SEVEN
publicações acadêmicas

BIOCOMPATIBILITY OF ALTICRN+SI3N4 PROSTHETIC COATING IN A SUBCUTANEOUS ANIMAL MODEL OF MICE

BIOCOMPATIBILIDADE DE REVESTIMENTO PROTÉTICO ALTICRN+SI3N4 EM MODELO ANIMAL SUBCUTÂNEO DE CAMUNDONGOS

BIOCOMPATIBILIDAD DEL RECUBRIMIENTO PROTÉSICO ALTICRN+SI3N4 EN UN MODELO ANIMAL SUBCUTÁNEO DE RATONES

https://doi.org/10.56238/sevened2025.037-016

Marco Antonio Schueda[1], Gustavo Emiliano Falcão Ferreira[2], Hercílio Fronza Júnior[3], Juliana Lins Maués[4], Suzana Bastos Batista[5], Karina Munhoz de Paula Alves Coelho[6], Moisés Cohen[7]

ABSTRACT

Nanotechnology used as a coating for orthopedic, neurological, and dental implants is increasingly important for their fixation and durability. Biocompatibility with bone tissue is therefore essential. After evaluating the results through histological analysis of 46 subcutaneous tissue samples from mice with and without AlTiCrN+Si3N4 coating (Titanium Aluminum Nitride enveloped in a silicon nitride layer), it was found that eosinophilic infiltration, intensity of the inflammatory process, and infiltration by multinucleated giant cells showed no statistical difference between the two groups. Fibrosis was found in the coated group, which was 1.7 times greater (RR = 1.7, 95% CI = 0.83 to 3.7), and the presence of black granular pigment characterized wear, displacement, and/or corrosion of the coating in all samples. It is concluded that such material has no space to be used in nanotechnological coatings of metal implants.

Keywords: Nanotechnology. Coating. Implant.

RESUMO

A nanotecnologia utilizada como revestimento de implantes ortopédicos, neurológicos e odontológicos tem importância, cada vez maior, para fixação e durabilidade dos mesmos. A biocompatibilidade com o tecido ósseo é, portanto, fundamental. Após avaliação dos resultados feita através por análise histológica em 46 amostras de tecido subcutâneo de camundongos com e sem recobrimento de AlTiCrN+Si3N4 (Nitreto de Cromo alumínio titânio envelopadas em uma camada de nitreto de silício) verificou-se que infiltração eosinofílica, intensidade do processo inflamatório, infiltração por células gigantes multinucleada não apresentarem diferença estatística entre os dois grupos. Verificou-se fibrose no grupo com

[1] Dr. in Orthopedics and Traumatology. Universidade Federal de São Paulo (UNIFESP).
E-mail: schueda.sc@gmail.com
[2] Master of Science in Materials Engineering. Universidade Federal de Santa Catarina (UFSC)
E-mail: gustavo@bioecco.com.br
[3] Specialist in Pathology. Universidade Federal do Paraná (UFPR). E-mail: hfronza@hotmail.com.br
[4] Doctor. Universidade Regional de Blumenau. E-mail: julianamaues9@gmail.com
[5] Doctor. Universidade Centro Universitário do Maranhão.E-mail: suzanabastos94@hotmail.com
[6] Specialist in Pathology. Hospital Municipal São José (HMSJ). E-mail: karinamunhoz@hotmail.com
[7] Full Professor of Orthopedics and Traumatology. Universidade Federal de São Paulo (UNIFESP).
E-mail: m.cohen@uol.com.br

revestimento foi 1,7 vezes maior (RR = 1,7, IC 95% = 0,83 a 3,7) e presença de pigmento granular negro que caracterizou desgaste, desplacamento e/ou corrosão do cobrimento em todas as amostras. Conclui-se que tal material não tem espaço para para ser utilizado em cobrimentos nanotecnológicos de implantes metálicos.

Palavras-chave: Nanotecnologia. Cobrimento. Implante.

RESUMEN
La nanotecnología utilizada como recubrimiento para implantes ortopédicos, neurológicos y dentales es cada vez más importante para su fijación y durabilidad. Por lo tanto, la biocompatibilidad con el tejido óseo es esencial. Tras evaluar los resultados mediante análisis histológico de 46 muestras de tejido subcutáneo de ratones con y sin recubrimiento de AlTiCrN+Si_3N_4 (nitruro de titanio, aluminio y cromo envuelto en una capa de nitruro de silicio), se observó que la infiltración eosinofílica, la intensidad del proceso inflamatorio y la infiltración de células gigantes multinucleadas no presentaron diferencias estadísticas entre los dos grupos. Se observó fibrosis en el grupo recubierto, que fue 1,7 veces mayor (RR = 1,7; IC del 95 % = 0,83 a 3,7), y la presencia de pigmento granular negro caracterizó el desgaste, el desplazamiento y/o la corrosión del recubrimiento en todas las muestras. Se concluye que este material no tiene cabida para su uso en recubrimientos nanotecnológicos de implantes metálicos.

