# EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

# Effect of Combined Injection of Hydroxyapatite and Estrone on Dental Well Healing

**I. N. Ivasenko, M. M. Solov'ev, G. M. Mel'tsova, D. A. Ivasenko, and V. A. Almazov**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 6, pp. 693-697, June, 1997
Original article submitted January 29, 1996

Combined injection of hydroxyapatite and estrone stimulates bone tissue regeneration in albino rats with tunnel defects of bone tissue after removal of mandibular incisors. $^3$H-thymidine autoradiography demonstrates that bone tissue regenerates due to proliferation and differentiation of osteogenic bone marrow precursor cells and connective tissue pericytes.

**Key Words:** *bone marrow; connective tissue; reparative osteogenesis; hydroxyapatite; estrone*

Reparative osteogenesis is an urgent problem of clinical dentistry and requires the search for effective treatment of diseases developing during repair of the alveolar edge of the jaws after removal of teeth and treatment of cysts of the jaws [5,7,13].

The presence of osteogenic precursor cells in human and animal bone marrow is a known fact, but bone marrow is not yet widely used as the substrate of reparative osteogenesis [4,9,10].

Hydroxyapatite (HA) and HA-based preparations have been widely used both in experiments and clinical practice [7,8,12,14]. Estrogens, potent stimulants of proliferation in target organs, cause similar effects in virtually all tissues [1,3].

Estrogen receptors are present on human and animal osteogenic bone marrow precursor cells [11]. Although estrogens have been proposed as stimulants of reparative osteogenesis for filling bone tissue defects [4], they have not been used for this purpose in clinical practice.

Our purpose was experimental validation of combined use of bioprostheses with 25% of HA and estrone for accelerating mandibular well filling in albino rats.

## MATERIALS AND METHODS

Forty-eight male Wistar rats weighing 150 g were used. Tunnel defect in the mandibular bone was created by removal of the left mandibular incisor, so that bone marrow elements could enter the tunnel.

After the tooth had been removed, gingival edges were drawn closer with sutures to create conditions for the formation of blood clot in the dental well. In experimental animals the defect was filled with bioprosthesis containing the tested components.

The animals were divided into 4 groups, 12 rats in each. In group 1 (control) no bioprostheses were used, in group 2 dental well was filled with bioprosthesis, in group 3 dental well was filled with bioprosthesis with 25% of HA [7], and in group 4 crystalline estrone was inserted in dental well in a dose of 0.5 mg/kg and bioprosthesis with 25% HA.

On days 4, 7, 14, and 30 postoperation three animals from each group were sacrificed. One hour before sacrifice the animals were injected $^3$H-thymidine in a dose of 1 μCi/g intraperitoneally. The

Isotope Laboratory, Department of Pathology, Research Center, Surgical Dentistry Chair and Faculty Therapy Chair, I. P. Pavlov St. Petersburg Medical University

