SUMARY PhD THESIS

Evaluation of tissue integration of titanium screws in the femur of male rabbits

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INTRODUCTION

When an orthopedic implant is inserted into the bone, the bone tissue is faced with an event for which it has no prior preparation. Although a discontinuity is created in the bone as in the case of fractures, here the implant material occupies most of the surgically excised bone and thus the gap between the damaged bone surfaces is significantly reduced. In the case of the inserted implant, a high stability of the implant with the bone is ensured. Even in this situation, the bone comes into intimate contact with a foreign surface that is unknown to the bone environment. The surface of the implant comes into direct contact with its own structures, and its properties play an essential role in the behavior of the cells that come into contact with it (PALMQUIST AND COL., 2010).

Researchers investigating the role of the implant surface have concluded that it significantly influences protein absorption, platelet adhesion, homeostasis, complement activation, inflammatory response, and osteogenic cell activity (PARK AND DAVIES, 2000). The physical surface properties of the implant allow bone formation directly on the surface of the implant, a process called contact osteogenesis. (DAVIES, 2003)

To achieve osteogenesis, cells with osteogenic potential such as osteoprogenitor cells from the periosteum and endosteum, to which perivascular pericytes are added, must migrate through the fibrino-platelet network of the blood clot formed in the space between the subsurface of the implant and the wall of the bone defect. Those that reach the surface of the implant, differentiate into osteoblasts and begin the synthesis of the bone matrix that is deposited on the surface of the implant as a cement line (DAVIES, 1996). Cells that during migration differentiate into osteoblasts before reaching the surface of the implant, stop migrating and begin the synthesis of bone matrix. From the synthesized bone matrix, bone spicules are formed that advance towards the surface of the implant, and the process is called distant osteogenesis (DAVIES ȘI HOSSEINI, 2000).

In each bone healing site, both types of osteogenesis take place, both contact and distance (SHAH AND COL., 2018). Most of the time, autogenous bone fragments resulting from the surgical drilling necessary to insert implants, serve as a target surface for cells with osteogenic potential, which deposit bone matrix directly on their surface (FRANCHI AND COL., 2004; SHAH AND PALMQUIST, 2017; RATIU AND COL., 2022).

The biomaterials used at catal time are divided into autologous, heterologous and xenografts, which, in addition to the beneficial effects, also trigger a series of adverse reactions in the body. To overcome certain problems related to the use of biomaterials, tissue engineering is used, which aims to discover new materials with superior qualities. Despite all efforts, the use of state-of-the-art biomaterials still leaves many question marks on the arduous path between hope and certainty.

THESIS STRUCTURE

The doctoral thesis ENTITLED "Evaluation of the tissue integration of titanium screws in the femur of male rabbits" is presented on 112 pages and contains a rich imaging materialized through 65 figures, of which 10 macroscopic, 53 microscopic and two graphic representations. By the ratio between the components and the way of writing, the methodology of writing doctoral theses was respected, the work being divided into two parts, as follows:

The first part is presented on 36 pages and includes 2 chapters in which the current state of knowledge is presented, with references to the chosen topic.

In the first chapter entitled "Biomaterials used in orthopedics" detailed information about biomaterials used in orthopedics is presented. The chapter contains information about the three generations of biomaterials, pointing out the advantages, limitations, certainties and hopes of specialists regarding the use of these biomaterials.

In the second chapter, with the title "The response of tissues to biomaterials", it is presented how tissues perceive contact with biomaterials which are nevertheless a foreign material for the body. Reference is made to the degree of tolerance and the events triggered in tissues upon direct contact with each type of biomaterial, each exerting an effect on tissues both by presence and by products that may be released upon contact with body fluids.

The second part of the paper spans 76 pages and is divided into 9 chapters containing information on objectives, materials and methods, histological research on the process of osseointegration of titanium screws inserted into the femur of male rabbits in holes with different diameters, the conclusions general, aspects of originality and innovative contributions of the thesis.

THE OBJECTIVES OF THE WORK

- inserting some titanium screws into the femur of male rabbits in the hole with a larger diameter than the screw axis;

--insertion of titanium screws into the femur of male rabbits in the hole with a smaller diameter than the screw shaft;

- the histological and histomorphometric evaluation of the osseointegration process in the case of the two experimental variants, 6 weeks after the insertion of the screws.

MATERIALS AND METHODS

The biological material was represented by 10 one-year-old male rabbits, in which titanium screws with a diameter of 2 mm were inserted into the femur, in two postures, respectively in the left femur in a 1.8 mm hole and in the right of 1 mm. After 6 weeks the animals were euthanized and the implanted area was processed for histological investigations.

