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## **CELLULAR MICROENVIRONMENT AND COLLAGEN DESTRUCTION DURING PE- RIODONT INFLAMMATION**

**ABSTRACT. Background.** The association between periodontitis and collagen damage with immune cells is an actual problem. Periodontitis is a bacterially induced exacerbation of chronic process and chronic inflammatory disease that destroys teeth supporting connective tissue. Bacteria initiate periodontitis and destruction of the alveolar periodontal connective tissue. **Objectives.** Immune cells location between damaged collagen fibers remains obscure and this is the purpose of the current study. **Results.** We have determined five variants of immune cells microenvironment: nodular, trabecular, diffuse, mixed. We have observed five types of collagen structures destruction in exacerbation of chronic process and chronic periodontal inflammation. They are characterized by swelling, pulping and insignificant necrosis. **Conclusion.** Connective tissue has signs of swelling and destruction during inflammation; edema is observed between collagen fibers. Collagen fiber damage during periodontitis is caused by neutrophils. Widespread edema of collagen fibers increasing of depth cells infiltration during chronic inflammation. Nodular type of immune cells microenvironment is observed during outcome of chronic inflammation. Trabecular type of immune cells microenvironment is observed during exacerbation of chronic process. Diffuse type of immune cells microenvironment is observed during chronic inflammation process.

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**Кузенко Є., Дяченко О., Кузенко О., Олішкевич А. Клітинне мікрооточення і руйнування колагену при запаленні пародонта.**

**Реферат.** Зв'язок між періодонтитом, пошкодженням колагену з мікрооточенням з імунних клітин залишається актуальною проблемою в стоматології, якій присвячена дана робота. Нами було виділено п'ять варіантів мікрооточення з імунних клітин: вузловий, дифузний, трабекулярний, змішаний. Вузловий тип мікрооточення спостерігається під кінець хронічного запалення; трабекулярний тип мікрооточення - при загостренні хронічного процесу; дифузний тип - при хронічному запальному процесі. При загостренні хронічного процесу та хронічному пародонтиті спостерігається руйнування колагенових структур, що супроводжується набряком і незначним некрозом.

**Ключові слова:** пародонтит, клітинне мікрооточення, колаген.

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### **Introduction**

Gingivitis and its treatment have been with mankind at least since the days of the Pharaohs [1]. Periodontitis is a group of inflammatory diseases that affect the connective tissue attachment and supporting bone around the teeth [2]. The periodontium includes the vasculature, epithelium, non-mineralized and mineralized connective tissues, as well as the inflammatory and immune cells that have infiltrated into the periodontal tissues. The cellular components of periodontitis are 70–80% granulocytes, 10–20% monocytes/macrophages, 5% mast cells and 5% T lymphocytes [3]. Pathogenesis of periodontal diseases has two major aspects: microorganisms and host response. Interactions between microbial plaque and host immune system play a critical role in the initiation and progression of peri-

odontal diseases [4]. Monocytes, macrophages and other cells (including fibroblasts and endothelial cells) respond to the dental plaque microorganisms. The primary hallmark of periodontitis, the destruction of periodontal tissue, is widely accepted to be a result of the host immune inflammatory response caused by periodontal microorganisms [5].

One important effector mechanism of the inflammatory mediators present in periodontal tissue is stimulation of the monocytes, macrophages formation, this cells believed to be the major cell type responsible for collagen destruction. Destruction of the collagen may occur as a result of acute inflammatory, mechanical stimulation, or neoplastic processes.

In some of the non-plaque induced gingival lesions, loss of attachment and destruction of alveolar

bone may occur. In addition, seven major categories of destructive periodontal diseases were listed: Chronic periodontitis, Localized aggressive periodontitis, Generalized aggressive periodontitis, Periodontitis as a manifestation of systemic disease, Necrotizing ulcerative gingivitis/periodontitis, Abscesses of the periodontium, Combined periodontic-endodontic lesions [6].

The aim of this study is to compare the levels of cologne destruction and variants of immune microenvironment in gingival tissues on exacerbation of chronic process and chronic stages of periodontal disease.