0007-4888/97/0006-0601$18.00 

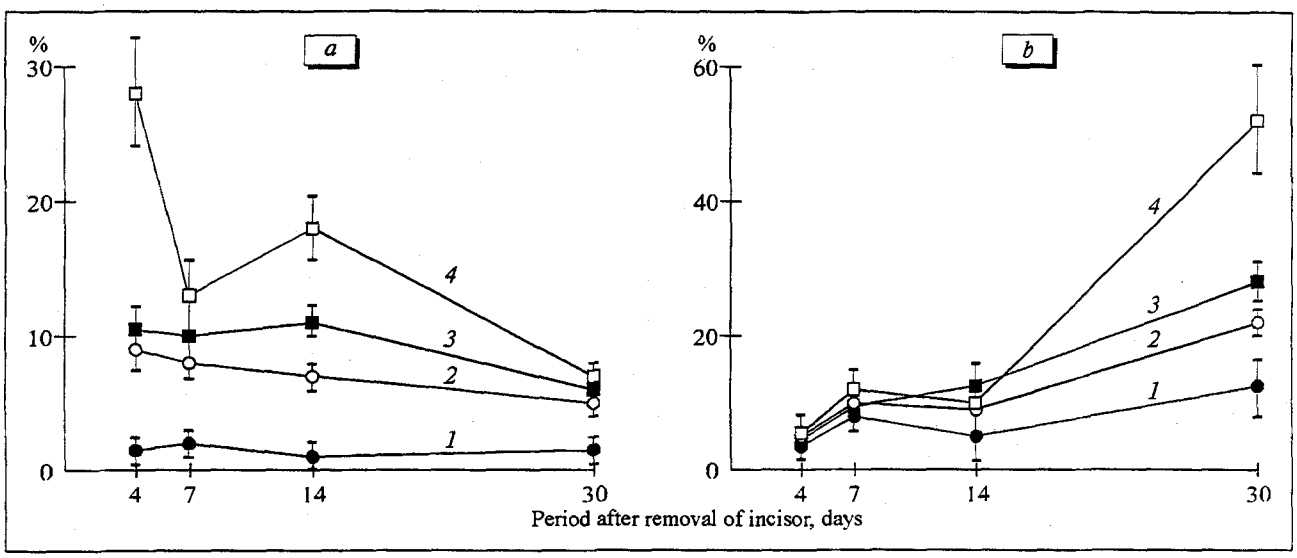


**Fig. 1.** Changes in the labeled nuclei index in rat mandibular connective tissue (*a*) and bone marrow (*b*) during reparative osteogenesis after removal of incisor. *1*) control; *2*) bioprosthesis; *3*) bioprosthesis with hydroxyapatite; *4*) bioprosthesis with hydroxyapatite and estrone.

mandible from which the tooth was removed was fixed and decalcified in 6.5% nitric acid on 10% neutral formalin and embedded in paraffin. Type M emulsion was applied onto paraffin-free 5 μ slices of the mandible, exposed for 30 days, developed, and stained with hematoxylin and eosin.

For assessing the proliferative activity of tissues, the labeled nuclei index (LNI) was estimated in percent for the spongy bone marrow tissue near the dental well and for the connective tissue filling the dental well.

Morphological assessment of reparative osteogenesis in mandibular spongy bone tissues was carried out at all steps of the study.

The results were processed using the Student—Fisher's test [2].

## RESULTS

Insertion of a bioprosthesis into the dental well markedly increased the LNI at the first steps of the study; by the end of experiment this index significantly decreased (Fig. 1, *a*). This proliferation-stimulating effect was observed with the bioprosthesis inhibiting the release of bone marrow elements from the well; the bioprosthesis resolved on day 14 of the experiment. The morphology of tissues did not change.

The use of HA for dental well filling significantly increased the LNI values in connective tissue cells starting from day 7 postoperation (Table 1).

Combined insertion of estrone and HA bioprosthesis into dental well markedly stimulated the proliferative activity of connective tissue cells, as

**TABLE 1.** Changes in the Proliferative Activity of Spongy Bone After Removal of Mandibular Incisor in Rats and Filling of the Defect with Estrone and HA

| Tissue | Treatment | LNI, % | | | |
|---|---|---|---|---|---|
| | | period postoperation, days | | | |
| | | 4 | 7 | 14 | 30 |
| Connective | Control | 1.20±0.18 | 1.6±0.4* | 0.83±0.3 | 1.0±0.1 |
| | Bioprosthesis | 9.13±0.50 | 8.21±0.3 | 7.62±0.6 | 4.10±0.2 |
| | HA bioprosthesis | 10.0±0.50 | 9.84±0.4 | 10.50±0.5 | 5.53±0.3 |
| | Estrone+HA | 28.0±2.5 | 15.42±1.2 | 18.2±1.5 | 6.62±0.6 |
| Bone marrow | Control | 3.43±0.26 | 8.30±0.9 | 4.93±0.8 | 11.82±1.1 |
| | Bioprosthesis | 4.95±0.42 | 12.30±1.2 | 9.46±0.24 | 21.42±0.9 |
| | HA | 4.90±0.57 | 9.20±0.7 | 12.41±1.1 | 28.0±1.42 |
| | Estrone+HA | 5.43±0.86 | 11.40±0.52* | 9.53±1.3 | 51.24±3.62* |

**Note.** *$p<0.01$ versus the control.

Fig. 2. Beginning of focal osteogenesis in dental well after insertion of bioprosthesis with hydroxyapatite (HA): transformation of crystalline HA in vacuolized amorphous state on day 7 of experiment; ×200. Here and on Figs. 3 and 4: hematoxylin-eosin staining.

shown by the LNI, this stimulation being particularly pronounced during the early stages of the study. In addition, the two waves of the curve reflecting the changes in LNI of connective tissue cells after insertion of HA become even more expressed after insertion of HA and estrone (Fig. 1, *a*).

