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Research Paper

Autologous Stem Cell Transplantation Promotes Mechanical Stretch Induced Skin Regeneration: A Randomized Phase I/II Clinical Trial

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ABSTRACT

Background: Mechanical stretch, in term of skin expansion, can induce effective but limited in vivo skin regeneration for complex skin defect reconstruction. We propose a strategy to obtain regenerated skin by combining autologous stem cell transplantation with mechanical stretch.
Methods: This randomized, blinded placebo-controlled trial enrolled 38 adult patients undergoing skin expansion presenting with signs of exhausted regenerative capacity. Patients randomly received autologous bone marrow mononuclear cell (MNC) or placebo injections intradermally. Follow-up examinations were at 4, 8 weeks and 2 years. The primary endpoint was the volume achieved in relation to the designed size of the expander (expansion index, EI). Secondary endpoints were surface area, thickness and texture of expanded skin. This trial is registered with ClinicalTrial.gov, NCT01209611.
Findings: The MNC group had a significantly higher EI at 4 weeks (mean difference 0.59 [95% CI, 0.03–1.16]; $p = 0.039$) and 8 weeks (1.05 [95% CI, 0.45–1.66]; $p = 0.001$) versus controls. At 8 weeks, the MNC group had significantly thicker skin (epidermis: $p < 0.001$, dermis: $p < 0.001$) and higher subjective scores for skin quality/texture (24.8 [95% CI, 17.6–32.1]; $p < 0.001$). The MNC group had more skin surface area (70.34 cm^2 [95% CI, 39.75–100.92]; $p < 0.001$). Patients in the MNC group gained up to the quadrupled surface area of expanded skin compared to pre-expansion at the end of expansion. No severe adverse events occurred.
Interpretation: Intradermal transplantation of autologous stem cells represents a safe and effective strategy to promote in vivo mechanical stretch induced skin regeneration, which can provide complex skin defect reconstruction with plentiful of tissue.

1. Introduction

Skin losses as results of a number of common diseases and injuries affect over 11 million people worldwide annually (Clark et al., 2007; Peck, 2011). Repairing massive skin deficiencies remains as a major clinical challenge, due to lack of suitable cutaneous tissue. Mechanical stretch is a stimulus that can initiate both in vivo and in vitro cell proliferation and regeneration (Mihic et al., 2014; Li et al., 2013). The clinical application of mechanical stretch, skin expansion, is a reliable reconstructive method for complex wounds. Constant mechanical stretch induced by inflating silicone expander stimulates in vivo skin regeneration (Handschel et al., 2013) that creates additional well-vascularized cutaneous tissue that is well-matched to the color, texture and contour of surrounding skin (Weng et al., 2010; Xie et al., 2013).

Often times in large area of tissue reconstruction, more skin is needed for reconstruction than tissue expansion can provide, because skin does not have the growth capacity to be expanded beyond two to three times its original area. Overexpansion results in thin, poorly vascularized tissue, which leads to skin necrosis and reconstructive failure (Huang et al., 2011; Elshahat, 2011). Bone marrow stem cell therapies have showed promise in promoting tissue regeneration, as applications have been reported in wound healing, critical limb ischemia, bone growth and cardiac tissue remodelling (Backly and

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Cancedda, 2010; Liu et al., 2012). We proposed the hypothesis that the transplantation of stem cells could possibly promote mechanical stretch induced skin regeneration and mitigate traditional limitations of skin expansion. Our recent preclinical findings supported this hypothesis (Yang et al., 2011). Additionally, combining mechanical stretch and stem cell transplantation for skin regeneration may wider the clinical implications for other types of in vivo tissue regeneration.

In this phase 1/2 trial, we aimed to evaluate the safety and efficacy of intradermal injection of autologous bone marrow mononuclear cells (MNCs) in promoting mechanical stretch-induced skin regeneration in adult patients.

2. Methods

2.1. Study Design

This 2-year study was a parallel, randomized, blinded, placebo-controlled clinical trial.

The study consisted of two phases. Phase 1, the pilot, was used to determine the sample size for Phase 2 and randomized 10 patients (5 to treatment and 5 to control). It showed that with 15 patients per study arm, the statistic power would be >90% (two-sided, alpha = 0.05). Assuming an attrition rate of 25% over the course of the study, we totally enrolled 38 patients (19 per group.)

2.2. Patients

The recruitment began on March 8th, 2011. Eligible patients were 18 to 60 years of age, had received surgical placement of an expander on the face, neck, anterior chest wall, abdominal wall or back that had been inflated to volumes of 80 to 600 cm^3. All patients had deterioration in expanded skin texture and thin/papery skin overlying the expander that did not improve after a 2 weeks' suspension of expansion, had elevated pressures in the tissue expander for at least 2 weeks, and needed further skin expansion to complete reconstruction. All patients were assessed by two independent plastic surgeons for eligibility.

Exclusion criteria included a history of severe illnesses, including any cancers, hepatitis, coronary artery disease, arteriosclerosis, diabetes, obesity (BMI > 30), anemia, myelodysplastic syndromes, and a history of bone marrow transplantation. Patients with risk factors for tissue necrosis such as steroid use and smoking within the previous 6 months were excluded.