### **Materials and Methods**

#### ***Patient selection and of gingival tissues collection***

The study samples have included the periodontal and epulis tissues of the patients. Group consisted of 34 patients who had died in Sumy Regional Hospital. The patients had various somatic diagnoses (not atherosclerotic complications) and dental – periodontitis. 17 males and 13 females, age range 43 to 69 years, mean age,  $57.33 \pm 8.31$  years have been investigating. The specimens of overgrown gingiva were collected during jaws sawing procedures. After group tissue samples stained by hematoxylin eosin for, all samples were divided in two groups (acute and chronic). Group has 3 subgroups.

Exacerbation of chronic process - 13 (5 males and 3 females, age range 43 to 69 years, mean age,  $54.38 \pm 7.9$  years).

Chronic subgroups - 17 (7 males and 15 females, age range 44 to 68 years, mean age,  $59.58 \pm 8.11$  years).

Normal periodontal tissues - 4 (1 males and 3 females, age range 18 to 23 years, mean age,  $20 \pm 2.16$  years)

Informed written consent was obtained from all study subjects in accordance with guidelines established by the Ukraine Health Council. The present study was approved by the Sumy State University (Protocol no. 5/2012).

Periodontal tissues samples have stained according to the methods specific for Hematoxylin and eosin (H&E) staining respectively.

Periodontal tissues were immersed in 10% paraformaldehyde pH 7.4 fixative at 18° C. These were then dehydrated with increasing concentration of ethanol before being embedded in paraffin.

Paraffin sections were prepared for acridine orange staining by mounting on the slides, dried on a hot plate, and then immersed into three sets of xylene for 2 minutes each followed by three sets of absolute ethanol for 5 minutes and finally rinsed with tap water. The aim was to remove the wax and dehydrate the sections. Slides (paraffin) were placed into acridine orange staining solution for 15 minutes, and rinsed with phosphate-buffered saline (PBS).

Then the slide was soaked in 0.1% calcium chloride solution for 3 minutes and was washed with PBS once again. Cover glass was mounted for observation under a fluorescence microscope to observe and read the result.

#### ***Immunostainings***

For MPO have been performed formalin fixed (pH 7,4) tissue. Paraffin-embedded tissue sections have been treated by mouse monoclonal anti-myeloperoxidase (Thermo Fisher Scientific UK). Briefly, 4 µm thick tissue sections were dewaxed in xylene and were placed in to water through graded alcohols. Antigen retrieval has been performed by microwaving slides in 10 mM citrate buffer (pH 6.2) for 30 min at high power, according to the manufacturer's instructions. To remove the endogenous peroxidase activity, the sections have been treated with freshly prepared 1.0 % hydrogen peroxide in the dark for 30 min at 37 °C temperature. Non-specific antibody binding was blocked by means of blocking serum.

The sections were incubated for 30 min, at 37 °C temperature, with the primary antibodies against myeloperoxidase diluted 1:100 in phosphate buffered saline (PBS) pH 7.2 then a triple washing with PBS follows. Anti-(mouse IgG)–horseradish peroxidase conjugate (1:40 000 dilution) has been fulfilled for the detection of the MPO primary antibodies, then the sections were incubated for 20 min, at 37 °C temperature. The colour was visualized by DAB. The appearance of the positive factors was detected semiquantitatively by counting of positive giant cells in visual field.

The data were analysed using STATISTICA 8.0 software, user version STA862D175437Q. The results have been presented as mean ± SD. The normalize test have been used before analysis of the data. Also, the nonparametric Student method was applied to perform a simple comparative analysis. The value of  $P < 0.05$  have been considered to be a significant.

#### **Results**

The periodontal measurements of participants recruited for this study have been summarized in Table 1.

The normal periodontal tissues a shown in Fig. 1(1). Connective tissue gingival fibres support the gingival margin as a cuff around the tooth.

The results of the exacerbation of chronic process a shown in Fig. 1(2). Connective tissue have swelled destruction of marginal gingiva (Fig. 1(2A)) and then of deeper supporting tissues. Influence of alternative factor acceleration of secondary acute inflammation as are result periodontal fibrous tissue destruction. Connective tissue destruction have secondary exacerbation of chronic process with more complex patterns of destruction Fig. 1(3B). Edema have observed between collagen fibers Fig. 1(3A).

