Thrombosis Research 151 (2017) 36–40

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Fibrin improves skin wound perfusion in a diabetic rat model

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ARTICLE INFO

Article history:
Received 1 August 2016
Received in revised form 23 December 2016
Accepted 5 January 2017
Available online 10 January 2017

Keywords:
Fibrin
Diabetes
Perfusion
Wound healing

ABSTRACT

The fibrin matrix of the thrombus that is formed directly after wounding, is an important determinant of the success of the early phase of wound healing. This phase is often impaired in patients with diabetes. A promising approach to improve skin wound healing is the application of a pro-angiogenic fibrin matrix onto the wound. We studied this in 59 female WAG/RijCrl diabetic rats, in which we created two dorsal full-thickness wounds of which one was treated with a human physiological fibrin matrix (2 mg/ml) and one with PBS as control. Wound healing parameters were determined at different time points. The wound closure was significantly improved in fibrin-treated wounds on day 3 and 7. Also, fibrin-treated wounds showed a significantly higher perfusion on day 28 and 35 compared to control wounds ($p < 0.05$). CD68 staining revealed that human fibrin did not induce an immune response. In conclusion: the application of a fibrin matrix on a diabetic wound showed improved perfusion and an increased early closure rate of the wound area.

1. Introduction

An important first step in wound healing is the formation of a fibrin clot. Fibrin is formed from the soluble plasma protein fibrinogen, a molecule of 340 kDa that is generated in the liver and circulates in blood with a concentration of approximately 2 mg/ml. It consists of six polypeptide chains connected by disulfide bonds; two Aα, two Bβ and γ chains [1–3]. Fibrin is the end product of the coagulation cascade, formed by the cleavage of fibrinopeptides A and B from fibrinogen by thrombin [3–5]. The primary function of the fibrin matrix is to stop bleeding. In addition to its role in hemostasis, the fibrin matrix also stimulates the attraction, migration, adhesion and proliferation of a variety of cells that are important in wound healing, such as inflammatory cells, fibroblasts [6] and endothelial cells [1,7].

Patients with Diabetes mellitus (DM) often have disturbed wound healing, which is a growing clinical problem since the prevalence of DM is increasing in Western societies. Worldwide, about 9% of all adults suffer from DM and every year about 1.5 million people die from the effects of DM [8]. These numbers show a dramatic increase over the last 30 years [9]. One of the major limitations in the healing of wounds in DM patients is circulatory insufficiency due to, among others, stiffening of the microvessels. When blood circulation is improved, the delivery of nutrients and oxygen to the wound area will be enhanced. Since oxygen is one of the most important molecules in angiogenesis, increased circulation and oxygenation will improve wound healing [10].

Fibrin has been used in studies on wound healing, However, these studies used fibrin glue consisting of fibrinogen with a high concentration of 20–40 mg/ml with a high concentration of 4 U/ml thrombin is used as a delivery tool of growth factors in wound healing [11–13]. Fibrin matrices from this high-concentration fibrinogen have a very tight matrix structure that allows none to only very limited angiogenesis and fibrinolysis. In our previous research we have already shown that using an optimized fibrin matrix in a wound, based on a concentration of fibrinogen of 2 mg/ml, creates a fibrin matrix with the highest capacity of endothelial cell ingrowth in vitro and in vivo in a normal rat wound healing model [14]. As patients with DM often have circulatory insufficiency, we now hypothesize that a fibrin matrix can also improve angiogenesis in delayed diabetic wound healing.

We studied the effect of a physiological fibrin matrix on delayed wound healing in a diabetic delayed wound healing rat model.

2. Material and methods

2.1. Animals

Sixty-five female WAG/RijCrl rats (6–8 weeks old), purchased from Charles River (l'Arbresle, France), were housed in pairs under standard 12 h light/dark cycles. Food and water were available ad libitum. The rats were allowed to acclimatize to their environment for one week prior to diabetes induction. The experimental protocol was approved by the Animal Experiments Committee under the Dutch national

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http://dx.doi.org/10.1016/j.thromres.2017.01.002
0049-3848/

Experiments on Animals Act and adhered to the rules laid down in this national law that serves the implementation of "Guidelines on the protection of experimental animals" by the Council of Europe (1986), Directive 86/609/EC. All surgery was performed under isoflurane anesthesia and the animals received Temgesic 2 days after surgery to minimize suffering.

2.2. Diabetes induction

Animals (weight > 120 g, 8–10 weeks of age) were fasted overnight and received an intraperitoneal injection of 60 mg/kg Streptozotocin (STZ, Sigma-Aldrich, St. Louis, MO) in 0.05 mol/L sodium citrate buffer (pH = 4.5). The diabetic condition was carefully monitored for at least 5 weeks before the start of the experiment. The diabetic state of the animals was monitored via physiological changes (e.g. elevated blood glucose levels and urine production, thinner skin and weight loss).

