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REVIEW ARTICLE

Bioactive Medications for the Delivery of Platelet Derivatives to Skin Wounds

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Abstract: Chronic wounds are the result of alterations in the complex series of events of physiological wound healing. In particular, the prolonged inflammation results in increased protease activity, in the degradation of extracellular matrix (ECM) and of growth factors (GFs). The relevance of platelet GFs in maintaining and restoring the complex equilibrium of different moments in wound healing is well recognized. Moreover, the observed decrease of their levels in chronic wounds suggested a possible therapeutic role of the external application to the wounds. It has been also pointed out that tissue regeneration can be more efficiently obtained by the synergic use of different GFs. Platelet derivatives such as platelet-rich plasma (PRP) and platelet lysate (PL) are able to release GFs in a balanced pool. Their therapeutic use in regenerative medicine and wound healing has been therefore more and more frequently proposed in clinical trials and in the literature. The development of a suitable formulation able to control the GFs release rate, to protect the GFs, and to assure their prolonged contact with the wound site, is of paramount importance for the therapeutic success. The present review considers some formulation approaches for PRP and PL application to wounds

Keywords: Platelet-rich plasma, platelet lysate, drug delivery systems, skin regeneration, wound healing, extracellular matrix (ECM).

1. THE WOUND HEALING PROCESS

Immediately after the occurring of a wound, a complex series of events is activated, with the aim to protect the organism from pathogen microorganism invasion and to restore tissue integrity. All these events are usually classified in four phases, that are hemostasis, inflammation, proliferation and tissue remodeling. Hemostasis occurs in a few minutes to avoid blood loss. It involves the activation of clotting cascade and the activation of platelets, that change morphology, adhere to collagen to form a plug, and release the content of their alpha granules, consisting in Growth Factors (GFs) and cytokines, that are signaling molecules essential for the regulation of further healing phases. In particular, it is possible to remember Transforming Growth Factor (TGF), Platelet-Derived Growth Factor (PDGF), Vascular Endothelial Growth Factor (VEGF), Tumor Necrosis Factor (TNF), Prostaglandin (PGE2), Fibroblast Growth Factor (FGF), Epidermal Growth Factor (EGF), Matrix Metalloproteinases (MMP), and Interleukin-1 [1, 2]. Vascular disruption and clotting induce moreover hypoxia, that stimulates Hypoxia-Inducible Factor-1 (HIF-1) [3, 4].

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The second phase is represented by inflammation, in which, thanks to TGF-β and interleukin chemotactic signaling, in about 48 hours, neutrophils infiltrate the tissues around the wound. Neutrophils can release, in turn, substances such as lactoferrin, proteases, neutrophil elastase and cathepsin, which will destroy bacteria and dead host tissue [1], and oxygen free radicals (ROS) that contribute to the antibacterial activity. Messenger molecules from platelets attract also macrophages rich in TGF-β and EGF, that stimulate angiogenesis and granulation tissue formation. However, if the inflammation phase is too prolonged, it can result in tissue damage and delayed proliferation typical of a chronic wound, characterized by healing process lasting up to three months or more [5]. It has been pointed out that also lack of oxygenation restoring contributes to the induction of chronic wounds that are, therefore, usually hypoxic [6, 2].

The proliferation phase involves angiogenesis, fibroblast migration, the formation of granulation tissue, epithelialization and wound retraction [1]. Angiogenesis starts within the first few minutes after the wound occurrence favored by the release of TGF-β, PDGF and FGF from platelets, and by VEGF, released in response to hypoxia, that induces angiogenesis during all the proliferative phase [7-9]. TGF-β and PDGF also stimulate the proliferation of fibroblasts and their

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migration to wound, where they lay down extracellular matrix proteins and produce collagen and fibronectin [1, 10, 11], and promote their differentiation to myofibroblasts, that play a role in wound contraction [12]. After 16-24 h from the wound occurrence, keratinocytes, activated by growth factors, chemokines and cytokines, proliferate and move to the healing area. A re-epithelization complex series of events starts, that will result in the regeneration of a functional epidermis [13]. A simplified scheme of wound healing time course is reported in Fig. (**1**).

When some kind of alteration occurs in the complex series of events of physiological wound healing, it results in chronic wounds, whose healing is impaired and can take from 12 weeks [14] up to 12-13 months to resolve [15]. Impaired wound healing can be due to several concomitant conditions such as malnutrition or depression, and pathologies such as chronic inflammation, diabetes mellitus, hormonal dysregulation, vascular diseases and thrombocytopenia related to chronic liver disease [2, 16-18]. Especially in the case of diabetes mellitus and vascular diseases, the gravity of the ulcers can lead to lower extremity amputation. Chronic wounds are characterized by the failure of the passage from the inflammatory to the proliferative phase so that ulcers lasting even for years can be formed. Prolonged inflammation induces increased protease activity resulting in the degradation of Extracellular Matrix (ECM) and of growth factors [19, 20].

2. THE PLATELET-DERIVED GFS AND THEIR ROLE IN WOUND HEALING

Among the most representative platelet GFs, PDGF is released by platelets immediately after the injury and has chemotactic effect for neutrophils, monocytes and fibroblasts. It promotes the production of ECM and collagen by fibroblasts and their change to myofibroblasts, inducing the occurrence of granulation tissue and the contraction of the collagen matrix. It stimulates further production of GFs such as TGF-β by macrophages and contributes to the inflammatory phase [10-12, 21, 22]

EGF is produced in the hemostasis phase by platelets although it is also released in later stages by macrophages and fibroblasts. It exerts a chemotactic effect on keratinocytes and promotes their proliferation playing an essential role in re-epithelialization [11].

