Study on the Application of PRP Enriched with Hyaluronic Acid to Skin Wounds-Macroscopic Result

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ABSTRACT: Tissue healing is a complex process of replacing damaged tissue structures, being a dynamic process with spatial and temporal involvement. In practice, there are three types of healing: primary, secondary and tertiary. Since even tertiary healing can cause different problems depending on the individual patient, the medical world has always strived for new, easier, more effective, faster, and low cost-effective methods to cover skin wounds. This experiment aims to find a method that is as efficient and fast as possible, with minimal costs, of native healing of skin wounds and the development of a protocol that is as reliable and safe as possible for the patient. The experiment was carried out within the animal facility of the University of Medicine and Pharmacy of Craiova, with the agreement of our institutional Ethics Committee. The experimental group consisted of 30 individuals (Wistar laboratory rats), clinically healthy, male and female, being divided into 3 sublots of 10 individuals each. Each sublot was sacrificed at different time intervals: 7, 14 and 21 days, respectively. The local evolution of each individual was examined macroscopically, following their size, whether or not the infection was present and the presence of other self-inflicted lesions later. Macroscopically, a faster wound healing was found where PRP enriched with hyaluronic acid was applied from the 7th day, so we can say that at first glance the "free" healing period in the case of enhanced epithelialization was about 7 days shorter. This fact will be confirmed or refuted following histopathological examinations and immunohistochemistry.

KEYWORDS: Hyaluronic acid, PRP, Wistar rats, healing, skin wound.

Introduction

Tissue healing is a complex process of replacing damaged tissue structures, being a dynamic process with spatial and temporal involvement [1].

This process has a special complexity, with the involvement of a multitude of factors, both internal and external, being organized in three stages: inflammatory, proliferative and remodeling, successive and overlapping stages [2].

In practice, there are three types of healing: primary, secondary and tertiary. If it is not possible to resort to primary healing, one can opt for secondary healing, which is often long-lasting and with significant sequelae, or tertiary, involving various methods of coating that follow the "reconstructive scale" [3,4].

Since even tertiary intentional healing can cause different problems depending on the individual patient, the medical world has always strived for new, easier, more effective, faster, and low cost-effective methods to cover skin wounds.

Thus, various tissue repair protocols have been developed, such as healing using negative vacuum pressure therapy [5], the use of copper nanoparticle dressings for chronic wounds [6], matrices derived from porcine bladder used in diabetic wounds [7], use of chitosan nanoparticles and honey-based compounds [8], topical based on Mentha piperita [9], balm based on Brassica oleracea [10], use of hyaluronic acid in various forms [11], as well as the widespread use of PRF [12] and PRP [13,14,15].

Hyaluronic acid is a biopolysaccharide that is part of the extracellular matrix, being found in higher concentrations in the skin, joints and cornea [16]. Dermal fibroblasts and epidermal keratinocytes are responsible for its synthesis, hyaluronic acid having a high molecular weight of 600,000 to 1,000,000 kDa. [16]. Being a very versatile compound, hyaluronic acid has a wide range of uses in various medical fields, such as ophthalmology [17], sports medicine [18], facial aesthetics [19].

PRP is obtained by centrifuging peripheral blood according to specific protocols, being rich in growth factors. Due to its composition, as well as its non-allergic properties, it is a good
treatment option in many medical areas, with good results, such as: dentistry [20], orthopedics [21], cosmetic surgery and dermatology [22], sports medicine [23].

**Material and Method**

This experiment aims to find a method that is as efficient and fast as possible, with minimal costs, of native healing of skin wounds and the development of a protocol that is as reliable and safe as possible for the patient.

The experiment was carried out within the animal facility of the UMF Craiova, with the approval of the institutional Ethics Committee.

The experimental group consisted of 30 individuals (Wistar laboratory rats), clinically healthy, male and female, being divided into 3 sublots of 10 individuals each.

