Original Research

Immunoreactivity of CD31 Protein in Endothelial Cells of Microcirculation in Oral Soft Tissues in Patients with Intra-Osseous Titanium Implants Subjected to Extremely Low-Frequency Magnetic Fields: the First Report in Prosthetic Patients

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Abstract

Our study objective was to assess the influence of extremely low-frequency magnetic fields (ELF MF) with induction similar to Earth's field, i.e. corresponding to the natural electromagnetic field, on the process of angiogenesis, with special regard to the degree of immunoexpression of the endothelial marker CD31 in soft tissues covering the alveolar process, determined using the Viofor JPS system in patients with intra-osseous titanium Alpha-Bio implants. CD31 labeling indicates that exposure to ELF MF with low value of induction (1) markedly stimulates angiogenesis and (2) widens the vascular bed in the oral soft tissues, which may significantly accelerate the regeneration processes.

Keywords: endothelial marker CD31, oral soft tissues, angiogenesis, titanium implants Alpha-Bio, ELF MF

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Introduction

It is well known that the external environment, filled with a natural magnetic field generated by the Earth, affects the function of the respective organs and systems of the human body. Extremely low-frequency magnetic fields (ELF MF) with low value of induction corresponding to the Earth's field can be also generated in an artificial way, which in the Polish nomenclature is defined as magnetostimulation [1-4]. Exposure to ELF MF, widely applied in modern clinical medicine, induces biological phenomena in the human body, similar to those that occur during physical activity. These are bioelectric phenomena that lead to the normalization of the cell membrane potential, biochemical processes increasing the enzyme activity and bioenergetic phenomena that stimulate cell regeneration [3-8].

Dynamic advances in dentistry have made clinicians search for new therapeutic surgical-prosthetic methods [9-12]. Due to the introduction of intra-osseous implants to therapy, patients have received extremely functional, aesthetic, and biocompatible prosthetic restorations [9-11, 13]. Lately, the intra-osseous implant healing process has been frequently aided with the use of local physiotherapeutic procedures involving exposure to extremely low-frequency magnetic fields with low value of induction [4, 13, 14].

It should be noted that the pathomorphological pattern of the oral soft tissues following the application of such therapy has not been fully investigated. And although the beneficial effect of ELF MF on microcirculation within various systems and organs [3, 7, 8, 15], including the oral soft tissues [4, 13, 14, 16], has been known, the sequence of morphological events concerning the oral vascular bed has not yet been presented. Thus the microscopic evaluation of angiogenesis (with special regard to immunohistochemistry) in soft tissues covering the alveolar process after stimulation of low-frequency magnetic fields (with induction similar to Earth's field) in patients with intra-osseous titanium implants, seems to be important both for pathomorphologists and dentists. It should be added that the application of ELF MF or ELF MF-like procedures, by some authors referred to as safe or at least nearly safe [4, 7, 14-18], is more and more common both in clinical practice and in home conditions.

Therefore, our main study objective was to determine the degree of immunoreactivity and distribution of the angiogenesis marker CD31 in vascular endothelial cells within microcirculation in the soft tissues covering the alveolar process in patients with intra-osseous titanium Alpha-Bio type implants subjected to the influence of extremely low-frequency magnetic fields with low induction values as compared to the expression of the marker prior to this procedure.

The current study is a continuation of our research on the application of this noninvasive physico-medical method in dental practice [13, 14, 19].

Material and Methods

This study was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. It was approved by the ethical committees of the Medical University of Białystok and Pomeranian Medical University in Szczecin, Poland.

The immunohistochemical (IHC) investigations involved 19 patients (9 women, 10 men) aged 19-59 years, qualified for surgical-prosthetic procedures in the Department of Propedeutics of Dentistry and Dental Physiodiagnostics of Pomeranian Medical University in Szczecin.

