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Utilization of Human Umbilical Cord–Placental Junction Mesenchymal Stem Cell Secretome in a Glucomannan Hydrogel for Regeneration and Anti-Inflammatory Effects in a Murine Model of Grade IIIB Burns

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ABSTRACT

Background: Mesenchymal stromal cell (MSC) secretomes offer a cell-free strategy for burn repair, but cord–placental junction MSC (CPJ-MSC) secretome and glucomannan-based delivery remain poorly characterized. We compared CPJ-MSC with Wharton's jelly MSC (WJ-MSC) secretome in murine full-thickness burns and examined proteomic differences.

Methods: Passage-6 MSCs isolated from six term umbilical cord–placental tissues were characterized, and undiluted serum-free conditioned media were loaded into a xanthan gum–glucomannan hydrogel. Hydrogel properties and Franz-cell protein permeation were evaluated. Forty-five male C57BL/6 mice with grade IIIB burns received standard treatment, WJ-MSC secretome hydrogel, or CPJ-MSC secretome hydrogel (n = 5/group/time point). Wound closure and histological outcomes were assessed on days 3, 7, and 14. Pooled secretomes were compared using LC-HRMS proteomics.

Results: The transparent, flexible hydrogel had a 2.6 ± 0.2-fold swelling ratio, withstood 4.4 ± 0.4 N compression and more than 200 folds, and achieved an apparent secretome-protein permeation rate of 8.90 μg/cm²·h. CPJ-MSC secretome increased wound closure versus standard treatment on day 7 ($p < 0.05$) and, on day 14, versus WJ-MSC secretome ($p < 0.01$) and standard treatment ($p < 0.001$). It also reduced inflammatory-cell infiltration on days 7 and 14 ($p < 0.05$ versus WJ-MSC; $p < 0.0001$ versus standard), increased day-14 epidermal thickness ($p < 0.01$ versus WJ-MSC; $p < 0.001$ versus standard), and improved re-epithelialization and dermal–collagen organization. Of 224 retained proteins, 177 were shared; 49 shared proteins were at least twofold enriched in CPJ-MSC secretome, compared with two in WJ-MSC secretome. CHI3L1 showed the greatest CPJ enrichment (approximately 20.1-fold).

Conclusion: CPJ-MSC secretome delivered through a xanthan gum–glucomannan hydrogel promoted wound closure, attenuated inflammatory-cell infiltration, and enhanced early tissue regeneration more effectively than WJ-MSC secretome. These findings support further mechanistic validation of this source-specific, cell-free treatment for severe burns.

Keywords: burn wound; cord–placental junction; glucomannan hydrogel; mesenchymal stromal cell secretome; proteomics; Wharton's jelly

INTRODUCTION

Burn injuries remain a major global health problem, causing an estimated 180,000 deaths annually, predominantly in low- and middle-income countries. Nonfatal burns also impose a substantial burden through prolonged hospitalization, disability, disfigurement, and socioeconomic loss [1]. Full-thickness or grade IIIB burns are particularly challenging because extensive destruction of the epidermis, dermis, vascular structures, and skin appendages disrupts the protective barrier and limits spontaneous regeneration. Tissue necrosis, prolonged inflammation, impaired vascularization, and dysregulated extracellular-matrix remodelling can further delay wound closure and increase the risks of infection, contracture, and hypertrophic scarring [2]. Treatments capable of coordinating inflammatory resolution with tissue regeneration are therefore needed.

Mesenchymal stem/stromal cells (MSCs) have emerged as promising regenerative candidates because of their immunomodulatory, angiogenic, and tissue-reparative properties. Increasing evidence indicates that these effects are mediated predominantly through paracrine signalling rather than permanent cellular engraftment or differentiation. The MSC secretome contains soluble cytokines, chemokines, growth factors, extracellular-matrix proteins, and extracellular vesicles that can modulate immune-cell activity, stimulate fibroblast and keratinocyte responses, promote angiogenesis, and regulate matrix deposition [3]. In a severe-burn model, human umbilical cord MSC treatment reduced inflammatory-cell infiltration and proinflammatory cytokine expression while increasing angiogenesis and improving collagen organization [4]. More recently, MSC secretome delivered in a hydrogel accelerated repair of partial-thickness thermal burns by reducing the residual burn area, increasing fibroblast proliferation, collagen deposition, and angiogenesis, and modulating polymorphonuclear and mononuclear inflammatory-cell responses [5]. Secretome-based treatment may therefore reproduce many of the paracrine benefits of MSCs while avoiding the need for survival, engraftment, and long-term persistence of transplanted cells [3]. Wharton’s jelly-derived MSCs (WJ-MSCs) are among the most extensively investigated perinatal MSC populations [6]. Their conditioned medium has been shown to enhance dermal-fibroblast proliferation and migration and to accelerate re-epithelialization and wound repair in a murine full-thickness wound model [7]. WJ-MSC secretome therefore represents an appropriate active comparator for evaluating secretomes obtained from less extensively studied regions of the umbilical cord.

The cord–placental junction is a distinct anatomical and developmental interface between the umbilical cord and placenta and represents another potentially valuable MSC source [8]. CPJ-MSCs have demonstrated high colony-forming efficiency, proliferation, and self-renewal capacity compared with MSCs isolated from other umbilical-cord regions, including Wharton’s jelly [8]. MSCs derived from different perinatal anatomical regions can also retain distinct transcriptomic, secretory, and immunomodulatory profiles [9]. CPJ-MSCs have shown strong immunoregulatory activity, although their biological effects may vary according to the experimental and

disease context [9]. Despite these promising characteristics, the regenerative activity and protein composition of CPJ-MSC secretome remain insufficiently characterized in full-thickness burn wounds, particularly in direct comparison with WJ-MSC secretome.

