

## Estimation of osteogenesis with AgNOR technology

Nunu Khetsuriani

Department of Surgical Dentistry, Tbilisi State Medical University, Georgia

### ABSTRACT

The odontogenic cysts are considered as one of the most frequent lesions among jaw and facial diseases. There are radicular and follicular (coronary) odontogenic cysts. The epidemiological investigations show that they constitute 94-96% of jaw diseases. The surgical treatment is a method of choice. A cystotomy or cystectomy may be performed. Despite of widely investigated treatment strategies for jaw odontogenic cysts, a great majority of questions remains disputable: 1. Which is a method of choice, if it is necessary to preserve teeth, roots of which are connected to the cyst wall? 2. Which is a method of choice, when dental roots are close but not etiologically related to the cyst wall by side surface or apex? 3. Is a resection of radicular apex always necessary? 4. How to stimulate bone regeneration after surgery to prevent complications? Despite of large number of osteostimulating medicaments, osteostimulation is considered as one of the most important and actual problems for scientists and clinicians. The results of clinical-experimental examinations showed that the mixture of chicken egg shell and vitamin D<sub>3</sub> (cholecalciferol) characterizes with advantage compared with hydroxylapatite. It is more effective osteostimulator, cost-effective, easily acceptable. It allows the termination of osteogenesis in 6-8 months or 1 year. The use of magnifying glass (binocular glass x6) facilitates a cystectomy, prevents to develop side effects and extend the indications for cystectomy (despite of size and localization of cysts).

**KEYWORDS:** *vitamin D<sub>3</sub>, cholecalciferol, powder of egg's shell, odontogenic cyst, cystectomy, osteostimulation*

**P**urpose of study: extension of indications for cystectomy – surgical treatment of jaw odontogenic cysts; The use of this method for any size cysts, in which the roots of intact teeth are located, allows a preservation of these teeth; The osteogenesis stimulation by effective and less expensive substances in a cyst cavity after cystectomy; To avoid a resection of important parts of radicular apex by osteogenesis stimulation to stabilize teeth located in a cyst cavity.

**Tasks of study:** a comparative characterization of experimental results obtained by osteogenesis investigation after filling of a bone cavity by hydroxylapatite and a sterile disperse powder of chicken egg shell and vitamin D<sub>3</sub> (cholecalciferol). The histological investigation and comparative characterization of bone regeneration after use of hydroxylapatite and a sterile disperse powder of chicken egg shell and vit.D<sub>3</sub> (CES+Vit.D<sub>3</sub>). To perform cystectomy, despite of size and localization of odontogenic cysts; To remove a cyst wall under the magnifying glass (x6) to prevent an injury of neurovascular bundles of intact teeth and mucosa of maxillary and nasal sinus. A stimulation of osteogenesis in a bone cavity by CES+Vit.D<sub>3</sub> prepared ex tempore after cystectomy. A roentgenological examination and dynamical evaluation of osteogenesis.

### MATERIAL AND METHODS

As the material of study have been used 60 adult rats of 180-200 g. The animals were distributed into three groups. Each group included 20 rats. The samples were studied 8 days, 16 days, 1 month and 6 months after surgery.

The operation was performed under anesthesia by sodium thypental. The animals were fixed by abdomen on the table, hairs were removed from thigh area, skin was treated by 96% ethanol and 5% iodine solution. A wound was made on back surface of thigh region and was extended to bone (up to 1 cm). Periosteum was removed by dental drill (with low rotation and permanent cooling by running water). In thigh cortical bone 2x1 cm size defect was made.

In the experimental group 1 a bone defect was filled by hydroxylapatite (20 cases). In the group 2 this defect was filled by the mixture of a sterile disperse powder of

chicken egg shell and oil solution of vitamin D<sub>3</sub> prepared ex tempore (20 cases). In the group 3 bone defects remained without osteostimulation.

In all cases the wounds were closed by 5/0 atraumatic threads. The operated animals have been under common regimen and diet. In the initial days the movement of feet was restricted due to pain. The swelling of soft tissue was moderate. In 52 cases the wounds were healed by primary extension, in 8 cases a secondary wound healing was observed, but this did not have any impact on the terminal results of surgery.

8 days, 16 days, 1 month and 6 months after surgery a decapitation of experimental animals has been performed. Afterwards the histological specimens were taken (60 samples).

The bone specimens were fixed in 10% neutral buffered formalin solution and embedded in paraffin. The paraffin sections were stained by hematoxylin-eosin, van Gieson and AgNOR technique to detect nucleolar organizer regions (Bio-Optica Milano).

The cytomorphometric study was performed. In H&E-stained slides osteoblasts, osteocytes, osteoclasts, chondrocytes and blood vessels were counted in 10 randomly selected fields of view. In AgNOR-stained slides AgNOR+cells and AgNOR dots were counted. The statistical significance of differences was evaluated by t test.

