EDITORIAL

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The Role of Calcified Tissues in the Different Stages of Implant Osteointegration*

Znaczenie tkanek zmineralizowanych w różnych stadiach osteointegracji implantu

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Abstract

The replacement of mineralized tissues lost because of trauma or lesion by using exogenous materials has been considered as an accepted treatment for the last 25 years. The replacement materials should, however, integrate with the osseous structure. The osseointegration process implies different factors, as are the shape and composition of the implant, the method and the timing of the implantation and all the various biological and biomechanical phenomena that take place in the bone-implant surface. The present study is aimed to describe the different stages of the osteointegration, and the presence and the role that the different osseous calcified tissues play all the long of this complex process. Our results show that the role of the chondroid tissue in the stabilization of the implant is essential for an effective osseointegration. That proves that the osseointegration process involves a membranous ossification mechanism (**Dent. Med. Probl. 2004, 41, 2, 179–191**).

Key words: osseous calcified tissues, chondroid tissue, osseointegration, membranous ossification, bone anatomy.

Streszczenie

Odbudowa tkanek zmineralizowanych, utraconych z powodu urazu lub resorpcji patologicznej po zastosowaniu materiałów alloplastycznych, jest akceptowana od 25 lat. Materiały odtwórcze powinny integrować się z tkanką kostną. Na proces osteointegracji mają wpływ różne czynniki, takie jak: kształt i skład implantu, metoda oraz czas implantacji, a także różne procesy biologiczne i biochemiczne zachodzące na granicy kości i implantu. Celem pracy było przedstawienie różnych etapów osteointegracji i ukazanie roli różnych zmineralizowanych tkanek kostnych przez cały okres tego procesu. Badania wykazały, że najważniejszą rolę w stabilizacji implantu, która jest niezbędna do efektywnej osteointegracji, odgrywa tkanka chondroidalna. Świadczy to, że w procesie osteointegracji jest najważniejszy mechanizm kostnienia na podłożu błoniastym (**Dent. Med. Probl. 2004, 41, 2, 179–191**).

Słowa kluczowe: zmineralizowane tkanki kostne, tkanka chondroidalna, osteointegracja, kostnienie na podłożu błoniastym, anatomia kości.

Reconstruction of local bone defects resulting from trauma, bone tumors, and spinal fusion is a major problem both in dental and in orthopedic surgery. The replacement of lost mineralized tissues using implant materials has been considered as an accepted treatment for at least 25 years. The requirements for these replacement materials were at first bioinertness and nontoxicity; afterwards a new

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concept that the materials must enhance cell adhesion, growth and differentiation was proposed [1].

Currently, autogenous bone grafts are considered as the gold standard method for treating these problems [2]. Although fresh autogenous bone grafts, which combine osteoconductive, osteogenic, and osteoinductive properties [3–5], are used to treat bone defects, this procedure involves the second operation and the amount of autogenous bone available is limited [6]. Additionally, the harvesting of autogenous bone may cause the patient's discomfort, surgical morbidity, infection, and impairment at donor sites [7]. Because of these limitations, allogenic bone, either frozen or freeze-dried, could be used as an alternative or supplement to autogenous bone [8]. However, the use of this material also has some limitations, including the high cost of bone-banking [9], the potential for graft-transmitted diseases and allograft fractures, the possible immunologic rejection and high rates of nonunion and infection [10, 11].

The biomaterials for bone-implant applications were initially classified as being either "bioinert" or "bioactive" [12]. Initially, the local biological reaction to the implant is often characterized by its fibrous tissue encapsulation. However, in bioactive or osteoconductive materials, new bone tissue is formed in direct or intimate contact with the implant surface. Hydroxyapatite (HA) that makes up the majority of the inorganic component of human bones and teeth [13, 14] is bioactive and directly bonded on the bone *in vivo* [15–17], whereas titanium (TI), which is bioinert, does not bond directly but has close contact with the bone [18]. Nevertheless, the force of the union linking the bone to the metal is smaller than the one linking it to a bioceramic like HA [19]. Both those materials have been used clinically during the last 35 years, clearly proving their biocompatibility; however, to this moment, it is not clear which material is the most favourable to the cells of the host tissue and what exactly is the cell response and the material interface activity of bone-bonding and non-bonding materials.

Hydroxyapatite implants may be block or granular, dense or porous, and its pores may be large or small. Porous and dense ceramics obtained from different sources have been the subject of extensive investigations describing their biological incorporation when implanted both intra- and extraskeletally in a variety of animal models [20, 21]. It is well documented that porous hydroxyapatites have osteoconductive properties when implanted next to viable bone [22]. Since the first reports of substantial bone differentiation in porous hydroxyapatites in extraskeletal sites of the baboon [23], other studies have confirmed this remarkable phenomenon consisting in the hydroxyapatite inducing bone differentiation [24]. Chu et al. found that the implant internal architecture and design controls the degree of bone regeneration. Moreover, they influence the process of bone regeneration, and determine the geometry and the mechanical properties of the regenerated tissue [25]. Nevertheless, the use of this type of implants has been limited to applications not destined to support loads because of its extremely low resistance to fracture, far below these displayed by other ceramics or by titanium.

