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Wound repair in rabbits using autologous biomaterials combined with rosuvastatin

Cicatrização de feridas em coelhos com biomateriais autólogos associados à rosuvastatina

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Abstract: Autologous platelet-rich plasma (aPRP) and autologous platelet-rich fibrin (aPRF) are blood-derived biomaterials that potentially enhance wound healing. Rosuvastatin (RSV), a lipid-lowering statin, exhibits pleiotropic effects that may promote tissue repair, warranting investigation into its use alone or combined with biomaterials for wound healing. This study aims to evaluate the wound repair effects of aPRP and aPRF, with or without adding 1.2% rosuvastatin. Sixteen clinically healthy adult male New Zealand rabbits were randomly assigned to two groups of eight, each receiving one of the biomaterials either with or without 1.2% rosuvastatin. The biomaterials used were of autologous origin, specifically aPRP and aPRF. Surgical wounds were induced and treated with biomaterials and 1.2% rosuvastatin over 17 days. Macroscopic assessments of wound area and epithelial gap distance were conducted, supplemented by histological analysis. A significant inverse correlation was observed between wound area and epithelial thickness with the use of aPRF ($r = -0.5500$). No significant difference was found in epithelial thickness between treatment groups ($p > 0.05$). In terms of the wound area, aPRP alone ($p = 0.001$), aPRF alone ($p = 0.021$), and aPRP+RSV ($p = 0.016$) treatments yielded smaller wound areas compared to aPRF+RSV at 14 days post-treatment. These findings suggest that the addition of 1.2% rosuvastatin to aPRP resulting in a smaller wound area compared to aPRF, enhances wound repair.

Keywords: biocompatible materials; statins; lipid-lowering drug; wound repair.

Resumo: O plasma rico em plaquetas autologo (PRPa) e a fibrina rica em plaquetas autologa (FRPa) são biomateriais derivados do sangue com potencial promissor para uso na otimização do processo de cicatrização de feridas. A rosuvastatina (RSV), uma estatina hipolipemiante, apresenta efeitos pleiotrópicos que podem melhorar a cicatrização, justificando o estudo de seu uso isoladamente ou em combinação com outros materiais para tais fins. Este estudo teve como objetivo avaliar a reepitelização de feridas com o uso do PRPa e FRPa autólogos associados ou não à rosuvastatina 1,2%. Foram utilizados dezesseis coelhos machos adultos da raça Nova Zelândia, clinicamente saudáveis, alocados aleatoriamente em dois grupos de 8 animais, cada grupo representando um biomaterial associado ou não à rosuvastatina 1,2%. As feridas cirúrgicas foram induzidas e tratadas

com biomateriais e rosuvastatina 1,2% por 17 dias. Avaliação macroscópica da área total das feridas e a distância do epitélio em cortes histológicos foram realizadas. A correlação entre área de ferida e espessura de epitélio foi inversamente proporcional e com maior intensidade com o uso da FRPa (r = -0,5500). Não foi observada diferença na espessura de epitélio entre os grupos (p > 0,05). A área da ferida do PRPa isolado (p=0,001), FRPa isolada (p=0,021) e PRPa+RSV (p=0,016) foram menores comparadas ao FRPa+RSV aos 14 dias. Esses achados sugerem que a rosuvastatina associada ao PRPa resultou em áreas menores de ferida comparada à PRFa, otimizando a cicatrização.

Palavras-chave : materiais biocompatíveis; estatinas; fármaco hipolipemiante; cicatrização.

1. Introduction

Tissue repair is a complex, multi-phase process. Re-epithelialization, essential for effective wound closure, relies on the balanced and synchronized mechanisms of progenitor cell proliferation, differentiation, and maturation into epithelial cells [1]. Prolonged inflammation can disrupt these processes, potentially leading to chronic wound conditions and impaired healing [2].

Blood-derived biomaterials are increasingly studied for their potential to optimize the healing process [3,4,5,6]. Platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) are notable for their high platelet and growth factor concentrations [7, 8], which support healing by enhancing collagen synthesis [9], stimulating fibroblast activity, and promoting angiogenesis [10]. The combined use of biomaterials and pharmacologic agents may further enhance wound repair efficacy.

