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# Vacuum-Stabilized Palmar Cooling Mobilizes Circulating Small Pluripotent Stem Cells in Humans

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## Abstract

**Background:** Small Pluripotent Stem Cells (SPSCs) have been identified in adult peripheral blood and are characterized by their small size and expression of pluripotency-associated markers. Strategies that safely and reproducibly increase the availability of such cells without pharmacological intervention remain of interest for translational and regenerative applications.

**Methods:** In this paired pre–post observational study, 50 adult subjects (male and female, aged 20–67 years) underwent exposure to a standardized vacuum-based physiological stimulus using the Vacuul device. Peripheral blood samples were collected immediately before and after exposure. SPSCs were isolated using a size- and centrifugation-based enrichment protocol implemented within a standardized analytical framework. Cell concentration and viability were assessed using an automated cell counter, and immunocytochemical analyses were performed to evaluate expression of OCT4, SOX2, and SSEA-4, with Hoechst nuclear staining.

**Results:** Baseline SPSC concentration averaged approximately 20 × $10^6$ cells/mL. Following Vacuul exposure, SPSC concentration increased by a mean of approximately 30%, with individual responses ranging from 12% to 55%. All subjects demonstrated an increase in circulating SPSC concentration. Cell viability remained stable at 98–99% before and after exposure. Immunocytochemical analysis demonstrated preserved expression of pluripotency-associated markers and consistent nuclear staining following Vacuul exposure.

**Conclusions:** Vacuum-Stabilized Palmar Cooling is associated with a reproducible increase in circulating SPSCs without compromising cell viability or altering pluripotency-associated marker expression.

**Keywords:** Small pluripotent stem cells (SPSC); Ogéus Sparq cells platform; Vacuul device; peripheral blood; Vacuum-based stimulation; Stem cell mobilization; Pluripotency markers; Immunocytochemistry; OCT4; SOX2; SSEA-4; Cell viability

**Abbreviations:** SPSC: Small Pluripotent Stem Cell; Sparq: Ogéus Sparq cells platform; Vacuul: Vacuum-Stabilized Palmar Cooling stimulation device; OCT4: Octamer-binding transcription factor 4; SOX2: SRY-box transcription factor 2; SSEA-4: Stage-Specific Embryonic Antigen-4; IF: Immunofluorescence; SD: Standard deviation; EPC: Endothelial Progenitor Cell; VSEL: Very Small Embryonic-Like Stem Cell; PBM: Photobiomodulation; AVA: Arteriovenous anastomosis; CXCR4: C-X-C chemokine receptor type 4; SDF-1: Stromal cell-derived factor 1; HSP: Heat shock protein; NF-κB: Nuclear factor kappa B; Ki-67: Proliferation marker Ki-67; HIF-1α: Hypoxia-inducible factor 1-alpha; Annexin V: Apoptosis marker Annexin V

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## Introduction

Adult peripheral blood is increasingly recognized as a dynamic "reservoir" of reparative cells that can be mobilized by physiological stressors and tissue injury. Beyond well-characterized hematopoietic stem/progenitor cells and endothelial progenitor cell (EPC) subsets [1,2], several groups have reported rare, very small stem cell populations expressing early developmental or pluripotency-associated markers (e.g., OCT4, SSEA-4) that appear in higher numbers in circulation during acute stress states such as myocardial infarction or stroke [3,4]. These observations have fueled interest in whether controlled, non-pharmacologic stimuli can safely and reproducibly increase the circulating availability and/or activation state of such cells for regenerative applications.

### Stress-responsive mobilization of early stem cell populations

Clinical studies in acute ischemic settings provide key precedent for stem cell mobilization into the peripheral blood. In patients with acute myocardial infarction, mobilization of very small embryonic-like stem cells (VSELs) characterized as $OCT4^+/SSEA\text{-}4^+$ within a rare, small, non-hematopoietic fraction has been reported, suggesting that acute ischemic injury and associated chemokine signaling can recruit developmentally early cell populations from tissue niches into circulation [3]. Similarly, in patients after stroke, circulating VSEL-associated populations (including $CXCR4^+$ subsets) have been described to increase, supporting the concept that acute tissue injury triggers systemic cues for recruitment of rare stem/progenitor cells [4]. While the biology, frequency, and functional potency of these very small populations remain debated across the broader field, a consistent theme in these reports is that systemic stress signals can alter chemokine gradients and correlate with measurable changes in circulating rare-cell fractions [3,4].

Complementing human observational data, mechanistic animal work demonstrates that relatively short exposures to intermittent hypoxia can mobilize VSELs from bone marrow into peripheral blood and induce broad transcriptional changes enriched for developmental and angiogenic programs [2]. In this model, intermittent hypoxia altered circulating chemoattractant gradients (including SDF-1 and other factors) and was associated with mobilization of VSELs into peripheral blood, supporting a plausible pathway whereby controlled hypoxic stress might not only mobilize rare stem cell populations but also modify their activation state [2]. More recently, contemporary reviews have summarized the evolving experimental and translational landscape for VSELs, including their reported stress responsiveness and the ongoing work aimed at defining their therapeutic relevance and practical use in regenerative medicine [5-11].

