Article Type: Research Article

Journal of Neurology and Neurological Sciences

Minimally invasive diagnostics of alzheimer's disease. The potential of using buccal cells

Alena Sidenkova*
Ural State Medical University, Yekaterinburg, Russia.

***Corresponding author:** **Alena Sidenkova**
Ural State Medical University, Yekaterinburg, Russia.
Email: sidenkovs@mail.ru

Received: Dec 10, 2025; **Accepted:** Feb 25, 2026;
Published: Mar 06, 2026

Journal of Neurology and Neurological Sciences
Volume 2 Issue 1 - 2026
www.jnans.org

Citation: Sidenkova A. Minimally invasive diagnostics of alzheimer's disease. The potential of using buccal cells. J Neurol Neuro Sci. 2026; 2(1): 1015.

Abstract

The high prevalence of Alzheimer's disease necessitates large-scale and affordable screening using minimally invasive biomarkers. This review aims to determine the potential value of buccal epithelium as a noninvasive biomarker of neurodenegeneration to further develop an accessible diagnostic tool for the early detection of Alzheimer's disease.

Materials and methods: A full-text search of publications in the PubMed medical publication database for the period from 2000 to 2025 was conducted using the keywords "buccal cell" and "Alzheimer's disease." Meta-analyses, original articles, and analytical reviews containing the outcomes of patients with Alzheimer's disease diagnosed according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 5th edition, and the NINCDS-AD&DA criteria (2018, 2024) were analyzed.

Results: Buccal epithelial cells and cells of the central nervous system undergo changes during the aging process. Changes observed in buccal epithelial tissue in patients with Alzheimer's disease and mild cognitive decline differ from age-related changes in individuals with normal aging. In pathologically aging, buccal epithelial cells exhibit elevated concentrations of phosphorylated proteins, abnormal nuclear- cytoplasmic ratios, and altered telomere length. The susceptibility of buccal epithelial cells to degenerative changes, which correlates with the stages and severity of Alzheimer's disease, the involvement of buccal epithelium in the mechanisms of this disease, and the shared embryogenetic origin of buccal epithelial cells and hippocampal cells from the ectodermal germ layer suggest that peripheral buccal epithelial tissue may be a potential marker of neurodegeneration. The relevance of studying buccal epithelium as a component of the laboratory-clinical complex for the early diagnosis of Alzheimer's disease, along with indicators of cognitive processes and the results of neuroimaging studies, is due to the simplicity, non-invasiveness and economic availability of obtaining this biomaterial.

Keywords: Buccal cell; Alzheimer's disease; Minimally invasive diagnostics.

Abbreviations: NINCDS-ADRDA: National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association; IWG: International Working Group; NIA-AA: National Institute on Aging and the Alzheimer's Association; PET: Positron Emission Tomography; Aβ: Amyloid-Beta p-tau-tau Protein; MRI: Magnetic Resonance Imaging; FDG: F-2-fluoro-2-deoxy-D-glucose; CSF: Cerebrospinal Fluid; MCI: Mild Cognitive Impairment; BE: Buccal Epithelium; SD: Vascular Dementia; ELISA: Enzyme-Linked Immunosorbent Assay; LSC: Laser Scanning Cytometry; Leukocytes: White Blood Cells; PCR: Polymerase Chain Reaction; ORF: Oral Fluid; MN: MicroNucleus – Micronucleus; PD: Parkinson's Disease; ASD: Autism Spectrum Disorder; MMSE: Mini-Mental State Examination.

Introduction

The medical and social significance of Alzheimer's Disease (AD), resulting in its high prevalence and severe public health and family history, necessitates early diagnosis. According to the World Health Organization's annual report (2025), more than 57 million people worldwide are registered with dementia, the majority of whom suffer from Alzheimer's disease. According to the same report, approximately 1.85 million people in the Russian Federation suffer from dementia [1].

Periodic updates to research and similar AD-related topics reflect evolving scientific understanding of the nature of this entity. Thus, the concept of AD in the early to mid-1980s was

associated with its outcome and the designation of dementia, as specified in the 1984 diagnostic criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA). According to these criteria, the precise diagnosis of AD developed from manifestations of severe cognitive deficit and indicated that the diagnosis cannot be established using laboratory tests [2].

In 2007, an amendment to the current NINCDS-ADRDA was presented by the International Working Group (IWG), which for the first time proposed a view of AD as a clinical and biological entity based on differential differences in phenotypes and in vivo biomarkers. This expanded the definition of AD to include prodromal (pre-dementia) stages [3], paving the way for earlier, pre-dementia diagnosis of AD.

In 2010, the IWG introduced a classification of pre-symptomatic stages into the AD glossary, including an asymptomatic stage at risk for individuals with biomarker evidence based on AD and a pre-symptomatic stage for individuals with monogenic AD mutations [4].

In 2011, the National Institute on Aging-Alzheimer's Association (NIA-AA) criteria defined three distinct pre-clinical stages using the amyloid cascade hypothesis: the first is amyloid lesions, the second is tau pathology causing neurodegeneration, and the third is the occurrence of minor cognitive changes [5-7].

In 2016, the IWG and NIA–AA consensus expanded the research criteria for Alzheimer's disease to include AD diagnosed at the preclinical stage based on in vivo amyloid β-tau positivity, indicating a high risk of progression to disease [8].

In 2018, a shift occurred from clinical and clinical-biological diagnostics to a biological indicator of AD, which was reflected in the 2018 NIA-AA diagnostic criteria, which underlie the search for biomarkers: Amyloid-β (Aβ), tau protein (p-tau), and neurodegeneration (A/T/N). This makes it possible to determine Alzheimer's disease upon detection of abnormal biomarkers of Aβ and tau positivity (amyloid-positive and tau-positive), regardless of international cognitive symptoms [9].