To take advantage of both the titanium and HA characteristics, numerous methods have been developed to cover the metallic implants with HA coating layers of various characteristics: plasma spraying [26–28], hot isostatic pressing [29], sol-gel deposition [30], biomimetic deposition, as proposed by Kokubo [31], HVOF-High velocity oxygen-fuel spraying [32], ion beam assisted deposition [33], magnetron sputtering [34], ion beam sputtering [35] and lately, with some degree of success, pulsed laser deposition [36–45].

To understand the biochemical mechanism of bone formation, it was proposed that, at least strategically, five factors must be taken into consideration [46, 47]. They are cells directly involved in bone formation; matrices produced by the cells; body fluids; regulators of general cellular activities as well as the calcification process; and mechanical stress. These five factors should be analyzed individually, the interactions between them elucidated, and finally they should be integrated into the whole picture of bone formation.

The clinical evolution of the osteointegrated implants has been the object of numerous studies since Albrektsson et al. [48], defined osteointegration as "the direct contact between implants and living osseous tissue". In the process of osteointegration numerous factors are implied, as are the form and the composition of implant, the various methods and chronology of implantation, and the biologic and biomechanic phenomena that take place in the bone-implant interface [49], such as the micromovements and the complex interactions between the bone calcified tissues and the implant [50].

The histological studies of the tissues around the implants, initiated in 1969 by Branemark by using decalcified material [51], were followed eight years later by Schroeder [52], using techniques without decalcification. Those articles presented the first evidences of direct bonding between bone and implant. The literature dealing with the histology of bone-implant interface has been abundant and extremely well documented, developing the essential concepts of osteointegration, osteocon-

duction and osteoinduction, as they are used at the present time [53, 54]. In the late years of the XX century the concept of "bone quality" was proposed as one of the main determinants of the stability of the implants [55].

The process of osteointegration is a process of bone repair that advances following pre-programmed phases [56], and it implies different structures and tissues [57] as it progresses.

The initial response of bone structure to the presence of implants as well as to a bone fracture, is a humoral reaction in which a series of Growth Factors play an essential role. Between them, we can mention the Transforming Growth Factor (TGF) superfamily [58], the Fibroblast Growth Factor (FGF) [59] and the Insulin-like Growth Factor (IGF) [60], all of them present in some measure in the cells of skeletal tissues.

Bone inducing activity has been described for other biological factors, like Interleukin-1 (IL-1) or Tumor Necrosis Factor (TNF) and even hormones, like Parathormone [61] or Growth Hormone. Osteogenin, described by Lacroix [62], or Bone Morphogenetic Protein (BMP) by Urist [63], in addition to cytokines produced by other non-osseous cellular lines [64], also have been shown to participate in the humoral phase of the osseous repair.

However, papers studying in detail the presence or the functional significance of the different osseous calcified tissues in the osteointegration process [57], or proposing in addition a chronological schedule of the phenomena involved [56] are sparse.

Material and Methods

In the first experimental study, 12 dogs of both sexes, younger than 2-years were used. Two different commercial implants, non HA-coated, were surgically implanted in the anterior surface of both tibiae of the animals. As the aim of the study was to test whether local and general treatments acted as enhancers of the osteointegration, the animals were grouped randomly and received a local treatment (ultrasounds or pulsating laser), added or not with a general, pharmacological treatment (Calcitonin), as presented in Table 1. In order to evaluate the influence of the treatments in the process of osteointegration, the animals were sacrificed one, two and three months after the intervention. The comparison of the results of the diverse adjuvant treatments in the process of osteointegration has been the object of one recent publication [65]. In the present work, the results obtained in the control, non-treated animals are shown, because those better represent the process of osteointegration without significant alterations.

The second experimental design was carried out on a total of six male dogs, also younger than two-year-old. A total of five rectangular (2 × 5 mm) Titanium plates covered by diverse types of Calcium-phosphate coatings were implanted in surgically drilled beds in the anterior surface of the dogs tibiae and were covered by the *periostium*, without any added fixation method. The various coatings were deposited by pulsed laser ablation, which permitted to choose the thickness and the degree of cristallinity of the diverse layers of the coating [41–45]. Moreover, in order to evaluate the influence of the characteristics of the coatings on the progression of the process of osteointegration, the animals were sacrificed one, two and three months after the intervention. The distribution of the samples and the periods of study are presented in Table 2.

The samples were processed following the protocol of study standardized by authors' unit [66], adapted from the one previously described by Donath [67]. In essence, it consists in a process of fixation, followed of the dehydration and preinclusion of the undecalcified samples within a mixture of metacrylate monomers and polymers (TECHNOVIT®, Exact-Kultzer, Wehrheim, Germany). The samples are then photopolymerized, obtaining a rigid block, that successively is mounted, cut with a diamond-coated sawband, and mechanically grinded until allowing its systematic study, starting with its observation by Scanning Electron Microscopy (SEM), either with secondary or with

Table 1. Sample distribution according to the surgical protocol of experiment

Tabela 1. Rozkład próbek w zależności od postępowania chirurgicznego

Time	Surgery	Surg + Calc	Surg + US	Surg + La	Surg + Ca + US	Surg + Ca + La
1 month	1Left, 5Left	2Left, 8Left	1Right	5Right	2Right	8Right
2 months	3Left, 7Left	4Left, 10Left	3Right	7Right	4Right	10Right
3 months	5Left, 9Left	6Left,12Left	5Right	9Right	6Right	12Right

Time – time after surgery (czas po implantacji), Calc – Calcitonin treatment (kalcytonina), US – Ultrasounds local treatment (miejscowe leczenie ultradźwiękami), La – Laser local treatment (leczenie laserem).

