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In Vivo Morphological and Morphometric Evaluation of Wound Healing Treated with Chitosan/Xanthan/ β-Glucan Biopolymer and Autologous Platelet-Rich Plasma

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Abstract

Promising results in the regeneration of skin lesions have been demonstrated with the use of natural (organic) products, such as dermal dressings made of polysaccharides (chitosan complexed with xanthan), as they promote a hydrated and thermally insulating microenvironment, allowing gas exchange; and sources rich in growth factors such as autologous platelet-rich plasma (PRPa), which contains transforming growth factor beta (TGF-β), vascular endothelial (VEGF) and platelet-derived (PDGF), responsible for stimulating the inflammatory cascade and healing. The aim of this study was to evaluate the action of a polymeric membrane (chitosan, xanthan and β-glycan) and PRPa on healing in vivo, used alone or in combination. For the tests, rabbits underwent a surgical procedure to induce the lesion and were distributed into a control group (GC), membrane group (GM), PRPa group (GPa) and membrane group associated with PRPa (GMPa), evaluated at moments M0, M7, M14, M21 and M28 (28 days). Wound color and exudation, presence of infection and inflammation, formation of granulation, scarring and necrotic tissue, morphological and morphometric analysis were evaluated. Statistical analyzes of the results were performed using the Software R® software, adopting a significance level of 5%. While statistical differences between treatments in healing time were not significant ($p>0.05$), all wounds achieved 100% retraction by M21. Notably, at M7, PRPa alone and in combination with the membrane contributed to higher wound retraction percentages (29.71% and 21.65%, respectively) compared to the control group (16.96%). These findings suggest that the complexed membrane, alone or combined with PRPa, fosters a humid environment, gas exchange, and antimicrobial activity crucial for healing, with PRPa further enhancing early wound retraction. It is concluded that the treatment of experimental surgical wounds with biodressings such as PRPa alone or associated with a complexed membrane of chitosan, xanthan, and β-glycan significantly contributes to wound retraction, in addition to offering a propitious and indispensable environment for the healing cascade, such as a humid environment, gas exchange, and antibacterial action. Future studies should consider a larger number of animals per group, histological evaluation for global tissue assessment, and collagen quantification.

Keywords: chitosan; xanthan; β-glucan; healing; PRP

1. Introduction

Skin grafts (autologous or not) are a therapeutic modality used in cases of tissue lesions such as severe wounds (burns and ulcers), since they provide connective tissue and stimulate the development of blood vessels [1,2], contributing to an orderly healing process (inflammation:

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humoral and cellular, characterized by hemostasis, leukocyte migration and the onset of the tissue repair cascade, with the release of vasoconstrictor substances, mainly thromboxane A2 and prostaglandins, by cell membranes; proliferation: fibroplasia, angiogenesis and formation of granulation tissue; and tissue remodeling: involving the synthesis of new collagen mediated by transforming growth factor beta (TGF-β) and the breakdown of old collagen by platelet-derived growth factor (PDGF), with lower susceptibility to infections)[3,4].

However, grafting techniques may provide only temporary wound coverage because of rejection and the high incidence of partial graft loss with recurrence of the lesion, which limits their use, in addition to other factors such as the size of the donor area, the clinical condition of patients, donor scarcity and the need for the wound to present a bed with healthy granulation tissue, absence of necrotic tissue, infection and foreign bodies, so that the graft heals properly[5–7].

As alternative therapeutic proposals, the use of bioactive dermal dressings based on natural polysaccharides (chitin, chitosan, alginate, pectin, xanthan, cellulose and its derivatives: methylcellulose, carboxymethylcellulose), used alone or complexed (chitosan complexed with xanthan), has proved promising in the healing processes of severe skin lesions[8–11], since in addition to being non-toxic, with high biocompatibility and biodegradability[12–14], they offer a favorable microenvironment, hydrated and thermally insulating, removing excess exudate and promoting gas exchange[15–17]. They also exhibit antimicrobial activity and promote the growth of probiotic microbes, thus improving skin immunity and promoting wound healing[18].

