WILLIAM CÉSAR CAVAZANA

MODELO EXPERIMENTAL PARA ESTUDO DE MÉTODOS DE FIXAÇÃO DE RETALHO PERICRÂNIO-CUTÂNEO. ANÁLISE RADIOLÓGICA E HISTOLÓGICA, EM RATOS.

EXPERIMENTAL MODEL TO STUDY FIXATION METHODS FOR PERICRANIUM-CUTANEOUS FLAP. RADIOLOGICAL AND HISTOLOGICAL ANALYSIS, IN RATS.

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Faculdade de Ciências Médicas

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Tutor: Prof. Dr. Luis Augusto Passeri
Co-tutor: Prof. Dr. Roberto Kenji Nakamura Cuman

EXPERIMENTAL MODEL TO STUDY FIXATION METHODS FOR PERICRANIUM-CUTANEOUS FLAP. RADIOLOGICAL AND HISTOLOGICAL ANALYSIS, IN RATS.

Tese de Doutorado apresentada à Pós-Graduação da Faculdade de Ciências Médicas da Universidade Estadual de Campinas - Unicamp para Obtenção do Título de Doutor em Ciências.

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Este exemplar corresponde à versão final da tese defendida pelo aluno ***William César Cavazana*** e orientada pelo Prof. Dr. ***Luis Augusto Passeri.***

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Dedico este trabalho...

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Resumo

Objetivo: Desenvolver um modelo experimental para estudar e radiologicamente monitorar o deslocamento de retalhos pericrânio-cutâneos de ratos submetidos à tração e fixação cirúrgica com sutura ancorada em um túnel ósseo e com o adesivo cirúrgico n-butil-2-cianoacrilato (Histoacryl®), com ênfase sobre a resposta inflamatória celular e a produção de colagénio tipo I e III. **Métodos:** Um marcador radiológico foi colocado no tecido subcutâneo de ratos Wistar submetidos a descolamento subperiosteal do pericrânio com tração e fixação do retalho. Foram constituídos aleatoriamente, quatro grupos: controle, sutura ancorada em túnel ósseo, adesivo cirúrgicoe e sham. Os dados foram coletados nos dias 3, 7, 14, 21 e 45 de pós-operatório, por radiografias e coleta de amostras teciduais da região cefálica, minutos antes da eutanásia, fixados em formalina, e submetidos ao preparo histológico. As lâminas foram coradas com hematoxilina-eosina e picrossírius. Foram realizadas contagens padronizadas de polimorfonucleares, monomorfonucleares, fibroblastos e macrófagos e foram determinadas as percentagens de colágeno tipos I e III. Um valor de $p < 0,05$ foi considerado significante. **Resultados:** A análise qualitativa dos dados indicou que os retalhos do grupo de adesivo cirúrgico permaneceram no lugar, sem alteração da posição pós-operatório imediato. Entretanto os retalhos dos grupos sutura ancorada em túnel ósseo e controle apresentaram

cicatrização semelhante, com uma perda de fixação na ordem de 9,7% e 22%, respectivamente, em comparação com a posição pós-operatória imediata. Também foi demonstrada maior resposta celular no grupo adesivo cirúrgico, com predomínio de polimorfonucleares,do dia 3 até o dia 45, e de macrófagos nos dias 3 e 7. A quantidade de colágeno tipo I superou 80% nos grupos tratados e controle ao final do experimento. A análise quantitativa mostrou maior quantidade de fibroblastos no grupo do adesivo cirúrgico do que no grupo sutura ancorada em túnel ósseo (p=0,0211). **Conclusão:** Este modelo experimental criou condições experimentais aceitáveis para testar diferentes métodos de fixação de tecidos, na região da calota craniana de ratos Wistar. O uso de fixadores teciduais contribuiu para um melhor posicionamento de retalhos periósteo-cutâneos e o adesivo cirúrgico foi superior à sutura ancorada em túnel ósseo para a fixação de retalhos pericrânio-cutâneos. O descolamento subperiostal desencadeou uma resposta inflamatória celular que foi amplificada com o uso de fixadores teciduais. O adesivo n-butil-2-cianoacrilato foi mais reativo que o fio de nylon monofilamentar ancorado em túnel ósseo.

Descritores: Ritidoplastia, Cianoacrilatos, Adesivos, Nylons, Cicatrização.

Abstract

Purpose: To develop an experimental model to study and radiologically monitor displacement of skin flaps in the pericranium of rats subjected to traction and surgical fixation using suture anchored in a skull bone tunnel or with N-butyl-2-cyanoacrylate (Histoacryl TM) surgical adhesive with emphasis on the cellular inflammatory response and the production of types I and III collagen. **Methods:** Radiological markers were placed in the subcutis of Wistar rats undergoing subperiosteal detachment of the pericranium with pulling and fixation of the flap. Four groups were randomly formed: control, suture anchored in the skull bone tunnel, surgical adhesive and sham. Data collection occurred on days 3, 7, 14, 21, and 45 postoperatively by radiographs and collection of specimens from the cephalic removed minutes before euthanasia, fixed in formalin, and subjected to histological preparation. Slides were stained with hematoxylin–eosin and Siriusred. Standardized counts of polymorphonuclear and mononuclear cells, fibroblasts, and macrophages were performed, and the percentages of types I and III collagen were determined. A p-value of <0.05 was considered significant. **Results:** Qualitative analysis of the data indicated that the flaps in the surgical adhesive group remained in place with no change from the immediate postoperative position. However, the flaps in the suture anchored in the skull bone tunnel group and in the control group showed similar healing, with a loss of attachment of 9.7% and 22%, respectively, compared with the immediate

postoperative position. Also was demonstrated higher cellular response in the adhesive group, with a predominance of polymorphonuclear cells from days 3−45 and macrophages from days 3−7. The amount of type I collagen exceeded 80% in the treated and control groups at the end of the experiment. Quantitative analysis showed more fibroblasts in the surgical adhesive group than in suture anchored in the skull bone tunnel group ($p = 0.0211$). **Conclusion:** This experimental model created acceptable experimental conditions for testing different soft tissue fixation methods on skull of Wistar rats. The use of tissue fixatives contributed to better placement of pericranium-cutaneous flaps, and surgical adhesive was superior to suture anchored in the skull bone tunnel for fixation of pericranium-cutaneous flaps. Subperiosteal detachment triggered a cellular inflammatory response that was amplified using soft tissue fixation methods. The adhesive n-butyl-2-cyanoacrylate was more reactive than the nylon monofilament thread anchored in the skull bone tunnel.

Key words: Rhytidoplasty, Cyanoacrylates, Adhesives, Nylons, Wound Healing.

1.Introdução

Procedimentos cirúrgicos subperiostais resultam na realização de retalhos periósteo-cutâneos para diversos propósitos. Nos casos que visam um reposicionamento tecidual, a aderência desta estrutura no pós-operatório passa a ter importância para a manutenção dos resultados obtidos no ato operatório.

Para Krietet al., (1) o efeito da cicatrização na aderência do periósteo ao crânio, em coelhos, foi significante no 3º e no 5º dia pós-operatório, em comparação aos coelhos não operados. Romo et al., em 2000 (2), também relataram que após uma fixação mínima inicial, a aderência se intensificou a partir da 6ª semana, completando-se no decorrer da 12ª semana, nos grupos de coelhos tratados com fixação semi-permanente, através de sutura ou de parafusos absorvíveis de copolímero de ácidos polilático e poliglicólico.

Com o desenvolvimento da ritidoplastia endoscópica frontal (3,4,5) e do terço médio da face (6,7), a busca por um meio de fixação ideal, capaz de manter as estruturas tracionadas e reposicionadas levou à necessidade de ampliar os estudos laboratoriais, para melhor conhecimento da ação de dispositivos de tração já consagrados.

Os meios de fixação utilizados para manter o retalho na posição desejada, em cirurgia plástica facial, podem variar. Os mais comuns são pontos de sutura, que podem ser ancorados em túneis ósseos da calota craniana, parafusos ou dispositivos metálicos ou absorvíveis, ou a aplicação de adesivos cirúrgicos (8-16).

Os fios de sutura de nylon monofilamentar são tradicionalmente empregados em operações envolvendo tecidos moles, sendo frequentemente comparados com adesivos cirúrgicos (17-19).

Os cianoacrilatos têm sido utilizados em operações abdominais, ginecológicas, ortopédicas, neurológicas, plásticas, urogenitais, vasculares, torácicas e buco-maxilo-faciais, dentre outras (20). São ésteres do ácido cianoacrílico, com uma cadeia alquil lateral. Seus monômeros líquidos se solidificam por polimerização aniônica, após o contato com uma base fraca, liberando pouco calor (21).

Batista et al. (19) estudando a reparação da parede abdominal anterior de ratos, que tiveram a síntese feita com nylon monofilamentar 3.0 e adesivo n-butil-2-cianoacrilato, não encontraram diferenças significantivas entre os grupos.

Justificativa

O desenvolvimento da ritidoplastia frontal endoscópica promoveu um avanço no tratamento dos efeitos do envelhecimento da região frontal, porém em virtude do tempo necessário para a aderência pós-operatória do periósteo

ao osso frontal, a previsibilidade de resultados ainda é deficiente, pois não há consenso sobre o meio de fixação ideal para os retalhos pericrânio-cutâneos. Os autores esperam desenvolver um modelo experimental em ratos, que permita o estudo dos meios de fixação e a reatividade tecidual a estes meios.

