

Clinical and histoenzymatic interrelations of the edentulous ridge's mucosa

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Abstract

The edentulous ridge's mucosa presents various histological aspects and different histoenzymatic equipment. These aspects are determined by constitutional factors (genetic, nutritional), as well as by age and by the associated pathology. The purpose of this paper is to evidenciate, through histochemical techniques, the aerobic (NADH₂-cytochrome-c reductase) and anaerobe (lactatdehydrogenase) oxidoreducing enzymatic activities, as well as the hydrolytic enzyme's activity (ATP-ases, pH 9.4), corroborated with the histological aspects of the edentulous ridge's mucosa. The enzymatic activity and the morphological alterations present various aspects, depending on the metabolic type (aerobe oxidative, anaerobe oxidative, hydrolytic), according to the mucosal area (epithelium, chorion), to the different cell types, or even within the same cellular type (especially at the level of the connective lamina propria cells). They also vary from one edentulous patient to another, depending on the existence of a previous prosthetic treatment and on the type of this treatment. The local clinical examination, sustained by a histoenzymatic investigation, reveals the importance of the structural alterations and they can offer guidance for the proper prosthetic treatment and for the achievement of functional prosthetic restorations that provide the oral mucosa its necessary trophicity for as long as possible.

Keywords: edentulous ridge, oral mucosa, enzymatic activity.

Introduction

Under normal circumstances, the oral mucosa's enzymatic equipment varies from one region to another [1, 2]. The oral mucosa presents metabolic alterations in the course of different pathologic conditions, better known being those that appear in the context of inflammatory lesions or tumoral pathology [3]. It is interesting to study the tissular enzymatic behavior of the edentulous ridge's mucosa in the cases of patients with existing previous prosthetic restorations and in the cases of patients without prosthetic treatment [4, 5].

Schroeder realized a histological research regarding the topographical structure of the edentulous ridge's mucosa. According to his research, the adherent fibromucosa of the edentulous ridge has a thick keratinized epithelium that intimately mingles with the submucosa. The submucosa presents a dense network of collagen fibers that penetrates the edentulous ridge's chorion. In the passive-mobile mucosa, the elastic fibers are dominant. The epithelial morphology and the morphology of the epithelium/submucosa limit are similar in the fixed mucosa as well as in the buccal one.

The fibromucosa of edentulous patients, wearers or not of prosthetic restorations, a keratinization process caused either by food impact in the case of unrestored edentulism, or by pressure in the case of patients wearers of prosthetic appliances, can be noticed. The connective

tissue of the edentulous ridge's fibromucosa has a nutritive function as well as the role of receiving forces in the case of edentulous patients. With the passing of time, in some of the cases of patients' wearers of mobile prosthetic restorations, the edentulous ridge's fibromucosa becomes thinner or, on the contrary, it becomes hyperplasic, losing its adherence to the periosteum.

Materials and Methods

The present study was made on a number of 32 partially or completely edentulous patients, of which 14 were men and 18 were women and who presented alterations of the edentulous ridge's mucosa. Twenty-three patients presented prosthetic restorations and nine had not undergone previous prosthetic treatment.

In the cases that needed surgical proprosthetic treatment, fragments of modified oral mucosa were prelevated from the edentulous ridge's area and they were processed for histoenzymatic determinations, as well as through the paraffin histological technique.

The fragments that were prelevated for histoenzymatic determinations were frozen in liquid nitrogen and afterwards cut into 3–4 μm thick slices using a cryotome (-5°C). On these sections, histochemical techniques were performed with the purpose of evidencing the *in situ* activity of the following enzymes: NADH₂-cytochrome-c reductase (diaphorase), the Dicusescu

method (1964); Lactatdehydrogenase, the Pearse method (1972); ATP-ases pH 9.4, the Padykula and Hermann method (1967).

The sections obtained through the paraffin histological technique were stained with Hematoxylin–Eosin (HE) and trichromic Goldner–Szeckelly (GS).

☐ Results

The enzymatic activity varies from the epithelium to the lamina propria, according to the enzyme type.

NADH₂-cytochrome-c reductase (diaphorase)

In the edentulous ridge's mucosa of partially edentulous patients, at epithelial level, the diaphorase's activity is intense in the basal cells, weak or medium in the polyedric cells and negative in the superficial cells (Figure 1).