The data indicate that bone marrow and connective tissues differently react to the insertion of bioprosthesis with the studied substances. This is seen not only from the amplitude of the maximum response, but also from the time of its development. The maximum LNI values are observed only on day 30 in bone marrow cells, when the proliferative activity of connective tissue cells is decreased. It is noteworthy that the amplitude of the maximum response of bone marrow elements is much higher than that of connective tissue cells (Fig. 1).

The biphasic pattern of the curve reflecting LNI changes in bone marrow tissue was observed throughout the entire experiment. It should be noted that the maximum rise of LNI in the cells was observed by day 30 (up to 51.2±3.6), when in the controls just the first slight increase of LNI in the same tissue was observed. This indicates that the proliferative activity of tissues normally varies during reparative osteogenesis (Fig. 1, *b*).

Such changes in the proliferative activity of mandibular tissue after insertion of osteogenesis inductor and/or stimulant is quite probable. The time course of tissue proliferative activity indicates that this succession of changes is normal in regulation of reparative osteogenesis in the rat mandible spongy bone.

Morphological analysis showed that after insertion of HA bioprosthesis in the well crystalline HA is vacuolized, becomes amorphous, and easily available for calcification of the forming bone as early as by day 14 of experiment (Fig. 2).

Combined insertion of HA bioprosthesis and estrone results in virtually complete filling of the well with trabeculae of the forming spongy bone 30 days after removal of the tooth (Fig. 3). In this case there is less connective tissue between the trabeculae than after insertion of HA bioprosthesis alone.

Moreover, when osteogenesis inductor and stimulant are used together, the connective tissue LNI markedly increases during the early stages of experiment, predominantly in the cells located along

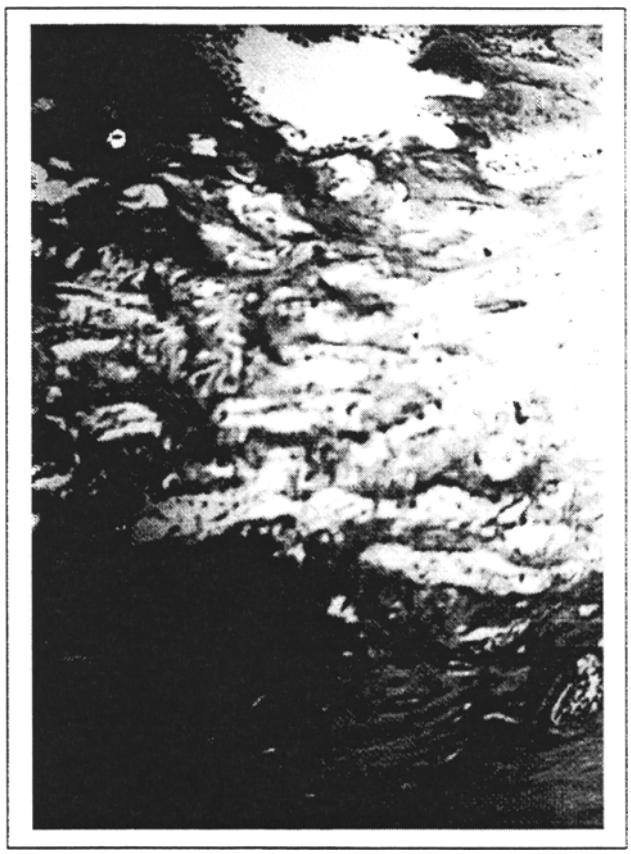

Fig. 3. Dental well filled with osteogenic tissue 30 days after joint insertion of estrone and hydroxyapatite bioprosthesis; ×70.