2. Objetivos

1) Desenvolver um modelo experimental em ratos, que simule as condições teciduais de uma cirurgia subperiostal.

2) Monitorizar radiologicamente o deslocamento de retalhos pericrânio-cutâneos, submetidos à tração cirúrgica e fixação, por meio de ponto ancorado em túnel ósseo e de adesivo n-butil-2-cianoacrilato, durante o processo de reparação.

3) Realizar análise histológica, quantificando as células inflamatórias e o colágeno tipos I e III.

3.Publicações

2 – ORIGINAL ARTICLE
MODELS, BIOLOGICAL

Experimental model for the study of soft tissue fixation methods on skin-pericranium flaps in rats[1]

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[II]PhD, Associate Professor, Department of Pharmacology and Therapeutics, Center of Health Sciences, UEM, Maringa-PR, Brazil. Interpretation of data.
[III]PhD, Associate Professor, Department of Odontology, Center of Health Sciences, UEM, Maringa-PR, Brazil. Interpretation of data.
[IV]PhD, Full Professor, Department of Surgery, School of Medical Sciences, UNICAMP, Campinas-SP, Brazil. Interpretation of data, manuscript writing, critical revision.

ABSTRACT

PURPOSE: To develop an experimental model to study and radiologically monitor displacement of skin flaps in the pericranium of rats subjected to traction and surgical fixation using suture anchored in a skull bone tunnel or with N-butyl-2-cyanoacrylate (Histoacryl™) surgical adhesive.

METHODS: Radiological markers were placed in the subcutis of Wistar rats undergoing subperiosteal detachment of the pericranium with pulling and fixation of the flap. We performed radiography on postoperative days 3, 7, 14, 21, and 45. A p-value of <0.05 was considered significant.

RESULTS: Qualitative analysis of the data indicated that the flaps in the surgical adhesive group remained in place with no change from the immediate postoperative position. However, the flaps in the suture anchored in the skull bone tunnel group and in the control group showed similar healing, with a loss of attachment of 9.7% and 22%, respectively, compared with the immediate postoperative position. There was no quantitative difference between the groups.

CONCLUSIONS: This experimental model created acceptable experimental conditions for testing different soft tissue fixation methods. The use of tissue fixatives contributed to better placement of pericranium-cutaneous flaps, and surgical adhesive was superior to suture anchor in the skull bone tunnel for fixation of pericranium-cutaneous flaps.

Key words: Rhytidoplasty. Surgical Fixation Devices. Wound Healing. Adhesives. Rats.

Introduction

Skin-periosteal flaps resulting from subperiosteal surgical procedures are used for various purposes and maintaining flap adherence is important postoperatively to preserve the achieved results.

Facial plastic surgery uses various fixation methods to maintain the flap in its new position. The most common are: a) sutures, for retraction and anchoring in a bone tunnel in the skull, or in the temporal fascia, the aponeurotic galea, or periosteal bridges; b) absorbable or metallic devices, such as screws; and c) surgical adhesives[1–16].

The authors aim to develop an experimental rat model to study and radiologically monitor the movement of pericranial cutaneous flaps during healing and compare flaps which underwent surgical traction and fixation with suture anchored in a skull bone tunnel or surgical adhesive (Histoacryl 0.5g, B Braun, Melsungen, Germany).

Methods

Approved under protocol number 018/2010 by the Ethics in the Use of Animals Experimentation Committee of the State University of Maringa (Brazil).

A sample of 35 male rats (*Rattus norvegicus albinus*, Wistar), 120 days of age, weighing 280–484g (average, 365.7g) was used. The rats were randomly distributed into three groups of 10 animals as follows: group A: control; group B: suture anchored in a skull bone tunnel; and group C: surgical adhesive. The last group of five animals constituted the Sham group (surgical control).

Beginning 24h prior to the experiments, the animals were acclimatized in a *vivarium* and maintained in plastic cages containing a maximum of four animals. The rats received water and food *ad libidum*, and were maintained at room temperature with 12-h light/dark cycles.

After weighing the animals, the experimental procedure was initiated with intramuscular anesthesia, with chlorhydrate 2-(2.6 xilidine)-5.6-dihidro-4H-1.3-thiazine (Rompun 2%, Bayer, Sao Paulo-SP, Brazil) at a dosage of 1 mg/kg and ketamine chlorhydrate (Ketalar, Pfizer, Guarulhos-SP, Brazil) at a dosage of 20 mg/kg, in a 1:1 ratio. Based on the dose of each drug,1 ml/kgbody weight of the mixture was used for anesthesia[1].

With the animal anesthetized, the head was shaved followed by antisepsis of the shaved skin with an alcohol solution including 0.5% clorhexidine (Riohex, Rioquimica, Sao Jose do Rio Preto-SP, Brazil). Using a 30G × 7 mm needle (PrecisionGlide™, BD, Sao Paulo-SP, Brazil), the skin was pierced in the frontal region, paramedian, 5 mm posterior to the nasal root, without transfixing the skin on the contralateral side. A 3-0, 5–10 mm steel thread (Aciflex, Ethicon, Sao Paulo-SP, Brazil) was then introduced through the eye of the needle to the subcutaneous tissue of the punctured region. This steel thread was exposed when the puncture needle was retracted posteriorly, permitting sectioning of the thread with a scissor and the final introduction of the same thread in the subcutaneous tissue using Halsted nippers (Figure 1).

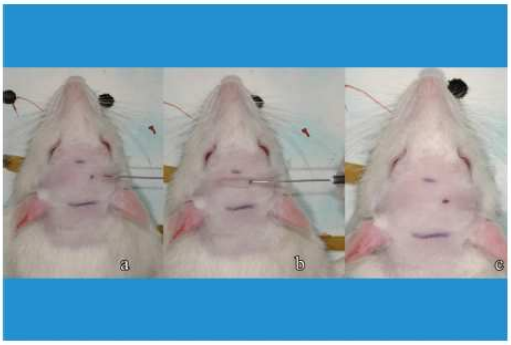

FIGURE 1 – A: 3-0 steel thread segment introduced through the eye of the needle; **B**: puncture needle retracted posteriorly; **C**: radiological marker insertion completed.

The function of this implanted thread was to serve as a dynamic radiological marker to observe the mobility of the flap during the study period.

After introducing the radiological marker, the cranium of the animal was immobilized in a cephalostat adapted from a portable computer metal support over a wooden board, to permit pre- and postoperative radiography (Figure 2).

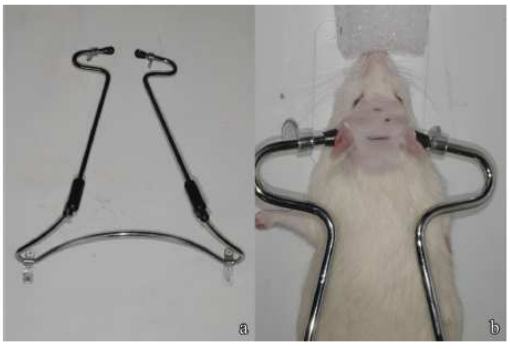

FIGURE 2 – A: cephalostat, **B**: immobilized cranium.

Radiographs were taken with the following technique: 27 kVp and 7 mA; 20-cm focal length; and exposure time of 0.06 s, with the central beam perpendicular to the film. We used 57 × 76 mm occlusal films (Kodak, Sao Jose dos Campos-SP, Brazil). The films were developed in an automatic processor (Revell, Xtec-Revell, Sao Paulo-SP, Brazil).

The distance corresponding to the measurements obtained in the study was stipulated as the distance between the midpoint of the radiological marker (steel thread) and the most distal point of the left nasal bone of the animal (Measure 1: Preoperative) (Figure 3).

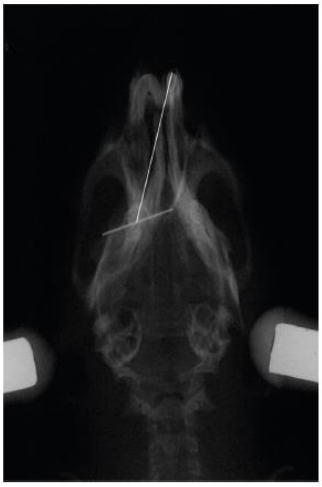

FIGURE 3 – Indicated line shows the reference for the measurements.

Following radiography, the animal was returned to the surgical board to elevate the periosteal-cutaneous flap, apply traction, reposition, and fix using one of the two methods. The skin was again disinfected with a 0.5% clorhexidine alcohol solution and a 15-mm transverse cutaneous incision was made, corresponding to the posterior border of the auricular pavilion, allowing access to the deep planes and the subsequent incision of the periosteum and thus, the pericranium (Figure 4).

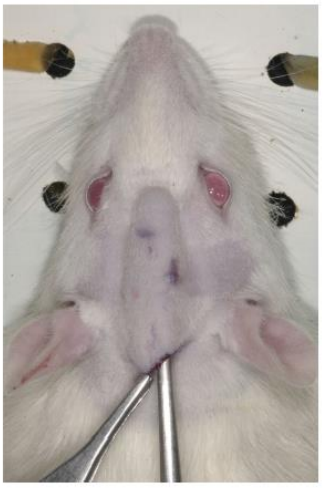

FIGURE 4 – **A**: incision in the skin and pericranium, **B**: skull exposure.

The subperiosteal flap was detached from the skull using a Freer elevator, with the nasal dorsum and lateral orbits of the animal as the distal limits. Next, the elevated flap was suspended with the help of an Adson nipper. At this stage, the experimental model is ready to test the various means of fixation (Figure 5).