In order to prepare the bed for the titanium implant Alpha-Bio (two-stage method implant), each patient underwent necessary surgical incision, and a full-thickness flap covering the mucosa and periosteum was prepared. Then a small section of soft tissue (5 mm in diameter), serving as the control material for morphological investigations, was obtained, fixed in 10% buffered formalin, placed in paraffin blocks and finally routinely stained with Mayer's hematoxylin and eosin (H,E). Immunohistochemistry for the endothelial marker CD31 used monoclonal mouse antibody (Clone JC 70A, Dako, Denmark) directed against the above protein. After the reaction performed with the use of the detection system En Vision (K 4007 kit), color reaction was carried out with the use of DAB chromogen (Dako, Denmark) in order to visualize the antigen-antibody complex.

One day after the surgical procedure, the same patients were exposed to extremely low-frequency magnetic fields generated by a magnetostimulator Viofor JPS (with frequency ranging between 180-195 Hz and value of induction 45 μT), using an elliptic applicator (mean value of a 10 min procedure – 62 μT) and a point applicator S (mean value of a 10 min procedure – 55 μT), according to the therapeutic program M1P3 (constant intensity 4 with ionic cyclotron resonance) [4, 17, 20, 21]. Specimens for analysis after ELF MF applications were collected after 10, 20, and 30 ELF MF procedures (successively from 6, 7, and 6 patients who previously underwent implant insertion procedure).

Following ELF MF application, a specimen 5mm in diameter (i.e. like the control) was collected from the same patient at the height of the cover screw, so not in line of postsurgical scar (i.e. beyond the implantation site).

The method of tissue material preservation and staining modes used were analogical to those of the control material, i.e. the samples collected prior to the use of ELF MF.

Histological and IHC investigations were performed in the Department of Medical Pathomorphology, Medical University of Białystok.

Results

The results of histological investigations indicate that routine staining with Mayer's hematoxylin and eosin (H, E) with reference to microcirculation within soft tissues covering the alveolar process in patients with Alpha-Bio titanium implants subjected to a series of treatments with the Viofor JPS system that stimulated the action of ELF MF showing low value of induction, i.e. after 10, 20, and 30 such procedures, was insufficient and did not always fully demonstrate the newly forming blood vessels. Dense networks of capillaries often got lost in the poorly contrasting connective tissue stroma. However, immunohistochemical investigations using the endothelial marker CD31 identified the network of the newly forming and already formed microcirculatory vessels, i.e. fine blood vessels (including capillaries and vascular buds) embedded in soft tissues covering the alveolar process in implanted patients following ELF MF (Figs 2-6B). It should be emphasized here that IHC CD31 staining was of great help in the assessment of the sequence of the morphological events observed in the course of angiogenesis.

The process of vessel neoformation in patients after application of ELF MF, as compared to the same patients not subjected to physiotherapy (Fig. 1), was already observed after 10 ELF MF procedures (Figs. 2, 3), increased with higher numbers of procedures (Figs. 4A, 4B, 5) and was most pronounced after 30 such applications (Figs. 6A and 6B). The proliferating network of the microcirculatory vessels within the soft tissues covering the alveolar process was seen mostly focally, mainly in the connective tissue stroma directly beneath the epithelium or even showed deeper location (Figs. 4A, 4B, 5). Sometimes, however, the process of angiogenesis was also observed in subepithelial connective tissue stroma and in soft tissues of markedly deeper location. The product of IHC reaction was distinctly accumulated on the surface of endothelial cells of the microcirculation studied, well identifying the respective types of vessels (Figs. 4A, 4B, 5, 6B). The intensity of IHC reaction for CD31 was most pronounced in budding vessels and in blood capillaries, especially those which still had no lumen, as shown in Figs. 2 and 3.

In patients after 30 exposures to extremely low-frequency magnetic fields, relatively numerous microcirculatory vessels were quite well differentiated, with a relatively wide and patent lumen; however, the intensity of IHC reaction in them was found to be weaker as compared to very young vessels, i.e. budding vessels and capillaries without a lumen (Figs. 6A and 6B).

