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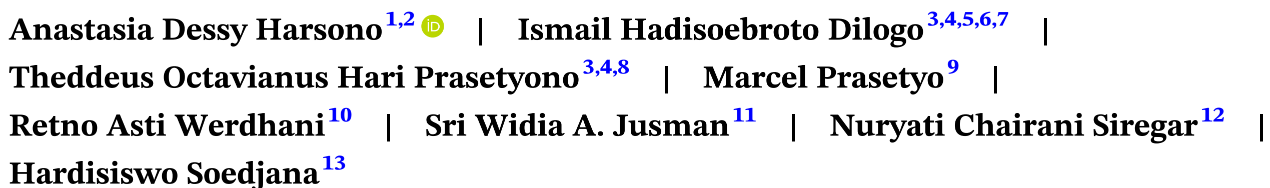
Clinical evaluation of intralesional umbilical cord-derived mesenchymal stem cells, conditioned medium and triamcinolone acetonide injection for keloid treatment: A pilot study



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Abstract

Topical keloid therapy is performed with triamcinolone acetonide (TA) intralesional injection. However, the recurrence rate is high with various side effects. Mesenchymal stem cells (MSCs) have high proliferative abilities and reduce the activity and proliferation of fibroblast cells in keloids. To overcome the costs and limitations, conditioned medium (CM) is used. This study aims to evaluate feasibility of intralesional injection of umbilical cord MSC (UC-MSC) and conditioned medium (UC-CM) compared to TA for keloid therapy. Twenty-four patients with keloids who met the inclusion criteria were included, randomized into three

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treatment groups and then got assessed for the sociodemographic data, keloid volume, histopathology (type 1:3 collagen ratio), interleukin-10 (IL-10) levels and Patient and Observer Scar Assessment Scale (POSAS) score during visits. Largest volume regression occurred in the UC-MSC group, followed by UC-CM and then the TA group (UC-MSC: 45.32% ± 2.61%; UC-CM: 43.61% ± 3.67%; TA: 28.34% ± 3.81%; $p = 0.003$). Similar pattern was also observed in increase in IL-10 levels, the decrease in POSAS scores and the reduction of type 1:3 collagen ratio. Hence, UC-MSC and UC-CM are promisingly more effective than TA for keloid therapy, showcasing their superiority in reducing keloid volume, symptoms and type 1:3 collagen ratio, as well as increasing the levels of IL-10.

KEYWORDS

collagen ratio, interleukin-10, keloid, mesenchymal stem cells, treatment

Key Messages

- Recently, mesenchymal stem cells (MSCs), specifically umbilical cord-derived MSCs (UC-MSCs), are recognized as highly promising allogeneic cell therapy agents, and their role in reducing fibroblast cell activity and proliferation in keloids is significant.
- This study aims to assess the feasibility of intralesional injection of UC-MSC and its conditioned medium (CM) for keloid therapy with its relation to clinical improvement, namely, a decrease in the volume of keloids, which was assessed by CT scan, symptoms by the Patient and Observer Scar Assessment Scale (POSAS), type 1:3 collagen ratio and IL-10 levels.
- UC-MSC and UC-CM are significantly more effective than TA in decreasing keloid volume, symptoms and type 1:3 collagen ratio, as well as increasing the IL-10 levels.
- UC-MSC and UC-CM are pretty promising in keloid therapy, although further studies over a more extended period are needed to determine their cost-effectiveness.

1 | INTRODUCTION

Keloids are excessive proliferation of scar tissue that forms on incisional scars or trauma to the skin that do not regress and grow beyond the initial wound margin. In addition to disrupting appearance, keloids can cause itching, pain and psychological stress.[1–3] Subjects with colour skin have a higher risk of keloids than those with white skin. Epidemiological data on keloids in African descent recorded as much as 6%–16% of the incidence of the total population. Hispanic and Mongoloid races also have a high incidence of keloids.[4,5]

Basically, the problems caused by keloids cause discomfort and significantly reduce the sufferer's quality of life. However, the results of keloid treatment are often unsatisfactory with a recurrence rate of 45%–100%.[3,5,6] Various therapeutic methods have been used, ranging from surgical to non-surgical approaches.[5] Surgical therapy includes excision followed by primary suturing, wound closure with skin grafts and/or flaps, while non-surgical therapies include triamcinolone acetonide (TA) injection, pressure garments, silicone gel or sheet, cryotherapy, radiotherapy and laser.[6,7] Considering the non-invasive nature and relatively low cost of topical therapy, new topical therapeutic methods for treating keloids with higher efficacy and fewer side effects are necessary.[6,8]

Mesenchymal stem cells (MSCs) are known to function as the most promising allogeneic cell therapy agents because they have high proliferative abilities, paracrine effects, multipotential differentiation and function as immunomodulators (including for interleukin-10 (IL-10)).[9] MSC can be obtained from various sources, such as umbilical cord, spinal cord, fatty tissue, peripheral blood cells and dental pulp. Besides being easy to propagate, MSC also has a function in reducing the activity and

proliferation of fibroblast cells in keloids. This was shown in a study by Sato[10] et al, in which keloid, mature and normal fibroblasts were harvested from patients and amnion-derived MSC was given alongside transforming growth factor β (TGF-β) to the cells. The administered amnion-derived MSC significantly suppressed the TGF-β-induced upregulation of α-smooth muscle actin (α-SMA) in keloid fibroblasts and collagen I in keloid fibroblasts, but not in mature fibroblasts.[10]

To overcome the production limitations and costs of cell-based therapy, conditioned medium (CM) is used, which is a metabolic product of the secretion of MSC. The secreted factors are referred to as secretomes, microvesicles or exosomes (which contains IL-10), which can be found in cell culture medium. The CM of MSCs has advantages such as suppressing local immune responses, reducing free radicals, inhibiting fibrosis formation and healthy cell death, stimulating angiogenesis and stimulating endogenous stem cell proliferation and differentiation in healthy tissues.[10]

Based on the existing potential, it is necessary to research on the effectiveness and mechanism of keloid regression in umbilical cord MSC (UC-MSC) therapy and umbilical cord MSC conditioned medium (UC-CM) compared to TA. This study aims to assess the feasibility of intralesional injection of UC-MSC and UC-CM for keloid therapy with its relation to clinical improvement, namely, a decrease in the volume of keloids, which was assessed by CT scan, symptoms by the Patient and Observer Scar Assessment Scale (POSAS), histopathology (type 1:3 collagen ratio) and quantitative in vitro IL-10 levels.

