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Nicotine pouches, oral epithelium, histopathology, oral potentially malignant disorders, in-vitro, *in vivo*.

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Nicotine Pouches and Oral Mucosal Health: A Review of Cellular, Histopathological, and Potentially Malignant Changes

Abstract
Nicotine pouches (NPs) have seen a swift rise in adoption as tobacco-free nicotine delivery mechanisms, often marketed as harm-reduction substitutes for combustible tobacco. While their global prevalence continues to grow, the enduring impacts of NPs on oral mucosal health are still not fully understood. Preliminary experimental findings from both *in vitro* and *in vivo* investigations suggest that nicotine exposure, irrespective of combustion-related toxic substances, can trigger considerable cellular dysregulation and structural modifications within the oral epithelium. This review critically evaluates the current evidence regarding NP-related cellular alterations, histopathological changes, and their potential contribution to the emergence of oral potentially malignant disorders (OPMDs). Furthermore, the review examines the underlying biological processes, contrasts the oral effects of NPs with those of other nicotine delivery systems, and identifies significant deficiencies in the current body of research. A thorough comprehension of these impacts is crucial for the enhancement of clinical protocols and the formulation of public health strategies grounded in empirical evidence.

1. Introduction

Nicotine pouches (NPs) are tobacco-free, flavoured delivery products of nicotine as a pharmaceutical-grade product in the oral vestibule, which combines pharmaceutical-grade nicotine, stabilizers and alkalizing agents.[1] In contrast to conventional smokeless tobacco products, NPs are not made of tobacco leaf material and thus can be commonly promoted as being cleaner and having lower exposure to toxicants. The marketability and distribution of these products throughout the world in the past few years have been on rapid growth. The NP use of NPs is promoted under the harm-reduction models, and in the last five years, their prevalence has risen significantly among young adults and former smokers who seek the use of NP products as substitutes for combustible cigarettes and traditional oral tobacco.[2] These products partially decrease the exposure to tobacco-specific nitrosamines (TSNAs) and combustion-based toxicants; however, they cannot be considered biologically inert. Their popularity creates significant issues concerning their effects on oral tissues in the long term, particularly with the oral cavity being the major location of placement and exposure.

The first biological barrier that is exposed to nicotine pouch constituents is the oral mucosa. Direct absorption through epithelial tissues causes a direct delivery to the system, thus, at a high speed. Nevertheless, constant and prolonged use of the same intraoral location leads to consecutive local chemical contact, mechanical friction, and mucosal irritation. Notably, most NPs are developed at high alkalinity (pH > 8.5) to maximise absorption of free-base nicotine, this latter quality being itself a potential chronic chemical irritant and a contributor to focal mucosal alterations also known as nicotine pouch keratosis.[3,4] Such lesions can be clinically expressed as white thickened formations or localised epithelial changes that cast doubt as to whether the

changes are reversible irritation or a dysplastic change that is at its early stage. These mucosal alterations are Besides nicotine and alkalising agents, NPs often have flavouring additives that are meant to enhance the appeal of consumers. Specific flavouring agents, such as menthol and cinnamaldehyde, have been shown to exert an independent cytotoxic and pro-inflammatory activity on oral soft tissues, such as epithelial and gingival fibroblasts.[5] These additives could enhance mucosal damage through augmenting oxidative stress, the release of inflammatory mediators, and the disturbance of epithelial barrier stratification. In this way, the effect of NPs even in the absence of tobacco-derived carcinogens can have biologically relevant effects via both chemical irritation and inflammatory responses.

Oral potentially malignant diseases (OPMDs) are a clinically relevant category of mucosal changes that have been demonstrated to have a high risk of developing oral squamous cell carcinoma (OSCC).[6] The conditions which are classified as OPMDs include the leukoplakia, the erythroplakia, and the oral epithelial dysplasia, which are known to be early stages in the multistep carcinogenic process. Since NPs work periodically in direct contact with the oral mucosa, the risk of chronic exposure to the development of OPMD should be examined closely. Although NP is considered to be safer than smoking, the oral cavity can still record local effects which are not detected by systemic toxicant measures.

On a molecular level, nicotine has mitogenic and anti-apoptotic effects, oxidative stress enhancement via reactive oxygen species (ROS) production and plays a role in pro-inflammatory microenvironment formation processes that play a role in epithelial dysregulation and dysplastic transformation.[7] Nicotine is able to bind to nicotinic acetylcholine receptors (nAChRs) on epithelial cells and induce pathways that mediate proliferation, angiogenesis, and survival. Continuous oxidative stress and chronic inflammation can further generate environments that are conducive to genomic instability and malignant evolution. Nicotine alone is not considered to be a direct carcinogen, but its tumour-promoting activity and capacity to stimulate cellular growth indicate a higher level of evaluation should be done in the context of chronic exposure to the mucosa.

