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Discordance in Cytokine Expression -IL-1 β and IL-17 in Variety of Disease Including Hepatocellular Carcinoma (HepG2) Cells in Different Culture Conditions

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ABSTRACT

The comprehensive study has been designed to carry the immunomodulatory, in the young females (age group-22-28 years) from the blood in the presence post biotic compound designated Z-compound. These healthy female (n= 39) rich in stem-cells also known as Men SCs. Present study compromise five experimental group, and each group consist of (n=9) individuals - 1) morphological analysis of PBMC-MenSCs in co-cultures by fluorescence microscopy, 2) ELISA-based cytokine profiling of IL-1 β and IL-17 using monoclonal antibodies, 3) MTT cell viability in hepatocellular carcinoma cells, and 4) isolation and functional characterization of MenSCs-derived exosomes with and without Z-compound post biotic treatment. The findings demonstrates that MenSCs in combination with Z-compound significantly suppress the pro-inflammatory cytokine IL-1 β (from 0.448 to 0.339 O. D units) and modulate IL-17 levels across three experimental days. Microscopically, PBMC morphology showing notable transformation upon co-culture with MenSCs derived exosomes, with cell diameter increasing from 167 μ m to 192.17 μ m, consistent with monocyte-to-macrophage (M2 phenotype) differentiation. Exosomes isolated from Z-compound-treated MenSCs exhibited inhibitory properties against cancer cells, while exosomes from non-post-biotic-treated MenSCs demonstrates anti-aging effects due to promoting growth factors and hair follicle stimulation. Preliminary findings become relevant and support the therapeutic adaptability of MenSCs exosome in cancer for regenerative medicine.

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Introduction

Mesenchymal Stem Cells (MSCs) have emerged as powerful therapeutic agents to regulate potent immunomodulatory, regenerative, and paracrine signaling capabilities. Among various MSCs sources, Menstrual Blood-derived Stem Cells (MenSCs) represent a particularly advantageous population: they are non-invasively obtained, ethically accessible, highly proliferative, and possess robust immunosuppressive potential [1]. MenSCs secrete a diversified bioactive molecule including cytokines, growth factors, and extracellular vesicles (exosomes) that enhance their therapeutic effects in a paracrine fashion [2]. Post biotics are defined as preparations of inactivated microorganisms and their metabolic by-products that confer health benefits upon the host without requiring viable microbial cells.

The Z-compound investigated in this study is a post biotic formulation whose secondary metabolites including Short-Chain Fatty Acids (SCFAs), bacteriocins, exopolysaccharides, and bioactive peptides exert antimicrobial, anti-inflammatory and immunomodulatory effects. Synergistic manner with MenSCs and Z-compound was hypothesized to enhance the anti-inflammatory and regenerative activity of the stem cells [3].

Peripheral Blood Mononuclear Cells (PBMCs), comprising lymphocytes and monocytes, serve as the primary immune modulator of cytokine secretion profile - specifically IL-1 β (a key pro-inflammatory mediator) and IL-17 (a Th17-lineage cytokine implicated in autoimmunity during chronic inflammation). The present study employs PBMC co-culture as in- vitro models to characterize how MenSCs and Z-compound, individually or in synergistic manner regulate the inflammatory cytokine milieu [4]. Exosomes derived from MenSCs serve as nano scale vehicles for the intercellular transfer of proteins, nucleic acids, and lipids. Present study further evaluates, how the post biotic conditioning of MenSCs alters the composition and biological activity of their secreted exosomes, revealing distinct functional profiles and anticancer properties, exosomes are derived from Z-compound-treated MenSCs, and anti-aging with growth-promoting properties, derived from untreated MenSCs [5].

Materials and Methods

Cell Lines and Primary Cells Culture

Human Peripheral Blood Mononuclear Cells (PBMCs), isolated from healthy donor (n= 69) peripheral blood by density gradient centrifugation. Females belong to young age group (age 22-28 years) during menstrual cycle, blood is rich in Stem cells known as MenSCs) were harvested non-invasively from menstrual effluent; characterized by fibroblast-like morphology and immunosuppressive phenotype activity were observed in

Hepatocellular Carcinoma Cell Line (HepG2) used as a cancer model in MTT proliferation and cytotoxicity assays with maintaining cell density $\times 10^6$.