2 | MATERIALS AND METHODS

2.1 | Research design

This study is a double-blind randomized controlled pilot study examining the effect of UC-MSC, UC-CM and TA on keloids carried out using CONSORT statement. Research is directed at studying keloid volume reduction, changes in POSAS score, type 1:3 collagen ratio and IL-10 levels. The laboratory staff prepared the substances in identical syringes without informing the researchers according to randomization. Data processing was carried out by statisticians and clinicians other than researchers.

2.2 | Research population

Patients aged 18–55 years old with keloids attending the Gatot Soebroto Army Hospital, Jakarta, from October 2021 were taken as research subjects after obtaining ethical approval, until the sample size of 24 (8 for each intervention, with power of 0.95) was met in April 2022.

Consecutive sampling was used as subject selection method, and then, the subjects were allocated according to computerized block randomization with block size of 3. The random allocation sequence was generated by administrative staff, the participants were enrolled by one of the researchers, and participants were assigned to interventions by laboratory staff. Patients should have keloids with a length of 2–10 cm and a thickness of 3–5 mm that were located on the chest, back, abdomen and extremities. Patients who have hypertrophic scars; history of kidney failure, hypertension, blood disorders or malignancy; were pregnant or breastfeeding; and had received keloid therapy were excluded.

2.3 | Materials and workflow

The research timeline is shown in Figure 1. Patients were screened initially by measuring the length and thickness of the keloids using a ruler. Patients who met the study inclusion criteria were randomly divided into three groups. Each patient in the groups was given the same injection volume (1 mL) in every cm^3 keloid volume using a 1-mL syringe and 27G needle. The injections were ultrasound-guided into the centre of the lesion with a 30–45° angle using an in-plane technique and with the same pressure. Group 1 was given UC-MSC 2 million cells/mL/cm^3, group 2 was given UC-CM 1 mL/cm^3, and group 3 was given TA 40 mg/mL/cm^3. To generate UC-MSC and UC-CM, 10 cm of umbilical cord was collected in 50 mL of transport medium containing alpha minimal essential medium (MEM [GIBCO 12000–0221]), amphotericin B (final concentration 7500 ng/mL [JR Scientific 50 701]) and penicillin/streptomycin (final concentration 300 U/mL [GIBCO 15 140-122]) and processed in less than 8 hours after collection. The umbilical cord was dissected and washed briefly with 0.5% povidone-iodine (betadine) with phosphate-buffered saline of pH 7.4 (PBS [Sigma P3813]), followed by washing with PBS to remove blood and betadine. In addition, the umbilical arteries and umbilical vein were dissected and discarded, and the umbilical cord was minced in a complete medium. Alpha-MEM and Dulbecco's modified Eagle's medium (DMEM [GIBCO 31600-034]) were used to create UC-CM. The complete medium contained amphotericin B (final concentration 2500 ng/mL), penicillin/streptomycin (final concentration 100 U/mL), 10% TC (Indonesian Red Cross) and 1% L-glutamine (Lonza 17-605C). For MEM, cultures were also supplemented with 10% autologous or allogeneic cord blood serum and 10% human AB serum (GIBCO 34 005-100). Three

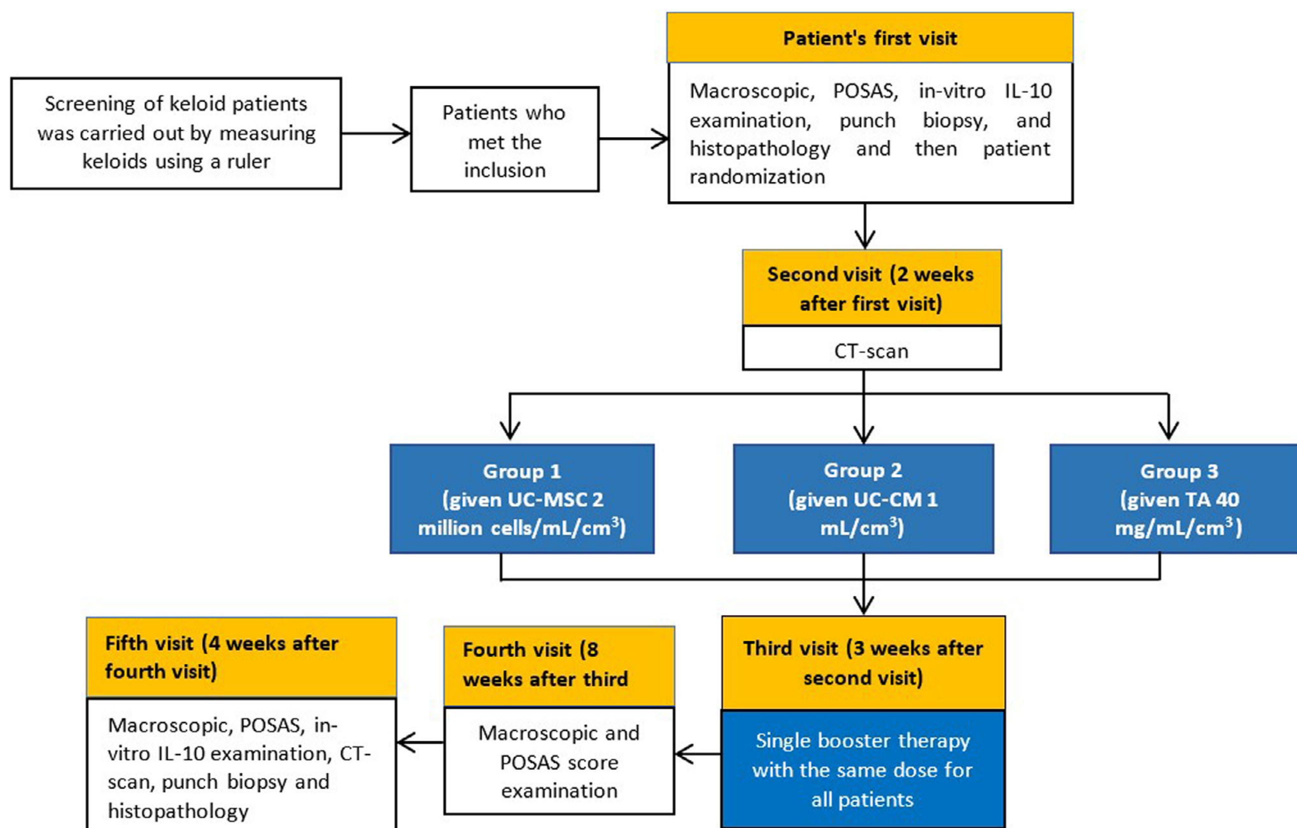

FIGURE 1 Research flow diagram. POSAS: Patient and Observer Scar Assessment Scale; UC-MSC: umbilical cord mesenchymal stem cells; UC-CM: umbilical cord mesenchymal stem cell conditioned medium; TA: triamcinolone acetonide.

