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How Should We Deal with the Failure of Antibiotics Draft I

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Abstract: The discovery and development of antibiotics have revolutionized modern medicine, saving millions of lives and reshaping global public health. However, antibiotic resistance has emerged as a formidable challenge, driven by clinical misuse, agricultural overuse, and environmental neglect. This review outlines the historical trajectory of antibiotics, from Fleming's discovery of penicillin to the current AI-assisted discovery approaches. We classify commonly used antibiotics, summarize their mechanisms of action, and discuss the societal and medical significance of antibiotics. Moreover, we analyze the underlying causes of the growing antibiotic resistance crisis and propose targeted strategies—including regulation, education, and technological innovation—to safeguard the efficacy of antibiotics in the future.

Keywords: Antibiotic resistance; Antimicrobial discovery; Public health; Misuse of antibiotics; Artificial intelligence; Drug development

Online publication: September 26, 2025

1. Introduction

The discovery of antibiotics marked one of the most significant medical advances of the 20th century. In 1928, Alexander Fleming observed the inhibitory effects of a mold (*Penicillium*) on surrounding bacteria, leading to the discovery of penicillin. This breakthrough revolutionized the treatment of infectious diseases, particularly during World War II. Since then, humans have embarked on a journey to explore, synthesize, and optimize antimicrobial agents. Excluding accidental ancient uses, the history of antibiotic discovery can be categorized into four major eras^[1].

The Golden Age of Antibiotics (1920s–1950s) began with Fleming's discovery and was followed by systematic isolation of soil-derived antibiotics by Waksman and others, including streptomycin. The Synthetic Modification Period (1950s–1970s) introduced structural refinements through medicinal chemistry to enhance efficacy and reduce toxicity. The Discovery Drought (1980s–2000s) saw reduced innovation as pharmaceutical companies redirected investments to chronic diseases (Aminov, 2010). The Modern Era (2000s–present) integrates genome mining, CRISPR, AI-assisted drug design, and phage therapy to combat antibiotic-resistant pathogens (Lewis, 2020)^[2].

Table 1. Classification of common antibiotics

Catagory ^[3]	Examples	Mechanism ^[4]	Common Uses for treatment ^[5]
B-Lactams (4-membered ring)	Penicillin, Amoxicillin	Destroy bacteria, by breaking cell walls	Strep throat ear infections
Macrolides (large rings)	Erythromycin, Azithromycin	Destroy bacterial ribosomes to stop the bacteria from producing proteins	Pneumonia, whooping cough
Aminoglycosides (sugar groups)	Streptomycin, Gentamicin	Sabotage genetic code translation	Tuberculosis, severe gut infections
Tetracyclines (4 benzene rings)	Doxycycline	block proteins + Block bacteria from gaining energy	Acne, Lyme disease
Quinolones (synthetic)	Ciprofloxacin	Destroy bacterial DNA during replication	UTIs, food poisoning
B-Lactams (4-membered ring)	Penicillin, Amoxicillin	Destroy bacteria, by breaking cell walls	Strep throat ear infections
Macrolides (large rings)	Erythromycin, Azithromycin	Destroy bacterial ribosomes to stop the bacteria from producing proteins	Pneumonia, whooping cough

2.1. The Significance of Antibiotics

Antibiotics have reduced bacterial infection–related mortality by over 80%, contributing to an increase in global life expectancy by approximately 23 years^[6]. They are essential for treating conditions such as sepsis and post-surgical infections^[7]. Additionally, antibiotics prevent fatal neonatal infections, although overuse can disrupt gut microbiota and immune development^[6]. In agriculture, antibiotics reduce animal disease and enhance productivity by up to 20%^[8]. Furthermore, they are integral to the safety of consumer products, such as contact lens disinfectants^[9].

2.2. Mechanisms of Bacterial Resistance

Bacteria resist antibiotics through several mechanisms:

- (1) Cell wall thickening^[10];
- (2) Production of β -lactamases to degrade antibiotics^[11];
- (3) Ribosomal modification, rendering macrolides ineffective^[12];
- (4) Horizontal gene transfer via plasmids and bacteriophages^[13].

2.3. Purpose of the Research

Despite modern technologies, the effective antibiotic arsenal is shrinking due to overuse and bacterial adaptation. This study investigates the multifactorial causes of antibiotic efficacy decline and proposes actionable strategies to prolong antibiotic utility.

2.4. Causes and Solutions to the Current Antibiotic Crisis

3. Agricultural Misuse

The overuse of antibiotics in livestock fosters resistant bacteria that may transfer to humans. Zhang et al demonstrated that industrial farming practices lead to unintentional antibiotic ingestion, gut microbiome imbalance, and colonization by resistant strains^[14]. To mitigate this:

- (1) Implement vertically integrated poultry operations with disease control;

- (2) Substitute antibiotics with probiotics, organic acids, enzymes;
- (3) Develop effective vaccines;
- (4) Enforce regulations on antibiotic residues and restrict contaminated meat sales.

4. Clinical Misuse

Medical misuse, including incorrect prescriptions and premature discontinuation, exacerbates resistance. Alrasheedy et al^[15] reported high rates of prescription errors, often involving unnecessary antibiotics. Solutions include:

- (1) Involving pharmacists in treatment plans;
- (2) Restricting over-the-counter antibiotic sales;
- (3) Monitoring hospital antibiotic use;
- (4) Isolating patients with resistant infections to prevent spread.

5. Lack of Public Awareness

Public understanding of antibiotic resistance is limited. Narmeen Mallah found that misuse correlates more with accessibility than education level^[16]. Additionally, Rossi highlighted that dairy products harbor *Pseudomonas* spp., which carry resistance genes^[17]. Addressing this requires:

- (1) Mandatory public education on antibiotic use;
- (2) Legal accountability for food and medical product manufacturers.

6. Future Directions: AI-Assisted Discovery

Antibiotic R&D is costly and high-risk, deterring investment. Zavaleta-Monestel proposed AI-assisted platforms to identify effective compounds and simulate clinical trials^[18]. These technologies promise to reduce costs and timelines, thus revitalizing antibiotic discovery.

7. Discussion

Antibiotic resistance is a multifaceted issue, deeply entangled with human behavior, economic interests, and global health governance. Agricultural and clinical misuse are widely recognized contributors, but the role of environmental and industrial contributors is equally critical. As evidence shows, bacteria do not require human aid to develop resistance—they naturally acquire and share resistance traits across species and environments. Therefore, even perfect clinical practice cannot halt resistance without broader systemic changes.

Policy changes must be globally harmonized. High-income countries must support low-income regions in monitoring and regulating antibiotic use. Surveillance systems should be expanded to track antibiotic use and resistance in both healthcare and agricultural settings. Investment in diagnostics is essential to ensure antibiotics are only used when necessary. Most importantly, public engagement must be improved—public campaigns, labeling regulations, and transparent food supply chains can raise awareness and promote responsible antibiotic use.

8. Conclusion

To prolong antibiotic effectiveness, a multi-pronged strategy is essential:

- (1) Reduce agricultural dependence on antibiotics, substitute with alternatives, and enhance regulation.

- (2) Enhance clinical practices through pharmacist involvement and prescription oversight;
- (3) Increase public awareness and corporate accountability via education and legislation;
- (4) Leverage AI and technology to accelerate the discovery and development of new antibiotics.

By coordinating across these domains, we can safeguard antibiotics for future generations and counter the escalating threat of resistant pathogens.

Disclosure statement

The author declares no conflict of interest.

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Application of Bioprinting Technology in the Construction of Vascularized Tissue-engineered Breasts

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Abstract: Reconstruction of the breast provides challenges to restore both form and function post-mastectomy. Traditional prostheses suffer from limitations such as periosteal contracture, tactile distortion, and the significant barrier of lacking a functional vascular supply in tissue-engineered breasts. 3D bioprinting represents a novel solution to the biomimetic construction of 3D breast models, and the hierarchical vascular network bioprinted into space can be precisely controlled through the ordered deposition of cells and biomaterials. The value of 3D bioprinting stems from its ability to transcend the physical constraints of nutrient infiltration and blood perfusion in vascularized tissue engineering and convert static scaffolds into living, metabolically-active tissue. The current trajectory of bioprinting research includes bionic design of multi-scale vascular topology, functional induction of endothelialized microchannels, and kinetic mechanisms of in vivo integration of printed tissue, all of which aspire to reduce the gap between mere morphological mimicry and reconstruction of physiological function.

Keywords: Bioprinting; Vascularization; Tissue engineering; Breast reconstruction

Online publication: September 26, 2025

1. Introduction

Loss of breast tissue following radical mastectomy not only provides a significant loss of physical identity for a woman, but also, very significantly, her psychological identity. Current silicone breast reconstruction is very challenging to intend to reproduce the real tissue the body has lost in terms of biological physiology and physical characteristics such as elasticity and mobile adaptability, and respond to foreign body reactions. Tissue-engineered replacement breast solutions can provide biocompatible replacements - however engineered tissues for breast reconstruction will remain a challenge to maintain cellular activity once implanted without vascular support again after a period of time - vascular flow is needed for cell and tissue development - ultimately leading to core necrosis. The innovation with bioprinting technology is combining the biology of angiogenesis and precision manufacture - using a multi-material layered printing strategy to shape the breast adipose stroma together with the vasculature, and by using the smart response properties of bio-ink to guide the endothelial cells self-assembly. This manufacturing technological path not only confronts the conventional sequential method of scale and 'shape first, vascularize later, building logic, but proposes making the tissue having its' intrinsic life-support system from and also at its' manufacturing source^[1].

2. Theoretical Foundations of Bioprinting Technology and Vascularized Tissue Engineering

2.1. Core Principles of Bioprinting Technology

The ability of a bioprinter to manipulate bioinks in three dimensions forms the physical basis of the technology, where a precision printhead or laser device deposits a composite system of cells and biomaterials layer by layer in accordance with a predefined digital model. The special rheological properties of bioinks allow them to maintain their structural shape at the moment of extrusion while providing the microenvironment necessary for cell survival, while temperature or photocross-linking mechanisms encourage the liquid ink to rapidly solidify into a stable scaffold. Engineers programmatically control the deposition paths and spatial and temporal sequences of different inks to confer heterogeneous structural features directly during the printing process, a simultaneous micro-to-macro construction strategy that breaks through the structural complexity limitations of traditional tissue fabrication methods. The intelligent release of bioactive factors in the ink further guides the directional migration and functional differentiation of the printed cells, allowing the static scaffolds to gradually evolve into metabolically active living systems.

2.2. Critical Needs for Vascularized Tissue Engineering

Cell populations within tissue-engineered structures require a continuous supply of oxygen and nutrients to avoid necrosis, and the capillary network must be densely distributed to a distance of no more than two hundred micrometers from each cell in order to meet the basic survival requirements. The ability of vascular endothelial cells to spontaneously assemble in engineered tissues is significantly limited by the artificial material environment, and the pore connectivity and surface chemistry of the scaffold material directly affects the extension pathways and branching efficiency of neovascular sprouts. The spatiotemporal concentration gradients of specific growth factors in the microenvironment are decisive for directing the directed growth of blood vessels, which is a central aspect of the precise regulation of the release kinetics of vascular endothelial growth factor and angiopoietin to become a central aspect of maintaining vascular stability. The degree of acceptance of the implant by the host immune system profoundly affects the integration process of the engineered vasculature with the body's circulatory system, and immunocompatibility modification of the material surface is crucial for mitigating the foreign body reaction and facilitating vascular anastomosis ^[2]. The engineered vascular network has to match the mechanical response of the natural breast tissue while assuming the function of material transportation. The coordination of the elastic modulus of the vascular wall with the deformation of the surrounding fat matrix determines the tactile realism of the reconstructed breast.

2.3. Biological Properties of Breast Tissue Constructions

The breast tissue is dominated by a soft matrix of adipocytes, which are packed in a honeycomb structure of lipid vesicles that form a characteristic elastic cushioning layer, giving the breast the ability to absorb energy and return to its original shape when under pressure. An intricate system of vasculature runs through the fat matrix as a channel of life, with microarterioles forming a dense tangle in the nipple region and branching out towards the base, a gradient pattern that ensures that lactating follicles have rapid access to the nutrients carried by the bloodstream. A three-dimensional network of collagen and elastin fibers wraps around the fat lobules and vascular bundles, and its unique relaxation structure allows the breast to change volume over the course of the physiological cycle without damaging the internal tissues. The precise spatial positioning of the ductal epithelial cells and the surrounding capillary endothelium maintains a constant exchange of signaling molecules that directly regulates the physiological rhythms of milk synthesis and secretion, while the patrolling activity of the immune cells in the adipose stroma constitutes a dynamic line of defense against the invasion of pathogens.

3. Key Challenges in Vascularized Tissue Engineering Breast Construction

The engineered vascular network is difficult to reproduce the finely graded structure of natural breast capillaries at the

microscopic scale, and small differences in the diameter of microchannels during the printing process may lead to uneven distribution of local blood flow or even the formation of ineffective circulation. The mechanical stress response of the host tissue to the implant is significantly different from the compliance of the engineered vascular system, and the sustained tensile force generated by daily activities may easily lead to structural deformation or leakage of the neovascular network. The slow release of metabolic wastes within the fat matrix gradually accumulates in the absence of efficient drainage channels, and this imbalance in the chemical microenvironment will inhibit the normal migration of endothelial cells and the process of lumen formation. The threshold of the body's immune system to recognize foreign biomaterials directly influences the intensity and duration of the inflammatory response in the implantation area, and overactive macrophages may attack the maturing endothelial junctions. Reconstructed breast tissue faces the potential risk of slow biomaterial degradation mismatched with the rate of neoplastic tissue regeneration after prolonged implantation, and premature scaffold disintegration will lead to collapse of the preconstructed vascular network ^[3].

4. Specific applications of bioprinting technology in vascularized breast construction

4.1. Multi-material bioink design

The formulations of bio-ink must account for the softer elasticity of the fat matrix in addition to the structure rigidity of the vascular network. The composite hydrogel system can provide precision zoning control of mechanical properties by manipulating the gelatin-sodium alginate gradient ratio. The vascular endothelial growth factor microspheres encapsulated within the endothelial cell only ink dissolve slowly and disburse at physiological temperature where the endothelial cells will migrate by crawling through the inked preset channels (in total a distance of about 12 mm to finally produce a continuous lumen after printing). The hyaluronic acid molecular chains entered in the adipocyte-laden ink create a three-dimensional spatial site-blocking effect that diminishes the chances of aberrant fusion of lipid droplets while hindering the structural shift of the newly generated tissue during the cell culture protocol. The smart-responsive cross-linking agent utilizes the wavelengths of this. Additively, the entanglement of molecular chains allows for free movement of adjacent cells at the interface between the two distinct materials for migration through the inked partition. The surface of the nano-adhesive peptide-modified functional ink matrix determines how the embedded cells interact with each other and the final density of the cells throughout the purposely created structure to resemble the natural extracellular matrix with topological features promoting ordered placement of lipid precursor cells to, eventually generate microfollicular structures ^[4].

4.2. Controlled printing strategies for 3D vascular networks

The precision printing system produces multi-stage branching path planning based on the anatomical data of breast vasculature, and the coaxial extrusion nozzle applies the hydrogel shell while infusing the temperature-sensitive sacrificial material, and the continuous cavity created with the later dissolution can be converted directly into microchannels for endothelial cell attachment. The print nozzle continuously adapts the interactive combinations of extrusion pressure and travel speed such that the transition region from millimeter-sized main blood vessels to micrometer-sized capillaries for diameter keeps a smooth inner surface, and turbulence loss from blood flow passing through avoids. Since the vascular patch is printed with a pulsed photocrosslinking device, localized energy curing is applied to vascular bifurcation nodes in order to ensure mechanical stability of the junction while allowing morphologic plasticity at the ends of the branches. Spatially localized magnetically responsive nanoparticles are preembedded in the vessel wall ink, so neovascular sprouts will have an external magnetic field gradient directing them toward the established vasculature of the host tissue. The biologically active interfacial layer in the printed construct allows active embedding of host capillaries into the engineered vascular network, and the two vascular systems link through luminal fusion and perfusion over time via molecular signaling ^[5].

4.3. Pre-vascularization in vitro and vascularization induction in vivo

The in vitro culture system mimics the pulsatile pressure and shear force environment inside the human body in a bioreactor, thereby stimulating the endothelial cells within the printed structure to quickly form dense junctions through stimulation from dynamic flow field, thus developing a prototype capillary network with basal perfusion function in two weeks. After implantation, the degradable scaffold material is designed to release the sequestered angiogenic factors in a time-regulated programmed fashion, while the chemotactic signals released by the decellularized matrix attract host vascular endothelial cells towards the engineered tissue on the implantation zone. The pre-embedded photosensitive cross-linking agents in the hydrogel network that were executing a live release-following modification curb on-demand can locally degrade the hydrogel under near-infrared laser irradiation, thus precisely opening up space for the neovascular sprouts to elongate into the interstitial space and to conduct branching topology. Autologous vascular endothelial cells already in the host tissue came to recognize specific adhesion molecules that were modified on the surface of the engineered vasculature and formed intercellular bridging structures by extending their pseudopods across the interface between the implant and the host tissue that eventually achieved lumen advancement and redirected blood flow. Slowly-released immunomodulatory molecules in the adipose matrix continued to suppress neutrophil over-infiltration and provided a stable microenvironment for the neovascular network being sustained very carefully and protected from inflammatory storms ^[6].

4.4. Synergistic optimization of biomechanical properties and tissue function

When engineered breast implants are subjected to compressive and shear stresses from daily activities, the energy dissipation properties of the fat matrix must be coordinated with the stress cushioning capacity of the vascular wall to avoid fatigue rupture of the vascular network during repeated deformation. The smooth muscle contraction response triggered by vascular endothelial cells sensing changes in blood flow velocity needs to precisely match the physiological regulatory rhythms of the natural breast, and overly strong luminal contraction may impede microcirculatory perfusion. The progressive degradation behavior of hydrogel scaffolds with incubation time should be synchronized with the rate of deposition of nascent extracellular matrix to ensure that the perivascular support structure does not undergo a sudden change in stiffness during mechanotransduction. When the concentration of lactate molecules released by the metabolic activity of adipocytes exceeds the clearance threshold of the vascular system, the acidic microenvironment will compromise the endothelial barrier function and induce a local inflammatory storm. The innervation process to re-establish tactile signals on the surface of the breast is dependent on the directional migration of Schwann cells around the vascular bundle, and the gradient of neurotrophic factor concentration released by the vascular endothelium directly affects the precision of the growth path of nerve axons ^[7].

5. Optimization directions for vascularized tissue-engineered breast constructs

5.1. Development and functionalized modification of novel bioinks

Bioink formulations need to break through the inherent contradiction between the mechanical strength and cytocompatibility of traditional hydrogels. Temperature-responsive polymers maintain smooth extrusion properties at low printing temperatures and rapidly form anti-deformation network structures in body temperature environments to support large volume fat deposition. Functional molecular components continue to play a precise regulatory role after ink curing, with enzyme-sensitive peptide-linked angiogenic factors dissociating and releasing only at specific matrix metalloproteinase concentrations, enabling synchronized coordination of neovascular extension and host tissue invasion. The ink matrix modified with nanoclay particles endows adipocytes with thixotropic properties required for three-dimensional culture, and the cyclic mechanical stresses generated by daily activities are converted into positive stimulatory signals that promote lipid droplet maturation. The covalent coupling of immunomodulatory molecules to the polymer chains in the inks is designed to ensure that their slow-release kinetics cover the critical post-implantation inflammatory

phase, consistently neutralizing tumor necrosis factor secreted by over-activated macrophages^[8]. Photosensitive decellularized matrix particles carry breast tissue-specific extracellular matrix components, which are triggered by near-infrared light to sequentially expose binding sites and guide endothelial cells to build functional luminal structures along the physiological vascular course. The weak bio-current generated by the conductive polymer network embedded in the ink of the vessel wall mimics the electrical signaling microenvironment during natural vascular development and significantly enhances the precision of smooth muscle cell circumferential alignment.

