# Guidelines on wound assessment methods and cell characterization

Project: PROMOS – Strenghtenning the biomedical ecosystem

WP3.1-Deliverable 2: Achievements by ICGEB and MUI under PROMOS WP3 and guidelines for wound assessment modalities and cell characterization in the development of cell therapies based on the Stromal Vascular Function (SVF) of the adipose tissue to promote the healing of chronic wounds.

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## Abstract

Il Deliverable 2 riassume il quadro metodologico e sperimentale definito nell'ambito del WP3 di PROMOS, finalizzato a standardizzare le strategie di valutazione delle ferite e la caratterizzazione della frazione vascolare stromale (SVF) nel contesto delle terapie rigenerative cellulari. Sono stati esaminati gli strumenti clinici per la valutazione della guarigione delle ferite, sottolineando la necessità di misure armonizzate e riproducibili negli studi che prevedono interventi basati sulla SVF. L'integrazione di scale cliniche strutturate (come le classificazioni PUSH, BWAT e DFU) con misurazioni quantitative, quali il tasso di guarigione della ferita (WHR), la planimetria digitale e le tecnologie non invasive (OCT, DNIRS, bioimpedenza e termografia), offre una piattaforma di valutazione multidimensionale e oggettiva. Un quadro standardizzato di questo tipo è essenziale per consentire la comparabilità tra gli studi e una solida valutazione dell'efficacia terapeutica. Sono state ottimizzate le condizioni di coltura per l'espansione delle SVF in contesti compatibili con le norme GMP. Tra i supporti testati, l'HEM ha dimostrato una capacità superiore di espandere le EC e FAP senza alterare i profili dei marcatori chiave. Questi risultati supportano la scelta dell'HEM come mezzo preferenziale per l'espansione delle SVF di grado clinico e contribuiscono alla riproducibilità e alla trasferibilità dei risultati. Abbiamo messo a confronto i metodi di prelievo del tessuto adiposo e abbiamo dimostrato che la SVF derivata dalla lipoaspirazione è fenotipicamente paragonabile alla SVF derivata dal prelievo in blocco per

quanto riguarda le cellule EC e FAP. Data la sua ridotta invasività, la maggiore scalabilità e la compatibilità con i sistemi chiusi, la lipoaspirazione rappresenta un approccio clinicamente fattibile ed eticamente vantaggioso per le applicazioni autologhe. Inoltre, la profilazione mediante citometria a flusso multiparametrica di 59 donatori ha rivelato che la composizione del SVF è influenzata da variabili specifiche del paziente, quali l'età e la storia di perdita di peso. Questi dati suggeriscono che le caratteristiche del donatore possono influenzare il potenziale rigenerativo dei sottogruppi di ASC, in particolare delle popolazioni pro-angiogeniche e $CD26^{+}/CD55^{+}$, rafforzando l'idea di stratificare i donatori nei futuri protocolli terapeutici. Abbiamo inoltre esaminato l'espansione e la crioconservazione delle cellule. Sebbene l'espansione induca noti cambiamenti nell'espressione dei marcatori di superficie (ad esempio, la riduzione di CD34), le caratteristiche mesenchimali essenziali rimangono in gran parte invariate. La crioconservazione, compresa quella a lungo termine, preserva la vitalità e il potenziale funzionale, a condizione che si applichino protocolli controllati, il che sostiene la fattibilità della creazione di una biobanca di SVF nell'ambito del progetto PROMOS. In conclusione, il Deliverable 2 definisce una piattaforma standardizzata e basata su dati scientifici per la valutazione delle ferite e la caratterizzazione delle SVF, che rafforza il potenziale traslazionale delle terapie rigenerative basate sulle SVF. Sulla base di questi risultati, si delineano diverse strategie per l'applicazione clinica futura.

# Part 1

## 1. Wound healing assessment

Effective wound assessment is critical for managing chronic ulcers, enabling clinicians to establish baseline severity, monitor healing progression, and tailor treatment strategies. Chronic wounds, full-thickness skin lesions that fail to heal within four weeks, typically remain stalled in the inflammatory phase. They are often associated with vascular, diabetic, and pressure ulcers, and exacerbated by comorbidities such as diabetes and peripheral arterial disease, particularly in elderly patients. Despite growing interest in cell-based therapies, standardized tools for objective wound evaluation remain limited. To address this gap, we reviewed validated assessment methods currently used in clinical practice and evaluated their applicability for monitoring outcomes in cell-based treatments.