explants (diameter 2–5 mm) with Wharton's jelly down were placed in each well of a 12-well plate (growth area 3.8 cm^2 [Biolite]), and several drops of each complete medium were added. Cultures were performed in triplicate for all media. In addition, the plate was incubated at 37°C and 5% CO_2. Cultures were observed daily to check for cell growth or contamination. In the event of contamination, the contaminated wells were removed. When the explant was attached to the plastic, 200–500 μL of the appropriate medium was added. Medium changes were performed every 2–3 days by removing half of the medium and adding half of each medium. When the cells outgrew the explants and were 90% confluent, they were harvested with TrypLE Select (GIBCO 12563-011) and the viable/non-viable cell yield was counted using the dye exclusion method. With the explant still attached, new appropriate medium was added and the plate was reincubated at 37°C and 5% CO_2. The second and subsequent cultures were observed daily to assess cell growth, and when the cells reached 90% confluence, they were harvested. Therefore, an explant could be harvested multiple times.[11] All patients received a single dose and one booster dose.

2.4 | Keloid volume and POSAS examination

The calculation of the percentage of keloid volume regression in each treatment group was carried out by calculating the difference in volume before and after therapy with a three-dimensional CT scan and then expressed as a percentage. POSAS examination will be carried out for subjective and objective assessment.

2.5 | Histopathology and IL-10 quantitative in vitro examination

Punch biopsies of the keloid tissues were then conducted twice, in the first meeting and 17 weeks after. Parameters to be evaluated in anatomic pathology examination are Sirius red staining to evaluate collagen structure under a polarizing lens and in vitro quantitative examination using the ELISA method to examine IL-10. Calculation of changes in the ratio of type 1 to type 3 collagen levels was carried out by dividing the ratio of collagen before treatment from the ratio of collagen after each treatment.

When visualized under a polarizing lens, type-3 collagen will appear as green-birefringence and type-1 collagen will appear as yellow-birefringence (Figure 2). The collagen ratio was obtained by dividing the composition of collagen type 1 to collagen type 3 in Sirius red staining under polarizing lenses using ImageJ program. The percentage of IL-10 increase is calculated in each intervention.

This quantitative in vitro examination uses a quantitative sandwich enzyme immunoassay technique. The preparation of the tissue homogenate will vary depending on the tissue type, as follows: The tissue is rinsed with ice-cold PBS to thoroughly remove excess blood and weighed prior to homogenization. Next, the tissue is chopped into small pieces and homogenized in fresh lysis buffer (MBS catalogue 2 090 451). Different lysis buffers need to be selected based on the subcellular location of the target protein (wy = 120–1:50, e.g., 1 mL of lysis buffer added in a 20–50-mg tissue sample) with a glass homogenizer on ice. The resulting suspension is sonicated with an ultrasonic cell disruptor until the solution becomes viscous. Then, the homogenate was centrifuged for 5 min at 10000×*g*. Collect supernates and test immediately or store at < −20°C. Cells should be lysed prior to testing according to the following instructions: Adhered cells should be gently washed with cold PBS, then separated with trypsin and collected by centrifugation at 1000 mg for 5 min (suspended cells can be collected by direct centrifugation). The cells were washed three times with cold PBS and then resuspended in fresh lysis buffer at a concentration of 10′ cells/mL. If necessary, the cells can be ultrasonicated until the solution is settled. Next, the cells were centrifuged at 1500×*g* for 10 min at 2–8C to remove cellular debris. Test immediately or aliquots and store at −20C.[12]

2.6 | Research ethics and funding

The research protocol has received a letter of passing ethical review by the Health Research Ethics Committee, Faculty of Medicine, University of Indonesia, with the number KET-1206/UN2.F1/ETIK/PPM.00.02/2021. Subjects' identity was maintained confidential, and informed consents were gathered from all the subjects. This trial is registered in clinicaltrials.gov with identifier of NCT05887804. The pilot trial was single-centred, which was conducted in Gatot Soebroto National Army Hospital, Jakarta. The trial was funded by International Indexed Publication (*Publikasi Terindeks Internasional/PUTI*)