An effective delivery system is required to retain the secretome at the wound surface, protect its bioactive constituents, and provide sustained local exposure [10]. Hydrogels offer a hydrated three-dimensional environment that can conform to the wound, maintain moisture, absorb exudate, and facilitate localized delivery of therapeutic molecules [10]. Natural polysaccharides are particularly attractive for this purpose because their abundant hydrophilic functional groups enable water absorption, polymer–protein interactions, and formation of flexible networks under relatively mild preparation conditions [10, 11]. Glucomannan is a high-molecular-weight, water-soluble heteropolysaccharide composed primarily of β-(1→4)-linked D-mannose and D-glucose residues, with acetyl substitutions that influence its solubility and intermolecular interactions [11]. Its abundant hydroxyl groups promote extensive hydration and hydrogen bonding, producing high viscosity, swelling capacity, water retention, and film-forming behaviour [11]. These properties allow glucomannan to form soft, moist, and conformable matrices suitable for contact with irregular wound surfaces [11]. In Indonesia, glucomannan can be obtained from porang (*Amorphophallus muelleri*), providing a locally available biomaterial with potential for scalable wound-dressing development [12]. Glucomannan has also been successfully formulated as a hydrated gel in konjac-based products, demonstrating its processing versatility and capacity to maintain functional viscosity [13]. When combined with xanthan gum, glucomannan forms a cooperative polysaccharide network through hydrogen bonding and intermolecular association [14]. This interaction can improve gel strength, elasticity, structural integrity, and water-holding capacity compared with either polymer alone [14]. Xanthan gum–konjac glucomannan blends have consequently demonstrated physicochemical and biological characteristics suitable for wound-dressing applications [14]. The polymer network may also entrap proteins and other secretome constituents while permitting their gradual diffusion into the wound environment, making the combination attractive for localized delivery of complex biological preparations [15]. Beyond its physical function as a hydrogel-forming polymer, glucomannan has demonstrated potentially relevant immunobiological activity. Porang-derived glucomannan increased murine macrophage viability and phagocytic activity, particularly at concentrations of 0.05–0.1%, supporting its capacity to interact with innate immune-cell responses [16]. In Western diet-fed mice, combined inulin–glucomannan supplementation preserved colonic mucosal architecture, reduced inflammatory severity, and produced a more favourable IL-6/IL-10 balance [17]. A separate study found that combined inulin and glucomannan attenuated metabolic liver injury while downregulating hepatic IL-6, Lbp, Nfkb1, and Cd36 expression [18]. The most recent related publication further showed that glucomannan-containing dietary-fiber supplementation modulated glial inflammatory signalling and reduced IL-6 expression in high-fat, high-sucrose diet-fed mice [19]. Although these studies used cellular or dietary models rather than topical

wounds, their convergent findings support the broader biological compatibility and inflammation-modulating potential of glucomannan and provide an additional rationale for investigating it as a functional wound-delivery material.

The combination of glucomannan with xanthan gum may therefore offer complementary advantages: glucomannan contributes hydration, swelling, protein association, and potential biological activity, whereas xanthan gum improves viscosity and stabilizes the polymer network [14]. Incorporation of MSC secretome could further transform this hydrated matrix into a bioactive dressing capable of maintaining wound moisture while delivering immunomodulatory and regenerative factors directly to the damaged tissue [3, 10]. Previous evidence that human umbilical cord MSC-conditioned medium incorporated into a hydrogel enhanced re-epithelialization, reduced inflammation, promoted vascularized granulation tissue, and accelerated healing in third-degree burns provides additional support for this strategy [20].

Accordingly, this preliminary study evaluated human CPJ-MSC secretome incorporated into a xanthan gum–glucomannan hydrogel in a murine model of grade IIIB burn injury, using WJ-MSC secretome as an established active comparator. The hydrogel was characterized for its physical properties, protein-loading capacity, morphology, and release profile. Its regenerative and anti-inflammatory effects were evaluated through macroscopic wound closure, inflammatory-cell infiltration, epidermal regeneration, and dermal and collagen organization. Comparative LC-HRMS proteomic profiling was also performed to identify source-dependent protein patterns that might explain differences in biological activity. Undiluted secretome was deliberately selected for this initial investigation to maximize the likelihood of detecting a biological effect. The study was designed to investigate preliminary efficacy and provide a mechanistic foundation for further studies, such as dose–response, protein-normalized, and targeted functional studies.

MATERIAL AND METHODS

Human Tissue Procurement and Ethical Approval

Human umbilical cord–placental tissues were obtained from 6 (six) healthy donors following uncomplicated term deliveries at Gotong Royong Hospital, Surabaya-Indonesia. Written informed consent was obtained from each donor before tissue collection. The study protocol was approved by the health research ethics committee of Widya Mandala Surabaya Catholic University under approval number 0008/WM12/KEPK/DSN/T/2026 Wharton’s jelly and cord–placental junction tissues were collected aseptically and transported to the laboratory in DMEM-High Glucose containing 4% Penicillin/Streptomycin/Amphotericin B at room temperature. All samples were processed within 30 minutes after collection.

Isolation and Culture of WJ-MSCs and CPJ-MSCs

Wharton’s jelly and cord–placental junction tissues were separated under sterile conditions, washed with phosphate-buffered saline, and cut into approximately 2 mm^3

fragments. Mesenchymal stem cells were isolated using the explant method/enzymatic digestion with enzyme, concentration, and duration as described previously [21]. The isolated cells were cultured in DMEM supplemented with 10% Human AB-Serum, and 2% Penicillin/Streptomycin/Amphotericin B. Cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2. The culture medium was replaced every 3-4 days. At approximately 80--90% confluence, the cells were detached using Trypsin-EDTA and sub-cultured. Passage-6 cells were used for MSC characterization and secretome production.