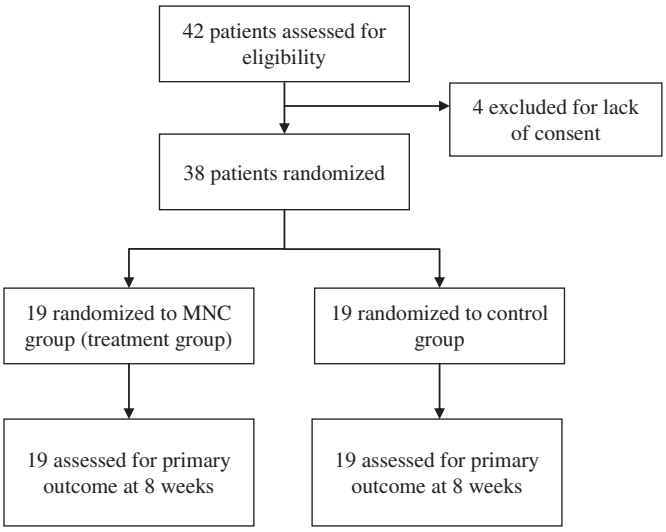
2.3. Randomization and Masking

After patients signed the informed written consent forms, they were randomized into two study groups, the MNC Group and the Control Group, in a 1:1 ratio by a computer-generated randomization schedule. The patient recruiting team randomized participants along the schedule and the conduct team performed the assigned treatment. Participants, data collectors, laboratory staff were masked during the trial. See Table 1 for the trial profile.

2.4. Intervention and Tissue Expansion Protocol

After randomization, the treatment group had bone marrow aspirate harvested from the anterior iliac crest under general or local anesthesia. MNCs were isolated using density gradient centrifugation (COBE 2991 Cell Processor, Gambro BCT, Lakewood, CO). An automated haemocytometer was used to confirm uniform cell counts in each MNC preparation; nucleated cell viability was assessed using trypan blue exclusion. The cell suspension including a total of $2.4–12.5 \times 10^7$ cells was adjusted to a final volume of 6–20 ml with saline. The flow cytometry analysis showed that MNCs samples contained an average of 2.37% CD34 cells, 0.92% CD90 cells, 3.51% CD105 cells and 0.95% CD133 cells. Approximately 0.1 ml cell suspension was injected

Table 1
Clinical trial protocol.



intradermally into per cm^2 expanded skin via a 27-gauge needle (approximately $0.5–1 \times 10^6$ cells/cm^2). An equal volume of saline was injected in the control group.

All patients continued with expander inflation immediately after cell transplantation, every three days to a target inner pressure of 100 mmHg. The inner pressure of the tissue expander was measured during each inflation, with the inner pressure meter reported previously (Sheng et al., 2013). The inflation stopped while the inner pressure of expander reached 100 mmHg. Inflation continued for at least 8 weeks, until sufficient tissue for reconstruction was obtained, or until skin had reached its maximal regenerative capacity. At that point, the expanded flap was surgically transferred to the recipient site.

2.5. Assessment and End Points

Outcomes were assessed at baseline (immediately before treatment) and 4 weeks, 8 weeks and two years after treatment. The primary end point was the absolute change in the expansion index measured at 4 weeks and 8 weeks after treatment. Secondary end points were changes in skin surface area, thickness and texture at 8 weeks versus baseline. Safety evaluations were conducted at two years.

2.5.1. Expansion Index (EI)

The volumes inflated at each visit were summed to obtain the total inflation volume. Each patient's tissue expander had a slightly different maximum capacity. To account for these sizes differences, we calculated an expansion index (EI), the total volume inflated divided by the designed volume of the expander.

2.5.2. Expanded Skin Surface Area

Before treatment and at 8 weeks post-treatment, all patients underwent 3-dimenssional laser scanning (Vivid 910, Minolta, Japan, with RapidForm 2006 software, INUS Technology) to measure the expanded skin's surface area. The surface area gained during the treatment phase was calculated by comparing the surface area at baseline versus 8 weeks.

2.5.3. Expanded Skin Thickness

The epidermal and dermal thicknesses of the expanded skin were measured at eight evenly spaced, marked points along the longitudinal axis using a duplex ultrasonic scanner (GE E8, 14 MHz) at baseline, 4 weeks and 8 weeks. Epidermal thickness was defined as the distance between the air-epidermal and epidermal-dermal high echo-level

bands. Dermal thickness was defined as the distance between the epidermal-dermal and dermal-subdermal high echo-level bands.

2.5.4. Expanded Skin Quality/Texture

We constructed a Visual Analogue Scale (VAS) for patients to self-assess the texture of their expanded skin (Paul-Dauphin et al., 1999). The left anchor of the VAS was set as "significantly deteriorated" and the right anchor was set as "significantly improved". All patients assessed the texture of their expanded skin at baseline, 4 weeks and 8 weeks.

2.5.5. Histological Examination

Tissue specimens were harvested during flap transfer surgery and included the expanded skin with adjacent unexpanded skin. Specimens were fixed with 4% paraformaldehyde and embedded in paraffin. 6 μm sections were stained with hematoxylin-eosin (H&E) and immunohistochemistry with an anti-PCNA antibody was used to identify proliferating cells. The number of PCNA-positive cells was counted in 5 random high-power fields (HPFs, 400X) using laser scanning confocal microscopy (Leica, Germany). Anti-CD31 antibody was applied to evaluate skin vascularization.

2.5.6. Safety

At two-years post treatment, all patients were brought to the clinic for a complete blood count with differential, history and physical exam.

2.6. Study Oversight

This study, registered with ClinicalTrial.gov (number NCT01209611), was approved by the institutional review board of Shanghai Ninth People's Hospital and conducted in accordance with the Declaration of Helsinki. Sponsors had no role in the study design, data collection, data analysis, data interpretation, or writing of this report.

