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ARTICLE

The measurement of wound tensile strength and the effect of PRP on wound tensile force: an experimental investigation on rabbits

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ABSTRACT

Platelets in PRP are used for their functions in the initiation and regulation of the wound healing process and are used for the repair of injured tissues and the rejuvenation of healthy tissues. In this study, we evaluated the effect of a single dose platelet-rich plasma on skin wound healing and we demonstrated the effect of platelet-rich plasma on skin wound healing by measuring changes in the wound tensile strength.

Material and methods: A total of 8 incisions, each 3 cm long, were made on the back skin on both sides of the vertebral column of 12 rabbits. After suturing their backs with staples, platelet rich plasma (PRP) was injected into the edges of the wounds on the left side and saline solution (saline) was injected into the edges of the wounds on the right side. The tensile force that causes wounds to rupture by applying tension was measured on the 7th, 14th, 21st, and 28th days with the help of a special home-designed device.

Results: The mean PRP enrichment was 3.19 fold over peripheral blood. The saline to PRP tensile strength ratios on the 7th, 14th, 21st, and 28th days were calculated as 75.7%, 104.0%, 105.3% and 86.5%, respectively. Overall, the difference in the tensile strength for wounds that had received saline or PRP was in-significant.

Conclusion: The application of PRP increases the tensile strength of the wound in the early period. It is possible to measure the tensile strength precisely in in vivo studies with economical home-designed devices.

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KEYWORDS
Platelet-rich plasma; PRP; tensile strength; wound healing

Introduction

Platelets start the inflammation phase of wound healing and manage it. Inflammatory, proliferative and remodeling are stages of wound healing. After activation by tissue damage, platelets initiate hemostasis and wound healing. Activated platelets promote wound healing by releasing biologically active proteins and growth factors (such as platelet-derived growth factor, transforming growth factor-b, fibroblast growth factor, epidermal growth factor, keratinocyte growth factor, and vascular endothelium growth factors) resulting in connective tissue healing, epithelial development, angiogenesis, and deposition of the collagen matrix. During the wound healing process, the tensile strength of the wound increases progressively. This increase is greater in the first week; later on, it is directly related to the increase in collagen production, and it stabilizes when collagen production and destruction are in balance. Weak crosslinks change to strong crosslinks and tensile strength increases progressively until the tensile force reaches an optimum strength. The stages of wound healing directly affect the tensile strength of the wound.

Wound healing is affected by endogenous or exogenous factors. Use of sealants, sutures, clips, many materials that are expected to accelerate wound healing or contrary many objects that weaken the wound healing, such as radiation therapy, steroids, etc. Research is carried out to enhance or accelerate wound healing. There are a lot of methods used for evaluating the changes in wound healing. Breaking strength analysis of skin incisions reflects mechanical properties of the wound. Measurement of tensile strength is an important assessment in many studies that involve wounds. Considering measurement of wound healing, the most reliable data is changed in wound tensile strength. So a reliable method needed to be used to measure the tensile strength of wounds. Biomechanical assays are useful techniques to evaluate the skin, tendons, ligaments wound healing. Several types of biomechanical assays (the static tensile, biaxial tensile, rheological properties) have been used for different purposes. Tensile strength measurement of repaired skin compared with intact skin is the most common and simplest assay which is applied to tensile failure load or stress or the modulus (stiffness) of skin under different conditions.

It is not easy to perform a standardized tensile strength measurement on live subjects. Because wound-healing studies need invasive procedures, in most of the studies tensile strength on the skin is measured *in vitro*. These studies often depend on the animal model used, and the region of the body of the skin being excised. This study concerns live tensile strength measurement on animal skin incisions with primary closure to serve as an experimental model for controlled clinical surgical settings. In the study, we measured the tensile strength changes during the healing of incisional skin wounds. We compared the normal wound healing with PRP injected wound healing on rabbit skin.