Table 2. Pulsed laser ablation deposit parameters for each sample type and covering layer characteristics

Tabela 2. Parametry pulsacyjnej ablacji laserowej dla każdego typu próbki i charakterystyki warstwy pokrywającej

Sample (Próbka)	Laser wavelength (Długość fali laseru) nm	H_2O Pressure (Ciśnienie H_2O) Pa	Substrate temperature (Temperatura substratu) °C	Thickness (Grubość) μm	Covering layer phases (Fazy warstwy pokrywającej) XRD
IAMO	KrF/248	0.5	20	1.0	ACP
ICRI	KrF/248	45	600	1.0	HA
IMIX	KrF/248	150	600	3.0	α-TCP + β-TCP
IYAG	Nd:YAG/355	45	600	9.0	HA
IBIC					
internal layer (warstwa wewnętrzna)	KrF/248	45	600	1.5	HA
external layer (warstwa zewnętrzna)	KrF/248	0.5	20	1.0	ACP

KrF – excimer-laser (laser eksymerowy), Nd:YAG – Nd:YAG laser (laser neodymowo-jogowy), ACP – amorphous calcium phosphate (bezpostaciowy fosforan wapnia), HA – hydroxyapatite (hydroksyapatyt), TCP – tricalcium phosphate (fosforan trójwapniowy).

backscattered electrons [68, 69]. Afterwards, the more significative samples were sectioned anew, mounted in transparent plastic slides and grinded again to allow its coloration and histological observation.

Results

Regarding calcified tissues, the first observation that affects its structure is the appearance in the zone of implants of an intense osteoclastic activity, as it is observed in Figures 1 and 2. The numerous biochemical factors present in the fracture or implant area have an obvious attractive effect for the osteoclasts [70], that play a fundamental role in the beginning of the bone repair [61, 71]. In authors experience, the osteoclasts appear during the first month after the positioning of metal implants, covered or not with hydroxyapatite. As previously described by Dhem [71], Roberts [56] and Schenk [64], the osteoclasts are followed by a line of bone-forming cells (lining cells), as it is shown in Figure 2.

Nevertheless, in presented results, in implants unlike in those described by Roberts [56] and Schenk [64], these cells do not form lamellar bone but chondroid tissue in from of thin trabeculae. In Figures 3 and 4, the autrhors observed these trabeculae formation, constituted by chondroid tissue that presents a characteristic aspect of a strongly calcified tissue, with a sparse, irregular extracellular matrix which sometimes appears striated due to the presence of bundles of collagen fibers. As described by Goret-Nicaise and her co-workers in the formation of the skull vault bones, the cellular lacunae of this tissue are big, irregular and confluent [72]. The difference in orientation of the trabeculae visible in Figures 3 and 4 depends on the different degree of osteoconductivity of the hydroxyapatite layers deposited by laser ablation on the implant surface [73].

The following phase of the osteointegration implies the apposition of woven bone upon the original chondroid tissue trabeculae, also as was described for the formation of the skull vault bones [72]. Figures 5 and 6 show the characteristic aspect of the woven bone, with a slightly more regular and less calcified extracellular matrix and isolated polygonal, big cellular lacunae. This tissue is located upon pre-existing trabeculae of chondroid tissue. This apposition usually is observed at the end of the first month after the implantation, although in implants covered by amorphous hydroxyapatite the deposition could be more rapid [73]. As in Figures 3 and 4, the different direction of the trabeculae shown in Figures 5 and 6, which are formed by chondroid tissue and woven bone, is related to the osteoconductivity of the various hydroxyapatite covering layers deposited by laser ablation [73].

The following phase of the implant osteointegration consists in an intense remodelling process of the area around the implant that happens at the end of the second month after the intervention. As described previously in the repair fracture process [69], the vascular elements, forming what was called “cutting cones”, play an essential role. In this phase of the implant osteointegracion (Fig. 7) numerous vascular elements (CC) appear, some originated in the original cortical bone vessels, while others, described by Roberts et al. [56] as “cutting--filling cones”, are originated in the subperiosteal

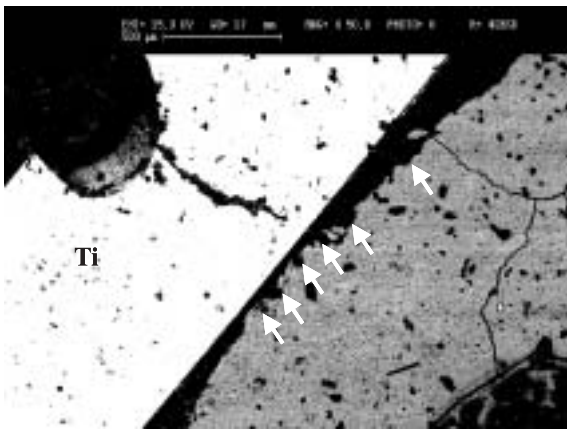

Fig. 1. Backscattered Electrons Scanning Electron Microscopy (BS-SEM). Dog Tibia, 1 month after surgery: Ti – titanium. Arrows: Howship's lacunae