Palabras clave: Nanotecnología. Recubrimiento. Implante.

1 INTRODUCTION

Nanotechnology is a practice of modern medicine that involves the use and manipulation of matter at molecular and anatomical levels. Its use in health derives from the concept known as Nanoscience.[1] The material coating of orthopedic, neurological and dental implants is increasingly important for their fixation and durability based on biocompatibility with bone tissue.[2,3]

The viability of the implant and the post-surgical prosthetic quality depend on the interaction between the exogenous material and the organism, being perfect if absent. The material must be biocompatible. In this context, coated prostheses emerged, which, theoretically, would maximize their physicochemical characteristics and allow for better acceptance by the organism as well as greater durability of the implant.[3,4,5,6,7,8]

The literature presents conflicting data regarding the real efficacy of coated prostheses. As the study by Lazarinis *et al.*, which demonstrates that coated femoral nails, in addition to having a survival similar to those that are not coated, also have similar rates in relation to the need for surgical revision.[9]

As well as the meta-analysis outlined by Voigt and Mosier, in 2011, which reviewed the results of 926 knee arthroplasties. This study suggested that coated implants achieved greater long-term durability in patients under the age of 70 years.[10] In this scenario, the plurality of characteristics of the materials implanted evokes the need to expand studies that evaluate the biocompatibility of materials and their coatings.

The objective of the present study is to evaluate the biocompatibility of the AlTiCrN+Si3N4 coating (Chromium Nitride Aluminum Titanium Enveloped in a Silicon Nitride Layer), using mice as an animal model, comparing its efficacy with uncoated prostheses.

2 MATERIALS AND METHODS

To carry out the study, 30 mice of the Swiss lineage, weighing between 30 and 35g, were used. A total of 23 mice remained for statistical analysis after the exclusion criteria and analysis of the anatomopathological material.

The animals were kept in the Chronic Treatment Laboratory (Health II) of the CCE - Experimental Surgical Center of UNIVILLE. They were individually accommodated in polypropylene boxes with sawdust, size 300x190x130 mm, with chrome-plated wire lid, without sharp corners. These boxes were then arranged on shelves in the laboratory, where the temperature was kept at 22 ± 1°C constantly and the lighting was controlled, maintaining

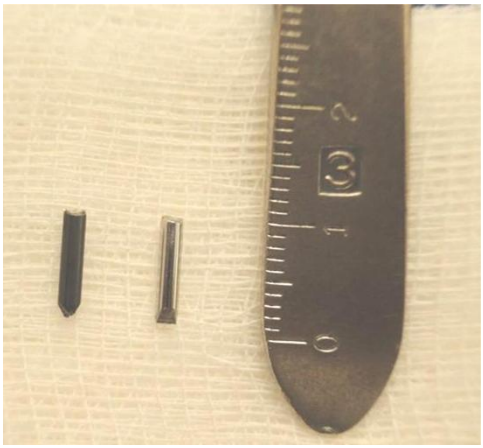
12 hours of light and 12 hours of darkness, always leaving the dark at night. Cleaning was carried out at three-day intervals. The water is disposed of in its own bottle and left available to the animals, being changed every week. The feed was placed on the wire lid and also left at the will of the animals, always being added when necessary.

The coating used was AlTiCrN+Si3N4 (Chromium Nitride, aluminum, titanium, enveloped in a layer of silicon nitride), with 2 multilayer fillers and 2 gradient fillers in bars.

The material to be tested for biocompatibility was inserted, by surgical incision, on both sides of the animal's back. As a standard sample, 1cm bars composed of (bar material) were used. Half of the samples were coated with the study material, (coated material studied). Figures 1 and 2.