Among the polysaccharides highlighted in the formulation of these dermal dressings, the following stand out: chitosan (a polysaccharide composed of D-glucosamine and N-acetyl-D-glucosamine monomers)[2,8,19], due to its antimicrobial activity and ability to accelerate healing, in addition to high biocompatibility, biodegradability and absorption and adsorption properties[3,11,18,20–22]; and xanthan (obtained by fermentation with the bacterium Xanthomonas campestris), a non-toxic polysaccharide which, due to its emulsifying, stabilizing and flocculating activity and its ability to form gels, films and membranes[23], enables the production of matrices with high absorption of aqueous solutions and proven stability in biological fluids[10]. Chitosan-based dressings stimulate closure, neovascularization and permanent dermal regeneration; they reduce the risk of amputations and infections by microorganisms such as Pseudomonas aeruginosa*[24]. Chitosan-based hydrogels have antibacterial efficacy against Gram-negative *E. coli strains, which is attributed to the interaction of chitosan with the bacterial plasma membrane[14].

β-Glucan, a structural polysaccharide of the cell wall of yeasts, fungi and some cereals, has demonstrated several beneficial effects, including immunomodulatory, anti-inflammatory, antitumor, antimutagenic and antioxidant activities[25,26]. This compound is considered hypocholesterolemic and hypoglycemic, in addition to exerting a protective effect against infections[27–30].

The contribution of these dermal dressings to the quality of the healing process does not involve only a shorter healing time, but also the prevention of possible infections[21,22,31] and a potential effect on collagen deposition in the tissue remodeling phase (involving the synthesis of new collagen mediated by TGF-β and the degradation of old collagen by PDGF), which, when disorganized, can result in healing disorders or excessive scarring (keloid and hypertrophic scar), due to exaggerated fibroblast activity and accumulation of extracellular matrix (ECM), stemming from an excessive inflammatory response during healing, with loss of normal control between synthesis and degradation[3,32].

Resulting from an imbalance of type I and III collagens, hypertrophic scars have been a major challenge for medical science; however, the use of chitosan-based dressings promotes better aesthetic quality of scar tissue and greater wound contraction due to its strong adhesive property to tissue and does not leave scars when applied to open wounds in animals[24]. It is noteworthy that chitosan can, by adsorption, increase the local concentration and interaction of important negatively charged molecules such as proteoglycans and glycosaminoglycans (GAGs), essential ECM components (relevant in tissue morphogenesis, differentiation and homeostasis)[11].

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It is also important to highlight that a source rich in growth factors is essential in tissue repair. Recently, autologous platelet-rich plasma (PRPa) (a platelet concentrate obtained from the patient's own blood) has shown significant results in several fields of medicine, including studies on the treatment of wound healing. In rabbits, PRPa application evidenced, by magnetic resonance imaging, the formation of new cartilage, indicating its ability to reconstruct and regenerate cartilage[33]. According to Ferraciolli et al. (2018), studies have shown a reduction in fine wrinkles and improvement of skin texture with the use of PRPa for facial and neck rejuvenation, probably due to stimulation of hyaluronic acid production.

As an organic, non-toxic and non-immunoreactive product that stimulates and accelerates wound healing, PRPa may help the integration of skin grafts in chronic wounds, because its therapeutic action is linked to PDGF (a universal initiator of the healing process and the main factor responsible for accelerating tissue regeneration)[6,34,35]. Plasma contains fibrinogen, which acts as a network, capturing platelets at the wound to form the clot, which limits bleeding and initiates cytokine signaling. Platelet α-granules release growth factors (TGF-β, VEGF and PDGF), which are responsible for stimulating the inflammatory and healing cascades[3,36]. Thus, platelets initiate and regulate the basic aspects of lesion healing and represent the most important component when the objective is scar modulation[37].

Due to the pathological and physiological complexity of the healing process, guaranteeing perfect tissue regeneration in cases of severe skin lesions using new treatments has been a challenge for researchers, since in addition to requiring a refined and systematized study on the production of these bioactive agents (dermal dressings and PRPa), based on their physicochemical properties, a thorough evaluation of the healing process through in vivo experimental assays is essential.

The aim of this study was to evaluate, through clinical-morphological and morphometric analysis (percentage of wound retraction), skin recovery in in vivo tests using a polymeric membrane (complexation of chitosan, xanthan and β-glucan), alone and associated with PRPa application.