The Z-compound is a post-biotic preparation containing inactivated microbial metabolites including short-chain fatty acids, organic acids, bacteriocins, exopolysaccharides, and bioactive peptides. It was applied to MenSCs cultures using RPMI 1640 media with 5% foetal calf serum and antibiotics under sterile conditions at 37 degrees inside CO2 incubator. The culture conditions are same for all the experimental groups to evaluate its immunomodulatory interactions and to condition MenSCs-derived exosomes for anticancer applications. Exosomes were isolated from conditioned media of two MenSCs populations: (1) MenSCs treated with Z-compound post biotic, and (2) untreated MenSCs. Isolation was performed by sequential ultracentrifugation. The resulting exosome fractions were characterized and applied to cancer cell lines (HepG2) and regenerative models respectively.

PBMC Co-Culture Experimental Design

PBMCs were co-cultured under five conditions for morphological and cytokine analysis- Control: PBMCs alone (baseline). PBS: PBMCs + Phosphate-Buffered Saline (vehicle control), Z-Compound: PBMCs + Z-compound post biotic, MenSCs: PBMCs co-cultured with MenSCs, MenSCs + Z-Compound: PBMCs co-cultured with MenSCs in presence of Z-compound.

Quantitative Analysis of Cytokines

Enzyme-Linked Immunosorbent Assay (ELISA) was performed using same culture conditions during collection of supernatants over three consecutive days to quantify cytokines IL-1 β and IL-17 levels (reported as O.D units at 450 nm). Each condition was performed in triplicate to reduce standard error.

MTT Assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was conducted for HepG2 cells seeded at $\times 10^6$ cells/well in 96-well plates, treated with Beet root, Beta Xanthine, and Rx compounds for 24,48,72 hours (three days) and absorbance was measured at 570 nm in triplicate for antitumor activity.

Fluorescence Microscopy

Phase-contrast fluorescence microscopy at 10 $\times$ magnification was used to assess PBMC morphological features and size across all co-culture conditions. ImageJ-based measurements were taken from representative fields to document cell diameter changes.

Results

Morphological Analysis of PBMC Cultures and Control

The baseline PBMC control displayed numerous small, relatively round cellular structures dispersed across the field with occasional clustering. These morphological features were characterized by compact, spherical cells that represents the 'normal' resting phenotype of PBMCs in the absence of any treatment stimulus. Cell density is distributed and served as the reference point for all experimental groups. PBMCs treated with the morphology largely comparable to the untreated control, confirming that the vehicle itself did not introduce significant artefactual changes to cell appearance or density. This validation is critical for attributing observed differences in other groups to the specific biological treatments.

Z-Compound Treatment

Exposure to the Z-compound post biotic resulted in observable changes in PBMC morphology, when compared to control and PBS groups. Changes in cell shape, clustering pattern, and density were assessed to characterize the direct immunological impact of the post biotic compound on circulating immune cells. This group established the baseline interaction between Z-compound and PBMCs in the absence of MenSCs. The combined condition (Z-compound + MenSCs) demonstrated the most robust PBMC viability, with cells appearing morphologically healthy. The presence of both MenSCs and Z-compound appeared to confer a protective effect on PBMCs, suggesting that MenSCs may mitigate or modulate the immunological impact of cytokines during the post biotic compound.

MenSCs Co-Culture

PBMCs co-cultured with MenSCs displayed a distinctive two-population morphology: smaller, rounder PBMCs overlaying a background of larger, flatter, spindle-shaped MenSCs forming a characteristic monolayer. The PBMCs maintained a healthy morphological profile in this setting, consistent with the known immunosuppressive and supportive microenvironment created by MenSCs due to rich in stem cells. This observation aligns with MenSCs-mediated immune tolerance. A critical and novel finding of this study concerns the morphological transformation of PBMCs upon exposure to MenSCs derived exosomes. Quantitative image analysis revealed a measurable increase in PBMCs diameter from approximately 167 μ m (baseline condition) to 192.17 μ m in exosome-treated cells. This expansion of cell size is consistent with the differentiation of monocytes into macrophages, specifically the anti-inflammatory M2 macrophage phenotype is characterized by increased cytoplasmic volume, and enhanced phagocytic capacity during .M2 stage of cell cycle has significant therapeutic implications: and repair and suppress pro-inflammatory cascades, suggesting that MenSCs-derived exosomes may serve as potent drivers of beneficial macrophage reprogramming, with applications in inflammatory diseases, wound healing, and post-transplant immunomodulation.

