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PROMOS

Potential Investigational Medicinal Product Dossier (IMPD)

Dossier del Medicinale Sperimentale (IMPD)

Prüfpräparat-Dossier (IMPD)

ASC Heal

Prodotto cellulare derivato da ASC autologhe incorporato nella matrice dermica INTEGRA Dermal Regeneration Template per l'applicazione nel trattamento di ferite croniche non cicatrizzanti

Autologes, aus ASC gewonnenes Zellprodukt, integriert in die INTEGRA® Dermal Regeneration Template, zur Anwendung bei chronischen, nicht heilenden Wunden

Autologous ASC-derived cell product incorporated into INTEGRA Dermal Regeneration Template for application to chronic non-healing wounds

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[EN] The IMPD describes ASC Heal as an investigational regenerative medicine product designed for the treatment of chronic non-healing cutaneous wounds, such as diabetic, venous, and pressure ulcers. The product is obtained from stromal/stem cells derived from the patient's autologous adipose tissue, expanded under GMP conditions and subsequently incorporated into the INTEGRA dermal matrix, which serves as a scaffold to support cell retention and distribution within the wound bed. The therapeutic objective is to stimulate tissue repair processes by promoting the formation of new blood vessels, modulating local inflammation, and supporting extracellular matrix regeneration. The dossier outlines the key aspects required for the product's clinical and regulatory development, including the manufacturing process, quality controls, traceability, preliminary release criteria, risk management, and stability, thereby defining a structured pathway towards the next stages of validation and regulatory assessment.

[ITA] L'IMPD descrive ASC Heal come un prodotto sperimentale di medicina rigenerativa pensato per il trattamento di ferite cutanee croniche che non guariscono, come ulcere diabetiche, venose e da pressione. Il prodotto è ottenuto da cellule stromali/staminali derivate dal tessuto adiposo autologo del paziente, espanse in condizioni GMP e successivamente incorporate nella matrice dermica INTEGRA, che funge da supporto per favorirne la permanenza e la distribuzione nel letto della ferita. L'obiettivo terapeutico è stimolare i processi di riparazione tissutale, promuovendo la formazione di nuovi vasi, modulando l'infiammazione locale e sostenendo la rigenerazione della matrice extracellulare. Il dossier descrive gli aspetti essenziali per lo sviluppo clinico e regolatorio del prodotto, tra cui processo produttivo, controlli di qualità, tracciabilità, criteri preliminari di rilascio, gestione dei rischi e stabilità, delineando un percorso strutturato verso le successive fasi di validazione e valutazione regolatoria.

[DE] Das IMPD beschreibt ASC Heal als ein Prüfpräparat der regenerativen Medizin, das für die Behandlung chronischer, nicht heilender Hautwunden wie diabetischer, venöser und Druckulzera entwickelt wurde. Das Produkt wird aus stromalen/Stammzellen gewonnen, die aus dem autologen Fettgewebe der Patientin bzw. des Patienten stammen, unter GMP-Bedingungen expandiert und anschließend in die dermale Matrix INTEGRA eingebracht werden. Diese dient als Trägerstruktur, um den Verbleib und die Verteilung der Zellen im Wundbett zu unterstützen. Das therapeutische Ziel besteht darin, Prozesse der Gewebereparatur anzuregen, indem die Bildung neuer Blutgefäße gefördert, die lokale Entzündungsreaktion moduliert und die Regeneration der extrazellulären Matrix unterstützt wird. Das Dossier beschreibt die wesentlichen Aspekte für die klinische und regulatorische Weiterentwicklung des Produkts, darunter Herstellungsprozess, Qualitätskontrollen, Rückverfolgbarkeit, vorläufige Freigabekriterien, Risikomanagement und Stabilität, und skizziert damit einen strukturierten Weg zu den nächsten Schritten der Validierung und regulatorischen Bewertung.

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Abbreviations and acronyms

The abbreviations and acronyms used throughout this IMPD are listed in Table 1.

Table 1. Abbreviations and acronyms.

Abbreviation	Definition
ASC	Adipose-derived stromal/stem cell
ATMP	Advanced therapy medicinal product
BWAT	Bates-Jensen Wound Assessment Tool
CoA	Certificate of analysis
CQA	Critical quality attribute
CTD	Common Technical Document
DFU	Diabetic foot ulcer
DNIRS	Diffuse near-infrared spectroscopy
DP	Drug product
DS	Drug substance
EC	Endothelial cell
FAP	Fibro-adipogenic progenitor
GMP	Good manufacturing practice
HEM	Human Endothelial Medium
HPL	Human platelet lysate
HSA	Human serum albumin
IPC	In-process control
IMP	Investigational medicinal product
ISCT	International Society for Cell and Gene Therapy / International Society for Cellular Therapy
NANT XL	Closed automated bioreactor platform for cell expansion
OOS	Out-of-specification
PER	Pericyte-enriched mural/perivascular population
QP	Qualified Person
PUSH	Pressure Ulcer Scale for Healing
TSE	Transmissible spongiform encephalopathy
WHR	Wound healing rate

1. Introduction

This potential IMPD describes ASC Heal, an autologous ASC-derived cell product intended for topical/local treatment of chronic, non-healing cutaneous wounds. This document is written as a quality-focused ATMP IMPD and should be aligned with the final clinical protocol, Investigator Brochure, GMP manufacturing records, QP declaration and national competent authority requirements before submission. (Lee et al., 2022; Andia et al., 2019; Bura et al., 2014).

1.1 Product name and classification

The investigational product is designated as adipose-derived stem cell (ASC). ASC Heal is an autologous, ex vivo-manipulated adipose tissue-derived cellular product and is expected to fall within the Advanced Therapy Medicinal Product (ATMP) framework. The product is developed as a combined cell-matrix product in which autologous ASC-derived cells are seeded onto INTEGRA Dermal Regeneration Template for application to the wound bed.

1.2 Proposed indication

ASC Heal is intended for the treatment of chronic, non-healing cutaneous wounds, including diabetic foot ulcers, venous leg ulcers and pressure injuries, where impaired perfusion, persistent inflammation and defective tissue remodeling limit durable closure under standard of care (Lee et al., 2022; Andia et al., 2019).

1.3 Product definition

The operational definition of ASC Heal and the related batch configuration are summarized in Table 2.

Table 2. Product definition and batch configuration.

Element	Definition
Starting material	Autologous subcutaneous adipose tissue/lipoaspirate collected from the enrolled patient under aseptic surgical conditions.
Active substance	Viable autologous ASC-derived cells isolated from adipose tissue, expanded ex vivo under GMP-compliant conditions, harvested, washed and characterized before incorporation into the matrix.
Final product	A single-use sterile cell-seeded INTEGRA Dermal Regeneration Template containing the autologous cellular active substance on a defined matrix area for topical/local application to the wound bed.
Mode of administration	Topical/local implantation of the cell-seeded matrix onto a surgically debrided wound bed.
Batch definition	Each batch is patient-specific and corresponds to one autologous product manufactured from one tissue procurement event for the same recipient.

1.4 Expected mechanism of action

The expected therapeutic activity is mediated by complementary direct and paracrine mechanisms. The ASC product contains endothelial, stromal/progenitor and mural/perivascular populations able to support vascular repair, local trophic signaling and extracellular matrix remodeling. Preclinical work with ASC seeded on INTEGRA in ischemic wounds supports a mechanism involving donor-cell contribution to vascular structures and stimulation of host vascularization. The proposed pharmacological activities include (Mizuno et al., 2012; Bora and Majumdar, 2017; Vuerich et al., 2023): (Mizuno et al., 2012; Bora and Majumdar, 2017; Vuerich et al., 2023).

- promotion of angiogenesis and revascularization through endothelial cell persistence, host vessel recruitment and release of pro-angiogenic mediators such as VEGF and FGF family members (Andia et al., 2019; Vuerich et al., 2023);
- immunomodulation of the wound microenvironment, including support of transition from persistent inflammation toward a pro-resolving/pro-repair phenotype;
- support of fibroblast and keratinocyte activity, granulation tissue formation and extracellular matrix remodeling;
- local cell retention and spatial delivery provided by the INTEGRA collagen-glycosaminoglycan matrix.

1.5 Dosage form and administration

ASC Heal is administered as a cell-seeded dermal regeneration matrix. Following wound debridement, the ASC Heal matrix is placed directly onto the prepared wound bed and secured according to the clinical protocol. A secondary sterile dressing may be applied. Dose should be expressed as viable cells per cm^2 of wound/matrix area. The preliminary dose range of 1-30 x 10^6 viable cells and should be re-evaluated and harmonized with the final manufacturing yield, matrix size, loading efficiency and clinical protocol.

1.6 Cell-Matrix product configuration

ASC Heal is defined as an autologous ASC-derived cell product incorporated into INTEGRA Dermal Regeneration Template for topical/local application onto the surgically debrided wound bed. INTEGRA is used to support local cell retention, wound-bed coverage and regenerative activity. The final regulatory classification of the cell-matrix product will be confirmed with the competent authority; for the purpose of this IMPD, the product is described and controlled as a combined cell-matrix ATMP. The cellular drug substance consists of viable autologous ASC-derived cells isolated from adipose tissue, expanded ex vivo under GMP-compliant conditions, harvested, washed and formulated before seeding onto INTEGRA. The cellular drug substance may be cryopreserved as an intermediate prior to final drug product preparation. The final drug product, consisting of cells seeded onto INTEGRA, is intended to be prepared fresh and applied within the validated in-use hold time. Final GMP manufacturing site details, site responsibilities, QP declaration, tissue establishment authorizations, raw-material specifications and certificates of analysis will be provided in the relevant quality sections and appendices. Clinical experience with autologous cultured adipose-derived stromal/stem cells in critical limb ischemia and the regulatory assessment of darvadstrocel (Alofisel) provide supportive context for the safety and regulatory positioning of adipose-derived stromal cell-based ATMPs; however, ASC Heal remains product-specific and requires its own quality, non-clinical and clinical justification (Bura et al., 2014; EMA, 2018).

2. Information on biological, chemical and pharmaceutical quality concerning biological investigational medicinal products in clinical trials

The following sections use a common technical document (CTD) structure for biological investigational medicinal products. Because ASC Heal is an autologous cell-matrix ATMP, the separation between drug substance (DS) and drug product (DP) is operational and should be confirmed with the competent authority. In this document, the DS is the harvested and characterized autologous ASC-derived cell preparation, and the DP is the cell-seeded INTEGRA matrix ready for topical/local clinical application.

S. Drug Substance

The drug substance of ASC Heal consists of viable, autologous adipose-derived stem cells isolated from subcutaneous adipose tissue obtained from the same patient by lipoaspiration and expanded ex vivo under GMP-compliant conditions. The cellular drug substance is intended to provide the biological activity of the investigational medicinal product through pro-regenerative, pro-angiogenic and immunomodulatory mechanisms. Following manufacture and release testing, the drug substance is incorporated into INTEGRA Dermal Regeneration Template to generate the final drug product for topical/local application onto the surgically debrided wound bed. The cells are not genetically modified and are not derived from an established cell bank. Each batch is patient-specific and corresponds to the cellular material obtained from one autologous adipose tissue procurement and processed as a single manufacturing run.