Figure 1

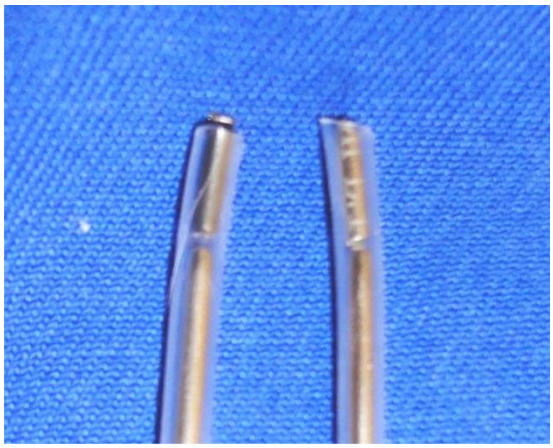
1 cm blocks with and without coating



Source: The authors

Figure 2

Introducers with blocks for subcutaneous inoculation



Source: The authors

The mice were previously anesthetized with 2% lidocaine without vasoconstrictor after local antisepsis with alcoholic chlorhexidine for this procedure (Figure 3). On the right side, the sample coated with the material to be studied was implanted, and on the left side, the same uncoated sample was implanted. After surgery, the surgical wound was sutured with 3.0 mononylon thread.

Figure 3

Introduction of the Subcutaneous Blocks

Source: The authors

The mice were randomly categorized into five groups of ten mice each. The first group of animals was euthanized through cervical displacement after 7 days of implantation of the material.

By means of a surgical procedure, the block containing the skin, subcutaneous tissue and the implanted materials was removed for anatomopathological evaluation with a study of the degree of tissue reaction, envisioning the evolution of the local reaction and the biocompatibility of the implant. After 7 days, a new group of animals were sacrificed and submitted to the same evaluation as the previous group. This was the case with the other groups, always respecting the previously established time interval and the methods of euthanasia, material collection and anatomopathological analysis.

Samples with and without coating (Figures 4 and 5) were sent for anatomopathological study. We comparatively analyzed the tissue reaction caused by the material through the following variables:

INFLAMMATORY PROCESS PATTERN, EOSINOPHILIC INFILTRATION, INFLAMMATORY PROCESS EXTENSION, INTENSITY OF THE INFLAMMATORY

PROCESS, MULTINUCLEATED GIANT CELL INFILTRATION, CONCENTRIC FIBROSIS, ANGIOFIBROBLASTIC PROLIFERATION, AND GRANULAR BLACK PIGMENT.

Figure 4

Sample captur

Source: The authors

Figure 5

Referral for analysis in 10% buffered formaldehyde

Source: The authors

We divided the analysis of the samples into three groups. In the first group, the analysis was performed after 3 weeks after implant implantation, in the second group, after 5 weeks, and in the third group, after 7 weeks. We obtained 8 samples in the first group, 7 samples in the second and 8 samples in the third group.

Samples that did not have sufficient material or were damaged to the material were excluded, making it impossible to perform anatomopathological studies.

3 RESULTS

All data were obtained and checked by the researcher.

The measures of central tendency considered in the statistical analysis were absolute and percentage frequencies. The estimate of the difference between the proportions was estimated using Pearson's chi-square test and Fisher's exact test.

The risk estimate was performed by calculating the relative risk and respective 95% confidence intervals.

The sample was calculated considering a type I error of 5%, with an estimated minimum test power of 85%. Type II error may have occurred for associations estimated to be non-significant due to the sample size.

The sample of this study consisted of 46 samples of subcutaneous tissue from mice tested for biological reaction to metallic wire without and with nanotechnological coating 3 (8 samples), 5 (7 samples) and 7 weeks (8 samples) after implantation.

3.1 INFLAMMATORY PROCESS PATTERN

In the group without nanotechnological coating, there was progression from a predominantly absent inflammatory pattern or chronic proliferative at 5 weeks to fibrosis at 7 weeks (p = 0.01). In the coated group, the same phenomenon was observed (p < 0.001). There was no difference in evolution between the two groups (p > 0.05) (Table 1, Figure 6).