5.2. Innovations in multiscale printing technology

The precision printing system integrates an array of microfluidic nozzles to synchronize the manufacturing of cross-scale structures. While the main nozzle extrudes the hydrogel containing adipocytes to construct the macroscopic contour of the breast, the adjacent micron-sized nozzles synchronously deposit endothelial cell suspensions to form the initial vascular plexus, and the two form a mechanically interlocked interface at the instant of cross-linking. The rotating printing platform is coupled with a dynamically focused laser system that varies the energy density layer by layer in the vertical direction, with low-energy curing of large fat matrix areas to maintain soft porosity and high-energy scanning of fine vascular bifurcation zones to ensure the structural integrity of the tube wall. The electric field-assisted microdroplet injection module accurately deposits vascular smooth muscle cells at preset coordinates, and electrostatic traction causes the cells to be arranged in an orderly manner along the radial direction of the lumen to form a myofibrillar layer with contractile function. The acoustic focusing assembly technology guides the nanofibers to self-organize around the vasculature to form a mesh-like stress barrier layer, effectively dispersing the localized pressure transmitted to the capillary network segments by daily limb activities. The real-time near-infrared spectral monitoring device intervenes in the continuous printing process to dynamically adjust the concentration of cross-linking agent in the subsequent ink according to the change of tissue light transmittance, preventing curing defects in the deep structure due to insufficient light. Vascular tree generation algorithm embeds fractal geometry rules in the print path planning, so that the spatial distribution density of terminal capillaries automatically adapts to the metabolic demand intensity of local fat cells. The periodically applied pulsating fluid force field in the bioreactor induces the nascent vascular network to establish the physiological response memory to blood flow shear stress in advance of the *in vitro* culture stage, which enhances the maturity of immediate post-implantation perfusion function. The enzyme-triggered self-repair hydrogel releases embedded thrombospondin upon the appearance of printed microcracks, inducing *in situ* deposition of fibrin for autonomous repair of microstructural defects^[9].

5.3. Dynamic culturing and application of bioreactor systems

Periodic fluid shear in the bioreactor chamber mimics the human blood flow washout effect, continuously stimulating engineered vascular endothelial cells to enhance connexin expression and optimize luminal antithrombotic properties. A three-dimensional stretching device applies radial tension to the adipose substrate synchronized with the respiratory rhythm, which induces adipose precursor cells to differentiate into mature adipocytes and form functional lipid droplets in an orderly manner under the guidance of mechanical signals. The intelligent sensing module in the culture fluid circulation loop monitors the lactate accumulation concentration in real time and dynamically adjusts the perfusion rate to keep the metabolic waste concentration within the threshold of vascular clearance capacity. The physiological oxygen gradient maintained on the surface of the gas exchange membrane drives the capillaries to extend naturally to the low-oxygen area, reproducing the oxygen-tropic growth pattern of angiogenesis *in vivo*. Multi-axial rotational scaffolds slowly change the spatial orientation of the engineered tissue during the incubation process to uniformly distribute the structural stress of gravity on the neovascular network to avoid accumulation of localized deformation. The electrical stimulation electrode array generates a weak bioelectric current at specific segments matching the heart rate, accelerating the establishment of a coordinated contractile conduction pathway in the smooth muscle layer of the vasculature.

5.4. Establishment of clinical translational pathways and standardized evaluation systems

Regulators need to establish a special approval pathway for bioprinted breast products, clarifying that key validation nodes from laboratory prototype to clinical implant include long-term functional stability testing and immune risk stratification assessment. Morphological maintenance of engineered breast implants should be quantified by 3D optical scanning combined with haptic feedback scales, and the trajectory of volume change during daily activities should meet preset tolerances before structural reliability is deemed adequate. A team of pathologists has developed a uniform vascular maturity grading scale that integrates core parameters such as capillary density, uniformity of blood flow velocity distribution, and endothelial barrier integrity into a composite evaluation index. The surgeon's operating manual must standardize the intraoperative procedures for the management of vascular anastomotic interfaces, in particular the control of safety thresholds for the length of time microvascular clips are used versus the concentration of anticoagulant local perfusion. The assessment of functional rehabilitation of the reconstructed breast by the health insurance system incorporates an index of psychosocial adaptation of the patient, and changes in dynamic scores on breast-specific quality of life scales reflect the true clinical value of the technique. A cross-center research database continuously collects data on vascular network remodeling after implantation in patients with different body types, providing a predictive model of physiological response for personalized printing protocols^[10].

6. Conclusion

Bioprinted vascularized breasts mark a paradigm leap in regenerative medicine from structural mimicry to living system reengineering. Current technologies have demonstrated the critical role of biomimetic vascular networks in the survival of engineered tissues, but multiple barriers still need to be broken through for clinical translation: the perfusion efficiency of capillary networks at the microscopic scale is still inferior to that of natural tissues, the mechanism of mechanical stress on the maturation of the printed vasculature in dynamic culture has not yet been clarified, and the long-term functional stability of the integration of the cross-scale structures has yet to be verified. Future breakthroughs will rely on the deep convergence of materials science and developmental biology - for example, the development of smart bioinks with chemokine gradient response, or self-tissue printing strategies that mimic embryonic angiogenesis. The true value of the technology lies not only in organ reconstruction, but also in providing a universal vascularization blueprint for complex human tissue fabrication.

Disclosure statement

The author declares no conflict of interest.

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Research Progress and Future Prospects of Mesenchymal Stem Cell-Derived Exosomes in the Treatment of Neurodegenerative Diseases

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Abstract: Mesenchymal stem cell-derived exosomes carry maternal bioactive components, which are of great significance in promoting tissue and organ development as well as tissue regeneration. In the clinical treatment of neurodegenerative diseases, mesenchymal stem cell-derived exosomes has significant value, can not only promote nerve repair, but also regulate the patient's immune function, which has important value in improving the patient's prognosis. This article will conduct a research review on the basic characteristics of mesenchymal stem cell, combine literature research to study the advantages, biogenesis and composition. This article introduces the research progress of mesenchymal stem cell-derived exosomes in the treatment of neurodegenerative diseases, analyzes the limitations and future prospects, and provides a better reference for clinical treatment.

Keywords: mesenchymal stem cell(MSC)-derived exosomes; neurodegenerative diseases; research progress; future prospects

Online publication: September 26, 2025

1. Introduction

Neurodegenerative diseases(NDDs) are a type of degenerative disease of the nervous system, where patient's neurons and other parts undergo pathological changes, leading to cognitive and speech dysfunction, which affects the patient's normal life^[1,2]. Currently, common neurodegenerative diseases in clinical practice include Alzheimer's disease, Huntington's disease, and others. Research has found that mesenchymal stem cell (MSC)-derived exosomes have important clinical application value in NDDs treatment, and have become a key focus of research by scholars both domestically and internationally^[3,4]. MSCs are a type of multipotent stem cell with self-renewal ability and the ability to regulate immune function. They have been widely used in the treatment of neurological and cardiovascular diseases^[5-6]. MSC-derived exosomes are of great significance in promoting neural repair and improving neurological function in patients, with high safety, and are an important method for clinical treatment of NDDs^[7,8]. Overall, in order to better promote the high-quality development of clinical treatment, further understanding of the clinical application of mesenchymal stem cell exosomes is needed, providing scientific guidance for clinical disease treatment.

2. Overview of MSC-derived exosomes

2.1. Basic characteristics of MSCs

MSCs have basic characteristics such as multi-directional differentiation and tissue repair. Adipose tissue, amniotic fluid,

and umbilical cord are important sources of MSCs^[9,10]. In clinical treatment, the biological function of MSCs can facilitate the migration of damaged tissues, and further achieve the therapeutic effect of tissue repair. The immunomodulatory function and pluripotent differentiation potential of MSCs make them have broad application prospects in the treatment of NDDs and better promote the development of clinical treatment techniques.

2.2. Biological functions of MSCs

The biological functions of MSCs include multi-directional differentiation, self-renewal, and immune regulation, making them an important method for the clinical treatment of NDDs^[11-13]. Firstly, the function of multi-directional differentiation. It can differentiate into various mesenchymal tissue cell lines, such as cardiomyocytes, etc, which can promote vascular tissue recovery, nerve repair, etc. Secondly, self-renewal ability. MSCs can maintain an undifferentiated state and genomic stability while being cultured and expanded in vitro for a long time, and still possess stem cell characteristics even after isolation and expansion. Thirdly, immune regulatory function. MSCs have unique immune regulatory functions, which can release cytokines and inhibit the function of other immune cell subsets.

2.3. Advantages of MSC-derived exosomes

The advantage of MSC-derived exosomes is that they exhibit higher repair ability in clinical treatment, while also ensuring the safety of clinical treatment. MSC-derived exosomes have demonstrated significant clinical advantages in reducing validation reactions, improving neurological function, and promoting neural repair^[14,15]. From clinical treatment experience, MSC-derived exosomes are rich in miRNA in the treatment of neurodegenerative diseases. They can reduce cell apoptosis, promote inflammation improvement in patients, and have high clinical treatment safety advantages, reducing the occurrence of immune complications.

2.4. MSC-derived exosomes Biogenesis and Composition

Exosomes are lipid bilayer membrane-bound exosomes composed of lipids, proteins, nucleic acids, and small amounts of metabolites. Exosomes are involved in the pathological processes of various diseases and are also of great significance in intercellular signal transduction. They are rich in various bioactive substances and affect the synthesis and expression of related proteins by regulating the transcription, translation, and expression of receptor cell genes^[16,17]. As shown in **Figure 1**, from the perspective of the biogenesis process of exosomes derived from MSCs, the contents of polycystic vesicles can be degraded or released into the extracellular environment. When proteins become targets for lysosomal degradation, polycystic vesicles fuse with lysosomal membranes, leading to the degradation of intracellular vesicles in lysosomes. In addition, polycystic cells can fuse with the plasma membrane and release intracellular vesicles in the form of exosomes from the cell, ultimately generating exosomes^[18].

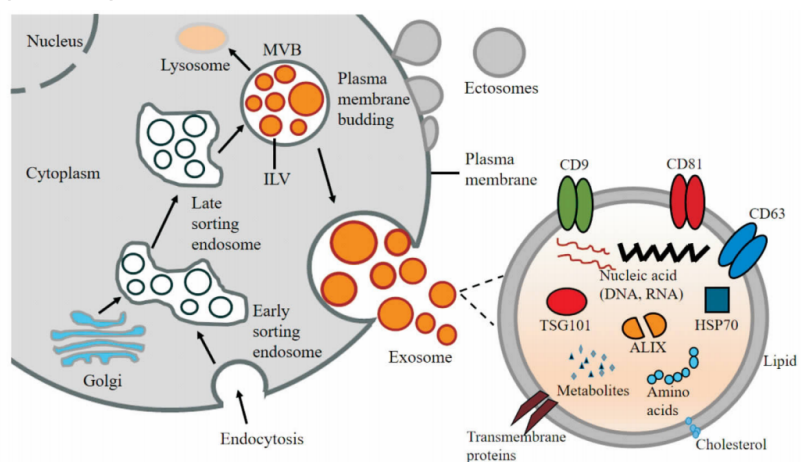


Figure 1. Biogenesis and composition of exosomes^[19]

2.5. The mechanism of action of MSC-derived exosomes

In the clinical treatment of NDDs, the mechanism of action of MSC-derived exosomes has immune regulation, promotion of neuronal repair, inhibition of validation cell activation, improvement of brain energy metabolism, et al, to better achieve neuroprotective effects^[20-22]. MSC-derived exosomes can activate specific T cell responses, effectively regulate immune responses, and better promote the occurrence of immune responses in patients. At the same time, exosomes from MSCs also have the value of promoting inflammatory responses, which can alleviate nerve damage caused by inflammation and achieve the effect of disease treatment. In terms of promoting neural repair and improving cell apoptosis, MSC-derived exosomes can increase the cell survival rate, promote neuronal repair, and further improve patients' neurological function.

3. Research progress on MSC-derived exosomes for the treatment of neurodegenerative diseases

3.1. Alzheimer's disease

Alzheimer's disease(AD) is a neurodegenerative disease characterized by cognitive impairment, and its main pathogenesis includes neuronal death, pro-inflammatory factors, et al^[23]. MSC-derived exosomes are of great significance for the treatment of AD, mainly by promoting anti-inflammatory and nerve cell axon growth, improving patients' immune function, and thus better delaying the occurrence of AD^[24]. NAKANO et al^[25] pointed out in their study that miR-146a can achieve the value of reducing inflammation, better improving cognitive function, and promoting neurological recovery. SHIN et al^[26] pointed out in their study that MSC-derived exosomes have demonstrated superior clinical effects, thereby better achieving the therapeutic effect of Alzheimer's disease cognitive impairment. Overall, they can promote the growth of neuronal axons, thereby better delaying the development of cognitive dysfunction in AD.

3.2. Parkinson's disease

Parkinson's disease(PD) patients are relatively common, and most patients experience symptoms such as neurological dysfunction and bradykinesia. From the perspective of pathogenesis, PD is closely related to mitochondrial damage, neuroinflammation, oxidative stress, and other factors. Multiple studies have shown that MSC-derived exosomes are mainly used in Parkinson's disease treatment by promoting endothelial cell growth, reducing neuronal apoptosis, and promoting the secretion of biomolecules in patients^[27,28]. Teixeira et al.^[29] found in their study that in Parkinson's disease treatment, MSC-derived exosomes can regulate the dopaminergic system, trigger neural mechanisms, and better achieve therapeutic effects in improving cognitive and motor dysfunction. Xue et al.^[30] pointed out in their study, MSC-derived exosomes are of great significance for the generation of endothelial cells in patients. For Parkinson's disease patients, they can increase dopamine levels, better increase cytokine expression, and achieve the effect of relieving cell damage. Overall, in Parkinson's disease treatment, MSC-derived exosomes can affect the activity of target cells, better protect neurological function, and achieve the goal of improving clinical symptoms.

3.3. Spinal Cord Injury

Spinal Cord Injury(SCI) is a neurodegenerative disease with severe trauma, causing local ischemia, edema, et al., which can have adverse effects on the patient's neurological and motor functions, and even lead to complications^[31]. MSC-derived exosomes can achieve the goal of inhibiting inflammatory response and reducing cell apoptosis by regulating cytokines in patients, which helps improve neurological function and promote spinal cord cell recovery. Gu et al.^[32] found in their study that miRNA-128 can activate autophagy in cells. Not only can it reduce neuronal apoptosis, but it can also achieve the effect of inhibiting cellular inflammation, thereby better promoting the recovery of spinal cord nerve function in patients. Nakazaki et al.^[33] point out MSC-derived exosomes can effectively regulate the TGF- β pathway, construct a stable spinal cord microenvironment. Therefore, MSC-derived exosomes can reduce cell apoptosis, promoting neuronal axon generation, improving BSCB and other mechanisms.

3.4. Huntington's Disease

Huntington's disease causes serious harm to the health of patients. Patients may present with symptoms such as dementia, involuntary movement, and mental abnormalities, and even exhibit pathological features of brain tissue atrophy^[34]. MSC-derived exosomes promote cell survival in Huntington's disease treatment by inhibiting inflammatory cell activation, reducing neurological dysfunction in patients, and ultimately promoting cell survival^[35]. Carmela et al.^[36] pointed out in their study that in the treatment of Huntington's disease, MSC-derived exosomes can achieve neuroprotective effects, reduce neuronal damage, better regulate inflammatory cell levels, and thereby improve the neurological dysfunction of Huntington's disease.

3.5. Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a motor neuron degeneration disease. Based on the clinical symptoms of patients, it mainly manifests as muscle weakness, and as the symptoms worsen, it can also cause hemiplegia. From the perspective of pathological mechanisms, impaired RNA metabolism, oxidative stress, and mitochondrial dysfunction are all pathological mechanisms of ALS, which affect the progression of the disease in patients^[37]. Debora et al.^[38] pointed out in their study that MSC-derived exosomes can improve mitochondrial dysfunction and inhibit cell apoptosis, thereby achieving the treatment of Amyotrophic Lateral Sclerosis. Therefore, in disease treatment, MSC-derived exosomes have good anti-apoptotic effects, and thus better promote the recovery of neuronal cells.

3.6. Multiple Sclerosis

Multiple Sclerosis is commonly found in the brainstem and spinal cord of patients^[39]. In clinical practice, many patients may exhibit cognitive and visual impairments. MSC-derived exosomes can improve neuroinflammation, regulate microglial differentiation, and achieve the effect of immunotherapy. In their study, Zhang et al.^[40] believed that MSC-derived exosomes have important value in increasing protein levels. By increasing the number of microglia in patients' bodies, they can achieve therapeutic effects in reducing neuroinflammation, better promoting myelin regeneration, and significantly improving patients' cognitive function. Xiao et al.^[41] think MSC-derived exosomes can enhance the protective ability of myelin sheaths in clinical treatment, achieve the effect of reducing neuroinflammation in patients, and better promote their health recovery.

4. Challenges and limitations of MSC-derived exosomes in the treatment of ND

MSC-derived exosomes therapeutic efficacy continues to be demonstrated across various experimental models. However, there are still some limitations that affect the high-quality implementation of clinical treatment. Firstly, there are certain limitations in the extraction of exosomes derived from MSCs. The existing techniques for extracting exosomes are relatively outdated and cannot achieve efficient and low-cost extraction, which affects the effectiveness of clinical treatment. Secondly, from the perspective of clinical research, the mechanism of action of exosomes derived from MSC in regulating immune response, promoting neuronal axon generation, and other aspects needs to be further clarified. Existing studies only discuss the important value of exosomes derived from MSCs in these aspects.

In the treatment of ND, MSC-derived exosomes have demonstrated significant therapeutic effects through their diverse biological functions. In order to better promote the high-quality development of clinical therapies based on MSC-derived exosomes, it is essential to actively advance the technology of exosome isolation through the research and development of extracellular vesicle-based drugs, and use biotechnology to improve the efficiency of exosome extraction, thereby improving the clinical treatment effect. In the future, it is necessary to further deepen the research on the mechanism underlying the action of MSC-derived exosomes in the treatment of neurodegenerative diseases, providing scientific guidance for clinical practice.

5. Conclusion

This review article found that MSC-derived exosomes have important clinical value in treating neurodegenerative diseases. They can achieve good clinical therapeutic effects through inhibiting neuroinflammatory responses, promoting vascular cell regeneration, and reducing cell apoptosis.

Disclosure statement

The author declares no conflict of interest.