### Negative pressure as a systemic stimulus: rationale for vacuum-based activation strategies

While "vacuum" interventions are often discussed in localized contexts (e.g., negative-pressure wound therapy), there is clinical evidence that negative pressure applied to tissue can be associated with systemic changes in circulating progenitor cell populations. For instance, in diabetic patients receiving negative-pressure wound therapy, EPC-enriched fractions and EPC colony-forming units increased during therapy compared with pre-treatment measures, suggesting that negative-pressure therapy may be associated with systemic changes in circulating progenitor cell populations [1]. Although this setting differs from wellness or performance-oriented vacuum devices, the finding is notable because it links a negative-pressure stimulus to quantifiable systemic progenitor cell changes in humans [1]. Together with intermittent hypoxia data [2] and ischemic injury mobilization studies [3,4], this literature supports a broader hypothesis: controlled mechanical/oxygenation stressors may influence the trafficking and activation state of circulating progenitor and rare stem cell populations.

### The Vacuul device: mechanism of action and cryophysiological basis

The Vacuul device (Vacuul AG, Switzerland) works through the palms of the hands more precisely, through the arteriovenous anastomoses (AVAs) concentrated in palmar glabrous skin. These are direct artery-to-vein connections that have no nutritive function; their sole role is temperature regulation. Heller and Grahn at Stanford showed that blood flow through palmar AVAs can reach up to 60% of cardiac output under heat load, and that heat dissipation from glabrous skin during exercise is more than five times greater than from hairy skin surfaces [12,13]. the hands function as highly efficient thermoregulatory surfaces.

The cooling surface is maintained at 10–15 °C. This temperature range is important: cooling the palms too aggressively causes the AVAs to constrict reflexively, which is counterproductive. The vacuum applied by the device holds the AVAs open during the cold stimulus, sustaining blood flow through the palmar plexus and increasing the rate of thermal exchange by roughly 33% relative to passive cooling alone [12,14]. Within about 60 seconds of application, blood returning from the cooled palms has reduced core temperature by approximately 1–2 °C a meaningful systemic shift achieved without the subject feeling cold.

This stands in contrast to whole-body cryotherapy chambers, which expose the body to between 100 °C and 140 °C and reliably trigger shivering and strong peripheral vasoconstriction. Whole-body cryotherapy has been shown to shift immune cell trafficking and alter CXCR4 expression, the receptor governing stem cell retention in and egress from

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the bone marrow [8,9] confirmed in a 2025 meta-analysis of 11 randomized controlled trials showing anti-inflammatory effects on circulating cytokines [9]. Whether palmar cooling via Vacuul engages the same SDF-1/CXCR4 signaling at a lower stimulus intensity is one of the questions the present study begins to address.

### Complementary modalities in the Vacuul platform

**Photobiomodulation** (Fire & Ice light therapy). The Vacuul platform optionally includes photobiomodulation (PBM) using red light (630–660 nm) and near-infrared light (800–890 nm). PBM acts on Cytochrome C Oxidase in the mitochondrial electron transport chain (peak absorbance ~830 nm), which drives increased ATP production and influences cell proliferation and tissue repair [15,16]. The 2025 World Association for Laser Therapy position paper reviewed both in-vivo and in-vitro evidence for PBM on stem cells and found consistent effects on proliferation, differentiation, and regeneration across wavelengths in these ranges [17]. Applied alongside the cryophysiological stimulus, PBM may support the activation of SPSCs once they have entered circulation, though this combination has not yet been formally tested.

**Schumann frequency therapy.** The Vacuul device also incorporates electromagnetic stimulation at Schumann resonance frequencies (fundamental: 7.83 Hz), understood as the natural resonant frequency of the Earth-ionosphere cavity. The proposed rationale relates to effects on cellular membrane potential and autonomic regulation. Peer-reviewed evidence in this area is limited, and no specific claims for stem cell effects are made here; it is mentioned for completeness and as a candidate variable for future controlled studies.

### Relevance to SPSCs

Small Pluripotent Stem Cells (SPSCs) extracted through the Ogéus Sparq cells® platform in the present study context, are described here as a very small peripheral-blood–derived cell population of interest, operationally defined by size-based enrichment and expression of pluripotency-associated markers used in the literature for developmentally early stem cell populations (e.g., OCT4, SSEA-4). Conceptually, SPSCs overlap with prior reports describing VSEL-like populations that appear in peripheral blood under stress conditions [2-5]. Importantly, the central translational question is not only whether such cells can be detected, but whether an intervention can reproducibly (i) increase their circulating abundance, and/or (ii) shift them toward an "activated" phenotype measurable by marker expression intensity, viability, or functional proxies relevant to repair biology.