### RESULTS

In order to characterize bone regeneration after use of sterile disperse powder of chicken egg shell and vitamin D<sub>3</sub> and hydroxylapatite, an experimental study was carried out. A bone defect was made in thigh bone of rats. This defect was filled by hydroxylapatite in group 1 and our osteostimulator CES+Vit.D<sub>3</sub> in group 2. The bone defect remained without osteostimulation in group 3. In each group a histological study was performed 8 days, 1 month and 6 months after the experiment.

The histological specimens were studied by haematoxylin-eosin, van Gieson and AgNOR technique to detect nucleolar organizer regions. The cytomorphometric study was performed (Tab.1,2,3).

Morphometric data	After 8 days	After 16 days	After 1 month	After 6 months
Compact bone	+	+	-	-
Spongy bone	-	+	+	+
Cartilaginous tissue	-	-	-	-
Osteoblasts	-	12,3±1,2	26,4±1,2	21,5±1,4
Osteoblasts	40,2±0,9	21,2±1,4	11,5±0,7	9,6±0,8
Osteoclasts	-	-	2,1±0,3	-
Chondrocytes	-	-	-	-
Blood vessels	6,4±0,7	9,2±0,7	2,8±0,2	2,4±0,1

**Tab.1** Results of morphometric study in group 1.

Morphometric data	After 8 days	After 16 days	After 1 month	After 6 months
Compact bone	-	-	-	+
Spongy bone	+	+	+	-
Cartilaginous tissue	+	+	+	-
Osteoblasts	24,6±0,9	25,4±0,9	32,6±1,3	38,6±0,8
Osteoblasts	18,8±0,7	12,3±1,1	9,3±0,7	-
Osteoclasts	9,8±0,6	8,4±0,5	7,3±0,4	-
Chondrocytes	39,6±1,6	31,4±1,6	46,8±1,4	-
Blood vessels	2,2±0,2	7,4±0,2	9,2±0,5	2,2±0,4

**Tab.2** Results of morphometric study in group 2.

Morphometric data	After 8 days	After 16 days	After 1 month	After 6 months
Compact bone	+	+	-	-
Spongy bone	-	-	+	+
Cartilaginous tissue	-	-	+	+
Osteoblasts	-	-	24,8±1,3	16,9±1,1
Osteoblasts	37,8±1,3	41,8±1,7	7,8±0,4	11,5±0,8
Osteoclasts	-	-	3,5±0,6	-
Chondrocytes	-	-	93,2±1,4	34,6±1,4
Blood vessels	3,4±0,5	3,2±0,4	1,2±0,2	1,5±0,4

**Tab.3** Results of morphometric study in group 3.

The comparative analysis of cytomorphometric results in group 1 (bone defect was filled by HA) and group 2 (bone defect was filled by CES+vit.D<sub>3</sub>) showed that 8 days after the experiment in group 1 the small fragments of compact bone were observed, but in group 3 the fragments of spongy bone and cartilaginous tissue were found. In group 2 the mean number of osteoblasts was 24,6±0,9, but group 1 lacked these cells. In group 2 the mean number of osteocytes was 2,1 times less ( $p < 0,05$ ),

the mean number of blood vessels was 2,8 times less ( $p < 0,05$ ), than in group 1. In group 2 the mean number of osteoclasts was 9,8±0,6, but group 1 lacked these cells. In group 2 the mean number of chondrocytes was 39,6±1,6, but group 1 lacked these cells.

16 days after the experiment in group 2 the fragments of spongy bone and cartilaginous tissue were observed, but in group 1 the fragments of compact and spongy bone

were found. In group 2 the mean number of osteoblasts was 2,1 times more ( $p<0,05$ ), the mean number of osteocytes was 1,7 times less ( $p<0,05$ ), the mean number of blood vessels was 1,2 times less ( $p<0,05$ ), than in group 1. In group 2 the mean number of osteoblasts was  $8,4\pm 0,5$ , but group 1 lacked these cells. In group 2 the mean number of chondrocytes was  $31,4\pm 1,6$ , but group 1 lacked these cells.

1 month after the experiment both groups lacked a compact bone. in group 1 the fragments of spongy bone were found, in group 2 the fragments of spongy bone and cartilaginous tissue were observed. In group 2 osteoblasts was 1,2 times more ( $p<0,05$ ), the mean number of osteocytes was 1,2 times less ( $p<0,05$ ), the mean number of osteoclasts was 3,5 times more ( $p<0,05$ ), the mean number of blood vessels was 3,3 times more ( $p<0,05$ ), than in group 1. In group 2 the mean number of chondrocytes was  $46,8\pm 1,4$ , but group 1 lacked these cells.