TGF-β is made by three isoforms, TGF-β 1-3, among which, the TGF-β1 is the most abundant during the wound healing [2]. It is released by platelets, neutrophils, macrophages and fibroblasts, and strongly promotes angiogenesis [10]. It regulates matrix deposition by fibroblasts, stimulating the synthesis of ECM components such as collagen, fibronectin, and hyaluronic acid [2].

Insulin-like Growth Factor (IGF) is released by platelets at the early stages of hemostasis phase. It attracts leukocytes, and participates in the inflammatory and proliferative phases, playing a regulatory role in the fibroblast proliferation [2]. The reduced expression of IGF has been observed in impaired wound healing diabetic patients [23]. However, high levels of IGF may be associated with the hypertrophic scar occurrence [11].

VEGF, together with TGF-β and FGF-β, is one of the mediators that are more involved in angiogenesis promotion, in neo-vasculogenesis, and in vascular permeabilization [10]. It is responsible for the migration into the wound area of inflammatory cells and of marrow-derived progenitor cells [24]. The main production of VEGF during wound healing occurs by keratinocytes in the epidermis, but VEGF is released after injury also by fibroblasts and macrophages. VEGF is also involved in the regulation of scar tissue deposition [25].

The recognized relevance of platelet GFs in maintaining and restoring the complex equilibrium of different moments in wound healing and the observed decrease of their levels in chronic wounds suggested in the last years, a possible therapeutic role of GFs when externally applied to wounds. However, this therapeutic approach requires the development of suitable carriers and formulations. The main challenge is, in this case, to protect GFs, improving their stability in the wound environment, and to favor their controlled release once they are applied at the wound site. In this perspective, in the literature, different advanced Drug Delivery Systems (DDS) are proposed with enhanced characteristics with respect to conventional semisolid formulations [26]. The most frequent formulations that have been proposed for the delivery of GFs to wounds according to the literature are represented by polymeric micro and nanoparticles, lipid nanoparticles, nanofibrous dressings, hydrogels and scaffolds [26]. Attention is also given in the literature, in the development of GFs carriers, to use new materials that are chosen in a range of biocompatible bioactive materials such as chitin and chitosan, hyaluronic acid, chondroitin sulfate, dextran, gelatin and collagen [27, 14].

Considering the complex interplay of mediator and growth factor effects during wound healing phases, it was

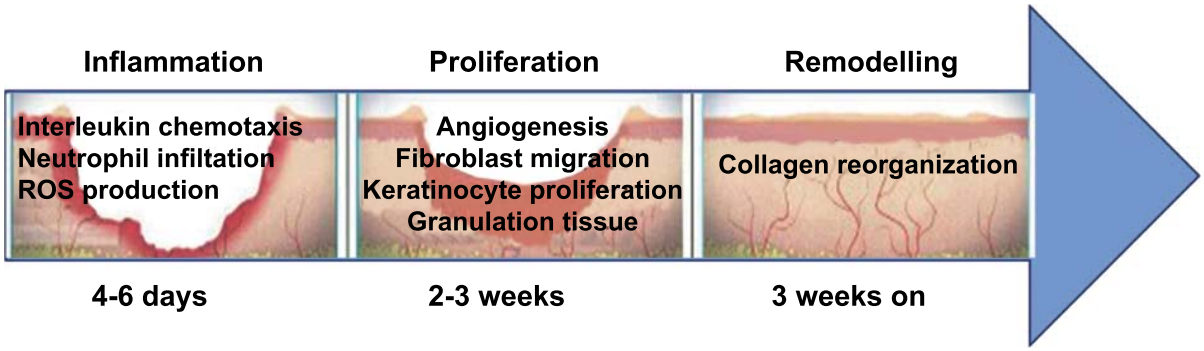

Fig. (1). Schematic representation of wound healing phases.

observed that no single exogenous agent can efficiently support, alone, all the aspects involved in repair [28]. Tissue regeneration can be more efficiently obtained by the synergic use of different GFs, as demonstrated by the more complete angiogenesis that followed the sequential administration of both VEGF and PDGF in comparison with the administration of VEGF alone [29].

In this perspective, as an alternative strategy to the administration of single or combined GFs, the therapeutic use in regenerative medicine and wound healing of platelet-derived hemoderivatives has been more and more proposed in clinical trials and in the literature [30, 31]. Among these derivatives, Platelet-Rich Plasma (PRP) [32-37], Platelet Gel (PG) [38-41], and Platelet Lysate (PL) [42-46] have been largely described in the literature. All of them can be either autologous or allogeneic. The use of autologous platelet derivatives reduces the risks of viral infection and it is better accepted by patients. Limitations can, on the other hand, arise in the case of patients with variable or poor quality platelets [47, 28].

The different platelet derivatives are produced by means of procedures based on different centrifugation steps. Although preparative details can be the subject to some variations and can impact on product composition and performance [30, 31], a simplified scheme of the different derivatives and of their preparation is illustrated in Fig. (**2**).

The centrifugation of whole blood results in the separation of an upper layer of plasma and platelets. Provided an anticlotting agent is previously added to the whole blood, the upper layer does not coagulate spontaneously but remains liquid, and it can be collected and further centrifuged to be separated into Platelet-Poor Plasma (PPP) and a pellet of Platelet-Rich Plasma (PRP). PRP is generally defined as a volume of autologous plasma with a platelet concentration above blood baseline (between $1.5 \times 10^5/\mu L$ and $3.5 \times 10^5/\mu L$) [31]. Platelet Gel (PG) originates by the activation of PRP by means of either thrombin [48], thromboplastin [49], calcium salts [48, 50] or collagen [50] resulting in activated platelets embedded in a fibrin network [31]. The platelets of PRP alternatively can be disrupted by freeze/thaw cycles or ultrasound treatment, originating a solution of Platelet Lysate (PL) [31, 51].

