

THEORY, RESEARCH AND DISCUSSIONS IN HEALTH SCIENCES

Editor

Assoc. Prof. Dr. Neslihan YILMAZ ÇIRAKOĞLU



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Chapter 1

Evidence-Based Approaches to Reducing and Managing Abnormal Repetitive Behaviors in Horses

Fatih YILDIRIM¹

Abstract

Abnormal repetitive behaviors in horses-such as crib-biting, weaving, and box-walking-are multifactorial behaviors arising from the interaction of genetic predisposition, early-life experiences, and current management practices, resulting from a mismatch between modern housing conditions and natural behavioral needs. This chapter addresses the classification, etiology, and risk factors associated with these major abnormal behaviors and presents a comparative overview of current, evidence-based methods for their reduction and management, including environmental enrichment, nutritional management, behavioral and training-based approaches, mechanical devices, and pharmacological or surgical interventions. Furthermore, it emphasizes that the complete "elimination" of an established stereotypy is not possible in most cases; instead, the current approach focuses on addressing the underlying environmental deficiencies and improving animal welfare.

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1. Introduction

Horses are social and active animals that have retained much of their natural behavioral repertoire throughout the domestication process, yet are unable to fully express this repertoire under modern housing and management conditions. While horses in the wild spend a significant portion of the day grazing, moving, and engaging in social interactions, those in stable environments are subjected to limited space for movement, restricted social contact, and intermittent, high-energy rations. This mismatch paves the way for the emergence of a range of behaviors referred to in the literature as "abnormal repetitive behaviors" (ARB) or, traditionally, as "stable vices" (Wickens and Heleski, 2010).

The aim of this chapter is to comprehensively address the classification of major abnormal behaviors observed in horses, the underlying causal mechanisms, and current, evidence-based methods for reducing, controlling, or managing these behaviors.

1.1. Classification of Abnormal Behaviors

Abnormal behaviors observed in horses are examined in the literature under two main categories: oral stereotypies and locomotor stereotypies. These behaviors are characterized by being repetitive, invariant, and seemingly functionless, and they are almost never observed in natural, free-living populations (Mason, 1991).

1.1.1. Oral Stereotypies: Crib-biting and Wind Sucking

Crib-biting is defined as a behavior in which the horse grasps a fixed surface (such as a manger edge, stall partition, or fence) with its incisors, arches and tenses its neck, and swallows air while producing a characteristic grunting sound. When the same behavior is exhibited without contact with an object, it is termed "wind sucking" and is considered a variant of the same behavioral phenotype. Although prevalence varies by population, it is reported to range from approximately 2.1% to 10.5% in general horse populations, with rates reaching up to 15% under certain management conditions (Wickens and Heleski, 2010).

1.1.2. Locomotor Stereotypies: Weaving and Box-walking

Weaving is defined as the rhythmic swaying of the head, neck, and sometimes the body from side to side while the horse keeps its forelimbs stationary. Box-walking (or stall-walking) involves the horse moving around inside the stable along a repetitive path in a circular or fixed pattern. Both behaviors are observed more frequently, particularly in small paddock areas and under conditions of restricted exercise (Cooper et al., 2000).

1.2. Etiology and Risk Factors

The development of abnormal behaviors cannot be attributed to a single cause; it is a multifactorial process resulting from the interaction of genetic predisposition, early-life experiences, and current management conditions. The higher prevalence of crib-biting in certain bloodlines suggests the existence of a hereditary component; however, this predisposition typically manifests in conjunction with environmental risk factors. The age and method of weaning emerge as critical risk factors; abrupt and early weaning, combined with a transition to rations characterized by a high concentrate-to-roughage ratio, significantly increases the risk of crib-biting (Waters et al., 2002). Individual housing during the foal stage has also been linked to the development of crib-biting and box-walking; the incidence of stereotypic behavior in individually housed foals was found to be significantly higher compared to those housed in pairs (McGreevy et al., 1995a).

Higher cortisol and endorphin levels have been reported in horses exhibiting crib-biting behavior compared to control groups, suggesting that this behavior may serve as a coping mechanism involving both stress and addiction-like processes. Current research indicates that a neural phenotype associated with basal ganglia dopamine physiology underlies stereotypic behaviors across various species (McBride and Hemmings, 2005).

1.3. Prevalence and Epidemiology

The incidence of abnormal behavior varies significantly by race, discipline and management system. In a large-scale study conducted on British Thoroughbred horses, the prevalence of stereotypic behavior in horses competing in dressage, eventing and endurance disciplines was reported as 32.5%, 30.8% and 19.5%, respectively (McGreevy et al., 1995b). In a study conducted in the Italian Thoroughbred population, the incidence of stereotypic behavior was reported as 7.4%, and suggesting a genetic component to the predisposition to stereotypic behaviour. (Vecchiotti and Galanti, 1986). In a population study conducted in Finland, a higher than expected prevalence of crypto sucking was detected in certain Finnhorse lines, and this was evaluated as supporting a hereditary component of behavioral predisposition (Hemmann et al., 2014a).

The prevalence of crypto sucking at the general population level varies between 2.8-15% depending on race; It is reported that Thoroughbred and warmblood breeds carry a higher risk compared to other breeds (Wickens and Heleski, 2010). These differences may reflect the combined effect of both genetic predisposition and intense training/feeding protocols specific to these breeds.

1.4. Genetic and Neuroendocrine Foundations

The first systematic evidence regarding the heritability of crib-biting was presented by Vecchiotti and Galanti (1986) in Italian Thoroughbred horses the more frequent occurrence of the behavior in specific lines within the breed suggested a genetic component. In a more recent study conducted in Finnhorses, the estimated heritability of crib-biting was 0.68, indicating a substantial genetic contribution to variation in the trait within that population (Hemmann et al., 2014a). Candidate gene studies have examined genes encoding ghrelin, the ghrelin receptor, leptin, the dopamine receptor, the μ -opioid receptor, N-cadherin, the serotonin receptor, and semaphorin; however, none of these candidates showed a statistically significant association with the crib-biting phenotype, suggesting that the behavior may have a complex nature shaped by polygenic and environmental interactions (Hemmann et al., 2014b).

At the neuroendocrine level, it has been shown that mesoaccumbens and nigrostriatal dopamine physiology is altered in horses exhibiting crib-biting, and that dopaminergic transmission within the basal ganglia circuitry is impaired (McBride and Hemmings, 2005). It has been suggested that dopamine receptor sensitivity in the caudate nucleus is reduced in these horses (a hypodopaminergic profile) and that this condition may represent a form of adaptation to chronic stress (Parker et al., 2008). Furthermore, it has been reported that plasma leptin levels are lower and gastrin levels are higher in crib-biting horses; However, no significant difference in gastric anatomy was detected compared to the control group (Hemmann et al., 2013). It has been shown that circadian fluctuations in ghrelin and stress hormones (ACTH, cortisol) following feeding follow a different pattern in crib-biting horses compared to control horses (Hemmann et al., 2012).

2. Behavioral, Psychological, and Health Consequences

Abnormal behaviors should be evaluated not merely as aesthetic or management issues, but also in terms of their clinical consequences directly affecting animal health and welfare. Crib-biting has been associated with gastric ulcers (Nicol et al. 2002), an increased risk of colic (Curtis et al., 2019), and dental wear (Grenager et al., 2010; Curtis et al., 2019); furthermore, an epidemiological link to neurological conditions such as temporohyoid osteoarthropathy (Grenager et al., 2010) has been reported. From a psychological perspective, it has been demonstrated that horses exhibiting crib-biting learn certain tasks faster than the control group, yet the extinction of a learned behavior takes longer in these horses (Roberts et al., 2015); this finding indicates the

particular importance of applying training protocols based on errorless learning principles for individuals exhibiting oral stereotypies.

2.1. Ethological Foundations of Stable Design

Stable design plays a decisive role in the prevention and management of abnormal behaviors. The presence of openings allowing horses to see one another (barred partitions, open-top partitions) reduces the risk of stereotypy compared to complete isolation. It has been experimentally demonstrated that even placing an image of a horse's head with pricked ears in front of a horse known to exhibit weaving behavior reduces the frequency of weaving and head-tossing; This finding suggests that visual social stimuli alone can provide a certain degree of protective effect (Cooper et al., 2000). In stable design, the provision of opportunities for social contact, access to turnout areas, and the placement of roughage delivery systems (such as slow feeders or hay nets) should be planned using an approach based on ethological principles (Rochais et al., 2018).

2.2. Methods for Reducing Abnormal Behaviors

The methods discussed below are classified according to their level of evidence and degree of acceptance in clinical practice. It is important to note that the complete "elimination" of an established (chronic) stereotypic behavior may not be possible in most cases; therefore, current literature prefers the concepts of "reducing frequency and severity" and "welfare-based management addressing the functional origin of the behavior" over "elimination."

2.2.1. Environmental Enrichment and Housing Arrangements

Increasing turnout time (12 hours or more per day) reduces the likelihood of developing stereotypic behaviors by providing horses with opportunities for natural grazing and movement. Facilitating social contact with compatible herd mates lowers the prevalence of crib-biting by reducing stress associated with isolation (Benhajali et al., 2009). In group-housed horses, the use of outdoor enrichment elements (structural, sensory, and nutritional enrichment) has been associated with a marked reduction in weaving and box/fence walking behaviors, according to owner reports. Indoor enrichment objects (hanging toys, salt licks) can somewhat reduce the frequency of crib-biting, but this effect is generally partial and temporary; enrichment alone should not be considered a universal solution for an established stereotype.

2.2.2. Nutrition Management-Based Interventions

Increasing the proportion of roughage and the frequency of meals plays a central role in preventing oral stereotypes and reducing their severity. It has been shown that providing concentrated feed in small, hourly portions reduces the frequency of crib-biting compared to the traditional practice of feeding twice daily (McCall et al., 2009). The use of devices such as slow-feeders and hay nets extends feeding duration, resulting in a pattern that more closely resembles natural grazing behavior (Rochais et al., 2018). Implementing a weaning protocol that involves a gradual transition emphasizing roughage is recommended as a management strategy to reduce the risk of stereotypic behavior development in foals (Nicol, 1999).

2.2.3. Behavioral and Training-Based Interventions

Approaches based on learning and reinforcement principles rely on reinforcing alternative, functional behaviors rather than directly suppressing stereotypic behaviors. It has been shown that horses exhibiting crib-biting learn certain tasks faster than control group horses, yet the extinction of a learned behavior takes longer in these horses (Roberts et al., 2015). The motivational strength of cribbing has also been demonstrated experimentally, with horses showing a high demand for opportunities to perform the behavior (Houpt, 2012). It has been reported that following short-term mechanical prevention, the behavior returns to its previous frequency—or even a higher one—supporting the view that the behavior is not merely a simple habit but possesses a strong motivational component (McGreevy and Nicol, 1998a, 1998b). Punitive approaches are reported to be ineffective because they do not address the functional origins of the behavior.

2.2.4. Mechanical and Physical Restraint Devices

Devices such as cribbing collars and muzzles aim to prevent the behavior by physically restricting neck muscle contractions or mouth and lip movements. Although these devices may suppress the behavior, they do not eliminate the underlying motivation; once the device is removed, the behavior typically returns at the same—or sometimes a higher—frequency. Furthermore, even a well-fitted collar or muzzle can cause pressure sores around the head and lead to sensitivity in the poll area. Although mechanical devices may suppress the expression of stereotypic behaviour, they do not necessarily address its underlying motivation and may entail welfare risks if improperly fitted or used for prolonged periods.

2.2.5. Pharmacological Interventions

Experimental studies have shown that opioid antagonists (e.g., naloxone) transiently reduce crib-biting behavior, a finding that supports the hypothesis that the behavior may be linked to the endogenous opioid system (Dodman et al., 1987). The effect of the NMDA receptor blocker dextromethorphan on crib-biting frequency has also been experimentally investigated; however, the clinical use of such agents remains limited due to their short duration of action, the requirement for parenteral administration, and cost. It has been suggested that a pharmacological approach, when combined with environmental or behavioral interventions, could yield a faster and more sustained therapeutic response; nevertheless, the number of controlled clinical studies specifically involving horses in this area remains limited.

Surgical interventions: In severe and treatment-resistant cases, the surgical removal of specific muscles in the lower neck (myectomy) and associated nerves (neurectomy) has been described. These procedures aim to prevent the physical execution of the behavior; however, due to their invasive nature, the risk of complications (such as wound healing issues, cosmetic alterations, and the possibility of recurrence), and the fact that they do not address the underlying motivational component of the behavior, they should be considered only as a last resort after all other management and behavioral options have been exhausted (Turner and White, 1984).

2.2.6. Comparative Evaluation of Methods

The table below summarizes the main approaches to managing abnormal behaviors in horses in terms of level of evidence, durability of effects, and risks to welfare.

Method	Level of Evidence	Durability of Effect	Welfare Risk
Turnout / increased social contact	High	Potentially sustained with continued management	Low
Roughage / change in feeding frequency	High	Potentially sustained with continued management	Low
Behavioral/training-based intervention	Moderate	Variable; requires continued management	Low
Mechanical restraint (collar, muzzle)	Low-Moderate	Temporary/device-dependent	Moderate-High
Pharmacological intervention	Low (limited clinical data)	Usually temporary	Moderate
Surgical intervention (myectomy)	Low (last resort)	Potentially long-lasting, but invasive	High

2.3. Welfare and Ethical Dimension

Current behavioral science literature emphasizes that stereotypic behaviors may serve as a coping mechanism developed by the horse in response to an adverse environment; consequently, it is suggested that forcibly suppressing the behavior could increase, rather than decrease, the animal's stress levels. In this context, current recommendations focus on addressing the underlying environmental and physiological deficiencies and minimizing the behavior's adverse health consequences, rather than simply "eliminating" the behavior itself.

Surveys of horse owners have reported that crib-biting horses possess a less anxious temperament compared to non-crib-biting horses and show no difference in terms of trainability (Nagy et al., 2010); this finding supports the view that the behavior may function as a self-directed stress management strategy for the horse. A comprehensive survey comparing the management practices of breeders in Europe, North America, and Australia revealed that early weaning age, a low roughage-to-concentrate ratio, and limited social contact were the factors most strongly associated with the development of abnormal behaviors, and that addressing these three factors should be a priority in breeding programs (Parker et al., 2008).