MSC Characterization

The morphology of WJ-MSCs and CPJ-MSCs was examined using Mayer's hematoxylin staining, observed with Nikon Trinocular Microscope Eclipse SI light microscope followed by documentation with Nikon digital sight microscope camera system DS-1000 (Nikon Corp., Tokyo, Japan). Immunophenotypic characterization was performed by flow cytometry with reference to the International Society for Cell and Gene Therapy minimal criteria [22]. Passage-6 cells were incubated with fluorochrome-conjugated antibodies against the positive MSC markers CD73 (Clone AD2), CD90 (Clone 5E10), CD105 (Clone SN6), and CD44 (Clone IM7) and the hematopoietic marker CD45 (Clone HI30) from Elabscience (New jersey, USA). The antibodies, manufacturers, catalog numbers, dilutions, cytometer model, gating strategy, and number of analyzed events were used in accordance with a previously published protocol [21]. Unstained cells were used to establish fluorescence thresholds.

Preparation of MSC Secretomes

Passage-6 WJ-MSCs and CPJ-MSCs were cultured until they reached approximately 90-95% confluence. The cells were washed 2 times with phosphate-buffered saline and incubated in serum-free DMEM-F12 for 72h. The conditioned media were collected and centrifuged at 500 x g for 20 minutes at 4°C to remove cells and debris. The supernatants were subsequently filtered through a 0,22µm filter. The secretome total protein concentrations were measured using the Bradford assay with bovine serum albumin (BSA) as the calibration standard [23]. Aliquots were stored at -80°C and subjected to no more than 2 freeze–thaw cycles before use.

Proteomic mapping of WJ-MSC Secretome and CPJ-MSC Secretome

Proteomic samples (100 mg) were dissolved in 500 µL of MS-grade water (Fisher Chemical), vortexed for 2 min, and incubated at room temperature for 1 h. Proteins were precipitated by adding 1 mL of ice-cold acetone and incubating at −20°C overnight, followed by centrifugation at 12,000 × g for 10 min at 4 °C. The resulting pellet was resuspended in 500 µL of 50 mM ammonium bicarbonate, reduced with 10 µL of 50 mM dithiothreitol for 1 h at 55°C, and alkylated with 10 µL of 50 mM iodoacetamide for 1 hour at 37°C in the dark. Proteins were digested with MS-grade trypsin (Pierce Thermo, Illinois, USA) at an enzyme-to-substrate ratio of 1:50 (w/w) for

16 h at 37°C. The digests were acidified to pH 3 with trifluoroacetic acid, centrifuged at 14,000 × g for 15 min at 4°C, and filtered through a 0.2 µm PVDF membrane before analysis. Peptides were separated using a Thermo Scientific Vanquish Horizon UHPLC system equipped with a binary pump (Germering, Germany) and an Acclaim PepMap 100 C18 analytical column (150 mm × 1 mm, 3 µm; Lithuania) maintained at 30°C. The mobile phases consisted of MS-grade water containing 0.1% formic acid (A) and MS-grade acetonitrile containing 0.1% formic acid (B). Samples (10 µL) were injected at a flow rate of 75 µL/min using a 45-min gradient comprising 5% B for 1 min, a linear increase from 5% to 50% B over 30 min, 50% B for 2 min, 90% B for 2 min, and re-equilibration at 5% B until 45 min. Mass-spectrometric analysis was performed using a Thermo Scientific Orbitrap Exploris 240 high-resolution mass spectrometer (Bremen, Germany) coupled to an OptaMax NG heated electrospray-ionization source operating in positive-ion mode at a spray voltage of 4,000 V. The sheath, auxiliary, and sweep gases were set to 10, 5, and 1 arbitrary units, respectively, and the ion-transfer-tube temperature was 300°C. Data were acquired in full-MS/data-dependent MS^2 mode over an m/z range of 350–1,200 at a full-MS resolution of 120,000 FWHM, with a maximum injection time of 100 ms, an intensity threshold of 8,000, charge states of 2–6, and a mass tolerance of 5 ppm. Data-dependent MS^2 spectra were acquired at a resolution of 15,000 FWHM using a normalized collision energy of 30 and nitrogen as the collision gas. The resulting data were processed using Thermo Scientific Proteome Discoverer version 2.5 (San Jose, CA, USA), with Sequest HT as the search engine and UniProt as the protein-sequence database [24].

Preparation of the Glucomannan Hydrogel

A xanthan gum–glucomannan hydrogel was prepared using the optimized glucomannan-to-xanthan gum weight ratio of 9:8, as described in Indonesian Patent No. IDS000014445 [25]. Pure Glucomannan powder were obtained from the manufacturer Ambico,LLC (Surabaya-Indonesia). Briefly, 1.125 g of glucomannan, 1.000 x g of xanthan gum, and 2 mg of methylparaben (Nipagin) were dispersed in distilled water, and the final volume was adjusted to 100 mL. The components were mixed until a homogeneous aqueous dispersion was obtained. The mixture was thermally cross-linked at 121°C for 1 h. The resulting hydrogel was then placed in a sterile, closed container and subjected to cold evaporation at 4°C for 72 h to generate a hydrogel structure. All subsequent procedures were performed under aseptic conditions. Mixture of WJ-MSC and CPJ-MSC secretome were separately reconstituted in phosphate-buffered saline at pH 6.8-7.4. The hydrogels were immersed in the respective secretome solutions at 2-4°C for 24 h to allow protein absorption into the hydrogel matrix. BSA-loaded hydrogels were used as protein controls in the absorption and release experiments.