2. Materials and Methods

2.1. Preparation of the Membranes

Dense and porous chitosan membranes complexed with xanthan and β-glucan were prepared according to the procedures described by Bellini et al. (2012) and Nakasse et al. (2020), at a chitosan:xanthan:β-glucan mass ratio of 1:0.85:0.15. A total of 90 mL of 1% (w/v) chitosan (Q) solution in 2% (v/v) acetic acid was dripped into a solution containing 0.765 g of xanthan (X) and 0.135 g of β-glucan.

To obtain the membranes, the chitosan solution was added by a peristaltic pump at a flow rate of 10 mL/min to the xanthan plus β-glucan solution, previously mixed under constant stirring at 1000 rpm for 2 minutes before chitosan addition. During preparation of the polymer complex, the temperature was maintained at 25 °C in a jacketed glass reactor with an internal diameter of 11 cm, external diameter of 12 cm and total capacity of 7.5 L.

After polymer complexation, the suspension was transferred to a 15-cm diameter polystyrene plate and dried in a forced-air oven at 37 °C for approximately 24 hours, until a dry membrane was formed. At the end of drying, for pH neutralization, the membranes were subjected to successive washings with distilled and deionized water and with 10 mM Hepes buffer for 30 minutes (three repetitions) and then dried again in a forced-air oven at 37 °C.

2.2. In Vivo Wound Healing Tests and Preparation of PRPa

The experimental procedures were approved (protocol no. 6115) by the Animal Use Ethics Committee (CEUA) of the Universidade do Oeste Paulista. New Zealand rabbits (n = 24, weighing 3.0 to 4.0 kg), housed in individual cages with free access to drinking water and commercial feed, were anesthetized intramuscularly with 2% xylazine hydrochloride (Xilazin® 2%) and zolazepam hydrochloride (Zoletil® 50) in association, at a dose of 15 mg/kg[38]. To calculate sample size, the

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following equation was used: $n = \frac{z^2.s^2.N}{e^2.(N-1)+(z^2.s^2)}$ where n = sample size; N = population size; z = standardized normal variable associated with confidence level; s = standard deviation; e = sampling error. The calculation used a 95% confidence level and an error rate of 3.5%, and considered that the total number of rabbits in the animal facility was 50 (N). Regarding the standard deviation, s = 0.0469 was used, considering the standard deviation obtained in a similar study[39]; thus, the sample size obtained was 6 animals, i.e., each of the 4 groups in the study contained 6 animals.

To obtain PRPa, 10 mL of blood were collected from the marginal ear vein of the rabbits by cannulation with a 20-gauge catheter[40]. The collected sample was divided into two sterile tubes containing 3.2% sodium citrate (Greiner Bio-one Brazil – sterile tube with 3.2% sodium citrate), resulting in 5 mL of whole blood in each tube, protected from light and kept at approximately 22 °C. An aliquot from each tube was taken for platelet counting (automatic counter Sysmex Poch Diff 100iV – Roche®). Centrifugation was started after 30 minutes to minimize platelet aggregation.

In the first centrifugation step, blood was centrifuged at 200 g (centrifuge Excelsa II, model 206-BL, Fanem®) for 10 minutes, allowing the formation of three layers: the upper or supernatant layer (cell-free plasma, i.e., platelet-poor plasma – PPP), yellowish; the leucoplatelet or buffy coat layer (where most platelets are found; also called the "hazy" zone), whitish; and the lower layer (red blood cells), red. The supernatant plasma and the leucoplatelet layer from each of the two tubes were pipetted and transferred to dry, sterile tubes for the second centrifugation step, at 400 g for 10 minutes. Of the supernatant, two-thirds were discarded as PPP, and the remaining one-third was homogenized to disperse the platelets and form PRP. At this moment, platelet count was performed, using as a minimum reference 1,000,000/mm³ to be considered platelet-enriched plasma[41,42].

After blood collection for PRPa preparation, the dorsal region of each animal was shaved with an electric clipper (AGC®), blade number 40. After skin antisepsis with 70% alcohol, the wound area was previously demarcated (with a marker pen), and four circular wounds of 0.8 cm in diameter were made with an 8-mm dermatological punch; they were identified as wound 1 (F1), 2 (F2), 3 (F3) and 4 (F4)[33,43]. For postoperative pain control, tramadol hydrochloride was administered intramuscularly at 0.5 mg/kg[44].