Ryc. 1. Rozproszenie wsteczne elektronów w skaningowym mikroskopie elektronowym (BS-SEM). Kość piszczelowa psa miesiąc po implantacji: Ti – tytan, strzałki oznaczają zatoki Howshipa

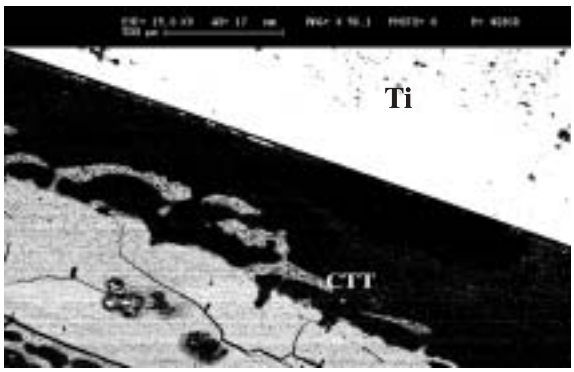

Fig. 3. Backscattered Electrons Scanning Electron Microscopy (BS-SEM). Dog Tibia, 1 month after surgery: Ti – titanium implant. CTT – chondroid tissue trabeculae, parallel to the implant surface

Ryc. 3. Rozproszenie wsteczne elektronów w skaningowym mikroskopie elektronowym (BS-SEM). Kość piszczelowa psa miesiąc po implantacji: Ti – implant tytanowy, CTT – beleczki tkanki chondroidalnej równoległe do powierzchni implantu

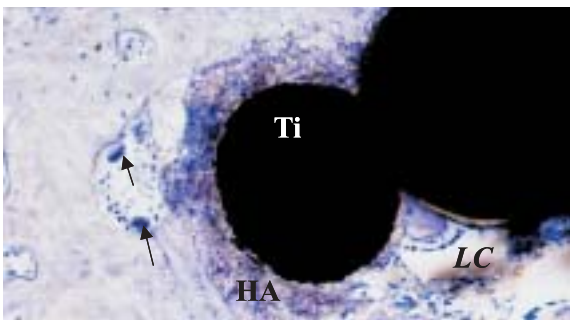

Fig. 2. Non-decalcified sample, Dog Tibia, coloured with a modified Toluidin Blue method. Magnification ×80. Ti – titanium microspheres. HA – plasma-sprayed hydroxyapatite covering layer. The arrows signal the osteoclast cells. LC – lining cells

Ryc. 2. Preparat tkanki niezmineralizowanej. Kość piszczelowa psa, barwienie zmodyfikowanym błękitem toluidyny. Pow. 80×: Ti – mikrosfery tytanu, HA – pokryta osoczem warstwa hydroksyapatytu, LC – komórki wyścielające. Strzałki oznaczają osteoklasty

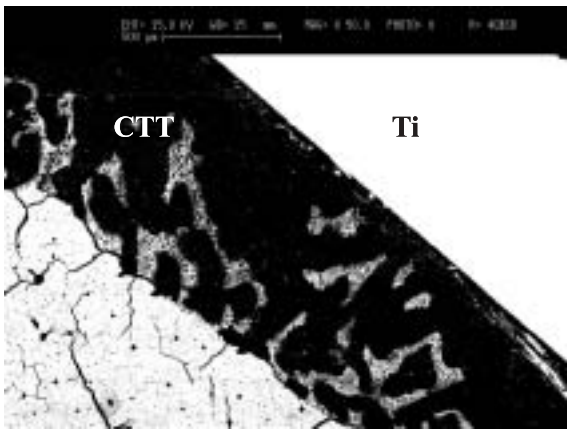

Fig. 4. Backscattered Electrons Scanning Electron Microscopy (BS-SEM). Dog Tibia, 1 month after surgery: Ti – titanium implant. CTT – chondroid tissue trabeculae, perpendicular to the implant surface

Ryc. 4. Rozproszenie wsteczne elektronów w skaningowym mikroskopie elektronowym (BS-SEM). Kość piszczelowa psa miesiąc po implantacji: Ti – implant tytanowy, CTT – beleczki tkanki chondroidalnej prostopadłe do powierzchni implantu

callus. In presented results, visible in Figures 7 and 10, these cutting-filling cones have a group of osteoclasts, followed by a line of bone-forming cells, the already mentioned lining cells.

