The Use of Fibroblasts in Periodontology and Implantology

I. I. Stepanova

Translated from *Kletochnye Tehnologii v Biologii i Medicine*, No. 3, pp. 165-168, July, 2007
Original article submitted March 12, 2007

Progress in modern esthetic dentistry is largely determined by the development of new technologies and their introduction into practice. One of the most perspective research trends are cell technologies based on the use of cell cultures for tissue repair in various abnormalities. Published data demonstrate the possibility of using autologous fibroblasts for repair of periodontal tissues and correction of postoperative retraction of the alveolar processes.

Key Words: *implantology; periodontology; tissue engineering; autologous fibroblasts; fibroblast growth factors*

Modern implantology and periodontology reached a qualitatively new level. Functional and highly esthetic results can now be attained, which is very important for reconstruction of the natural smile.

Progress in dental implantology, high level of implant healing and of its functioning, and the efficiency of therapy for inflammatory diseases of the periodontium largely depend on adequate status of soft tissues of the marginal periodontium [34].

Many failures of dental implantology are caused by problems with soft tissues [13], which can manifest by inflammation of the mucosa [16], gingival hyperplasia [28], peri-implantitis with gingival recession and simultaneous bone loss in the zone adjacent to the implant [20], denudation of the thread on the implant body [15]. Soft tissue around the abutment heads or implant pins are modified after implantation.

The efficiency of regeneration of lost periodontal structures cannot be guaranteed in case of periodontal inflammation development during surgical intervention on the periodontal pouches and on the bone of the alveolar process [3,12,17].

The most prevalent complication in the treatment of periodontal inflammation is, in addition to atrophy of the hard structures and the ligamentous system, is gingival atrophy presenting as recession, leading to functional and cosmetic problems in 30-65% cases [12].

A new trend of reconstructive surgery started active development at the end of the 1990s: tissue engineering, making use of growth factors produced by cells and cell cultures for stimulation of regeneration of tissue structures. This trend seems to be most promising for restoration of lost structures in functionally active tissues [10]. The use of fibroblast cultures or bioactive substances produced by them seems to be perspective for restoration of periodontal tissues and repair of postoperative retraction of the alveolar process after interventions on the periodontium and implantations.

Special attention is paid to fibroblasts, cells of mesenchymal origin and the main component of the connective tissue, producing procollagen, fibronectin, glycosaminoglycans, proelastin, enzymes, growth factors (*i.e.* components serving as fibrous backbone for the connective tissue and forming the extracellular matrix). Fibroblasts also produce enzymes and proteins playing an important role in the regulation of local homeostatic processes and cell-cell interactions [1,5-8].

Regeneration of the periodontal tissue is regulated by transforming growth factor-α (regulating angiogenesis), transforming growth factor-β (stimulating type 1 collagen, fibronectin, and osteonectin synthesis, bone matrix formation) [11], basic fibroblast growth factor (bFGF; exhibits a positive im-

Institute of Dentistry, Federal Agency for Health Care and Social Development, Moscow

0007-4888/07/1441-0147

pact on the growth of cells of all types in a wound, stimulates the production of extracellular matrix components (fibronectin and collagen) by fibroblasts).

Fibroblasts introduced into the wound release bFGF stimulating angiogenesis, as a result of which cells associated with new vessels (so-called pericytes) appear in periodontal tissues. Pericytes retain the potential of mesenchymal cells and, being pluripotent stem cells, are capable of differentiation into fibroblasts and osteoblasts. In addition, regeneration osteogenesis is realized at the expense of stimulation of periosteal osteogenic cells with obligatory participation of newly formed blood vessels.

Numerous *in vivo* experiments demonstrated a pronounced stimulatory effect of bFGF on angiogenesis [23]. It was experimentally shown *in vitro* and *in vivo* that proliferation of endotheliocytes in capillaries and main vessels increased significantly in the presence of bFGF; the presence of this factor was also associated with intensification of endotheliocyte migration and growth of these cells into collagen matrix with the formation of tubular structures, similar to blood capillaries [24]. This factor stimulates proliferation of smooth-muscle cells and pericytes playing an important role in the formation of vessels.

bFGF is a potent mitogenic factor for mesodermal cells considerably reducing their doubling time. bFGF stabilizes the phenotypical expression of cultured cells, due to which the cells can be cultured during a long time without changes in their phenotype. This phenomenon can be explained by the capacity of bFGF to regulate the synthesis and formation of extracellular matrix components essential for the gene expression [23].