Physicochemical Characterization of the Hydrogel

The hydrogels were visually evaluated for color, transparency, odor, homogeneity, and gross structural integrity. Surface and cross-sectional morphology of hydrogels with

and without MSC secretome was examined by scanning electron microscopy (Hitachi TM 300). Before imaging, samples were frozen at -80°C overnight, lyophilized with a freeze dryer (Taitec, Tokyo, Japan) for 24 hours, and then mounted onto alumunium stubs using conductive double-sided carbon tape. To ensure adequate conductivity, the samples were coated with a thin layer of gold and sputter-coated for 120 seconds at accelerating voltage of 10-15 kV. Representative images were acquired with a 200-µm scale reference. The swelling ratio was determined according to ASTM F2900-11 [26]. Pre-weighed dry hydrogel specimens were immersed in DMEM at room temperature for 12h. After excess surface liquid was removed, the swollen specimens were weighed, and the subtracting the weight before and after incubation and dividing the difference by the initial dry weight of the sample. Mechanical properties were assessed using a compression test performed with Ohaus Dynamometer MN-8008 (Ohaus Corp., New Jersey, USA). The maximum compressive force was recorded in newtons. Folding endurance was determined by repeatedly folding each hydrogel specimen at the same location until structural failure occurred or more than 200 folds had been completed. Protein absorption capacity was evaluated by incubating hydrogel specimens of a defined surface area with either MSC secretome or BSA solution. After incubation for 2 h, the remaining protein in the solution was quantified using the Bradford assay. Protein absorption per unit area was calculated from the difference between the initial and residual protein quantities. All physicochemical tests were performed using 5 (five) independent hydrogel specimens.

Experimental Animals and Ethical Approval

A total of 45 C57/BL6 male mice, aged 8-10 weeks and weighing 18-20 g, were obtained from Pusvetma, Ministry of Agriculture, Surabaya-Indonesia. The animals were housed under controlled conditions at 24°C, with 50-70% humidity, and a 12-h light/dark cycle, with free access to standard chow-diet and water. Animals were acclimatized for 7 days before the experiment. All animal procedures were approved by the Ethic Health comitee of widya mandala catholic university under approval number 0008/WM12/KEPK/DSN/T/2026 and animal-study reporting was guided by ARRIVE 2.0 [27]. The method used for sample-size calculation was executed using G*Power software (Heinrich Heine University, Düsseldorf, Germany) [28] utilizing an alpha level of 0.05 and a power of 0.80.

In Vitro Protein-Release Study with C57/BL6 mouse skin.

Protein release from the hydrogel was evaluated using a vertical (Franz) single chamber diffusion-cell system (Corning corp., Arizona, USA). Excised C57/BL6 mouse skin was mounted between the donor and receptor compartments, with the appropriate surface facing the donor chamber. Hydrogels loaded with either MSC secretome or BSA (Himedia ultra, India) purewere placed in the donor compartment. The receptor chamber contained PBS, maintained at 37°C using a circulating water jacket and stirred at 780 rpm. At predetermined intervals over 6 h, 200µL samples were collected from the receptor chamber and replaced with an equal volume of fresh

medium. Protein concentrations were determined using the Bradford assay. Cumulative protein permeation was expressed as milligrams per square centimeter, and the release rate was calculated from the slope of the cumulative permeation-versus-time curve. The release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas kinetic models [29]. The correlation coefficient and kinetic constant were calculated for each model. The model providing the highest correlation coefficient was considered to best describe protein release from the hydrogel.

Induction and Treatment of Grade IIIB Burn Wounds

Mice were anesthetized using using 100 mg/kg Ketamin (Mahakam Beta Farma, Jakarta, Indonesia) and 10 mg/kg Xylasine (Intechemie, Netherland) intraperitoneally, and the dorsal hair was removed. After skin disinfection, a full-thickness grade IIIB burn was induced on day 0 using a metal probe measuring 8 mm, heated to 120°C and applied to the dorsal skin for 10 second. On day 1, necrotic tissue was surgically debrided under anesthesia. The animals were then assigned to the following groups: Standard-treatment group: wounds received DMEM-F12 soaked Glucomannan hydrogel. In WJ-MSC group, wounds received a glucomannan hydrogel containing WJ-MSC secretome. CPJ-MSC group: wounds received a glucomannan hydrogel containing CPJ-MSC secretome. Each hydrogel dressing measured 1 x 1 cm and contained. Dressings were secured using hypafix and changed every 3 days. Five mice from each group were terminated on days 3 (no change), 7 (1x change), and 14 (2x change) after treatment.

Macroscopic Wound Assessment

Wounds were photographed under standardized lighting and camera-distance conditions on days 3, 7, and 14. A metric scale was included in each image for calibration. Wound areas were measured using ImageJ [30] to group allocation. The percentage of wound closure was calculated comparing the mean wound area H+1 after debriment (as 100% Value) and the wound area at the specified observation time. Wound-closure percentages on days 7 and 14 were used for between-group comparisons.

Tissue Collection and Histological Examination

At each termination time point, mice were euthanized using 100 mg/kg Ketamin and 10 mg/kg Xylasine intraperitoneally followed by cervical dislocation. A full-thickness skin specimen containing the wound and a margin of surrounding healthy tissue was excised. Samples were fixed in 4% neutral buffered formalin, dehydrated through graded ethanol, cleared in xylene, and embedded in paraffin. Sections of 5µm were prepared and stained with hematoxylin and eosin and Masson’s trichrome. Hematoxylin-and-eosin-stained sections were examined for epidermal regeneration, inflammatory-cell infiltration, granulation tissue formation, and overall tissue architecture. Inflammatory-cell infiltration was graded using the Hematoxylin-and-eosin-stained skin sections evaluation with a predefined 0–5-point semiquantitative

scoring system for mononuclear inflammatory-cell infiltration. Infiltration was scored as follows: 0, absent; 1, minimal, with rare scattered cells occupying ≤5% of the evaluated wound area; 2, mild focal or multifocal infiltration occupying >5–15%; 3, moderate multifocal infiltration occupying >15–30%; 4, marked and partly coalescing infiltration occupying >30–50%; and 5, severe diffuse infiltration occupying >50% of the wound area [31]. Five non-overlapping high-power fields covering the wound center and margins were assessed for each section. Evaluations were performed independently by two observers who were blinded to the treatment groups, and the median field score was used as the score for each specimen. Scores from days 7 and 14 were used for statistical comparisons. Masson's trichrome-stained sections were examined for collagen deposition, organization, and remodeling. Epidermal thickness was measured on day 14 at 10 (ten) representative, non-overlapping fields per specimen using ImageJ. Measurements were performed at 100x by 2 (two) investigators blinded to the treatment groups using Microscope Nikon Eclipse Si RS and Digital Camera DS1000. The mean value for each animal was used in the analysis.