Table 1

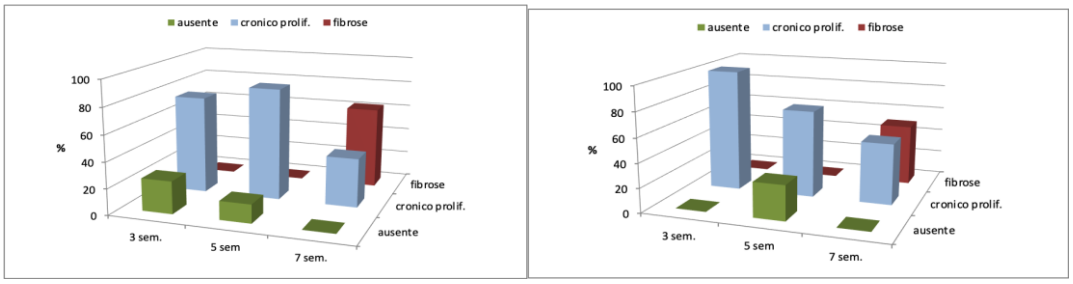
Inflammatory Process Pattern

	UNCOATED				COATED				
	Absent	Crô pro	Fibros	p*	Absent	Crôn pro	Fibros	p**	p***
3 s	02 25,0%	06 75,0%	00 0,0%	0,01	00 0,0%	08 100,0%	00 0,0%	< 0.001	0,13
6 s	01 14,3%	06 85,7%	00 0,0%		02 28,6%	05 71,4%	00 0,0%		0,51
7 s	00 0,0%	03 37,5%	05 62,5%		00 0,0%	04 50,0%	04 50,0%		0,61

NOTE: Pearson's Chi-square test * comparison between groups (uncoated) ** comparison between groups (with coated) ***comparison between uncoated and coated.
Source: Authors.

Figure 6

A - Pattern of Inflammatory Process without Coating B - Pattern of Inflammatory Process with Coating



The risk of fibrosis was 7 times higher in the uncoated group when comparing the 3rd and 7th weeks (RR = 7.0 95% CI = 1.09 to 44.6). In the coated group, it was 1.7 times higher (RR = 1.7, 95% CI = 0.83 to 3.7) at week 7.

3.2 EOSINOPHILIC INFILTRATION

No significant eosinophilic infiltration was observed in any of the groups ($p > 0.05$) at any of the evaluation moments ($p > 0.05$) (Table 2, Figure 7).

Table 2

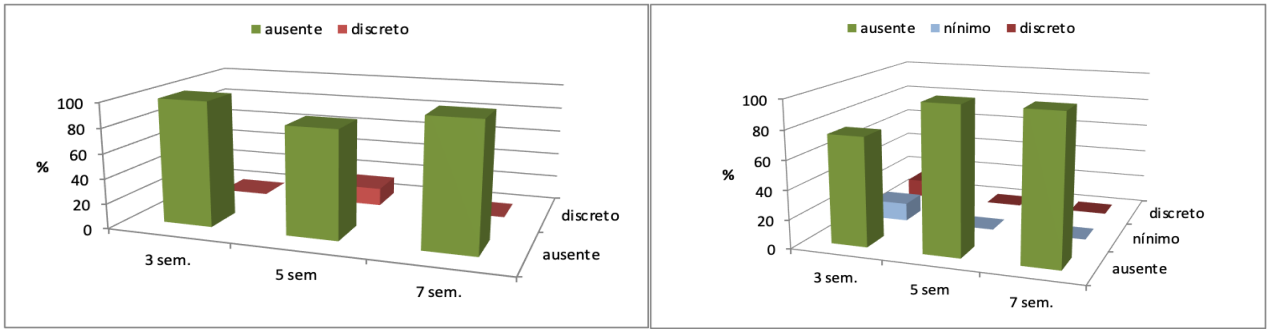
Eosinophilic Infiltrate

	UNCOATED				COATED				
	Absent	Minimum	Discreet	p*	Absent	Minimum	Discreet	p**	p***
3 s	08 100,0%	00 0,0%	00 0,0%	0,3	06 75,0%	01 12,5%	01 12,5%	0,39	0,31
6 s	06 85,7%	00 0,0%	01 14,3%		07 100,0%	00 0,0%	00 0,0%		0,29
7 s	08 100,0%	00 0,0%	00 0,0%		08 100,0%	00 0,0%	00 0,0%		1,00

NOTE: Pearson's Chi-square test * comparison between groups (uncoated) ** comparison between groups (coated) ***comparison between uncoated and coated
Source: Authors.

Figure 7

A Eosinophilic infiltrate without coating B - Eosinophilic infiltrate with coating



3.3 EXTENT OF THE INFLAMMATORY PROCESS

In the group without nanotechnological coating, no significant difference was observed according to time (p = 0.17). In the group with coating, there was a change in predominance from moderate to mild or minimal extension (p = 0.04). In the comparison of the two groups, it was observed that at 3 weeks the extent of the inflammatory process was greater in the group with nanotechnological coating (p = 0.02) (Table 3, Figure 8).