2.7. Statistical Analysis

Continuous variables are presented as mean(standard deviation), unless noted. Categorical variables are presented as counts and percentages. Chi-squared and Student's t-tests were used to compare groups. Analysis of end points was performed on an intention-to-treat basis. Fisher's Exact Test was used when expected values of the cell counts were low while evaluating group associations at different time points. Safety analysis was conducted for all patients who had undergone randomization and treatment. The repeated-measure linear model was used to identify changes in the continuous variables over time for each group. Significance was set at alpha < 0.05. SPSS (version 16.0) was used for analyses.

3. Results

3.1. Patient Characteristics

Of the 42 patients recruited between March 2011 and March 2013, four were excluded because of refusing to sign inform consent. 38 patients were enrolled, with 19 randomly assigned to treatment and 19 to placebo. No patient dropout occurred after randomization. Baseline demographic and disease characteristics were not significantly different between the groups (Table 2).

Table 2
Demographic and baseline characteristics of patients.

Characteristic	Treatment group		Control group	
	Count[a] (N = 19)	%	Count[a] (N = 19)	%
Age in years, mean(SD)	34.32(12.73)		34.89(12.86)	
Male	15	78.95	14	73.68
Smoker (last 6 months)	5	26.32	6	31.58
Cause of defect				
Post-burn scar	17	89.47	18	94.74
Nevus	2	10.53	1	5.26
Site of expanded skin				
Face	4	21.05	5	26.32
Neck	7	36.84	4	21.05
Trunk	8	42.11	10	52.63
Time from tissue expander placement to study enrollment (months)	13.42(2.36)		13.26(2.62)	
Maximum size of tissue expander(ml)				
<100	2	10.53	3	15.79
100–250	9	47.37	9	47.37
>250	8	42.11	7	36.84
Previous expansion at the same site				
None	13	68.42	15	78.95
Once	6	31.58	4	21.05
Baseline expander inflation volume	595.39(464.97)		533.82(369.14)	
Baseline Expansion Index (EI), mean(SD)	2.55(0.86)		2.44(0.68)	
Baseline appearance of expanded skin				
Papery	13	68.42	11	57.89
Stretch marks	4	21.05	7	36.84
Consistent High Inner Pressure	3	15.79	2	10.53

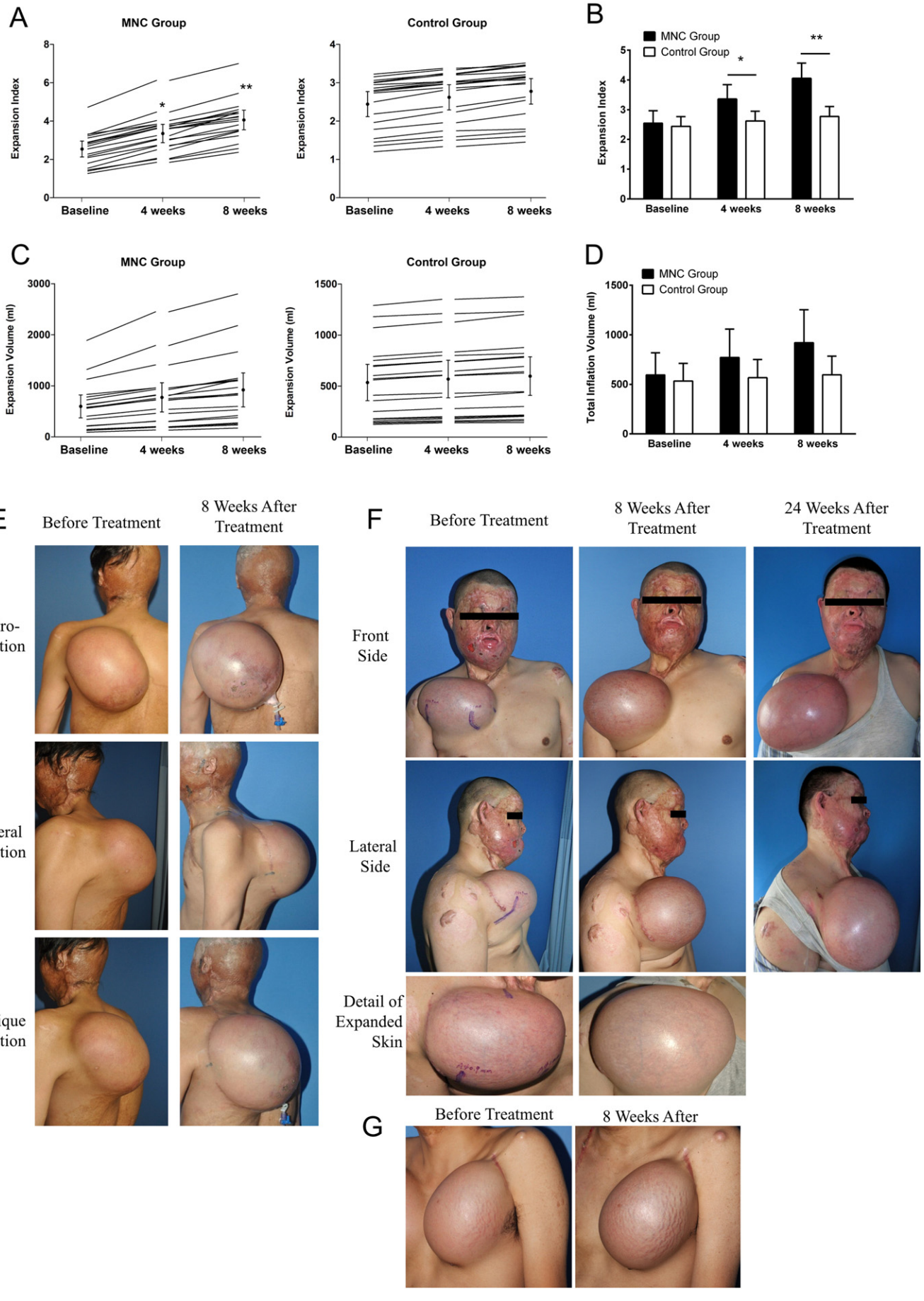
[a] Unless otherwise defined.

