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Treatment of human chronic wounds with autologous extracellular matrix/stromal vascular fraction gel

A STROBE-compliant study

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Abstract

Stem cell therapy is considered as the most promising treatment for chronic wounds. Extracellular matrix/stromal vascular fraction gel (ECM/SVF gel), an adipose-derived stem cell-based cytotherapy, has shown healing potential in experimental wounds in animal models. However, the effects of ECM/SVF gel on human chronic wounds have not been investigated. The aim of the present study is to investigate the therapeutic effect of ECM/SVF gel on human chronic wounds.

Autologous ECM/SVF gel was prepared and used to treat patients with chronic wounds in clinics, with negative pressure wound therapy as the positive control. Wound healing rate per week and histological changes were performed.

The average wound healing rate per week in the ECM/SVF gel group was 34.55 ± 11.18% compared with 10.16 ± 2.67% in the negative pressure wound therapy group ($P < .001$). Histological analysis with hematoxylin and eosin, Masson's trichrome staining, and CD31 immunohistochemistry showed less lymphocyte infiltration, more collagen accumulation, and more newly formed vessels in the ECM/SVF gel group treated skins compared to the control.

ECM/SVF gel is an effective therapeutic option for chronic wound healing in clinics.

Abbreviations: ASCs = adipose-derived stem cells, ECM = extracellular matrix, EGF = epidermal growth factor, FGF = fibroblast growth factor, HGF = hepatocyte growth factor, MSCs = mesenchymal stem cells, NPWT = negative pressure wound therapy, SVF = stromal vascular fraction, VEGF = vascular endothelial growth factor.

Keywords: adipose-derived stem cell, extracellular matrix, negative pressure wound therapy, stromal vascular fraction, wound healing

1. Introduction

Chronic wounds are those that have not proceeded through a systematic and timely repair process to produce anatomic and functional integrity after 3 months.[1] Currently, chronic wounds present a noteworthy social and economic burden due to the increasingly aging population and the prevalence of cardiovascular and metabolic diseases worldwide. It has been estimated that approximately 1% to 2% of individuals at any given time could be affected by chronic wounds.[2,3] It has been reported that chronic wounds affect around 6.5 million patients.[4] Moreover, the costs for the treatment of venous leg ulcers are 1% of the total annual health care budget, and health care costs for chronic venous diseases are spiraling.[5,6] Therefore, it is very important, although difficult, to manage and monitor chronic wounds.

Currently, the therapies for chronic wounds may be roughly divided into 4 categories based on trauma to the patients and clinical efficacy: conventional, novel, plastic surgery, and cell based.[7–9] Conventional therapies include active dressings through topical application of growth factors, and various biological dressings such as silver and alginate, and hyperbaric oxygen, among others.[10] These therapies are noninvasive and especially suitable for outpatients under nursing supervision, while the clinical efficacy is moderate. Novel therapies include the use of platelet-rich plasma, negative pressure wound therapy (NPWT), and artificial dermis, among others.[11–13] These require surgical debridement and are minimally invasive with much better healing efficacy than conventional therapies. Plastic operations, such as skin and flap grafting, are invasive and require the sacrifice of healthy skin tissue, which has strict indications.

Adipose-derived stem cell-based therapy is one of the most promising therapeutic strategies for wound healing based on precise physiologic requirements, such as re-epithelization, angiogenesis, and immunomodulation.[14–16] However, several drawbacks still need to be resolved. Application of collagenase in the adipose-derived stem cells (ASCs) harvesting increases the risk

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of biological contamination. Additionally, culture and purification of ASCs still require specific laboratory equipment and experience. Moreover, in most studies, stromal vascular fraction (SVF) cells and ASC suspensions are used in isolation without the protection of the extracellular matrix (ECM) components, leaving the cells at the recipient site vulnerable to the immune system. Rapid elimination of SVF cells or ASCs by the immune system causes inadequate cell retention after injection, thereby failing therapeutically.[17–20] All these factors limit further application of ASC/SVF cell therapies.