2.4. Practical Management Protocol

In clinical practice, a stepwise approach is recommended for managing a horse exhibiting abnormal behavior. The first step involves conducting a comprehensive management assessment—covering turnout time, level of social contact, roughage-to-concentrate ratio, meal frequency, and stable design—while a veterinarian rules out underlying medical causes such as gastric ulcers, pain, or neurological disorders. In the second step, turnout duration should be gradually increased, compatible companions provided, barn design modified to allow for visual and tactile contact, and feeding frequency raised by increasing the roughage ratio. In the third step, nutritional enrichment tools—such as slow feeders or hay nets—should be introduced, environmental enrichment elements added to the stall, and behavioral progress monitored regularly (e.g., via a weekly behavior log). In the fourth step, if the behavior remains severe or poses a serious health risk despite management interventions, the use of mechanical restraint devices (temporarily and accompanied by welfare monitoring) and pharmacological support (under veterinary supervision) may be considered appropriate. In the fifth and final step, when all previous measures have been exhausted and the behavior seriously threatens the animal's health, surgical options should be considered as a last resort, with the animal's overall welfare as the primary concern.

At every stage of this stepped protocol, objectively monitoring the effectiveness of the intervention using a standardized behavioral observation form (e.g., recording daily behavior frequency or the number of stereotypic episodes per hour) will facilitate both the clinical decision-making process and data collection for future research.

3. Conclusion

Abnormal behaviors seen in horses are behaviors that arise from a complex interaction of genetic predisposition, early life experiences and current management conditions, rather than being a simple "bad habit" due to a single cause, often reflecting the animal's effort to adapt to an adverse environment. Current scientific evidence supports a holistic approach based on addressing the environmental deficiencies underlying the behavior, improving well-being, and minimizing the negative health consequences of the behavior, rather than forced "elimination" of the behavior. Preventive management strategies such as environmental enrichment, social contact, forage-based feeding, and appropriate weaning are generally preferred because they address underlying management-related risk factors while minimizing welfare risks associated with restrictive interventions.

Declaration of use of AI

AI-assisted tools were used during manuscript preparation for language editing, readability enhancement, and proofreading. The author reviewed and approved the final manuscript and assumes full responsibility for its content.

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Chapter 2

Global Meat Production and Food Security: Production Trends, Trade Rebalancing, and Policy Implications for 2025-2034

Fatih YILDIRIM¹

Abstract

Livestock-derived foods underpin three of the four internationally recognised dimensions of food security -availability, access, and stability- yet the medium-term trajectory of global meat supply and its environmental implications remain insufficiently integrated into the food security literature. This study provides an integrated, food-security-oriented empirical analysis of global meat production, trade, productivity and price dynamics for 2025-2034, and tests whether regional livestock emission intensities have historically converged. We used a secondary-data framework drawn from the OECD-FAO Agricultural Outlook 2025-2034, the FAO Meat Market Review 2025, FAOSTAT, and the World Integrated Trade Solution database, assessing availability, access, stability, and environmental sustainability trends across four meat categories (poultry, pork, beef and sheep meat). A supplementary regression tested historical (1990-2010) convergence in regional livestock emission intensity.

Global meat production reached 381.5 million tonnes (Mt) in 2025 (+2.5%), led by poultry (+3.7% to 156 Mt), and is projected to reach 406 Mt by 2034 (+13%), with Asia contributing 55% of growth. Greenhouse gas emissions are projected to grow by only 6% despite 13% production growth, a decoupling that varies sharply by region and income group. China's share of global meat imports is projected to fall from 20% to 16%. The historical regression found no automatic convergence in emission intensity ($R^2 = 0.825$, $p = 0.005$, $n = 7$): Sub-Saharan African regions improved far less than Asian regions despite much higher starting points.

Productivity gains and emissions growth can decouple in the meat sector, but this decoupling and the associated trade rebalancing are highly uneven across regions and income groups, and historical evidence argues against automatic technological catch-up by lower-income regions.

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We propose a productivity-sustainability nexus framework and recommend differentiated climate targets, targeted technology transfer to Sub-Saharan Africa, expanded biosecurity investment, trade-policy coherence, and food-security-aware dietary guidance.

Keywords: food security, meat production, livestock trade, greenhouse gas emissions, trade policy, biosecurity, food policy.

1. Introduction

The global meat sector is one of the most strategically significant components of the global food system, particularly in regions where livestock contributes to farm income, dietary quality, and trade exposure (FAO, 2026a; Ederer et al., 2023). It accounts for approximately 40% of global agricultural production value, employs around 1.3 billion people, and underpins food security, rural livelihoods, and international agricultural trade (FAO, 2026a; Ederer et al., 2023). Traditional livestock systems support the livelihoods of 70% of the world's rural poor (FAO, 2026a). Yet the sector simultaneously faces mounting pressure from climate policy, disease outbreaks, trade protectionism, and shifting dietary norms.

Meat production bears directly on at least three of the four internationally recognised dimensions of food security. It is a core determinant of physical availability of animal-source protein at the global and regional levels; it shapes economic access, since real producer and consumer prices govern affordability for low-income households; and, through its dependence on cross-border trade, it is exposed to stability risks arising from disease shocks, climate regulation, and shifting bilateral trade relationships. These dimensions are analytically inseparable: a change in production composition (availability) propagates through trade structure (stability) into price dynamics (access), with distributional consequences that differ sharply by country income group. Treating meat production, trade, and price trends in isolation, as much of the existing literature does, therefore risks understating the food security stakes of the developments described below.

The 2025-2034 period is particularly consequential for both global meat markets and African import-dependent economies. Several structural forces are converging: post-pandemic supply chain normalisation; China's partial retreat from global meat import markets following African Swine Fever (ASF) recovery; rising biosecurity risks from Highly Pathogenic Avian Influenza (HPAI) and Foot-and-Mouth Disease (FMD); and increasing stringency of climate-related agricultural regulations in the European Union. These forces are reshaping who produces meat, where it is traded, and at what environmental cost.

Despite the scale of these developments, an integrated food security synthesis of current evidence on the global meat system remains lacking. Existing analyses tend to focus narrowly on individual commodities, single regions, or isolated policy levers, and rarely connect production and trade trends explicitly back to the availability, access, and stability dimensions of food security, limiting the ability of policymakers to form a coherent picture of system-level trade-offs. This study addresses that gap by offering a comprehensive food-security-oriented analysis across four analytical dimensions, aligning production, trade, prices, and

emissions with food policy concerns (FAO, 2026a; OECD/FAO, 2025; WITS, 2026): (1) current production and livestock population trends, as a determinant of availability; (2) international trade structures and bilateral imbalances, as a determinant of access and stability; (3) medium-term projections (2025-2034) for production, productivity, and prices, bearing on the future trajectory of all three dimensions; and (4) GHG emission trajectories relative to production growth, bearing on the long-run sustainability of availability.

The analysis makes three principal contributions to the food security literature, especially by connecting trade rebalancing and sustainability with food access in low- and middle-income countries (Ederer et al., 2023; Van der Laan et al., 2024). First, it links the availability dimension of food security directly to trade structure by providing an updated empirical synthesis of the 2025 global meat production landscape and its 2034 projections, integrating commodity-level data with country-level trade patterns. Second, it shows that the environmental sustainability of future food availability is more nuanced than commonly portrayed, with productivity-driven decoupling of GHG emissions from production growth varying significantly by income group and region- a finding relevant to the design of climate policies that do not compromise food availability. Third, and most directly relevant to the access and stability dimensions of food security, it identifies a structural mismatch between growing nutritional demand in low- and middle-income countries and persistent affordability constraints despite declining real meat prices.

2. Materials and Methods

2.1. Data Sources

This study employs an integrated secondary data framework combining four international sources, each of which is widely used in agricultural policy analysis and market surveillance (OECD/FAO, 2025; FAO, 2026a; OECD, 2026; WITS, 2026). The OECD-FAO Agricultural Outlook 2025-2034 (OECD/FAO, 2025) provides internationally harmonised projections for agricultural commodity markets using a 2022-2024 base period. The FAO Meat Market Review 2025 (FAO, 2026b) supplies current-year (2025) production and trade estimates across all major meat categories. Livestock population data were sourced from FAOSTAT (FAO, 2026a) covering 2000-2024. Bilateral trade statistics for 2023 were drawn from the World Integrated Trade Solution (WITS, 2026) database of the World Bank. Per capita consumption data were sourced from OECD (2026) harmonised statistics. GHG emission projections are expressed in gigatonnes of CO₂ equivalent (Gt CO₂e) using IPCC AR6 global warming potentials (IPCC, 2022).

2.2. Analytical Approach

The analysis proceeds in four sequential steps, following a commodity-trade-projection framework commonly used in food system and agricultural outlook studies (OECD/FAO, 2025; USDA FAS, 2026). Step 1 characterises the 2025 production landscape and compares it against FAOSTAT livestock population trends over 2000-2024. Step 2 analyses WITS bilateral trade data to identify key surplus and deficit economies. Step 3 presents OECD-FAO medium-term projections for production, productivity, and prices, disaggregated by species and income group. Step 4 integrates GHG emission projections with production growth trajectories to assess emission intensity dynamics. All production and trade data are reported in carcass weight equivalent (cwe) unless otherwise noted. Country income classifications follow World Bank analytical groupings; regional groupings follow FAO/OECD standard definitions.

2.3. Ethics Statement

This study relied exclusively on secondary, publicly available aggregate datasets (OECD/FAO, 2025; FAO, 2026a, 2026b; WITS, 2026; OECD, 2026) and the published regional estimates of Otte et al. (2019). No animals or human participants were used, sampled, or contacted in the conduct of this research, and no primary data were collected. Ethical approval was therefore not required for this study.

3. Results

3.1. Global Meat Production in 2025

Total global meat production in 2025 reached 381.5 Mt, representing a 2.5% increase over 2024 (Table 1). Three structural drivers underpinned this recovery: increased slaughter rates linked to herd liquidation in major producing countries; lower feed input costs relative to 2024; and persistently strong consumer demand for animal protein across middle-income markets. Poultry achieved the highest growth rate at 3.7% (+5.6 Mt), reaching 156.1 Mt and cementing its position as the largest single meat category by volume. Pork production grew 2.7% to 128.8 Mt, driven partly by China's post-ASF herd rebuilding (Ceruti et al., 2025). Beef grew marginally (+0.7% to 77.7 Mt), whilst sheep meat recorded a slight decline (−0.5% to 18.9 Mt).

Table 1. Global Meat Production, 2024-2025 (million tonnes, carcass weight equivalent)

Meat Category	2024 (Mt)	2025 Estimate (Mt)	Annual Change (%)
Poultry	150.5	156.1	+3.7
Pork	125.4	128.8	+2.7
Beef & Veal	77.2	77.7	+0.7
Sheep & Goat Meat	19.0	18.9	-0.5
Total	372.1	381.5	+2.5

Note. cwe = carcass weight equivalent; Mt = million tonnes. Source: FAO (2026b); OECD/FAO (2025). Totals may not sum due to rounding.

3.1.1. Livestock Population Dynamics (2000-2024)

The 2025 production trends are embedded in longer-run livestock population dynamics (Table 2). Between 2000 and 2024, the global chicken population nearly doubled (+96.0%), from 14.1 billion to 27.7 billion birds. Goat populations grew 57.6%, reflecting their comparative advantage in marginal agroecological zones. Buffalo (+29.0%), sheep (+28.1%), and cattle (+19.6%) grew more modestly. Pig populations, affected by successive ASF waves in Asia from 2018, grew by only 7.1% over the 24-year period.

Table 2. Changes in Global Livestock Populations, 2000-2024 (million head)

Species	2000	2024	Change (%)
Cattle	1,319.7	1,578.6	+19.6
Buffalo	164.3	212.0	+29.0
Sheep	1,063.8	1,362.6	+28.1
Goats	753.5	1,187.5	+57.6
Pigs	898.7	962.4	+7.1
Chickens	14,120.5	27,680.7	+96.0
Ducks	919.7	1,274.8	+38.6

Note. Source: FAOSTAT (FAO, 2026a). All figures in million head.

3.2. International Meat Trade: Structure and Bilateral Patterns

Global meat trade expanded 3.4% in 2025 to reach 41.9 Mt, recovering from two years of decline (FAO, 2026b). Growth was led by beef (+7.0% to 13.9 Mt), followed by pork (+2.3% to 10.0 Mt) and poultry (+2.0% to 16.7 Mt). Sheep and goat meat trade contracted marginally (-0.8% to 1.3 Mt) (Table 3).

Table 3. Global Meat Trade Volume by Category, 2025

Meat Category	2025 Trade Volume (Mt)	Annual Change (%)
Poultry	16.7	+2.0
Beef & Veal	13.9	+7.0
Pork	10.0	+2.3
Sheep & Goat Meat	1.3	-0.8
Total	41.9	+3.4

Note. Mt = million tonnes. Source: FAO (2026b).

Analysis of 2023 bilateral trade data (Table 4) reveals significant structural asymmetries amongst major economies. China is the world's largest livestock product importer at USD 53.4 billion, yet exports remain limited at USD 14.0 billion—a net import position of USD 39.4 billion. However, China became a net chicken meat exporter in 2024, reflecting post-ASF supply recovery (USDA FAS, 2026). The United States, despite being a major exporter (USD 35.5 billion), is simultaneously a net importer, with imports of USD 42.5 billion and a net position of -USD 7.0 billion. Brazil maintains the most favourable export surplus (USD 20.9 billion net), consistent with its status as the world's leading beef exporter.

Table 4. Livestock Product Trade Values by Country, 2023 (billion USD)

Country	Exports (bn USD)	Imports (bn USD)	Net Position (bn USD)
United States	35.5	42.5	-7.0
China	14.0	53.4	-39.4
Germany	25.7	27.7	-2.0
Netherlands	32.2	17.2	+15.0
Brazil	24.1	3.2	+20.9
France	17.2	20.1	-2.9
Japan	2.2	22.4	-20.2
Italy	9.8	21.2	-11.4
Turkey	3.4	2.1	+1.3
World Total	214.4	226.5	n/a

Note. Net position = exports minus imports; negative values indicate net import positions. Trade values are reported in current USD; exports are valued on an FOB basis and imports on a CIF basis, as reported in WITS. Source: WITS (2026).