The process of neoangiogenesis was frequently accompanied by variously enhanced inflammatory reactions – from poor to intense (Figs. 2, 3, 4A, 4B, 5). Initially, i.e. after 10 ELF MF procedures, it manifested as acute infiltration, with high content of neutrophils, whereas after 20

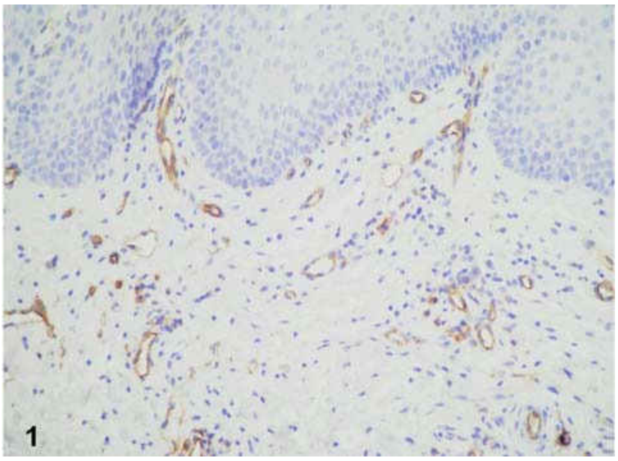
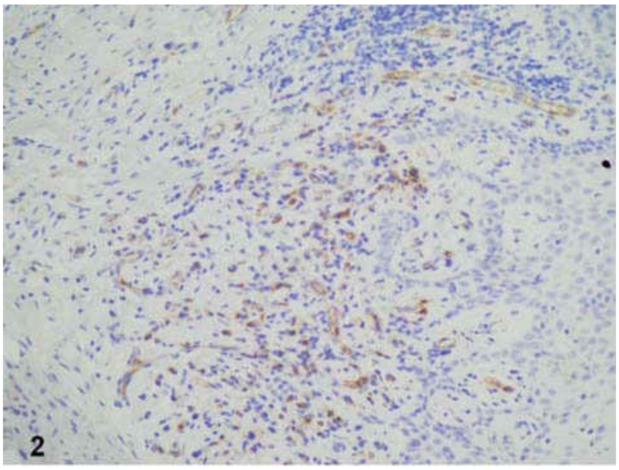
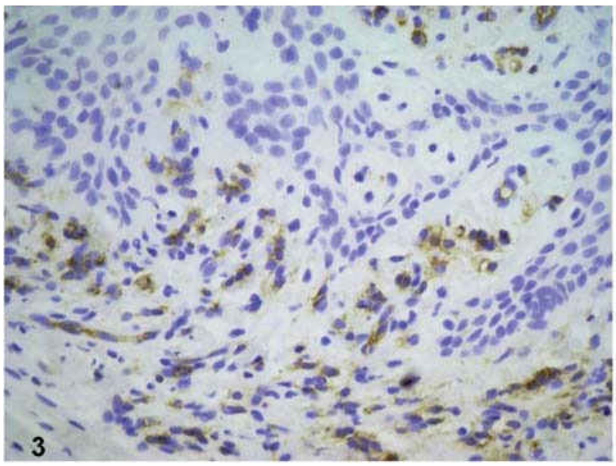


Fig. 1. The immunohistochemical (IHC) picture of microcirculation within the connective tissue stroma beneath the stratified squamous epithelium lining the alveolar process.
Microcirculatory vessels are well differentiated. Most vessels showed a positive or strongly positive reaction for the biomarker CD31. The immunoreactive material accumulates distinctly on the surface of endothelial cells, well identifying the respective types of vessels.
Reference group. Soft tissues covering the alveolar process collected from a patient during the preparation of the bed for a titanium implant (prior to ELF MF procedures). Magn. 200 x.





Figs. 2; 3. A picture of the proliferating, very poorly differentiated network of microcirculatory vessels – mainly vascular buds and capillaries – in subepithelial connective tissue stroma of the alveolar process. Note the large number of inflammatory cells around the newly formed vessels.
We recorded a strongly positive reaction for CD31 in the majority of proliferating microcirculatory vessels, especially in budding vessels and in some of the blood capillaries.
Patient after 10 ELF MF procedures. Fig. 2 – magn. 200x; Fig. 3 – magn. 400x.

and 30 procedures it became chronic, with the predominance of inflammatory mononuclear cells. Following 30 ELF MF procedures, the inflammatory infiltration was usually weakly expressed or even lacking, as shown in Figs. 6A and 6B.

A thorough morphology of the inflammatory reaction coexisting with the process of neoangiogenesis and changes in stratified squamous epithelium covering the alveolar process in the study patients will be presented in the subsequent paper.