Table 3

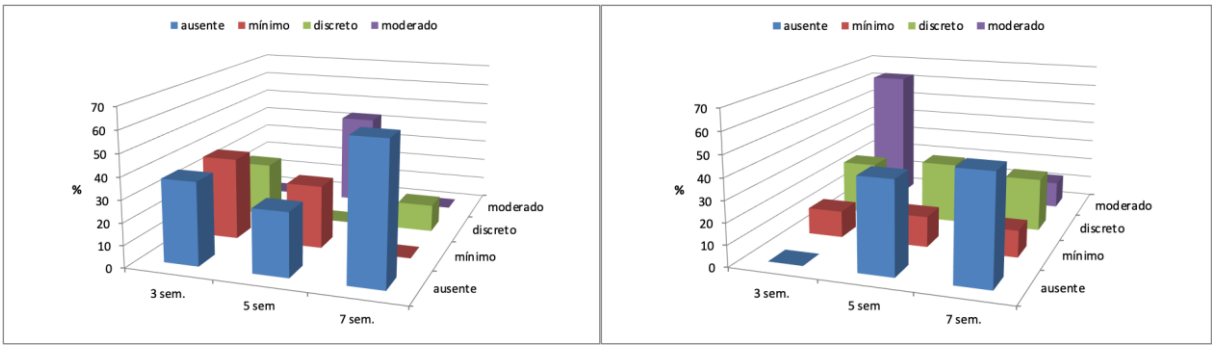
Extent of the Inflammatory Process

	UNCOATED					COATED					
	Ausen	Mini	Discre	Mod	p*	Ausen	Minimum	Discre	Mod	p**	p***
3 s	03 37,5%	03 37,5%	02 25,0%	00 0,0%	0,17	00 0,0%	01 12,5%	02 25,0%	05 62,5%	0,04	0,02
6 s	02 28,6%	02 28,6%	00 0,0%	03 42,9%		03 42,9%	01 14,3%	02 28,6%	01 14,3%		0,31
7 s	05 62,5%	00 0,0%	01 12,5%	00 0,0%		04 50,0%	01 12,5%	02 25,0%	01 12,5%		0,61

NOTE: Pearson's Chi-square test * comparison between groups (uncoated) ** comparison between groups (coated) ***comparison between uncoated and coated
Source: Authors.

Figure 8

A - Extent of the uncoated inflammatory process. B - Extension of the inflammatory process with coating



3.4 INTENSITY OF THE INFLAMMATORY PROCESS

Regarding the intensity of the inflammatory process, no difference was observed between the evaluation moments or between the groups (p > 0.05) (Table 4, Figure 9).

Table 4

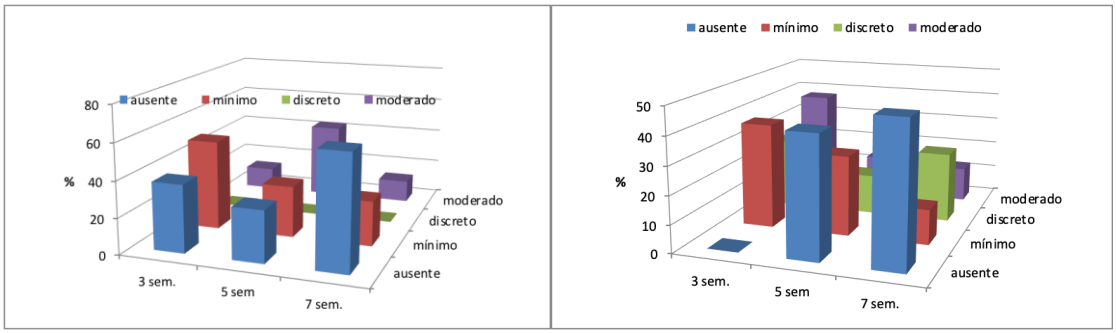
Intensity of the Inflammatory Process

	UNCOATED					COATED					
	Ausent	Minimum	Discr	Mod	p*	Ausent	Minimum	Discr	Mod	p**	p***
3 s	03 37,5%	04 50,0%	00 0,0%	01 12,5%	0,4	00 0,0%	03 37,5%	02 25,0%	03 37,5%	0,3	0,10
6 s	02 28,6%	02 28,6%	00 0,0%	03 42,9%		03 42,9%	02 28,6%	01 14,3%	01 14,3%		0,53
7 s	05 62,5%	02 25,0%	00 0,0%	01 12,5%		04 50,0%	01 2,5%	02 25,0%	01 12,5%		0,48

NOTE: Pearson's Chi-square test * comparison between groups (uncoated) ** comparison between groups (with coated) ***comparison between uncoated and coated.
Source: Authors.