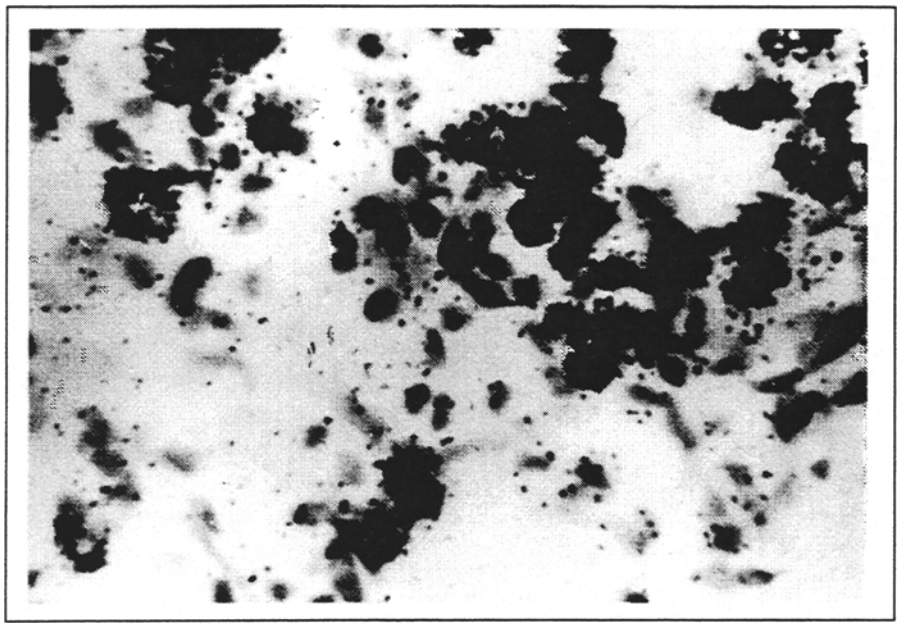

**Fig. 4.** Labeled nuclei of pericytes in vascular walls of regenerating connective tissue on day 4 of experiment; ×1440.

the vessels growing into the regenerating connective tissue (Fig. 4). Among these cells there are pericytes with osteogenic potential; stimulation of these cells enhances reparative osteogenesis [6].

Combined use of HA and estrone permits a more effective utilization of the bone marrow (osteogenic precursor cells) and connective tissue (pericytes) as sources of reparative osteogenesis during healing of mandibular dental well in experimental animals.

## REFERENCES


1. V. A. Almazov, G. E. Arkad'eva, A. Yu. Zaritskii, and I. N. Ivasenko, *Byull. Eksp. Biol. Med.*, **101**, No. 6, 753-755 (1986).
2. E. V. Gubler, *Computation Methods for Analysis and Recognition of Pathological Processes* [in Russian], Leningrad (1978).
3. O. I. Epifanova, *Hormones and Cell Multiplication* [in Russian], Moscow (1965).
4. I. N. Ivasenko, D. A. Ivasenko, and V. A. Almazov, *Byull. Eksp. Biol. Med.*, **119**, No. 1, 72-75 (1995).
5. F. Z. Mirsaeva, "Prevention of atrophic processes in the jaws after removal of ontogenic cysts and teeth," Author's Synopsis of Dissertation [in Russian], Moscow (1989).
6. L. N. Mikhailova and A. A. Pal'tsyn, *Byull. Eksp. Biol. Med.*, **101**, No. 6, 755-757 (1986).
7. M. M. Solov'ev, T. M. Alekhova, G. M. Mel'tsova, *et al.*, in: *Pressing Problems of Clinical Dentistry* [in Russian], St. Petersburg (1993), pp. 77-78.
8. M. M. Solov'ev, I. N. Ivasenko, T. M. Alekhova, *et al.*, *Stomatologiya*, No. 3-6, 755-757 (1992).
9. A. Ya. Fridenshtein and K. S. Lalykina, *Induction of Bone Tissue and Osteogenic Precursor Cells* [in Russian], Moscow (1973).
10. C. C. Alling, *J. Prosthet. Dent.*, **52**, No. 6, 827-831 (1984).
11. D. S. Colvard, *Proc. Natl. Acad. Sci. USA*, **86**, No. 3, 854-857 (1989).
12. J. R. Hupp and S. J. McKehna, *J. Oral Maxillofac. Surg.*, **46**, No. 7, 538-545 (1988).
13. J. Loeb, F. Cuveller, and M. Putz, *Acta Stomatol. Belg.*, No. 2, 111-115 (1985).
14. P. R. Mehliseh, T. D. Taylor, D. G. Leibold, *et al.*, *J. Oral Maxillofac. Surg.*, **46**, No. 10, 839-843 (1988).