Discussion

As shown by the current IHC investigations regarding CD31 immunoexpression in soft tissues covering the alveolar process in patients with intra-osseous titanium Alpha-Bio type implants subjected to exposure of extremely low-

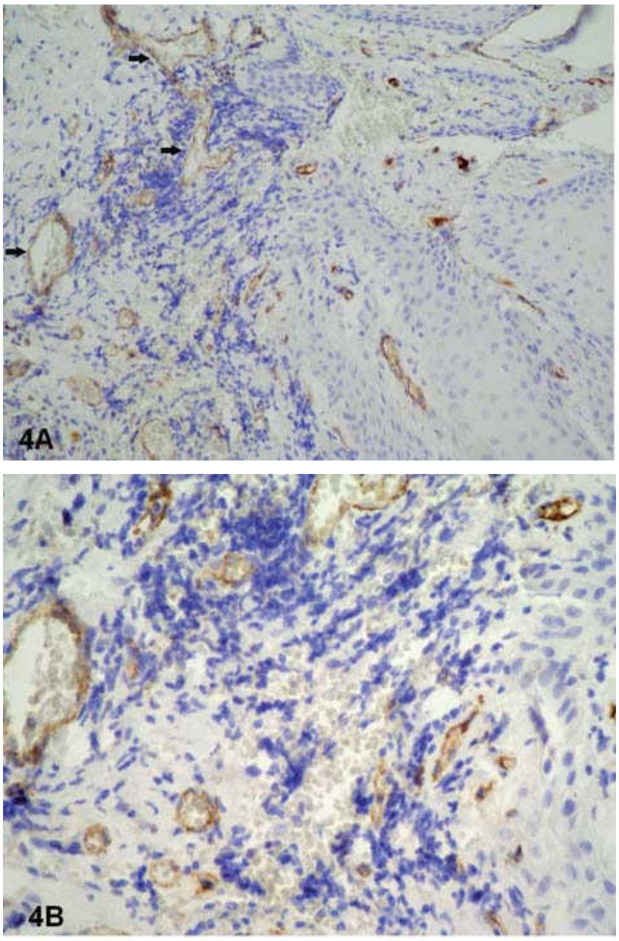


Fig. 4A, B. The IHC picture of strongly pronounced angiogenesis referring to the area directly beneath epithelium and to slightly deeper lying connective tissue of the alveolar process; neoformation of blood vessels is accompanied by intense inflammatory infiltration. Microcirculatory vessels are well differentiated; among them fine arterioles with a relatively wide lumen (->); however, most of them contain blood morphotic elements, mainly erythrocytes.
Positive or strongly positive reaction for CD31 in the majority of proliferating microcirculatory vessels.
Patient after 20 ELF MF procedures. Fig. 4A – magn. 200x; Fig. 4B – magn. 400x.

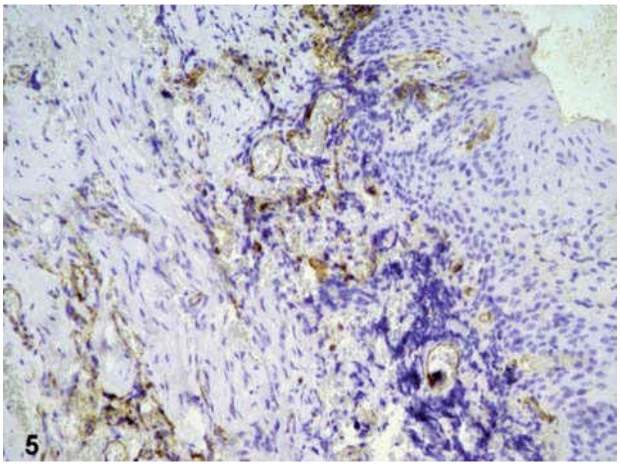


Fig. 5. Strongly positive reaction for CD31 in the majority of proliferating microcirculatory vessels in subendothelial and slightly deeper lying connective tissue stroma of alveolar processes. Intense inflammatory infiltration around the growing capillaries. Patient after 20 ELF MF procedures. Magn. 200 x.