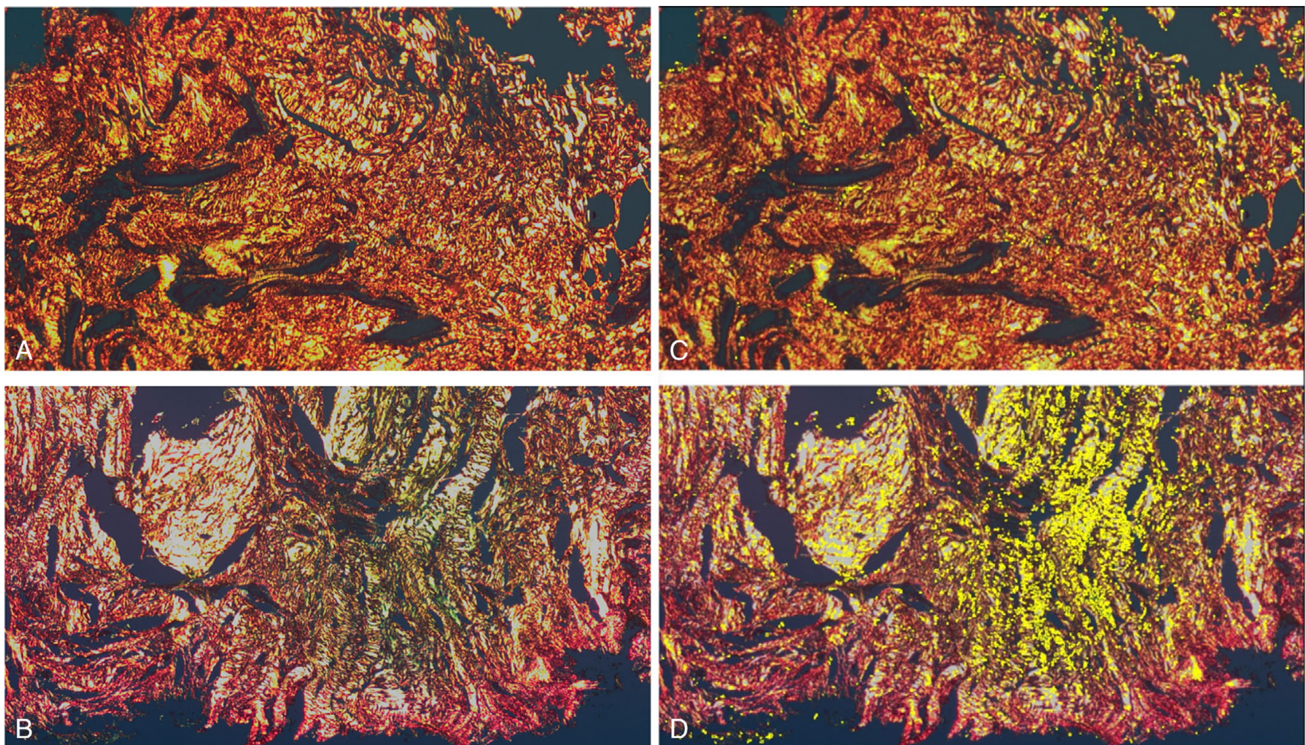

FIGURE 2 Histopathology of Sirius red keloid tissue staining viewed with a polarized lens. (A) Before being given treatment dominated by type-1 collagen, which looks orange yellow under a polarizing lens. (B) After being given UC-MSC, areas of type-3 collagen shown in green (middle). (C) and (D) When done, the type-3 collagen area was selected with the ImageJ application, and the selected area is shown in yellow.

research grant from the Directorate of Research and Development University of Indonesia.

2.7 | Data analysis

After completing data collection, data processing will then be carried out consisting of editing, coding, tabulating, data entry and cleaning using Microsoft Excel 2016 and IBM SPSS Statistics 25. Data analyses used in this study were univariate analysis and bivariate analysis. Univariate analysis was performed to determine the frequency distribution of the research variables. Bivariate analysis was performed for each hypothesis. The statistical test in bivariate analysis will use the ANOVA test if the data were normally distributed, otherwise Kruskal–Wallis test. Subgroup analyses were conducted using independent T-test if the distribution is normal, otherwise the Mann Whitney test.

3 | RESULTS

A total of 24 research subjects were recruited according to the inclusion and exclusion criteria with eight subjects in each group, shown in Figure 3. The recruitment process was conducted from 6th January 2022 until 10th February 2022 until the sample size was met. No subjects dropped out; therefore, all data could be involved in the analysis. The basic characteristics of the research subjects prior to the intervention are given in Table 1. There were no significant differences between groups in terms of age, BMI, blood pressure and keloid location. The proportions of sex, smoking status and age were balanced between

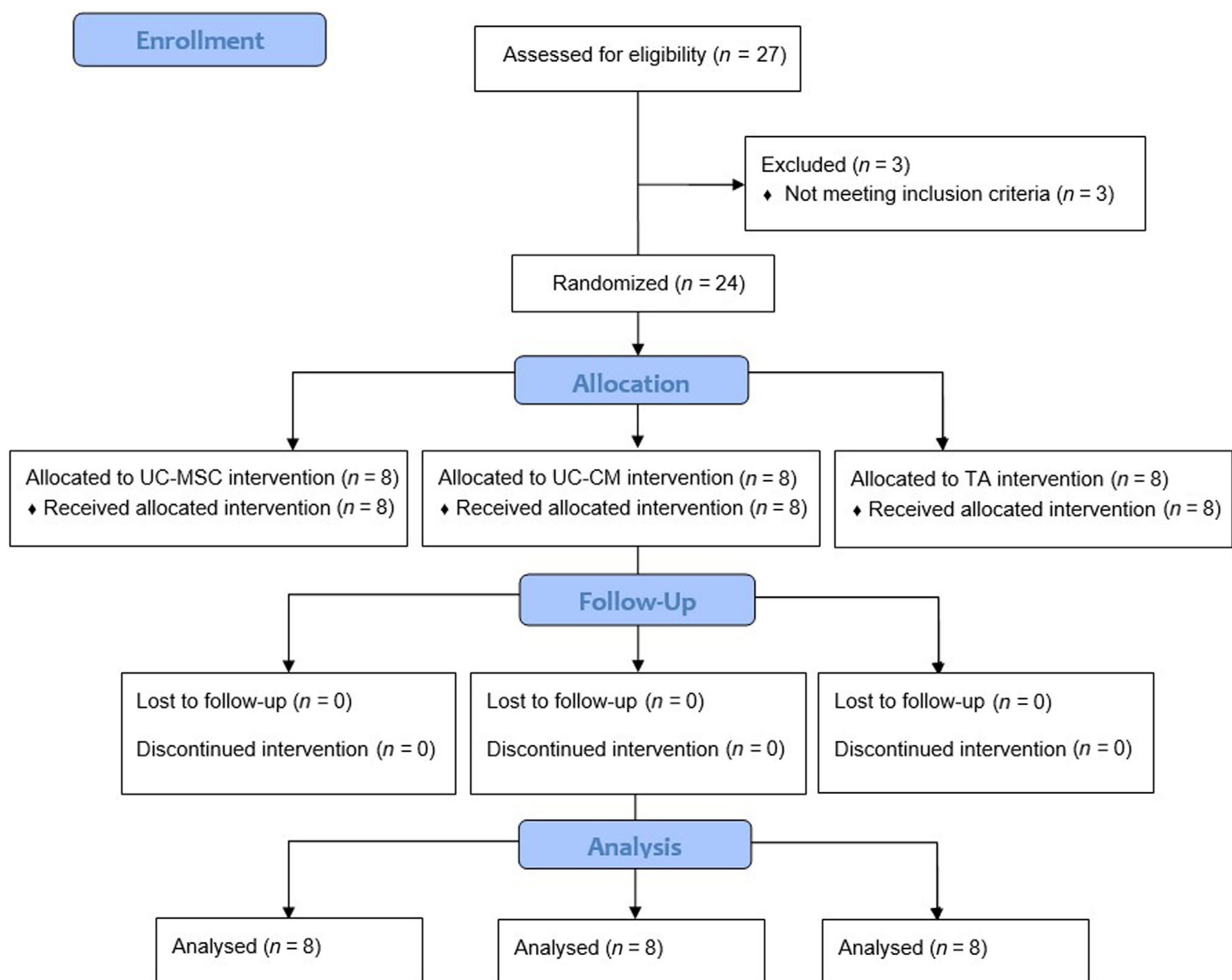

FIGURE 3 CONSORT flow diagram. UC-MSC: umbilical cord mesenchymal stem cells; UC-CM: umbilical cord mesenchymal stem cell conditioned medium; TA: triamcinolone acetonide.

treatment groups. The location of keloids among the three treatment groups does not differ statistically.

