Research Article

SCISET
www.sciset.com

Research Chronicle in Health Sciences

2026
Vol. 12 No. 1: 104

Quality of Care Assessment in Contemporary Healthcare Organizations

Stephen Brigido[1]*, Nicole Protzman[2], Chila Ferdinand[3], Nancy Lisa[2,3], Biadge Kefale[3]

[1]Research and Technology, Lonza, Walkersville, MD, USA
[2]Process Development, Lonza, Walkersville, MD, USA
[3]Research and Technology, Lonza, Walkersville, MD, USA

Corresponding author:
Stephen Brigido, Research and Technology, Lonza, Walkersville, MD, USA. E-mail: St.brigido@gmail.com

Received: May 11, 2026; **Accepted:** May 28, 2026; **Published:** June 10, 2026

Citation: Brigido S, et al. Quality of Care Assessment in Contemporary Healthcare Organizations. Research Chronicle in Health Sciences, 2026, Vol. 12 No. 1: 104

Abstract

Effective wound healing using biomaterials depends on the presence of extracellular matrix (ECM) components that support and guide the body's natural regenerative processes. Macrophages, key immune cells derived from monocytes, play a pivotal role in both the initiation and resolution phases of tissue repair. During early wound healing, macrophages drive the inflammatory response, whereas in later stages, they contribute to tissue remodeling and resolution. Chronic wounds often result from dysregulated macrophage activation, which impairs normal healing. This study aimed to evaluate the influence of a decellularized, dehydrated human amniotic membrane (DDHAM) on monocyte-to-macrophage differentiation and activation in vitro. Human monocytes were isolated from peripheral blood of healthy donors and cultured on standard tissue culture plates (CB), collagen type I-coated plates (COL), and DDHAM-coated plates. Proinflammatory (M1) macrophage differentiation was induced using granulocyte-macrophage colony-stimulating factor (GM-CSF) followed by activation with a proinflammatory stimulus comprising lipopolysaccharide (LPS) and interferon-gamma (IFN-ɣ). The results demonstrated that DDHAM significantly enhanced monocyte differentiation compared to CB and COL controls, as evidenced by increased cell size, viability, expression of macrophage-specific genes, and secretion of soluble factors. Notably, macrophages differentiated on DDHAM and activated with inflammatory signals exhibited a marked reduction in expression of several LPS-inducible NF-κB target genes, with IL12β, encoding the IL12p40 subunit, showing the most pronounced downregulation ($p < 0.001$). Mechanistic studies revealed that DDHAM-mediated effects on differentiation were dependent on β2 integrins. Collectively, these findings provide the first evidence that DDHAM can modulate macrophage behavior by promoting polarization toward an M2 phenotype, which is associated with tissue regeneration, vascular remodeling, and resolution of inflammation, supporting its potential as a scaffold to enhance wound repair.

KEYWORDS: Biomaterials; Decellularization; Human amniotic membrane; DDHAM; Extracellular matrix; Macrophages; Monocytes; Differentiation; Polarization; Scaffold; Wound healing.

INTRODUCTION

Monocytes are large, phagocytic white blood cells that serve as essential components of the innate immune system [1]. They function as the body's first line of defense against infection and injury and play a pivotal role in tissue repair and remodeling [2]. During wound healing, monocytes are among the earliest cells recruited to sites of injury, where they contribute to all overlapping phases of tissue repair: inflammation, proliferation, and maturation [3]. Upon stimulation by damage signals, monocytes exit the bone marrow, circulate in the bloodstream, and differentiate into macrophages once they enter the tissue microenvironment.

Macrophages are highly plastic immune cells capable of adopting a range of functional phenotypes depending on environmental cues [4]. They are critical regulators of tissue regeneration, orchestrating both inflammatory responses and subsequent repair processes [4,5]. During wound healing, macrophages dynamically shift their phenotype in response to temporal and spatial signals within the wound microenvironment [6,7].

Traditionally, macrophages are categorized into classically activated M1 and alternatively activated M2 phenotypes [8]. M1 macrophages dominate early in the healing process, promoting inflammation to combat pathogens and clear debris [9]. As healing progresses, macrophages transition toward the M2 phenotype, which supports tissue repair, remodeling, and resolution of inflammation [9-11]. A higher M2-to-M1 ratio has been linked with more constructive tissue remodeling outcomes [12-15]. While the M1/M2 classification aids in conceptual understanding, it oversimplifies the complexity of macrophage biology. In reality, macrophage polarization exists on a spectrum, encompassing specialized subsets such as M2a, M2b, M2c, and M2d, each activated by distinct stimuli [16-18]. For example, M1 macrophages are induced by interferon-gamma (IFN-γ), lipopolysaccharide (LPS), tumor necrosis factor (TNF), or granulocyte-macrophage colony-stimulating factor (GM-CSF) [16]. M2a macrophages respond to interleukin-4 (IL-4) and interleukin-13 (IL-13); M2b is activated by immune complexes, Toll-like receptor (TLR) agonists, and Fc receptor signaling; M2c is triggered by IL-10, transforming growth factor-beta (TGF-β), and glucocorticoids; and M2d activation occurs via TLR and adenosine A2A receptor signaling [16,19]. Once polarized, macrophages display distinct cytokine profiles, gene expression patterns, and surface marker signatures [14]. For instance, M1 macrophages express pro-inflammatory genes such as TNF, IL1β, IL6, and IL8, whereas M2 macrophages are associated with markers including CD206, CCL22, and CCL18 [14,20].

