Experimental study of the biointegration of dental implants fabricated by laser sintering In-Vivo

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Rehabilitation of patients with dental implants is becoming an more and more acceptable and affordable option for dental treatment every year, both for the doctor and the patient. Such popularity is due to the high aesthetic and functional results of dental treatment with implants, while the rate of complications and implant loss has fallen to a minimum in recent years. According to a research report by Moraschini V. Et al. (2015) approximately 94%-97% of dental implant surgeries have a successful outcome and the 10-year implant "survival rate" is over 89.7% [2]. These results have been achieved by the implementation of modern implantation protocols based on a better understanding of bone regeneration and soft tissue structures, as well as their interaction with various modifications of the titanium implant surface, into the practice of clinicians.

Currently, titanium remains the most sought-after material for dental implants [26]. There are several types of modern titanium implant surfaces, which are created by exposing titanium to various physical and physicochemical factors. The most common methods of implant surface modification include sandblasting, acid etching, sandblasting and acidizing (SLA), anodizing (TiO_2) and laser treatment of the implant surface. The effect of these factors on the titanium surface gives it a micro- and nano-roughness, which is represented as “Peaks and walleyes” (peaks and pits) under electron microscopy. Such relief of the titanium surface allows mesenchymal osteogenic cells to be in direct contact with the inorganic implant surface and their further differentiation into osteoblasts, which facilitates the closest possible production of bone tissue to the surface of the placed implant. It has been repeatedly demonstrated experimentally that it is the micro- and nanostructure of the implant surface that forms a tight contact between the implant surface and the bone tissue (Bone-implant contact, BIC) without a soft tissue layer at an earlier date than in the case of the previous generation of implants [6]. It is important to mention that not only the micro- and macrostructure of the implant allows a more effective interaction with the bone tissue, but also the electrochemical modification of the surface, which strengthens the bond between the artificial material and the bone tissue. Anodised implant surfaces have these properties, as shown experimentally (Albrektsson et al., 2000; Henry et al., 2000) [24]. That is why the main task of the various surface modifications of modern implants is to intensify the osseointegration process by modifying the 3D structure of the implant surface in a different way from that of the previous generation of implants [3]. However, although there is a significant difference between the results of experimental robotic studies (invivo and invitro) investigating the osseointegration of previous and current generation implants, the number of clinical research with long-term results is not enough to draw a definitive conclusion about the dominant effectiveness of one or another surface of modern implants in the osseointegration process [25]. As a consequence, the lack of research data (clinical and preclinical) is causing some controversies between specialists and often leaves the clinician at a loss regarding the type of implant to rehabilitate patients.

The research of implants with new surfaces or new architecture, with which it is hoped to optimize or even improve the osseointegration processes, follows certain phases: technical study of the implant surface, study of the reaction of cell cultures to the implant surface (InVitro), experimental study in animal models (InVivo) and then moving on to the clinical phase of the research. In this way a complete picture is obtained and conclusions drawn regarding the effectiveness of a particular material implanted into the human body. It is also important to mention that by using material that has already been researched in advance, it is acceptable to omit some of the research phases of new implants, given the availability of sufficient data from research studies that have already been carried out. One of the most important fields of research in evidence-based medicine, however, is still experimental modeling in the animal body. In such scientific research, there is the possibility of finding new properties of various materials (including titanium) after they have been introduced into the animal body. As a striking classic example, we can offer the experimental study of Professor I. Brånemark's experimental research on intraosseous blood flow in rabbits, in which the phenomenon of titanium osseointegration was accidentally discovered and resulted in the active development of implantology as a discipline [1]. The importance of experimental research on animals is also determined by the closeness of the experimental model to the human body. Even InVitro researches with human cell culture do not fully simulate the reaction of all tissues and fluids with which the implant comes into contact (blood, tissue fluid, oral fluid and its components) as it is inserted into the formed bone bed (21). Several different species can be used as experimental animals to study the osseointegration of implants: rats, rabbits, pigs, dogs and sheep. The PubMed biomedical literature base provides more than 2,000 results for animal experimental studies in the field of implantology[4]. However, considering the peculiarities of animal housing, bioethical regulations in different countries and regions, the availability of animals, the speed of regeneration processes and the reproductive potential of the animal, rabbits are one of the most popular experimental animals, which is determined by the relative easiness of animal maintenance, fast metabolism, high reproductive potential and low aggressiveness [27]. Moreover, among rodents, rabbits are the most suitable animals for modeling alveolar healing processes after removal of lower incisors; in other rodents, the size of the jaws and the anatomy of the tooth tissues do not allow efficient and complete alveolar surgery, particularly tooth extraction. The most common bones used for implants in rabbits are long bones, particularly the femur and tibia. But it is important to mention that the origin and regeneration characteristics of these bones are different from those of the jaws. Also, the operation of implant placement in the limb bone of rabbits is a highly sterile procedure, unlike in the jawbone (particularly for immediate implants after tooth extraction). This characteristic does not allow to display the closest to reality conditions using the long bone model and does not provide for the possibility of different amounts and composition of oral specific microorganisms entering the implanted area, what can subsequently seriously affect the difference in the study results between implants placed in the jaw and the extraction site from the same implants placed in the sterile bone tissue of the limbs. Few osseointegration researches are known that have been performed on the jaws of rabbits [22, 23]. As for the content of the research results in recent years, we should highlight those focused on comparing the effectiveness of implants with different surfaces for the osseointegration process. As a criterion, most of the authors used twist tests, RFA (resonance frequency analysis) values and morphometric examination of bone preparations with implants placed at different times. In the experimental research by Jung-Woo Koh at al. (Seol, 2009), the author compared the data on the osseointegration of the implants made by milling (without modification) with the modified surfaces: SLA, anodized and Ca-P implant surfaces. As a criterion of osseointegration the values of RFA and implant unscrewing test were used, as well as the examination of the bone macrostructure in the area of inserted implants. This research showed a significant difference between the osseointegration data in favour of implants with modified surfaces, although no significant difference was found between implants with anodised and SLA surfaces [6].

There is disagreement about the effectiveness of the macrostructure of the implant. In an experimental research on mini-pigs, Yee-Seo Kwon et al. (Seoul, 2013) and Osstem Implant Co (Busan, 2013) found that implant neck micro whorls designed for increased stability in the cortical bone layer did not show a significant difference in stability and osseointegration data at different times (from 2 to 14 weeks). These results should also be taken into consideration in the search for a more effective implant surface macro-relief [8].

It should be mentioned that experimental research of the effectiveness of the implants surface treated by the laser (Nd:YAG laser) has been carried out. This modification gives the implant surface a specific micro-roughening, which according to the experimental study on rabbits considerably increases morphometric and mechanical indices of integration of the implants so modified [10].