3.1 | Keloid volume reduction

From the Shapiro–Wilk normality test, it was found that the data distribution was normal in all three groups (UC-MSC: $p = 0.322$; UC-CM: $p = 0.127$; TA: $p = 0.136$); therefore, one-way ANOVA test was used, showing statistically significant results. The volume measured by CT scan showed a significant difference in the UC-MSC group with respect to TA and UC-CM with respect to TA (Table 2).

3.2 | Changes in POSAS scores

There were significant differences between treatment groups in the subjective components of pain and stiffness, as well as the objective components of vascularity, relief, flexibility and area (Table 3). The graph of the pattern of changes in the total POSAS score in each treatment is shown in Figure 4.

3.3 | Ratio of collagen level type-1: type-3

From the Shapiro–Wilk normality test, it was found that the data distribution was not normal in the UC-MSC group ($p = 0.01$) and TA ($p = 0.00$); therefore, it was continued with the Kruskal–Wallis hypothesis test with statistically significant results. The Mann–Whitney post hoc test showed a significant difference in the UC-MSC to TA and UC-CM to TA groups (Table 4).

3.4 | IL-10 levels

In the Shapiro–Wilk normality test, normal data distribution was found in all three treatment groups (UC-MSC: $p = 0.185$; UC-CM: $p = 0.130$; TA: $p = 0.075$); therefore, one-way ANOVA hypothesis test was used. There was a significant difference between groups ($p = 0.001$), and the post hoc one-way ANOVA test showed a significant

TABLE 2 The percentages of keloid CT volume regression.

Treatment	CT volume regression (%)	*p*-value	Ratio	*p*-value
UC-MSC	45.32 ± 2.61	**0.003****	MSC-CM	1000
UC-CM	43.61 ± 3.67		MSC-TA	**0.006**
TA	28.34 ± 3.81		CM-TA	**0.014**

Note: Bold indicates significant value ($p < 0.05$).
Abbreviations: TA, triamcinolone acetonide; UC-CM, umbilical cord mesenchymal stem cell conditioned medium; UC-MSC, umbilical cord mesenchymal stem cells.
**One-way ANOVA test.

TABLE 1 Basic characteristics of research subjects between groups.

Characteristics	UC-MSC ($n = 8$)	UC-CM ($n = 8$)	TA ($n = 8$)	*p*-value
Gender; *n* (%)				
Man	1 (12.5)	0 (0)	0 (0)	0.352^
Woman	7 (87.5)	8 (100)	8 (100)	
Age (years)	30.38 ± 1.99	29.88 ± 3.54	27.88 ± 1.83	0.770**
BMI (kg/m^2)	21.93 ± 0.87	24.30 ± 1.56	24.42 ± 1.57	0.338*
Smoking status; *n* (%)				
Yes	1 (12.5)	0 (0)	0 (0)	0.352^
No	7 (87.5)	8 (100)	8 (100)	
Blood pressure (mmHg)				
Systolic	112.88 ± 2.64	114.25 ± 3.28	114.12 ± 3.31	0.942**
Diastolic	70.12 ± 2.69	72.12 ± 2.92	75.75 ± 2.55	0.352**
Keloid location				
Trunk	3	5	5	0.511^
Upper extremities	5	3	3	

Abbreviations: BMI: body mass index; TA, triamcinolone acetonide; UC-CM, umbilical cord mesenchymal stem cell conditioned medium; UC-MSC, umbilical cord mesenchymal stem cells.
*Kruskal–Wallis test.
**One-way ANOVA test.
^χ^2 test.

TABLE 3 POSAS score improvement after treatment.

POSAS components	Score improvement UC-MSC	UC-CM	TA	p-value
Subjective				
Pain	3.50 (2–4)	2 (1–5)	1 (0–3)	**0.036***
Itch	4.37 ± 0.82	1.5 (0–10)	2.12 ± 0.40	0.119*
Colour	3.87 ± 0.44	3.62 ± 0.62	2.37 ± 0.65	0.172**
Stiffness	3.62 ± 0.42	3.12 ± 0.29	2.12 ± 0.35	**0.023****
Thickness	4.62 ± 0.37	4.37 ± 0.56	3.62 ± 0.84	0.511**
Regularity	4 (2–6)	4.12 ± 0.74	3.00 ± 0.71	0.225*
Comparison to normal skin	4.25 ± 0.56	5 (1–5)	2.62 ± 0.46	0.075*
Objective				
Vascularity	3.00 ± 0.27	2.50 (2–3)	1.75 ± 0.25	**0.012***
Relief	3 (3–5)	3 (2–4)	2.12 ± 0.29	**0.007***
Flexibility	4 (4–7)	4.25 ± 0.25	3.5 (2–4)	**0.021***
Area	4.00 ± 0.27	4 (3–4)	2.37 ± 0.50	**0.023***

Note: Bold indicates significant value ($p < 0.05$).
Abbreviations: POSAS, Patient and Observer Scar Assessment Scale; TA, triamcinolone acetonide; UC-CM, umbilical cord mesenchymal stem cell conditioned medium; UC-MSC, umbilical cord mesenchymal stem cells.
*Kruskal–Wallis test.
**One-way ANOVA test.

difference between the UC-MSC with TA groups and the UC-CM with TA groups (Table 5).

3.5 | Correlation of the increased IL-10 levels with the ratio reduction of collagen type-1:type-3

To determine the strength of the effect of the percentage increase in IL-10 on the decrease in the ratio of collagen type-1 to collagen type-3, a correlation test was performed. Because the data on the ratio of collagen type-1 to collagen type-3 were not normally distributed, the Spearman correlation test was performed with significant positive correlation result ($r_s = 0.876$, $p = 0.000$).