Those structures are responsible for the remodelling of the osseous tissues around the implant, by means of an osteoclastic resorption of the calcified tissues deposited in the initial phases, followed by an apposition of lamellar bone, whose *lamellae* are characteristically parallel to the bone-implant interface (Fig. 8 and 9). These new *lamellae* are therefore oriented perpendicular to the osteons of the pre-existing cortical bone, as observed in Figure 9. During this phase the cutting-filling cones show that they are turned aside to form a secondary osteon. Figure 10 shows one of these cutting-filling cones that has turned aside 90 degrees respect of its original orientation, to locate itself parallel to the interface, in an inverse process to the originally described by Roberts [56].

The final phase of osteointegration includes as much the remodelling of the bone-implant interface as these of the pre-existing cortical bone and the subperiosteal callus, which is visible in the spread of the Howship's lacunae in Figure 11. This image shows the presence of an intense osteoclastic acti-

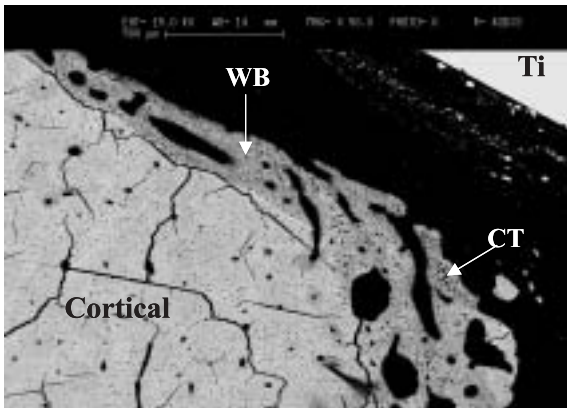

Fig. 5. Backscattered Electrons Scanning Electron Microscopy (BS-SEM). Dog Tibia, 2 months after surgery: Ti – titanium implant, CT – chondroid tissue, WB – woven bone

Ryc. 5. Rozproszenie wsteczne elektronów w skaningowym mikroskopie elektronowym (BS-SEM). Kość piszczelowa psa 2 miesiące po implantacji: Ti – implant tytanowy, CT – tkanka chondroidalna, WB – kość falista

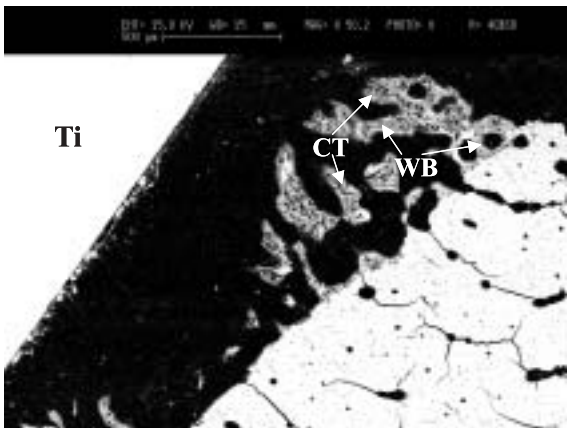

Fig. 6. Backscattered Electrons Scanning Electron Microscopy (BS-SEM). Dog Tibia, 2 months after surgery: Ti – titanium implant, CT – chondroid tissue, WB – woven bone

Ryc. 6. Rozproszenie wsteczne elektronów w skaningowym mikroskopie elektronowym (BS-SEM). Kość piszczelowa psa 2 miesiące po implantacji: Ti – implant tytanowy, CT – tkanka chondroidalna, WB – kość falista

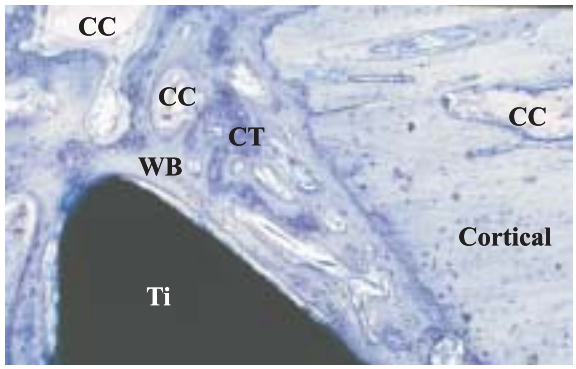

Fig. 7. Non-decalcified sample, Dog Tibia 2 months after surgery, coloured with a modified Toluidin Blue method. Magnification × 40. Ti – titanium impant, Cortical – pre-existing bone cortical, CT – chondroid tissue, WB – woven bone, CC – cutting cones

Ryc. 7. Preparat tkanki niezmineralizowanej. Kość piszczelowa psa 2 miesiące po implantacji. Barwienie zmodyfikowanym błękitem toluidyny. Pow. 40 ×: Ti – implant tytanowy, Cortical – kość korowa, CT – tkanka chondroidalna, WB – kość falista, CC – przecięte granule

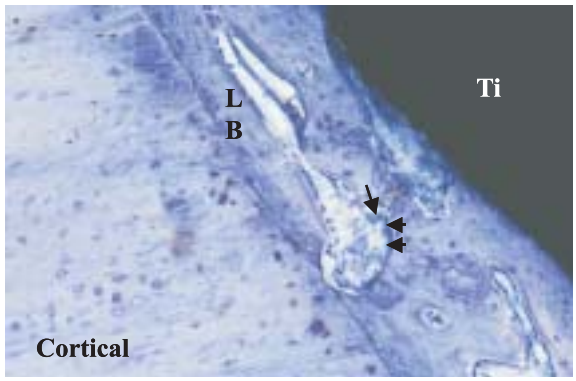

Fig. 8. Non-decalcified sample, Dog Tibia 2 months after surgery, coloured with a modified Toluidin Blue method. Magnification × 80. Ti – titanium implant, Cortical – preexisting bone cortical, LB – lamellar bone. The arrows signal the osteoclast cells in the extreme of the cutting-filling cone