3.2. Primary Outcomes

The treatment group had significantly higher EI versus controls at 4 weeks (mean difference 0.59 [95% CI 0.03–1.16]; p = 0.039) and 8 weeks (mean difference 1.05 [95% CI 0.45–1.66]; p = 0.001) (Fig. 1). At baseline, patients from both groups had similar expander inflation volumes (595.39(464.97) ml vs. 533.82(369.14) ml, p = 0.65) and EIs (2.55(0.86) vs. 2.44(0.68), p = 0.67). At 4 weeks post treatment, patients in the treatment group had a total inflation volume of 771.63(594.13) ml and EI of 3.36(1.00), versus patients in the control group with a total inflation volume of 595.55(392.19) ml and EI of 2.76(0.69). At 8 weeks post treatment, the treatment group had an inflation volume and EI of 920.95(688.70) ml and 4.05(1.06), respectively; the control group had inflation an expansion volume and EI of 640.68(412.69) ml and 3.00 (0.74), respectively (Fig. 1).

All patients from the treatment group were able to continue expander inflation longer than 4 weeks (Fig. 1), whereas only 5 patients in the in control group were able to do so.

Fig. 1. Changes in the EI and expansion volume. The MNC group had significant an increase in EI at both 4 and 8 weeks. (A), higher EI than the control group at 4 and 8 weeks. p = 0.039 and p < 0.001, respectively (B). The increase in total inflation volume in treatment versus control groups was showed (C–D). Patient 1: prior to enrollment, this patient's expanded skin had deteriorated in thickness and quality. At 8 weeks, photographs show a significant increase in the expanded skin surface area and quality, and was successfully used for face and neck reconstruction (E). Patient 2: prior to study enrollment, the patient had stretch marks throughout the expanded skin and could not continue expansion due to thinness of the skin. After MNC injection, stretch marks diminished and skin growth improved. Photographs show a significant increase in the expanded skin surface area at 8 weeks after treatment, which continued to grow for an additional 16 weeks. At the end of the expansion process, the donor site skin had quadrupled in surface area, from 15 * 13 cm^2 to 35 * 32 cm^2 (F). Patient 3: case from the Control Group. The patient presented with stretch marker at the baseline. At 8 weeks, the expanded skin became deteriorated with more severe stretch markers (*p = 0.039, **p < 0.001).


A
MNC Group
Control Group
Expansion Index
Baseline
4 weeks
8 weeks
B
MNC Group
Control Group
Expansion Index
C
MNC Group
Control Group
Expansion Volume (ml)
D
MNC Group
Control Group
Total Inflation Volume (ml)
E
Before Treatment
8 Weeks After Treatment
Retro-position
Lateral Position
Oblique Position
F
Before Treatment
8 Weeks After Treatment
24 Weeks After Treatment
Front Side
Lateral Side
Detail of Expanded Skin
G
Before Treatment
8 Weeks After