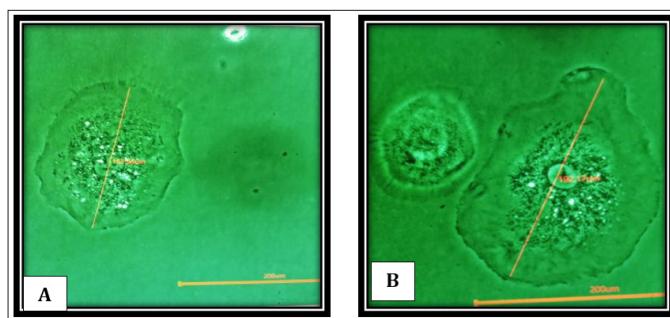


Figure 1 A&B: Fluorescence microscopy images (10 $\times$) showing PBMC size transformation. Left (1A): Baseline PBMC with ~ 167 μ m diameter; Right (1B): PBMC after MenSCs derived exosome treatment showing enlarged cell size at 192.17 μ m, suggesting monocyte-to-M2 macrophage differentiation. Scale bar = 200 μ m.

Cytokines Analysis of IL-1 β and IL-17

ELISA-based quantification of IL-1 β (a canonical pro-inflammatory cytokine) and IL-17 (a Th17-lineage cytokine linked to autoimmunity) was performed over three consecutive days. The following quantitative data represent as mean $\pm$ O.D values in triplicate measurements.

Table 1: Day 1 Mean ± Values for IL-1β & IL-17 in Different Treatment Groups

Treatment Group	IL-1β OD (Mean)	IL-17 OD (Mean)
Control (PBMC)	0.565	1.314
PBS	0.561	0.896
MenSCs	0.628	0.744
Z-Compound	0.952	1.051
MenSCs + Z-Compound	0.535	0.794

The findings of day 1, Z-compound alone showed the highest IL-1β (0.952), suggesting an initial pro-inflammatory response. However, when combined with MenSCs, IL-1β was reduced to 0.535, below even the PBS baseline of 0.561. The IL-17 was showing highest value in the PBMC control (1.314), with Z-compound and MenSCs +Z-compound both showing elevated, but moderated IL-17 levels. MenSCs alone demonstrated the lowest IL-17 (0.744), consistent with their inherent immune regulatory capacity.

Table 2: Day 2 Mean ± O.D Values (Triplicates) for IL-1β and IL-17

Group	IL-1β Mean (OD)	IL-17 Mean (OD)
PBMC Control (Initial)	0.591	0.785
2-Compound	0.556	1.072
MenSCs (Cells Alone)	0.448	1.305
MenSCs + Z-Compound	0.339	0.975
PBMC Control	0.628	0.672

The findings of the day2 showing the highest IL-1β (~0.866), suggesting vehicle-induced inflammatory baseline stimulation. The 2-compound reduced IL-1β (~0.556), indicating an anti-inflammatory modulatory effect. MenSCs alone further suppressed IL-1β (~0.448), demonstrating their inherent anti-inflammatory potency. MenSCs + Z-compound combination achieved the lowest IL-1β (0.339), suggesting synergistic suppression. Basal PBMC controls (~0.59–0.628) confirmed stable reference cytokine levels. Similarly, IL-17 Trends MenSCs alone induced the highest IL-17 (~1.305), which may reflect immune regulatory Th17 axis engagement.2-Compound alone elevated IL-17 (~1.072) above baseline, indicating Th17 stimulatory activity. Combining MenSCs

with 2-compound reduced IL-17 (~0.975) relative to MenSCs. PBMC act as controls showing lower values for IL-17 (~0.668) and confirming the vehicle’s neutrality for this cytokine.