In the same way, experimental research has been carried out regarding the hydrophilic properties of the implant surface. In the experiment 32 implants with different hydrophilic surface properties and degrees of hydrophilicity Neoporos and Acqua (Titamax CM; Neodent, Curitiba, PR, Brazil) were placed on 16 rabbits. As the result of the morphometric research performed there was no significant difference between the rates of osseointegration of the implants with different degrees of hydrophilicity in the early period (2-4 weeks). The author concludes that the most important factor for osseointegration is the surface microroughness and the quality of the osteotomy of the receiving implant bed [14].

Goal

To experimentally compare the qualitative and quantitative indices of osseointegration of implants made by titanium rod milling and laser sintering of titanium powder.

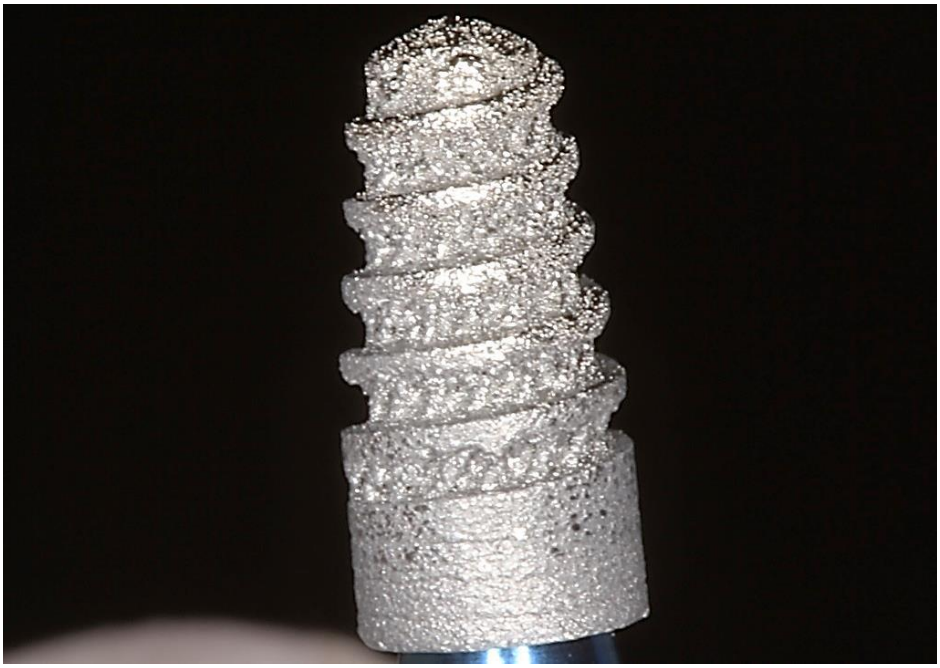
Materials and methods

A total of 12 rabbits of the .... breed were taken for the experiment (Pic. 1, Pic. 2). The plan and structure of the experiment were discussed by the bioethics committee of N.I. Pirogov ENMU and an opinion was received, as well as permission to carry out this study.

The animals were divided into 2 groups: a control group (n=6) and a main group (n=6). In the control group, implants with an anodized surface made by milling from Bio3 Progressive titanium rod (Germany) were placed. In the main group, we used anodized implants made by sintering the titanium powder Bio3 SIM? (Germany) (Pic. 3). For the implantation procedure, the animals underwent combined intramuscular anesthesia with ketamine solution (10 mg/kg of animal weight) and local anesthesia with Ultracaine DS-Forte. After anesthesia, the surgical field was treated with soapy water and Betadine. The anesthetized animal underwent a typical mandibular incisor extraction and curettage of the extraction socket (Pic. 4), after which a skin incision was made along the alveolar process of the lower jaw in the submandibular area on the side of the extracted tooth (Pic. 5) and the soft tissue was bluntly and acutely prepared to expose the external compact plate of the extracted tooth socket (Pic. 6). This access was chosen because of the lack of space in the animal's mouth and the inability to insert the dental instrument needed for the implantation with the classic intraoral approach. Then, according to the classic protocol, an osteotomy of the implant socket was performed perpendicular to the alveolar process through the external cortical plate of the extraction socket until the lingual surface, which has a spongy substance layer, is resting on it. The implant was inserted manually into the socket and was inserted to the cortical layer using a torque wrench (Pic. 6). At the end of the operation, the wound was irrigated with 0.9% NaCl and sutured layer by layer with Vicryl (Jonson&Jonson) atraumatic suture. The animals were removed from the experiment by overdosing with Thiopental sodium solution (60 mg/kg animal weight) on 15/30/45/60 days in 2 individuals in each group. After cardiac and respiratory arrest and signs of biological death, the jaw preparations with implants were dissected and removed (Pic. 7). At the same time, cone beam computed tomography (CBCT) was performed with a Gendex CB-500 (KaVo) with the following interpretation of the tomography data in the i-CATVisionTM (Imaging Science International) software interface (Pic. 8). The jaw preparations were fixed with 10% isotonic formalin solution, followed by dehydration in increasing concentrations of 70%-96% ethanol (Pic. 9). Morphological research was carried out using electron microscopy of a series of slides of prepared preparations…

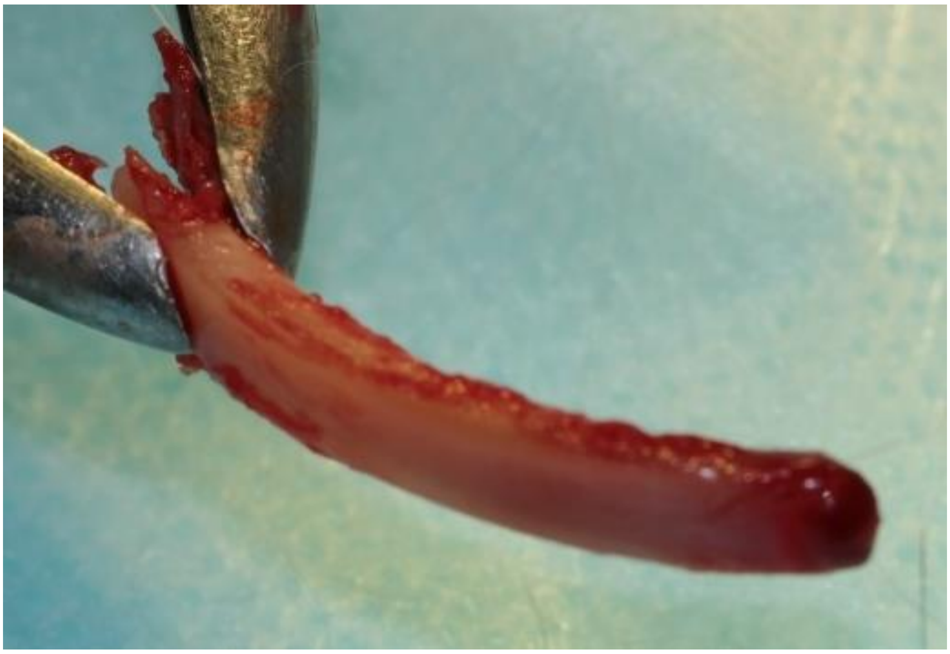
Pic. 1. Type of experimental animal: rabbit breed...

Pic. 2. Lower incisors of the rabbit: the most accessible teeth for removal in rodents



Pic. 3. Titanium implant with double-layered mesh-like surface macrostructure.

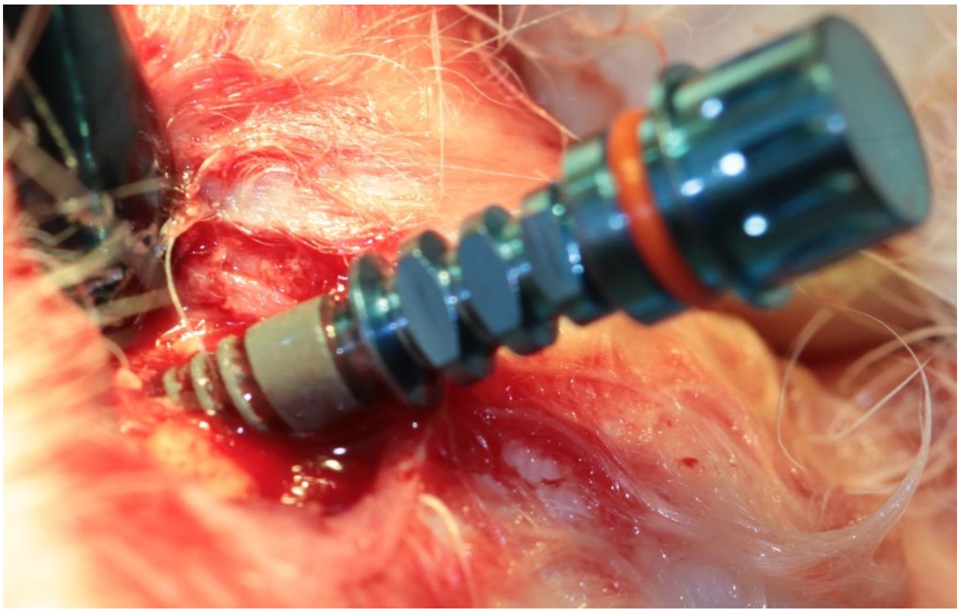
Pic. 2. Rabbit mandibular incisors most suitable for removal procedures in experimental rodents



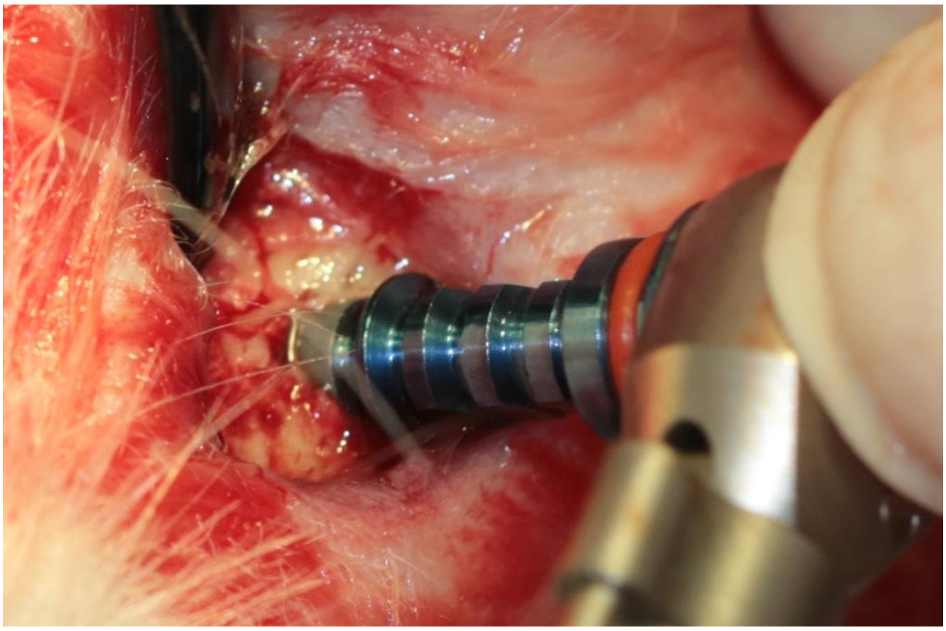
Pic. 4. View of an extracted mandibular incisor in a rabbit. On the top of the tooth there is a growth area, which must be removed before inserting the implant.

Pic. 5. View of the external operating radius in the projection of the extraction site

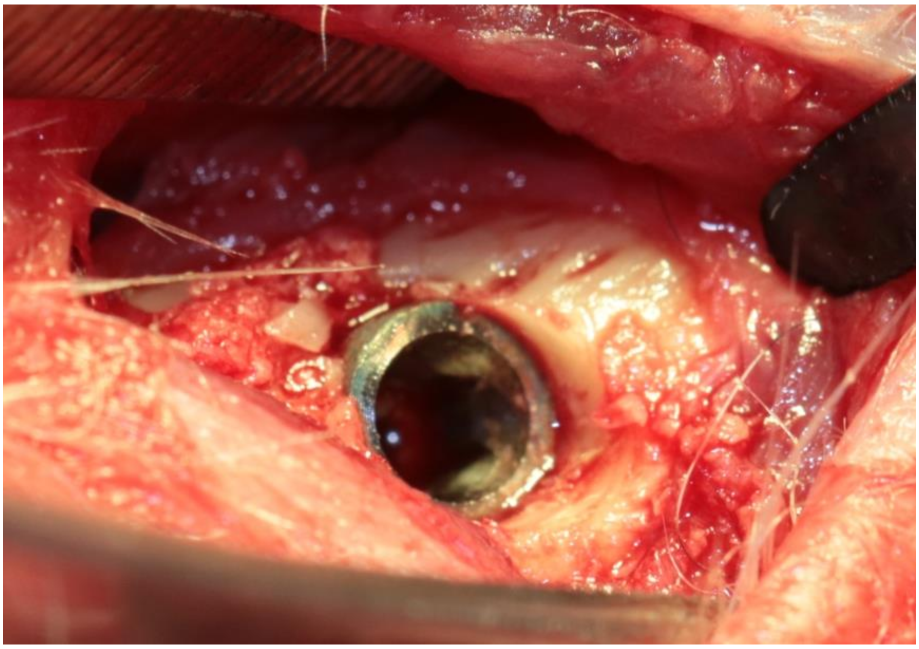
Pic. 6. View of the exposed lower-external alveolar wall of the extracted crib tooth in the percutaneous access



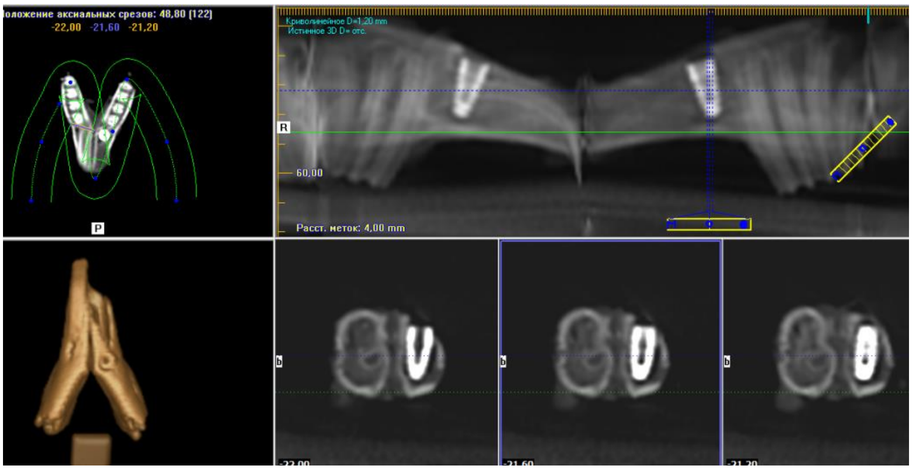
Pic. 5. Manual insertion of the implant into the osteotomy area



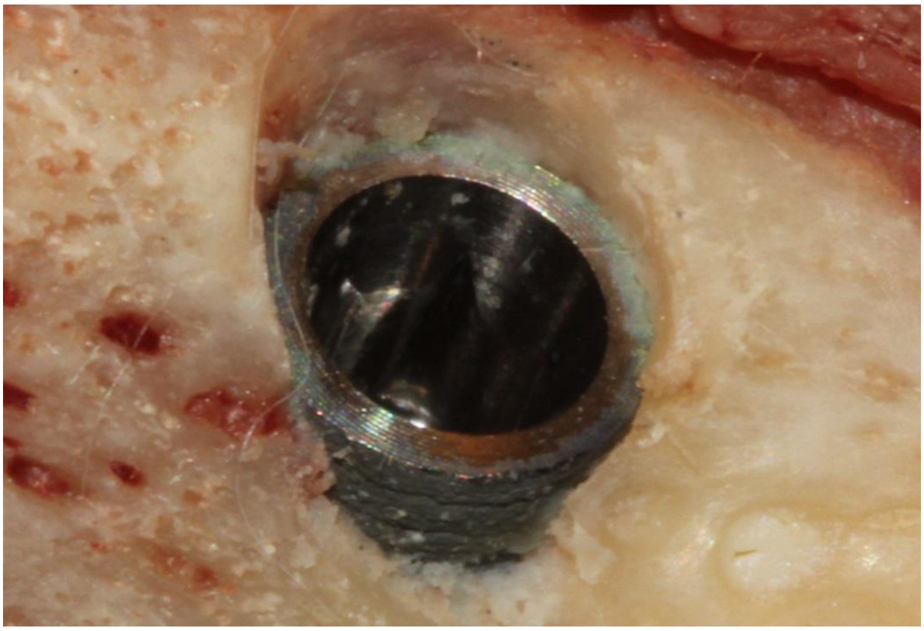
Pic. 6. Final fixation of the implant with the torque wrench



Pic. 7. View of the removed preparation after the animal was removed from the experiment (15 days)



Pic. 8. 3D reconstruction of the rabbit jaw preparation with inserted implant in the interface of i-CATVisionTM.


Pic. 9. View of the installed implant in the rabbit jaw preparation extracted on day 15

Results of the experiment

As a result of the experiment, successful integration of laser sintered implants was achieved, which was shown by electron microscopy of a series histological sections of rabbits jaws with implants in different terms. The results of the experiment, as well as literature data, show a perspective of successful using of laser sintering implants applying into clinical practice in order to more effectively rehabilitate patients with partial or complete edentulism.

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Report on the results of the implant sample research

1. RESEARCH METHODS

Sample preparation for microscopic research

Drying

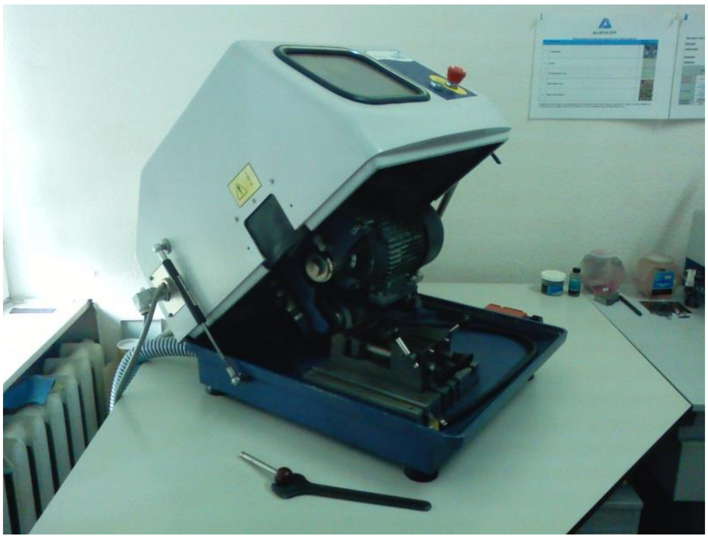
Drying was done in a vacuum drying oven for 4 hours.

Previous fixation of the sample for cutting

The size of the provided sample exceeded the maximum allowable dimensions of the press form for pressing into bakelite for further preparation of the sample for research. Therefore, the sample had to be trimmed. To preserve its integrity, the sample was poured into epoxy resin and kept in a vacuum oven for 30 minutes.

Cutting

The sample was cut on a Delta Abrasimet cutting machine (Picture 1.2) manufactured by Buehler (Germany) using a BuehlerMet II Wheels- HRC35-50 abrasive wheel with a cooling liquid supply.



Picture 1.2 - Delta Abrasimet Buehler cutting machine (Germany)

Pressing into the cage

For further automatic grinding and polishing, the cut sample was pressed into a bakelite cage. This operation was done on a SimpliMet 1000 automatic press (Picture 1.3) by Buehler (Germany).

Picture 1.3 - SimpliMet 1000 automatic press by Buehler (Germany)

Grinding

The sample was grinded on an automatic grinding and polishing machine GP beta (Picture 1.4) with a multi-position grinding and polishing gear Vector POWER HEAD with a load of 10 N and a rotation speed of the abrasive disc of up to 120 rpm. During the grinding process, BuehlerMet silicon carbide abrasive papers with grit sizes P120, P320, P600, and P1200 were used in sequence. After grinding, the sample was rinsed and wiped with alcohol.

Picture 1.4 - Automatic grinding and polishing machine GP beta by Buehler (Germany)

Polishing

The sample was polished on the same grinding and polishing machine (Picture 1.4) using felt and a corundum suspension with a particle size of 50 nm. The rotation speed of the polishing surface did not exceed 100 rpm.

The electron microscopic researches

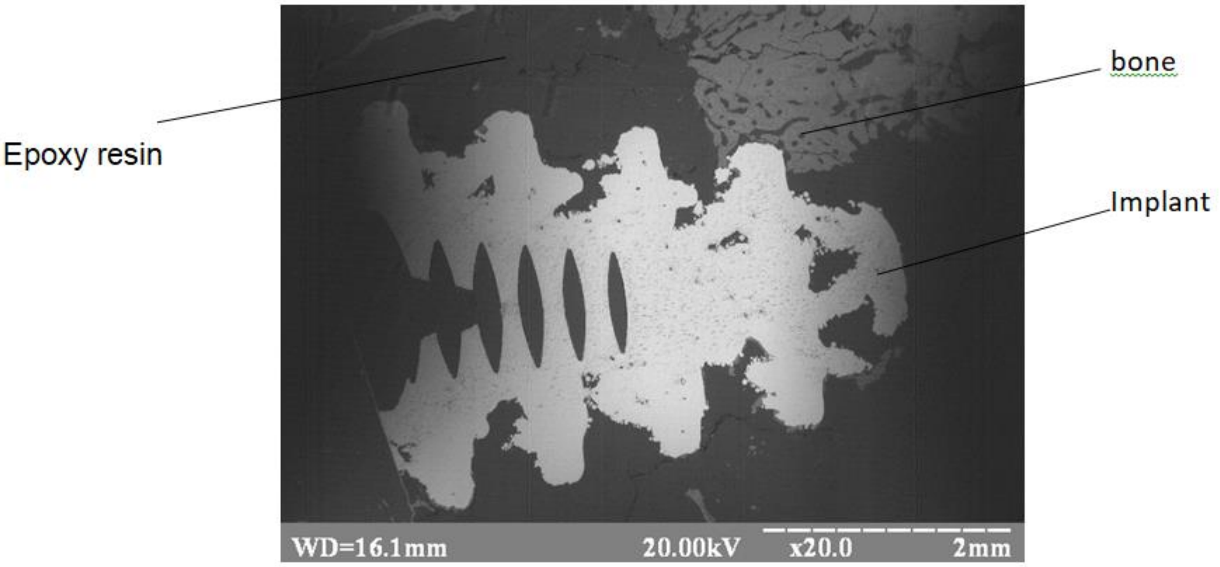
The researches were carried out on a scanning electron microscope SEM 106I (Picture 1.5) of Selmi (Ukraine) with an attachment for energy dispersive X-ray microanalysis EDS. The window of the EDS detector is made of beryllium, which does not allow to determine the content of chemical elements with an atomic number lower than that of magnesium (O, N, C. B, H, Li). The resolution of the obtained images reached 200 nm, and the accuracy of chemical composition determination significantly depends on the concentration of the element in the material.

Microstructure analysis was carried out using a reflected electron detector (COMPO mode) and in a low controlled vacuum mode (this was necessary to remove the charge from the non-conductive epoxy).

Picture 1.5 - Scanning electron microscope REM-106I of Selmi (Ukraine)

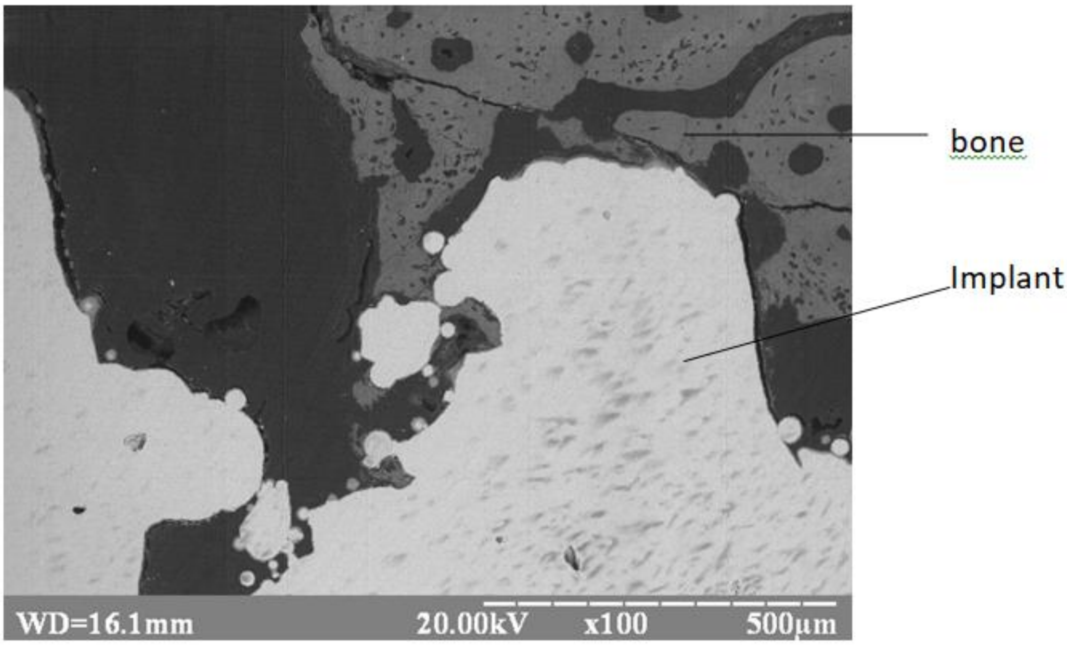
2. RESEARCH RESULTS

According to the results of electron microscopic research of the sample, it was found that most of the implant (Picture 2.1) is in contact only with epoxy resin.



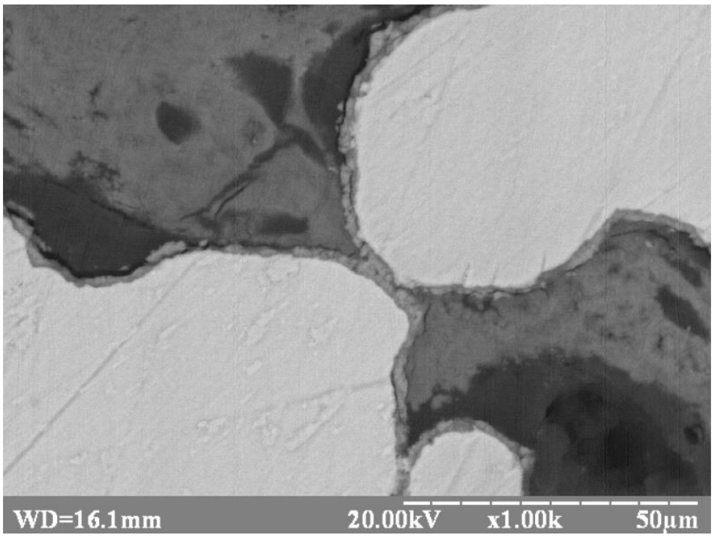
Picture 2.1 - Sample structure

In this case, a strong connection appears at the site of bone ingrowth into the implant relief because of mechanical adhesion to it (Picture 2.2).



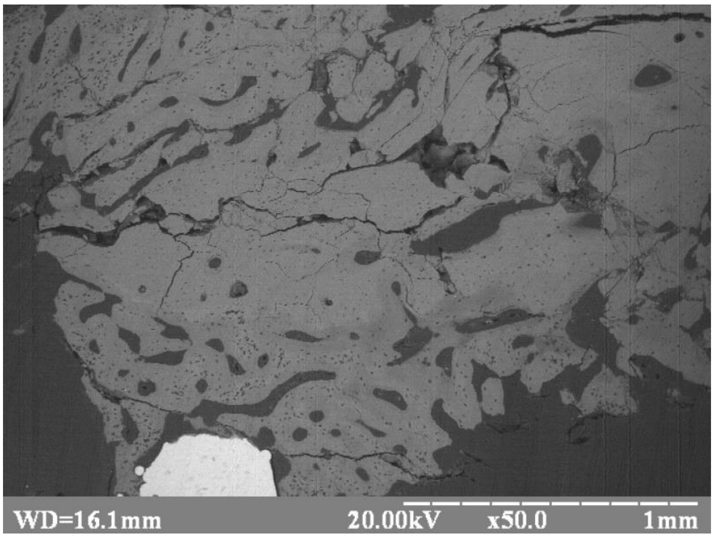
Picture 2.2 - Bone ingrowth into the implant

The research also revealed a coating (Picture 2.3), the thickness of which is about 2 - 4 microns.



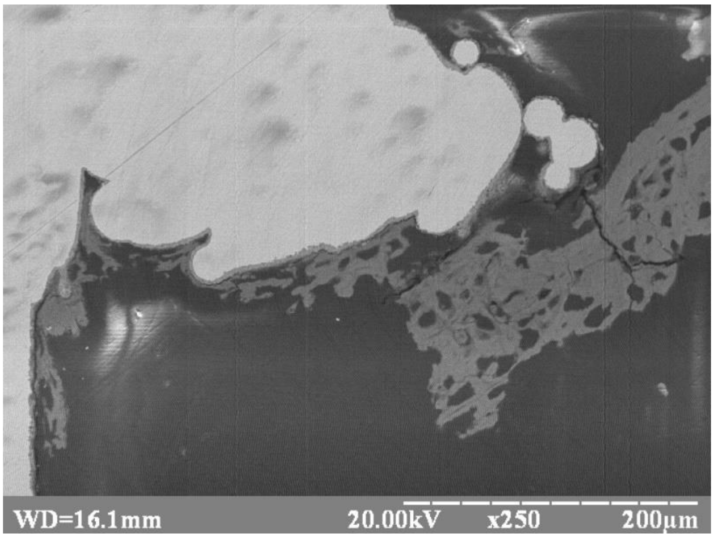
Picture 2.3 - Interaction between bone and implant coating

The composition of the bone near the implant (Picture. 2.4) is different from the main mass of bone (in the picture, this part of the bone has a darker shade).



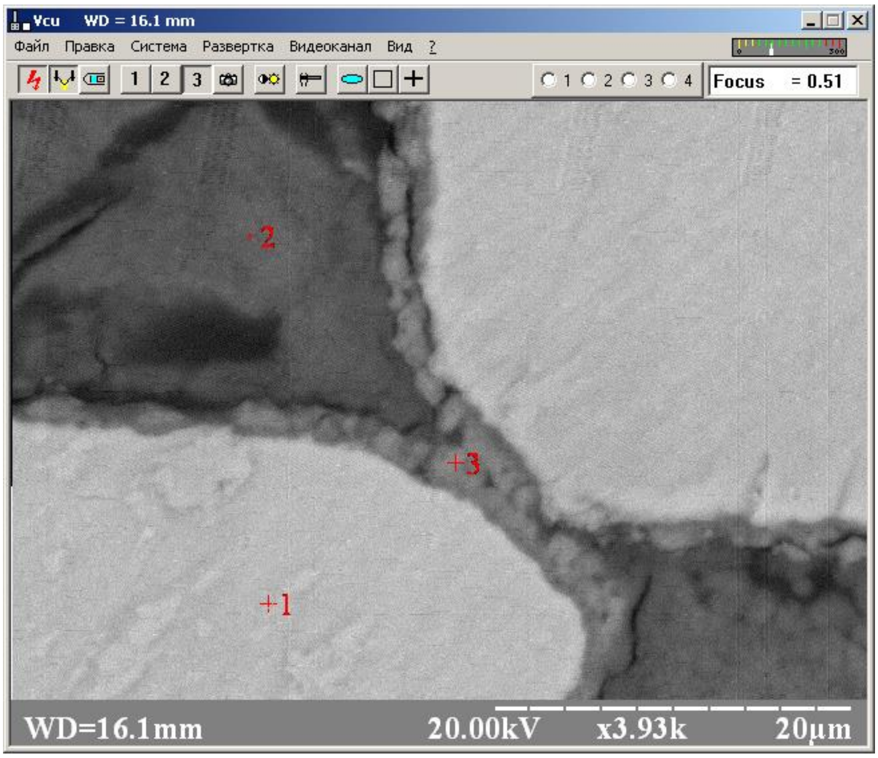
Picture 2.4 - Bones near the implant

Bone ingrowth along the surface of the implant coating and adhesion to it (Picture 2.5) is very dense.



Picture 2.5 - Bone on the surface of the implant coating

Based on the results of micro-X-ray spectral analysis, the chemical composition of the implant, coating and bone was compared (Picture 2.6).



1.

Элемент		Инт.	C %
Al	K	150	1.61
Ti	K	6577	98.39
Ti	L	0	0.00

2.

Элемент		Инт.	C %
Al	K	29	0.88
P	K	549	18.65
Ca	K	1766	60.94
Ca	L	1	0.00
Ti	K	315	19.52

3.