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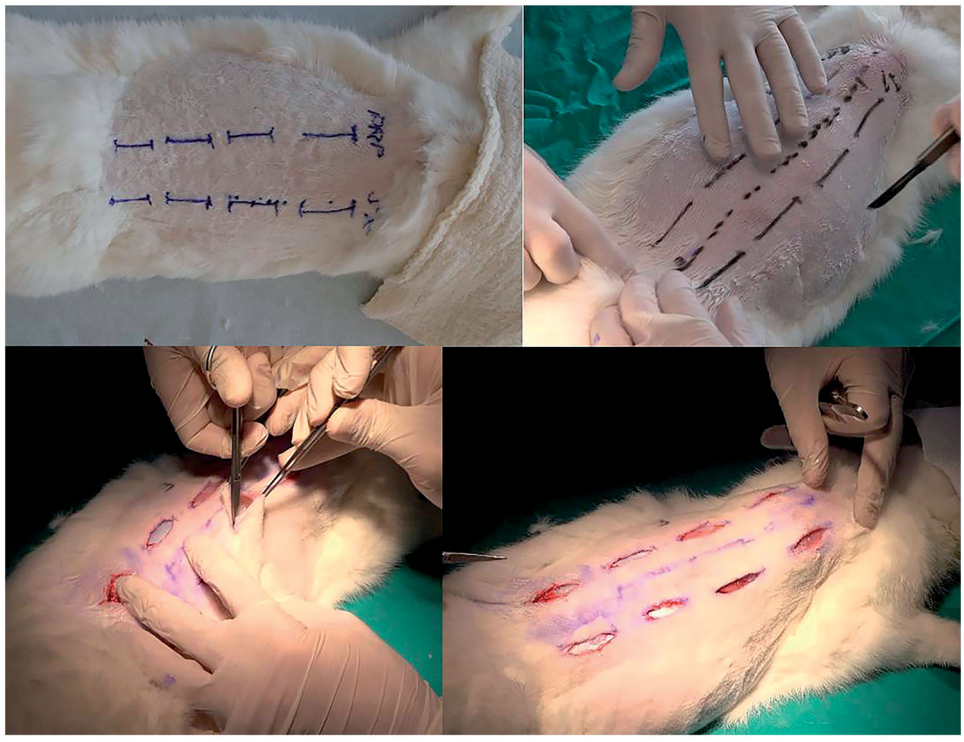

Figure 1. Planning, incision, note that when the cut in skin layer by scalpel is incomplete, scissor is used to cut remaining layers.

In the current study, we aimed to evaluate objectively the effect of applying platelet-rich plasma (PRP) to the wound healing by measuring the tensile strength of the developing scars.

Material and methods

The experiment was carried out on the back skin of 12 female New Zealand rabbits that weighed 2–3 kg. This study was approved by the local Ethics Committee of Animal Research (AIBU 30.12.2016/86). Anaesthesia was administered subcutaneously with 40 mg/kg ketamine hydrochloride (Ketalar® 50 mg/ml vial, Pfizer) and 5 mg/kg xylazine (Rompun® vial, Bayer). Depth of anaesthesia was evaluated by muscle tonus and corneal reflex. Following the settlement of anaesthesia, surgicals sites' hair was removed using an electrical shaver. Then, the area was cleaned with 10% povidone–iodine solution (wiped with saline) and made ready for surgical intervention. This procedure was repeated before each operation every week. On the back skin of the rabbits, 4 incisions that included the panniculus carnosus were made at each side of the vertebral column, parallel to the column, 2 cm away from the column and with 1 cm of space between them (Figure 1). The incisions were sutured with staples (Figure 1). By using 26 g needles, a total of 4 cc of PRP (4 × 1 cc) was injected into the edges of each wound to the left side of the vertebral column and 4 cc saline (4 × 1 cc) was injected into the edges of each wound on the right side (Figure 2). The wounds were checked under a magnifying loupe to verify that sufficient eversion of wound edges had occurred and that the edges were in full contact with each other. Starting with the first 2 incisions on the cephalic end, on the 7th, 14th, 21st, and 28th days, a progressive force was applied to the scars. The force that caused a rupture in the scars was measured and recorded as the kilogram-force (kgf) (Figures 3–5). The tensile strength was evaluated as the force measured at the first moment of rupture in the scar (Figures 3–5). The progressively increasing force was applied to each wound with the controlled manual mechanism of the device until the wound edges ruptured (Figures 3–6). The exact time when the wound edges ruptured was recorded by the device and also verified with the help of a camera (Figures 3–6). After the surgery, each subject was kept in a 22–24 °C temperature and 45–50% humid room, in a 12-h period of light–dark cycle, in individual cages separated from each other to prevent the intervention of surgical sites. Metamizole sodium at a dose of 50 mg/kg was administered through drinking water after surgical interventions for analgesia. Subjects were able to access water and food *ad libitum*.

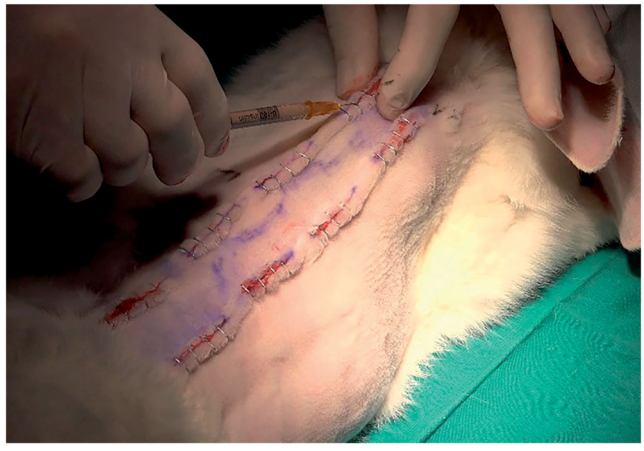

Figure 2. Wounds are repaired by staplers and PRP or saline solution is injected into wound edges.

The preparation of PRP

PRP was prepared using the double spin method. After measuring the basal blood levels of the rabbit platelets, 8.5 cc of blood was drawn from the dorsal ear vein of each rabbit and it was put into tubes containing 1.5 cc of acid-citrate-dextrose A (ACD-A), as an

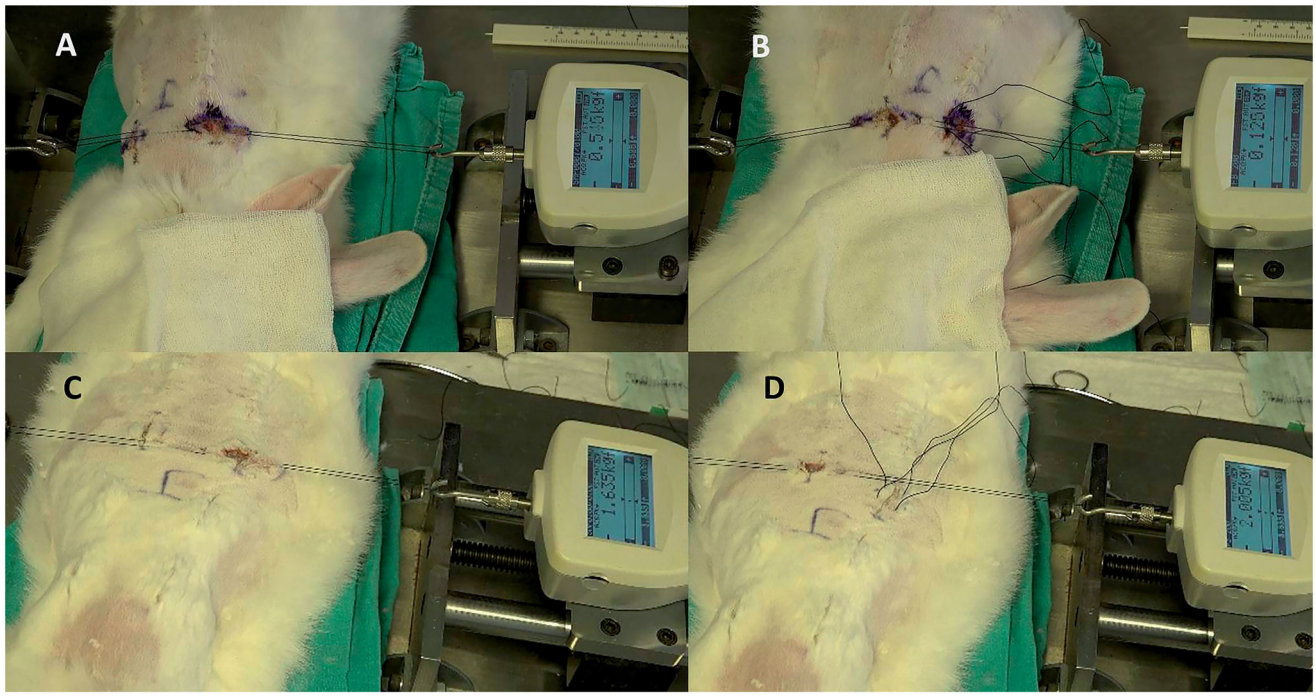

Figure 3. Tensile strength measurement; day 7 PRP (A), saline (B), day 21 PRP (C), saline (D).

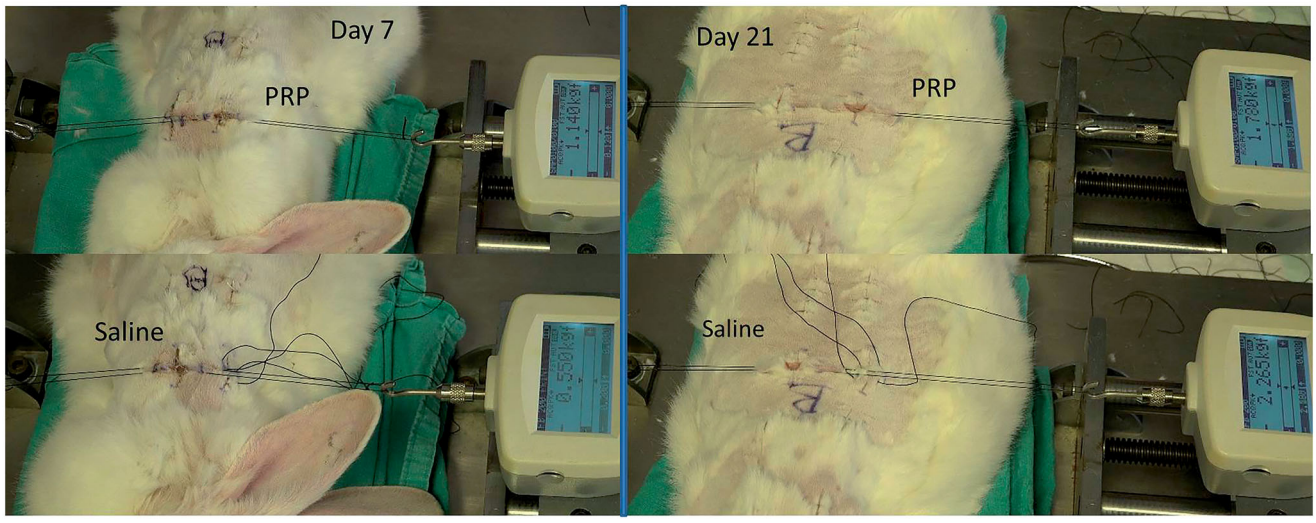

Figure 4. Tensile strength measurement; day 7 and day 21.

