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Nanofibrous rhPDGF-eluting PLGA-collagen hybrid Scaffolds Enhance Healing of Diabetic Wounds

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Abstract

Patients with chronic, non-healing diabetic ulcers extend hospital stays and increase the financial burden than non-diabetics. This investigation developed nanofibrous growth factor-eluting poly (lactic-co-glycolic acid) (PLGA)-collagen hybrid scaffolds that enabled the sustainable release of recombinant human platelet-derived growth factor (rhPDGF) to treat diabetic wounds. RhPDGF, collagen, and PLGA were dissolved in hexafluoroisopropanol, and then electrospun into nanofibrous scaffolds. An enzyme-linked immunosorbent assay kit and an elution method were utilized to evaluate the rates of *in vivo* and *in vitro* release of growth factors from the scaffolds. High concentrations and effectiveness of rhPDGF were documented for over three weeks. The water contact angles of nanofibrous rhPDGF-eluting PLGA-collagen hybrid scaffolds were less (97.2 ± 0.7^{o}) than those of PLGA-collagen hybrid mesh (107.6 ± 1.0^{o}) or virgin PLGA (113 ± 3.3^{o}) (all *post hoc* $p < 0.05$). The nanofibers with rhPDGF-eluting PLGA-collagen hybrid scaffold also exhibited significantly higher water-retaining capacity than those in other groups (all *post hoc* $p < 0.001$). Furthermore, the scaffolds caused more re-epithelialization and contained more collagen I in rats with rhPDGF-eluting scaffolds than controls, as determined from the expressed matrix metalloproteinase 9 in the hair canals in developing hair follicles. These results revealed for the first time, the application of rhPDGF as a growth factor adjuvant, and developed a new collagen-based composite with potent cell infiltration and epithelialization properties.

Keywords

Nanofibrous scaffolds; electrospinning; release profile; diabetic wound; growth factor; rhPDGF

Introduction

Patients with chronic, non-healing ulcers may extend longer hospital stays and increase the financial burden on hospitals than others.[1] Diabetes mellitus is a serious, lifelong metabolic condition that is a leading cause of chronic wound resulting in non-traumatic amputation in both developed and developing nations.[2] Numerous biochemical abnormalities may accelerate neuropathy and cause vascular changes in wounds, including disturbances in collagen metabolism, delay in re-epithelialization, impairment in fibroblasts proliferation and keratinocytes migration.[3-4] The earliest intervention can help promptly to heal such a wound, reducing the risk of amputation of the affected limb.[5] Development of biochemical correction treatment methods to enhance the wounds repair is therefore urgently required.

Collagen and its producing cells, including fibroblasts and keratinocytes, are important in skin development and play an essential part in the wound healing and tissue remodeling.[6] In use of electrospun poly (lactic-co-glycolic acid) (PLGA)-collagen hybrid nanofibers as a dressing for wounds is an operative means of regeneration of skin that stabilizes vascular and cellar apparatuses in the wound bed to deactivate numerous harmful factors.[7-10] Upon damage, platelet-derived growth factor (PDGF) is released from degranulated platelets into the wound fluid. Notably, decreased actions of PDGF and its receptors are seen in the impaired wounds resulting from hyperglycemia, showing that the expression of PDGF and its receptors is an essential component of wound healing.[11-12] The biological activity of recombinant human PDGF-BB (rhPDGF-BB) is similar to that of naturally occurring PDGF, and can promote the chemotactic recruitment, following the formation of granulation tissue, the proliferation of cells and the facilitation of epithelialization that participate in wound repair.[13] The rhPDGF-BB gel has been approved by the US Food

and Drug Administration for treating diabetic foot ulcers.[14] But, the gel is relative costly and short acting, leading to poor compliance and certain uneasiness for local treatment by patients or nursing care.[15]

This fact provides a new idea on using rhPDGF and collagen to promote diabetic wound healing. We posit that the treatment of an rhPDGF/collagen/PLGA dressing can improve production of collagen content, and accelerate the wound healing rate comparing with PLGA-collagen hybrid or virgin PLGA scaffolds. In this work, rhPDGF-eluting PLGA-collagen hybrid mesh was electrospun into nanofibrous scaffolds for diabetic wounds care.

Methods and Methods

Materials

The PLGA that was utilized herein was a commercially material (Resomer RG 503, Boehringer, Germany) and had a ratio of lactide and glycolide (50:50). rhPDGF-BB and hexafluoroisopropanol (HFIP) were obtained from Future Health Biotechnology (Beijing, China) and Sigma-Aldrich (Saint Louis, U.S.A.), respectively.

The electrospinning apparatus included a needle and syringe, an aluminum collection plate, a grounding electrode, and a high-voltage direct current (DC) power supply.[7] Three groups, including rhPDGF-BB-eluting PLGA-collagen hybrid scaffolds (Group A) (PLGA, 280 mg; rhPDGF, 5 mg; collagen 140mg), PLGA-collagen hybrid scaffolds (PLGA, 280 mg; collagen 140mg) (Group B) and virgin PLGA scaffolds (PLGA, 280 mg) (Group C) were separately mixed in HFIP (1 ml) and then fabricated. Nanofibrous scaffolds with 200 μm in thickness were produced.

Scanning electron microscopy (SEM)

The surface of nanofibrous morphology was studied using a Hitachi S3000N SEM (Tokyo, Japan) following gold coating. The average fiber diameter and pore size of each sample were acquired from the SEM images using Image J software. Measurements of diameter and pore size were made at 100 random positions in each sample test (n=5). The nanofibrous-scaffolds porosity was also measured.[16]

Mechanical propertie and Water contact angle

The mechanical characteristics of both group A and B were tested on a Lloyd

tensiometer (AMETEK, U.S.A.) following the ASTM D638 standard.[17] The water contact angles of nanofibrous scaffolds were recorded by a contact angle measurement instrument (First Ten Angstroms, U.S.A.) (n= 5).

Water absorption capacity of nanofibrous scalffolds

The water absorption volume of the three different scaffolds was obtained. The nanofibrous scaffolds were studied by immersing in distilled water at 28℃ and measured for an half, one, two, three, eight, 24, and 48 hours. The water content (WC, %) was measured.

***In vivo* wound healing study**

Sprague-Dawley male rats (n=18; mean body weight 306 ± 17 g; age 12 weeks) were prepared and randomized to three groups. All procedures related to animal were institutionally approved, and all animals were cared for under the direct supervision of a licensed veterinarian, according to the principles of the National Institute of Health (Taiwan). All rats were received standard rodents chow with free access to *ad libitum* drinking water, with a cycle of 12h light: 12h darkness and controlled humidity and temperature; they were kept in individual cages in a central animal care facility before ,during, and after the experiments. A single 70 mg/kg intraperitoneal injection of sterile streptozotocin (STZ) (Sigma, U.S.A.) in 0.1 mol/L sodium citrate buffer at pH 4.5 was used to induce experimental diabetes in the rates before they were treated. The measurement of blood sugar levels of over 300 mg/dl 72 h after STZ injection confirmed the diabetic state.

After anesthesia, two circular 0.8 cm dorsal full-thickness wound were prepared. In the healing process, no other topical drug was used and no dressing was replaced.

The wound areas were analyzed using Image J image software with tracing onto glass microscope slides. The rate of wound closure that denotes the percentage wound reduction from the initial size was determined.

Blood was sampled daily from tail veins of each rat and an OneTouch strips (LifeScan, Milpitas, U.S.A.) was using for measurement of its glucose content. Insulin glargine (Sanofi-Aventis, Frankfurt, Germany) was administered to any rat if they had exhibited "high" glucose levels or significant weight lost.

On days three, seven, and 14, each wound was excised down to the layer of fascia along a 5 mm border of unwounded skin. Intra-dermis tapping (below the dressings) was performed with 19-gauge fine-needle aspiration on wounds were treated with rhPDGF-eluting scaffolds for *in-vivo* release measurement on four different periods.

In vivo and *in vitro* release of rhPDGF

Active *in vivo* and *in vitro* rhPDGF-BB concentrations were acquired by Quantikine PDGF-BB Enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, U.S.A.). A multiple detection plate reader (Tecan SAFIRE, Durham, U.S.A.) at an absorption wavelength of 450 nm using a reference wavelength of 570 nm was used for sample analysis. The standards in each kit were used to plot standard curves.