Statistical Analysis

Data were analyzed using Graphpad Prism 11. Continuous data were expressed as the mean ± standard deviation. Normality and homogeneity of variance were evaluated using the Shapiro–Wilk respectively. Normally distributed continuous outcomes, including wound closure and epidermal thickness, were compared among groups at each time point using one-way analysis of variance followed by Tukey's multiple-comparison test. Non-normally distributed data were analyzed using the Kruskal–Wallis test followed by Dunn's multiple-comparison test. As an ordinal outcome, inflammatory-cell infiltration scores were analyzed using the Kruskal–Wallis test with Dunn's post hoc test. Release-model parameters were estimated by regression analysis. All tests were two-sided, and ($p<0.05$) was considered statistically significant. Statistical significance was denoted as follows: (*$p<0.05$), (**$p<0.01$), (***$p<0.001$), and (****$p<0.0001$).

RESULTS

Morphological and Phenotypic Characteristics of WJ-MSC and CPJ-MSC

Cells isolated from Wharton's jelly and the cord–placental junction adhered to the culture surface and exhibited the spindle-shaped, fibroblast-like morphology characteristic of mesenchymal stromal cells (Supplementary Figures 1 and 2). Flow-cytometric analysis of passage-6 WJ-MSCs demonstrated high expression of the MSC-associated surface markers CD105, CD90, CD73, and CD44, whereas CD45 expression was substantially lower (Supplementary Figure 3).

Physical and Morphological Characteristics of the Hydrogel

The xanthan gum–glucomannan hydrogel formed a clear, transparent, flexible, and odorless sheet (Figure 1A). Scanning electron microscopy demonstrated differences

between unloaded and secretome-loaded hydrogels. The unloaded hydrogel exhibited an irregular, folded surface and a visibly layered cross-sectional structure. Following secretome loading, the hydrogel showed a comparatively more homogeneous and compact surface and cross-sectional morphology (Figure 1B and C).

The secretome-loaded hydrogel had a swelling ratio of 2.6 ±0.2-fold and withstood a compressive force of 4.4 ±0.4 N. The measured protein-absorption capacity was 357.38 ± 8.47 μg/cm² for the MSC secretome, compared with 306.41±30.21 μg/cm² for BSA. The hydrogel also retained its structural integrity for more than 200 folding cycles (Table 1).

Protein Release and Release-Kinetic Profile

Protein permeation from both secretome-loaded and BSA-loaded hydrogels increased rapidly during the initial phase of the diffusion experiment and subsequently approached a relatively stable level (Figure 2A and B). The calculated protein-release rate was 8.90 μg/cm²·h for the MSC secretome and 8.00 μg/cm²·h for BSA.

The release profiles were fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas models. For the MSC secretome, the corresponding correlation coefficients were 0.8655, 0.8395, 0.9150, and 0.9469, respectively. The Korsmeyer–Peppas model therefore provided the best fit for secretome release, with a reported kinetic coefficient of 0.229. The BSA release profile showed the same pattern, with correlation coefficients of 0.8261, 0.8142, 0.8699, and 0.9053 for the zero-order, first-order, Higuchi, and Korsmeyer–Peppas models, respectively (Table 2). These findings indicated that the Korsmeyer–Peppas model most closely described protein release from the xanthan gum–glucomannan hydrogel.

Macroscopic Burn-Wound Healing

Macroscopic observations demonstrated progressive differences in wound healing among the three treatment groups (Figure 4A). On day 3, open wounds remained visible in the standard-treatment, WJ-MSC secretome, and CPJ-MSC secretome groups. By day 7, wounds treated with the CPJ-MSC secretome hydrogel showed greater contraction and a smaller residual wound area than wounds receiving standard treatment or the WJ-MSC secretome hydrogel. By day 14, wounds in the CPJ-MSC secretome group were almost completely closed, whereas residual wound areas remained visible in the other groups. On day 7, wound closure in the CPJ-MSC secretome group was significantly greater than that in the standard-treatment group ($p<0.05$). The differences between the standard-treatment and WJ-MSC groups and between the WJ-MSC and CPJ-MSC groups were not statistically significant at this time point. By day 14, the WJ-MSC secretome hydrogel produced significantly greater wound closure than standard treatment ($p<0.05$). Wound closure was further increased in the CPJ-MSC secretome group compared with the WJ-MSC secretome group ($p<0.01$)and the standard-treatment group ($p<0.001$) (Figure 4D).

Histological Wound Repair

Hematoxylin-and-eosin and Masson's trichrome staining revealed treatment-dependent differences in tissue repair (Figure 4B and Supplementary Figure 4). At day 14, wounds receiving standard treatment continued to show incomplete epithelial coverage, disruption of the dermal architecture, and prominent cellular infiltration. The WJ-MSC secretome group showed improved re-epithelialization and granulation-tissue organization compared with standard treatment. The CPJ-MSC secretome group exhibited the most complete epithelial coverage and a more organized dermal and collagen architecture, with histological features approaching those of normal skin.

Inflammatory-Cell Infiltration

Both secretome-loaded hydrogels reduced inflammatory-cell infiltration relative to standard treatment (Figure 4C). On day 7, the infiltration score was significantly lower in the WJ-MSC secretome group than in the standard-treatment group ($p<0.001$). The CPJ-MSC secretome group had a lower infiltration score than both the standard-treatment group ($p<0.0001$) and the WJ-MSC secretome group ($p<0.05$). A similar treatment effect was observed on day 14. The WJ-MSC secretome hydrogel significantly reduced inflammatory-cell infiltration compared with standard treatment ($p<0.0001$). The CPJ-MSC secretome group again showed the lowest infiltration score, with significant differences relative to both standard treatment ($p<0.0001$) and the WJ-MSC secretome group ($p<0.05$).