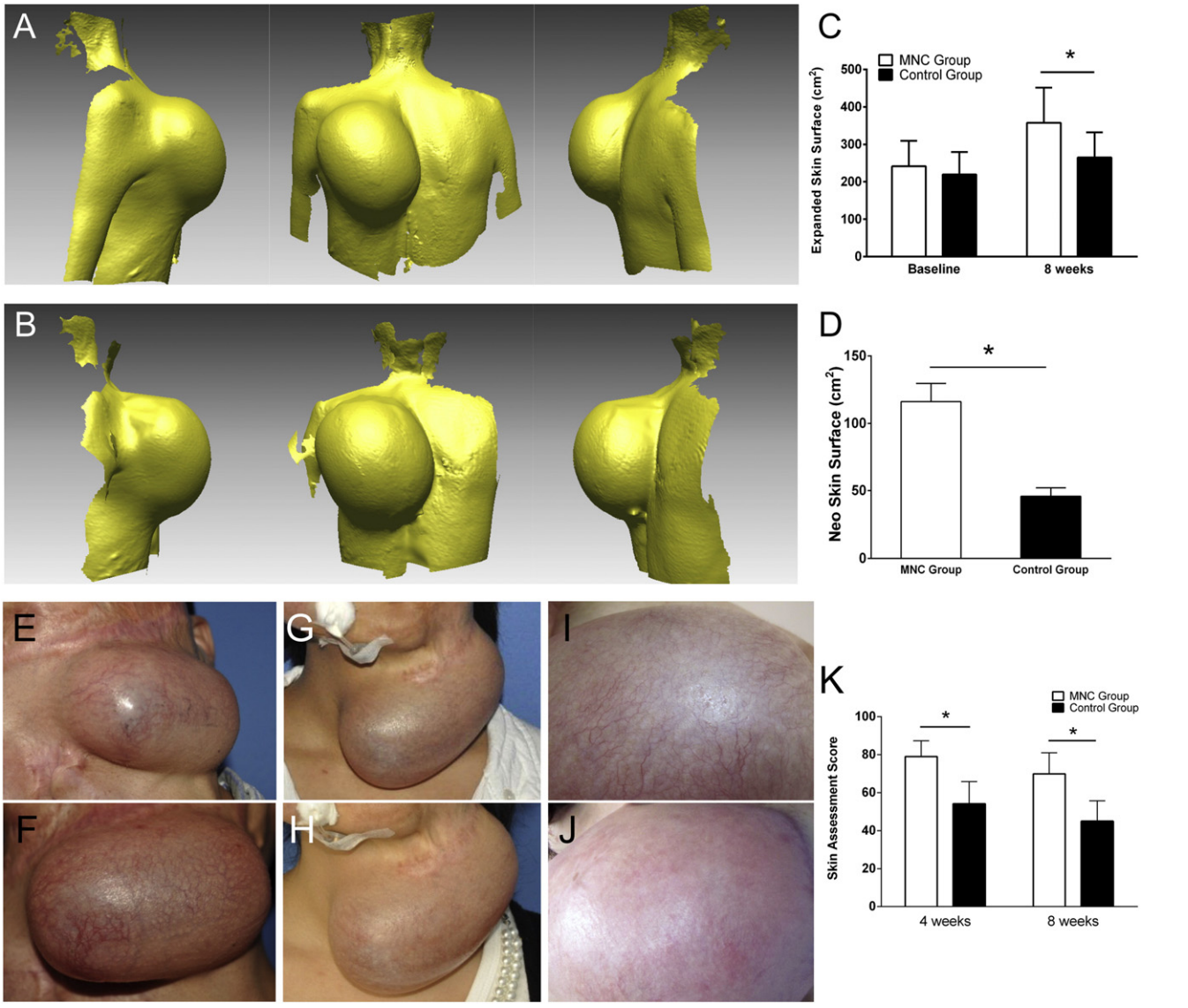


Fig. 2. Evaluation of skin surface area and texture. The expanded skin surface at baseline (A) and 8 weeks (B) was evaluated by 3-Dimensional laser scanning. The results showed that the MNC group had significantly more skin surface area at 8 weeks versus controls ($p < 0.001$), despite starting with similar amounts of skin surface area at baseline ($p = 0.612$) (C). Patients in the MNC group gained significantly more skin surface area after 8 weeks of treatment (D). Photographs show significant improvement in expanded skin texture at 4 weeks post treatment compared to baseline. E G, I, before treatment; F, H, J, 4 weeks post treatment. Patients in the MNC group scored higher in their assessment of their expanded skin quality versus patients in the control group at both 4 weeks and 8 weeks (*$p < 0.001$).

3.3. Secondary Outcomes

3.3.1. Surface Area of Expanded Skin

3D scanning indicated that patients in the treatment group had significant increases in expanded skin surface area compared to the control group (Fig. 2). At baseline, the expanded skin surface of the treatment and control groups were 241.30(141.42) cm^2 and 219.19(124.44) cm^2, respectively ($p = 0.612$). At 8 weeks, skin surface area increased to 357.41(194.40) cm^2 in the treatment group (an increase of 116.11(58.69)cm^2) and 265.96(138.81)cm^2 in the control group (an increase of 45.77(27.48)cm^2) (mean difference 70.34 cm^2 [95% CI, 39.75–100.92]; $p < 0.001$).

3.3.2. Skin Texture

The treatment group had higher VAS scores at 4 weeks (79.0(8.4) versus 54.2(11.6)) in the control group (mean difference 24.8 [95% CI, 18.2–31.5]; $p < 0.001$). The MNC group continued to score higher at 8 weeks (69.8(11.2) versus 45.0(10.7) in the control group, mean difference 24.8 [95% CI, 17.6–32.1]; $p < 0.001$). Fig. 2 highlights improved skin textures in the MNC group at their 4-week assessment. At 8 weeks, significantly fewer patients in the treatment group complained of thin, papery/transparent skin versus controls (1 versus 7, $p = 0.04$).

3.3.3. Skin Thickness

The MNC group had significantly thicker epidermis and dermis versus controls at 4 weeks (epidermal mean difference of 0.06 mm [95% CI, 0.04–0.08]; $p < 0.001$; dermal mean difference of 0.36 mm [95% CI, 0.15–0.57]; $p < 0.001$) and 8 weeks (epidermal mean difference of 0.05 mm [95% CI, 0.03–0.08]; $p < 0.001$; dermal mean difference of 0.42 mm [95% CI, 0.22–0.62]; $p < 0.001$).

At baseline, the MNC and the control groups had similar epidermal and dermal thicknesses (epidermis: 0.16(0.03) mm and 0.17(0.02) mm, respectively ($p = 0.526$); dermis: 0.75(0.33)mm and 0.74(0.32)mm, respectively ($p = 0.91$)). At 4 weeks, the epidermal thickness of treatment patients increased by 0.04(0.03) mm to 0.21(0.03) mm ($p < 0.001$), and the dermal thickness increased 0.31(0.12) mm to 1.01(0.38) mm ($p = 0.029$). Improvement continued at 8 weeks: epidermal thickness