Immunofluorescence imaging

The compounds that were carried out in the investigative procedure were purchased from Sigma. Type I collagen or matrix metalloproteinase 9 (MMP-9) (Abcam, Cambridge, MA) labeling index in dermis was tested related to DAPI

labeling nuclei.

Statistical analysis

All data in this study are presented as mean ± standard deviation. One-way ANOVA was used to find statistically significant differences among groups. For making multiple comparisons, as part of the ANOVA, the *post hoc* Bonferroni procedure was implemented to detect significant differences between the means of the two related groups. If the p value is < 0.05, results were considered to be statistically significant. The data were calculated using Statistical Package for Social Sciences software (version 17.0, Chicago, U.S.A.).

Results

Figure 1 displays SEM images of the biodegradable nanofibers (3,000× magnification) (Fig. 1a and 1b). The pore space of the nanofibrous scaffolds in the two groups were comparable (Fig. 1c and 1d) (94.5±55.8 x 10^4 nm^2 for group A and 85.5 ±41.0 x 10^4 nm^2 for group B) (P = 0.241), but the nanofibers diameters in group A (206.9±120.1 nm) was significantly smaller than group B (282.7±114.2 nm) (Fig. 1e and 1f) (p < 0.001). The nanofibrous-scaffolds porosities were similar (group A 89.9±0.9% and group B 88.5±0.7%) (p = 0.510).

The results in Fig. 2 display that the tensile strength of the virgin PLGA nanofibers (2.12±0.10 MPa) was close to that of the rhPDGF-BB-eluting (2.04±0.08 MPa) or PLGA-collagen hybrid nanofibers (2.07±0.13 MPa) (Anova p = 0.683). However, virgin PLGA nanofibers had higher elongation at breakage than did rhPDGF-BB-eluting and virgin PLGA-collagen hybrid nanofibers (All *post hoc* p<0.001), and the PLGA-collagen hybrid nanofibers exhibited higher elongation at breakage (60.3±1.8 %) than did the rhPDGF-BB-eluting PLGA-collagen hybrid nanofibers (50.0±2.0 %) (*post hoc* p =0.003).

The measured contact angles of water of group A, B, and C scaffolds were 97.2±0.7^o, 107.6±1.0^o, and 113±3.3^o, respectively (Fig. 3). Clearly, nanofibers in group C revealed less hydrophilicity than those in group A or B (*post hoc* p < 0.001 and p =0.048, respectively). Moreover, adding rhPDGF-BB significantly reduced the hydrophobicity of the electrospun PLGA-collagen hybrid nanofibrous scaffolds (*post hoc* p =0.002).

The results in Fig. 4 show that nanofibers in groups A and B had much higher capacities than those in group C following submersion, reaching their peak water absorption at 24 hours (193.3 ± 30.8 and 137.2 ± 19.7% respectively). Additionally,

virgin PLGA nanofibers (group C) achieved their peak values of water content (79.7 ± 1.1%) at three hours. The water-retaining volume of group A and B surpassed that of group C nanofibers, mostly due to the hydrophilic characteristic of rhPDGF-BB and collagen in the matrix (All *post hoc* $p < 0.001$). After eight hours of immersion, the nanofibers in group A also exhibited significantly higher water-retaining capacity than those in collagen group (*post hoc* $p < 0.001$).

The rhPDGF-BB-eluting scaffolds continuously released the growth factors for 21 days, with a burst release in the initial period on day one (102± 3 ng/ml), and release as a second peak from days five to seven (> 24 ng/ml) (Figure 5). After that, the concentration slowly declined (around 5 ng/ml). *In vivo* the levels of growth factor were measured. The measurements indicate that the concentration of the reached growth factors reached a peak on day three (89± 7 ng/ml), after which it decreased slowly to 3± 1 ng/ml on day 21.

Figure 6 presents wounds on a diabetic rat in three groups on different time following therapy. The proportions of the wounds areas that were treated by group B and C fell slowly to 8.3± 1.6% and 9.2± 0.9%, respectively, by day 14 (*post hoc* $p = 0.500$). The proportions of wound regions protected by the rhPDGF-BB-eluting membranes decreased to about 3.6± 0.5% by day 14. Group A promoted wound repairing more than that achieved by both other groups (*post hoc p* all < 0.001).

The nanofibrous scaffolds were extensively developed into integrated ambient skin without causing any significant production of inflammatory cells (Figure 7). The nanofibrous groups presented thicker collagen (double arrow) following recovery in the layer between epidermis and subcutaneous tissue than was found in PLGA only group. At 14 days after operation, almost entirely healed and protected by re-epithelialization in epidermis in all cases and produced sparse infiltrate of

inflammatory cells and fibrous connective tissue both in the dermis and subcutaneous layer were noted in the wounds of three groups. On week two, substantial differences in epithelialization and healing of stratum corneum were found among the wounds in histology across the groups, caused by migration of keratinocytes across the wounds. Cell proliferation and migration of the outermost layer was most rapidly in the wounds in group A. After 14 days, rhPDGF-BB-eluting PLGA-collagen hybrid scaffolds exhibited full re-epithelialization and the highest proliferation of keratinocytes in the epidermis layer (arrow).

The results in Fig. 9 suggest that the content of collagen in PLGA-collagen hybrid scaffolds with rhPDGF-BB-eluting group (0.93± 0.02) or without rhPDGF-BB-eluting group (0.75± 0.01) were considerably higher than that in PLGA only (group C, 0.57± 0.01) and the collagen content in rhPDGF-BB-eluting PLGA-collagen hybrid scaffolds group also remarkably exceeded that in PLGA-collagen hybrid scaffolds group on day seven (all *post hoc* $p < 0.001$).

On day 14, MMP-9 content index in the corium of rhPDGF-BB-eluting PLGA-collagen hybrid scaffolds (0.65± 0.02) was up-regulated than that in PLGA-collagen hybrid group (0.49± 0.03) or PLGA only group (0.07± 0.02) (all *post hoc* $p < 0.001$) (Fig. 10). Additionally, MMP-9 expression was greater in the hair canals of developed hair follicles in group A than in other groups (circular area).

Discussion

Deficiency of growth factors in diabetic wound areas is the key issue, which result in delayed wound repair. In use of rhPDGF-BB PLGA-collagen hybrid scaffolds significantly promoted the proliferation of cells in the wound bed area, markedly improved the wound repair, and enhanced the quality of repairing in rats associated with diabetes. Furthermore, we confirmed that treatment with high concentrations of rhPDGF-BB in PLGA-collagen hybrid membranes enhanced MMP-9 expression in the hair canals of developed hair follicles and collagen content in the diabetic wound areas during the healing process for more than three weeks. These findings indicate that the enhancement of MMP-9 function and collagen content by rhPDGF-BB would contribute to the acceleration of diabetic wound repair.

Because viscosity of a polymer solution depends on concentration, the presence of rhPDGF-BB in PLGA will reduce solution viscosity. Upon a particular applied force, applied by the particular electric field in the electrospinning and collection process, lower viscous solutions stretched higher resulting in decreased diameters of electrospun rhPDGF-BB-eluting PLGA-collagen hybrid nanofibers. These features could be explained with reference to changes in the viscosity and diameter of the spinning solution. Fibers become interconnected as they dry only following they reach the collector.[18] Additionally, the overall tension in fibers depends on self-repulsion of the excess charges on the ejected jet during electrospinning. Adding rhPDGF-BB resulted in the accumulation of a greater charge density on the surface of the jet. The total electric charge that was carried by the electrospinning jet significantly increased during the fabricated process because rhPDGF-BB is a heat-stable positively charged hydrophilic protein.[19] As the number of charges that were carried by the jet increased, higher thinning and elongation powers that could overcome the surface tension force

resulting in a charged liquid jet that were exerted on the jet in the electrical field. Therefore, the final diameters of the fibers decreased markedly and their distribution became narrower with increasing the charge density. Furthermore, the fabricated scaffolds were high porosity with regularly distributed and fully interlocked geometry than previously obtained mats.[20-21] Additionally, the examined materials were shown to be compatible with the nearby tissue without noted adverse reactions and inflammatory response, which is in line with the results of Schneider *et al*.[22] The rhPDGF-BB PLGA-collagen hybrid nanofibers in this work served as ultimate scaffolds for cell seeding prior to cells ingrowth and tissue regeneration.[23] Additionally, the electrospun composite rhPDGF-BB/collagen or collagen nanofibrous mat had inferior mechanical properties when compared to the pure PLGA nanofibers, perhaps on account of macromolecular chains rearrangement and structural change in the mixed solution.[24] Nevertheless, the rhPDGF-BB/collagen or collagen scaffolds herein had adequate material extensibility and strength leaded to tolerate the variations of structure and morphology that occur in wound healing process.[25]