The Revised Criteria for the Diagnosis and Staging of Alzheimer's Disease (2024) reflect current concepts that AD is a continuous biological process that begins with the manifestation of pathological changes in the brain, long before the onset of clinical symptoms. Early biomarkers of AD include the wear of amyloid deposits detected by assessing the results of Positron Emission Tomography of the brain (PET); Aβ 42/40, p-tau 181/Aβ 42, p-tau/Aβ 42, detected in the cerebrospinal fluid; phosphorylated p-tau 217, which is found in blood plasma. In addition, the integrated scheme of biological and clinical staging of AD observes comorbidity and cognitive reserve, which can modify the relationship between the biological and clinical stages of AD [10,11].

The possibility of early preclinical diagnosis of asthma allows for the formation of a risk group for development, which determines the need for regular screening, identifying the disease before the first symptoms appear, i.e., searching for available and diagnostically effective markers of the body.

Modern intravital diagnosis of AD can be divided into two categories: brain imaging and liquid biopsy. Brain imaging (structural Magnetic Resonance Imaging (MRI), functional MRI, 18F-2- Fluoro-2-Deoxy-D-Glucose (FDG) PET, and amyloid-PET) plays a pivotal role in diagnosis be- cause neurodegeneration often parallels or precedes cognitive decline, which is a symptom of AD. Structural or coma abnormalities can be captured by MRI scanning, while FDG-PET tracks glucose positioning mechanisms for the area of decreased brain activity. Of the various imaging modalities, amyloid-PET is the most reliable diagnostic imaging technique due to its ability to detect aggregated amyloid-β in the brain. The high cost of imaging diagnostics and the potential for its widespread use [12].

Cerebrospinal Fluid (CSF), which constantly circulates in the ventricles of the brain, cerebrospinal fluid pathways, and the subarachnoid space of the brain and spinal cord, has been proven to be a diagnostically significant biomarker of AD, but the invasive nature of collecting CSF biosamples limits the possibility of widespread use of this method [13,14].

Previously, proteinopathies were considered to be exclusively CNS-specific, but recent studies have demonstrated the presence of tau and amyloidopathy in peripheral tissues, suggesting a systemic nature of proteinopathies [15-17]. The use of blood bioassays and minimally invasive tissue samples containing neurodegeneration biomarkers is expected to enable large- scale, inexpensive screening and risk stratification for AD development. This supports interest in studying peripheral tau for their involvement in normal aging and AD.

Thus, the relevance of searching for a minimally invasive biological marker for AD underlies modern concepts of the body as a continuous process, including a pre-phenomenological stage at which biomarker diagnostics are possible. Given the general prevalence and medical and social significance of AD, predicting the risk of its development is clearly necessary within the overall framework of healthcare and social service resource planning.

This review aims to determine the potential value of buccal epithelium as a noninvasive biomarker of neurodenegeneration to further develop an accessible diagnostic tool for the early detection of Alzheimer's disease.

Materials and methods

A full-text search was conducted in the PubMed medical publications database from 2000 to 2025 using the keywords: buccal cell, Alzheimer's disease, minimally invasive diagnostics.

Inclusion criteria:

1. Full-text publications (meta-analyses, original studies, reviews);
2. Publications contain descriptions, analyses, or studies involving patients with AD diagnosed according to the criteria of the Diagnostic and Statistical Manual of Diseases, 5th edition (DSM- 5), and NINCDS–AD&DA (2018, 2024).

Non-inclusion criteria:

1. The diagnostics of the study participants included in the publication were not verified according to DSM-V requirements.

As a result, keywords were identified, in accordance with the inclusion/exclusion criteria and a 25-year search depth of scientific publications for keywords in the Russian-language medical database PubMed. 14 full-text publications were found, the analysis of which forms the basis of this descriptive review.

Table 1: Buccal epithelium as a material for research of Alzheimer's patients.

