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PKHALADZE MAIA

ULTRASTRUCTURAL ASPECTS OF GINGIVAL SOFT TISSUES CELLS POPULATION UNDER EXPERIMENTAL GINGIVITIS

14.00.15- Pathological anatomy

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Scientific Supervisor – Professor ***Zurab Tsagareli.*** M.D., Ph.D., Sc.D.

Official Opponents - Professor ***Teimuraz Jorbenadze.*** M.D., Ph.D., Sc.D. (14.00.15)

- Professor ***Tamar Okropiridze.*** M.D., Ph.D., Sc.D. (14.00.21)

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Secretary of Scientific Qualifying council,
M.D., Ph.D., Sc.D., Professor R.Rukhadze

THE GENERAL CHARACTERISTICS OF RESEARCH

Problem actuality

Establishing new treatment technologies in the dental health service centers intensified the interest of investigators and stomatologists to the different kinds of pathological process in oral cavity, especially to the gingival regeneration problems. Detailed research of gingival soft tissue trauma and its outcomes are very actual problem, which enquires to include morphological methods in research. Numerous articles have been devoted to the problem of gingival epithelium injury and healing ?Oshio K., Shiomi N. et al. 2000; Wiberg M., Hazari A. et al. 2003; Silvestri M., Sartori S. et al. 2003).

In the latest decade there was revised knowledge about regeneration process in mammals (Sarkisov D.S. 1993; Wang H.H., Carroll W.J. 2000; Bikov V.P 2005), including surgical wound healing as one of the types of regeneration process ?Polimeni G., Albandar J.M.,Wikesjo U.M. 2004; Cheung H.K., Zhang J. 2004), also skin type mucous membrane healing. It is established, that the main stage of mucous membrane restoration is epithelium regeneration, followed by perfect recovery. Process is characterized by hypertrophy and hyperplasia of cell and tissue structural elements.

Problems of the gingival epithelium reparation are closely connected with the pathogenesis and clinics of parodont system diseases. In the latest years there were intensified investigations on pathogenesis of parodont system diseases and their morphological substrate (Tatishvili N.2002; Kipiani G. 2003; Lobjanidze T2005; Galogre A. et.al 2005; Kassab E.A. 2003). It provides to estimate each cell population, its role in inflammatory and reparation process. The microcirculatory system damage is very important; It is an independent factor of initiation and development of parodontitis ?Johnsson et al. 2004; Zhao et al. 2005; Tsagareli Z. et.al 2005).

Purpose and Tasks of Research

Purpose of research was to study reparation process of gingival mucous membrane epithelium and in its different layers during the experimental gingivitis, take in to consideration hypertrophy, hyperplasia and cell (nucleus) dying process; To establish the equation of these processes during the wound healing without complication; To investigate current ultrastructural changes in gingival histological elements and tissue-capillary relationship based on these findings.

The tasks of research

1. To establish morphological changes in epithelium and the time of its restoration;
2. To study degree of hypertrophy and hyperplasia (changes in cells size, quantity and their structural elements) process in multilayer flat epithelium.
3. To reveal relationship between cells mitotic activity and nuclear dying processes during the experimental gingivitis.

The Theoretical Value of Research

It is established the injury and reparation process dynamic in the germinative and granular layers of the mucous membrane, based on comparing of the cell area, mitotic index and nuclear dying coefficient. It was realized comparison of the cell area, quantity and proliferation activity with intensity of the dying process in the individual layers and concluded about presence of the mitotic gradient comparison with cell dying process during first 5 days. In the same terms there was increased number and area of double-nucleus and nucleolus cells. Significant changes

were revealed in the lamina proprea of mucosal membrane. Particularly decreased number of macrophages, immunocompetentive cells and fibroblasts, which reduced granulation process and caused persistention of perivascular swelling.

The weak immunohistochemical expression of cytoceratins 5.18 and 10.13 display a epithelium and mucosal membrane dissociation and disturbance of the terminal differentiation.