A wet environment plays an essential role for recruitment and proliferation of fibroblasts or keratinocytes as well as collagen synthesis during wound healing; it also disfavors scar formation because healing commonly proceeds more quickly under humid conditions than dry conditions.[26] Therefore, an ideal wound dressing for wound must offer a moist wound environment, be absorbent to carbonic anhydride and oxygen, and aid in faster healing of wound. The management of biomaterial hydrophobicity at the surface of polymers for making them enhanced cell-substrates interaction within scaffolds is promising. The surface of nanofiber was relatively smooth and might have the similar hydrophilicity due to compatible roughness.[27]

However, the effect of hydroscopic membrane also depends on the polymer characteristics of the fiber. Despite electrospun PLGA mats exhibited hydrophobic characteristic[28], the presence of collagen and rhPDGF-BB made the nanofibrous membranes more hydrophilic.[29] Furthermore, rhPDGF-BB-eluting collagen scaffolds retained more water than collagen-only scaffolds herein. Therefore, cell-adhering proteins can be competitively adsorbed by surfaces moderate wettability, resulting in cell adhesion.[30] Moreover, the nanofibrous membranes has the benefit of reducing the necessity for frequent wound dressing and cleaning and, allowing the skin to enhance better on wound repair and for pain relief.[31]

The sustainable release of rhPDGF-BB from nanofibrous membranes herein proceeds through a three-stage kinetic mechanism: an initial drug-burst, diffusion-limited and degradation-controlled release.[32-33] The *in vivo* and *in vitro* drug delivery profiles showed that growth factors were almost released after electrospinning of rhPDGF-BB-eluting PLGA-collagen hybrid platform. Nevertheless, the molecules placed on the nanofibrous outsides resulted in the initial burst of rhPDGF-BB delivery for five days. Following the rhPDGF-BB released, the rhPDGF-BB nanofibrous scaffolds proceeds through two more stages - diffusion-limited release on days five to seven and degradation-controlled release after day seven.[33-34] Therefore, the nanofibrous membranes released rhPDGF-BB for more than three weeks, favoring the diabetic wounds treatment.

Diabetes has been demonstrated to impair the production of collagen and delay wound repair, both are serious to the phases of proliferation and maturation after a wound damage.[35] PDGF-BB can stimulate the synthesis and accumulation of collagen in wound healing to an extent that is controlled by its chemoattactant activity for collagen-producing cells or macrophages.[36-37]

Health care practitioners have reported that hair-bearing skin tends to heal more rapidly than those that lack hair follicles.[38-39] The diabetic population has a significantly higher rate of hair loss and significantly thinner hair than the non-diabetic population because people with diabetics have fewer new strands and filaments parts of follicles.[40] MMP-9, one of a family of zinc-dependent endoproteases[34], participates in regulating the formation of hair canals and promotes the formation of the initial lumen process by actively remodeling the extracellular matrix, preparing the hair canal for emergence of the hair shaft and preventing damage to its walls by a growing hair fiber.[41] PDGF is an effective mitogen that is generated in keratinocytes and endothelial cells, and is essential in the cell differentiation, proliferation and growth.[42] During wound healing, re-epithelialization process was promoted by migrating epithelial stem cells in the hair follicle to the epidermis. This fact reveals that PDGF-BB is involved in initiating wound repair, suggesting that rhPDGF-BB-eluting PLGA-collagen hybrid scaffolds may be utilized in an innovative treatment to accelerate would healing.

Changed expression of PDGF-BB has been related to problems of possible cancer promotion because of PDGF natural activity.[43-44] However, the external application of rhPDGF-BB has been evaluated for potential problems in a variety of studies without suggestion of carcinogenicity, toxicity, or mutagenicity after administration as an implantable device or drug combination product.[45-47]

In conclusion, the developed rhPDGF-BB–eluting PLGA-collagen hybrid nanofibrous membranes made treated diabetic wound contain more collagen content and increase the MMP-9-induced expression of hair follicles than collagen-PLGA hybrid dressing, owing to delivery of effective rhPDGF-BB. Further studies are warranted to confirm these results in other animal models for the study of hair follicle

to clarify the complex association between hair follicle function and wound healing.

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Duality of interest

The authors report no duality of interest with respect to this work.

References

1. Alva, M. L.; Gray, A.; Mihaylova, B.; Leal, J.; Holman, R. R., The impact of diabetes-related complications on healthcare costs: new results from the UKPDS (UKPDS 84). *Diabet Med* **2015,** *32* (4), 459-66.
2. Alavi, A.; Sibbald, R. G.; Mayer, D.; Goodman, L.; Botros, M.; Armstrong, D. G.; Woo, K.; Boeni, T.; Ayello, E. A.; Kirsner, R. S., Diabetic foot ulcers: Part I. Pathophysiology and prevention. *Journal of the American Academy of Dermatology* **2014,** *70* (1), 1 e1-18; quiz 19-20.
3. O'Loughlin, A.; Kulkarni, M.; Creane, M.; Vaughan, E. E.; Mooney, E.; Shaw, G.; Murphy, M.; Dockery, P.; Pandit, A.; O'Brien, T., Topical administration of allogeneic mesenchymal stromal cells seeded in a collagen scaffold augments wound healing and increases angiogenesis in the diabetic rabbit ulcer. *Diabetes* **2013,** *62* (7), 2588-94.
4. Falanga, V., Wound healing and its impairment in the diabetic foot. *Lancet* **2005,** *366* (9498), 1736-43.
5. O'Loughlin, A.; McIntosh, C.; Dinneen, S. F.; O'Brien, T., Review paper: basic concepts to novel therapies: a review of the diabetic foot. *Int J Low Extrem Wounds* **2010,** *9* (2), 90-102.
6. Bermudez, D. M.; Herdrich, B. J.; Xu, J.; Lind, R.; Beason, D. P.; Mitchell, M. E.; Soslowsky, L. J.; Liechty, K. W., Impaired biomechanical properties of diabetic skin implications in pathogenesis of diabetic wound complications. *Am J Pathol* **2011,** *178* (5), 2215-23.
7. Liu, S.-J.; Kau, Y.-C.; Chou, C.-Y.; Chen, J.-K.; Wu, R.-C.; Yeh, W.-L., Electrospun PLGA/collagen nanofibrous membrane as early-stage wound dressing. *Journal of Membrane Science* **2010,** *355* (1–2), 53-59.
8. Chen, G.; Sato, T.; Ohgushi, H.; Ushida, T.; Tateishi, T.; Tanaka, J., Culturing of skin fibroblasts in a thin PLGA–collagen hybrid mesh. *Biomaterials* **2005,** *26* (15), 2559-2566.
9. Holmes, C.; Wrobel, J. S.; Maceachern, M. P.; Boles, B. R., Collagen-based wound dressings for the treatment of diabetes-related foot ulcers: a systematic review. *Diabetes Metab Syndr Obes* **2013,** *6*, 17-29.
10. Cullen, B.; Watt, P. W.; Lundqvist, C.; Silcock, D.; Schmidt, R. J.; Bogan, D.; Light, N. D., The role of oxidised regenerated cellulose/collagen in chronic wound repair and its potential mechanism of action. *Int J Biochem Cell Biol* **2002,** *34* (12), 1544-56.
11. Gao, Z.; Sasaoka, T.; Fujimori, T.; Oya, T.; Ishii, Y.; Sabit, H.; Kawaguchi, M.; Kurotaki, Y.; Naito, M.; Wada, T.; Ishizawa, S.; Kobayashi, M.; Nabeshima, Y.; Sasahara, M., Deletion of the PDGFR-beta gene affects key fibroblast functions important for wound healing. *J Biol Chem* **2005,** *280* (10), 9375-89.
12. Cox, O. T.; Simpson, D. A.; Stitt, A. W.; Gardiner, T. A., Sources of PDGF expression in murine retina and the effect of short-term diabetes. *Mol Vis* **2003,** *9*, 665-72.
13. Grotendorst, G. R.; Martin, G. R.; Pencev, D.; Sodek, J.; Harvey, A. K., Stimulation of granulation tissue formation by platelet-derived growth factor in normal and diabetic rats. *J Clin Invest* **1985,** *76* (6), 2323-9.
14. Papanas, N.; Maltezos, E., Benefit-risk assessment of becaplermin in the treatment of diabetic foot ulcers. *Drug Saf* **2010,** *33* (6), 455-61.
15. Sibbald, R. G.; Torrance, G.; Hux, M.; Attard, C.; Milkovich, N., Cost-effectiveness of becaplermin for nonhealing neuropathic diabetic foot ulcers.