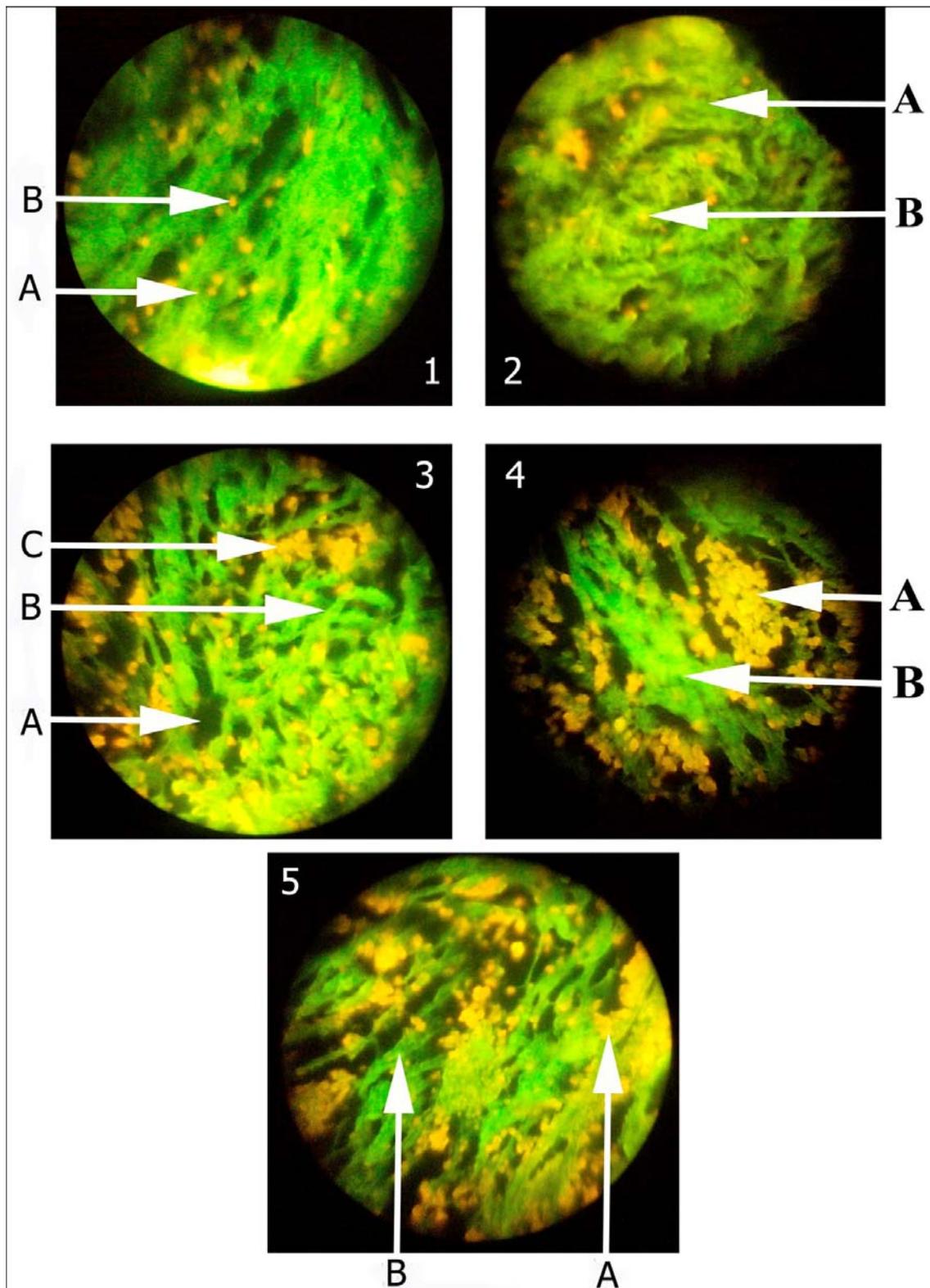


Fig. 1. Changes in collagen during inflammation.  
 1 - normal connective tissue: A – connective tissue, B – immune cells;  
 2 - exacerbation of chronic inflammation: A – swelled destruction of connective tissue B – immune cells;  
 3 - chronic inflammation: A – collagen fibers edema B – complex patterns of collagen destruction C - immune cells form the layers or trabeculae;  
 4 - outcome of the chronic inflammation: A – leukocytes accumulate in foci B – trabeculae of connective tissue;  
 5 - outcome of the chronic exacerbation inflammation: A – different forms of inflammatory infiltration B – trabeculae of connective tissue.