Author	Purpose of the study	Study characteristics	Scope and characteristics of the sample. Research biomaterial. Research methods.	Research results
Hattori H. et al., 2002 [18]	Determination of protein levels in oral epithelial cells in AD	Comparative study of tau protein content in oral epithelial cells in patients with AD, vascular dementia and healthy controls	The patients were male. 11 patients had AD, 29 had vascular dementia (VD). The average age of the study group was 74.8 years. The control group consisted of 33 "young" cognitively healthy men (aged under 50 years), and 34 "older" control subjects (aged over 50 years). The average age of the control group participants was 55.1 years. Biomaterial: exfoliated oral epithelial cells. Research methods: Western blot to determine the molecular weight of oral tau protein. Enzyme-linked immunosorbent assay (ELISA) to determine tau protein concentration in CSF. Comparison of tau protein content in CSF and buccal epithelial cells.	Tau protein levels in oral epithelium were positively correlated with CSF levels ($p<0.05$). Patients with Alzheimer's disease had significantly higher tau protein levels than patients with vascular dementia and healthy controls ($p<0.01$). Young patients with Alzheimer's disease had higher baseline tau protein levels than older patients ($p<0.05$).
Thomas P. et al., 2007 [23]	Determination of the ratio of buccal cell populations and micronuclei in AD	Comparative study of buccal cells and micronuclei in patients with AD and healthy controls	Study groups: Main group: 54 patients with AD-associated dementia (mean age 76.9 years); Comparison group: 56 cognitively healthy individuals (mean age 68.7 years). Biomaterial: buccal epithelium. Research methods: AD cytological analysis.	The increase in the frequency of basal cells ($P<0.0001$), condensed chromatin cells ($P<0.0001$), and karyorrhexigenic cells ($P<0.0001$) is significantly lower in patients with AD. The odds ratio for diagnosing AD in individuals with a frequency of basal cells and karyorrhexigenic cells <41 per 1000 cells is 140, with a specificity of 97% and a sensitivity of 82%.
Thomas P. O. et al., 2008 [20]	In studying age-related changes in telomere length in leukocytes (WBCs) and buccal epithelium, the answer to the question of whether excessive telomere shortening is a common feature of leukocytes, buccal epithelium, and brain tissue in patients with AD, diagnosed with the disease or confirmed by histopathology, or in healthy age- and sex- matched controls, remains unknown.	A comparative study of the body length of leukocytes, cheek epithelium and hippocampus during aging and degeneration	Study groups: Main group – 54 participants with asthma (ages 58–93); comparison group: "younger group" – 30 participants (ages 18–26); "elderly group" – 26 partici- pants (ages 64–75). Biomaterial: buccal epithelial cells, white blood cells. Research methods: quantitative Polymerase Chain Reaction (PCR) for absolute measurement of telomere length in buccal epithelium, leukocytes, and the hippocampus.	The WBC body length ($P<0.0001$) and BE ($P<0.01$) were significantly shorter in AD compared to healthy individuals of the same age (31.4% and 32.3%, respectively). The hippocampal cells of the brain of patients with AD had a significantly longer body length ($P=0.01$) compared to the control samples (49.0%). The body length in buccal cells was 52.2–74.2% shorter than that of WBC ($P<0.0001$). The odds ratio for diagnosing AD was 10.8 (95% CI 1.19–97.85) if the WBC body length was less than 115 kbp, per diploid genome with a specificity of 46% and a sensitivity of 92.9%. The odds ratio for diagnosing AD was 4.6 (95% CI 1.22–17.16) if the buccal epithelial telomere length was less than 40 kb per did genome with a specificity of 72.7% and a specificity of 63.1%.
Fenech M. et al., 2011 [25]	Primary objectives: 1. To collect databases of baseline micronucleus (MN) frequencies and their interactions with methodological, demographic, genetic, and exposure variables; 2. To identify functions influencing MN pathways; 3. To develop standardized assay protocols for reliable comparison of data between laboratories and differences; 4. To assess the relationship of the MN cycle with future cross-sectional event outcomes.	The Human Micronucleus (HUMN (xL)) International Project(www.humn. org), found- ed in 2007 to serve as a coalition of global research trends, aims to use micronucleus (MN) assays to study contemporary DNA in buccal epithelial cells of human populations.	Currently, a database of over 7,000 subjects worldwide has been created for the HUMN (xL) project. Biomaterial: cytokinesis-blocked micronucleus (CBMN) assay in human peripheral blood lymphocytes and exfoliated buccal epithelial cells. Image analysis, flow cytometry, and laser scanning cytometry, standardization of Micronucleus (MN) counting protocols in buccal epithelial cells.	Future activities will focus on defining global concepts affecting the micronucleus cycle; validating various automated scoring systems based on other tissues, such as red blood cells and nasal cells; and prospectively investigating associations with pregnancy, developmental defects, childhood cancer, cardiovascular disease, and neurodegenerative diseases.