2.3. Full-thickness ulcer wound model and treatment

After a stable diabetic period of at least 5 weeks, the rats received two dorsal full-thickness wounds. The left wound was covered with 300 μl PBS pH 7.4 and served as a control. The right wound was covered with 300 μl fibrin matrix, consisting of human fibrinogen (2 mg/ml in PBS pH 7.4, Enzyme Research Laboratories, South Bend IN, USA), containing 0.43 U/ml FXIII (determined photometrically with Berichrom Factor XIII, Siemens Healthcare diagnostics, Deerland, IL), depleted of plasminogen, von Willebrand Factor and fibronectin mixed with human thrombin (1 U/ml, Siemens Healthcare Diagnostics, Breda, the Netherlands) immediately before application to the wound. Before surgery, animals were anesthetized with a mixture of isoflurane and oxygen. After removal of the dorsal hair, two full-thickness wounds with a diameter of 15 mm (3 cm apart, 4 cm caudal from the scapulae) were created with surgical scissors as previously described by Tong et al. [15]. Initial wound sizes in control and fibrin-treated wounds were similar. After application of the fibrin matrix, the animals were left in a dorsolateral position for 15 min to allow the fibrin matrix to form. All animals received Temgesic (0,05 mg/kg, Rekitt Benckiser Healthcare, Ltd., Hull, East Yorkshire, UK) i.m. before surgery and twice a day for 2 days after surgery. Animals were followed for either 3 (n = 6), 7 (n = 20), 21 (n = 20) or 42 (n = 19) days, randomly assigned, and sacrificed for wound histology and gene/protein expression assays.

2.4. Perfusion measurements

Perfusion in the wounded area and the normal (unwounded) skin was measured weekly from 14 days after surgery onwards using O2C (LEA Medizintechnik, Giessen, Germany). Measurements were performed according to manufacturer's recommendations. Four perfusion parameters were measured: oxygen saturation (SO2), relative hemoglobin (rHb), blood flow in the microcapillary network and blood flow

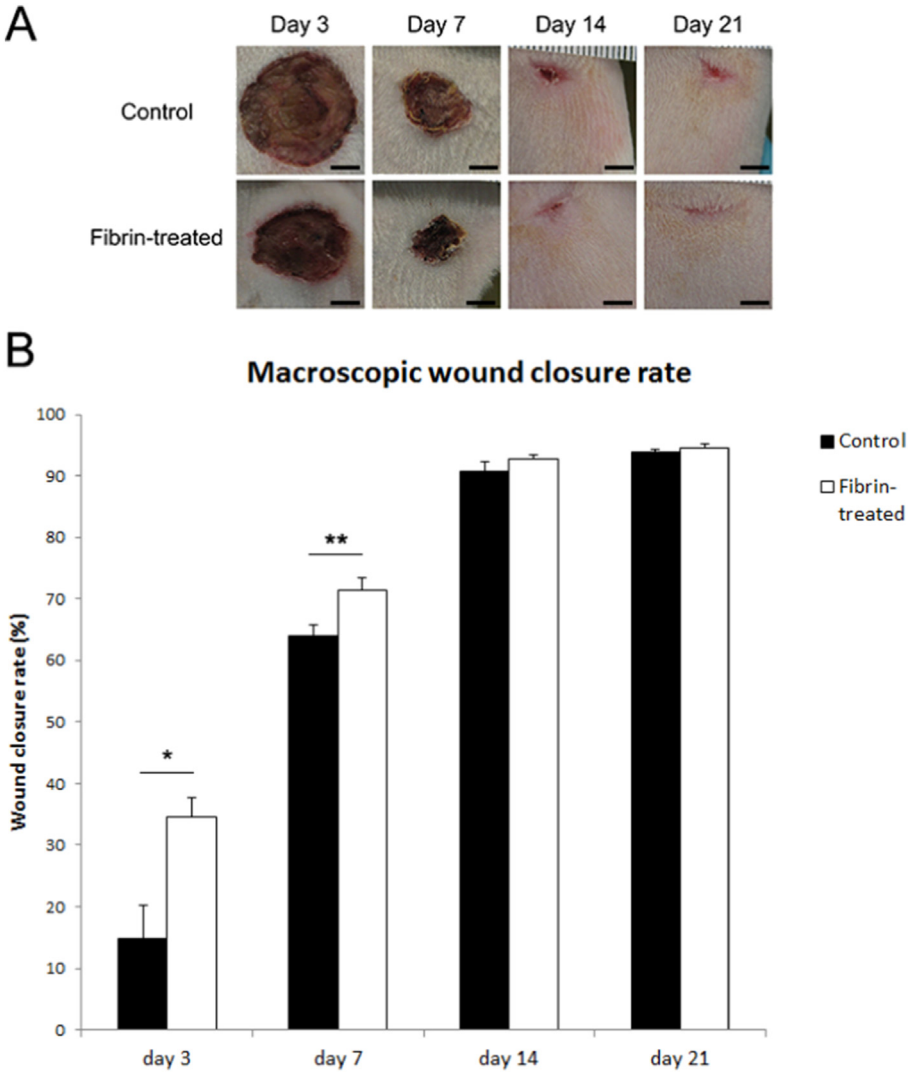

Fig. 1. Improved wound closure rate (A and B) on day 3 and 7 of fibrin-treated wounds compared to control wounds. Scale bars are 5 mm. *p < 0.05, **p < 0.01.