The Practical Value of Research

The knowledge about the terms of gingival mucous membrane epithelization and reparation is one of the main components in the treatment of gum and parodontal system diseases.

Approbation of Research

Approbation of Research has been leaded in the scientific council′s expended session of the department of general pathology and human ecology, A.Natishvili Institute of the Experimental Morphology, Georgian Academy of sciences (23 March, 2005 year, N8).

Structure and Volume of Research

Dissertation is stated on 112 typed pages and contains: introduction, the review of the literature, methods and materials of research, chapters of own research results, diction of received results, conclusions, practical recommendation and index of used literature, which contains 133 items. The material is illustrated with 27 micro photos and 6 tables.

PUBLICATIONS around the dissertation have been published 4 scientific works (The list of publications is introduced at the end of the work).

MATERIALS AND METHODS OF RESEARCH

The experiment has been done on 80 nonlinear white mature rats (age 2-3 months, weight 120-160 g). The rats were kept in common vivarium conditions in several small groups (5 rats). Their feeding ration correspond the recommendation of Zapadnuk I. P et.al (1983).

As a rule, the experiment was carried out in the first half of the day, in 18-22C^0 conditions.

Experimental animals were divided in two groups:

I group - 10 rats – without operative intervention, they spent 14 days in vivarium quarantine (control groups).

II group – 70 rats, on which experimental gingivitis was designed by using wide spread method (Volojin A.I., Vinogradova S.I. 1991). After peeling the gingiva around the dental neck we put the ligature which caused the mechanical irritation.

All painful manipulations have been done with ether anesthesia. The animals were killed after 300 mg/kg hexanal injection, after 12-24 hours and 3, 5, 14, 28, 45 days of operation.

3-5 mm pieces of gingival mucous were always taken from the same place: in experimental animals – from the area between the wound and frontal teeth gingiva. In control group animals – from upper general dental gingiva.

Taken materials for the morphological research were fixed in 10% neutral formalin, embedded in paraffin; Specimens were stained by hematoxylin and eosin. Stained specimens were used for estimation of the cell structure of gingival mucosal membrane.

5 mkm paraffin section were used for immunohistochemical study provided by standard streptavidin – biotin – peroxidas method. Anti cytoceratin 10.13 and 5.18 (Novastain Super ABC system Great Britain) monoclonal antibodies were used. To reveal the products of immunoreaction was used DAB (USA) visualization system. Specimens were post stained by hematoxilin. For results estimation we took into consideration the localization and intensity of immunostain.

For electron-microscopic investigations, material was fixed in the 2.5% glutaraldehyde solution (pH 7.3-7.4). Post-fixation was made in OsO_4 1% solution (pH 7.35). After dehydratation in ascending alcohol series material was embedded in Epon resin. Ultrathin sections were obtained using Reichert-OmU3 ultramicrotome and stained with uranyl acetate and lead citrate solution, viewing and photographing with Tesla BS-500 electron microscope (accelerating voltage 70Kv).

We measured the whole thickness of the epithelial regenerate on the gingival mucous epithelium specimens by the microscope (Obx20) and ocularmicrometer (Obx15) to determine regenerative potential of the epithelium. Also we separately measured thickness of the basal, spinous, granular and corn layers. Besides we count the granular and spinous layers cell rows number.

In the same sections we studied area of the whole cell, cytoplasm, nucleus and nucleolus, also number of the nucleus and nucleolus in the separate cells by planimetric method.

Average value was calculated from 20-100 measurement for each parameter. Statistical analysis showed, that such number of measurement guaranteed repeatable results.

In the epithelial regenerates we counted: amount of the mitotic figures in the basal and spinous cells (prophase, metaphase, anaphase, telophase), double-nucleated cells in the spinous layer, amount of the dying nucleus (picnosis, karyolisis) among 5000 cells on the 3-5 specimens taken from 25-30 mkm intervals. Cell counting was done with binocular microscope (OcX7, ObX90). In the ocular was placed 4X4mm diaphragm. We determined mitotic coefficient (mc), double nucleated cell coefficient of the spinous layer (dcc) and dying nucleus coefficient (dnc). The results were expressed in per cents (%).