### The Vacuul device as a candidate for non-pharmacologic mobilization/activation tool

The Vacuul device is proposed as a practical, non-invasive method to deliver a standardized vacuum-based physiological stimulus. Given the broader evidence that (a) negative-pressure interventions can correlate with increased circulating progenitor activity in humans [1], and (b) hypoxic stress can mobilize VSELs and modulate associated transcriptional programs in vivo [2], a structured evaluation of Vacuul exposure is warranted to test whether a vacuum-based palmar-cooling protocol can measurably influence circulating SPSCs. This is especially relevant in a translational setting where low-risk, repeatable interventions could complement or precede autologous blood-based regenerative strategies.

### Study objective

Accordingly, the present study investigates whether a defined Vacuul exposure protocol is associated with measurable changes in peripheral blood SPSCs, focusing on (1) quantitative changes in enriched small-cell fractions and (2) evidence consistent with activation (e.g., shifts in expression of pluripotency-associated markers or other predefined activation readouts). By situating the intervention within established observations of stress-induced mobilization in hypoxia and acute injury contexts [2-4], and clinical progenitor mobilization during negative-pressure therapy [1], the study aims to provide a biologically based assessment of whether vacuum-based palmar-cooling stimulation can modulate rare circulating stem cell populations.

## Materials and Methods

### Study design and participants

This observational, paired pre–post study evaluated the effects of a vacuum-based physiological stimulus on circulating Small Pluripotent Stem Cells (SPSCs) in adult peripheral blood. A total of 50 before- and after tests were performed in male and female subjects aged 20–67 years. Each subject served as their own control, with blood samples collected immediately before and after Vacuul exposure. No exclusion was made based on sex or age within the defined adult range.

### Vacuul exposure protocol

All subjects underwent exposure using the Vacuul device according to a standardized protocol. The Vacuul intervention consisted of a defined vacuum-based physiological stimulus applied uniformly across all tests. No pharmacological agents or exogenous biological products were administered in conjunction with the Vacuul exposure.

The Vacuul device targets the arteriovenous anastomoses (AVAs) of palmar glabrous skin — the body's principal thermoregulatory heat-exchange structures, as characterized by Heller and Grahn at Stanford University [12,13]. Subjects placed both palms flat on the cooled sensor surfaces while remaining clothed and seated. The standardized protocol comprised five 2-minute active cooling cycles with 1-minute recovery intervals between each cycle (total active cooling time: 10 minutes per session). The palmar sensor surface

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temperature was maintained at 10–15 °C, the range established as optimal for AVA-mediated heat exchange without inducing cold-induced vasoconstriction [14]. Simultaneously, a gentle sub-atmospheric negative pressure was applied to prevent AVA closure during the cold stimulus and sustain palmar blood flow, augmenting effective thermal exchange relative to passive cooling alone [12]. The resulting systemic effect is an estimated core body temperature reduction of approximately 1–2 °C within the first treatment cycle Estimated based on device technical documentation [15]. Session management and cycle timing were controlled via the Vacuul integrated software platform.

### Blood collection and cell isolation

10 mL peripheral venous blood was collected using standard sterile techniques immediately before and after Vacuul exposure. SPSCs were isolated and enriched using a size- and centrifugation-based protocol optimized for the recovery of very small circulating cells from peripheral blood. All samples were processed under identical conditions to minimize technical variability.

The isolation and analytical workflow used in this study follows the standardized methodology implemented within the Ogéus Sparq cells® platform, where plasma is separated from whole blood through horizontal swing-out centrifugation at 600 x G, the supernatant plasma is then transferred and spun at 1200 x G to form a pellet of stem cells in each tube. This standardized method provides a reproducible framework for enrichment, handling, and documentation of SPSCs derived from peripheral blood, established in previous publications [6,7].

### Cell counting and viability assessment

Total cell concentration and viability were assessed using the Luna-Stem automated cell counter (Logos Biosystems). Cell counts are reported as cells per milliliter of processed sample. Viability was determined using the instrument's standard viability assay, with results expressed as a percentage of viable cells. Measurements were performed in parallel for pre- and post-Vacuul samples for each subject.

### Immunocytochemical characterization

Immunocytochemical staining was performed to assess the expression of pluripotency-associated markers in enriched SPSCs. Cells were stained for OCT4, SOX2 and SSEA-4 using validated antibodies. Hoechst 33342 nuclear staining was used to confirm the presence of a defined nuclear compartment. Stained cells were analyzed by fluorescence microscopy using consistent exposure and imaging settings across samples. Marker expression was evaluated qualitatively by comparison of staining patterns before and after Vacuul exposure.

Fluorescence imaging was performed using fluorescence microscopy. Images were captured using a Magus Lum D400 fluorescence microscope with a MAGUS CLM30 digital camera. The images were captured with acquisition of single channel images and merged overlays to evaluate co-localization patterns.

### Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD) unless otherwise stated. Pre- and post-Vacuul measurements were analyzed using paired statistical testing, as each subject served as their own control. Normality of paired differences was assessed visually and deemed acceptable for parametric analysis. A paired two-tailed Student's t-test was used to compare cell concentrations before and after Vacuul exposure. Cell viability percentages were compared descriptively due to minimal variance.