S.1 General information

S.1.1 Nomenclature

The drug substance is defined as viable autologous ASC-derived cells isolated from adipose tissue and expanded ex vivo under controlled GMP-compliant conditions. Working names include ASC Heal cellular active substance, autologous ASC-derived cells, or ASC Heal DS. The product does not currently have an International Non-proprietary Name.

S.1.2 Structure

As a cellular active substance, ASC Heal DS does not have a single molecular structure. The DS is a heterogeneous viable cell preparation derived from the stromal vascular fraction of adipose tissue. Relevant cellular components include endothelial cells (ECs), fibro-adipogenic progenitors (FAPs), ASC/stromal populations and mural/perivascular populations (PERs). The identity and composition of these populations are evaluated by multiparameter flow cytometry and selected orthogonal characterization assays (Bourin et al., 2013; Zimmerlin et al., 2010). (Bourin et al., 2013; Zimmerlin et al., 2010; Crisan et al., 2012).

S.1.3 General properties

The DS is expected to support wound repair through angiogenic, paracrine, trophic and matrix-remodeling activities. The key biological properties relevant to the proposed mode of action are viability, cell number, identity/composition, sterility/microbiological safety, endotoxin/mycoplasma status, absence/control of process-related impurities, and potency related to angiogenesis and regenerative signaling. (Mizuno et al., 2012; Vuerich et al., 2023).

S.2 Manufacture

S.2.1 Manufacturers

The activities for the ASC Heal cellular drug substance will be performed in accordance with applicable GMP requirements at the sites listed below. At the current stage of development, the final GMP manufacturing sites, testing laboratories, QP release responsibilities and tissue establishment authorizations are under sponsor confirmation. Final site names, addresses, responsibilities, GMP status, QP declaration and applicable tissue establishment permits will be inserted before clinical trial submission and before manufacture of clinical batches. The drug substance manufacturing chain will include, as applicable, adipose tissue procurement, transport to the manufacturing site, ASC isolation, ex vivo expansion, harvest, washing, formulation, in-process control testing, quality control testing, storage if applicable, batch documentation review and release for drug product manufacture. If the same site is responsible for both drug substance and drug product manufacture, this will be indicated and cross-referenced to section P.3.1. The planned responsibilities for drug substance manufacture and testing are summarized in Table 3.

Table 3. Planned manufacturers and testing sites for the ASC Heal cellular drug substance.

Site / organization	Address	Activity	GMP / authorization status	Responsibility
Clinical procurement site	To be confirmed	Autologous adipose tissue procurement by lipoaspiration	Tissue establishment permit / clinical site authorization to be confirmed	Starting material collection, donor/patient identification, chain-of-custody
Transport provider / logistics	To be confirmed	Transport of adipose tissue to manufacturing site	Qualified/validated transport system to be confirmed	Controlled transport, temperature monitoring, traceability
GMP manufacturing site	To be confirmed	ASC isolation, cell expansion, harvest, washing and DS formulation	GMP manufacturing authorization / QP declaration to be confirmed	Drug substance manufacture
Quality control laboratory	To be confirmed	Cell count, viability, flow cytometry, endotoxin, sterility, mycoplasma, potency-related assays	GMP/qualified testing laboratory status to be confirmed	Drug substance testing
Qualified Person / release site	To be confirmed	Review of batch documentation and release for DP manufacture	QP responsibility to be confirmed	Batch certification/release decision

Final manufacturer information will be updated once the clinical manufacturing model has been confirmed. No clinical batch will be manufactured or released before confirmation of the relevant GMP authorizations, quality agreements, QP responsibilities and tissue establishment requirements.

S.2.2 Description of manufacturing process and process controls

The proposed DS manufacturing process comprises the following high-level steps. Parameters, acceptable ranges and decision points should be transcribed from the final GMP batch record/SOPs. The proposed high-level drug substance manufacturing process and process controls are summarized in Table 4.

Table 4. Proposed drug substance manufacturing process and process controls.

Step	Description and controls
1. Tissue procurement	Autologous adipose tissue is obtained by lipoaspiration under aseptic surgical conditions. Manual lipoaspiration is preferred in this draft based on preservation of in vivo angiogenic potency in the comparative dataset; PureGraft or MicroAire alternatives require comparability justification.
2. Transport to GMP site	Lipoaspirate is transported in a validated sterile container under controlled temperature and time limits. Chain-of-identity and chain-of-custody are maintained.
3. Tissue washing and digestion	Adipose tissue is washed and enzymatically digested using GMP-grade collagenase, with DNase and buffered solution as applicable. Digestion time, temperature, enzyme concentration and quench conditions are critical process parameters.
4. ASC recovery	The digested suspension is centrifuged and filtered to obtain the ASC cell suspension; oil, liquid layers, debris and residual enzymes are removed.
5. Expansion	ASC cells are seeded and expanded in HEM-based GMP-compatible medium in a closed NANT XL bioreactor workflow. Culture duration, confluency, medium change, temperature and CO_2 are monitored.
6. Harvest and wash	Cells are detached using a GMP-compatible detachment reagent such as TrypLE Select CTS or equivalent, washed and collected. Cell count and viability are determined.
7. DS testing and release for DP manufacture	DS is sampled for identity/composition, viability, purity, microbiological tests, endotoxin, mycoplasma and potency/functional assays as feasible.
8. DS storage or direct transfer	Depending on final strategy, DS is either used fresh for INTEGRA seeding or cryopreserved as an intermediate/DS.

S.2.3 Control of materials

S.2.3.1 Raw and starting materials

The proposed raw and starting materials for drug substance manufacture are listed in Table 5.

Table 5. Raw and starting materials for drug substance manufacture.

Material	Type	Use	Control strategy
Autologous adipose tissue / lipoaspirate	Starting material	Collected from the same patient. Donor screening and infectious-disease testing according to applicable tissue/cell regulations and national requirements.	Chain-of-identity, chain-of-custody, procurement records, time/temperature limits, donor eligibility testing.
Collagenase NB6	Raw material	Enzymatic tissue digestion.	Qualified supplier, CoA, sterility/endotoxin where applicable, residual enzyme control or process clearance.
DNase I	Raw material	Reduction of viscosity and support of ASC recovery during digestion.	CoA, biological-origin assessment, residual impurity strategy.
BFHH / HBSS / PBS and wash buffers	Raw materials	Tissue processing, washing and dilution.	Sterile/compendial grade where available, CoA, endotoxin and suitability for cell therapy manufacturing.
Human platelet lysate	Biological raw material	Culture supplementation.	Human-origin material control, viral/TSE risk assessment, donor pool testing, CoA, batch qualification.
HEM	Raw material / process medium	Selected compatible expansion medium to support EC and FAP preservation/expansion.	CoA, adventitious-agent assessment.
TrypLE Select CTS or equivalent	Raw material	Cell detachment after expansion.	CoA, residual impurity risk assessment.
HSA 1% or final carrier component	Excipient / formulation component	Cell resuspension and final loading onto INTEGRA.	Pharmacopoeial/medicinal product grade if possible, viral/TSE safety documentation.
INTEGRA Dermal Regeneration Template	Non-cellular matrix / device component of DP	Cell delivery scaffold and dermal regeneration matrix for local retention on wound bed.	CE/approved device documentation.

S.2.3.2 Source, history and generation of the cellular substrate

The cellular substrate is autologous adipose tissue collected from the same patient intended to receive the product. The use of lipoaspirate is preferred over en bloc excision because it is less invasive, scalable and compatible with closed/aseptic processing workflows. Donor characteristics such as age, BMI, weight-loss history and comorbidities may influence ASC composition and should be captured in the clinical/manufacturing dataset to support future donor-stratification and comparability analyses (Iyyanki et al., 2015; Chen et al., 2017; Duscher et al., 2016).

S.2.3.3 Cell bank system, characterization and testing

A conventional master/working cell bank system is not expected for this patient-specific autologous product. If autologous DS is cryopreserved for delayed preparation or repeat dosing, each cryopreserved patient-specific lot should be treated as a patient-specific intermediate/DS with full traceability, qualified storage conditions and defined post-thaw acceptance criteria. No allogeneic cell bank is envisaged in this draft.

S.2.4 Control of critical steps and intermediates

The critical drug substance manufacturing steps and controls are summarized in Table 6.

Table 6. Critical drug substance manufacturing steps and controls.

Critical step	Critical parameters	Controls / acceptance approach
Adipose tissue collection	Procurement method, cannula/suction parameters, aseptic collection, volume, time to processing.	Collection record; tissue volume; macroscopic acceptance; donor testing; transport temperature/time.
Enzymatic digestion	Collagenase/DNase concentration, digestion time, temperature, mixing, quench/neutralization.	Digestion record; recovery yield; residual enzyme/process impurity strategy.
Filtration/centrifugation	Filter pore size, centrifugation speed/time, washing efficiency.	Cell count, debris removal, viability.
Expansion in HEM	Seeding density, medium lot, culture duration, confluency, medium changes.	Confluency monitoring; morphology; contamination checks; cell count/viability; flow cytometry.
NANT XL closed expansion if used	Closed sterile cartridge, automated seeding, imaging every 8 h, medium change, detachment and harvest.	System logs, cell culture report, temperature/CO_2/confluency, operator traceability.
Harvest	Detachment reagent, incubation time, wash steps, residual reagent removal.	Cell count, viability, identity/composition, residual impurity assessment as applicable.
Cryopreservation/thawing if used	Cryoprotectant composition, controlled cooling, storage temperature, thawing procedure.	Post-thaw viability, recovery, identity, potency and in-use hold time.

S.2.5 Process validation and-/or evaluation

For an early clinical ATMP, full process validation may be limited, but process evaluation, engineering runs and aseptic process simulation are expected. The aseptic processing steps, including tissue handling, digestion, expansion, harvest, formulation and cell seeding onto INTEGRA, should be supported by media fills or process simulations representative of the final workflow. For NANT XL, process qualification should include cartridge integrity, aseptic connection/disconnection, automated medium exchange, detachment, harvest and log/report generation. Acceptance criteria and deviation procedures should be predefined.

S.2.6 Manufacturing process development

The process-development package should support the clinical process by linking the material used in non-clinical studies with the material intended for clinical use:

- HEM was selected as the preferred GMP-compatible medium because comparative work showed enhanced endothelial and mural/perivascular support relative to DMEM/F12 and comparable translational performance to the laboratory grade EGM-2, while being more suitable for GMP-oriented workflows.
- Manual lipoaspiration is preferred as the baseline procurement approach. PureGraft filtration and MicroAire power-assisted lipoaspiration may remain process alternatives but require comparability and potency justification if adopted clinically.
- Expanded ASC cells were seeded onto INTEGRA in preclinical work and implanted into ischemic wound models, supporting INTEGRA as the selected matrix/delivery platform (Vuerich et al., 2023).

- NANT XL provides a closed, automated and traceable expansion environment with controlled temperature/CO2, confluency monitoring and final run reports, and may reduce operator-dependent variability. Clinical implementation requires site-specific qualification (Fitzgerald et al., 2022).

Integrated quality risk analysis and control strategy

The integrated quality risk analysis and control strategy are summarized in Table 7.