Rosuvastatin (RSV), a lipid-lowering statin, has demonstrated anti-inflammatory properties beneficial to wound healing and dentistry [11]. It promotes endothelial proliferation [12], angiogenesis, lymphangiogenesis [13], and fibroblast recruitment [10], all of which contribute to tissue repair. Topical administration offers several advantages over oral delivery, including reduced hepatic degradation, lower systemic side effects, easy application, suitability for large surface areas, accelerated healing, and reduced bacterial resistance [14, 15].

This study aims to assess the healing efficacy of autologous platelet-rich plasma (aPRP) and autologous platelet-rich fibrin (aPRF), with or without 1.2% rosuvastatin, by evaluating wound contraction and epithelial thickness. We also explore correlations between these parameters, hypothesizing that biomaterials combined with rosuvastatin will promote faster wound closure.

2. Material and methods

This study was approved by the Ethics and Animal Use Committee (CEUA) of the University of Oeste Paulista (UNOESTE) under protocol number 3840, adhering to the guidelines of the National Council for the Control of Animal Experimentation [16], the Guide for the Care and Use of Laboratory Animals [17], and ARRIVE guidelines [18].

Sixteen clinically healthy adult male New Zealand rabbits (average weight 3 ± 1 kg) were housed individually and provided with food and water ad libitum. The rabbits were

randomly divided into two groups of eight, with each group representing one biomaterial, used alone or combined with 1.2% rosuvastatin, and a control wound. The biomaterials included autologous PRP and PRF. Four equidistant dorsal wounds were induced on each rabbit: one treated with 0.9% sodium chloride as a control, one with an autologous biomaterial (aPRP or aPRF) alone, one with 1.2% rosuvastatin gel alone, and one with a biomaterial combined with 1.2% rosuvastatin (aPRP+RSV or aPRF+RSV).

Rosuvastatin 1.2% was prepared in both liquid and gel forms, with 20% glycerin and methylcellulose polymer (Aristofelx® Gel, São Paulo, Brazil) as vehicles, homogenized in a water bath at 50–60°C, following Grover et al. [19]. Autologous biomaterials were prepared by collecting an average of 4 mL of blood from the auricular vein using a 23 G scalp needle. For autologous platelet-rich plasma (aPRP), blood was collected into tubes with sodium citrate anticoagulant and subjected to a double centrifugation protocol [20,9]: initially at 200 G for 10 minutes (Excelsa Baby 206R, Fanem) to separate platelet-poor plasma (PPP) from other blood constituents. PPP was then mixed with 200 µL of the red cell fraction and centrifuged again at 400 G for 10 minutes, yielding aPRP. In another tube, 400 µL of aPRP was combined with 100 µL of 10% calcium gluconate for activation. For treatments requiring aPRP with 1.2% rosuvastatin, 200 µL of each component was used, along with 100 µL of 10% calcium gluconate, for a final treatment volume of 0.5 mL in both cases [21,22].

For autologous platelet-rich fibrin (aPRF), blood was collected into anticoagulant-free tubes, allowed to coagulate naturally, then centrifuged at 200 G for 10 minutes as described by Azevedo (2014) [23]. The upper portion containing aPRF was separated for use alone or combined with 1.2% rosuvastatin gel [10].

The anesthesia protocol involved intramuscular administration of 2% xylazine hydrochloride (Xilazin®) and tiletamine hydrochloride with zolazepam (Zoletil® 50) at doses of 5 mg/kg and 15 mg/kg, respectively, followed by 0.1 mL of 2% lidocaine hydrochloride with vasoconstrictor per injection site for local blockage during wound induction and biopsies [9]. Post-procedural analgesia was provided using intramuscular tramadol hydrochloride at 0.5 mg/kg twice daily for 3 days [24]. Wounds were created using an 8 mm punch, and biopsies were performed with a 2 mm punch at specific wound locations over time. Biopsies were taken at 1 o'clock on day 7, at 5 o'clock on day 14, and at 8 o'clock on day 17.

With manual restraint, dorsal trichotomy was performed to create a sufficiently large area for the four wounds. The upper-left wound served as the control, treated with 0.9% sodium chloride; the lower-left was treated with 1.2% rosuvastatin gel; the upper-right received one of the autologous biomaterials (aPRP or aPRF), and the lower-right received the biomaterial combined with 1.2% rosuvastatin. Sterile rayon bandages and adhesive dressings (Band-Aid®) were applied after treatment; the dressings typically detached the following day, leaving the wounds open until the next treatment. Rabbits and wounds were inspected daily throughout the study.