In the chorion of the oral mucosa, the NADH₂-cytochrome-c reductase aerobic enzyme has a higher activity in all of its connective tissue components, compared to the one in the epithelium. The reaction is intense in the endothelium of the capillary loops. Pericapillary, the fibroblasts, which are the most frequent cells, present different intensity degrees (very weak, weak, medium, intense and very intense). In the profound chorion, there is intense activity in arterioles, capillaries, connective cells (Figure 2). In the entire lamina propria, there are rare, isolated and intensely reactive macrophages and plasmocytes with medium reaction.

The enzymatic activity is more intense in the cases of partially edentulous patients.

In the chorion of the edentulous ridge's mucosa, there are different number of unequal inflammatory microsources, rich in lymphoplasmocytes and macrophages with intense and very intense reactions.

Lactatdehydrogenase (LDH)

At epithelial level, there is an intense and very intense reaction in the first layers (basal layer and profound horny cells layer) and a lack of activity in the superficial epithelial cells.

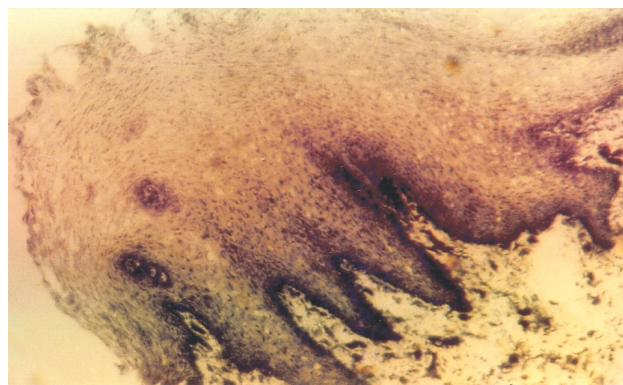


Figure 1 – Completely edentulous patient. NADH₂-cytochrome-c reductase. Edentulous ridge's mucosa. The epithelium presents intense positive reaction in the basal layer, very weak reaction in the profound spinous cells and negative reaction in the superficial cells.

In the chorion, the capillary loops are intensely positive. They are surrounded by numerous fibroblasts with different intensity reactions, from weak to very intense. The relatively frequent macrophages and the rare mastocytes are also intensely reactive (Figure 3). In the profound lamina propria, there are intense reactions in all the blood vessels and in the connective tissue cells.

In the cases of some of the completely edentulous patients, the epithelium is completely non-reactive. The connective tissue cells of the chorion of these patients appear mostly with medium reactions that are a reduced compared to the ones of the partially edentulous patients.

ATP-ases pH 9.4

The epithelium of the edentulous ridge's mucosa is non-reactive. The chorion sends thin and elongated papillae.

In the profound and in the superficial chorion, blood vessels of all calibers have a very intense endothelial activity. At arteriolar level, the same reaction appears in the endothelium, but also in the smooth muscular fibers of the “tunica media”. Surrounding the blood vessels, there are frequent fibroblasts, medium and intensely positive (Figure 4).

We resumed the researched enzymatic activity from the epithelium, as well as from the cellular population and the blood vessels of the chorion (Table 1).

On the examined paraffin sections, we encountered epithelial alterations, as well as modifications at lamina propria level. The edentulous ridge's epithelium is ortho- or para-keratinized (Figure 6, A–C). It is thicker than the gingival epithelium and the palatal epithelium. In the connective tissue of the lamina propria an inflammatory process takes place. The inflammatory process has a different intensity from one case to another and even from one area to another (Figure 5, A–D). On some of the sections, we encountered an intense fibroblastic reaction indicating a stimulation of the fibroblastic synthesis activity and a remodeling of the connective tissue (Figure 6D).

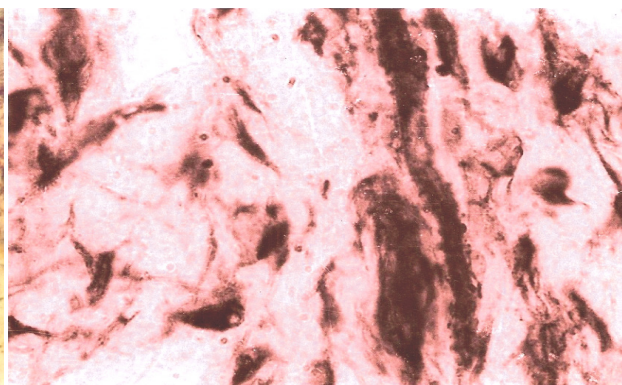


Figure 2 – Partially edentulous patient. NADH₂-cytochrome-c reductase. The chorion of the mucosa has very reactive blood cells in the endothelium and intensely positive fibroblasts, macrophages and mastocytes.