Although some differences in composition can be found between these derivatives, a physiologically balanced mixture of growth factors is always present. Their release presents, therefore, clinical advantages with respect to single GF administration as they more closely respond to the complex necessity of the mediators that are involved in the tissue repair process [28, 30].

In the perspective of the optimization of the clinical use of hemoderivatives as therapeutic agents, some drawbacks should, however, be overcome, among which the poor stability of GFs, and especially in the case of PRP and PL, the short retention times at the application site. This aspect could result especially critical in case of ophthalmic delivery [52, 53], but it can play a relevant role also in the case of cutaneous application in skin wounds.

A direct comparison of PL and Platelet Gel (PG) on the rat wounds was carried out [54], suggesting the better performance of PG, as it can be seen in Fig. (**3**). This result can be attributed to a gradual discharge of GFs from platelets and to the semisolid texture of PG that supports its longer persistence at the wound site. However, among the advantages of PL with respect to PG is the ease preparation of ready to use single doses that can be stored frozen until use.

A possible strategy to improve PRP and PL permanence at the wound site, and their GFs stability, is represented by the development of suitable formulations, able to protect the GFs from degradation and to maintain them for longer times in contact with the wound bed. A suitable formulation could moreover sustain the release of GFs secreted by platelet alfa granules according to therapeutic requirements, and, if it is based on bioactive materials, it could provide a synergistic effect in tissue reparation.

Like in the case of GFs, formulations suitable for hemoderivatives can be represented, as illustrated in Table **1**, by hydrogels, sponge-like dressings, powders and beads,

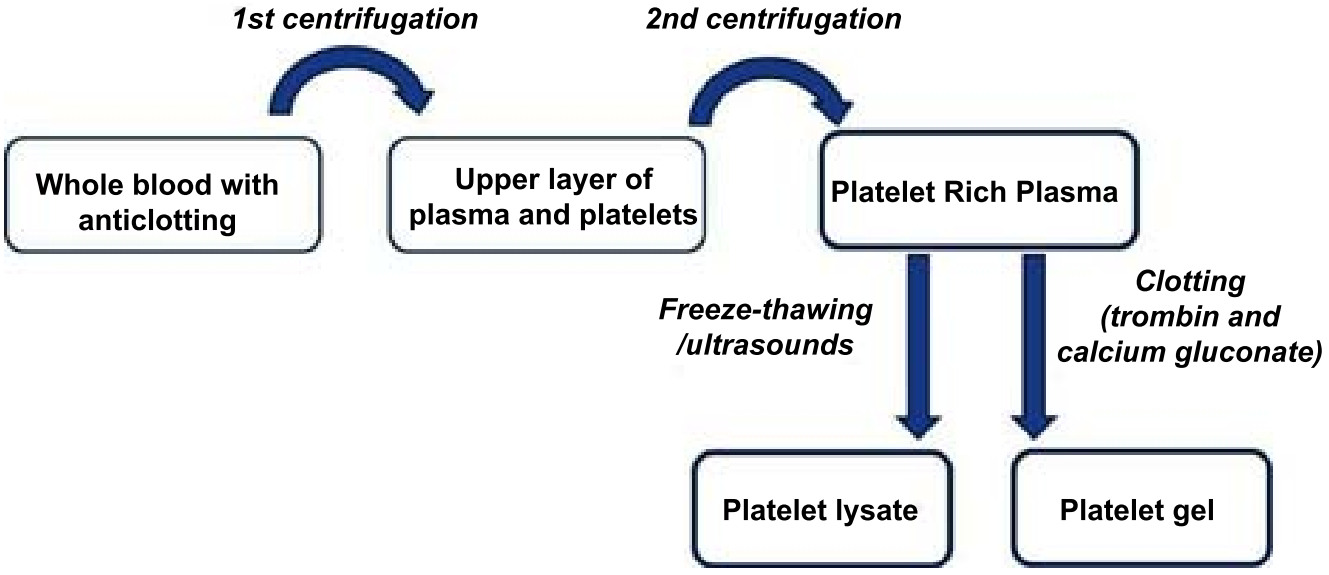

Fig. (2). Scheme of platelet derivative preparation methods.