Ostomy Wound Manage **2003,** *49* (11), 76-84.
16. Espinal, L., Porosity and Its Measurement. In *Characterization of Materials*, John Wiley & Sons, Inc.: **2002**.
17. ASTM Standard D638-10, 2010. Standard Test Methods for Tensile Properties of Plastics. ASTM International, West Conshohocken, PA, 2010.
18. Fashandi, H.; Karimi, M., Pore formation in polystyrene fiber by superimposing temperature and relative humidity of electrospinning atmosphere. *Polymer* **2012,** *53* (25), 5832-5849.
19. Treiber, G.; Wex, T.; Schleyer, E.; Troeger, U.; Hosius, C.; Malfertheiner, P., Imatinib for hepatocellular cancer – Focus on pharmacokinetic/pharmacodynamic modelling and liver function. *Cancer Letters* **2008,** *260* (1–2), 146-154.
20. Meng, Z. X.; Wang, Y. S.; Ma, C.; Zheng, W.; Li, L.; Zheng, Y. F., Electrospinning of PLGA/gelatin randomly-oriented and aligned nanofibers as potential scaffold in tissue engineering. *Materials Science and Engineering: C* **2010,** *30* (8), 1204-1210.
21. Polymeric Scaffolds in Tissue Engineering Application: A Review. *International Journal of Polymer Science* **2011,** *2011*.
22. Schneider, O. D.; Weber, F.; Brunner, T. J.; Loher, S.; Ehrbar, M.; Schmidlin, P. R.; Stark, W. J., In vivo and in vitro evaluation of flexible, cottonwool-like nanocomposites as bone substitute material for complex defects. *Acta biomaterialia* **2009,** *5* (5), 1775-84.
23. Hild, N.; Schneider, O. D.; Mohn, D.; Luechinger, N. A.; Koehler, F. M.; Hofmann, S.; Vetsch, J. R.; Thimm, B. W.; Muller, R.; Stark, W. J., Two-layer membranes of calcium phosphate/collagen/PLGA nanofibres: in vitro biomineralisation and osteogenic differentiation of human mesenchymal stem cells. *Nanoscale* **2011,** *3* (2), 401-9.
24. Chen, V. J.; Ma, P. X., The effect of surface area on the degradation rate of nano-fibrous poly(L-lactic acid) foams. *Biomaterials* **2006,** *27* (20), 3708-15.
25. Zhang, Y. Z.; Venugopal, J.; Huang, Z. M.; Lim, C. T.; Ramakrishna, S., Crosslinking of the electrospun gelatin nanofibers. *Polymer* **2006,** *47* (8), 2911-2917.
26. Vogt, P. M.; Andree, C.; Breuing, K.; Liu, P. Y.; Slama, J.; Helo, G.; Eriksson, E., Dry, moist, and wet skin wound repair. *Ann Plast Surg* **1995,** *34* (5), 493-9; discussion 499-500.
27. Schneider, O. D.; Loher, S.; Brunner, T. J.; Schmidlin, P.; Stark, W. J., Flexible, silver containing nanocomposites for the repair of bone defects: antimicrobial effect against E. coli infection and comparison to tetracycline containing scaffolds. *Journal of Materials Chemistry* **2008,** *18* (23), 2679-2684.
28. Lee, C. H.; Hsieh, M. J.; Chang, S. H.; Lin, Y. H.; Liu, S. J.; Lin, T. Y.; Hung, K. C.; JH, S. P.; Juang, J. H., Enhancement of Diabetic Wound Repair Using Biodegradable Nanofibrous Metformin-Eluting Membranes: in vitro and in vivo. *ACS Appl Mater Interfaces* **2014,** *6* (6), 3979-3986.
29. Proctor, W. R.; Bourdet, D. L.; Thakker, D. R., Mechanisms underlying saturable intestinal absorption of metformin. *Drug Metab Dispos* **2008,** *36* (8), 1650-8.
30. Arima, Y.; Iwata, H., Effect of wettability and surface functional groups on protein adsorption and cell adhesion using well-defined mixed self-assembled monolayers. *Biomaterials* **2007,** *28* (20), 3074-82.
31. Pangilinan, R.; Tice, A.; Tillotson, G., Topical antibiotic treatment for uncomplicated skin and skin structure infections: review of the literature. *Expert Rev Anti Infect Ther* **2009,** *7* (8), 957-65.

32. Kou, J. H.; Emmett, C.; Shen, P.; Aswani, S.; Iwamoto, T.; Vaghefi, F.; Cain, G.; Sanders, L., Bioerosion and biocompatibility of poly(d,l-lactic-co-glycolic acid) implants in brain. *Journal of Controlled Release* **1997,** *43* (2–3), 123-130.
33. Anderson, J. M.; Shive, M. S., Biodegradation and biocompatibility of PLA and PLGA microspheres. *Advanced Drug Delivery Reviews* **2012,** *64, Supplement* (0), 72-82.
34. Birkedal-Hansen, H.; Moore, W. G.; Bodden, M. K.; Windsor, L. J.; Birkedal-Hansen, B.; DeCarlo, A.; Engler, J. A., Matrix metalloproteinases: a review. *Crit Rev Oral Biol Med* **1993,** *4* (2), 197-250.
35. Spanheimer, R. G.; Umpierrez, G. E.; Stumpf, V., Decreased collagen production in diabetic rats. *Diabetes* **1988,** *37* (4), 371-6.
36. Pierce, G. F.; Brown, D.; Mustoe, T. A., Quantitative analysis of inflammatory cell influx, procollagen type I synthesis, and collagen cross-linking in incisional wounds: influence of PDGF-BB and TGF-beta 1 therapy. *J Lab Clin Med* **1991,** *117* (5), 373-82.
37. Ivarsson, M.; McWhirter, A.; Borg, T. K.; Rubin, K., Type I collagen synthesis in cultured human fibroblasts: regulation by cell spreading, platelet-derived growth factor and interactions with collagen fibers. *Matrix biology : journal of the International Society for Matrix Biology* **1998,** *16* (7), 409-25.
38. Ito, M.; Cotsarelis, G., Is the hair follicle necessary for normal wound healing? *J Invest Dermatol* **2008,** *128* (5), 1059-61.
39. Ito, M.; Liu, Y.; Yang, Z.; Nguyen, J.; Liang, F.; Morris, R. J.; Cotsarelis, G., Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. *Nat Med* **2005,** *11* (12), 1351-4.
40. Klam, W. P.; Roeder, L. M.; Rosebrough, R. H.; Heald, F. P., Scalp hair morphology in normal and diabetic children and adolescents. *Journal of adolescent health care : official publication of the Society for Adolescent Medicine* **1983,** *4* (2), 91-3.
41. Sharov, A. A.; Schroeder, M.; Sharova, T. Y.; Mardaryev, A. N.; Peters, E. M.; Tobin, D. J.; Botchkarev, V. A., Matrix metalloproteinase-9 is involved in the regulation of hair canal formation. *J Invest Dermatol* **2011,** *131* (1), 257-60.
42. Heldin, C. H.; Westermark, B., Mechanism of action and in vivo role of platelet-derived growth factor. *Physiol Rev* **1999,** *79* (4), 1283-316.
43. Mantur, M.; Snarska, J.; Sidorska, A.; Ostrowska, H.; Kruszewska-Wnorowska, K.; Wojszel, J., Changes in PDGF concentration in surgically treated colorectal carcinoma. *Advances in medical sciences* **2008,** *53* (1), 37-41.
44. Kim, S. J.; Uehara, H.; Yazici, S.; Langley, R. R.; He, J.; Tsan, R.; Fan, D.; Killion, J. J.; Fidler, I. J., Simultaneous blockade of platelet-derived growth factor-receptor and epidermal growth factor-receptor signaling and systemic administration of paclitaxel as therapy for human prostate cancer metastasis in bone of nude mice. *Cancer research* **2004,** *64* (12), 4201-8.
45. Nevins, M.; Giannobile, W. V.; McGuire, M. K.; Kao, R. T.; Mellonig, J. T.; Hinrichs, J. E.; McAllister, B. S.; Murphy, K. S.; McClain, P. K.; Nevins, M. L.; Paquette, D. W.; Han, T. J.; Reddy, M. S.; Lavin, P. T.; Genco, R. J.; Lynch, S. E., Platelet-derived growth factor stimulates bone fill and rate of attachment level gain: results of a large multicenter randomized controlled trial. *Journal of periodontology* **2005,** *76* (12), 2205-15.
46. Daniels, T.; DiGiovanni, C.; Lau, J. T.; Wing, K.; Younger, A., Prospective clinical pilot trial in a single cohort group of rhPDGF in foot arthrodeses. *Foot & ankle international* **2010,** *31* (6), 473-9.