The statistical analysis was made by student t-criteria. The difference was confident if $p<0.015$.

RESULTS OF RESEARCH AND DISCUSSION

There was epithelial injury after 12 hours of the procedure (rat model of the gingivitis). After 12 hours of operation we found reepithelisation signs in the defect, though wall thickness of the regenerated epithelium was not changed by this time, however during this period the thickness of the spinous layer was increased by 6%. Our study reveals hypertrophy and hyperplasia of the multilayer flat epithelium in 3-5 days.

After 3 days of operation there was hypertrophy of the wall epithelium – 9%. Basal layer thickness was increased by 19%, spinous layer – 6%, number of cell lines was increased by 31%. In the granular layer we observed as layer thickness, as increasing number of cell lines. Epithelium thickness reaches its maximum after 5 days – 12% (from 134,26mkm to150,

68 mkm). At this time the basal layer is thickened by 18%, spinous layer – 11%, amount of cell rows is increased by 33%, granular layer was thickened by 18 %.

After 14 days from operation basal layer of epithelium maintained tendency of thickening (11%), these parameters normalized after only 28 days.

During the experiment the corn layer remained unchanged.

After operation, made on mucosal membrane, the epithelium examination revealed, that changed not only the thickness of layers, but the area of the cell structural components.

From the beginning of the experiment, after 12 hours of operation, the basal cell area was increased by 14%, which is due by increasing of cell cytoplasm area (34%). It can be explained by postoperative oedema.

Increasing of basal cells and their cytoplasm area is not statistically confident. On this term nucleolus area was increased by 24%. Pikkok et al. (2004) suggest that increasing of number and size of nucleolus associated with intensification of anabolic process in the cells.

This suggestion is proved by the fact, that after 5 days from modeling if gingivitis the basal cell area reached its maximum – 20%, which is caused by increasing of nuclear are – 32%. Increasing of cytoplasm area is not statistically confident. At the same time number of nucleolus was increased – 8%, the number of double-nucleolus cells – 3 times. The basal cell layer, which was increased by 12% at the fifth day, was gradualy decreased at 14th day. This was due by changed nuclear area. Number of the double-nucleolus cells was two times increased by the 14th day of experiment (table 1).

Increasing rate of the cell area in the spinous layer was the same as in the basal layer – after 12 hour about 22% (maximal mean). Such hypertrophy of the cell is caused generally by sharp increasing of the nucleus area (53%).

There was no considerable difference in cell areas in the control and experimental groups on the third day of experiment; the same time nucleolus area was 48% increased. On the third day the double-nucleolus cell number was increased by 2.5 times.

On the 5th and 14th days the spinous cell area was not changed. At this time the area and number of the nucleolus was increased by 4%, number of double nucleolus cells – 1.5–2 times respectively.

On the 28th day of experiment parameters of cell, cytoplasm and nucleus area normalized. At the same time the number of double-nucleolus cells remained 2.6 times increased, but on the 45th day – 1.8 times in comparison with control group.

The changes in the granular layer were significant on the 5th day. Cell area increased by 9%, reached maximum on 3rd day (17%) and due by increasing of the cytoplasm area: maximum mean on the 3rd day (22%). On the 3rd day also icreased the nuclear area - 10%, and reached the normal levels on the 5th day after operation. The nucleolus is not changed during the experiment. After 14 day from operation areas of all structures were normalized.

During the wound healing changes of cell and nuclear areas was strongly associated with mitotic activity, which is the main source of the tissue restoration and which is realized by the one of the cell divission form – mitosis.

There is no consensus among the investigators about the localization of the mitosis in the epithelial layers. According Baliabin A. A. et al. (1978) the mitosis presented in all rows of the basal and spinous layers of the pathologically changed gingiva.