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Tumour Microtubes and Therapy Resistance in Gliomas Molecular Mechanisms and Therapeutic Opportunities

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Abstract: Gliomas are highly infiltrative primary brain tumours characterised by poor prognosis and frequent recurrence due to therapy resistance. Conventional treatments, including surgery, radiotherapy, and chemotherapy, are often ineffective in achieving long-term control, particularly in glioblastoma, the most aggressive subtype. Recent discoveries have revealed that glioma cells form extensive networks of actin- and myosin-rich membrane structures, termed tumour microtubes (TMs), which enable long-range intercellular communication, calcium wave propagation, and metabolic exchange via connexin 43 (Cx43) gap junctions. These networks facilitate tumour cell survival by supporting self-repair, maintaining calcium homeostasis, and conferring resistance to radiotherapy and temozolomide chemotherapy. GAP-43, a neuronal growth-associated protein, has been identified as a key driver of TM formation, linking glioma biology to neural developmental pathways. Targeting TM networks or Cx43-mediated signalling—through monoclonal antibodies, gap junction inhibitors, or peptide disruptors—has shown promise in preclinical models, particularly when combined with PI3K pathway inhibition to overcome temozolomide resistance. This review synthesises current molecular insights into TM biology, highlights their contribution to glioma therapy resistance, and discusses emerging strategies to translate these findings into effective, tumour-selective therapeutic approaches.

Keywords: glioma; astrocytoma; glioblastoma; tumour microtube; connexin 43; GAP-43; calcium wave; therapy resistance; radiotherapy; chemotherapy; intercellular communication; monoclonal antibody; PI3K pathway; temozolomide resistance

Online publication: September 26, 2025

1. Introduction

Gliomas, which include astrocytomas, oligodendrogliomas, and glioblastomas, represent a diverse group of malignant brain tumours arising from glial cells within the central nervous system. Their diffuse infiltration along blood vessels and axonal tracts renders complete surgical resection virtually impossible, necessitating reliance on radiotherapy and chemotherapy for disease control. Despite multimodal treatment, gliomas—especially glioblastomas—remain highly lethal, with median survival often measured in months. A major barrier to improved outcomes is the ability of glioma cells to resist therapeutic interventions and adapt to cellular stress, enabling recurrence even after initial tumour debulking and treatment.

The molecular and cellular mechanisms underlying this resistance have been a subject of intense investigation. While early research focused on genetic drivers, such as IDH1/2 mutations and 1p/19q chromosomal codeletion, more recent work has uncovered the critical role of intercellular communication in sustaining tumour survival. In 2015, Osswald et al. described a previously unrecognised form of tumour connectivity: long, stable, actin- and myosin-rich protrusions

termed tumour microtubes (TMs). These structures form extensive multicellular networks that allow glioma cells to exchange calcium signals, organelles, and metabolic resources, and to rapidly repair damage via nuclear transfer. TMs are particularly abundant in high-grade astrocytomas lacking 1p/19q codeletion, correlating with poor prognosis and pronounced therapy resistance.

This review examines the discovery, structure, and function of TMs in gliomas, with a focus on their molecular regulation by GAP-43 and functional integration via Cx43 gap junctions. It also explores how TMs contribute to radiotherapy and temozolomide resistance, and evaluates emerging therapeutic strategies aimed at disrupting TM networks or targeting Cx43-mediated signalling, with the goal of improving clinical outcomes for patients with these devastating tumours.

2. Therapy Resistance in Gliomas: Challenges and Molecular Insights

Gliomas, including astrocytoma and oligodendrogliomas, are malignant brain tumours that arise from glial cells in brain and spinal cord^[1]. These tumours exhibit highly infiltrative growth patterns, spreading along blood vessels and nerve tracts, making complete surgical removal nearly impossible^[2]. Consequently, glioma treatment relies on a combination of radiotherapy and chemotherapy. However, gliomas frequently develop therapy resistance, leading to recurrence and poor patient prognosis^[3,4,5].

A major challenge in glioma treatment is understanding the molecular mechanisms underlying therapy resistance. Glioma cells use intercellular gap junction communication^[6], but the precise mechanisms remained unclear. Osswald et al., (2015) aimed to investigate this process and its role in therapy resistance^[7].

Gliomas are classified based on molecular markers, particularly IDH1/2 (Isocitrate Dehydrogenase) mutations and of 1p/19q chromosomal codeletion^[8]. Oligodendrogliomas with 1p/19q codeletion respond better to treatment, whereas astrocytomas without this codeletion exhibit higher resistance^[2]. Glioblastomas, the most aggressive gliomas, are IDH wildtype and highly treatment resistant^[4,9]. The reasons behind this variation in therapy sensitivity remain a major research focus.

3. Membrane Extensions and Discovery of Tumour Microtubes

Membrane-bound intercellular connections have been identified in biological systems. In *Drosophila*, actin-rich protrusions called cytonemes facilitate long-range intercellular signalling^[10]. In mammalian cells, tunnelling nanotubes (TNTs), actin-based projections enabling intercellular transfer of signals and organelles, were discovered by Rustom et al., (2004)^[11]. This revealed a novel mode of cellular communication, expanding understanding beyond previously recognised mechanisms involving direct contact and signalling molecule. These raised the possibility that glioma cells might use similar structures for intercellular communication and therapy resistance.

Osswald et al., (2015) hypothesised that astrocytoma cells form long membrane extensions to establish intercellular networks^[7]. They monitored tumour growth through a cranial window implanted in mice skulls and by using multiphoton laser-scanning microscopy (MPLSM), they observed that glioma cells extended long, thin protrusions containing actin and myosin into brain tissues. Further analysis using three-dimensional scanning electron microscopy (3D SEM) revealed that these extensions contained mitochondria, suggesting ATP production and vesicle trafficking. Unlike previously identified TNTs, these glioma-specific extensions were longer, thicker, and more stable, and followed axonal tracts for glioma cell invasion, leading to their designation as tumour microtubes (TMs)^[7].

4. TMs in Human Astrocytomas

To determine if TMs exist in human gliomas, Osswald et al., (2015) analysed resected human glioma samples and

stained them with IDH1R132H mutation-specific antibodies to carry out immunohistochemistry analysis^[7]. With precise identification of tumour-derived membrane extensions within the dense brain tissue, they found 63% of 1p/19q non-codeleted astrocytoma cells with intercellular TMs, whereas 0.7% codeleted oligodendroglioma cells exhibited similar structures (**Figure 1**). Further analysis revealed that TM formation varied by tumour type and grade, with longer TMs correlating with poorer prognosis^[7]. These findings established TMs as defining features of gliomas.

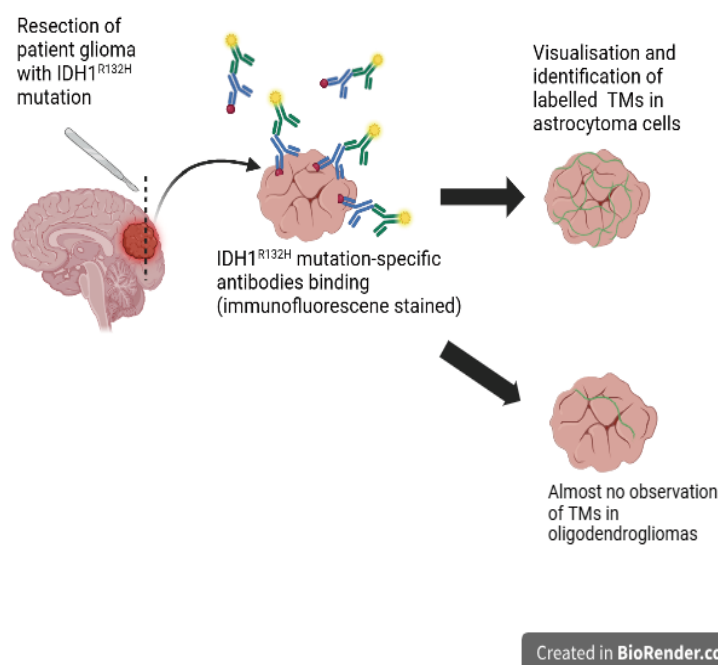


Figure 1. Identification of Tumour Microtubes (TMs) in Human Gliomas using IDH1 R132H Mutation-Specific Antibodies.

Patient glioma samples with IDH1 mutations were resected and analysed using immunohistochemistry (IHC) and immunofluorescence staining (green). IDH1 mutation specific antibodies were used to selectively bind glioma cells, enabling the visualization of TMs within brain tissue. TMs were identified in astrocytoma cells, but not in oligodendrogliomas. Labelled TMs were then analysed, providing evidence for their presence in human gliomas.

Own work, created in Biorender.com. Information from (Osswald et al., 2015)^[7].

5. TM-mediated communication and the role of Connexin 43 (Cx43)

Given that intercellular calcium waves (ICWs) play a crucial role in astrocyte and neuronal communication^[12], Osswald et al., (2015) hypothesised that TMs facilitate similar ICW propagation in gliomas^[7]. Time-lapse imaging confirmed calcium waves travelled along TMs in glioblastoma stem-like cells (GBMSCs), indicating bidirectional communication along TMs. Heat maps showed TM-connected tumour cells exhibited synchronized calcium transients, but not in TM-unconnected cells. TM intersections with simultaneous calcium peaks act as relay sites, forming a tumour-wide communication network^[7].

To investigate whether gap junctions mediate ICWs along TMs, Osswald et al., 2015 applied gap junction inhibitor carbenoxolone to GBMSCs and normal astrocytes, which significantly reduced ICW frequency in GBMSCs, but had minimal effect on normal astrocytes^[7]. A dye transfer assay confirmed selective dye spread between TM-connected tumour cells, which was abolished upon gap junction blockage^[7].

Analysis of glioma transcriptomic data from The Cancer Genome Atlas (TCGA) and confirmation by confocal microscopy revealed that connexin 43 (Cx43), a key protein forming gap junctions, was the only connexin highly

expressed at TM junctions in 1p/19q non-codeleted gliomas. 3D SEM imaging showed direct membrane contact at these junctions with ICW propagation. Knocking down Cx43 in GBMSCs using short hairpin RNA (shRNA) disrupted ICW propagation, reduced TM connectivity, and decreased tumour size in mouse models. Mice with Cx43-deficient tumours exhibited increased survival, suggesting that Cx43 is essential for TM network stability and glioma progression^[7].

6. TM networks enable self-repair and radiation resistance

To test whether TM networks contribute to glioma survival, Osswald et al., (2015) selectively ablated single TM-connected GBMSCs using laser irradiation^[7]. Following cell death, neighbouring tumour cells extended new TMs toward the damaged site, with a nucleus travelling through TMs to replace lost cells (**Figure 2**). Similarly, when multiple tumour cells were ablated, TM-connected cells rapidly repopulated the damaged area, whereas non-TM-connected cells exhibited minimal repair. In contrast, non-TM-connected cells exhibited minimal repair.

TMs also confer radiation resistance. Following radiotherapy, TM-connected cells were protected from apoptosis, whereas non-connected tumour cells died. Knockdown of Cx43 reduced this radioprotective effect, leading to non-TM-connected tumour regression. Researchers also found that basal intracellular calcium levels remained stable in TM-connected cells during radiation, whereas non-connected cells exhibited highly variable calcium levels, suggesting that TMs are involved in maintaining calcium homeostasis and redistributing calcium to mitigate cytotoxic effects of radiation^[7]. These findings demonstrated that TMs are involved in tumour self-repair and resist radiotherapy. Disrupting TM formation or Cx43-mediated communication could be a therapeutic strategy.

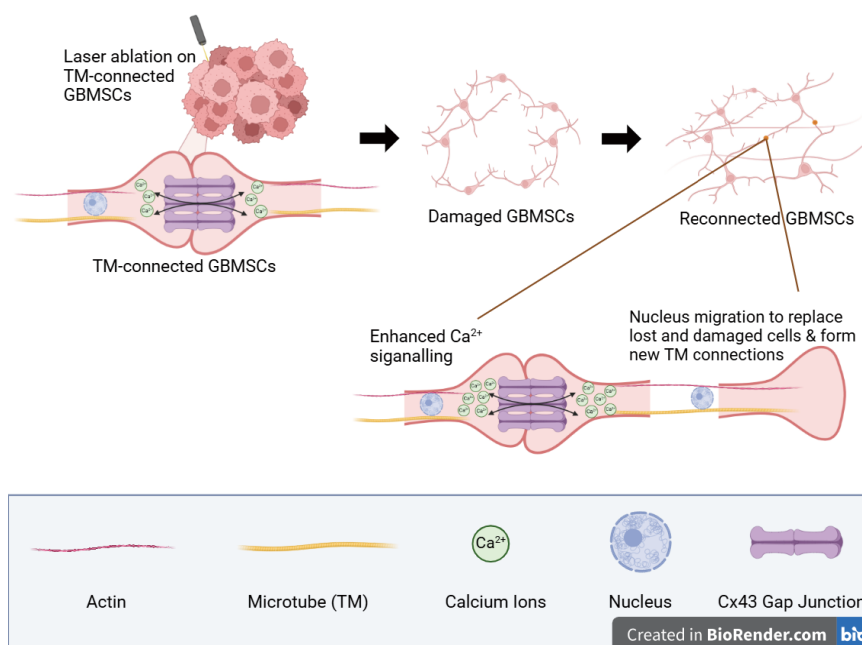


Figure 2. Tumour microtubes (TMs) facilitate glioblastoma repair and resistance to therapy. Laser ablation of TM-connected glioblastoma stem-like cells (GBMSCs) results in local cell loss. In response, surviving TM-connected cells enhance calcium signalling and initiate nuclear migration to replace lost cells, extending new TMs to re-establish network connectivity. This repair mechanism supports tumour survival following therapy and highlights TMs as a key driver of glioblastoma resistance.

Own work, created in Biorender.com. Information from (Osswald et al., 2015)^[7].

7. Growth-associated protein 43 (GAP-43) as a key driver of TM formation

To identify the molecular drivers of TM formation to better understand their role in tumour progression and therapy

resistance, Osswald et al., (2015) compared transcriptomic profiles of 1p/19q non-codeleted astrocytoma and codeleted oligodendrogliomas. They found that GAP-43, a protein involved in neuronal axon growth, was overexpressed in astrocytomas, which is hypothesised as driver of TM formation^[7]. To investigate its functional role, they engineered GBMSCs with GAP-43 knockdown. These GAP-43 deficient cells showed impaired TM formation, reduced Cx43 expression, and disrupted ICW propagation, leading to enhanced tumour regression after radiotherapy. Conversely, overexpressing GAP-43 in TM-lacking oligodendrogliomas induced a TM-rich phenotype with increased invasion and radiation resistance. These findings confirm GAP-43 as a key regulator of TM formation and therapy resistance^[7].

8. Future Directions

8.1. Are TMs unique to glioblastomas?

TMs are well-characterised in gliomas, but evidence for their presence in other tumours is limited. However, TNT-like structures have been observed in various cancers cells, including bladder cancer^[13], colon^[14], ovarian^[15], breast cancer^[16], etc. Further studies should determine whether TMs contribute to therapy resistance in other malignancies and influence metastatic potential.

8.2. Targeting Cx43 in Glioblastoma

As discovered by Osswald et al., (2015), glioblastoma cells form extensive TM networks and gap junctions with astrocytes through Cx43, making them highly invasive^[7]. Cx43-targeting therapies, such as the monoclonal antibody MAbE2Cx43, that targets the extracellular loop (E2) of Cx43, could disrupt TM networks and reduce glioma invasion^[17]. By disrupting gap junction intercellular communication, MAbE2Cx43 inhibits the transfer of ICWs between glioma cells and astrocytes. In glioma-bearing rats, combining MAbE2Cx43 with radiotherapy significantly prolonged survival compared to standard radiotherapy or chemotherapy. However, delivering Cx43-targeting agents across the blood-brain barrier remains a challenge. Intraoperative or intrathecal administration may enhance drug delivery to brain lesions^[17].

Cx43 inhibitors, including tonabersat^[18] and meclofenamate^[19], are under clinical trials for glioblastomas. These inhibitors enhance chemotherapy and radiotherapy effectiveness, but Cx43's widespread expression in the heart, brain, and skin raises concerns about off-target effects, including cardiac arrhythmias, neurological dysfunction, and impaired wound healing^[20]. Future research should focus on tumour-specific Cx43-targeting strategies, such as phosphorylation site inhibitors or monoclonal antibodies, to maximise therapeutic efficacy while minimising toxicity.

8.3. Overcoming Temozolomide resistance

Temozolomide (TMZ) is the standard chemotherapy for glioblastoma, but at least 50% patients develop resistance driven by intercellular communication and DNA repair mechanisms^[21,22,23].

In glioblastomas, after growth factors bind to their receptors, Cx43 selectively binds to the PI3K catalytic subunit β (p110 β /p85) to activate AKT kinase. Activated AKT phosphorylates and inhibits pro-apoptotic factors such as Bcl-2 (B-cell leukaemia/lymphoma 2 protein) from generating cell apoptosis^[24,25], leading to TMZ resistance independent of methylguanine-DNA methyltransferase (MGMT), a well-known mediator of this resistance^[23]. Targeting this Cx43-p110 β /p85 interaction is a promising strategy (**Figure 3**). The Cx43 peptide inhibitor α CT1 disrupts this interaction, blocking PI3K/AKT activation and restoring glioblastoma sensitivity to TMZ. Preclinical studies have demonstrated that combining α CT1 with PI3K inhibitors (e.g. TGX-221 or GSK2636771) enhances tumour cell apoptosis, reduces tumour growth, leading to increased glioblastoma responsiveness to TMZ and prolongs survival in mouse models^[23]. These findings support the clinical development of this combined therapy as a strategy to overcome TMZ resistance in glioblastoma.

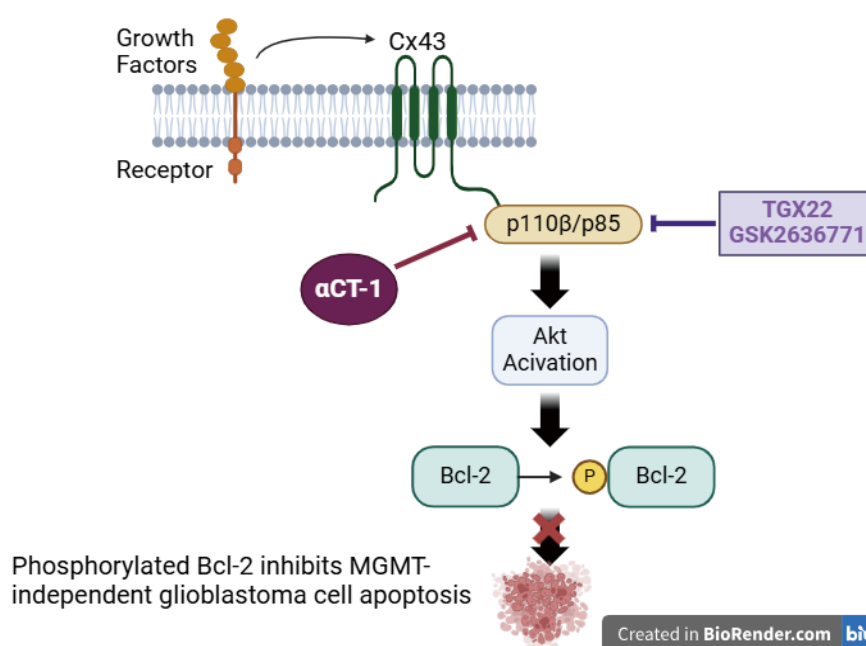


Figure 3. Cx43-mediated activation of PI3K/AKT pathway contributes to TMZ resistance in Glioblastoma.