Epidermal Regeneration

Epidermal thickness was assessed on day 14 as an indicator of re-epithelialization (Figure 4E). Treatment with the WJ-MSC secretome hydrogel significantly increased epidermal thickness compared with standard treatment ($p<0.05$). The CPJ-MSC secretome hydrogel produced a further increase compared with the WJ-MSC secretome group ($p<0.01$) and standard treatment ($p<0.001$). Thus, among the treatments evaluated, the CPJ-MSC secretome hydrogel produced the greatest improvement in epidermal regeneration. Overall, the undiluted CPJ-MSC secretome delivered using a xanthan gum–glucomannan hydrogel produced greater wound closure, reduced inflammatory-cell infiltration, and enhanced epidermal regeneration compared with standard treatment and the established WJ-MSC secretome comparator.

Proteomic profiling of WJ-MSC Secretome and CPJ-MSC Secretome

LC-HRMS database searching initially returned more than 27,000 protein entries across the two MSC secretomes. After excluding low-abundance and uncharacterized entries, 224 protein entities were retained for descriptive analysis. A comprehensive breakdown of the proteomic analysis is provided in a shared repository (see data availability statement). Comparison with plasma-protein annotations indicated minimal overlap with commonly reported plasma proteins. Among the retained proteins, 177 were detected in both CPJ-MSC and WJ-MSC secretomes, whereas 6 were detected only in the CPJ-MSC secretome. Among the 177 shared proteins, analysis of median-

centred LFQ abundance identified 49 proteins that were at least twofold enriched in CPJ-MSC secretome, 2 that were at least twofold enriched in WJ-MSC secretome, and 126 that differed by less than twofold. CHI3L1 showed the greatest enrichment in CPJ-MSC secretome ($log_2FC = 4.33$; approximately 20.1-fold; eight unique peptides), whereas THBS1 showed the strongest enrichment in WJ-MSC secretome ($log_2FC = -1.12$; approximately 2.2-fold; seven unique peptides). FN1, COL1A1, COL1A2, SERPINE1, and COL6A1 were among the most abundant shared proteins, demonstrating that both secretomes contained a prominent extracellular-matrix and tissue-remodelling component. A heatmap of the 36 proteins with the highest LFQ abundances revealed distinct profiles between the two secretomes (Figure 5A). Among the displayed proteins, the transgelin variant showed the highest abundance in both secretomes, with a stronger signal in the CPJ-MSC secretome. Pyruvate kinase, L-lactate dehydrogenase A, desmin, alpha-internexin, neurofilament-related proteins, and fructose-bisphosphate aldolase were comparatively more abundant in the WJ-MSC secretome. In contrast, the CPJ-MSC secretome showed higher abundances of an ACTN4-related protein, inter-alpha-trypsin inhibitor heavy chain 2, several actin isoforms, ACX136, G protein-coupled receptor 77, ferroxidase, laminin subunit beta-1, nidogen-1, and calreticulin. Six selected CPJ-enriched proteins (ACX136), G protein-coupled receptor 77, ferroxidase, laminin subunit beta-1, nidogen-1, and calreticulin—are highlighted in Figure 5B.

DISCUSSION

This study combines a xanthan gum–glucomannan hydrogel with undiluted CPJ-MSC secretome and directly compares its activity with the more established WJ-MSC secretome in a grade IIIB burn model. The hydrogel successfully absorbed and released secretome proteins while remaining transparent, flexible, and mechanically stable. Both secretome-loaded hydrogels promoted wound repair, confirming the regenerative potential of perinatal MSC secretomes. Nevertheless, CPJ-MSC secretome produced the strongest response, characterised by earlier improvement in wound closure, consistently lower inflammatory-cell infiltration, more organised dermal repair, and greater epidermal regeneration. The proteomic findings provide a coherent explanation for this source-dependent difference, indicating that CPJ-MSC secretome combines the shared MSC regenerative core with a quantitatively enriched set of matrix-remodelling, cytoprotective, and immunoregulatory proteins.

The physical characteristics of the hydrogel support its potential as a topical wound dressing. Transparency may allow wound observation without frequent dressing removal, whereas flexibility and resistance to more than 200 folding cycles facilitate handling and conformity to the wound surface. Its 2.6-fold swelling ratio indicates an ability to absorb fluid while maintaining a hydrated environment [10]. These findings are consistent with previous evidence that xanthan gum–konjac glucomannan blends can form hydrated and cytocompatible wound-dressing matrices [14]. Secretome

loading also produced a more homogeneous and compact hydrogel morphology, suggesting integration of secretome constituents within the polysaccharide network. The greater protein uptake observed for MSC secretome than for BSA may reflect the diverse molecular sizes, charges, conformations, and polymer affinities within the secretome rather than molecular size alone [15].

The Franz diffusion-cell experiment confirmed early protein transfer from the hydrogel, with a release rate of 8.90 μg/cm²·h for MSC secretome compared with 8.00 μg/cm²·h for BSA. The Korsmeyer–Peppas model provided the best descriptive fit among the four tested models. Because the reported value of 0.229 was defined as a kinetic coefficient, it should not be interpreted as the release exponent n unless confirmed by the original equation [29]. Moreover, the receptor-compartment profile reflects both release from the hydrogel and permeation across the intervening membrane [32]. These results establish the ability of the hydrogel to deliver secretome proteins during the early phase after application. Extending the experiment across the complete three-day dressing interval in simulated wound fluid would further determine the duration of release and preservation of representative bioactive proteins.