3.3. Medium-Term Projections, 2025-2034

3.3.1. Production and Species Composition

Global meat production is projected to reach 406 Mt by 2034, a 13% increase over the 2022-2024 base period (Table 5). Asia will account for 55% of total growth, with China contributing approximately 10% and India approximately 8%, followed by the United States (8%) and Vietnam (7%). At the species level, poultry will account for 69% of the projected production increase-growing from 156.1 Mt to 173 Mt-increasing its share of total meat production from 40.9% to 42.6%. Pork will remain largely stable at 130 Mt, whilst sheep meat is projected to remain stable, edging up from 18.9 Mt to 19 Mt.

Table 5. Projected Global Meat Production by Species, 2025 vs. 2034

Species	2025 (Mt)	Share 2025 (%)	2034 Proj. (Mt)	Share 2034 (%)	Growth Contrib. (%)
Poultry	156.1	40.9	173	42.6	69
Pork	128.8	33.8	130	32.0	5
Beef & Veal	77.7	20.4	84	20.7	26
Sheep & Goat	18.9	5.0	19	4.7	0
Total	381.5	100.0	406	100.0	100

*Note. Mt = million tonnes carcass weight equivalent. Base period: 2022-2024.
Source: OECD/FAO (2025), adapted by the authors.*

3.3.2. Productivity Gains by Country Income Group

The projections identify significant productivity improvements over 2025-2034 (Table 6). Upper-middle-income countries are projected to achieve a 24% improvement in beef carcass weights, whilst lower-middle-income countries are projected at +13%. Notably, on the metric of output per animal across all meat types, lower-middle-income countries are projected to achieve a larger relative improvement (+17%) than upper-middle-income countries (+10%), suggesting that the rate of catch-up in production efficiency may be accelerating-a finding with significant implications for investment targeting in agricultural development policy (Rauw et al., 2025; Ojo et al., 2024).

Table 6. Projected Productivity Improvements by Country Income Group, 2034 vs. Base Period

Indicator	Upper-Middle Income (%)	Lower-Middle Income (%)
Beef carcass weight improvement	+24	+13
Output per animal, all meat types	+10	+17

Note. Source: OECD/FAO (2025). Percentage changes relative to 2022-2024 base period average.

3.3.3. Price Trajectories

In the short term (2025-2027), ruminant meat prices (beef, sheep) are projected to rise due to supply constraints associated with herd rebuilding cycles. Non-ruminant prices (pork, poultry) are projected to decline, driven by production expansion and reduced Chinese import demand. Over the medium term (2027-2034), real prices across all categories are projected to fall: ruminant real prices by approximately 8% below the base period average, and non-ruminant real prices by approximately 20%. Whilst favourable for consumers in low-income countries where meat affordability constrains dietary quality (Ederer et al., 2023), these trajectories create significant margin pressure for producers in high-cost environments.

3.4. GHG Emissions and the Productivity-Emissions Nexus

Despite 13% projected production growth, GHG emissions from the global meat sector are projected to increase by only 6%, rising from 3.4 to 3.5 Gt CO₂e over the projection period (OECD/FAO, 2025). This partial decoupling is driven by three mechanisms: a compositional shift toward poultry (the least emission-intensive category, generating approximately 4.08 kg CO₂e per kg of product, compared with approximately 27 kg for beef); productivity improvements that reduce GHG per unit of output; and national-level low-carbon livestock initiatives in major producing countries (Goswami et al., 2026). However, the distribution of emission changes is highly unequal across regions (Table 7). Africa shows the largest projected relative increase (+18%), driven by population growth, low baseline productivity, and limited access to improved technologies. Europe is the only region projected to achieve an absolute emissions reduction (−7%), attributable to regulatory pressure and precision livestock management. Capper (2023) demonstrates that disease control alone-reducing FMD prevalence in beef cattle from 45% to 5%-could reduce GHG emission intensities by 9.1%, underscoring the climate co-benefits of biosecurity investment.

Table 7. Projected Changes in Meat-Related GHG Emissions by Region, Base Period vs. 2034

Region	Projected GHG Change (%)
Africa	+18
Asia	+8
Latin America	+4
North America	+1
Europe	−7

Note. GHG emissions measured in Gt CO₂e using IPCC AR6 global warming potentials. Base period: 2022–2024. Source: OECD/FAO (2025).

At the country income level, emission intensity (GHG per kg of protein produced) is declining across all groups except low-income countries. Annual improvements are: high-income countries −0.6%; upper-middle-income −0.3%; lower-middle-income −1.6%-suggesting that the fastest emission intensity improvements are occurring in rapidly developing livestock sectors where productivity catch-up is most pronounced.

3.5. Historical Evidence on Regional Emission-Intensity Convergence, 1990–2010

To test whether the productivity-driven decoupling projected for 2025–2034 has a historical precedent, and whether regions with the highest current emission intensities can be expected to converge toward lower-intensity trajectories without targeted intervention, we examined historical regional data on livestock GHG emission intensity (kg CO₂-eq per kg of animal protein) for seven Asian and Sub-Saharan African sub-regions over 1990–2010, compiled from FAOSTAT-based estimates reported by Otte et al. (2019) (Table 8).

Table 8. Regional Livestock GHG Emission Intensity, 1990 and 2010 (kg CO₂-eq per kg animal protein)

Region	1990	2010	Change (%)
South Asia	129	75	−41.9
East Asia	52	31	−40.4
Southeast Asia	110	57	−48.2
Western Sub-Saharan Africa	244	219	−10.2
Eastern Sub-Saharan Africa	281	230	−18.1
Central Sub-Saharan Africa	290	255	−12.1
Southern Sub-Saharan Africa	103	62	−39.8

Note. Source: authors' calculations based on Otte, Pica-Ciamarra and Morzaria (2019), derived from FAOSTAT livestock production, protein content, and emissions data.

A simple ordinary least squares regression of the percentage change in emission intensity (1990–2010) on the 1990 baseline level shows a strong positive relationship ($\beta = 0.150$, $R^2 = 0.825$, $p = 0.005$, $n = 7$): sub-regions with higher initial emission intensity experienced systematically smaller relative improvements. The three Asian sub-regions, starting from comparatively low baselines (52–129 kg CO₂-eq/kg protein), achieved reductions of 40–48% over the two decades, whereas the four Sub-Saharan African sub-regions, starting from far higher baselines (244–290 kg CO₂-eq/kg protein), achieved reductions of only 10–18%.

This pattern is the opposite of the automatic convergence that a simple catch-up hypothesis would predict, and it bears directly on the productivity-sustainability nexus proposed in this study. It suggests that Asia's historical intensity gains reflect targeted investment in feed quality, breed improvement and animal health over the period, rather than a generic process that Sub-Saharan African systems can be expected to replicate spontaneously. Combined with the 2025–2034 projections in Table 7, which show Africa facing the largest absolute increase in emissions (+18%), this historical evidence reinforces the case for differentiated, deliberately targeted technology transfer to Sub-Saharan Africa (Policy Implication b) rather than reliance on the market-driven diffusion process observed in Asia.

Given the small number of sub-regional observations ($n = 7$), this exercise is intended as an illustrative empirical test of the convergence hypothesis rather than a definitive causal estimate; unobserved factors such as initial livestock species composition, agroecological constraints, and differential rates of GDP growth likely account for part of the observed relationship.

4. Discussion

The results reported above bear directly on all three food security dimensions identified in the Introduction. The production and productivity findings (Sections 3.1 and 3.3.1–3.3.2) speak to availability; the trade and price findings (Sections 3.2 and 3.3.3) speak to access and stability; and the emissions findings (Section 3.4) speak to the long-run environmental sustainability of that availability. The following subsections interpret these results jointly, rather than as isolated market phenomena, and draw out their implications for food security policy.

4.1. The Productivity-Sustainability Nexus: Challenging the Trade-off Framing

The prevailing framing in much of the academic and policy literature presents sustainability and productivity in the livestock sector as fundamentally in tension

(Van der Laan et al., 2024; Rauw et al., 2025). Our findings challenge this empirically. The projected decoupling of production growth (+13%) from GHG emissions growth (+6%) over 2025-2034 demonstrates that productivity-driven intensification-particularly the compositional shift toward poultry and improved output per animal-can materially reduce the emissions intensity of meat production, even in the absence of radical technological change. Rauw et al. (2025) provide the mechanistic basis for this finding, showing that selection for feed efficiency in livestock species operates through energy-sparing and metabolic flexibility mechanisms that do not inherently require trade-offs with productivity. Ojo et al. (2024) extend this to beef cattle, demonstrating that genomic tools can identify animals with both higher carcass weight and better feed efficiency.

This has a critical implication for food policy, because affordability and micronutrient adequacy remain central constraints in many low- and middle-income settings (Ederer et al., 2023; Stadnik, 2024). Climate policy instruments targeting absolute emissions reductions in the livestock sector-via production limits, carbon taxes, or mandatory dietary shifts-risk undermining food security in precisely the regions where nutritional demand is growing fastest. The nutritional case for moderate consumption of animal-source protein, including its superior bioavailability for critical micronutrients including vitamin B12, haem iron, and zinc, is well established (Stadnik, 2024). Policy instruments should therefore be differentiated: emissions intensity targets, rather than absolute caps, may be more appropriate in low- and middle-income country contexts.

4.2. China's Structural Transition and Global Trade Rebalancing

The projected decline in China's share of global meat imports from 20% to 16% by 2034 represents one of the most significant structural shifts in global food trade of the coming decade, with implications for exporters and import-dependent economies alike (USDA FAS, 2026; WITS, 2026). This shift reflects three concurrent dynamics: post-ASF domestic pork supply recovery (Ceruti et al., 2025); active government investment in domestic meat production capacity; and China's transition to a net exporter in chicken meat as of 2024 (USDA FAS, 2026). For exporting countries-particularly Brazil, the United States, and EU member states-this rebalancing requires strategic reorientation toward alternative import markets in the Near East, Southeast Asia, and Sub-Saharan Africa. The EU's projected export share decline from 19% to 13% compounds this challenge: compliance costs associated with the Farm to Fork strategy are simultaneously reducing EU production competitiveness. As China's import demand historically anchored price stability in global meat markets, its partial withdrawal may

increase price volatility in beef and pork markets, with asymmetric consequences for import-dependent low-income countries (Ederer et al., 2023).

4.3. Biosecurity as a Structural Food Policy Challenge

Animal diseases represent the most acute and least predictable risk to the production and trade trajectories projected in this study, and the market effects of ASF and related shocks are well documented in prior research (Ceruti et al., 2025; Niemi, 2020). Ceruti et al. (2025) document the full scope of ASF's One Health implications, whilst Niemi (2020) quantifies the market consequences in European contexts, showing that ASF-related production disruptions reduced pig meat exports by approximately 15% in affected countries. The re-emergence of FMD in EU member states in 2025, for the first time since 2011, highlights the fragility of disease-free status as a trade asset. Capper (2023) demonstrates that controlling AIB (avian infectious bronchitis) in poultry from 75% to 20% prevalence would reduce emission intensities by 11.3%, indicating that biosecurity investment carries direct environmental co-benefits. Biosecurity should therefore be positioned not as a purely veterinary concern but as a core food system policy priority with returns spanning food security, trade stability, and climate objectives.

4.4. Relevance for Southern African Economies

The trade and price dynamics documented above have particular relevance for Southern African economies, several of which combine substantial poultry import dependence with recurring policy debates over anti-dumping tariffs and regional trade protection (FAO, 2026b; WITS, 2026). As China and other major exporters reallocate surplus production in response to shifting import demand, Southern African markets are among those exposed to the resulting price and volume swings, with direct consequences for the affordability of animal protein for lower-income consumers in the region. The productivity-sustainability nexus proposed here is also directly applicable to Southern African livestock systems, where productivity gains could similarly help reconcile rising domestic demand with emissions and resource constraints. We see this as a priority area for future country- and region-level research building on the global patterns identified in this study.

4.5. Policy Implications

Our findings generate five interconnected policy implications for international food system governance, especially for regions balancing food access, producer viability, and climate goals (OECD/FAO, 2025; FAO, 2026b).

a. Differentiated climate targets for the livestock sector. The heterogeneous regional and income-group distribution of GHG emission trajectories makes a strong empirical case against uniform global emissions caps for livestock production. Differentiated emission intensity targets-with wealthier regions pursuing absolute reductions and lower-income regions pursuing intensity improvements-should be calibrated to ensure that climate objectives do not undermine food security in nutritionally vulnerable populations.

b. Technology transfer as a food policy priority. The productivity gap between income groups (Table 6) represents both a food security risk and an environmental opportunity, supporting greater investment in genetics, feed efficiency, and livestock management (Rauw et al., 2025; Ojo et al., 2024). Bridging this gap through targeted technology transfer-precision livestock management tools, improved genetics, optimised nutrition-would simultaneously reduce emissions intensity, improve farm incomes, and expand food availability in supply-constrained regions.

c. Biosecurity governance as a global public good. International disease surveillance and response capacity generates returns across food security, trade stability, and climate dimensions. The current fragmentation of global biosecurity governance represents a structural gap in the international food policy architecture that warrants a dedicated financing mechanism.

d. Trade policy coherence to protect import-dependent economies. As China's structural transition reshapes global meat trade flows, World Trade Organization (WTO) agricultural negotiating frameworks should be reinvigorated to ensure that trade flow reorientation does not systematically disadvantage food-insecure countries in Sub-Saharan Africa, South Asia, and the Near East (WITS, 2026; USDA FAS, 2026).

e. Evidence-based dietary guidance reflecting nutritional realities. The documented role of animal-source proteins in providing critical micronutrients with superior bioavailability (Stadnik, 2024) must be integrated into dietary guidance in low- and middle-income countries, alongside affordability considerations (Ederer et al., 2023). Policies promoting wholesale substitution with plant-based alternatives, without accounting for dietary context and micronutrient adequacy, risk creating nutritional harms alongside any environmental benefits.

5. Conclusions

This study has provided a comprehensive food-security-oriented analysis of global meat production, trade, and consumption dynamics for the 2025-2034 period, yielding five principal findings relevant to the availability, access, and

stability dimensions of food security: global meat production (availability) reached 381.5 Mt in 2025, with poultry driving growth and projected to account for 42.6% of total production by 2034; Asia will account for 55% of projected production growth, fundamentally reshaping the geography of global meat availability; GHG emissions are projected to increase by 6%, less than half the 13% projected increase in production; however, this relative decoupling is highly uneven across regions, with Africa experiencing the largest absolute increases and, consequently, facing the greatest long-term challenges to sustainable availability; China's structural transition from dominant meat importer to increasingly export-active economy will reshape global trade flows (stability), with asymmetric access risks for import-dependent low-income countries; and animal diseases-ASF, HPAI, FMD-remain the most acute systemic risks to all three dimensions simultaneously, with biosecurity investment yielding co-benefits across food security, trade stability, and climate objectives.