### 1.2 Clinical evaluation and assessment scales

Wound assessment relies on multiple parameters, including size (length × width), depth, wound edges, undermining, necrotic tissue type and amount, exudate characteristics, surrounding skin color, peripheral edema, induration, granulation tissue, and epithelialization. Additional measures such as thermometry, oxygen saturation, and blood perfusion provide further insight into tissue viability.

**Size measurement**

Manual measurement with a sterile ruler remains the simplest and most widely adopted method. However, digital imaging has become increasingly important, offering standardized photographic documentation for longitudinal analysis. High-quality wound photography requires strict standardization: the camera should be positioned perpendicular to the wound, lighting must be diffuse to avoid glare, and a reference scale (e.g., ruler) should be included.

Dedicated software can then convert pixel counts into surface area ($cm^2$), ensuring reproducible quantification.

**Wound Healing Rate**

The Wound Healing Rate (WHR) or Ulcer Healing Rate (UHR) provides a quantitative measure of re-epithelialization, calculated as:

$$(Ai-Af)/Ai \text{(Ai−Af)/Ai}$$

where Ai is the initial wound area and Af the final measurement. WHR values range from −1.0 to 1.0 or as percentage closure, with 100% indicating complete healing. This metric is widely used in preclinical studies and offers a standardized approach for tracking progress [1].

**Standardized Tools**

**PUSH**

Developed by the National Pressure Ulcer Advisory Panel, PUSH assesses surface area, exudate amount, and tissue type. It demonstrates strong validity, high inter-rater reliability, and sensitivity to clinical change [3-5].

**BWAT**

The Bates-Jensen Wound Assessment Tool evaluates 13 wound characteristics, providing a comprehensive profile across chronic wound types. While more time-intensive than PUSH, BWAT is widely used in clinical trials and offers robust outcome measures [6-11].

**Diabetic Foot Ulcer Scales**

In the context of Diabetic Foot Ulcers (DFU), multiple classification and severity systems have been proposed. Tools such as the Wagner Scale, University of Texas (UT) system, SAD, and PEDIS classifications provide structured frameworks for grading ulcer severity, infection status, and ischemia, aiding treatment planning and prognostic assessment [12-19].

### 1.3 Biophysical assessment

Non-invasive technologies complement clinical evaluation by providing objective physiological data:

- **Optical Coherence Tomography (OCT)**: Offers high-resolution imaging comparable to histology, enabling assessment of inflammation and tissue remodeling without biopsy [20].
- **Diffuse Near-Infrared Spectroscopy (DNIRS)**: Measures oxygenation trends in the wound bed, predicting healing trajectories [21].
- **Bioimpedance**: Reflects tissue composition and hydration, serving as a marker for wound progression [22].
- **Thermography:** Captures temperature gradients to calculate a thermal index, correlating with inflammatory activity and healing potential [23].

### 1.4 Histopathological analysis

When deeper insight is required, biopsy followed by hematoxylin and eosin staining remains the gold standard for characterizing cellular composition and excluding malignant transformation. This approach identifies key immune cell populations, such as M1 pro-inflammatory and M2 anti-inflammatory macrophages, which influence healing dynamics [1].

## 2. Selection of culture media for SVF expansion

Standardizing culture conditions is essential for ensuring reproducibility and regulatory compliance in cell-based therapies. Within the PROMOS framework, ICGEB focused on optimizing the expansion of Stromal Vascular Fraction (SVF) cells derived from human adipose tissue. These cells include endothelial cells (ECs) and fibro-adipogenic progenitors (FAPs), both critical for tissue regeneration and wound healing.

**Comparative Analysis of Culture Media**

We evaluated three GMP-grade media to identify the most effective formulation for expanding SVF-derived cells:

- **Human Endothelial Medium (HEM)** – specifically designed for endothelial cell culture.
- **DMEM/F12** – a widely used medium for primary cells and cell lines.
- **EGM-2** – commonly employed for endothelial cell expansion in research settings.

**Performance Assessment**

Cell expansion was assessed using immunofluorescence and flow cytometry:

- **Endothelial Cells**: HEM demonstrated superior performance, achieving the highest CD31+ area (5.7%) and ERG+ nuclei count (15.2%), compared to DMEM/F12 (1.5%, 5.2%) and EGM-2 (4.4%, 8.3%).
- **Smooth Muscle Cells**: No significant differences were observed among media for α-SMA expression.
- **Flow Cytometry** confirmed that HEM promotes robust expansion of ECs (CD45−CD31+CD146+CD34+) and FAPs (CD45−CD146−CD31−CD34+), supporting its suitability for clinical-grade cell production.