4 | DISCUSSION

Advances in science and technology provide an alternative to intralesional injection of triamcinolone as the first line of keloid therapy, namely, MSC or CM from MSC (MSC-CM) as an alternative to avoid the side effects caused without reducing the effectiveness of the therapy obtained. MSCs are characterized by their regenerative capacity and have been recognized for their role in accelerating the wound healing process. MSC can develop at the site of injury, transdifferentiate into epidermal or dermal line cells, and possess immunomodulatory, antifibrotic and angiogenic properties by secreting large quantities of paracrine growth factors.[13] In this study, MSC from the umbilical cord was used, which has many advantages compared to MSC taken from other locations. Genomic studies, cell behaviour and stem characterization showed that UC-MSC extracted from Wharton's jelly in the umbilical cord has primitive and unique properties that are different from bone marrow and other MSC related to embryological migration of the yolk sac and aortic-gonadal mesonephros.[14,15] Protected in the umbilical cord, UC-MSC is not polluted from the adult environment with a very high differentiation and self-renewal capacity, easily accepted ethically, does not cause pain during extraction and is easily obtained from medical waste in the form of umbilical cord.[16]

4.1 | Keloid volume reduction

In this study, it was found that the largest volume regression occurred in the UC-MSC group, followed by UC-CM and then the TA group (UC-MSC: 45.32% ± 2.61%; UC-CM: 43.61% ± 3.67%; TA: 28.34% ± 3.81%; $p = 0.003$). When compared between groups, UC-MSC to TA ($p = 0.006$) and UC-CM to TA ($p = 0.014$) showed significant differences, while UC-MSC to UC-CM was not significantly different. Several studies have reported that UC-MSC has unique tumoricidal properties.[14,17] There is high expression of tumour suppressor genes

and pro-apoptotic genes in UC-MSC. UC-CM and cell lysate from UC-MSC were found to inhibit the growth of breast and ovarian adenocarcinoma and osteosarcoma cells in vitro. Furthermore, congenital abdominal hernias have been successfully treated by attaching the baby's own umbilical cord containing Wharton's jelly to the hernia without scarring and keloid formation due to the tumoricidal nature of UC-MSC.[14]

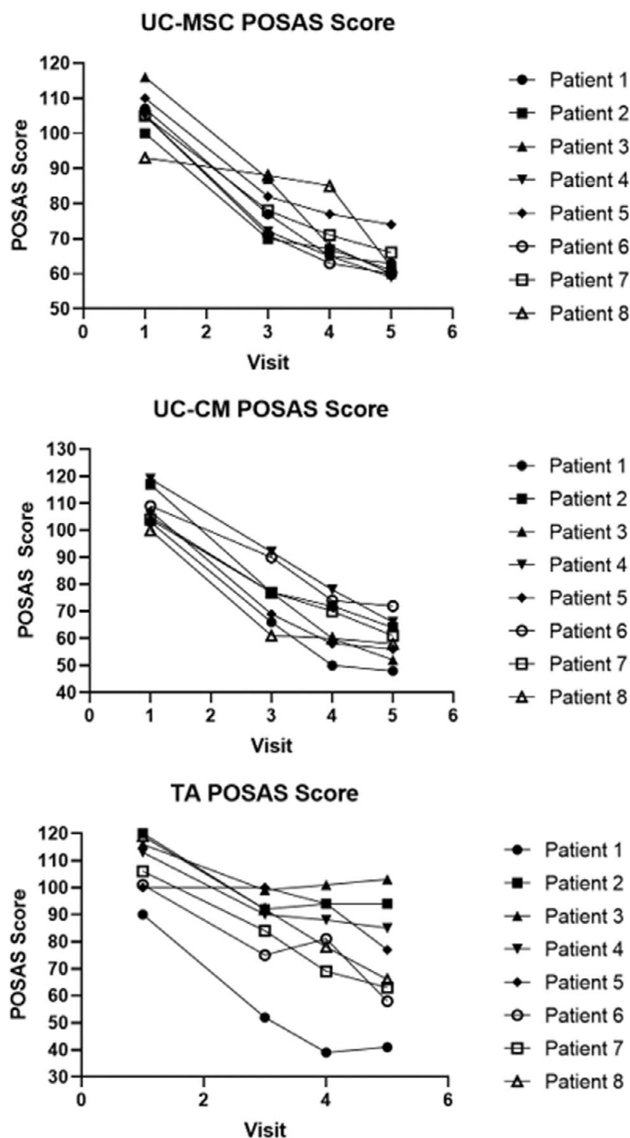

FIGURE 4 Graph of the pattern of changes in the total POSAS score in each treatment at each visit. POSAS: Patient and Observer Scar Assessment Scale; UC-MSC: umbilical cord mesenchymal stem cells; UC-CM: umbilical cord mesenchymal stem cell conditioned medium; TA: triamcinolone acetonide.

This is in line with a study by Arjunan[14] et al, which found a decrease in keloid volume and weight after 30 days in mice with congenital immune disease injected with UC-CM compared to controls (CM of human skin fibroblasts) in vitro and in vivo. This shows that UC-MSC has tumoricidal properties, inhibiting the growth of various cancers both in vitro and in vivo. Another study by Liu[17] et al in 2018 proved that UC-MSC has increased the expression of tumour suppressor genes and anti-apoptotic genes compared to other types of stem cells such as human embryonic stem cells and bone marrow MSC. UC-CM also inhibits the growth of lymphoma cells indicating the presence of anti-cancer molecules secreted by UC-CM. Antifibrotic effects have also been found in CM of adipose tissue and bone marrow MSC.[17] UC-CM and UC-MSC contain tumoricidal molecules that inhibit the growth of keloid cells given the fact that keloids behave like benign tumours with uncontrolled growth.[14] This is evident from the results of research by obtaining a significant decrease in keloid tissue volume in the UC-CM and UC-MSC treatment group. In a study of keloid cells isolated from Asian populations, the tumoricidal effect of UC-CM and UC-MSC was associated with increased expression of proapoptotic and autophagy genes (BECLIN-1, BAX, ATG5, ATG7) and decreased anti-apoptotic genes (SURVIVIN), which works in the mitotic phase, thereby inhibiting the proliferation of keloid cells.[14,17]

The insignificant difference shown by UC-CM against UC-MSC in reducing keloid volume on keloid CT scans shows that UC-CM provides a keloid volume reduction effect that is no worse than UC-MSC. Until now, there have been no studies that have compared the effects of UC-MSC, UC-CM and TA in reducing keloid volume. However, a study by Sato[10] et al observing the effect of amnion-derived MSC-CM on normal fibroblasts and keloid fibroblasts showed results that were in line with

TABLE 4 The ratio reduction of type 1:3 collagen ratio in the three treatments.