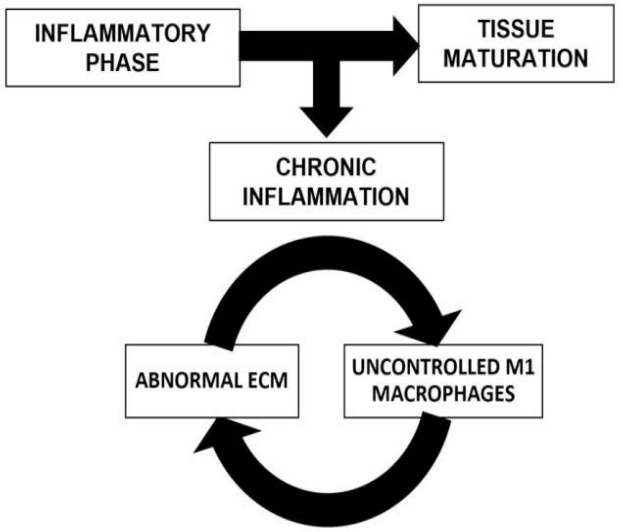

Figure 1: Abnormal ECM and uncontrolled M1 macrophage cycle.

In wound healing, macrophages ensure proper progression through the inflammatory, proliferative, and maturation phases. While initial inflammation is critical, prolonged M1 activation impedes healing, leading to chronic wounds [21-23]. Chronic, non-healing wounds often exhibit a persistent pro-inflammatory macrophage phenotype, where macrophages fail to transition into reparative M2 states [23-25]. Furthermore, in chronic wounds, the extracellular matrix (ECM)—normally essential for regulating macrophage behavior—is often degraded due to excessive protease activity by M1 macrophages [26]. This creates a feedback loop where an abnormal ECM perpetuates uncontrolled M1 activation, further impairing tissue repair (Figure 1).

Application of decellularized ECM scaffolds represents a promising strategy to restore a regenerative environment. Functional ECM scaffolds actively guide macrophage polarization, support recruitment of endogenous cells, stimulate angiogenesis, and dampen excessive inflammation, thereby facilitating tissue remodeling [15,27-29]. However, the tissue source and processing methods significantly influence the scaffold's capacity to modulate macrophage behavior [30].

BIOVANCE® (Celularity Inc., Florham Park, NJ) is a decellularized, dehydrated human amniotic membrane (DDHAM) allograft. The decellularization process removes cells, cell debris, and DNA while preserving the native ECM, producing a scaffold largely free of cellular material. The resulting DDHAM retains key ECM components, including collagen, fibronectin, laminin, glycosaminoglycans, and elastin [31], without extraneous growth factors or cytokines that could trigger an unpredictable immune response [31]. DDHAM provides a biologically active ECM scaffold that supports cell attachment, proliferation, and tissue restoration while minimizing inflammation and scarring [32].

The present study aimed to investigate the effects of DDHAM on macrophage differentiation and activation from human monocytes in vitro. We hypothesize that DDHAM will favorably modulate macrophage behavior, promoting M2 polarization to support tissue repair, resolution of inflammation, and regenerative healing.

MATERIALS AND METHODS

Decellularized Dehydrated Human Amniotic Membrane (DDHAM)

BIOVANCE® (Celularity Inc., Florham Park, NJ) is a commercially available decellularized, dehydrated human amniotic membrane allograft used for advanced wound management across a wide range of indications. It is regulated by the U.S. Food and Drug Administration as a human tissue-based product under Section 361 of the Public Health Service Act.

Human Subject Protections

As this study utilized commercially available DDHAM and involved only in vitro experiments with cells from anonymized healthy donors, no direct human subject participation occurred. Therefore, institutional review board approval was not required.

Monocyte Isolation

Peripheral blood monocytes were obtained from healthy donor LeukoPaks (New Jersey Blood Services, Scotch Plains, NJ; a division of New York Blood Center, New York, NY) (N = 2). Blood samples were diluted 1:2 with sterile phosphate-buffered saline (PBS) and carefully layered over Ficoll-Paque Plus (GE Healthcare, Chicago, IL). After density gradient centrifugation, peripheral blood mononuclear cells (PBMCs) were collected. Monocytes were then isolated from PBMCs using CD14+ MicroBeads via positive selection, following the manufacturer's instructions (Miltenyi Biotec, Bergisch Gladbach, Germany).

Co-Culture of Monocytes with DDHAM and Controls

Monocytes were seeded at a density of 0.5 × 10^6 cells/mL in 24-well plates (Corning) containing complete RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS; Gibco®, Thermo Fisher Scientific, Waltham, MA). For DDHAM co-culture, 2 × 3 cm pieces of the membrane were bisected and placed at the bottom of each well using sterile forceps, stabilized with sterile Teflon inserts. The DDHAM was pre-equilibrated in RPMI medium for 1–2 hours, and medium was removed prior to adding monocytes. Control conditions included CellBind plates (CB) and collagen type I-coated plates (COL), with Teflon inserts similarly used [33].

Monocyte Differentiation and M1 Polarization

To induce differentiation, monocytes were cultured with 100 ng/mL recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF). For classical M1 polarization, monocytes were incubated with 100 ng/mL GM-CSF for 3 days, after which medium was replaced with RPMI containing 10 ng/mL lipopolysaccharide (LPS) and 50 ng/mL interferon-gamma (IFN-γ). Cell lysates for gene expression and culture supernatants for cytokine profiling were collected at 4 and 24 hours post-stimulation.

β2 Integrin Blocking Experiments

To evaluate the role of β2 integrins, monocytes were pre-incubated in Falcon tubes (Corning, Glendale, AZ) with either an isotype control antibody or a β2-specific blocking antibody (azide-free, ultra-low endotoxin, clone TS1/18; BioLegend®, San Diego, CA) at concentrations of 10–25 μg/mL for 30–60 minutes at room temperature prior to culture with DDHAM or control surfaces.