Treatments and dressing changes were conducted every 4 days for 16 days, except for the initial change, which was done 3 days after wound induction [25, 22]. Biopsies and wound measurements were taken with a digital caliper on days 0, 7, 14, and 17 to calculate wound area [26]. Biopsy samples were fixed in 10% formalin for 24 hours, embedded in paraffin, sectioned into 4 µm slices, and stained with Hematoxylin-Eosin (HE). Following the final biopsy, animals were anesthetized and euthanized using a carbon dioxide chamber [24]. Epithelial thickness measurements images were obtained using Motic® software at 40x magnification, and with Image J® software calibrated measure thickness in µm. Three measurements of epithelial thickness—minimum, maximum, and average—were taken by a blinded analyzer [27,28].

For statistical analysis, Jamovi® software was used, and graphics were generated with GraphPad Prism®. Wound area and epithelial thickness data were assessed for normality using the Shapiro-Wilk test, revealing a non-normal distribution. Subsequently, the Kruskal-Wallis test with post hoc Dwass-Steel-Critchlow-Fligner (DSCF) analysis was applied to compare groups, while the Friedman test with Durbin-Conover post hoc analysis evaluated temporal changes, with a significance level of 5%. The Spearman test assessed correlations among variables, categorizing correlation strength as weak ($0.2 \leq |r| < 0.4$), moderate ($0.4 \leq |r| < 0.7$), or strong ($0.7 \leq |r| < 0.9$). To compare biomaterials, data for isolated autologous biomaterials and those combined with 1.2% rosuvastatin were re-evaluated. Normality was assessed using the Shapiro-Wilk test, confirming a non-normal distribution. Kruskal-Wallis and DSCF tests were used for group comparisons, while the Friedman and Durbin-Conover tests analyzed changes over time, maintaining a 5% significance level.

3. Results

No significant differences were observed between groups in the average wound areas when using aPRP, with or without RSV 1.2%, at any evaluation point. All groups showed smaller wound areas on days 14 and 17 compared to day 7 ($p < 0.05$). For wounds treated with aPRF, whether or not combined with RSV 1.2%, wound areas were also smaller on days 14 and 17 relative to day 7 ($p < 0.05$). No differences were found between groups on days 7 and 17. On day 14, wounds treated with aPRF alone ($p = 0.016$) and the control group ($p = 0.008$) displayed smaller wound areas than the aPRF+RSV group, which had a larger wound area.

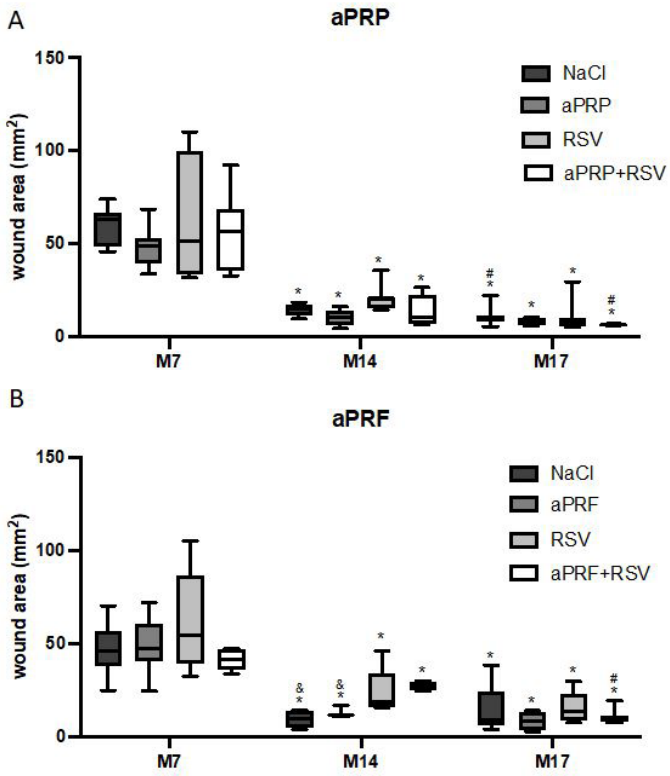

Figure 1. Data of wound area (mm^2) of surgically induced wounds treated with autologous Platelet-rich plasma (aPRP) (A) and autologous platelet-rich fibrin (aPRF) (B) biomaterials isolated or associated with rosuvastatin 1.2% for 17 days. NaCl: 0.9% sodium chloride solution; aPRF: autologous platelet-rich fibrin; aPRP: autologous platelet-rich plasma and RSV: rosuvastatin. M7, M14 and M17 are the days of wound induction. Kruskal Wallis Test followed by post hoc Dwass-Steel-Critchlow-Fligner for groups and Friedman test by Durbin-Conover for moments, 5% of significance, *: difference from M7; #: difference from M14. &: difference from aPRF+RSV.