Together, these findings challenge the conventional sustainability-productivity trade-off framing and support a productivity-sustainability nexus framework as the more appropriate guide for food security policy internationally. They also demonstrate that meat production, trade, and price trends cannot be adequately assessed as a matter of market economics alone: each of the developments documented here operates directly on one or more pillars of food security, and policy responses that ignore this interconnection risk unintended consequences for the food security of the most vulnerable populations. Future research should incorporate household-level dietary transition data from middle-income countries, model alternative China demand scenarios, and assess the feasibility of differentiated emission intensity targets under existing international climate frameworks.

Data availability statement

This study uses only third-party, publicly available secondary data; no new primary data were collected. The data supporting this study are openly available from the OECD-FAO Agricultural Outlook 2025-2034 at <https://doi.org/10.1787/601276cd-en>, from FAOSTAT and the FAO Meat Market Review at <http://www.fao.org/faostat>, from the World Bank World Integrated Trade Solution at <https://wits.worldbank.org/>, and from OECD Statistics at <https://stats.oecd.org>.

Ethics statement

This study relied exclusively on secondary, publicly available aggregate datasets; no animals or human participants were used, and no primary data were collected. Ethical approval was therefore not required.

Declaration of use of AI

During the preparation of this manuscript, the author used Claude (Anthropic) to assist with organising and drafting sections of the text, formatting references and tables, and checking internal consistency between in-text citations and the reference list. The author reviewed and edited all AI-assisted content, and takes full responsibility for the accuracy, originality, and conclusions of this work.

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Chapter 3

Surgical Approaches to Temporomandibular Joint Disorders

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Introduction

Temporomandibular joint disorders (TMDs) comprise a heterogeneous group of musculoskeletal and functional conditions affecting the temporomandibular joint (TMJ), masticatory muscles, and associated structures [1]. TMDs represent one of the most common causes of chronic orofacial pain and functional limitation, significantly affecting mastication, speech, quality of life, and psychosocial well-being.

The etiology of TMJ disorders is multifactorial and may involve biomechanical dysfunction, trauma, internal derangement, degenerative joint disease, inflammatory arthropathies, parafunctional habits, psychosocial stress, and systemic conditions [2]. Because of this complex pathophysiology, management strategies frequently require multidisciplinary evaluation and individualized treatment planning.

Most patients with TMJ disorders respond favorably to conservative treatment modalities including patient education, occlusal appliances, physiotherapy, pharmacological therapy, behavioral modification, and minimally invasive interventions [3]. However, a subset of patients develops persistent pain, joint locking, progressive degeneration, or severe functional impairment requiring surgical management.

Surgical treatment of TMJ disorders has evolved substantially over the last decades. Historically, open arthrotomy procedures represented the primary surgical approach for severe TMJ pathology [4]. Advances in minimally invasive surgery, endoscopic techniques, imaging, and regenerative medicine have significantly expanded treatment options and improved outcomes.

Arthrocentesis and arthroscopy have become increasingly important minimally invasive procedures for management of internal derangements, inflammatory conditions, and closed-lock disorders [5]. These techniques aim to

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reduce inflammation, eliminate adhesions, improve joint mobility, and decrease pain with lower morbidity compared with open surgery.

Open surgical procedures remain necessary in selected cases involving ankylosis, severe degenerative joint disease, neoplasms, developmental deformities, traumatic injuries, and failed minimally invasive treatments [6].

Contemporary TMJ surgery additionally incorporates regenerative strategies including platelet concentrates, stem-cell-based therapies, viscosupplementation, tissue engineering, and biologic joint preservation approaches [7].

Despite advances in surgical management, controversies remain regarding indications, timing of intervention, optimal surgical techniques, and long-term outcomes. Careful patient selection and accurate diagnosis therefore remain essential [8].

This chapter reviews the indications, surgical anatomy, diagnostic evaluation, arthrocentesis, arthroscopy, open surgical procedures, total joint replacement, regenerative therapies, complications, and future directions in surgical management of temporomandibular joint disorders.

Anatomy and Functional Considerations of the TMJ

The temporomandibular joint is one of the most complex synovial joints in the human body because of its bilateral function, fibrocartilaginous surfaces, and combined rotational and translational movements [9].

The joint consists of the mandibular condyle, glenoid fossa, articular eminence, fibrocartilaginous articular disc, synovial membrane, capsule, and supporting ligaments [10].

The articular disc divides the joint into superior and inferior compartments. The inferior compartment primarily permits rotational movement, whereas translational movement occurs mainly within the superior compartment [11].

The posterior attachment tissues, commonly termed the retrodiscal tissue, contain vascular and neural structures and may become important pain sources in internal derangement disorders [12].

Muscles of mastication, particularly the lateral pterygoid muscle, additionally influence joint biomechanics and disc-condyle coordination [13].

Normal TMJ function depends on harmonious interaction among osseous structures, articular disc position, muscular activity, occlusion, and neuromuscular control [14].

Disruption of these relationships may result in internal derangement, inflammation, degenerative change, pain, and functional limitation [15].

Understanding TMJ anatomy is critically important for safe surgical access because of the close proximity of facial nerve branches, superficial temporal

vessels, auriculotemporal nerve, external auditory canal, and cranial base structures [16].

Classification of Temporomandibular Joint Disorders

Temporomandibular disorders encompass multiple pathological entities with varying clinical presentations and treatment requirements [17].

The Research Diagnostic Criteria for Temporomandibular Disorders and the more recent Diagnostic Criteria for TMD provide widely accepted classification systems integrating clinical and psychosocial assessment [18].

Muscle-related disorders include myalgia, myofascial pain, and muscle spasm. These conditions are generally managed conservatively and rarely require surgery [19].

Internal derangement refers to abnormal positional relationships between the articular disc and mandibular condyle. Disc displacement with reduction typically presents with joint clicking, whereas disc displacement without reduction may cause closed lock and restricted mouth opening [20].

Degenerative joint disease includes osteoarthritis and osteoarthrosis characterized by cartilage degeneration, osseous remodeling, crepitus, pain, and reduced function [21].

Inflammatory conditions such as rheumatoid arthritis, juvenile idiopathic arthritis, psoriatic arthritis, and ankylosing spondylitis may additionally involve the TMJ [22].

Fibrous or bony ankylosis represents severe joint fusion causing significant functional limitation and facial deformity [23].

Traumatic injuries, condylar fractures, developmental abnormalities, neoplasms, and hyperplastic disorders may also require surgical intervention [24].

Accurate diagnosis and classification are essential for selecting appropriate surgical or nonsurgical management strategies.

Diagnostic Evaluation and Imaging

Comprehensive evaluation of TMJ disorders requires detailed history taking, physical examination, and appropriate imaging studies [25].

Clinical assessment should include evaluation of pain characteristics, joint sounds, mandibular range of motion, occlusion, muscle tenderness, locking episodes, parafunctional habits, and functional limitation [26].

Maximum mouth opening, lateral excursions, protrusive movement, and deviation patterns provide important diagnostic information [27].

Magnetic resonance imaging remains the gold standard for evaluation of soft tissue structures and disc position [28]. MRI allows visualization of disc displacement, joint effusion, inflammatory changes, retrodiscal tissue pathology, and marrow abnormalities.

Cone-beam computed tomography and conventional CT are particularly useful for osseous evaluation including condylar erosion, osteophytes, fractures, ankylosis, and degenerative remodeling [29].

Ultrasonography has also been investigated as a low-cost imaging modality for evaluation of joint effusion and superficial disc position, although its diagnostic accuracy remains limited compared with MRI [30].

Diagnostic arthroscopy may occasionally provide direct visualization of intra-articular pathology and facilitate simultaneous therapeutic intervention [31].

Imaging findings must always be correlated with clinical symptoms because structural abnormalities may occasionally exist in asymptomatic individuals [32].

Conservative Management Before Surgery

Most TMJ disorders are initially treated conservatively because many patients improve without surgical intervention [33].

Patient education and behavioral modification are fundamental components of treatment. Avoidance of parafunctional habits, soft diet, stress reduction, and jaw rest may reduce joint loading and muscular tension [34].

Occlusal splints are frequently used to decrease parafunctional activity and improve neuromuscular balance [35].

Pharmacological management may include nonsteroidal anti-inflammatory drugs, muscle relaxants, corticosteroids, antidepressants, and intra-articular medications [36].

Physical therapy modalities such as jaw exercises, ultrasound therapy, manual therapy, posture correction, and transcutaneous electrical nerve stimulation may improve function and reduce pain [37].

Minimally invasive procedures including intra-articular corticosteroid injection, hyaluronic acid injection, prolotherapy, platelet-rich plasma, and botulinum toxin have also gained popularity [38].

Surgical intervention is generally reserved for patients with persistent pain, functional limitation, progressive degeneration, ankylosis, or failed conservative management [39].

Arthrocentesis

Arthrocentesis is one of the least invasive surgical procedures used in TMJ management and is commonly indicated in patients with internal derangement and closed-lock disorders [40].

The technique involves lavage of the superior joint space using needles inserted into the upper compartment under local anesthesia or sedation [41].

Arthrocentesis aims to remove inflammatory mediators, eliminate adhesions, improve lubrication, and reduce negative intra-articular pressure [42].

The procedure is relatively simple, cost-effective, and associated with low morbidity compared with more invasive surgical approaches [43].

Several studies have demonstrated significant improvement in pain, mouth opening, and joint function following arthrocentesis [44].

Various adjunctive agents may additionally be injected following lavage, including hyaluronic acid, corticosteroids, platelet-rich plasma, and injectable platelet-rich fibrin [45].

Complications are generally uncommon but may include transient facial nerve weakness, hematoma, infection, extravasation, or perforation of adjacent structures [46].

Arthrocentesis is particularly valuable as an early minimally invasive intervention before consideration of arthroscopy or open surgery [47].

Arthroscopy

TMJ arthroscopy represents a minimally invasive endoscopic surgical technique that allows direct visualization and treatment of intra-articular pathology [48]. Since its introduction into maxillofacial surgery, arthroscopy has become one of the most important surgical modalities for management of internal derangement and inflammatory TMJ disorders.

The procedure is typically performed under general anesthesia using small-diameter endoscopes inserted into the superior joint compartment [49]. Arthroscopic systems permit both diagnostic evaluation and therapeutic intervention.

Diagnostic arthroscopy allows direct assessment of synovitis, adhesions, cartilage degeneration, disc mobility, vascularity, and intra-articular fibrosis [50]. Visualization often reveals pathological changes not fully appreciated on imaging studies alone.

Therapeutic arthroscopic procedures may include lysis of adhesions, lavage, synovectomy, disc mobilization, electrocoagulation, debridement, and intra-articular medication delivery [51].

Compared with arthrocentesis, arthroscopy offers improved visualization and greater therapeutic capability while maintaining relatively low surgical morbidity [52].

Several studies have demonstrated favorable outcomes in patients with closed lock, painful clicking, synovitis, and degenerative joint disease [53]. Improvements in pain, mandibular mobility, and joint function are commonly reported.

Advanced arthroscopic techniques additionally allow disc repositioning and suturing procedures in selected patients [54].

Complications are uncommon but may include facial nerve injury, bleeding, perforation of the external auditory canal, infection, intracranial penetration, or instrument breakage [55].

Appropriate surgical training and familiarity with TMJ anatomy are essential because of the small operative field and proximity to critical structures [56].

Arthroscopy currently occupies an important intermediate position between arthrocentesis and open surgical procedures in the management algorithm of TMJ disorders.

Open Surgical Procedures

Open TMJ surgery remains necessary for selected patients with severe structural pathology, failed minimally invasive treatment, ankylosis, neoplasms, trauma, or advanced degenerative disease [57].

Common open surgical approaches include preauricular, endaural, postauricular, and modified temporal incisions [58]. Surgical access must balance adequate visualization with protection of facial nerve branches and cosmetic outcomes.

Disc Repositioning Surgery

Disc repositioning procedures aim to restore functional relationships between the articular disc and condyle in patients with symptomatic internal derangement [59].

Techniques may involve disc mobilization, plication, suturing, anchoring systems, or retrodiscal tissue modification [60].

Successful outcomes depend largely on disc condition, duration of displacement, and degree of degenerative change [61].

Discectomy

Discectomy involves partial or complete removal of the articular disc and is generally indicated in severely deformed, perforated, or nonfunctional discs [62].

Interpositional grafts such as temporalis fascia, auricular cartilage, dermis, or alloplastic materials may be used to reduce postoperative fibrosis and degenerative change [63].

Although discectomy may improve pain and function, concerns remain regarding long-term degenerative remodeling following disc removal [64].

Eminectomy

Eminectomy involves removal or reduction of the articular eminence and is primarily indicated in recurrent TMJ dislocation or hypermobility disorders [65].

Reduction of the mechanical obstruction allows freer condylar translation and decreases dislocation risk [66].

Condylotomy

Condylar procedures may be indicated in severe degenerative disease, trauma, ankylosis, condylar hyperplasia, or neoplastic conditions [67].

High condylectomy may additionally be performed in selected patients with severe osteoarthritis or condylar deformity [68].

Open surgical procedures generally provide greater structural correction than minimally invasive techniques but are associated with higher morbidity and longer recovery periods [69].

Ankylosis Surgery

TMJ ankylosis represents one of the most challenging TMJ disorders and may result from trauma, infection, inflammatory disease, or previous surgery [70].

Fibrous or bony fusion between the mandibular condyle and cranial base may severely restrict mandibular movement and produce facial asymmetry, malocclusion, and airway compromise [71].

Children with ankylosis are particularly vulnerable because mandibular growth disturbance may lead to severe craniofacial deformity [72].

Surgical treatment generally involves gap arthroplasty, interpositional arthroplasty, reconstruction, or total joint replacement [73].

Gap arthroplasty involves resection of ankylotic bone to create separation between the mandibular ramus and cranial base [74].

Interpositional arthroplasty additionally places biological or alloplastic material between resected surfaces to reduce recurrence risk [75].

Temporalis muscle and fascia flaps are among the most commonly used autogenous interpositional materials because of proximity and vascularity [76].

Postoperative physiotherapy is critically important because recurrence frequently results from inadequate mobilization and fibrosis [77].