Элемент		Инт.	C %
Al	K	123	2.91
P	K	303	7.46
Ca	K	188	3.23
Ca	L	1	0.00
Ti	K	2704	86.41

Picture 2.6 - Chemical composition of bone, coating and implant (wt%)

Results of the use of dental implants made by the method of direct laser sintering (DMLS) after the surgical stage of dental implantation

Resume.

The accumulated significant clinical experience in the use of dental implants has demonstrated that the topography and roughness of the implant surface plays a major role in many cellular and molecular mechanisms, and more effectively contributes to the speed and quality of osseointegration. As a way to overcome the problem of porous structure with fully controlled design of the external shape and internal architecture, manufacturers have proposed the option of additive manufacturing of dental implants by direct laser sintering (DMLS). By comparing the radiological picture, the dynamometric force of implant placement and measuring the indicators of primary implant stability (ISQ), we studied the experience of using dental implants made by the DMLS method after the surgical stage of dental implantation in comparison with classical milled implants.

Keywords.

Dental implants, DMLS, torque test, Alpha Dent Superior Active, Alpha Dent Cell.

Introduction.

Dental implantation as a method of treatment of secondary edentulousness has been growing in popularity since its invention to the present day. The process of osseointegration, as a direct structural and functional connection between the structured living bone and the implant surface, is the basis of the global popularity of this technology. This biological phenomenon gives a foreign body the ability to exist stably in a living organism, withstand heavy mechanical loads, have long-term clinical success and, as a result, patient satisfaction. Since implant surface features have long been studied and characterised as an important factor in promoting osseointegration [1,2], current research is focused on optimising the potential of the implant surface for osseointegration. Currently, the most widely used dental implants are those made by machining a titanium rod with subsequent modification of the product surface: sandblasting [3], acid etching [4,5], anodising [6,7], and discrete deposition of calcium phosphate crystals [8].

These surface treatment methods are aimed to improve stability and enhance osseointegration [1-9]. Extensive clinical experience with dental implants has demonstrated that the topography of the implant surface plays a key role in many cellular and molecular mechanisms. Rough surfaces demonstrate better adsorption of biomolecules from biological fluids, which start a cascade of biological events that regenerate bone, improve the initial cellular response, including cytoskeleton organisation and cell differentiation [1-3,8,9].

Histologically, rough surfaces have been shown to be more effective in improving the speed and quality of osseointegration compared to untreated surfaces. There are researches that clearly demonstrate significantly higher success rates of dental implantation of samples with a rough surface [5,7,10,12,13]. According to modern knowledge of osseointegration, it has become known that the surface roughness is optimal for bone ingrowth when the distance between the peaks of the material structure and the pore size are in the range of 200 - 400 μm [11]. As osseointegration is a key factor in the success of dental implants, it may be biologically justified to use porous implants, expanding their functions by promoting osseointegration throughout the product body.

Improved fixation can be achieved by bone growth into or through the porous structure of the implant, connecting not only to the rough surface of the implant but to the entire implant body.

However, from a mechanical point of view, such an implant-bone connection must be sufficiently rigid to withstand the mechanical pressure exerted on it by the orthopaedic structure. It is known that classical methods cannot produce a porous structure with a fully controlled design of the external shape and internal architecture. Producers have proposed methods of additive production of dental implants as a way to overcome this problem [14, 15]. This approach of directly generating physical objects with a distinctive structure and shape based on three-dimensional modelling has opened up a wide area for the study of the processes of osseointegration of a printed implant in a living organism.

Goal.

To study the experience of using dental implants made by direct laser sintering (DMLS) after the surgical stage of dental implantation in comparison with classical milled implants.

Objective.

- To determine the difference in the dynamometric force of installation (torque) during the installation of implants made by direct laser sintering in comparison with implants made by the classical method.
- To compare the stability of dental implants made by the direct laser sintering method and those made by the classical method by measuring the ISQ indicators.
- To investigate the radiological picture of osseointegration of dental implants made by direct laser sintering and those made by the

classical method.

- To analyse the clinical experience of using dental implants made by direct laser sintering.

Materials and methods of the research.

22 people aged 22-54 years, both sexes, took part in the research, who simultaneously, side by side, were implanted with at least two implants of the same size, where the first one was made by the classical method of machining a titanium rod with the further modification of the product surface by sandblasting and acid etching, and the second implant was printed in layers by selective laser melting. The last ones, received by additive manufacturing, were made using laser fusion technology in a powder layer with different consecutive layers growing in the vertical direction - the direction of load application. For the production of the samples, 30 nm thick layers of Grade-4 titanium powder with an average particle size of 45±10 μm were used, fused by two fibre lasers with a power of 200 W each (weld length 1070 nm). The samples were formed with the Trumpf TruPrint 1000 device. Samples of Alpha Dent Superior Active (21 samples) and Alpha Dent Cell (21 samples) implants were used. The process of implant surface treatment of the studied samples is identical (wet SLA surface). The initial stability of all implants (ISQ) and the end face were measured. The implant torque was measured with a wrench during implant placement. ISQ was determined using a Penguin RFA device at the time of implantation and at 15, 30, and 60 days. Some implants were immediately fitted with gingival moulders. Intraoral contact radiography was done using a Pluto super HD visiograph. Radiological control was done at the time of implantation, at 30 and 60 days.

Results of the study.

1. Determination of the difference in the dynamometric force of insertion (torque) of implants made by direct laser sintering and those made by the classical method during their installation.

It should be noted that the torque value is crucial when deciding on the placement of a gingival moulder or early orthopaedic loading, so achieving high values of the dynamic insertion force is a priority for the implantologist. At the time of implant placement, we fixed the value of the dynamometric force (torque) in Newtons per cm2, the data obtained are shown in Table 1.

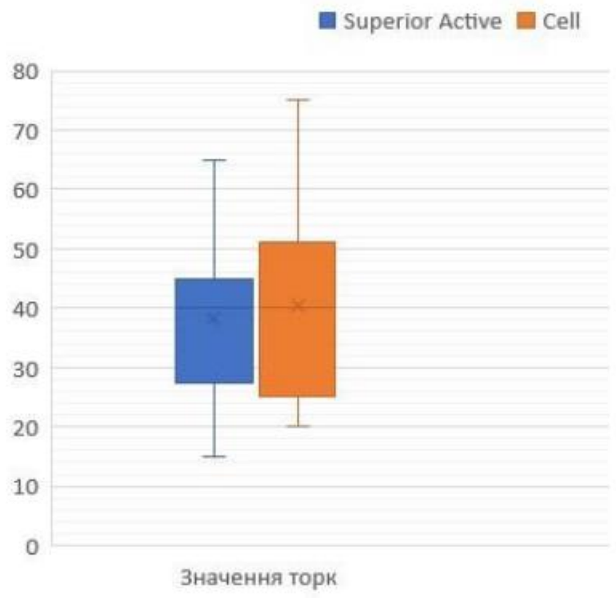


Table 1: Values of the dynamometric force at the time of placement for different types of implants of identical size.