Flow Cytometry Analysis

Cells were detached using Accutase™ Cell Detachment Solution (Innovative Cell Technologies, San Diego, CA) and stained with fluorescent antibodies from BioLegend® (San Diego, CA) and BD Biosciences (San Jose, CA). Data were acquired on a BD LSRFortessa™ Cell Analyzer (BD Biosciences) to assess cell surface marker expression [34].

Multiplex Cytokine Profiling

Culture supernatants were analyzed for cytokine, chemokine, and growth factor secretion using magnetic bead-based multiplex kits (Millipore Sigma, Burlington, MA), according to manufacturer instructions. Targets included IL-6, IL-8, TNF-α, IL-1β, MCP-1, GRO, VEGF, and FGF-2.

RNA Isolation and Gene Expression

Cells were lysed in 350 μL of Buffer RLT (Qiagen, Hilden, Germany) for 10 minutes, and RNA was isolated using RNeasy Mini Kits (Qiagen). Complementary DNA (cDNA) was synthesized using SuperScript® III reverse transcriptase (Invitrogen, Thermo Fisher Scientific, Waltham, MA). Quantitative reverse transcription PCR (RT-qPCR) was performed using primers and reagents from SABiosciences (Frederick, MD) to evaluate M1- and M2-associated gene expression.

Monocyte Viability Assessment

Viability of monocytes cultured on CB, COL, CB+GM-CSF, or DDHAM was measured on day 4 using the CyQuant™ Cell Proliferation Assay (Thermo Fisher Scientific, Waltham, MA). Data are expressed as percent viability relative to input cell numbers (N = 4).

Cytokine, Chemokine, and Growth Factor Analysis of Activated Macrophages

To assess M1 activation, supernatants and lysates were collected at 24 and 72 hours following stimulation with LPS and IFN-γ. Multiplex profiling evaluated inducible cytokine and chemokine secretion (IL-12p40, IL-12p70, IL-1α, IL-1β, TNF-α, RANTES, GRO, MCP-1, IL-8) and growth factor expression. Gene expression analyses of 24- and 72-hour samples included M1 markers (TNF, IL1β, IL6, IL8) and M2 markers (CD206, CCL22, CCL18).

β2 Integrin Functional Analysis

The impact of β2 integrin blockade on monocyte differentiation and M1 polarization was assessed by pre-incubating monocytes with β2 antibodies and co-culturing with DDHAM or control surfaces. Cytokine and chemokine secretion was measured via multiplex analysis, while CD11b expression was evaluated by flow cytometry. RT-qPCR was performed to quantify IL1β, IL6, and TNIP3 gene expression under β2 inhibition.

Statistical Analysis

All experiments were conducted in triplicate or greater, with gene expression experiments repeated twice. Data were analyzed

using GraphPad Prism® (Version 4, San Diego, CA). Parametric, unpaired t-tests were used to compare experimental groups, with a significance threshold set at $p < 0.05$. Data are reported as pooled results from multiple independent experiments.

RESULTS

Isolation and Characterization of Human Monocytes

Flow cytometry was employed to assess the impact of DDHAM on monocyte differentiation and macrophage maturation (Figure 2). Monocytes were cultured on DDHAM, CellBind plates (CB), collagen type I-coated plates (COL), and CB with GM-CSF. Type I collagen was included as a control to mimic the three-dimensional extracellular matrix (ECM) structure present in vivo. After 3–4 days of culture, adherent cells were collected for flow cytometric analysis [35].

On day 4, monocytes cultured on DDHAM displayed significantly larger cell size and higher granularity compared with CB, indicative of differentiation toward macrophage-like cells. Similarly, viability assays revealed that monocytes on DDHAM exhibited greater survival relative to CB ($p < 0.001$, $N = 4$). Analysis of macrophage-specific surface markers (CD11b, CD16, CD206, HLA-DR) demonstrated that DDHAM enhanced expression of all markers except HLA-DR compared with CB and COL controls. Collectively, these findings suggest that DDHAM promotes monocyte differentiation, viability, and macrophage-specific marker expression, similar to GM-CSF-driven differentiation.

DDHAM Effects on Cytokine, Chemokine, and Growth Factor Secretion

The influence of DDHAM on factors implicated in wound healing was examined by multiplex analysis of 24-hour culture