Periodontal measurements of patients

Clinical parameters	Exacerbation of chronic process	Chronic subgroups	Normal periodontal tissues
$\Sigma$	N = 13	N = 17	N=4
probing pocket depth $\leq 4$ mm	14.61 $\pm$ 0.58***	3.51 $\pm$ 0.12***	
probing pocket depth 4-6 mm	26.19 $\pm$ 8.55*	0.00	
probing pocket depth $\geq 7$ mm	18.34 $\pm$ 3.62*	0.00	
clinical attachment level	5.62 $\pm$ 0.56**	3.98 $\pm$ 0.14*	
bleeding on probing	4.86 $\pm$ 0.43*	15 $\pm$ 0.11	
number of teeth	26.76 $\pm$ 2.12**	6.27 $\pm$ 0.52***	

Note: p value – \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001.

The results of the chronic a shown in Fig. 1 (4). Consists of dilated thin-walled vessels in loose edematous stroma with superimposed edema. Widespread edema of collagen fibers increasing of depth cells infiltration (Fig. 1 (4A)). The results of the chronic exacerbation inflammation a shown in Fig. 1 (5). Destruction have progressed until tooth support becomes inadequate and more complex patterns of collagen fibers loss develop. Despite the deep extension of inflammation it may have recovery of alveolar collagen fibers complex.

We have determined five variants of immune cells microenvironment:

1. Nodular – the leukocytes accumulate in foci, sometimes with the formation of pseudo-follicles. Fig 1(4A)
2. Trabecular – the immune cells form the layers or trabeculae Fig. 1(2)
3. Diffuse - the leukocytes are located around

the collagen fibers as single scattered cells Fig. 1(3C).

4. Mixed – a combination of different forms of inflammatory infiltration. Fig. 1(5A).

In the stroma of the control group tissue the immune cells were also presented, because they are a part of the fibrous tissue of any organs, including the oral cavity. But they are located singly and very rare.

The immunoexpression of MPO have been confirmed by the presence of brown stained cytoplasm in cell infiltration. Myeloperoxidase was expressed in 98,2  $\pm$  5,9 % (P < 0.01) positive cells in exacerbation of chronic process. Figure 2 shows the results of the MPO expression in chronic and exacerbation of chronic process. Myeloperoxidase 72,8  $\pm$  4,5 % P < 0.01 was expressed in chronic plasmatic cell infiltration during inflammation. Normal periodontal tissues single neutrophil have been detected.