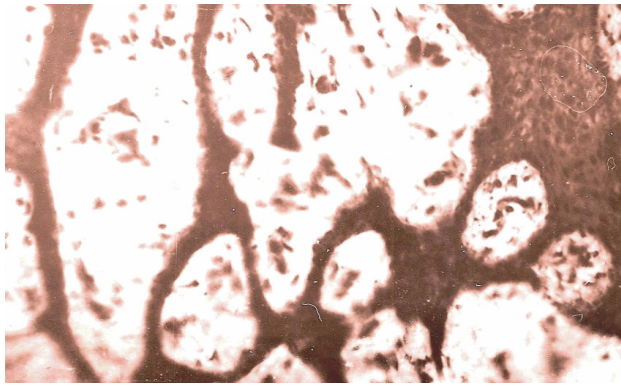


Figure 3 – Partially edentulous patient. Lactatdehydrogenase. Edentulous ridge's mucosa. The epithelium presents intense or very intense enzymatic reaction in the basal and in the profound polyhedral layers. Very intense reactions in the capillary loop's endothelium and in frequent fibroblasts and rare macrophages in lamina propria.

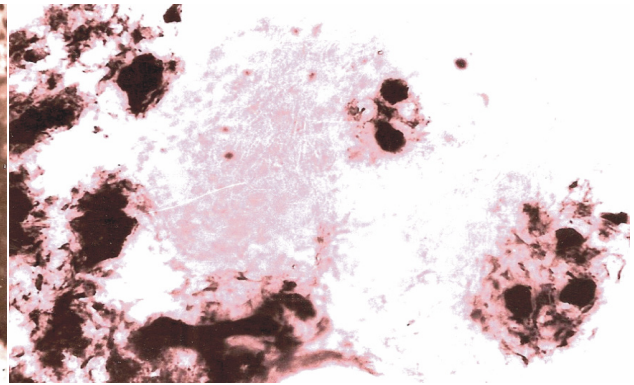


Figure 4 – Partially edentulous patient. ATP-ases pH 9.4. Edentulous ridge's mucosa. A reactive epithelium. In the conjunctival papillae, on a transversal section, very intense reactions can be observed in capillaries and also intense reactions are noticed in the pericapillary fibroblasts.

Table 1 – Researched enzymatic activity

Enzyme	Epithelium			Chorion					
	B	P	Sup	Fb	Mac	Mas	PI	PMN	VS
NADH ₂ -cytochrome-c reductase	+	-+	-	+++					
	+	-	-	++	+++	++	++	+++	+++
	-+	-	-	+	++			++	++
				-+					
Lactatdehydrogenase	+	-+	-	+	++	+	+	++	+
				-+	+			+	
ATP-ases pH 9.4	-	-	-	+	+	-	-+	+	+++
				-+	-+			-+	

- - Negative reaction; +- Weak reaction; + - Medium reaction; ++ - Intense reaction; +++ - Very intense reaction; B - Basal cells; P - Polyhedral cells; Sup - Superficial cells; F - Fibroblasts; Mac - Macrophages; Mas - Mastocytes; PI - Plasmocytes; PMN - Polymorphonuclear cells; VS - Blood vessels.