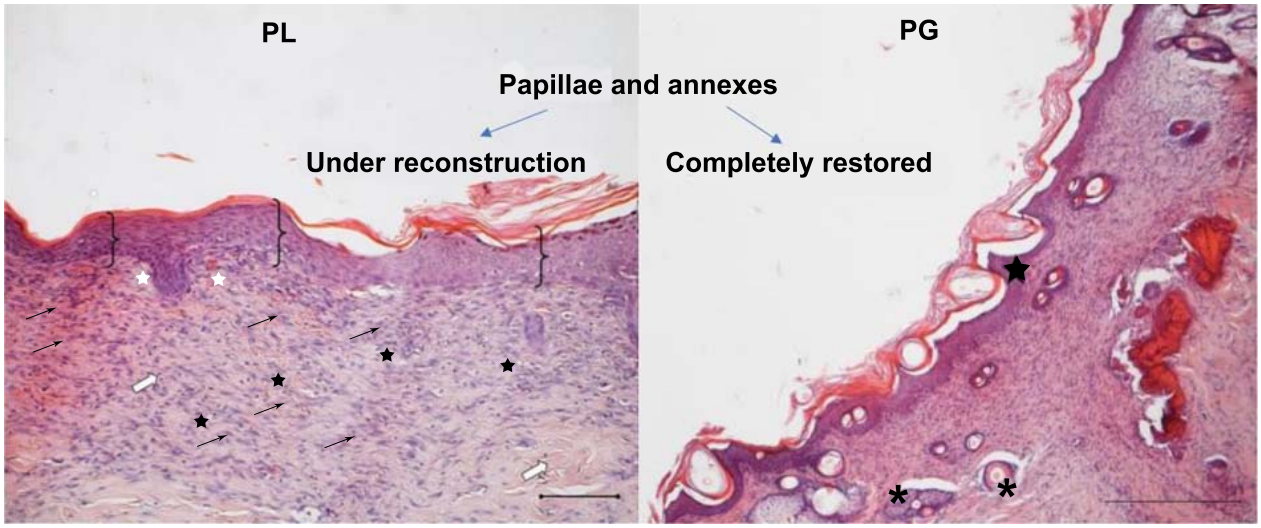

Fig. (3). (**a**) Hematoxylin-eosin staining of histological sections of wounds in a rat model after 18 days of treatment with platelet lysate and (**b**) platelet gel. In PG treated wound papillae (stars), and cutaneous annexes (asterisks) can be seen. Modified from [54] (Creative Commons CC-BY 4.0 license).

Table 1. Classification of the platelet derivative delivery systems by formulation type.

-	Materials	Association of Actives	Evaluation Model	Platelet Derivative	References
Hydrogels	chitosan	Tigecycline in chitosan-TPP NPs	Fibroblast cell line Drosophila melanogaster infection model	Human PRP freeze dried and added to hydrogel as powder	[70]
	Gelatin, GA crosslinked		mice	Murine PRP releasate dropped into the hydrogel to complete absorption	[71]
	Gelatin, freeze dried granules	bFGF	Injection in leg ischemia murine model	PRP absorbed in hydrogel granules	[50]
	Methacrylate modified Gellan gum, photo-crosslinkable		HUVEC	PL gradient obtained by microfluidic	[72]
Sponge-like dressings	Chitosan glutamate, sodium hyaluronate		Fibroblasts	PL loaded before freeze drying	[73]
	Chitosan/sericin		Fibroblasts and human skin biopsies	PL loaded before freeze drying and absorbed on the dressings	[74]
Powders/beads	Pectin/chitosan particles by ionotropic gelation with $CaCl_2$	Manuka honey fractions	Murine wound model	PL absorbed into particles	[75]
	Collagen beads		CAM chicken model	PL in collagen solution during beads preparation	[76]
	Hyaluronic acid/calcium alginate core-shell particles in the alginate matrix	Vancomycin	Fibroblast cell culture Human skin biopsies	PL in the core of the particles	[77]
Micro nanoparticles	LMW heparin and protamine microparticles		Intradermal injection in rat wounds	PRP	[78]

Table (1) contd….

-	Materials	Association of Actives	Evaluation Model	Platelet Derivative	References
	Porous silicon microparticles		Fibroblast cell culture Human skin biopsies	PL	[79]
	SLN associated with chondroitin sulfate and sodium hyaluronate in chitosan dressings	Silver sulfadiazine	Fibroblast cell culture	PL	[80]
	Chitosan oleate polymeric micelle and nano-emulsions in chitosan glutamate dressings	Silver sulfadiazine and alfa tocopherol	Fibroblast and keratinocytes cell culture, murine wound model	PL	[54, 81, 82]
Electrospun membranes	Silk Fibroin		Fibroblast cell culture	PL added before electrospinning	[83]
	Chitosan and poly-(ethylene oxide)		Keratinocyte and fibroblast cell cultures	PRP electrospun in the polymer solution	[84]
Scaffold	Silk Fibroin		Murine wound model	PL	[85]
	Chitosan		*In vitro* characterization	PRP loaded during preparation and absorbed on the prepared scaffold	[86]
	Collagen/gelatin		Murine wound model	PL	[87]
	Chondroitin sulfate and sodium alginate		fibroblasts	PL	[88]

nano-microparticles, electrospun membranes. Hydrogels can be based on different polymers such as chitosan, gelatin and alginate, and they have in common a three-dimensional structure based on crosslinking occurrence, that can in some cases mimic the extracellular matrix network improving the cell growth [55-58]. This structure is important for the mechanical properties of the hydrogel and is responsible for its ability to absorb wound exudates [14, 59]. Modulation of exudate absorption is a relevant feature also in the case of sponge-like dressings, generally obtained from hydrogels by means of a freeze-drying process [60-62]. Nano and microparticles have been largely studied in the last decades also in wound healing applications [63-66] been proposed either to be injected into the wounds or to be applied on them associated to other drug delivery systems, that can be again represented by hydrogels or dressings [54].

More recently, electrospun membranes have been more and more often proposed as versatile drug delivery systems [67, 68], and specifically for wound healing in which the peculiar three-dimensional structure, resembling the extracellular matrix, seems especially useful to favor the cell growth and therefore, the wound re-epithelization [69].

A further and particular approach is represented by the association of hemoderivatives to scaffolds. Scaffolds, thanks to the presence of an interconnected porous network, represent a three-dimensional support for the growth of regenerative tissue. Scaffolds can either have a cellular component and be populated by cells before application to the wound, or they can be acellular and act as support for population by cells at the application site [89]. They should, in any case, show mechanical resistance but adequate porosity to allow cell growth inside the pores, and to allow oxygen and nutrients exchange. Optimal porosity is also relevant for the development of new vascularization inside the regenerated tissue [90].

The development of suitable formulations by the delivery of hemoderivatives allows moreover their association with drugs. Because infections are often responsible for a further delay in healing of chronical wounds, bioactive dressings with associated drugs having antibacterial activity have been quite often studied, aimed to be loaded either with PRP or with PL.

3. PLATELET REACH PLASMA DELIVERY SYSTEMS

A chitosan-Platelet-Rich Plasma (PRP) hydrogel, containing the antibiotic tigecycline loaded in nanoparticles was proposed to treat *S. aureus* infection in chronic wounds.