Treatment	Ratio reduction of type 1:3 collagen ratio	*p*-value	Comparison	*p*-value
UC-MSC	4.60 (4.15–8.05)	**0.002***	MSC-CM	0.529^
UC-CM	4.80 ± 0.26		MSC-TA	**0.001^**
TA	3.96 (1.63–4.14)		CM-TA	**0.009^**

Note: Bold indicates significant value ($p < 0.05$).
Abbreviations: TA, triamcinolone acetonide; UC-CM, umbilical cord mesenchymal stem cell conditioned medium; UC-MSC, umbilical cord mesenchymal stem cells.
*Kruskal–Wallis test.
^Mann–Whitney test.

TABLE 5 The percentage increase in IL-10 levels in the three treatments.

Treatment	IL-10 percentage increase	*p*-value	Comparison	*p*-value
UC-MSC	78% ± 15%	**0.001****	MSC vs CM	0.349
UC-CM	53% ± 3%		MSC vs TA	**0.011**
TA	17% ± 7%		CM vs TA	**0.004**

Note: Bold indicates significant value ($p < 0.05$).
Abbreviations: TA, triamcinolone acetonide; UC-CM, umbilical cord mesenchymal stem cell conditioned medium; UC-MSC, umbilical cord mesenchymal stem cells.
**One-way ANOVA test.

the present study. Amnion-derived MSC-CM was observed to increase the proliferation of mature fibroblasts but significantly suppressed the proliferation of keloid fibroblasts. Amnion-derived MSC-CM significantly suppresses TGF-β-induced α-SMA upregulation in keloid fibroblasts and normal fibroblasts and suppresses collagen I in keloid fibroblasts but not in mature fibroblasts. Amnion concentrate significantly suppresses TGF-β-induced α-SMA expression.[10]

It has been confirmed that UC-MSC mostly exerts its therapeutic effect via soluble paracrine bioactive factors and extracellular vesicles, especially the secretome present within the UC-CM. These secreted components play an important role in modulating the immune response, enhancing UC-MSC survival and enhancing damaged tissue regeneration. Oxidative stress is one of the conditions that negatively affect the quality of stem cell therapy and regenerative medicine. High levels of reactive oxygen species (ROS) produced during stem cell isolation, cell culture and transplantation cause oxidative stress that can induce apoptosis and limit the regenerative ability of stem cells.[18] In summary, ROS accumulation in MSC contributes to loss of homeostasis, leading to premature senescence and decline in the quality of MSC.[19] This can lead to the insignificant keloid volume reduction difference between UC-CM and UC-MSC groups. Lifestyle factors such as smoking and psychological stress are important modulators of oxidative stress, which both decreases MSC biofunction and proliferation capabilities.[20,21]

4.2 | Changes in POSAS score

The POSAS score consists of subjective parameters and objective parameters. The UC-MSC group experienced the most decrease in POSAS scores for all objective parameters, followed by the UC-CM and TA groups with significant differences. For subjective parameters, only the parameters of pain and stiffness were significantly different between groups, with the greatest decrease in pain and stiffness in the UC-MSC group followed by UC-CM and TA. The decrease in the stiffness and flexibility of keloid tissue is probably related to the composition of collagen type-1 and collagen type-3, in which collagen type-1 is more rigid and larger in size than collagen type-3.[22]

For pain and itch parameters, UC-CM and UC-MSC showed a greater reduction than TA. This was due to the greater anti-inflammatory effect and slower growth of keloids in the UC-CM and UC-MSC groups. Macrophages in keloids have M2 polarization and respond to inflammation in keloids by triggering Th2 cells to mount a chronic immunological response. Dermal mast cells are also found more in keloids than in normal tissue. Immediately after injury, macrophages secrete TGF-β, which causes fibroblasts to proliferate and synthesize collagen. In addition, TGF-β and histamine from mast cells stimulate dermal fibroblasts to produce periostin, a matricellular protein that contributes to tissue remodelling and more to pathological scarring. Periostin was found to be a pruritogen, which induces itching via the Th2 cytokine cascade. It has also been suggested that neuronal dysfunction and the phenomenon of compression neuropathy in keloids contribute to the itching and pain. Increased thermosensory thresholds for heat, cold, pain, allokinesis and allodynia are found in keloids, suggestive of neuropathy. Fibroblast proliferation and excessive collagen deposition lead to compression of nociceptive afferent neurons. This compression can then induce a neuropathic pain response similar to compression neuropathy.[23]

In Figure 4, it can be seen that in the UC-MSC and UC-CM treatments, a decrease in the POSAS score always occurred in each measurement. However, the POSAS score reduction in UC-MSC began to decline during the fourth and fifth visits, concluding that a UC-MSC booster injection is needed between the third and fourth visits (3–11 weeks from the first visit). In contrast to the TA group, there were several subjects who experienced an increase in POSAS scores when measured at the fourth and fifth visits. This suggests that for intralesional TA injections, a repeat injection is needed 3–11 weeks after the first injection to maintain a subjective therapeutic effect in the patient, in line with the theoretical basis, which states that intralesional TA injections should be repeated once every 4 weeks.[24]

4.3 | Histopathology (type 1:3 collagen ratio)

In this study, it was found that the ratio of type-1 to type-3 collagen decreased the most in keloid cells that received UC-CM injection, followed by UC-MSC and TA