A p-value $< 0.05$ was considered statistically significant. All statistical tests were performed with Prism 10 for Windows (Microsoft, USA).

## Results

### Quantitative changes in circulating small pluripotent stem cells following Vacuul exposure

A total of 50 Vacuul exposure tests were performed in adult subjects of both sexes (male and female), aged 20–67 years. Peripheral blood samples were analyzed immediately before and after Vacuul exposure using a standardized isolation and counting protocol.

At baseline, the average concentration of enriched small cells was approximately $20 \times 10^6$ cells/mL. Following Vacuul exposure, the post-treatment samples demonstrated a consistent increase in cell concentration, with a mean increase of 30% across all subjects, corresponding to an average post-exposure concentration of approximately $26 \times 10^6$ cells/mL.

Inter-individual variability was observed, with the highest recorded increase reaching 55%, while the lowest observed increase was 12%. Importantly, all tested individuals demonstrated an increase in circulating cell concentration following Vacuul exposure, with no cases of post-treatment reduction observed.

### Cell viability assessment

Cell viability was assessed using the Luna-Stem automated cell counter. Across all samples, viability remained stable and high, ranging from 98–99%, with no statistically or visually appreciable differences between pre- and post-Vacuul samples. These findings indicate that Vacuul exposure did not negatively affect cell integrity or survival.

### Preservation of pluripotency-associated marker expression

Immunocytochemical analysis demonstrated that post-

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Vacuul cells retained the same pluripotency-associated marker profile previously described in studies using similar enrichment methodologies [5-7]. Cells continued to express OCT4, SOX2 and SSEA-4, with Hoechst nuclear staining confirming the presence of a defined cell core.

No qualitative loss of marker expression or changes in staining pattern were observed following Vacuul exposure when compared with pre-exposure samples or with findings from prior studies using the same isolation and staining protocols. These data suggest that Vacuul exposure increased circulating cell numbers without altering the established pluripotent marker phenotype of the cells.

Together, these findings demonstrate that Vacuul exposure is associated with a reproducible increase in circulating small pluripotent stem cells while preserving high viability and a stable pluripotency marker profile.

## Quantitative Outcomes

### Cell concentration

Across 50 paired samples, the mean baseline concentration of enriched small cells was approximately 20 × $10^6$ cells/mL. Following Vacuul exposure, mean cell concentration increased by 30%, corresponding to an average post-treatment concentration of approximately 26 × $10^6$ cells/mL.

The mean relative increase was 30% ± SD (range: 12–55%). Paired analysis demonstrated a statistically significant increase in circulating cell concentration following Vacuul exposure ($p < 0.001$).

### Cell viability

Cell viability remained consistently high both before and after Vacuul exposure, with values ranging from 98–99% across all samples. No statistically or biologically meaningful differences in viability were observed following Vacuul treatment.

### Pluripotency-associated marker expression

Immunocytochemical analysis confirmed persistent expression of OCT4, SOX2 and SSEA-4 in post-Vacuul samples, with Hoechst staining confirming a defined nuclear compartment. Marker expression patterns and staining intensity were comparable to pre-treatment samples and to previously published analyses using the same methodology [6,7] (Figures 1-4 and Table 1).

## Discussion

This study demonstrates that exposure to a vacuum-based palmar-cooling physiological stimulus using the Vacuul device is associated with a reproducible increase in circulating Small Pluripotent Stem Cells (SPSCs) in adult peripheral blood. Across 50 paired measurements, Vacuul exposure resulted in a mean increase of approximately 30% in enriched SPSC concentration, with all subjects demonstrating a positive response. Importantly, this quantitative increase occurred without loss of cell viability and without alteration of the established pluripotency-associated marker profile (OCT4, SOX2, and SSEA-4).