Table 7. Integrated quality risk analysis and control strategy.

Risk / QA	Source of risk	Control strategy	Tests / records	Residual risk / note
Microbial contamination	Autologous tissue procurement, enzymatic digestion, cell culture, matrix loading, short in-use manipulation.	Aseptic procurement; qualified sterile raw materials; closed system where feasible; environmental monitoring; sterility, endotoxin and mycoplasma tests; aseptic process validation/media fill.	Sterility, endotoxin, mycoplasma, bioburden where applicable; final release or staged release strategy.	If final sterility result is not available before application, use risk-based staged release and post-administration reporting procedure.
Low viability / poor recovery	Donor variability, transport delay, digestion, expansion, cryopreservation/thawing, matrix seeding.	Time/temperature limits; validated digestion; optimized HEM expansion; post-thaw controls.	Viability and viable cell dose at DS and DP stage.	Final thresholds to be justified by batch data and clinical dose requirements.
Loss of identity / altered composition	Expansion-induced marker shifts, detachment, donor variability.	Defined passage/duration; HEM medium; flow cytometry panel; morphology/confluency monitoring.	CD45, CD31, CD146, CD34, CD90 and ASC markers; EC/FAP/PER composition.	CD34 changes during expansion should be interpreted in the context of ASC biology and predefined acceptance/characterizatio n strategy.
Insufficient potency	Low pro-angiogenic cell populations, donor factors, process changes, matrix incompatibility.	HEM process; marker-based characterization; potency assay reflecting angiogenesis/paracrine activity.	VEGF/angiogenic factor secretion, tube formation, EC/FAP/PER characterization, cell retention on INTEGRA.	Potency assay acceptance criteria need qualification with manufacturing and preclinical correlation.
Process-related impurities	Residual collagenase/DNase/TrypLE /HPL components, leachables from disposables.	Validated washes; qualified materials; residual impurity testing where risk-relevant.	Residual enzyme/protein/DNA if applicable; endotoxin; compatibility/leachables assessment.	Set impurity tests based on risk and exposure.
Matrix-related failure	Incorrect INTEGRA lot/dimensions, loss of sterility, poor cell loading, poor retention.	Qualified device component; sterility/expiry checks; validated loading procedure; visual inspection.	INTEGRA identity/integrity, lot/expiry, cell loading efficiency, dose per cm^2, in-use hold time.	Clarify regulatory status and documentation requirements for INTEGRA as DP component.
Administration error / chain-of-identity failure	Patient-specific autologous workflow.	Unique identifiers, double checks, batch record, clinical site verification.	Chain-of-identity/custody checks at procurement, release, shipment and application.	Critical for autologous product; require reconciliation before application.

S.3 Characterization

S.3.1 Elucidation of structure and other characteristics

Characterisation should define the cellular composition, biological activity and product profile of the DS. The proposed phenotyping strategy is based on viable singlet CD45-negative cells and analysis of endothelial, fibro-adipogenic and perivascular subsets (Bourin et al., 2013; Zimmerlin et al., 2010; Crisan et al., 2012). (Bourin et al., 2013; Lazarski and Hanley, 2024). The proposed characterization strategy for drug substance cellular composition and function is summarized in Table 8.

Table 8. Characterization strategy for drug substance cellular composition and function.

Attribute / population	Method / markers	Relevance
Viability	Live/dead dye, 7-AAD/PI, trypan blue or equivalent.	Ensures minimum viable cell dose and suitability for matrix loading.
Hematopoietic exclusion	CD45.	Exclusion/quantification of hematopoietic populations.
Endothelial cells (ECs)	CD45- CD31+ CD146+ CD34+.	Direct angiogenic component; associated with human endothelial persistence within INTEGRA in preclinical work.
Fibro-adipogenic progenitors (FAPs)	CD45- CD31- CD146- CD34+ CD90+.	Paracrine support of host vascularization and tissue regeneration.
Pericyte-enriched mural/perivascular population (PERs)	CD45- CD31- CD146+ CD34- CD90+.	Mural/perivascular support and vessel maturation.
ASC/stromal markers	CD73+, CD90+, CD105+.	Alignment with ASC/MSC characterization, with interpretation adapted to heterogeneous ASC-derived product.
Functional subsets	Exploratory CD26, CD55, CD146, CD9, CD140a.	Support donor/product profiling and future potency or stratification; not proposed as release tests unless qualified.
Potency-related secretome	VEGF, FGF-2, ANGPT1/2, TGF-beta, IL-10, MMP/TIMP ratio as applicable.	Links to angiogenesis, immunomodulation and matrix remodeling.

S.3.2 Impurities

Potential impurities include residual digestion enzymes (collagenase, DNase), detachment reagent, culture medium/HPL components, host/tissue debris, dead cells, and leachable from single-use systems. The impurity-control strategy should combine qualified material sourcing, defined wash steps, process evaluation and targeted residual testing where risk-relevant. Product-related impurities include non-viable cells, unwanted cell subpopulations and senescent/aberrant cells; these should be controlled by viability, passage/culture duration, morphology and flow cytometry.

S.4 Control of the active substance

Given the autologous nature of the product and the expected inter-donor variability of adipose-derived ASC-derived cells, control of the active substance relies on a combination of starting-material controls, in-process controls, final substance testing and batch record review. The main quality attributes monitored include cell number, viability, identity, ASC phenotype, cellular composition, purity, microbiological safety, endotoxin, mycoplasma and potency-related angiogenic activity. The cellular drug substance is tested before and after expansion in bioreactor. Testing is intended to confirm that the cell suspension meets predefined acceptance criteria and is suitable for manufacture of the final cell-seeded matrix drug product. Where full microbiological results are not available before final product preparation or clinical use, release may be supported by a staged control strategy including rapid microbiological methods, validated aseptic processing, environmental monitoring, raw-material controls and post-release review of final sterility results. Acceptance criteria are established based on available process development data, published experience with ASC-based products, and the intended mechanism of action of ASC Heal. Specifications will be refined during clinical development as additional manufacturing, stability, comparability and clinical safety data become available.

S.4.1 Specification - drug substance

The proposed ASC Heal cellular drug substance specification is provided in Table 9.

Table 9. Proposed specification for ASC Heal cellular drug substance.

Parameter	Acceptance criterion	Method
Appearance	Cell suspension, free of visible clumps/particulates outside predefined acceptance.	Visual inspection
Viable cell count / dose	Sufficient to meet DP dose and matrix loading target.	Automated counter/Burker chamber + viability dye
Viability	> 70% post-harvest / post-thaw; final threshold to be justified.	Trypan blue, 7-AAD/PI or live/dead flow cytometry
Identity / composition	ASC-derived phenotype; EC, FAP, PER and ASC marker panel.	Flow cytometry
Purity / unwanted cells	CD45+ and other negative markers within predefined range; dead cells controlled.	Flow cytometry
Sterility	No growth / negative.	Ph. Eur. 2.6.1, BacT/Alert or rapid method
Mycoplasma	Negative.	Ph. Eur. 2.6.7 or validated NAT/PCR
Endotoxin	Within clinically acceptable limit based on route/dose.	LAL or recombinant Factor C
Potency	Angiogenic/paracrine activity demonstrated.	VEGF secretion, endothelial tube formation, or qualified surrogate assay
Genetic stability	No clinically relevant abnormality for expanded cells if applicable.	Karyotype or alternative assay on selected lots/passages

S.4.2 Analytical procedures

Analytical procedures used for control of the ASC Heal cellular drug substance are described in the same order as the drug substance specification. Pharmacopoeial methods, including sterility, mycoplasma and endotoxin testing, are performed according to the relevant Ph. Eur. chapters unless otherwise stated. Any deviation from or modification to pharmacopoeial procedures will be described and justified. Cell count and viability are assessed using an automated cell counter or Bürker chamber in combination with a viability dye, or by flow-cytometry-based viability assessment. Visual inspection is performed to confirm the absence of visible aggregates, particulates, discoloration or other macroscopic abnormalities. Flow cytometry is used to assess identity, stromal phenotype, purity and cellular composition. The method includes appropriate antibody panels, viability exclusion, singlet gating, fluorescence-minus-one and/or unstained controls, predefined gating strategy, minimum event count and standardized data-analysis procedures. The core marker panel includes CD73, CD90 and CD105 for the stromal phenotype; CD45 and HLA-DR for hematopoietic and immunogenicity-associated markers; and CD31, CD34, CD146 and CD90 combinations for characterization of endothelial, fibro-adipogenic and perivascular/mural subpopulations. CD31-positive cells are quantified as a relevant ASC-derived pro-angiogenic subpopulation and are not treated as an impurity. Potency-related activity is assessed using one or more angiogenic/paracrine functional assays, such as VEGF secretion by ELISA, endothelial tube formation, cytokine profiling or another qualified surrogate assay reflecting the intended mechanism of action. Potency methods will define cell input, assay conditions, positive and negative controls, readouts, acceptance criteria and qualification status. The analytical methods used in early clinical development may be qualified rather than fully validated. Method validation and refinement of acceptance criteria will continue during development as additional manufacturing, stability and clinical data become available (Lazarski and Hanley, 2024).

S.4.3 Validation of analytical procedures

Full validation may not be complete for early-phase clinical trials.

S.4.4 Batch analyses

At the current stage of development, no GMP clinical batches of the ASC Heal cellular drug substance have yet been manufactured using the final intended clinical process. Therefore, full batch analysis data according to the proposed drug substance specification are not yet available. The proposed drug substance specification is based on available process development data, preclinical experience, analytical feasibility and the intended control strategy. Results from representative engineering, development, non-clinical and stability batches will be tabulated in this section as soon as they become available.

Available non-clinical and process development studies have supported the feasibility of SVF/ASC isolation, expansion, phenotypic characterization and incorporation into INTEGRA Dermal Regeneration Template. However, these data are considered supportive and are not presented as formal GMP batch release data unless the batches were manufactured under conditions representative of the intended clinical process. For future batches, results will be reported in the same order as the drug substance specification and will include appearance, viable cell count/dose, viability, identity/stromal phenotype, cellular composition, purity/unwanted cells, sterility, mycoplasma, endotoxin, potency-related activity and, where applicable, genetic stability. Any result outside the proposed acceptance criteria will be investigated and justified. Batch analysis data will be updated as additional representative manufacturing runs are completed. The first GMP clinical batches will be reviewed against the proposed specification and used to refine acceptance criteria, confirm analytical suitability and support the final control strategy.

S.4.5 Justification of specification

The proposed specifications for the ASC Heal cellular drug substance are provisional for early clinical development and are based on available process development data, preclinical experience, analytical feasibility, safety considerations, and the intended mechanism of action of the product. The specification is designed to ensure that each patient-specific autologous cellular batch is suitable for incorporation into INTEGRA Dermal Regeneration Template and subsequent topical/local application onto the wound bed.

Given the autologous nature of the product, inter-donor variability in cell yield, expansion capacity and cellular composition is expected. Therefore, the specification combines fixed safety-related acceptance criteria with reportable characterization parameters that will be further refined as additional GMP manufacturing and clinical data become available.