Galankin V. N. et al. (1987) noted the presence of mitosis in the granular cells too. Our experiment revealed that most mitosi were localized in the basal and law rows of the spinous layer. There was no evidence of the presence of mitosis in the granular and the upper rows of the spinous layer. Our findings are similar to the Tsepov. P. M et al. (1999) and Struev I. V. et al dates, who suggest that mitosi are located in the basal layer and bordering spinous cell rows. Mitosis is located as “nests” – several mitosi in the epithelial processus.

After 12 hours from operation there were solitary mitosi in the basal layer of the gingival epithelium. After 1 day mitotic activity reached 17.62% and maintained this increased rate before 14^{th} day of observation. This date correspond with Jarnbring et al. (2002) findings. In our experiment the maximal number of the divided cells were revealed on the 3rd day, mitotic coefficient reached 26.64%, on the 5th day - 22,84%. From the 28th day mitotic activity was decreased and reached control group rate in the basal layer (table 2).

Increasing of mitotic activity in the spinous cells was already evident after 12 hours from operation; it reached 5.74% which exceed the control group by 54%.

There was maximum mitotic activity in the spinous cells on the 3rd day – from 4.7% (control group) to 8.4%, on the 5th day – from 3.86% (control group) to 7.18%.

From the 14th day the mitotic activity decreased and reached control group level.

However basal and spinous layers are germinative layer (Hahmouzi J., 1999), That′s why it is very interesting total mitotic activity of these cells.

In the germinative layer mitotic activity reached maximum on the 3rd day, it is increased from 10.86% (control group) to 16.68%. On the 5th day – 14.52%.

Paralelly to the cell division processes (mitosis) there are evident cell dying events. Tissue restoration speed of epithelisation depends how much the division processes exceed cells death.

There are not quantitative data in the literature about the cell dying processes during a wound healing in the epithelium after gingival injure.

In the basal layer NDC reached maximum on the 3rd day when also was noted the maximum of mitotic activity. At this term NDC reached 8.32% (in control group 5.84%). On the 5th day there was increased rate of the NDC – 11%. Piknotic nucleus in the spinous layer was located in all cell rows. In this case NDC was increased as in the basal layer on 3rd day by 59%, from 3.42% (control group) to 5.44%, which was the same on the 5th day (table 3).

On the 3rd day total NDC in the germinative layer was increased about 46%, from 4,56% (in control group) to 6,69%. On the other terms of experiment there no any evidence for increasing the NDC. Presence of the double-nucleated cell indicated the reparation process (Simain-Sato et al. 1999, Kassab et al. 2003). The origin and function of these cells is not completely clear yet.

Double-nucleated cell coefficient increased from the beginning of the observation by 3,9 times (after 12 hours from operation) and remained increased till 5th day of experiment, when it is equals to controls parameters. We can compare our resultes with Kassab et al. (2003) data about presence of the double-nucleated cell in the spinous layer of the tongue mucosal membrane epithelium. Author remarks presence of maximal number of double-nucleated cells from the first hours after trauma, though minimal number of these cells coincided with maximal mitotic activity.

The total mitotic activity increased by 28% in the germinative layer.

So that the 3^{rd}-5^{th} days of experiment is critical moment in the gingival wound healing process, there is complete wound epithelization. On this very term significantly qualitative and quantitative changes were evident.

Some investigators, for example Hahmouzi et al. (1999) consider that area of regenerated epithelial cells reach their maximum at the moment of wound closing, but they do not give data about the size of the cell and its structural components. We suggest, that it is necessarily to reveal the relationship of all parameters for imagining complete picture. Changed parameters of area are more informative and exact, than mitotic coefficient, with which is connected.

Thickening of the basal layer can be explained by increased cell area, which reaches its maximum level on the 5^{th} day. At the same time nucleus size is enlarged maximum, number of the nucleoli and double- nucleolus cells significantly increased. It is significant that mitotic activity increased by 48% at this time, NDC – by 43%, but proliferate processes significantly exceed the dying process, because increasing of MC, which was 26.64%, considerable more than NDC, which finally cause tissue reparation. Cell row number increased by 31% in the spinous layer exactly due to cells, which area has the growth tendency. In these cells the

nuclear area and number of nucleoli, as a double-nucleated cells number was increased. Double-nucleated cells number increased 2.2 times.