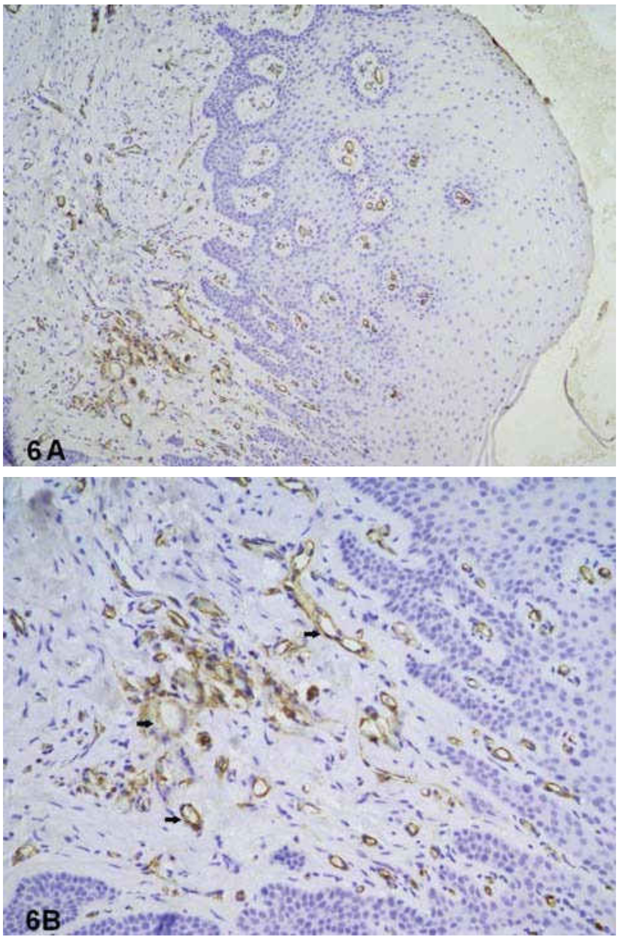


Fig. 6A, B. The IHC picture of the newly formed, well differentiated microcirculatory vessels. Among them – numerous patent blood vessels of relatively wide lumen (->). Stratified squamous epithelium slightly dilated. Both epithelium and connective tissue stroma free of inflammatory infiltration. Positive or strongly positive reaction for CD31 in very young microcirculatory vessels; in larger blood vessels, expression of this marker is less pronounced. Patient after 30 ELF MF procedures. Fig. 6A – magn. 100 x; Fig. 6B – magn. 200x.

frequency magnetic fields with induction similar to Earth's field, this physical technique markedly stimulates the process of angiogenesis and widens the vascular bed in the oral soft tissues studied. The results are consistent with our preliminary observations reported previously [13]. The beneficial effect of ELF MF was most pronounced after the largest number of procedures, i.e. 30.

It should be noted that IHC studies for the endothelial marker – the CD31 protein – by far better than routine histological staining with H,E identified the network of the newly forming and already formed microcirculatory vessels, i.e. fine blood vessels including capillaries and vascular buds within soft tissues covering the alveolar process. They also greatly facilitated the assessment of the sequence of the morphological events observed in the process of angiogenesis in our prosthetic patients subjected to ELF MF.

The application of ELF MF with low value of induction is commonly known as one of the new noninvasive physical-medical methods to cause changes in the body similar to those observed during physical activity, i.e. aiding biological regeneration [1, 3-7, 18].

The beneficial effects of this method involve analgesic, anti-inflammatory, anti-edematous, sedative actions, facilitate vasodilatation and angiogenesis, and stabilize the vegetative nervous system. Hence, low-frequency magnetic field exposure has been used in a number of disorders, including vegetative neurosis, locomotor organ pain with degenerative background (inflammatory and post-traumatic), Parkinson's disease, Alzheimer's disease, multiple sclerosis, drug-resistant depression, and migraines, as well as in rehabilitation after cerebral strokes and improvement of peripheral circulation [1-3, 7, 15, 22-27]. This type of therapy has been used successfully in clinical plastic surgery, e.g. in the management of postoperative pain and edema, in the treatment of chronic wounds, and to improve microcirculation [8].