Appearance

Appearance is included as a routine release parameter to confirm that the cellular drug substance is a homogeneous cell suspension and free of visible clumps, particulates, discoloration or signs of contamination. Visible abnormalities may indicate cell aggregation, contamination, inappropriate handling or formulation failure and therefore represent a relevant quality and safety concern.

Viable cell count

Viable cell count is included to ensure that the cellular drug substance is sufficient to meet the intended final drug product loading target on INTEGRA. The target dose is expressed as viable cells per cm^2 of INTEGRA matrix or wound area, according to the assigned clinical dose cohort.

Cell yield is expected to vary between patients due to donor-specific factors and starting material variability. For this reason, the acceptance criterion is linked to the ability to manufacture the assigned dose cohort rather than to a fixed total cell number. Batches that do not provide sufficient viable cells to meet the intended dose and matrix loading requirement will not proceed to final drug product manufacture unless justified and approved according to predefined procedures.

Viability

Viability is considered a critical quality attribute for the cellular drug substance because the biological activity of ASC Heal depends on the presence of viable ASC-derived cells capable of exerting pro-angiogenic, paracrine and immunomodulatory effects. A provisional acceptance criterion of ≥80% viable cells prior to seeding onto INTEGRA is proposed for the cellular drug substance. This threshold is considered appropriate for early clinical development to ensure sufficient viable cellular material before final drug product preparation. A lower threshold may be justified for the final cell-seeded matrix or in-use product if supported by cell recovery, retention, viability and potency data after seeding onto INTEGRA.

Identity/ASC-stromal phenotype

Identity testing is based on flow-cytometry confirmation of an adipose-derived stromal/ASC phenotype. Positivity for CD73, CD90 and CD105 is used to confirm the presence of the expanded stromal/ASC component within the heterogeneous ASC-derived drug substance. The provisional criterion of ≥70% CD73/CD90/CD105-positive cells within the viable population is considered acceptable for early clinical development and will be refined based on additional manufacturing data. Because ASC Heal is not intended to be a purified ASC population, identity is interpreted together with the broader cellular composition profile (Bourin et al., 2013).

Cellular composition

The cellular drug substance is a heterogeneous ASC-derived product. Therefore, EC, FAP and PER populations are characterized by flow cytometry and reported as part of the product profile. At this stage, fixed acceptance limits for EC, FAP and PER subpopulations are not proposed, because sufficient GMP batch data are not yet available to define meaningful ranges. Results will be collected during development to establish expected ranges and evaluate correlation with potency and clinical outcome.

Purity

Purity testing is included to control unwanted or potentially undesirable cellular populations. CD45-positive hematopoietic cells are limited to ≤10% of viable cells, unless otherwise justified by process and clinical data. This criterion is intended to reduce excessive hematopoietic/inflammatory cell contamination while recognizing the heterogeneous nature of SVF-derived products. HLA-DR expression is monitored as an immunogenicity-associated marker and is expected to be low or negative. A provisional limit of ≤10% HLA-DR-positive cells is proposed. This is considered appropriate for an autologous product in early clinical development and will be reassessed as additional data become available.

Cell aggregates

Control of visible cell aggregates is included to reduce the risk of non-uniform seeding onto INTEGRA, inconsistent matrix loading, reduced cell recovery and potential loss of product quality. The cellular drug substance should be free of visible aggregates before seeding. If a quantitative aggregate assay is implemented, a provisional limit such as ≤5% aggregates may be applied and refined during process qualification.

Sterility

Sterility is a critical safety attribute for a cell-based product intended for application to chronic wounds. Testing is performed using Ph. Eur. 2.6.1, BacT/Alert, Bactec or another validated method. Due to the limited in-use shelf life of the final cell-seeded INTEGRA product, final sterility results may not always be available before clinical application. In such cases, release may follow a staged or conditional strategy based on validated aseptic processing, environmental monitoring, raw-material controls, in-process microbiological controls and available rapid test results. Final sterility results will be reviewed retrospectively according to predefined quality and clinical risk-management procedures.

Mycoplasma

Mycoplasma testing is included as a mandatory microbiological safety parameter. The acceptance criterion is negative. Testing is performed according to Ph. Eur. 2.6.7 or by a validated NAT/PCR-based rapid method. Given the relevance of mycoplasma contamination for cell culture products, a negative result should be available before release whenever compatible with the manufacturing timeline.

Endotoxin

Endotoxin is included as a critical safety parameter. The proposed provisional acceptance criterion is ≤0.5 EU/mL, with total endotoxin exposure remaining below the clinically acceptable limit based on route of administration, product volume and patient dose. The limit will be harmonized between the drug substance and final drug product specifications. The selected criterion is intended to provide a conservative safety margin for topical/local application to a wound bed and will be reassessed based on final formulation volume, matrix loading procedure and clinical dose.

Potency

Potency testing is included to demonstrate biological activity relevant to the intended mechanism of action of ASC Heal. The proposed potency strategy focuses on angiogenic and paracrine activity, using assays such as VEGF secretion, endothelial tube formation, cytokine profiling or another qualified surrogate assay. At this stage of development, the potency assay and acceptance criteria are under qualification. A provisional requirement for demonstrable angiogenic/paracrine activity compared with an appropriate negative control is considered acceptable for early clinical development. Quantitative results will be collected across development and GMP batches to define a more robust potency specification. The selected potency approach is justified by the proposed mechanism of action of ASC Heal, which relies on promotion of angiogenesis, support of host vascularization, modulation of the wound microenvironment and paracrine stimulation of tissue repair (Andia et al., 2019; Vuerich et al., 2023).

Genetic stability

Genetic stability is included as a development and characterization parameter for expanded cells, particularly where culture expansion is performed. The acceptance criterion is absence of clinically relevant chromosomal abnormalities in selected lots or passages. Routine karyotype testing of every autologous batch is not proposed at this stage, given the short-term expansion, autologous use, absence of genetic modification and absence of immortalization. Genetic stability testing will be performed on selected representative batches or passages as part of process characterization and risk assessment.

Stability-indicating parameters

Viability and cellular composition and potency-related activity are considered stability-indicating parameters for the cellular substance. Where the drug substance is cryopreserved as an intermediate, post-thaw viability, recovery, phenotype and potency-related activity will be assessed to support the proposed storage conditions and shelf life.

For fresh cells used directly for seeding onto INTEGRA, in-use stability will be assessed by monitoring the viability, matrix loading/retention and potency-related activity over the proposed hold time before clinical application.

S.5 Reference standards or materials

No compendial reference standard is available for the ASC Heal cellular drug substance. Due to the autologous and heterogeneous nature of the ASC-derived cell population, establishment of a conventional reference standard is not considered feasible at this stage of development. Reference and control materials used for analytical testing will include well-characterized representative development or engineering batches of ASC Heal cellular drug substance, where available, and assay-specific positive and negative controls. These materials will be used to support method qualification, assay performance monitoring, flow-cytometry gating strategy, potency assay interpretation and comparability during process development. Any representative cellular reference material will be manufactured using a process comparable to the intended clinical manufacturing process and characterized using the analytical methods described in sections S.3 and S.4, including cell count, viability, morphology, flow-cytometry phenotype, cellular composition, sterility, mycoplasma, endotoxin and potency-related activity. For flow cytometry, appropriate controls will include unstained cells, fluorescence-minus-one controls, viability controls and compensation controls, as applicable. For potency-related assays, reference or control materials may include a qualified ASC/SVF-derived cell batch, positive control cells or conditioned medium, and negative controls such as assay medium alone or non-cell-seeded matrix, depending on the assay format. Commercial reference standards or qualified materials will be

used for compendial or biochemical assays where applicable, including endotoxin standards for LAL or recombinant Factor C testing, molecular controls for mycoplasma NAT/PCR, and calibrated standards for ELISA-based quantification of VEGF or other soluble factors. The qualification status, storage conditions, stability, intended use and acceptance criteria for each reference or control material will be documented in the relevant analytical method SOPs. As development progresses, a representative reference batch may be established to support assay trending, comparability assessments and refinement of potency specifications.

S.6 Container closure system

The DS container closure system depends on whether the DS is used fresh or cryopreserved. Fresh DS may be transferred in sterile closed bags/syringes/vials compatible with short-term hold. Cryopreserved DS may be stored in sterile cryobags or cryovials validated for the selected cryopreservation conditions. Container integrity, extractables/leachables and compatibility with cells and formulation should be documented.

S.7 Drug substance stability

The stability program should be defined on cellular DS under fresh hold or refrigerated/ambient transport when applicable, cryopreserved storage and post-thaw hold. Stability-indicating attributes should include viability, viable cell recovery, identity/composition, potency and microbiological status. The previous statement of 6-month storage at ≤ -80 °C should be retained only if supported by product-specific real-time stability data. For long-term storage, liquid nitrogen vapor-phase storage may be considered depending on the final cryopreservation strategy.

S.7.1 Protocol and methods, results, shelf life

Stability summary and conclusion

The ASC Heal cellular drug substance consists of autologous ASC-derived cells prior to seeding onto INTEGRA Dermal Regeneration Template. Drug substance stability will be assessed to support the proposed storage condition, transport condition, post-thaw handling, and maximum hold time before final drug product manufacture. The final drug product, consisting of cells seeded onto INTEGRA, is intended to be prepared fresh and used within a validated in-use hold time; stability of the final cell-seeded matrix is described in section P.8.

Two drug substance handling scenarios are considered during development:

- Scenario A: fresh cellular drug substance used directly for seeding onto INTEGRA;
- Scenario B: cryopreserved cellular drug substance stored as an intermediate and thawed before seeding onto INTEGRA.

For the current early clinical development stage, the preferred strategy is to cryopreserve the cellular drug substance as an intermediate, where required, and to prepare the final cell-seeded INTEGRA product fresh immediately before clinical use. The final cell-seeded INTEGRA product is not intended to be cryopreserved.

Stability protocol

Stability studies will be performed on batches manufactured using a process comparable to the intended clinical manufacturing process. Where possible, stability batches will include material representative of the final clinical cell population, formulation, cell concentration and container closure system. The cellular drug substance will be stored in sterile cryogenic containers or bags suitable for cell therapy products. The container closure system used in stability studies will be identical to, or representative of, the system intended for clinical storage. Compatibility of the cellular drug substance with the container closure system will be evaluated as part of the stability program. For cryopreserved drug substance, the proposed storage condition is ≤ -80 °C for short- to medium-term storage, with continuous temperature monitoring. Transfer to vapor-phase liquid nitrogen may be considered for longer-term storage if required. Cryopreservation will be performed using a controlled-rate or validated stepwise freezing procedure and a defined cryoprotective formulation.

Proposed stability time points

The proposed stability testing schedule for cryopreserved cellular drug substance is summarized below. The time points may be adapted based on manufacturing feasibility and available batch numbers.

The proposed drug substance stability time points are summarized in Table 10.

Table 10. Proposed drug substance stability testing time points.