We previously introduced an injectable adipose tissue-derived product, extracellular matrix/stromal vascular fraction gel (ECM/SVF-gel), which is rich in ASCs and adipose native ECM and can be rapidly fabricated by pure mechanical force.[21] With the support of native ECM, ECM/SVF-gel maintains the optimal cell (i.e., SVF cells) retention. It has been reported that the superior healing effect of ECM/SVF gel on wound healing than SVF suspension was observed.[22] However, the effects of ECM/SVF gel on human chronic wounds have not been investigated. The aim of the present study is to investigate the therapeutic effect of ECM/SVF gel on human chronic wounds and NPWT treatment served as positive control.

2. Materials and methods

2.1. Subjects

The study was approved by the Ethical Committee of Southern Medical University and Affiliated Hospital of Zunyi Medical College and investigations were conducted per the Declaration of Helsinki. All subjects provided written informed consent. Subjects were >18 and < 80 years of age and had wounds of duration >3 months. Subjects were excluded if they were in critical condition, such as shock, resulting from various disorders, multiple organ dysfunction, or serious infections; had any hematological diseases or psychiatric conditions; had severe undernourishment or low body weight; were pregnant; or were participating in another clinical trials.

2.2. Fat harvesting and ECM/SVF gel procedure

The ECM/SVF gel was prepared according to previously reported methods.[21] Briefly, under spinal anesthesia, liposuction was performed in the medial thigh with a 3-mm multiport cannula that contained several sharp side-holes of 1-mm in diameter (Tulip Medical Products, San Diego, CA), at –0.75 atm of suction pressure. After sedimentation, the fat was centrifuged at 1200*g* for 3 minutes, the liquid portion was discarded, and the oil layer collected and saved for further use. The Coleman fat was then mechanically emulsified by shifting between the two 10-mL syringes connected by a female-to-female Luer-Lok connector, at a stable speed (10 mL/s) for 1 minute. The resulting emulsion was filtered using a Nano Transfer filter (Tulip Medical Products, San Diego, CA) to remove connective tissue remnants. Oil (0.5 mL) was added and mixed gently by shifting between syringes 3–5 times until a flocculate was observed within the emulsion. The mixture was then centrifuged at 2000*g* for 3 minutes resulting in 2 different layers: an oily layer and the ECM/SVF gel layer (Fig. 1).

2.3. Wound intervention

ECM/SVF group: Conventional wound debridement was performed and the ECM/SVF gel was applied. The volume used was based on the area of the wound, and the ratio was approximately 0.25 mL/cm^2. Approximately three-fourths of the prepared ECM/SVF gel was injected directly into the base and edges of the wound and the remaining gel covered the wound as a dressing, which was then covered with physiological saline gauze. After 3 days, the first dressing change was performed, and the gauze was reapplied to retain moisture. The dressings were changed at 2- to 3-day intervals. After discharge, the subjects returned weekly so the wound could be monitored; the remaining wound area was measured and evaluated for complications.