Table 3: Day 3 ELISA Mean ± O.D Values for IL-1β and IL-17

Group	IL-1β Mean (OD)	IL-17 Mean (OD)
PBMC Control (Initial)	0.637	1.463
PBMC	0.788	0.795
PBMC + Z-Compound	0.637	0.668
PBMC + MenSCs	0.650	0.542
PBMC + MenSCs + Z-Compound	0.500	1.034

The findings of day 3 showing IL-1β: MenSCs + Z-compound combination maintained the lowest IL-1β (0.500) across all groups, confirming persistent anti-inflammatory synergy.IL-1β activity. By Day 3, MenSCs alone achieved the lowest IL-17 (0.542), suggesting progressive immunomodulatory maturation over time. IL-17: The MenSCs + Z-compound group showed elevated IL-17 on Day 3 (1.034), that may indicate a compensatory Th17 response upon dual treatment.

Interestingly, to conclude present findings after three days coulter for quantitative analysis of antigen using ELISA with specific antibodies (antiIL17 with antiIL1β and suggests that co-treatment of PBMCs with MenSCs and the Z-compound post biotic produces a dual modulatory effect: a consistent and significant suppression of IL-1β with a moderate, context-dependent modulation of IL-17 as details are summarized in table-4. The IL-1β suppression trajectory (0.565 → 0.448 → 0.339 in the MenSCs + compound arm) indicates enhanced anti-inflammatory action with sustained treatment. IL-17 dynamics were more complex, reflecting the well-known dual roles of this cytokine in both pro-inflammatory and tissue-protective signaling. These findings align with known MSCs immunomodulatory mechanisms, wherein IL-1β-priming enhances MSC anti-inflammatory potency through upregulation of IDO, TSG-6, and PGE2 secretion, IL-17 interactions shape broader immunosuppressive programs. The Z-compound post biotic appears to synergistically amplify these effects, potentially through its SCFA and modulating PBMC cytokine receptors. The details.

Table 4: Showing 3-day Consolidated ELISA Mean ± O.D Values for IL-1β and IL-17 for Different Treatment Groups

Group	Day 1 IL-1β	Day 2 IL-1β	Day 3 IL-1β	Day 1 IL-17	Day 2 IL-17	Day 3 IL-17
PBMC Control	0.565	0.591	0.637	1.314	0.785	1.463
PBS	0.561	0.866	0.788	0.896	0.668	0.795
Z-Compound	0.952	0.556	0.637	1.051	1.072	0.668
MenSCs	0.628	0.448	0.650	0.744	1.305	0.542
MenSCs + Z-Compound	0.535	0.339	0.500	0.794	0.975	1.034

Table- 5.0 showing the MTT assay was conducted on the HepG2 (hepatocellular carcinoma cell line) to evaluate the effect of three candidate compounds i.e. Beet root, Beta Xanthine, and Rx on cancer cells to evaluate metabolic activity and viability of proliferating cells after 24 hours of treatment. The control mean ± O.D at 570 nm was 0.417, representing 100% cell viability.

Table 5: Drug Stock Concentrations used in the MTT Assay

Drug Sample	Stock Concentration
Beta Xanthine	118.6 ng/ μ L
Beet Root	24.6 ng/ μ L
Rx	422.4 ng/ μ L
Reagent Blank	0.084 OD
Control Mean	0.417 OD

The MTT assay on HepG2 cells revealed predominantly pro-proliferative effects for all three compounds at the tested concentrations, with cell viabilities frequently exceeding 200% of control. No clear dose-dependent cytotoxic curve with calculable IC50 was established for any compound under these conditions. The only inhibitory signal was observed with Rx at the 05 μ L dose (91.8% viability), suggesting that true cytotoxic activity for this compound exists at concentrations below 10 μ L. These findings necessitate further validation including: testing of lower concentration ranges (especially for Rx), ruling out non-specific MTT interference (e.g., direct reduction of tetrazolium by plant compounds), and extending the assay duration beyond 24 hours to observe longer-term cytotoxicity kinetics.

Table-6 showing details findings of the beta Xanthine demonstrated a strong pro-proliferative effect on HepG2 cells at 10 and 50 μ L concentrations, with O.D values approximately 2.4 $\times$ the control (viability >240%). At the highest dose (100 μ L), viability returned toward 160% of control, suggesting partial dose-dependent attenuation but no cytotoxicity.