anticoagulant. Three tubes of blood were taken from each rabbit. After the tubes were centrifuged in the first spin at 180 g × 10 min, all plasma of the 3 tubes was combined by transferring them to an empty tube. This new tube was centrifuged at 180 g × 10 min as the second spin. The pellet was diluted with 4 cc of plasma. After homogenizing the PRP, samples were taken from two different points of the tube and a platelet count was performed and recorded (Table 1). Four cc of PRP was prepared for each rabbit.

Measuring the tensile strength

We used a home-designed device (Figures 6 and 7) for measuring the tensile strength of the wound (the force at the moment of rupture). After the rabbits were placed inside the device, the sutures were made on both sides of the wound, 1 cm away from the center and 1 cm in length. Sutures were connected to the device (Figures 3–5). The position of the rabbit was adjusted carefully in the device to eliminate any angulation of the sutures so as to measure the rupture force correctly (Figures 3 and 4). Using the device, progressively increasing force was applied to the wound edges. The first moment that the wound separated was recorded automatically by the power meter of the device (Figure 6) and was also recorded by a video camera. The special feature of the power meter was that it recorded automatically any pause in the resistance against the applied force (Figure 6). The procedure for tensile strength measurement was applied to the 4 pairs of incisions (4 right and 4 left) from the cephalic end towards the tail on the 7th, 14th, 21st, and 28th days, respectively (Figures 3–5). The rupture forces for wounds to which saline and PRP had been applied were recorded and compared (Table 2). Statistical analysis was also performed (Table 3). Since the two groups are compared and the distribution of data and scale level meet the conditions for using parametric tests, an independent *t*-test (a parametric test) was used for the statistical analysis. The *p*-value of 0.05 is considered

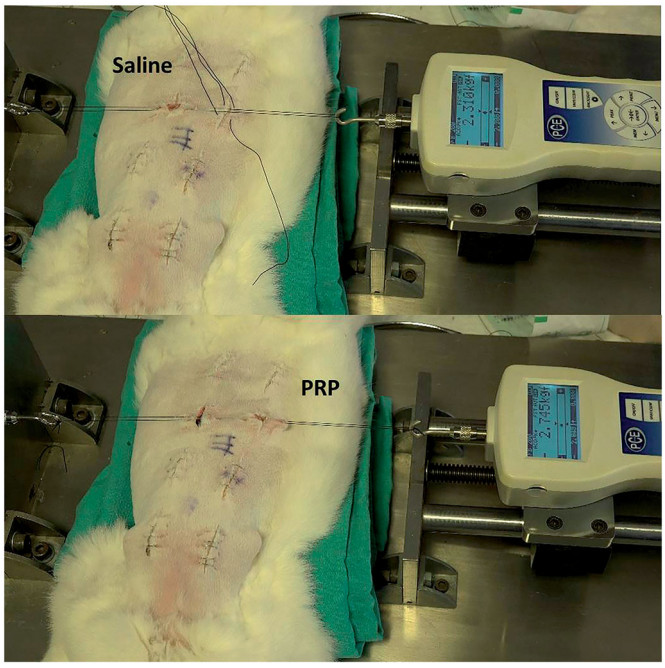

Figure 5. Tensile strength measurement; day 21.

for the confidence interval of 95% and the results that had a *p*-value less than 0.05 were accepted as significant.

Results

The rabbits' blood platelet levels ranged between 368,000 and 720,000/μL, with a mean of 516,000/μL. PRP platelet counts ranged between 1,258,000 and 2,118,000/μL, with a mean of 1,616,000/μL, the enrichment ranged between 2.61- and 3.98-fold,

Table 1. Platelet counts of rabbits; base blood level and PRP ($\times 10^3$/μL).

Rabbit	Blood	PRP	Fold
1	390	1285	3.29
2	480	1790	3.73
3	525	1580	3.01
4	670	1750	2.61
5	368	1258	3.42
6	400	1590	3.98
7	534	1672	3.13
8	720	1889	2.62
9	486	1347	2.77
10	610	1645	2.70
11	562	2118	3.77
12	445	1468	3.30
Mean	516	1616	3.19