Ryc. 8. Preparat tkanki niezmineralizowanej. Kość piszczelowa psa 2 miesiące po implantacji. Barwienie zmodyfikowanym błękitem toluidyny. Pow. 80 ×: Ti – implant tytanowy, Cortical – kość korowa, LB – kość blaszkowata. Strzałki wskazują osteoklasty w pobliżu przeciętych granul wypełniających

vity, which begins to be evident around the end of the second month after the intervention and increases with time. Indeed, as described by Roberts [56], once osteointegration takes place and the implant becomes stable, the natural processes of bone remodelling start again, and the cortical part of the bone experiences the last and definitive phase of differentiation [61], with the formation of Haversian osteons, like those observed in Figure 12. These osteons will evolve in the same form that the rest of the osteons of the organism, in a process of constant remodelling that implies also the osseous interface with the implant.

Discussion

The calcified tissues of the bone arise from a common origin, the so-called non-differentiated skeletogenic mesenchyme that can be originated

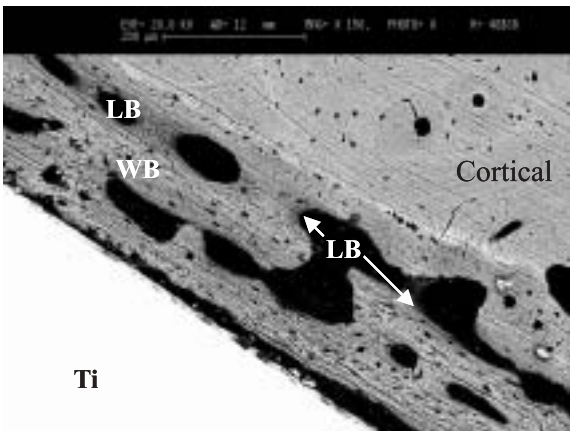

Fig. 9. Backscattered Electrons Scanning Electron Microscopy (BS-SEM). Dog Tibia, 3 months after surgery: Ti – titanium implant, Cortical: pre-existing bone cortical, WB – woven bone, LB – lamellar bone. The arrows signal the sense of the bone matrix apposition

Ryc. 9. Rozproszenie wsteczne elektronów w skaningowym mikroskopie elektronowym (BS-SEM). Kość piszczelowa psa 3 miesiące po implantacji. Ti – implant tytanowy, Cortical – kość korowa, WB – kość falista, LB – kość blaszkowata. Strzałki wskazują miejsca apozycji kostnej

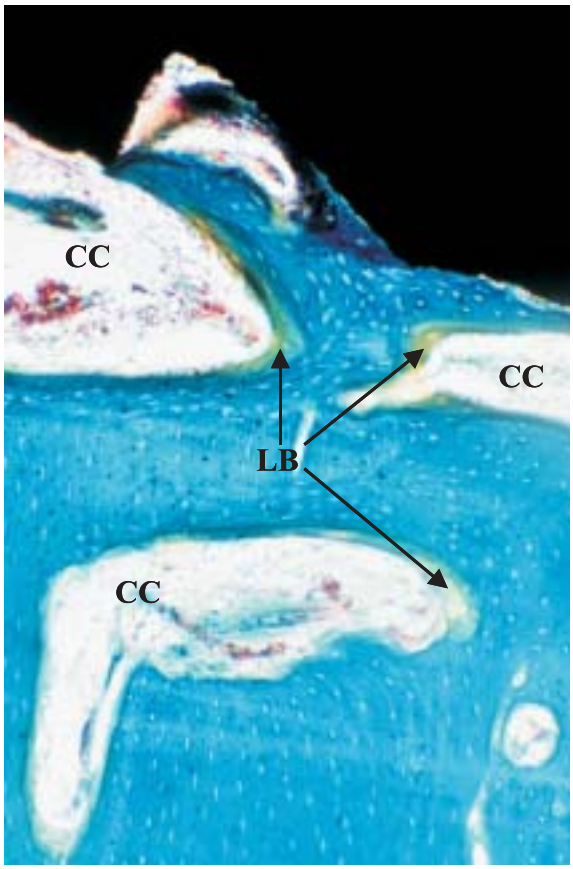

Fig. 10. Non-decalcified sample, Dog Tibia 2 months after surgery, coloured by Masson's Trichrome method. Magnification × 80. LB – lamellar bone, CC – cutting-filling cone. The arrows signal the sense of the bone matrix apposition

Ryc. 10. Preparat tkanki niezmineralizowanej. Kość piszczelowa psa 2 miesiące po implantacji. Barwienie metodą Massona trójbarwną. Pow. 80 ×. LB – kość blaszkowata, CC – przecięte ziarna wypełniające. Strzałki wskazują miejsca apozycji kostnej

either from structures of mesodermal or mesectodermic origin [74]. Depending on the influence of a wide variety of factors such as blood flow or oxygenation of the zone, the forces that are applied, or the presence of growth factors, the mesenchyme will differentiate in the diverse types of calcified tissue: calcified cartilage, chondroid tissue, woven bone or lamellar bone [75].