François M. et al., 2014 [19]	Development and validation of an automated high-potency laser scanning cytometry (LSC) protocol for assessing cytoplasmic, nuclear, and lipid parameters of buccal cells to identify individuals at increased risk of developing AD.	Comparative analysis of the ratios of buccal cell types, nuclear DNA content and neutral lipid content of buccal epithelia in BA, MCI and external controls	The study group consisted of 13 men and women with Alzheimer's disease (mean age 77.7 years) and 13 patients with Mild Cognitive Impairment (mean age 75.3 years). The control group consisted of 26 cognitively healthy individuals (mean age 76.1 years). Biomaterial: buccal epithelial cells. Study methods: Buccal Cell Count (BCC) to determine the ratio of buccal cell types, nu- clear DNA content and form, and neutral lipid content in buccal cells. Correlation analysis.	DNA content is significantly higher in all cell types in MCI ($p<0.01$) and AD ($p<0.05$) compared to controls, due to an increase in nuclei >2N. Abnormal nuclear shape (roundness) is significantly higher in transitional cells in MCI ($p<0.001$) and AD ($p<0.01$) compared to controls. Neutral lipid content (measured by Oilred O "ORO" staining) in buccal epithelium is significantly lower in the MCI group ($p<0.05$) compared to controls. DNA/ORO ratio in buccal epithelium is significantly higher in MCI and AD compared to controls, with values for MCI being approximately 2.8-fold higher ($p<0.01$) and for AD approximately 2.3-fold higher ($p<0.05$) than in controls. A strong negative correlation was found between buccal epithelial DNA content and ORO content in the AD group ($r(2)=0.75$, $P<0.0001$), but not in the MCI or control group.
Mathur S. et al., 2014 [29]	To study changes in the three-dimensional nuclear telomeric architecture of buccal cells from patients with AD depending on the severity of the disease based on five three-dimensional parameters: 1. Telomere length; 2. Telomere number; 3. Telomere aggregation; 4. Nuclear volume; 5. Telomere spatial distribution	Comparative analysis of telomeres of mucosal treated cells during aging and AD	Study groups: Main group: 41 patients with AD (mean age 75.8 years); comparison group: 41 healthy controls (mean age 74.3 years). Biomaterial: buccal epithelium Methods: Three-dimensional (3D) analysis of telomeres in buccal epithelial cells.	In healthy individuals matched for age, 3D telomere profiles were significantly different in shape in patients with mild, moderate, and severe AD ($p<0.0001$)
Thomas P. et al., 2015 [24]	To study the correlations between plasma concentrations of B vitamins and buccal biomarkers in AD	Comparative study of the structure of buccal epithelium and plasma concentration of B12 in patients with AD.	Study groups: the main group consisted of patients with AD; the comparison group consisted of cognitively healthy individuals. Biomaterial: buccal epithelium. Methods: Plasma vitamin B12 concentrations were determined using the ARCHI- TECT® and AxSYM® systems. Genomic stability was measured using buccal micronucleus cytome analysis.	AUC for AD tumor basal cells was 0.96 ($p<0.0001$), for karyorrhexic cells 0.88 ($p<0.0001$), and for basal and karyorrhexic cells 0.91 ($p<0.0001$). Plasma vitamin B12 in the control group showed a positive correlation with pyknosis ($r=0.5365$, $p=0.004$), karyolysis ($r=0.5447$, $p=0.004$), and condensed chromatin ($r=0.5238$, $p=0.006$). Plasma B12 level in AD patients showed a positive correlation with micronuclei ($r=0.3552$, $p=0.04$) and basal cells ($r=0.3448$, $p=0.04$).
François M. et al., 2016 [26]	To determine the typology of buccal epithelial cells taking into account the structure of nuclear DNA, the content of lipids and tau protein and Aβ in the buccal epithelium.	A comparative descriptive study of buccal cytome in aging and AD.	Study groups: main group – 20 patients with AD, 20 patients with MCI, comparison group – 20 healthy controls. Biomaterial: cells based on the results of the Australian Flagship Study of Aging in Imaging, Biomarkers, and Lifestyle. Methods: visual assessment of buccal cytomeme, laser scanning cytometry.	DNA content, aneuploidies, neutral lipids, and all other levels were consistent across all groups. Tau protein levels were significantly lower in basal and karyolytic buccal cell types compared to differentiated buccal cells. Aβ, dependent on the frequency bin containing the Aβ signal, as well as the area and integral of the Aβ signal, were significantly higher in the AD group compared to the control group. Buccal Aβ correlated with Mini-Mental State Examination (MMSE) scores ($r=-0.436$, $P=0.001$) and several blood- based biomarkers.
Garcia A. et al., 2017 [30]	To study the structure of DNA in buccal cells in AD.	A comparative study of quantitative assessment of DNA structure of buccal epithelium in aging and AD	Study groups: main group – 37 patients with asthma; comparison group – 37 healthy controls. Biomaterial: buccal epithelium Methods: ultra-high-resolution microscopy for quantitative assessment of DNA structure.	In patients with AD, the buccal cells showed an increase in the interchromatin compartment and disruption of the DNA structure; this was not detected in healthy controls.
KoseOzlece H. et al., 2018 [28]	Evaluation of changes in the oral mucosa in patients with AD and Parkinson's Disease (PD) using a stereological method.	Comparative analysis of changes in the oral cavity membrane in AD and PD	Study groups: main group – 29 patients with AD, 30 patients with Parkinson's disease, comparison group – 30 healthy controls. Biomaterial: brush biopsies. Methods: cell volume nucleator transmitted from electronic electric cheeks. Cytomorphometric analysis.	The nuclear volume was 484.39±117.10 μm3 in the AD group, 509.71±132.26 μm^3 in PD patients, and 509.30±100.21 μm^3 in the control group. The cytoplasmic volume was 115,456.60±30,664.98 μm3 in the AD group, 103,097.93±25,034.65 μm3 in PD patients, and 109,528.45±28,381.43 μm3 in the control group. The nuclear and cytoplasmic volumes did not differ significantly between the parameters ($P>0.05$).
Zuev V.A. et al., 2019 [21]	The expression of pathological proteins in the buccal epithelium of patients with AD is studied.	Comparative analysis of the expression of Aβ42, τ-protein, p16 and p53 proteins in the buccal epithelium of elderly and non-elderly patients with Alzheimer's disease.	Study groups: Main group – participants with AD; comparison group – matched by gender and age. Biomaterial: buccal epithelial cells. Study methods: Immunocytochemistry of Aβ42, tau protein, and caspase-dependent apoptosis markers p16 and p53.	Increased synthesis of the Alzheimer's markers Aβ42 (15-30 times) and τ protein (5 times) in children compared to individuals of the same age without neurodegenerative disease. In AD, the synthesis of aging and apoptotic proteins p16 (6-10 times) and p53 (2-3 times) is increased compared to individuals of the same age without neuropathology.

Siddiqui M.S. et al., 2020 [27]	Assessing the regenerative potential of the brain by assessing the buccal epithelium	A comparative study of endogenous γH2AX in buccal cell nuclei in patients with MCI, AD, and healthy individuals	Study groups: main group - 16 patients with AD, 18 patients with MCI, comparison group - 17 healthy controls. Biomaterial: buccal epithelium Methods: Laser Scanning Cytometry (LSC).	Decreases in nuclear diameter and buccal epithelial cell diameter with age. Elevated γH2AX levels were recorded in the nuclei of neurons in AD. γH2AX levels were significantly increased in the nuclei of the AD group compared to MCI and control conditions. Nuclear roundness (a measure of irregular nuclear shape) was significantly higher in the nuclei of buccal cells in the AD group compared to MCI and control conditions. A positive correlation was found between nuclear roundness and γH2AX signals.
Reimann H. et al., 2020 [31]	To study the role of DNA damage in the pathogenesis of AD.	Comparative study of DNA damage in the form of micronuclei in connective tissue samples in AD..	Study groups: 425 subjects in the main group (with AD) and healthy controls in the comparison group. Biomaterial: buccal epithelium Methods: Ultra-high-resolution microscopy for DNA quantification. Binucleate cells, karyolytic cells, and karyolytic cells of the buccal epithelium were quantified.	No significant differences in DNA damage or cytotoxicity markers were found in patients compared to healthy participants. This study did not reveal a direct link between cheek cell changes and neurogenerative diseases. It revealed the influence of lifestyle factors and diseases on the human cheek cytomeme.
Bazarny V.V. et al., 2021[22]	To assess the level of proinflammatory and anti-inflammatory factors in oral fluid (OF) and buccal epithelium in patients with Alzheimer's-type cognitive impairment	A comparative study of the state of oral fluid and buccal epithelium in AD.	Study groups: The main group consisted of 12 patients with AD-associated dementia (mean age 76.2 years); The comparison group consisted of 12 cognitively healthy individuals (mean age 75.3 years). Biomaterial: Oral Fluid (OF), buccal epithelium. Research methods: clinical, psychopathological, neuropsychological, and cytological assessments; the stability of salivary and serum biomarkers was assessed using multiparametric fluorescence analysis with magnetic microspheres.	Correlations have been established between amnesia, speech impairments, praxis, gnosis, and oral fluid pathology and buccal epithelium (severity of karyopyknosis and karyorrhexis). Direct correlations have been established between the neurodegenerative process of systemic inflammation and buccal epithelial degeneration. A relationship has been established between serum and salivary BDNF levels and neuropsychological parameters of immediate memory and nominative speech regulation.