47. Digiovanni, C. W.; Baumhauer, J.; Lin, S. S.; Berberian, W. S.; Flemister, A. S.; Enna, M. J.; Evangelista, P.; Newman, J., Prospective, randomized, multi-center feasibility trial of rhPDGF-BB versus autologous bone graft in a foot and ankle fusion model. *Foot & ankle international* **2011,** *32* (4), 344-54.

Legends for figures

Figure 1: Scanning electron microscope (SEM) pictures of rhPDGF-BB-eluting PLGA-collagen (a), and virgin PLGA-collagen hybrid scaffold (b) (Scale bar: 5 μm).

Figure 2: Stress-strain curves of three groups. Virgin PLGA: tensile strength 2.16 MPa, elongation at breakage 91.4 %; rhPDGF-BB-eluting PLGA-collagen hybrid scaffold: tensile strength 2.07 MPa, elongation at breakage 48.9 %; PLGA-collagen hybrid scaffolds: tensile strength 2.07 MPa, elongation at breakage 61.9%.

Figure 3: Measured contact angles. rhPDGF-BB-eluting PLGA-collagen hybrid (a), PLGA-collagen hybrid (b), and virgin PLGA scaffolds (c). Contact angles were 98.0°, 107.6°, and 112.7°, respectively.

Figure 4: Various water content of three groups. (*Group A versus Group B, †Group A versus Group C, ‡Group B versus Group C, $p<0.05$ in *post hoc* analysis)

Figure 5: *In vitro* rhPDGF-BB release.

Figure 6: The process of wound repair on different days. Day zero (a,b,c), three (d,e,f), seven (g,h,i), and 14 (j,k,l) following treatment with group A, B, and C. (Scale bar = 5 mm).

Figure 7: Wound repair analysis for three groups.

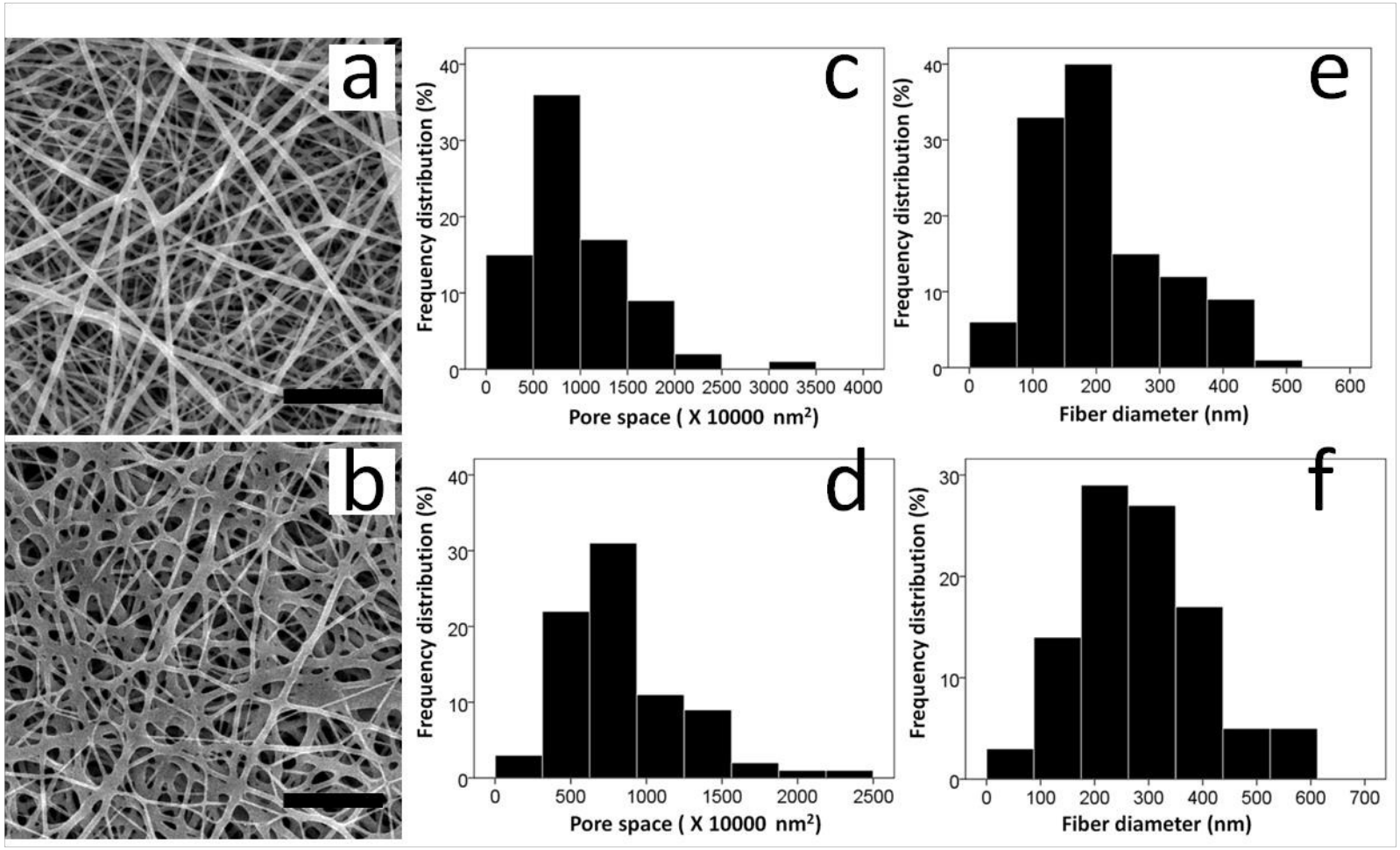
Figure 8: Wound histological images (hematoxylin/eosin, H&E stain) in three groups

on day 14. Double arrow indicates dermal layer. Scale bar = 50μm.

Fig. 9: Expression of collagen on day seven. DAPI-labeled nuclei (blue) (d,e,f). Cy3-conjugated secondary antibody (orange) (g,h,i). rhPDGF-BB-eluting PLGA-collagen hybrid scaffold increases collagen I in dermis (double arrow). Dashed lines indicate dermal–epidermal junction. Scale bar = 75μm.

Fig. 10: Effect of three groups on MMP-9 content on day 14. DAPI-labeled nuclei (blue) (d,e,f). Cy3-conjugated secondary antibody (orange) (g,h,i). rhPDGF-BB-eluting PLGA-collagen hybrid scaffolds up-regulates MMP-9 in dermis (double arrow).