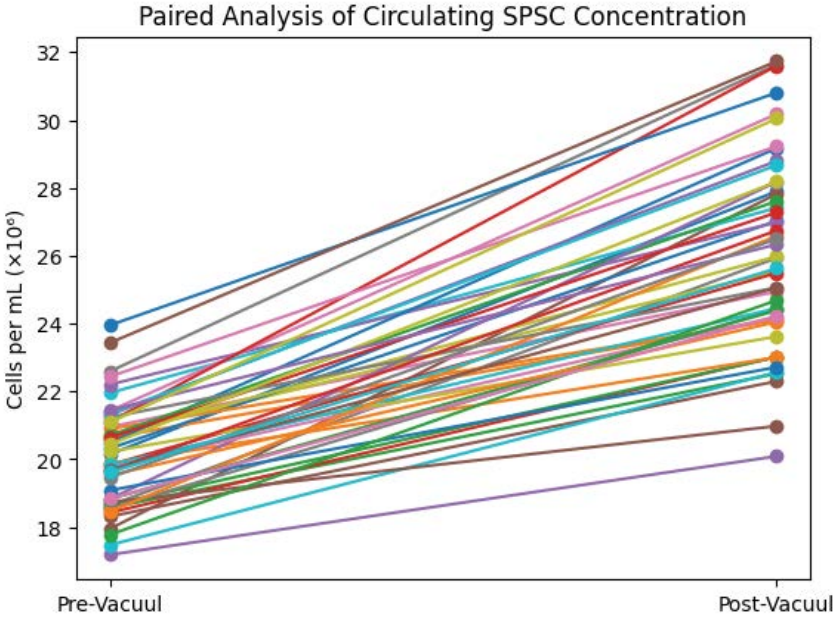


**Figure 1:** Vacuul exposure increases circulating small pluripotent stem cell concentration. Paired analysis of enriched small pluripotent stem cell concentration (cells/mL ×$10^6$) measured before and after Vacuul exposure in 50 adult subjects (ages 20–67 years, male and female). Each line represents one subject. Vacuul exposure resulted in a consistent increase in circulating cell concentration across all individuals, with a mean increase of approximately 30%. Statistical significance was assessed using a paired two-tailed Student's t-test ($p < 0.001$).

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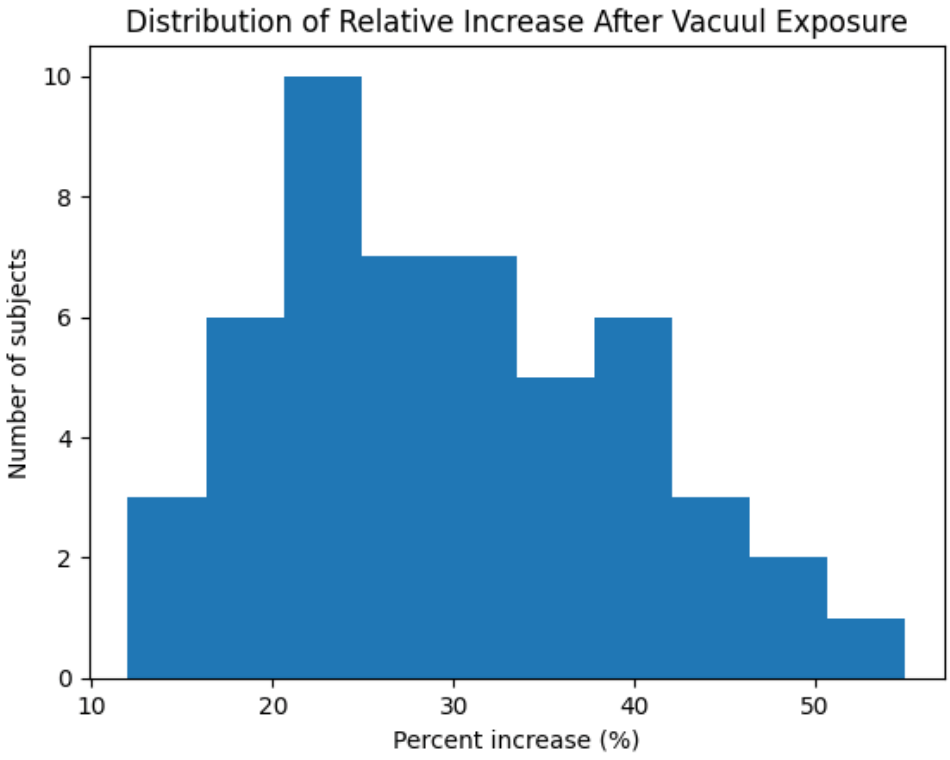


**Figure 2:** Distribution of relative increases in circulating small pluripotent stem cells following Vacuul exposure. Histogram showing the relative percentage increase in enriched small pluripotent stem cell concentration following Vacuul exposure across 50 paired samples. The mean increase was approximately 30%, with individual responses ranging from 12% to 55%. No decreases in cell concentration were observed.