Early diagnosis and aggressive rehabilitation significantly improve long-term outcomes.

Total Temporomandibular Joint Replacement

Total TMJ replacement has become increasingly important in management of end-stage TMJ disease, severe ankylosis, failed previous surgeries, advanced degenerative disease, and extensive joint destruction [78].

Both stock prostheses and patient-specific custom prosthetic systems are currently available [79].

Modern TMJ prostheses generally consist of titanium mandibular components and ultra-high-molecular-weight polyethylene fossa components [80].

Computer-assisted planning and CAD/CAM technologies have significantly improved prosthesis design, surgical precision, and anatomical adaptation [81].

Indications for total joint replacement include severe inflammatory arthropathy, recurrent ankylosis, congenital deformity, neoplasms, traumatic destruction, and failed autogenous reconstruction [82].

Several studies have demonstrated substantial improvements in pain, mandibular function, diet, and quality of life following total TMJ replacement [83].

Potential complications include infection, prosthetic loosening, heterotopic bone formation, facial nerve injury, material wear, and limited long-term prosthesis lifespan [84].

Despite these concerns, total joint replacement remains one of the most predictable options for restoration of function in severe TMJ pathology [85].

Regenerative and Biologic Therapies

Regenerative medicine has become an increasingly important field in TMJ management because conventional surgical approaches may not fully restore native joint structure and function [86].

Platelet-rich plasma and platelet-rich fibrin have been investigated extensively because of their anti-inflammatory and regenerative properties [87].

Intra-articular PRP injections may reduce pain and improve joint mobility through modulation of inflammation and stimulation of tissue repair [88].

Hyaluronic acid viscosupplementation is another widely used biologic treatment aimed at improving joint lubrication and reducing friction [89].

Stem-cell-based therapies represent one of the most promising future directions. Mesenchymal stem cells may potentially contribute to cartilage regeneration and modulation of inflammatory pathways [90].

Tissue engineering approaches involving biomimetic scaffolds, hydrogels, growth factors, and bioactive materials are additionally under investigation [91].

Three-dimensional bioprinting and customized scaffold fabrication may eventually permit biologically functional joint reconstruction [92].

Although regenerative therapies demonstrate promising preliminary results, standardized protocols and long-term clinical evidence remain limited [93].

Complications of TMJ Surgery

Although most TMJ surgical procedures are successful when appropriately indicated, complications may occur and require careful management [94].

Facial nerve injury is among the most significant complications because of the close anatomical relationship between surgical approaches and temporal or zygomatic branches [95].

Transient facial weakness is relatively common following open surgery, whereas permanent deficits are less frequent [96].

Other complications include hematoma, infection, salivary fistula, auriculotemporal nerve injury, hearing changes, malocclusion, fibrosis, heterotopic bone formation, and recurrence of ankylosis [97].

Intracranial perforation is a rare but potentially catastrophic complication associated particularly with arthroscopy or ankylosis surgery [98].

Persistent pain and limited functional improvement may additionally occur despite technically successful surgery [99].

Careful patient selection, surgical expertise, and postoperative rehabilitation are therefore essential for optimizing outcomes and minimizing complications [100].

Postoperative Rehabilitation and Physiotherapy

Postoperative rehabilitation represents one of the most critical determinants of long-term success following TMJ surgery. Regardless of the surgical technique performed, inadequate physiotherapy may result in fibrosis, recurrent hypomobility, pain, and functional deterioration [101].

Early mobilization is particularly important following arthroplasty, ankylosis surgery, and total joint replacement procedures [102]. Prolonged immobilization increases the risk of scar formation and restricted mandibular movement.

Physiotherapy protocols generally include passive stretching exercises, active range-of-motion exercises, jaw-opening devices, muscular relaxation techniques, and posture correction [103].

TheraBite systems, wooden tongue depressors, and customized physiotherapy appliances are frequently used to improve mandibular opening gradually [104].

Patient compliance is critically important because long-term rehabilitation often requires daily exercises for several months [105].

Pain management also influences rehabilitation success. Adequate postoperative analgesia facilitates early mobilization and improves patient cooperation [106].

Multidisciplinary rehabilitation involving oral surgeons, physiotherapists, pain specialists, psychologists, and prosthodontists may significantly improve long-term outcomes in complex TMJ disorders [107].

Outcomes and Prognostic Factors

Surgical outcomes in TMJ disorders vary according to diagnosis, disease severity, patient characteristics, surgical technique, and postoperative rehabilitation [108].

Arthrocentesis and arthroscopy generally demonstrate favorable outcomes in patients with internal derangement, closed lock, and inflammatory joint disease [109]. Improvements in pain and mandibular mobility are commonly reported.

Open surgical procedures may provide substantial benefit in selected patients with structural pathology; however, outcomes are more variable in chronic pain-dominant conditions [110].

Ankylosis surgery outcomes depend largely on early diagnosis, adequate resection, interpositional material selection, and aggressive postoperative physiotherapy [111].

Total joint replacement generally demonstrates high patient satisfaction and predictable functional improvement in severe end-stage disease [112].

Several prognostic factors influence treatment success. Younger patients, shorter disease duration, lower psychosocial burden, and better preoperative function are generally associated with more favorable outcomes [113].

Psychological factors such as anxiety, depression, catastrophizing behavior, and chronic pain sensitization may significantly affect postoperative pain perception and satisfaction [114].

Careful patient selection and realistic preoperative counseling therefore remain essential components of successful TMJ surgery.

Future Perspectives

The future of TMJ surgery is expected to involve increasingly personalized, minimally invasive, and regenerative approaches integrating advanced imaging, digital planning, biologic therapies, and tissue engineering [115].

Artificial intelligence-assisted imaging analysis may improve diagnostic accuracy and facilitate earlier identification of progressive joint pathology [116].

Three-dimensional virtual surgical planning and CAD/CAM technologies are likely to become increasingly integrated into TMJ reconstruction and prosthetic joint replacement workflows [117].

Customized patient-specific prostheses generated through additive manufacturing may further improve anatomical adaptation and functional outcomes [118].

Regenerative medicine represents one of the most promising future directions. Stem-cell-based therapies, scaffold engineering, biologic matrices, and molecular signaling modulation may eventually permit true biologic regeneration of cartilage and joint tissues [119].

Minimally invasive endoscopic procedures are also expected to continue evolving with improved instrumentation and visualization systems [120].

Robotic-assisted surgery and augmented reality-guided procedures may additionally improve surgical precision and reduce operative morbidity [121].

Molecular research investigating inflammatory pathways, genetic predisposition, and pain modulation mechanisms may contribute to more targeted therapeutic approaches [122].

Long-term multicenter studies and standardized treatment protocols remain critically important for improving evidence-based decision-making in TMJ surgery [123].

Conclusion

Temporomandibular joint disorders represent complex multifactorial conditions that may significantly impair function, quality of life, and psychosocial well-being. Although most patients respond favorably to conservative treatment, selected cases require surgical intervention because of persistent pain, functional limitation, structural degeneration, or ankylosis.

Surgical management of TMJ disorders has evolved substantially from traditional open arthrotomy procedures toward increasingly minimally invasive and biologically oriented approaches. Arthrocentesis and arthroscopy now occupy central roles in management of internal derangement and inflammatory joint disorders because of their favorable outcomes and relatively low morbidity.

Open surgical procedures including disc repositioning, discectomy, eminectomy, ankylosis surgery, and total joint replacement remain essential for severe structural pathology and advanced joint destruction.

Advances in imaging, digital planning, CAD/CAM technologies, and customized prosthetic systems have significantly improved surgical precision and predictability. Regenerative medicine and biologic therapies further expand the potential for minimally invasive and tissue-preserving treatment strategies.

Successful TMJ surgery requires accurate diagnosis, careful patient selection, meticulous surgical technique, and comprehensive postoperative rehabilitation. Psychological and functional factors must additionally be considered because chronic pain and psychosocial influences frequently contribute to symptom severity.

Future developments involving artificial intelligence, tissue engineering, stem-cell-based regeneration, robotic surgery, and personalized digital workflows are expected to further transform TMJ management.

Overall, contemporary TMJ surgery continues to move toward more precise, minimally invasive, regenerative, and patient-specific therapeutic approaches aimed at improving both functional and quality-of-life outcomes.

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Bone Regeneration Strategies in Maxillofacial Defects

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Introduction

Reconstruction of maxillofacial bone defects remains one of the most challenging areas in oral and maxillofacial surgery. Craniofacial bone loss may result from trauma, congenital deformities, tumor resection, infection, cystic lesions, periodontal disease, medication-related osteonecrosis, or severe alveolar atrophy [1].

Successful regeneration of maxillofacial bone is essential not only for restoration of facial aesthetics but also for recovery of mastication, speech, airway support, implant rehabilitation, and overall quality of life [2].

Traditional reconstructive approaches relied primarily on autogenous bone grafting and vascularized free flaps. Although these techniques remain highly effective, donor site morbidity, limited graft availability, unpredictable resorption, prolonged surgical time, and increased patient morbidity have stimulated development of alternative regenerative strategies [3].

Bone regeneration in maxillofacial surgery has therefore evolved toward biologically driven approaches integrating tissue engineering, biomaterials, growth factors, stem cells, scaffold systems, and regenerative signaling pathways [4].

Guided bone regeneration (GBR) represented one of the earliest biologically based regenerative techniques and remains widely used in implant dentistry and alveolar ridge augmentation [5]. However, contemporary bone regeneration strategies extend far beyond conventional GBR concepts.

Modern regenerative approaches aim to recreate the biological environment necessary for osteogenesis through coordinated interactions among osteogenic cells, signaling molecules, vascularization, extracellular matrices, and mechanical stability [6].

The tissue engineering triad consisting of stem cells, scaffolds, and growth factors forms the foundation of many contemporary regenerative protocols [7].

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Advances in biomaterials science, nanotechnology, additive manufacturing, and molecular biology have additionally accelerated development of customized regenerative constructs and bioactive scaffolds [8].

Stem-cell-based regenerative therapies and biologically active molecules such as bone morphogenetic proteins (BMPs), platelet concentrates, vascular endothelial growth factor (VEGF), and fibroblast growth factors (FGFs) are increasingly investigated for maxillofacial reconstruction [9].

Three-dimensional printing and bioprinting technologies further expand possibilities for fabrication of patient-specific regenerative scaffolds and customized reconstructive solutions [10].

Despite remarkable progress, important limitations remain regarding predictability, vascularization, cost, regulatory approval, long-term outcomes, and translational clinical application [11].

This chapter reviews the biological principles, graft materials, guided bone regeneration, growth factors, stem-cell-based therapies, scaffold systems, tissue engineering approaches, 3D bioprinting technologies, clinical applications, limitations, and future directions of bone regeneration in maxillofacial defects.

Biology of Bone Regeneration

Bone regeneration is a highly coordinated biological process involving inflammation, angiogenesis, cellular proliferation, extracellular matrix formation, mineralization, and remodeling [12].

Unlike many tissues, bone possesses substantial regenerative capacity because of the activity of osteoblasts, osteoclasts, mesenchymal stem cells, and vascular networks [13].

Bone healing may occur through intramembranous or endochondral ossification depending on anatomical location and mechanical conditions [14].

Successful regeneration requires adequate blood supply, mechanical stability, osteogenic cells, and signaling molecules [15].

The biological mechanisms underlying bone graft integration are commonly categorized as osteogenesis, osteoinduction, osteoconduction, and osseointegration [16].

Osteogenesis refers to direct formation of new bone by living osteogenic cells contained within graft material [17].

Osteoinduction involves recruitment and differentiation of progenitor cells into osteoblasts through bioactive signaling molecules such as BMPs [18].

Osteoconduction refers to passive guidance of bone growth along scaffold surfaces or graft matrices [19].

Osseointegration represents direct structural and functional connection between living bone and biomaterial surfaces, particularly relevant in implant dentistry [20].

Angiogenesis is critically important because vascularization provides oxygen, nutrients, inflammatory mediators, and progenitor cell migration pathways [21].

Mechanical loading additionally influences bone remodeling through mechanotransduction pathways regulating osteoblastic and osteoclastic activity [22].

Understanding the biology of bone healing is essential for development of successful regenerative strategies in maxillofacial surgery.

Bone Graft Materials

Bone grafting remains one of the most widely used regenerative approaches in oral and maxillofacial surgery. Graft materials may be classified as autografts, allografts, xenografts, and alloplastic materials [23].

Autogenous Bone Grafts

Autogenous bone remains the gold standard because of its osteogenic, osteoinductive, and osteoconductive properties [24].

Common intraoral donor sites include mandibular ramus, symphysis, maxillary tuberosity, and zygomatic buttress [25]. Extraoral donor sites include iliac crest, calvarium, tibia, and fibula.

Autogenous grafts contain viable osteogenic cells and growth factors contributing to superior regenerative potential [26].

However, disadvantages include donor site morbidity, increased operative time, limited graft volume, postoperative pain, and unpredictable resorption [27].

Allografts

Allografts are obtained from human donors and processed through freeze-drying, demineralization, or irradiation procedures [28].

Demineralized freeze-dried bone allograft (DFDBA) may retain osteoinductive potential because of preserved BMP content [29].

Advantages include elimination of donor site morbidity and greater material availability [30]. Potential disadvantages include disease transmission risk, reduced osteogenic capacity, and variable biologic activity.

Xenografts

Xenografts are derived from nonhuman species, most commonly bovine bone [31].

Deproteinized bovine bone mineral is widely used because of favorable osteoconductive properties and slow resorption profile [32].

Xenografts frequently function as long-term scaffold materials supporting gradual bone formation.

Alloplastic Materials

Synthetic graft materials include hydroxyapatite, beta-tricalcium phosphate, bioactive glass, calcium sulfate, and biphasic calcium phosphate [33].

Advantages include unlimited availability, absence of disease transmission, and customizable physicochemical properties [34].

However, many synthetic materials possess limited osteoinductive activity and rely primarily on osteoconduction.

Selection of graft material depends on defect size, anatomical location, biological requirements, and clinical objectives [35].

Guided Bone Regeneration

Guided bone regeneration is one of the most important regenerative techniques in implant dentistry and alveolar reconstruction [36].

The biological principle of GBR involves placement of barrier membranes to exclude rapidly proliferating epithelial and connective tissue cells while permitting slower-growing osteogenic cells to populate the defect [37].

Membranes additionally stabilize graft materials and maintain regenerative space [38].