Results

The search for biomarkers for Alzheimer's Disease (AD) has drawn attention to peripheral tissues, which, like the brain, have an ectodermal structure. Based on this, scientists hypothesized that during aging (including pathological aging), these tissues would undergo pathological changes similar to neurodegenerative ones. An example of such tissue is the buccal epithelium, which develops early in embryogenesis from the same germ layer as the neural tube. According to a publication in PubMed, buccal epithelium has become a starting point for studies in patients with AD or Mild Cognitive Impairment (MCI) (Table 1).

A comprehensive analysis of the results of 14 published comparative studies, which involved a total of more than 7,500 participants, identified several pathological patterns of BE in patients with BA and/or MCI:

High levels of proteins in the epithelium ensure oral health;

Involvement of the buccal epithelium in systemic proteinopathy in AD;

Acceleration of the synthesis of aging and apoptotic proteins in the buccal epithelium in AD;

The concentration of the phosphorylated form of the histone protein γH2AX in the nuclei of the buccal epithelium in AD is higher than in MCI and in cognitively healthy individuals;

- Increased intracellular lipid content in the buccal epithelium;
- A strong negative correlation between the DNA content in the nuclei of the buccal epithelium and increased intracellular lipid content in the buccal epithelium in patients with AD;
- Genomic instability of buccal epithelial cells in patients with asthma; - the presence of micronuclei (MN) in the buccal epithelial cells of patients with AD, being a manifestation of increased genomic instability, indicates a decrease in their regenerative capabilities;
- Shortening of telomere length in buccal epithelial cell nuclei in patients with AD correlates with a decrease in telomere length in hippocampal cells, erythrocytes, and leukocytes;
- Changes in the nuclear-cytoplasmic index, an increase in the number of abnormally shaped nuclei in the buccal epithelium, and irregular nuclear shape and cell diameter in patients with AD. Thus, researchers interested in buccal epithelial cells in patients with neurodegenerative pathology identified pathological patterns in the buccal epithelium in this patient.

To achieve the goal of our study, we utilized the mechanisms of neurodegeneration that affect buccal epithelial tissue (Table 2).

1. The external series of pathogenetic links in the formation of Alzheimer's disease with the development of buccal epithelium:
2. The external series of pathogenetic links in the formation of Alzheimer's disease with the development of buccal epithelium:
3. Decreased choline intake during fetal development promotes intense amyloid formation in adulthood. High levels of tau protein in buccal epithelial cells have been detected in early childhood in individuals at high risk for Alzheimer's disease, as confirmed by animal studies

4. The early ontogenetic commonality, the mesodermal structure of the brain and oral cells from the ectodermal germ layer, determines the conditions for balanced structural changes in the late stages of ontogenesis, including during the degenerative process.

5. Buccal epithelial and hippocampal cells undergo similar morphological changes during aging, including telomere shortening, intense tau protein synthesis, and increased expression of hyperphosphorylated tau and amyloid peptide β.

6. In cognitive disorders, a decrease in the expression of a number of peptides has been established in buccal epithelial cells: amyloid peptide β, NF-kB, tau protein, claudin, and S100 protein, which have been proven to be plasma protein markers of neurodegeneration.

Table 2: Potential mechanisms of pathogenesis involving buccal epithelium in AD.

Author	Concept
Migliore L. et al., 2011 [36]	Micronuclei (MN), formed as a result of chromosome breakages or chromosome segregation abnormalities, are ideal biomarkers for studying genomic instability. In AD and PD, increased MN frequency in peripheral lymphocytes occurs due to chromosome segregation abnormalities and breaks. In premature aging, MN increases with aging in cultured cells. The frequency of aneuploidy is increased in AD, and the risk of AD is high. Analysis of buccal MN cytomas in AD revealed additional changes in MN frequency and other cellular modifications reflecting reduced regenerative capacity.
Bolognesi C. et al, 2015[38]	Evaluation of inhalation and local exposure to genotoxic agents, diet, and lifestyle factors. Local oral lesions, early tumor biomarkers, and various chronic diseases were assessed. In AD, the number of buccal epithelial cells with micronuclei increased following surgical interventions.
Paltsev M.A. et al., 2017 [34]	In AD, hyperphosphorylated τ-protein, BACE1, and Aβ42 peptide are produced in the CSF. Increased expression of hyperphosphorylated τ-protein in blood lymphocytes in AD, and the content of high-molecular forms of phosphorylated T-protein and amyloid precursor protein (APP) in platelets of AD patients. Increased number of associated factors, synthesis of Aβ40 and Aβ42 peptides, and τ-protein in skin fibroblasts and buccal epithelium in AD. Increased expression of hyperphosphorylated τ-protein and Aβ peptide in the olfactory and buccal epithelium in AD.
Block J., 2019 [32]	The multifactorial pathogenesis of asthma begins in the epithelia associated with the situation. Infection, which disrupts the integrity of the epithelium, proceeds in such a way that the infectious agent penetrates the epithelial barrier and enters the environment. AD as a model for the slow progression of infectious encephalitis
Avramouli A. et al., 2020 [35]	Buccal cells contain numerous proteins associated with brain processes. They are the best candidates for the discovery of AD biomarkers. A brief description of relevant laboratory methods for identifying AD-related protein structure changes is provided.
Paraskevaidi M. et al,2020 [37]	Saliva and buccal cells have been shown to have great potential to provide a completely noninvasive alternative to current CSF and blood sampling procedures. This review presents findings and analytical approaches, including proteomics, metabolomics, spectroscopy, and microbiome analysis, that have been used to study and detect AD using saliva and buccal cell samples. Aβ, T-tau, and P-tau proteins, metabolites found in saliva and buccal epithelia, hold potential as diagnostic tools for AD.
Myakotnykh V.S. et al., 2022 [33]	A study of buccal epithelium and oral fluid as surrogate models of degenerative lesions of cerebral structures. Degenerative lesions of cerebral structures caused by slow movements, initiation, and retention, resulted in abnormal changes in opportunistic oral microflora.