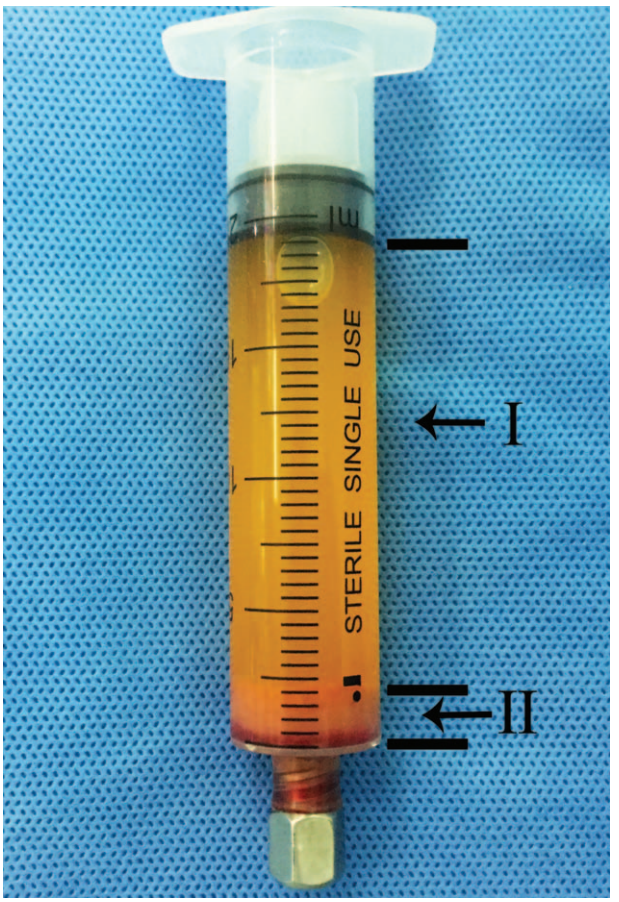

Figure 1. The continuous mechanical dissociation procedure results in 2 different layers: I, oily layer; II, ECM/SVF gel layer. ECM = extracellular matrix, SVF = stromal vascular fraction gel.

Control group: Conventional wound debridement was performed and NPWT was applied. At 2 weeks postsurgery, the negative pressure drainage equipment was removed, and the wound condition was examined and recorded.

For all subjects, before and at 2 weeks after surgery, digital photographs of the wounds were taken, and peri-wound tissues were harvested by punch biopsy and fixed in 10% paraformaldehyde for histological analysis. All subjects received the same conventional treatments during this study, including pressure offload of the wound, blood pressure management, blood glucose monitoring, and antibiotics.

2.4. Observational indices

2.4.1. Wound healing rate. Two weeks after surgery, the residual wound area was calculated using a pixel/area ratio program (Adobe Photoshop CS6.0). The healing rate was evaluated as follows: (Original wound area—residual wound area)/original wound area × 100%.

Table 1

Summary of patient characteristics (mean ± standard deviation).

Treatment group	Number of cases	Age	Duration of wound, months	Original wound size, cm^2	Wound healing rate per week (%)
ECM/SVF gel group	10	60.70 ± 11.10	6.00 ± 4.19	8.79 ± 5.65	34.55 ± 11.18
NPWT group	10	60.20 ± 10.18	8.30 ± 4.85	23.36 ± 12.54	10.16 ± 2.67

ECM = extracellular matrix, NPWT = negative pressure wound therapy, SVF = stromal vascular fraction gel.

2.4.2. Histological analysis. Peri-wound tissues were fixed in 10% paraformaldehyde, then embedded in paraffin and cut into approximately 4-mm sections that were processed and stained with hematoxylin and eosin (H&E). To observe collagen deposition, Masson's trichrome staining was performed, and relative collagen expression was calculated by Image J. Evaluation of neovascularization was performed using CD31 immunostaining and the number of new blood vessels per 5 × high magnification were counted.

2.4.3. Statistical analysis. Results are expressed as mean ± standard deviation. Data were analyzed using a *t* test of independent samples by SPSS ver. 19.0 (Armonk, NY). $P < .05$ was considered statistically significant.

3. Results

3.1. Wound healing rate

Twenty patients who were admitted to the affiliated Hospital of Zunyi Medical College between March of 2016 and September of 2017 were enrolled and evaluated (14 men and 6 women, 40–74 years of age). Wound types included 9 venous stasis ulcers, 5 traumatic infections, 3 diabetic ulcers, 2 scar ulcers, and 1 sarcoidosis. All wounds had remained unhealed for more than 3 months and were unresponsive to standard wound care or skin grafting. Ten subjects were assigned to each treatment group. All patients treated with autologous ECM/SVF gel had a good final outcome. The average wound healing rate was 34.55 ± 11.18%. In the NPWT group, the rate was 10.16 ± 2.67%. The difference was statistically significant ($P < .001$) (Table 1). There were no donor site complications in the patients who had fat harvested from their thighs (Figs. 2 and 3).

3.2. Histological analysis

H&E staining revealed decreased lymphocyte infiltration into the deep dermis layers following ECM/SVF gel administration on day 14 (Fig. 4). Masson's trichrome staining indicated a higher level of collagen accumulation in the ECM/SVF gel group compared with NPWT ($P < .001$); the collagen was also thicker in the ECM/SVF specimens (Fig. 5). CD31 staining indicated more newly formed vessels in the ECM/SVF specimens than for NPWT ($P < .001$) (Fig. 6).