Figure 1:



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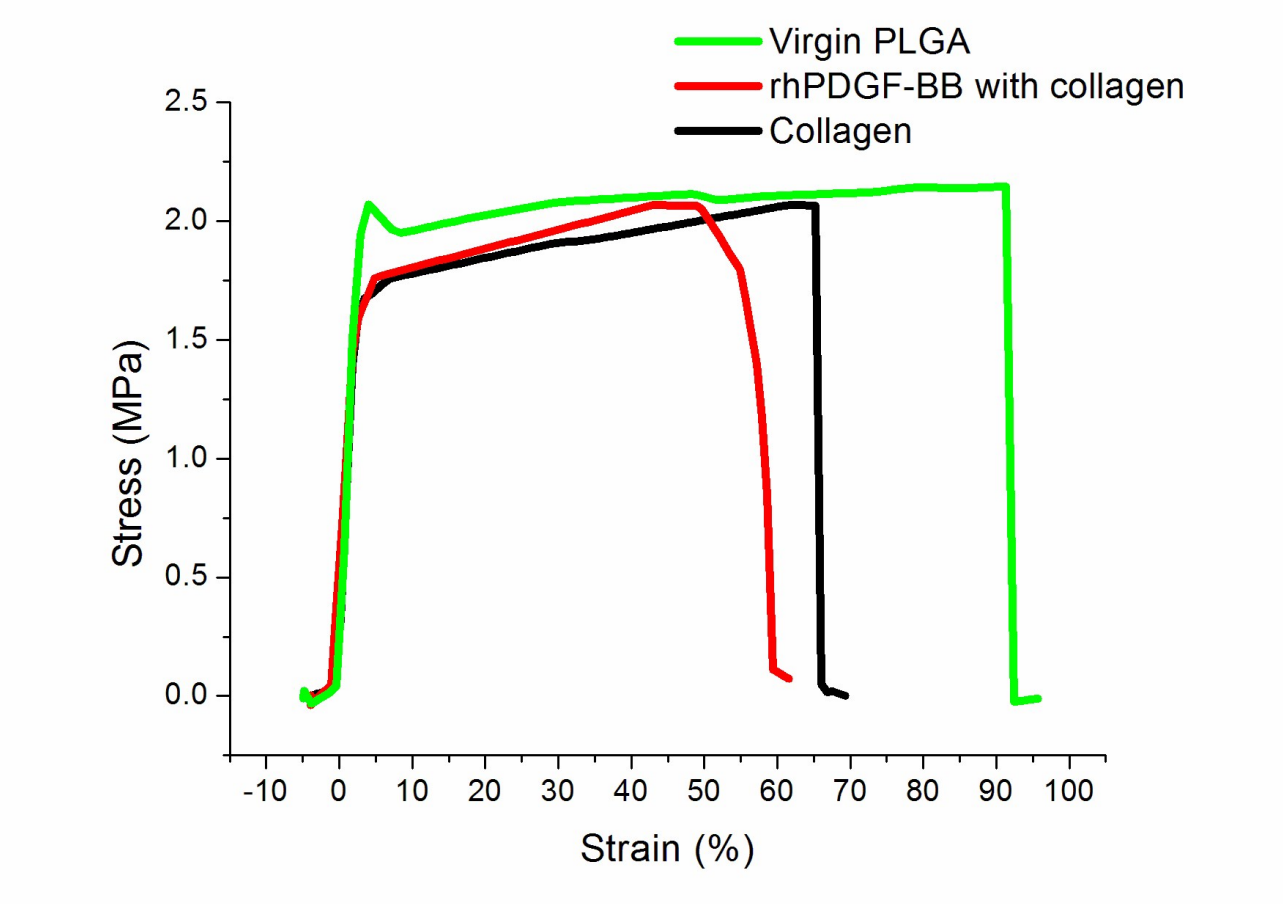

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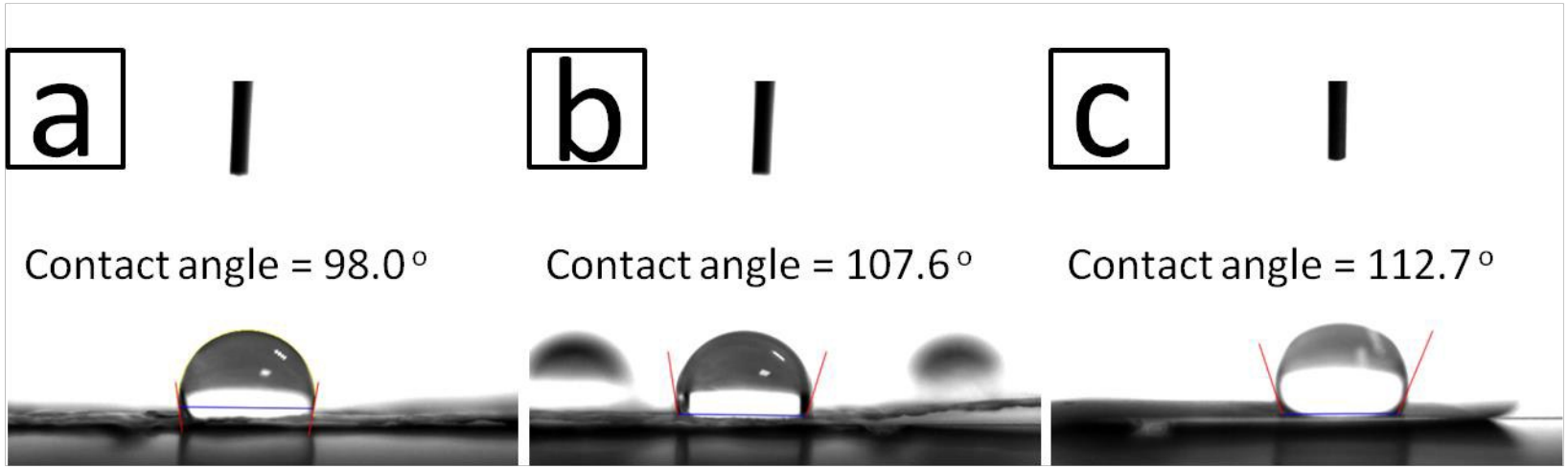

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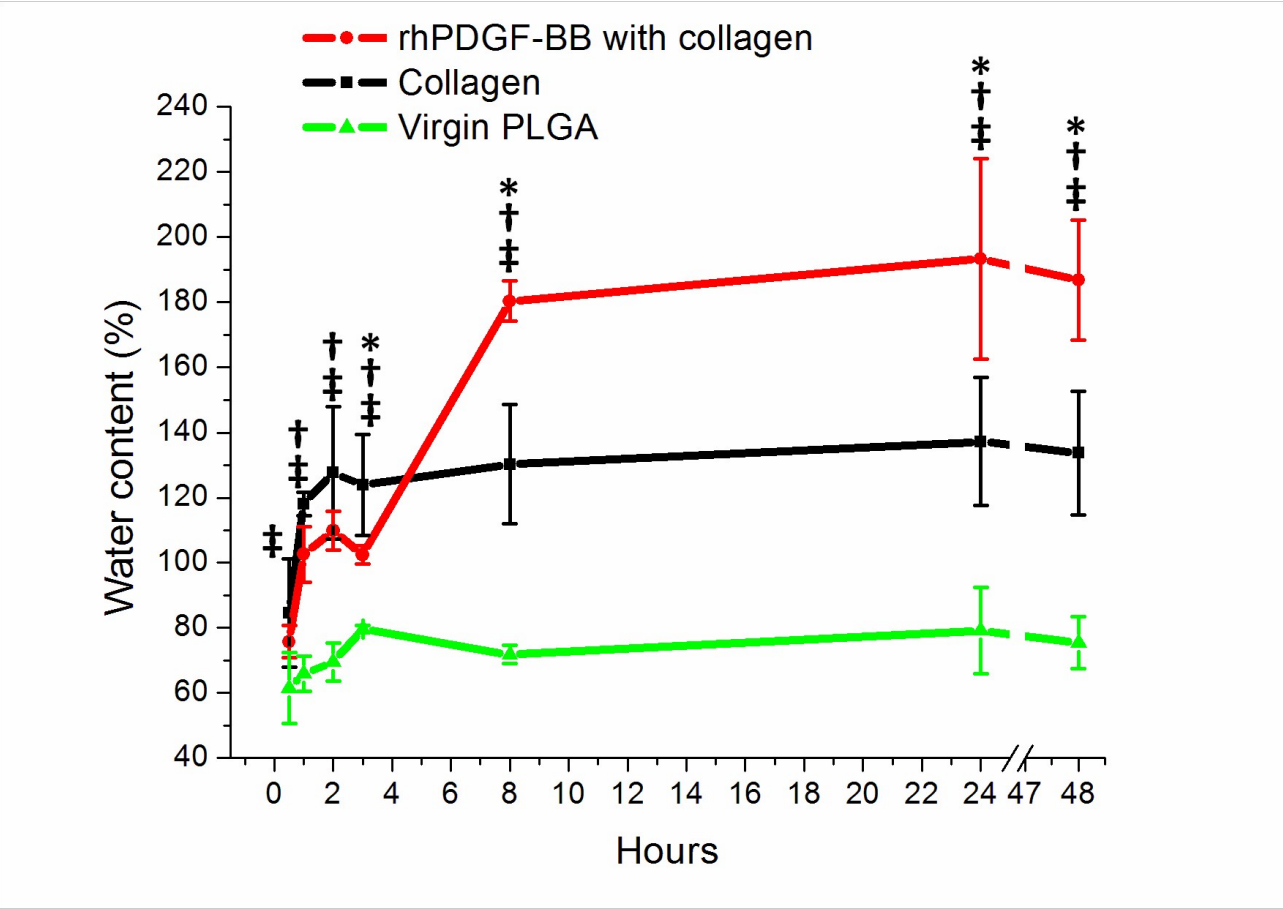

