



Evaluation of protein adsorption in rats submitted to nanotextured and polyurethane foam-coated silicone mini-implants

Avaliação da absorção de proteínas em ratas submetidas à colocação de mini-implantes de silicone nanotexturizados e revestidos pela espuma de poliuretano

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■ ABSTRACT

Introduction: The control of protein absorption is necessary to define biomaterials' properties and their specific uses. Blood plasma contains several different proteins, including fibrinogen, which plays an important role in cell adhesion and biocompatibility results in implants. This study's objectives were to evaluate in the laboratory rats subjected to the placement of nano-textured silicone mini-implants and polyurethane foam-coated mini-implants based on the measurement of serum fibrinogen and plasma proteins. **Methods:** Sixty albino rats were used, divided into two groups of 30 animals for each type of silicone mini-implant (nanotextured and polyurethane foam) and subdivided into three subgroups, according to the animals' euthanasia time (30, 60 and 90 days). The mini-implants were inserted in the animals' backs below the Panniculus carnosus. At the time of euthanasia, blood samples were obtained by cardiac puncture. The thermal precipitation technique was used to determine total and serum plasma proteins, and the difference between the latter two obtained the fibrinogen value. **Results:** When the groups were compared, it was observed that the nanotextured group presented a higher amount of fibrinogen and plasma protein in the 90-day subgroup, with statistical significance ($p=0.004$). When comparing the subgroups among themselves, a significant difference was evidenced ($p<0.001$). **Conclusion:** The nanotextured mini-implants showed a lower protein absorption concerning polyurethane foam-coated implants in the 90-day subgroup. **Keywords:** Blood proteins; Experimental implants; Breast implants; Mammoplasty; Rats.

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■ RESUMO

Introdução: O controle da absorção de proteínas é necessário para a definição das propriedades dos biomateriais e de seus usos específicos. O plasma sanguíneo contém diversas proteínas diferentes, dentre elas o fibrinogênio, que apresenta importante papel na adesão celular e nos resultados de biocompatibilidade em implantes. Os objetivos deste estudo foram avaliar laboratorialmente as ratas submetidas à colocação de mini-implantes de silicone nanotexturizados e revestidos por espuma de poliuretano a partir da aferição do fibrinogênio sérico e mensuração da proteína plasmática. **Métodos:** Foram utilizadas 60 ratas albinas, divididas em dois grupos de 30 animais para cada tipo de mini-implante de silicone (nanotexturizado e espuma de poliuretano) e subdivididas em 3 subgrupos, conforme o tempo de eutanásia dos animais (30, 60 e 90 dias). Os mini-implantes foram inseridos no dorso dos animais abaixo do Panniculus carnosus. No momento das eutanásias, amostras de sangue foram obtidas por punção cardíaca. Utilizou-se a técnica de precipitação térmica para determinação das proteínas plasmáticas total e sérica, e o valor do fibrinogênio foi obtido mediante a diferença entre estas duas últimas. **Resultados:** Quando comparados os grupos entre si, observou-se que o grupo nanotexturizado apresentou uma maior quantidade de fibrinogênio e da proteína plasmática no subgrupo de 90 dias, com significância estatística ($p=0,004$). Ao comparar os subgrupos entre si, em ambos os grupos, evidenciou-se uma diferença significativa ($p<0,001$). **Conclusão:** Os mini-implantes nanotexturizados mostraram uma menor absorção de proteínas em relação aos implantes revestidos pela espuma de poliuretano, no subgrupo de 90 dias. **Descritores:** Proteínas sanguíneas; Implantes experimentais; Implantes de mama; Mamoplastia; Ratos.

INTRODUCTION

The advancement of materials' functions and performance is the main objective of surface engineering, aiming to produce implants that trigger increasingly controlled biological responses¹. When an alloplastic material is placed in contact with the living organism, the proteins present in the blood interact with the surface identified as foreign. Protein absorption in these biomaterials is one of the main points during the development of technologies in bio-devices².