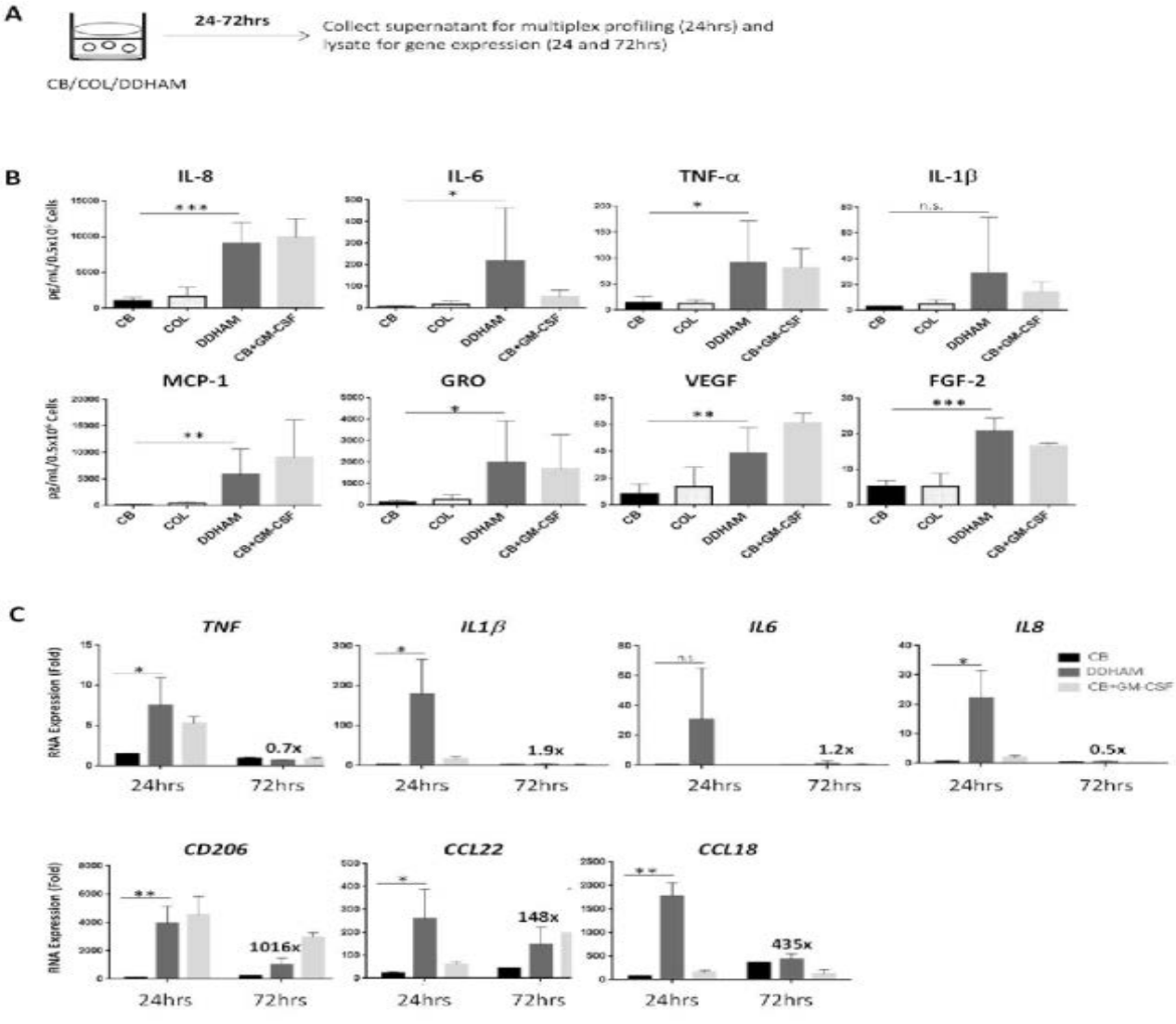

Figure 2: Monocytes cultured on DDHAM displayed changes in cytokine and M1/ M2 gene expression profiles.

supernatants (Figure 3). Cytokines (IL-6, IL-8, TNF-α, IL-1β), chemokines (MCP-1, GRO), and growth factors (VEGF, FGF-2) were measured in monocytes cultured on DDHAM versus CB. DDHAM significantly enhanced secretion of all factors compared with CB ($p < 0.05$), except IL-1β ($p = 0.1$) [36].

Temporal gene expression analysis was conducted at 24 and 72 hours to assess M1 and M2 polarization. DDHAM transiently increased the expression of M1-associated genes (TNF, IL1β, IL8; $p < 0.05$) at 24 hours, while IL6 expression did not differ significantly ($p = 0.2$). By day 3, M1 gene expression returned to baseline levels. In contrast, M2-associated genes (CD206, CCL22, CCL18) were not only upregulated at 24 hours ($p < 0.05$) but also maintained elevated expression through day 3, suggesting a more sustained M2 polarization. These findings indicate that DDHAM supports a transient pro-inflammatory M1 response while promoting long-term M2-associated regenerative signaling, mimicking GM-CSF effects more closely than CB or COL.

Impact of DDHAM on Activated M1 Macrophages

To investigate whether DDHAM modulates inflammatory macrophage responses, monocytes were differentiated on DDHAM or CB and then stimulated with LPS and IFN-γ to mimic chronic wound conditions (Figure 4). M1 macrophages derived from DDHAM-cultured monocytes displayed significant suppression in secretion of pro-inflammatory cytokines, including IL-12p40 ($p < 0.001$), IL-12p70 ($p < 0.01$), IL-1α ($p < 0.05$), IL-1β ($p < 0.01$), TNF-α ($p < 0.01$), and RANTES ($p < 0.05$), compared with CB. In contrast, secretion of GRO, MCP-1, and IL-8 was either unaffected or significantly increased (GRO: $p < 0.01$; MCP-1: $p < 0.05$). At the gene expression level, IL12β (encoding IL-12p40) was significantly downregulated in DDHAM-derived M1 macrophages ($p < 0.05$). Overall, these results demonstrate that DDHAM suppresses key NF-κB target inflammatory cytokines while selectively preserving chemokine secretion important for tissue repair [37].