The biological effects developed progressively. By day 7, CPJ-MSC secretome had already increased wound closure relative to standard treatment, whereas by day 14 both secretome preparations were effective and the CPJ-MSC secretome produced an additional improvement over WJ-MSC secretome. This temporal pattern suggests that both preparations initiated repair, but the CPJ advantage became most apparent during the proliferative and early remodelling phases [33]. The activity of the WJ-MSC comparator agrees with studies showing that WJ-MSC conditioned medium promotes angiogenesis and cutaneous repair [34] and that umbilical-cord MSC conditioned medium delivered using a thermosensitive hydrogel improves experimental burn healing [20]. The positive WJ-MSC response therefore strengthens the comparison and highlights the greater activity of CPJ-MSC secretome under the same experimental conditions.

Histological findings supported the macroscopic results. Both secretome hydrogels reduced inflammatory-cell infiltration, but CPJ-MSC secretome produced the lowest scores on days 7 and 14, indicating more effective progression from inflammation toward tissue reconstruction. CPJ-treated wounds also showed more complete epithelial coverage and better-organised dermal and collagen architecture. The greater epidermal thickness at day 14 is compatible with enhanced keratinocyte proliferation and re-epithelialisation during this early stage of repair [33]. Subsequent evaluation of CD68/CD163 macrophage phenotypes, Ki-67, angiogenic markers, collagen subtypes, and fibre organisation should help define how CPJ-MSC secretome coordinates inflammatory resolution with epidermal and dermal regeneration.

Proteomic analysis provides a strong biological rationale for the superior CPJ response. The analysis used pooled secretomes from three WJ-MSC donors and

three CPJ-MSC donors, providing an integrated representation of each cell source while reducing the influence of an individual donor. Of the 224 retained protein entities, 177 were shared, 6 were detected only in CPJ-MSC secretome, and 41 were detected only in WJ-MSC secretome. The shared proteins included abundant FN1, COL1A1, COL1A2, COL6A1, and SERPINE1, demonstrating that both secretomes possessed a prominent extracellular-matrix and tissue-remodelling component. However, 49 shared proteins were enriched by at least twofold in CPJ-MSC secretome, whereas only 2 showed equivalent enrichment in WJ-MSC secretome. Thus, regenerative potency appeared to be associated more closely with the abundance and functional coordination of specific proteins than with the total number of proteins detected.

The CPJ-enriched matrix-associated proteins provide a plausible link to its stronger histological effect. COL5A1, COL6A1, SERPINH1, LUM, BGN, TIMP1, and LAMC1 collectively participate in collagen synthesis and folding, fibril organisation, basement-membrane formation, cell–matrix interaction, and control of extracellular-matrix proteolysis [24, 35]. This coordinated module may support the formation and stabilisation of granulation tissue while maintaining balanced matrix turnover. Type VI collagen is a functional regulator of dermal matrix assembly and fibroblast behaviour rather than merely a structural component [36], while lumican can modulate fibroblast signalling and collagen organisation [37]. The enrichment of laminin subunit beta-1 and nidogen-1 may further facilitate reconstruction of the dermo–epidermal junction. Nidogen-1 connects laminin with other basement-membrane components, and its experimental loss alters laminin deposition and epidermal differentiation during wound repair [38].

Calreticulin may complement these structural proteins by promoting the cellular phase of repair. Extracellular calreticulin stimulates keratinocyte and fibroblast migration and has improved wound closure and tissue regeneration in experimental diabetic wounds [39]. Its enrichment in CPJ-MSC secretome may therefore facilitate cellular entry into the wound bed and subsequent re-epithelialisation. The ferroxidase signal may indicate support for iron efflux and protection from iron-mediated oxidative damage [40], whereas G protein-coupled receptor 77-associated signalling may contribute to local immune regulation [41]. Together, these proteins suggest that CPJ-MSC secretome provides not only extracellular-matrix building blocks but also signals supporting cell migration, stress adaptation, and inflammatory control.

CHI3L1 was the most strongly CPJ-enriched shared protein, with a $\log_2$FC of 4.33, corresponding to an approximately 20-fold difference. CHI3L1 is associated with tissue remodelling and communication between macrophages and healing-associated fibroblasts. CHI3L1-expressing fibroblast populations have been associated with successful healing of diabetic wounds [42], while MSC-derived CHI3L1 has demonstrated immunomodulatory activity by suppressing T-cell activation and inflammatory cytokine production [43]. Its marked enrichment may therefore help connect the lower inflammatory-cell infiltration with the improved matrix organisation observed in CPJ-treated wounds. CHI3L1 is consequently a strong candidate for

targeted validation through neutralisation, depletion, or recombinant-protein add-back experiments.

The distinct CPJ profile is also biologically compatible with its anatomical origin. CPJ-MSCs arise from the transition between the umbilical cord and placenta, a developmentally active region involved in tissue attachment, vascular support, and maternal–fetal communication [8]. Direct regional comparisons have shown that CPJ-MSCs possess higher proliferative and self-renewal capacity than WJ-MSCs during serial culture [8], while other same-donor studies have identified distinct transcriptomic and immunoregulatory properties [9]. These observations support the interpretation that CPJ-MSCs retain a source-specific paracrine programme favouring matrix reconstruction, cellular protection, and immune regulation.

Several aspects of this preliminary study provide a clear direction for further development. The cohort size and day-14 endpoint were sufficient to identify an early efficacy signal; larger studies with longer follow-up can further determine scar maturation, tensile strength, pigmentation, restoration of skin appendages, and normalisation of epidermal thickness. Further, donor-resolved proteomics and targeted validation by ELISA, immunoblotting, or parallel-reaction monitoring will could confirm the reproducibility of the CPJ-enriched protein signature. The use of glucomannan also offers regional and translational relevance because “porang” (*Amorphophallus muelleri*) is cultivated abundantly in Indonesia and can provide a locally available source of this polymer [12]. Future development will require pharmaceutical-grade control of purity, molecular weight, endotoxin, sterilization stability, and batch consistency [26].