This is because, when a material is implanted, the absorption of proteins is one of the first steps in the biological process, as well as the coagulation cascade, which triggers immune and inflammatory reactions^{2,3}. Therefore, the protein absorption phenomenon's control is necessary to define biomaterials' properties and their specific uses³. Blood plasma contains several different proteins, including fibrinogen, which is a signaling molecule with a wide spectrum of functions, which can lead from a balance between

coagulation and protection against infections to processes of fibrosis and intense inflammation⁴. Thus, fibrinogen shows an important role in cell adhesion and, consequently, in the biocompatibility results of implants³.

During the evolution of breast implants, several coating surfaces were proposed to minimize tissue reactions⁵, of which nanotextured implants, through nanotechnology, proved to be safer concerning anaplastic large cell lymphoma⁶ and polyurethane foam if proved effective in reducing the rate of capsular contracture⁷.

Segmented polyurethane is a thermoplastic elastomer that has been used extensively in surgical procedures due to its excellent physical and mechanical properties, thermoplasticity and biocompatibility⁸⁻¹⁰.

Currently, with the advancement of nanotechnology, materials with surfaces of smaller and smaller sizes and more similar to biological structures have been proposed, thus allowing increasingly natural tissue reactions between the implant and adjacent tissues, which should decrease the intensity of the

inflammatory response and influence the absorption of proteins^{1,11}.

OBJECTIVES

The present study aims to evaluate in the laboratory the rats submitted to the placement of nanotextured silicone implants and coated with polyurethane foam, with the following parameters:

- measurement of fibrinogen;
- measurement of plasma protein.

METHODS

The research was carried out in the Universidade Estadual de Ponta Grossa (UEPG) experimental surgery vivarium after being approved by the Ethics Committee on the Use of Animals (CEUA) UEPG. CEUA process - 041/2018. UEPG Protocol: 16450/2018. All procedures strictly followed the existing regulations for animal research.

The study design was a primary study (randomized clinical trial), interventional, experimental in animals (rats), prospective, analytical, controlled, randomized, double-blind and single-centered.

A total of 60 albino rats (*Rattus norvegicus albinus*, *Rodentia mammalia*) weighing between 190 to 250 grams and 30 to 60 days old had free access to water and a species-specific diet, with room temperature and 12-hour circadian cycles.

They were randomly divided into two groups of 30 animals for each type of silicone mini-implant (nanotextured and polyurethane foam), and subdivided into 3 subgroups, according to the animals' euthanasia time (30, 60 and 90 days).

In the nanotextured group, n = 30, mini-implants with nanotextured surfaces (Silimed[®], Rio de Janeiro, Brazil) were placed. In the polyurethane group, n = 30, mini-implants with polyurethane foam coating (Silimed[®]) were placed.

The implanted materials had the same layers as a human breast implant, discoid in shape, with 22 +/- 1 mm (mm) in diameter and 9 +/- 1 mm in height in mini-implants with a nanotextured surface, and with 24 +/- 1 mm in diameter and 11 +/- 1 mm high in mini-implants coated with polyurethane foam. The height was defined as the point of greatest implant projection on the vertical axis (Figure 1). Concerning the pores on the surface of the mini-implants, those with a nanotextured surface had the following dimensions: diameter 0.3 to 8.7 micrometers (300 to 8700 nanometers); average roughness (Ar) 4.12 micrometers (4120 nanometers); and depth 3.08 to 10.74 micrometers. The mini-implants coated with

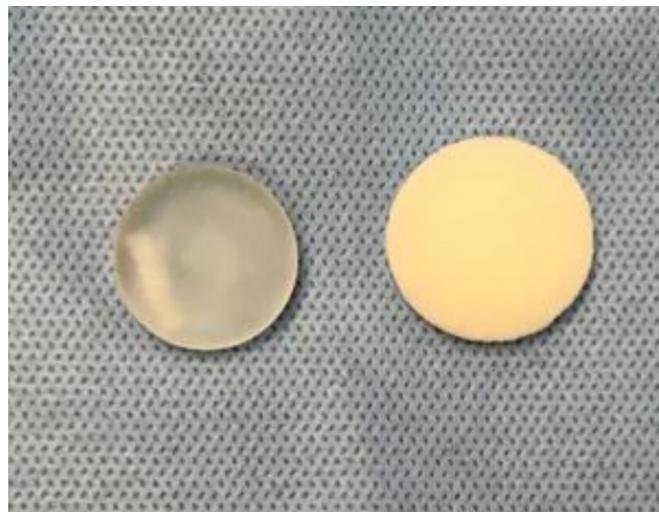


Figure 1. Mini-implants of nanotextured silicone and coated by polyurethane foam.