Although the use of NPs is increasing rapidly, there is a lack of robust longitudinal human data to assess their carcinogenicity. The existing data are mostly based on short-term observational research, case studies, and *in vitro* experimental studies. The gap in the research is huge on whether NP-associated mucosal lesions are temporary and reversible or whether they are early precancerous changes with clinical implications in the long term. In addition, the majority of available research is on the detection of systemic harm reduction relative to cigarettes, whereas the local mucosal results in oral regions are not fully studied. There are also no standardised diagnostic criteria for nicotine pouch keratosis, and there are no long-term follow-up studies, which makes it even more difficult to assess the risk.

To this end, there exists an acute necessity to combine the upcoming experimental and clinical data to resolve the biological effects of NPs on oral epithelial tissues. long-term in nature, and thus their persistence is not well comprehended.

Knowledge of the cellular, histopathological, and mechanistic pathways that are involved is crucial to dental practitioners, oral pathologists, and other regulators of public health to ascertain whether such products hold previously underestimated oral health risks.

Therefore, the objective of this narrative review is to integrate current evidence regarding nicotine pouch-related oral mucosal alterations, with a specific focus on:

(i) Cellular and molecular alterations identified in *in vitro* models;

(ii) Histopathological changes observed in *in vivo* studies; and

(iii) Mechanistic pathways linking nicotine exposure, mucosal injury, and early malignant transformation.

By highlighting existing knowledge gaps and future research priorities, this review aims to provide a clearer understanding of the potential oral health consequences associated with the growing use of nicotine pouches.

2. Biological Basis of Nicotine-Oral Mucosal Interaction

Nicotine has a biological action by activating nicotinic acetylcholine receptors (nAChR) that are present on oral keratinocytes, fibroblasts, endothelial cells, and immune cells.[8,9] The non-neuronal nAChRs control such important processes as epithelial proliferation, differentiation, inflammatory signalling, and angiogenesis and therefore can directly impact oral mucosal homeostasis.

2.1 Oxidative Stress and Genomic Instability

Cross-sectional *in vitro* evidence invariably reveals that exposure to nicotine increases the levels of intracellular ROS in oral epithelial cells that leads to oxidative DNA damage, lipid peroxidation, and oxidative protein damage.[10,11] Prolonged oxidative stress impairs DNA repair, causing genomic instability, a primary and crucial initial event in OPMD pathogenesis.[12]

2.2 Inflammatory Signalling

The effect is that nicotine induces pro-inflammatory cytokines (IL-6, TNF- a, and IL-1 beta) production via the activities of NF- KB- dependant pathways.[13,14] Low-grade chronic inflammation impairs epithelial homeostasis and promotes dysplastic development in tissues that have high cellular turnover, like the oral mucosa.

2.3 Dysregulation of Proliferation, Apoptosis, and Angiogenesis

Experimental models have shown that nicotine stimulates the growth of the basal cells as well as the suppression of apoptosis, and thus genetically altered cells survive.[15,16] Simultaneously increasing the endothelial growth factor (VEGF) promotes angiogenesis and tissue remodelling, which forms a microenvironment that facilitates the development of dysplastic and neoplastic transformation.[17,18]

The activation of nicotinic acetylcholine receptors (nAChRs) is highlighted by a mechanistic overview of nicotine-oral mucosa interactions, as shown in Figure 1. It describes low stream biological impacts like oxidative stress, discharge of inflammatory cytokines, proliferative imbalance, and angiogenesis. Altogether, the figure outlines the role of these pathways that can cause epithelial dysplasia and early malignant transformation.