**Implications**

Selecting HEM as the preferred medium ensures optimal yield and quality of regenerative cell populations under GMP-compliant conditions. This standardization is a critical step toward translating SVF-based therapies into clinical trials, improving consistency and therapeutic outcomes.

# 3. Methods of adipose tissue collection and SVF extraction

Adipose tissue is a rich source of regenerative cells, particularly the Stromal Vascular Fraction (SVF), which contains endothelial cells, fibro-adipogenic progenitors, and other supportive cell types. Standardizing collection and processing methods is essential to ensure cell viability, reproducibility, and compliance with clinical-grade requirements.

## 3.1 Evolution of Collection Techniques

Historically, adipose tissue was harvested via en bloc excision [24-26], primarily for reconstructive purposes. While this approach preserves tissue architecture and minimizes shear stress, it is invasive, associated with higher morbidity, and unsuitable for large-scale or minimally invasive applications. Modern practice favors liposuction-based techniques, which are less invasive, scalable, and compatible with closed-system processing workflows.

## 3.2 Current Harvesting Methods

Three main techniques dominate clinical and experimental practice:

**Coleman technique**

The Coleman method involves gentle manual aspiration using syringes without suction, followed by reinjection in small aliquots [24].
Advantages:

- High cell viability due to minimal mechanical trauma.
- Lower risk of complications such as hematomas or fibrosis.
- Faster recovery for patients.

Limitations:

- Labor-intensive and time-consuming.

- Limited scalability for large-volume harvesting.
- Operator-dependent reproducibility.

**Power-Assisted Liposuction (PAL) - Microaire system**

PAL uses an electrically driven handpiece that imparts rapid reciprocating motion to the cannula (4,000–5,000 cycles/min), reducing surgeon fatigue and operative time [27].

Advantages:

- Efficient for large-volume harvesting.
- Improved cannula advancement in fibrous tissue.
- Lower postoperative discomfort compared to manual methods. Considerations:
- Aggressive settings may increase tissue trauma and risk of hematoma or contour irregularities.
- For regenerative applications, conservative suction and cannula design are recommended to preserve delicate cell populations.

**Puregraft Closed Filtration System**

Puregraft provides a sterile, single-use filtration chamber for washing and purifying lipoaspirate [28] .

Advantages:

- Removes blood, tumescent fluid, and free lipids without centrifugation.
- Minimizes shear stress, preserving cell viability.
- Closed system reduces contamination risk and ensures regulatory compliance.

Limitations:

- Higher per-procedure cost due to disposable kits.
- Limited capacity (250–850 mL), making it more suitable for small- to medium-volume procedures.

## 3.3 SVF Extraction and Processing

Following collection, adipose tissue or lipoaspirate undergoes enzymatic digestion and mechanical dissociation:

- Tissue is rinsed with calcium- and bicarbonate-free Hank's solution (CBFHH) and minced.
- Digestion is performed using collagenase and DNase II in a buffered solution, followed by neutralization in endothelial medium supplemented with platelet lysate.
- Sequential centrifugation and filtration (70 µm strainer) yield a purified SVF cell suspension.
- Cells are plated at a density of $5 \times 10^4$ cells/cm² for expansion.

This standardized workflow ensures high cell recovery and viability, supporting downstream applications in regenerative medicine.

## 3.4 In Vitro Comparison of Lipoaspiration and En Bloc Tissue Methods

Given the superior clinical feasibility of lipoaspirate as a starting material for autologous cell therapy, ICGEB evaluated the expansion potential of Stromal Vascular Fraction (SVF) cells derived from adipose tissue collected using different liposuction techniques, compared to the traditional en bloc excision method.

**Comparative Analysis**

Flow cytometry performed on freshly isolated cells revealed no significant differences in endothelial cell (EC) populations across harvesting methods:

- Manual: 0.9%
- PureGraft: 0.5%
- MicroAire: 0.9%
- En bloc: 3.8%

Similarly, fibro-adipogenic progenitor (FAP) populations were comparable:

- Manual: 24.1%
- PureGraft: 23.6%
- MicroAire: 29.2%
- En bloc: 42.9%

**Immunofluorescence Findings**

Immunofluorescence confirmed consistent expression of key markers across all methods:

- **CD31** (endothelial marker): Manual 4.8%, PureGraft 8.7%, MicroAire 5.7%, En bloc 5.1%
- **ERG** (endothelial nuclear marker): Manual 23.6%, PureGraft 9.9%, MicroAire 15.5%, En bloc 21.6%
- **α-SMA** (smooth muscle marker): Manual 3.6%, PureGraft 5.3%, MicroAire 3.5%, En bloc 1.7%

These results indicate that the choice of adipose tissue harvesting method, whether lipoaspiration or en bloc excision, does not significantly impact SVF composition or marker expression. Consequently, lipoaspirate represents a clinically viable and less invasive alternative for SVF isolation, supporting its use in regenerative applications.