4. Discussion

Although bone marrow-derived mesenchymal stem cells (MSCs) were the first stem cells used to treat wounds,[23,24] adipose-derived stem cells (ASCs) may be a superior alternative. ASCs can be easily harvested from abundant adipose tissue, and have advantages in terms of proangiogenic properties and immunomodulatory effects.[25,26] Numerous animal and clinical works have confirmed the healing effects of ASCs or stromal vascular fraction (SVF), which are heterogeneous cells containing ASCs, on wound healing.[27–32] Our study demonstrates that adipose ECM/SVF gel can support healing of human chronic wounds; this therapy promoted the neoangiogenesis, synthesis of collagen tissue, and induce a favorable immunomodulatory effect on chronic wounds.

The reason of this superior therapeutic effect may be attributed to the concentrated SVF cells and adipose-derived ECM in ECM/SVF gel. According to our previous study, the density of ASCs and endothelial cells (ECs) is approximately $1.9 \pm 0.2 \times 10^5$ cells/mL and $7.7 \pm 2.4 \times 10^4$ cells/mL, respectively, in ECM/SVF gel.[21] Therefore, the first factor for accelerating wound healing may be the condensed SVF cells (e.g., ASCs, ECs, pericytes, etc.). There are many studies reported that ASCs have the potential to promote re-epithelization, angiogenesis, and immunomodulatory effects by secretion of many growth factors such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), epidermal growth factor (EGF), and fibroblast growth factor (FGF), among others.[33–35] Additionally, the differentiation pluripotency of ASC, such as differentiating to epidermal cells and ECs, is also important for re-epithelization and angiogenesis in chronic wounds.[36] It has been reported that ECs directly form capillary cells and are important in angiogenesis.[37]

Another factor for accelerating wound healing is ECM that contains collagen, elastin, mucopolysaccharides, and fibronectin.

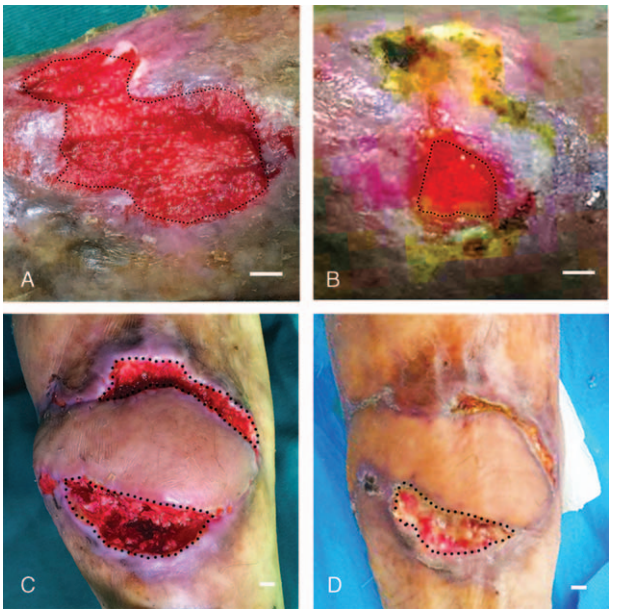

Figure 2. Changes in wounds after ECM/SVF gel grafting. (A) A chronic left foot wound with an original area of 5.70 cm^2. (B) Residual area 14 days post-treatment was 1.04 cm^2. (C) A chronic left leg wound with an original area of 11.03 cm^2. (D) Residual area 14 days post-treatment with ECM/SVF gel grafting was 5.14 cm^2. Scale bar = 1 cm. ECM = extracellular matrix, SVF = stromal vascular fraction gel.