The effects of bFGF on osteogenetic processes were also studied. It was shown on the model of experimental disorders in the bone formation process in 5-week-old mice [21] that bFGF treatment normalized the synthesis of the cartilage in animals and led to the edgewise growth of the bones; signs of enchondrial ossification were detected in chondrocytes, intensification of the osteogenetic process was noted in the distal third of the femoral bone, the endosseal osteoblasts intensely proliferated, presumably differentiating from the bulk of fibroblast-like mesenchymal cells. Hence, systemic treatment with bFGF can activate osteogenesis.

The chondrogenic potential of mature chondrocytes from the hyaline cartilage can be restored by means of bFGF treatment [18]. The content of chondrocytes increased by 2000 times in the presence of bFGF. Addition of this factor to the medium leads to a drastic accumulation of chondrocytes and formation of cartilaginous tissue.

Administration of bFGF to rats with bone defects of the jaws resulted in limited osteogenesis *in vivo* [33].

Treatment with bFGF led to the formation of a periodontal ligament with the formation of new tooth cement and active osteogenesis [29].

Clinical trials of bFGF as a drug for the treatment of periodontal destruction were started in Japan in 2005 [31].

Hence, the use of bFGF in dentistry holds much promise. The optimistic predictions of many scientists are based on the direct use of fibroblasts, producing not only bFGF, but many other bioactive substances, which leads to more pronounced and long-lasting clinical effect [27,33].

Osteoplastic material introduced into mandibular bone wound in a rabbit in combination with M-22 human embryonic fibroblast culture intensely stimulated proliferative processes in the population of fibroblastic elements in comparison with injection of osteoplastic material alone into bone defects. The Japanese scientists in experiments on beagle dogs demonstrated the possibility of restoring the dental root by creation of artificial cavities on the buccal surfaces of the second premolar and first molar and introducing titanium, populated with pre-cultured autologous fibroblasts of the periodontal ligament, into these cavities. The results indicate the possibility of construction of periodontal tissues including the tooth cement, periodontal ligament, and bone around titanium by populating it with cultured fibroblasts and introducing into dental bone defects [22].

Bone defects in experimental rats were repaired by means of subcutaneous injections of a mixture of β-tricalcium phosphate (β-TCP), mesenchymal stem cells from rat femoral bone, and fibrin glue, used for the formation of a 3D template for bone growth [32]. The results of histological and morphological studies confirmed the formation of the new bone in all biopsy specimens, but not in the control group, in which only fibrin glue with β-TCP was used.

Transplantation of cultured autologous cells from the periodontal ligament on nano-Hap-collagen (nHAC) into damaged periodontium led to the growth of new bone, periodontal ligament, and cement [17].

Published data indicate that application of cell cultures in clinical dentistry for repair of the periodontal tissues is based on the use of allogenic (donor) and autologous (patient's own) cells. The use of autologous human fibroblasts in dentistry can become an alternative to allogenic fibroblasts, because the use of autofibroblasts is not associated

with side effects and allergic reactions; in addition, growth factors are released, protein synthesis is stimulated, angiogenesis and capillary endotheliocyte proliferation are accelerated, and hence, the regeneration of periodontal tissues is stimulated.

The use of allogenic human fibroblasts in dentistry improves wound healing due to activation of protein synthesis and release of growth factors [2, 11]. Methods of transplantation of these cells on biopolymer films, dura mater, and osteoplastic material are described [10].