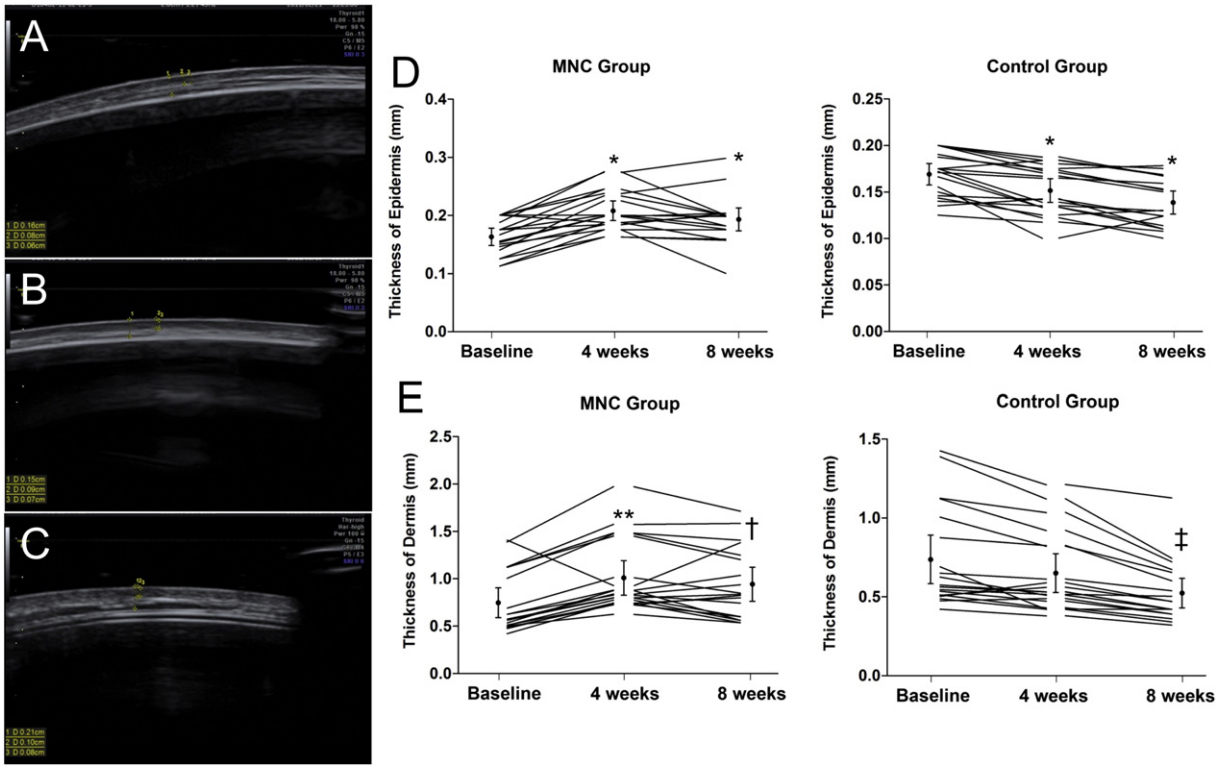


Fig. 3. Thickness of expanded skin in each group over time. Ultrasound was used to assess the thickness of the expanded skin in both groups (A–C, results from the MNC group at baseline, 4 weeks, and 8 weeks). Epidermal thickness in the treatment group increased significantly at 4 weeks and 8 weeks, whereas the epidermal thickness in the control group steadily decreased (D). A similar downward trend was found in dermal thickness at 4 and 8 weeks for the control group (E) (*$p < 0.001$, **$p = 0.029$, †$p = 0.076$, ‡$p = 0.017$).

in the MNC group was 0.19(0.04) mm (a 0.03(0.03) mm increase from baseline ($p < 0.001$)), and dermal thickness was 0.95(0.38) mm, (a 0.23(0.14) mm increase from baseline ($p = 0.076$)) (Fig. 2).

In controls, both the epidermis and the dermis decreased in thickness throughout follow-up: the epidermis was 0.15(0.03) mm at 4 weeks ($p < 0.001$) and 0.14(0.03) mm at 8 weeks ($p < 0.001$); the dermal was 0.65(0.24) mm at 4 weeks ($p = 0.36$) and 0.52(0.19) mm ($p = 0.017$) at 8 weeks (Fig. 3).

3.3.4. Histological Examination

On HE staining, skin samples from the MNC group were thicker than those of the control group. Epidermal thickness of in the MNC group was 0.17(0.03) mm versus 0.10(0.02)mm in the control group (mean difference 0.07 mm [95% CI, 0.05–0.08]; $p < 0.001$). Dermal thickness in the treatment group was 3.21(0.52) mm versus 1.51(0.45) mm in the control group (mean difference 1.58 mm [95% CI, 1.50–2.14]; $p < 0.001$) (Fig. 4). Due to elastic contraction of the skin after expander

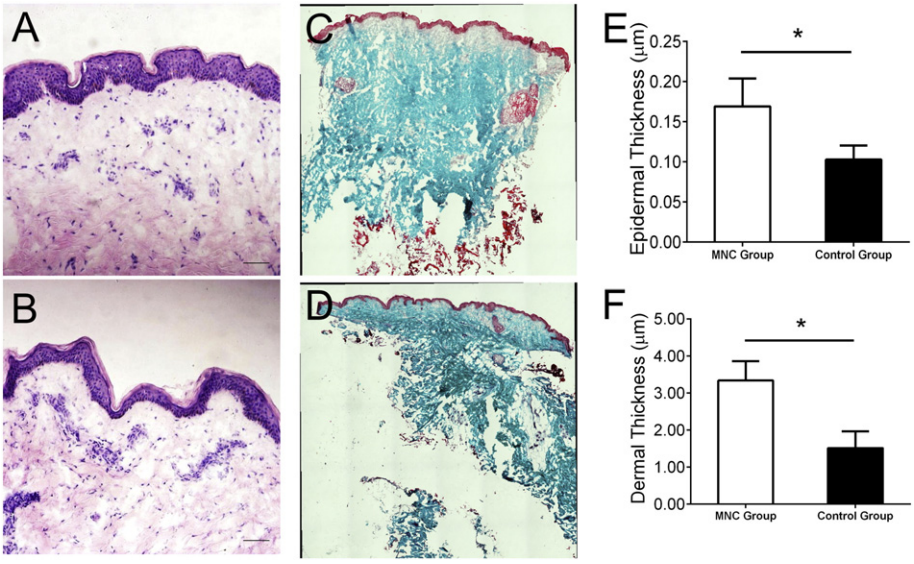


Fig. 4. Histology of expanded skin. HE staining and Masson staining was used to examine the expanded skin sections from each group. The treatment group (A, C), had significantly thicker epidermis and dermis versus the control group (B, D). The results showed that skin sections from the MNC Group were significantly thicken than those from the Control Group in both epidermal and dermal thickness (E–F) (*$p < 0.001$).

removal, dermal thickness was higher on histological analysis than ultrasound examination in all patients.