Worthy of note are interesting publications by Polish authors – Sieroń et al. [3, 23, 28, 29], summing up the results of their own 15-year experimental and clinical research on the application of variable magnetic fields in medicine [3]. The authors emphasize the influence of these fields on enzymatic and hormonal activity, free oxygen radicals, carbohydrates, protein and lipid metabolism, dielectric and rheological properties of blood, and activity of the central dopamine receptor in experimental animals [3, 28, 29]. The researchers confirm high therapeutic efficacy not only of clinical magnetotherapy but also of ELF MF application in the treatment of numerous ailments, which apart from the above-mentioned disorders also include osteoarthrosis, abnormal ossification, osteoporosis, nasosinusitis, spastic paresis, diabetic polyneuropathy, and retinopathy [3, 4].

It should be emphasized here that dentistry is a separate and important sector in which low-frequency magnetic fields are used [4, 13, 14, 19-21, 30]. Polish researchers, Opalko et al., have contributed a lot to the growing interest in ELF MF procedures. The authors give a long list of indications for local application of ELF MF with low value of induction in dental practice, the major being post-traumatic conditions of soft and hard tissues, pain relief mainly after surgical procedures on periodontium, after conduction anesthesia, in diseases of the pulp and periapical tissue [4, 16, 20, 21]. In one of the papers, Opalko and Dojs [20] report that the use of slow variable magnetic fields generated by Viofor JPS to aid the treatment of the teeth chosen for extraction and to preserve teeth with recorded endo-perio syndrome contributes to bone structure regeneration. They note that the endodontic treatment of replanted teeth, aided with ELF MF, has stopped the osteolysis process, which has been confirmed radiologically [20].

The application of ELF MF as an effective therapy aiding conventional dental treatment is also suggested, with special emphasis put on prosthetic indications, including post-implantation conditions [4]. The use of this noninvasive method is also recommended as an effective therapy in various diseases of the temporomandibular joint, in the treatment of neuralgic pain and in neuralgia n. V and n. VII [4, 14, 31].

The beneficial effect of ELF MF applied in dentistry can be explained by the influence on the development of lateral circulation in damaged tissues with possible acceleration of post-extraction wound healing, the formation of bone union after surgical procedures, and maxilla and mandible injuries [4, 14, 20, 21]. This fact is also reflected by our IHC investigations in the current pioneer study concerning the assessment of immunoreactivity of the biomarker CD31 in the endothelial cells of the newly formed microcirculatory blood vessels in soft tissues enclosing the alveolar process in patients with titanium intra-osseous implants.

It is well known that CD31, a 130 kD glycoprotein, belongs to the immunoglobulin superfamily called PECAM (platelet endothelial cell adhesion molecule). This antigen is localized on the surface of vascular endothelial cells in many organs and systems. It takes part in the migration of blood cells through the vascular wall and in the process of angiogenesis [32, 33].

It should be noted here that vascular endothelium is a universal multifunction tissue producing many substances, fulfilling metabolic functions and being involved in interactions between blood and tissues. Structurally intact endothelial cells may react to various physiological and pathological factors by adapting their normal structure and function, and due to the newly acquired properties [32, 34, 35-38]. This suggests the direction of the use of clinical ELF MF in the treatment of vascular disorders [7, 8, 15, 16, 26].

According to some authors, the biological effect of low-induction magnetic fields, based on ionic cyclotron resonance, significantly enhances blood flow in arteries and capillaries, and improves venous drainage [15]. It mobilizes microcirculation, increases oxygen uptake by tissues and stimulates peripheral circulation [7, 8, 15].

In conclusion, our immunohistochemical investigations for the endothelial marker CD31, the first in literature, indicate that the application of extremely low-frequency magnetic fields with induction similar to Earth's field in patients with dental titanium implants (1) markedly stimulates angiogenesis and (2) dilates the vascular bed, thus improving blood supply in the soft tissues that cover the alveolar process. Improvement of microcirculatory effects may significantly accelerate regenerative processes in soft tissues and at the same time may enhance the bone union process. This would explain the beneficial effect of ELF MF procedures on the healing of intra-osseous Alpha-Bio type implants that we have been witnessing for a few years now in clinical examinations.

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