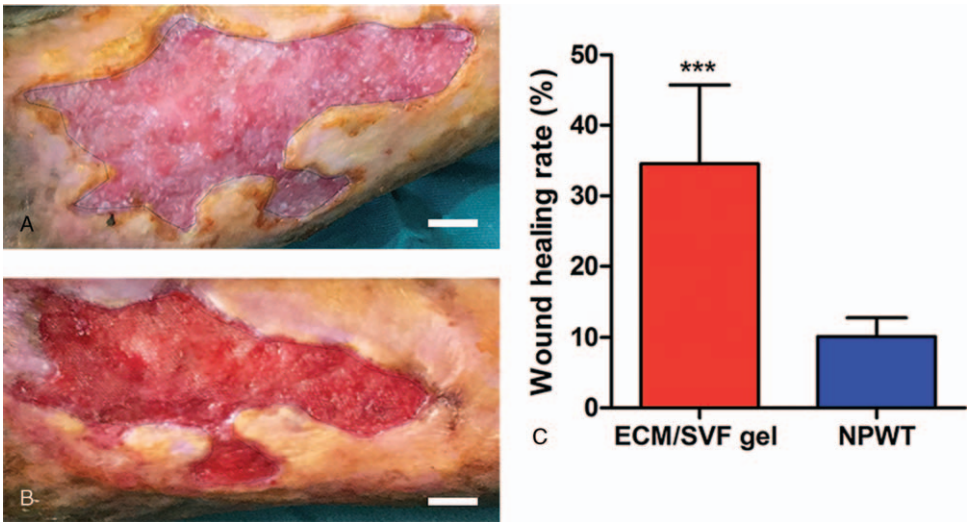

Figure 3. Changes in wounds after treatment with negative pressure wound therapy. (A) A chronic right leg wound with an original area of 19.65 cm^2. (B) Residual area 14 days post-treatment with negative pressure wound therapy was 17.34 cm^2. (C) Quantification of the wound area of the 2 treatment groups. ***$P < .001$. Scale bar = 1 cm.

It has been reported that ECM facilitates migration and morphogenesis of angiogenesis,[38] while collagen and fibronectin are important components during the wound healing process.[39] Moreover, the synergistic interaction among SVF cells, ECM, and the growth factors in the ECM/SVF gel may be the unique factor for accelerating wound healing. For example, the combined application of ECs and pericytes has been proven to augment angiogenesis compared with their individual use.[40] It has already been reported that adipose native ECM may provide a favorable cellular microenvironment for SVF cells adhesion and survival, leading to an accelerated wound healing.[41]

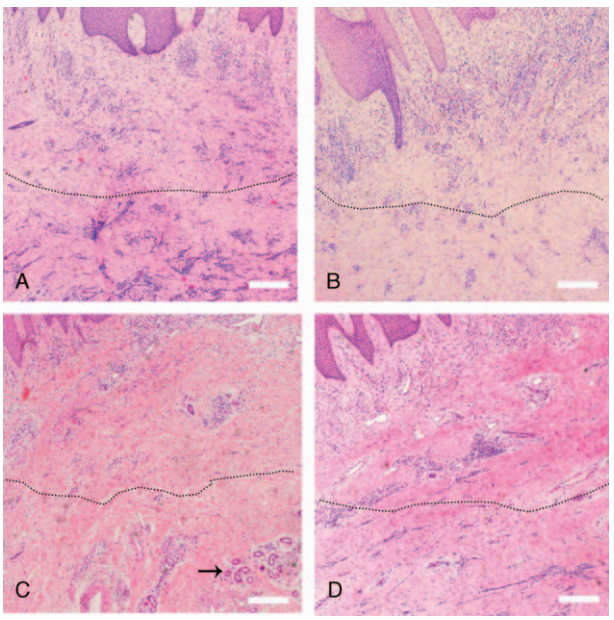

Figure 4. Hematoxylin and eosin staining of tissue from the wound area. (A) Numerous inflammatory cells infiltrate the deep dermal layers (below dotted line) before treatment with ECM/SVF gel grafting. (B) Numerous inflammatory cells infiltrate the deep dermal layers (below dotted line) before negative pressure wound therapy. (C) Decreased inflammatory cell infiltration in the dermis deep layer (below dotted line) and new vascular structures (black arrow) after ECM/SVF gel grafting. (D) The inflammatory cell infiltration had not significantly changed (below dotted line) after negative pressure wound therapy. Scale bar=20 μm. ECM = extracellular matrix, SVF = stromal vascular fraction gel.