polyurethane foam had the following dimensions: diameter 120 to 320 micrometers; average roughness (Ar) 1500 micrometers; and pore depth 480 to 1200 micrometers. After distribution to groups, the rats were randomly removed from the cages and anesthetized by intraperitoneal injection, composed of an association of ketamine hydrochloride 1% (Dopalen[®], Hertape, Belo Horizonte, Brazil) at a dose of 40mg/kg and hydrochloride of xylazine 2% (Dopasen[®], Hertape) at a dose of 8mg/kg according to the guide for anesthesia and analgesia of laboratory animals - UNIFESP/CEUA (2017)¹². The effectiveness of anesthesia was assessed by the absence of movement, corneal-eyelid reflex, and motor reaction after grasping the fat pad of one of the hind legs, in addition to a good ventilatory pattern. With the rats positioned in the prone position, trichotomy was performed on the dorsal region, with subsequent antisepsis and sterile surgical field placement.

The incision's delimitation was performed regarding a subcostal horizontal line, following the posteroinferior costal edge, which was met with the middle sagittal line. With a scalpel cable no. 3, coupled with a blade no. 15, a horizontal incision was made, with an extension of 20 mm at the intersection of these reference lines.

The pocket was made for the mini-implants in a retromuscular plane (below the *Panniculus carnosus*), and, later, the mini-implant was introduced vertically, being positioned horizontally according to the group (nanotextured or polyurethane). The skin's suture was intradermal with mononylon 5-0 (Ethicon[®]) with buried knots. There was no removal of the stitches in the postoperative period, and the surgical wound was kept exposed (Figure 2).



Figure 2. Immediate post-op.

Postoperative analgesia was with a single intramuscular application of sodium dipyrone (20mg/kg) in the posterior limb's lateral region. No postoperative dressings or stitches were performed.

Euthanasia occurred according to subgroups of 30, 60 and 90 days by applying four times the therapeutic dose of Dopalen® and Dopasen® and subsequent cervical dislocation. There was no death, infection of the surgical site or extrusion of the implants, so no rats were excluded.

Evaluation methodology

Blood samples were obtained on the day of euthanasia of the animals, according to each subgroup, by intracardiac puncture performed by the veterinarian ([Video 1](#) ) and were placed in tubes without and with anticoagulant ethylenediaminetetraacetic acid (EDTA)¹³. The triplicate thermal precipitation technique was used for each animal, consisting of filling six capillary tubes with blood up to ¾ of the capacity, properly closed at one end. After that, they were centrifuged at 8.0rpm in a microhematocrit centrifuge to separate the plasma for 5 minutes. After being centrifuged, three of the capillaries were randomly chosen and broken to obtain a drop, which was later placed on the Goldberg refractometer to measure total plasma protein (TPP)^{13,14}. The remaining three capillary tubes were taken to the water bath (temperature of 56-58 °C, for three minutes) and afterward, again centrifuged, as previously described, obtaining this time the serum, which was measured in the refractometer, resulting in serum protein (SP)¹³.

The fibrinogen value was obtained by the difference between the total plasma protein and the serum protein. The result was multiplied by 1,000, as fibrinogen is evaluated in mg.dL⁻¹¹³.

Statistical evaluation

The results were described by median, minimum and maximum values. For the comparison of groups (nanotextured and polyurethane), in each subgroup (30, 60 and 90 days), the Mann-Whitney non-parametric test was used. The comparisons between the subgroups for each group were made using the Kruskal-Wallis non-parametric test. Values of $p < 0.05$ indicated statistical significance. The data were analyzed with the computer program Stata/SE v.14.1. StataCorpLP, USA.