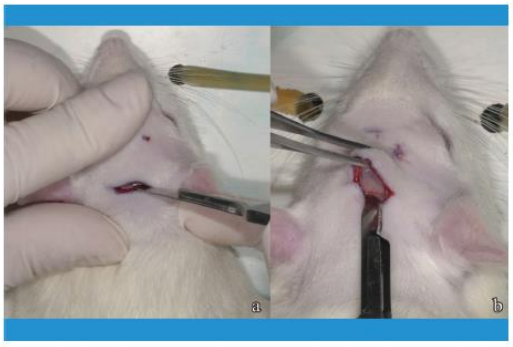

FIGURE 5 – Periosteal-cutaneous flap elevation.

Group A underwent detachment and elevation of the flap, without traction. Group B underwent the creation of two orifices in the skull 4 mm apart, using a 2-mm round drill bit, preserving the dura-mater, and a 4.0 nylon monofilament (Mononylon, Ethicon) thread was passed through from one opening to the other anchored with a simple suture. The suture was also passed through the periosteum and subcutaneous tissue of the flap to promote and sustain traction on the flap (Figure 6).

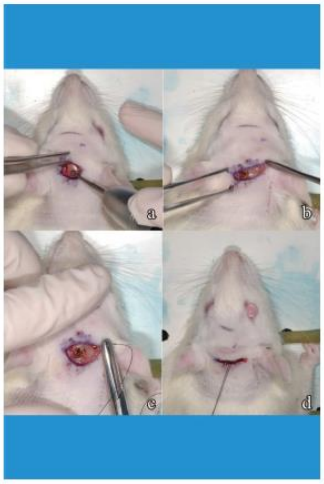

FIGURE 6 – A: round drill bit, **B**: bone tunnel, **C**: 4-0 nylon monofilament thread through the bone tunnel, **D**: retracted flap.

Group C underwent flap traction and received 0.25 ml (0.25 mg) per animal of n-butyl 2-cyanoacrylate surgical adhesive (Histoacryl 0.5 g; B Braun) over the exposed bone of the skull, using the actual ampule with an applicator tip (Figure 7).

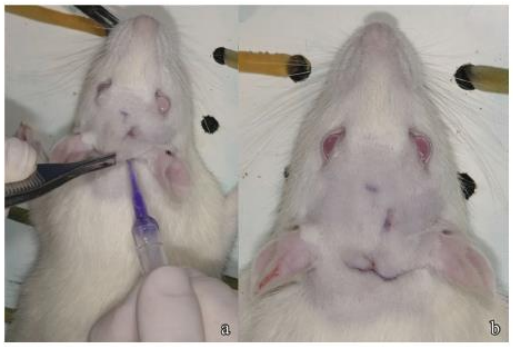

FIGURE 7 – A: n-butyl 2-cyanoacrylate glue application, **B**: retracted flap.

The Sham group received only the radiological marker in the subcutaneous tissue to indicate the normal mobility of the tissues covering the skull. The skin incisions were sutured with three simple interrupted sutures of 5-0 monofilament nylon (Mononylon, Ethicon).

Radiographs and measurements were repeated postoperatively (Measure 2: Immediate Postoperative). Measure two corresponds to the detachment of the flap, promoted by the traction and maintained by fixation.

The animals received a single oral dose of paracetamol solution (Tylenol, Janssen-Cilag, Sao Paulo-SP, Brazil) at a dosage of 10 mg/kg by gavage. Recovery occurred in the animals' cages.

The animals operated on underwent radiological evaluations, anesthetized as for surgery, on postoperative day 3, 7, 14, 21, and 45, providing long-term measurements that indicated whether the initial traction of the flap was maintained or not by the fixation.

The radiographs were digitized and the distances between the stipulated points for Measures 1 and 2 were obtained using the software, Image Tool® (Dental Diagnostic Science, UTHSCSA, San Antonio, TX, USA). Measure 2 was then compared with the postoperative measurements (Figure 8).

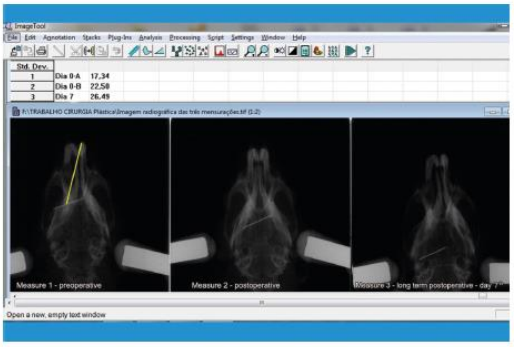

FIGURE 8 – Image analysis using the software, ImageTool® (goup B, rat 4).

Two animals per group were euthanized in ascending numerical sequence using deep anesthesia once the final radiographs were taken and after collecting tissue for histological analysis.

Qualitative data analysis was performed and also ANOVA analysis of variance and Tukey's test with a significance level of 5%.

Results

No macroscopic tearing of the dura was detected in Group B from the skull perforations and, in most cases, these surgeries were bloodless.

There were no significant differences between the groups over time and between radiological time-points (Table 1).

TABLE 1 – Mean values of the displacement in millimeters of the radiological markers positioned in the subcutaneous tissue of the cephalic region of Wistar rats, since pre-operative time, for groups A, B, C and Sham, at seven time-points.

	Measure 1	Measure 2	Day 3	Day 7	Day 14	Day 21	Day 45
Group A	19	19.9	22.3	23.1	19.6	21.9	15.6
Group B	20.8	25.4	24.6	26.1	23.4	27.8	22.95
Group C	20.8	22.7	21.8	27	25.9	21	23.05
Sham	23.5	23.5	14.7	21.8	23.9	27.1	19.1

** ANOVA significance level of 5%

Qualitative data analysis showed that:

a) Treatment caused 17.1% greater flap stretching in Group B than in Group A, and 4.3% greater in Group C than in Group A.

b) On day 3, Groups B and C had a setback in traction of 3.2% and 5.7%, respectively, while Group A had a progressive increase in average initial distance of approximately 11.5% and skin movement in the Sham group tended towards contracture, 37.5% below the average initial level.

c) On day 7, Groups B and C regained the previous decline in distance by 5.7%, and 24%, respectively, while Group A continued to increase the distance, but to a lesser extent, at 3.1%. The movement of the markers in the Sham group tended to return to the initial measurement in amounts similar to those in Group A, while Groups B and C exhibited scar stretching from 2.6% and 20.9%, respectively.

d) On day 14, skin stretching slowed and decreased 10% in Group B, 3.7% in Group C, and 14.8% in Group A. The Sham group markers essentially returned to their initial positions.

e) On day 21, the scar in Groups A and B stretched 18.8% and 11.7%, respectively, and at this point Group B reached its maximum stretch of 9.4% above the immediate postoperative value. Stretching in Group C regressed 19.3%; 9.1% below the immediate postoperative value.

f) On day 45, Groups A and B lost the stretch obtained during healing by day 21 and the flaps contracted, causing a decline of 22% and 9.7%, respectively, far short of the initial average postoperatively. The skin marker in the Sham group also tended to contract, falling 18.7% compared with the initial mean. Group C restored the stretch at 9.8%, approximating the immediate postoperative value (Figure 9).

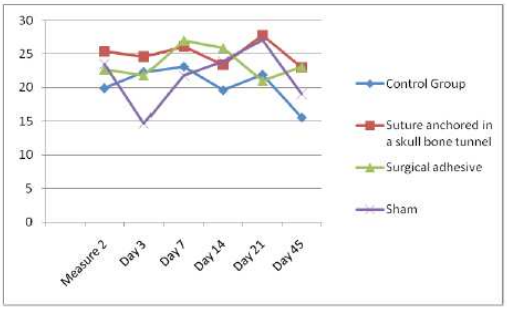

FIGURE 9 – Mean values in millimeters for tissue stretching in groups A, B, C, and Sham, from the time of the operation to the five assessed time points.

Discussion

The experimental model proposed is easily reproduced and uses easily obtained tools and instruments, therefore filling a void in experimental research in plastic surgery with previous results only in either clinical cases or less commonly, in rabbits[17]. We chose Wistar rats because these animals have a low risk of anesthetic death, are commonly available, are maintained in several research centers, and allow our model to be reproduced with other types of fixation.

The radiological marker is the essential element in the collection of the dynamic data and its clinical application in rats was also efficient. It was completely inserted in the soft tissues and not free-floating between the periosteum and the skull, which is a concern. It was visible on the radiographs and all of the measurements were taken from its midpoint to the most distal point on the left nasal bone. Its midpoint could also be considered a central axis because of its three-dimensional positioning in the soft tissues. Therefore, minor irregular displacement of its ends or even its length did not influence the measurements.

The need to create a cephalostat led us to create a practical and trustworthy model using a set of mobile supports for a portable computer. This set, attached to a wooden board, allowed for the immobilization of the rat's head and, as a result, the standardization of the radiographs.

Occlusal dental 57 × 76 mm radiograph films have dimensions compatible with the anatomical area we studied, facilitating the imaging and allowing for repeatability.

The animals we used were purposefully larger with weights in the range of 350g. This was important for the surgical procedures and also for imaging records and specimen collection.

An initial number of 15 animals for Group A and for each treated group was not approved by the Ethics in the Use of Animals in Experimentation Committee, reducing the statistical significance.