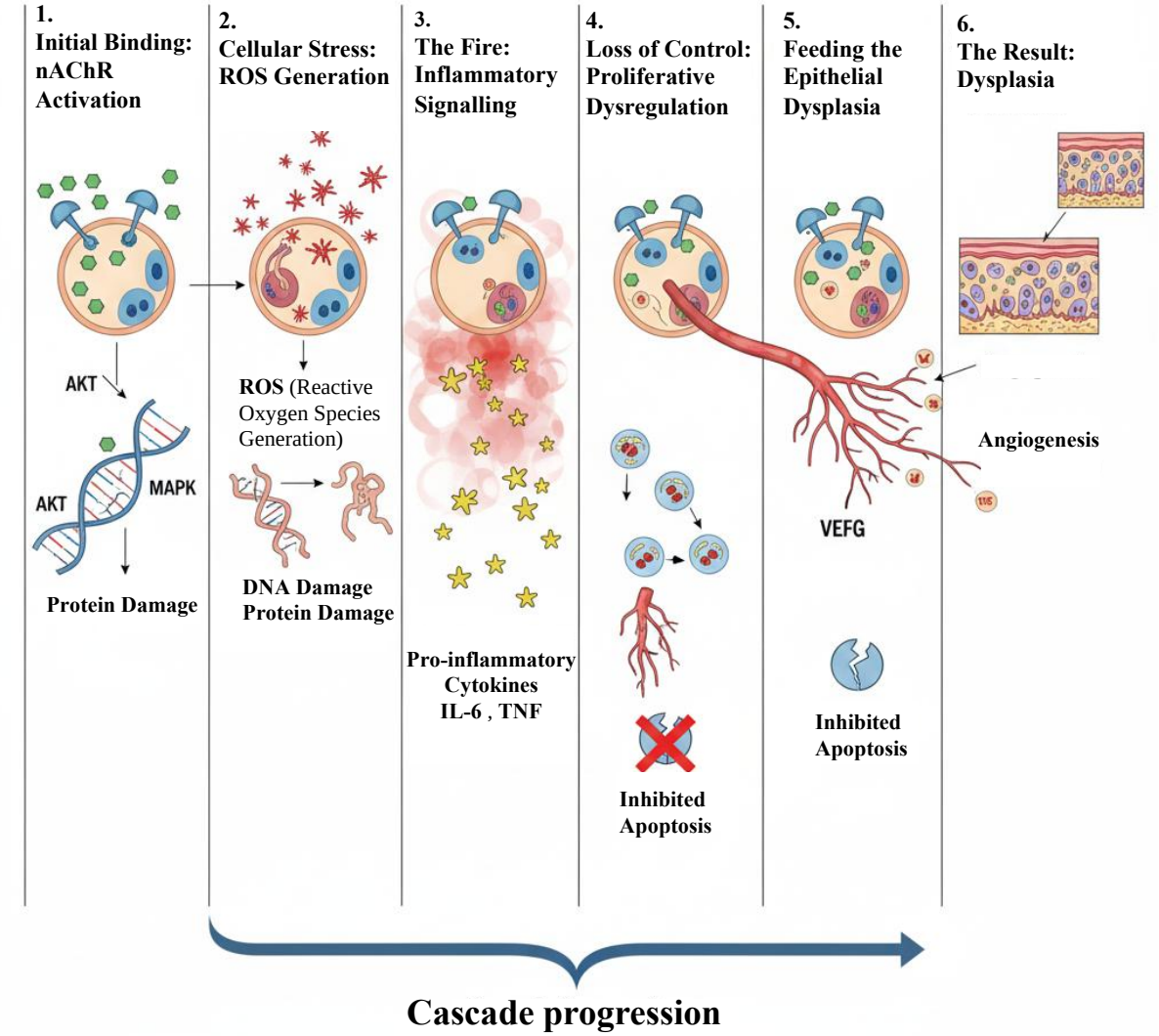

Figure 1: Mechanistic overview of nicotine-oral mucosa interaction illustrating nAChR activation, ROS generation, inflammatory signalling, proliferative dysregulation, angiogenesis, and progression toward epithelial dysplasia.

Table 1 provides an overview of the key biological processes by which nicotine influences oral tissues, which are oxidative stress, inflammation, imbalance in apoptosis, and angiogenesis. It connects outcomes of the cells, like DNA damage and cytokine upregulation, to clinical outcomes such as dysplasia and malignant progression. The following table gives a brief outline of how nicotine may lead to mucosal pathology.

Table 1. Mechanisms of Nicotine-Induced Oral Mucosal Alterations

Mechanism	Cellular Effect	Key Evidence	Clinical Relevance
Oxidative stress	DNA damage, lipid peroxidation	*In vitro* oral keratinocyte studies	Genomic instability, dysplasia
Inflammation	↑ IL-6, TNF-α, IL-1β	Animal and human studies	Chronic mucosal inflammation
Proliferation/apoptosis imbalance	↑ Proliferation, ↓ apoptosis	Cell culture and *in vivo* models	OPMD initiation
Angiogenesis	↑ VEGF, neovascularisation	Endothelial and tumor models	Malignant progression

3. *In vitro* Cellular Alterations Induced by Nicotine Pouches (NPs)

In vitro culture models that allow isolating specific cellular reactions to nicotine and flavouring additives are essential in the preliminary assessment of the toxicological profile of NPs. The predominant cells that are utilised in the current studies to replicate the localised effects of NP constituents include human gingival fibroblasts (HGFs), oral epithelial cells (OECs), and periodontal ligament fibroblasts.