RESULTS AND CONCLUSIONS

Chapter 5 entitled "Evaluation of the tissue integration of some titanium screws, in a hole larger than the screw shaft" looked at how the osseointegration process unfolds in the most recommended situation, i.e. which ensures primary stability but without exerting additional pressure on the surrounding bone . The experiment started from the following objectives:

-insertion of titanium screws into the femur of clinically healthy male rabbits in a 1.8 mm hole;

-verification by histological study of the process of tissue integration of titanium screws, 6 weeks after insertion.

Following this investigation, the following conclusions were drawn:

At 6 weeks after screw insertion, the interface is covered with new proliferated bone whose thickness is different from one area to another, the thickest being in the endosteal area, followed by the periosteal area and then the central part.

In the central area of the interface, the layer of newly proliferated bone does not have the same thickness, being relatively thin near the screw turns and significantly thicker in the grooves between the screw turns, taking on a particular "sawtooth" appearance

The appearance is due to the fact that at the time of milling and self-tapping, bone particles that were incorporated into a blood clot reached the level of the grooves, together constituting the best autologous augmentation material.

The areas initially occupied by autologous augmentation material are occupied at 6 weeks by new proliferated bone, because this material occupies the space to avoid being invaded by connective tissue and is easily replaced by new proliferated bone.

The proliferated bone at the interface has a modest degree of organization and remodeling, being predominantly composed of primary bone, areas of osteoid, and even small and rare areas where small amounts of material remaining from the time of screw insertion still persist.

There are also small areas where incipient bone remodeling processes are present, materialized by the presence of a small number of bone lamellae sketches, but overall bone remodeling does not exceed 5%.

In this context, we can say that the existing bone on the interface 6 weeks after the insertion of the screws, is only in the early stage of remodeling towards lamellar bone, being still far from ensuring adequate mechanical resistance.

The devitalized bone from the depth of the interface is involved at this moment of the experiment in intense remodeling processes, but an unbalanced one in which bone resorption clearly predominates at the expense of bone proliferation.

The most and largest resorption spaces are between the proliferated bone at the interface and the underlying bone, so the degree of attachment between the two types of bone is significantly affected, making the interface still weak.

If we consider all the aspects captured by us, it is clear that 6 weeks after the insertion of the screws, the peri-implant area is occupied by still fragile structures, even if the osseointegration has evolved naturally.

We believe that at 6 weeks the post-implant structures are weak and can yield at the slightest mechanical stress, which is an advantage for temporary implants because they are easily extracted, but not for permanent implants.

Chapter 6 entitled "Evaluation of tissue integration of titanium screws in a hole smaller than the screw shank" followed the osseointegration process of titanium screws forcibly inserted into bone, by self-tapping, to investigate the influence of this insertion mode on the adjacent bone and of reparative processes. To verify these aspects, the following objectives were set:

Insertion by self-drilling of a screw with a diameter of 2 mm and a shaft of 1.5 mm in a hole with a diameter of 1 mm, causes the turns of the screw to be completely buried in the bone that occupies intimately the grooves between the turns.

In this variant, maximum contact is obtained between the bone and the surface of the screw, and if we judge only from the point of view of mechanical stability, we can say that we have obtained maximum stability.

The somewhat forced insertion of the screw, however, exerts additional pressure on the hard component of the bone, amplifying the injuries generated by the breaking of some bone components and ischemia following the breaking of the zonal vessels.

At 6 weeks, most of the newly proliferated tissues are present in the periosteal and endosteal areas, while near the bone wall the newly proliferated structures are much less and in some places at a very early stage.

In the periosteal and endosteal areas, new proliferated bone is present arranged in the form of a thick layer that has expanded on the interface, outward in the case of the periosteal and inward in the endosteal, determining the growth of the interface that takes on the appearance of a fan.

This relatively intense proliferation benefited from favorable conditions given primarily by the short distance from the sources of osteoblasts and the fact that the pressures felt by the hard bone structures are not exerted on these areas.

Considering the time interval that has passed since the insertion of the screws we can say that the bone proliferation in the periosteal and endosteal areas took place at the appropriate level without being influenced by the way of inserting the screws.

On the interface portion next to the bone wall there are newly proliferated structures but in very small quantity arranged in a very thin state and in a relatively initial stage of organization, respectively as a cement or osteoid line.