Following elevation, the periosteal-cutaneous flap delineates a space similar to the optical cavities obtained in endoscopic frontal rhytidoplasty to receive the fixation devices. No skin resection was performed in the flap traction. These three elements (broad subperiosteal detachment similar to an optical cavity, fixation, and no skin resection) reinforce this model for testing fixation in endoscopic frontal rhytidectomy. Gravity could be a confounding variable in human studies; however, gravity appears to have less influence in this model despite the horizontal position of the flap.

The incremental stretching of the pericranium-cutaneous flap in Group A, from days 3–7 suggests that fixation could be disposable. Troilius[8] advocates that the means of fixation could be expendable in endoscopic frontal rhytidectomy to achieve more natural results. In our study, we found that the control group did not reach the levels of stretching achieved by the treated groups, neither in the immediate postoperative period, nor at the end of the experiment, contrary to the study by Troilius[8].

Kriet *et al*.[18] studied the tensile strength of pericranium-cutaneous flaps using a tensiometer in rabbits that did not receive periosteal fixation, and observed a decrease in tension starting on day 3 postoperatively and a recovery by day 8 postoperatively, which remained until the last measurement on day 28 postoperatively. Romo *et al*.[17] observed minimal periosteal adherence in the early postoperative period, which progressed up to 12 weeks in rabbits. The minimum time for periosteal adherence was six weeks. In our study, after a decrease near day 3, we observed recovery of the amount of stretching of the flaps in the treated groups on day 7. This confirms an effect of tissue fasteners on this recovery, possibly creating a greater inflammatory response and, therefore, greater stretch of the flap in the long term. We saw this in Group B on day 21 and in Group C on days 14 and 45 postoperatively. The greater effect seen in Group C can also be explained by the exothermic reaction generated in the polymerization of this tissue adhesive[16,19]. Contrary to cyanoacrylates, Embucrilate™ is less toxic with a long chain and a gradual-release reaction[20]. Romo *et al*.[17] compared rabbits subjected to subperiosteal coronal rhytidectomy with skin resection and, using an absorbable screw fastener endoscopically and without skin resection, they concluded that the use of continuous semi-permanent fastening means improved accuracy and maintenance of early postoperative results. Although the study was conducted in mice, it showed similar scar behavior to our study, where the final position of the radiological markers after day 21 for the suture anchored in a skull bone tunnel group and after day 45 for the surgical adhesive group surpassed the initial position obtained with surgery, supporting our experimental model.

Graf *et al*.[7] analyzed a group of 72 patients who underwent endoscopic frontal rhytidectomy and found that in 38 patients analyzed between 3.5 and 8.5 months, there was a progressive and spontaneous elevation of the medial, central, and lateral areas in both eyebrows and saw the same effect in another 24 patients studied between 3.5 and 24 months. In this study, was found spontaneous stretch in the control group and the Sham group already present in the immediate postoperative period, which progressed by days 3 and 7, when it reached its peak. However, the treated groups also showed peak stretch, at days 7 and 45 in Group C, and at day 14 in Group B, suggesting an effect of the reactivity of the fixation method. Our experimental model appears to reflect situations encountered clinically.

Conclusion

This experimental model created acceptable experimental conditions for testing different soft tissue fixation methods. The use of tissue fixatives contributed to better placement of pericranium-cutaneous flaps, and surgical adhesive was superior

to suture anchor in the bone tunnel for fixation of pericranium-cutaneous flaps.

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Comparative study of tissue reactivity to n-butyl-2-cyanoacrylate and nylon monofilament thread on pericranium-cutaneous flaps in rats[1]

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ABSTRACT

PURPOSE: To study the repair of pericranium-cutaneous flaps fixed with suture anchored in a skull bone tunnel or N-butyl-2-cyanoacrylate adhesive in Wistar rats with emphasis on the cellular inflammatory response and the production of types I and III collagen.

METHODS: The operated region in the cephalic region of Wistar rats was removed minutes before euthanasia, fixed in formalin, and subjected to histological preparation. Slides were stained with hematoxylin–eosin and Picrosirius. Standardized counts of polymorphonuclear and mononuclear cells, fibroblasts, and macrophages were performed, and the percentages of types I and III collagen were determined. Data collection occurred on days 3, 7, 14, 21, and 45 postoperatively. A value of $p<0.05$ was considered statistically significant.

RESULTS: Quantitative analysis of the data showed more fibroblasts in the surgical adhesive group than in the nylon monofilament thread groups ($p=0.0211$). Qualitative analysis showed higher reactivity in the adhesive group, with a predominance of polymorphonuclear cells from days 3−45 and macrophages from days 3−7. The amount of type I collagen exceeded 80% in the treated and control groups at the end of the experiment.

CONCLUSIONS: Subperiosteal detachment triggers a cellular inflammatory response that is amplified using soft tissue fixation methods. The adhesive n-butyl-2-cyanoacrylate was more reactive than the nylon monofilament thread anchored in the skull bone tunnel.

Key words: Rhytidoplasty. Cyanoacrylates. Adhesives. Nylons. Wound Healing. Rats.

Introduction

Traction and fixation of soft tissue flaps in facial plastic surgery is performed by means of soft tissue fixation methods, the most common being sutures, screws (metallic or absorbable), and surgical adhesives[1-20].

Cyanoacrylates are cyanoacrylic acid esters with adhesive properties that polymerize at room temperature without a solvent or catalyst and that have one alkyl side chain in their molecular structure. Long chains confer less toxicity than do short chains. Cyanoacrylates thus demonstrate slow degradation in cyanoacetate and formaldehyde, limiting the accumulation of toxic byproducts that must be eliminated by the organism[8-12].

Nylon monofilament threads are traditionally used as surgical sutures in soft tissues and are often compared with surgical adhesives[13-15].

This work aimed to study the repair of pericranium-cutaneous flaps pulled and fixed with a nylon monofilament thread anchored in the skull bone tunnel or with a surgical adhesive, in Wistar rats. The histological tissue reactivity and production of types I and III collagen were also evaluated.

Methods

This study was approved under protocol number 018/2010 by the Ethics in the Use of Animal Experimentation Committee of the State University of Maringa (Brazil).

Thirty-five male Wistar rats (*Rattus norvegicus albinus*) at 120 days of age and weighing 280 to 484g (average, 365.7g) were used. The rats were randomly distributed into three groups of ten animals as follows: Group A, control; Group B, suture anchored in a skull bone tunnel; and Group C, surgical adhesive. The last group of five animals constituted the Sham group (surgical control).

Beginning 24 hours prior to the experiments, the animals were acclimatized in a vivarium and maintained in plastic cages containing a maximum of four animals each. The rats received water and food *ad libitum* and were maintained at room temperature with a 12-h light/dark cycle.

After weighing the animals, the experimental procedure was initiated with intramuscular anesthesia using chlorhydrate 2-(2,6-xylidine)-5,6-dihydro-4H-1,3-thiazine (Rompun 2%; Bayer, Sao Paulo-SP, Brazil) at a dose of 1mg/kg and ketamine chlorhydrate (Ketalar; Pfizer, Guarulhos-SP, Brazil) at a dose of 20mg/kg in a 1:1 ratio. Based on the dose of each drug, the mixture was used for anesthesia at a dose of 1ml/kg of body weight.

With the animal anesthetized, the head was shaved followed by antisepsis of the shaved skin with an alcohol solution including 0.5% chlorhexidine (Riohex; Rioquimica, Sao Jose do Rio Preto-SP, Brazil).

A 5 to 10-mm 3-0steel thread (Aciflex; Ethicon, Sao Paulo-SP, Brazil) was then introduced into the subcutaneous tissue as a dynamic radiological marker to observe the mobility of the flap during the study period. After introducing the radiological marker, the cranium of the animal was immobilized in a cephalostat to permit preoperative radiographs.

A 15-mm transverse cutaneous incision corresponding to the posterior border of the auricular pavilion was created. The incision allowed access to the deep planes and subsequent incision of the periosteum and thus the pericranium.

The subperiosteal flap was detached from the skull using a Freer elevator. The nasal dorsum and lateral orbits of the animal were the distal limits. Next, the elevated flap was suspended with the help of an Adson nipper. At this stage, the experimental model was ready for evaluation of the various fixation methods.

Group A underwent detachment and elevation of the flap without traction. Group B underwent the creation of two skull orifices 4 mm apart using a 2-mm round drill bit and preserving the duramater. A 4-0 nylon monofilament (Mononylon; Ethicon, Sao Paulo-SP, Brazil) thread was passed through from one opening to the other and anchored with a simple suture. The suture was also passed through the periosteum and subcutaneous tissue of the flap to promote and sustain traction on the flap (Figure 1).

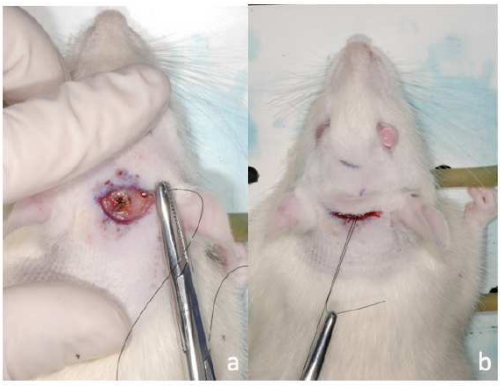

FIGURE 1 – (A) 4-0 nylon monofilament thread through the bone tunnel and **(B)** retracted flap.

Group C underwent flap traction and received 0.25 ml (0.25 mg) per animal of n-butyl-2-cyanoacrylate surgical adhesive (Histoacryl 0.5g; B. Braun Melsungen AG, Melsungen, Germany) over the exposed bone of the skull using the actual ampule with an applicator tip (Figure 2).