Comparing the areas between groups treated with isolated autologous biomaterials and those combined with RSV 1.2%, wound areas on days 14 and 17 were significantly smaller than on day 7 ($p < 0.05$). On day 14, isolated aPRP ($p = 0.001$), isolated aPRF ($p = 0.021$), and aPRP+RSV ($p = 0.016$) groups showed smaller wound areas compared to the aPRF+RSV group.

For epithelial thickness (Figure 2), wounds treated with aPRP, with or without RSV 1.2%, consistently showed greater epithelial thickness than normal skin ($p < 0.05$), which had not been subjected to injury or treatment. Only the aPRP and RSV 1.2% groups showed increased thickness on day 14 ($p < 0.001$) and day 17 ($p = 0.014$) compared to day 7. The aPRP group was the only one exhibiting a decrease in epithelial thickness on day 17 compared to day 14 ($p = 0.003$).

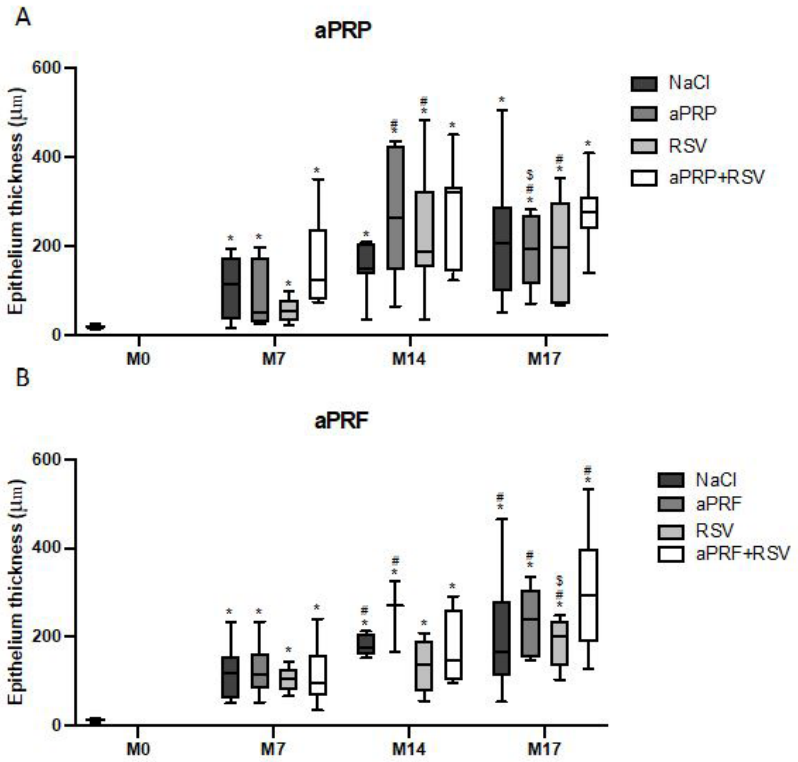

Figure 2. Data of epithelium thickness (µm) of surgically induced wounds treated with autologous Platelet-rich plasma (aPRP) (A) and autologous platelet-rich fibrin (aPRF) (B) biomaterials isolated or associated with rosuvastatin 1.2% for 17 days. NaCl: 0.9% sodium chloride solution; aPRF: autologous platelet-rich fibrin; aPRP: autologous platelet-rich plasma and RSV: rosuvastatin. M7, M14 and M17 are the days of wound induction. Kruskal Wallis Test followed by post hoc Dwass-Steel-Critchlow-Fligner for groups and Friedman test by Durbin-Conover for moments, 5% of significance, *: difference from M0; #: difference from M7; $ difference from M14.