Table 6: Beta Xanthine Absorbance Values Showing Pro-Proliferative Activity at Two Different Concentrations (10 μ L & 50 μ L)

Concentration	Absorbance (OD 570nm)
Control	0.417
10 μ L (1.18 mg)	1.006
50 μ L (5.93 mg)	1.016
100 μ L (11.86 mg)	0.668

The findings reveals that Beet root showed a moderate proliferative effect at 10 μ L and 50 μ L doses, with O.D values approximately double the control at 10 μ L. At 100 μ L, viability approached control levels (0.486 vs 0.417), suggesting neither significant proliferation nor cytotoxicity at higher doses as documented in table-7.

Table 7: Findings Showing Beet Root Absorbance Values in Mild Pro-Proliferative at Different Concentrations

Concentration	Absorbance (OD 570nm)
Control	0.417
10 μ L (0.24 mg)	0.866
50 μ L (1.23 mg)	0.907
100 μ L (2.46 mg)	0.486

Table-8 showing the details absorbance of Rx compound showed the most complex dose-response relationship. Notably, the lowest dose (05 μ L) produced slight inhibition (OD 0.383 vs control 0.417, ~91.8% viability), while intermediate doses (10–0.996 and 20–1.021 μ L) induced marked proliferation. Higher concentrations progressively dampened proliferative activity, returning toward control-level OD values, suggesting a putative cytotoxic window at sub-10 μ L doses.

Table 8: Rx Compound Absorbance Showing Concentration Dependent Biphasic Response

Concentration	Absorbance (O.D 570nm)
Control	0.417
05 μ L (2.11 mg)	0.383
10 μ L (4.22 mg)	0.996
20 μ L (8.44 mg)	1.021
30 μ L (12.67 mg)	0.496
40 μ L (16.89 mg)	0.616
50 μ L (21.12 mg)	0.456
100 μ L (42.24 mg)	0.446

Exosomes Isolation and Characterization

Exosomes nano sized extracellular vesicles (typically 30–150 nm in diameter) secreted by cells that serve as critical mediators of intercellular communication. MenSCs-derived exosomes were isolated from two experimental conditions and investigated for distinct functional roles, revealing a striking bifurcation in biological activity depending on whether the parent MenSCs were conditioned with the Z-compound post biotically [6]. Exosomes isolated from MenSCs treated with the Z-compound post biotic demonstrated inhibitory properties against cancer cells. This anticancer activity is hypothesized to arise from the incorporation of bioactive metabolites from the Z-compound including SCFAs such as butyrate, bacteriocins, and exopolysaccharide fragments are well documented as to Inhibit histone deacetylase (HDAC) activity, inducing epigenetic silencing of oncogenic pathways, activate apoptotic cascades in cancer cells through caspase-dependent mechanisms down-regulate pro-survival signaling (PI3K/AKT, NF- κ B) via exosomal miRNA delivery and enhance immune-mediated reprogramming in tumor-associated macrophages [7-11]. The anticancer exosomes from Z-compound-conditioned MenSCs thus represent a novel biologically derived nano carrier system with potential applications in oncological therapeutic strategies, particularly for cancers with inflammatory microenvironments where immunomodulation is paramount [12].

Anti-Aging and Growth Factor Activity of Untreated MNSCs Exosomes

The exosomes derived from MenSCs that were not conditioned with the post biotic Z-compound exhibited a distinct regenerative and anti-aging functional profile. These properties of exosomes has been well characterized as to promote growth factor secretion, stimulation of EGF (Epidermal Growth Factor), VEGF (Vascular Endothelial Growth Factor), and KGF (Keratinocyte Growth Factor) expression in recipient cells [13]. Hair follicle stimulation: activation of Wnt/ β -catenin signaling pathways in dermal papilla cells, promoting antigen phase induction and hair growth, anti-aging dermal effects: upregulation of collagen type I and III synthesis, reduction of MMP-1 (matrix metalloproteinase-1) activity, and protection against oxidative stress-induced senescence. Wound healing acceleration and promotion of fibroblast proliferation, keratinocyte migration, and angiogenesis has also been well documented [14-16]. These properties position untreated MenSCs exosomes act as valuable tool for bio therapeutics in regenerative medicine contexts, including treatment of androgenetic alopecia, photo aging, chronic wound repair, and dermal volume restoration [17]. Post biotics preparations of inactivated microorganisms and their metabolic products have recently emerged as stable, safe, and clinically versatile alternatives to traditional probiotics in dermatological applications. Their biological activity is primarily mediated by secondary metabolites including short-chain fatty

acids (SCFAs), organic acids, bacteriocins, exopolysaccharides (EPS), extracellular vehicles (EVs), and bioactive peptides. These metabolites exert antimicrobial, anti-inflammatory, antioxidant, and barrier-enhancing effects relevant to a wide spectrum of cutaneous conditions [18].