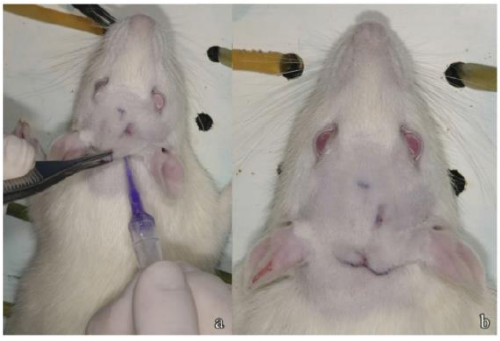

FIGURE 2 – **(A)** Application of n-butyl-2-cyanoacrylate glue and **(B)** retracted flap.

The skin incisions were sutured with three simple interrupted suturesof 5-0 monofilament nylon (Mononylon; Ethicon, Sao Paulo-SP, Brazil).

Postoperative radiographs were taken immediately after the surgery, and control radiographs were taken on postoperative days 3, 7, 14, 21, and 45.

The animals received a single oral dose of paracetamol solution (Tylenol; Janssen-Cilag, Sao Paulo-SP, Brazil) at a dose of 10mg/kg by gavage. Recovery occurred in the animals' cages.

Collection of tissue specimens for histological analysis was performed on days 3, 7, 14, 21, and 45 postoperatively after radiographs, even in anesthetized animals.

A skin incision edge bordering the upper eyelid allowed the removal of radiological marker not to damage the microtome during histological preparation. Proceeded another incision proximally located above the surgical incision and with the aid of a 2-mm round drill bit, there were four holes adjacent the proximal edge of the skull of the animal. Then two other incisions were made in the skin, bordering the edge of the eyelid contralateral arm, and the anterior border.

The four holes in the adjacent proximal edge of the skull facilitated introduction of Metzenbaum scissors to finish sectioning the bone along the path marked by the skin incisions to remove the specimen en bloc for histological study.

The material was fixed in formalin, and a central segment covering the greater extension of the operated region was embedded in paraffin after routine histological preparation. Histological sections 4 µ thick were prepared on two slides for each animal, and then stained with hematoxylin–eosin and Picrosirius[16].

The cellular inflammatory response was examined in the sections stained with hematoxylin–eosin. The same pathologist performed cell counts on five microscopic fields of 330 µm^2 corresponding to one sample from each animal in regions with a predominance of inflammatory cells adjacent to the pericranium at x400 magnification. Cells were sorted in terms of whether they were polymorphonuclear (PMN) or mononuclear (MN), and the numbers of fibroblasts (FIB) and macrophages (MAC) present in the microscopic fields were analyzed (Figure 3).

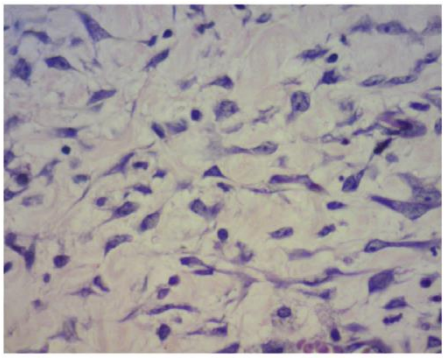

FIGURE 3 – Photomicrograph from Group C on postoperative day 7 (hematoxylin–eosin, x400 magnification).

Images of sections stained with Picrosirius were used to determine the percentages of types I and III collagen. Five different regions of each histological section were measured to obtain the average value of the collagen fibers present. The type I collagen fibers were thick and stained orange to red, and the type III collagen fibers were thin and stained green. Image-Pro Plus 4.0 software (Media Cybernetics Co., Roper Industries, Inc., Sarasota, FL, USA) allowed for the determination of the percentage of fibers of each color and enabled us to highlight them to better illustrate their tissue concentrations (Figure 4).

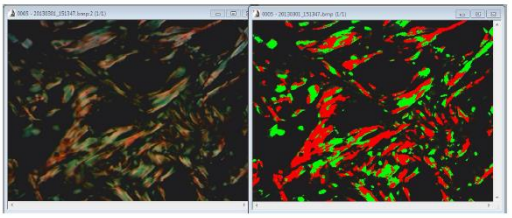

FIGURE 4 - Photomicrograph from Group B on postoperative day 7 with color highlighting of the fibers to the left (Sirius red, x400 magnification).

Five digital photomicrographs of each rat and of each staining were obtained with a Dino-Eye camera AM423X (Dino-Lite, An Mo Electronics Corporation, New Taipei City, Taiwan).

Histological analysis was performed at the Laboratory of Experimental Pathology, Pontifical Catholic University of Parana in Curitiba, Brazil.

Quantitative data analysis, ANOVA analysis of variance, Tukey's test, and a T-test with a significance level of 5% were performed.

Results

Surgery with subperiosteal detachment led to an increase in the number of inflammatory cells at the surgical site. Both PMN and MN cells were present, the latter in larger quantities, but without a significant difference (Table 1).

TABLE 1 - Mean values of inflammatory polymorphonuclear (PMN) and mononuclear (MN) cells in Groups Sham, A, B, and C at five time points.

		Day 3	Day 7	Day 14	Day 21	Day 45
Sham	PMN	0.2	0.2	0.2	0.2	0.2
	MMN	1.8	2	2	0.8	2.2
Group A	PMN	0.2	2.5	2.5	0.7	0.2
	MMN	8.2	6.2	5.9	3.7	1.2
Group B	PMN	2.4	3.1	2.1	0	0
	MMN	4.4	4.6	5.8	1.5	0.8
Group C	PMN	3.4	3.6	2.5	1.5	0.2
	MMN	8	5.5	4.4	3.5	0.8

* ANOVA significance level of 5%

The qualitative data analysis demonstrated the effect of the surgery. An inflammatory response was triggered in Groups A, B, and C; it peaked on postoperative day 7 and declined from postoperative day 14 onward. Throughout the repair process, there was a predominance of MN cells in Groups Sham, A, B, and C; however, from postoperative day 3, the PMN count showed an inflammatory effect caused by the soft tissue fixation method used (suture anchored in the skull bone tunnel was less reactive than surgical adhesive). The PMN count remained higher in Group C from postoperative days 3–45, than in the other groups (Figure 5).

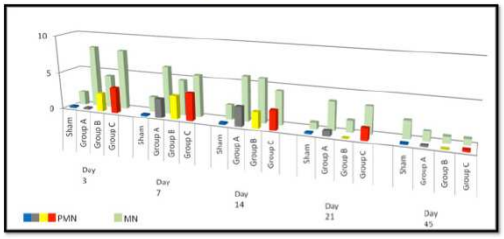

FIGURE 5 – Distribution of inflammatory polymorphonuclear (PMN) and mononuclear (MN) cells in Groups Sham, A, B, and C at five time points.

Subperiosteal detachment triggered tissue repair with fibroblast migration from postoperative days 3–7 and a decrease from postoperative day 14 on ward. This effect was more intense in Group C, which was treated with surgical adhesive (Table 2, Figure 6). There was a significant difference in the number of fibroblasts between Groups C and B on postoperative day 7 (p=0.0211) (Table 2, Figure 7).

TABLE 2 - Mean values of fibroblasts in Groups Sham, A, B, and C at five time points.

	Day 3	Day 7	Day 14	Day 21	Day 45
Sham	15	17	25.4	24	20.2
Group A	32.8	38	36.6	23.1	21.8
Group B	29.6	19.3**	26.4	19.7	14.8
Group C	32	47.7**	32.3	27.8	24.2

* ANOVA significance level of 5%
** T-test p=0,0211

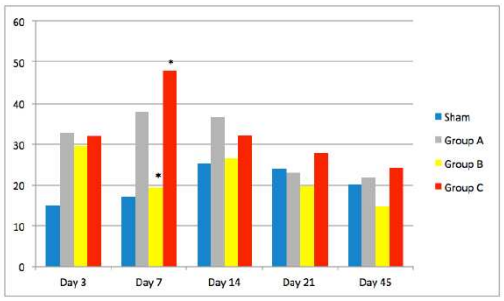

FIGURE 6 – Distribution of fibroblasts in Groups Sham, A, B, and C at five time points.

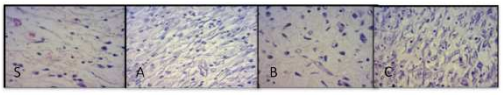

FIGURE 7– Photomicrographs from Groups Sham (S), A, B, and C on postoperative day 7 (hematoxylin–eosin staining, x400 magnification).

There was a predominance of macrophages in Group C on postoperative days 3 and 7, but without statistical significance (Table 3).

TABLE 3 - Mean values of macrophages in Groups Sham, A, B, and C at five time points.

	Day 3	Day 7	Day 14	Day 21	Day 45
Sham	1.4	1	1	0.4	0.4
Group A	2.3	2.6	2.9	3	1.6
Group B	2.3	2.3	2.5	0.8	1.4
Group C	3.5	3.3	2.3	2.6	0.8

*ANOVA significance level of 5%

At the end of the experiment, there was a predominance of type I collagen fibers in the three operative groups without significant differences, but in excess of 80%. This result highlights the role of subperiosteal detachment as a stimulus for repair (Figure 8).