Discussion

Early diagnosis of Alzheimer's Disease (AD) currently relies on invasive and/or expensive methods, which reduces its availability and timeliness for excluding the majority of patients and necessitates the search for readily available biomarkers [39].

Identified pathological findings in the buccal epithelium in AD and/or MCI include both structural abnormalities of cells (decreased levels of basal, karyolytic, and condensed chromatin cells, increased numbers of buccal epithelial cells with micronuclei, and abnormal cytoplasmic proportions) and abnormalities in the genetic and chromosomal structure of these cells (increased numbers of shortened telomeres and their aggregation, nuclear DNA mobility, and abnormal DNA/neutral lipid content).

Some authors point out that parallelism of cytological findings between brain cells, particularly the hippocampus and epithelium, develops in the oral cavity and is detected both in neurodegeneration and in normal aging [40]. For example, during normal aging, large ratios of nuclei and cell diameters are formed in buccal epithelial cells, which, according to researchers, correlates with an age-related decline in the body's regenerative potential [41].

Some authors point out that parallelism of cytological findings between brain cells, particularly the hippocampus and epithelium, develops in the oral cavity and is detected both in neurodegeneration and in normal aging [40]. For example, during normal aging, large ratios of nuclei and cell diameters are formed in buccal epithelial cells, which, according to researchers, correlates with an age-related decline in the body's regenerative potential [41].

By studying histopathological data obtained in buccal epithelium with impaired hippocampal function, confirmed neuropsychologically, researchers are attempting to establish parallels in the process of buccal epithelial tissue in the pathological process of AD [47]. There is evidence of the involvement of buccal epithelium in some mechanisms of neurological diseases and health disorders, which allows the use of buccal epithelial tissue to study the principles of the development of these disorders. A precedent for the use of buccal epithelium as a biomarker is the study of DNA methylation processes in buccal epithelial cells in individuals with Autism Spectrum Disorders (ASD). Through the use of succal epithelium, some differences in methylated regions of genes were discovered between individuals with ASD and healthy controls [48].

A series of studies of various biological tissues in cognitive disorders demonstrate the presence of protein changes in buccal epithelial cells similar to those found in blood lymphocytes, where these protein markers are detected along with other

pathological findings, demonstrating the presence or presence of a neuronal risk for degeneration [49,50].

A number of authors, using immunohistochemistry and flow cytometry in a study of Caucasians and Mexican mestizos, found no relationship between the concentration of tau protein in cells responsible for the formation of the oral cavity and age [18]. Moreover, François M et al. (2016), using ELISA, found no differences between control subjects, MCI subjects, and Alzheimer's disease patients in an Australian population [26].

The question of such a wide dissemination of research results is logical. One possible reason is the mobility of buccal epithelium in a non-specific climate of various factors. It has been established that the condition of the buccal epithelium is influenced by a variety of causal and pathological processes, which, according to some authors, may indicate the onset of non-specific pathological phenomena reflecting mitotic disturbances caused by intoxication, harmful environmental factors, smoking, oral diseases, reactions to dental prosthetics, and, finally, processual aging, etc. [51,52].

Currently, examples of the use of buccal epithelium analysis in neuropsychological diagnostics in patients with Alzheimer's dementia are presented, which allows for a more accurate determination of the nature of the pathological process [53]. For example, ELISA results showed that a higher initial p-tau status in AD patients than in age-matched healthy controls led to the development of oral Cerebrospinal Fluid (CSF) p-tau-positive cells in the inner cheek epithelium, which resulted in a higher percentage of p-tau-positive cells in the inner cheek epithelium found in individuals with a low Mini-Mental State Examination (MMSE) score. The authors concluded that patients with cognitive impairment and a high percentage of p-tau-positive cells develop proteinopathy and, very likely, neurodegenerative diseases [54].

The involvement of the buccal epithelium, as a peripheral tissue, in the process of pathologically accelerated aging and the contribution of buccal epithelium to the mechanisms of Alzheimer's disease pathogenesis have been demonstrated in various studies. Consequently, direct regulation of pathological findings in peripheral and central tissues is impossible due to the different dynamics of these tissues and the constant influence of various factors throughout life. Incorporating buccal epithelium assessment into diagnostic systems and methods likely offers an affordable option for developing a minimally invasive early marker of AD.

Conclusion

During aging, buccal epithelial cells undergo changes similar to those occurring in the central tissue. Changes observed in buccal epithelial tissues in AD and MCI include normal aging processes in the buccal epithelium, a form of apoptosis, increased concentrations of phosphorylated theoretical proteins, damaged DNA in the nuclei of the buccal epithelium, genomic instability, changes in telomere length, abnormalities in the nuclear-cytoplasmic index ratio, the emergence of protein markers, neurodegeneration, etc. Epithelial effects in the pathogenesis of AD are also evident, including the involvement of the buccal epithelium in neuroinflammatory cells as a peripheral link in the infectious process; and the involvement of the buccal epithelium in proteinopathies, including tauopathy with peripheral amyloid plaques. The common embryogenetic origin of buccal epithelial and hippocampal cells from the ectodermal germ layer determines the universal changes in these two different tissues during aging.