# Part 2

## 1. Outline of the different marker

The stromal vascular fraction (SVF) of adipose tissue comprises a heterogeneous mixture of hematopoietic, endothelial, and mesenchymal cell populations, including adipose-derived stem/stromal cells (ASCs). Within this compartment, multiparameter flow cytometry enables the identification of cellular subsets based on surface marker expression profiles, allowing a refined characterization of present cell populations. While hematopoietic cells are commonly identified by CD45 expression, endothelial cells typically express CD3. In contrary, ASCs must lack expression of hematopoietic and endothelial markers, but express CD34. According to the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT), ASCs are recommended to be identified by a surface marker profile including CD34, CD73, CD44, CD90, and CD105.

In addition, ASCs must be plastic-adherent in standard culture conditions and display in vitro differentiation capacity toward adipogenic, chondrogenic, and osteogenic lineages [29].

Recent studies have described several distinct ASC subpopulations in SVF [30]. These ASC subsets differ in their regenerative potential; however, their precise phenotypic definition and functional relevance, particularly in the context of wound healing and therapeutic applications, remain only partially understood and are the focus of ongoing investigation.

$CD55^{+}CD26^{+}$ double-positive ASCs represent a functionally enriched subset within human adipose tissue that appears especially well suited to promote wound healing. These cells retain a typical mesenchymal immunophenotype but exhibit a distinct transcriptional and secretory profile compared with $CD55^{-}$ and/or $CD26^{-}$ ASC fractions, including increased expression of genes associated with angiogenesis, extracellular matrix remodeling, and immune modulation. Functionally, $CD55^{+}CD26^{+}$ ASCs enhance wound closure by acting on several key phases of repair: they promote early angiogenesis, accelerate re-epithelialization, and support deposition and remodeling of granulation tissue. In murine skin-wounding models, transplantation of this subset, or SVF products enriched for $CD55^{+}CD26^{+}$ (DPP4) ASCs,

results in faster wound contraction, thicker and better organized neodermis, and increased vascular density compared with unfractionated ASCs, effects that correlate with elevated local levels of VEGF and other pro-regenerative mediators [31, 32]. CD55 is thought to contribute by protecting transplanted cells and surrounding tissues from complement-mediated damage and by modulating the inflammatory milieu to favor a pro-resolving macrophage phenotype, thereby facilitating the transition from inflammation to proliferation (PMID: 37357314). CD26/DPP4, in turn, modulates the bioavailability of chemokines and growth factors involved in cell recruitment, angiogenesis, and matrix turnover; altered CD26 activity within the wound bed has been linked to changes in revascularization, fibroblast behavior, and overall wound closure dynamics [33-35]. Together, coexpression of CD55 and CD26 defines an ASC subset whose combined complement-regulatory and protease activities appear to fine-tune the inflammatory and angiogenic environment, thereby potentiating the intrinsic pro-regenerative functions of ASCs in cutaneous wound healing.

CD55 expression has also been associated with augmented differentiation capacity, supporting the concept that complement-regulatory molecules expressed on ASCs may stratify subpopulations with distinct lineage commitment profiles and trophic properties [36].

CD26 marks ASC subsets with altered proliferative and differentiation behavior and has been implicated in the regulation of cellular senescence [37, 38]. Emerging evidence links $CD26^+$ ASCs to obesity-associated alterations in adipose tissue biology. In obese mice, the proportion and phenotype of $CD26^+$ ASCs within the SVF are decreased compared with lean controls [39].

Additional marker combinations further refine the functional heterogeneity of ASCs and related SVF populations. For example, $CD140a^+$ ASCs stratified by high or low CD9 expression have been associated with fibrotic remodeling, suggesting that tetraspanin–integrin signaling modules may identify progenitor pools that either promote or restrain extracellular matrix deposition [40].

In parallel, endothelial-committed fractions within the SVF, defined by $CD31^+CD146^+$ expression, contribute significantly to wound repair through their angiogenic capacity and paracrine support of surrounding cells [41].