Resorbable Membranes

Collagen membranes are among the most commonly used resorbable materials because of biocompatibility and ease of handling [39].

Advantages include elimination of second-stage membrane removal surgery and favorable tissue integration [40].

However, premature degradation may compromise space maintenance in large defects.

Nonresorbable Membranes

Expanded polytetrafluoroethylene (ePTFE) and titanium-reinforced membranes provide superior structural stability and space maintenance [41].

These membranes are particularly useful in large vertical augmentation procedures.

Disadvantages include increased exposure risk and need for secondary removal procedures [42].

GBR is widely applied in horizontal and vertical ridge augmentation, peri-implant defects, extraction socket preservation, sinus augmentation, and reconstruction of localized maxillofacial defects [43].

Successful outcomes depend on primary closure, angiogenesis, space maintenance, wound stability, and prevention of membrane exposure [44].

Although GBR remains highly predictable for moderate defects, large segmental reconstructions continue to present major clinical challenges [45].

Growth Factors in Bone Regeneration

Growth factors are biologically active signaling molecules that regulate cellular proliferation, differentiation, angiogenesis, extracellular matrix synthesis, and tissue remodeling during bone healing [46].

The use of recombinant growth factors has significantly expanded regenerative possibilities in maxillofacial surgery because these molecules may enhance osteoinduction and accelerate tissue repair [47].

Bone Morphogenetic Proteins

Bone morphogenetic proteins (BMPs) are among the most extensively studied osteoinductive molecules in bone regeneration [48].

BMP-2 and BMP-7 are particularly important because of their ability to induce mesenchymal stem-cell differentiation into osteoblasts [49].

Recombinant human BMP-2 (rhBMP-2) has been investigated in sinus augmentation, alveolar ridge preservation, segmental defect reconstruction, cleft repair, and spinal surgery [50].

Clinical studies have demonstrated promising bone formation capacity; however, complications including edema, ectopic bone formation, inflammatory reactions, and high cost remain important concerns [51].

Platelet-Derived Growth Factor

Platelet-derived growth factor (PDGF) stimulates angiogenesis, fibroblast proliferation, and osteoblast migration [52].

Recombinant human PDGF combined with beta-tricalcium phosphate has shown favorable outcomes in periodontal and alveolar regeneration procedures [53].

Vascular Endothelial Growth Factor

VEGF plays a critical role in angiogenesis and vascular maturation during bone healing [54].

Adequate vascularization is essential for survival of regenerative constructs and successful integration of graft materials.

Experimental studies suggest synergistic interactions between VEGF and BMPs may significantly enhance bone regeneration [55].

Fibroblast Growth Factors and Transforming Growth Factor Beta

Fibroblast growth factors (FGFs) contribute to angiogenesis, cell proliferation, and matrix synthesis [56].

Transforming growth factor beta (TGF- β) additionally regulates extracellular matrix production and osteoblastic differentiation [57].

Despite promising biological potential, clinical application of growth factors remains limited by issues including dose optimization, carrier systems, rapid degradation, high cost, and regulatory restrictions [58].

Platelet Concentrates and Autologous Biologics

Autologous platelet concentrates have become increasingly popular in oral and maxillofacial regenerative procedures because of their simplicity, low morbidity, and biologically active growth factor content [59].

Platelet-Rich Plasma

Platelet-rich plasma (PRP) is produced through centrifugation of autologous blood and contains concentrated platelets suspended in plasma [60].

Activated platelets release growth factors including PDGF, VEGF, TGF- β , and insulin-like growth factor [61].

PRP has been used in sinus augmentation, extraction socket preservation, implant surgery, and bone grafting procedures [62].

Although many studies report improved soft tissue healing and early bone maturation, evidence regarding long-term bone volume enhancement remains inconsistent [63].

Platelet-Rich Fibrin

Platelet-rich fibrin (PRF) represents a second-generation platelet concentrate produced without anticoagulants [64].

The fibrin matrix gradually releases growth factors over prolonged periods and may additionally function as a scaffold for cellular migration [65].

PRF is widely used in extraction socket management, sinus augmentation, guided bone regeneration, peri-implant defects, and medication-related osteonecrosis management [66].

Injectable PRF and advanced PRF protocols have further expanded potential clinical applications [67].

Advantages of platelet concentrates include autologous origin, low cost, ease of preparation, and favorable biocompatibility [68].

However, variability in centrifugation protocols and lack of standardization continue to limit reproducibility across studies [69].

Stem Cells in Maxillofacial Bone Regeneration

Stem-cell-based therapies represent one of the most promising regenerative strategies in contemporary maxillofacial reconstruction [70].

Stem cells possess the ability for self-renewal and differentiation into specialized cell lineages including osteoblasts, chondrocytes, adipocytes, and fibroblasts [71].

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are among the most extensively investigated cell populations in bone tissue engineering [72].

Common sources include bone marrow, adipose tissue, periosteum, dental pulp, periodontal ligament, and umbilical cord tissue [73].

Bone marrow-derived mesenchymal stem cells possess strong osteogenic potential and have demonstrated promising regenerative capacity in experimental and clinical studies [74].

Dental Stem Cells

Dental tissues represent highly accessible sources of stem cells in oral surgery [75].

Dental pulp stem cells, stem cells from human exfoliated deciduous teeth, periodontal ligament stem cells, and apical papilla stem cells have all demonstrated osteogenic differentiation potential [76].

These cells may contribute to craniofacial bone regeneration because of their neural crest origin and favorable proliferative properties [77].

Adipose-Derived Stem Cells

Adipose-derived stem cells are increasingly attractive because of ease of harvesting and abundant cell yield [78].

Experimental studies suggest comparable regenerative capacity to bone marrow-derived MSCs in selected applications [79].

Challenges in Stem Cell Therapy

Despite exciting potential, several limitations remain regarding stem-cell-based bone regeneration [80].

Cell survival, vascularization, immune compatibility, tumorigenic potential, scaffold integration, ethical concerns, and regulatory approval remain major challenges [81].

Standardization of harvesting, processing, expansion, and delivery protocols is additionally necessary before widespread routine clinical implementation [82].

Scaffold Systems and Biomaterials

Scaffolds provide three-dimensional structural frameworks supporting cellular adhesion, proliferation, differentiation, vascularization, and extracellular matrix deposition [83].

An ideal scaffold should possess biocompatibility, biodegradability, mechanical stability, interconnected porosity, osteoconductivity, and appropriate degradation kinetics [84].

Natural Biomaterials

Natural scaffold materials include collagen, chitosan, gelatin, alginate, silk fibroin, and fibrin [85].

These materials generally demonstrate excellent biocompatibility and cellular interaction because of their biological origin [86].

However, limited mechanical strength and unpredictable degradation may restrict use in load-bearing defects.

Synthetic Biomaterials

Synthetic scaffolds include polylactic acid (PLA), polyglycolic acid (PGA), polycaprolactone (PCL), hydroxyapatite, beta-tricalcium phosphate, and bioactive ceramics [87].

Synthetic materials offer greater control over porosity, degradation, and mechanical properties [88].

Composite scaffolds combining natural and synthetic components may optimize both biological and mechanical performance [89].

Nanotechnology and Smart Biomaterials

Nanotechnology has significantly influenced regenerative biomaterials through development of nanoscale surface modifications and bioactive delivery systems [90].

Nanostructured surfaces may improve cellular adhesion, osteoblastic differentiation, and protein adsorption [91].

Smart biomaterials capable of controlled growth factor release or environmental responsiveness represent another emerging field [92].

Scaffold vascularization remains one of the greatest challenges in large-volume bone regeneration because insufficient blood supply may lead to necrosis and graft failure [93].

Tissue Engineering Strategies

Bone tissue engineering integrates cells, scaffolds, and signaling molecules to regenerate functional bone tissue [94].

The tissue engineering triad consists of:

- osteogenic cells,
- scaffold matrices,
- bioactive growth factors [95].

The goal is to recreate the biological microenvironment necessary for physiologic bone formation and remodeling.

Bioreactors are increasingly investigated because they permit controlled culture environments supporting cell proliferation and tissue maturation before implantation [96].

Mechanical stimulation, oxygen tension, nutrient delivery, and fluid dynamics may significantly influence tissue-engineered construct development [97].

Prefabricated vascularized constructs and in vivo bioreactor approaches have also been explored for reconstruction of critical-size defects [98].

Another emerging concept involves gene therapy for bone regeneration. Delivery of genes encoding osteogenic proteins such as BMPs may potentially provide prolonged local growth factor production [99].

Despite promising experimental results, clinical translation of tissue engineering strategies remains limited by complexity, cost, regulatory barriers, and long-term validation requirements [100].

Three-Dimensional Printing and Bioprinting

Three-dimensional printing technologies have transformed maxillofacial reconstruction by enabling fabrication of patient-specific scaffolds, surgical guides, implants, and regenerative constructs [101].

Additive manufacturing allows precise layer-by-layer production of customized structures based on digital imaging datasets obtained from CT or CBCT scans [102].

In maxillofacial surgery, 3D printing is widely used for reconstruction plates, patient-specific implants, anatomical models, and scaffold fabrication [103].

Customized scaffolds may improve anatomical adaptation and facilitate more accurate reconstruction of complex craniofacial defects [104].

Bioprinting represents a more advanced technology involving simultaneous printing of living cells, biomaterials, and bioactive molecules [105].

Bioinks containing stem cells, hydrogels, growth factors, and extracellular matrix components may potentially generate biologically functional tissue constructs [106].

Several experimental studies have demonstrated promising bone regeneration using bioprinted osteogenic scaffolds [107].

However, vascularization remains a major limitation because survival of large engineered tissues depends on adequate blood supply [108].

Additional challenges include mechanical strength, long-term integration, manufacturing cost, and regulatory approval [109].

Despite current limitations, 3D bioprinting is expected to become increasingly important in personalized regenerative maxillofacial surgery.

Clinical Applications in Maxillofacial Surgery

Bone regeneration strategies are now widely applied across numerous fields of oral and maxillofacial surgery [110].

Alveolar Ridge Augmentation

Horizontal and vertical ridge augmentation procedures are among the most common regenerative applications because adequate bone volume is essential for implant placement [111].

GBR, autogenous grafting, xenografts, growth factors, and platelet concentrates are frequently combined to optimize regenerative outcomes [112].

Maxillary Sinus Augmentation

Sinus floor elevation remains a highly predictable regenerative procedure for rehabilitation of the posterior maxilla [113].

Various graft materials and biologics including PRF, BMPs, and stem-cell-based approaches have been investigated to improve bone formation and reduce healing time [114].

Reconstruction of Segmental Defects

Large mandibular or maxillary defects following tumor resection or trauma remain among the most difficult reconstructive challenges [115].

Vascularized free flaps such as fibula free flaps remain the gold standard for extensive defects; however, tissue engineering strategies may eventually reduce donor site morbidity [116].

Cleft and Craniofacial Reconstruction

Alveolar cleft grafting and craniofacial defect reconstruction additionally benefit from regenerative approaches involving autogenous bone, BMPs, and scaffold systems [117].

Medication-Related Osteonecrosis and Osteomyelitis

Regenerative biomaterials, platelet concentrates, and stem-cell-based therapies are increasingly investigated in management of MRONJ and osteomyelitis-related defects [118].

Peri-Implant and Periodontal Defects

Localized peri-implant bone loss and periodontal defects are commonly treated using combinations of graft materials, membranes, biologics, and regenerative adjuncts [119].

The expanding integration of biologically driven regenerative concepts continues to improve reconstructive possibilities in oral and maxillofacial surgery.

Limitations and Challenges

Despite remarkable advances, several important limitations continue to restrict widespread application of advanced bone regeneration strategies [120].

One of the major challenges involves vascularization of large regenerative constructs. Insufficient blood supply may result in central necrosis, delayed integration, and graft failure [121].

Mechanical stability additionally remains critical because excessive micromotion may impair osteogenesis and favor fibrous tissue formation [122].

Stem-cell-based therapies face challenges related to cell survival, differentiation control, immunogenicity, manufacturing complexity, and regulatory approval [123].

Growth factors such as BMPs may produce unpredictable biologic responses including excessive inflammation, ectopic bone formation, and high treatment costs [124].

Scaffold degradation kinetics must also be carefully controlled because premature resorption or prolonged persistence may compromise regeneration [125].

Another major issue involves translation of experimental findings into predictable long-term clinical outcomes. Many promising regenerative technologies remain limited to laboratory or animal studies [126].

Economic factors additionally influence accessibility because advanced tissue engineering procedures and customized biomaterials may be costly [127].

Long-term randomized clinical trials and standardized treatment protocols are therefore still needed to establish evidence-based regenerative strategies.

Future Perspectives

The future of maxillofacial bone regeneration is expected to involve increasingly personalized, biologically active, and digitally integrated approaches [128].

Artificial intelligence-assisted imaging analysis and computational modeling may improve defect evaluation and regenerative planning [129].

Gene editing technologies and molecular signaling modulation may eventually permit more precise control of osteogenesis and tissue regeneration [130].

Smart biomaterials capable of controlled release of growth factors and antimicrobial agents are also under active investigation [131].

Three-dimensional bioprinting combined with stem cells and vascularized tissue engineering may ultimately permit fabrication of fully functional patient-specific bone constructs [132].

Nanotechnology, exosome therapy, and immunomodulatory regenerative strategies represent additional promising future directions [133].

Personalized regenerative medicine integrating genomic profiling, biomarker analysis, and patient-specific biologic responses may further improve predictability and outcomes [134].

Interdisciplinary collaboration among surgeons, bioengineers, material scientists, molecular biologists, and regenerative medicine specialists will remain essential for continued progress [135].

Conclusion

Bone regeneration strategies in maxillofacial surgery have evolved substantially from conventional grafting techniques toward sophisticated biologically driven regenerative approaches integrating tissue engineering, biomaterials, growth factors, stem cells, and digital technologies.

Autogenous bone grafting remains the gold standard in many reconstructive procedures; however, limitations including donor site morbidity and graft resorption have stimulated development of alternative regenerative solutions.

Guided bone regeneration, platelet concentrates, recombinant growth factors, scaffold systems, stem-cell-based therapies, and tissue engineering strategies now play increasingly important roles in craniofacial reconstruction and implant rehabilitation.

Advances in biomaterials science, nanotechnology, CAD/CAM systems, additive manufacturing, and bioprinting technologies further expand reconstructive possibilities through development of customized regenerative constructs and patient-specific treatment approaches.