Wounds treated with aPRF, with or without RSV 1.2%, also showed greater epithelial thickness than normal skin at all evaluation points ($p < 0.05$). In the NaCl group, day 14 ($p = 0.014$) and day 17 ($p = 0.014$) differed from day 7, as did the aPRF group on day 14 ($p = 0.005$) and day 17 ($p = 0.013$) compared to day 7. In the RSV 1.2% group, day 17 showed greater thickness than day 7 ($p = 0.004$) and day 14 ($p = 0.028$). In the aPRF+RSV group, only day 17 differed from day 7 ($p = 0.034$), with consistently greater thickness over time. When comparing the isolated autologous biomaterials with those combined with RSV 1.2%, no significant differences in epithelial thickness were observed across all evaluation points. In the aPRP group, day 14 ($p < 0.001$) and day 17 ($p < 0.001$) were different from day 7, with day 17 also differing from day 14 ($p = 0.018$). The aPRF group showed differences on day 14 ($p = 0.024$) and day 17 ($p = 0.047$) compared to day 7.

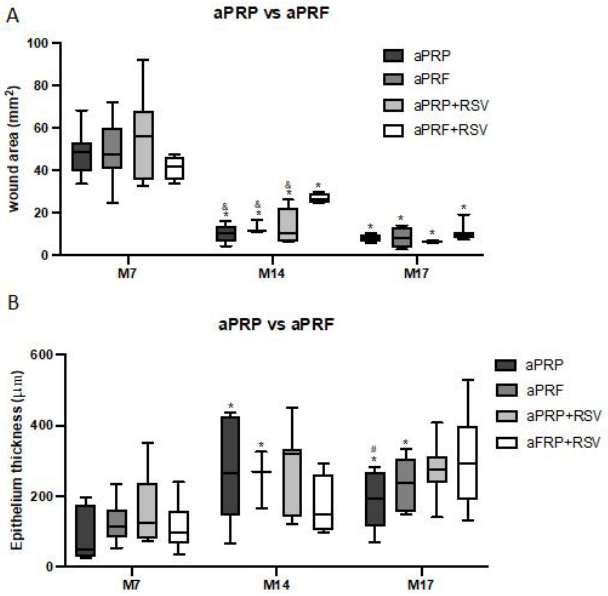

Figure 3. Data of wound area (mm²) (A) and epithelium thickness (µm) (B) of surgically induced wounds treated with autologous biomaterials isolated or associated with rosuvastatin 1.2% for 17 days. NaCl: 0.9% sodium chloride solution; aPRF: autologous platelet-rich fibrin; aPRP: autologous platelet-rich plasma and RSV: rosuvastatin. M7, M14 and M17 are the days of wound induction. Kruskal Wallis Test followed by post hoc Dwass-Steel-Critchlow-Fligner for groups and Friedman test by Durbin-Conover for moments, 5% of significance, *: difference from M7; #: difference from M14; &: difference from aPRF+RSV.

A negative correlation between wound area and epithelial thickness was found, indicating that smaller wound areas tend to be associated with greater epithelial thickness. In this study, the correlation was stronger for aPRF (r = -0.5500) than for aPRP (r = -0.4436), whether or not these biomaterials were combined with RSV 1.2%.

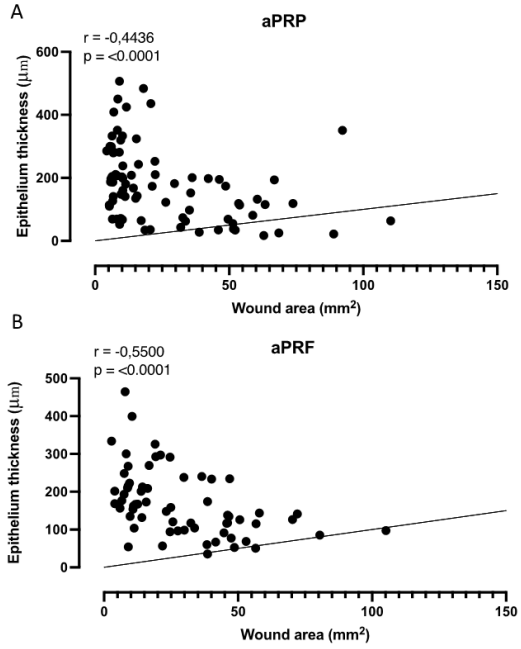

Figure 4. Data of Spearman correlation of wound area (mm²) and epithelium thickness (µm) of surgicaly induced wounds treated with autologous Platelet-rich plasma (aPRP) (A) and autologous platelet-rich fibrin (aPRF) (B) biomaterials isolated or associated with rosuvastatin 1.2% for 17 days. NaCl: 0.9% sodium chloride solution; aPRF: autologous platelet-rich fibrin; aPRP: autologous platelet-rich plasma and RSV: rosuvastatin. M7, M14 and M17 are the days of wound induction. The matrix of correlation ≤ 0.2 $|r|$ < 0.4 was a weak correlation, 0.4 ≤ $|r|$ < 0.7 moderated and 0.7 ≤ $|r|$ < 0.9 a strong correlation, considering a 5% significance level.