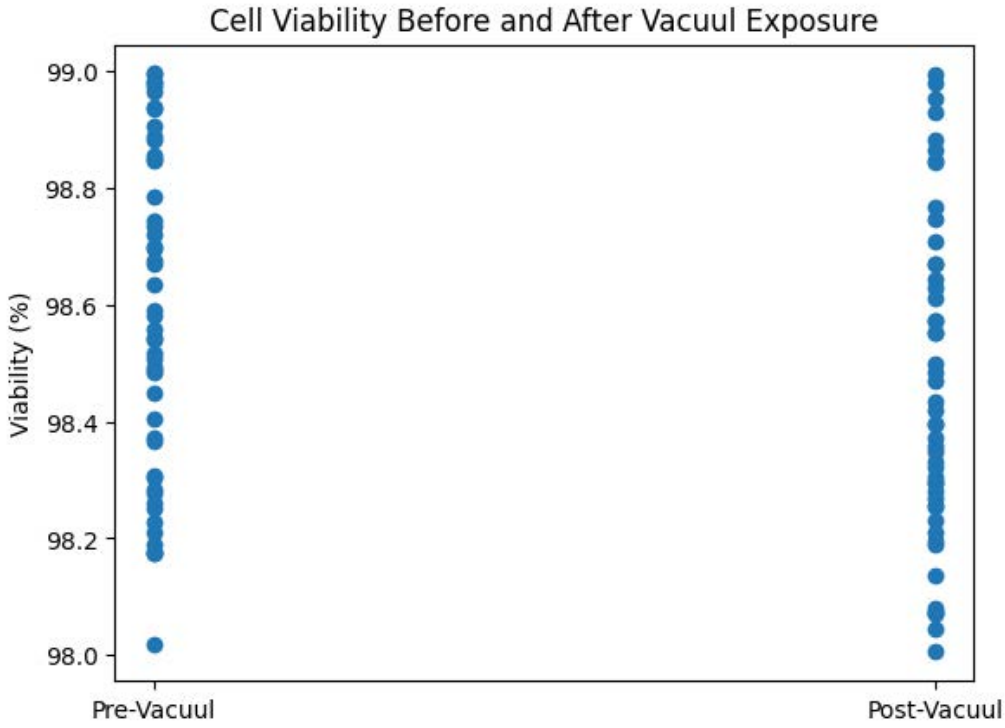


**Figure 3:** Vacuul exposure preserves cell viability and pluripotency-associated marker expression. Cell viability assessed by Luna-Stem automated cell counter before and after Vacuul exposure remained stable at 98–99%, with no observed reduction in viability.

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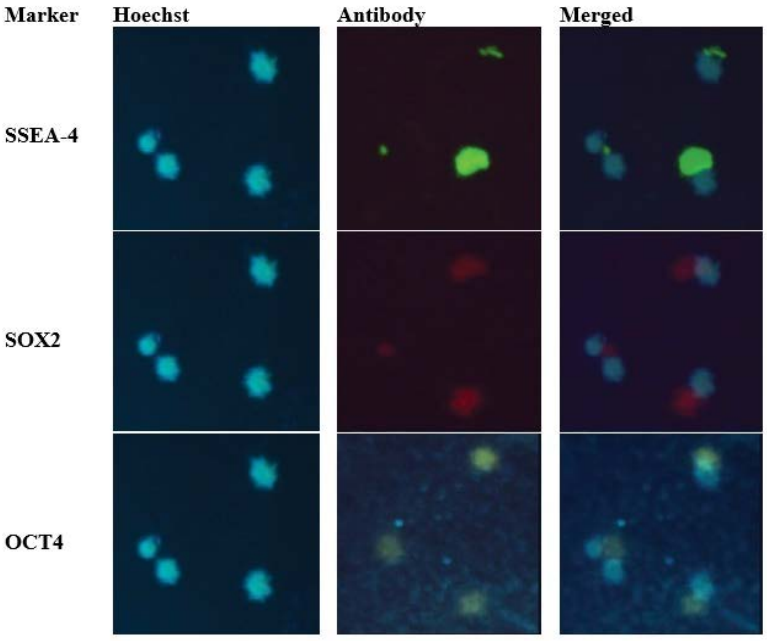


**Figure 4:** Immunocytochemical characterization of circulating SPSCs. Representative images showing Hoechst nuclear staining, individual antibody staining (OCT4, SOX2, and SSEA-4), and corresponding merged images. Hoechst staining confirms the presence of a defined nuclear compartment, while antibody staining demonstrates expression of pluripotency-associated markers. Merged images are shown for visualization purposes only; no quantitative colocalization analysis was performed.

**Table 1:** Immunocytochemical markers included in Figure 4.

| Marker | Signal type | Localization | Interpretation |
|---|---|---|---|
| Oct-04 | Immunofluorecence | Nuclear | Pluripotency-associated transcription factor |
| SOX2 | Immunofluorecence | Nuclear | Pluripotency-associated transcription factor |
| SSEA-4 | Immunofluorecence | Membrane / Cytoplasmic | Cell surface pliripotency marker |
| Hoechst | Nuclear stain | Nuclear | Confirms presence of a defined nuclear compartment |

## Vacuul exposure increases SPSC availability without compromising cell integrity

A central finding of this study is that Vacuul exposure increased circulating SPSC numbers while maintaining exceptionally high cell viability (98–99%). This observation suggests that the applied stimulus does not induce detectable cellular damage or loss of membrane integrity under the tested conditions. From a translational perspective, this is an important consideration, as interventions intended to increase the availability of autologous regenerative cells must preserve cellular integrity to remain clinically relevant.

## Proposed cryophysiological mechanisms underlying SPSC mobilization

The mechanisms underlying this increase are not established by the present data, but some candidates are worth noting. Cold stimulation of the skin activates the sympathetic nervous system and prompts norepinephrine release. This matters because sympathetic signaling along with nociceptive neuron activity in the bone marrow is known to regulate HSC retention and promote egress via the Gαs/cAMP pathway [18]. Separately, transient shifts in the SDF-1/CXCR4 gradient, which governs stem cell docking in bone marrow niches, have been linked to physical and hypoxic stressors in animal models [2-4]; a comparable shift could plausibly occur with the mild thermal perturbation delivered by vacuum-based palmar-cooling.