Overall, the convergence of wound closure, inflammatory-cell infiltration, epidermal regeneration, dermal organization, and proteomic enrichment provides coherent preliminary efficacy evidence. CPJ-MSC secretome appears to enhance healing through the coordinated action of matrix-assembly, matrix-protection, immunoregulatory, cytoprotective, and pro-migratory proteins rather than through a single mediator. CHI3L1 and the COL5A1/COL6A1/SERPINH1/LUM/BGN/TIMP1/LAMC1 module represent priority candidates for functional validation. These findings support continued development of the xanthan gum–glucomannan carrier and position CPJ-MSC secretome as a promising source-specific, cell-free treatment for burn-wound repair.

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AUTHOR CONTRIBUTIONS

Yudy Tjahjono: Conceptualization, methodology, investigation, formal analysis, data curation, visualization, supervision, project administration, funding acquisition, and writing-original draft. Jopy Wikana: Methodology, investigation, data collection, and formal analysis. Relly Yanuari Primariawan, Sianty Dewi: Resources, clinical coordination, tissue procurement, and writing-review and editing. Hendy Wijaya: Methodology, formal analysis, data interpretation, and writing-review and editing. Bernadette Dian Novita: Methodology, inflammatory-response interpretation, formal analysis, and writing-review and editing. Suryo Kuncorojakti: Animal-study methodology, investigation, histological analysis, supervision, and writing-review and editing. Lucia Hendriati: Hydrogel formulation, physicochemical characterization, methodology, validation, and writing-review and editing. Chun-Ming Huang, Chi-Ying F. Huang, Takuya Sugahara: Supervision, validation, data interpretation, and writing-review and editing. Jie Tang, Mengke Zhang: Proteomic investigation, data curation, proteomic investigation, bioinformatic analysis, visualization, and writing-review and editing. Hevi Wihadmadyatami: Histopathological methodology, investigation, validation, and data interpretation. Brilliant Dwi Putra, Mei Shanti Huang, Christian Charlie Wijaya, Felix Christ Antono: Investigation, data collection, and laboratory assistance. All authors reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

AUTHOR DECLARATIONS

The Authors declare no conflict of statement.

DATA AVAILABILITY

The complete data generated during the LC-HRMS analysis are available in the following Google Drive repository: https://drive.google.com/drive/folders/1PWBmCRncK-X4Q6abYjSeQtT5t2dZWf9S?usp=sharing

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used ChatGPT (OpenAI) to assist in language refinement, grammar correction, and improving technical clarity. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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FIGURE LEGENDS

Figure 1. **Physical and morphological characteristics of the xanthan gum–glucomannan hydrogel.** (A) Gross appearance of the transparent, flexible hydrogel sheet. Representative scanning electron micrographs of the surface and cross-section of hydrogels (B) without and (C) with MSC secretome. Scale bars = 200 μm.

Figure 2. **Protein permeation from the secretome-loaded hydrogel.** (A) Schematic of the Franz diffusion-cell experiment using mouse skin as the membrane. (B) Protein permeation profiles and apparent fluxes of MSC secretome and bovine serum albumin (BSA). (C) Linearized fitting of MSC-secretome release data to zero-order, first-order, Higuchi, and Korsmeyer–Peppas models with their respective correlation coefficient (r). Korsmeyer-Peppas shows higher r (r=0,9469).

Figure 3. **Experimental design of the murine burn-wound study.** Full-thickness burns were induced on day 0, followed by debridement and hydrogel application on day 1. Animals received standard treatment, WJ-MSC secretome hydrogel, or CPJ-MSC secretome hydrogel. Wound assessment and tissue collection were performed on days 3, 7, and 14 (n = 5 per group per time point).

Figure 4. **Effects of MSC-secretome hydrogels on burn-wound healing.** (A) Representative wound photographs on days 3, 7, and 14. (B) Representative hematoxylin–eosin (H&E) and Masson's trichrome-stained sections on day 14, with normal skin as reference. Quantification of (C) immune-cell infiltration, (D) wound closure, and (E) epidermal thickness. STD, standard treatment; WJ, WJ-MSC secretome hydrogel; CPJ, CPJ-MSC secretome hydrogel. Scale bars: 4 mm (A) and 100 μm (B). ns, not significant; *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

Figure 5. **Comparative proteomic profiles of WJ-MSC and CPJ-MSC secretomes.** (A) Heatmap showing the relative LC-HRMS abundances of proteins detected in WJ-MSC and CPJ-MSC secretomes, with cooler and warmer colors representing lower and higher relative abundance, respectively. (B) Focused visualization of six proteins enriched in the CPJ-MSC secretome: ACX136, G protein-coupled receptor 77, ferroxidase, laminin subunit beta-1, nidogen-1, and calreticulin. These proteins are described as CPJ-enriched rather than CPJ-specific because corresponding signals are also present in the WJ-MSC column in panel A.

Supplementary Figure 1. **Isolation of WJ-MSCs and CPJ-MSCs from human umbilical-cord tissue.** (A) Identification of the Wharton's jelly (WJ) and cord–placental junction (CPJ) sampling regions. (B–E) Sequential tissue dissection, processing, and explant culture.

Supplementary Figure 2. **Morphology of WJ-MSCs and CPJ-MSCs.** Representative bright-field images showing adherent, spindle-shaped cells. Scale bar = 20 μm.

Supplementary Figure 3. **Surface-marker expression of passage-6 WJ-MSCs.** A. The gating strategy of WJ and CPJ-MSC to analyze CD105, CD90, CD73, CD44, and CD45 expression. B. Flow-cytometric analysis demonstrated high expression of CD105, CD90, CD73, and CD44, with substantially lower CD45 expression for both WJ- and CPJ-MSC. ****p < 0.0001.

Supplementary Figure 4. **Histological appearance of burn wounds during early healing in day 3 and 7**. Representative H&E- and Masson's trichrome-stained sections from the standard-treatment, WJ-MSC secretome-hydrogel, and CPJ-MSC secretome-hydrogel groups on days 3 and 7. Scale bar = 100 μm.

Supplementary Files

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