6 months after the experiment in group 2 the fragments of compact bone were found, in group 1 the spongy bone and cartilaginous tissue were observed. In group 1 the mean number of osteoblasts was  $21,5\pm 1,4$ , but group 2 lacked these cells. In group 2 the mean number of osteocytes was  $9,6\pm 0,8$ , but group 1 lacked these cells. In group 2 the mean number of blood vessels was 1,1 times less ( $p>0,05$ ), than in group 1. Both groups lacked osteoclasts and chondrocytes.

The comparative analysis of AgNOR results (Tab.4) showed that in group 1 (bone defect was filled by HA) AgNOR-positivity was not found in any dates of the experiment.

In group 2 (bone defect was filled by CES+vit.D<sub>3</sub>) AgNOR-positivity was observed 8 days, 16 days and 1

month after the experiment. 6 months after the experiment all cells were AgNOR-negative. 8 days after the experiment the mean number of AgNOR+ cells was  $38,7\pm 1,4$ , the mean number of AgNOR dots per cell was  $1,7\pm 0,2$ . 16 days after the experiment the mean number of AgNOR+ cells was  $30,5\pm 1,6$ , the mean number of AgNOR dots per cell was  $2,2\pm 0,3$ . 1 month after the experiment the mean number of AgNOR+ cells was  $45,3\pm 1,5$ , the mean number of AgNOR dots per cell was  $1,8\pm 0,4$ .

In group 3 (bone defect remained without osteostimulation) AgNOR-positivity was found 1 and 6 months after the experiment. 8 days and 16 days after the experiment all cells were AgNOR-negative. 1 month after the experiment the mean number of AgNOR+ cells was  $88,4\pm 2,3$ , the mean number of AgNOR dots per cell was  $1,0\pm 0,1$ . 6 months after the experiment the mean number of AgNOR+ cells was  $33,5\pm 1,4$ , the mean number of AgNOR dots per cell was  $1,2\pm 0,2$ .

A comparative analysis of morphometric results showed that experimental application of CES+vit. D<sub>3</sub> stimulates a reparative regeneration of bone tissue. The regeneration of bone defects is more intensive after use of CES+vit.D<sub>3</sub> compared with hydroxylapatite.

#### CONCLUSION

The histological study suggests a progressive course of reparative regeneration. 8-16 days after the experiment the numbers of osteoblasts, osteoclasts and osteocytes were increased. After 1 month the bone defect was filled by spongy bone, after 6 months the compact bone may be found. After use of HA regeneration and proliferation progress less intensively.

Experiment dates	AgNOR+ cells		AgNOR dots	
	Group 2	Group 3	Group 2	Group 3
After 8 days	$38,7\pm 1,4$	-	$1,7\pm 0,2$	-
After 16 days	$30,5\pm 1,6$	-	$2,2\pm 0,3$	-
After 1 month	$45,3\pm 1,5$	$88,4\pm 2,3$	$1,8\pm 0,4$	$33,5\pm 1,4$
After 6 months	-	$1,0\pm 0,1$	-	$1,2\pm 0,2$

**Tab.4** Results of AgNOR study.

#### REFERENCES:

1. Бернадский Ю.И. – Основы челюстно-лицевой хирургии. . 2000г. с303-313.
2. Робустова Т.Г., Безрукова – Основы челюстно-лицевой хирургии. . 2000г. 127-202.
3. Woner H. – Rontgen beim Zahnarzt. – Koln: Quintessenz, 1990.s. 262-300 (2003) 305-331

## Анализ остеогенеза AgNOR технологией

*Нуну Хецуриани*

Кафедра хирургической стоматологии Тбилисского государственного медицинского университета, Грузия

### РЕЗЮМЕ

Одной из важных проблем современной хирургической стоматологии является заполнение дефектов костной ткани, образующихся при хирургическом лечении ряда заболеваний и повреждений кости. Различные биологические материалы, применяемые для замещения дефектов костной ткани, в настоящее время не в полной мере отвечают требованиям хирургии. 36 больным с одонтогенической костной челюстью провели цистэктомию. Полости кисты заполняли смесью порошка яичной скорлупы и витамина Д<sub>3</sub> для стимулирования остеогенеза на участке послеоперационного костного дефекта. Химический состав яичной скорлупы идентичен химическому составу человеческих костей и зубов, а витамин Д<sub>3</sub> стимулирует обмен ионов Са и Р. Раны во всех случаях зажили первичным натяжением. После 2-3 месяцев, при рентгенологическом контроле отмечалась отчетливая тенденция к уменьшению кистозной полости.

**Ключевые слова:** *колекальциферол, стерильный порошок яичной скорлупы, одонтогенная киста, цистэктомия*