Signals from growth factors activate Cx43, which binds to p110β/p85, leading to AKT activation. Activated AKT phosphorylates Bcl2, preventing apoptosis in MGMT-independent glioblastomas. The Cx43-targeting peptide inhibitor αct1 disrupts this interaction, inhibiting PI3K/AKT signalling, leading to apoptosis. Additionally, PI3K inhibitors (TGX-221, GSK2636771) enhance apoptosis and sensitize glioblastoma cells to TMZ.

Own work, created in Biorender.com. Information from (Datta et al., 1997; Pridham et al., 2022)^[23,25].

9. Conclusion

The discovery of TMs has advanced our understanding of glioma progression, invasiveness, and therapy resistance. These actin- and myosin-rich membrane extensions form stable, interconnected networks that facilitate long-range calcium signalling via Cx43 gap junctions. Through this structural and functional integration, glioma cells can maintain calcium homeostasis, coordinate stress responses, and rapidly repair damage through nuclear transfer and cell replacement.

The identification of GAP-43 as a critical regulator of TM formation links glioma biology to neuronal growth programs and offers a promising molecular target. Strategies to disrupt TMs or interfere with Cx43-mediated signalling—whether through monoclonal antibodies, small-molecule gap junction inhibitors, or peptide disruptors—show potential in preclinical models, especially when combined with PI3K pathway inhibition. Future research should determine whether TMs are unique to gliomas or a more general mechanism of therapy resistance across cancers. The development of tumour-selective Cx43 inhibitors will be key to translating these findings into safe and effective treatments with minimal off-target effects, potentially transforming the therapeutic landscape for this devastating disease.

Disclosure statement

The author declares no conflict of interest.

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Coenzyme A and Its Derivatives in Metabolic Dormancy: A Multifaceted Regulatory Network

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Abstract: Metabolic dormancy is a survival strategy that can be found in bacteria, fungi, plants, and hibernating animals, to survive under unfavorable conditions, such as nutrient shortage and oxidative stress. Coenzyme A (CoA), a central metabolic molecule, plays an important role during dormancy by integrating energy metabolism, redox homeostasis, and post-translational modifications. This essay discusses the different roles of CoA and its derivatives in metabolic dormancy, particularly in enzyme activity control, stress adaptation, and gene expression. By integrating energy metabolism, gene regulation, and redox balance, CoA functions as a metabolic switch that helps cells enter, maintain, and exit dormancy and as an antioxidant to defend against oxidative stress. Understanding these mechanisms may provide evidence of bacterial persistence, hibernation, and metabolic diseases, with potential applications in biotechnology and medicine.

Keywords: Coenzyme A Metabolism; Bioprocess Engineering; Metabolic Dormancy; Systems Biology; Oxidative Stress Response

Online publication: September 26, 2025

1. Introduction

Coenzyme A (CoA) is an important molecule found in a wide range of living cells. Coenzyme A (CoA) and its derivatives, such as acetyl-CoA, malonyl-CoA, and succinyl-CoA, participate in essential biochemical pathways, including carbohydrate metabolism, lipid metabolism, fatty acid oxidation, and protein modification through CoAlation^[1]. Protein CoAlation is a post-translational modification (PTM), the thiol group of coenzyme A (CoA) covalently binds to the side chain of cysteine residues of proteins, forming a reversible disulfide bond which acts as an antioxidant in oxidative stress.

The biosynthetic pathway of CoA involves 5 enzymatic pathways. CoA is synthesized from pantothenic acid (vitamin B5), Adenosine triphosphate (ATP), and cysteine^[2]. Pantothenate kinase (Pank) catalyzes the phosphorylation of pantothenate into 4'-phosphopantothenate; this reaction is a rate-limiting step due to the feedback inhibition of the product CoA and its other thioester derivative^[2]. Exogenous substrates and hormones can influence the efficacy of CoA biosynthesis. The study has shown that substrates like glucose, pyruvate, fatty acid, and insulin can reduce the rate of CoA biosynthesis by inhibiting Pank^[2].

Acetyl-CoA, acyl-CoA, and malonyl-CoA are key CoA thioester derivatives formed from distinct biochemical pathways with different metabolic functions. Acetyl-CoA can be synthesized from pyruvate catalyzed by the pyruvate dehydrogenase complex (PDH) in mitochondria or from citrate, linking carbohydrate and lipids metabolism to the

tricarboxylic acid (TCA) cycle^[3,4]. Both reactions bind the acetyl group to the cysteine residue of CoA, generating a thioester bond^[5]. Similarly, this thioester bond also exists in malonyl-CoA and acyl-CoA, where a malonyl group and an acyl group, respectively, are linked to the thiol (-SH) group of CoA. These CoA thioesters act as the central regulator of metabolism, allowing organisms to adapt to the varying nutrient conditions.

Metabolic dormancy is usually described as a reversible physiological state of organisms in adverse environmental conditions like unfavorable temperatures, pressure, pH value, oxidative stress, and nutrient shortage^[6]. Such adaptation patterns can be observed in seed dormancy, bacterial sporulation, and mammals during hibernation, enabling them to survive under harsh conditions.

Dormancy is usually characterized by reduced metabolic activity, allowing organisms to conserve energy. Specifically, the key features include decreased respiration and biosynthesis, developmental arrest, and increased resistance to stress. During dormancy, species usually rely on stored nutrients such as fats, starch or glycogen, and proteins. Therefore, glycolysis, which uses glucose as a carbon source, is modulated during dormancy. In yeast, key glycolytic enzymes include glucokinase (Glk1) and phosphofructokinase (PFK1), which can transform into filamentous or punctate assemblies when glucose is absent^[7,8,9]. Such conformational transition allows cells to regulate the enzyme activity to meet the metabolic demand. Specifically, Glk1, which catalyzes the phosphorylation of glucose to glucose-6-phosphate, is inhibited by filamentation in the presence of glucose^[10]. This may indicate that Glk1 catalyzes the first step of glycolysis if glucose levels rise. Moreover, the energy level controls the two conformations of PFK1, the inhibited and activated forms. High-energy signals like ATP and citrate accumulation will result in their allosteric interaction with PFK1, locking PFK1 at the inhibited state. In contrast, low-energy signals like AMP and F6P activate glycolysis when energy is needed, balancing inhibition and activation^[11].

2. CoA and Its Derivatives as a Key Regulator of Energy Conservation in Metabolic Dormancy

Efficient energy production is important for maintaining cell function. The universal energy currency, ATP, can be generated from two carbon sources: fatty acids and glucose. As shown in **Figure 1**, mitochondrial metabolism converts these fuels into ATP through sequential processes, including glycolysis, the tricarboxylic acid (TCA) cycle, β -oxidation of fatty acid, and oxidative phosphorylation. Decarboxylation and carboxylation of pyruvate produced from glycolysis generate acetyl-CoA and oxaloacetate (OAA) respectively. Moreover, acyl-CoA is synthesized from fatty acid and CoA by fatty acyl-CoA synthase. The acyl group is transported into the mitochondrial matrix as acylcarnitine, which is regenerated into Acyl-CoA for β -oxidation. The reduced equivalents produced from these pathways, including NADH and FADH₂, donate electrons to the electron transport chain (ETC) and drive ATP synthesis through oxidative phosphorylation.

Acetyl-CoA is mainly produced from carbohydrates and fatty acids. Glucose is catalyzed into pyruvate through glycolysis. Pyruvate is converted into oxaloacetate (OAA) by pyruvate carboxylase (PC), which may enter the tricarboxylic acid (TCA) cycle₂ or be catalyzed back to phosphoenolpyruvate (PEP) by mitochondrial phosphoenolpyruvate carboxykinase (PECK-M) through gluconeogenesis. The acetyl group can also be transferred from pyruvate to CoA, generating acetyl-CoA by pyruvate dehydrogenase (PDH). The acyl group of fatty acid is transported to the matrix by carnitine palmitoyltransferase 1 (CPT1), carnitine-acylcarnitine translocase (CACT), and carnitine palmitoyltransferase 2 (CPT2). The acetyl-CoA enters the TCA cycle to generate NADH/FADH, which produces ATP in the electron transport chain (ETC). Acetyl-CoA carboxylase (ACC) catalyzes the acetyl-CoA into Malonyl-CoA in lipogenesis, which uses the citrate from the TCA cycle as the substrate. The figure was produced from bio render and was adapted from^[12,13].

AMP kinase (AMPK) is a key energy sensor that responds to cellular ATP/AMP ratio fluctuations. The canonical regulation of AMPK involves activation when its Thr172 residue is phosphorylated by liver kinase B1 (LKB1). AMP binding to AMPK triggers three activation mechanisms. First, the direct binding of AMP to AMPK can cause allosteric activation of Thr172-phosphorylated AMPK^[14]. Secondly, the phosphorylation action of LKB1 is promoted^[15]. Moreover,

dephosphorylation catalyzed by phosphatases on Thr172 is inhibited by the direct binding of AMP. This system allows AMPK to sensitively monitor the fluctuation of the AMP to ATP ratio^[16].

AMPK links fatty acid biosynthesis to the current energy status by inactivating ACC through phosphorylation. The study has shown that Ser79, Ser1200, and Ser1215 of human ACC1 are the critical sites where AMPK phosphorylates^[17]. In contrast, Ser1157 in yeast ACC1 has been reported to be phosphorylated by AMPK-equivalent enzymes^[18]. The ACC phosphorylation results in significant conformational changes that inhibit ACC activity, which decreases fatty acid synthesis and redirects acetyl-CoA toward energy production during starvation states. By contrast, in nutrient-rich states, the citrate generated from the TCA cycle enhances the activity of ACC by stabilizing active conformation by filament formation^[19].

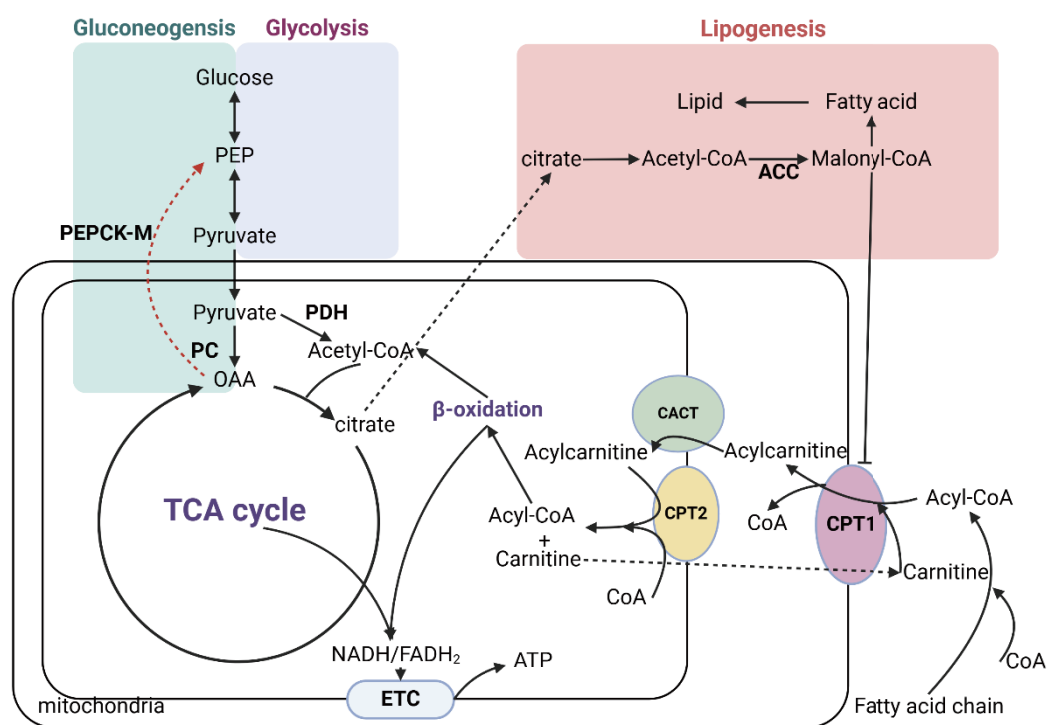


Figure 1. Mitochondrial metabolism and the CoA control sites

2.1. Allosteric Inhibitor Role of Malonyl-CoA

The regulation of ACC activity is essential for fatty acid metabolism, which controls the biosynthesis of lipids and regulates malonyl-CoA levels to prevent the suppression of fatty acid oxidation. The β -oxidation of fatty acids generally includes 2 steps (**Figure 1**): translocation and oxidation. The fatty acid is first converted to acyl-CoA by acyl-CoA synthase. The transportation of acyl groups into the mitochondrial matrix for β -oxidation is carried out by the carnitine shuttle system, including 3 key proteins^[20]: carnitine palmitoyltransferase 1 (CPT1), carnitine-acylcarnitine translocase (CACT), and carnitine palmitoyltransferase 2 (CPT2). CPT1 is found on the external mitochondrial membrane of mitochondria; it catalyzes the transfer of the acyl group from acyl-CoA to carnitine, producing acylcarnitine and releasing CoA. The acylcarnitine is then transported into the mitochondrial matrix by CACT. CPT2 catalyzes a reverse reaction inside the mitochondria, the acyl group is reattached to CoA and therefore generates acyl-CoA^[20]. This system transports the long-chain acyl groups across the membrane.

Malonyl-CoA is a key molecule that suppresses the β -oxidation of fatty acids by inhibiting the CPT1, which blocks the transport of the acyl group, which is the rate-limiting step of β -oxidation^[20]. This process decreases the uptake of fatty acid from the cytosol, which ensures the cell favors lipid synthesis over fatty acid oxidation when nutrients and energy

are abundant. Conversely, during metabolic dormancy, when nutrients are deficient, AMPK phosphorylates and inhibits ACC, which lowers the concentration of malonyl-CoA and reduces the CPT1 inhibition. Increasing the level of fatty acid oxidation to generate ATP. The physiological study has shown the CPT1 inhibition by malonyl-CoA in CPT1c knockout (KO) mice, which have decreased body weight and food intake. In addition, CPT1c KO mice have shown increased susceptibility to high-fat diet-induced obesity, suggesting CPT1c may protect against fatty accumulation^[21]. This energy use strategy dynamically controls lipids synthesis and degradation, optimizing energy use.

2.2. Allosteric Activator Role of Acetyl-CoA

Oxaloacetate (OAA) is a key metabolic intermediate involved in both the TCA cycle and gluconeogenesis (**Figure 1**). Pyruvate carboxylase catalyzes the carboxylation of pyruvate using bicarbonate (HCO_3^-) as a CO_2 donor to generate OAA, which is the first step of gluconeogenesis and provides OAA to sustain glucose synthesis, particularly in the liver and kidneys, where it plays a crucial role in maintaining blood glucose levels during fasting or metabolic dormancy. OAA is then reduced to malate to cross the mitochondrial membrane into the cytosol, where the malate is oxidized back to OAA. Decarboxylation and phosphorylation then occur to convert OAA to phosphoenolpyruvate (PEP) by phosphoenolpyruvate carboxykinase (PECK).

Certain tissues, such as the brain and red blood cells, are highly dependent on glucose for energy. The brain depends on glucose from gluconeogenesis, especially in the early stages of fasting before ketone bodies become a primary fuel source^[22]. Red blood cells lack mitochondria; therefore, their energy production relies entirely on glycolysis. The pyruvate in gluconeogenesis may be synthesized from some non-carbohydrate precursors, including lactate and alanine, catalyzed by lactate dehydrogenase and alanine transaminase in the liver^[12].

As a result, the activity of PC is tightly controlled to make sure that the gluconeogenesis adapts to the metabolic needs. Acetyl-CoA acts as an allosteric activator of PC, which binds to the regulatory site and causes conformational change^[23]. The α_4 quaternary structure of most PCs includes a biotin carboxylation domain in each subunit. This domain is responsible for catalyzing the ATP-dependent carboxylation of biotin, a necessary step for transferring the carboxyl group to pyruvate^[23]. The catalytic efficiency of this domain is enhanced during acetyl-CoA binding. This may be because of the stabilization of the PC structure, which avoids the dissociation of PC into monomers or dimers^[23]. A study carried out by Mayer et al.^[24] suggests that in chicken, rat, and sheep livers, the tetrameric structure of the enzyme was much better preserved when acetyl-CoA was present during preparation for electron microscopy. Moreover, the structural study^[24] also found that the PC tetramer exists in a more compact conformation when acetyl-CoA binds to it.

2.3. The Role of Acetylation in Gene Regulation and Metabolic Control

Acetylation is a reversible protein post-translational modification that occurs to regulate protein function and gene expression. The reaction involves binding the acetyl group to the lysine residues of the protein (**Figure 2**). This process is catalyzed by acetyltransferases such as lysine acetyltransferases (KATs) and histone acetyltransferases (HATs). The reverse reaction deacetylation removes the acetyl group, which is catalyzed by deacetylases, including histone deacetylases (HDACs) and lysine deacetylases (KDACs)^[25]. Acetyl-CoA is the universal acetyl group donor, it has been proven that the acetyl-CoA concentration can affect the extent of acetylation in *Drosophila* S2 cells and in mammalian cells^[26], where a decline in CoA levels corresponds with decreased acetylation of histones and tubulin.

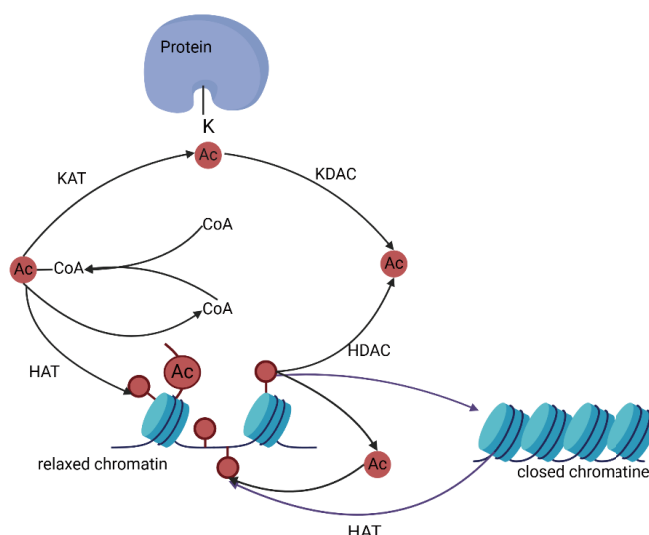


Figure 2. Acetylation and Deacetylation of Protein and Histone

The acetylation and deacetylation are catalyzed by acetyltransferases including lysine acetyltransferases (KATs) and histone acetyltransferases (HATs); and deacetylases such as histone deacetylases (HDACs) and lysine deacetylases (KDACs) respectively. Ac standard for Acetate. The figure was produced from biorender and was adapted from ^[25]

2.3.1. Gene Level Control by Histone Acetylation

Histone acetylation is a crucial PTM that was discovered about 60 years ago. The positively charged amino group on the side chain of lysine can interact strongly with negatively charged DNA, which stabilizes chromatin in a closed structure (**Figure 2**) ^[27]. HATs catalyze the binding of the acetyl group to the lysine residues, which neutralize its positive charge and reduce the interaction between histones and DNA molecules, resulting in a relaxed chromatin conformation available for transcription. Conversely, the deacetylation removes the acetyl groups returns chromatin to the closed structure, and inhibits transcription ^[27].