A key discovery of the recent studies is the fact that NPs induce dose-dependent cytotoxicity and oxidative stress, even though they do not present the heavy metal and combustion-generated profile typical of the conventional tobacco. Reactive oxygen species (ROS) are generally overexpressed, and they may lead to the splitting of DNA and the hindering of cell repair mechanisms.[19] In addition, it has also been shown that the alkaline environment induced by NPs to enhance the penetration of nicotine alters cellular viability irrespective of the concentration of nicotine.

3.1 Comparative Analysis of *In vitro* Evidence

The most notable experimental *in vitro* studies assessing the cellular toxicity induced by nicotine in the functions of gingival fibroblasts, keratinocytes, and epithelial cells are summarised in Table 2. Results are always consistent, showing an increase in ROS, cytokine induction, apoptosis regulation, and MAPK activation. It shows dose-dependent cellular stress reaction to early mucosal dysregulation.

Table 2: Summary of *In vitro* Studies on Oral Cellular Responses to NPs

Author's	Cell Type	Nicotine Exposure	Key Findings & Molecular Outcomes
Kang et al.[20]	Human Gingival Fibroblasts	Nicotine (various concentrations)	Decreased viability; increased ROS, caspase-3 activation, and apoptosis via MAPK pathways.
Kashiwagi et al.[21]	Human Gingival Epithelial Cells	1×10^{-3} M Nicotine ± LPS	Nicotine enhanced IL-8 expression via ERK signalling and nAChR activation.
Johnson et al.[22]	Human Gingival Keratinocytes	1 μM - 1 mM Nicotine	Nicotine modulated IL-1α and IL-8 production, especially with LPS.
Argentin & Cicchetti[23]	Human Gingival Fibroblasts	~1 μM Nicotine	Increased ROS and genotoxic effects; attenuated apoptosis in some contexts.
Nguyen et al.[24]	Human Periodontal Ligament Fibroblasts	Nicotine + *P. gingivalis* lysate	Enhanced ROS production and oxidative stress biomarkers.

3.2 Mechanistic Insights: From Exposure to Dysplasia

In vivo information shows that the disturbance of the oral epithelial barrier triggers the emergence of a potentially malignant phenotype. Exposure to high levels of nicotine, particularly in its form as nicotine products (NPs), has been shown to initiate the epithelial-mesenchymal transition (EMT) characterised by the increased expression of mesenchymal markers, such as vimentin, and the reduced expression of epithelial markers, such as E-cadherin.[25,26] It is generally accepted that loss of epithelial integrity and augmentation of cellular motility are a pivotal initial circumstance in oral dysplasia and carcinogenesis.[27]

Moreover, chronic inflammatory signalling pathways, including nuclear factor kappa B (NF-κB) and tumour necrosis factor-alpha (TNF-α), apparently triggered by long-term exposure to nicotine, have been associated with malignant transformation.[28] The stimulation of these pathways supports the oncogenic change and epithelial malregulation through amplifying oxidative stress, establishing a pro-inflammatory environment, and disrupting the normal regulation of cell cycles.[29] Taken together, these molecular alterations provide a biologically plausible explanation of the clinical development of oral potentially malignant disorders (OPMDs) in individuals who use non-pharmacological NPs in the long run.[30]

4. *In vivo* and Animal Studies

Localised histopathological changes of prolonged nicotine pouches (NPs) use on the oral mucosa have been studied widely with the help of animal models, particularly Syrian golden hamsters and rabbits. These models provide valuable information on the tissue-level responses of long-term nicotine exposure and are very similar to the human oral epithelial responses. In line with a recent experimental study, high alkalinity of commercially available NPs (median pH approximately 8.8) has a significant effect on mucosal irritation, epithelial stress and the ensuing structural remodelling at the site of placement.[31,32]

Key Histopathological Findings

A multiplicity of histopathological alterations have been associated with long-term exposure to NP, and reflect the chronic responses of the epithelium or sub-epithelium to chronic chemical insult:

- **Acanthosis and Hyperkeratosis**: Acanthosis occurs due to the marked proliferation of stratum spinosum, and is usually accompanied by hyperkeratosis or parakeratosis and may have a characteristic wavy or chevron-like surface appearance.[33,34]
- **Basal Cell Hyperplasia**: Acanthosis occurs due to the marked proliferation of stratum spinosum, and is usually accompanied by hyperkeratosis or parakeratosis

and may have a characteristic wavy or chevron-like surface appearance.[35,36]

- **Subepithelial Inflammatory Infiltration:** Subepithelial dense lymphocytic and macrophage infiltration in the lamina propria is an effect of the chronic inflammatory response that is caused by continuous exposure to NPs, which signifies the presence of immune activation at the point of contact.[36]
- **Localised Vascular Changes:** Local tissue injury and inflammation-mediated increases in vascular permeability may result in histopathological changes, including epithelial spongiosis (intercellular oedema) and endothelial cell swelling with dilatation of superficial capillaries within the lamina propria.[37]

All these findings indicate that there is a trend of dose- and time-dependent histopathological progression that includes the early dysplastic changes in long-term exposure environments and adaptive changes that are reversible.

Table 3 summarises the histopathological changes of the oral mucosa following exposure to nicotine pouches in the short and long-term. It characterises the epithelial hyperplasia, hyperkeratosis, inflammatory infiltration and sometimes dysplastic atypia with increasing chronic exposure models. This table strengthens the fact that mucosal injury and remodelling are time-dependent.

Table 3. Histopathological Progression in Oral Mucosa Following Nicotine Pouch Exposure

Parameter	Control	Short-term Exposure (<4 weeks)	Long-term Exposure (>12 weeks)
Epithelial Architecture	Normal stratification	Mild acanthosis; intraepithelial oedema	Marked epithelial hyperplasia; elongated and anastomosing rete ridges
Keratinization	Thin, orthokeratotic layer	Mild parakeratosis with whitish surface film	Extensive hyperkeratosis with leathery plaque formation
Inflammatory Profile	Minimal or absent	Moderate lymphocytic infiltration	Dense, chronic subepithelial inflammatory infiltrate
Cellular Atypia	Absent	Rare reactive cellular changes	Occasional focal epithelial dysplasia with nuclear hyperchromasia

5. Nicotine Pouches and Oral Potentially Malignant Disorders (OPMDs)

Although the nicotine pouches (NPs) have commonly been marketed as a harm-reduction alternative to smoking harmful combustible tobacco, recent studies have shown that chronic use of NPs can be associated with oral mucosal changes that are consistent with oral potentially malignant disorders (OPMDs). Recent case series and observational reports have reported the development of localised white lesions at locations of repeat NP placement as a so-called nicotine pouch keratosis.[38,39] The morphologies of these lesions have a high morphological overlap with oral leukoplakia clinically and histopathologically, with specific keratotic plaques and different levels of epithelial thickening.[40]

5.1. Mechanistic Drivers of Malignant Transformation

Several biological processes have been suggested in order to explain the possible development of nicotine pouch (NP)-related lesions into malignant transformation and dysplasia. Among the most outstanding issues is the occurrence of epithelial dysplasia, which involves enlargement of the nucleus, hyperchromasia, maturation of epithelial cells and a heightened mitosis. These changes can be caused by the chronic chemical irritation of the location where the pouch is placed, as well as the occurrence of nicotine-induced mitogenic stimulation, which increases the excessive proliferation of the epithelial cells. Histopathological studies have indicated that about 5-25% of biopsy samples of enduring NP-related lesions would bear different levels of epithelial dysplasia and indicate that a part of the lesions with persistent lesions have premalignant potential.[41,42] Moreover, with age, long-term Kerato-otic lesions have been demonstrated to exhibit loss of epithelial polarity and loss of normal stratification, which has been previously recognised as one of the hallmarks of early dysplastic transformation and indicative of impaired epithelial differentiation.[43] In addition to these structural alterations, the pro-oncogenic microenvironment can be created due to the chronic exposure to NP, which leads to the persistent activation of inflammatory pathways. High local expression of tumour necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6) leads to genomic instability and loss of DNA repair ability coupled with incurred oxidative stress, which predisposes to malignant progression.[44,45] Despite the fact that most NP-associated lesions related to the keratotic lesion demonstrate some partial or complete regression after discontinuation of the use of products, the presence of focal epithelial dysplasia is reported, which emphasises the necessity of clinical monitoring and surveillance over time. This issue is especially pertinent to heavy users, who are usually characterised by exceeding 10 NP units per day of usage over five years; cumulative exposure could raise oncogenic risk and the probability of permanent mucosal changes.[46]