Tigecycline nanoparticles of 95±13 nm dimensions were obtained by ionic cross-linking of chitosan with Tripolyphosphate (TPP) and dispersed in a PRP loaded chitosan hydrogel. The chitosan hydrogel was prepared aseptically by dissolving 2% (w/v) chitosan in 1% (v/v) acetic acid. The solution was neutralized with NaOH to pH 7.4, centrifuged and washed in PBS. PRP obtained from a blood bank was activated by addition of 10% (w/v) $CaCl_2$, freeze-dried and crushed into fine powder and added to the chitosan hydrogel.

Rheological characterization confirmed shear thinning behavior. The gel system showed thermal stability and injectability and released the drug in a sustained manner. Cell proliferation and migration assay performed on fibroblast

cell line demonstrated the bioactivity of PRP loaded chitosan gel.

The antibacterial activity due to tigecycline was demonstrated *in vitro* and *in vivo* in a Drosophila melanogaster infection model [70].

Another gel system proposed in the literature was based on a biodegradable gelatin hydrogel impregnated with murine PRP releasate (PRPr). PRPr represented the supernatant obtained by PRP after activation with $CaCl_2$ and centrifugation, rich in growth factors such as VEGF, PDGF and TGFβ1, released by platelets. Acidic gelatin with an isoelectric point of 5.0 was used to prepare hydrogel sheets by heating at 40°C and crosslinking with Glutaraldehyde (GA) for 24 h at 4°C. After treatment with glycine to block the residual GA, the sheets were washed with double-distilled water, freeze-dried and sterilized with ethylene oxide gas. PRP was obtained by activation with $CaCl_2$ and centrifugation. Volumes of 100 µl of the supernatant (PRPr) were dropped onto gelatin disks until complete absorption.

The percentage wound area calculated with respect to the original lesion, the neo-epithelialization and the wound contraction were compared on days 1, 5, 7, 14 and 21 post-wounding on 4 groups of 45 mice (90 wounds in total) each treated with saline (control), gelatin sheets, PRPr and PRPr absorbed on gelatin sheets. After 5, 7 and 14 days, for PRPrG, PRPr and gelatin treatments wound area was statistically smaller than that of the control group. On day 14, a new capillary formation was evaluated by anti-von Willebrand factor immune-histological staining. It was observed that the treatment with PRPr loaded gel reduced wound area, increased epithelialization length, prevented wound contraction and stimulated angiogenesis more than PRPr. Moreover, the authors suggest that gelatin sheets acting as a sustained-release system of PRP GFs might be useful to reduce the variability of GFs concentration in autologous PRP preparations [71].

A combination of chitosan and gelatin was performed to obtain a sponge crosslinked with tannic acid. The sponge showed good stability and mechanical resistance, and high porosity. Antibacterial properties were found against *E. coli* and *S. aureus* and were attributed to chitosan and tannic acid presence. The sponges were loaded with PRP obtained by double centrifugation from rabbit blood and activated by 0.25 M $CaCl_2$. Loading was obtained by soaking the sponge in PRP and by freeze drying. Rabbit wounds healing resulted quicker, with almost complete reduction (about 90%) of the wound area after 9 days, with PRP loaded sponges than with unloaded ones, that in turn showed a better repairing effect (around 70% of wound reduction after 9 days) than PRP alone or control [91].

Chitosan scaffolds obtained by freeze-drying of chitosan solution in 0.2M acetic acid were associated with PRP by means of two different methods. In a first case PRP was added to chitosan solution before freeze-drying (scaffolds called as "GEL"), in the second case it was added drop by drop to freeze-dried chitosan scaffold previously obtained (scaffolds named "SPONGE"), in different volumes (20, 40 and 80 µl). A study of PRP characteristics depending on the centrifugation method was also performed, demonstrating that the centrifugation procedure did not affect platelet activation. The biological activities of all GFs released were preserved both for GEL and for SPONGE scaffolds. Differences were observed in release profiles, that resulted prolonged to 20 days for PDGF-BB, IGF-1, and TGF-β1 from GEL scaffold, while a sharp burst release was obtained for the same GFs in the case of the SPONGE scaffold. GEL scaffold was therefore proposed as a promising vehicle for PRP in regenerative treatments [86].

Some heparin-conjugated biomaterials have been proposed in GF delivery systems [92, 93]. according to the hypothesis that heparin is structurally close to heparin sulfate, a component of the extracellular matrix with an important role in GFs regulation during wound healing [94]. In particular, it has been demonstrated that Heparin-Conjugated Fibrin (HCF) is able to sustain the release of growth factors that show high affinity for heparin [93].

More recently, a mixture of PRP and HCF has been therefore proposed to treat skin wounds, showing a sustained release of FGF, PDGF-BB, and VEGF lasting for a longer period than that obtained with PRP, Calcium-activated PRP (C-PRP), or a mixture of Fibrin and PRP (F-PRP). HCF-PRP resulted in much faster wound closure and dermal and epidermal regeneration of full-thickness skin wounds in mice and enhanced angiogenesis with respect to C-PRP or F-PRP. Moreover, HCF reduced GF activity loss. This effect and the sustained release can be attributed to electrostatic interactions between several types of GFs contained in PRP and heparin contained in HCF [95].

It was more recently evaluated the treatment of a skin wound with a mixture of PRP and poly (lactic-co-glycolic acid) nanospheres (HCPNs) in fibrin gel (FG-HCPN-PRP). Results similar to those previously observed with HCF were obtained with HCPNs that provided long-term delivery and preserved bioactivity of several growth factors contained in PRP. FG-HCPN-PRP showed a release more prolonged than PRP mixture with FG alone (FGPRP).