Figure 9

A - Intensity of the Inflammatory Process without coating. B - Intensity of the inflammatory process with coating



3.5 INFILTRATION BY MULTINUCLEATED GIANT CELLS

Regarding infiltration by multinucleated giant cells, no difference was observed between the evaluation moments or between the groups ($p > 0.05$) (Table 5, Figure 10).

Table 5

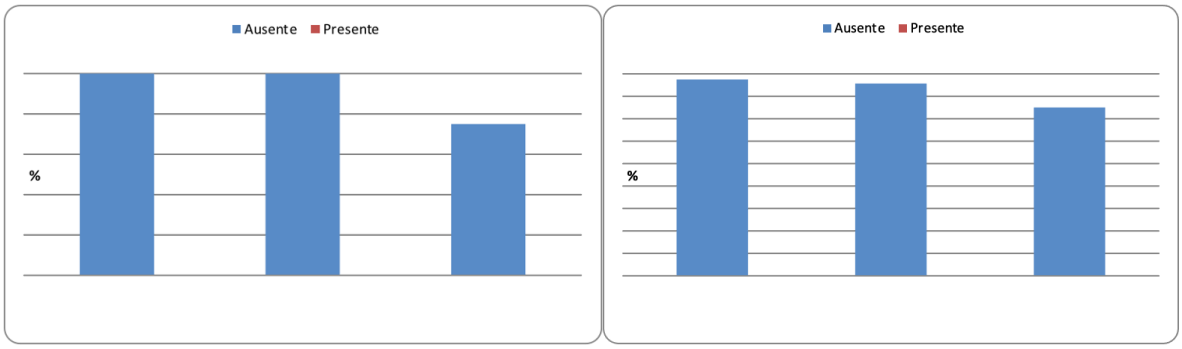
Infiltrated by Multinucleated Giant Cells

	UNCOATED			COATED			
	Absent	Present	p*	Absent	Present	p**	p***
3 s	08 100,0%	00 0,0%	0,1	07 87,5%	01 12,5%	0,77	1,00
6 s	07 100,0%	00 0,0%	2	06 85,7%	01 14,3%		1,00
7 s	06 75,0%	02 25,0%		06 75,0%	02 25,0%		0,98

NOTE: Pearson's Chi-square test * comparison between groups (uncoated) ** comparison between groups (coated) *** comparison between uncoated and uncoated
Source: Authors.

Figure 10

A - infiltration by uncoated multinucleated giant cells B - infiltration by multinucleated giant cells with coating



3.6 COCENTRIC FIBROSIS

In the group without nanotechnological coating, no significant difference was observed according to time (p = 0.52). In the coated group, there was progression from absence to a minimum of 3 to 7 weeks (p = 0.01). In the comparison of the two groups, it was observed that at 7 weeks, fibrosis was more frequent in the group with nanotechnological coating (p = 0.02) (Table 6, Figure 11).

Table 6

Concentric Fibrosis

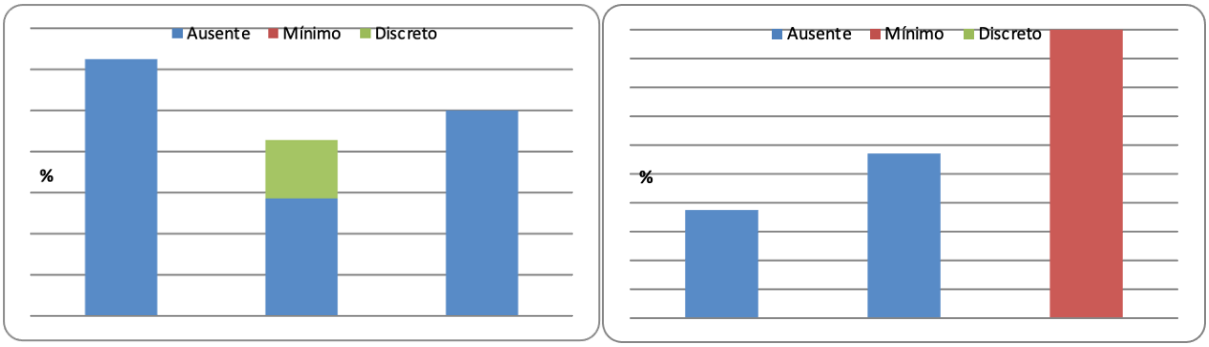
UNCOATED WITH COATING

	Absent	Minimum	Discreet	p*	Absent	Minimum	Discreet		p***
3 s	05 (62,5%)	02 (25,0%)	01 (12,5%)	0,52	03 (37,5%)	02 (25%)	03 (37,5%	0,01	0,47
6 s	02 (28,6%)	02 (28,6%)	03 (42,8%)		04 (57,1%)	02 (28,6%)	01 (14,3%)		0,43
7 s	04 (50,0%)	03 (37,5%)	01 (12,5%)		00 (0,0%)	08 (100%)	00 (0,0%)		0,02

NOTE: Pearson's Chi-square test * comparison between groups (uncoated) ** comparison between groups (coated) *** comparison between uncoated and uncoated
Source: Authors.