RESULTS

The groups (nanotextured and polyurethane) were compared for the variables fibrinogen and plasma protein in the subgroups of 30, 60 and 90 days.

When the groups were compared to each other, it was observed that the nanotextured group had a greater amount of fibrinogen and plasma protein in the 90-day subgroup, with statistical significance ($p = 0.004$) (Tables 1 and 2 and Figures 3 and 4).

Table 1. Comparison of fibrinogen in the nanotextured and polyurethane groups over time.

Subgroups	Groups		p*
	Nanotextured median (min-max)	Polyurethane median (min-max)	
30d	5.6 (5-5.6)	5.5 (4.8-6)	0.962
60d	9.2 (8.8-9.8)	9.2 (8.8-10)	0.673
90d	7.2 (6.4-7.8)	6.3 (5-7.4)	0.004
p** (30 x 60 x 90d)	<0.001***	<0.001***	

*Mann-Whitney nonparametric test, $p < 0.05$; **Non-parametric test of Kruskal-Wallis, $p < 0.05$; ***Nanotextured: 30D x 60D: $P < 0.001$; 30D x 90D: $P < 0.001$; 60D x 90D: $P < 0.001$; Polyurethane: 30D x 60D: $P < 0.001$; 30D x 90D: $P < 0.001$; 60D x 90D: $P < 0.001$.

Table 2. Comparison of plasma protein in the nanotextured and polyurethane groups over time.

Subgroups	Groups		p*
	Nanotextured median (min-max)	Polyurethane median (min-max)	
30d	5.6 (5 - 6)	5.6 (4.8 - 6)	0.813
60d	9.4 (9 - 10)	9.4 (8.2 - 10)	0.393
90d	7.9 (6.4 - 8)	7 (5.2 - 7.4)	0.002
p** (30 x 60 x 90d)	<0.001***	<0.001***	

*Mann-Whitney nonparametric test, $p < 0.05$; **Non-parametric test of Kruskal-Wallis, $p < 0.05$; ***Nanotextured: 30D x 60D: $P < 0.001$; 30D x 90D: $P < 0.001$; 60D x 90D: $P < 0.001$; Polyurethane: 30D x 60D: $P < 0.001$; 30D x 90D: $P < 0.001$; 60D x 90D: $P < 0.001$.

When comparing the subgroups, a significant difference was observed ($p < 0.001$) (Tables 1 and 2 and Figures 3 and 4).

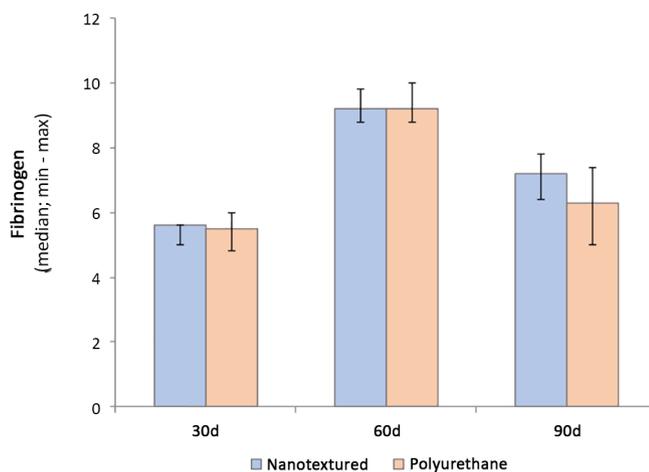


Figure 3. Comparison of fibrinogen in the nanotextured and polyurethane groups over time.