6. Comparative Analysis: Nicotine Products and Oral Health

A comparative analysis reveals that there are some significant variations in the biological effects of nicotine delivery systems on tissues in the mouth. Even though Nicotine pouches (NPs) eliminate the carcinogens related to the combustion process, the local administration method has distinctive cellular and histological outcomes that must be considered.[47]

On the cellular level, NP exposure has been associated with high levels of reactive oxygen species (ROS), moderate levels of cellular cytotoxicity, and apoptosis activation in oral keratinocytes, particularly following long or high-dose exposure.[48] These changes are mainly manifested histopathologically as hyperkeratosis, acanthosis and sometimes in a few cases as focal epithelial dysplasia. This is equivalent to a dose-dependent moderate risk of oral potentially malignant disorders (OPMDs).[49]

On the contrary, conventional smokeless tobacco products show high levels of genotoxicity, cytotoxicity, and DNA adducts. These are histologically proven by the gross dysplasia of the epithelium and a population at substantial risk of malignant neoplasm.[50] Even the conventional cigarettes, which have a high ROS load but are also exposed to multiple carcinogens, lead to serious dysplasia, widespread keratosis, and a considerably high risk of oral cancer.[51]

Electronic cigarettes appear to be comparatively less cytotoxic and less inflammatory in the oral cavity, with only minimal-to-moderate changes recorded in histopathological observations, which are mostly low-to-moderate cytotoxicity and inflammatory responses. But recent studies show that there can be some moderate dangers to oral health with long-term use, particularly in people at risk.[52]

In general, it can be stated that, even though NPs can be a lower-risk alternative in comparison to the traditional smokeless tobacco, chronic exposure cannot be considered risk-free, at least when it comes to the development of OPMD.

7. Integrated Mechanistic Insights

Multi-biological pathways that interact in a cascade, producing nicotine-induced changes of the oral epithelium that favour a pro-dysplastic microenvironment. Nicotine exposure activates intracellular reactive oxygen species (ROS) production and causes oxidative DNA damage, lipid peroxidation and mitochondrial dysfunction, early phenomena in epithelial stress and genomic instability, in oral epithelial cells.[53] Simultaneously, nicotine triggers essential inflammatory signalling transduction, specifically, the NF- 00B and mitogen-activated protein kinase (MAPK) pathways, which leads to protracted cytokine release and permanent inflammatory impairment of epithelial homeostasis.[54] It is also known that Nicotine fosters the imbalance of cell proliferation and cell death by enhancing basal keratinocyte proliferation and suppressing programmed cell death, therefore leading to abnormal epithelial maturation and tissue thickening.[55] In addition, the angiogenesis, extracellular matrix remodelling and stromal support are augmented by the upregulation of vascular endothelial growth factor (VEGF), which provides the environment promoting early neoplastic progression.[56] These mechanisms are interconnected and constitute an integrated network of pathogenic processes assessing and associating chronic nicotine pouch exposure with epithelial dysregulation and the possibility of early oncogenic conversion.

Figure 2 offers a composite mechanistic model of the cellular and tissue-level effects of exposure to nicotine pouch on the oral mucosa. It links molecular changes, including ROS production and NF- 0 activation, to pathological events, including hyperkeratosis and dysplastic development. This number highlights the multi-pathway network that associates prolonged exposure to potentially cancerous alterations.