Full-thickness skin wound closure occurred much faster with FG-HCPN-PRP (wound size 67.4 ± 5.3% and 41.3 ± 3.2%, respectively at days 6 and 9) than with FG-HCPN or FG-PRP [96].

Bioactive gelatin hydrogel granules have been studied to support the combined delivery of PRP Growth Factor Mixture (PGFM) together with basic Fibroblast Growth Factor (bFGF). bFGF demonstrated angiogenic effects on ischemic legs and on heart, while PRP was expected to exert a stabilization effect on the capillary network. PRP was obtained by treatment of blood with different concentrations of $CaCl_2$. The obtained PRP (100 µg of bFGF (10 µl) and 100 µl of PGFM) was absorbed into gelatin granules previously freeze dried. *In vitro* release of bFGF and of PGFM GFs from gelatin granules was verified comparing the GFs release and the hydrogel degradation. The relevance of collagenase addition to PBS solution was also considered. The complete release was observed within about 30 h with PGFM obtained with 2% w/v $CaCl_2$, in good correlation with the degradation rate of gelatin granules. Special attention was paid to the angiogenic effects of the formulation, evaluated by injecting the hydrogel granules *in vivo* in a leg ischemia murine model.

The angiogenic effect was evaluated by Laser Doppler Blood Perfusion Image (LDPI) analysis and by histological examination, confirming the positive action of bFGF and PGMF loaded granules [50].

Low-molecular-weight heparin (Fragmin) and protamine, have been proposed to prepare water-insoluble micro-nanoparticles with dimensions between 0.1 e 3 μm (F-P M-NPs), whose ability to support both the formation of granulation tissue and the neovascularization effects of PRP was demonstrated [78, 97]. The association of PRP and F-P M-NPs was made by mixing the two components and was based on the ability of heparin and fragmin to interact with different functional proteins, in particular with GFs. F-P M-NPs, alone and associated with PRP, were injected intradermally into the wounds in a rat model. PRP and saline (control) were used for comparison. It was observed that the treatment with PRP associated with the F-P M-NPs induced more effective epithelialization of the wounds, and neovascularization compared with PRP, F-P M-NPs, and control (saline), confirming the ability of F-P M-NPs to protect GFs and sustain their release at the injection site [78].

Some of the papers that consider the use of PRP describe delivery systems loaded with the hemoderivative previously subjected to freeze-thawing cycles resulting in platelet lysis, therefore with characteristics close to those of platelet lysate.

Some authors [84] designed a delivery system obtained by electrospinning, loaded with human PRP previously subjected to a freezing-thawing procedure to obtain lysis of platelets applicable in the treatment of chronic wounds. The optimal concentration of the hemoderivative in growth medium to stimulate proliferation of both fibroblasts and keratinocytes resulted in 2% (v/v), while at higher concentrations cell morphology was altered and cell mobility and proliferation were reduced.

Mixtures of polymers represented by 2% (w/w) chitosan and 3% (w/w) poly-(ethylene oxide) 50:50 were obtained in 3% (v/v) acetic acid in water, with the addition of 0.3% (w/v) Triton X-100 and 10% (v/v) DMSO. The lysate was added to the polymer mixture before electrospinning and it was shown that the electrospinning process did not impair the biological activity of PRP.

Nanofibers loaded with PRP stimulated cell proliferation but reduced cell mobility and caused changed cell morphology. PRP loaded nanofibers induced significant cell proliferation, and a minimal improvement of cell motility, showing a synergic effect of PRP with the support due to the peculiar structure of the nanofibers.

The morphology of the electrospun nanofibers was maintained for at least 72h, while the GFs release was quite fast, and its effect was exerted on the cells in the first 24h [84].

A PRP coated electrospun scaffold based on Poly-(ε-caprolactone) (PCL) nanofibers (PRP-PCL scaffolds) was proposed for tissue regeneration applications by Diaz-Gomez *et al*. [98] Human PRP was subject to freeze-thaw cycles and centrifuged, and the supernatant rich in GFs was used. PCL nanofibers were immersed in PRP lysate under stirring for 4 h at 4°C, and freeze-dried. The PRP coating was quantified in 2.85 mg/mg.

Mesenchymal Stem Cells (MSC) adhesion and proliferation resulted higher on the PRP-PCL scaffolds than on plain PCL nanofibers, confirming that PRP coating improved the hydrophilicity and the wetting properties of the scaffold and the availability of cell recognition sites. The adsorbed PRP stimulated angiogenesis, as demonstrated by the neovascularization that was observed in a chicken chorioallantoic membrane (CAM) model [98].

4. PLATELET LYSATE DELIVERY SYSTEMS

Some work is reported in the literature about the development and the characterization of dressings loaded with PL. Sponge-like dressings intended for the treatment of chronic skin ulcers were obtained by freeze-drying mixtures of PL and either chitosan glutamate (CSG) or sodium hyaluronate [73]. They contained glycine (GLY) as a cryoprotectant agent and water as a plasticizer and were loaded with different amounts of PL. Depending on their composition, dressings showed different mechanical and hydration properties that make them suitable for the treatment of wounds characterized by different exudate amounts. In particular, when placed in contact with phosphate buffer pH 7.2 (medium simulating wound exudate), HA-based dressing immediately gelified and dissolved in 12 min, while CSG-based formulations maintained their structure after 6 days. When glycerol-phosphate was added to CGS dressings, they underwent a complete gelification after 24 h. Dressings containing PL, when subjected to a proliferation test on human fibroblast cells, showed percentage proliferation values comparable to those obtained with fresh PL, indicating that the freeze-drying process and the excipients employed did not disturb the activity of PL Growth Factors (GFs). Such results were also confirmed by PDGF AB content, chosen as representative of the pool of GFs present in PL.