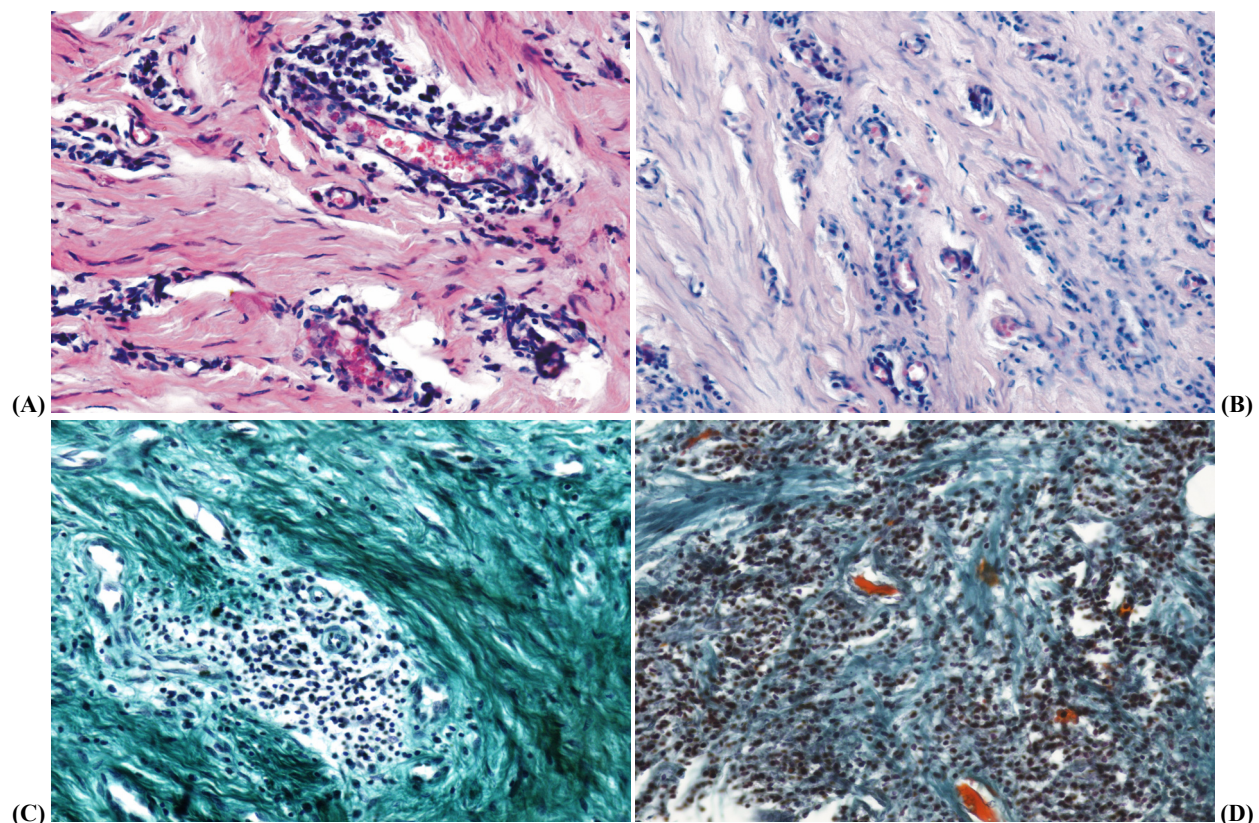


Figure 5 – Edentulous ridge: lamina propria. Inflammatory perivascular infiltrate consisting of lymphocytes, plasmocytes, macrophages. HE staining, ob. ×20 (A, B) and GS trichromic staining, ob. ×20 (C, D).