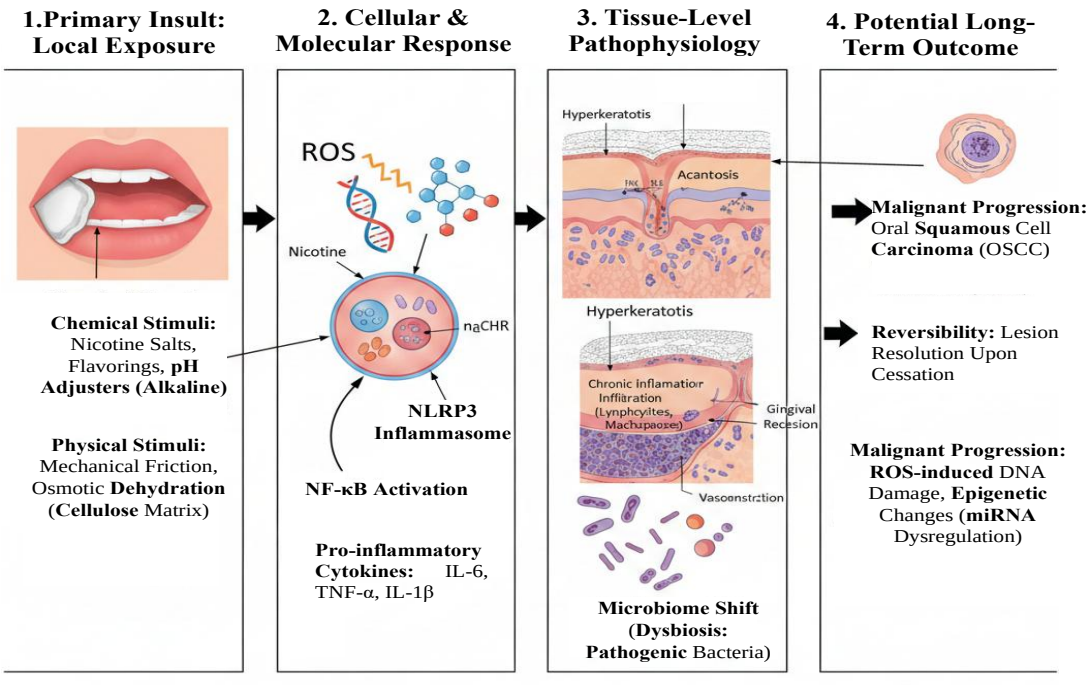

Figure 2. Integrated mechanistic model illustrating cellular, inflammatory, and tissue-level effects of nicotine pouch exposure on oral mucosa.

Table 4 sums up the molecular and cellular processes activated by exposure to nicotine pouches and correlates them with the clinical and histological manifestations of these processes. It identifies major dysregulatory mechanisms that include ROS formation, activation of NF-KB, the increase of inflammatory cytokines, stress responses of the keratinocytes, and the formation of lesions. In this table, the mechanistic events have been linked with the observable changes in the oral mucosa.

Table 4. Multilevel Mechanistic and Clinical Markers Associated with Nicotine Pouch-Induced Oral Mucosal Alterations

Level	Key Mechanisms	Clinical / Histological Markers
Molecular	ROS, NF-κB activation, miRNA dysregulation, elevated IL-6, TNF-α	Nuclear shrinkage, acantholysis
Cellular	Keratinocyte stress, fibroblast repression, vasoconstriction, dysbiosis	White "snus-like" lesions, gingival recession

8. Discussion and Future Perspectives

The recent proliferation of nicotine pouches as an easily sold product that is supposedly free of tobacco requires a review of their oral health consequences to be critically evaluated. Even though these products commonly feature as contemporary harm-reduction products, especially since they contain reduced or no tobacco-specific nitrosamines (TSNAs), the overall evidence examined in this section suggests that nicotine pouches (NPs) are not biologically neutral.[57] Its growing popularity across the world, particularly among youth and people who have been looking for a substitute to combustible tobacco, has been faster than its strong clinical evidence on their safety in the long run. Although the toxicant load in NPs is considerably lower than that of cigarettes or even the traditional smokeless tobacco, the localised character of nicotine delivery creates certain biological and histopathological issues that should be given special attention.

Nicotine pouches, in contrast to inhaled nicotine products, get into direct contact with the oral mucosa, which leads to an uninterrupted exposure of epithelial tissues to nicotine, alkalizing agents, and flavouring additives. This repetitive site-specific exposure can potentially favour chronic mucosal irritation, epithelial remodelling and activation of inflammation. Notably, mucosal tissues of the oral cavity are the most sensitive, and cellular remodelling occurs promptly, and the latter tissue becomes especially susceptible to chronic chemical damage. New clinical findings of focal keratotic lesions in the sites of placement of the pouch further heighten the need to be more vigilant and mechanistic. These results dispel the notion that being "tobacco-free" only translates into being risk-free and therefore show the need to consider oral health outcomes and not just the harm-reduction measures using a systemic harm-reduction level.

8.1 Impact of pH and the Local Oral Microenvironment

Product alkalinity is one of the strongest factors that influence NP-associated mucosal changes. The high pH, which was specifically developed to make nicotine bioactive and bioavailable in free-base form, seems to be a chronic chemical irritant that has the independent effects of causing localised hyperkeratotic lesions commonly referred to as nicotine pouch keratosis.[58] Numerous products on the market exhibit a pH of greater than 8.5, and this alkaline microenvironment is sufficient to break epithelial barrier integrity and cause adaptive thickening of the keratin layer.