The potential mechanism for accelerating wound healing from ECM/SVF gel was further verified by histology analysis. H&E staining revealed that lymphocyte infiltration into the dermis layers was obviously decreasing after ECM/SVF gel injection, which confirms immunomodulation of ASCs in the lymphocyte-mediated chronic inflammatory response. It has been reported that collagen synthesis and neovascularization are essential for wound healing. Collagen is essential for wound healing as it provides a biological scaffold for the migration of various repair cells and the generation of new vessels.[42] Additionally, increasing the number of type I collagens in the early stages of wound healing can promote healing and reduce scarring.[43] In our study, Masson's trichrome and CD31 staining demonstrated an increased collagen accumulation and newly formed vessels in the ECM/SVF gel group compared with NPWT group.

Recently, Lafosse et al[44] reported the implantation of ASCs seeded a biological dressing (human acellular collagen matrix) in 3 patients with chronic wounds. The results revealed that the matrix supports the cellular adhesion and spreading and improves local ASCs delivery without a significant local or systemic prolonged inflammatory reaction. Although the therapy of this biological dressing is promising, the time-consuming manufacturing remains to be improved. The advantages of using ECM/SVF gel as a cytotherapy tool in treating chronic nonhealing wounds can be summarized as follow. First, ECM/SVF gel therapy is a minimally invasive procedure. The surgical incision is only 3 to 5 mm on the ipsilateral or contralateral thigh for liposuction. Unlike skin grafting or skin flaps, ECM/SVF gel is useful for treating intractable skin ulcers on the lower leg in the elderly who are unable to tolerate general anesthesia without the need to sacrifice healthy skin tissue. Second, there are no ethical issues associated with the clinical applications of ECM/SVF gel. Without collagenase digestion and cultured-in vitro, special permits are not required, which is especially suitable for countries

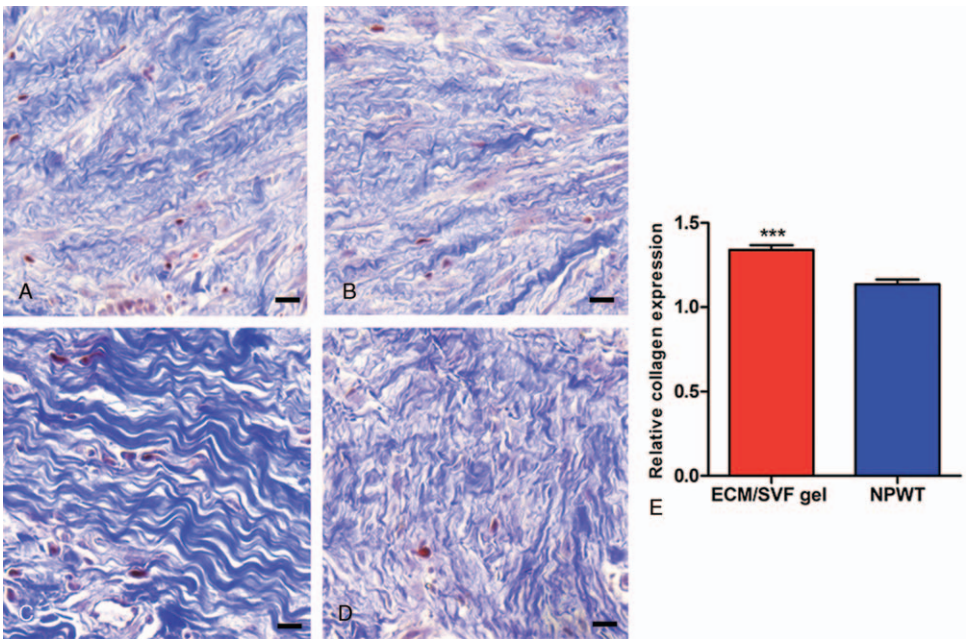

Figure 5. Masson's trichrome staining of tissue from the wound area. (A) Thin collagen layer before ECM/SVF gel grafting. (B) Thin collagen layer before negative pressure wound therapy. (C) The thickest collagen layer was seen after ECM/SVF gel grafting. (D) The collagen layer is thicker after negative pressure wound therapy. (E) Relative quantification of collagen density. ***$P < .001$. Scale bar=20 μm. ECM = extracellular matrix, SVF = stromal vascular fraction gel.

with strict regulation in the use of stem cell therapy. Third, the preparation and injection of ECM/SVF gel is simple and time-saving without much finical burden. The total operation time could be < 2 hours, and the patients can be discharged several days postsurgery and return to the hospital weekly to monitor the wound.

However, a standard ECM/SVF gel-based chronic wound treatment scheme is required. For example, the ratio between transplanted ECM/SVF gel volume and wound bed area should be developed. In addition, more clinical cases and clinical trials are needed to support efficacy, side effect profiles, and long term outcomes.

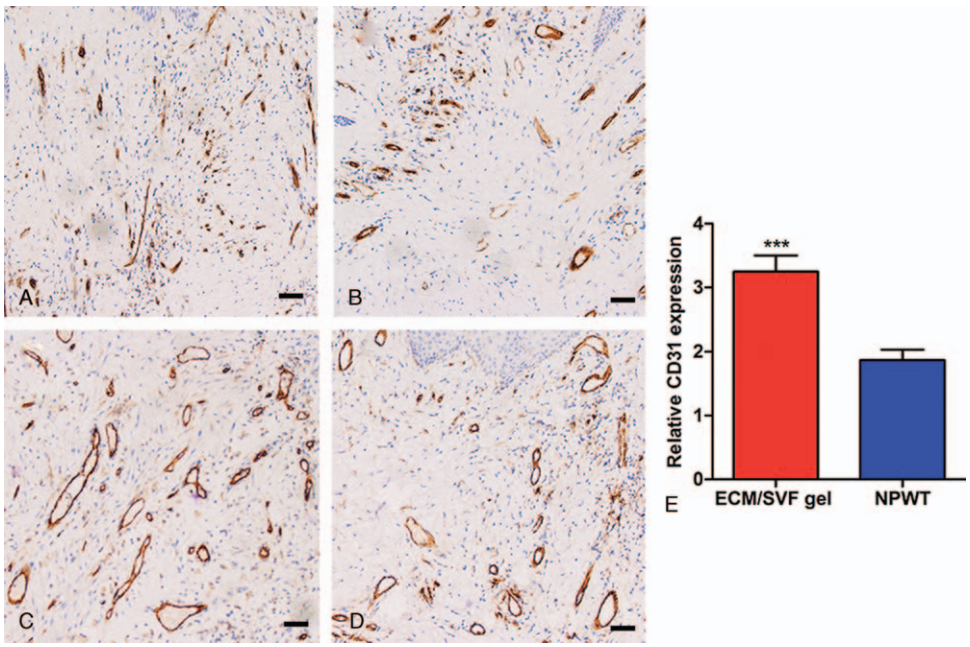

Figure 6. Immunohistochemistry with CD31 of tissue from the wound area. (A) Scant neovascularization before ECM/SVF gel grafting. (B) Scant neovascularization before negative pressure wound therapy. (C) The greatest extent of neovascularization was seen after ECM/SVF gel grafting. (D) Neovascularization increased after negative pressure wound therapy. (E) Relative quantification of neovascularization. ***$P < .001$. Scale bar=20 μm. ECM = extracellular matrix, SVF = stromal vascular fraction gel.

5. Conclusions

ECM/SVF gel can exert a therapeutic effect on human chronic wounds, which is likely attributed to a favorable immunomodulatory effect, increased collagen accumulation, and improved neovascularization. This therapy is simple, safe, and minimally invasive but should be further improved in terms of the establishment of a standard treatment scheme.

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Investigation: Chengliang Deng.
Methodology: Jingwei Feng, Feng Lu.
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Writing – original draft: Chengliang Deng.
Writing – review & editing: Liangyue Wang.

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