The cartilaginous tissue shows the first signs of calcification in the form of hydroxyapatite crystals in extracellular matrix vesicles. As the calcification progresses, the cells show necrosis signs. Later on, the calcified cartilaginous matrix is reabsorbed by the chondroclasts, which is followed by a cell-vascular invasion that later will allow the apposition of bone tissue on the remnants of the calcified extracellular matrix [76]. Its aspect is very characteristic, in the microradiography [75] as in the MEB-BS image [68, 69]: big cellular lacunae, round or oval, ordered in form of columns and surrounded by pillars of highly mineralized extracellular matrix. This level of mineralization, which represents 90% of these present in the dental enamel, is deposited at a speed from 35 to 39 microns/day [75] and progresses, like in the primary cartilages in a centrifugal sense [76]. Its extracellular matrix is characterized to contain two types of collagen that appear successively: type I in the periphery of the cellular lacunae and type II in the extracellular matrix [78]. In spite of its relevance in the development and growth of the bones of endochondral origin, either of the appendicular skeleton or the axial one, the presence of areas of cartilage calcified around the implants is, in presented studies, very infrequent. The authors have observed it in a form of small cell clusters, surrounded by other calcified tissues, in the interior of the periosteal callus [73]. As proven in a previous study, these processes are formed because of alterations of the vascular supply [69].

Chondroid tissue was first described at the end of the XIX century, by Brock [79], Schaffer [80], von Brunn [81] and Grohé [82], who gave it different names, all referring to its intermediate aspect between cartilage and bone. It was not until the last third of the XX century when authors like Enlow [83], Beresford [84] and Hall [85] described it as an independent tissue, simultaneously different and similar to the bone and to the cartilage. However, the decisive contribution of Goret-Nicaise [86–89] was capital for the definition of this peculiar calcified tissue.

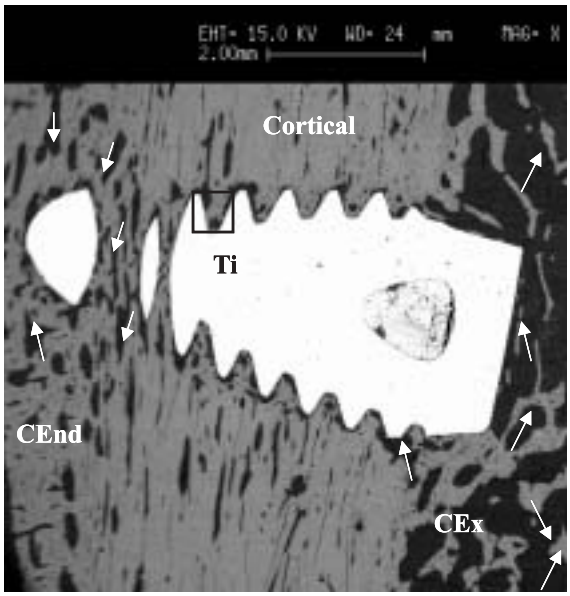

Fig. 11. Backscattered Electrons Scanning Electron Microscopy (BS-SEM). Dog Tibia, 3 months after surgery: Ti – titanium implant, Cortical: pre-existing bone cortical, CEnd – endosteal callus, Cex – exosteal callus. The arrows signal the Howship's lacunae

Ryc. 11. Rozproszenie wsteczne elektronów w skaningowym mikroskopie elektronowym (BS-SEM). Kość piszczelowa psa 3 miesiące po implantacji: Ti – implant tytanowy, Cortical – kość korowa, CEnd – kostnina śródkostna, CEX – kostnina pozakostna. Strzałki wskazują zatoki Howshipa

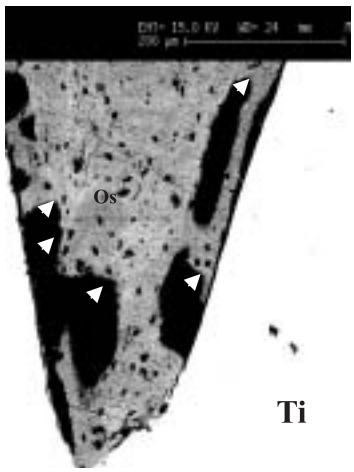

Fig. 12. Backscattered Electrons Scanning Electron Microscopy (BS-SEM). Dog Tibia, 3 months after surgery, bigger magnification of the marked area in Fig. 11. Os – remodelling osteon. The arrows signal the Howship's lacunae

Ryc. 12. Rozproszenie wsteczne elektronów w skaningowym mikroskopie elektronowym (BS-SEM). Kość piszczelowa psa 3 miesiące po implantacji, większe powiększenie miejsca zaznaczonego na ryc. 11. Os – remodelowany osteon. Strzałki wskazują zatoki Howshipa