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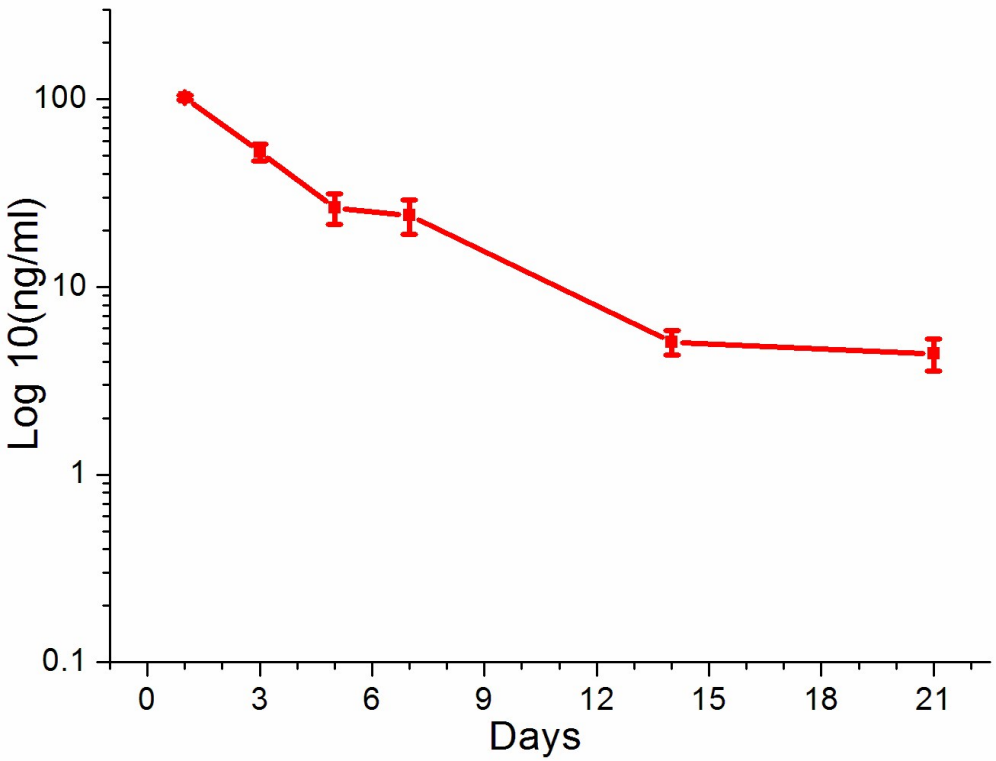

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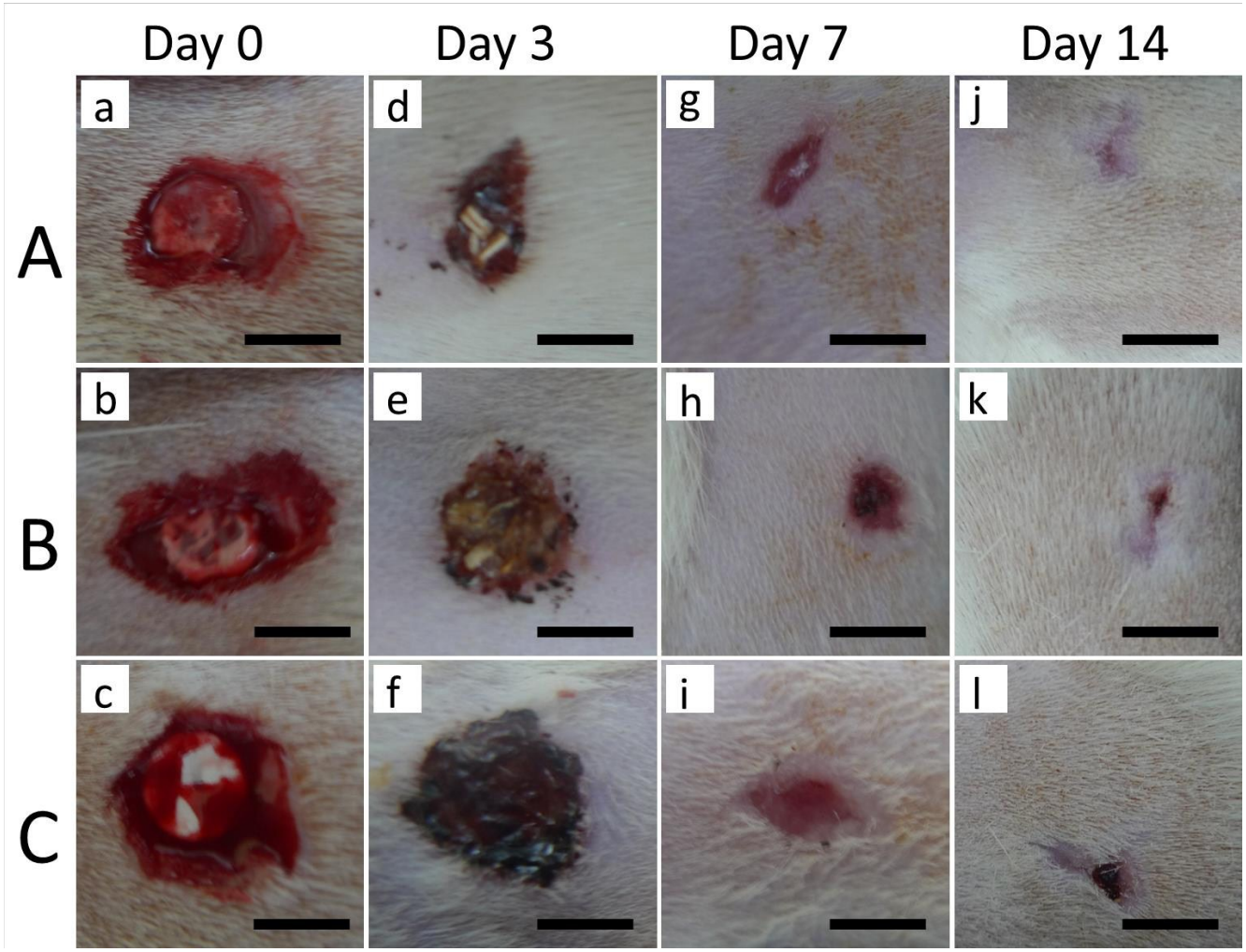