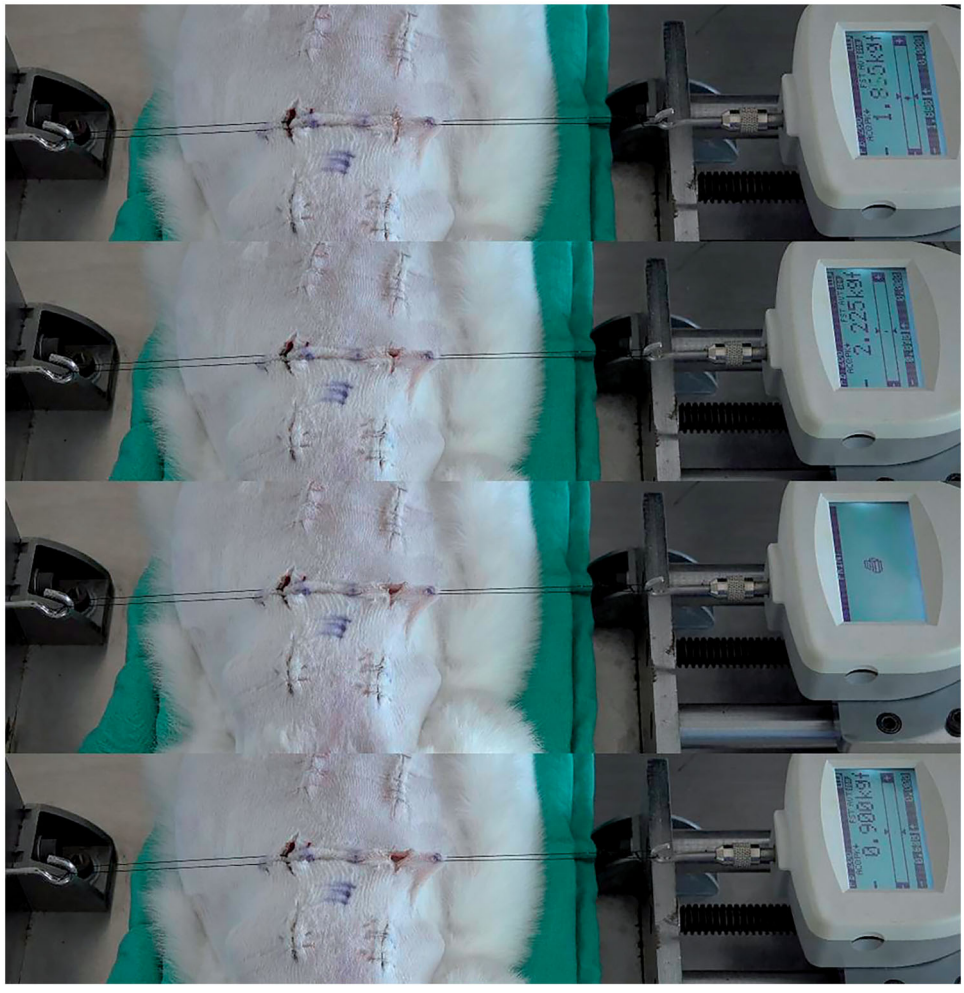

Figure 6. Measurement steps; note that when a sudden relaxation occurs during progressive increasing force, power-meter automatically records the pike force measurement.