Table 9: Mechanistic Comparison of Anticancer vs. Regenerative MenSCs Exosome Populations

Feature	Z-Compound-Conditioned Exosomes	Untreated MenSC Exosomes
Primary Activity	Anticancer / Immunosuppressive	Anti-aging / Regenerative
Key Cargo	SCFA metabolites, bacteriocin peptides, anti-tumor miRNAs	Growth factors (EGF, VEGF, KGF), Wnt ligands
Target Pathway	HDAC inhibition, NF-κB suppression, apoptosis induction	Wnt/β-catenin, TGF-β, MAPK/ERK
Target Cells	Cancer cells, tumor-associated immune cells	Dermal fibroblasts, keratinocytes, hair follicle papilla cells
Clinical Application	Cancer immunotherapy adjuvant	Alopecia treatment, anti-aging cosmeceuticals

Significance of Secondary Metabolites in Skin Short-Chain Fatty Acids (SCFAs)

Acetate, propionate, and butyrate regulate inflammation through G-protein-coupled receptors (GPR41/43/109A) and epigenetic modulation via HDAC inhibition. In the skin, SCFAs improve barrier integrity, reduce pro-inflammatory cytokine secretion, and enhance keratinocyte differentiation. Butyrate in particular shows promise in inducing apoptosis in melanoma cells through epigenetic mechanisms [19]. Lactic acid, phenyl lactic acid, and indole-lactic acid exert antimicrobial effects by lowering skin pH and disrupting pathogen membranes. Lactic acid additionally promotes hydration and keratinocyte turnover, making it a cornerstone of cosmeceutical formulations [20].

Antimicrobial Peptides, Bacteriocins and Exopolysaccharides
Bacteriocins such as *planta ricin* have recurring selectively that inhibit *Staphylococcus aureus* and *cutibacterium acnes* key pathogens in eczema and acne respectively and reducing biofilm formation critical to chronic skin infection resolution. Exopolysaccharides (EPS) produced by *Lactobacillus* and *Bifidobacterium* species enhance immune tolerance, act as antioxidants, and improve skin hydration upon topical application. Their film-forming properties offer promising cosmetic formulation potential [21-22]. Microbial extracellular vesicles EVs carry proteins, nucleic acids, and lipids that modulate immune responses and facilitate metabolite delivery into skin cells. Probiotic-derived EVs have been shown to accelerate wound closure and reduce oxidative stress in preclinical models [23].

Table 10: Mechanisms of Post Biotic Secondary Metabolites in Skin Biology

Mechanism	Mediating Metabolites	Skin Outcome
Antimicrobial activity	Organic acids, bacteriocins	Pathogen inhibition, pH control
Anti-inflammatory action	SCFAs, EPS	NF-κB/NLRP3 suppression, ↓ IL-1β/TNF-α
Barrier function improvement	SCFAs, bioactive peptides	↑ Filaggrin, loricrin, lipid synthesis
Wound healing promotion	EVs, peptides, SCFAs	↑ Angiogenesis, fibroblast proliferation
Antioxidant defense	Bioactive peptides, enzymatic metabolites	ROS neutralization, ↓ Oxidative damage

Clinical Applications Across Skin Disorders Atopic Dermatitis (AD)

SCFAs, bacteriocins, and EPS reduce *S. aureus* colonization, enhance ceramide synthesis, and modulate Th2-driven inflammation. Pilot clinical trials show improvements in erythema, pruritus, and barrier function. Acne vulgaris phenyl lactic acid, reuterin, and bacteriocins inhibit *C. acnes*, reduce biofilm formation, and lower inflammatory cytokine release. Early human studies suggest topical postbiotics may reduce lesion count and sebum oxidation [24, 25]. Psoriasis (SCFAs & EPS) regulate keratinocyte hyper proliferation and modulate. The activity of Th17/Treg regulate immune imbalance, suggesting may reduce psoriatic inflammation [26].

