase of the cycle. Progesterone stimulates the formation of pinopods, which facilitate implantation. The most important effects of pinopods on implantation are to remove cell surface glycoprotein MUC1 is a molecule that suppresses intercellular adhesion in the implantation window, allowing implantation to occur. The early embryo at the morula stage develops into a blastocyst consisting of approximately 32 to 256 cells before implantation. The loss of the zona pellucida (hatching), which occurs within a few days after the morula reaches the uterus, is considered the first biological step towards implantation of the embryo.

Implantation Stages

Implantation consists of three stages:

1. Apposition: The blastocyst's contact with the implantation site in the endometrium is considered the first step in the implantation process. In humans, implantation generally occurs in the midsagittal plane of the uterus, on the superior and posterior walls. This process is considered a proinflammatory response resulting from increased endometrial vascular permeability at the implantation site, influenced by cyclooxygenase-mediated prostaglandins. In humans, elevated prostaglandin E_2 levels in the epithelium and underlying stroma at the implantation site play a key role in both embryonic attachment and increased vascular permeability. Therefore, prostaglandin E_2 is considered a key regulator of trophoblast cell attachment to the endometrium and tissue invasion.

2. Adhesion: It is defined as the attachment of trophoblast cells to the endometrial epithelium. Adhesion between trophoblast cells and the endometrial cells of the uterus occurs through cell adhesion molecules such as integrins, cadherins, selectins, and the immunoglobulin family. Cell adhesion proteins expressed by the trophoblast during invasion interact with specific ligands present in the decidua's extracellular matrix, regulating adhesion and progression.

3. Invasion: Invasive trophoblast cells penetrate the basal lamina of the endometrial epithelial layer and migrate into the stromal tissue. Trophoblasts play a key role in invasion. Trophoblasts differentiate into cytotrophoblasts and syncytiotrophoblasts at this stage. Syncytiotrophoblast cells destroy the walls of maternal arteries, turning them into loose sinusoidal sacs (lacunae) lined with trophoblasts. The primary goal of endometrial invasion is to replace small, high-resistance vessels

with larger, low-resistance vessels; otherwise, the blood circulation between the mother and the fetus functions to ensure that blood flow remains high throughout pregnancy. Abnormalities in trophoblast invasion may predispose to various pregnancy complications, such as fetal growth restriction or the development of preeclampsia.

Plasminogen activators (PAs) are closely associated with trophoblastic invasion. PAs are enzymes that convert plasminogen to plasmin to enable proteolytic degradation of the extracellular matrix. Trophoblast cells carry receptors known as plasminogen activator receptors on their surfaces. The MMP family of matrix metalloproteinases plays an important role in the disruption of the ECM during trophoblast invasion. This enzyme family is divided into three main classes based on the substrates they target: collagenases, gelatinases, and stromelysins. Type IV collagen is a key building block of the uterine extracellular matrix. The invasive ability of human trophoblast cells is largely related to the enzymes type IV collagenases, namely MMP-2 and MMP-9, that degrade this structure. Invasion of trophoblastic cells is a characteristic feature of malignant cells. However, it is crucial that the invasion area of trophoblasts remains restricted to the placenta throughout pregnancy and is controlled. This process is controlled by the stimulatory and inhibitory effects of various growth factors, cytokines, and enzymes. Decidual cells synthesize PAI-1, the primary inhibitor of PA. Decidua-derived TGF-β serves as one of the key regulatory factors limiting the invasion of human trophoblast cells by increasing the expression of both TIMPs and PAI-1. TGF-β also induces antiproliferative signals at the fetal-maternal interface, promoting the transformation of invasive and proliferative cytotrophoblasts into noninvasive and multinucleated syncytiotrophoblasts.

Mediators Effective in Implantation

1. Cytokines

Cytokines consist of various proteins that regulate the mother-embryo interaction and play critical functions in the progression of implantation. They also regulate immune adaptation and tissue remodeling for this interaction. Cytokines are among the key regulatory molecules in the process of embryo adhesion to the endometrium, facilitating physical contact between the embryo and the endometrium. A molecular communication complex mediated by cytokines, various growth factors, prostaglandins, matrix-degrading enzymes, and adhesion molecules is crucial for successful implantation. Cytokines, which are regulatory peptides or glycoproteins, unlike hormones, generally exert local paracrine or autocrine effects in tissues.

1.1. Interleukin-6 (IL-6)

The IL-6 family plays a crucial role in blastocyst development and implantation. Leukemia inhibitory factor (LIF) is a cytokine belonging to the IL-6 family. IL-6, a proinflammatory inflammatory response, plays an important role in fertility. IL-6, also secreted by epithelial and trophoblastic cells, is an important cytokine of estrogen action. IL-6 production peaks during the luteal phase of the menstrual cycle and the implantation phase. This increase suggests a central role in increasing the endometrium's capacity to accept embryos, the development of placental trophoblast layers, and the maintenance of immunological stability during pregnancy.

1.2. Interleukin-1 (IL-1)

IL-1 is one of the important paracrine factors regulating the interaction between the embryo and the endometrium. In the endometrium, it is secreted by epithelial and stromal cells, and cytotrophoblasts. IL-1 expression peaks in the first period of pregnancy. IL-1 induces the transformation of stromal cells, which is important for decidualization. IL-1 stimulates the VEGF and contributes to implantation by regulating matrix metalloproteinases (MMPs) and tissue metalloproteinase inhibitors (TIMPs).

1.3. Leukemia Inhibitory Factor (LIF)

LIF is an important immunoregulatory molecule classified within the IL-6 cytokine family due to its properties. Abnormal production of LIF, which plays a crucial role in embryo implantation, can lead to implantation failure. LIF plays a critical regulatory role in preparing the uterus for implantation,

facilitating decidualization, supporting blastocyst growth and development, regulating the interaction between the embryo and the endometrium, controlling trophoblast invasion, and modulating the immune response. LIF also promotes stromal proliferation by regulating epidermal growth factor (EGF) expression. LIF is also effective in the emergence of pinopodes in epithelial cells, which facilitate the implantation.

2. Cell Adhesion Molecules

2.1. Colony-stimulating factor-1 (CSF-1)

Both the embryo and endometrium express CSF-1. The biological interaction between this endometrial epithelial-derived factor and the trophectodermal receptor contributes to the efficient initial contact and adhesion between the embryo and the uterus.

2.2. Integrins

Formed by the association of α and β subunits, integrins are important transmembrane glycoprotein receptors that enable cells to interact with the external environment. Menstrual cycle-specific integrins, synthesized in the endometrium in the mid-secretory (luteal) phase, are considered markers of the implantation window. Trophoblasts express integrins during implantation, contributing to implantation.

2.3. Cadherins

The glycoprotein family that plays a fundamental role in the mechanisms where intercellular communication occurs in a Ca^2-dependent manner is known as cadherins. They are found in both trophoblasts and endometrial epithelia.

2.4. Selectins

The selectin family includes various members, including P-selectin, L-selectin, and E-selectin. Of these, L-selectin is known to be expressed both in trophoblast tissue and on the surface of pinopodic structures. The first step in implantation is the interaction between L-selectin in trophoblasts and its ligands in the endometrial epithelium. L-selectin is the most important one in the implantation process. The attachment and penetration of cytotrophoblast cells into decidual tissue is promoted by mechanisms mediated by ligands that interact with L-selectin.

2.5. Mucin-1 (MUC-1)

MUC-1 is a molecule belonging to a family of transmembrane glycoproteins expressed on the apical epithelial surface of the endometrium and modified with numerous sugar molecules. In women, the level of this molecule increases significantly, particularly during the secretory phase, and remains elevated during the receptive period, when the embryo is ready for implantation. High glycosylation protects MUC-1 from proteolytic degradation, increasing its stability.

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BÖLÜM 3

STRUCTURAL AND HİSTOLOGİCAL PERSPECTİVES ON THE ANTİOXİDANT ROLE OF QUERCETİN

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Introduction

Natural polyphenols are defined as a broad group of phytochemicals commonly found in plant-derived foods and beverages (1–3). Structurally, polyphenols consist of aromatic rings functionalized with one or more hydroxyl groups (2). In particular, flavonoids and other phenolic compounds constitute an important part of the regular human diet (4,5). The average daily intake of flavonoids varies between approximately 1–2 g depending on the type and quantity of fruits, vegetables, and beverages consumed (6). Diets rich in polyphenols have been associated with inverse relationships with various pathological conditions, including cancer, cardiovascular diseases, and degenerative disorders (7,8). Moreover, polyphenolic compounds have been reported to exhibit numerous pharmacological activities, including antioxidant, anti-inflammatory, anticarcinogenic, antiviral, and antiallergic effects

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(9,7,10–12). Natural compounds—particularly flavonoids—have attracted considerable attention as therapeutic candidates due to their low toxicity and pleiotropic mechanisms of action (13,14). The anticancer effects of flavonoids are associated with multiple mechanisms, including the regulation of reactive oxygen species (ROS) and antioxidant enzyme activities, modulation of intracellular signaling pathways, induction of cell-cycle arrest, activation of apoptosis and autophagy, inhibition of cancer cell proliferation and invasion, downregulation of glycolytic metabolism, and reduction of metastasis risk (15–17).

The aim of this section is to comprehensively discuss the effects of Quercetin (QRC), a polyphenolic flavonoid, and to evaluate its anticancer, antioxidant, and anti-inflammatory potentials in light of molecular and cellular mechanisms.

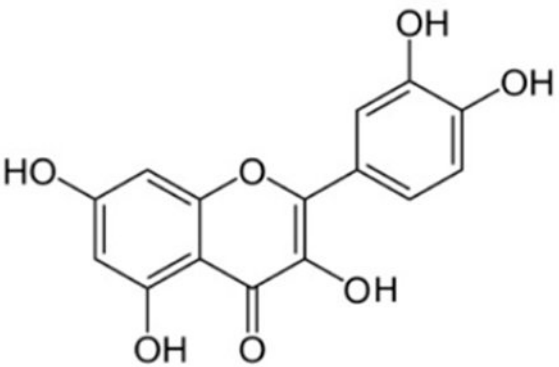
Information About Quercetin

QRC (3,3′,4′,5,7-pentahydroxyflavone) is a polyphenolic flavonoid abundantly present in various dietary sources, including apples, red grapes, onions, raspberries, honey, cherries, citrus fruits, and leafy green vegetables. QRC exhibits a wide range of biological activities, including antioxidant, anticancer, antiviral, apoptosis-inducing, protein kinase C-inhibitory, cell-cycle-regulatory, and anti-angiogenic effects (18). This flavonoid, commonly found in nuts, tea, vegetables, and aromatic herbs, has been extensively investigated by researchers due to its broad biological activities (19–21). QRC predominantly occurs in two main forms: the free aglycone form and conjugated derivatives bound to various molecules. These conjugates include QRC glycosides, prenylated QRC, QRC ethers, and QRC sulfates. Such structural diversity not only enhances the solubility and stability of QRC but also contributes to the modulation of its biological effects (22).

Sources and Metabolism of Quercetin

QRC is a natural flavonoid compound characterized by its multiple ring structures and the presence of hydroxyl groups. It possesses a distinctive molecular framework consisting of a C6–C3–C6 carbon skeleton and, in many dietary forms, a glucose moiety attached to one of its hydroxyl groups. This unique molecular architecture confers strong biological activity to QRC and plays a crucial role in determining its solubility, metabolic transformation, and functional behavior within biological systems.

Figure 1. *Structure of Quercetin*



QRC possesses a total of five functional hydroxyl groups, each of which has the potential to undergo conjugation. The phenolic hydroxyl groups of QRC act as electron donors and are primarily responsible for its free radical scavenging activity. In particular, the catechol moiety formed by two adjacent hydroxyl groups exhibits markedly superior electron-donating capacity compared with other structural arrangements (23).

QRC metabolism primarily occurs in the small intestine, colon, liver, and kidneys (24). Following oral ingestion, QRC interacts with proline-rich salivary proteins through hydrogen bonding or hydrophobic interactions, forming soluble QRC–protein aggregates that do not hinder absorption. In the stomach, only a small proportion of QRC is degraded into phenolic acids. In the small intestine, quercetin glycosides (QGs) are efficiently absorbed by intestinal epithelial cells via sodium-dependent glucose co-transporters (SGLTs) and subsequently hydrolyzed to the QRC aglycone by cytosolic β-glucosidase (CBG) (25). Alternatively, some quercetin glycosides can be deglycosylated by lactase–phlorizin hydrolase (LPH) (26).

Thereafter, QRC and its metabolites undergo extensive phase II metabolism in the liver and small intestine, including glucuronidation, sulfation, and methylation, resulting in derivatives such as QRC-3-glucuronide, QRC-3′-sulfate, and 3′-O-methylquercetin (isorhamnetin) (27). Some of these metabolites enter the bile and systemic circulation through multidrug resistance-associated proteins (MRPs) and organic anion transporters (OATs), ultimately being excreted in urine and feces. Non-absorbed QRC and its metabolites are also eliminated through feces. Additionally, the gut microbiota plays an important role in QRC metabolism by degrading it into low-molecular-weight phenolic compounds, such as 3,4-dihydroxyphenylacetic acid and 4-hydroxybenzoic acid, which are more readily absorbed (28).

Quercetin and Apoptotic Mechanisms

Apoptosis is an energy-dependent physiological process that governs programmed cell death in response to intracellular and extracellular stimuli. This mechanism is critical for maintaining tissue homeostasis, eliminating abnormal cells, and preventing tumor formation. The apoptotic process proceeds through two major

pathways: the extrinsic (death receptor-mediated) pathway and the intrinsic (mitochondrial) pathway (29,30).

QRC, a plant-derived flavonoid with potent antioxidant, anti-inflammatory, and anticancer properties, has been increasingly recognized for its multifaceted regulatory role in apoptotic processes. Recent studies demonstrate that QRC can activate both the extrinsic pathway initiated by cell-surface death receptors and the intrinsic pathway mediated by mitochondrial signaling (31–33).

Effects on the Extrinsic Apoptotic Pathway

QRC promotes programmed cell death by enhancing the transcription of death receptors and their ligands associated with the extrinsic pathway. Notably, quercetin significantly upregulates the expression of tumor necrosis factor (TNF), death receptors DR4 and DR5, Fas, Fas ligand (FASL), and TRAIL. TRAIL and TNF receptors serve as key signaling nodes for initiating extrinsic apoptosis (32).

Initiator caspases (caspases-2, -8, -9, and -10) are activated in a dose-dependent manner in response to QRC exposure. In particular, caspase-8 expression increases by approximately 3.6-fold following 48-hour treatment with 50 μM quercetin. As caspase-8 activation represents a major step in the TNF-induced extrinsic apoptotic cascade, this increase indicates that QRC effectively activates the extrinsic pathway (34,35).

Contribution of the Intrinsic (Mitochondrial) Pathway

The mitochondrial apoptotic pathway is activated by internal stimuli such as cellular stress, DNA damage, and oxidative imbalance. This pathway is characterized by the release of cytochrome c from mitochondria into the cytoplasm, activation of caspase-9, and subsequent activation of effector caspases (caspases-3, -6, and -7) (36,37). QRC treatment has been shown to cause a

slight increase in caspase-9 gene expression and cytochrome c levels, suggesting that the intrinsic pathway plays a partial role in QRC-induced apoptosis (37).

Activation of Effector Caspases

Effector caspases, including caspases-3, -6, and -7, are upregulated in a dose-dependent manner following QRC treatment, indicating coordinated progression of the apoptotic response. Caspase-3 holds particular importance due to its central role in poly(ADP-ribose) polymerase (PARP) cleavage and DNA fragmentation (34). QRC significantly enhances both caspase-3 transcription and active caspase-3 protein levels, demonstrating effective initiation of the apoptotic cascade.

In QRC-treated HeLa cells, a pronounced increase in caspase-3 activity has been observed, confirming strong activation of the caspase cascade. Additionally, the upregulation of caspase-2—a key mediator of DNA damage response—further supports the activation of caspases-3 and -8, correlating with DNA fragmentation and comet assay findings (36).

Conclusion

These findings demonstrate that QRC is a potent bioactive flavonoid capable of inducing apoptosis through both the extrinsic pathway and, to a lesser extent, the intrinsic mitochondrial pathway. By coordinately activating initiator and effector components of the caspase cascade, QRC exhibits substantial therapeutic potential as a natural compound that promotes programmed cell death, particularly in cancer cells.

Quercetin and the Mechanism of Ferroptosis

Ferroptosis is a programmed form of cell death characterized by iron-dependent lipid peroxidation, arising from disruptions in iron metabolism and insufficiency of antioxidant defense systems.

This process is morphologically, biochemically, and genetically distinct from classical apoptosis, necrosis, and autophagy. Ferroptotic cells typically exhibit excessive accumulation of reactive oxygen species (ROS), elevated levels of lipid peroxides, and reduced activity of glutathione peroxidase 4 (GPX4) (22). A recent study investigated the ferroptosis-inducing effects of QRC in gastric cancer (GC) cells in detail (36). The findings demonstrated that QRC treatment significantly increased lipid peroxidation levels in GC cells. Transmission electron microscopy (TEM) analyses confirmed hallmark ferroptotic morphological alterations, including condensed mitochondria, loss of cristae, and shrunken mitochondrial membranes.

Further molecular analyses revealed that QRC directly interacts with the sodium-dependent glutamine transporter SLC1A5 (Solute Carrier Family 1 Member 5). Specifically, QRC binds to Ser-343, Ser-345, Ile-423, and Thr-460 residues of SLC1A5, thereby inhibiting its expression. This interaction impairs the nuclear translocation of nuclear factor erythroid 2-related factor 2 (NRF2), one of the key regulators of the cellular antioxidant defense system. Suppression of NRF2 activity leads to a reduction in the expression of the cystine/glutamate antiporter (xCT) and GPX4, thereby markedly weakening the glutathione (GSH)-dependent antioxidant capacity of the cells.

Simultaneously, QRC/SLC1A5 signaling activates the phosphorylated calcium/calmodulin-dependent protein kinase II (p-CaMKII) and phosphorylated dynamin-related protein 1 (p-DRP1) pathway, enhancing ROS production. The resulting oxidative stress accelerates the peroxidation of cellular lipids and promotes ferroptosis.

Moreover, inhibition of SLC1A5 increases intracellular iron levels, which intensifies free radical generation through the Fenton reaction. Together, these three mechanisms—suppression of

antioxidant defenses, enhanced ROS production, and intracellular iron accumulation—collectively induce ferroptotic cell death in GC cells (22).

Conclusion

QRC suppresses the NRF2/xCT/GPX4 axis through SLC1A5 inhibition, amplifies oxidative stress via CaMKII/DRP1-mediated pathways, and contributes to iron accumulation, thereby promoting ferroptosis. These findings indicate that QRC functions not only as an inducer of apoptosis but also as a versatile anticancer agent capable of activating alternative programmed cell death pathways such as ferroptosis.

Quercetin and the Mechanism of Autophagy

Autophagy is a highly conserved lysosome-mediated cellular degradation and recycling process. It involves the sequestration of cytoplasmic components including damaged organelles, misfolded proteins, and toxic metabolites within double-membrane autophagosomes, which subsequently fuse with lysosomes for degradation and recycling into reusable biomolecules. As a key regulator of cellular energy balance and stress adaptation, autophagy plays a dual role in cancer biology. On one hand, it functions as a tumor-suppressive mechanism by eliminating potentially oncogenic factors, damaged mitochondria, and reactive oxygen species, thereby maintaining genomic stability and cellular homeostasis. On the other hand, in established or advanced tumors, autophagy is frequently repurposed as a survival mechanism, enabling cancer cells to cope with nutrient deprivation, hypoxia, or chemotherapy-induced stress and contributing to metabolic adaptation and treatment resistance (22).

Among natural polyphenols, QRC has drawn considerable scientific interest for its ability to modulate autophagy in human gastric cancer cells. Functional studies have demonstrated that QRC

induces autophagy; however, inhibition of autophagy markedly enhances QRC-induced apoptosis (38). These data indicate that QRC-induced autophagy functions as a protective cellular response.

At the molecular level, QRC activates autophagy by suppressing the Akt/mTOR signaling cascade. Dephosphorylation of Akt and mTOR results in the inactivation of downstream effectors such as p70 S6 kinase (p70 S6K) and 4E-BP1. Additionally, QRC promotes the stabilization of hypoxia-inducible factor-1α (HIF-1α), a critical regulator of cellular metabolism, invasion, and survival. Accumulation of HIF-1α not only inhibits the mTOR pathway but also upregulates BCL2 interacting protein 3 (BNIP3) and BNIP3-like (BNIP3L). These proteins disrupt the interaction between Beclin-1 and Bcl-2/Bcl-xL, thereby promoting autophagic flux (22).

In other oncogenic models, QRC has been shown to induce excessive autophagy, leading to autophagy dependent cell death (39,40). This dual behavior indicates that QRC exerts context-dependent effects, capable of supporting cancer cell survival or inducing lethal autophagy depending on factors such as intracellular microenvironment, drug concentration, exposure duration, and cancer-specific signaling networks (22).

Conclusion

QRC exerts a bidirectional regulatory role on autophagy by modulating both the Akt/mTOR–HIF-1α–BNIP3 axis and Beclin-1–dependent pathways. Thus, QRC is considered a context-dependent bioactive flavonoid capable of inducing either protective or death-promoting autophagic responses in cancer cells.

Quercetin and Anti-Inflammatory Mechanisms

QRC is a natural flavonoid distinguished by its potent anti-inflammatory properties among its diverse biological activities. Considering the central role of chronic inflammation in the

development of gastric cancer, QRC's regulatory effects on inflammatory signaling pathways hold significant therapeutic value.

At the molecular level, QRC suppresses tumor necrosis factor-α (TNF-α) induced inflammatory responses by modulating the TNF-α–Src family tyrosine kinase c-Src extracellular signal-regulated kinase 1/2 (ERK1/2) c-Fos proto-oncogene axis. This regulation results in the inhibition of matrix metalloproteinase-9 (MMP-9) gene and protein expression. As MMP-9 is a key enzyme responsible for extracellular matrix degradation and metastatic progression within the tumor microenvironment, QRC's inhibitory effect on this pathway represents an important mechanism limiting tumor invasion and inflammation-associated tissue destruction.

Additionally, QRC inhibits activation of the nuclear factor kappa-B (NF-κB) pathway. NF-κB is a central regulator of inflammatory cytokines and adhesion molecules and plays a major role in promoting pro-inflammatory gene expression in gastric cancer cells. By suppressing NF-κB activation, QRC reduces cytokine release, oxidative stress responses, and cancer-associated inflammatory signaling.

Another key anti-inflammatory mechanism of QRC involves modulation of gastric epithelial injury induced by Helicobacter pylori (HP) infection, a major trigger of gastric carcinogenesis. QRC inhibits the Sp1 transcription factor (SP1) /Lipocalin-2 (LCN2) axis, thereby reducing HP-induced epithelial apoptosis and inflammatory tissue damage. This demonstrates that QRC not only attenuates inflammatory signaling but also contributes to the preservation of mucosal integrity (22).

Conclusion

QRC exhibits multilayered regulatory effects on TNF-α–c-Src–ERK1/2–c-Fos, NF-κB, and SP1/LCN2 signaling pathways, functioning as a protective and therapeutic flavonoid agent in

inflammation-associated gastric cancer. These findings indicate that QRC has the capacity to restore homeostatic balance in the tumor microenvironment by modulating both molecular mediators and transcriptional regulators of inflammation.

Quercetin and Its Anti-Angiogenic Effects

Quercetin (QRC) exhibits potent anti-tumor activity by suppressing angiogenesis, a critical process required for tumor growth and progression. Angiogenesis within tumor tissues is essential for providing cancer cells with nutrients and oxygen, facilitating metastatic dissemination, and maintaining the continuity of the tumor microenvironment. Consequently, inhibition of angiogenic pathways is considered a strategic therapeutic target in cancer management.

At the molecular level, QRC exerts its anti-angiogenic effects primarily by targeting the vascular endothelial growth factor receptor-2 (VEGFR-2) pathway, thereby inhibiting endothelial cell proliferation, migration, and tube formation. VEGFR-2 is one of the principal regulators of tumor neovascularization, and its suppression results in the inhibition of neoangiogenesis and consequently the restriction of tumor growth.

QRC also exerts regulatory effects on the downstream phosphatidylinositol 3-kinase (PI3K) /AKT signaling axis by inhibiting the AKT (Protein Kinase B) component of the VEGFR-2 pathway. Inhibition of this pathway reduces the proliferative and anti-apoptotic capacity of tumor cells, thereby significantly limiting cell growth and tumor volume in malignancies such as breast and prostate cancers.

Notably, QRC maintains its anti-angiogenic efficacy even in drug-resistant cancer cells. This characteristic suggests that QRC may enhance the therapeutic efficacy of conventional chemotherapeutic agents and overcome treatment failures associated

with chemotherapy resistance. Therefore, QRC holds potential both as an adjuvant molecule that increases chemosensitivity and as an independent anti-angiogenic agent (41).

Conclusion

QRC suppresses tumor angiogenesis at multiple levels by targeting VEGFR-2 and the PI3K/AKT signaling pathway. This mechanism highlights QRC as a significant phytochemical compound capable of inhibiting vascular development within the tumor microenvironment, limiting metastatic spread, and slowing cancer progression.

Experimental and Cellular Studies on Quercetin

A wide range of experimental studies has demonstrated that QRC exhibits diverse biological activities across different cell lines, including induction of apoptosis, reduction of oxidative stress, and preservation of histological integrity.

Chien et al. (42) reported that QRC administration induced apoptotic cell death in breast cancer cells accompanied by a marked reduction in p53 expression. This effect was associated with decreased metabolic activity, suppression of anti-apoptotic proteins, and increased expression of the pro-apoptotic factor Bax. These findings suggest that QRC may activate apoptotic pathways even in a p53-independent manner.

Morphological analyses using light and fluorescence microscopy showed that PI-stained HeLa cells exposed to QRC exhibited classical apoptotic features, including cell shrinkage, nuclear fragmentation, and apoptotic body formation. Similarly, in leukemia cells (NALM6), QRC activated the intrinsic (mitochondrial) apoptotic pathway by increasing cytochrome c release and stimulating caspase-9 activity (43). In contrast, in breast cancer cells, QRC triggered the extrinsic apoptotic pathway by

stimulating caspase-8 and caspase-3 activities without altering mitochondrial membrane potential (44). These differences indicate that QRC initiates apoptosis through cell type-specific signaling mechanisms (37).

Another study conducted on HepG2 hepatocellular carcinoma cells demonstrated that QRC exerted anticancer effects by activating p53-mediated apoptotic signaling. This was characterized by increased p53 protein expression and an elevated Bax/Bcl-2 ratio. Considering the transcriptional regulatory role of p53 on Bax and Bcl-2, QRC appears to enhance both the sensitivity and effectiveness of apoptosis mediated by these molecules (45).

QRC has also been shown to attenuate oxidative stress and inflammation. In renal ischemia/reperfusion (I/R) injury models, QRC significantly decreased malondialdehyde (MDA) levels, a major marker of lipid peroxidation, while restoring glutathione (GSH) levels (46). These findings demonstrate that QRC mitigates oxidative tissue damage through its antioxidant capacity and preserves renal functional integrity (47).

Experimental evidence further highlights the protective effects of QRC in reproductive system injuries. In cyclophosphamide (CP)-induced testicular toxicity models, increased reactive oxygen species and oxidative stress led to degenerative changes in seminiferous tubules, Leydig cell damage, and impaired steroidogenesis. QRC treatment reduced ROS production, preserved mitotic activity of germinal epithelial cells, and maintained testosterone synthesis in Leydig cells, thereby sustaining the histological integrity of testicular tissue (48).

QRC is a potent plant-derived flavonoid antioxidant with well-documented histoprotective effects across various experimental models. The key findings related to its actions on reproductive,

hepatic-renal, cardiovascular, and neuronal tissues are summarized below.

Histological Effects of Quercetin on the Reproductive System

QRC demonstrates significant protective effects against cellular and tissue damage caused by oxidative stress. In various experimental models—including type 2 diabetes mellitus (DM) (49), lead toxicity (50), and organophosphate pesticide-induced testicular injury such as diazinon exposure (51) QRC administration resulted in marked histomorphological improvements.

Histological evaluations showed that QRC preserved seminiferous tubule structure and reduced degeneration, vacuolization, and basement membrane disruption in germ cells. Spermatogenesis was maintained, and the Johnsen score increased significantly. Leydig and Sertoli cells displayed more organized morphology, with preserved cytoplasmic and structural integrity. TUNEL staining revealed a substantial reduction in apoptotic cell numbers, confirming the anti-apoptotic effects of QRC. Additionally, Masson's trichrome staining showed decreased collagen deposition and reduced fibrosis, suggesting that QRC limits stromal remodeling and fibrotic progression in testicular tissue.

Conclusion

QRC protects testicular tissue against oxidative or toxic injury by preserving structural integrity, supporting germinal epithelial organization, and limiting fibrotic changes, thus acting as an effective histoprotective agent.

Histological Effects of Quercetin on Hepatorenal Tissues

QRC exerts pronounced protective effects on hepatic and renal tissues subjected to oxidative stress and toxic injury. In experimental models such as non-alcoholic fatty liver disease (NAFLD) (52), co-exposure to aluminum oxide nanoparticles

(Al_2O_3NPs) and lead acetate (Pb) (53), and cyclophosphamide-induced toxicity (54), QRC administration has been shown to improve tissue morphology.

Histopathological analyses demonstrated reduced hepatocyte vacuolization, nuclear pyknosis, and sinusoidal dilation in QRC-treated groups compared to control and toxicity groups. Hematoxylin-eosin (H&E) staining revealed preservation of hepatic cord architecture, while Masson's trichrome staining showed significant reductions in fibrosis and collagen accumulation. Periodic acid–Schiff (PAS) staining indicated restoration of normal glycogen storage in hepatocytes.

In renal tissue, QRC treatment reduced degeneration and necrosis in tubular epithelial cells and improved glomerular structure. These findings support the conclusion that QRC reduces oxidative damage and preserves cellular integrity in hepatic and renal tissues through both antioxidant and anti-inflammatory mechanisms.

Conclusion

QRC limits fibrosis, cellular degeneration, and necrosis in liver and kidney tissues subjected to oxidative or toxic stress, thereby contributing to the preservation of tissue structure and function.

Cardiovascular and Neuroprotective Histological Effects of Quercetin

QRC is a potent flavonoid antioxidant that protects cardiovascular and nervous system tissues against oxidative stress and ischemic injury. Numerous experimental studies have demonstrated its beneficial effects on brain health (55), ischemic stroke (56), and myocardial damage (57).

In myocardial tissue, QRC treatment preserved the organization of muscle fibers and reduced cellular infiltration and

edema, indicating protection of cardiac structural integrity and attenuation of inflammation.

In brain tissue, histological analyses revealed reduced nuclear condensation, decreased perineuronal vacuolization, and preservation of neuronal integrity. QRC also significantly reduced astrocyte activation, as demonstrated by decreased GFAP staining, supporting its neuroprotective and anti-inflammatory effects within the central nervous system.

Conclusion

QRC exerts protective histological effects on cardiovascular and neural tissues by mitigating oxidative stress and inflammatory injury, thereby supporting tissue integrity and contributing to functional recovery.

Conclusion

QRC, a naturally occurring flavonoid, exhibits potent antioxidant, anti-inflammatory, and cytoprotective properties that confer protective effects across various organ systems. Histological studies consistently demonstrate that QRC reduces oxidative damage, inhibits apoptotic pathways, and attenuates inflammatory responses, thereby preserving tissue structural integrity. Through modulation of key cellular signaling pathways such as Nrf2/HO-1, NF-κB, and MAPK, QRC supports histo-architectural organization and enhances functional recovery following metabolic or toxic insults.

Overall, current histopathological evidence indicates that QRC is a highly promising bioactive compound for protecting against oxidative and inflammation-mediated tissue injury. However, comprehensive dose-dependent, long-term, and clinical studies are required to fully establish its safety profile and clinical applicability.

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DISSECTOR METHOD IN STEREOLOGY

BÖLÜM 4

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Introduction

Stereological analysis methods, which are used in all scientific studies involving quantitative data, have gained increasing importance in recent years due to their reliability, impartiality, low cost, and rapid results. Their reproducibility and the resulting closer approach to accurate results with repeated analysis make this method preferable. Obtaining realistic data from planned studies is crucial for the study's acceptability.

Stereology has a wide range of applications, encompassing not only biological materials but also inanimate objects. Numerical data on the geometric and statistical structure of three-dimensional samples are obtained from two-dimensional cross-sections. Three-dimensional analysis of structural changes in tissues from histological sections is one of the applications of stereology. A time-consuming process such as cell counting may be achieved practically and effectively through stereological approaches. Objectivity and efficiency are fundamental concepts in stereology.

This study provides information about stereology, which has a wide range of applications, and its dissector method.

1. What is Stereology?

Stereology is a method frequently used in quantitative studies in disciplines such as medicine, veterinary medicine, mathematics, and engineering, where digital data is extensively used (Akalan & Demirkan, 2013; Keleş, 2019). Stereology, which provides accurate and reliable results and has a high scientific acceptance rate, is a set of techniques that enable information about the true properties of structures from data obtained from two-dimensional cross-sections of three-dimensional samples (keleş, 2019; Yurt et al., 2018). Quantitative description of three-dimensional samples from two-dimensional images may be achieved using stereological methods (Cruz-Orive, 1993; Yurt et al., 2018). A key aspect of these methods is that the selected samples represent the entire structure. Structural changes in tissues from very small components to systemic dimensions can be observed (Mouton, 2002). It encompasses many methods, including optical and physical dissectors, fractionators, nucleators, and point sampling (Gundersen et al., 1988a; Keleş, 2019). Data such as the morphology of structures of interest, volumetric components, cell number, length, volume, and surface area can be calculated with this method (Akalan & Demirkan, 2013; Keleş, 2019; Odacı et al., 2004). Furthermore, in recent years, stereological analysis has gained importance in the quantitative analysis of regeneration and degeneration in the central and peripheral nervous systems in the clinic. Stereology is frequently used in the diagnosis of diseases, determination of their effects and post-treatment follow-up (Yurt et al., 2018).

In stereology, issues such as objectivity, efficiency, systematic random sampling, and preliminary studies are crucial (Akalan & Demirkan, 2013; Kaplan, 2006; Keleş, 2019). One of the most important advantages of this method is that it does not systematically deviate from the true value even when the study is repeated and that results that are even closer to the true value are obtained (Gundersen et al., 1988b; Keleş, 2019; Odacı et al., 2004). Furthermore, with optimally used resources, fewer errors are achieved in a short time (Gundersen & Jensen, 1987; Keleş, 2019; Mouton, 2002). The most strategic basis of stereological methods is systematic random sampling. Taking a random number of sections

from the structure of interest at specific intervals within the sampling interval yields results that are statistically closer to reality (Cruz-Orive, 1999). Another important point in stereology is the clear visualization of the counted elements in the tissue. Sectioning and staining are crucial in stereological analyses (Kalkan, 2009). Stereology is a combination of mathematical and statistical methods that eliminates the loss of information and dimensions that occur during sectioning (Mouton, 2002).

2. Dissector Method

The dissector method, one of the unbiased stereological methods, is considered the first of the modern design-based stereological methods. The dissector method, a three-dimensional sampling method, was developed to unbiasedly calculate the number of particles present in a given volume. It provides data on the numerical density of particles in three-dimensional structures (Sterio, 1984; Yurt et al., 2018). Numerical density is a crucial data point for assessing the relationship between structure and function (Boyce et al., 2010). The basic logic of the method is to find the first and last visible parts (ends) of particles along the sectioning direction. A virtual three-dimensional stereological probe uses in this method (Figure 1). This probe detects the "ends" or "tops" of particles in one direction (Gundersen et al., 1988a). This method achieves accurate and unbiased results by calculating the number of particles, such as cells, cell organelles, and neurons, within a virtual three-dimensional volume in consecutive serial section planes taken within a specific "t" interval without individually evaluating all components of the investigated structure (Sterio, 1984; Yurt et al., 2018).

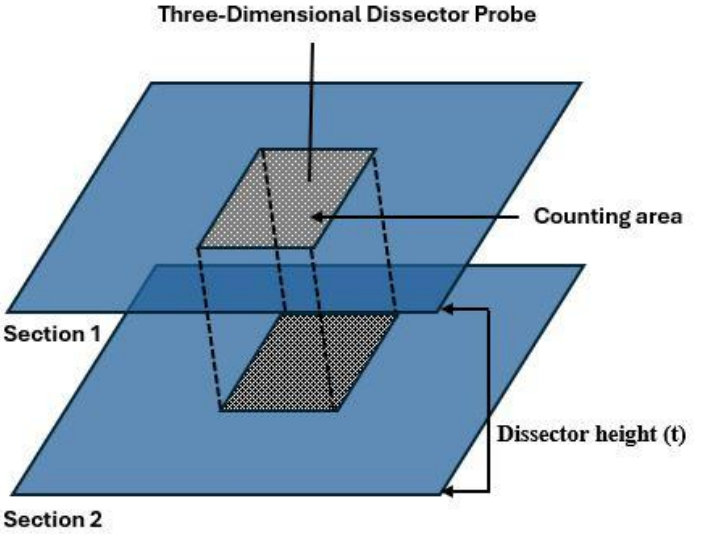

Figure 1. Schematic Representation of Three-Dimensional Dissector Probe.

The number of particles in a tissue or organ may be estimated objectively and independently of the section orientation, particle size and orientation, and tissue shrinkage or swelling (Sterio, 1984). The dissector method utilizes sections prepared using histological techniques. Because this method samples the particles to be counted based on their presence, it allows reliable results to be obtained from high-quality histological sections meticulously prepared (Kalkan, 2009). This method is applied in two ways: physical and optical dissector. The physical dissector counting technique is based on taking two consecutive sections of tissue. The optical dissector method is applied by using consecutive optical sections within a single thick section volume and performing particle counts in a selected sampling area (Gundersen, 1986; Odacı et al., 2004; Sterio, 1984).

In dissector methods that require working with section surfaces, it is essential to sample sections of the biological structure

to be studied using a systematic random sampling method at a predetermined frequency. For example, if a 1/55 random sampling is to be performed for the biological structure under study, two consecutive sections are selected from the first 55th section, followed by each subsequent 55th pair of sections, until the tissue is studied. This method ensures that every point on the structure under study has an equal chance of sampling. This ensures a systematic random sampling appropriate for dissector counting (Ünal et al., 2002a; 2002b).

Instead of counting the projections of all particles of varying sizes and numbers within the tissue, counting the ends of the particles facing either the top or bottom of the section planes will yield more accurate results. Each particle should be sampled only once during particle counting. When counting particles in structures whose normal boundaries do not interfere with other areas, if the entire area is visible within the image field at once, the particles within the studied area are counted individually without any two-dimensional restriction (Duman, 2010; Sterio, 1984; Ünal et al., 2002a) (Figure 2). However, counting all particle projections intersected by frame boundaries or ignoring the intersected particles leads to erroneous calculations of the actual number. Following the use of different counting frames, Gundersen (1977) developed a model for an unbiased counting frame: all particle projections whose projections lie entirely within the counting frame, do not touch the edges of the frame, and are in contact with free lines are included in the counting (Figure 3).

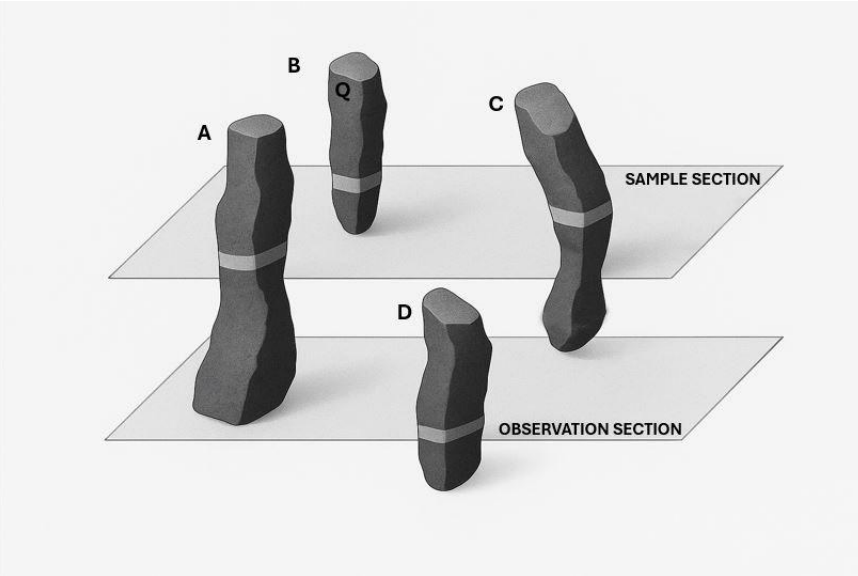

Figure 2. Particle counting using the dissector method. Particle A is definitely not counted because its projection is visible in the sample and observation sections. Particle B is counted because it is observed only sample section and is assigned Q. Particle C is definitely not counted because its projection is visible in the sample and observation sections. Particle D is definitely not counted because it is not observed in the sample section.

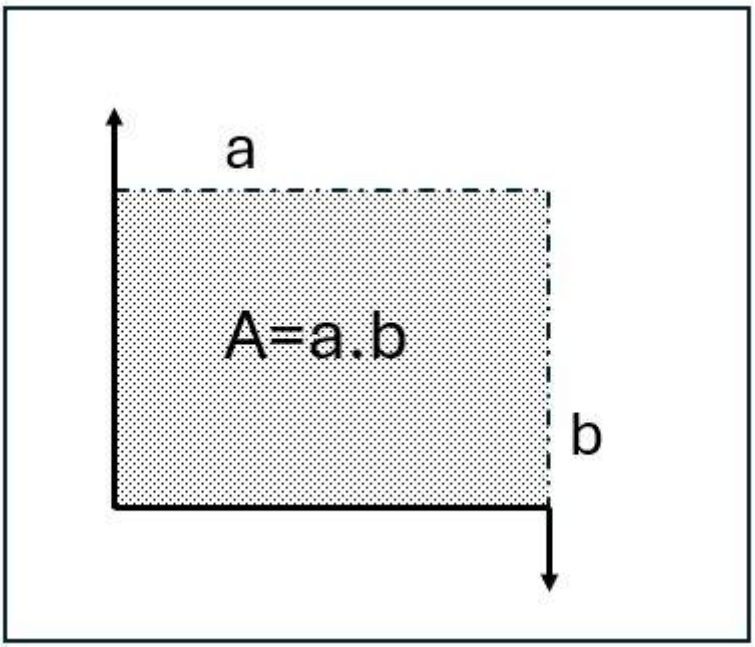

Figure 3. Neutral Counting Frame. While solid lines indicate prohibited edges, dotted lines represent free edges.

In studies, particle counts or densities determined in sections of biological components of interest are often misjudged due to various factors, including section thickness, changing tissue volume, and differences in particle size and location within the tissue. Knowing the distance between sections, i.e., the dissector height, is crucial in determining the dissector volume to be used in calculating particle counts. Dividing the total number of particles counted within a dissector volume by the dissector volume provides an unbiased calculation of particle count/numerical density per unit volume (Sterio, 1984; Ünal et al., 2002a).

2.1. Physical Dissector

Particle counting is performed using a physical dissector applied to two consecutive sections of the same thickness used as samples and observations. In this counting method, the parallelism

of the serial section planes and the distance between consecutive sections are important when calculating particle numerical density. The distance between pairs of sections should be 1/3 or 1/4 of the average height of the particles to be counted relative to the sectioning plane (Gundersen et al., 1988a; Sterio, 1984; Ünal et al., 2002a, 2002b). The opposite areas in the pairs of sections selected for dissector counting are limited by the counting frame. The physical dissector counting technique, which is based on taking two consecutive sections of tissue, is based on the principle that a structure considered a particle is visible in one section but not in the adjacent section. The particles counted accordingly are called dissector particles (Duman, 2010; Sterio, 1984). Because it can be difficult to observe identical particles in consecutive sections, this method may not be practical in some applications. The physical dissector method is more easily applicable in electron microscopic studies (Yurt et al., 2018).

2.2. Optical Dissector

The optical dissector is a stereological method that creates virtual three-dimensional counting boxes under a microscope. As the optical dissector moves optically along the z-axis within a tissue section approximately 20–30 μm thick, a virtual cube is created (Gundersen, 1986; Gundersen et al., 1988a; Yurt et al., 2018). This method eliminates the need for comparisons between two separate sections, as with the physical dissector. An unbiased counting frame is applied to consecutive optical sections taken within a single thick section volume. A three-dimensional virtual dissector probe is created, and grain counting is performed within the selected sampling area (Akalan & Demirkan, 2013; Coggeshall, 1992; Odacı et al., 2004).

In recent years, the optical dissector method has been reported to be more effective in light microscopic studies due to its ease of application and time savings (Yurt et al., 2018). The fact that it does not require comparison between two physically separated serial sections and that it is much easier to meet the prerequisite conditions for using the optical dissector makes the optical dissector method more practical and effective than the

physical dissector method (Dorph-Petersen, Nyengaard & Gundersen, 2001; Yurt et al., 2018). The optical dissector technique utilizes serial optical sections within a single thick section volume for particle counting. This technique facilitates particle counting using the objective's optical focal plane, and the application of a systematic random sampling approach allows for equal sampling of each particle in the tissue of interest. This eliminates particle sampling bias (Gundersen, 1986).

The number of particles per unit volume (Nv) is determined by counting the number of particles (Q=number of dissector particles) observed in the distance between two selected optical planes in the counted thick section. The reference volume (Vref) of an organ or structure is calculated using the Cavalieri method. The dissector volume (V(ref)) is determined by the distance between corresponding surfaces of two optical sections (h = dissector height) and the area of the sampled sections (a). The total number of particles (or their numerical density, N) in a given volume is calculated using the formula (Kaplan, 2006).

$$N = N_v . V_{ref}$$

$$N = \frac{\sum Q -}{h . \sum a} . V\ (ref)$$

Conclusion

The dissector counting method, one of the stereological analysis methods, is considered one of the most unbiased and effective methods for calculating numerical biological abundances, independent of tissue-related factors. In conclusion, this analysis provides reliable, acceptable, accurate, and realistic results at low cost and in a short time.

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