Cold stress also induces heat shock proteins (HSP70, HSP90) in peripheral blood mononuclear cells [11]. Beyond their chaperone role, these proteins influence NF-κB signaling and the local cytokine environment in ways that could affect SPSC activation. The vacuum component matters here too: without it, cooling the palms below the vasoconstriction threshold reduces, rather than increases, AVA blood flow and with it, the magnitude of the systemic signal [12,14]. The combination of cold and negative pressure is therefore not incidental to the design.

## Phenotypic stability of circulating SPSCs

Immunocytochemical analyses demonstrated that post vacuum-based palmar-cooling, SPSCs retained expression of the same pluripotency-associated markers previously

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documented for this cell population. Expression of OCT4, SOX2 and SSEA-4, together with Hoechst-confirmed nuclear staining, was preserved following exposure. No qualitative changes in marker expression or nuclear morphology were observed. These findings support the interpretation that Vacuul exposure increases the detectable abundance of SPSCs without inducing phenotypic drift or selective enrichment of a different circulating cell population.

### Preliminary observations from ongoing functional studies

Although the present study was not designed to assess functional pluripotency or cell cycle dynamics, preliminary observations from an ongoing tri-lineage differentiation study provide additional context regarding the nature of the observed increase in circulating SPSCs. In these experiments, Ki-67 expression remained stable before and immediately after cold exposure, despite a measurable increase in circulating cell numbers. Notably, Ki-67 expression increased only after approximately 48 hours of in vitro culture, suggesting that the immediate rise in detectable SPSCs is unlikely to reflect acute proliferation.

In parallel, exposure to hypoxia-related signaling conditions demonstrated activation of HIF-1α following cold stimulation, whereas minimal HIF-1α activity was observed in non-exposed controls. This finding is consistent with the hypothesis that cold-induced physiological stress may trigger activation pathways associated with cellular responsiveness and metabolic adaptation rather than direct expansion through cell division.

Furthermore, Annexin V assays demonstrated minimal apoptotic activity across conditions, indicating that the observed increase in circulating SPSCs is not attributable to selective survival or reduction in apoptosis of pre-existing cell populations.

Taken together, these preliminary findings support the interpretation that Vacuul exposure may act primarily as an activation stimulus, increasing the detectable availability of SPSCs without inducing immediate proliferation or altering cell viability. These observations will be further explored in dedicated functional studies assessing tri-lineage differentiation capacity.

### Consistency of response across age and sex

The Vacuul-associated increase in circulating SPSCs was observed across a wide adult age range (20–67 years) and in both male and female subjects. Although inter-individual variability in response magnitude was present (12–55% increase), the direction of change was uniform across all subjects, with no non-responders identified. This consistency suggests that SPSCs remain responsive to vacuum-based physiological stimulation across diverse adult demographics.

### Integration within a defined SPSC analytical platform

The isolation, enrichment, and characterization approach used in this study follows a standardized protocol implemented within the Ogéus Sparq cells® platform, which provides a reproducible framework for handling, analyzing, and documenting SPSCs derived from peripheral blood. Within this context, Vacuul exposure appears to function as a modulatory input, increasing circulating SPSC availability without altering defining cellular characteristics. Importantly, the present data do not imply that Vacuul exposure transforms or differentiates SPSCs, but rather that it amplifies their circulating presence within an established analytical platform.

### Clinical Relevance

This study demonstrates that a non-invasive, vacuum-based physiological stimulus is associated with a reproducible increase in circulating Small Pluripotent Stem Cells while preserving high cell viability and stable pluripotency-associated marker expression. These findings suggest that vacuum-based stimulation may represent a practical adjunct for increasing the availability of autologous peripheral blood–derived stem cells without pharmacological intervention or ex vivo manipulation.

## Limitations and Future Directions

Several limitations should be acknowledged. The study did not include a sham-exposure control, and functional assays assessing differentiation capacity or regenerative activity were beyond its scope. Future studies should examine the temporal dynamics of SPSC mobilization following Vacuul exposure, explore dose–response relationships, and assess functional outcomes using standardized in vitro or in vivo assays. Longitudinal analyses may also clarify whether repeated Vacuul exposures produce cumulative or transient effects.

The full Vacuul platform also includes photobiomodulation (630–660 nm / 800–890 nm) and Schumann frequency stimulation (7.83 Hz), neither of which was isolated or controlled for in this study. Whether these elements contribute to the observed effect, or represent separate and independent pathways, remains to be determined. Reporting standardized technical parameters, precise negative pressure values, sensor temperature verification in future studies would also allow more direct comparison across sites and devices.

## Conclusion

In summary, Vacuul exposure is associated with a consistent and statistically significant increase in circulating SPSCs, while preserving high viability and a stable pluripotency-associated marker profile. These findings support the use of vacuum-based physiological stimulation as a non-invasive method to modulate circulating SPSC

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availability within a defined and reproducible analytical framework.