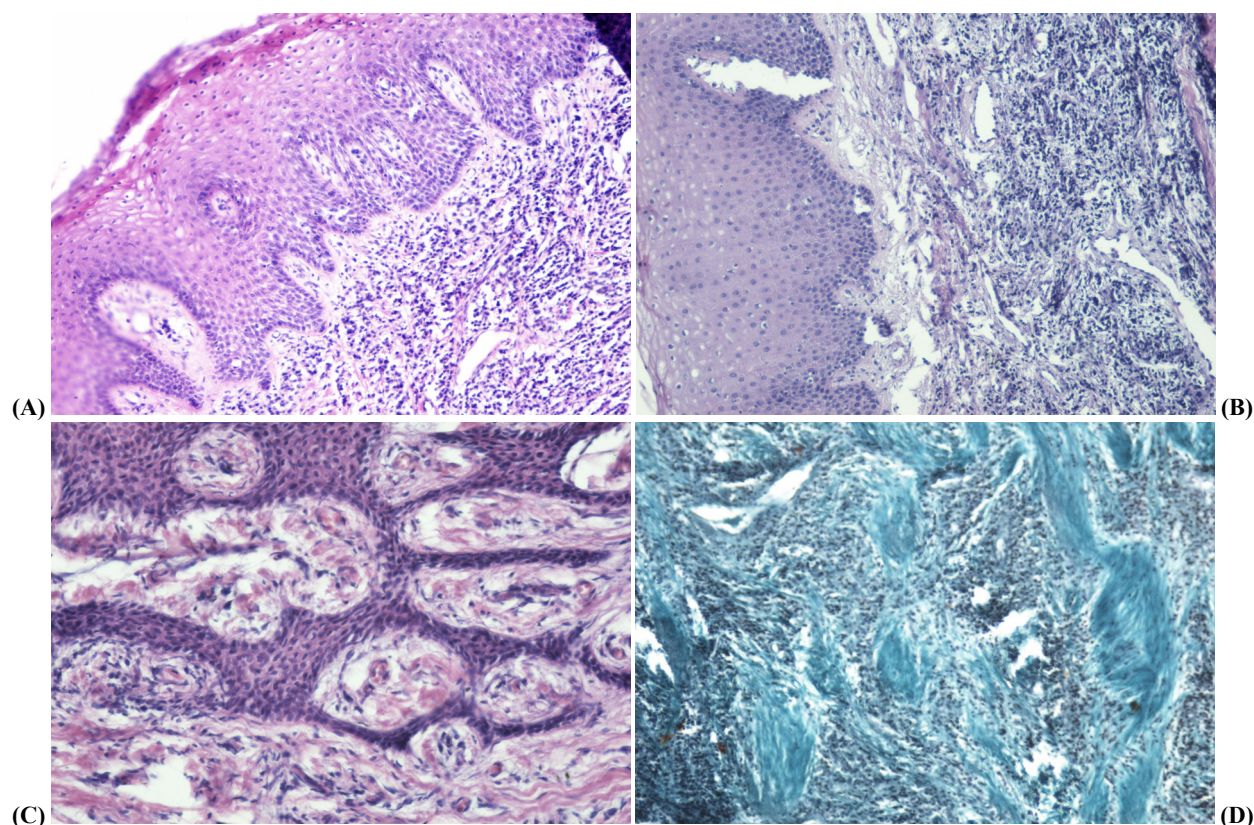


Figure 6 – *Acanthosis, discrete focal papillomatosis, parakeratosis, perivascular diffuse inflammatory infiltrate (A–C) and intense fibroblastic reaction (D). HE staining, ob. ×20 (A–C) and GS trichromic staining, ob. ×20 (D).*

Discussion

The histoenzymatic observations show morphophysiological aspects characteristic to the studied partially edentulous patients [6, 7]. These aspects are supported by the histological alterations observed on the sections obtained through the paraffin histological technique.

The followed aerobic (NADH₂-cytochrome-*c* reductase) and anaerobe (lactate dehydrogenase) oxidoreducing enzymatic activities, as well as the hydrolytic enzyme (ATP-ases), have indicated reactions of different intensities in the cases of partially or completely edentulous patients wearers or not of prosthetic restorations.

On some of the sections, the histoenzymatic aspects are identical to those met in normal gingival mucosa, where energetic cellular processes actively take place in the epithelium as well as in the chorion, the aerobic metabolism being more accentuated than the anaerobe one [8]. Thus, on these sections, at epithelial level, the aerobic and the anaerobe enzymes have an intense activity, indicating an active cellular metabolism in the germinating area, which ensures the regenerating processes. In the superficial epithelial areas, simultaneously with the involution of the cellular organelles, the enzymatic activity takes place with lower intensity, especially for the oxidoreducing enzyme [9, 10]. In the lamina propria, the fibroblasts are the most enzymatically active and, they insure, through their synthesis capacity, the structural components of the fibers and of the fundamental substance.

On the sections that came from patients wearers of prosthetic restorations, there are various aspects, according to the metabolic type (aerobe oxidative, anaerobe

oxidative, hydrolytic), to the mucosal area (epithelium or chorion), to the different cellular type or even among the same cellular type (especially in the case of the cells of the connective lamina propria). These aspects differ from one patient to another [11].