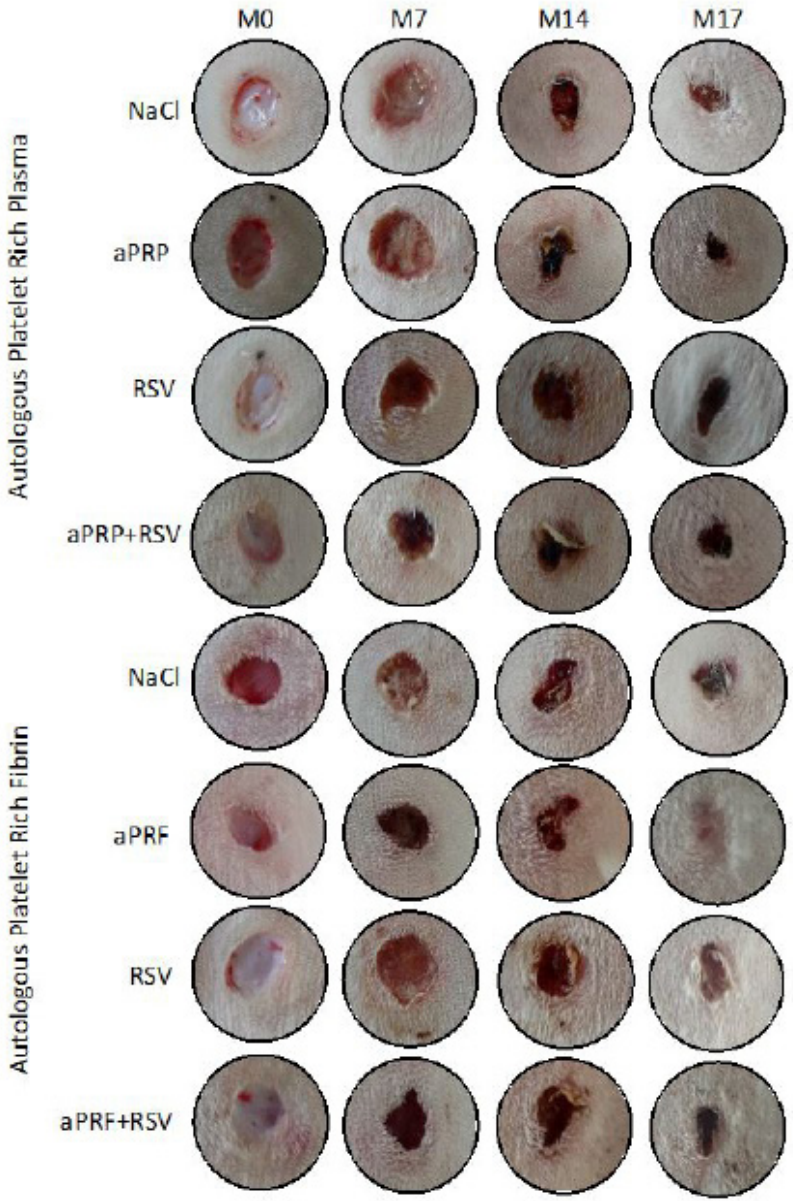

Figure 5. Schematic representation of the macroscopic appearance of surgically induced wounds in rabbits treated with autologous biomaterials isolated or associated with rosuvastatin 1,2% for 17 days. NaCl: 0.9% sodium chloride solution; aPRF: platelet-rich fibrin; aPRP: platelet-rich plasma and RSV: rosuvastatin. M7, M14 and M17 are the days of wound induction. Observe that the only group who has a total closure of the wound was the aPRF.