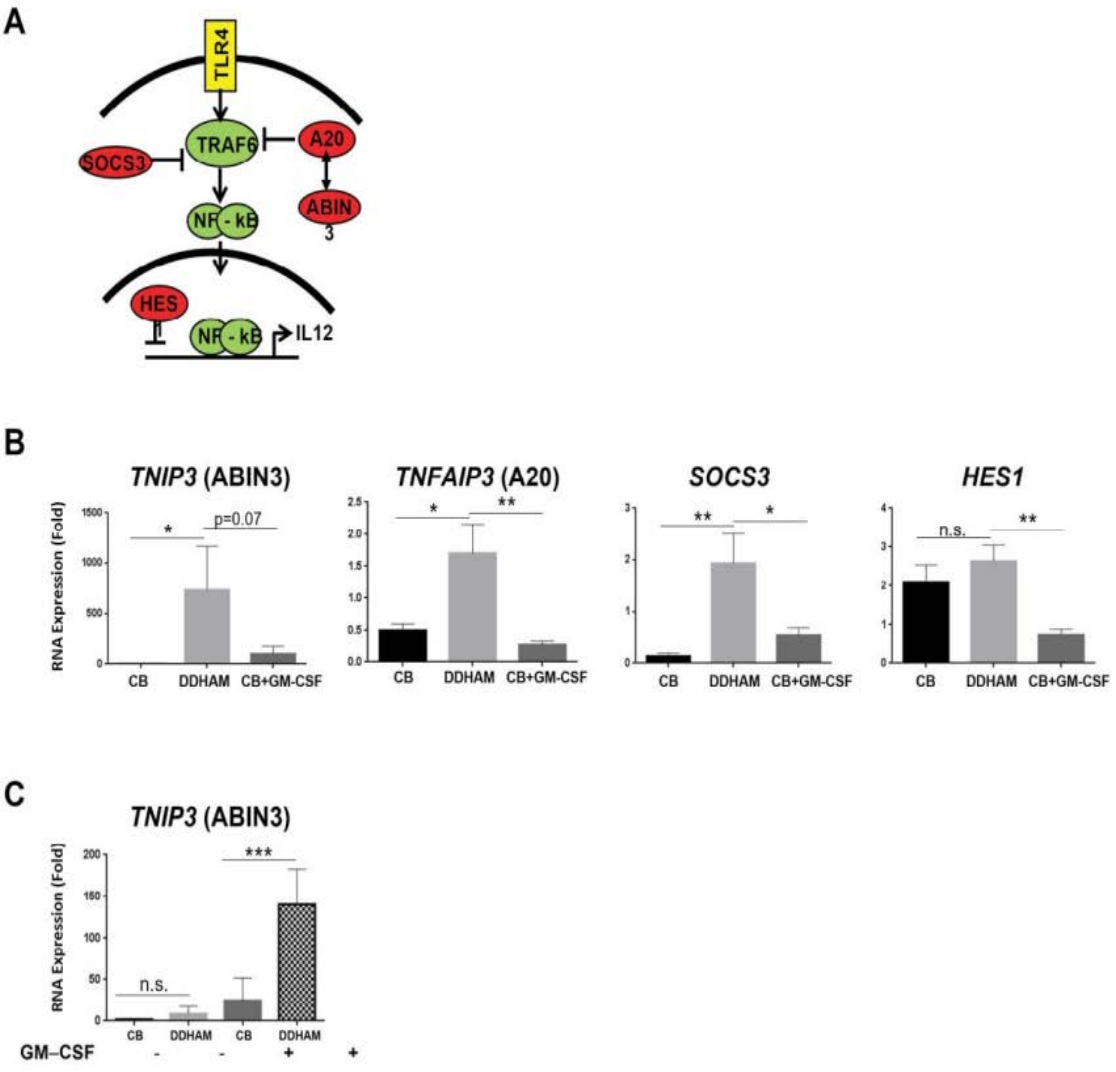

Figure 2: DDHAM induces significantly higher expression cytokine inhibitors and sustains greater expression of the NF-κB inhibitor ABIN3 when used together with GMCSF.

DDHAM Induces Expression of NF-κB Inhibitory Proteins

Gene expression analysis of monocytes cultured on DDHAM revealed upregulation of known inhibitors of cytokine expression. At 24 hours, DDHAM significantly increased TNIP3 (ABIN3; $p < 0.05$), TNFAIP3 (A20; $p < 0.05$), SOCS3 ($p < 0.05$), and HES1 ($p < 0.01$ versus CB+GM-CSF). By 72 hours, TNIP3 (ABIN3) expression remained elevated when DDHAM was combined with GM-CSF ($p < 0.001$). These findings suggest that DDHAM promotes early and sustained expression of negative regulators of NF-κB, contributing to controlled inflammatory responses.

Role of β2 Integrins in Monocyte Differentiation and M1 Activation

To examine the mechanistic role of β2 integrins, monocytes were pre-treated with a β2-blocking antibody prior to co-culture with DDHAM. Blocking β2 integrins impaired DDHAM-mediated upregulation of CD11b ($p < 0.05$) and reduced gene expression of IL1β ($p < 0.05$) and TNIP3 (ABIN3; $p < 0.01$), demonstrating that DDHAM's effects on monocyte differentiation and cytokine modulation are integrin-dependent.

In M1 macrophages, β2 integrin blockade reversed DDHAM-induced suppression of IL-12p40 ($p < 0.05$), IL-12p70 ($p < 0.01$), TNF-α ($p < 0.001$), RANTES ($p < 0.01$), and IL-1α, while secretion of GRO remained unaffected ($p > 0.05$). These results indicate that β2 integrins are critical mediators for DDHAM-driven regulation of pro-inflammatory cytokine expression but not for chemokines that are not suppressed by DDHAM [38,39].

Summary

Overall, DDHAM enhances monocyte survival, promotes macrophage differentiation, transiently induces M1-associated factors, and sustains M2-associated gene expression. It selectively suppresses pro-inflammatory cytokine secretion in activated M1 macrophages, upregulates NF-κB inhibitors, and exerts its effects in a β2 integrin-dependent manner, highlighting its potential to modulate the inflammatory response and support regenerative wound healing.

DISCUSSION

Chronic wounds are characterized by a prolonged inflammatory state, which prevents tissue repair and impairs wound closure [40, 41]. Therapeutic strategies that can correct the cellular and molecular imbalances contributing to persistent inflammation are critical to restoring normal wound healing. Macrophages, with their dynamic phenotypic shifts throughout the wound healing process, serve as a key target for such interventions [18, 42]. This study investigated how DDHAM influences monocyte differentiation and macrophage activation in vitro.