The presence of a thin layer of newly proliferated bone at the interface next to the bone wall demonstrates that contact osteogenesis took place with difficulty due to the very small space between the screw surface and the bone wall

The bone in the depth of the interface still presents at 6 weeks the appearance of mostly non-viable bone, with polymorphous, empty spaces that are mostly fissures and discrete, early-stage remodeling processes.

Inserting the screw into a hole smaller than the diameter of the screw core is disadvantageous in the case of permanent implants, but does not constitute a major problem in the case of temporary implants.

Chapter 7 "Histomorphometric evaluation of peri-implant bone proliferation" compared the peri-implant bone proliferation, to see the degree of coverage of the implant with bone in the two experimental variants. To verify these aspects, the objectives were set:

- Insertion by self-tapping of some titanium screws in holes with different diameters, respectively larger and smaller than the screw shank;

-Comparative histomorphometric verification of newly proliferated bone in the periosteal and endosteal areas of the interface.

The conclusions that emerged from this investigation were:

6 weeks after the insertion of the titanium screws, intense bone proliferation was observed in the periosteal and endosteal areas leading to a significant increase in the interface surface with the appearance of a fan.

Morphometry demonstrated that the interface surface is comparable in the two experimental variants, even if there are some small differences, they are not statistically significant.

The proliferation of new bone on the interface is different depending on the area, the most intense being in the endosteal area, followed by the periosteal and the most modest in the osteal area, without significant differences between the two experimental variants.

The largest amount of newly proliferated bone in the endosteal area demonstrates that the proliferation of new bone started in this area and only then in the periosteal area, so we can say that osseointegration takes place from the depth to the outside.

In the case of both experimental variants, the newly proliferated bone occupies the entire depth of 1 mm of the interface, both in the periosteal and in the endosteal area, which demonstrates that it was formed by contact osteogenesis.

The very good bone proliferation in the periosteal and endosteal areas of the interface in both experimental variants demonstrates that the size of the insertion hole does not significantly influence bone proliferation in these areas of the interface.

Chapter 8 entitled "General conclusions" briefly presents the issues resulting from the investigations carried out, as follows:

In the version with a 1.8 mm hole, 6 weeks after the insertion of the screws, the interface is covered with new proliferated bone whose thickness is different from one area to another, the thickest being in the endosteal area, followed by the periosteal area and then the hostile one.

In the osteal area of the interface, the newly proliferated bone forms a continuous layer but uneven in thickness, being relatively thin near the screw turns and significantly thicker in the grooves between turns, giving the overall "sawtooth" appearance

The degree of organization and remodeling of the proliferated bone at the interface is relatively modest, consisting of primary bone, with areas of osteoid and even small and rare areas where small amounts of material left over from the time of drilling and self-tapping still persist.

In the version with a 1 mm hole, there is also bone proliferation over the entire surface of the interface, the most intense in the endosteal area, then in the periosteal area and much more modest in the osteal area.

The level reached by the bone proliferation in the endosteal and periosteal areas after 6 weeks, demonstrates that in these areas the bone proliferation took place at the appropriate level without being influenced by the method of screw insertion.

On the osteal portion of the interface, newly proliferated bone is present but in very small quantity and arranged in a very thin state, in a relatively initial stage of organization, namely as a cement line and some amount of osteoid.

The presence of a thin layer of newly proliferated bone at the interface in the osteal area demonstrates that contact osteogenesis took place with difficulty only in this area, due to the very small space between the screw surface and the bone wall

Morphometry demonstrated that intense bone proliferation in the endosteal and periosteal areas led to a significant increase in the interface, which at 6 weeks has a fan-like appearance, in the case of both experimental variants.

The comparative assessment of the interface in the two experimental variants demonstrated that the interface surface is comparable in the two experimental variants, and the small differences found are not statistically significant.

The most intense proliferation of new bone was found in the endosteal area of the interface, which proves that it started in this area, so we can say that osseointegration takes place from the depth to the outside.

The very good bone proliferation in the periosteal and endosteal areas of the interface in both experimental variants demonstrates that the size of the insertion hole does not significantly influence bone proliferation in these areas of the interface.

The only area where there are differences in the thickness of newly proliferated bone between the two experimental variants is the osteal, but it does not occupy more than one third of the height of the interface, so it is not a problem for temporary implants.

Chapter 9 "The originality and innovative contributions of the thesis" includes the most interesting aspects captured in the investigations carried out, respectively:

- Reparative bone proliferation begins at the endosteum level and not at the periosteum level as most orthopedists claim;
- Titanium stimulates bone proliferation so that the interface surface doubles;
- Inserting implants into a hole smaller than the diameter of the screw stem is not a problem for temporary implants but only for permanent ones, such as dental ones.