Histone acetylation provides the transcription controls for plant development and seed dormancy. In *Arabidopsis thaliana*, the expression of *DOG1* (DELAY OF GERMINATION 1) regulates the states of seed in maturation or dormancy, which involves dynamic histone modifications mediated by transcriptional repressors and histone deacetylases (**Figure 3**) ^[28]. The HIGH-LEVEL EXPRESSION OF SUGAR INDUCIBLE2 (HSI2) and HSI2-LIKE1 (HSL1) are the two transcriptional repressors that interact with the histone deacetylases HD2B and HD2A, forming a complex that directly inhibits the expression of *DOG1* during seed maturation by removing the acetyl group on histone H4 lysine 5 (H4K5ac) ^[28]. Conversely, when the histone acetylation level is high, *DOG1* remains activated and promotes seed dormancy ^[28].

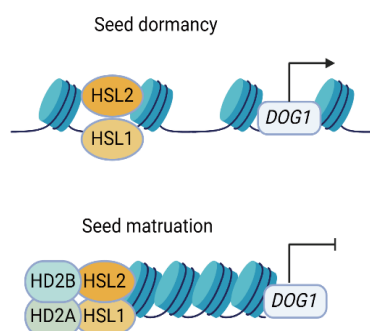


Figure 3. Epigenetic Regulation of *DOG1* Expression During Seed Dormancy and Maturation

Aberration: HIGH-LEVEL EXPRESSION OF SUGAR INDUCIBLE2 (HSI2); HSI2-LIKE1 (HSL1); DELAY OF GERMINATION 1 (DOG1) The figure was produced from biorender and was adapted from^[27].

VERNALIZATION5/VIN3-LIKE 3 (VEL3) is a chromatin-associated protein that provides the maternal epigenetic control of seed dormancy, making sure that progeny adapt to environmental conditions experienced by the mother^[29]. As a subunit of histone deacetylase complex, VEL3 combines with MULTICOPY SUPPRESSOR OF IRA1 (MSI1) and SIN3-ASSOCIATED PROTEIN 18 (SAP18) to decrease the acetylation level of histone. VEL3 also suppresses a key transcriptional regulator gene *ORESERAI* (ORE1), which will induce seed dormancy in the endosperm^[29].

Overall, histone acetylation acts as a cell dormancy switch in gene expression level by controlling the transcription. Providing a survival mechanism for cells to adapt to the environment by deciding to enter the dormancy or development states.

2.3.2. Activity Control in Enzyme-Level Acetylation

Histone acetylation is widely known for its transcription regulation, but acetylation also directly affects enzyme activity. At the enzyme level, lysine acetylation regulates enzyme activity and interaction with metabolic pathways. Mass spectrometry analyses carried out by Wang, Q et al.^[30], demonstrate that about 90% of the key enzymes involved in metabolism show acetylation in *Salmonella enterica*. Depending on the nutrient source of the *S. enterica* grows, the acetylation is different in cells, which is higher in glucose-source cells than in citrate-grown cells. The two distinct nutrient sources represent the different metabolic pathways to produce energy: glucose-based glycolysis and citrate-dependent gluconeogenesis.

The single forms of acetyltransferase and NAD-dependent deacetylase that exist in *S. enterica* are Pat and CobB respectively^[31]. The two mutants generated in the study knock out Pat (Δ pat) and CobB (Δ cobB) respectively^[30], which tests the influences of the metabolic pathway. A pattern of increased glycolysis rate and decreased citrate utilization is observed in Δ cobB mutants. While an opposite growth pattern is found in Δ pat mutants with lower acetylation levels^[30]. This experiment evidence suggests that acetylation may promote glycolysis and inhibit gluconeogenesis and oxidative metabolism.

2.3.3. Acetylation in Regulating the Initiation of Autophagy

Autophagy is a highly conserved cellular degradation process that removes the damaged organelles and cytoplasmic proteins by the lysosome. During metabolic dormancy, autophagy is important to break down macromolecules to generate energy. The key steps of autophagy involve initiation, autophagosome formation, and fusion with the lysosome. Acetylation on the molecules in these processes helps activate or inactivate the signaling pathway.

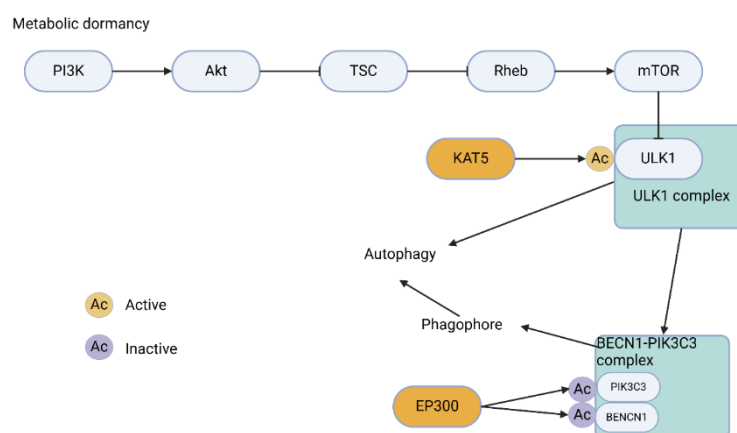


Figure 4. The activation step of autophagy through the PI3K-mTOR pathway.

Acetylation occurs on ULK1 catalyzed by KAT5 and activates autophagy, while acetylation catalyzed by EP300 at PIK3C3 and BECN1 inhibits autophagy. The figure was produced from biorender and was adapted from^[32,33]

When autophagy is initiated by the low mTOR (mammalian target of rapamycin kinase) signal during metabolic dormancy, the TOR complex activity is reduced at low-nutrient conditions including amino acid shortage, which leads to the activation of unc-51 like autophagy activating kinase 1 (ULK1) (**Figure 4**)^[33]. The ULK1 complex stimulates another protein complex: BECN1-PIK3C3/VPS34 complex, which stands for beclin 1 and phosphatidylinositol 3-kinase catalytic subunit type 3, which is responsible for producing phosphatidylinositol-3-phosphate (PtdIns3P), facilitating the production of the phagophore^[32], the early form of the autophagosome.

The lysine acetyltransferase 5 (KAT5) catalyzed the acetylation on the ULK1 at the residues K162 and K606, enhancing ULK1 activity and promoting autophagy (**Figure 4**). Interestingly, this activation is isolated to the mTOR-AMPK phosphorylation, meaning that it provides an alternative way for autophagy activation^[32].

2.4. Antioxidant Role of Protein CoAlation

Oxidative stress is a state in a situation where the generation of reactive oxygen species (ROS) cannot balance with the cellular defense ability to neutralize or eliminate ROS. When oxidative stress occurs, excess ROS can damage cell components, including lipids, proteins, and DNA, leading to impaired function and potential cell death. This damage may result in a wide range of diseases, including aging, inflammation, cancer, cardiovascular diseases, and neurodegenerative disorders^[34].

Protein CoAlation is a post-translational modification where the thiol group of coenzyme A (CoA) covalently binds to the thiol group of cysteine residues in proteins, forming a reversible thioester bond. Due to the slow oxidation rate of CoA, which is 4 times slower than glutathione (GSH) and 720 times slower than cysteine with a copper catalyst^[35], protein CoAlation provides good protection against oxidative stress. Because of the location of cysteine usually at the catalytic site due to its hydrophobicity, CoA also helps maintain the activity of enzyme, by covalently forming the thioester bonds with the cysteine and glutathione^[2].

2.4.1. Protein CoAlation in Bacteria Sporulation

Bacterial sporulation is a survival strategy of bacteria such as *Bacillus* and *Clostridium* species, forming highly resistant and dormant spores in harsh environments. They remain in metabolic dormancy until the conditions are favorable. Experimental results have shown that protein CoAlation is induced when the oxidizing agent, including H₂O₂, NaOCl, and diamide, is introduced and during carbon starvation. In addition, the CoAlation level increases according to the increase in the concentration of the oxidizing agent and the time length.

By comparing the CoA proportion in the developmental and dormant state of the spores, a high percentage of acetyl-CoA in growing spores, while only 1.5% of that is found in dormant state. In contrast, 43% of CoA in dormant spores are covalently linked to protein^[36], which is rapidly degraded during spore germination, leaving only 2% of CoA bound to protein with the disulfide bond^[36].

Key CoAlated proteins in *Bacillus subtilis* spores include alkyl hydroperoxide reductase AhpC, alcohol dehydrogenase AdhB, phosphopentosemutase Drm, and YneT which are related to redox regulation, metabolism, and stress responses. The reversible CoAlation of AhpC observed in *Staphylococcus aureus* occurs at the residue Cys168, which may protect its catalytic site, and the deCoAlation during germination might restore enzymatic function^[37].

In general, the experiment results illustrate that protein CoAlation widely exists in dormant spores of *Bacillus* species. The difference in CoA/CoA thioester proportion between dormant and germinated spores indicates the adaptation to the environment, high acetyl-CoA level and activated enzymes are necessary for early cell growth. Whereas the high percentage coaled protein helps against oxidative stress.

3. Discussion and Summary

Coenzyme A is a key metabolic cofactor involved in energy production, enzyme activity regulation, and redox balance. Playing a crucial role in metabolic dormancy across bacteria, plants, and animals. This essay has discussed how CoA and its derivatives regulate energy conservation, oxidative stress resistance, and gene expression to enter or exit dormancy. Similar patterns of metabolic dormancy are observed across distinct species.

One of the functions of CoA in dormancy is controlling metabolic pathways to conserve energy. The further study in this area can provide metabolic evidence for oncology, which helps in understanding the influences of lipids metabolism and histone acetylation on cancer proliferation^[38] and the survival mechanism of the dormant cancer cells^[33].

Additionally, CoAlation acts as an antioxidant protection mechanism during dormancy. Binding the CoA to the side chain of cysteine residue protects it from irreversible oxidative damage. This modification may have potential applications in aging and age-related diseases, where oxidative stress and metabolic decline play a crucial role.

CoA derivatives also contribute to gene regulation by acetylation. In plants, histone acetylation and deacetylation regulate seed dormancy and germination. In bacteria, acetylation and deacetylation affect enzyme activity and transcription for the most efficient energy consumption, and control of autophagy.

Future research of the role of CoA in metabolic dormancy could explore areas like aging, metabolic regulation, and stress resistance. A deeper comprehensive of protein protection by CoAlation is needed to develop possible therapies and drug targets.

Disclosure statement

The author declares no conflict of interest.

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Study on the Effects of AKG on Amino Acid Metabolism in Growing Pigs Fed a High-Fat Diet

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Abstract: To observe the effects of different doses of α -ketoglutarate (AKG) on amino acid metabolism in the tissues of growing pigs fed a high-fat diet. Forty growing pigs were selected and divided into 4 groups with 10 pigs in each group. The control group was fed only a basal diet, while the experimental groups were fed a high-fat diet: Experimental Group I (0.5% AKG + high-fat diet), Experimental Group II (1% AKG + high-fat diet), and Experimental Group III (1.5% AKG + high-fat diet). After the feeding period, the small intestine, cecum, colon, serum, liver, and leg muscle were collected to detect amino acid composition. Compared with the control group, the addition of AKG significantly increased the serum content of some glucogenic amino acids, the branched-chain amino acid Ile, and the aromatic amino acid Trp ($p < 0.05$); in the liver, AKG significantly decreased the content of some glucogenic and aromatic amino acids ($p < 0.05$); in the leg muscle, the addition of AKG extremely significantly decreased the content of branched-chain and aromatic amino acids ($p < 0.01$). The addition of AKG delayed weight gain in growing pigs by reducing the content of glucogenic amino acids in the liver and muscles, and had a protective effect on the intestinal mucosal morphology of growing pigs fed a high-fat diet..

Keywords: High-fat diet; Growing pigs; Intestinal mucosa; Amino acid metabolism

Online publication: September 26, 2025

1. Introduction

The intestine not only undertakes the task of absorbing nutrients in mammals but also plays a role in resisting exogenous harmful substances. The intestinal barrier is mainly composed of the normal intestinal flora, mucus layer, intestinal epithelial cells, and the intestinal immune system. In the process of amino acid metabolism, α -ketoglutarate (AKG), as a precursor of glutamate and glutamine, not only directly enters the TCA cycle as an energy substance to produce energy but also combines with ammonium ions produced during glutamine metabolism to form glutamate^[1]. Current results from metabolic experiments indicate that approximately 40% of AKG disappears in the intestine, suggesting that AKG can be utilized by cells of various intestinal systems and exert biological functions^[2]. Currently, there are relatively few reports on the effects of AKG on amino acid metabolism and its relationship with intestinal absorption in obese model growing pigs. Therefore, this experiment investigated the effects of adding different doses of AKG to the diet of growing pigs fed a high-fat diet on the amino acid metabolomics of some tissues and intestinal mucosal morphology, aiming to reveal the mechanism of action of AKG from the perspective of energy metabolism, and to provide an experimental basis for the development and utilization of AKG as a new feed additive and its application as a lipid-lowering regulator.

2. Materials and Methods

2.1. Experimental Animals and Design

A single-factor experimental design was used. Forty healthy Duroc × Landrace × Yorkshire crossbred growing barrows with similar body weight (approx. 25-30 kg) were selected. They were randomly divided into 4 groups with 10 replicates per group and 1 pig per replicate.

Control group (CON group): Fed a basal diet.

High-fat group (HF group): Fed a high-fat diet.

High-fat + 0.5% AKG group (HF+0.5A group): Fed a high-fat diet + 0.5% AKG.

High-fat + 1.0% AKG group (HF+1.0A group): Fed a high-fat diet + 1.0% AKG.

High-fat + 1.5% AKG group (HF+1.5A group): Fed a high-fat diet + 1.5% AKG.

High-fat diet formulation: 10% lard + 10% egg yolk powder + 4% cholesterol + 1% cholic acid + 75% ordinary feed.

2.2. Measurement Indicators and Methods.

2.2.1. Sample Collection and Preparation

Serum collection: At eight weeks, 10 mL of blood was collected from the anterior vena cava of the experimental pigs into clean centrifuge tubes. After standing at room temperature for 1 hour, it was centrifuged at 4 °C, 3500 r/min for 15 min. The serum was collected, aliquoted into 500 µL sterile centrifuge tubes, and stored at -80°C.

2.2.2. Measurement Methods

Serum biochemical indicators: Serum-related biochemical indicators were measured using a Shenzhen Mindray BS-190 automatic biochemical analyzer, with reagent kits from Shenzhen Mindray.

Amino acid indicators: The method for measuring serum amino acids was as follows: take 300 µL of serum, add an equal volume of 8% sulfosalicylic acid, vortex, let stand at 4°C for about 8 hours, centrifuge at 10000 rpm for 10 min, take the supernatant, filter through a 0.45 µm membrane into the inner tube of an amino acid sample vial, and detect the contents of Glutamate (Glu), Serine (Ser), Histidine (His), Glycine (Gly), Threonine (Thr), Alanine (Ala), Arginine (Arg), Tyrosine (Tyr), Cysteine (Cys), Valine (Val), Methionine (Met), Phenylalanine (Phe), Isoleucine (Ile), Leucine (Leu), Lysine (Lys), Proline (Pro), etc., using an L-8800 automatic amino acid analyzer (Beckman). For liver and leg muscle amino acid measurement: take tissue samples, homogenize with physiological saline, centrifuge at 3000 r/min for 20 min, take the supernatant according to the acetonitrile deproteinization method, filter through a 0.45 µm membrane into the inner tube of an amino acid sample vial, and detect with the amino acid analyzer.

2.3. Data Processing

Experimental data were preliminarily sorted using Excel 2010, and then one-way T-test in SPSS 19.0 statistical software was used to compare the significance of differences between treatment groups. $P < 0.05$ was considered a significant difference, and $0.05 < P < 0.10$ was considered a trend towards significance. Results are expressed as mean ± standard error ($\bar{x} \pm SE$).

3. Results and Analysis

3.1. Effects of AKG on Serum Amino Acid Metabolism in Growing Pigs Fed a High-Fat Diet

As shown in **Table 1**, after adding AKG to pigs fed a high-fat diet, compared with the control group, the three doses of AKG treatment (Experimental Groups I, II, and III) significantly increased the serum content of glucogenic amino acids such as Asp, Ser, and Gly ($P < 0.05$), and also significantly increased the content of the aromatic amino acid Trp ($P < 0.05$). These results suggest that the glutamate precursor AKG can significantly increase the content of some glucogenic amino acids, branched-chain amino acids, and aromatic amino acids in the serum of obese model pigs.

Table 1. Effects of AKG on Serum Free Amino Acids in Growing Pigs Fed a High-Fat Diet

AA Classification		Control Group	Group I	Group II	Group III
Glucogenic AA	Asp	51.27±20.85a	69.09±6.87b	88.69±1.58b	72.19±11.97b
	Glu	137.68±10.09	211.27±20.32	80.97±16.85	141.08±39.43
	Asn	13.98±2.39	18.59±5.85	16.28±0.98	22.98±3.68
	Ser	115.23±14.29a	176.39±21.59b	115.26±7.64a	205.97±48.69b
	Gln	497.68±76.38	398.67±5.29	351.27±20.85	428.14±2.49
	Gly	279.69±54.39a	350.61±47.23b	297.77±11.79b	400.39±8.76b
	Thr	215.69±39.85a	249.78±19.42b	297.39±11.95b	303.58±12.66b
	Arg	57.69±21.36	86.95±18.64	79.69±12.09	100.27±20.06
	Ala	259.27±78.49	296.27±72.51	248.75±8.20	324.70±44.29
Branched-Chain AA	Val	41.39±18.69a	66.78±8.36b	51.37±8.69b	54.78±13.94b
	Ile	35.10±2.89	39.67±11.96	38.74±10.02	69.68±11.88
	Leu	36.66±5.68	35.74±10.85	30.89±9.85	44.48±9.72
Aromatic AA	Tyr	307.25±90.56	278.69±20.09	319.59±10.29	306.42±10.57
	Trp	19.36±1.77a	30.96±7.85	39.40±7.99	41.26±7.68
	Phe	40.56±10.25	31.27±4.25	33.60±2.21	51.17±6.99

3.2. Effects of AKG on Liver Amino Acid Metabolism in Growing Pigs Fed a High-Fat Diet

As shown in **Table 2**, after adding AKG to pigs fed a high-fat diet, compared with the control group, the three doses of AKG treatment (Experimental Groups I, II, and III) extremely significantly decreased the liver content of glucogenic amino acids such as Asn, Gly, Thr, and Ala ($P<0.01$), and also significantly decreased the liver content of aromatic amino acids Tyr, Trp, and Phe ($P<0.05$). However, for the content of branched-chain amino acids, no significant changes occurred in the three groups ($P>0.05$). These results suggest that the glutamate precursor AKG can significantly reduce the content of some glucogenic and aromatic amino acids in the liver of obese model pigs.