Nicotine pouch keratosis can be clinically seen as whitish, thickened plaques where the pouch is repeatedly placed. Even though these lesions often disappear after the use is discontinued, their presence leaves a structural and biological platform that can potentially predetermine the development of dysplastic progression with ongoing exposure.[59] The irritable persistent effect caused by the alkaline compounds can also enhance the release of inflammatory cytokines, oxidative stress, and epithelial growth, which are components of the aetiology of oral potentially malignant disorders (OPMDs). Moreover, the hypothesis is that the local pH adjustment can impact the oral microbiome, which can be followed by dysbiosis and indirect inflammatory outcomes. These tissue-level effects of nicotine infection underline the necessity to look beyond nicotine effects as the cause of mucosal pathology, and also the formulation chemistry of pouches as a major cause of mucosal pathology.

Moreover, repetitive mechanical trauma due to prolonged location of pouches in the same area of the oral vestibule can result in a compounding effect of the effects of chemical irritation. This alkalinity, frictional stress, and inflammatory activation can eventually lead to epithelial remodelling that is similar to that observed in early leukoplakic or dysplastic changes. Therefore, the oral microenvironment is a critical contact point in which NP exposure can have long-term pathological effects that have not been adequately defined.

8.2 Gaps in Current Knowledge

Although there are more and more emerging experimental and clinical observations, there are a number of gaps in knowledge that are critical. Commercialisation of nicotine pouches has become significantly faster than the development of long-term epidemiological evidence, so long-term oral effects of nicotine pouches are still largely unclear.

- Lack of Longitudinal Human Studies: There is an absence of 10-20-year prospective studies evaluating malignant transformation rates of NP-associated oral

lesions. Most available evidence is derived from short-term case series, observational reports, and experimental models, which cannot fully establish the long-term carcinogenic risk profile.

- Synergistic Risk Factors: The combined effects of NP use with alcohol consumption, human papillomavirus (HPV) infection, periodontal disease, or genetic susceptibility are poorly characterised. Such co-factors are well-established contributors to oral carcinogenesis, and their interaction with chronic nicotine pouch exposure remains an important unanswered question.
- Flavouring Agent Toxicity: The long-term impact of flavouring additives on oral epithelial cells, the oral microbiome, and gingival health requires detailed molecular and toxicological investigation. Certain compounds, such as menthol and cinnamaldehyde, have demonstrated cytotoxic and pro-inflammatory potential in experimental studies, yet their cumulative effects in chronic users remain unknown.

In addition to these gaps, there are no standard diagnostic criteria for nicotine pouch keratosa and as such, comparisons of results between studies are hard to make, and so no clear guidelines for clinical management can be made. In addition, the severity of NP-associated lesions, such as reversible irritation or early premalignant change, is still debatable. The need to address these gaps is the necessity to properly define the long-term safety profile of NPs and use evidence-based regulatory and clinical recommendations.

In subsequent studies, a large-scale cohort study, molecular diagnostic markers and an extended history of histopathological monitoring will be essential in establishing whether nicotine pouches are indeed a reduced-risk product or a new cause of oral mucosal pathology.

9. Conclusion

Nicotine pouches present a multifaceted and dynamic issue of oral medicine and population health. This review illustrates that the range of biological responses to NP use is quite broad, involving oxidative stress and inflammatory signalling, to epithelial acanthosis, hyperkeratosis, and dysplastic transformation in some instances. There is no doubt that the overall risk of carcinogenesis is lower than with classical smokeless tobacco products, but the amount of evidence concerning the role of NPs as the possible cause of oral potentially malignant disorders is growing, particularly under the impact of chronic and high-frequency exposure. This localised administration of nicotine, with product alkalinity and flavouring additives, generates a local environment that has the potential to cause mucosal irritation, epithelial remodelling, and pro-inflammatory dysregulation. Clinicians must be very suspicious when assessing persistent white lesions or keratotic plaques in the oral vestibule of the NP users. Clinical surveillance and patient counseling along with non-resolving lesion biopsy, should be carried out frequently so that dysplastic change is spotted early. Notably, the tone of the communication regarding health to the population should change to underline that the absence of tobacco does not mean the absence of danger, and the decreased levels of systemic toxicants do not exclude the presence of localised damage in the mouth. There is an urgent need to have robust longitudinal human studies, standardised diagnostic criteria and mechanistic biomarker-based research to demystify the long-term potential malignant transformation of the lesions with NP involvement. This evidence will be instrumental in the formulation of informed clinical guidelines and proper regulatory policies regarding the use of nicotine pouches.

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Consent for Publication

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Competing Interests

The authors declare that they have no competing interests.

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