Study type	Storage condition	Time points	Purpose
Real-time stability	-80 °C	T0, 1 month, 3 months, 6 months, 9 months, 12 months	Support proposed DS shelf life
Accelerated	-20 °C or controlled temperature excursion, if justified	Short defined intervals, e.g. 24-72 h	Assess sensitivity to temperature deviations
Post-thaw hold stability	2-8 °C and/or room temperature, as clinically applicable	0 h, 2 h, 4 h, 6 h	Define maximum hold time before seeding onto INTEGRA
Fresh DS hold stability	2-8 °C and/or room temperature	0 h, 2 h, 4 h, 6 h	Support use of fresh DS if no cryopreservation is performed

Stability-indicating tests

The stability-indicating tests will include selected release and characterization assays relevant to safety, identity and biological activity. Where the same methods are used for release testing, this will be stated and cross-referenced to section S.4. The stability-indicating tests for the cellular drug substance are listed in Table 11.

Table 11. Stability-indicating tests for the cellular drug substance.

Parameter	Method	Purpose
Appearance	Visual inspection	Detect visible aggregates, discoloration or contamination
Cell recovery	Cell count	Assess post-storage or post-thaw cell loss
Viability	Trypan blue, 7-AAD/PI or live/dead flow cytometry	Critical stability-indicating attribute
ASC/stromal phenotype	Flow cytometry: CD73, CD90, CD105	Confirm maintenance of stromal/ASC identity
Cellular composition	Flow cytometry: CD45, CD31, CD34, CD146, CD90 +/- additional markers	Monitor EC, FAP, PER and unwanted cell populations
Sterility / bioburden	Ph. Eur. 2.6.1, BacT/Alert/Bactec or equivalent	Confirm microbiological safety
Mycoplasma	Ph. Eur. 2.6.7 or validated NAT/PCR	Confirm absence of mycoplasma
Endotoxin	LAL or recombinant Factor C	Confirm endotoxin remains within specification
Potency-related activity	VEGF secretion, endothelial tube formation, cytokine/paracrine assay or qualified surrogate	Confirm maintenance of angiogenic/paracrine activity
Genetic stability, if applicable	Karyotype or alternative method on selected lots/passages	Developmental characterization, not routine stability testing

Acceptance criteria

The cellular drug substance should remain within the proposed drug substance specifications throughout the assigned shelf life. The following provisional stability acceptance criteria are proposed for early clinical development:

The provisional drug substance stability acceptance criteria are summarized in Table 12.

Table 12. Provisional drug substance stability acceptance criteria.

Parameter	Provisional stability acceptance criterion
Appearance	No visible clumps, particulates, discoloration or signs of contamination
Post-thaw viability	> 70% viable cells; target > 80% before seeding where feasible
Cell recovery	Sufficient to meet assigned INTEGRA loading dose
ASC/stromal phenotype	CD73/CD90/CD105-positive phenotype maintained within predefined range
CD45+ cells	< 10% or within predefined development range
HLA-DR	< 10% or negative
EC/FAP/PER composition	Report result; no fixed limit at this stage
Sterility	Negative / no growth
Mycoplasma	Negative
Endotoxin	< 0.5 EU/mL or within clinically justified limit
Potency	Angiogenic/paracrine activity maintained relative to T0 or reference control

Available data

Available development data and literature support the feasibility of cryopreservation of ASC-derived cell populations. Cryopreservation may reduce total viable nucleated cell recovery, particularly affecting CD45-positive hematopoietic cells, but remaining cells can retain adhesive, proliferative and colony-forming properties. Preliminary data from cryopreserved ASC-derived cells indicate that post-thaw viability remained stable independently of storage duration, supporting the feasibility of ASC banking under controlled conditions. Previous draft data for ASC Heal indicated post-thaw testing of viability, identity and potency and proposed stability for up to 6 months at ≤ -80 °C; this will be confirmed using representative clinical-process batches and the final drug substance formulation.

Proposed shelf life

Based on the available development information and pending confirmation with representative GMP batches, an initial provisional shelf life of up to 6 months at ≤ -80 °C is proposed for cryopreserved cellular drug substance. This shelf life will be confirmed or revised based on real-time stability data, post-thaw recovery, viability, identity, cellular composition and potency-related activity. For fresh or thawed cellular drug substance before seeding onto INTEGRA, a conservative maximum hold time of up to 4 hours at the defined handling temperature is proposed pending completion of in-use stability studies. The final validated hold time will be based on cell viability, recovery, absence of visible aggregation, maintenance of phenotype and suitability for INTEGRA seeding.

Stability conclusion

The proposed drug substance stability strategy is considered appropriate for early clinical development. It focuses on attributes most relevant to safety and biological activity: viability, recovery, identity, cellular composition, microbiological safety and potency-related activity. Stability acceptance criteria and shelf life will be refined as additional representative GMP batch data become available.

P. Investigational Medicinal Product

P.1 Description and composition of the medicinal product

The DP is the final ASC Heal cell-seeded INTEGRA matrix. It consists of the autologous ASC-derived cellular active substance distributed within INTEGRA Dermal Regeneration Template in a defined matrix area and carrier solution. The product is supplied as a single-use sterile product for topical/local application to the debrided wound bed. The final composition, matrix dimensions and cell dose per cm² should be aligned with the clinical protocol and validated manufacturing process. The main components of the ASC Heal drug product are described in Table 13.

Table 13. Description of ASC Heal drug product components.

Component	Function	Description
Autologous ASC-derived cells	Active substance	Viable cell dose per cm² and total viable cells per product; final target to be defined.
INTEGRA Dermal Regeneration Template	Matrix/device component	Sterile collagen-glycosaminoglycan dermal scaffold supporting cell retention and wound bed delivery.
Physiological carrier solution	Formulation vehicle	Supports cell suspension and matrix loading; composition to be defined.
Human serum albumin 1% or equivalent	Excipient	Cell stabilization during preparation/loading; final concentration to be defined.
Secondary dressing	Clinical procedure component	Applied after ASC Heal placement; not part of the active DP unless defined in protocol.

P.1.1 Composition of the drug product

The ASC Heal drug product consists of viable autologous ASC-derived cells incorporated into INTEGRA Dermal Regeneration Template. The drug product is intended for single-use topical/local application onto the surgically debrided wound bed.

The cellular component is manufactured from autologous adipose tissue, expanded ex vivo under GMP-compliant conditions, harvested, washed, formulated in a physiological carrier solution and seeded onto INTEGRA under aseptic conditions. INTEGRA acts as a biocompatible dermal regeneration matrix supporting local retention and distribution of the cellular drug substance at the wound site.

The drug product is not administered as an injectable cell suspension. No additional diluent is currently foreseen for clinical administration. If a thawing or resuspension step is required before seeding onto INTEGRA, the relevant solution will be described in P.3 and controlled as part of the manufacturing process.

The qualitative and quantitative composition of the ASC Heal drug product is provided in Table 14.

Table 14. Qualitative and quantitative composition of ASC Heal drug product.

Component	Quantity	Quality standard	Function
Autologous ASC-derived cellular drug substance	Dose according to clinical cohort: proposed 1 x 10^6, 5 x 10^6 or 10 x 10^6 viable cells/cm^2 of INTEGRA matrix and/or wound area	Manufactured under GMP; controlled according to DS specification in S.4.1	Active cellular component; provides pro-angiogenic, paracrine, immunomodulatory and regenerative activity
INTEGRA Dermal Regeneration Template	Matrix size selected according to wound dimensions; final size to be defined in the clinical protocol	Sterile medical device; manufacturer specification and CoA; suitability for intended use to be documented	Biocompatible dermal scaffold; supports cell loading, local retention and wound-bed coverage
Physiological carrier solution	Quantity sufficient for cell resuspension and matrix seeding; final volume to be defined according to matrix size and cell dose	Sterile, injectable-grade / Ph. Eur. where applicable	Maintains cells in suspension before and during seeding onto INTEGRA
Human serum albumin, if used	1% in formulation solution	Ph. Eur. / GMP-grade / CoA; viral safety documentation	Protein supplement; supports cell viability and reduces cell adhesion to processing surfaces
Cryoprotectant, if DS is cryopreserved before DP manufacture	Not intended to be part of the final DP after washing/resuspension; residual level to be controlled or justified if applicable	GMP-grade / CoA	Used only for DS cryopreservation; removed or diluted before final drug product preparation
Secondary sterile dressing / fixation material, if supplied with product	As required by clinical procedure	CE-marked / clinical-grade; manufacturer specification	Protects and secures the cell-seeded INTEGRA matrix after application; not part of the active cellular component

Container closure

The final drug product is prepared as a sterile, single-use cell-seeded INTEGRA matrix. The matrix is maintained in a sterile container or tray suitable for short-term in-use handling and transfer to the clinical site or operating/procedure room. The container closure system used for the final drug product will be compatible with the cell-seeded matrix, maintain sterility during the validated in-use hold time, and allow aseptic application according to the clinical procedure. If the cellular drug substance is cryopreserved before drug product manufacture, it will be stored separately in sterile cryogenic vials or bags as described in S.6/S.7. The final cell-seeded INTEGRA drug product is prepared fresh and is not intended to be cryopreserved after seeding.

P.2 Pharmaceutical development

P.2.1 Product development rationale

The product concept is based on the need to retain regenerative cells locally within a chronic wound environment that is characterized by impaired vascularization and insufficient trophic support. INTEGRA provides a clinically established porous collagen-glycosaminoglycan matrix designed to support cell infiltration, neovascularization and dermal regeneration. Preclinical work used expanded human ASC cells seeded onto INTEGRA and implanted in ischemic wounds, supporting the use of INTEGRA as a delivery platform for ASC Heal (Vuerich et al., 2023).

P.2.2 Manufacturing process development

Development studies should characterize cell loading onto INTEGRA, including loading volume, cell density, incubation time, temperature, matrix orientation, distribution uniformity, cell retention, viability after loading and in-use hold time. The preclinical procedure used 5 x 10^5ASC cells seeded onto an INTEGRA scaffold for approximately 30 minutes at 37 C and 5% CO_2 before implantation; clinical scaling must be justified based on matrix area, wound size and dose cohort.

P.3 Manufacture

The manufacture of the ASC Heal drug product consists of the final preparation of the cellular drug substance and its aseptic incorporation onto INTEGRA Dermal Regeneration Template. The cellular manufacture, including adipose tissue procurement, ASC-derived cell isolation, ex vivo expansion, harvest, washing, formulation and drug substance controls, is described in section S.2.

The final drug product is defined as viable autologous ASC-derived cells seeded onto INTEGRA Dermal Regeneration Template for topical/local application onto the surgically debrided wound bed. The final cell-seeded INTEGRA matrix is prepared fresh and is not intended to be cryopreserved after seeding. The process is performed aseptically under controlled conditions and is designed to maintain cell viability, cell retention on the matrix, sterility, traceability and suitability for clinical application.

P.3.1 Manufacturers

All activities are performed according to approved procedures and under the applicable GMP, tissue establishment and clinical trial requirements. Site responsibilities include final drug substance preparation, INTEGRA handling, cell seeding onto INTEGRA, in-process controls, final product testing where applicable, packaging, labelling, storage during the in-use hold period, batch certification and release by the Qualified Person. If any final preparation steps are performed at the clinical site after batch release, such as thawing of the cellular drug substance, transfer into the seeding solution, or application of the cell-seeded INTEGRA matrix to the patient, these steps will be described in the clinical protocol and qualified as in-use/reconstitution activities rather than as independent manufacturing steps, where applicable.