Sometimes, the epithelium presents a significant decrease of the oxidative enzymatic activity, which causes a prevalence of the anaerobe glycolytic metabolism over the aerobic one [12, 13]. This aspect suggests that the mitochondrial functions are reduced where the diaphorase is located. However, in the same time, lactate dehydrogenase, a cytolytic enzyme with anaerobe glycolytic oxidative activity, is much more intense. These aspects are correlated with a reduced enzymatic activity, even a negative one, for the ATP-ases, a hydrolytic enzyme and an indicator of much reduced cellular exchanges. We have encountered these aspects in some of the completely edentulous patients who had been wearers of prosthetic restorations and in edentulous patients suffering from diabetes mellitus, endocrinopathies, cardiovascular diseases, etc. They correspond to mucosal areas with atrophic aspect [14, 15]. The oxidative aerobic and anaerobe enzymatic activities decrease very much in the atrophic mucosa, signaling a reduced metabolism and, by default, a decreased mucosal defense capacity.

In other cases, the epithelial enzymatic reaction is different in the basal area, compared to the superficial one. Thus, in the profound area of the epithelium, where there is normally an intense mitotic activity, the activity of the mitochondrial enzymes is also intense, in order to energetically sustain this process also encountered

in edentulous patients with aclinically obvious mucosal hipertrophy [16].

The structural changes of the basal membrane, consisting of pathway irregularities and thickness irregularities observed on some of the sections, represent some of the causes of the epithelial modifications because of the alteration of its changes and nutrition.

In "lamina propria", the enzymatic activity is more intense, but it is different, according to its various structural elements [17]. The most enzymatically active for the oxidative endothelial enzymes, as well as for the membrane ATP-ases, are the blood vessels. This indicates the existence of active nutritional substances and metabolites exchanges between blood and tissues.

At fibroblastic level, the enzymatic activity varies from quasi-normal to very intense, because of the glycosaminoglycans (GAGs) and collagen fibers synthesis that caused the fibrous aspect of the mucosa in edentulous patients wearers of prosthetic restorations and in patients suffering from chronic diseases such as diabetes or chronic hepatitis [18, 19]. The increased collagen synthesis in the oral mucosal areas situated under mobile prosthetic restorations is the fibroblastic response to the local mechanic factor.

Various macrophages with intense and very intense enzymatic activities are present. Their principal function, phagocytation, joins the function of lymphocytes and plasmocytes. The enzymatic activities are intense in plasmocytes, indicating an active synthesis process in these cells [20]. Thus, in the connective tissue of the oral mucosa's chorion a phagocytation activity, associated with an immune lymphoplasmocytary process, takes place.

The presence of a number of inflammatory cells on some of the sections that came from patients without clinical signs of inflammation verifies the existence of a defense barrier, which ensures the mucosal structural integrity.

The enzymatic activities researched in this paper present different reactional intensities in younger healthy patients facing their first prosthetic rehabilitation, compared to older patients who suffer or not from general diseases, where the enzymatic reactions are very diverse, being either increased or decreased, indicating a reduced local defense capacity.

In the presence of local factors (e.g., prosthetic restorations) or general factors (e.g., different pathological conditions), the edentulous ridge mucosa's defense capacity is stimulated through the increase of the enzymatic activity and through the presence of macrophages, lymphocytes and plasmocytes. The observations made can conclude that there is a local potential for regeneration of the surface epithelium, as well as one at the level of "lamina propria".

The encountered metabolic disturbances can be remedied by intervening on some adjustment mechanisms of the oxidative metabolism and on some products with a role in cellular metabolism (vitamins, proteins, ions, etc.).

These different cytophysiological and enzymatic aspects are important for the therapeutic decision regarding the edentulous patients, as the local metabolic

parameters influence the option for a certain kind of behavior towards the prosthetic treatment.

Conclusions

The histoenzymatic observations in conjunction with histological aspects evidenced on sections show characteristic metabolic aspects that define the edentulous ridge mucosa's morphophysiology, depending on the clinical situation before prosthetic treatment and thus providing important therapeutic data.

The organism, through constitutional factors (genetic, nutritional), through associated disease and through age, plays an important part in the appearance of enzymatic changes.

The edentulous ridge's mucosa presents modifications that interest all its structural elements (epithelium, basement membrane, lamina propria) in different proportions, according to every patient and to the existence of a previous prosthetic treatment and to the specific type of prosthetic treatment.

Local clinical examination completed by histoenzymatic investigation evidences the importance of the structural modifications and may orient towards a certain prosthetic conduct.

Knowing the metabolic behavior and the structural aspect of the edentulous ridge's mucosa helps individualize the prosthetic treatment in order to obtain functional prosthetic restorations and ensuring the necessary mucosal trophicity for as long as possible.

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