The animals were identified, kept in individual cages (with free access to drinking water and commercial feed), and randomly divided into four experimental groups: control group (GC), membrane group (GM), PRPa group (GPa) and membrane plus PRPa group (GMPa). These animals underwent treatments as described in Table 1, were monitored daily, and the healing process was evaluated at moments M0, M7, M14, M21 and M28 (28 days) after the experimental procedure[45].

Table 1. Distribution of animals used in the experiment.

Group	Animals	n (lesions)	Treatment
GC	6	24	0.9% NaCl solution
GM	6	24	Membrane
GPa	6	24	PRPa
GMPa	6	24	Membrane + PRPa

In GC, the wounds were covered with a band-aid-type dressing; in GM, with dense and porous membranes (chitosan/xanthan/β-glucan) of 0.8 cm in diameter, previously hydrated in 0.9% saline; in GPa, with PRPa gel (topical) plus band-aid-type dressing; and in GMPa, the wounds were covered with topical PRPa gel and a previously hydrated membrane.

2.3. Morphological and Morphometric Evaluation of Healing

Morphological, morphometric and photographic evaluations were carried out immediately after the experimental lesion (M0) and at M7, M14, M21 and M28 (28 days) after the beginning of the experiment[45].

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In the clinical-morphological analysis, the following characteristics were recorded: wound exudation, presence of infection and inflammation, granulation and scar tissue, and necrotic tissue[46] (score: 1-present, 0-absent); and wound color (score: 1-pink, 2-yellowish, 3-pale, 4-cyanotic)[47].

For clinical-morphometric analysis, the width and height of the wound edges in millimeters were measured with a digital caliper (DC-60 Western®). The measurements obtained were used to calculate the percentage of remaining wound area relative to the initial area (PAFR), using the following formula: PAFR = (AM/AI) × 100%, where PAFR is the percentage of wound area relative to the initial area, AI = original wound area on day zero and AM = area measured at each evaluation moment[42].

2.4. Statistical Analysis

Descriptive analysis of quantitative variables was performed considering mean and standard deviation and, for qualitative variables, absolute frequency. The effect of treatments and moments on the quantitative variable PAFR was assessed using a Generalized Linear Mixed Model, assuming a Poisson distribution and treating animal as a random effect, since standardized residuals did not meet the assumptions of normality and homoscedasticity, verified by the Shapiro–Wilk and Levene tests, respectively. The effect of treatment and moment on dichotomous variables (presence/absence) was verified using a Generalized Linear Mixed Model with binomial distribution, again treating animal as a random effect. In all models, contrasts within factors were obtained using the Bonferroni multiple comparison test. All analyses were performed in R® software (R Core Team, 2020), adopting a significance level of 5%.

3. Results and Discussion

The wound healing process is complex and consists of four phases: hemostasis (activation of the coagulation cascade and platelet aggregation), inflammation (characterized by secretion of cytokines by immune cells and removal of necrotic tissue, infection control by phagocytosis and production of free radicals by macrophages and neutrophils), proliferation (re-epithelialization and formation of granulation tissue and extracellular matrix (ECM), due to migration and proliferation of fibroblasts and endothelial cells to the wound site) and tissue remodeling (in which both wound contraction and collagen remodeling occur — degradation of type III collagen and formation of type I collagen — and tissue tensile strength increases), which would require dressings with different characteristics for each period of healing. The biological dressing obtained from the complexation of chitosan, xanthan and β-glucan showed gas permeability, water absorption and other properties, indicating potential for different stages of wound healing[14,17,18,48].

The structural characteristics of chitosan are similar to glycosaminoglycans (GAGs), which are components of the extracellular matrix; therefore, this feature supports the use of chitosan in skin tissue engineering[19,31,49]. Studies indicate that chitosan contributes to granulation tissue formation and re-epithelialization, accompanied by angiogenesis and regular collagen fiber deposition, limiting scar formation and tissue retraction. In vivo assays have demonstrated that combining biopolymers (membranes made by associating chitosan with xanthan or chitosan with alginate) or chitosan alone can improve the properties of biodressings, such as greater fluid absorption, and thereby favor potential healing[11,50].