P.3.2 Batch formula

One ASC Heal drug product batch is patient-specific and corresponds to the final cell-seeded INTEGRA matrix prepared from one autologous cellular drug substance batch derived from a single adipose tissue procurement. Batch size may vary according to wound area, assigned dose cohort and INTEGRA matrix size. Quantities may be adjusted on a pro rata basis within predefined ranges to achieve the required viable cell dose per cm² of INTEGRA matrix and/or wound area. The proposed batch formula for ASC Heal drug product is summarized in Table 15.

Table 15. Proposed batch formula for ASC Heal drug product.

Component	Quantity per batch	Function
ASC Heal cellular drug substance	Sufficient to achieve assigned dose cohort: proposed 1 x 10^6, 5 x 10^6 or 10 x 10^6 viable cells/cm² of INTEGRA matrix or wound area	Active cellular component
INTEGRA Dermal Regeneration Template	Size selected according to wound dimensions; matrix may be trimmed under aseptic conditions according to clinical need	Dermal regeneration scaffold
Seeding solution	Volume sufficient to uniformly distribute cells onto the matrix; final range to be defined according to matrix size	Supports cell suspension and seeding onto INTEGRA
Human serum albumin	1% in seeding solution	Supports cell viability and reduces non-specific adhesion
Secondary sterile container	One sterile container per final product	Maintains sterility during in-use hold and transfer
Secondary sterile dressing	As required by clinical procedure	Secures and protects the applied matrix

The final batch formula will be confirmed based on seeding validation, matrix loading capacity, cell retention, viability and in-use stability data. The final cell dose will be expressed as viable cells/cm² of INTEGRA matrix and wound area.

P.3.3 Description of manufacturing process and process controls

Drug product manufacture begins after the cellular drug substance has met the applicable pre-seeding acceptance criteria. The drug substance is prepared as a viable cell suspension at the required concentration in the defined seeding solution. INTEGRA Dermal Regeneration Template is inspected, identified, and prepared aseptically according to the manufacturer's instructions and the approved manufacturing procedure. The cellular drug substance is seeded onto INTEGRA using a defined cell-loading procedure. The procedure controls cell concentration, seeding volume, matrix size, seeding surface, incubation time, temperature and handling conditions. Development and preclinical studies used seeding of expanded cells onto INTEGRA Dermal Regeneration Template for 30 minutes at 37 °C and 5% CO2 before implantation in the ischemic wound model; this provides a scientific basis for the final clinical seeding procedure, which will be qualified under GMP-relevant conditions. After seeding, the cell-seeded INTEGRA matrix is visually inspected and maintained under defined in-use conditions until clinical application. Samples may be taken from the cell suspension before seeding, residual seeding solution after loading, or representative process controls to assess dose, viability, cell recovery/retention, sterility, endotoxin and/or other predefined quality attributes. The final cell-seeded matrix is packaged in a sterile container/tray, labelled with the unique batch and patient identifier, and released according to the staged release strategy described in P.5 (Vuerich et al., 2023).

Proposed process flow

The proposed drug product manufacturing process flow is shown in Table 16.

Table 16. Proposed drug product manufacturing process flow.

Step	Process description	Controls
1. DS release for DP manufacture	Cellular DS reviewed against pre-seeding criteria	DS release review; cell count, viability, identity, sterility status, endotoxin, mycoplasma
2. Preparation of cell suspension	Cells adjusted to required concentration in carrier/seeding solution	Cell concentration, viability, volume, visual inspection
3. INTEGRA preparation	Matrix inspected, identified, trimmed if needed and transferred aseptically	Matrix identity, expiry, package integrity, size, sterility documentation
4. Cell seeding onto INTEGRA	Defined cell dose applied to the matrix under aseptic conditions	Seeding volume, cell dose/cm^2, time, temperature
5. Incubation / cell loading	Matrix maintained under defined conditions to allow cell retention	Incubation time and temperature; visual inspection
6. Post-seeding handling	Cell-seeded matrix transferred to sterile container/tray	In-use hold start time; container closure check
7. Final product labelling and release	Product labelled and reviewed for clinical use	Batch record, label check, QP/authorized release
8. Clinical transfer/application	Product transferred to clinical area and applied to wound bed	Chain-of-custody, time/temperature log, application record

The final product is administered by topical/local application of the cell-seeded INTEGRA matrix onto the surgically debrided wound bed. No injection or periwound infiltration of the cellular product is performed.

P.3.4 Control of critical steps and intermediates

The main critical steps in ASC Heal drug product manufacture are preparation of the cellular suspension, aseptic handling of INTEGRA, cell seeding onto the matrix, in-use hold before application, packaging/labelling and release. These steps are controlled because they may affect cell dose, cell viability, matrix loading, sterility, traceability and consistency of product application. The critical drug product manufacturing steps and controls are summarized in Table 17.

Table 17. Critical drug product manufacturing steps and controls.

Critical step	Critical process parameter	Control / test	Proposed acceptance criterion
Cellular DS preparation	Cell concentration, viability, formulation volume	Cell count, viability, visual inspection	Meets DS specification and assigned DP dose requirement
INTEGRA preparation	Correct product, size, expiry, package integrity	Visual/documentary check	Correct matrix, intact sterile packaging, within expiry
Matrix trimming	Aseptic handling, final size	Batch record, size documentation	Size matches prescribed wound/matrix area
Cell seeding	Cell dose/cm2, seeding volume, uniform distribution	Calculation, operator check, process record	Within predefined dose and volume range
Cell loading/incubation	Time, temperature, handling conditions	Time/temperature record	Within validated limits
Post-seeding hold	Time and temperature before use	In-use hold log	Within validated maximum hold time, provisionally up to 4 hours pending validation
Packaging/labelling	Patient-specific identification and chain-of-custody	Independent label check	Correct patient/batch ID, no mismatch
Microbiological safety	Aseptic process, environmental monitoring, sterility status	EM review, sterility/mycoplasma/endotoxin status	Meets staged release requirements

The drug product manufacturing process is intended to be continuous from cell seeding onto INTEGRA to final clinical application, with no extended holding times. If a hold time is applied, it will be defined by in-use stability data evaluating cell viability, cell retention, appearance, sterility assurance and potency-related activity. Pending completion of validation, a conservative maximum in-use hold time will be applied.

P.3.5 Process validation and/or evaluation

Full process validation is not expected for early clinical development; however, critical manufacturing steps will be evaluated and controlled. Process evaluation will focus on reproducibility of the final cell-seeding step, cell dose accuracy, cell retention on INTEGRA, post-seeding viability, maintenance of sterility, traceability and ability to meet the final product specification. Preparation or mixing steps involving matrices after batch release and before administration will be qualified and described where applicable. Aseptic process validation will be performed for the drug product manufacturing process. Media fill/process simulation studies will be designed to simulate the worst-case aseptic operations associated with preparation of the cell suspension, handling of INTEGRA, cell seeding, incubation/hold, transfer to the final sterile container and labelling. At least three successful process simulations will be performed, as applicable, before clinical use or according to the approved GMP validation plan. Process evaluation runs will be performed using representative cellular material or surrogate material, as appropriate, to confirm the robustness of the cell-loading procedure. The following parameters will be evaluated. The drug product process evaluation parameters are listed in Table 18.

Table 18. Drug product process evaluation parameters.

Evaluation parameter	Proposed assessment
Cell dose accuracy	Comparison between target and delivered viable cell dose/cm^2
Seeding efficiency	Cell recovery in residual seeding solution and/or matrix-associated cell estimate
Uniformity of distribution	Visual/microscopy-based or surrogate distribution assay, if feasible
Post-seeding viability	Viability after seeding and after proposed in-use hold
In-use hold time	Viability, appearance, cell retention and potency-related activity over time
Microbiological safety	Media fill, environmental monitoring, sterility testing strategy
Traceability	Batch record, chain-of-custody and label reconciliation

The final cell-seeded INTEGRA product has limited in-use shelf life; therefore, not all microbiological results may be available before clinical application. The release strategy will rely on a combination of qualified aseptic processing, environmental monitoring, raw-material controls, available rapid microbiological results and retrospective review of final sterility data. Any positive or out-of-specification microbiological result will trigger predefined clinical notification, investigation and corrective/preventive actions. Development data support the use of INTEGRA as a cell delivery matrix: preclinical studies used SVF cells seeded on INTEGRA in a murine ischemic wound model, and the SVF + INTEGRA combination was evaluated for revascularization and tissue regeneration endpoints. The final clinical manufacturing process will be qualified to ensure that this cell-matrix preparation can be performed reproducibly under controlled GMP conditions (Vuerich et al., 2023).

P.4 Control of excipients

The excipients and non-cellular materials used for preparation of the ASC Heal drug product are selected to be suitable for GMP manufacture and clinical use. Whenever available, compendial, injectable-grade or medical-device-grade materials are used. For non-compendial materials, supplier qualification, in-house specifications, certificates of analysis and suitability-for-use assessments are applied. For the purpose of this IMPD, excipient control covers the seeding solution and HSA components that may remain in the final cell-seeded INTEGRA product. INTEGRA Dermal Regeneration Template is controlled through manufacturer documentation, sterility assurance, package integrity, expiry and suitability for the intended cell-seeding procedure. Cryoprotectant used for cells storage, is not intended to be part of the final drug product after washing/resuspension; any residual carry-over will be assessed and controlled or justified.

P.4.1 Specification

Excipients are controlled according to pharmacopoeial standards. A preliminary list of excipients and relevant non-cellular materials is provided below and will be updated once the final formulation, seeding solution and clinical presentation are confirmed. The specifications for excipients and non-cellular materials are summarized in Table 19.

Table 19. Specifications for excipients and non-cellular materials.

Material / component	Proposed quality standard	Key controls	Function
Seeding solution	Sterile injectable-grade / Ph. Eur. where applicable	Sterility, endotoxin, appearance, pH/osmolality where applicable, CoA	Maintains the cellular DS in suspension and supports seeding onto INTEGRA
Human serum albumin (HSA)	Ph. Eur. / licensed plasma-derived medicinal product / GMP-grade	CoA, viral safety documentation, TSE/plasma-derived product documentation, sterility/endotoxin as applicable	Protein supplement supporting cell viability and reducing non-specific adhesion
Cryoprotectant, if DS is cryopreserved before DP manufacture	GMP-grade / Ph. Eur. or highest available grade	CoA, sterility/endotoxin as applicable, residual carry-over assessment if not fully removed	Used only for cellular DS cryopreservation; not intended as final DP excipient
INTEGRA Dermal Regeneration Template	Sterile medical device; manufacturer specification and CoA/sterility documentation	Identity, package integrity, expiry, sterility assurance, lot traceability, suitability for cell seeding	Dermal scaffold supporting cell loading, retention and wound-bed coverage
Secondary dressing / fixation material, if supplied with the product	CE-marked or clinical-grade sterile material	Manufacturer specification, sterility, package integrity, expiry	Secures and protects the applied cell-seeded matrix

P.4.2 Analytical procedures

For compendial excipients, analytical procedures are performed according to the relevant pharmacopoeial monographs or are covered by supplier release testing documented in the certificate of analysis. For sterile injectable-grade materials, batch release documentation is reviewed before use. For non-compendial materials, in-house specifications and supplier qualification procedures will define the required tests and acceptance criteria. INTEGRA is controlled primarily through manufacturer documentation, sterile package integrity, expiry, lot traceability and visual inspection before use. Any additional in-house testing or suitability assessment required for the cell-seeding procedure will be described in the relevant SOPs and qualification reports.