Figure 11

A - Uncoated Concentric Fibrosis. B - Concentric fibrosis with lining



3.7 ANGIOFIBROBLASTIC PROLIFERATION

In the group without nanotechnological coating, a variation between 3 and 5 weeks (p = 0.04) was observed, but with a return of the pattern of absence of angiofibroblastic proliferation at 7 weeks. In the coated group, no significant change was observed (p = 0.27.) In the comparison of the two groups, no significant difference was observed (p > 0.05) (Table 7, Figure 12).

Table 7

Angiofibroblastic Proliferation

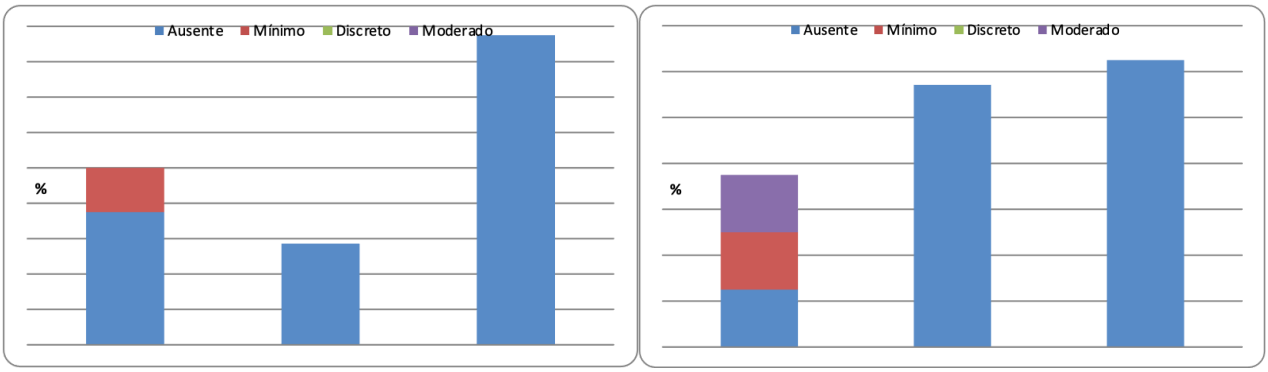
	UNCOATED					COATED					
	Ausent	Minimum	Discret	Mod.	p*	Ausent	Minimum	Discret	Mod.	p**	p***
3s	03 37,5%	04 50,0%	00 0,0%	01 12,5%	0,04	01 12,5%	02 25,0%	02 25,0%	03 37,5%	0,2	0,19
6s	02 28,6%	01 14,3%	02 28,6%	02 28,6%		04 57,1%	00 0,0%	01 4,3%	02 28,6%		0,57
7s	07 87,5%	01 12,5%	00 0,0%	00 0,0%		05 62,5%	01 12,5%	02 25,0%	00 0,0%		0,31

NOTE: Pearson's Chi-square test * comparison between groups (uncoated) ** comparison between groups (with coated) ***comparison between uncoated and coated.

Source: Authors.

Figure 12

A - angiofibroblastic proliferation without coating. B - angiofibroblastic proliferation without coverage



3.8 BLACK GRANULAR PIGMENT

Black granular pigment was seen in all cases and in all weeks with nanotechnological coating.

4 DISCUSSION

The evaluation of the results was performed through histological analysis. The organic reactions of the mice to the implants were divided into categories and the intergroup comparison was made based on these characteristics.[11]

The sample of this study consisted of 46 samples of subcutaneous tissue from mice tested for biological reaction to metallic wire without and with nanotechnological coating 3 (8 samples), 5 (7 samples) and 7 weeks (8 samples) after implantation.

We will discuss the behavior of the studies that showed statistical difference, since the analyses of EOSINOPHILIC INFILTRATION, INTENSITY OF THE INFLAMMATORY PROCESS, MULTINUCLEATED GIANT CELL INFILTRATION did not present statistical difference between the two groups.