Morphometric evaluation, in which the epithelialized area was considered macroscopically, showed no significant difference between groups regarding wound exudation, presence of infection and inflammation, necrotic tissue, granulation tissue and scar tissue; similarly, it was not possible to obtain significant differences for the variables wound edge and crust because the generalized linear mixed model applied to the data showed singularity in the covariance matrix (Table 2). None of the groups showed abnormalities in healing, and in none of the four groups was any wound observed with development of an infectious process, as evidenced by studies that identified the ability of biodressings to control infection[14,24,31]. It is important to note that ideal dressings should have the

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following functions: be non-toxic and non-allergenic, with hemostatic and antibacterial properties, allowing gas permeability, moisturizing capacity, exudate absorption, promotion of cell proliferation and inhibition of scarring[18,31]. Currently, limitations associated with the use of some common dressings include difficulty in removal, inability to protect the wound against microbial infection, poor gas exchange between the wound and the environment, deficient mechanical properties, lack of sterility, induction of allergic reactions, poor absorption of wound exudates and inability to maintain a moist environment for accelerated healing[14,51].

Table 2. Absolute frequency of qualitative variables in the different treatments and at moments M0, M7, M14, M21 and M28 days.

Treatment	Moment	Wound edges – Presence	Wound edges – Absence	Crust – Presence	Crust – Absence
Control	0	-	-	-	-
	7	0	6	6	0
	14	0	6	0	6
	21	0	6	0	6
	28	0	6	0	6
Membrane	0	-	-	-	-
	7	0	6	6	0
	14	0	6	2	4
	21	0	6	0	6
	28	0	6	0	6
PRPa	0	-	-	-	-
	7	5	1	6	0
	14	0	6	3	3
	21	0	6	0	6
	28	0	6	0	6
PRPa + Membrane	0	-	-	-	-
	7	0	6	6	0
	14	5	1	5	1
	21	0	6	0	6
	28	0	6	0	6

The ability to absorb wound exudate and permeability are important in the initial phase of wound healing. Gas permeability determines the capacity to control fluid loss, a fundamental physical characteristic of wound dressing materials, and directly controls the moist environment during healing, which benefits this tissue repair process. Dressings with gas permeability in the range of 2000–2500 g/24 h·m² per day are ideal for maintaining a moist healing environment, since low gas permeability can increase the risk of bacterial infection by leaving exudate at the wound edge[17].

At 7 days post-surgery, a gradual reduction (retraction percentage) in wound diameter was observed in all experimental groups, with complete healing (100%) at 21 days (Figure 1). Statistical analysis showed no significant differences ($p > 0.05$) in wound retraction percentage between the different treatments at 7 and 14 days (Table 3 and Figure 2). However, other studies have indicated that using chitosan in biodressings of different physical forms (membranes, complexed solutions, hydrogels, bandages and others), alone or in association, can synergistically accelerate healing when compared with other treatments[11,44,50–52], by inducing the expression of genes that regulate angiogenesis, promoting early wound granulation and collagen deposition, and creating a moist, breathable external environment along with appropriate physical compression[51]. Healing of deep second-degree burn wounds can also be accelerated when chitosan is complexed with cellulose and silver nanoparticles, as it plays an important role in regulating growth and inflammatory factors, including VEGF, EGFL-7, TGF-β1, bFGF, TNF-α and IL-1β[55]. Moreover, studies using autologous platelet-rich plasma (PRPa) gel in wound healing have demonstrated its effectiveness in accelerating

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the healing process[39,54–56], contributing to increased angiogenesis in surgical wounds in rabbits43,59 and to the production of more organized collagen fibers when compared to heterologous PRP[42].