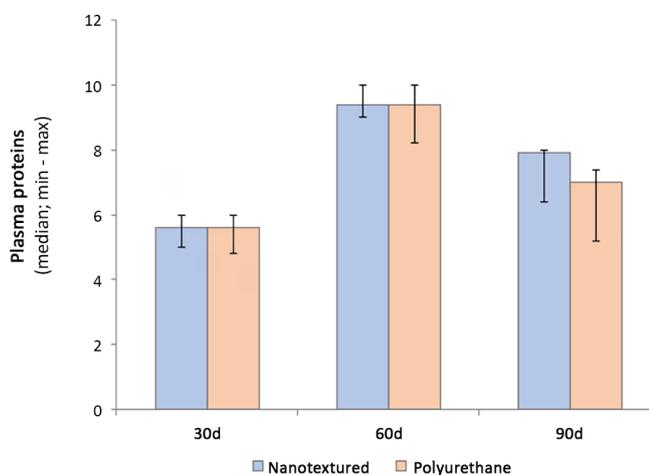


Figure 4. Comparison of plasma protein in the nanotextured and polyurethane groups over time.

DISCUSSION

The foreign body reaction is the inflammatory sequence triggered by the implantation of biomaterials¹⁵, corresponding to the absorption of proteins on the implant surface, infiltration of inflammatory cells, fusion of macrophages and giant cells, activation of fibroblasts and, finally, formation of a fibrous capsule¹⁶.

The acute phase of healing is closely related to the activation of macrophages, which produce a variety of growth factors (IGF-1, VEGF- α , TGF- β and Wnt) that are proteins that regulate the proliferation of endothelial and epithelial cells, activate myofibroblasts, and can differentiate into progenitor cells and neovessel formation. Macrophages, therefore, recover tissue homeostasis by activating anti-inflammatory cells and regulating the deposition of collagen and fibrin¹⁷.

When there is inflammation, the presence of fibrinogen and fibrin is frequent. Like the macrophage, fibrinogen exerts its effects depending on the context in which it is, both in inflammation of the tissue and in its repair, in wound healing or in the development of fibrosis¹⁸.

Thus, the mechanisms that regulate these different activations of macrophages and fibrinogen have become active areas of research¹⁸. It is known today that these responses vary according to the characteristics of the implanted material, such as its size, biological behavior, pore size, surface topography and sterilization techniques¹⁷.

Biomaterial's engineering has focused on creating implants that more accurately simulate human tissues, both physically and chemically¹⁵. With the annual growth in the number of mammoplasty surgeries, the ideal implant's choice remains a challenge, aiming for natural results and safety procedures¹⁹.

Currently, breast implants can be classified according to their filling (silicone or saline), shape (round or anatomical) and surface texture (smooth, micro and macrot textured)²⁰. The surface texture is determined from the material's roughness: smooth (less than $10\mu\text{m}$), microtextured ($10\text{-}50\mu\text{m}$) and macrot textured (greater than $50\mu\text{m}$)⁵.

The silicone implant coated with polyurethane foam is considered a macrot textured implant. Its use began in 1970, motivated by a supposed reduction in capsular contracture²¹. The polyurethane foam forms a foamy mass in situ containing pores; this porosity allows for cell growth inside, leading to the incorporation of the implant lining into the adjacent tissue. After forming the capsule around the implant, the polyurethane coating degrades and merges with the capsule²².

More recently, with the advent of nanotechnology, greater tissue mimicry has become reality²³, making it possible to build surfaces with specific nano protrusions, depending on the need, both for promotion and to prevent the absorption of proteins².

Nanotextured surfaces demonstrated the ability to more efficiently control the interactions between the recipient tissue and the implant surface, decreasing the foreign body reaction, inflammation and scar tissue formation, in addition to greater control of colonization by pathogens²⁴.

CONCLUSION

The nanotextured implants showed a lower protein absorption in relation to polyurethane foam-coated implants in the 90-day subgroup.

COLLABORATIONS

ENS	Analysis and/or data interpretation, Conception and design study, Conceptualization, Data Curation, Final manuscript approval, Formal Analysis, Investigation, Methodology, Project Administration, Resources, Software, Validation, Visualization, Writing - Original Draft Preparation, Writing - Review & Editing
GHP	Funding Acquisition, Realization of operations and/or trials, Resources
BZL	Realization of operations and/or trials, Writing - Original Draft Preparation
AYK	Realization of operations and/or trials
LMF	Supervision
LCL	Realization of operations and/or trials

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