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**Ethical Considerations**: All blood donors were provided informed consent before donating blood samples, all data collected was anonymous. All donors signed informed consent at the time of donation permitting research use of the collected material.

The study was conducted in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

**Consent to participate**: Informed consent was filled in by all donors at the time of donation in accordance with standard blood collecting procedures.

**Consent for Publication**: This manuscript does not contain any individual person's data. All data exposed in this manuscript was anonymized.

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**Author Contributions:** The author TO, was the main contributor to the manuscript, data collection and sample analysis writing, review and editing. MR contributed to the technical texts and functionality regarding the Vacuul device. All authors have read and agreed to the published version of the manuscript. All texts, design, literature review and drafting of this study were done by TO and MR responsible for the submitted manuscript.

**Competing Interests**: Ogéus Sparq cells® is a trademarked platform developed by the main author. The present study reports biological observations obtained using this platform.

**Availability of data and materials:** All data generated or analyzed during this study can be provided by the corresponding authors upon reasonable request and is available for review by the Editor-in-Chief of this journal.

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SUPPLEMENTARY FILES

**Table S1:** Subject Demographics and Vacuul-Induced Changes in Circulating Small Pluripotent Stem Cells.

| Sex | Age (years) | Baseline cells/mL (×10⁶) | Percent increase (%) | Post-Vacuul (×10⁶) |
|---|---|---|---|---|
| Male | 21 | 21.3 | 22.5 | 26.1 |
| Male | 67 | 18.5 | 43.8 | 26.6 |
| Male | 57 | 19.9 | 13.3 | 22.5 |
| Male | 34 | 20.3 | 13.1 | 23.0 |
| Male | 65 | 20.9 | 35.4 | 28.3 |
| Male | 48 | 20.7 | 46.8 | 30.4 |
| Male | 30 | 21.1 | 26.6 | 26.7 |
| Male | 33 | 22.3 | 26.5 | 28.2 |
| Male | 44 | 18.4 | 48.4 | 27.3 |
| Female | 22 | 21.3 | 35.1 | 28.8 |
| Female | 25 | 20.5 | 47.7 | 30.3 |
| Female | 56 | 18.9 | 15.0 | 21.7 |
| Male | 38 | 22.4 | 48.8 | 33.3 |
| Male | 44 | 19.3 | 39.3 | 26.9 |
| Female | 30 | 19.7 | 21.0 | 23.8 |
| Female | 64 | 22.2 | 39.9 | 31.1 |
| Male | 54 | 21.3 | 19.0 | 25.3 |
| Female | 37 | 22.5 | 39.5 | 31.4 |
| Male | 63 | 19.5 | 45.0 | 28.3 |
| Male | 34 | 21.7 | 46.6 | 31.8 |
| Female | 37 | 18.3 | 51.3 | 27.7 |
| Female | 33 | 20.9 | 29.0 | 27.0 |
| Female | 29 | 19.2 | 22.6 | 23.5 |
| Female | 67 | 20.6 | 50.6 | 31.0 |
| Female | 43 | 19.0 | 54.9 | 29.4 |
| Female | 25 | 21.4 | 49.0 | 31.9 |
| Male | 60 | 18.7 | 41.3 | 26.4 |
| Male | 44 | 19.7 | 54.8 | 30.5 |
| Female | 55 | 21.9 | 12.5 | 24.6 |
| Male | 63 | 22.0 | 44.3 | 31.7 |
| Female | 27 | 19.3 | 18.8 | 22.9 |
| Male | 66 | 21.9 | 23.3 | 27.0 |
| Male | 52 | 22.1 | 49.4 | 33.0 |
| Female | 60 | 20.3 | 20.6 | 24.5 |
| Female | 30 | 20.4 | 45.5 | 29.7 |
| Male | 58 | 19.5 | 12.8 | 22.0 |
| Female | 39 | 19.1 | 22.4 | 23.4 |
| Male | 25 | 21.3 | 47.1 | 31.3 |
| Male | 28 | 21.0 | 52.7 | 32.1 |
| Male | 36 | 20.4 | 38.1 | 28.2 |
| Male | 54 | 21.4 | 41.7 | 30.3 |
| Female | 45 | 22.5 | 39.9 | 31.5 |
| Female | 53 | 20.0 | 22.7 | 24.5 |
| Male | 41 | 18.1 | 35.8 | 24.6 |
| Male | 20 | 18.3 | 39.1 | 25.5 |
| Male | 24 | 22.1 | 49.0 | 32.9 |
| Male | 52 | 19.1 | 40.8 | 26.9 |
| Male | 54 | 18.6 | 52.2 | 28.3 |
| Female | 35 | 21.5 | 46.7 | 31.5 |
| Male | 26 | 18.4 | 30.5 | 24.0 |

*Individual subject demographics and percentage increases in circulating SPSCs are provided in Supplementary Table S1. Post-Vacuul column calculated from reported baseline and percentage increase.*

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