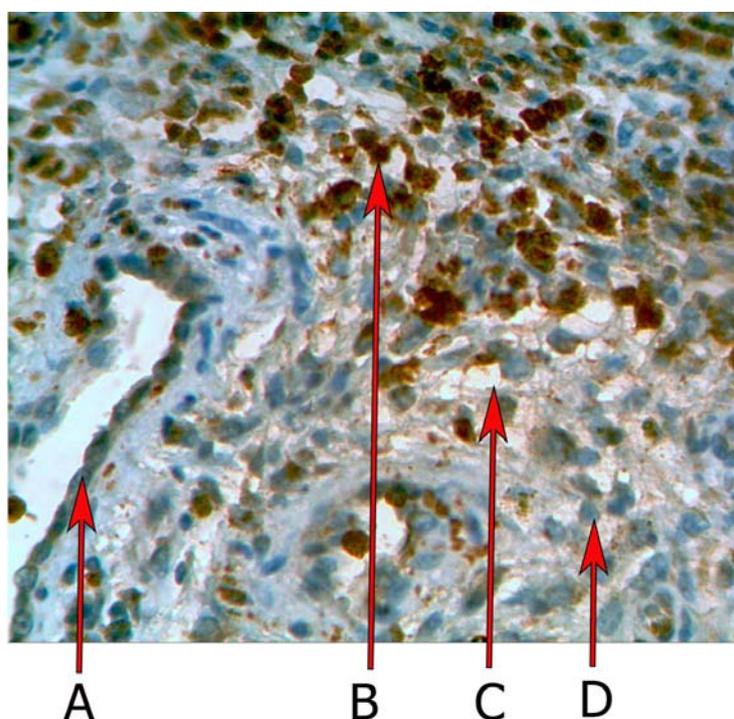


Fig. 2. Immunoexpression of MPO in chronic inflammation. A – vessel; B – MPO positive cells; C – edema; D - MPO negative cells.  $\times 320$ .

## Discussion

Periodontal disease have caused by selected species of bacteria that colonize the tooth surface and invade the adjacent tissue, causing inflammation and, ultimately, connective tissue destruction and bone resorption. Most patients with plaque-induced periodontitis will have the chronic form [7]. In most cases the disease is slowly progressing [8], but short periods of rapid attachment loss can occur [9]. Although chronic periodontitis can occur in localized or generalized patterns, the two forms appear to be identical with regards to their etiology and pathogenesis. However, evidence that tissue loss caused by periodontal disease is reduced by inhibitors of prostaglandins and inhibitors of matrix metalloproteinases indicates that much of the tissue damage is caused by up-regulation of the host response [10, 11] We have reported that MMP1 significantly simulat tissue destruction and inflammation [12]. MMPs are the main collagen-degrading enzymes in gingival and they are believed to be mainly responsible for collagen degradation in inflamed tissue during periodontitis. They produced by fibroblasts, macrophages and neutral metalloproteinase such as pro collagenase. The cells participating in exacerbation of chronic and chronic inflammation are circulating leucocytes, plasma cells and tissue macrophages.

Myeloperoxidase (MPO) have catalyzed the synthesis of microbicidal hypochlorous acid enabling the defence against bacteria [13]. Furthermore, plasma cells synthesize hypochlorous acid from  $H_2O_2$  and NaCl. Hydroxyl radical ( $-OH$ ) is mostly active in damaging important molecules such as

DNA proteins and lipids [14]. Hydrogen peroxide ( $H_2O_2$ ) being a potent agent of oxygen species, is capable of crossing the nuclear membrane and damaging the DNA [15]. There is growing support for the claim that inflammation induces DNA damage which leads to apoptosis in periodontal cells [16].

Inflammation is typically characterised by vascular and cellular events with emigration of neutrophils leukocytes, not all examples of acute inflammation show infiltration by neutrophils. On the other hand, some chronic inflammatory conditions are characterised by neutrophilic infiltration. Osteomyelitis is an example of chronic inflammation but the cellular response in this condition is mainly neutrophilic. The morphological variation in inflammation depends upon a number of factors and processes. This testifies to the formation of different morphological forms of infiltration which we first described in periodontal disease.

## Conclusion

Connective tissue have swelled and destruction during inflammation. Edema have observed between collagen fibers. The collagen damage during periodontitis is the action of neutrophils. Widespread edema of collagen fibers increasing of depth cells infiltration during chronic inflammation. Nodular type cells microenvironment have observed during outcome of chronic inflammation. Trabecular – the immune cells form have observed during exacerbation of chronic process. Diffuse type cells microenvironment have observed during chronic inflammation process.

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**Кузенко Е., Дяченко Е., Кузенко Е., Олишкевич А. Клеточное микроокружение и разрушение коллагена при воспалении пародонта.**

**Реферат.** Связь между периодонтитом, повреждением коллагена с микроокружением из иммунных клеток остается актуальной проблемой в стоматологии, которой посвящена данная работа. Нами было выделено пять вариантов микроокружения из иммунных клеток: узловой, диффузный, трабекулярный, смешанный. Узловой тип микроокружения наблюдается на исходе хронического воспаления; трабекулярный тип микроокружения – при обострении хронического процесса; диффузный тип - при хроническом воспалительном процессе. При обострении хронического процесса и хроническом пародонтите наблюдается разрушение коллагеновых структур, что сопровождается отеком и незначительным некрозом.

**Ключевые слова:** пародонтит, клеточное микроокружение, коллаген.