Collectively, these observations illustrate that specific marker constellations such as $CD55^{+}CD26^{+}$, $CD26^{+}$, $CD140a^{+}CD9$^high/low, and $CD31^{+}CD146^{+}$ can be used to functionally describe SVF and ASC populations with respect to differentiation potential, fibrotic propensity, and pro-regenerative efficacy. Nevertheless, the molecular mechanisms linking surface phenotype to functional output remain incompletely defined. Systematic studies integrating high-resolution phenotypic profiling with standardized in vitro and in vivo functional assays are still required to translate these findings into robust, marker-guided cell selection strategies for wound healing and other therapeutic applications.

Within the PROMOS framework, MUI aimed to profile SVF and its cellular composition to assess cell types present and evaluate donor potential for wound healing therapies. The work was done applying a multi-gate flow cytometry analysis for cell surface markers of endothelial cells (CD45-/CD31+), hematopoietic cells (CD45+), and ASCs (CD45-/CD31-/CD90+/CD34+) as well as ASC subtypes (CD26+, CD55+, CD146+, CD9+, CD140a+). Data from 59 patients were further analyzed according to patient characteristics and compared by sex (female vs. male), method of weight loss, smoking status (non-smokers vs. smokers), and subjected to linear regression analyses with respect to age, extent of weight loss (%), and body mass index (BMI) at the time of abdominoplasty.

Age positively correlated with higher hematopoietic cells and certain ASC subtypes like CD26+ and CD146+. Additionally, we found that weight loss method is associated with pro-angiogenic ASC markers.

These results indicate patient factors may affect ASC regenerative quality, warranting further donor optimization research.

## 2. Impact of SVF expansion on marker expression

In vitro expansion of SVF cells leads to notable shifts in surface marker expression, particularly among adipose-derived stem cells (ASCs). In general, it is well known that ASCs retain the expression of many markers, including CD73+, CD105+ and CD90+, while CD34+ is quickly lost over time in culture. The loss of CD34 expression may reflect ASC commitment to

differentiation, though other researchers contend that variable ex vivo CD34 levels arise from suboptimal culture conditions lacking the factors needed to sustain in vivo expression [42].

Initial adherence selects for fibroblast-like ASCs, with proliferation rates being influenced by culture conditions, donor specifity and seeding density. Media choice, such as DMEM with FBS versus endothelial media like EGM-2, modulates subtype selection and surface marker expression profiles (e.g., higher perivascular markers in DMEM).

## 3. Impact of cryopreservation

Cryopreservation of stromal vascular fraction (SVF) enables long-term storage of adipose-derived cell populations for repeated or delayed cell culture or analysis. SVF is typically frozen in a cryoprotective medium containing serum (e.g., FBS or human serum) and a permeating cryoprotectant such as dimethyl sulfoxide (DMSO), followed by controlled-rate or stepwise cooling to −80 °C and subsequent transfer to liquid nitrogen for long-term storage.

It has been shown that cryopreservation typically decreases the number of viable SVF cells but mainly affecting hematopoietic CD45+ cells. However, the remaining cells retain adhesive, proliferative properties, and colony-forming ability similar to fresh samples and therefore also expendable in vitro 43. Consistent with expectations and prior reports, cryopreserved cells required slightly longer to reach confluence, likely attributable to DMSO exposure [43]. SVF stem cell potency remains largely intact post-cryopreservation, as shown by comparable surface marker expression (e.g., CD34+, CD105+, CD73+, CD29+), and adipogenic and osteopogenic differentiation.

When comparing short- (1-2 months) versus long-term (12-13 years) cryopreservation, it was found that the extended cryopreservation reduces stemness but retains partial wound-healing potential [44].

Within the PROMOS project, MUI utilized an extensive cell bank comprising cryopreserved SVF samples from over 55 patients undergoing elective abdominoplasty between September 2019 and December 2025. Flow cytometry analyses yielded critical insights into SVF composition and post-thaw cell viability; Although cryopreservation typically is expected to result in reduced viable nucleated cell counts, using a simple viability dye, no correlation was

observed between storage duration and viability. The percentage of viable cells remained stable regardless of extended cryostorage time.

These findings, along with current evidence, indicate that cryopreserved SVF retains sufficient functional properties for clinical use - when appropriate protocols and controlled conditions are applied. Thus, SVF banking remains a valid option for preserving its therapeutic potential over longer storage periods. In parallel, freezing adipose tissue as an intact graft is technically feasible and has been clinically applied in some settings; however, it is associated with more variable cell survival and graft retention compared to isolated SVF, suggesting less predictable regenerative performance [45].