Bio-OSS bone-replacing material and Bio-Gide resorbed double-layer collagen membrane (Geistlich) were used at Central Institute of Dentistry in experiments and clinically [9,10] for surgical treatment of inflammatory destructive periodontitis. Suspension of M-22 cells (human skin diploid fibroblasts) was used in surgical treatment at the stage of introduction of osteoplastic material. Before use the Bio-OSS granules were impregnated with M-22 cell suspension, inserted into bone defects, and closed by the Bio-Gide membrane. After 3 months stable remission was observed in the operated area, persisting during up to 6 months (maximum period of observation). X-Ray examination showed the formation of new bone structures, disappearance or sharp reduction of osteoporosis, better discernible clear-cut contour of the bone. The depth of periodontal pouches decreased by 5.3±0.6 mm.

Human allofibroblast cultures populating the dura mater are also used for the treatment of periodontium. The use of this transplant in combination with allofibroblasts in the treatment of chronic generalized periodontitis has certain advantages: the regeneration of periodontal tissues, including the bone tissue, is more intense than after transplantation of synthetic materials.

Clinical use of Astra Tech screw implants in combination with human diploid cells HEL 4/81 (human embryonic lung 4/81) promotes rapid healing of the postoperative wounds and full-value osteointegration.

Recombinant morphogenetic protein in combination with mesenchymal bone marrow stem cells was used (with good results) in the sinus lifting operation for reconstruction of the maxillary alveolar processes in rabbits. Cultured epithelial cells were transplanted for increasing the volume of soft tissues [30].

Autologous fibroblasts from the gingival mucosa on a hydroxyapatite carrier were transplanted into damaged periodontium [14]. Clinical observations were carried out during 3-6 years after the treatment. The depth of the pouches and bone defects decreased significantly in comparison with the control (transplantation of hydroxyapatite alone). These observations indicate that transplantation of hydroxyapatite in complex with autologous gingival fibroblasts led to more pronounced regeneration of periodontal tissues.

The Isolagen Company at the beginning of 2004 presented the results of the first phase of clinical trials (6-month double blind clinical trial). Clinical studies of 176 periodontal zones were carried out in 21 patients in Dentistry Department of Research Health Center at the University of Texas. Patients with periodontitis received transplantations of cultured autologous fibroblasts into the "black triangle" zones (gingival interdental papillae). The clinical status of the marginal periodontium improved significantly due to increase in the volume of interdental papillae. Paired *T* test (statistical method for evaluation of results) showed that clinical response to autologous cells was obviously superior to response to placebo. The gingival mucosa thickened by 1.1 mm; positive results were attained even in the gravest cases. It is noteworthy that the patients received no other treatment. The effect was clinically significant in 81% cases and higher than the placebo effect in the rest cases (p=0.0113). The gingival status improved significantly in 20 of 21 patients. No side effects were observed.

Autologous mucosa with transplantation of autologous fibroblasts was used in a clinical experiment with surgical repair of the gingival recession. Autotransplant was used in one jaw for replacing one tooth and autologous fibroblasts were applied in the zone of another tooth [19]. Examinations were carried out after 3, 5, 7, 9, and 12 months. Better clinical results were observed after autofibroblast transplantation.

Hence, the use of cultured fibroblasts for stimulation of periodontal tissue healing and for repair of lost interdental gingival papillae and restoration of sufficient gingival volume gave satisfactory results. The results suggest that the use of autologous fibroblasts in periodontology and implantology is a perspective method, because this treatment modality completely rules out the risk of allergic reactions and side effects: autofibroblasts do not conflict with their own immune system and hence, are not rejected, and provide a lasting clinical effect.

REFERENCES

1. L. I. Bobro, *Arkh. Patol.*, **52**, No. 12, 65-68 (1991).
2. V. N. Vol'khina, *Meditsina i Biotekhnologiya*, 6 (2003).
3. I. S. Gerasimovich and Yu. A. Boldyrev, *Ibid.*, 1-6 (2002).
4. B. K. Gavrilyuk, *Cell Culture and Tissue Reconstruction (as Exemplified by the Skin)* [in Russian], Pushchino (1988).