The calcification of chondroid tissue is defined by its granular, confluent and concentric evolution, accompanied by a reduction of the volume of the cells, similar to which it is observed in the osseous tissue [77]. This process is in opposition to the cell hypertrophy described for the calcification of the cartilages both primary [90] and secondary [91]. It confers a very specific aspect to this tissue, initially described by microrradiography [75, 89] and later confirmed [68, 69] by MEB-BS: numerous big, irregular cell spaces, characteristically confluent, often immersed in an extracellular matrix with a high degree of calcification, around 80% of the enamel calcium content [87]. This calcification is deposited at a speed of 44 to 67 microns per day, ten times superior to the one measured for the lamellar bone [75, 89], and it begins by the appearance of crystalline nodules in the extracellular matrix. This is similar to what has been described for dentin [92], woven bone and calcified cartilage [93], tissues that, like chondroid tissue, are characterized by a very fast and abundant deposit of the calcium salts [77]. These calcium salts, however, present an almost identical molecular composition in all calcified tissues [13, 14].

In the extracellular matrix of chondroid tissue, the type I collagen is arranged in the intercellular septa, whereas simultaneously type II collagen is found in the immediate periphery of the cellular lacunae [75, 86]. Once again on the contrary that happens in the calcified cartilage. Long hydroxyapatite crystals are deposited upon those matrix collagen fibers [77], which will represent the vector of the slowest phase of the calcification of the chondroid tissue.

Initially described in the mandibular synfisis [89] and later in the totality of the mandibular structure [75] and in the skull sutures [94], chondroid tissue participates in the formation of all the skeletal parts: cephalic [72], axial [77] and appendicular [77]. It participates both in the processes of endochondral and membranous ossification. Moreover, in the membranous ossification, chondroid tissue is the essential intermediate step for the ossification [72, 77].

Chondroid tissue appears in locations in which the bone growth is accelerated in addition to suffer special mechanical stress: in the embryo, the mandibular synfisis [89], the skull sutures [94], and the membranous wall of the long bones [75]. In the postnatal life, chondroid tissue has been proved to play an essential role in processes such as fracture callus repair [67], dental eruption [95], skeletogenesis induced by bone distraction [77] and the osteointegration of metallic implants covered by hydroxyapatite [73].

From a biomechanical point of view [96], it

seems evident that the application of forces in the sense of the distraction is one of the environmental factors that influences the differentiation of the osteoprogenitor cells [77] towards the chondroid tissue production, as well as the one that determine the direction of its trabeculae [73].

On the other hand, the particular characteristics of its mineralization, which is more rapid and intense than in the other skeletal tissues [75, 87], although homogenous as far as their molecular characterization [13], results in a three-dimensional tissue structure able to better support divergent forces [96]. In addition, chondroid tissue constitutes a calcified and stable superstructure, acting simultaneously as a reservoir rich in phosphocalcic salts and a quickly stabilized base allowing the apposition of woven or lamellar bone. These biomechanical characteristics made it an essential element to ensure the structural stability in the processes in which participates, either during the development, or the postnatal life.

In presented results, the accelerated chondroid tissue deposit that provides a mineralized and stable bed to implants, is followed by the apposition of woven bone. With an extracellular matrix composed essentially by type I collagen [97], its calcification level is similar to the one of the chondroid tissue: around the 85% of the level of the enamel [87]. However, the speed of the deposit of the calcic salts is from 25 to 28 microns/day for the woven bone, half the value obtained for the chondroid tissue [89], although their molecular structure is similar [13]. Throughout the calcium deposit in the extracellular matrix, the cell lacunae acquire an aspect similar to the one described for the cells of the chondroid tissue, which sometimes makes its identification difficult, even more because they both can appear simultaneously during processes that imply a membranous ossification [98]. However, the morphology of its cellular lacunae is more polygonal and its extracellular matrix shows a more regular composition. Moreover, it does not present the striated images caused by inserted collagen fibers visible in the chondroid tissue. Woven bone, where calcification is more linear than the one of the chondroid tissue [77], contributes to stabilize the callus around the implant, although its mechanical properties do not allow it to support the forces that could be applied to the lamellar bone [56].

The final phase of the osteointegration the so-called “maturation phase” by Roberts [56], constitutes the adaptation of the bone-implant interface to the continuous remodelling cycle that the bones carry out to maintain their integrity throughout life. In presented experiments, this process is re-started at the end of the third month after surgery in form of the reappearance of Haversian remodelling osteons, either in direct continuity with those of the pre-existing cortical (Fig. 11) or, in the periphery of implants, parallel to their surface.

Conclusions

The participation of the chondroid tissue in the osteointegration of the metallic implants, covered or not by hydroxyapatite, results in a greater stability and speed of calcification of the bone callus around the implant. Because of its biomechanical characteristics, the chondroid tissue confers to the callus a greater stability and a greater content in calcium salts that the other calcified tissues. The chondroid tissue is quickly covered by woven bone, which is replaced by osteonal remodelling, contributing significantly to the final stabilization of implants. The presence and the essential role of the chondroid tissue in the osteointegration incline authors to consider it a process in which the main mechanism implied is membranous ossification.

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