P.4.3 Validation of the analytical procedures

Validation of analytical procedures for compendial excipients is not repeated by the sponsor when the materials are used according to their approved specification and the relevant certificate of analysis is available. For non-compendial materials or modified analytical procedures, suitability for intended use will be demonstrated through method qualification, supplier qualification, incoming material control and, where applicable, compatibility studies with the cellular drug substance and the INTEGRA matrix.

P.4.4 Justification of specification

The selected specifications are justified by the intended use of the materials in a cell-based ATMP applied to a wound bed. Materials in contact with the cells are required to be sterile and of appropriate quality. For HSA and other human-derived components, specifications are justified by their established clinical use, pharmacopoeial quality where applicable and viral/TSE safety documentation. For any non-compendial material, the in-house specification will be justified based on supplier information, risk assessment, compatibility with the cell-seeded matrix and control of patient-safety-related attributes such as sterility and endotoxin. If a cryoprotectant such as DMSO is used for storage of the cellular drug substance, it will be removed

or diluted during the post-thaw washing/formulation procedure before final product preparation. Residual carry-over into the final drug product will be controlled or justified based on the final process and clinical exposure.

P.4.5 Excipients of human origin

Human serum albumin and/or human platelet lysate may be used during manufacture or formulation. Human-derived materials are controlled through supplier qualification, certificates of analysis, donor-screening documentation where applicable, viral safety statements, TSE risk documentation and compliance with applicable requirements for plasma-derived or human-derived materials. Detailed control of adventitious agents is summarized in Appendix 3.A.1. If HSA is used in the final seeding solution, it will be sourced as a licensed plasma-derived medicinal product or equivalent GMP-grade material suitable for clinical use. Its use is justified by its role in maintaining cell viability and reducing non-specific cell loss during handling and matrix seeding.

P.4.6 Novel excipients

No novel excipient is currently proposed for the ASC Heal drug product. If any excipient not previously used in medicinal products or not previously used by the proposed route of administration is introduced during development, full information on manufacture, characterization, control and safety will be provided and cross-referenced to non-clinical and clinical safety data as applicable.

P.5 Control of Investigational Medicinal Drug Product

Control of the ASC Heal drug product is based on a staged, risk-based strategy combining cellular drug substance testing, drug product process controls, final product checks, aseptic process controls and QP review. Because the final drug product is a fresh cell-seeded INTEGRA matrix with a limited in-use shelf life, some tests may be performed on the cellular drug substance, pre-seeding suspension, residual seeding solution or representative process samples rather than destructively on the final matrix. This approach will be justified by process qualification and risk assessment. The proposed specification for the investigational medicinal product is summarized in Table 20.

Table 20. Proposed specification for ASC Heal investigational medicinal product.

Parameter	Proposed specification	Method / test substrate	Release status
Appearance / integrity	Cell-seeded INTEGRA matrix visually intact, uniformly wetted/loaded, free of visible particulates, discoloration, damage or signs of contamination.	Visual inspection of final matrix	Required before application
Identity / traceability	Correct patient-specific cellular DS, correct INTEGRA lot, correct batch label and chain-of-custody reconciliation.	Documentary check; batch record; label verification	Required before release
Viable cell dose / matrix loading	Assigned dose cohort achieved: 1 x 10^6, 5 x 10^6 or 10 x 10^6 viable cells/cm_2 of INTEGRA matrix and/or wound area. Provisional acceptable delivered dose: >80% of target or within qualified process range.	Calculated from pre-seeding cell count, viability, seeding volume and residual seeding assessment where applicable	Required before release
Viability	Representative pre-seeding/post-seeding sample >70% viable cells at release or end of validated in-use hold; target >80% before seeding where feasible.	Trypan blue, 7-AAD/PI or live/dead flow cytometry on representative sample	Required; sample-based
Cell retention / seeding efficiency	Provisional target >70% cell retention on matrix or within qualified process range; result reported during development.	Residual seeding solution cell count, surrogate retention assay or qualified process control	Under qualification
Sterility	No growth / negative. Final result may be available after application under staged release strategy.	Ph. Eur. 2.6.1, BacT/Alert/Bactec or validated rapid method on DS, residual fluid or representative sample	Required; staged/conditional release may apply
Mycoplasma	Negative.	Ph. Eur. 2.6.7 or validated NAT/PCR, preferably on DS/pre-seeding sample	Required before release where feasible
Endotoxin	<0.5 EU/mL and total endotoxin exposure below clinically acceptable limit, e.g. <5 EU/kg/hour.	LAL or recombinant Factor C on DS, carrier/seeding solution or representative DP sample/extract	Required before release
Potency-related activity	Demonstrable angiogenic/paracrine activity. Provisional criterion: positive activity versus negative control; VEGF secretion and/or tube formation result reported.	VEGF ELISA, endothelial tube formation, cytokine/paracrine assay or qualified surrogate on DS/post-seeding representative sample	Potency-indicating parameter under qualification
In-use shelf life	Clinical application within validated in-use hold time; provisional maximum 4 hours after completion of seeding pending validation.	Time/temperature log and batch record review	Required before application

P.5.1 Specifications

The proposed drug product specifications are provisional for Phase I/II clinical development. They are intended to ensure patient safety, correct dose delivery, sterility assurance, traceability and maintenance of biological activity. The final specification will be refined as additional manufacturing, process qualification, stability and clinical data become available.

P.5.2 Analytical procedures

Analytical procedures used for drug product control are described in the same order as the specification. Visual inspection is performed on the final cell-seeded INTEGRA matrix before release to verify matrix integrity, wetting/loading appearance and absence of visible contamination or damage. Identity and traceability are verified by review of patient-specific identifiers, cellular drug substance batch number, INTEGRA lot number, labels, chain-of-custody documentation and batch records. Cell dose and viability are determined primarily on the cellular drug substance or representative pre-seeding/post-seeding samples, as destructive testing of the final cell-seeded matrix may not be feasible. Cell retention or seeding efficiency may be estimated by comparing target cell input with residual cells recovered from the seeding solution or by a qualified surrogate method. Microbiological testing is performed using pharmacopoeial or validated rapid methods on the most representative available sample, such as the cellular drug substance, residual seeding fluid, carrier solution, or final product. Potency-related testing will use assays reflecting the intended angiogenic/paracrine mechanism of action, such as VEGF secretion, endothelial tube formation, cytokine profiling or another qualified surrogate assay. The method will define test substrate, cell input, assay controls, readouts, acceptance criteria and qualification status.

P.5.3 Validation of analytical procedures

For Phase I/II clinical development, analytical methods will be qualified for their intended use rather than fully validated, except where pharmacopoeial or microbiological methods require validation or suitability demonstration. Sterility, mycoplasma and endotoxin assays will be performed according to pharmacopoeial methods or validated rapid methods, with method suitability demonstrated for the relevant product matrix or representative sample. Flow cytometry, viability and potency-related assays will be qualified to demonstrate adequate specificity, precision, robustness and suitability for the autologous ASC-derived product. Full validation will be implemented progressively as development advances and as sufficient batch data become available.

P.5.4 Batch analysis

Batch analysis data will be collected for development, engineering and GMP clinical batches. Results will identify the tested material clearly, including whether testing was performed on the final cell-seeded matrix, cellular drug substance, pre-seeding cell suspension, residual seeding solution, carrier solution or other representative sample. Batch data will be used to refine acceptance criteria and demonstrate process consistency.

P.5.5 Characterization of impurities

Potential drug product impurities include process-related residues, product-related impurities and matrix-related or handling-related contaminants. Process-related residues may include residual collagenase, DNase, detachment reagent, culture medium components, human platelet lysate proteins, HSA, cryoprotectant carry-over if the drug substance was cryopreserved, and leachable from single-use materials. These are controlled mainly at the drug substance stage through washing, formulation controls, raw-material qualification and process controls. Product-related impurities include non-viable cells, cell debris, visible cell aggregates and unintended cellular populations outside predefined ranges. These are controlled through viability testing, visual inspection, flow-cytometry characterization and process controls. Matrix-related risks include incorrect matrix identity, compromised sterile packaging, particulate contamination or incompatibility with the cell-seeding procedure; these are controlled through incoming material checks, manufacturer documentation and visual inspection.

P.5.6 Justification of specification (release criteria)

The proposed drug product specification is justified by the fresh, patient-specific and cell-matrix nature of ASC Heal. The specification focuses on attributes directly linked to patient safety and correct product use: identity/traceability, appearance, dose, viability, microbiological safety, endotoxin and in-use shelf life. Acceptance criteria are provisional for early clinical development and will be refined based on manufacturing experience, in-use stability data, assay qualification and clinical safety data. Some release tests may be performed on cells, pre-seeding suspension, residual seeding solution or representative process samples because destructive testing of the final cell-seeded INTEGRA matrix may not be feasible. This testing strategy is justified by the continuous nature of the process, the limited in-use shelf life of the final drug product, and the use of defined process controls for seeding, cell retention, aseptic handling and release. The staged release strategy will identify which results are available before administration and which may be reviewed retrospectively. If final sterility results are not available before

application, release will be supported by validated aseptic processing, environmental monitoring, raw-material controls, available rapid microbiological data and QP risk assessment. Any post-administration out-of-specification or positive microbiological result will trigger immediate quality investigation, clinical notification and predefined corrective/preventive actions according to the clinical protocol and pharmacovigilance procedures. The EMA assessment of darvadstrocel (Alofisel) is considered a relevant regulatory precedent for an adipose-derived stromal cell-based ATMP, particularly for the need to justify potency, microbiological safety, product-specific release strategy and comparability; direct extrapolation to ASC Heal is not assumed (EMA, 2018). The overall safety rationale is further supported by published clinical experience with autologous adipose-derived stromal/stem cells and by manufacturing/regulatory experience with adipose-derived MSC/ASC products (Bura et al., 2014; Sensebe et al., 2011; EMA, 2018).

P.6 Reference standards or materials

No compendial reference standard is available for the ASC Heal final drug product. Due to the fresh, autologous and cell-seeded matrix format, retention of a reference sample identical to the administered drug product may not be feasible. Where possible, representative samples such as cellular drug substance aliquots, residual seeding solution, conditioned medium, matrix extract or a process-simulated cell-seeded matrix may be retained for investigation, assay trending or comparability purposes. Assay-specific reference and control materials will be used as applicable, including endotoxin standards, mycoplasma NAT/PCR controls, ELISA calibration standards, positive and negative controls for potency assays, and characterized cellular control material for flow-cytometry or functional assay trending. The storage conditions, stability and intended use of retained materials will be documented in the relevant SOPs.