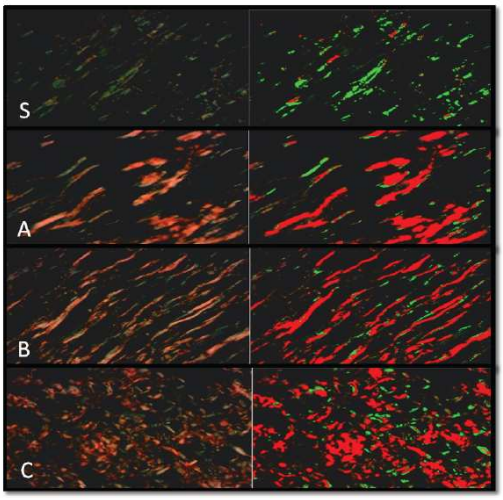

FIGURE 8 – Photomicrographs from Groups Sham (S), A, B, and C on postoperative day 45, with color highlighting of the fibers to the left (Sirius red, x400 magnification).

The surgical adhesive resulted in the highest collagen production at the end of the experiment in the operative groups to the order of 70.4% relative to the values on postoperative day 3 (Table 4, Figure 9).

TABLE 4 - Mean percentages of type I collagen in Groups Sham, A, B, and C at five time points.

	Day 3	Day 7	Day 14	Day 21	Day 45
Sham	34,38	24,68	82,54	72,74	70,74
Group A	75,72	40,95	75,57	78,23	86,63
Group B	65,36	58,45	72,36	64,26	86,81
Group C	49,26	69,46	72,4	65,65	83,92

* ANOVA significance level of 5%

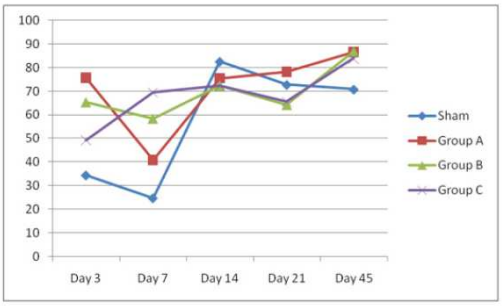

FIGURE 9 – Distribution of percentages of type I collagen in Groups Sham, A, B, and C at five time points.

Discussion

The study of tissue reactivity in surgery indirectly contributes to the prediction of results, so that measures taking and surgical planning, especially in plastic surgery. A successful outcome occurs in the proportion that the inflammatory response, which is individual, single, singular, manifests itself in a positive way for that individual, that is also unique, even within a group of the same species.

In this experimental study, subperiosteal detachment resulted in a more intense inflammatory response from postoperative day 3 in the groups that underwent flap fixation. The same effect was seen in Group A (control) from postoperative day 7. In all three operative groups, the inflammatory response declined on postoperative day 21.This demonstrates the need to perform displacements that promote wound healing, when repositioning the flap in the desired position. Likewise, the presence of soft tissue fixation methods increased the inflammatory reaction, thereby increasing collagen deposition and scar maturation. This was shown in the treated groups, in which the deposition of type I collagen was higher than that in the control group on postoperative day 7. Although the degree of collagen deposition was similar among the three groups at the end of the experiment, when the flap displacement was analyzed with a radiological control, we found the need to use soft tissue fixation methods to maintain the proposed tissue repositioning[18].

The initial number in the study design of 15 animals in Group A and for each treated group was not approved by the Ethics in the Use of Animal Experimentation Committee, reducing the statistical significance.

Saltz and Zamora[7]reported that endoscopic frontal rhytidectomy avoids a bicoronal incision. However, the ideal soft tissue fixation method was controversial and the authors

reported the use of fibrin glue with good hemostatic function and for tissue fixing under a light pressure dressing. In the same year, Mixter[19] reported the use of adhesive n-butyl-2-cyanoacrylate in endoscopic frontal rhytidectomy as a safe and inexpensive fixative, and although he saw foreign body reaction in some patients, this resolved with the removal of the adhesive fragments. In our study, we emphasized the importance of developing an experimental model for the study of soft tissue fixation methods and their tissue reactions. We found that the adhesive n-butyl-2-cyanoacrylate is more reactive than suture anchored in a skull bone tunnel; thus, adhesive promotes increased migration of PMN cells and fibroblasts corresponding to the higher repositioning of the flaps in the long term[18]. We also observed during the collection of specimens for histological analysis the presence of cover slip adhesive polymerized between the soft tissue and the bone plate. This finding corresponded to that of Mixter[19]and caused a chronic foreign body reaction. In our study, we found a larger amount of fibroblasts in Group C on postoperative days 3 and 7, but an absence of multinucleated giant cells.

Mastieri *et al*.[12] studied the inflammatory response of n-butyl-2-cyanoacrylate implanted in the subcutaneous tissue of rats and observed no significant differences compared with controls, concluding that this adhesive is biocompatible. Mugali *et al*.[14] showed that in rats, this adhesive causes less stimulation of TNF alpha and IL-1beta than does catgut; it is also less immunogenic. We observed that the number of MN cells was high in all groups during the five days of measurement, but the humoral inflammatory response was not assessed.

Batista *et al*.[15]compared sutures in the abdominal wall of rats that underwent operations using 3-0 nylon monofilament thread and adhesive n-butyl-2-cyanoacrylate. The suture time was faster in the adhesive group, and although there was one case of wound dehiscence and abscess formation, this group showed higher tensile strength than did the suture group on postoperative day 14. In our study, we observed easy flap fixation and less surgical time when using adhesive and no occurrences of dehiscence or abscess formation. We believe that these reactions may be associated with the amount of adhesive applied.

Sadato *et al*.[20] studied the effect of dilutions of n-butyl-2-cyanoacrylate/ethiodol in proportions of 20:80 and 50:50 for embolization of the renal arteries of rabbits and quantified the macrophages and neutrophils. No significant differences were seen throughout the study, nor were there differences in the degree of injury to the vascular wall. We believe that dose-control studies may be able to identify less reactive doses of n-butyl-2-cyanoacrylate. The development of devices with gradual adhesive-releasing capabilities or even less reactive adhesive isomers for use within soft tissues may also be possible.

Conclusions

Subperiosteal detachment triggers a cellular inflammatory response that is amplified when soft tissue fixation methods are used. The adhesive n-butyl-2-cyanoacrylate is more reactive than nylon monofilament thread anchored in a skull bone tunnel.

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4.Discussão

O modelo experimental proposto é de fácil reprodutibilidade e utiliza instrumentais e materiais de fácil obtenção. Desta forma, buscou-se preencher uma lacuna na pesquisa no campo da Cirurgia Plástica. A escolha de ratos para compor as amostras, foi feita pelo fato destes animais serem resistentes ao procedimento anestésico, havendo baixo risco de morte, são comumente criados em biotérios de vários centros de pesquisa, o que torna o nosso modelo experimental mais reprodutível, ampliando as possibilidades de estudo de fixadores para tecidos moles.

O marcador radiológico foi elemento essencial na coleta de dados dinâmicos. Neste estudo, ela foi inserida completamente na tela subcutânea e não ficou dispersa entre o pericrânio e a calota craniana, como poderíamos supor, pois era visível a elevação do retalho. As medidas radiológicas foram feitas do ponto médio do marcador radiológico até o ponto mais distal do osso nasal esquerdo. O ponto médio da marca radiológica pode ser considerado o eixo central da posição tridimensional que este elemento assume entre os tecidos moles, de forma que deslocamentos em suas extremidades não influenciaram nas medidas obtidas.

A necessidade de se criar um cefalostato, nos fez compor diversos modelos. Por fim, chegou-se a um modelo prático e confiável utilizando um conjunto de hastes móveis, destinadas ao suporte de computador portátil. Esta, fixada sobre uma prancha de madeira, permitiu a imobilização da cabeça do rato e, consequente, padronização das radiografias.

O filme oclusal, para radiografias odontológicas, apresenta dimensões compatíveis com a área anatômica a ser estudada, facilitando a obtenção de imagens. Também deram praticidade ao modelo experimental pela facilidade de se repetir uma radiografia, caso necessário.

Os animais utilizados foram propositalmente maiores, com peso na faixa de 350g, de modo a facilitar os procedimentos cirúrgicos, a documentação radiológica e a coleta de amostras para exame histológico (anexo 1). Um número de 15 animais para os grupos tratados não foi autorizado pelo Comitê de Ética no Uso de Animais para Experimentação, o que diminuiu a significância estatística.

O retalho pericrânio-cutâneo elevado delimita um espaço similar à cavidade óptica obtida nas ritidoplastias frontais endoscópicas, estando assim pronto para receber os fixadores teciduais a serem estudados. Também não foram realizadas ressecções cutâneas nos animais deste estudo. Desta forma, estes três elementos: descolamento subperiostal amplo, semelhante a uma cavidade óptica, fixadores teciduais e ausência de ressecção cutânea reforçam este modelo para o estudo de fixadores teciduais utilizados em ritidoplastias frontais endoscópicas. Segundo Ramirez, 1994 (4), com o passar da idade, a ação contínua dos músculos depressores e a força da gravidade promovem a

ptose da cauda dos supercílios. Assim sendo, a força da gravidade pode ser um fator interveniente no estudo de casos clínicos, mas este modelo experimental desenvolvido parece ser menos influenciado por esta força, em virtude da posição horizontal do retalho.

A operação foi na maioria dos casos exangue. Não houve laceração da dura-máter dos animais nos quais foram realizados túneis ósseos. Isto foi devido à característica da broca e diminuição da rotação, com a progressão da mesma no delgado osso da calota craniana do rato. O túnel ósseo obtido nos ratos se assemelha aos descritos na literatura em humanos (Figura 1).