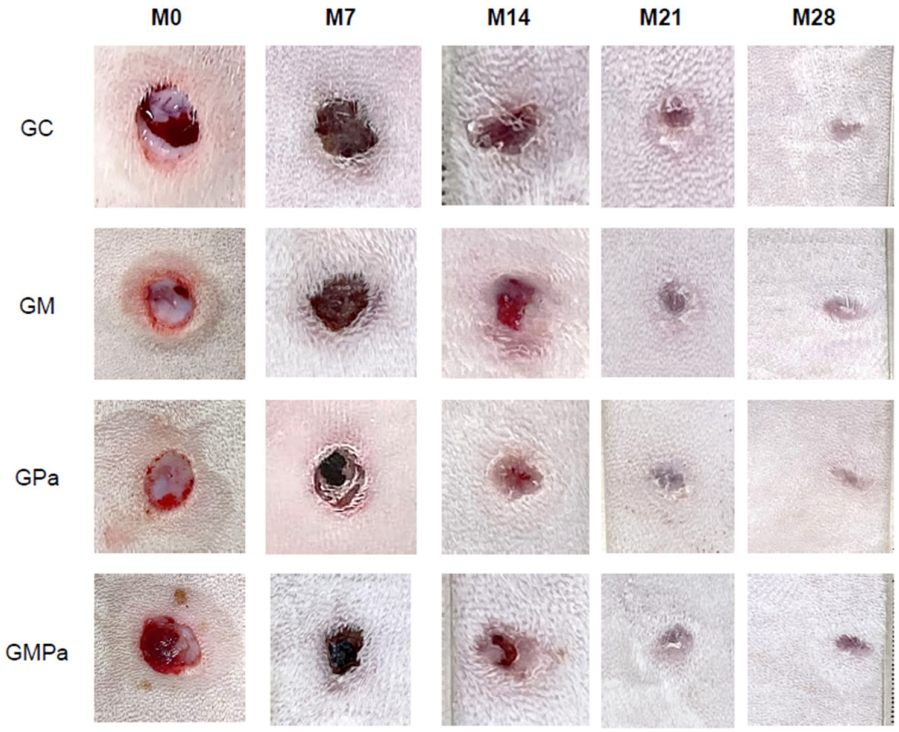

Figure 1. photographic images showing the wound healing process at moments M0, M7, M14, M21 and M28 days.

It is suggested that the small number of animals evaluated in this study may explain the absence of differences between treated and control groups. Studies with about eight rabbits per group, each subjected to four surgical wounds using an 8-mm punch in the dorsal region, have shown significant differences for treatments with biomaterials (PRP and platelet-rich fibrin – PRF)[43], whereas other studies with dermo-epidermal surgical wounds on the left elbow region and treatment with PRP also showed significant differences between the treated and control groups, each with 15 animals[59]. Additionally, it is important to consider several factors in experimental conditions involving wound induction and healing, such as methodological standardization of wound induction; wound location, number and size per animal; postoperative treatments; the animal's behavior in the enclosure; formation of exuberant granulation tissue[60]; among others.

Table 3. Descriptive analysis of the percentage of remaining wound area relative to the initial area (PAFR) for the different treatments and moments.

Treatment	Moment	n	Mean	SD	Min	Max
Control	0	6	0.00a	0.00	0.00	0.00
	7	2	16.96b	13.69	7.28	26.64
	14	5	72.62c	16.89	50.11	89.62
	21	6	100d	0.00	100	100
	28	5	100d	0.00	100	100
Membrane	0	6	0.00a	0.00	0.00	0.00
	7	4	16.63b	14.06	6.62	37.02
	14	5	71.51c	22.84	40.02	96.16

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	21	6	100d	0.00	100	100
	28	6	100d	0.00	100	100
	0	6	0.00a	0.00	0.00	0.00
	7	5	29.71b	9.97	17.59	43.01
PRPa	14	6	68.09c	10.54	56.64	81.72
	21	6	100d	0.00	100	100
	28	6	100d	0.00	100	100
	0	6	0.00a	0.00	0.00	0.00
	7	4	21.65b	15.05	8.46	41.24
PRPa + Membrane	14	6	61.87c	19.49	33.35	80.12
	21	6	99.06d	2.31	94.34	100
	28	6	100d	0.00	100	100

n: number of observations; SD: standard deviation; min: minimum; max: maximum; different letters indicate significant difference ($p < 0.05$) between moments within each treatment.