Chronic Wounds & Ulcers

SCFAs, peptides, and EVs accelerate re-epithelialization, enhance angiogenesis, and reduce bacterial burden. Promising results reported in diabetic wound models. Cosmetic and anti-aging EPS, lactic acid, and antioxidant peptides improve hydration, reduce wrinkle formation and protect against UV-induced oxidative stress [27,28]. SCFAs and antimicrobial peptides suppress overactive TLR2 signaling implicated in rosacea flare-ups. Post biotic metabolites may rebalance skin microbiome and reduce demodex-associated inflammation [29]. In seborrheic dermatitis, organic acids and bacteriocins combat *Malassezia* over-growth, while restoring microbial homeostasis. Lactic acid bacteria metabolite formulations show symptomatic improvement in dandruff [30]. Skin Infections that includes bacteriocins, organic acids, and EVs exhibit broad-spectrum antimicrobial activity against *S. aureus*, *S. pyogenes*, and *Candida albicans*, preventing biofilm formation [31]. Photo aging & UV damage includes SCFAs, EPS, and antioxidant enzymes protect keratinocytes from UV-induced oxidative stress, DNA damage, and inflammation. Anti-photo aging cosmeceuticals based on lysates and metabolites are in development and in skin cancers SCFAs, particularly butyrate, may induce apoptosis and inhibit proliferation in melanoma and non-melanoma cancer cells via inhibition of HDAC [32,33]. In vitiligo, antioxidant metabolites (glutathione, EPS, SCFAs) may reduce oxidative injury driving melanocyte loss, while immunomodulatory metabolites dampen autoimmune response [32]. The convergence of findings across this multi-modal study reveals a coherent and compelling narrative about the therapeutic versatility of MenSCs -exosome platforms potentiated by post biotic conditioning [33].

Immunomodulatory Synergistic Activity of MenSCs and Z-Compound

The three-day ELISA data consistently demonstrated that the MenSC + Z-compound combination produced the most pronounced IL-1 β suppression across all time points. This is clinically significant because IL-1 β is a master regulator of the inflammatory cascade, acting upstream of TNF- α , IL-6, and prostaglandin pathways. Its suppression by the MenSCs-compound combination suggests a potentially disease-modifying effect in chronic inflammatory conditions including rheumatoid arthritis, inflammatory bowel disease, and autoimmune skin disorders [34]. The IL-17 dynamics were more nuanced. While MenSCs alone elevated IL-17 — a cytokine traditionally associated with Th17-mediated inflammation — this may represent a paradoxical immunoregulatory mechanism. Emerging evidence suggests that moderate IL-17 elevations during MSC immunosuppression may reflect Treg-Th17 axis recalibration, ultimately promoting more durable immune tolerance. The addition of Z-compound dampened this IL-17 signal (Day 1-2) and allowing controlled re-emergence by Day 3, suggesting a temporally regulated immunomodulatory program [35].

Morphological Evidence of M2 Macrophage Polarization

The observed size increase of PBMCs from approximately 167 μ m to 192.17 μ m upon MenSCs exosome treatment is a hallmark of monocyte-to-macrophage differentiation. This transformation is accompanied by characteristic ultrastructural changes including cytoplasmic expansion, increased organelle density (particularly lysosomes and mitochondria), and nuclear morphological changes from horseshoe-shaped to kidney-shaped or round configurations [36]. The M2 macrophage phenotype, specifically promoted by MSC-derived paracrine signals including PGE2, IL-10, and TGF- β delivered via exosomes, is distinguished by expression of CD163, CD206, and Arg-1 surface markers. M2 macrophages play critical roles in tissue repair, anti-inflammatory cytokine production (IL-10, IL-4), and phagocytosis of apoptotic bodies and cellular debris. The demonstration that MenSCs exosomes can drive this phenotypic shift in PBMCs suggests their potential utility in conditions characterized by pathological M1 macrophage dominance, such as sepsis, chronic wounds, and fibrotic diseases [35]. Exosome Functionality in Cancer vs. Regeneration properties scientifically novel finding of this study is the demonstration that the functional identity of MenSCs exosomes is fundamentally altered by post biotic conditioning. Z-compound-conditioned exosomes acquire anticancer properties likely through loading of SCFA-derived epigenetic regulators and bacteriocin-derived antimicrobial peptides into their lipid bilayer and luminal cargo. These conditioned exosomes may represent a safe, cell-free cancer therapeutic with tumor-targeting potential that could be harnessed for drug delivery [37]. Untreated MenSCs exosomes, by contrast, retain and amplify the stem cells native regenerative secretome, including variety of growth factors like EGF, VEGF, FGF, HGF to regulate Wnt pathway activities. This positions them as natural bio stimulants for hair follicle regeneration, anti-aging dermal remodeling, and wound repair — effectively creating two distinct therapeutic products from the same stem cell source depending on culture conditions [38]. Post biotic secondary metabolites in Z-compound's secondary metabolites provide the mechanistic bridge linking all experimental observations. SCFAs acting through GPR43/41 on PBMCs suppress IL-1 β transcription through NF- κ B pathway inhibition. Bacteriocins modulate pathogen-associated molecular pattern (PAMP) recognition by immune cells, reducing baseline inflammatory tone. When these metabolites are incorporated into MenSCs exosomal cargo through cellular