Table 1. **Area of the rat gingival mucose membran epithelium basal layer cells and other structural elementes (mkm^2)**

Terms of observation and animal groupes		Cell area M±m	p	Cytoplasm area M±m	p	nuclear area M±m	p	nucleoli area M±m	p
12 hour	Experimental	73,08±1,22	0,004	34,20±0,80	0	38,88±1,08	0,7	1,96±0,06	0,347
	control	63,72±1,93		25,56±0,667		38,16±1,74		1,88±0,06	
1 day	Experimental	75,94±1,55	0,94	35,90±0,86	0,69	40,00±2,03	0,394	3,14±0,11	0,006
	control	68,12±3,86		32,04±1,57		36,08±2,44		2,54±0,11	
3 day	Experimental	81,00±4,29	0,011	31,56±2,06	0,631	49,32±2,31	0,001	2,18±0,00	0,393
	control	67,56±0,37		30,46±0,87		37,10±0,52		2,10±0,01	
5 day	Experimental	75,24±0,67	0	29,88±0,91	0,923	45,36±0,35	0	2,1±0,03	1
	control	67,08±0,40		29,88±0,73		37,10±0,52		2,1±0,04	
14 day	Experimental	70,38±0,29	0	29,18±0,25	0,565	41,0±0,58	0	2,14±0,02	0,565
	control	66,06±0,27		29,8±0,38		36,28±0,22		2,12±0,02	
28 day	Experimental	68,90±0,37	0,02	29,3±0,56	0,264	39,52±0,35	0,303	2,12±0,02	0,631
	control	66,78±0,61		28,3±0,57		38,52±0,87		2,10±0,03	
45 day	Experimental	67,52±0,58	0,303	28,94±0,51	0,264	38,58±0,28	1	2,10±0,00	1
	control	66,78±0,33		28,28±0,22		38,58±0,50		2,10±0,00	

Table 2. Changes of thickness of the **rat gingival mucose membran Epithelium layers (in microns)**

Terms of observation and animal groupes		Basal layer M±m	p	Spinous layer M±m	p	Granular layer M±m	p	Corn layer M±m	p	Whole epithelium M±m	p
12 hour	Experimental	8,96±0,14	0,303	67,80±1,48	0,027	33,04±0,98	1	28,06±0,78	0,846	138,06±4,35	0,264
	control	8,78±0,08		63,70±0,47		33,00±0,35		27,88±0,69		133,36±0,88	
1 day	Experimental	9,03±0,12	0,199	68,28±2,88	0,199	36,80±1,94	0,23	28,70±2,26	0,846	142,82±2,26	0,11
	control	8,82±0,10		64,02±0,19		34,04±0,73		28,02±0,78		134,90±0,73	
3 day	Experimental	10,56±0,49	0,008	68,24±2,11	0,199	38,26±2,00	0,059	29,16±0,86	0,23	146,20±3,03	0,017
	control	8,78±0,12		64,06±2,03		33,34±1,02		27,80±0,62		133,90±2,87	
5 day	Experimental	10,40±0,32	0,001	70,98±0,98	0,017	39,54±1,18	0,005	29,72±1,50	0,264	150,68±2,87	0,04
	control	8,80±0,10		64,14±2,05		33,54±1,06		27,78±0,55		134,26±2,85	
14 day	Experimental	9,86±0,32	0,015	66,40±1,77	0,199	37,24±1,44	0,069	28,86±0,70	0,394	142,36±3,14	0,05
	control	8,82±0,09		63,94±0,15		33,80±0,81		28,14±0,36		134,70±1,09	
28 day	Experimental	9,10±0,09	0,059	64,52±0,58	0,347	34,92±0,56	0,347	28,36±0,36	0,631	136,90±0,42	0,094
	control	8,82±0,08		63,84±0,38		33,78±1,07		28,08±0,38		134,50±1,20	
45 day	Experimental	9,00±0,812	0,303	64,28±0,34	0,631	34,08±0,59	0,983	28,32±0,41	0,631	135,74±1,29	0,631
	control	8,84±0,07		64,04±0,27		34,00±0,		28,04±0,4		134,92±0,	