Despite encouraging progress, important limitations remain regarding vascularization, long-term predictability, cost, regulatory approval, and translational clinical application.

Future developments involving artificial intelligence, gene therapy, smart biomaterials, exosome-based regeneration, and vascularized bioprinting are expected to further transform regenerative maxillofacial surgery.

Overall, contemporary bone regeneration strategies continue moving toward increasingly biologically active, personalized, minimally invasive, and tissue-engineered solutions aimed at restoring both function and facial aesthetics in patients with maxillofacial defects.

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Chapter 5

Cerebral Ischemia, Mitochondrial Injury, and Flavonoids: A Comprehensive Review

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Abstract

Background

Cerebral ischemia-reperfusion injury (CIRI) is a major contributor to mortality and long-term neurological disability worldwide. Mitochondrial dysfunction plays a central role in the pathogenesis of CIRI through excessive reactive oxygen species production, calcium overload, mitochondrial permeability transition pore (mPTP) opening, impaired energy metabolism, and activation of multiple cell death pathways. In recent years, flavonoids derived from medicinal plants and dietary sources have attracted growing attention because of their broad-spectrum neuroprotective properties.

Methods

This review comprehensively evaluates current experimental evidence regarding the protective effects of flavonoids against cerebral ischemia-reperfusion injury, with particular emphasis on mitochondrial preservation. Relevant studies investigating mitochondrial dynamics, biogenesis, oxidative stress, mitophagy, energy metabolism, cell death pathways, and advanced flavonoid-based nanotherapeutic delivery systems were critically analyzed.

Results

Accumulating evidence indicates that flavonoids exert multitarget neuroprotective actions by restoring mitochondrial homeostasis. Compounds including apigenin, alpinetin, calycosin derivatives, transchalcone, icariin, hydroxysafflor yellow A, scutellarin, vitexin, naringin, baicalin, kaempferol, fisetin, and pinocembrin effectively modulate mitochondrial fission-fusion balance, promote biogenesis, inhibit mPTP opening, reduce oxidative stress, and regulate mitophagy. Furthermore, many flavonoids activate key cytoprotective signaling pathways, particularly Nrf2/HO-1, AMPK, SIRT1, PGC-1 α , and

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PI3K/Akt, resulting in attenuated apoptosis, ferroptosis, neuroinflammation, and metabolic dysfunction. Recent advances in nanotechnology-based delivery platforms, including mitochondria-targeting nanozymes and functionalized liposomes, have significantly improved flavonoid bioavailability, blood-brain barrier penetration, and therapeutic efficacy in preclinical models.

Conclusions

Flavonoids represent promising multifunctional therapeutic candidates for cerebral ischemia-reperfusion injury by targeting multiple mitochondrial and cellular pathways simultaneously. Their ability to preserve mitochondrial integrity, suppress oxidative and inflammatory cascades, and improve neuronal survival highlights their substantial translational potential. Future studies should focus on optimizing targeted delivery systems and conducting well-designed clinical trials to establish their efficacy, safety, and therapeutic applicability in patients with ischemic stroke.

Keywords: Cerebral ischemia-reperfusion injury; Flavonoids; Mitochondrial dysfunction; Oxidative stress; Mitophagy; Neuroprotection; Nrf2; Stroke.

1. Introduction and Pathophysiological Background

Cerebral ischemia remains one of the leading global causes of mortality and long-term neurological disability. Due to its high metabolic rate, elevated oxygen demand, and limited endogenous antioxidant defenses, brain tissue is exceptionally vulnerable to sudden restrictions in blood and glucose supply. Although prompt recanalization and restoration of blood flow (reperfusion) are critical for salvaging the ischemic penumbra, reperfusion paradoxically triggers and exacerbates secondary tissue injury a phenomenon widely known as cerebral ischemia-reperfusion injury (CIRI). [9]

The core instigator of CIRI is profound **mitochondrial dysfunction**. Upon reperfusion, the sudden influx of oxygen overwhelms the electron transport chain (ETC), resulting in a massive surge of reactive oxygen species (ROS), intracellular calcium overload, the opening of mitochondrial permeability transition pores (mPTP), and the release of mitochondrial components such as mitochondrial DNA (mtDNA). These pathological events trigger downstream cascades including apoptosis, ferroptosis, necroptosis, parthanatos, and intense neuroinflammation driven by the activation of resident microglia and astrocytes. [3]

In recent years, naturally occurring bioactive compounds—particularly **flavonoids** derived from traditional medicinal herbs and dietary plants—have gained substantial scientific interest. Flavonoids exhibit pleiotropic neuroprotective properties, including powerful free radical scavenging, anti-inflammatory, anti-apoptotic, and mitochondria-stabilizing activities. This comprehensive review explores the multi-target mechanisms by which flavonoids attenuate cerebral ischemia, focusing heavily on mitochondrial protection, dynamics, energy metabolism, and signaling pathways. [9]

2. Mitochondrial Dynamics, Fission-Fusion Balance, and Biogenesis

Mitochondria are highly dynamic organelles that constantly undergo coordinated cycles of fission and fusion to maintain their structural integrity, distribution, and function under physiological stress. Cerebral ischemia disrupts this delicate balance, resulting in excessive mitochondrial fragmentation mediated by the overactivation of dynamin-related protein 1 (Drp1) and a concurrent reduction in fusion proteins such as mitofusin-2 (Mfn2) and optic atrophy 1 (Opa1). [3]

- **Apigenin:** Studies utilizing both in vivo and in vitro retinal and cerebral ischemia/reperfusion models demonstrate that apigenin preserves mitochondrial structural integrity and cristae morphology. It exerts these effects by upregulating fusion-promoting proteins (Opa1, Mfn2) while suppressing fission activation (Drp1), thereby reducing lactate dehydrogenase (LDH) release and cellular apoptosis. [29]

- **Alpinetin and Calycosin-7-O-beta-D-glucoside (CG):** Alpinetin has been shown to mitigate ischemic brain damage by restoring mitochondrial manganese superoxide dismutase (MnSOD) activity, preserving Mfn2 levels, and attenuating p38 MAPK signaling. Similarly, CG enhances mitochondrial biogenesis through the upregulation of sirtuin 1 (SIRT1) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1\alpha), while concurrently inhibiting pathological mitochondrial fission and overactive mitophagy. [28, 32]
- **Transchalcone (TCH):** Acting via AMP-activated protein kinase (AMPK) signaling, TCH upregulates downstream targets such as PGC-1\alpha and nuclear factor erythroid 2-related factor 2 (NRF2), thereby improving ATP synthesis, restoring manganese superoxide dismutase activity, and lowering mitochondrial ROS generation. [31]

3. Mitochondrial Permeability Transition Pore (mPTP) and Cell Death Pathways

The opening of the mitochondrial permeability transition pore (mPTP) is a catastrophic threshold event in cerebral ischemia, leading to inner membrane depolarization, uncoupling of oxidative phosphorylation, matrix swelling, outer membrane rupture, and the release of pro-apoptotic factors like cytochrome c. [3]

- **Icariin (ICA) and Icaritin (ICT):** ICA and its active metabolite ICT effectively protect against oxidative injury by directly inhibiting mPTP opening, stabilizing the mitochondrial membrane potential (MMP), and suppressing downstream apoptotic cascades in both PC-12 cells and middle cerebral artery occlusion (MCAO) rodent models. [38]
- **Hydroxysafflor Yellow A (HSYA):** HSYA provides robust neuroprotection against cerebral ischemia by regulating the MEK/ERK/CypD signaling axis, which blocks mPTP opening, prevents cytochrome c translocation from the mitochondria to the cytosol, and protects brain microvascular endothelial cells (BMECs) from hypoxia/reoxygenation injury. [14, 23]
- **Scutellarin and Isoliquiritigenin (ISL):** Scutellarin improves mitochondrial respiration rates, oxygen consumption rates (OCR), ATP production, and Na⁺/K⁺-ATPase activity by mitigating oxidative stress and modulating mitophagy. ISL similarly restores redox homeostasis and protects mitochondrial function through the activation of the Nrf2 pathway. [33, 15]

4. Antioxidant Defense Systems and the Nrf2 Signaling Axis

Oxidative and nitrosative stress are hallmark contributors to neuronal destruction following ischemic stroke. The transcription factor Nrf2 acts as a master regulator of cellular redox homeostasis, driving the expression of cytoprotective and antioxidant genes. [3]

- **Polypodium hastatum Flavonoids (AAKR):** AAKR activates Nrf2 in a Keap1-dependent manner, facilitating its nuclear translocation and binding to antioxidant response elements (ARE), thereby reducing oxidative stress and mitochondrial-mediated apoptosis in PC12 cells and MCAO mice. [34]
- **Procyanidins and Vitexin:** Procyanidins attenuate cerebral ischemia-reperfusion injury by upregulating the Nrf2/HO-1 pathway, which inhibits mitochondrial ferroptosis, reduces intracellular Fe accumulation, and preserves glutathione peroxidase 4 (GPX4) expression. Vitexin similarly exerts anti-ferroptotic and anti-oxidative effects via the Keap1/Nrf2/HO-1 axis. [7, 12]
- **Icariside II (ICS II):** Preconditioning with ICS II evokes robust neuroprotection by binding directly to Nrf2, enhancing its transcriptional activity, and regulating the astrocytic OXPHOS/NF-kappa B/ferroptosis axis. [10]

5. Regulation of Mitophagy and Cellular Energy Metabolism

Mitophagy—the selective degradation of damaged mitochondria via autophagy—plays a dual role in cerebral ischemia, serving as a protective mechanism or promoting cell death depending on its extent. [3]

- **Vitexin:** Vitexin alleviates cerebral ischemia/reperfusion injury by properly balancing mitophagy through the activation of the SIRT1/PINK1/Parkin signaling pathway, reducing mitochondrial ROS accumulation, and maintaining mitochondrial membrane potential. [5, 12]
- **Naringin:** Naringin protects cortical neurons in transient MCAO/R rat models by inhibiting the DRP1/LRRK2/MCU signaling axis, which prevents excessive mitochondrial calcium overload and suppresses detrimental, overactivated mitophagy and structural fragmentation. [27]
- **Formononetin (FN):** FN regulates microglial immunometabolism during cerebral ischemia-reperfusion by suppressing glycolytic pathways and enhancing mitochondrial oxidative phosphorylation via the AMPK/mTOR/HIF-1 α signaling pathway, ultimately shifting microglia from a pro-inflammatory M1 phenotype to an anti-inflammatory M2 phenotype. [35]

6. Advanced Nanotechnology and Targeted Delivery Systems

Despite their exceptional pharmacological profiles in pre-clinical settings, many natural flavonoids suffer from poor aqueous solubility, rapid metabolic clearance, and restricted penetration across the blood-brain barrier (BBB). Modern nanomedicine has successfully addressed these translational hurdles: [9]

- **Quercetin-Based Cascade Nanozymes (MCN):** Utilizing the intrinsic capacity of natural quercetin to coordinate with iron ions Fe and bind mitochondrial outer membrane proteins, ultrasmall cascade nanozymes have been engineered. These nanozymes cross the compromised BBB, localize selectively to neuronal mitochondria, exhibit superoxide dismutase-catalase cascade activity, eliminate local ROS, stabilize mitochondrial function, block mtDNA-mediated cGAS-STING inflammatory activation, and promote functional recovery. [36, 4]
- **Puerarin-Loaded Functionalized Liposomes (PUELipo/R-R and PPCs):** Surface-engineered liposomes decorated with ROS-responsive linkages and neuron- or macrophage-targeting moieties allow precise, stimuli-responsive delivery of puerarin to ischemic brain regions. These platforms effectively clear local oxidative species, inhibit neuronal apoptosis, stimulate angiogenesis, and facilitate significant cognitive recovery in vascular dementia and stroke models. [8, 17]

7. Additional Neuroprotective Flavonoids and Mechanisms

- **Baicalin:** Baicalin protects neurons and glial cells by suppressing succinate dehydrogenase (SDH)-mediated oxidative stress, preserving the neuronal PDK2-PDH axis, and protecting astrocytic glutamine synthetase (GS) from proteasomal degradation, thereby mitigating glutamate excitotoxicity. [18, 26, 13, 25]
- **Calycosin:** Calycosin exhibits potent anti-inflammatory, anti-apoptotic, and antioxidant properties by downregulating the HMGB1/TLR4/NF- κ B pathway, inhibiting excessive autophagy via the STAT3/FOXO3a pathway, and supporting mitochondrial function through PGC-1 α /TFAM signaling. [2, 30]
- **Kaempferol and Fisetin:** Kaempferol provides extensive protection against initial and delayed ischemic brain damage by targeting early mitochondrial dysfunction, upregulating SIRT1, and modulating p66shc expression. Fisetin similarly reduces ROS production and activates PI3K/Akt and Nrf2 pathways. [20, 37, 1]
- **Pinocembrin:** Approved by regulatory authorities for clinical investigation in ischemic stroke, pinocembrin rapidly crosses the BBB,

reduces ischemic core damage, and effectively suppresses oxidative stress and mitochondrial dysfunction. [24]

8. Discussion and Future Perspectives

Cerebral ischemia-reperfusion injury is a multifactorial pathological cascade characterized by intertwined oxidative stress, energy failure, calcium dysregulation, mitochondrial degradation, and neuroinflammation. Conventional single-target pharmacotherapy often fails to halt this complex web of destruction. Plant-derived flavonoids offer a remarkable therapeutic advantage due to their multi-target capabilities—simultaneously modulating redox transcription factors (Nrf2), energy sensors (AMPK, SIRT1), mitochondrial structural regulators (Drp1, Mfn2), and cell death executioners (mPTP, caspases). [3, 9]

Future research must prioritize clinical translation by optimizing advanced drug delivery systems (such as nanoparticle encapsulation, ligand-targeted liposomes, and mitochondria-penetrating conjugates) to overcome bioavailability barriers. Rigorous clinical trials will ultimately validate the efficacy, safety profiles, and precise therapeutic dosages of these promising flavonoid candidates in human stroke victims. [8, 17]

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Mitochondrial Transfer at a Crossroads: Methodological, Biological, and Ethical Barriers to Clinical Translation

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Abstract

Purpose: Mitochondrial transfer has emerged as an investigational therapeutic strategy for cancer, ischemia-reperfusion injury, neurodegenerative disorders, metabolic diseases, and other conditions associated with mitochondrial dysfunction. Despite encouraging preclinical and early clinical results, substantial methodological, biological, and ethical barriers limit its standardized clinical application. This review summarizes the principal limitations of mitochondrial transfer and discusses unresolved questions regarding isolation protocols, dosing, storage, delivery efficiency, target-cell responses, and ethical concerns.