(UC-MSC: 4.60 (4.15–8.05); UC-CM: 4.8 ± 0.26; TA: 3.96 (1.63–4.14); $p = 0.002$) with a significant difference, and UC-MSC to TA and UC-CM to TA with UC-MSC to UC-CM is not significantly different. The keloid genetic marker identified is the TGF-β or SMAD family. Its role in pathological fibrogenesis includes promoting fibroblast proliferation and increasing the synthesis and deposition of type-1 collagen more than type-3 collagen.[25] In keloids, it was found that, in addition to increased collagen production, the ratio of type-3 collagen compared to type-1 collagen was lower than normal skin, which is also one of the causes of denser and stiffer tissue in keloids.[3] Although the composition of procollagen type-1 mRNA in keloids is much increased compared to normal skin, the composition of procollagen type-3 mRNA is not changed. Thus, the procollagen type-1/type-3 mRNA ratio in keloids increased significantly (22.1) compared to the ratio in normal skin (5.2). On the other hand, in hypertrophic scars, the ratio of type-1/type-3 collagen averaged 7.73; this was significantly lower than the ratio in keloids (17.28).[24] In this study, it was found that the ratio of type-1 collagen to type-3 collagen was in accordance with the theoretical basis, which was 15.44. According to researchers, the decrease in the ratio of type-1 collagen to type-3 collagen that occurred in the group that was given UC-MSC and UC-CM was due to the presence of IL-10 and other antifibrotic factors produced by UC-MSC. This decrease in type-1 collagen ratio is in line with a study by Sato[10] et al, which proved that UC-CM can reduce type-1 collagen production in keloid fibroblasts by lowering TGF-β levels.

4.4 | Quantitative in vitro IL-10 levels

In this study, the highest increase in IL-10 levels was found in the UC-MSC group, followed by the UC-CM and TA groups, respectively, with significantly different results. There are no other studies that have compared IL-10 levels in keloid tissue treated with UC-MSC or UC-CM against TA. IL-10 is a cytokine that is produced by a variety of cell types, including monocytes, macrophages and T cells. Keratinocytes have also been shown to be capable of producing IL-10 after injury. The pleiotropic effect of IL-10 as a keloid treatment is achieved through the following mechanisms: (1) modulating the recruitment and differentiation of inflammatory cells and reducing their secretion of pro-inflammatory cytokines, (2) down-regulating the TGF-β/SMAD signalling pathway, (3) increasing matrix breakdown extracellular by increasing proteolytic enzymes and (4) degrading the extracellular matrix.[25] IL-10 is a potent immunosuppressive that is known to be upregulated in foetal tissues to increase their regenerative capacity. Contrary to the occurrence of keloid scars in adults, the foetus in the second trimester can restore the injured skin architecture to be unmarked. A cytokine hypothesis was proposed as the theoretical basis, which refers to the regenerative capacity of foetal tissue shown to be produced by relatively higher levels of anti-inflammatory cytokine (IL-10) than pro-inflammatory cytokine (IFN-γ, TNF-α, IL-2, IL-6, IL-8 and IL-12). The lower foetal pro-inflammatory response is not a result of deficiency or immaturity of foetal immune cells, but rather due to suppression of chemo-attractant signals in foetal injury. By inhibiting TGF-β/SMAD signalling, concomitant administration of IL-10 reduces collagen expression and promotes extracellular matrix degradation because TGF-β1 inhibits matrix metalloproteinase (MMP) activity.[25,26] When IL-10 is administered to adult mice and the inflammatory response decreases, scar-free results are obtained.[3] However, considering IL-10 levels found in the subjects of this study tended to be small in quantity, other antifibrotic factors (IDO, PGE2) should also be considered and investigated further.[27]

A very strong and significantly different positive correlation was shown between IL-10 levels and a decrease in the ratio of type-1 collagen to type-3 collagen. IL-10 can directly suppress the growth of keloid fibroblasts in a dose-dependent manner.[26] Keloid fibroblast proliferation was suppressed from day 2 ($p < 0.05$) by increasing the IL-10 concentration gradient (5, 10 and 20 ng/mL) relative to the positive control, which was 0.2 mg/mL 5-FU. Morphological changes in keloid fibroblasts were also observed at 20 ng/mL IL-10, whereby keloid fibroblasts were drastically shorter and smaller with wider intercellular spaces.[26] By suppressing the growth of keloid fibroblasts, the ratio of type-1 collagen to type-3 collagen will also be reduced.[25]

4.5 | Research implications

The first part of this study compared the reduction in keloid tissue volume from CT scans in the three treatments. It is proven that UC-MSC and UC-CM reduce keloid volume more significantly than TA. However, the symptomatic improvement measured by POSAS scale was declining in the UC-MSC group as observed during the fourth and fifth visits, also showing no statistical difference towards UC-CM treatment group in several POSAS scale components. Therefore, it is necessary to carry out further research on the cost-effectiveness of UC-MSC and UC-CM with a longer study period considering that UC-MSC can be given only once, while UC-CM needs to be given repeatedly to maintain its

effectiveness as a keloid therapy. Research that compares the cost-effectiveness of biological agents, either UC-MSC or UC-CM with more conventional methods such as surgery, can also be carried out. Apart from that, it can also be important to educate patients about the importance of not smoking, exercising regularly and avoiding stress and pollutants so that the quality of the implanted UC-MSC remains optimal in providing the effect of keloid therapy.

This study has limitations, namely, the cost-effectiveness between UC-CM and UC-MSC cannot be determined because the research period is only 17 weeks due to limited research funds and the high cost of UC-MSC; therefore, the difference in the effectiveness of UC-MSC to UC-CM is not significant. As a result, UC-MSC is not significantly more effective at reducing type 1:3 collagen ratios or increasing IL-10 levels than UC-CM. However, in this study, we can already see the promising effect of UC-MSC on keloids. The price of UC-CM is much cheaper than UC-MSC; hence, UC-CM is expected to be a cheap alternative to keloid therapy, if it is proven better after testing its cost-effectiveness against UC-MSC. In this study, it is suggested to repeat UC-CM injection in the range of 3–11 weeks after the last injection. Type 1:3 collagen ratios decreased significantly when the IL-10 level increased. This shows the effectiveness of IL-10 as a keloid therapy agent, but there are still other factors/cytokines such as IDO and PGE2 that affect the size and ratio of keloid collagen so that it must be studied further. Therefore, it is necessary to carry out further observations to determine the exact UC-CM booster period.

5 | CONCLUSION

This pilot trial found that UC-MSC and UC-CM are significantly more effective than TA in decreasing keloid volume, symptoms and type 1:3 collagen ratio, as well as increasing the IL-10 levels. This study is only limited to looking at the effectiveness of UC-MSC, and the obtained side effects are more comfortable for patients. Hence, UC-MSC and UC-CM is pretty promising as a keloid therapy. Further studies over a more extended period are needed to determine their cost-effectiveness.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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