Table 3. Mitotic coeficient and nuclear dayng coefficient basal layer of the rat gingival mucose membran epithelium

Terms of observation and animal groupes		Mitotic coeficient ?:%		nuclear dayng coefficient ?:%	
		M±m	P	M±m	P
12 hour	Experimental	18,10±1,25	0,11	2,86±0,32	0,631
	control	15,40±1,49		3,26±0,76	
1 day	Experimental	17,62±0,22	0	6,40±0,40	0,7
	control	14,42±0,20		6,52±0,15	
3 day	Experimental	26,64±0,37	0	8,32±0,52	0,005
	control	18,00±0,48		5,84±0,35	
5 day	Experimental	22,84±0,25	0	7,76±0,16	0,005
	control	15,76±0,13		7,00±0,13	
14 day	Experimental	19,26±0,59	0	6,38±0,58	0,303
	control	15,36±0,22		7,08±0,34	
28 day	Experimental	13,92±0,10	0,7	6,28±0,28	0,565
	control	13,50±0,13		6,78±0,43	
45 day	Experimental	13,76±0,10	0,394	6,66±0,07	0,394
	control	13,58±1,14		6,56±0,07	

Therefore gingival wound reparation due by intensification of the mitotic activity in the germinative layer, by new cells generation. Number of the dividing cells exceeds the number of the dying ones.

Increasing of the granular layer cell rows, the area of this cells, nucleus and cytoplasm area also reflect activity of the reparative processes in the submerged layers of epithelium. Toward the surface the spinous layer cells are more flattened and transformed in to the flattened cells.

Area of the granular layer cells are greater than in control group, but comparison with submerged spinous layer cells they are less than 1,5 times. Area of the cytoplasm of the granular layer cells are increased, bat they are 1,6 times less than spinous layer cells cytoplasm area., and area of the nucleus is lees - by 1,2 times.

At this time we can see some tendencies of the thickening of corn layer, but differences between control and experimental groups was not statistically confident.

On the 14^{th} day after operation all parameters are normalized. Epithelial layer is slightly thickened. There marked significant thickening of basal, and slight thickening of spinous and granular layers, reduced number of rows in spinous and granular layers but basal cell and its

nuclear area is not changed yet. Number of double nucleoli cells is increased, because MC is 20% more in comparison with control group. Area of spinous cells exceeds control group parameters. Their nucleoli and nucleus area is slightly increased, the same changes are marked with double-nucleolus cell number, but double-nucleus amount decreased and exceed the control group parameters only by 60%. MC and NDC equal to control parameters. According this fact we suppose, that the hypertrophy of spinous cells is independent phenomenon, because it disappears after MC decreasing. Though, cell hypertrophy is not associated with mitosis. Increased MC of germinative layer dues basal cells mitosis.

On the 28th day of experiment whole epithelium and its layers thickness is normalized.

On the 45th day of observation all studied parameters are in norm. Also increased number of nucleolus and double-nucleoli cells is not confident. According our and other investigators′ dates (Bartold P.M., Walsh H.J. 2000; Марченко В.Т. et al. 2004) crucial moment in the gingival healing is the beginning of the wound epithelisation in 3-5 days. If in this moment epithelisation process is disturbed, it points presence of coincident diseases, such as: alveolitis, osteomielitis and etc. (Быков В.П. 2005).

It is known, that main gingival function is – protective, which is provided by its immunocompetentive cells system, including lymphocytes, macrophages, antigen-presenting, dendritic and plasma cells.

In the realization of the immune reactions in gingival actively participate mast cells, fibroblasts and activated epithelial cells (Быков В.П., 2005).