Different types of bone in patients of different age groups and sexes led to the heterogeneity of the data obtained, but it should be noted that in almost all patients, under identical conditions, the torque value is higher for printed samples (Cell implants), indicating a larger total implant surface area. In the upper jaw, the torque value was relatively lower than in the lower jaw, which is due to the biological characteristics of the jaw bone structure. The only drawback of torque testing is the impossibility, for obvious reasons, of further measurements at different stages of osseointegration, but nevertheless, this method gives a comprehensive picture of the interaction of the implant with the bone at the time of implantation.

2. Comparison of the stability of dental implants made by the direct laser sintering method and those made by the classical method by measuring ISQ indicators.

All patients who had dental implants were fixed ISQ Penguin RFA at the time of implantation, at 15, 30 and 60 days (Table 2). According to the data obtained, in most cases, there is an increase in ISQ during the first 14 days, which probably indicates the effect of straightening the trabeculae of the spongiosis layer of bone.

From 14 to 30 days, a recession of indicators is fixed, which may signal the activation of osteoclasts and active restructuring of the bone matrix around the implant. From 30 to 60 days, we can observe a dynamic increase in implant stability. It should be noted that the speed of this stage most likely confirms the quality of interaction between the implant surface and osteoblasts, which in these terms transform the constructed protein matrix into immature bone tissue. When comparing the numerical data of the studied implants, a rapid increase in values in the period from 20 to 60 days for implants of the Cell type is noted (Table 3).

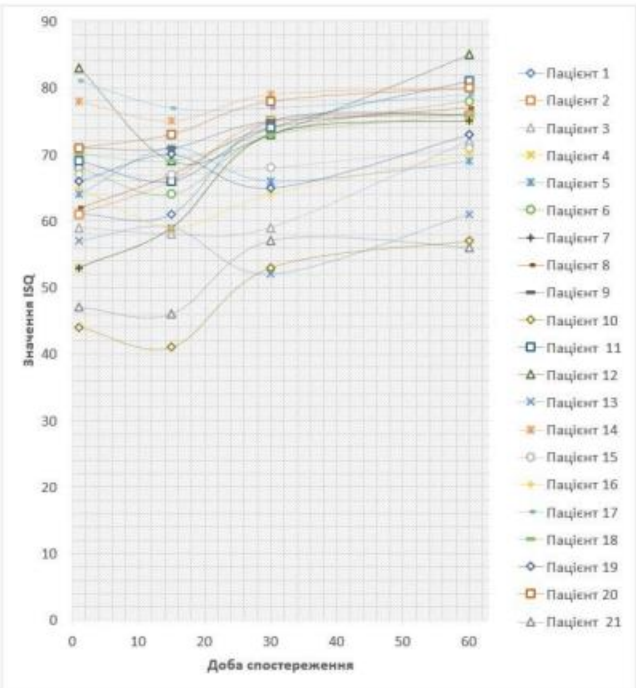
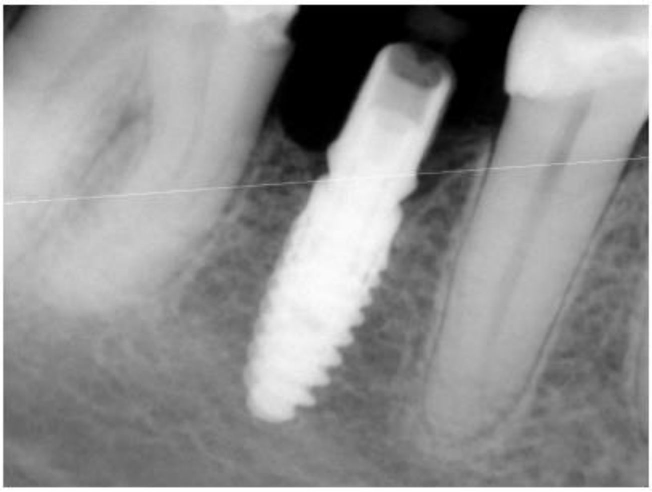


Table 2. Summary table of the stability values of Cell implants at different stages of observation (ISQ-test).

3. Radiological picture of dental implants made by direct laser sintering and those made by the classical method at the stages of osseointegration.

Intraoral contact radiography allows to objectively assess the bone pattern around the implant and is a leading method in the diagnosis and prognosis of early complications of dental implantation. During the observation period, no complications were observed in any patient. Radiological control was carried out at the time of implantation, on the 30th and 60th day. The radiological picture had a similar uniform character, which changed little even with orthopaedic loading (Fig. 1). The clear borders of the implant profile with no signs of intensity reduction at the border with bone tissue indicate true osseointegration.



Picture 1. Radiological picture of an Alpha Dent Cell printed implant with a provisional crown.

Conclusions.

Based on the analysis of the data presented above, comparing different types of implants, a number of interesting conclusions can be drawn. The values of the dynamometric force of the implants installed in all pairs of the investigated implants were close to each other, which is explained by identical bone supply conditions and the same size of the implants. Their average value ranged from 25-55 N\cm2.

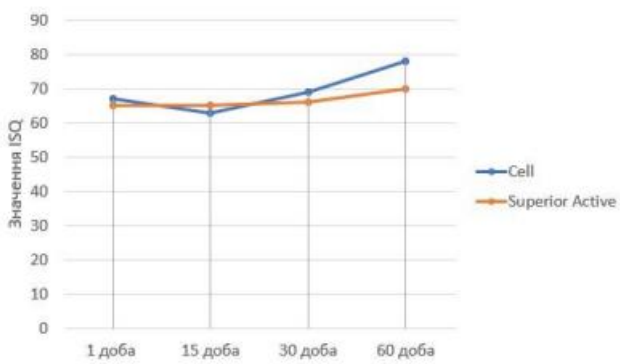


Table 3: Comparison of the average ISQ values of implants made by the classical method (Superior Active) and DMLS method (Cell).

There were no significant statistical differences. But it should still be noted that under identical conditions, a slightly higher torque value can be developed with Cell implants. The ISQ-test demonstrates similar values for the compared implant samples, but special attention should be paid to the dynamic increase in the ISQ value from 20 to 60 days, which indicates the participation of the printed frame of the implant body as a structure with a more developed area of contact with the bone and, as a result, somewhat faster and better osseointegration. This is also confirmed by X-rays. Thus, studying the experience of using dental implants made by direct laser sintering in comparison with classical milled samples, we can conclude that DMLS implants are promising and have clinical advantages.

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