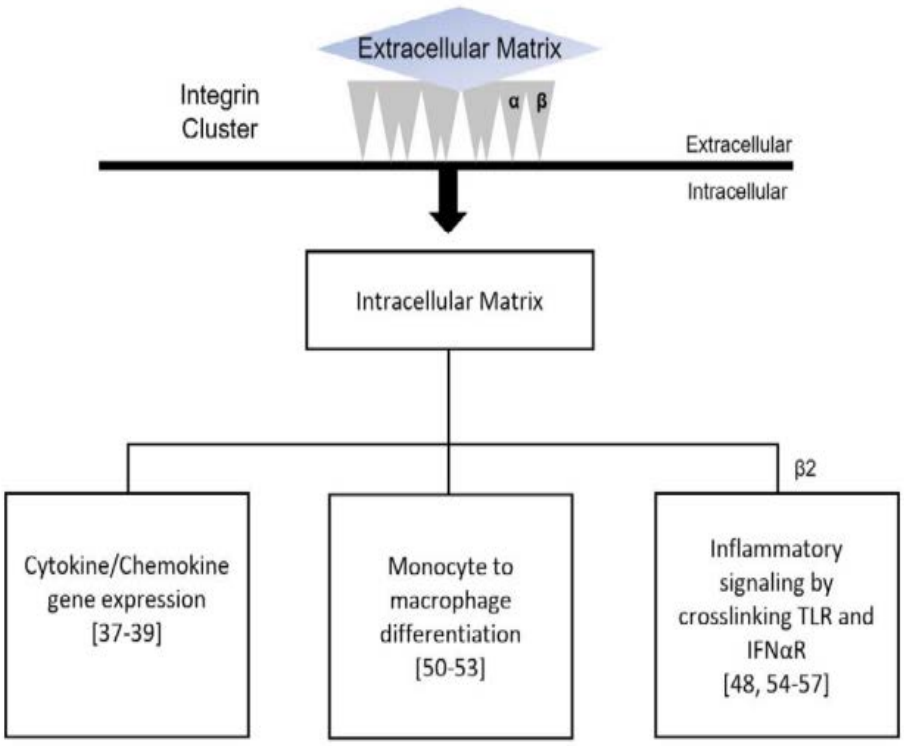

Figure 3: Beta 2 integrins are required for the effects of DDHAM on monocyte differentiation and proinflammatory cytokine regulation.

The findings demonstrate that DDHAM, a decellularized extracellular matrix (ECM) scaffold, enhances monocyte differentiation, as evidenced by increases in cell size, granularity, viability, and macrophage-specific gene expression. Furthermore, DDHAM modulates the secretion of cytokines, chemokines, and growth factors that are essential for wound healing. Specifically, monocytes cultured on DDHAM exhibited elevated levels of IL-6, IL-8, TNF-α, MCP-1, GRO, VEGF, and FGF-2, whereas IL-1β levels remained unchanged. The upregulation of VEGF and FGF-2 suggests that DDHAM may facilitate angiogenesis and epithelialization, processes critical for effective tissue repair [43–46]. The limited change in IL-1β aligns with observations that acute wound fluids contain relatively low amounts of this cytokine [47]. Overall, these results indicate that DDHAM supports the release of molecular mediators necessary for proper wound healing.

In addition, DDHAM promotes differentiation of monocytes into macrophages with a dual pattern of activation. Culturing on DDHAM led to a transient increase in both M1-associated genes (TNF, IL1β, IL8, IL6) and cytokine signaling inhibitors (A20, ABIN3, SOCS3, HES1), comparable to or exceeding the effects of GM-CSF. Notably, DDHAM induced a sustained upregulation of M2-associated genes (CD206, CCL22, CCL18), promoting a regenerative, anti-inflammatory phenotype. This temporal pattern—initial pro-inflammatory M1 activation followed by sustained M2 polarization—mirrors the natural progression observed in acute wound healing and suggests that DDHAM can support a balanced immune response conducive to tissue repair.

When examining M1 macrophages differentiated with GM-CSF and activated with LPS + IFN-γ, DDHAM significantly suppressed pro-inflammatory cytokines, including IL-12, IL-1α, IL-1β, TNF-α, and RANTES. These effects were not observed with monocytes cultured on collagen type I. Moreover, DDHAM enhanced the expression of cytokine inhibitors and sustained elevated ABIN3 levels in the presence of GM-CSF, supporting the notion that DDHAM restricts excessive M1 activation. Notably, IFN-γ did not override DDHAM's anti-inflammatory modulation, consistent with prior reports of NF-κB inhibitory regulation [36, 48].

Integrin-mediated interactions play a pivotal role in monocyte and macrophage behavior. β1 and β2 integrins are abundantly expressed on monocytes and regulate differentiation, cytokine production, and inflammatory signaling through TLRs and interferon receptors [37–39, 48–57]. Blocking β2 integrins in DDHAM-monocyte co-cultures significantly impaired monocyte differentiation, cytokine secretion, and gene expression of IL1β and TNIP3 (ABIN3). These findings indicate that DDHAM's effects on both differentiation and inflammatory regulation are β2 integrin-dependent, likely mediated through negative feedback loops and induction of NF-κB inhibitors [48].

The immunomodulatory properties of human placental cells are well-documented [58]. Studies have shown that human amniotic mesenchymal tissue cells (hAMTCs) and their conditioned media can promote the M1-to-M2 switch in macrophages, enhancing anti-inflammatory profiles and accelerating wound closure in diabetic mouse models [59]. The present study demonstrates that DDHAM, an acellular product derived from human amniotic tissue, similarly promotes transient M1 activation followed by sustained M2 polarization and limits pro-inflammatory cytokine expression. These parallels suggest that both cellular and acellular amniotic-derived products can guide macrophages toward a pro-regenerative phenotype, although further in vivo studies are necessary to confirm DDHAM's clinical efficacy.