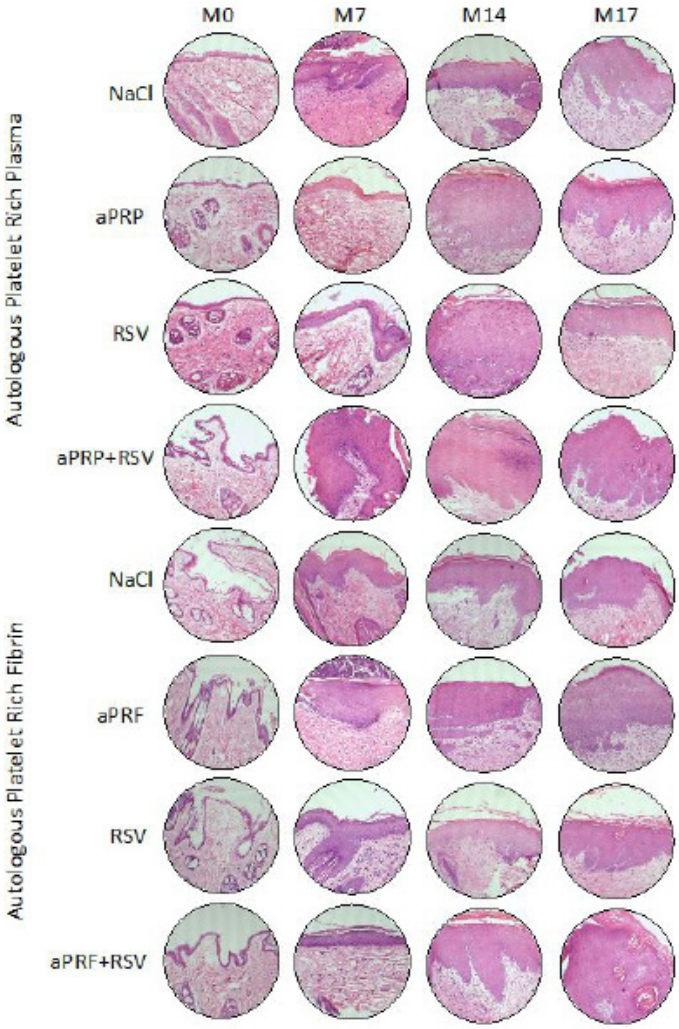

Figure 6. Schematic representation of histological sections of wounds surgically induced in rabbits treated with autologous biomaterials isolated or associated with rosuvastatin 1.2% for 17 days. HE stain, 40x. NaCl: 0.9% sodium chloride solution; aPRF: autologous platelet-rich fibrin; aPRP: autologous platelet-rich plasma and RSV: rosuvastatin. M7, M14 and M17 are the days of wound induction. Observe the increase of the epithelium thickness in all groups, and the regression on the aPRP group on M17.

4. Discussion

This study assessed wound healing by measuring wound area and epithelium thickness, investigating the effects of autologous aPRF and aPRP, either alone or combined with 1.2% RSV. The results showed that both the isolated aPRF group and the NaCl group exhibited smaller wound areas compared to the aPRF+RSV group at 14 days. Similarly, isolated aPRP and aPRP+RSV groups also demonstrated smaller areas than aPRF+RSV. No significant differences were found in epithelium thickness across the groups. Previous research by Xu et al. [28] highlighted that both the thickness and length of newly formed epithelium serve as viable measures for quantifying re-epithelialization. Similarly, Sun et al. [27] utilized epithelium thickness to measure this parameter in burn wounds. Our findings indicated that aPRP use led to earlier re-epithelialization, as evidenced by a decrease in epithelial thickness from M14 to M17 ($p=0.018$), consistent with the progression from the granulation phase to the

remodeling phase of healing [2]. This effect may be attributed to the earlier release of growth factors by PRP compared to PRF [29].

Khalifa et al. [30] compared the effects of aPRP and aPRF on wounds induced in dogs, finding that both treatments facilitated complete re-epithelialization. However, the use of aPRP resulted in a greater epithelium thickness compared to aPRF. Despite this, the authors found no statistical difference in overall healing outcomes between these biomaterials. This supports our findings, which also showed no significant differences in epithelium thickness between aPRP and aPRF.

Snowden [31] concluded in 1984 that both wound contraction rate and re-epithelialization are linear constants and, when evaluated together over an adequate period, serve as appropriate parameters for a quantitative comparison of incisional wound behavior. More recently, in 2022, Bull et al. [32] studied venous leg ulcers, focusing on gross area reduction (GAR), percentage area reduction (PAR), and wound margin advance (WMA). They determined that these healing metrics exhibited a substantially linear and similar trajectory over a four-week period.