Each subplot was sacrificed at different time intervals: 7, 14 and 21 days, respectively. The local evolution of each individual was monitored during the experimental days, as well as their general state of health. Before slaughter, the lesions were examined macroscopically, following their size, whether or not the infection was present and the presence of other self-inflicted lesions later.

After macroscopic observation of the lesions, they were excised, up to the superficial surface, under general anesthesia. The pieces were being sent to the laboratory for histopathological and immunohistochemical examination. After sampling, the rats were slaughtered.

Classical surgical instruments were used to create skin defects in the posterior thorax. Two defects were performed per individual, 1cm in diameter each, one of the defects representing the control site and the other the working variable (Figures 1 and 2).

**Figure 1. Preoperative drawing-Control site (left), Working variable (right).**

**Figure 2. Surgical skin defects.**

At the working variable, the experimental product was applied, and at the control site, the healing was done freely. During the surgical procedure, the animals were under general anesthesia with ketamine and xylazine.

The experimental product applied at the working variable was a mixture of PRP with granular hyaluronic acid. PRP was obtained from venous blood collected from the jugular vein, on syringes with 3.8% sodium citrate, using a 26G peripheral venous catheter (Figure 3).

**Figure 3. The experimental product obtained from the combination of granular hyaluronic acid and PRP.**

We compared the degree of healing between the control site and the working variable by a 2-tailed t test at 7, 14 and 21 days postoperatively. Statistical significance was established at p<0.05.

For the statistical analysis Microsoft Excel and GraphPad Prism 9 were used.
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Result

On the first day, after the defects were surgically produced and the PRP enriched with hyaluronic acid has been applied, no change was observed in the wounds, in the case of any of the individuals.

On 7th day, there were no signs of sepsis or alteration in the general condition of the animals in the experiment, which behaved normally throughout this period.

At the level of the lesions, macroscopically the individuals of the sublot showed no signs of local infection, edema or perilesional erythema. It also showed no other injuries caused by self-harm.

From the point of view of wound healing, there were no major macroscopic changes between the control site and the working variable, the contraction of the wound being minimal, 0.1-0.2cm in diameter, with a stable blood crust on their surface, no bleeding, no interstitial fluid (Figure 4).

At the level of the control site, the diameter of the wound remained 1cm, while at the level of the working variable in 3 individuals no changes were observed, in 4 individuals a reduction of 0.1cm was observed and in the other 3 individuals a reduction of 0.2cm (Figures 5-8).

On the 14th day, none of the individuals showed signs of sepsis, they had a preserved appetite, and their general condition is good, showing a normal behavior during the 2 weeks.

Macroscopically, there were no signs of local infection or self-inflicted scratch lesions on the surgically produced lesions.

Regarding the macroscopic healing of the wounds, major changes were observed compared to the appearance at 7 days, both in control site and in working variable. Thus, at the level of the control site, where no product was

Figure 4. 7th day post-surgery.

Figure 5. Diameter of unhealed wound (cm) 7th day post-surgery.

Figure 6. Data distribution with standard deviation at the 7th day.

Figure 7. Control site (1cm diameter) 7th day post-surgery.

Figure 8. Working variable (diameter) 7th day post-surgery.
applied and the healing followed its natural course, there was a 50% reduction in wound diameter on average, the surface of the wound being still covered by a stable blood crust, no active bleeding, no extravasation of interstitial fluid. At the level of the control lesion, an average reduction of 85% of the initial diameter was observed, thus remaining an unepithelialized surface of 0.1-0.3 cm, covered by a stable, bloody crust (Figures 9-11).

At the level of the control site, at 14 days, there was a cure of 50% of the wound in 30% of the individuals, half of them showing a reduction in diameter by 0.4 cm, and 2 individuals a reduction of 0.6 cm (Figure 12).