It is important to note that this study focused on IL12β as a marker of M1 differentiation, and additional M1 cytokines should be examined in future investigations for a more comprehensive understanding of macrophage activation. Additionally, while the in vitro model provides insight into DDHAM's effects on monocytes, it cannot fully replicate the complex cellular and biochemical interactions present in vivo. Therefore, further research, including preclinical and clinical studies, is required to determine whether DDHAM can effectively modulate macrophage responses and promote tissue repair in chronic wound settings.

CONCLUSION

This study demonstrates that DDHAM significantly enhances the differentiation of human monocytes compared with standard tissue culture plastic or collagen type I-coated surfaces. Monocytes cultured on DDHAM exhibited increased cell size, improved viability, elevated expression of macrophage-associated genes, and higher secretion of soluble factors. When exposed to pro-inflammatory stimuli (LPS and IFN-γ), macrophages differentiated on DDHAM showed reduced expression of several NF-κB target genes, with IL12β (encoding the IL-12p40 subunit of IL-12/23) being the most strongly suppressed. The modulatory effects of DDHAM on monocyte differentiation were shown to be dependent on β2 integrins. Collectively, these findings suggest that DDHAM can effectively regulate macrophage behavior, promoting a shift toward a regenerative phenotype that supports tissue repair through dampening excessive inflammation and enhancing angiogenic signaling.

REFERENCES

1. Badylak, S. F., Taylor, D., & Uygun, K. (2011). Whole-organ tissue engineering: Decellularization and recellularization of three-dimensional matrix scaffolds. Annual Review of Biomedical Engineering, 13, 27–53.
2. Brown, B. N., Valentin, J. E., Stewart-Akers, A. M., et al. (2009). Macrophage phenotype and remodeling outcomes in response to biologic scaffolds with and without a cellular component. Biomaterials, 30(8), 1482–1491.
3. Crapo, P. M., Gilbert, T. W., & Badylak, S. F. (2011). An overview of tissue and whole-organ decellularization processes. Biomaterials, 32(12), 3233–3243.

4. Badylak, S. F., Freytes, D. O., & Gilbert, T. W. (2009). Extracellular matrix as a biological scaffold material: Structure and function. Acta Biomaterialia, 5(1), 1–13.
5. Spiller, K. L., Anfang, R., Spiller, K. J., et al. (2014). The role of macrophage phenotype in vascularization of tissue engineering scaffolds. Biomaterials, 35(15), 4477–4488.
6. Sadtler, K., Estrellas, K., Allen, B. W., et al. (2016). Developing a pro-regenerative biomaterial scaffold microenvironment requires T helper 2 cells. Science, 352(6283), 366–370.
7. Xue, J., Sharma, V., Hsieh, M., et al. (2014). Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. Immunity, 40(2), 274–288.
8. Daley, J. M., Brancato, S. K., Thomay, A. A., et al. (2010). The phenotype of murine wound macrophages. Journal of Leukocyte Biology, 87(1), 59–67.
9. Wynn, T. A., Chawla, A., & Pollard, J. W. (2013). Macrophage biology in development, homeostasis and disease. Nature, 496(7446), 445–455.
10. Mosser, D. M., & Edwards, J. P. (2008). Exploring the full spectrum of macrophage activation. Nature Reviews Immunology, 8(12), 958–969.
11. Mantovani, A., Biswas, S. K., Galdiero, M. R., et al. (2013). Macrophage plasticity and polarization in tissue repair and remodeling. Journal of Pathology, 229(2), 176–185.
12. Chen, F. M., & Liu, X. (2016). Advancing biomaterials of human origin for tissue engineering. Progress in Polymer Science, 53, 86–168.
13. Magatti, M., De Munari, S., Vertua, E., et al. (2008). Human amnion mesenchymal cells inhibit the response of naive and memory lymphocytes. Stem Cells, 26(3), 638–649.
14. Toda, A., Okabe, M., Yoshida, T., et al. (2007). The potential of amniotic membrane/amnion-derived cells for regeneration of various tissues. Journal of Pharmacological Sciences, 105(3), 215–228.
15. Koob, T. J., Lim, J. J., Massee, M., et al. (2013). Properties of dehydrated human amnion/chorion composite grafts: Implications for wound repair and soft tissue regeneration. Journal of Biomedical Materials Research Part B, 101(1), 178–188.
16. Niknejad, H., Peirovi, H., Jorjani, M., et al. (2008). Properties of the amniotic membrane for potential use in tissue engineering. European Cells and Materials, 15, 88–99.
17. Alviano, F., Fossati, V., Marchionni, C., et al. (2007). Term amniotic membrane is a source of stem cells with neural differentiation potential. Differentiation, 75(6), 451–463.
18. Tseng, S. C. G., Li, D. Q., & Ma, X. (1997). Suppression of T-lymphocyte proliferation by human amniotic membrane stromal cells. Investigative Ophthalmology & Visual Science, 38(3), 706–713.
19. Hao, Y., Ma, D., Hwang, D., et al. (2000). Identification of antiangiogenic and antiinflammatory proteins in human amniotic membrane. Cornea, 19(3), 348–352.
20. He, H., Liu, L., & Zhou, Y. (2012). Decellularized extracellular matrix scaffolds promote macrophage polarization toward M2 phenotype in vitro. Tissue Engineering Part A, 18(9-10), 1017–1025.
21. Brown, B. N., Valentin, J. E., Stewart-Akers, A. M., et al. (2010). Macrophage phenotype and remodeling outcomes in response to biologic scaffolds. Biomaterials, 31(23), 6393–6401.
22. Badylak, S. F., & Gilbert, T. W. (2008). Immune response to biologic scaffold materials. Seminars in Immunology, 20(2), 109–116.
23. Spiller, K. L., & Koh, T. J. (2017). Macrophage-based therapeutic strategies in regenerative medicine. Advanced Drug Delivery Reviews, 122, 74–83.
24. Mantovani, A., Sica, A., Sozzani, S., et al. (2004). The chemokine system in diverse forms of macrophage activation and polarization. Trends in Immunology, 25(12), 677–686.
25. Martinez, F. O., Sica, A., Mantovani, A., et al. (2008). Macrophage activation and polarization. Frontiers in Bioscience, 13, 453–461.
26. Daley, J. M., Brancato, S. K., Thomay, A. A., et al. (2010). The phenotype of murine wound macrophages. Journal of Leukocyte Biology, 87(1), 59–67.
27. Goren, I., Allmann, N., Yogev, N., et al. (2009). A transgenic mouse model of inducible macrophage depletion. American Journal of Pathology, 175(1), 132–147.
28. Mantovani, A., & Locati, M. (2009). Orchestration of macrophage polarization. Trends in Immunology, 30(12), 1–7.
29. Benoit, M., Desnues, B., & Mege, J. L. (2008). Macrophage polarization in bacterial infections. Journal of Immunology, 181(6), 3733–3739.
30. Kim, J. H., & Kim, H. W. (2013). Macrophage polarization and biomaterials. International Journal of Molecular Sciences, 14(1), 102–114.
31. Spiller, K. L., Koh, T. J. (2017). Macrophage-based therapeutic strategies in regenerative medicine. Advanced Drug Delivery Reviews, 122, 74–83.
32. Chen, F. M., & Liu, X. (2016). Advancing biomaterials of human origin for tissue engineering. Progress in Polymer Science, 53, 86–168.
33. Hodde, J., Hiles, M., & Badylak, S. F. (2001). The influence of biologic scaffolds on wound healing in a canine model. Journal of Surgical Research, 99(2), 322–327.
34. Sivaraj, K. K., & Adams, R. H. (2016). Blood vessel formation and function in skeletal development. Development, 143(15), 2706–2715.
35. Brown, B. N., Badylak, S. F. (2013). Expanded applications of extracellular matrix scaffolds for regenerative medicine. Expert Review of Medical Devices, 10(1), 55–66.
36. MacNeil, S. (2007). Progress and opportunities for tissue-engineered skin. Nature, 445(7130), 874–880.
37. Wang, H., & Li, Y. (2012). The role of M1/M2 macrophages in wound healing. Journal of Tissue Engineering and Regenerative Medicine, 6(5), 420–430.