In subsequent work, sponge-like dressings based on CSG (high molecular weight), GLY and Sericin (SER) intended for the treatment of chronic skin ulcers were developed. Dressing development was assisted by employing a DoE approach. In particular, a simplex centroid design was used. The optimized formulation was characterized by optimal mechanical properties and by cell proliferation and antioxidant activity on human fibroblast cell line [61]. It was loaded with PL and a synergic effect of SER and PL was proved *in vitro* on fibroblast proliferation. PL was loaded by using two different approaches: the first one consisted of freeze-drying a mixture of CSG, GLY, PL and SER, while the second one was based on the extemporaneous loading of PL in the CSG/GLY/SER freeze-dried dressings (0.25, 0.35, and 0.5 ml on 25 mg weight dressing). Dressings obtained with the first approach were able to absorb a high amount (about 8-fold of dry weight) of a fluid mimicking wound exudate (PBS), forming a pseudoplastic and elastic gel. Dressings prepared with the second approach enabled to load 90 μL/cm^2 PL. The formulations developed were proved to increase *in vitro* the number not only of viable fibroblasts but also of those in the proliferative phase. Moreover, histological evaluation of human skin strips placed in contact with the PL-loaded dressings indicated their positive effect on dermal matrix reconstruction [74].

More recently, a powder formulation for the delivery of Manuka Honey (MH) bioactive components and Platelet Lysate (PL) in chronic skin ulcers was developed [75]. It consists of pectin (PEC)/Chitosan (CS) particles prepared by ionotropic gelation in the presence of $CaCl_2$. The particles were characterized for particle size, hydration properties and mechanical resistance. Different experimental conditions, such as CS and calcium chloride concentrations and rest time in the cationic solution were investigated. Two different fractions of MH were also considered: Fr1, rich in methylglyoxal, and Fr2, rich in polyphenols. Fr1 was proved to be the fraction able to enhance *in vitro* proliferation of human fibroblasts. *In vivo* efficacy of PL- and Fr1-loaded particles was assessed on a rat wound model. Both treatments markedly increased wound healing to the same extent (remaining wound area about 30% *versus* about 60% for the control after 18 days).

Gellan Gum (GG), a polysaccharide widely used in tissue engineering [99, 100] can be easily crosslinked by divalent ions, resulting in hydrogels that are nevertheless characterized by poor mechanical properties. To improve this aspect, gellan gum was chemically modified with methacrylate groups obtaining photo-cross-linkable hydrogels (MeGG) [72]. The authors based their work on the consideration that in the extracellular matrix the presence of gradients, for example of bioactive molecules, pH, temperature and oxygen, regulates cell behavior and migration during numerous situations, among which wound healing. A PL gradient was realized inside the constructs by means of microfluidic tools, and its relevance for HUVEC growth was confirmed by higher cell number in the more concentrated sections [72].

Nonwoven silk fibroin scaffolds were obtained by autoclaving cocoon fibers for the removal of sericin components, by carding fibers, by applying an entanglement process through high-pressure water jets and by applying a drying step at 140 °C. Adipose Stem Cells (ASC) and Platelet Lysate (PL) were obtained from rabbits. Platelet lysate associated with adipose-derived stromal cells improved their growth and adhesion to scaffolds. *In vivo* studies were performed on wounds induced on rabbit back, that were treated with the application of scaffolds loaded by soaking either with PL or with PL associated with ASC. The control group was treated with scaffolds soaked in the physiological solution. Fast wound recovery was obtained with PL soaked scaffolds, comparable to that obtained with PL and ASC association [85].

Dressings based on silk fibroin and obtained by electrospinning were recently developed. Fibers were obtained starting from a fibroin aqueous solution to which PEO was added to adjust the solution viscosity and improve the electrospinning process. PL at 7% (w/w_{silk}) was added to fibroin solution before electrospinning. Fibroin crystallinity was investigated by Fourier Transform Infrared spectroscopy (FTIR) and put in relation with fibers degradation mechanism and release kinetics. For crystallinity percentages lower than 20%, degradation depends mainly on dissolution while, when the crystalline phase is increased over 40%, proteolysis becomes predominant. The authors confirmed that in case of high molecular weight molecules these different mechanisms impact on release profiles of PL growth factors, as demonstrated in the case of PDGF and TGF-β1. The viability evaluation performed on human dermal fibroblast cell culture demonstrated the ability of fibroin dressings to maintain GFs biological activity, inducing fibroblast elongated morphology typical of cells migrating to wound closure [83].

A Collagen/Gelatin Scaffold (CGS) previously developed for the sustained release of basic fibroblast growth factor (bFGF), was then assessed as a vehicle for the application of PL to skin wounds. PL was obtained from healthy donors and characterized by dosing transforming growth factor (TGF)-b1, Platelet-Derived Growth Factor (PDGF)-BB, Vascular Endothelial Growth Factor (VEGF), and bFGF as representative of the whole pool of platelet GFs. CGS (8-mm diameter, 1.5mm thick) was impregnated with 50 µl of PL at increasing concentrations and introduced in skin wounds on the backs of mice. Remaining wound area was measured during the time until 3 weeks after implantation and it was found reduced for all the treatments with respect to the control, but the lowest for the intermediate PL concentration. Histological evaluation of wound sections allowed to measure the neo-epithelium length and immune histological staining for von Willebrand factor was performed to quantify the area of newly formed capillaries. In all cases, the results suggested that there was an optimal concentration of PL for the improvement of tissue healing, above which a further PL concentration resulted in an inhibitory effect on cell and new capillary proliferation. This effect was probably due to the PL release of Thrombospondin (TSP)-1, an important angiogenesis inhibitor. However, in all cases, the wound healing effect was clearly higher with PL than for the controls [87].