The MNC group had significantly more PCNA+ proliferating cells in the basal layer of the epidermis ($p = 0.001$), and superior vascularization as shown by more CD31+ cells in skin sections ($p < 0.001$) versus controls (Fig. 5).

3.3.5. Safety

During the study, one patient in the MNC group had their expander damaged via puncture during routine inflation. The expander was immediately replaced and the patient completed the trial by successfully completing expander inflation and follow-up. Temporary ecchymosis within the injected treatment area was observed in all patients in both groups. This effect resolved within one week without residual complications. During the 8-week follow-up, no infection occurred, and no severe adverse events, such as ectopic mass formation, skin ulceration, or necrosis, were observed.

At the two-year follow-up blood test and physical exam, no patients had anemia, leukopenia or other signs of hematopoietic suppression.

4. Discussion

Large-scale skin deformity reconstruction remains a challenge in plastic surgery due to the lack of adequate adnexal soft tissue and skin. Our trial is to synergistically combine two methods of regenerative tissue stimuli, stem cells and mechanical stretch, to overcome the limitations of stretch-induced regeneration and create autologous tissue.

Mechanical stretch via tissue expanders stimulates proliferation and maturation in both embryonic and adult cells (Mihic et al., 2014; Banerjee et al., 2015). In adult skin, mechanical tension induces fibroblasts proliferation and collagen synthesis (Wong et al., 2012) while mechanotransduction can induce epithelial morphogenesis and wound healing (Wong et al., 2012; Zhang et al., 2011). Skin expanders use mechanical stretch to stimulate cell proliferation and activate fibrin and collagen synthesis to achieve in vivo regeneration of overlaying skin (Jaalouk and Lammerding, 2009; Zollner et al., 2013). However, the regenerative capacity of skin is limited. Mechanism for regenerative exhaustion in skin is still debated and previous attempts to sustain in vivo tissue regeneration using single growth factors have been ineffective (Sun et al., 2014). Recent findings suggest that stem cell deregulation or insufficiency is involved (Clevers et al., 2014; Pellettieri and Sanchez, 2007). Our recent preclinical studies showed that mechanical stretch in skin can recruit circulating bone marrow-derived stem cells migrating and participating in skin regeneration (Zhou et al., 2013; Zhou et al., 2014). Results also proved intradermal transplantation of bone marrow-derived stem cells to stretched rat skin effectively enhances skin growth (Yang et al., 2011).

MNC is a group of cells including multiple kinds of stem/progenitor cells, including bone marrow mesenchymal cells and epithelial progenitor cells, which have demonstrated a regenerative promoting effect (Assmus et al., 2006; Tateishi-Yuyama et al., 2002; Perin et al., 2012).