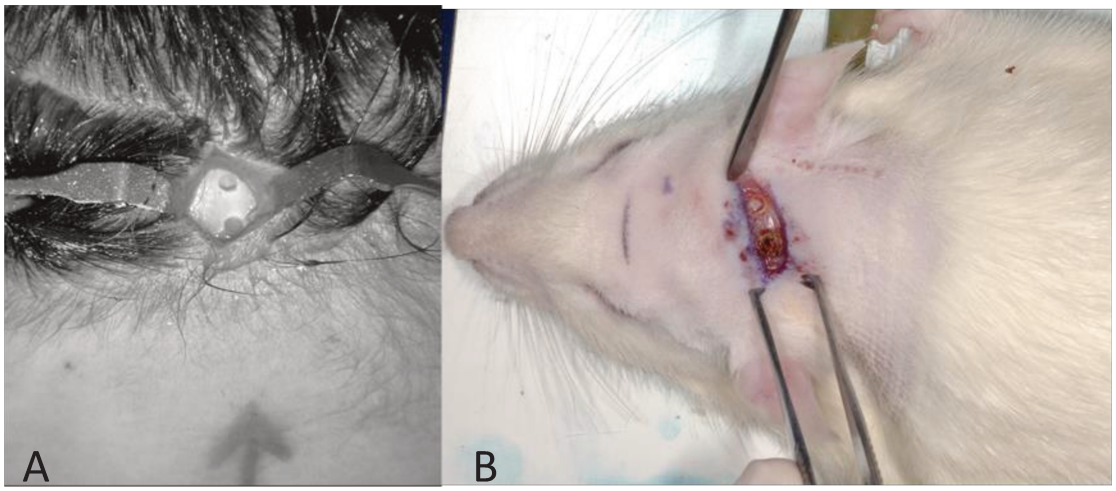

***Figura 1**. A) Túnel ósseo em humanos (Jones et al (10)). B) Túnel ósseo em animal do Grupo B.*

O estiramento progressivo do retalho pericrânio-cutâneo no grupo controle a partir do 3º dia, estendendo-se até o 7º dia, sugere que os meios de fixação poderiam ser dispensáveis. Troilius (11) advoga que os meios de fixação seriam dispensáveis nas ritidoplastias frontais endoscópicas, para a

obtenção de resultados mais naturais em pacientes. Neste estudo experimental em ratos, observamos que os grupos que receberam algum tipo de tração apresentaram, ao final do experimento, um posicionamento do retalho pericrânio-cutâneo superior às suas próprias medidas iniciais e superiores a controle, no mesmo momento.

Observamos nos grupos tratados, um retrocesso na tração feita no retalho pericrânio-cutâneo a partir do 3º dia, seguida de uma recuperação do estiramento no 7º dia. Kriet et al. (1) estudando a força tênsil, medida com tensiômetro em retalhos pericrânio-cutâneos de coelhos, que não receberam qualquer tipo de fixação periostal, observaram uma diminuição desta força a partir do 3º dia de pós-operatório e uma recuperação a partir do 8º dia de pós-operatório que se manteve, indiferentemente do tempo, até a última aferição feita no 28º dia pós-operatório. Romo et al. (2), observaram uma mínima aderência periostal nos primeiros dias de pós-operatório e uma aderência progressiva no decorrer do tempo, até 12 semanas, em coelhos, sendo que o tempo mínimo para a aderência periostal foi de seis semanas. No presente estudo não foi aferida a força tênsil, mas a recuperação das medidas do estiramento do retalho periósteo-cutâneo, reduzidas em algumas fases do processo cicatricial, demonstrou o provável efeito dos fixadores teciduais para esta recuperação, agregando, provavelmente, uma maior resposta inflamatória ao processo e consequentemente maior estiramento do retalho a longo prazo. Isto foi observado no grupo adesivo, que produziu uma resposta inflamatória mais intensa, provavelmente pelo efeito do calor dispersado nos tecidos na reação exotérmica, gerada na polimerização deste adesivo tecidual logo após a

operação, mas com repercussão até a fase tardia do reparo (20,22), mesmo que entre os cianoacrilatos, este tenha menor toxicidade tecidual, por ser de cadeia longa e ter uma liberação reacional gradativa (23). Também, Romo et al. (2) que compararam coelhos submetidos a técnicas de ritidoplastia subperiostal coronal, com ressecção de pele, e endoscópica com uso de parafuso absorvível, como fixador, e sem resecção de pele, concluem que o uso de fixador permanente ou semipermanente melhora a acurácia e a manutenção precoce dos resultados pós-operatórios. Neste estudo experimental, o efeito do descolamento sub-periostal resultou em uma reação inflamatória, mais intensa, a partir do 3º dia nos grupos B e C, acompanhada pelo grupo controle A. A partir do 7º dia e com declínio evidenciado nos três grupos ao 21º dia. Isto demonstra a necessidade de existir descolamentos que resultem em reparação, para que um objetivo de reposicionamento tecidual seja concretizado. Da mesma forma, a presença de fixadores teciduais incrementa a reação inflamatória, aumentando com isso a deposição de colágeno e a maturação cicatricial, como podemos ver nos grupos tratados, onde a deposição de colágeno tipo I é superior, em relação ao controle no 7º dia. Embora sejam equivalentes, nos três grupos, ao final do experimento, quando analisamos com controle radiológico o deslocamento dos retalhos, constatamos a necessidade do uso de fixadores teciduais, para a manutenção do reposicionamento tecidual proposto, pois observamos que a posição final do marcador radiológico nos grupos tratados supera a posição inicial obtida com a cirurgia, reforçando as qualidades do modelo experimental, ora descrito.

Graf et al. (7) analisaram um grupo de 72 pacientes submetidos a ritidoplastia frontal endoscópica distribuídos em dois grupos, um dos 38 pacientes, estudados no tempo pós-operatório entre 3,5 a 8,5 meses e outro de 24 pacientes, estudados entre 4,5 meses e 29 meses. Foi observado no grupo de 38 pacientes, um aumento progressivo e espontâneo da elevação dos supercílios a partir de 3,5 meses e aos 8,5 meses, o mesmo ocorrendo no grupo de 24 pacientes no intervalo médio de 24,5 meses. Em nosso estudo experimental, observamos o estiramento espontâneo, no grupo controle, já no pós-operatório imediato, quando comparado ao grupo Sham e progressivo no 3º e no 7º dia quando atinge seu pico. Os grupos tratados, também, apresentaram picos de estiramento, o grupo embucrilato no 7º dia e o grupo túnel ósseo no 14º dia. Isto nos sugere que esta variação está associada à reatividade ao descolamento subperiostal e ao meio de fixação, e o modelo experimental descrito parece reproduzir situações observadas em casuísticas clínicas.

Saltz e Zamora (16), em 1998, relataram que a ritidoplastia frontal endoscópica evita a incisão bicoronal, porém o ponto controverso seria o meio de fixação ideal e relatam o uso de cola de fibrina, com boa função hemostática, e como fixadora tecidual sob um curativo de leve pressão. Também, no mesmo ano, Mixter (26) relata uso de adesivode n-butil-2-cianoacrilato em ritidoplastia frontal endoscópica, como um fixador seguro e de baixo custo, embora tenha presenciado reação de corpo estranho em algumas pacientes, solucionada com a remoção de fragmentos do adesivo. Neste estudo, ressaltamos a importância do desenvolvimento de um modelo experimental para o estudo dos fixadores

teciduais e correspondentes respostas teciduais e pudemos constatar que o adesivo de n-butil-2-cianoacrilato foi mais reativo que o fio de sutura, promovendo maior migração de polimorfonucleares e fibroblastos, com correspondência à maior fixação dos retalhos a longo prazo. Também observamos na coleta dos especimes, para análise histologica, a presença de uma lamínula do adesivo polimerizado, entre o tecido mole e a tábua óssea, que corresponderia ao encontrado por Mixter (24), sendo o causador de reação de corpo estranho crônica. Em nosso estudo houve uma maior quantidade de fibroblastos no grupo C, no 3ºe 7º dia, mas com ausência de células gigantes polinucleadas.

Mastieri et al. (22) estudaram a reação inflamatória do n-butil-2-cianoacrilato implantado na tela subcutânea de ratos e não observaram diferenças significantivas em relação ao controle, concluindo que este adesivo é biocompatível na tela subcutânea de ratos. Muglaliet al.(18) demonstraram que este adesivo, em ratos, gera menor estímulo de TNF alfa e IL1 beta, que o fio de catgut, sendo também menos imunogênico. Pudemos observar que a celularidade, representada por monomorfonucleares, foi alta em todos os grupos, durante os cinco tempos de aferição, porém a resposta inflamatória humoral não foi aferida.

Batista et al. (19) compararam suturas da aponeurose da parede abdominal, em ratos, realizadas com fio monofilamentar 3.0 e adesivo n-butil-2-cianoacrilato. No grupo adesivo, o tempo de sutura foi mais rápido, houve um abcesso e uma deiscência de sutura, mas, no 14º dia este grupo demonstrou maior força tênsil que o grupo da sutura. Neste estudo não avaliamos a força

tênsil, mas observamos maior facilidade e menor tempo cirúrgico com o uso do adesivo, sem a presença de deiscências ou abcessos, porém acreditamos que estas reações possam decorrer da quantidade de adesivo aplicados.

Sadato et al. (25) estudaram o efeito de diluições do n-butil-2-cianoacrilato/etiodol, nas proporções 20:80 e 50:50, na embolização de artérias renais de coelhos e quantificaram, macrófagos e neutrófilos, sem diferenças significantes em todo o estudo, assim como não houve diferenças no grau de injúria da parede vascular. Acreditamos que estudos dose-controle possam identificar possíveis doses menos reativas de n-butil-2-cianoacrilato, ou possam ainda ser desenvolvidos dispositivos de liberação gradativa ou isômeros menos reativos do adesivo, para uso no interior de tecidos moles.

5.Conclusões

1) O modelo experimental desenvolvido permitiu criar condições experimentais para o estudo de diferentes fixadores de tecidos, na região da calota craniana de ratos Wistar.

2) O descolamento subperiostal desencadeou uma resposta inflamatória celular que foi amplificada pelo uso de fixadores teciduais, contribuindo para o melhor posicionamento de retalhos periósteo-cutâneos,.

3) O adesivo n-butil-2-cianoacrilato foi mais reativo que o fio de nylon monofilamentar ancorado em túnel ósseo.

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7.Anexos

7.1.Anexo 1. Tabelas.

Tabela 1: Descrição dos pesos dos animais nos grupos.

Amostra	Sham	Grupo A	GrupoB	Grupo C
R1	300	415	375	348
R2	393	485	325	395
R3	360	350	380	420
R4	378	365	372	302
R5	365	430	360	427
R6		280	375	310
R7		385	362	360
R8		395	345	365
R9		287	368	380
R10		303	335	336
Médias	359,2	369,5	359,7	364,3

Tabela 2 : Descrição do deslocamento em milímetros de marcas radiológicas posicionadas no tecido celular subcutâneo cefálico de ratos Wistar, em cinco momentos.

Amostra	Pré-operatório				Pós-operatório				Dia 3				Dia 7				Dia 14				Dia 21				Dia 45			
Estatísticas																												
	Sham	Contr.	T.Oss.+pon.	Embu.	Sham	Contr.	T.Oss.+pon.	Embu.	Sham	Contr.	T.Oss.+pon.	Embu.	Sham	Contr.	T.Oss.+pon.	Embu.	Sham	Contr.	T.Oss.+pon.	Embu.	Sham	Contr.	T.Oss.+pon.	Embu.	Sham	Contr.	T.Oss.+pon.	Embu.
R 1	16,3	20,2	18,8	27,4	16,3	23	24,5	24,3	14.7	21,4	21,2	21,2																
R 2	24,3	19	25,9	22,3	24,3	20	25,3	18,5		23,2	27,9	22,3	21.8															
R 3	23,6	21,8	19,9	24,7	23,6	22,1	20,5	35,2						23,6	25,4	27,5	23.9											
R 4	30,8	18,7	20	17,3	30,8	21	20,5	22,5						22,5	26,7	26,5					27.1							
R 5	22,5	17,1	18,2	21,1	22,5	18,3	24,5	20,9										15,8	22,4	19,9					19.1			
R 6		18,8	24,1	20,2		20,4	32,9	22,8										23,4	24,4	31,8								
R 7		18,1	14,3	16,3		23,1	23,9	17,5														22,4	26,1	17,7				
R 8		21,6	22,3	18,7		17,7	29,5	24,4														21,3	29,4	24,3				
R 9		15,7	22,5	20,5		15,3	26,6	21,1																		14,8	22,8	25,3
R 10	18.8		22,2	19,5		18,4	25,8	20																		16,3	23,1	20,8
n	5	10	10	10	5	10	10	10	1	2	2	2	1	2	2	2	1	2	2	2	1	2	2	2	1	2	2	2
Mínimo	16,30	15,70	14,30	16,30	16,30	15,30	20,50	17,50	_	21,40	21,20	21,20	_	22,50	25,40	26,50	_	15,80	22,40	19,90	_	21,30	26,10	17,70	_	14,80	22,8	20,8
Máximo	30,80	21,80	25,90	27,40	30,80	23,10	32,90	35,20	_	23,20	27,90	22,30	_	23,60	26,70	27,50	_	23,40	24,40	31,80	_	22,40	29,40	24,30	_	16,3	23,1	25,30
Média	23,50	19	20,82	20,80	23,50	19,93	25,40	22,72	_	22,30	24,55	21,75	_	23,05	26,05	27,00	_	19,60	23,40	25,85	_	21,85	27,75	21,00	_	15,55	22,95	23,05
Desvio Padrão	5,167	1,912	3,393	3,327	5,167	2,573	5,502	4,756	_	1,273	4,738	0,7778	_	0,7778	0,9192	0,7071	_	5,374	1,414	8,415	_	0,7778	2,333	4,667	_	0,7071	0,1	2,828
Erro Padrão	2,311	0,6046	1,073	1,052	2,311	0,8138	1,740	1,504	_	0,9000	3,350	0,5500	_	0,5500	0,6500	0,5000	_	3,800	1,000	5,950	_	0,5500	1,650	3,300	_	0,5000	0,1	2,000
Soma	117.5	171	208,2	208	117.5	199,3	254	227,2	14.7	44,60	49,10	43,50	21.8	46,10	52,10	54,00	23.9	39,20	46,80	51,70	27.1	43,70	55,50	42,00	19.1	31,1	45,9	46,1

Tabela 3 : Descrição das porcentagens médias de colágeno tipoI, aferidas em cinco campos microscópicos do tecido celular subcutâneo cefálico justaperiostalde ratos Wistar, em cinco momentos (aumento 400x).

	Dia 3				Dia 7				Dia 14				Dia 21				Dia 45			
Amostra Estatísticas	Sham	Controle	T.Oss.+sut	Adesivo	Sham	Controle	T.Oss.+sut	Adesivo	Sham	Controle	T.Oss.+sut	Adesivo	Sham	Controle	T.Oss.+sut	Adesivo	Sham	Controle	T.Oss.+sut	Adesivo
R 1	34,38	67,58	53,64	46,32																
R 2		83,86	77,08	52,20																
R 3					24,68	21,92	35,92	48,06												
R 4						59,98	80,98	90,86												
R 5									82,54	70,76	61,12	69,28								
R 6										80,38	83,60	75,52								
R 7													72,74	74,50	38,74	53,66				
R 8														81,96	89,78	77,64				
R 9																	70,74	86,28	86,26	83,48
R 10																		86,98	87,36	84,36
n	1	2	2	2	1	2	2	2	1	2	2	2	1	2	2	2	1	2	2	2
Mínimo	-	67,58	53,64	46,32	-	21,92	35,92	48,06	-	70,76	61,12	69,28	-	74,50	38,74	53,66	-	86,28	86,26	83,48
Máximo	-	83,86	77,08	52,20	-	59,98	80,98	90,86	-	80,38	83,60	75,52	-	81,96	89,78	77,64	-	86,98	87,36	84,36
Média	-	75,72	65,36	49,26	-	40,95	58,45	69,46	-	75,57	72,36	72,40	-	78,23	64,26	65,65	-	86,63	86,81	83,92
Desvio Padrão	-	11,51	16,57	4,158	-	26,91	31,86	30,26	-	6,802	15,90	4,412	-	5,275	36,09	16,96	-	0,4950	0,7778	0,6223
Erro Padrão	-	8,140	11,72	2,940	-	19,03	22,53	21,40	-	4,810	11,24	3,120	-	3,730	25,52	11,99	-	0,3500	0,5500	0,4400
Soma	34,38	151,4	130,7	98,52	24,68	81,90	116,9	138,9	82,54	151,1	144,7	144,8	72,74	156,5	128,5	131,3	70,74	173,3	173,6	167,8

**ANOVA: nível de significância a 5%

Tabela 5 : Descrição das porcentagens médias de monomorfonucleares neutrófilos, aferidas em cinco campos microscópicos do tecido celular subcutâneo cefálico justaperiostalde ratos Wistar, em cinco momentos (aumento 400x).

	Dia 3				Dia 7				Dia 14				Dia 21				Dia 45			
Amostra Estatísticas	Sham	Controle	T.Oss.+sut	Adesivo	Sham	Controle	T.Oss.+sut	Adesivo	Sham	Controle	T.Oss.+sut	Adesivo	Sham	Controle	T.Oss.+sut	Adesivo	Sham	Controle	T.Oss.+sut	Adesivo
R 1	1.8	7.2	6.2	8.6																
R 2		9.2	2.6	7.4	2															
R 3					1.6	5.4	3.4		2											
R 4					10.8	3.8	7.6						0.8							
R 5									1.2	5.8	5.8						2.2			
R 6									10.6	5.8		3								
R 7													2	1.8		2				
R 8													5.4	0.8		5				
R 9																	1.1	0.7		1
R 10																	1.3	0.9	0.6	
n	1	2	2	2	1	2	2	2	1	2	2	2	1	2	2	2	1	2	2	2
Média		8,200	4,400	8,000		6,200	4,600	5,500		5,900	5,800	4,400		3,700	1,500	3,500		1,200	0,8000	0,8000
Desvio Padrão		3,553	3,169	3,432		6,015	1,897	3,171		5,259	1,619	2,366		3,020	1,269	2,121		1,643	0,8367	1,095
Erro Padrão		1,123	1,002	1,085		1,902	0,6000	1,003		1,663	0,5121	0,7483		0,9551	0,4014	0,6708		0,7348	0,3742	0,4899
Soma	1.8	16.4	8.8	16	2	12.4	9.2	11	2	11.8	11.6	8.8	0.8	7.4	2.6	7	2.2	2.4	1.6	1.6