Chitosan contributes to wound healing by promoting hemostasis, increasing platelet adhesion and aggregation, reducing the inflammatory response at the wound, improving vascular endothelial proliferation and increasing the expression of growth factors and cytokines. In addition, it can improve chemotaxis and the function of inflammatory cells to accelerate granulation tissue formation and consequently the wound healing process[54,55].

According to Martins et al. (2013), healing time with the use of chitosan membrane for treating distal limb wounds in horses was longer compared to control due to exuberant granulation tissue formation, which, although beneficial for healing by inducing interleukins responsible for fibroblast and keratinocyte migration and proliferation, becomes detrimental to tissue repair when it exceeds the wound surface. However, it is important to note that current data suggest chitosan does not delay wound healing; it is also important to highlight that, despite the increasing number of trials with new chitosan dressings, studies on the relationship between chitosan and wound healing remain limited. The small number of available trials restricts appropriate interpretation of existing results. New research must be rigorously designed to confirm any clinically relevant effect of chitosan on wound healing[60].

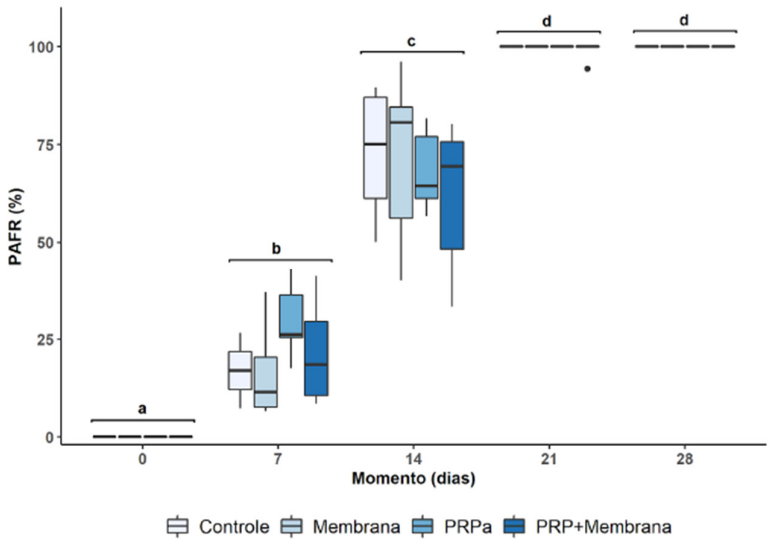

Figure 2. box-plot of the percentage of remaining wound area relative to the initial area (PAFR) for the different treatments and moments.

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From M7 to M14, significant differences ($p < 0.005$) were observed in wound retraction percentage for all groups, with reductions of 55.66%, 54.88%, 38.38%, and 40.22% for the GC, GM, GPa and GMPa groups, respectively. There was less retraction for the GPa and GMPa groups compared to the other groups, because, despite the lack of statistical significance between treatments, a higher retraction percentage can be observed for these groups at M7: 29.71% for GPa and 21.65% for GMPa (Table 3).

Considered an advanced therapy in wound treatment, PRP represents a source of growth factors and other proteins that have synergistic biological effects and act as mediators in the healing process. Growth factors stimulate the inflammatory response, angiogenesis, fibroblast proliferation and, consequently, increased collagen synthesis[39,56,61], which may contribute to the higher retraction percentages in the GPa and GMPa groups. It should also be emphasized that the macroscopic results for the treated groups GM, GPa and GMPa were considered positive, even in the absence of statistical differences between groups. In this regard, more consistent results could be obtained with a larger sample size.

Complexation of β-glucan into the chitosan-xanthan membrane[11] may contribute to healing quality, since β-glucan belongs to the group of physiologically active compounds collectively termed Biological Response Modifiers (BRMs)[62], which, because they are not synthesized by the human body, are not recognized by the immune system as self-molecules and consequently induce both innate and adaptive immune responses[63]. Evidence indicates that glucans exhibit biological properties characterized by a cascade of events, including specific binding to macrophages and their receptors and signaling to the cell nucleus, leading to expression of genes involved in regulating apoptosis of proliferating and invading cells[64]. Furthermore, studies indicate that β-glucan has antioxidant activity via free radical scavenging[65].