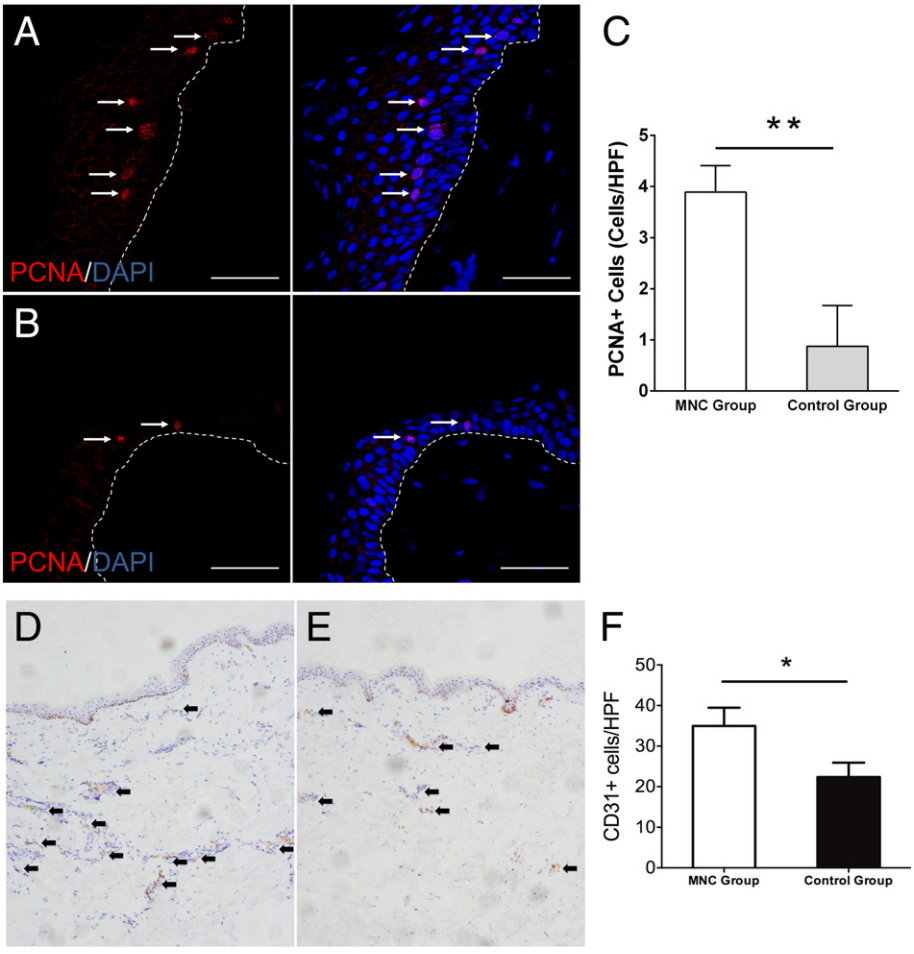


Fig. 5. Immunohistochemical staining of expanded skin. Staining showed that more PCNA+ cells were observed in expanded skin from the MNC group (A) compared to the control group (B). Statistical analyses showed that the PCNA+ cell number was significantly higher in treatment group compared with the control group (C). More CD31+ cells were observed in expanded skin from the treatment group (D) compared to the control group (E), indicating superior angiogenesis in the treatment group. Results showed that the PCNA+ cell number was significantly higher in MNC Group compared with the Control Group (F). Bar, 25 μm (*$p < 0.001$, **$p = 0.001$).

According to current studies, MNCs can secrete a wide spectrum of growth factors (Otsuru et al., 2012; Moniche et al., 2012; Usach et al., 2011) and clinical efficacy of MNC-assisted tissue regeneration has been found in boney (Otsuru et al., 2012), neural (Moniche et al., 2012; Usach et al., 2011) and cardiac tissue (Perin et al., 2012; Goncharova et al., 2014), likely through microenvironment regulation (Backly and Cancedda, 2010; Stappenbeck and Miyoshi, 2009; Laflamme and Murry, 2011). Moreover, the safety of MNC transplantation has been verified in these clinical trials, making MNCs transplantation a reliable option for clinical application. The minimal ex vivo manipulation of MNCs separation makes it feasible for use. In this trial, we discussed the application of MNCs transplantation in stimulating autologous tissue regeneration under mechanical stretch.

Results from this study indicate that intradermal transplantation of MNCs in humans can promote sustainable regeneration of mechanically stretched skin that had previously shown signs of regenerative exhaustion. The MNC group showed statistically significant improvement versus both controls and baseline for all primary and secondary outcomes. Surface area of expanded skin in the treatment group grew 48% from baseline, which was twice as much as controls. Our evidence showed that intradermal MNC injections reverse the trajectory of skin thinning and deteriorating texture while continuing skin expansion in patients who had reached regenerative exhaustion.

Though several experimental approaches have explored suitable biomaterials and tissue-engineering technologies for full-thickness skin reconstruction, limited clinical successes have been achieved (Sun et al., 2014; Russell, 2014). Our attempt to conjunct stem cell with in vivo mechanical stretch has broad implications for safer, larger-scale skin reconstruction. Mechanical stretch during tissue expansion creates a unique microenvironment (Zollner et al., 2013; Zhou et al., 2013) for skin regeneration. With the assist of MNCs, stretched skins are endorsed accelerated angiogenesis (Sheng et al., 2011; Zhou et al., 2013; Sasaki et al., 2008) and growth factor secretion (Yang et al., 2011; Stappenbeck and Miyoshi, 2009). Histological results of the present trial showed that there was a durable increase in the number of proliferating cells in the expanded skin after MNC transplantation ($p < 0.01$). Further investigations are required to understand the therapeutic mechanism by which MNCs promote cell proliferation and regeneration. Limitations of our study include the small sample size, as larger, multi-center trials are warranted. While the safety of MNC transplantation has been verified in clinical trials (Assmus et al., 2006; Assmus et al., 2007; Perin et al., 2011), longer-term follow-up in our cohort is needed and underway to assess safety and long-term results. More accessible autologous stem cells are needed; as such, we are currently evaluating the efficacy of more readily harvested stem cells, such as adipose derived stem cells. Defining contraindications for this type of therapy are important for translational purposes. Cancer patients comprise an important subset of patients requiring large-scale reconstruction; the safety of using stem cells in close proximity to a previously cancerous area must be investigated. Finally, the cellular mechanism by which the transplanted MNCs accelerate skin regeneration under stretch must be further explored. Our histological results showed significantly more proliferating cells and angiogenesis in the treatment group, but we could not track transplanted stem cells.

In conclusion, our study shows that intradermally transplanted MNCs in mechanical stretched skin is a safe and feasible clinical application that can overcome the regenerative limitations of skin to provide significant amounts tissue for surgical reconstruction. We believe that synergistically integrating stem cells and mechanical stretch stimuli will engender further advances in in vivo tissue regeneration.

Disclosure of Potential Conflicts of Interest

We indicate no potential conflicts of interest. The funders had no role in study design, data collection, data analysis, interpretation, writing of the report.

Author Contributions

SZ, TZ, YX, and QL initiated and designed the protocol, trial implementation strategy. SZ, GZ, CC and QL did the scientific literature search. CC, JW and MY assisted in the design of the study. TZ, YX, YG and BG participated in trial conduct. SZ, CC, JW and JZ participated in data collection. SZ, GZ and FX analyzed the data. SZ, QL, GZ, KL, GZ, HL, LQP, CAY and FX contributed in data interpretation. SZ, GZ, CAY and QF wrote the first draft of the manuscript, and LQP, MY and WPM critiqued and modified the manuscript. All authors reviewed and approved the work.

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