P.7 Container Closure System

The final drug product is presented as a sterile, single-use cell-seeded INTEGRA matrix maintained in a sterile container or tray suitable for short-term in-use handling and transfer to the clinical area. The container closure system must maintain sterility during the validated in-use hold period, protect the matrix from mechanical damage and allow aseptic application to the wound bed. INTEGRA is supplied in its original sterile packaging and is inspected before use for package integrity, expiry, correct product identity and lot traceability. After aseptic preparation and cell seeding, the cell-seeded matrix is transferred to the final sterile container or maintained in a qualified sterile system according to the manufacturing procedure. If any container or tray is not a CE-marked medical device or is used outside its intended purpose, suitability, biocompatibility and compatibility with the cell-seeded matrix will be documented. Labelling includes the unique batch identifier, patient identifier, product name, matrix size, dose cohort or cell dose, storage conditions, expiry or use-by time, and handling instructions. Chain-of-custody and chain-of-identity are maintained from drug substance preparation through final product release and clinical application.

P.8 Stability, storage conditions, transport and logging

The ASC Heal final drug product is a fresh cell-seeded INTEGRA matrix with limited in-use shelf life. It is not intended to be cryopreserved after cell seeding. Stability requirements therefore focus on the time between completion of seeding onto INTEGRA and topical/local application to the wound bed, including any transfer to the clinical site or procedure room. The drug product will be handled under defined temperature and time conditions, with continuous documentation of manufacturing completion time, end of seeding, start and end of in-use hold, transfer time and application time. Any deviation from the approved hold time or temperature range will be assessed before use and documented in the batch record.

P.8.1 Protocol and methods, results, shelf life

In-use stability studies will be performed on representative development, GMP batches to verify that the final cell-seeded INTEGRA matrix remains within specification during the proposed hold time. Stability studies will evaluate appearance, cell viability, cell retention/recovery, matrix integrity, sterility assurance and potency-related activity over time. The proposed in-use stability protocol for the drug product is summarized in Table 21.

Table 21. Proposed drug product in-use stability protocol.

Study condition	Proposed time points	Stability-indicating tests	Purpose
Cell loading condition	End of seeding / T0	Appearance, cell dose calculation, representative viability, residual cell counts if applicable	Confirm initial DP quality
In-use hold at controlled condition (provisionally 15-25 °C)	T0, 1 h, 2 h, 4 h	Appearance, viability, cell retention/recovery, potency-related activity where feasible	Define maximum hold time before application
Temperature excursion	Defined short intervals	Appearance, viability, cell retention, matrix integrity	Assess sensitivity to deviations
Transport	Start and end of transfer	Time/temperature log, appearance, viability on representative sample	Support transfer from GMP area to clinical area

The provisional in-use shelf life for the final cell-seeded INTEGRA matrix is up to 4 hours after completion of cell seeding, pending confirmation by in-use stability data. During development, the final validated hold condition will be selected based on cell viability, matrix retention, potency-related activity and practical clinical workflow. The cell-loading step may include a defined incubation period, for example 30 minutes at 37 °C and 5% CO_2, based on process-development and preclinical experience; the clinical procedure will be finalized and qualified under GMP-relevant conditions. For release, the final product must be applied within the approved use-by time. The minimum stability acceptance criteria are no visible matrix damage or contamination, maintenance of representative cell viability >70%, acceptable cell retention or dose delivery, endotoxin within specification, and no available evidence of microbial contamination at the time of release. Potency-related activity will be monitored during development and incorporated into the final stability specification once the assay is qualified. Transport and logging procedures will ensure chain-of-identity, chain-of-custody and temperature/time traceability from final product preparation to clinical application. The product must not be used if traceability is compromised, sterile container integrity is lost, the validated hold time is exceeded, or any critical release criterion is not met.

3. Appendices

3.A.1 Adventitious agents safety evaluation

All biological materials used in the manufacture of ASC Heal will be identified and assessed for adventitious agent risk. The main potential sources of adventitious agents are the autologous adipose tissue starting material, human-derived materials such as HSA or human platelet lysate, animal-derived or biological raw materials where used, and the INTEGRA matrix. The risk is mitigated by donor screening, raw-material qualification, GMP manufacture, aseptic processing, environmental monitoring, in-process testing and final/representative product microbiological testing. The adventitious-agent risk assessment and control strategy are summarized in Table 22.

Table 22. Adventitious-agent risk assessment and control strategy.

Material	Origin / risk consideration	Control strategy
Autologous adipose tissue	Human starting material; potential microbial or viral contamination risk	Donor eligibility and testing; aseptic procurement; traceability; transport controls; in-process and final microbiological testing
Human serum albumin, if used	Human plasma-derived material	Licensed/Ph. Eur. or GMP-grade material; CoA; viral safety and TSE documentation; supplier qualification
Human platelet lysate, if used	Human-derived culture supplement	Qualified supplier; donor screening/pooling controls; viral safety documentation; sterility/mycoplasma/endotoxin testing as applicable
Collagenase/DNase or other enzymes	Biological or recombinant material; potential impurity/adventitious risk	GMP-grade or qualified supplier; CoA; origin statement; sterility/endotoxin as applicable; residual control
INTEGRA Dermal Regeneration Template	Sterile biological/medical-device matrix; animal-origin risk if applicable	Manufacturer documentation; sterility assurance; package integrity; expiry; TSE/viral safety documentation as applicable

3.A.1.1 Non-viral adventitious agents

The principal non-viral adventitious agent risks are bacterial, fungal and mycoplasma contamination. These risks are controlled through aseptic tissue procurement, controlled transport, GMP cleanroom manufacture, closed or functionally closed processing where feasible, qualified raw materials, environmental monitoring, in-process controls and sterility/mycoplasma testing. Sterility testing is performed using pharmacopoeial or validated rapid methods. Mycoplasma testing is performed according to Ph. Eur. 2.6.7 or a validated NAT/PCR method.

TSE risk is addressed for all relevant animal-derived or biological materials, including the INTEGRA matrix and enzymes where applicable, through supplier documentation, certificates of suitability or equivalent TSE statements, and compliance with applicable guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents. Any material without adequate TSE documentation will be subject to risk assessment and may be rejected for clinical manufacture.

3.A.1.2 Viral adventitious agents

The main potential source of viral adventitious agents is the human starting material and any human-derived raw material used during manufacture. Donor screening and testing will be performed according to applicable tissue and cell requirements and national regulations, including at minimum HIV, HBV, HCV and syphilis testing, with additional testing as required by the clinical protocol and local requirements. Human-derived materials such as HSA or human platelet lysate are controlled through supplier qualification, donor-screening documentation, viral inactivation/removal information where applicable, certificates of analysis and viral safety statements. The risk of viral contamination introduced during manufacture is considered limited because processing is performed under controlled GMP conditions with qualified raw materials and aseptic procedures. Viral safety assessment will be updated if new human-derived or animal-derived materials are introduced into the process.

4. Substantial amendments

Not applicable at the initial clinical trial application stage. Substantial amendments will be documented in this section during clinical development, including changes to manufacturing site, manufacturing process, INTEGRA handling or matrix-seeding

procedure, release specifications, stability/shelf-life, potency assay, raw materials of biological origin, container closure system, dose, route/application procedure or any change with potential impact on product quality, safety or clinical use.

5. References

Andia I, Maffulli N, Burgos-Alonso N. Stromal vascular fraction technologies and clinical applications. Expert Opin Biol Ther. 2019;19(12):1289-1305. doi:10.1080/14712598.2019.1671970.

Bora P, Majumdar AS. Adipose tissue-derived stromal vascular fraction in regenerative medicine: a brief review on biology and translation. Stem Cell Res Ther. 2017;8(1):145. doi:10.1186/s13287-017-0598-y.

Bourin P, Bunnell BA, Casteilla L, Dominici M, Katz AJ, March KL, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture-expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science and the International Society for Cellular Therapy. Cytotherapy. 2013;15(6):641-648. doi:10.1016/j.jcyt.2013.02.006.

Bura A, Planat-Benard V, Bourin P, Silvestre JS, Gross F, Grolleau JL, et al. Phase I trial: the use of autologous cultured adipose-derived stroma/stem cells to treat patients with non-revascularizable critical limb ischemia. Cytotherapy. 2014;16(2):245-257. doi:10.1016/j.jcyt.2013.11.011.

Chen YW, Wang JR, Liao X, Li SH, Xiao LL, Cheng B, et al. Effect of suction pressures on cell yield and functionality of the adipose-derived stromal vascular fraction. J Plast Reconstr Aesthet Surg. 2017;70(2):257-266. doi:10.1016/j.bjps.2016.10.028.

Crisan M, Corselli M, Chen WCW, Péault B. Perivascular cells for regenerative medicine. J Cell Mol Med. 2012;16(12):2851-2860. doi:10.1111/j.1582-4934.2012.01617.x.

Duscher D, Luan A, Rennert RC, Atashroo D, Maan ZN, Brett EA, et al. Suction assisted liposuction does not impair the regenerative potential of adipose derived stem cells. J Transl Med. 2016;14:126. doi:10.1186/s12967-016-0881-1.

European Medicines Agency. Alofisel: EPAR - Public assessment report. EMA/CHMP/64055/2018. European Medicines Agency; 2018.

Fitzgerald JC, Duffy N, Cattaruzzi G, Vitrani F, Paulitti A, Mazzarol F, et al. GMP-compliant production of autologous adipose-derived stromal cells in the NANT 001 closed automated bioreactor. Front Bioeng Biotechnol. 2022;10:834267. doi:10.3389/fbioe.2022.834267.

Iyyanki T, Hubenak J, Liu J, Chang EI, Beahm EK, Zhang Q. Harvesting technique affects adipose-derived stem cell yield. Aesthet Surg J. 2015;35(4):467-476. doi:10.1093/asj/sju055.

Lazarski CA, Hanley PJ. Review of flow cytometry as a tool for cell and gene therapy. Cytotherapy. 2024;26(2):103-112. doi:10.1016/j.jcyt.2023.10.005.

Lee MH, Kang BY, Wong CC, Li AW, Naseer N, Ibrahim SA, et al. A systematic review of autologous adipose-derived stromal vascular fraction for the treatment of acute cutaneous wounds. Arch Dermatol Res. 2022;314(5):417-425. doi:10.1007/s00403-021-02242-x.

Mizuno H, Tobita M, Uysal AC. Concise review: adipose-derived stem cells as a novel tool for future regenerative medicine. Stem Cells. 2012;30(5):804-810. doi:10.1002/stem.1076.

Sensebé L, Bourin P, Tarte K. Good manufacturing practices production of mesenchymal stem/stromal cells. Hum Gene Ther. 2011;22(1):19-26. doi:10.1089/hum.2010.197.

Vuerich R, Groppa E, Vodret S, Ring NAR, Stocco C, Bossi F, et al. Ischemic wound revascularization by the stromal vascular fraction relies on host-donor hybrid vessels. NPJ Regen Med. 2023;8(1):8. doi:10.1038/s41536-023-00283-6.

Zimmerlin L, Donnenberg VS, Pfeifer ME, Meyer EM, Péault B, Rubin JP, et al. Stromal vascular progenitors in adult human adipose tissue. Cytometry A. 2010;77(1):22-30. doi:10.1002/cyto.a.20813.