Chondroitin Sulfate (CS) and Sodium Alginate (SA) were used to prepare acellular scaffolds showing good chemical stability to sterilization by γ-radiation, highly porous bubble morphology, limited swelling and high flexibility. MTT proliferation test and confocal laser scanning microscopy studies showed that fibroblasts and endothelial cells were able to populate the scaffold. A comparison of the cell proliferation, with and without PL, put in evidence the fundamental role of the hemoderivative presence to promote cell growth in scaffolds. It was moreover envisaged the presence of a synergic effect between PL and CS, that together favor better cell migration and growth on the bubble surface of the scaffold structure [88].

The selection of collagen as the basis for carriers of GFs aimed to wound healing is based on its presence in the extracellular matrix, with the support of tissue integrity. Based on this assumption, Collagen (Coll) millimetric gel beads were chosen to encapsulate PL. Solutions of collagen (2.5 mg/ml) and PL were deposited in droplets on superhydrophobic surfaces where they were allowed hardening by self-assembling. Different concentrations of PL were tested and the association with stem cells was evaluated. The effect of freeze-drying or cryopreservation was also studied. Release medium enriched with collagenase was used to simulate the environment at the wound site. The presence of encapsulated PL made the beads more stable towards the collagenase effect, probably for the reinforcing effect of fibrin. Cell proliferation effect of PL evaluated also by scratch test, was confirmed. As previously observed [87], also, in this case, an optimal PL concentration for maximum proliferation induc-

tion was identified. The pl-induced proliferation of stem cells loaded in the beads, resulting in a bead network interconnected by stem cells. Angiogenic effect of PL was confirmed on a CAM chick model [76].

Micro- and nanoparticles as DDS are widely explored in the literature in wound healing [64, 101], and in particular for the application of GFs at wound site [26].

PL obtained by centrifugation of PRP in the presence of heparin as an anticoagulant was associated with different kinds of Porous Silicon (PSi) microparticles. Loading was obtained by stirring PSi suspensions at different concentrations in a PL solution diluted 1:1 (v/v) with NaCl physiological solution. *In vitro* and *ex vivo* evaluation allowed to confirm the positive effect of the systems in comparison to the controls on wound healing mechanisms [79].

Recently, dressings intended for the combined delivery to chronic skin ulcers of PL and of the anti-infective model drug Vancomycin Hydrochloride (VCM) were developed [102]. In particular, such formulations consisted of HA core-shell particles, loaded with PL and coated with calcium alginate, embedded in a VCM containing alginate matrix. For the formation of HA/PL core-shell particles, two $CaCl_2$ concentrations were used. Dressings were characterized for hydration properties and for their biological activity on fibroblasts (*in vitro*) and on skin biopsies (*ex-vivo*). They were able to absorb a high amount of wound exudate, forming a protective gel on the lesion area. The $CaCl_2$ concentration did not influence VCM release, but strongly modified the release of the growth factor PGFAB from HA particles. The results of *in vitro* and *ex vivo* tests provided proof of concept of the ability of dressings to improve wound healing.

Also, powder formulations were developed for the combined delivery of platelet lysate and VCM in chronic skin ulcers. In particular, calcium alginate particles were prepared by freeze-drying beads obtained by ionic gelation method. They were able to absorb PBS, forming a gel, and to modulate VCM and PDGF AB release. The presence of PL was responsible for enhancement properties of human fibroblast proliferation [77].

Silver compounds, such as Silver Sulfadiazine (AgSD), are often first choice antibacterials thanks to their large spectrum activity and quite good activity even in case of antibiotic-resistant bacteria. As a drawback, however, they show cytotoxicity toward fibroblasts and keratinocytes and poor compatibility with GFs. Different approaches have been explored to associate PL with AgSD encapsulated in nanoparticles able to reduce negative effects on tissue and GFs in skin wound healing.

One of these approaches involved the development of solid lipid nanoparticles (SLNs) associated with chondroitin sulfate and sodium hyaluronate, chosen as bioactive polymers for their known tissue repairing properties. Nanoparticles were then embedded in either Hydroxypropyl-Methylcellulose (HPMC) or chitosan glutamate dressings. These results able to enhance the antimicrobial activity of AgSD maintaining the efficacy of PL [80].

Positive results were also obtained by encapsulating AgSD in polymeric micelles based on a recently developed chitosan oleate salt [103]. This amphiphilic derivative of chitosan was proposed to improve aqueous dispersion of poorly soluble drugs such as anti-infectives of suitable use in wound healing like clarithromycin [81], and antioxidant agents like alpha-tocopherol [82]. Both chitosan and oleic acid are described in the literature for their antimicrobial activity, that is maintained for the salt and can support the efficacy towards both bacterial and fungal strains of lipidic phases such as essential oils [104]. Chitosan oleate demonstrated good compatibility with PL and improved GFs release as confirmed by PDGF-AB quantification [103]. This effect can be attributed to chitosan, as previously described [105].

CONCLUSION

Chitosan oleate polymeric micelles, loaded with AgSD and alpha-tocopherol nanoemulsions, were therefore loaded into chitosan glutamate freeze-dried bioactive dressings aimed to extemporaneous embedding with PL at the wound site. Dressings of 2 mg total weight were embedded with 25 µl of PL. Dressings showed good compatibility with PL *in vitro* on fibroblast cell cultures and an accelerating wound healing effect *in vivo* on a murine wound model, supporting PL application to wounds. After 18 days, the remaining wound area was about 10% for the PL loaded dressing, compared to a remaining area of about 40% for the control. The study confirmed the relevance of a suitable formulation to improve the PL contact with the wound bed and its efficacy [54].

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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