Thus, the motility of buccal epithelial cells and the degenerative changes that correlate with AD stages and severity, as well as their synchronicity in the mechanisms of this disease, allow us to consider peripheral buccal epithelial tissue as a potential marker of neurodegeneration. The method for obtaining and studying buccal epithelial cells is minimally invasive, time-consuming, and cost-effective, with the exception of certain genetic tests. Buccal epithelium is an accessible biomaterial for the identification of AD markers.

References

1. World Health Organization. World health statistics. Geneva: World Health Organization.
2. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of the Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology. 1984; 34: 939–944.
3. Dubois B, Feldman HH, Jacova C, Dekosky ST, Barberger-Gateau P, Cummings J, et al. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. Lancet Neurol. 2007; 6: 734–746.
4. Dubois B, Feldman HH, Jacova C, Cummings JL, Dekosky ST, Barberger-Gateau P, et al. Revising the definition of Alzheimer's disease: a new lexicon. Lancet Neurol. 2010; 9: 1118–1127.
5. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. Science. 1992; 256: 184–185.
6. Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging–Alzheimer's Association workgroups. Alzheimers Dement. 2011; 7: 280–292.
7. Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease. Alzheimers Dement. 2011; 7: 270–279.
8. Jack CR Jr, Bennett DA, Blennow K, Carrillo MC, Feldman HH, Frisoni GB, et al. A/T/N: an unbiased descriptive classification scheme for Alzheimer disease biomarkers. Neurology. 2016; 87: 539–547.
9. Jack CR Jr, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, et al. NIA-AA research framework: toward a biological definition of Alzheimer's disease. Alzheimers Dement. 2018; 14: 535–562.
10. Jack CR Jr, Andrews JS, Beach TG, Buracchio T, Dunn B, Graf A, et al. Revised criteria for diagnosis and staging of Alzheimer's disease. Alzheimers Dement. 2024; 20: 5143–5169.
11. Scheltens P, Siemers E, Snyder HM, Sperling R, Teunissen CE, Carrillo MC. Revised criteria for diagnosis and staging of Alzheimer's disease. Alzheimers Dement. 2024; 20: 5143–5169.
12. Ramirez MK, Phipps CJ, Murman DL, Beadle JN, Phatak VS, Warren DE. Structural neuroimaging correlates of neuropsychiatric symptoms in Alzheimer's disease: a systematic review. Dement Geriatr Cogn Disord. 2025; 1–15.
13. Barichello T, Giridharan VV, Dal-Pizzol F. A cerebrospinal fluid biosignature for the diagnosis of Alzheimer's disease. Braz J Psychiatry. 2019; 41: 467–468.
14. Lehmann S, Gabelle A, Paquet C. Can we rely only on ratios

of cerebrospinal fluid biomarkers for Alzheimer's disease diagnosis? Alzheimers Dement. 2015; 11: 1125–1126.

15. Corbillé AG, Preterre C, Rolli-Derkinderen M, Coron E, Neunlist M, Lebouvier T, et al. Biochemical analysis of α-synuclein extracted from control and Parkinson's disease colonic biopsies. Neurosci Lett. 2017; 641: 81–86.

16. François M, Leifert W, Hecker J, Faunt J, Martins R, Thomas P, et al. Altered cytological parameters in buccal cells from individuals with mild cognitive impairment and Alzheimer's disease. Cytometry A. 2014; 85: 698–708.

17. Puig KL, Combs CK. Expression and function of APP and its metabolites outside the central nervous system. Exp Gerontol. 2013; 48: 608–611.

18. Hattori H, Matsumoto M, Iwai K, Tsuchiya H, Miyauchi E, Takasaki M, et al. The tau protein of oral epithelium increases in Alzheimer's disease. J Gerontol A Biol Sci Med Sci. 2002; 57: M64–M70.

19. François M, Leifert W, Hecker J, Faunt J, Martins R, Thomas P, Fenech M. Altered cytological parameters in buccal cells from individuals with mild cognitive impairment and Alzheimer's disease. Cytometry A. 2014; 85: 698-708

20. Thomas P, O'Callaghan NJ, Fenech M. Telomere length in white blood cells, buccal cells and brain tissue and its variation with ageing and Alzheimer's disease. Mech Ageing Dev. 2008; 129: 183–190.

21. Zuev VA, Dyatlova AS, Lin'kova NS, Kvetnaya TV. Expression of Aβ42, tau-protein, p16, p53 in buccal epithelium: prospects for Alzheimer's disease diagnostics. Bull Exp Biol Med. 2019; 166: 676–679.

22. Bazarny VV, Sidenkova AP, Rezaykin AV, Myakotnykh VS, Borovkova TA, Selkina EO, et al. Possibility of using oral fluid and buccal epithelium studies in the diagnosis of Alzheimer's disease. Adv Gerontol. 2021; 34: 550–557.

23. Thomas P, Hecker J, Faunt J, Fenech M. Buccal micronucleus cytome biomarkers may be associated with Alzheimer's disease. Mutagenesis. 2007; 22: 371–379.

24. Thomas P, Fenech M. Buccal cytome biomarkers and their association with plasma folate, vitamin B12 and homocysteine in Alzheimer's disease. J Nutrigenet Nutrigenomics. 2015; 8: 57–69.

25. Fenech M, Holland N, Zeiger E, Chang WP, Burgaz S, Thomas P, et al. The HUMN and HUMNxL international collaboration projects on human micronucleus assays in lymphocytes and buccal cells. Mutagenesis. 2011; 26: 239–245.

26. François M, Fenech MF, Thomas P, Hor M, Rembach A, Martins RN, et al. High content multi-parameter analyses in buccal cells to identify Alzheimer's disease. Curr Alzheimer Res. 2016; 13: 787–799.