Regarding the Inflammatory Process Pattern:

In the group without nanotechnological coating, there was progression from a predominantly absent inflammatory pattern or chronic proliferative at 5 weeks to fibrosis at 7 weeks ($p = 0.01$). In the coated group, the same phenomenon was observed ($p < 0.001$). There was no difference in evolution between the two groups ($p > 0.05$).

The extent of the inflammatory process did not vary with the analysis times in the uncoated group ($p = 0.17$). In the group with coating, there was a change in predominance from moderate to mild or minimal extension ($p = 0.04$). In the comparison of the two groups,

it was observed that at 3 weeks the extent of the inflammatory process was greater in the group with nanotechnological coating (p = 0.02).

When analyzing the intensity of the inflammatory process, there was a predominance of absence or minimal presence. There was no difference between the evaluation moments or between the groups (p > 0.05).

The risk of fibrosis was 7 times higher in the uncoated group when comparing the 3rd and 7th weeks (RR = 7.0 95% CI = 1.09 to 44.6). In the coated group, the risk was 1.7 times higher (RR = 1.7, 95% CI = 0.83 to 3.7) in the comparison between the 3rd and 7th week.

In all the samples analyzed from each series of the group with nanotechnological coating, black granular pigment was found, while the group without coating did not present this characteristic at any time.

The granular black pigment found in microscopy corresponds to the detachment of the implant material, which can occur as a result of wear or corrosion of the metal.

5 CONCLUSION

After evaluating the results by histological analysis in 46 subcutaneous tissue samples from mice with and without AlTiCrN+Si3N4 coating (Chromium Nitride, aluminum, titanium, enveloped in a layer of silicon nitride), it was found that eosinophilic infiltration, intensity of the inflammatory process, multinucleated giant cell infiltration, did not present statistical difference between the two groups.

Fibrosis in the group with 1.7 times the coating (RR = 1.7, 95% CI = 0.83 to 3.7) and the presence of black granular pigment characterize wear, detachment and/or corrosion of the coating.

It is concluded that such a material would not have space for use in nanotechnological coating in metal implants.

REFERENCES

1. Kumar, S. S. D., & Abrahamse, H. (2020). Advancement of nanobiomaterials to deliver natural compounds for tissue engineering applications. International Journal of Molecular Sciences, 21(18), Article 6752. https://doi.org/10.3390/ijms21186752

2. Silveira, S. R., Sahm, B. D., Kreve, S., & Reis, A. C. (2023). Osseointegration, antimicrobial capacity and cytotoxicity of implant materials coated with graphene compounds: A systematic review. Japanese Dental Science Review, 59, 303–311.

3. Bomfim, V. V. B. S., & cols. (2023). Inovações em cirurgia ortopédica: Avanços tecnológicos e estratégias para recuperação pós operatória. Revista Ibero-Americanas de Humanidades, Ciências e Educação, 9(12).

4. Navarro, M., Michiardi, A., Castaño, O., & Planell, J. A. (2008). Biomaterials in orthopaedics. Journal of the Royal Society Interface, 5(27), 1137–1158.

5. Dias, R. C., Kisaki, Y., Ungaretti, A., Bredemeier, F., Tarragô, R., & Schwartsmann, C. R. (1993). Estudo experimental da reação tecidual óssea ao titânio Ti6A14V recoberto com hidroxiapatita. Revista Brasileira de Ortopedia e Traumatologia, (June).

6. Lazarinis, S., Kärrholm, J., & Hailer, N. P. (2011). Effects of hydroxyapatite coating on survival of an uncemented femoral stem. Acta Orthopaedica, 82(4), 399–404.

7. Catalin, M., Adrian, P., & Stanca, C. (2009). Selection of biomaterials for orthopaedic applications using the ponderated properties method. The Romanian Review Precision Mechanics, Optics & Mechatronics, 19(36).

8. Voigt, J. D., & Mosier, M. (2011). Hydroxyapatite (HA) coating appears to be of benefit for implant durability of tibial components in primary total knee arthroplasty. Acta Orthopaedica, 82(4), 448–459.

9. Silva, V. V. (2017). Aplicação de biomateriais em ortopedia e engenharia de tecido ósseo. Revista Saúde Meio Ambiente, 5(2), 14–27.

10. Rodrigues, L. B. (2013). Aplicações de biomateriais em ortopedia. Estudos Tecnológicos em Engenharia, 9(2), 63–76.

11. Costa, T. O. A., Donato Neto, E. P., Sena, A. J. G., & Guimarães, A. L. S. (2024). Uso de biomateriais e técnicas regenerativas em cirurgias ortopédicas. Brazilian Journal of Implantology and Health Sciences, 6(11), 3104–3112.