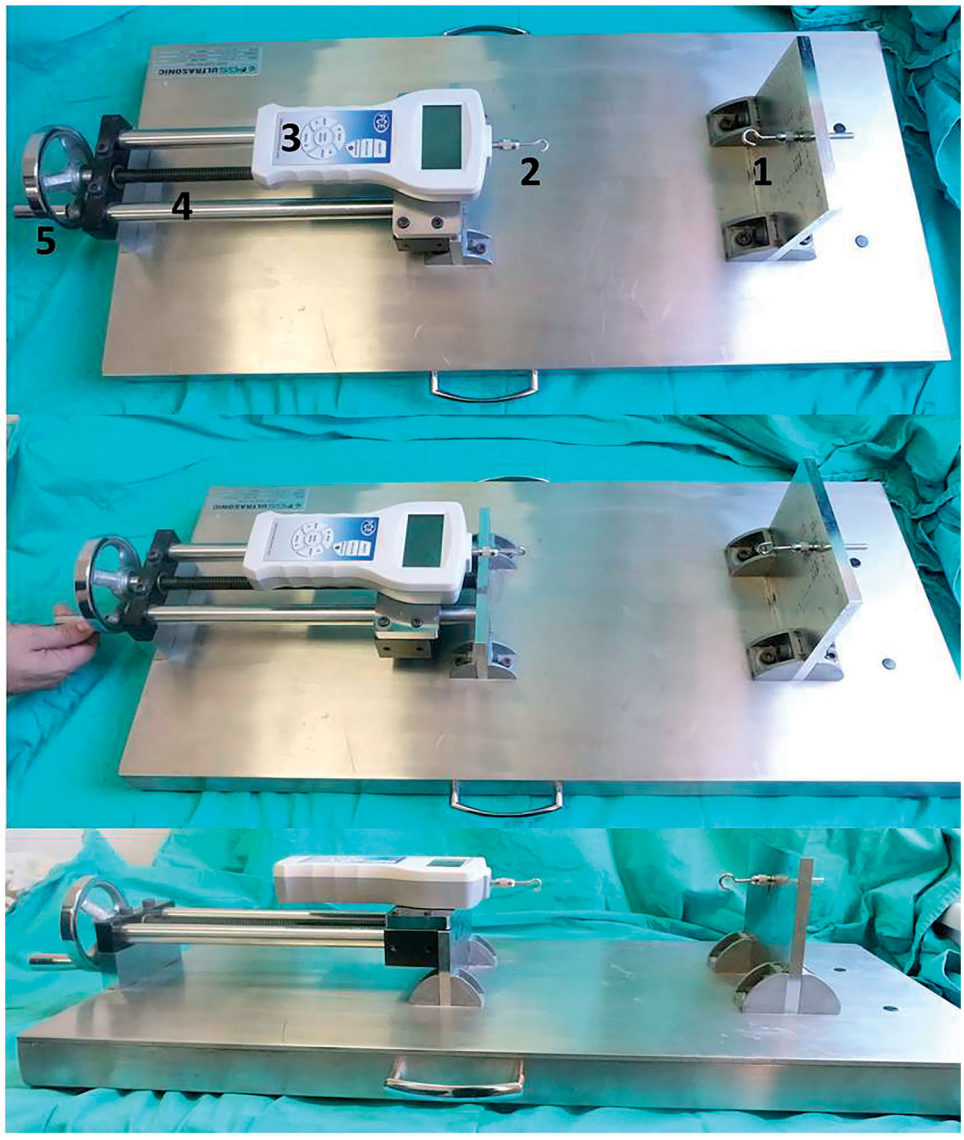

Figure 7. The home designed tensile force measurement device (by Mehmet Irfan Oktay); (1) stabile hook, (2) mobile hook, (3) power-meter, (4) rails, (5) manual movement control.

with a mean of 3.19-fold (Table 1). The tensile strengths during the rupture of the wounds that had received PRP and saline were compared and statistical analysis was performed (Tables 2 and 3).

The first result was that the tensile strength values obtained differed considerably across the rabbits (Tables 2 and 3). Therefore, the recorded tensile strength values were additionally evaluated in a ratio for each pair of incisions that had received a PRP or a saline injection for each rabbit (Table 2). The tensile strength difference was calculated by subtracting the values for the sides to which saline had been applied from the values for the sides to which PRP had been applied (Table 2). When the saline value is higher, the difference is recorded as minus signs in the table (Table 2).

Also, the ratio between saline/PRP was calculated by dividing the value for the saline by the value for the PRP. The saline to PRP ratios on the 7th, 14th, 21st and 28th days were 75.7, 104, 105.3 and 86.5%, respectively (Table 2). In addition, after removing the lowest and the highest values, the means of the remaining 10 values for the 7th, 14th, 21st and 28th days were calculated as 74.6, 102.7, 104.3 and 91.8%, respectively.

On the 7th day, the measured tensile force on the side where PRP had been applied was higher than the saline applied side, whereas the tensile forces due to the PRP and the saline were similar on the 14th and 21st days (Table 2). On the 28th day, the values of the measured tensile force for the sides to which PRP had been applied were slightly higher than saline applied sides (Table 2). But the statistical analysis did not yield any significant differences between the saline and the PRP data. An independent *t*-test was used for the statistical analysis and the results that had a *p*-value less than 0.05 were accepted as significant (Table 3).

Discussion

The effects of platelets on wound healing are well known: platelets are cells that are effective in initiating the wound healing process. Wound healing consists of the stages of inflammation, proliferation, and maturation, including components of coagulation, inflammation, angiogenesis, fibroplasia, contraction, epithelialization, and remodeling [1–4]. Damaged vessels become vasoconstrictive after injury, and contact with exposed tissue

Table 2. (A) Tensile strength measurements. (B) Graphic presentation.

Rabbit	Saline 7. day	PRP 7. day	Saline 14.day	PRP 14. day	Saline 21.day	PRP 21.day	Saline 28.day	PRP 28.day	Dif. 7. day	Dif. 14. day	Dif. 21. day	Dif. 28. day	%Dif. 7. day	%Dif. 14. day	% Dif. 21. day	% Dif. 28. day
1	0.820	0.940	2.930	2.225	2.310	2.745	1.125	2.400	0.120	−0.705	0.435	1.275	87.2	131.7	84.2	46.9
2	0.120	0.540	1.970	1.975	2.005	1.635	2.290	2.545	0.420	0.005	−0.370	0.255	22.2	99.7	122.6	90.0
3	0.550	1.140	1.815	1.695	2.265	1.780	2.065	2.695	0.590	−0.120	−0.485	0.630	48.2	107.1	127.2	76.6
4	0.110	0.170	1.090	1.000	2.040	1.315	3.135	2.290	0.060	−0.090	−0.725	−0.845	64.7	109.0	155.1	136.9
5	0.350	0.445	0.630	0.705	1.120	1.340	1.795	2.440	0.095	0.075	0.220	0.645	78.7	89.4	83.6	73.6
6	0.250	0.360	1.310	1.770	1.815	2.705	2.480	3.290	0.110	0.460	0.890	0.810	69.4	74.0	67.1	75.4
7	0.630	0.860	1.430	1.745	2.400	2.175	2.650	3.940	0.230	0.315	−0.225	1.290	73.3	81.9	110.3	67.3
8	0.945	0.835	2.250	1.990	2.515	2.095	3.155	3.575	−0.110	−0.260	−0.420	0.420	113.2	113.1	120.0	88.3
9	0.750	0.980	1.950	1.330	2.250	1.625	2.560	2.195	0.230	−0.620	−0.625	−0.365	76.5	146.6	138.5	116.6
10	0.310	0.355	1.105	1.045	2.650	2.380	3.815	3.050	0.045	−0.060	−0.270	−0.765	87.3	105.7	111.3	125.1
11	0.415	0.450	0.735	0.770	1.070	1.555	2.930	3.265	0.035	0.035	0.485	0.335	92.2	95.5	68.8	89.7
12	0.185	0.195	0.4	0.425	1.265	1.415	2.015	3.890	0.010	0.025	0.150	1.875	94.9	94.1	89.4	51.8
Mean	0.453	0.606	1.468	1.390	1.975	1.897	2.501	2.965	0.153	−0.078	−0.078	0.463	75.7	104.0	106.5	86.5

Values are in kgf. Dif.: Difference.
After removing highest and lowest mean of 10 values for differences 7,14, 21, and 28 days; 77.2, 102.7, 105.6, 85.4.

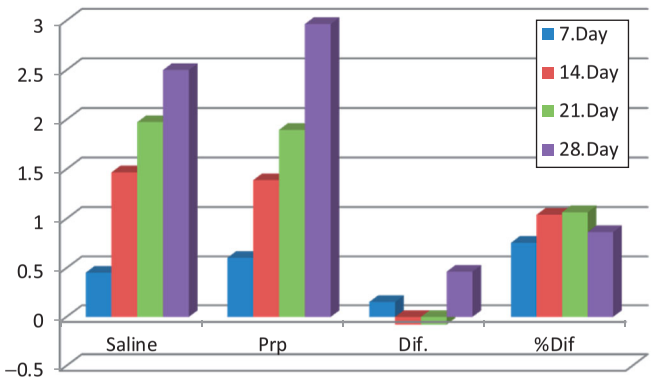

Table 3. Statistical analysis at 7, 14, 21 and 28 days, and *t*-test results.

	Mean	SD	Sig. (*p*)
Saline 7th day	0.4529	0.28281	0.235
PRP 7th day	0.6058	0.32901	
Saline 14th day	1.4679	0.74070	0.777
PRP 14th day	1.3896	0.58949	
Saline 21st day	1.9754	0.55705	0.723
PRP 21st day	1.8971	0.51135	
Saline 28th day	2.5013	0.71535	0.105
PRP 28th day	2,9646	0,62332	

activates platelets [1–4]. Platelets and hemostasis mechanisms start the inflammation phase. In the proliferation phase, which is the second stage of wound healing (3–10 days after injury), fibroblasts take on the main role and collagen production, extracellular matrix production, neovascularization, and angiogenesis occur. It is assumed that the remodeling phase starts on the 21st day and it takes about 1 year to complete [4]. A complete and firm scar formation is considered as the endpoint of wound healing.

In this study, we measured the effect of applying a single dose of PRP to the wound by evaluating weekly tensile strength changes. After injecting PRP and saline into the wounds' edges, we measured the changes in the tensile strength of the wound during the healing process with the help of a specially home-designed device. We objectively demonstrated the changes in tensile strength of wounds during the normal wound healing process and the effects of PRP on it. The aim of this study was to investigate the effects of PRP on skin wound healing by evaluating the tensile force changes. We thought that PRP would accelerate wound healing by affecting the stages of the wound healing process. Because the platelets initiate the inflammation phase, the effects of PRP application were expected to appear in the early period. Although the differences were statistically insignificant in the study, in the first week, the side of the vertical column on which PRP had been applied had a greater increase in tensile strength.

While the injections of saline and PRP showed more difference in the early days, this difference disappeared in the following days. We can say that platelets' positive effects on inflammation caused the tensile strength to increase in the early period of the healing process. However, as the elapsed time increased, the tensile strength equalized for the wounds that had received PRP and saline; this is an expected result because, in the progression of all wounds, the tensile strength becomes balanced in time. Since the aim of its application is to initiate inflammation, PRP does not have a positive effect on the late period of the wound. Perhaps additional PRP injections to wounds days or weeks after suturing can cause an increase in tensile strength. This option may be considered in further studies. Considering our observations in this study, it seems that an increase in tensile strength in the later days may be possible by additional delayed multiple injections of PRP to the wound rather than only at the creation of the wound.

The aim of the PRP application is to trigger the start of inflammation in normal tissues for rejuvenation and for wounds that do not heal or heal slowly. There may not be a benefit of applying PRP to the wound edges in an individual who has normal wound healing. Causing excessive platelet addition by applying PRP to the wound edges may not always be useful. PRP is considered to have a positive effect on tissue healing, but the possibility of the negative effect of excessive inflammation caused by excessive platelets on wound healing should also be considered [3].

In the study, in the first 7 days, in 11 rabbits, the tensile strength increased on the side to which PRP had been applied more than it did on the sides to which saline had been applied (Table 2), but the difference was statistically insignificant. At the end of week 2, the tensile strength was higher on the sides to which saline had been applied in 8 rabbits, but on the side to which PRP had been applied in 4 rabbits. At the end of week 3, tensile strength was higher on the sides to which saline had been applied in 7 rabbits, but on the sides to which PRP had been applied in 5 rabbits. At the end of week 4, tensile strength was higher on the sides to which saline had been applied in 3 rabbits and on the sides to which PRP had been applied in 9 rabbits. However, none of these differences was statistically significant. Although the increase in tensile strength in our study is not statistically significant, we observed that in the first week in all animals except one, the tensile strength was higher on the side to which PRP had been applied (Table 2). We believe that this situation is caused by platelets, especially their contribution to the inflammation phase. In the later weeks, the control data (the wounds injected with saline) and the PRP data showed similar progress. The study does not include 2- or 3-month periods, but considering the phases of the wound healing process, we believe that a positive effect of PRP on tensile strength in the longer term is not to be expected. However, we think that we have shown that the effect of injecting PRP is the rapid increase in the tensile strength of the wound in the early period.

An important contribution of the study is the home-designed device, used to measure the increase in the wound tensile force. The device we designed was similar to the device designed in 1970 by Thompson et al. that was used on pigs [5]. Measurement was made by manually adding weight to a scale mounted on the system [5]. Unlike that device, our device has the advantages of using modern technology to measure the force, using a digital power meter to make more precise and digital measurements, recording all changes in force, and recording the peak point of force during the rupture of the wound (Figures 3–5). Similar devices can easily be made and they can be used in different studies, especially where measurement of the tensile strength is necessary.

Several types of biomechanical assays have been used to measure tensile strength, but most of them are useful only for *in vitro* measurements [5–15]. Although very complicated devices are used in the *in vitro* environment, it is rare to have an inexpensive, sensitive, and ready, alternative technique that can be used *in vivo* [3, 5–15]. Our device's special abilities are its manual application of a very precise controlled force and its precise digital recording by its power meter of the first rupture force. Using the device for other purposes and studies is also possible. When applying a force, precisely recording the rupture moment and applying controlled manual force are important advantages. The device consists of a power meter and a manual lever mounted on a rail system, and its rail system is designed to eliminate the tremor effect on the power meter (Figure 6).

There are many factors in PRP applications that can cause differences in results. For effective PRP treatment, a PRP injection in a sufficient concentration and dose to initiate inflammation, activate platelets, and release growth factors is required [6, 16–27]. However, in clinical applications, major differences in results have been found [6, 16–27]. In our study, when evaluating the tensile strength measurements, the differences in the tensile strength from the different subjects over the same durations were striking. The differences were parallel for both the wounds that received PRP and those that received saline. We think that the differences

in wound tensile strength across rabbits for the same duration were caused by individual differences in healing and in platelets. Generally, it is accepted that the effective amount of platelet concentration for PRP applications is over 1,000,000/μL. However, because the average blood platelet level in rabbits is 500,000 (±100), that concentration is not suitable for PRP studies in rabbits. Therefore, in our work on rabbits, we used fold enrichment as a measure of the increase in the base blood level. The mean PRP concentration was 3.19-fold.

Today, PRP applications are not only for aesthetic purposes. They are also used in many areas, especially for tendon and muscle injuries [6, 16–27]. These studies consider the purpose of PRP to be to mimic the effects of platelets during wound injury. Therefore, PRP can be used effectively in areas without injuries (e.g. as part of aesthetic procedures). By extrapolating from platelet function in wounded areas, the benefit of PRP injections to wound healing would be expected to be higher when injected during the later, rather than the earlier, period of wound healing. According to the results of this study, PRP application in treating the injury is predicted to be useful during the follow-up period as in the acute phase.

Potential weaknesses of the study

We did not measure the rupture force of the normal skin of the rabbits. We just applied force to the center point, not the whole wound and we measured the force at the moment of the first rupture in the center part of the wound, not in the whole wound. Although in some articles, it is stated that more than a 4- to 5-fold concentration is required for an effective PRP treatment, in our study, the mean concentration of PRPs we injected into the wounds was 3.19-fold the blood base level. We sutured the wounds with staples. Although the full contact and the eversion of the wound edges were verified under magnification, staples are not as reliable as sutures. Also, in the study, PRP was applied only to the acute wound. Alternatively, for further studies, additional PRP doses could be administered in the wound area or on its edges and on later days after creating and suturing the wound. The study evaluates the effects of PRP on wound healing only in terms of tensile strength. This study does not comment on the effects of PRP on the other features of wound healing, such as the quantity and quality of the wounds.

Conclusion

The positive effects of platelets on inflammation and on the blood supply may increase the tensile strength in the early period. It is possible to measure precisely the tensile strength with an economical device designed in-house that has a counterpart in *in vivo* studies.

Acknowledgement

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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