This study monitored total wound area over 17 days, finding significantly smaller wound areas at 14 days post-lesion induction, irrespective of the treatment used. Although some studies achieved total wound closure within this period, the use of an 8mm punch and subsequent wound biopsies might have contributed to keeping the wounds open. Nonetheless, in this study, the isolated aPRF group exhibited complete wound closure by day 17. Balse and Baliga [33] reported that PRF enhanced soft tissue healing and bone regeneration more effectively than PRP when used as grafts following dental extractions. In contrast, Yerke et al. [34] observed no differences in macroscopic soft tissue healing between aPRP and aPRF at dental extraction sites. Our findings showed a moderate correlation ($0.4 \leq |r| < 0.7$) between wound area and epithelium thickness when using these biomaterials. At 14 days, we noted a smaller wound area compared to 7 days ($p<0.05$), but there was no significant difference between aPRP and aPRF treatments.

Khalaf and Salih [35] treated chronic wounds induced in goats with both PRP and PRF, noting that each promoted tissue repair and re-epithelialization faster than the control group. However, they did not distinguish which biomaterial performed better, a finding consistent with other studies [36,37,38] where it was also not possible to determine a preference between the biomaterials for the evaluated variables. This supports our results.

In this study, the performance of isolated biomaterials and combined with 1.2% rosuvastatin was reevaluated. At 14 days we observed, that isolated aPRP, isolated aPRF, and aPRP+RSV groups had smaller wound areas compared to aPRF+RSV, indicating that the combination of 1.2% rosuvastatin with aPRP was more effective than with aPRF. However, this finding contrasts with the results from Ferreira et al. [10], who noted significant improvements in fibroblast quantity and neovascularization with rosuvastatin combined with aPRF, rather than aPRP. Additionally, the pairing of RSV 1.2% and aPRP in wound healing was further

evaluated in a histopathological study [22] and regarding collagen quality (21), both of which yielded promising outcomes.

Pradeep et al. [39] found that combining 1.2% RSV gel with aPRF in bone grafts for mandibular defects was synergistic and significantly enhanced the evaluated parameters. However, our study did not observe such synergism. At 14 days, both the isolated 1.2% RSV and aPRF+RSV groups exhibited larger wound areas compared to isolated aPRF (p=0.016). At 7 and 17 days, there were no significant differences among the groups, which was consistent with results obtained when aPRP was used. In the field of dentistry, Gautam et al. [40], who also utilized 1.2% RSV gel combined with PRF, reported favorable outcomes in treating intraosseous defects in patients with chronic periodontitis. In our study, the same RSV concentration was used in both liquid form for blending with PRP and in gel form for individual use and combination with PRF. The varying performances of RSV associations might have been influenced by the different vehicles used in each formulation. The varying conclusions drawn from studies examining the association of biomaterials with RSV in the healing process highlight the need for further research. More sophisticated assessments of their mechanisms of action are essential to understand the interactions and potential benefits fully.

5. Conclusion

Combining rosuvastatin with autologous platelet-rich plasma or isolated autologous biomaterials resulted in smaller wound areas compared to combinations with autologous platelet-rich fibrin, suggesting a positive impact on wound healing. No significant differences in epithelium thickness were observed among the treatments. A moderate correlation was observed between wound area and epithelium thickness for treatments using either autologous biomaterial, with or without rosuvastatin. Further studies are essential to better understand the mechanisms through which these biomaterials and rosuvastatin influence wound repair.

Declaration of conflict of interest

The authors declare no conflict of interest.

Data availability statements

The data will be provided upon request.

Author contributions

Conceptualization: Y. F. Vicentini, C. L. Santarém, G. A. Nai and R. M. B. Nogueira. Data curation: Y. F. Vicentini and C. L. Santarém. Formal analysis: Y. F. Vicentini and C. L. Santarém. Methodology: C. L. Santarém, G. A. Nai and R. M. B. Nogueira. Supervision: C. L. Santarém, G. A. Nai and R. M. B. Nogueira. Investigation: Y. F. Vicentini and C. L. Santarém. Visualization: G. A. Nai and R. M. B. Nogueira. Writing (original draft): Y. F. Vicentini and C. L. Santarém. Writing (proofreading and editing): Y. F. Vicentini, C. L. Santarém, G. A. Nai and R. M. B. Nogueira

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