The results of this study highlight the many factors that may interfere with the rate and/or time of healing despite the different treatments, such as animal movement in cages, which allowed contact and probably excessive abrasion of the wound region; possible excessive granulation tissue formation; different experimental designs; among others. Studies have reported that chitosan membrane not only promotes good adhesion, excellent hemostasis and wound re-epithelialization, but also reduces itching and has analgesic action[14,51], which may contribute to reduced animal movement in cages and lower abrasion of wounds, as observed in the GM and GMPa groups.

The bioactivities of chitosan that facilitate wound healing can be explained by several mechanisms: antibacterial activity, according to hypotheses, results from electrostatic adhesion of chitosan to the bacterial cell wall and membrane, as well as interaction with intracellular targets to inhibit protein synthesis[19,31]; anti-inflammatory action stems from chitosan's ability to induce increased levels of anti-inflammatory cytokines such as IL-10 and TGF-β1 and decreased levels of pro-inflammatory cytokines; its antioxidant action results from stabilization and scavenging of free radicals (reactive oxygen species – ROS) by the amino and carboxyl groups of chitosan, which is vital in the inflammatory stage of healing, since moderate ROS levels can facilitate wound healing by stimulating cell migration and angiogenesis, whereas excess ROS exacerbate the inflammatory response and impair wound healing, especially in chronic wounds; promotion of tissue regeneration arises from modulation of growth factors that: promote macrophage migration to wounds; promote fibroblast proliferation; promote synthesis of proteoglycans and collagen; and promote angiogenesis. Chitosan induces the release of coagulation factors from platelets and red blood cells and their adhesion to tissues to form clots, contributing significantly to hemostasis; whereas scar reduction depends on its cationic properties: chitosan inhibits type I collagen production in wounds, promotes granulation tissue formation, reduces wound contraction and decreases scar formation[51].

Animals in GC showed more agitated behavior after surgery, which promoted greater contact of the wound with the containment cage and exposure of the lesions, as well as an increase in wound size in some animals, which was not observed in the other groups. Hemostatic and analgesic characteristics[14,18,31,52] can be attributed to the positive (cationic) charge of chitosan, which interacts with negatively charged red blood cells; therefore, the hemostatic effect of chitosan depends

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on electrostatic adhesion to blood cells. Studies have indicated the analgesic action of carboxymethyl chitosan in scalded rats, in which the concentration of bradykinin and 5-hydroxytryptophan (potent algogenic substances) monitored by immunoenzymatic assay was significantly lower than in the control group ($p < 0.005$), indicating analgesic action; whereas for chitosan the results were the same as in the control group[19].

The methodology used to prepare the complexed chitosan, xanthan and glucan membrane should be considered among the factors that contributed to the lack of accelerated healing, since solubilizing chitosan in acetic acid is disadvantageous: acetic acid, together with chemical crosslinkers such as carbodiimide or glutaraldehyde, among others, has cytotoxic effects on mammalian cells; therefore, this is a major limitation for wound healing[19].

Although differences between groups were not significant, the positive effect of the complexed membrane (chitosan, xanthan, β-glucan) and PRPa on reducing wound size should be considered when planning further studies for clinical application. Thus, new studies should be developed and well designed, considering analysis of granulation tissue formation, evaluation of analgesia through immunoenzymatic assay, standardized postoperative analgesic treatment, larger sample sizes and histological analysis to assess the quality of the healing process.

4. Conclusions

Treatment of experimental surgical wounds with biodressings such as PRPa alone or associated with a complexed membrane of chitosan, xanthan and β-glucan contributes significantly to wound retraction, in addition to offering a favorable and indispensable environment for the healing cascade, such as a moist environment, gas exchange and antibacterial action. For future studies, a larger number of animals per group is suggested, as well as histological evaluation for global tissue assessment and collagen quantification.

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Abbreviations

The following abbreviations are used in this manuscript:

PRP	Platelet-Rich Plasma
TGF-β	Transforming Growth Factor Beta
VEGF	Vascular Endothelial Growth Factor
PDGF	Platelet-Derived Growth Factor
ECM	Extracellular Matrix
GAGs	Glycosaminoglycans
GC	Control Group
GM	Membrane Group
GPa	PRP Group
GMPa	Membrane plus PRP Group
PAFR	Percentage of Remaining Wound Area Relative to the Initial Area

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