# Part 3

## 1. Conclusion and Perspective

This Deliverable 2 summarizes the methodological and experimental framework established within WP3 of PROMOS to standardize wound assessment strategies and stromal vascular fraction (SVF) characterization in the context of regenerative cell-based therapies.

We reviewed clinical tools for wound healing evaluation, highlighting the need for harmonized and reproducible outcome measures in studies involving SVF-based interventions. The integration of structured clinical scales (e.g., PUSH, BWAT, DFU classifications) with quantitative measurements such as wound healing rate (WHR), digital planimetry, and non-invasive technologies (OCT, DNIRS, bioimpedance, thermography) provides a multidimensional and objective assessment platform. Such a standardized framework is essential to enable cross-study comparability and robust evaluation of therapeutic efficacy. We optimized culture conditions for SVF expansion under GMP-compatible settings. Among the media tested, HEM demonstrated superior capacity to expand EC and FAP without altering key marker profiles. These findings support the selection of HEM as a preferred medium for clinical-grade SVF expansion and contribute to reproducibility and translational readiness. We compared adipose tissue harvesting methods and demonstrated that lipoaspirate-derived SVF is phenotypically comparable to en bloc derived SVF in terms of EC and FAP cells. Given its

reduced invasiveness, better scalability, and compatibility with closed systems, lipoaspiration represents a clinically feasible and ethically favorable approach for autologous applications. In addition, multiparametric flow cytometry profiling of 59 donors revealed that SVF composition is influenced by patient-specific variables such as age and weight loss history. These data suggest that donor characteristics may modulate the regenerative potential of ASC subsets, particularly pro-angiogenic and $CD26^+/CD55^+$ populations, reinforcing the concept of donor stratification in future therapeutic protocols. We further examined the cells expansion and cryopreservation. While expansion induces known shifts in surface marker expression (e.g., reduction of CD34), essential mesenchymal characteristics are largely retained. Cryopreservation, including long-term storage, preserves viability and functional potential when controlled protocols are applied, supporting the feasibility of SVF biobanking within PROMOS. Collectively, Deliverable 2 establishes a standardized, evidence-based platform for wound evaluation and SVF characterization that strengthens the translational potential of SVF-based regenerative therapies.

Building on these findings, several strategic directions emerge for future clinical translation:

1. **Marker-guided cell selection**
   The identification of functionally enriched ASC subpopulations (e.g., $CD55^+CD26^+$, $CD31^+CD146^+$) opens the possibility of refining SVF-based products through marker-guided enrichment strategies. Future work should integrate high-resolution phenotyping with functional in vitro and in vivo assays to define predictive biomarker signatures of regenerative efficacy.
2. **Donor stratification and personalized regenerative medicine**
   The observed correlations between donor characteristics and SVF composition suggest that patient profiling may optimize therapeutic outcomes. Developing predictive algorithms that incorporate age, BMI, metabolic status, and ASC subtype distribution could enable personalized cell therapy approaches.
3. **Standardization toward clinical trials**
   The methodological harmonization achieved in this deliverable provides a foundation for regulatory alignment and multicenter reproducibility. Future efforts should focus on establishing SOPs for GMP manufacturing, quality control release criteria, and potency assays tailored to wound healing endpoints.

4. **Integration with advanced wound monitoring technologies**
   Combining SVF therapy with quantitative biophysical monitoring tools may allow real-time assessment of treatment response and early identification of non-responders. This integrated approach could enhance adaptive trial design and improve therapeutic precision.
5. **Long-term biobanking and repeat treatments**
   The confirmation that cryopreserved SVF retains therapeutic properties supports the development of structured SVF biobanks. This strategy may enable staged or repeated administrations, particularly in chronic wound patients at high risk of recurrence.
6. **Mechanistic insights and microenvironment modulation**
   Future research should further dissect the molecular mechanisms linking surface marker expression to functional output, particularly in relation to complement regulation, angiogenesis, immune modulation, and extracellular matrix remodeling within the wound niche.

Deliverable 2 significantly advances the methodological standardization, biological understanding, and translational readiness of SVF-based regenerative therapies within PROMOS. By integrating rigorous wound assessment tools with refined cellular characterization and optimized processing workflows, PROMOS is positioned to move from experimental standardization toward clinically actionable regenerative strategies for chronic wound management.

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