Restoring Prohealing/Remodeling-Associated M2a/c Macrophages Using ON101 Accelerates Diabetic Wound Healing

Ching-Wen Lin1, Chih-Chiang Chen2,3, Wen-Yen Huang4, Yen-Yu Chen1, Shiou-Ting Chen4, Hung-Wen Chou1, Chien-Ming Hung1, Wan-Jiun Chen1, Chia-Sing Lu5, Shi-Xin Nian2, Shyi-Gen Chen1,6, Hsuen-Wen Chang7, Vincent H.S. Chang7,8,9, Li-Ying Liu2, Ming-Liang Kuo10 and Shun-Cheng Chang1

Diabetic wounds exhibit chronic inflammation and delayed tissue proliferation or remodeling, mainly owing to prolonged proinflammatory (M1) macrophage activity and defects in transition to prohealing/proremodeling (M2a/M2c; CD206+ and/or CD163+) macrophages. We found that topical treatment with ON101, a plant-based potential therapeutic for diabetic foot ulcers, increased M2c-like (CD163+ and CD206+) cells and suppressed M1-like cells, altering the inflammatory gene profile in a diabetic mouse model compared with that in the controls. An in vitro macrophage-polarizing model revealed that ON101 directly suppressed CD80+ and CD86+ M1-macrophage polarization and M1-associated proinflammatory cytokines at both protein and transcriptional levels. Notably, conditioned medium collected from ON101-treated M1 macrophages reversed the M1-conditioned medium–mediated suppression of CD206+ macrophages. Furthermore, conditioned medium from ON101-treated adipocyte progenitor cells significantly promoted CD206+ and CD163+ macrophages but strongly inhibited M1-like cells. ON101 treatment also stimulated the expression of GCSF and CXCL3 genes in human adipocyte progenitor cells. Interestingly, treatment with recombinant GCSF protein enhanced both CD206+ and CD163+ M2 markers, whereas CXCL3 treatment only stimulated CD163+ M2 macrophages. Depletion of cutaneous M2 macrophages inhibited ON101-induced diabetic wound healing. Thus, ON101 directly suppressed M1 macrophages and facilitated the GCSF- and CXCL3-mediated transition from M1 to M2 macrophages, lowering inflammation and leading to faster diabetic wound healing.

INTRODUCTION

Functional changes in macrophage subtypes from a proinflammatory (M1-like) phenotype to an anti-inflammatory/proremodeling (M2-like) phenotype are critical for the transition from inflammation to regeneration in wound healing (Gordon, 2003; Graney et al., 2020). M2 macrophages are either converted from M1 macrophages or from newly arriving monocytes with mixed phenotypes, primarily M2a, M2b, M2c, and M2d subtypes, characterized by distinctive surface markers and functions (Graney et al., 2020). M2a and M2c subtypes, obtained from M2 macrophages activated by exposure to different stimulators, are functionally characterized as prohealing and proremodeling, respectively, and are distinguished by their corresponding expression of the surface markers CD206 and CD206/CD163 (Abdelaziz et al., 2020; Graney et al., 2020).

Diabetic foot ulcers are a type of chronic wound in which hyperglycemic conditions impede the healing process. In patients with diabetes with such wounds, there is a prolonged inflammation phase featuring sustained levels of M1-like macrophages and a shortage of M2-like macrophages (quantified as a higher M1/M2 ratio) (Ganesh and Ramkumar, 2020; Huang et al., 2019; Kryszczyn et al., 2018). In particular, sustained secretion of IL-6, TNF-α, and IL-1 family
Figure 1. ON101 promotes diabetic and HFD-induced wound healing. Kinetic analysis of skin excisional wound healing (a, b) in the NFD-C57BL/6 (NFD/B6) mice and HFD-induced obesity mouse model and (c–g) in db/db mice. (a, c) Wound recovery rate measured at indicated time points given as a percentage of the change in area from D 0. Black arrows indicate the initiation of drug application (D 0 in HFD-C57BL/6 mice, D 3 in db/db mice). *P < 0.05 and **P < 0.01 analyzed by two-way repeated-measures ANOVA; n = 6 in HFD group and n = 12 in db/db mice. H&E staining represents (e) epithelization (dotted line) and (g) granulation tissue in the indicated treatment groups; # indicates wound bed. Bar = 250 µm. D, day; HFD, high-fat diet; NFD, normal-fat diet.

C-W Lin et al. ON101 Accelerates Diabetic Wound Healing JID Innovations (2022), Volume 2
Figure 2. IHC staining and quantification of serial sections of biopsies from ON101- or placebo-treated wounds. (a–e) Images of IHC results for (a) anti-Ly-6C/6G, (b) anti-CD3, (c) anti-MOMA2, (d) anti-MMP9, and (e) anti-K14 within wound biopsies of db/db mice at the indicated time points. Quantified results for each antibody are shown on the right (except for anti-K14) and are expressed as means ± SEMs (n = 8 per group). Bar = 250 μm. D, day; IHC, immunohistochemistry; K14, keratin 14; MMP9, matrix metalloproteinase 9.
Figure 3. ON101 alters the population of iNOS⁺, CD163⁺, and CD206⁺ cells in diabetic wound surroundings. Immunohistochemical staining of wound biopsies from db/db mice for detecting (a) iNOS, (c) CD163, and (e) CD206. (b) Quantification of iNOS-positive stain in the whole field compared with that in the total tissue area; n = 8 selected fields per group. (d) Quantification of the CD163-positive stain in the dermis compared with total nuclear stain. n = 16 selected fields per group. (e) Quantification of the CD206-positive stain in the wound edge compared with the nuclear stain in the field. n = 8 selected fields per group. All values represent means ± SEM. Statistical analysis is based on the Student’s t-test. *P < 0.05, **P < 0.01, and ***P < 0.001.
members caused by prolonged maintenance of M1 macrophages within the wound area impedes M2 macrophage polarization and damages surrounding tissues (Seraphim et al., 2020).

Considerable effort has been devoted to understanding the pathology of diabetic foot ulcers and identifying the possible strategies for resolving ulcer chronicity and avoiding severe consequences such as amputation (Frykberg and Banks, 2015). Accordingly, approaches designed to rebalance the M1/M2 ratio by reducing the proportion of M1 macrophages or minimizing proinflammatory cues possess therapeutic potential for promoting diabetic wound healing (Hu et al., 2020; Nguyen et al., 2020; Perrault et al., 2018). In line with this treatment rationale, bioengineered materials designed to enhance M2 macrophage infiltration or topically administered exosomes derived from M2 macrophages have been applied in patients (Gan et al., 2019; Ishida et al., 2019; Kim et al., 2019). Both approaches showed that enrichment of M2 macrophages and reversing imbalanced M1/M2 ratios accelerated diabetic wound healing.

ON101, a topical cream formulated using identified, defined fractions of Plectranthus amboinicus (PA-F4) and Centella asiatica (S1) in a proprietary ratio, has been reported to be capable of inhibiting NLRP3 inflammasome signaling and regulating macrophages (Huang et al., 2021; Leu et al., 2019). Having established the clinical efficacy of ON101 in promoting wound healing in randomized controlled studies (Huang et al., 2021; Leu et al., 2019), in this study, we further explore the details of the molecular mechanism by which ON101 regulates cell function and cellular networks to improve healing in diabetic wounds and gain insight into the macrophage phenotypes involved in diabetic foot ulcers.

RESULTS

ON101 promotes wound healing in two animal models of diabetic wound healing

We first confirmed ON101’s healing activity in two mouse models of type 2 diabetes: high-fat diet (HFD)-induced diabetes and genetically obese db/db mice. The wound recovery rate was slower in diabetic wounds than in normal wounds in both mouse models (Figure 1a and b for HFD and Figure 1c...
and dB for dB/db). For the HFD model, obesity-induced hyperglycemia and insulin resistance were established by feeding an HFD for 10 weeks. After wounding, mice from both diabetic models received either a placebo or ON101 cream topically. The wound recovery rate was significantly faster in the ON101-treated group than in the placebo group from day 6 to day 9 in the HFD mouse model (Figure 1a and b). Consistent with this, ON101 significantly promoted diabetic wound healing in dB/db mice compared with placebo from day 6 to day 12 after treatment (Huang et al., 2021) (Figure 1c and d). Histological examinations showed that more granulation tissue appeared on day 6, and faster epithelization was observed on day 9 in the ON101-treated group than in the placebo group (Figure 1e). Together, these results indicate that ON101 effectively accelerates wound healing in a diabetic milieu compared with placebo controls regardless of the pathological process by which diabetes is generated.

ON101 treatment alters the populations of macrophage subtypes and modulates gene expression profiles in diabetic wounds

Perturbed inflammatory cues are a major cause of impaired diabetic wound healing (Eming et al., 2014; MacLeod and Mansbridge, 2016). We first performed immunohistochemistry (IHC) to determine whether ON101 affected specific types of immune cells, including neutrophils (anti-Ly6G/6C), T cells (anti-CD3), and monocytes/macrophages (anti-MOMA2) (Figure 2a–c). The fibroblast marker matrix metalloproteinase 9 (Liu et al., 2009) and epithelial marker keratin 14 were also analyzed (Figure 2d and e). Quantifications revealed that neutrophils, fibroblasts, and T cells appeared in the first 3 days after wounding, and the expression of each marker decreased gradually during the healing process without a significant difference between ON101 and placebo groups (Figure 2a and b and d). On the other hand, total monocyte and macrophage counts increased from day 0 to day 6, with no significant differences in overall cell numbers stabilizing from day 6 to 12 (Figure 2c) between the two groups.

Among monocytes and macrophages, the inducible nitric oxide synthase (iNOS)-positive M1 subtype of macrophages decreased over time (Figure 3a and b and g). Specifically, the proportion of iNOS+ cells was reduced significantly around wound edges in the ON101-treated group on day 6 after wounding compared with that in the placebo group (Figure 3b). The increased number of CD163+ cells was observed on day 6 (Figure 3c and d and h), whereas the expression pattern of CD206+ cells was marked enriched after wounding on day 3 (Figure 3e and f), indicating earlier CD206+ enrichment than CD163+. In addition, the proportions of CD163+ and CD206+ cells were increased from day 6 in ON101-treated wounds compared with that in placebo groups (Figure 3d and f). The iNOS-MOMA2 or CD163-MOMA2 double-staining images showed that a decreased number of iNOS*MOMA+ double-positive staining in the ON101-treated group (Figure 3g) and a greater number of CD163*MOMA2+ could be observed in the ON101-treated group than in their corresponding placebo controls (Figure 3h). These results imply that ON101 treatment might modulate the proportion of M1 and M2 macrophages around the diabetic wound bed.

To quantify the dynamic changes in macrophage subtypes induced by ON101, macrophages isolated from wound-surrounding tissues of dB/db mice treated with either ON101 or placebo cream were analyzed by flow cytometry (Figure 4a). These analyses confirmed a gradual increase of total macrophages (F4/80+) around the wound but without significant differences between groups (Figure 4b). Notably, the proportion of CD163+ cells among all macrophages (CD163+ in F4/80+ cells) was significantly increased in the ON101-treated group compared with that in the placebo group (Figure 4c), indicating enhanced recruitment or increased numbers of M2c-like macrophages in the ON101-treated group. In addition, a lower non–M2 cells/M2 ratio was observed in the ON101-treated group on days 6 and 9 compared with that in the placebo group (Figure 4e), suggesting that ON101 may modulate the population of macrophage subtypes.

Next, we explored which genes might be affected by ON101 in diabetic wounds. A total of 86 wound healing–related genes were analyzed using a TaqMan Array Mouse Wound Healing Panel (Supplementary Table S1). Confirmed by qRT-PCR, several M1-associated genes, including Ifnγ, Il1a, Cxcl1, and Cxcl11, were downregulated, whereas the M2-associated gene Il4 was upregulated by ON101 treatment (Figure 4e and f). In addition, genes likely involved in lymphocyte recruitment and differentiation, such as Cxcl3 and Gcsf (Martin et al., 2021; Reyes et al., 2021), were also upregulated by ON101 treatment (Figure 4f). These results suggest that topical application of ON101 downregulates proinflammation-associated genes and upregulates M2-associated genes and other genes related to chemotaxis or cell differentiation in a diabetic wound.

ON101 directly suppresses M1 macrophage polarizing and restores CD206+ M2 macrophages by reversing M1-mediated M2 suppression

Poor glycemic control in patients with diabetes is often associated with chronic inflammation and elevated levels of proinflammatory cytokines (Chang and Yang, 2016). To test the M1 or M2 polarization under our conditions, the polarizable human monocyte cell line, THP-1, was used to polarize M1 or M2 macrophages under either normal-glucose or high-glucose (HG) conditions. The results show that the proportion of CD86 and CD80 (M1 markers) was higher among cells cultured in HG than in those cultured in normal-glucose conditions (Figure 5a and b). In contrast, the proportion of M2a/c markers (CD163+ and/or CD206+) after M2-polarizing conditions was lower in the HG medium than in the normal-glucose medium (Figure 5c and d).

After establishing this model and under the same experimental conditions, increasing concentrations of ON101 were cultured with the cells in the M1-polarizing condition, which led to a dose-dependent suppression in the proportion of CD86- and CD80-positive M1 macrophages (Figure 5e). This was not attributable to nonspecific cytotoxic effects (Figure 5g). Cd80 and Cd86 mRNA levels were also downregulated by ON101 (Figure 5f), suggesting that ON101 transcriptionally suppressed CD80 and CD86. In contrast, in
Figure 4. ON101 alters the dynamics of macrophage subtypes and the transcriptional expression profile of inflammation-associated cytokines in db/db mice. (a) Gating strategy for analyzing the macrophage subtypes in a diabetic wound. (b–d) FACS analysis of macrophage subtypes in diabetic wounds. n = 3 mice per group. (b) The number of F4/80-positive macrophages around wound beds in db/db mice after treatment with ON101 or placebo cream. A fixed number of cells (5 x 10⁶) was gated, and the F4/80-positive population was quantified after excluding dead cells using 7-AAD viability staining. (c) Quantification of the percentage of CD163+ cells among F4/80+ cells in each wound. (d) The ratio of cell numbers in the non-M2 population/M2+ population in each wound sample. (e, f) Relative expressions (normalized with GAPDH and compared with that on day 0) of (e) proinflammatory cytokine genes and (f) macrophage-associated
the in vitro M2 macrophage polarization model, ON101 did not affect the expression levels of CD163 or CD206, suggesting that ON101 does not directly affect M2 polarization (Figure 5h).

Ex vivo M1 polarization experiments isolated from human PBMCs further revealed that although basal amounts of CD14+/CD68+/CD86+ and CD14+/CD68+/CD80+ cells differed among six donors, the proportions of CD86+ and CD80+ levels appeared to be suppressed in a concentration-dependent manner by coadministering ON101 in HG medium (Figure 5i). Collectively, these findings show that ON101 directly inhibits M1 polarization by suppressing the expressions of CD80 and CD86.

To further explore whether ON101 alters M1 macrophage functions, we performed RNA sequencing (Figure 6a). A total of 121 genes changed after ON101 treatment (Supplementary Table S2). qRT-PCR validation showed that ON101 downregulated the M1-associated chemokines CXCL9, CXCL10, and CCL12, which function in T helper 1–mediated immune activation (Kuo et al., 2018) (Figure 6b). CCL2 and CCL3, cytokines involved in monocyte and/or macrophage recruitment and migration (Schaufelstatter et al., 2012; Zhuang et al., 2019), were significantly upregulated by ON101 treatment (Figure 6b). In addition, an examination of proinflammatory cytokines released from ex vivo–polarized M1 macrophages from six independent donors revealed that ON101 significantly suppressed the levels of IL-6, IL-1β, and TNF-α (Figure 6c). These findings show that ON101 treatment alleviates M1-associated inflammation and provides an environment that favors monocyte recruitment.

To determine whether M1-dominated proinflammatory cues impair the polarization of M2 macrophages, we collected conditioned medium (CM) from M1 polarization cultures (M1-associated CM [M1-CM]) and cotreated with M2-polarizing agents to see the extent of M2 polarization as shown in Figure 7a. Flow cytometry analyses showed that M1-CM dramatically suppressed the expression of both CD206 and CD163 during the M2-polarization process (Figure 7b). By contrast, ON101-treated M1-CM significantly rescued the levels of CD206-positive macrophages but not those of CD163 (Figure 7b). In addition, M1-CM increased CD80 and CD86 levels in M2 macrophages, whereas ON101-treated M1-CM downregulated both CD86 and CD80 expression (Figure 7c). These results reveal the repressive role of factors secreted from M1 macrophages to counter a microenvironment favoring M2 polarization. Thus, ON101 is likely to function in mitigating the M1-dominant milieu, changing it to an M2a-favorable environment.

ON101 enhances the activity of adipocyte progenitor cells to promote M1-to-M2 transition

Adipocyte progenitor cells (ADPCs), identified by their positive staining for Pref-1, are skin-resident mesenchymal stem cells with multiple regenerative potentials that mediate skin regeneration and diabetic wound repair (Gadelkarim et al., 2018; Sul, 2009). There was a noticeable increase in Pref-1–positive cells around the subcutaneous fat layer during the wound healing process in ON101-treated db/db mice compared with that in mice in the placebo group (Figure 8a), suggesting that ON101 might activate ADPCs.

Because ON101 treatment increased the expression of CD163+ cells during the healing process in mice with diabetes, we further evaluated whether ON101 promoted CD163+ M2 polarization through the activation of adipocyte stem cells. To test this, CM from cultures of ADPCs (ADPC-associated CM), with or without ON101 treatment, was applied to the M1 polarization assay (Figure 8b). ADPC-associated CM significantly suppressed CD80 and CD86 (Figure 8c) but only slightly increased the intensity of CD163 and CD206 (Figure 8d). Interestingly, the application of ON101-treated ADPC-associated CM significantly increased the intensities of both CD206 and CD163 markers (Figure 8d), suggesting that ADPCs play a significant role in ON101-induced promotion of the M1-to-M2 transition.

ON101 stimulates ADPC expression of GCSF and CXCL3, which are engaged in the M1-to-M2 transition

We next analyzed whether the genes altered by ON101 treatment in diabetic wounds could be produced by ADPCs during ON101 treatment and focused on candidate genes detected in wound tissues in ON101-treated db/db mice (Figure 4e and f). The qRT-PCR results showed that ON101 induced a concentration-dependent increased expression of GCSF and CXCL3 (Figure 5e) but not that of other genes in ADPCs (Figure 5f). To test whether GCSF and CXCL3 might be involved in the marker switch between M1 and M2 macrophages, recombinant GCSF and CXCL3 proteins, with or without their neutralizing antibodies, were coadministered with M1-polarizing cytokines. GCSF treatment caused a concentration-dependent increase in both CD163 and CD206 (Figure 8g), whereas CXCL3 treatment only induced CD163 expression (Figure 8h). Both GCSF- and CXCL3-mediated inductions of M2a/c markers were abolished by the corresponding neutralizing antibodies (Figure 8g and h). In addition, both GCSF and CXCL3 treatments activated signal transducer and activator of transcription 3 (STAT3) signaling pathways, whereas GCSF activated protein kinase B phosphorylation, which was again abolished by their corresponding antibodies (Figure 8i). These results reveal that ON101 can readily stimulate both GCSF and CXCL3 gene expression in ADPCs and that their expressions may subsequently promote the activation of CD206+ and/or CD163+ M2 subtypes in M1 macrophages.

M2 macrophages are critical for ON101-accelerated wound healing

Because ON101 exerts dual effects on M1/M2 ratios, downregulating M1 and promoting M2 macrophages, we first clarified whether M2 macrophages are critical for normal wound healing by a loss-of-function assay of M2 macrophages. Mannosylated clodronate (m-Clo) was applied to promote M1-to-M2 transition.
Figure 5. ON101 directly attenuates M1 markers under HG conditions. THP-1–derived (a, b) M1 and (c, d) M2 polarization models in culture medium containing NG (1,000 mg/l glucose) or HG (4,500 mg/l glucose), as determined by flow cytometry. (a) Representative histograms showing CD86 and CD80 intensity cultured under M1-polarizing conditions in NG or HG medium. (b) Summary data calculated from three independent experiments showing the MFIs for CD86 and CD80. (c) Representative histograms show CD163 and CD206 expression intensities under M2-polarizing conditions in NG or HG medium. (d) Summary data calculated from three independent experiments showing MFIs for CD163 and CD206. Data are shown as means ± SEM. Statistical analysis is based on the Student’s t-test. *P < 0.05, **P < 0.01, and ***P < 0.001. (e) MFI values and (f) fold changes in the gene expressions of CD86 and CD80 induced by ON101 in M1 macrophages (compared with 0 µg/ml of ON101). (g) Cell viability assays of THP-1–derived M0, M1, and M2 macrophages after ON101 treatment for 48 h. The results were standardized against the control group for each cell type. (h) MFIs of CD163 and CD206 after a 48-h treatment with an M2-polarizing cocktail and different concentrations of ON101 as indicated. Data are shown as means ± SEM of three independent experiments. (i) M1 macrophages derived from human PBMCs treated with ON101 for 96 h. Markers were analyzed by FACS (n = 6; paired Student’s t-test). Data are shown as means ± SEM; Student’s t-test. **P < 0.01 and ***P < 0.001. h, hour; HG, high glucose; iso, isotype control; MFI, mean fluorescence intensity; NG, normal glucose.

www.jidinnovations.org
pretreated with m-Clo or control liposomes (m-Encapsomes) for 7 days before wounding and then continued being treated for 6 days after wounding (Figure 9a). CD163⁺ M2 macrophages were efficiently depleted by m-Clo as determined by flow cytometry (Figure 9c). Compared with wound healing in the m-Encapsomes- treated group, wound healing was delayed in the m-Clo- treated group, suggestive of the vital role of M2 macrophages in normal wound healing (Figure 9b).

Further testing sought to evaluate whether ON101 treatment can promote diabetic wound healing by enriching the population of M2 macrophages. After the administration of m-Clo or m-Encapsomes, db/db mice were cotreated with ON101 or placebo cream after wounding (Figure 9d). ON101 treatment improved the wound healing rate compared with the placebo treatment; notably, this effect was abolished by reducing the proportion of M2 macrophages through m-Clo treatment (Figure 9e and f). Flow cytometry analysis of M2 macrophages further indicated that ON101-accelerated wound healing was accompanied by an increase in the population of CD163⁺ macrophages, an effect that was also effectively attenuated by m-Clo treatment (Figure 9g). IHC staining showed a thin dermis layer and a deficiency of CD163⁺ and CD206⁺ cells around the wound bed in the m-Clo-treated group (Figure 9h). In addition, the extracellular matrix remodeling protein, α-smooth muscle actin as well as Pref-1⁺ cells were markedly increased in the ON101-treated group, effects that were attenuated by M2 depletion (Figure 9h). On the basis of these results, we conclude that topical ON101 treatment promotes diabetic wound healing through dual pathways—attenuated M1-mediated proinflammatory cues and enhanced M2 macrophage populations—by enabling the M1-to-M2 transition through factors secreted by ADPCs. Thus, ON101 facilitates diabetic wound healing through an M2-macrophage-dependent pathway.

**DISCUSSION**

Dysregulation of macrophage subsets manifests as a defect in the M1-to-M2 transition and is recognized as the primary
factor that impedes diabetic wound healing (Boniakowski et al., 2017; Khanna et al., 2010). Research-based on reshaping the M1/M2 populations has failed to yield an effective solution (Ganesh and Ramkumar, 2020; Raziyeva et al., 2021). We identified ON101 as a drug that efficiently accelerates diabetic wound healing dually through attenuating M1 macrophage polarization and activating ADPCs, thereby creating an environment favoring the M1-to-M2 transition. ON101 simultaneously promotes this transition through multiple cellular pathways involving macrophages and ADPCs, altering their chemokine/cytokine profiles to promote polarization toward both prohealing M2a and proremodeling M2c macrophages. Such a mechanism has successfully improved diabetic wound healing clinically (Huang et al., 2021). These findings show proof of concept with respect to how medicinal plant ingredients can reshape macrophage subtypes that are critical in the coordinated process of healing complicated dermal wounds.

Figure 7. ON101 attenuates M1-mediated M2a marker expression. (a) Schematic depicting the experimental flowchart. MFI values for (b) CD163+ and CD206+ M2 macrophage populations and (c) CD80+ and CD86+ M1 macrophage markers under M2-polarization conditions were analyzed by flow cytometry 48 h after M2 polarization. Data are shown as means ± SEM of three experiments. Statistical analysis is based on the Student’s t-test. **P < 0.01 and ***P < 0.001. CM, conditioned medium; h, hour; MFI, mean fluorescence intensity.
Figure 8. ON101 promotes M1-to-M2 macrophage transition through ADPC-mediated production/secrections of GCSF and CXCL3. (a) Immunohistochemical detection of Pref-1 in wound biopsies from db/db mice and quantified results using an area quantification module with 16 selected fields per group. Bar = 100 μm. (b–d) Schematic of CM collected from ADPCs treated with ON101 for 24 h for the second round of M1 polarization. MFIs for (c) M1 markers and (d) M2 markers detected by FACS 48 h after M1 polarization. Expression of (e) GCSF and CXCL3 genes or other (f) indicated cytokines in human ADPCs after 24 h of ON101 treatment, determined by qRT–PCR. (g, h) Expression of CD163 and CD206 after coadministration of recombinant (g) GCSF or (h) CXCL3 protein and C-W Lin et al. ON101 Accelerates Diabetic Wound Healing
We showed in this study that CM from ON101-treated ADPCs induced the expression of CD206 and CD163, suggesting that ON101 might indirectly promote the generation of M2a/M2c macrophages by modulating the secretion of GCSF and CXCL3 from ADPCs. However, whether there are other factors secreted from ON101-challenged ADPCs needs to be further investigated. ON101 treatment may therefore be a more effective and convenient therapeutic for activating ADPCs in situ rather than transplating live ADPCs into wounds, which may be expensive and require considerable time to perform.

In summary, we found that ON101, a medicinal plant agent, exhibits a unique function in accelerating diabetic wound healing by attenuating chronic inflammation through the suppression of M1-macrophage polarization and activity. Then, ON101 generates a milieu favorable for the enrichment of M2 macrophages by stimulating the secretion of CXCL3 and GCSF from ADPCs. Our findings showed that ON101-mediated reorchestrating of the distribution of immune cells and their messengers increases diabetic wound healing abilities and may therefore be another therapeutic option for treating complicated diabetic foot ulcers.

**MATERIALS AND METHODS**

**Drug formulation**

ON101 cream and placebo cream were used in animal experiments. ON101 cream contains 1.25% of ON101 powder and was manufactured as previously described (Huang et al., 2021). Placebo cream is the cream-based vehicle control containing no ON101. For in vitro assays, ON101 powder was dissolved in DMSO (D12345, Thermo Fisher Scientific, Waltham, MA) at a stock concentration of 25 mg/ml.

**Animal models of diabetes**

All animal experiments were approved by the Institutional Animal Care and Use Committee of Oneness Biotech (Taipei, Taiwan). Mice were housed in standard conditions of 25 °C with a 12/12-hour light–dark cycle with ad libitum access to food and water. Male leptin receptor–deficient (db/db) mice aged 9 weeks (C57BLKS/J Iar–/+ Leprdb/Leprdb, Institute for Animal Reproduction, Ibaraki, Japan) with fasting blood glucose levels of 300–500 mg/dl and body weight >35 g were assigned randomly to groups for drug treatments. Two full-thickness wounds were created on the back of each mouse using a sterile 6-mm biopsy punch. The wounds were covered with silicone splints (inner/outer diameter of 10/14 mm) to anchor them and reduce skin contraction and then covered with a transparent occlusive dressing (Tegaderm, 3M, Saint Paul, MN). ON101 (1.25%) or a placebo cream were applied topically once daily from day 3 after wounding to the date the mice were sacrificed.

For HFD mouse model experiments, male C57BL/6 mice aged 6 weeks (BioLASC0, Taipei, Taiwan) were fed an HFD for rodents containing 60 kcal of fat for 10 weeks. An oral glucose tolerance test was used to evaluate whether an HFD-induced obesity model had been established, defined as a mean 60-minute oral glucose
Figure 9. M2 macrophages are required for normal as well as ON101-enhanced diabetic wound healing. (a–c) Subcutaneous injection of m-Clo or m-Enc during normal wound healing in C57BL/6 mice. (a) Schematic; n = 4 mice per group. (b) Wound recovery rate, calculated as the percentage change from the original size (day 0) and images of wounds at the indicated times. (c) Biopsies from the point of wounding were analyzed by flow cytometry to detect the proportions of M2 macrophages (F4/80<sup>+</sup>/CD163<sup>+</sup>) at indicated time point. Data represent the percentages of F4/80<sup>+</sup> or CD163<sup>+</sup> macrophages. (d–h) M2 macrophage dependence of ON101-promoted diabetic wound healing. m-Clo/m-Enc. (d) Schematic; n = 4 mice per group. (e) Wound recovery rate...
Images of wounds were acquired at the indicated time points, and wound areas in images were calculated using ImageJ software. The quantification procedure of all IHC slides was performed using Aperio AT2 (Leica, Wetzlar, Germany). The wound modules.

For M2 macrophage depletion experiments, C57BL/6N mice aged 8 weeks (BioLASCO) or db/db mice were fed standard chow and the HFD-induced obesity model was established, mice were randomly allocated to receive either mannosylated Clodrosome; WB, wound bed.

IHC staining and quantification

Full-thickness biopsy samples of skin tissue surrounding wound edges were fixed in 10% paraformaldehyde followed by embedding in paraffin wax and sectioned at 5 μm intervals. IHC was performed with a (with a routine antigen-retrieval procedure) using antibodies as listed in Table 1. Whole-tissue scans were analyzed with HALO software (Indica Labs, Albuquerque, NM) using Area Quantification, version 1.0; Cytonuclear, version 1.5; and Cytonuclear FL, version 1.4, modules.

The quantification procedure of all IHC slides was performed using HALO software (Indica Labs) after whole slide scanning was performed using Aperio AT2 (Leica, Wetzlar, Germany). The wound (percentage change from day 0) and images of wounds at the indicated times for each treatment group. (f) H&E staining represents re-epithelialization (dotted line) and the WB in the indicated treatment. (g) FACS analysis of the proportions of M2 macrophages (F4/80+/CD163-) in wound surroundings. * denotes wound bed. Bar = 250 μm. Data represent means ± SEM. P values in b and e were analyzed using two-way repeated-measures ANOVA, whereas in g was analyzed using Student’s t-test. *P < 0.05, **P < 0.01, and ***P < 0.001. A-SMA, α-smooth muscle actin; m-Enc, m-Encapsome; IHC, immunohistochemistry; iNOS, inducible nitric oxide synthase; NS, not significant. m-Clo, mannosylated Cloodosome; WB, wound bed.

Table 1. Antibody List

| Reactive Species | Application | Antibody List (Information) |
|------------------|-------------|-----------------------------|
| mouse            | IHC         | Against CD163 (ab182422, Abcam, Cambridge, United Kingdom), iNOS (E-AI-70051, Elabscience, Houston, TX), CD206 (ab6493, Abcam), cytoketatin 14 (ab181595, Abcam), Ly6G/6C (ab2557, Abcam), MMP9 (ab228402, Abcam), CD3 (ab16669, Abcam), MAMA2 (ab3451, Abcam), and αSMA (ab32575, Abcam). |
| mouse            | Flow        | APC-conjugated anti-mouse F4/80 (17-4801-82, eBioscience, San Diego, CA) and PE-conjugated anti-mouse CD163 (12-1631-82, eBioscience) and 7-AAD (A1310, Thermo Fisher Scientific, Waltham, MA). |
| human            | Western blot| p-STAT3 antibody (number 9131S), STAT3 antibody (number 9139S), p-Akt antibody (number 9272S), Cell Signaling Technology, Danvers, MA. Proteins were detected by enhanced chemiluminescence. |
| human            | Flow cytometry | PE CF594–conjugated anti-hC6D6 (BD Biosciences, Franklin Lakes, NJ), FITC-conjugated anti-hC6D6 (BioLegend, San Diego, CA), PE/Dazzle 594–conjugated anti-hC80 (BioLegend), Pacific Blue-conjugated anti-hCCR7 (BioLegend), PE-conjugated anti-hCD163 (BioLegend), BV605-conjugated anti-hCD206 (740417, BD Biosciences), and BV510-conjugated anti-FVS (564006, BD Biosciences). |

Table 2. Primer List

| Gene Name | Primer Sequence (5′ → 3′) |
|-----------|---------------------------|
| mIFNy     | F: CGCCGCAACGTACTGGAAGGACTA<br>R: GTTCGTAGTGCGCTGATGTTG |
| mIFNα     | F: ACGGCTGATGTTGAGTACCTT<br>R: AGGTGAAGTGTGTATCGAGT |
| mCXCL1    | F: GCTTAGAAAGTGTGGCCTCACGG<br>R: AACGTCCGGACATCCTT |
| mCXCL11   | F: AGGAGGTCACAGGCTATAGC<br>R: CGATCTGTCGGTATTGAG |
| mIL4      | F: AGATGGATATGTCGAAAAACACTG<br>R: AGATGACAAATTGCGACAGACA |
| mCSF3     | F: TGGCAGACATGAAAAACACTG<br>R: AGATGACAAATTGCGACAGACA |
| mCXCL3    | F: AGATCTCCACACGGCTTCTC<br>R: AACCCTGGTATGGTGTG |
| mGAPDH    | F: CGAATCTACACAGGACTCCTCC<br>R: TGGGATGTCTCAGGGAATCTT |
| hCD80     | F: CTCACTTCTCTGATTGTAAC<br>R: TCCCTTTGCCAGTAGCTGGA |
| hCD83     | F: TCCCTGACCTGCGCCATACAG<br>R: GCACCAAGACCCCATAC |
| hCD86     | F: CTTGAAATCTCGACTCGTCCGTA<br>R: GCCCAATAGTCTGGCTAGA |
| hCXCL9    | F: CAAACCAACCCACAGAGTGC<br>R: GCCGACACCTGCCTGAGAC |
| hCXCL10   | F: GAAGCTGAGCTCAGTACTGCA<br>R: TGTAGGGCTTGTAGTTGAC |
| hCL12     | F: TCAGCCTAGCCTACAGATGC<br>R: CTTAGTGCTCAGGTCCTG |
| hCL3      | F: TCAGCTTCAGAGGACACGG<br>R: CTGGAGTACTGACAGGCTT |
| hCXCL3    | F: AAGCCGTAATGAGTACGCC<br>R: GTGCTCCCCCTTGTCAGATC |
| hCXCL2    | F: AGTCCTGCGCCTGAGTAGT<br>R: GCCCTCCGCTGAGGAGCAG |
| hCSF3     | F: GTTCGTTGAGCACAATCCTC<br>R: GAAACCGCGTAGACACCCTC |
| hGAPDH    | F: ACATCGCCTACAGACACCATG<br>R: TGTAGGTAGGCGTAGAGG |

Abbreviations: F, forward; h, human; m, murine; R, reverse.

Table 1. Antibody List

| Reactive Species | Application | Antibody List (Information) |
|------------------|-------------|-----------------------------|
| mouse            | IHC         | Against CD163 (ab182422, Abcam, Cambridge, United Kingdom), iNOS (E-AI-70051, Elabscience, Houston, TX), CD206 (ab6493, Abcam), cytoketatin 14 (ab181595, Abcam), Ly6G/6C (ab2557, Abcam), MMP9 (ab228402, Abcam), CD3 (ab16669, Abcam), MAMA2 (ab3451, Abcam), and αSMA (ab32575, Abcam). |
| mouse            | Flow        | APC-conjugated anti-mouse F4/80 (17-4801-82, eBioscience, San Diego, CA) and PE-conjugated anti-mouse CD163 (12-1631-82, eBioscience) and 7-AAD (A1310, Thermo Fisher Scientific, Waltham, MA). |
| human            | Western blot| p-STAT3 antibody (number 9131S), STAT3 antibody (number 9139S), p-Akt antibody (number 9272S), Cell Signaling Technology, Danvers, MA. Proteins were detected by enhanced chemiluminescence. |
| human            | Flow cytometry | PE CF594–conjugated anti-hC6D6 (BD Biosciences, Franklin Lakes, NJ), FITC-conjugated anti-hC6D6 (BioLegend, San Diego, CA), PE/Dazzle 594–conjugated anti-hC80 (BioLegend), Pacific Blue-conjugated anti-hCCR7 (BioLegend), PE-conjugated anti-hCD163 (BioLegend), BV605-conjugated anti-hCD206 (740417, BD Biosciences), and BV510-conjugated anti-FVS (564006, BD Biosciences). |

Abbreviations: αSMA, α-smooth muscle actin; 7-AAD, 7-aminoactinomycin D; APC, allophycocyanin; Akt, protein kinase B; IHC, immunohistochemistry; iNOS, inducible nitric oxide synthase; MMP9, matrix metalloproteinase 9; p-Akt, phosphorylated protein kinase B; PE, phycoerythrin; p-STAT3, phosphorylated signal transducer and activator of transcription 3; STAT3, signal transducer and activator of transcription 3.

Table 2. Primer List

| Gene Name | Primer Sequence (5′ → 3′) |
|-----------|---------------------------|
| hGAPDH    | F: CGAATCTACACAGGACTCCTC<br>R: TGGGATGTCTCAGGGAATCTT |
| hCL12     | F: TCAGCCTAGCCTACAGATGC<br>R: CTTAGTGCTCAGGTCCTAG |
| hCL3      | F: TCAGCTTCAGAGGACACGG<br>R: CTGGAGTACTGACAGGCTT |
| hCXCL3    | F: AAGCCGTAATGAGTACGCC<br>R: GTGCTCCCCCTTGTCAGATC |
| hCXCL2    | F: AGTCCTGCGCCTGAGTAGT<br>R: GCCCTCCGCTGAGGAGCAG |
| hCSF3     | F: GTTCGTTGAGCACAATCCTC<br>R: GAAACCGCGTAGACACCCTC |
| hGAPDH    | F: ACATCGCCTACAGACACCATG<br>R: TGTAGGTAGGCGTAGAGG |

Abbreviations: F, forward; h, human; m, murine; R, reverse.
bed and surrounding tissue were first circled for quantification. For antigens that belong to membrane staining, such as CD163, CD206, CD3, and MOMA2, the multiplex module was used to calculate the relative percentage of positive cells. For antibodies against cytoplasmic proteins such as iNOS, matrix metalloproteinase 9, and Ly-6C/6G, an area quantification module was used to calculate the area of positive tissue/total area of the tissue of interest, expressed as a percentage. All of the quantifications were normalized with hematoxylin stain.

**qRT-PCR**

Skin tissue was first homogenized using a TissueLyser LT (Qiagen, Hilden, Germany) with homogenization beads. Total RNA from in vitro cultured cells and skin tissues was extracted at different time points using GENEzol TriRNA Pure kits (Geneaid Biotech, New Taipei City, Taiwan) and reverse transcribed into cDNA using First Strand cDNA Synthesis kits (Thermo Fisher Scientific). qRT-PCR analyses were performed on a QuantStudio 6 Flex Real-Time PCR System using SYBR Green qRT-PCR Master Mix (both from Thermo Fisher Scientific). qRT-PCR analyses were conducted by two-way ANOVA with Tukey’s post hoc test for multiple pairwise comparisons. Data are presented as means ± SEM, and differences with a P < 0.05 were considered statistically significant.

**Data availability statement**

No datasets were generated or analyzed during this study.

**ORCIDs**

Ching-Wen Lin: http://orcid.org/0000-0001-8984-1137
Chih-Chiang Chen: http://orcid.org/0000-0002-5723-6730
Wen-Yen Huang: http://orcid.org/0000-0001-9625-0802
Yen-Yu Chen: http://orcid.org/0000-0002-6716-3985
Shiou-Ting Chen: http://orcid.org/0000-0003-3010-3652
Hung-Wen Chou: http://orcid.org/0000-0003-3568-7845
Chien-Ming Hung: http://orcid.org/0000-0002-9218-7065
Wan-Jiun Chen: http://orcid.org/0000-0003-2339-3127
Chia-Sing Lu: http://orcid.org/0000-0001-5941-3406
Shi-Xin Nian: http://orcid.org/0000-0003-3423-5759
Shyi-Gen Chen: http://orcid.org/0000-0001-6796-8188
Hsuen-Wen Chang: http://orcid.org/0000-0002-0464-9822
Vincent H.S. Chang: http://orcid.org/0000-0002-9218-7065

**AUTHOR CONTRIBUTIONS**

Conceptualization: CWL, CCC, VHSC, MLK; Formal Analysis: CMH; Investigation: WYH, YYC, STC, HWC, SXN, CSL, SWC, LYL; Project Administration: CWL, WJC; Supervision: MLK, SCC, SGC; Writing – Original Draft Preparation: CWL; Writing - Review and Editing: WYH, MLK, SCC.
ACKNOWLEDGMENT
We acknowledge the support of Peggy Tseng (BOI HUI Biotech) for the characterization of the purity of adipocyte-derived stem cells. Adipocyte-derived stem cells and human PBMCs were conveyed through institutional review board number B202005048, Tri-Service General Hospital (Taipei, Taiwan). We thank DerMEDiit (www.dermedit) for language editing services that were paid for by Oneness Biotech. This work was funded by Oneness Biotech.

CONFLICT OF INTEREST
CWL, YYC, HWC, WJC, and SGC are employers in Oneness Biotech. WHY, STC, and CMH are employers in Microbio. MLK is an employer in Microbio Shanghai. The remaining authors state no conflict of interest.

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.xjid.2022.100138

REFERENCES
Abdelaziz MH, Abdelwahab SF, Wan J, Cai W, Huixuan W, Jianjun C, et al. Alternatively activated macrophages: a double-edged sword in allergic asthma. J Transl Med 2020;18:58.
Boniatkowski AE, Kimball AS, Jacobs BN, Kunkel SL, Gallagher KA. Macrophage-mediated inflammation in normal and diabetic wound healing. J Immunol 2017;199:17–24.
Chang SC, Yang WY. Hyperglycemia, tumorigenesis, and chronic inflammation. Crit Rev Oncol Hematol 2016;108:146–53.
Chu SY, Chou CH, Huang HD, Yen MH, Hong HC, Chao PH, et al. Mechanical stretch induces hair regeneration through the alternative activation of macrophages. Nat Commun 2019;10:1524.
Eming SA, Martin P, Tomic-Conic M. Wound repair and regeneration: mechanisms, signaling, and translation. Sci Transl Med 2014;6:265sr6.
Frykberg RG, Banks J. Challenges in the treatment of chronic wounds. Adv Wound Care (New Rochelle) 2015;4:560–82.
Gadelkarim M, Abushouk AI, Ghanem E, Hamaad AM, Saad AM, Abdel-Daim MM. Adipocyte-derived stem cells: effectiveness and advances in delivery in diabetic wound healing. Biomed Pharmacother 2018;107:625–33.
Gan J, Liu C, Li H, Wang S, Wang Z, Kang Z, et al. Accelerated wound healing in diabetes by reprogramming the macrophages with particle-induced clustering of the mannose receptors. Biomaterials 2019;219:119340.
Ganesh GV, Ramkumar KM. Macrophage mediation in normal and diabetic wound healing responses. Inflamm Res 2020;69:347–63.
Gordon S. Alternative activation of macrophages. Nat Rev Immunol 2003;3:23–35.
Goren I, Müller E, Schiefelbein D, Christen U, Pfeilschifter J, Mühl H, et al. Systemic anti-TNFalpha treatment restores diabetes-impaired skin repair in ob/ob mice by inactivation of macrophages. J Invest Dermatol 2007;127:2259–67.
Graney PL, Ben-Shaul S, Landau S, Bajpai A, Singh B, Eager J, et al. Macrophages of diverse phenotypes drive vascularization of engineered tissues. Sci Adv 2020;6:eaaq6391.
Hu J, Zhang L, Liechty C, Zghieb C, Hodges MM, Liechty KW, et al. Long noncoding RNA GASS regulates macrophage polarization and diabetic wound healing. J Invest Dermatol 2020;140:1629–38.
Huang SM, Wu CS, Chiu MH, Wu CH, Chang YT, Chen GS, et al. High glucose environment induces M1 macrophage polarization that impairs keratinocyte migration via TNF-α: an important mechanism to delay the diabetic wound healing. J Dermatol Sci 2019;96:159–67.
Huang YY, Lin CW, Cheng NC, Cazzell SM, Chen HH, Huang KF, et al. Effect of a novel macrophage-regulating drug on wound healing in patients with diabetic foot ulcers: A randomized clinical trial. JAMA Netw Open 2021;4:e2122607.
Ishida Y, Kuninaka Y, Nosaka M, Furuta M, Kimura A, Taruya A, et al. CCL2-mediated reversal of impaired skin wound healing in diabetic mice by normalization of neovascularization and collagen accumulation. J Invest Dermatol 2019;139:2517–27.e5.
Khanna S, Biswas S, Shang Y, Collard E, Azad A, Kauh C, et al. Macrophage dysfunction impairs resolution of inflammation in the wounds of diabetic mice. PLoS One 2010;5:e9539.
Kim H, Wang SY, Kwak G, Yang Y, Kwon IC, Kim SH. Exosome-guided phenotypic switch of M1 to M2 macrophages for cutaneous wound healing. Adv Sci (Weinheim) 2019;6:e1900513.
Kruger MJ, Conradie MM, Conradie M, van de Vyver M. ADSC-conditioned media elicit an ex vivo anti-inflammatory macrophage response. J Mol Endocrinol 2018;61:173–84.
Krzyszczyk P, Schloss R, Palmer A, Berthiaume F. The role of macrophages in acute and chronic wound healing and interventions to promote pro-wound healing phenotypes. Front Physiol 2018;9:419.
Kuo PT, Zeng Z, Salim N, Mattarollo S, Wells JW, Leggatt GR. The role of CXCR3 and its chemokine ligands in skin disease and cancer. Front Med (Lausanne) 2018;5:271.
Lachmann N, Ackermann M, Frenzel E, Liebhaber S, Brennig S, Happe C, et al. Large-scale hematopoietic differentiation of human induced pluripotent stem cells provides granulocytes or macrophages for cell replacement therapies. Stem Cell Rep 2015;4:292–96.
Leu WJ, Chen JC, Guh JH. Extract from Plectranthus amboinicus Inhibit Maturation and Release of interleukin 1beta Through Inhibition of NF-kB Nuclear Translocation and NLRP1 inflammasome Activation. Front Pharmacol 2019;10:573.
Liu Y, Min D, Bolton T, Nabé V, Twigg SM, Yue DK, et al. Increased matrix metalloproteinase-9 predicts poor wound healing in diabetic foot ulcers: response to Muller et al. Diabetes Care 2009;32:e137.
Lu CS, Lin CW, Chang YH, Chen HY, Chung WC, Lai WY, et al. Antimetabolite pemetrexed primes a favorable tumor microenvironment for immune checkpoint blockade therapy. J Immunother Cancer 2020;8:e001392.
MacLeod AS, Marsbridge JN. The innate immune system in acute and chronic wounds. Adv Wound Care (New Rochelle) 2016;5:65–78.
Martin KR, Wong HL, Witko-Sarsat V, Wicks IP. G-CSF – a double edge sword in neutrophil mediated immunity. Semin Immunol 2021;54:101516.
Mirza RE, Fang MM, Ennis WJ, Koh TJ. Blocking interleukin-1beta induces a healing-associated wound macrophage phenotype and improves healing in type 2 diabetes. Diabetes 2013;62:2379–87.
Mouritzen MV, Petkovic M, Qvisit K, Poulsen SS, Alarico S, Leal EC, et al. Improved diabetic wound healing by LFcinB is associated with relevant changes in the skin immune response and microbiota. Mol Ther Methods Clin Dev 2021;20:726–39.
Nakai K. Multiple roles of macrophage in skin. J Dermatol Sci 2021;104:2–10.
Nguyen VT, Farman N, Palacios-Ramirez R, Sheih M, Behar-Cohen F, Aractigi S, et al. Cutaneous wound healing in diabetic mice is improved by topical mineralocorticoid receptor blockade. J Invest Dermatol 2020;140:2223–34.e7.
Nolan GS, Smith OJ, Jell G, Mosaehebi A. Fat grafting and platelet-rich plasma in wound healing: a review of histology from animal studies. Adipocyte 2021;10:80–90.
Perrault DP, Bramos A, Xu X, Shi S, Wong AK. Local administration of interleukin-1 receptor antagonist improves diabetic wound healing. Ann Plast Surg 2018;80:S317–21.
Raziyeva K, Kim Y, Zharkinbekov Z, Kassymbek K, Jimi S, Saparov A. Chemokine CCL18 causes maturation of cutaneous monocytes to macrophages in the M2 spectrum. Immunology 2012;135:287–98.
Reyes N, Figueroa S, Tiwari R, Geliebter J. CXCL3 signaling in the tumor microenvironment. Adv Exp Med Biol 2021;1302:15–24.
Schaufstatter IU, Zhao M, Khalidoyanidi SK, Discipio RG. The chemokine CCL18 causes maturation of cutaneous monocytes to macrophages in the M2 spectrum. Immunology 2012;135:287–98.
Sul HS. Minireview: Pref-1: role in adipogenesis and mesenchymal cell fate. Mol Endocrinol 2009;23:1717–25.
Zarif JC, Hernandez JR, Verdone JE, Campbell SP, Drake CG, Pienta KJ. A phased strategy to differentiated human CD14+ monocytes into classically
and alternatively activated macrophages and dendritic cells. Bio-
Techniques 2016;61:33–41.
Zhao C, Medeiros TX, Sové RJ, Annex BH, Popel AS. A data-driven computa-
tional model enables integrative and mechanistic characterization of
dynamic macrophage polarization. iScience 2021;24:102112.
Zhao H, Shang Q, Pan Z, Bai Y, Li Z, Zhang H, et al. Exosomes from adipose-
derived stem cells attenuate adipose inflammation and obesity through
polarizing M2 macrophages and beiging in white adipose tissue. Diabetes
2018;67:235–47.
Zhuang Z, Yoshizawa-Smith S, Glowacki A, Maltos K, Pacheco C,
Shehabeldin M, et al. Induction of M2 macrophages prevents bone loss in
murine periodontitis models. J Dent Res 2019;98:200–8.
This work is licensed under a Creative Commons
Attribution-NonCommercial-NoDerivatives 4.0
International License. To view a copy of this license, visit
http://creativecommons.org/licenses/by-nc-nd/4.0/