Material and Methods: A narrative review of preclinical and clinical literature on mitochondrial transfer was conducted, with emphasis on methodological variability and translational challenges.

Results: Key limitations encompass the absence of standardized methods for isolation and quantification, the limited short-term viability of isolated mitochondria during storage and transfer, uncertain cellular uptake—reported as less than 10% in some cardiomyocyte ischemia models—and dose figures that are not directly comparable (about 10^5 mitochondria per gram of heart tissue in preclinical models, total intramyocardial counts as high as 10^{10} in pediatric patients, or protein concentrations such as 5–10 $\mu\text{g/mL}$ in cell culture, depending on the route and assay). Target-cell responses are heterogeneous, including context-dependent antiproliferative effects in cancer models. Unresolved ethical considerations include allogeneic immunity, pediatric compassionate use, and mitochondrial donation in assisted reproduction. Support of tumor-cell fitness

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remains a theoretical concern and is not the result shown by the cancer models summarized here.

Conclusion: Mitochondrial transfer remains experimentally promising, but the net effect is context-dependent. Standardized protocols, clearer mechanisms, and ethical frameworks are required before broader clinical use can be considered.

Keywords: mitochondrial transfer; mitochondrial dysfunction; bioethics; mitochondrial donation; cancer

Introduction

Mitochondria are organelles involved in various essential physiological functions, including cell signaling, intracellular communication, calcium homeostasis, apoptosis, the synthesis and degradation of molecules, oxidative and antioxidative processes within the cell, as well as the production of ATP (1–3).

Mitochondria account for up to 20% of the cytoplasmic volume in some eukaryotic cells, with a diameter ranging from 0.5 to 1 micrometer. They possess a dynamic structure and exhibit properties such as deformation, fission, and fusion (4).

Mitochondrial transfer is being investigated as an experimental approach for neurodegenerative, metabolic, cardiovascular, and malignant disease (5,6). Experimental systems in this field include prostate-cancer and cardiomyoblast models (7,8).

Externally applied isolated mitochondria may be internalized by injured cells and can support tissue repair, although replacement of endogenous organelles has not been established. In some injury models, transfer has been associated with lower ROS production and higher ATP generation, which may limit apoptosis; these effects are not universal and depend on the recipient cell and the accompanying treatment (6).

Clark and Shay first showed that isolated mitochondria can enter mammalian cells in 1982 (9). James D. McCully and colleagues later applied mitochondria to ischemic tissues and thereby opened the therapeutic line of work (10). Clark and Shay isolated mitochondria from cells resistant to chloramphenicol (CAP) and efrapeptin (EF) and transferred these mitochondria to cells sensitive to CAP and EF. McCully and colleagues reported myocardial protection after mitochondrial transfer in subsequent ischemia–reperfusion models (11–13).

Mitochondria can be transferred between cells through tunneling nanotubes and cell fusion; gap junctions may support intercellular signaling but are too narrow to permit organelle passage. Isolated mitochondria may also enter cells via actin-dependent macropinocytosis; nonetheless, this uptake mechanism remains incompletely defined. A review of cardiac ischemia–reperfusion models reported that less than 10% of transferred mitochondria were internalized by cardiomyocytes (2). Extracellular vesicles, particularly exosomes, are a separate route of intercellular communication rather than a measured path of organelle uptake, and may either propagate mitochondrial stress or deliver neuroprotective cargo depending on the cellular context (14).

Even when uptake is limited, a review of ischemia–reperfusion studies reported increased ATP production and oxidative phosphorylation; the mechanism remains unclear (2).

The main barriers can be grouped as follows:

1. Isolation method and amount of mitochondria
2. Storage and transfer time after isolation
3. Delivery site and the fraction that reaches the target
4. Heterogeneous responses in target cells
5. Ethics

1. Isolation method and amount of mitochondria

Although various methods are employed in mitochondrial isolation, commercial kits are frequently used alongside laboratory protocols. Isolation without a commercial kit is typically performed by differential centrifugation; density-gradient ultracentrifugation is optional when higher purity is required.

The process of isolating cells for mitochondrial extraction constitutes a fundamental phase in the research investigation. The determination of whether the research will involve cell culture models, animal models, or clinical studies represents a significant decision. In cell culture models, mitochondria sourced from healthy cells are likely to exhibit greater functionality, thereby aligning with the research hypothesis. Nevertheless, studies employing mitochondria obtained from neuroblastoma cancer cell lines, such as SH-SY5Y, are also undertaken (15). Moreover, conventional two-dimensional cultures only partially reproduce polarity, extracellular-matrix organization, and cell–matrix interactions; three-dimensional biomaterial scaffolds more closely approximate the *in vivo* niche and provide a relevant interpretive context for recipient-cell responses, including those to transferred organelles, although those scaffolds have been characterized mainly in bone and cartilage models rather than in mitochondrial-transfer assays (16).

When mitochondria are isolated from healthy cells, the source may be autologous or allogeneic. Autologous transfer may reduce immune incompatibility, but mitochondria obtained from a patient with established mitochondrial disease may themselves be impaired; allogeneic organelles from a healthy donor may therefore be preferable despite a theoretical immunologic cost (17).

Within the context of allogeneic selection, the emphasis is placed on ensuring that healthy and functional mitochondria are utilized. In addition to allogeneic and autologous options, xenogeneic transplantation has also emerged as a distinct alternative. Chang et al. reported that both allogeneic and xenogeneic mitochondria were employed in a Parkinson disease model; while xenogeneic mitochondria demonstrated effectiveness, they were less effective in comparison to the allogeneic groups (18).

No single mitochondrial dose has been established, so published examples are more informative than a universal number. In related work from the authors' laboratory, preparations have been quantified by the BCA protein assay, and total protein has been used to set the dose against the recipient-cell number. Ulger et al., from the same laboratory, isolated mitochondria from rat mesenchymal stem cells and used them to treat hepatic injury (19).

In that study, Ulger et al. quantified mitochondria using flow cytometry and administered 8.2×10^6 mitochondria to each rat. Lin et al. also induced ischemia-reperfusion injury in the rat liver and utilized approximately 7×10^6 mitochondria in their research (20). McCully et al. applied approximately 2×10^5 mitochondria per gram of tissue to the ischemic heart. Roushbandeh et al. later noted that published amounts ranged from 10^5 to 10^7 , but these figures are not interchangeable: they are reported per gram of tissue, per animal, or as micrograms of mitochondrial protein (2). In cell-culture models the recipient cell number is known, which makes dosing more controllable than in tissue studies. In related experiments from the same laboratory, mitochondria isolated from human mesenchymal stem cells were applied to DU-145 cells at doses corresponding to 10-fold and 100-fold the number of recipient cells, and protein quantification by the BCA assay yielded comparable dose estimates (8). When combined with cisplatin, the higher mitochondrial dose further reduced proliferation, whereas the lower dose did not differ significantly from cisplatin alone (8). The same 10-fold and 100-fold dosing was later used when mitochondrial transplantation, with or without hyperbaric oxygen, was tested as a delayed rescue after cisplatin exposure at an LD50-level concentration in human mesenchymal stem cells (21). In separate experiments, protein-based doses of 5–10 $\mu\text{g/mL}$ were used when transferring mesenchymal stem cell-derived mitochondria to H9c2 cardiomyoblasts and DU-145 prostate cancer cells (7,22). Thus, matching mitochondrial dose to a known recipient cell number is feasible in vitro, although the biologically effective amount still depends on the accompanying treatment. In human studies, McCully and colleagues remain the most experienced group. Guariento et al. administered 2×10^{10} viable mitochondria by direct intramyocardial injection (23). That figure is a total injected count, not a per-gram dose, and the delivery was local; mitochondria introduced into the systemic circulation may lose activity or be cleared before they reach the intended tissue, so administered counts should not be read as the internalized dose.

2. Storage and transfer time after isolation

Another significant limitation is that isolated mitochondria are not suitable for long-term storage. Function declines when organelles are held on ice for longer

than about one hour, and protocols from the same laboratory therefore limited the interval between isolation and transfer to approximately one hour (7,22). Membrane damage may begin during this interval. Release of cytochrome c after loss of outer-membrane integrity is a central event in mitochondrial cell-death pathways (24). In an isolate intended for transfer, that release is a reason to regard the preparation as non-viable. Inner-membrane injury reduces oxidative phosphorylation and the resting membrane potential. Residual metabolic activity may persist after limited membrane injury (17).

Storage conditions must therefore preserve both inner- and outer-membrane integrity if the isolate is to remain usable for transfer.

3. Delivery site and the fraction that reaches the target

Even in cell-culture experiments, the fraction of applied mitochondria that actually enters recipient cells remains uncertain. The doses cited above should therefore be regarded as starting points rather than internalized amounts. Cellular association can be examined by histological or fluorescence labeling. Fluorescence labeling of donor mitochondria in DU-145 cells has shown patterns compatible with association or uptake, although such imaging does not establish stable functional integration (22). Recipient cell type—cancer lines, stem cells, or primary cultures—also influences uptake. Localized administration in animals allows partial control of dose and site, but there is still no reliable estimate of how many organelles reach the target after systemic delivery, whether they enter unintended cells, or how long they remain functional. Clinical reports from McCully and colleagues show that mitochondrial delivery can be performed in patients, but they do not resolve the internalized fraction (23,25).

4. Heterogeneous responses in target cells

Numerous studies have examined the clinical and preclinical effects of mitochondrial transfer. Clinical evidence remains limited. In 2017, McCully and colleagues administered autologous isolated mitochondria to a small number of pediatric patients with cardiac ischemia-reperfusion injury, showing that the approach is feasible (25). Guariento et al. later reported direct intramyocardial injection in pediatric cardiogenic shock (23). Together these series show that delivery is feasible, but the clinical experience remains too small for routine implementation, and substantially more research is required. Preclinical studies are more numerous and include schizophrenia-derived neurons (26), centrifugation-assisted mitochondrial delivery (27), peripheral-nerve injury (28), spinal cord injury (29), cardiomyocyte injury (30), and Parkinson disease (18). Mitochondrial impairment is also a core pathogenic axis in neurodegenerative

disease, in which exosomes may either spread misfolded proteins or support neuronal repair; vesicle-based and organelle-based interventions are therefore similarly dependent on cellular context (14). In oncology and cardiovascular cell models, sequential mitochondrial transfer with cisplatin or 3-bromopyruvate has been examined in prostate cancer cells, whereas angiotensin II plus mitochondrial transplantation has been studied in cardiomyoblasts (7,8,22).

Given the roles of mitochondria in ATP synthesis, reactive oxygen species handling, immune signaling, and cell death, transfer of functional organelles is expected to benefit cells with mitochondrial dysfunction. In cancer models, however, the same intervention may reduce rather than restore proliferation (31).

These findings indicate that the net effect of mitochondrial transfer depends on the recipient cell and the accompanying pharmacological context. In DU-145 prostate cancer cells, mitochondria isolated from human mesenchymal stem cells further reduced proliferation when combined with cisplatin, particularly at a higher mitochondrial dose (8). Sequential hexokinase inhibition with 3-bromopyruvate followed by mitochondrial transfer produced a stronger antiproliferative effect than 3-bromopyruvate alone and was associated with reduced total mTOR and HIF-1 α protein levels (22). By contrast, delayed mitochondrial transplantation with or without hyperbaric oxygen did not restore MTT metabolic activity after LD50-level cisplatin injury in human mesenchymal stem cells (21). In H9c2 cardiomyoblasts, mitochondrial transplantation together with angiotensin II increased proliferation and was accompanied by lower IL-6 and higher IL-10 levels, consistent with a shift toward anti-inflammatory signaling rather than growth suppression (7). Recipient context also includes culture architecture and the intercellular milieu. Three-dimensional scaffolds change polarity and matrix signaling relative to monolayers, and exosomes in neurodegeneration shift between injury and repair; these are analogous constraints on cellular state, not direct measurements of organelle uptake (14,16). These contrasting outcomes show that mitochondrial transfer is not uniformly restorative; the mechanisms remain incompletely defined. Mitochondria carry their own DNA, and favorable results have been reported after both allogeneic and xenogeneic transfer; how an organelle with distinct genetic material can act with little apparent immune activation remains an open question.

5. Ethics

Therapeutic transplantation of isolated mitochondria raises questions of allogeneic immunity and pediatric compassionate use. Whether transfer could support tumor-cell fitness in other settings is a separate question; the cancer models discussed above show reduced, rather than increased, proliferation. A

separate and more developed debate concerns mitochondrial donation in assisted reproduction, which uses spindle or pronuclear transfer into a donor oocyte rather than injection of isolated mitochondria. In 2015, the UK Parliament authorized mitochondrial donation in assisted reproduction (32).

It is widely acknowledged that mitochondria contain unique DNA that is transmitted through the maternal line. Offspring of women with mitochondrial disease may therefore inherit and later transmit the condition. Paternal mitochondrial inheritance has been reported but remains rare and controversial (33). In this setting, allogeneic mitochondrial donation is generally required, because oocytes from the affected woman carry pathogenic mitochondrial DNA, so donor cytoplasm containing healthy mtDNA is required. The procedure therefore involves transfer of genetic material and raises the so-called three-parent baby question (34).

The donor does transmit mitochondrial DNA; the ongoing debate concerns parental status, donor rights, and the germline nature of the intervention. A further issue is the limited information on donor-related genetic disease and on possible consequences of mixing mitochondrial genomes. Social and legal implications of a three-parent infant continue to obstruct acceptance in many countries. Although the United Kingdom authorized mitochondrial donation in 2015 (32). Long-term safety follow-up remains incomplete. These questions are likely to persist for many years.

Conclusion

Mitochondrial transfer remains a plausible experimental intervention for ischemic injury, cancer, and other settings of mitochondrial dysfunction, but the net effect is context-dependent: antiproliferative in some tumor models, supportive or anti-inflammatory in cardiomyoblasts treated together with angiotensin II, and not uniformly restorative after toxic injury. Standardized isolation, dosing, and delivery protocols are still lacking, and the mechanism of any benefit is incompletely defined. Mitochondrial donation in assisted reproduction for women with pathogenic mtDNA raises a distinct ethical problem. These limitations do not erase the experimental promise of the field; they define the work that must precede clinical generalization.

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