Granulocytes play the pivot role in the nonspecifical immune reactions of gingival protective mechanism (Саркисов Д.С. 1993; Gmur R., Wiss C., et al. 2004. Trombelli H. 1999).

We supposed, that our findings about presence of the nonordered arrangement of the collagen fibers formations, which expressed by microclasmatosis and micropinocytosis from fibroblasts, are results of increased secretion from sensibilized fibroblasts. Presence of such formations proved by other authors (Mikhailova L. M., Barkhina T. G. et al, 2001; Van der Zee E., Vogels M.F. et al. 2004). Our electron microscopic investigations reveal that, in during gingival tissue reparation process, at the periphery of the plasmocytes clusters, which have tight junctions between each other, are located more big plasmocytes with dilatated endoplasmic reticulum, some times plasmocytes - with smooth endoplasmic reticulum. There are big amount of the lysosome, phagosomes, cytolysophagosomes in such cells. They also contain fragments of the membrane and granular structures. Characteristically, these plasmocytes population on the 14-28 days from experiment exceed of other cell population and occupies the big area of the investigative field. That is why part some plasmocytes take microphagal function. Other investigators found such changes in plasmocytes during the stress and infection (Joly J.C., Pelioto D.B. et al. 2002, Bimstein E., Matsson H., 1999; Wang H.H, Caroll W.J., 2000; Paolo antonio M., 2002).

Ultrastructural study reveals that from the 5th day of the experiment fibroblasts occupy most part of the mucosal membrane, which form groups or are solitarily located like plasmocytes. Fibroblasts have elongated forms; they have large elongated nuclei with dentated membrane and marginated chromatin. Cytoplasm occupies little area and commonly surrounds nucleus. It is poor with organelles. By the 14th-28th day big fields of collagen fibers, which are orientated in different direction, are progressing and gradually reach their maximum. In these fields we can see hypertrophy and hyperplasia of the collagen fibers.

Characteristical feature of the lamina proprea is activity of the mast cells. Mast cells on the 3th - 5th day are generally in the granule synthesis phase, and by the later terms they are on the different stages of exocytosis, which followed membrane destruction and cytoplasm lightening. In such cells we also can see features of pinocytosis and microclasmatosis.

Lymphocytes are located in groups (2-3 cells) or associated with other cell populations. In such cases, where lymphocytes are joined there are tight contacts, but when they join with different cell population, their connections are intermediate. These populations are presented by plasmocytes, granulocytes and mast cells. In some lymphocyte we can find dilatation of the

perinuclear space and chaotic distribution of heterochromatin. Similar events were described by other authors but at the late stages of gingivitis (Tsepov L. M., Levchenkova N.C. et al. 1999).

On the 28th day of the modeling experimental gingivitis there is reduction of multilayer flat epithelium swelling and is formed acanthosis and papilomatosis.In the lamina proprea we can found degranulation of mast cells and fibroblast activation. Characteristical feature of ultrastructural changes is limfoplasmocytar infiltration and recrutitation of macrophages. Some endothelial cells are dystrophic.

In the same time there are the prominent changes of the cellular structure in the plasmocytes. There are focal and sometimes diffuse dilatations of the rough endoplasmatic reticulum. There are focal or diffuse dilatations in perinuclear area. The same changes are noted during the chronic hypertrophy gingivitis (O. Khardzeishvili et al., 2001; Bikov V.P et al. 2005).

In the same term there a lot of cell associations: plasmocytes with lymphocytes, lymphocytes with granulocytes, fibroblasts with mast cells; there are different kinds of changes in these cells too.

Such a great number of plasmocytes are at the expense of other type of cells, it is very significant the reduction of imminocompetetive cells.

There are the same transformation in other tissues and organs. It indicates functional balancing of different kinds of cell populations. This fact is proved by light and electronmicroscopic method (Krijanovski G.N 2001; Mikhailova L. M et.al 2001; Kassab M.M., Cohen R.E. 2003).