5. G. Gaier, *Electron Histochemistry* [in Russian], Moscow (1974).
6. A. Ham and D. Cormack, *Histology* [in Russian], Moscow (1993), Vol. 2, pp. 61-63.
7. E. V. Glushchenko, T. L. Zayats, and G. G. Serov, *Byull. Eksp. Biol. Med.*, **121**, No. 5, 575-577 (1996).
8. V. P. Goncharov, *Fiziol. Zh.*, **80**, No. 9, 163-173 (1994).
9. A. I. Grudyanov, A. I. Erokhin, and S. F. Byakova, *Novoe v Stomatologii*, **98**, No. 8, 72-77 (2001).
10. A. I. Grudyanov, A. I. Erokhin, and L. L. Mironova, *Tsitologiya*, **43**, No. 9, 854 (2001).
11. I. A. Novikova, *Meditsina i Biotekhnologiya*, 1-5 (2003).
12. V. F. Chernysh, Yu. N. Shutov, and A. M. Kovalevskii, *Parodontologiya*, Nos. 4-6, 19-23 (1997).
13. D. C. Hoelscher and A. M. Simons, *J. Oral Implantol.*, **20**, No. 4, 282-291 (1994).
14. L. T. Hou, A. Y. Tsai, C. M. Liu, and F. Feng, *Cell Transplant.*, **12**, No. 7, 787-797 (2003).
15. G. M. Horning and M. P. Mullen, *Compendium*, **11**, No. 10, 604-609 (1990).
16. U. Lekholm, I. Ericsson, R. Adell, and J. Slots, *J. Clin. Periodontol.*, **13**, No. 6, 558-562 (1986).
17. H. Lu, Z. F. Wu, and Y. Tian, *Zhonghua Kou Qiang Yi Xue Za Zhi*, **39**, No. 3, 189-192 (2004).
18. I. Martin, G. Vunjak-Novakovic, J. Yang, *et al.*, *Exp. Cell Res.*, **253**, No. 2, 681-688 (1999).
19. M. K. McGuire and M. E. Nunn, *J. Periodontol.*, **76**, No. 6, 867-880 (2005).
20. R. M. Meffert, *Implant. Dent.*, **1**, No. 1, 25-33 (1992).
21. H. Nagai, R. Tsukuda, H. Jamasaki, and H. Mayahara, *J. Vet. Med. Sci.*, **61**, No. 8, 869-875 (1999).
22. T. Nakahara, T. Nakamura, E. Kobayashi, *et al.*, *Tissue Eng.*, **10**, Nos. 3-4, 537-544 (2004).
23. E. B. Nery, K. L. Lynch, and G. E. Rooney, *J. Prosthet. Dent.*, **40**, No. 6, 668-673 (1978).
24. Y. Ohgishi *et al.*, *J. Orthop. Res.*, No. 7, 568-578 (1976).
25. A. Palmon, H. Roos, and J. Edel, *J. Periodontal Res.*, **7**, No. 6, 974-980 (2000).
26. A. Saito, E. Saito, M. Kawanami, and A. Shimada, *Cell Transplant.*, **12**, No. 5, 519-525 (2003).
27. Y. Seibert and S. Nyman, *J. Periodontol.*, **61**, No. 3, 157-165 (1990).
28. A. D. Sones, *J. Prosth. Dent.*, **62**, No. 5, 581-585 (1989).
29. S. Takayama, S. Murakami, T. Nozaki, *et al.*, *J. Periodontal Res.*, **33**, No. 6, 315-322 (1998).
30. M. Ueda, I. Tohnai, and H. Nakai, *Artif. Organs*, **25**, No. 3, 164-171 (2001).
31. K. Yamazaki, *Niphpon Hotetsu Shika Gakkai Zasshi*, **49**, No. 4, 587-592 (2005).
32. Y. Yamada, J. S. Boo, R. Ozawa, *et al.*, *J. Cranio Maxillofac. Surg.*, **31**, No. 1, 27-33 (2003).
33. G. Zellin and A. Linde, *Bone*, **26**, No. 2, 161-168 (2000).
34. H. H. Zaden, *J. Calif. Dent. Assoc.*, **32**, No. 12, 1011-1020 (2004).