At the level of the working variable, it was found that 50% of the individuals had a wound of 0.2 cm, 40% had a wound healing rate of 90% and only one individual had a wound of 0.3 cm (Figure 13).

On 21st day, the subjects showed no changes in general condition or appetite, the behavior being normal and the signs of sepsis absent. There were no signs of local infection in the wounds, the rats showing clean wounds during the 3 weeks, without active bleeding or secondary self-harm.

Macroscopically, at the end of the experiment there was an important difference compared to the previous week, as well as between control site and working variable. At the level of the control site, on the 21st day, an incomplete epithelialization was found, of approximately 80-90%, with a minimum granulation area of 0.1-0.2 cm, while at the level of working variable the epithelialization was complete, being present only the postoperative scar (Figures 14-16).
At the level of the working variable, all the individuals from group 3, at 21 days postoperatively, no longer had granulation tissue, instead of the wound, a good quality postoperative scar could be observed (Figure 18).

Discussions

The use of PRP and hyaluronic acid has gained great popularity in recent years, with medical research trying to find as many and varied uses as possible.

Although initially the two were used separately, and comparisons were often made between them [24,25,26,27], following the positive results obtained in the use of both independent PRP [28] and hyaluronic acid [29,30], the medical world turned its attention to the effects of the combinations of the two.

Hyaluronic acid, used alone in skin abrasions or perianal wounds in rats, has shown an acceleration in their healing [29,30], while PRP has provided satisfactory results in tendon healing in rats [31], but also in oral surgery, being used mainly in bone regeneration [32].

Although the combination of PRP with hyaluronic acid is less popular, it is mainly used in the treatment of osteoarthritis of the knee, with good results especially at 6 months and 12 months [33,34,35], and in facial rejuvenation, with obvious clinical results in level of facial skin [36].

Given the satisfactory results obtained from the use of PRP, hyaluronic acid and their combinations in different structures, we considered that PRP enriched with hyaluronic acid can bring major benefits in healing skin wounds.

We decided to follow the macroscopic evolution of the wounds treated with PRP enriched with hyaluronic acid and to follow the possible side effects at local level, but also at...
systemic level, which may occur during the experiment.

The 30 individuals in the group survived and showed no signs of altered general condition during the experiment.

Also, at the local level, none of the lesions showed signs of infection, so the macroscopic healing followed its natural course.

The laboratory animals did not show any signs of local discomfort during the 21 days, as there were no lesions of perilesional scratches or wounds.

Although after the first 7 days there was no major change between the control site and working variable, on the 14th day the macroscopic differences were visible between the two wounds, so that the control site was closed in a proportion of 40-60%, and at the level of working variable in the proportion of 80-90%.

At the end of the experiment, on the 21st day, the macroscopic appearance of the control site was similar to the appearance of the working variable at 14 days, while the control was completely healed, with the presence of a good quality elastic scar.

It was decided to carry out both the control site and the working variable, at the level of the same subject, in order to minimize as much as possible the intervention of the individual factors in the healing process of the wounds that were to be examined.

An impediment to the use of this compound at this time is the sterility of the product.

Although PRP is obtained from its own biological product, blood, which is not normally contaminated with pathogens, harvested in sterile conditions, the granular hyaluronic acid used is not a sterile product.

Thus, the problem is to find a method of sterilizing the preparation, so as not to contaminate the wound, which does not affect the component properties and does not distort or destabilize the product obtained by combining the two.

Conclusions

Macroscopically, a faster wound healing was found when PRP enriched with hyaluronic acid was applied from the 7th day, so we can say that at first glance the “free” healing period in the case of enhanced epithelialization was about 7 days shorten.

This fact will be confirmed or refuted following histopathological examinations and immunohistochemistry.

If microscopic data prove the effectiveness of combining PRP with granular hyaluronic acid, this product could be a real help in healing skin wounds, both by reducing the actual healing time and by providing a solution where other methods of coating are not possible.

Conflict of interests
None to declare.

References


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