38. Siqueira, R. C., & Saldanha, R. (2011). Biologic dressings: A review. Burns, 37(4), 493–501.

39. Hiraoka, M., Sakaguchi, M., & Yamamoto, T. (2006). Effects of amniotic membrane on cutaneous wound healing in a rat model. Burns, 32(8), 930–936.

40. Eming, S. A., Krieg, T., & Davidson, J. M. (2007). Inflammation in wound repair: Molecular and cellular mechanisms. Journal of Investigative Dermatology, 127(3), 514–525.

41. Martin, P. (1997). Wound healing—aiming for perfect skin regeneration. Science, 276(5309), 75–81.

42. Singer, A. J., & Clark, R. A. (1999). Cutaneous wound healing. New England Journal of Medicine, 341, 738–746.

43. Barrientos, S., Stojadinovic, O., Golinko, M. S., et al. (2008). Growth factors and cytokines in wound healing. Wound Repair and Regeneration, 16(5), 585–601.

44. Diegelmann, R. F., & Evans, M. C. (2004). Wound healing: An overview of acute, fibrotic and delayed healing. Frontiers in Bioscience, 9, 283–289.

45. Clark, R. A. (1996). Wound repair: Overview and general considerations. Journal of Investigative Dermatology, 106(5), 1013–1016.

46. MacLeod, A. R., & Turner, R. M. (2008). Cytokine interactions and wound healing. Cytokine, 44(2), 123–129.

47. Gill, S. E., & Parks, W. C. (2008). Metalloproteinases and their inhibitors: Regulators of wound healing. International Journal of Biochemistry & Cell Biology, 40(6-7), 1334–1347.

48. Gordon, S., & Martinez, F. O. (2010). Alternative activation of macrophages: Mechanism and functions. Immunity, 32(5), 593–604.

49. Arnaout, M. A., Goodman, S. L., & Xiong, J. P. (2007). Structure and function of integrins. Immunity, 26(1), 17–28.

50. Lim, J. J., Koob, T. J., & Massee, M. (2013). Amniotic membrane in wound healing. Journal of Tissue Viability, 22(2), 32–38.

51. Rezaei, M., & Baradaran, B. (2011). Immunomodulatory properties of amniotic membrane in regenerative medicine. Journal of Cellular Physiology, 226(9), 2334–2341.

52. Sart, S., Tsai, A. C., Li, Y., et al. (2014). Three-dimensional microenvironment for stem cell therapy in tissue regeneration. Biotechnology Advances, 32(5), 770–784.

53. Gurtner, G. C., Werner, S., Barrandon, Y., et al. (2008). Wound repair and regeneration. Nature, 453, 314–321.

54. Smith, L. R., Cho, S., & Discher, D. E. (2012). Stem cell differentiation is regulated by extracellular matrix mechanics. Journal of Cellular Biology, 196(4), 641–651.

55. Choi, J. S., & Yoo, J. Y. (2010). Role of macrophages in skin wound healing. Yonsei Medical Journal, 51(6), 835–844.

56. Broughton, G., Janis, J. E., & Attinger, C. E. (2006). The basic science of wound healing. Plastic and Reconstructive Surgery, 117(7 Suppl), 12S–34S.

57. Galiano, R. D., Michaels, J. t., Dobryansky, M., et al. (2004). Quantitative and reproducible murine model of excisional wound healing. Wound Repair and Regeneration, 12(4), 485–492.