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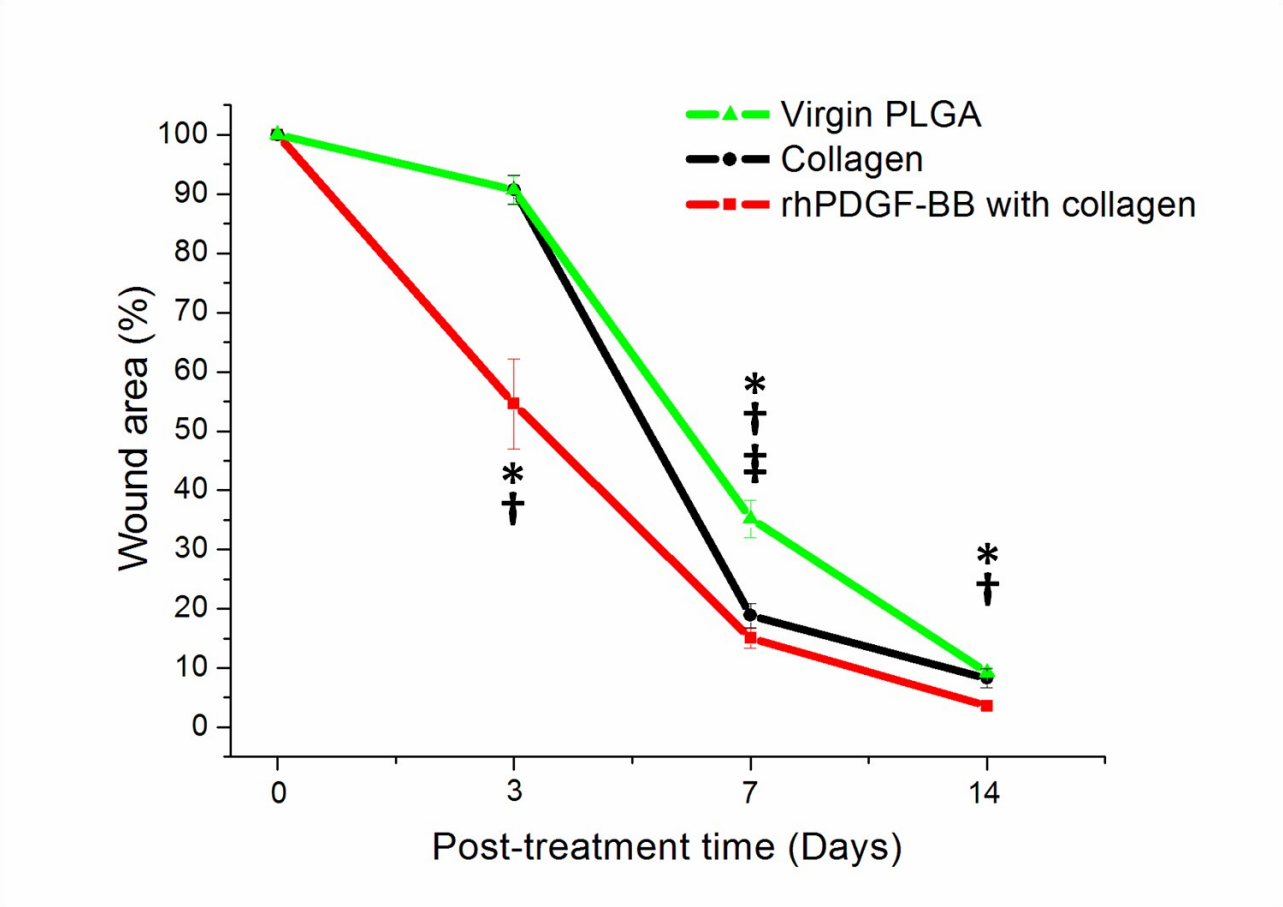

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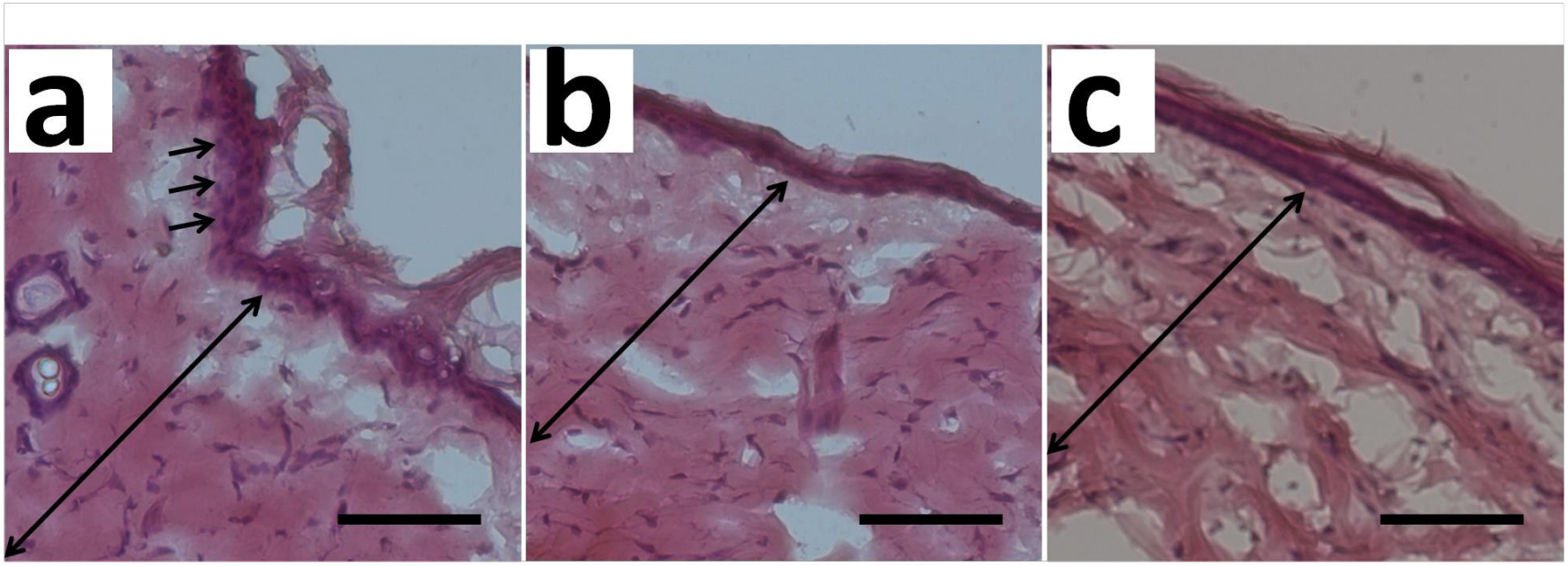

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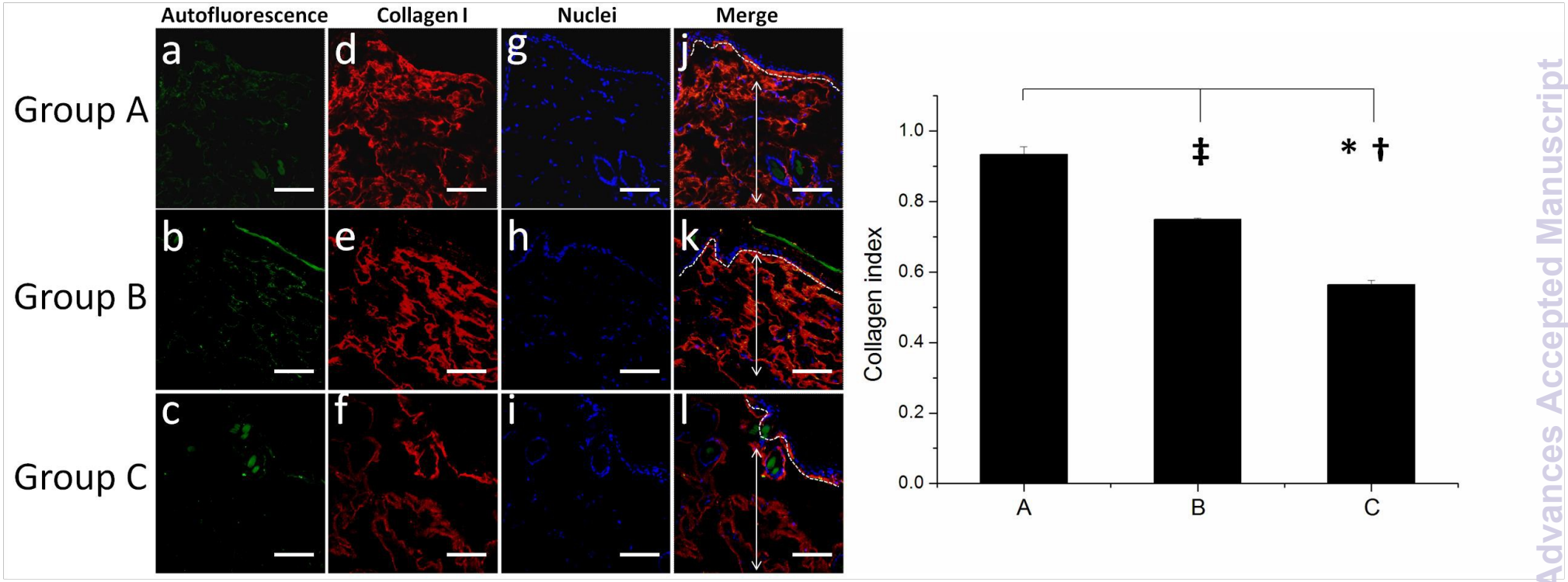

Figure 10: