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Comparison of interleukin 10 homologs on dermal wound healing using a novel human skin *ex vivo* organ culture model

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Abstract

Background—Anti-inflammatory cytokine interleukin (IL)-10 has been shown to induce regenerative healing in postnatal wounds. A viral homolog of IL-10 produced by human cytomegalovirus (CMV IL-10) similarly generates potent immunoregulatory effects, but its effects on wound healing have not been investigated. Currently, there are limited cost-effective methods of screening vulnerary therapeutics. Taken together, we aim to develop and validate a novel human *ex vivo* dermal wound model and hypothesize that CMV IL-10 will enhance dermal wound healing.

Methods—Full-thickness circular (6-mm) explants were taken from surgical skin samples and 3-mm full-thickness wounds were created. Explants were embedded in collagen I matrix and maintained in specially formulated media with the epidermis at air–liquid interface, and treated with human IL-10 or CMV IL-10 (200 ng/mL). The viability of cultured explants was validated by histology and lactate dehydrogenase (LDH) activity. Epithelial gap, epithelial height, basal keratinocyte migration, vascular endothelial growth factor levels, and neovascularization were measured at days 3 and 7 to determine IL-10 effects on wound healing.

Results—Culture explants at day 7 appeared similar to fresh skin in morphology, cell, and vessel density. By day 14, the epidermis separated from the dermis and the cell density diminished. Day

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7 wounds appeared viable with advancing epithelial and basal keratinocyte migration with no evidence of necrosis. Cytotoxicity analysis *via* the quantification of LDH revealed no differences between controls and treated groups. There was a slight increase in the quantity of LDH in media at day 3; however, this decreased at day 5 and continued to decline up to day 21. CMV IL-10 treatment resulted in a significant decrease in the epithelial gap and an increase in epithelial height. There were no differences in the rates of basal keratinocyte migration at day 7 between treated and control groups. Interestingly, human IL-10 increased vascular endothelial growth factor expression and neovascularization compared with controls.

Conclusions—The human *ex vivo* wound model provides a simple and viable design to study dermal wound healing. Both IL-10 homologs demonstrate vulnerary effects. The viral homolog demonstrates enhanced effects on wound closure compared with human IL-10. These data represent a novel tool that can be used to screen therapeutics, such as CMV IL-10, before preclinical studies.

Keywords

Wound healing; IL-10 homologs; Regenerative medicine; Organ culture

1. Introduction

The normal physiological postnatal response to cutaneous injury results in scar formation, which has diminished integrity compared with uninjured skin [1]. Compared with fetal regenerative healing, this inherently deficient repair process is exacerbated in several disease states, such as in diabetes, that can lead to nonhealing wounds and has implications for the quality of postsurgical healing [2,3]. Development of new cellular and molecular therapeutics that can recapitulate cutaneous regenerative repair [1,4–6] can save some of the \$25 billion spent annually on impaired wound healing and potentially improve patient outcomes [2].

In an effort to achieve this goal, we have previously demonstrated a significant role for interleukin (IL)-10 in the fetal scarless wound healing phenotype [7–9]. IL-10 is a pleiotropic immunoregulatory homodimeric cytokine product of CD4⁺ and CD8⁺ T cells, B cells, monocytes, macrophages, and keratinocytes [10]. In postnatal tissue repair, levels of IL-10 have been shown to be inversely correlated with increased fibrosis. We have demonstrated [7] and others have confirmed [11–13] that the overexpression of IL-10 can recapitulate the scarless wound healing phenotype in postnatal wounds. The mechanisms of IL-10 effects have been attributed to its well-studied anti-inflammatory and immunoregulatory properties. Recently, IL-10 has been shown to have other regulatory properties besides its anti-inflammatory effects. In a myocardial infarct murine model, IL-10 increases endothelial progenitor cell (EPC) survival and upregulates vascular endothelial growth factor (VEGF) expression in EPCs and EPC-mediated neovascularization in the ischemic myocardium, which corroborated with improved left ventricular function, reduced infarct size, and fibrosis in the myocardium [14]. The mechanisms of IL-10 effects are not completely understood and there are no data about the effects of IL-10 on cutaneous wound closure, granulation tissue formation, and neovascularization.

It has been recently demonstrated that the human cytomegalovirus (CMV) harbors a homolog of IL-10 (CMV IL-10) [15] that is a more potent anti-inflammatory agent and immunosuppressor [16]. The *CMV IL-10* gene has a unique structure, the position of the two introns within the *CMV IL-10* gene matches the position of the first and third introns of the *human IL-10* gene, but the protein is distinctive and has only 27% sequence identity to the human IL-10 cellular protein. Despite the limited homology between CMV IL-10 and human IL-10, CMV IL-10 binds to and induces signal transduction through the same IL-10 receptor complex as human IL-10 cellular protein and induces a stronger anti-inflammatory response. These findings suggest the potential that the CMV IL-10 may have unique vulnerary effects compared with human IL-10, but there are no studies that have looked at the effect of the CMV IL-10 on wound healing outcomes.

The development of biological therapeutics for clinical use is dependent on a successful experimental model that can recapitulate each phase of wound healing and is representative of the human anatomy. In this context, the use of human skin in organ culture has the complex anatomy and cellular elements of skin, and potentially provides a better alternative model to study wound healing outcomes [17]. To that end, we have developed a human *ex vivo* skin organ culture wound model that has the advantage of testing wound healing processes in an efficient and cost-effective process and also retains the human epitopes for translational capability.

Taken together, this has led us to hypothesize that IL-10 may have an effect on wound closure, and the viral homolog CMV IL-10 because of its known increased potency may have further advantageous effects on wound closure. To test this hypothesis, we optimized an *ex vivo* organ skin culture model and examined the effect of human and CMV IL-10 treatment on wound healing outcomes.

2. Materials and methods

2.1. Ethics statement

Adult human skin sections were obtained from the National Disease Research Interchange (Philadelphia, PA). The study protocol, which involves use of anonymous, de-identified, discarded human adult skin, was reviewed and granted an exempt status by The Cincinnati Children's Hospital Medical Center Institutional Review Board.

2.2. Human *ex vivo* model

Human skin from de-identified donors was shipped overnight in hanks buffer. Skin was disinfected in 70% ethanol, and several changes of Dulbecco's modified Eagle's medium buffer containing 2× penicillin and 2x streptomycin (Life Technologies, Carlsbad, CA). Subcutaneous fat was carefully removed with iris scissors. Full-thickness circular 6-mm explants were obtained from the skin using a 6-mm punch biopsy. These samples were embedded in a rat tail collagen I gel matrix (2.5 mg/mL) (BD Bioscience, San Jose, CA). The cell culture media consisted of serum-free Dulbecco's Modified Eagle's Medium (Life Technologies) supplemented with 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 50 µg/mL ascorbic acid, 100 µM adenine, 0.5 µM hydrocortisone, 0.1 µM cholera toxin, 100 IU/mL penicillin, and 10 µg/mL streptomycin (Sigma-Aldrich, St. Louis, MO). The *ex*

vivo organ cultures were cultured at the air–liquid interface and maintained in the cell culture incubator at 37°C with 5% CO₂. The media were changed every other day. The viability of the organ culture explants was tested upto 3 wk in culture by (1) lactate dehydrogenase (LDH) cytotoxicity assay performed on the cell culture media to determine cell viability, and (2) histologic evaluation of the explants to analyze the organization of the epidermis, dermis and cellular density.

Thereafter, the wound model was created. A 3-mm full-thickness (donut-like) wound was created using a 3-mm punch biopsy in the middle of the 6-mm explants obtained from the human skin. The full-thickness wounded explants thus formed were embedded in a rat tail collagen I gel matrix (2.5 mg/mL) (BD Bioscience), and maintained at the air–liquid interface in the serum-free media as previously described (Fig. 1). Treatment groups included wounds treated with 200 ng/mL of human IL-10 ($n = 5$) or 200 ng/mL of CMV IL-10 ($n = 5$), additional controls included treatment with phosphate-buffered saline (PBS; $n = 5$). The samples were maintained in *ex vivo* organ culture for upto 3 wk. Media were changed every other day. Viability of the treatment groups was tested similarly as previously described by LDH assay and histologic evaluation.

2.3. Validation of the organ culture model

The viability of cultured explants was analyzed using a cytotoxicity detection kit (LDH assay kit; Roche, Indianapolis, IN). This is a colorimetric assay for the quantification of cell death and cell lysis based on the measurement of LDH activity released into the supernatant (media) of organ culture by damaged cells. Media were collected from the organ cultures on days 1, 3, 5, 7, 14, and 21. The LDH measurement was executed according to the manufacturer's instructions. Relative cytotoxicity (in absorbance) is reported.

Additionally, the histologic structure of the organ culture samples has been evaluated as a marker for viability. The cultured skin grafts were harvested at days 0, 4, 7, 14, and 21 in *ex vivo* culture and fixed in 10% neutral-buffered formalin. The fixed tissues were then embedded in paraffin and 5 µm sections were obtained. The tissue sections were stained with hematoxylin and eosin (H&E) and observed under a Nikon Eclipse 80i light microscope with the images captured by a Nikon Digital Sight DS-U1 camera system (Nikon Instruments, Melville, NY).

2.4. Wound healing analysis

To determine differences in IL-10–mediated effects on wound healing, epithelial gap closure and re-epithelialization of the wounds, epithelial height, and basal keratinocyte migration were measured. Wound sections of 5 µm thickness were cut from paraffin embedded blocks. Epithelial gap and granulation tissue deposition were studied from H&E–stained sections and morphometric image analysis using Nikon Elements (Nikon Instruments). On a 4× edge-to-edge wound section image, epithelial gap was measured as the distance (in millimeters) between encroaching epithelial margins. Epithelial height was measured (in millimeters) at the highest point on both the encroaching epithelial margins of the wound. Wound sections were also analyzed for basal keratinocyte migration (in millimeters).

Wound extra cellular matrix composition was determined using the Masson trichrome staining as per the manufacturer's protocol (Polyscientific, Bay Shore, NY).

2.5. Immunohistochemistry analysis to quantify neovascularization

Paraffin sections of 5 μ m thickness were obtained from the middle of the wound, dehydrated, rehydrated to distilled water, and immersed in antigen retrieval solution at pH 6.2 for 20 min at 95°C and allowed to cool down for 20 min at room temperature (Dako Cytomation, Carpinteria, CA) to reactivate masked epitopes. Slides were washed with distilled water and transferred to PBS. Samples were blocked using normal 10% goat serum in PBS with 0.1% Tween-20 for 30 min at room temperature. Sections were immunostained with rat CD31 (1:20; BD Pharmingen, Franklin Lakes, NJ), followed by biotinylated goat anti-rat antibody (1:200; Vector laboratories, Burlingame, CA). In the *ex vivo* organ culture wound model, capillary lumen density was measured as the average number of CD31-positive lumens from eight randomly chosen 20 $\times$ sections across the sample.

2.6. VEGF enzyme-linked immunosorbent assay and quantification of total protein

VEGF levels were measured in media collected from organ culture samples at day 7 postwounding using a VEGF enzyme-linked immunosorbent assay (ELISA) kit as per the manufacturer's protocol (R&D systems, Minneapolis, MN). ELISA data were normalized to total protein analyzed by Coomassie Plus protein assay (Thermo Scientific, Logan, UT).

2.7. Statistical analysis

All data are presented as the mean $\pm$ standard deviation. The Student *t*-test was used to identify difference between individual groups. Analysis of variance and *post hoc* tests with Bonferroni corrections were used to determine the effects of the wound treatment with human IL-10, CMV IL-10, or PBS on measured parameters. $P < 0.05$ was considered to denote statistical significance.

3. Results

3.1. Validation of the human skin *ex vivo* organ culture model

Fresh human skin sample, which was processed immediately after collection from the patient at day 0, demonstrated an undulated epidermis, basket weave pattern of collagen in the dermis, blood vessels, and dermal appendages (Fig. 2A). Compared with day 0 human skin samples, organ culture samples at day 7 did not show any significant differences in histologic appearance in the dermal extra cellular matrix organization or epidermal stratification by H&E and trichrome staining, demonstrating that the epidermal and dermal structure of the skin was maintained under our organ culture conditions for at least 7 d (Fig. 2B). After 14 d in culture, detachment of epidermis from dermis was observed. Also, the thickness of the epidermis at day 14 was decreased and only a few keratinocytes were seen in the epidermis. The structure of the dermis appeared similar to fresh human skin from day 0, but the vasculature appeared reduced (Fig. 2C and D). Cytotoxicity analyzed by measuring the levels of LDH activity released into *ex vivo* organ culture media demonstrated that LDH activity declined from day 1 to day 21 in culture (Fig. 2E).

3.2. Validation of the *ex vivo* wound model

To determine if the *ex vivo* organ culture model can be used as a tool for full-thickness wound healing studies, 3-mm full-thickness wounds were created and different treatments were added to the culture media. H&E analysis (Fig. 3A, C, and D) demonstrated that all day 7 wounds appear viable with no evidence of necrosis and advancing epithelial margins (indicating metabolic activity). Trichrome analysis (Fig. 3B, D, and F) demonstrated thickened encroaching epithelial margins and basal keratinocyte migration. To validate viability, cytotoxicity analysis *via* the quantification of LDH activity released into the cell culture media revealed no differences between controls and treated groups after 7 d of serial measurements (Fig. 3G). There was a slight increase in the quantity of LDH in media at day 3; however, this decreased at day 5 and continued to decline up to day 7.

3.3. CMV IL-10 increases wound closure, epithelial height, and basal keratinocyte migration at day 7

To investigate the role of IL-10 in wound closure, epithelial height, and keratinocyte migration during wound healing in the human *ex vivo* wound model, human recombinant IL-10, CMV IL-10, or PBS was added to the wound beds. Combined H&E and trichrome analysis of wound healing parameters demonstrated that CMV IL-10 treatment resulted in a significant decrease in the epithelial gap compared with human IL-10 or PBS controls (Fig. 4A: PBS 2.98 ± 0.25 mm *versus* human IL-10 2.67 ± 0.4 , CMV IL-10 1.69 ± 0.6 ; $P < 0.05$). However, the difference between human IL-10 and PBS controls was not significant. Furthermore, CMV IL-10 resulted in a larger epithelial height in comparison with human IL-10 or PBS control (Fig. 4B: PBS 0.18 ± 0.03 mm *versus* human IL-10 0.18 ± 0.02 , CMV IL-10 0.29 ± 0.06 ; $P < 0.05$). There were no differences in the rates of basal keratinocyte migration at day 7 between treated and control groups (Fig. 4C and F: PBS 0.73 ± 0.15 mm *versus* human IL-10 1.03 ± 0.6 , CMV IL-10 0.70 ± 0.24 ; $P = \text{ns}$).

3.4. Human IL-10 overexpression increases VEGF expression and neovascularization

To determine the effect of different IL-10 homologs on neovascularization in the human *ex vivo* wound model, human IL-10, CMV IL-10, or PBS was added to the wound beds. VEGF expression in the organ culture media was analyzed at both days 3 and 7 postwounding by ELISA. At day 3, there was a significant increase in the VEGF protein expression in the human IL-10–treated wounds compared with PBS controls (Fig. 5A: PBS 60.3 ± 2.9 pg/μg *versus* human IL-10 72.04 ± 6.5 pg/μg; $P < 0.05$). However, the CMV IL-10–treated wounds demonstrated a huge variation in VEGF levels, with no statistically significant difference compared with either PBS-treated wounds or human IL-10–treated wounds (Fig. 5A: CMV IL-10 68.03 ± 24.5 pg/μg *versus* PBS 60.3 ± 2.9 pg/μg or human IL-10 72.04 ± 6.5 pg/μg; $P = \text{ns}$). At day 7, there were no statistically significant differences between the control and the IL-10 homologs in the VEGF expression (Fig. 5B: PBS 22.95 ± 2.5 pg/μg *versus* human IL-10 23.22 ± 1.2 pg/μg, CMV IL-10 24.09 ± 3.1 pg/μg; $P = \text{ns}$). Wound vessel density was assessed by CD31 immunohistochemical staining at day 7. Human IL-10–treated wounds demonstrated significantly increased capillary density compared with PBS controls at day 7 (Fig. 5C: PBS 12.5 ± 1.91 capillary lumens per high power field [CAPS/HPF] *versus* human IL-10 17.4 ± 3.7 CAPS/HPF; $P < 0.01$). Again, the CMV

IL-10–treated wounds demonstrated a huge variation in capillary density, with no statistically significant difference compared with either PBS-treated wounds or human IL-10–treated wounds (Fig. 5C: CMV IL-10 15.3 ± 3.4 CAPS/HPF *versus* PBS 12.5 ± 1.91 CAPS/HPF or human IL-10 17.4 ± 3.7 CAPS/HPF; $P = \text{ns}$).

4. Discussion

Our data suggest novel biologic mechanisms for IL-10 homologs that are in addition to its known inflammatory role. Our results demonstrate that IL-10 is a vulnerary therapeutic for cutaneous wound healing because of its effects on wound closure, granulation tissue formation, and neovascularization. The viral homolog CMV IL-10 has increased potency compared with the human cellular IL-10 cytokine in improving wound closure and epithelial height. The human IL-10 cytokine improves the VEGF expression and improves neovascularization of the wounds. Additionally, our results demonstrate that the human skin *ex vivo* organ culture model is a viable tool to study therapeutic targets to improve wound healing.

The focus of this study is the comparison of IL-10 homologs on dermal wound healing and neovascularization using the *ex vivo* human skin organ culture model. Although the human IL-10 cytokine is most commonly known to have pleiotropic effects in regulating the immune system and inflammation [10,18], our laboratory data have shown a role for IL-10 in exhibiting a scarless wound healing phenotype [7]. However, before this study no data existed regarding the effects of IL-10 on wound closure and granulation tissue formation. Our data show IL-10 increases wound closure and epithelial height. Interestingly, on comparing the differences in wound healing rates produced by the IL-10 homologs (CMV *versus* human), the viral homolog demonstrated a larger epithelial height and significantly decreased the epithelial gap of the wound after 7 d. These findings are novel because they suggest a new biological function of the viral IL-10 homolog produced by CMV, as there is no known role for viral IL-10 in a cutaneous application. Our data also demonstrate that the human IL-10 significantly increases VEGF expression in the *ex vivo* organ culture model at early time points, which leads to an increase in the vessel density observed at the later day 7 time point. Recent data from our laboratory demonstrated that human IL-10 overexpression significantly increases VEGF expression by dermal fibroblasts. As fibroblasts are the major cell type in the human dermis, this may be one of the possible mechanisms for increased VEGF levels in the *ex vivo* human skin organ culture wound model. Previous studies by Krishnamurthy *et al.* [14] demonstrated that IL-10 increases VEGF expression by EPCs. Although this may represent one of the mechanisms for IL-10 effects on increased cutaneous wound neovascularization, it is difficult to study the mechanism in this model because it is isolated from systemic circulation and potential effects thereof of the bone marrow-derived cells (including EPCs) on wound healing parameters. Future studies are necessary to determine the mechanism(s) used by different IL-10 homologs to regulate the rate of wound closure and neovascularization.

Several *in vitro* wound closure models, such as monolayer keratinocyte culture [19,20], keratinocyte-fibroblast cocultures [21,22], and organotypic cultures [23,24] are used in wound healing studies. Although these models are relatively cost effective, easy to maintain,

and reproducible, they cannot completely reproduce the physiology of human skin for wound repair studies and clinical translation. The fidelity of these models is further limited because of the over simplification of the matrix or wound environment, the lack of diverse cell types and adnexal elements of the skin, and the lack of multidimensional growth that effect the wound healing. *In vivo* models using animal subjects have the advantage of simulating wound healing that is affected by the host's vascular and immune systems and the external environment, similar to the human clinical cases. However, these models are physiologically different; for example, mice and rats heal cutaneous wounds by contraction of the subcutaneous muscle (panniculus carnosus) instead of granulation tissue deposition and re-epithelialization as seen in humans [25,26]. It is also very expensive to test larger animal models, such as the pig, because a number of replicates are required to minimize the effects of intraspecies differences. *Ex vivo* models offer an attractive option to study wound healing, and are an effective tool to determine the potency and toxicity of therapeutics. *Ex vivo* organ culture studies allow the researcher to examine the impact of a drug at the organ level and at the same time providing a means to analyze the individual parts causing the overall net effect. Ultimately, this equips the researcher with a better understanding of how the drug of interest reacts with a selected human system. Our results demonstrate that the *ex vivo* human skin organ culture was viable for at least 7 d. This provides an opportunity to study the early therapeutic effects of different drugs on wound healing. It is further interesting to note that we used a basic serum-free cell culture media formulation in these experiments. Recent studies by Bagabir *et al.* [27] demonstrated that by optimizing the cell culture media formulation, they were able to maintain their *ex vivo* keloid organ cultures intact and viable for up to 4 wk. We are in the process of optimizing the organ cultures for long-term wound healing studies.

5. Conclusions

Our novel human organ culture model provides an accurate representation of the anatomic structure and physiology of the human skin to study wound healing outcomes and the development of vulnerary therapeutics. It is important to note that although this model provides a vehicle for the rapid screening of drugs and toxicity, limitations exist regarding its application, such as the inability to evaluate the systemic adverse effects produced by therapeutic agents. Nonetheless, local toxicity and efficacy can be assessed efficiently. Future studies will use large animal models to further investigate the novel biology of the different IL-10 homologs in wound healing outcomes. These data may lead to the development of novel wound healing therapies for both the normal scarring process and impaired wound healing states.

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Fig. 1.

(A) Human skin was washed in several changes of PBS and maintained in Dulbecco's modified Eagle's medium at the time of sample collection. Adipose tissue was excised with iris scissors. (B) Dermal punch biopsy (6-mm) was used to core out circular human skin samples to establish the *ex vivo* organ culture model. (C) A full-thickness excisional wound of 3-mm diameter was made in the center of each of the 6-mm biopsies that were obtained. (D) Explants were cultured in 24-well tissue culture dishes to establish an *ex vivo* human skin organ culture model. Rat tail collagen I gel was formed on the bottom of the plates. The wound explants were placed on gel and additional collagen was added for support, media was added and organ cultures were maintained at the air-liquid interface. After 24 h, the media were changed and new media with or without the treatments were added to the cultures. (Color version of figure is available online.)

Fig. 2.

(A) H&E-stained fresh human skin at day 0. (B) Compared with fresh human skin sample at day 0, *ex vivo* organ culture samples at day 7 demonstrate no significant difference in appearance demonstrating that the epidermal and dermal structure and vessel density of the skin was maintained under our organ culture conditions for at least 7 d. (C and D) After 14 d in culture, detachment of epidermis from dermis was observed. Also, the thickness of the epidermis at day 14 was decreased and only a few keratinocytes were seen in the epidermis. The structure of the dermis looked similar to fresh human skin from day 0, but the vasculature appears reduced. (E) LDH activity released into *ex vivo* organ culture media indicates the viability of the model. All sections are at 10× magnification. Graph represents the average $\pm$ standard deviation. (Color version of figure is available online.)

Fig. 3.

(A and B) PBS control wounds, (C and D) human IL-10–treated wounds, (E and F) CMV IL-10–treated wounds. (A, C, and E) H&E–stained *ex vivo* human skin organ culture wound sections at day 7 with different treatments (edge-to-edge wound representation at 4× magnification). Epithelial gap is measured as the distance (in millimeters) between the encroaching epithelial margins (arrows). (B, D, and F) The Mason trichrome–stained wound sections (representative area at the encroaching epithelial margins at 20× magnification). Black dotted line represents the distance of basal keratinocyte migration. Epithelial height is measured as the height of the encroaching wound epithelium at the highest point. (G) Cytotoxicity assay (LDH) demonstrates no significant differences between the treatments and PBS ions control, suggesting no adverse effects of the treatments on organ culture. Graph represents the average $\pm$ standard deviation. hIL-10 = human interleukin 10. (Color version of figure is available online.)

Fig. 4.

CMV IL-10 treatment results in a significant decrease in the epithelial gap (A), and results in a larger epithelial height (B) compared with human IL-10 or PBS controls at day 7. There is no difference in the rate of basal keratinocyte migration at day 7 between treated and control groups (C). Bar plots represent the average $\pm$ standard deviation. *P* values are calculated by the *t*-test. hIL-10 = human interleukin 10. (Color version of figure is available online.)

Fig. 5.

(A) There is a significant increase in the VEGF protein expression at day 3 in human IL-10–treated wounds compared with PBS controls, but there is no significant difference between the human and viral IL-10 homologs. (B) By day 7, there are no differences in the VEGF expression between the control and various IL-10 homologs. (C) Human IL-10–treated wounds demonstrate significantly increased capillary lumen density compared with PBS controls at day 7, but there is no significant difference between the human and viral IL-10 homologs. CAPS/HPF (magnification, 20×) were measured in eight fields. Bar plots represent the average $\pm$ standard deviation. hIL-10 = human interleukin 10. (Color version of figure is available online.)