Table 2. Effects of AKG on Liver Free Amino Acids in Growing Pigs Fed a High-Fat Diet

AA Classification		Control Group	Group I	Group II	Group III
Glucogenic AA	Asp	366.82±58.56	136.87±29.57	254.98±32.24	153.57±38.09
	Glu	363.93±19.44	262.27±40.68	280.78±55.28	392.59±52.43
	Asn	84.69±9.87A	43.36±5.97B	56.28±0.98B	38.67±6.27B
	Ser	415.98±25.46	176.39±9.48	252.69±15.9	200.78±16.62
	Gln	264.67±11.38	303.127±18.26	323.36±20.85	196.39±12.49
	Gly	789.23±23.39A	485.69±7.70B	576.31±3.35B	398.69±11.57B
	Thr	80.08±39.85A	101.59±25.36B	152.39±26.87B	98.39±11.29B
	Arg	147.89±21.36	115.66±18.64	179.69±12.09	179.58±20.06
	Ala	1497.36±98.49A	1176.27±99.51B	1348.75±128.20B	324.70±44.29B
	Val	178.26±5.68a	58.36±4.59	79.59±5.72	69.89±3.25

Table 1 (Continued)

AA Classification		Control Group	Group I	Group II	Group III
Branched-Chain AA	Val	178.26±5.68a	58.36±4.59	79.59±5.72	69.89±3.25
	Ile	152.10±2.89	39.67±11.96	41.74±10.02	39.68±11.88
	Leu	232.66±5.68	60.74±10.85	96.89±9.85	85.48±9.72
Aromatic AA	Tyr	907.25±90.56a	1078.69±120.09	1019.59±1.29	896.42±10.57
	Trp	29.36±1.77a	78.96±7.85b	55.82±7.99b	43.26±7.68b
	Phe	144.56±10.25a	39.27±4.25b	68.60±2.21b	54.17±6.99b

3.3. Effects of AKG on Leg Muscle Amino Acid Metabolism in Growing Pigs Fed a High-Fat Diet

As shown in **Table 3**, after adding AKG to pigs fed a high-fat diet, compared with the control group, the three doses of AKG treatment (Experimental Groups I, II, and III) significantly decreased the muscle content of amino acids such as Asn, Glu, Asp, Ser, Gly, Val, and Thr ($P<0.05$), and also significantly decreased the muscle content of branched-chain amino acids Val, Ile, and Leu ($P<0.05$), and extremely significantly decreased the content of phenylalanine Phe ($P<0.01$). These results suggest that the glutamate precursor AKG can significantly reduce the content of some glucogenic and branched-chain amino acids in the leg muscle of obese model pigs, inducing the catabolism of blood sugar.

Table 3. Effects of AKG on Leg Muscle Free Amino Acids in Growing Pigs Fed a High-Fat Diet

AA Classification		Control Group	Group I	Group II	Group III
Glucogenic AA	Asp	356.49±44.93a	312.62±27.41b	264.28±32.2b	269.68±38.09b
	Glu	354.93±19.44a	297.27±40.68b	329.78±55.28b	287.59±52.43b
	Asn	52.69±9.87Aa	27.85±5.97Bb	21.97±0.98Bb	24.67±6.27Bb
	Ser	215.98±25.46a	176.39±9.48b	179.69±15.9b	198.78±16.62b
	Gln	233.67±11.38	225.127±18.26	219.36±20.85	152.39±12.49
	Gly	149.23±23.39a	85.69±7.70b	76.31±3.35b	98.69±11.57b
	Thr	380.08±39.85a	310.59±25.36b	258.39±26.87b	178.39±11.29b
	Arg	147.89±21.36	100.66±18.64	79.69±12.09	79.58±20.06
	Ala	497.36±8.49A	396.27±72.51B	348.75±8.20B	324.70±44.29B
Branched-Chain AA	Val	99.26±5.68a	42.36±4.59b	42.59±5.72b	37.89±3.25b
	Val	99.26±5.68a	42.36±4.59b	42.59±5.72b	37.89±3.25b
	Ile	52.10±2.89a	39.67±11.96b	41.74±10.02b	39.68±11.88b
	Leu	236.66±5.68a	135.74±10.85b	130.89±9.85b	144.48±9.72b
	Tyr	507.25±90.56	378.69±20.09	319.59±10.29	406.42±10.57
	Trp	219.36±71.09	178.96±7.96	155.82±8.99	141.26±7.07
	Phe	63.56±10.08A	34.27±4.96B	41.60±2.87B	38.17±6.45B

4. Discussion

Blood is an important amino acid pool in the body and a hub connecting nutrient absorption and metabolic utilization.

Because blood amino acid composition is easily affected by diet and body health status, measuring blood amino acid composition can be a valuable reference for disease diagnosis^[3]. Our experimental results confirm that the glutamate precursor AKG can significantly increase the serum content of some glucogenic amino acids, branched-chain amino acids, and aromatic amino acids in obese model pigs. Measurements of amino acid metabolism in the liver and muscles determined that adding AKG to the diet of SD obese model pigs reduces the aromatic amino acid content in the liver and muscle amino acid pools.

As an energy donor, AKG can serve as a substitute for amino acids in the small intestine. When the body's energy supply is severely insufficient, glucogenic amino acids in the body can be gluconeogenized into glucose in the liver through specific metabolic pathways to serve as an energy supply for the body^[4]. The results of this study show that in the high-fat diet groups treated with different concentrations of AKG, the serum Asp, Ser, and Gly content in growing pigs was significantly higher than in the high-fat diet control group, but different doses of AKG treatment significantly reduced the content of most glucogenic amino acids in the liver and leg muscle. Therefore, we speculate that under physiological conditions with sufficient energy to meet the body's energy demands, adding AKG to a high-fat diet may primarily regulate glucogenic amino acids to enhance body protein synthesis, but the specific biological mechanism requires further in-depth study. Muscle tissue not only uses carbohydrates and lipids as metabolic substrates but is also the main site for branched-chain amino acid (BCAA) metabolism in the body^[5]. Studies in obese humans and obese animal models have found that BCAA levels in serum are very high, indicating impaired BCAA catabolism. Simultaneously, elevated ceramide levels were found in muscle tissue; this lipid acts as a signaling molecule inhibiting insulin receptor activity^[6]. In this study, AKG addition significantly increased the content of the branched-chain amino acid Ile and extremely significantly reduced BCAA metabolism in the leg muscle, consistent with the above research results. Therefore, we speculate that AKG can accelerate BCAA decomposition in the intestine during its metabolism, reducing the concentration of BCAAs in circulation, thus potentially playing a role in remodeling metabolic pathways in muscle.

AKG is an intermediate of the TCA cycle and a precursor for glutamate family amino acids. Endogenous AKG is mainly generated by the oxidative decomposition of glucose. When circulating AKG is insufficient, glutamate acts as an anaplerotic substrate to participate in the cycle^[7]. One study using 24 pregnant sows as subjects found that dietary supplementation with AKG during the peripartum period significantly increased the birth weight of newborn piglets and the serum concentrations of aspartate, serine, glutamine, glycine, valine, ornithine, lysine, and arginine compared to the control saline group^[8]. Glutamate is a major energy source for upper intestinal epithelial cells and intestinal microorganisms. Even when providing more than 4 times the required amount of Glu, most Glu is oxidized for energy or converted into other non-essential amino acids in intestinal epithelial cells^[9]. The mechanism may be that the preliminary digestion of nutrients begins in the stomach, but the main site of nutrient absorption is the small intestine. As a precursor of glutamate, AKG is converted to Glu upon ingestion, while fat digestion begins in the small intestine. Because the dwell time of chyme in the duodenum is relatively short, fat does not exert its effect, while the massively increased monosodium glutamate causes the body to reduce Glu absorption by downregulating the expression of the EAAC1 gene^[10] to maintain amino acid balance in duodenal epithelial cells.

In this experiment, adding AKG to pigs fed a high-fat diet significantly reduced the muscle content of amino acids such as Asn, Glu, Asp, Ser, Gly, Val, and Thr. Under energy restriction, dietary fat does not have a significant effect on the preservation of body protein and branched-chain amino acids (including Leu, Ile, and Val)^[11]. However, under normal physiological conditions, fat intake can reduce the proportion of energy supplied by nutrients other than fat. The results of this experiment also show that adding high fat to the diet can reduce the level of glucogenic amino acids in the blood. Blood Val is a good predictor of amino acid preservation; studies have found that dietary fat supplementation can increase blood Val concentration, indicating that dietary fat can promote the preservation of body amino acids^[12].

5. Conclusion

In obese model pigs, the addition of AKG delayed weight gain by reducing the content of glucogenic amino acids in the liver and muscles and had a protective effect on the intestinal mucosal morphology of growing pigs fed a high-fat diet.

Funding

This study was supported by the 2024 Natural Science Foundation of Hubei Province Project, CHINA (Project No.: 2024AFB972).

Disclosure statement

The author declares no conflict of interest.

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Research Progress of Tracheal Stent in Vitro and Animal Experiments

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Abstract: Tracheal stent implantation is a common method in clinical treatment of benign tracheal bronchial stenosis and tracheal bronchial fistula. With the widespread application of tracheal stent implantation, mucosal ulcer, tracheal restenosis, granulation and other complications of tracheal stent implantation are increasing, and relevant tracheal stent implantation in vitro and animal experiments are needed to test the effect of tracheal stent implantation. This paper summarized the status and development of in vitro testing and animal experimental research on tracheal stents in recent years, which laid the foundation for subsequent research and development of related technologies.

Keywords: Tracheal stent; Tracheal stenosis; Animal experiments; In vitro experiment

Online publication: September 26, 2025

1. Introduction

With the development of interventional radiology, especially the rapid development of high-resolution HRCT, the diagnosis rate of airway diseases has also been greatly improved, especially tracheal stenosis and tracheal fistula. In order to solve the above problems, the rapid rise of interventional radiology and new interventional medical devices has led to the emergence of materials, designs, and performance of tracheal stents. The implantation and removal technology of tracheal stents is constantly improving and has been widely adopted. This article summarizes the research status and latest progress of tracheal stents in vitro and animal experiments by analyzing the anatomical structure of the human airway and the basic differences in airway structure of commonly used experimental animal breeds, and organizing previous animal experimental research literature on tracheal stents. It provides a reference for subsequent research and development.

2. Anatomical analysis of the human trachea

2.1. Anatomy of the human main trachea

The main trachea of the human body is a circular tube that extends from the throat to the bronchi, with a relatively flat posterior wall^[1]. The length of an adult's trachea is approximately 11 to 13 centimeters, which can be divided into two parts: the neck and chest. The cervical trachea descends along the midline of the neck, covering the sternohyoid muscle and sternocleidomastoid muscle in front, adjacent to the esophagus in the back, and accompanied by the inferior laryngeal nerve, blood vessels, and thyroid lobes on both sides. The anterior part of the thoracic trachea is adjacent to the aortic

arch and its branches (unnamed artery, left common carotid artery), unnamed vein, and thymus, while the posterior part is still close to the esophagus. In clinical routine tracheotomy, a midline incision is often chosen at the 3-4 or 4-5 tracheal cartilage rings^[2]. The tracheal cartilage ring is C-shaped, and its posterior notch is connected by smooth muscle and elastic fiber tissue, forming a dynamic structure with certain elasticity to maintain airway patency and provide anatomical basis for biomechanical research of tracheal stents and other implanted instruments.

2.2. Anatomy of human bronchi

The bronchus is a primary branch of the trachea, including the left and right main bronchi. The left main bronchus is relatively slender, with an average length of about 4 centimeters, forming an angle of about 35-36 degrees with the central axis of the trachea. It runs relatively obliquely and enters the left lung through the left pulmonary hilum. The right main bronchus is relatively short and thick, with an average length of about 2-3 centimeters, forming an angle of about 22-25 degrees with the axis of the trachea. It runs relatively vertically and enters the right lung through the right pulmonary hilum. Due to the anatomical characteristics of the right main bronchus, foreign bodies in the trachea are more likely to fall into that side. This structural feature has important reference value in the construction of foreign body models involving tracheal stent implantation and animal experiments^[3]. In addition, the bronchial branch structure and its epithelial cell types and functional differentiation provide key in vitro and in vivo experimental evidence for evaluating the cell biocompatibility and epithelial regeneration ability of tracheal stent materials.

3. Anatomy of related animal tracheas

3.1. Tracheal Anatomy of Rabbits

The trachea of rabbits is composed of 48-50 C-shaped cartilage rings, and the trachea below the cricoid cartilage narrows slightly after entering the chest cavity, and branches into the left and right main bronchi. The diameter of the right main bronchus is slightly larger than that of the left, and it branches into the upper lobe bronchus and enters the right lung apex lobe. The volume of rabbit lungs is relatively small, with the right lung being heavier than the left lung, weighing approximately 7-7.5 grams for the right lung and 5-5.5 grams for the left lung. The right lung is divided into 4 lobes, while there are different literature reports on the division of the left lung into 2 lobes or 3 lobes, mainly due to whether the left lung apex lobe is further divided into the anterior and posterior parts. The structure of rabbit tracheal cartilage and the composition of epithelial cells make it a commonly used in vivo model for evaluating the cell compatibility and epithelial regeneration ability of tracheal scaffolds.

3.2. Tracheal Anatomy of Small tailed Han Sheep

The trachea from the upper incisors to the glottis of the Small tailed Han sheep is about 18 centimeters long, and the subglottic trachea is about 30 centimeters long with a diameter of about 3 centimeters. The trachea is composed of a cylindrical long tube connected by a "C" - shaped cartilage ring and a cartilage membrane. It enters the chest cavity through the anterior opening and divides into left and right main bronchi at the level of the fifth thoracic vertebra, which enter the lungs. The right main bronchi also emit a small right apex lobe bronchus before branching off. The sheep trachea has a large size and stable cartilage ring structure, making it suitable for tracheal stent implantation surgery and medium - to long-term animal experimental studies on in vivo biocompatibility, epithelial differentiation, etc.

3.3. Tracheal Anatomy of Dogs

The tracheal structure of dogs includes the mucosal layer (pseudostratified columnar ciliated epithelium), submucosal layer, fibromuscular cartilage membrane, and outer membrane. The trachea starts from the throat, extends along the midline of the neck, enters the chest cavity through the anterior opening, and ultimately splits into the left and right main bronchi at approximately the 4th to 6th intercostal spaces. The right main bronchus is relatively short and thick, while the

left main bronchus is slightly slender. There are mucous glands and serous glands distributed within the tracheal mucosa, and the mucosal epithelium has dense cilia and secretory functions. The canine trachea is clearly divided into cervical and thoracic segments, with an anatomical structure similar to that of humans. The epithelial cell types and mucociliary clearance mechanisms are clear, making it a classic animal model for evaluating the biocompatibility of tracheal stent materials in vivo and their impact on respiratory epithelial cell differentiation^[4,5].

4. Current research status of tracheal stents

4.1. Classification of new tracheal stents

Tracheal stents can be classified into three types based on their geometric shape: Y-shaped, T-shaped, and straight tube shaped. The “Y” - shaped stent is suitable for narrow or large fistulas in the tracheal prominence area, with stable fixation but complex release; The “T” - shaped stent can be used for subglottic stenosis, preserving vocal function but requiring an stoma; The straight tube stent is suitable for upper and middle tracheal lesions and is easy to insert but prone to migration. According to their structure, they can be divided into circular, mesh, and complex biomimetic types. Among them, 3D printed “C” ring scaffolds can better match the mechanical environment of tracheal cartilage, mesh covered scaffolds are used to seal fistula openings, and woven biomimetic scaffolds serve tissue regeneration.

From the perspective of materials science, tracheal stents are divided into two categories: non degradable and degradable. Non degradable stents such as metal, silicone, etc. provide long-lasting support but require secondary surgery to remove, which can easily cause inflammation and epithelial abnormalities. Degradable materials such as poly (p-cyclohexanone) (PDS), poly (lactic acid) (PLA), poly (caprolactone) (PCL), etc. not only have suitable mechanical properties and elastic recovery ability, but also have good biocompatibility and controllable degradation cycle, which can match the process of airway tissue healing, reduce long-term foreign body reactions, and support normal epithelial cell regeneration and functional differentiation. Therefore, they have attracted much attention in tissue engineering and regenerative medicine research.

4.2. Application of new tracheal stent

4.2.1. Tracheal stent for treating airway stenosis

Airway stenosis is divided into benign and malignant types, and in adults it is mostly acquired (such as iatrogenic, infectious, or foreign body). At present, metal or silicone stents are commonly used in clinical practice to treat malignant stenosis. For example, Xiong Zhen and other comprehensive methods such as high-frequency electrocautery, balloon dilation, and stent placement are used to treat benign central airway stenosis, and stents are selected according to the degree and location of stenosis, achieving good long-term efficacy^[6]. However, long-term retention of non degradable materials can lead to impaired mucociliary clearance function, granulation hyperplasia, and abnormal epithelial cell differentiation. Therefore, current research focuses on biodegradable scaffolds, evaluating their effects on airway epithelial cell behavior, barrier function, and ciliary differentiation in vitro models and animal experiments, in order to promote physiological epithelial repair while maintaining mechanical support.

4.2.2. Tracheal stent for treating tracheoesophageal fistula

Tracheal esophageal fistula is often caused by congenital developmental abnormalities or acquired factors such as surgery, tumors, and infections. The treatment methods include surgery, stent placement, endoscopic techniques, and biological protein gel closure, among which tracheoesophageal stent placement has become mainstream due to its minimally invasive and efficient nature. Common types of stents include metal coated stents, silicone stents, 3D printed stents, and biodegradable stents. Straight stents are commonly used for the proximal trachea, while “L” or “Y” stents are suitable for the distal or protuberance regions. The outer diameter of the stent usually needs to be 10% -20% larger than the inner diameter of the trachea at the fistula site to achieve effective occlusion^[7].

Studies at the cellular and animal levels have shown that the biocompatibility and surface properties of scaffold materials directly affect epithelial cell migration, differentiation, and fistula closure quality. For example, biodegradable materials such as PCL/PLA blend scaffolds have shown potential in *in vivo* experiments to promote mucosal regeneration, reduce leakage, and improve ciliary epithelial distribution. Although self-expanding metal-coated scaffolds have good adhesion and are easy to place, their long-term retention may still interfere with normal epithelial function. Therefore, a new type of biodegradable scaffold with a cell-friendly interface has become a current research focus, aiming to achieve the dual goals of mechanical occlusion and tissue regeneration.

5. Current status of animal experimental research on tracheal stents

5.1. Preparation of animal models

In terms of animal model construction, domestic researchers have conducted multiple explorations. Li Zhaonan et al.^[8] selected New Zealand rabbits and studied the construction and performance of nano silver synergistic cisplatin eluting fiber scaffolds by inserting film-coated and drug-eluting scaffolds. They found that it has potential in drug release and provides a reference for the functionalization of scaffold coatings. However, this study did not evaluate the scaffold effect under pathological conditions and further *in vivo* experimental verification is needed. Another study successfully established a rabbit model of tracheal stenosis by combining tracheotomy with nylon brush scraping of mucosa, and found that magnesium alloy stents were superior to nickel titanium alloy in terms of radial support and biocompatibility^[9]. Zhao Chun et al.^[5] used beagle dogs and constructed a model of tracheal stenosis under bronchoscopy guidance through electric burns and low-power disruption of mucosal continuity. This type of thermal and electrical injury method has the advantages of precise operation, good repeatability, and few complications. In addition, the canine trachea is similar in length and diameter to the human body, making it more suitable for simulating the *in vivo* biological reactions and epithelial repair process after stent implantation.

The commonly used methods for constructing animal models of tracheoesophageal fistula include azithromycin induction, surgical incision, and magnetic compression. The surgical incision method is easy to operate and widely used. For example, Yang Xinyue et al.^[10] successfully established a tracheal fistula model through a midline incision in the neck of beagle dogs; Li Shuixiu et al.^[11] used magnetic compression to construct a rabbit model of tracheoesophageal fistula. This non-invasive method has fewer complications and provides new ideas for fistula repair and re-epithelialization research.

There is relatively little research abroad, and most of it focuses on models of tracheobronchial softening. Ha J et al.^[12] implanted stents into healthy pigs and validated the compression mechanical model of the stent and its effectiveness in the treatment of tracheobronchial softening. They found that nickel titanium spiral stents significantly improved stenosis. Mondal et al.^[13] established an *in vitro* softening model by excising sheep tracheal cricoid cartilage, providing a new method for optimizing the mechanical properties of scaffolds and connecting *in vivo* and *in vitro* models. Ruegamer JL et al.^[14] used piglets to evaluate the effectiveness of balloon-expandable metal stents in treating tracheal stenosis, indicating that the model is suitable for simulation studies on the treatment of tracheal stenosis in children.

Overall, domestic research often uses small animals such as rabbits and mice, which are low-cost and easy to operate; However, foreign countries tend to use large animals such as pigs, sheep, and dogs, whose airway structures are more similar to those of the human body, which is more conducive to simulating scaffold tissue interactions and epithelial differentiation processes at the physiological and cellular levels.

5.2. Current Status of Experimental Research

Currently, domestic research mainly focuses on material modification, drug release, and structural design of tracheal stents. Li Zhaonan et al.^[7] developed an electrospun-coated scaffold containing nano silver and cisplatin, which can significantly inhibit the growth of various pathogens and biofilm formation, demonstrating good antibacterial performance and cell

compatibility. Li Yahua et al.^[15] developed arsenic trioxide eluting nanofiber scaffolds and confirmed in a rabbit model that they can promote tracheal epithelial regeneration and fistula repair. Zhao Chun^[5] used electrospray technology to prepare paclitaxel eluting scaffolds, which effectively inhibited granulation tissue proliferation and restenosis, indicating the potential of drug sustained-release scaffolds in regulating local cell behavior and tissue repair.

In terms of stent structure, some scholars have explored the application of grass based composite materials and 3D printed concave hexagonal filling models in airway stents, and found that they have good mechanical adaptability and printing feasibility. Yuan Zhengchao et al.^[16] developed a composite aerogel scaffold by combining 3D printing and electrospinning technology. The scaffold showed stable mechanical properties, good blood compatibility and biocompatibility in both in vitro and in vivo experiments, and was suitable for tracheal cartilage repair.

Foreign research also focuses on the biological response and restenosis mechanism of scaffold materials. Arellano Orden et al.^[17] compared paclitaxel eluting stents, nickel titanium alloy, and cobalt based alloy stents in a rabbit model and found that early elevation of IL-8 in the blood after implantation can predict the progression of tracheal stenosis, providing a biomarker basis for evaluating the inflammatory response and epithelial abnormal differentiation caused by stents. Ruegemer JL et al.^[14] placed balloon expandable metal stents in young pigs and avoided complications caused by excessive dilation by adjusting balloon parameters. Chaure J et al.^[18] simulated the fluid dynamics interaction between the airway and vascular stent in rabbits and found differences in stress distribution among different metal materials.

It is worth noting that current research is increasingly introducing cellular biology evaluation indicators into animal models, such as epithelial barrier function, ciliary differentiation degree, inflammatory factor expression, and collagen deposition, in order to comprehensively evaluate the biocompatibility of tracheal stents and their impact on airway tissue regeneration in the in vivo environment.

6. Summary and outlook

With the continuous development and clinical application of new tracheal stents, stent implantation has become increasingly mature in the treatment of tracheal stenosis and tracheoesophageal fistula. However, postoperative complications such as stent displacement, mucus retention, restenosis, and epithelial repair still occur from time to time. The material characteristics, structural design, and surface coating of the stent not only affect its mechanical properties, but also directly regulate the behavior of host cells, such as epithelial cell proliferation and differentiation, inflammatory response, and ciliary function recovery, thereby determining long-term efficacy.

Future research needs to further combine in vitro cell models with animal experiments to systematically evaluate the biocompatibility of tracheal stents, especially biodegradable materials and biologically active coating stents. The focus should be on investigating the effects of scaffold materials on the differentiation and barrier function of airway epithelial cells, and verifying their effects on promoting tissue regeneration, inhibiting granulation tissue proliferation, and reducing fiber wrapping in an in vivo model. At the same time, it is necessary to optimize the selection of animal species and modeling methods to improve the clinical predictive value of experimental results.

Looking ahead to the future, developing new personalized tracheal stents with good cell compatibility and epithelial repair function based on different etiologies and lesion characteristics will be an important direction for achieving precise treatment and improving patients' long-term prognosis.

Disclosure statement

The author declares no conflict of interest.

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Publisher's note

Whioce Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Analysis of Influencing Factors on Medication Adherence in Patients with Allergic Rhinitis

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Abstract: Objective: To analyze the factors influencing medication adherence in patients with allergic rhinitis, and to provide a reference for improving patient compliance in clinical treatment. Methods: A total of 86 patients with allergic rhinitis who received medication treatment at our hospital from January 2022 to December 2023 were selected and divided into a high-adherence group and a low-adherence group based on their medication adherence assessment results, with 43 patients in each group. The criteria for high adherence were defined as taking medication on time and in the prescribed amount without unauthorized discontinuation, dose reduction, or missed doses, and being able to accurately recite the medication regimen and precautions during follow-up. The criteria for low adherence were defined as having ≥ 3 instances of unauthorized discontinuation, dose reduction, or missed doses during the medication period, or being unable to accurately recite the medication regimen during follow-up. Basic data, disease awareness, occurrence of adverse drug reactions, and family support were collected from both groups using a unified method, and factors influencing adherence were compared and analyzed. Results: The high-adherence group had a higher rate of meeting disease awareness standards, a lower incidence of adverse drug reactions, and a higher rate of family support compared to the low-adherence group, with all differences being statistically significant ($P < 0.05$). Conclusion: Insufficient disease awareness, occurrence of adverse drug reactions, and lack of family support are the main factors contributing to low medication adherence in patients with allergic rhinitis. Targeted interventions can be implemented clinically to improve patient adherence.

Keywords: Allergic rhinitis; Medication treatment; Adherence; Influencing factors; Disease awareness

Online publication: September 26, 2025

1. Introduction

Allergic rhinitis is a common clinical allergic disease characterized by symptoms such as nasal congestion, runny nose, nasal itching, and sneezing. The disease has a long course and is prone to recurrent episodes, requiring long-term medication to control symptoms. Patient adherence to treatment directly affects the effectiveness of disease control^[1]. In recent years, with changes in environmental factors and lifestyles, the incidence of allergic rhinitis has shown an annual increasing trend. It is particularly prevalent among adolescents and middle-aged individuals, causing numerous disruptions to patients' daily lives, work, and studies. Some patients, enduring prolonged symptoms, may develop adverse

emotions such as anxiety and irritability, further diminishing their quality of life. In clinical practice, some patients, due to insufficient understanding of the disease, concerns about medication side effects, or other reasons, often exhibit non-compliant behaviors such as unauthorized discontinuation or dose reduction of medications. These behaviors result in poor treatment outcomes or even exacerbation of the condition, not only increasing patients' medical expenses but also potentially leading to complications like sinusitis and asthma, thereby complicating treatment. Therefore, analyzing the factors influencing patients' adherence to pharmacological treatment is crucial. Based on this, this study aims to identify the primary factors affecting adherence by uniformly collecting relevant data from patients with allergic rhinitis exhibiting different levels of adherence, providing a basis for clinical interventions.

2. Materials and Methods

2.1. General Information

Eighty-six patients with allergic rhinitis who received pharmacological treatment at our hospital from January 2022 to December 2023 were selected and divided into a high-adherence group and a low-adherence group based on adherence assessment results, with 43 patients in each group. The criteria for determining adherence in both groups were as follows: Comprehensive assessment was conducted through patients' medication records (verification of remaining medication quantities and medication logs during outpatient follow-ups) and monthly follow-ups (telephone inquiries about medication usage). The high-adherence group was required to meet the following criteria: timely and appropriate medication usage during the treatment period, without any unauthorized discontinuation, dose reduction, or missed doses, and the ability to accurately recite the usage, dosage, and precautions of the medications during follow-ups. The low-adherence group was required to meet the following criteria: the presence of ≥ 3 instances of unauthorized discontinuation (each instance lasting ≥ 2 days), dose reduction (dosage reduced by more than 50% of the prescribed amount), or missed doses during the treatment period, or the inability to accurately recite the core content of the medication regimen (such as daily medication frequency and drug formulation) during follow-ups. In the high-compliance group, there were 23 males and 20 females, aged between 22 and 65 (41.25 ± 5.38) years; in the low-compliance group, there were 22 males and 21 females, aged between 23 and 64 (40.92 ± 5.16) years. There were no significant differences in general demographic data between the two groups ($P > 0.05$), indicating comparability. Inclusion criteria: (1) Meeting the diagnostic criteria for allergic rhinitis; (2) Requiring at least three months of pharmacological treatment; (3) Having clear consciousness and being able to cooperate with the investigation. Exclusion criteria: (1) Presence of severe heart, liver, or kidney diseases; (2) Presence of mental disorders preventing communication; (3) Concurrent receipt of other nasal treatments.

2.2. Data Collection Methods

A unified data collection process was employed for both groups, as follows: (1) Basic Data Collection: By reviewing patient medical records, basic information such as gender, age, disease duration, and types of medications used (e.g., intranasal corticosteroids, oral antihistamines) was recorded, ensuring accurate and complete information extraction; (2) Disease Cognition Survey: During patient visits or follow-ups, a face-to-face questionnaire survey was conducted. The questionnaire included three core questions regarding the etiology of allergic rhinitis, the necessity of long-term medication use, and the risks of unauthorized drug discontinuation. Healthcare professionals recorded patient responses on-site. Patients who correctly answered all three questions were considered to have met cognitive standards; (3) Monitoring of Adverse Drug Reactions: Through outpatient follow-up inquiries, telephone follow-ups, and active patient feedback, the presence of adverse reactions such as nasal dryness, dizziness, and gastrointestinal discomfort during medication use was recorded. Detailed records of the onset time, manifestations, and duration of adverse reactions were maintained. Suspected adverse reactions were promptly verified and their association with medication use confirmed; (4) Assessment of Family Support: Through telephone interviews with patients' family members or inquiries during patient follow-ups regarding family member accompaniment, it was determined whether family members regularly reminded patients to

take medications, monitored symptom changes, and observed reactions after medication use. If family members engaged in at least one of the aforementioned behaviors, it was considered indicative of family support. The entire data collection process was carried out by uniformly trained healthcare professionals to ensure consistent survey standards and minimize information bias.

2.3. Observation Indicators

The study compared the disease awareness attainment rate between the two groups (attainment was defined as patients correctly answering questions about the etiology of the disease, the necessity of medication, and the risks of discontinuing medication), the incidence of adverse drug reactions (including nasal dryness, dizziness, gastrointestinal discomfort, etc. during medication), family support rate (defined as family members regularly reminding patients to take medication and monitoring their symptoms), and the overall treatment effectiveness rate (symptoms completely disappeared or significantly alleviated were considered effective).

2.4. Statistical Methods

SPSS 24.0 was used for data analysis. Measurement data were analyzed using t-tests, while categorical data were analyzed using χ^2 tests. A P-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Comparison of Disease Awareness Attainment Rate and Family Support Rate Between the Two Groups

The high-compliance group demonstrated higher disease awareness attainment rates and family support rates compared to the low-compliance group ($P < 0.05$), as shown in **Table 1**.

Table 1. Comparison of Disease Awareness Attainment Rate and Family Support Rate Between the Two Groups [n (%)]

Group	Disease Knowledge Met Standard	High Family Support
High Adherence Group(n=43)	38 (88.37)	36 (83.72)
Low Adherence Group(n=43)	16 (37.21)	15 (34.88)
χ^2	24.088	21.247
p-value	0.000	0.000

3.2. Comparison of Incidence of Adverse Drug Reactions Between the Two Groups

The incidence of adverse drug reactions was lower in the high-compliance group than in the low-compliance group ($P < 0.05$), as shown in **Table 2**.

Table 2. Comparison of Incidence of Adverse Drug Reactions Between the Two Groups [n (%)]

Group	Nasal Dryness	Dizziness	Gastrointestinal Discomfort	Total Incidence
High Adherence Group(n=43)	3 (6.98)	1 (2.33)	0 (0.00)	4 (9.300)
Low Adherence Group(n=43)	10 (23.26)	8 (18.60)	0 (0.00)	18 (41.860)
χ^2				11.972
p-value				0.001

3.3. Comparison of Overall Treatment Effectiveness Rate Between the Two Groups

The overall treatment effectiveness rate was higher in the high-compliance group than in the low-compliance group ($P < 0.05$), as shown in **Table 3**.

Table 3. Comparison of Overall Treatment Effectiveness Rate Between the Two Groups [n (%)]

Group	Effective	Ineffective	Total Effective Rate
High Adherence Group (n=43)	40 (93.02)	3 (6.98)	40 (93.02)
Low Adherence Group (n=43)n	26 (60.47)	17 (39.53)	26 (60.47)
χ^2			12.770
p-value			0.000

4. Discussion

As a chronic disease, allergic rhinitis is primarily managed through medication to control symptoms and reduce recurrence. The effectiveness of treatment is directly determined by whether patients can adhere to long-term and regular medication use. Therefore, analyzing the factors influencing medication adherence and implementing targeted interventions are of great significance for improving patients' conditions^[2]. In this study, by using a unified method to collect and comparatively analyze data from patients with high and low medication adherence, it was found that disease awareness, adverse drug reactions, and family support were closely related to patient adherence. These findings align with clinical realities, and a detailed analysis will be conducted below from these perspectives.

From the perspective of disease awareness, the results of this study show that the disease awareness compliance rate in the high-adherence group (88.37%) was significantly higher than that in the low-adherence group (37.21%) ($P < 0.05$). This indicates that insufficient awareness of allergic rhinitis and its pharmacological treatment among patients is a major factor contributing to low adherence. In clinical practice, some patients, lacking a proper understanding of the disease, perceive allergic rhinitis as merely a "minor issue" and discontinue medication once symptoms subside, or they worry about dependency and side effects from long-term medication use, leading to unauthorized discontinuation or dosage reduction. During the data collection process, it was observed that most patients in the high-adherence group could clearly articulate the chronic nature of allergic rhinitis and understand that long-term medication use can effectively reduce the frequency of acute episodes. In contrast, many patients in the low-adherence group viewed the disease as merely "seasonal colds" and had a vague understanding of the importance of medication adherence, further confirming the significant impact of disease awareness on adherence. In clinical practice, patients with different educational levels exhibit varying degrees of disease awareness. Patients with lower educational levels often struggle to comprehend disease-related knowledge and provide incomplete responses to survey questions. Therefore, healthcare professionals should use more accessible language and incorporate real-life examples in disease education during routine consultations. For instance, comparing the long-term treatment needs of allergic rhinitis to those of hypertension, which requires long-term medication control, can facilitate better patient understanding.

Adverse drug reactions (ADRs) are another key factor affecting patient adherence. In this study, the incidence of ADRs in the high-adherence group (9.30%) was significantly lower than that in the low-adherence group (41.86%) ($P < 0.05$). Commonly used medications for allergic rhinitis treatment, such as intranasal corticosteroids, may cause nasal dryness, while oral antihistamines may lead to dizziness, gastrointestinal discomfort, etc. Although these ADRs are mostly mild, some patients, lacking prior knowledge or coping strategies, may perceive them as "severe side effects," leading to fear and discontinuation of medication. Analysis of ADR records revealed that patients in the high-adherence group often proactively used saline nasal irrigation to alleviate mild nasal dryness, whereas those in the low-adherence

group frequently discontinued medication directly upon experiencing similar symptoms without promptly informing healthcare providers, resulting in interrupted subsequent treatment. Notably, individual tolerance to ADRs varies among patients. During data collection, it was observed that some patients found even mild nasal dryness unacceptable, while others adapted spontaneously. This necessitates healthcare providers to thoroughly assess patients' psychological states and tolerance levels before prescribing medications, offering psychological counseling to sensitive patients in advance. Additionally, when prescribing medications, providers should prioritize formulations with lower ADR incidence rates, such as mometasone furoate nasal spray among intranasal corticosteroids, which exhibits relatively fewer local irritation reactions^[3-4].

The impact of family support on patient adherence cannot be overlooked either. Data from this study indicated that the family support rate in the high-adherence group (83.72%) was significantly higher than that in the low-adherence group (34.88%) ($P < 0.05$). Patients with allergic rhinitis often require long-term home medication, making the attitudes and behaviors of family members crucial in shaping patients' medication habits. Regular reminders from family members and attention to symptom changes can effectively reduce non-adherence due to "forgetfulness" or "laziness." Conversely, if family members show indifference towards patients' medication use or even endorse the idea that "medication can be stopped when symptoms improve," it further diminishes patient adherence. Interviews with family members revealed that most of those in the high-adherence group took the initiative to learn the medication schedules of the patients, using methods such as mobile phone alarms and sticky notes to remind patients to take their medications. Some family members even accompanied patients to follow-up appointments and promptly communicated with healthcare providers about the patients' responses to the medications. In contrast, family members in the low-adherence group often stated that they were "unaware of the specific medication situations of the patients" and only paid attention to medication issues when the patients' symptoms significantly worsened. This difference in support directly affected the patients' adherence. In clinical practice, we have also encountered special family situations, such as solitary patients lacking reminders from family members, resulting in generally low medication adherence. For such patients, strategies like guiding them to set medication reminders on their mobile phones and collaborating with community health service centers for regular home visits can compensate for the lack of family support and help patients develop regular medication habits. Additionally, this study found that the total treatment effectiveness rate in the high-adherence group (93.02%) was higher than that in the low-adherence group (60.47%) ($P < 0.05$). This result further confirms the positive impact of improving adherence on treatment outcomes. When patients take medications regularly, the drugs can continuously exert their effects, effectively controlling nasal mucosal inflammation and alleviating nasal symptoms. Non-adherence to medications, however, can lead to subtherapeutic drug concentrations in the body, failing to effectively control the condition, and may even increase treatment difficulty due to recurrent exacerbations. In recording the improvement of patients' symptoms, it was found that most patients in the high-adherence group adhered to using intranasal corticosteroids for three months as prescribed, resulting in a significant reduction in the frequency of symptoms such as nasal congestion and runny nose. In contrast, some patients in the low-adherence group discontinued their medications after one month due to symptom relief, leading to symptom recurrence within 2-3 weeks, with more severe symptoms than before. This also indicates that improving patient adherence is not only a means to enhance treatment compliance but also a crucial aspect of improving the treatment outcomes for allergic rhinitis^[5-6].

Based on the above analysis, it can be seen that inadequate disease awareness, adverse drug reactions, and lack of family support are the primary factors affecting medication adherence in patients with allergic rhinitis. In clinical practice, targeted measures can be implemented to address these issues, such as enhancing disease and medication education by using accessible language to help patients fully understand relevant knowledge about the disease and its treatment, thereby eliminating misconceptions; optimizing medication regimens by taking into full consideration individual patient circumstances when selecting drugs, prioritizing those with minimal side effects and ease of use, while proactively informing patients about potential adverse reactions and coping strategies, along with regular follow-up monitoring; emphasizing family support by involving family members in the patient's treatment process to foster a positive therapeutic

environment^[7]. Through these interventions, medication adherence among patients can be effectively improved, leading to enhanced treatment outcomes for allergic rhinitis, reduced disease recurrence, and improved quality of life. In clinical practice, it is also important to tailor intervention methods to the specific needs of each patient. For instance, for elderly patients, more visual demonstrations (such as on-site demonstrations of nasal spray usage) can be employed instead of relying solely on written instructions, while strengthening communication with family members to encourage them to take on more reminders responsibilities. For younger patients, disease education can be delivered through online platforms (such as WeChat official accounts and short videos) to enhance the appeal and acceptability of the information^[9-10]. Only by developing personalized intervention plans based on patient characteristics can we more effectively address issues affecting adherence, truly help patients establish a habit of regular medication use, and achieve long-term effective control of allergic rhinitis.