27. Siddiqui MS, François M, Rainey-Smith S, Martins R, Masters CL, Ames D, et al. Evaluation of gamma-H2AX in buccal cells as a molecular biomarker of DNA damage in Alzheimer's disease. Life (Basel). 2020; 10: 141.

28. Kose Ozlece H, Findik Guvendi G, Huseyinoglu N, Ehi Y, Kivrak Y. Cytological and cytometric analysis of oral mucosa in patients with Alzheimer's and Parkinson's disease. Neuropsychiatr Dis Treat. 2018; 14: 1901–1906.

29. Mathur S, Glogowska A, McAvoy E, Righolt C, Rutherford J, Willing C, et al. Three-dimensional quantitative imaging of telomeres in buccal cells identifies Alzheimer's disease severity. J Alzheimers Dis. 2014; 39: 35–48.

30. Garcia A, Huang D, Righolt A, Righolt C, Kalaw MC, Mathur S, et al. Super-resolution structure of DNA differs in buccal cells of controls and Alzheimer's patients. J Cell Physiol. 2017; 232: 2387–2395.

31. Reimann H, Stopper H, Polak T, Lauer M, Herrmann MJ, Deckert J, et al. Micronucleus frequency in buccal mucosa cells of patients with neurodegenerative diseases. Sci Rep. 2020; 10: 22196.

32. Block J. Alzheimer's disease might depend on enabling pathogens which do not necessarily cross the blood–brain barrier. Med Hypotheses. 2019; 125: 129–136.

33. Myakotnykh VS, Sidenkova AP, Kharitonova MP, Meshchaninov VN, Melnik AA, Khilyuk DA. Oral cavity condition in early diagnosis and prediction of cognitive disorders. Adv Gerontol. 2022; 35: 418–428.

34. Paltsev MA, Zuev VA, Kozhevnikova EO, Linkova NS, Kvetnaia TV, Polyakova VO, et al. Molecular markers of early diagnosis of Alzheimer's disease in peripheral tissues. Adv Gerontol. 2017; 30: 809–817.

35. Avramouli A, Vlamos P. Buccal mucosa biomarkers in Alzheimer's disease. Adv Exp Med Biol. 2020; 1195: 49–56.

36. Migliore L, Coppedè F, Fenech M, Thomas P. Association of micronucleus frequency with neurodegenerative diseases. Mutagenesis. 2011; 26: 85–92.

37. Paraskevaidi M, Allsop D, Karim S, Martin FL, Crean S. Diagnostic biomarkers for Alzheimer's disease using non-invasive specimens. J Clin Med. 2020; 9: 1673.

38. Bolognesi C, Bonassi S, Knasmueller S, Fenech M, Bruzzone M, Lando C, et al. Clinical application of micronucleus test in exfoliated buccal cells: a systematic review and meta-analysis. Mutat Res Rev Mutat Res. 2015; 766: 20–31.

39. Ausó E, Gómez-Vicente V, Esquiva G. Biomarkers for Alzheimer's disease early diagnosis. J Pers Med. 2020; 10: 114.

40. Oh G, Ebrahimi S, Wang SC, Cortese R, Kaminsky ZA, Gottesman II, et al. Epigenetic assimilation in the aging human brain. Genome Biol. 2016; 17: 76.

41. Yun MH. Changes in regenerative capacity through lifespan. Int J Mol Sci. 2015; 16: 25392–25432.

42. Lo RY. The borderland between normal aging and dementia. Tzu Chi Med J. 2017; 29: 65–71.

43. Bazarny VV, Mandra YV, Sidenkova AP, Polushina LG, Maksimova AY, Semenova EA, et al. Age-related features of the buccal epithelium in healthy individuals. Klin Lab Diagn. 2022; 67: 345–350.

44. Bazarny VV, Sidenkova AP, Sosnin DY. Lactoferrin of oral fluid in normal conditions and Alzheimer's disease. Klin Lab Diagn. 2022; 67: 207–213.

45. Moskaleva EY, Glukhov AI, Zhirnik AS, Vysotskaya OV, Vorobiova SA. Telomere length and telomerase activity as biomarkers in diagnostics and prognostics. Biochemistry (Mosc). 2025; 90: 700–724.

46. Morali O, Savagner P, Larue L. Epithelium–mesenchyme transitions during early development. Madame Curie Bioscience Database. Austin: Landes Bioscience. 2013.

47. Andrews SV, Sheppard B, Windham GC, Schieve LA, Schendel DE, Croen LA, et al. Blood DNA methylation and autism spectrum disorder. Mol Autism. 2018; 9: 40.

48. Dubois B, von Arnim CAF, Burnie N, Bozeat S, Cummings J. Biomarkers in Alzheimer's disease: role in early and differential diagnosis. Alzheimers Res Ther. 2023; 15: 175.

49. Talbot K. Brain insulin resistance in Alzheimer's disease and its treatment. Neurodegener Dis Manag. 2014; 4: 31–40.

50. Weinberg A, Naglik JR, Kohli A, Tugizov SM, Fidel PL Jr, Liu Y, et al. Innate immunity including epithelial host factors. Adv Dent Res. 2011; 23: 122–129.

51. Şenel S. Physical, microbiological and immune barriers of oral mucosa. Int J Mol Sci. 2021; 22: 7821.

52. Myakotnykh VS, Sidenkova AP, Bazarny VV, Melnik AA, Urazaeva AT, Khilyuk DA. Diagnostic value of buccal epithelium studies in elderly patients with cognitive impairment. Sib Vestn Psikhiatr Narkol. 2022; 114: 40–45.

53. Kandimalla RJ, Prabhakar S, Wani WY, Kaushal A, Gupta N, Sharma DR, et al. CSF p-tau levels in prediction of Alzheimer's disease. Biol Open. 2013; 2: 1119–1124.