On the early terms of experimental gingivitis, there is evidence activation of vascular endotheliocytes of the microcircular system: fibroblasts are located along the capillaries in groups. There are several changed and unchanged erythrocytes and trombocytes around the capillaries, which indicates activation of the nutritional transport. On the histological specimens blood vassals are filled. There is proliferation of the blood vassal contained no matured granular tissue, some eosinocytes are degranulated. It is proved by authors (Mikhailova L.M. et al. 2001; Kerdvongbundit V. et al. 2003; Anusaksatnien O., Webb S.A. et al. 2003).

Based on our and literature data we can suggest that gingival epithelium, fibroblasts and mast cells play a pivot role in the wound healing which functional activity become more diverse and increased secretory activity of lymphocytes and plasmocytes provides releasing of numerous anti-inflammatory mediators.

CONCLUSIONS

1. Reparation processes in the multilayer flat epithelium and mucosal lamina proprea in the experimental gingivitis model from the first days characterizes by active course. Double-nucleated cells, mitotic foci in the basal and spinous layer epitheliocytes. On the 45th day in the lamina proprea of the multilayer flat epithelium there is evidence of the multiplication of microcirculatory blood vassals.
2. Reparation processes after gingival injury presented generally in the basal and spinous layers: total mitotic coefficient of the germinative layer increased by 79% with comparison of norm and exceeds nucleus dying coefficient by 59%. Though before 5th day from operation filling the tissue defect mostly results from proliferation of the germinative layer cells.
3. Thickness of the multilayer flat epithelium basal layer is maximal on the 5th day after gingival injury at the expense of hypertrophies of the nucleus and nucleolus: the maximum rate of the nucleoli area and number was seen after 24 hours from operation. Tendency of growth of the nucleoli and nucleus area and number in the spinous layer is maintained until 5th day which cause maximal thickening of this layer on this term.

Decreasing of the mitotic activity in spinous layer became evident early than in basal layer.

4. On the 14th day in the lamina proprea of the mucous membrane during gingival wound reparation dominates fibroblasts, from the 28th day – plasma cells with hypertrophied channels of the rough endoplasmatic reticulum. In all parts of the microcircular vassals' endotheliocytes we found cells with bizarre nucleus, papillary processes of cytoplasm and thin peripheral cytoplasm, which made the structural precondition for improvement of the mucosal membrane feeding.
5. The hypertrophy and increasing of number of rows of the granular layer cells in the reparation process of the gingival mucosal epithelium coinciding with maximum level of the restoration processes in the germinative layer, which is due by migration of the spinous cell toward the epithelium surface. Granular layer cells hyperplasia was not revealed. The corn layer was not changed during the experiment (45 days).
6. In the dynamic of the reparation processes by the electron microscopic study in the subepithelial connective tissue was established the coarsening of the collagen fibers of the mucosal membrane of lamina proprea, activating of fibroblasts, also increasing density of the membrane of basal lamina hemidesmosomal and desmosomal contact surface of the epithelial cells.

   Significantly increased the expression of cytokeratin 5/18 and 10/13 in the gingival wound reparation processes, which are the markers of the terminal differentiation and strengthening of the junction of epithelium with lamina proprea.

LIST OF THE PRINTED SCIENTIFIC WORKS ON THE THEME OF DISSRTATION

1. Morphological characteristics of gingival mucous cellular composition in experimental gingivitis – "Experimental and Clinical Medicine", 2006, N3(28), p.15-18 (co-autor: Tsagareli Z., Gorgoshidze G., Tavzarashvili I.) (in Georgian).
2. Estimation of proliferative activity of gingival mucosa epiteliocytes under experimental gingivitis in rats - "Experimental and Clinical Medicine", 2006, N4(29), p.54-57 (in Georgian).
3. Morphological criteria of gingival mucous pathology in gingivitis - "Experimental and Clinical Medicine", 2006, N5(30), p.9-12 (co-autor: Tavzarashvili I., Gorgoshidze G., Kureli I., Tsagareli M.) (in Georgian).