endocytosis and re-secretion, they create a biologically amplified delivery vehicle that combines stem cell immunomodulatory signals with post biotic bioactive molecule [39,40].

Conclusion

The comprehensive study conclude with the remarks that MenSC + Z-compound Co-Treatment Achieves Superior IL-1 β Suppression: The combination produced the lowest IL-1 β across all three experimental days (nadir: 0.339 OD on Day 2), indicating synergistic anti-inflammatory activity superior to either agent alone or IL-17 Modulation is Temporally Dynamic: MenSCs alone drive IL-17 elevation (potentially immunoregulatory), while combination treatment moderates this elevation, suggesting a time-dependent cytokine balance program. The MenSC Exosomes Drive Monocyte-to-M2 Macrophage Differentiation: Quantitative microscopy confirmed PBMC size expansion from ~167 μ m to 192.17 μ m upon exosome treatment, consistent with M2 macrophage polarization and enhanced immune survival capacity with Z-Compound Conditioning Bifurcates MenSC Exosome Function: Post biotic-conditioned exosomes acquire anticancer inhibitory properties, while untreated MenSCs exosomes retain pro-regenerative, anti-aging and hair-growth-promoting activity. MTT Assay Reveals Context-Dependent Proliferative Effects: All three test compounds (Beet root, Beta Xanthine, Rx) showed primarily pro-proliferative effects on HepG2 cells at tested concentrations; Rx showed a narrow inhibitory window at the lowest dose (<10 μ L) requiring further exploration either through post biotic Secondary Metabolites Offer Broad Dermatological Utility: SCFAs, organic acids, bacteriocins, EPS, and EVs from post biotic preparations address a wide spectrum of skin conditions from atopic dermatitis to skin cancer through antimicrobial, anti-inflammatory, and regenerative mechanisms. There is still gap to complete future aspects that includes proteomic and transcriptomic characterization of Z-compound-conditioned vs. untreated MenSCs exosomes using LC-MS/MS and small RNA sequencing to identify specific anticancer and regenerative cargo molecules. In - vivo validation of MenSCs + Z-compound anti-inflammatory effects in murine models of colitis, arthritis, and psoriasis. Flow cytometric surface marker analysis (CD163, CD206, Arg-1) to formally confirm M2 macrophage polarization induced by MenSCs exosomes in PBMC cultures still lacking. Dose-optimization studies for the Rx compound in MTT assays at sub-10 μ L concentrations to identify true IC50 and cytotoxic mechanism in HepG2 and other cancer cell-lines and clinical translation studies with post biotic-MenSCs exosome formulations for alopecia and chronic wound healing in Phase I/II clinical trials]. Microbiome-metabolomics integration to define how the Z-compound post biotic microbiota shapes MenSCs exosome cargo composition and functional polarization. The MenSCs showing variety of physiological activity during variety of clinical lesion due to rich in stem cells that act as relevance of regenerative medicine. However, present study is small, but interesting in continuation for further characterization of stem cells using pluripotent markers —Oct4, Nanog and Sox4 have been reported in variety of cancers.

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Authors' Contributions

AKS is designed and implemented for data analysis, Priya help for technical analysis and VPS help for clinical analysis.

Ethics Approval and Consent to Participate

Study was approved by Savera Cancer and Multispecialty Hospital (EC26) and informed consent form was signed by guardian.

Competing Interests

There are no competing interests between the authors.

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