Disclosure statement

The author declares no conflict of interest.

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Application of Microbial Fermentation Technology in Improving Food Safety and Shelf Life

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Abstract: As a food processing technology with a long history, microbial fermentation has shown its unique advantages in safety control and quality extension in the modern food industry. The fermentation process produces antibacterial substances such as organic acids and bacteriocins through microbial metabolism, which can effectively inhibit the growth and reproduction of pathogenic bacteria and spoilage bacteria. At the same time, the reduction of pH value and the regulation of water activity caused by fermentation have created environmental conditions that are not conducive to the growth of microorganisms for food. In many fields, such as dairy products, meat products, fermented beverages and cereal and vegetable products, fermentation technology has achieved large-scale application, significantly improving the microbial safety and shelf life stability of products. By optimizing strain selection and controlling process parameters, fermentation technology is gradually replacing some chemical preservatives, providing a more natural and healthy preservation solution for the food industry, which has broad application prospects and research value.

Keywords: microbial fermentation; Food safety; Extended shelf life; Antibacterial substances; Organic acids

Online publication: September 26, 2025

1. Introduction

Food safety and shelf-life control have always been the core challenges facing the food industry. With the continuous growth of consumers' demand for natural and healthy food, the application of traditional chemical preservatives has been increasingly questioned and restricted. As a biological preservation method, microbial fermentation technology can not only maintain the nutritional value and sensory quality of food, but also produce a variety of natural antibacterial ingredients through microbial metabolic activities, so as to achieve the dual goals of improving food safety and extending shelf-life. The successful application of fermentation technology in traditional foods such as yogurt, pickles and sausage has proved its effectiveness. In recent years, with the rapid development of Microbiology, food science and biotechnology, the application scope of fermentation technology has been expanding and the process parameters have become increasingly accurate, playing an increasingly important role in the modern food industry, providing an innovative path to solve the problem of food safety and quality^[1].

2. Food safety and shelf life control mechanism of microbial fermentation technology

2.1. Mechanism of action between organic acids produced by fermentation and antibacterial substances

During fermentation, lactic acid, acetic acid and other organic acids produced by microorganisms metabolizing carbohydrates achieve antibacterial effect through dual mechanisms. Undivided organic acid molecules penetrate the microbial cell membrane by means of fat solubility and dissociate in the cell, releasing protons, leading to cytoplasmic acidification, destroying the proton kinetic potential and inhibiting the activity of key enzymes, so as to collapse the energy metabolism system of pathogenic bacteria and spoilage bacteria. Bacteriocin Produced by lactic acid bacteria, as an antimicrobial peptide synthesized by ribosomes, kills Gram-positive bacteria by forming pores on the target cell membrane or interfering with the synthesis of cell wall. The minimum inhibitory concentration of nisin against *Listeria monocytogenes* can reach 25 IU/ml. secondary metabolites such as hydrogen peroxide and diacetyl further strengthen the antibacterial barrier. Hydrogen peroxide oxidizes unsaturated fatty acids in the bacterial cell membrane, while diacetyl combines with arginine to block the amino acid metabolic pathway of microorganisms. This multi-target synergy mechanism enables fermented food to establish a more stable microbial control system than a single preservative, which effectively reduces the risk of tolerant strains^[2].

2.2. pH adjustment and water activity control mechanism

The changes of environmental parameters caused by fermentation constitute the physical and chemical barrier for food preservation. The accumulation of organic acids reduces the pH value of food from the initial 5.5-6.5 to 4.0-4.5. This acidification process significantly reduces the pH adaptation range of microbial growth, and reduces the growth rate constant of *E. coli*, *Staphylococcus aureus* and other moderate preference bacteria by more than 80%. According to Henderson hasselbalch equation, when the environmental pH is less than 1 unit of PKA, the molecular concentration of undivided acid increases by 10 times, and the antibacterial effect increases exponentially. During the fermentation process, the free water content decreases due to the binding water molecules of extracellular polysaccharides synthesized by microorganisms and proteolytic hydrolysates, and the water activity (AW) decreases from 0.98 to 0.92-0.95. The reduction of water activity limits the ability of microorganisms to obtain water for metabolism, prolongs the propagation delay of spoilage bacteria by 3-5 times, and inhibits the deterioration pathways such as lipid oxidation and Maillard reaction. The coordinated regulation of pH and water activity produces a fence effect. Even if a single parameter does not reach the complete antibacterial level, the combined effect of multiple factors can still achieve the goal of long-term preservation (see **Figure 1**).

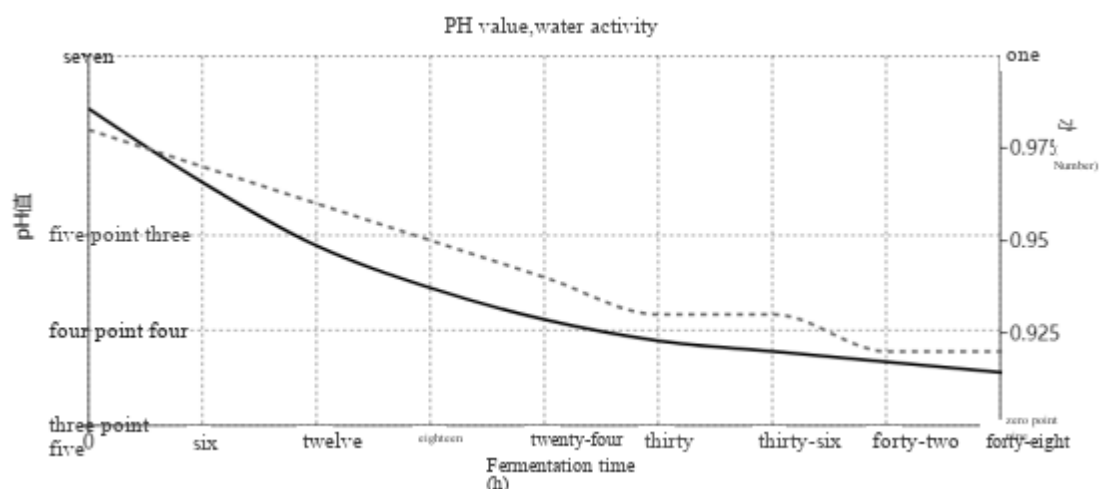


Figure 1. Effect of fermentation time on pH and water activity

Note: the data is based on the model of lactic acid bacteria fermented dairy products, the initial pH is 6.5, and the inoculation volume is 10 μ cfu/ml

2.3. Effect of active metabolites on food quality stability

Fermentation metabolites maintain the long-term stability of food quality by regulating the biochemical reaction kinetics. Protease and peptidase hydrolyze macromolecular proteins into bioactive peptides with molecular weight of 2-5 kDa. These peptides have antioxidant activity, and the thiobarbituric acid value (TBARS) of fermented meat products is maintained below 0.3 mg/kg by scavenging free radicals and chelating metal ions to inhibit lipid peroxidation. Galactose and glucose produced by β - galactosidase decomposing lactose participate in the early stage of Maillard reaction to produce a reducing Amadori compound, which competitively consumes oxygen and interrupts the free radical chain reaction, delaying the fatty acid corruption process. The coenzyme factors such as vitamin B and folic acid synthesized by the fermentation strain maintain the activity balance of the endogenous enzyme system. Glutathione peroxidase continuously removes hydrogen peroxide under the action of coenzyme to prevent its oxidation reaction with unsaturated fatty acids^[3]. The extracellular polysaccharides produced by some lactic acid bacteria form a three-dimensional network structure in the food matrix, which physically blocks the diffusion of oxygen to the interior, reducing the oxygen transmission rate by 40-60%. At the same time, the hydroxyl of polysaccharide molecules capture free radicals, cooperate with chemical and physical mechanisms to stabilize the food texture and flavor compounds, and ensure that the rate of sensory quality deterioration during shelf life is controlled within an acceptable range.

3. Research on application effect of fermentation technology in main food types

3.1. Safety improvement and shelf life extension of dairy fermentation technology

Dairy fermentation builds a microbial ecological barrier through the symbiotic metabolism of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Formic acid and CO₂ produced by *Streptococcus thermophilus* provide growth factors for *Lactobacillus*, while amino acids released by *Lactobacillus* stimulate the proliferation of *Streptococcus*^[4], making the total number of bacteria reach 10⁸ -10⁹ cfu/ml within 6 hours and occupy the niche quickly. The conversion rate of lactose was more than 90% by fermentation, and the pH value of lactic acid was reduced to 4.2-4.4. Under this acidity condition, the survival time of *Escherichia coli* o157: h7 was shortened from 72 hours to 8 hours, and the expression of *Staphylococcus aureus* enterotoxin gene was reduced by more than 95%. The bioactive peptide released by casein enzyme blocked the Fenton reaction by chelating Fe²⁺ and Cu²⁺ ions, which prolonged the induction period of lipid peroxidation to 3.2 times that of unfermented milk. The shelf life of fermented yogurt can reach 21-28 days under the storage condition of 4 °C. During this period, hydrogen peroxide continuously produced by lactic acid bacteria maintains the redox potential at +50 to +100 MV, inhibiting the metabolism of aerobic spoilage bacteria. At the same time, the gel network formed by extracellular polysaccharides reduces the rate of whey precipitation, so that the sensory score remains above 8.5 on the 21st day (see **Table 1**).

Table 1. Influence of fermentation on safety index and shelf life of dairy products

Evaluating indicator	Unfermented fresh milk	Fermented yogurt	Improvement range
pH value	6.6-6.8	4.2-4.4	Reduce by 34%
Lactobacilli count (CFU/mL)	<10 ³	10 ⁸ -10 ⁹	Increase by 10 ⁵ times
E. coli survival time (h)	72	8	89% shorter
Enterotoxin gene expression	Baseline level	Reduce by 95%	Strong suppression
Lipid peroxidation induction period (days)	3.5	11.2	Extended by 3.2 times
4 shelf life days	7-10	21-28	Extended by 2.5 times
Whey yield	-	<15	Texture stable
Sensory score (21 days)	not applicable, inadequacy, inapplicability	8.5/10	fine quality

3.2. Preservation technology and effect evaluation of fermented meat products

Fermented sausages rely on the co metabolism of *Lactobacillus plantarum* and *Pediococcus pentosaceus* to achieve biological preservation. *Lactobacillus plantarum* converts glucose into lactic acid, acetic acid and CO₂ through heterotypic fermentation, with an acid production rate of 0.8-1.2 mmol/(L h), rapidly reducing the pH value of mince to below 5.3, and reducing the spore germination rate of *Clostridium botulinum* from 78% to 12%. Pediocin PA-1 produced by *Pediococcus pentosaceus* blocked the synthesis of peptidoglycan by binding with lipid II. The bactericidal rate constant of *Listeria monocytogenes* reached 0.15 h^{-1} , and the colony number decreased by 4 logarithmic orders within 24 hours. During the fermentation process, the endogenous protease hydrolyzed myosin to small molecular peptides, and the content of amino nitrogen increased from 0.3 g/kg to 1.8 g/kg. The sulfhydryl group of free amino acids captured lipid free radicals, reducing the production rate of malondialdehyde by 62%. Under the conditions of fermentation temperature of 22-24 °C and relative humidity of 85-90%, the water activity was stable in the range of 0.88-0.90 after 14 days, which inhibited the germination of *Aspergillus* and *Penicillium* spores, and the shelf life was extended to more than 90 days. During this period, the volatile basic nitrogen value remained below 15 mg/100g, which met the national standard limit requirements.

3.3. Microbial control and quality maintenance technology of fermented beverages

Fermented beverages achieve microbial stability and flavor balance through the sequential fermentation of yeast and lactic acid bacteria. Yeast converts sucrose into ethanol and CO₂ in the pre fermentation stage (0-48h). When the ethanol concentration is accumulated to 0.5-2.5% (V/V), the minimum inhibitory concentration against Gram-negative bacteria is 1.2%. At the same time, CO₂ dissolves to form carbonic acid, which reduces the pH value to 3.8-4.2. This double barrier blocks the growth of *Escherichia coli* and *Salmonella*. In the post fermentation stage (48-96 h), lactic acid bacteria used residual monosaccharides to produce lactic acid, the acidity increased from 8 mmol/L to 25-30 mmol/L, the specific growth rate of spoilage bacteria decreased from 0.18 h^{-1} to 0.02 h^{-1} , and the lag period extended to more than 72 hours. The total amount of volatile components such as ethyl acetate and isoamyl alcohol produced by yeast metabolism reached 180-220 mg/L. The hydrolysis rate constant of these flavor compounds in the low pH environment decreased by 40%, and the aroma intensity remained above 85% of the initial value after 60 days of storage. The shelf life of fermented beverages can reach 6 months at room temperature (25 °C), during which the total acidity fluctuation is less than $\pm 5\%$, and the yeast and mold counts are always lower than the detection limit of 10 CFU/ml.

3.4. Application technology optimization of fermented grain and vegetable products

The fermentation of cereal and vegetable broke through the quality fluctuation of traditional natural fermentation through the targeted screening of substrate specific strains. The combination of *Lactobacillus acidophilus* and *Lactobacillus brevis* coupled amylase hydrolysis and lactic acid fermentation. The α -amylase activity reached 120-150 U/g, and the conversion rate of reducing sugar produced by hydrolysis exceeded 85%, which increased the yield of organic acids to 55-70 mmol/L, and the pH value was stable in the range of 3.6-4.0. The nitrate reductase system of *Lactobacillus plantarum* transformed nitrate in vegetables and further reduced it to nitrogen. The nitrite residue decreased from 180 mg/kg to less than 5 mg/kg, eliminating nitrosamine carcinogenic precursors. Ames test verified that the mutation was negative. The cellulase and pectinase released by fermentation degraded cell wall polysaccharides, which increased the soluble dietary fiber from 2.3% to 5.8%, and the bioavailability by 1.5 times. At the same time, the carotenoids and polyphenols released increased the antioxidant capacity to 8500 $\mu\text{mol TE}/100\text{g}$. Under the conditions of $10\text{ }\mu\text{CFU/g}$ inoculum and 30-32 °C fermentation temperature, the shelf life of fermented cereal and vegetable products can reach 90 days at 4 °C, the pH fluctuation is less than 0.2 units, and the retention rate of texture hardness is 78%, which provides a technical path for the development of clean label products (see **Table 2**).

Table 2. Changes in functional components of fermented grains and vegetable products

Surveillance Project	Unfermented	Fermented	Change Rate
Starch Hydrolysis Rate (%)	-	85	-
Nitrate (mg/kg)	180	5	-97%
Soluble Dietary Fiber (%)	2.3	5.8	+152%
ORAC Value (μmol TE/100g)	3500	8500	+143%
Shelf Life (days)	15-20	90	+350%

4. Process parameters and effect evaluation of microbial fermentation technology application

4.1. Application characteristics and selection strategies of different fermentation strains

The functional characteristics of fermentation strains determine their suitability in specific food substrates. *Lactobacillus plantarum* has become the preferred strain for vegetable fermentation due to its extensive substrate utilization ability and strong acid tolerance. The diameter of the inhibitory zone of its plantaricin bacteriocin against *Listeria monocytogenes* is 18-22 mm. The homotypic fermentation characteristics of *Pediococcus pentosaceus* make the lactic acid yield reach the theoretical conversion rate of 95%, which is suitable for meat products requiring rapid acidification. *Lactobacillus delbrueckii* subsp. *bulgaricus* protein hydrolase activity is up to 350 U/g, which is the key strain for the improvement of the texture of dairy products. The ethanol fermentation efficiency of *Saccharomyces cerevisiae* is close to the theoretical value, but it is sensitive to so a. So a tolerant *Kluyveromyces* should be selected for low sulfur drinks. It is necessary to. The selection of strains needs to comprehensively evaluate the acid production rate, antibacterial spectrum, metabolic by-products and environmental tolerance. The synergistic effect can be achieved by optimizing the proportion of multiple strains by response surface methodology^[5]. For example, the compound of *Lactobacillus plantarum* and *Pediococcus pentosaceus* at 3:1 can increase the pH decline rate of fermented sausage by 40%, and the bacteriocin production increases to 2.3 times that of a single strain.

4.2. Effect of fermentation process conditions on safety and shelf life

The fermentation temperature directly regulated the product safety by affecting the microbial growth kinetics. The specific growth rate of lactic acid bacteria reached the peak value of 0.42 h^{-1} at 37°C , but the risk of miscellaneous bacteria competition increased. Although the rate of lactic acid bacteria decreased to 0.28 h^{-1} at 30°C , the growth of *Escherichia coli* was completely blocked. The amount of seed was linearly correlated with the rate of pH decline, and the time for pH to reach 4.5 was shortened from 12 hours to 4 hours when it was increased from 10^6 to 10^8 CFU/g, and the time for pathogenic bacteria to be exposed to the appropriate environment was reduced by 67%. The salt concentration adjusts the water activity through osmotic pressure. Every 1% increase in salinity within the range of 2-4% will reduce the a_w value by 0.015 unit. At the same time, chloride ion inhibits the respiratory chain of Gram-negative bacteria, and the ATP synthesis efficiency will decrease by 55%. The fermentation time needs to balance safety and sensory. When the pH is lower than 3.8 due to excessive fermentation, the sour and astringent taste will burst out, which will reduce consumer acceptance by 40%. The optimal end point should be set in the range of pH 4.0-4.3.

4.3. Comparative analysis of fermentation technology and traditional anti-corrosion methods

The fermentation technology has significant advantages in the continuity of microbial control and consumer acceptance. The inhibition rate of potassium sorbate at 1000 ppm on yeast reached 99%, but the residue decreased to 62% after 30 days, and the inhibition rate decreased to 85%. The fermentation organic acid system maintained a steady state due to the

continuous metabolism of microorganisms, and the inhibition effect was stable within 90 days. Sodium benzoate is easy to induce tolerant strains only through a single target inhibition. It has been found that 20% of *Escherichia coli* is resistant to 500 ppm, while the barrier effect of multiple barriers of fermentation technology reduces the escape probability to 1/15 of chemical corrosion prevention. The direct cost of fermentation process is 0.8-1.2 yuan/kg, which is higher than 0.3-0.5 yuan/kg of chemical preservatives, but the market premium is 30-50%, 68% of consumers accept the price increase of more than 15%. The added value of fermented products such as bioactive peptides and B vitamins significantly affects the competitiveness, and the high-end market share increases from 12% in 2015 to 37% in 2024, which is in line with the development trend of clean labels and functional foods.

5. Epilogue

With its natural, efficient and multifunctional characteristics, microbial fermentation technology has become an important means to improve food safety and extend the shelf life. The active substances such as organic acids and bacteriocins produced in the fermentation process build a multiple microbial barrier, which effectively reduces the risk of foodborne diseases. The successful application in dairy products, meat products, beverages and vegetable foods shows that the collaborative improvement of food safety and sensory quality can be achieved by scientifically selecting fermentation strains and accurately controlling process parameters. Future research should focus on the development of new functional strains, in-depth analysis of fermentation mechanism, intelligent optimization of process parameters and collaborative application of fermentation technology and other preservation technologies, so as to further expand the application scope of fermentation technology.

Disclosure statement

The author declares no conflict of interest.

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