MASTERARBEIT | MASTER'S THESIS

Titel | Title

Formulation and characterisation of a hydrogel-based delivery system

verfasst von | submitted by

Dalia Mohamed Hamid BSc

angestrebter akademischer Grad | in partial fulfilment of the requirements for the degree of

Magistra pharmaciae (Mag. pharm.)

Wien | Vienna, 2025

Studienkennzahl lt. Studienblatt | Degree programme code as it appears on the student record sheet:	UA 066 605
Studienrichtung lt. Studienblatt | Degree programme as it appears on the student record sheet:	Masterstudium Pharmazie
Betreut von | Supervisor:	Univ.-Prof. Dr. Lea Ann Dailey
Mitbetreut von | Co-Supervisor:	Gianluca Bello PhD

Abstract

This thesis describes the development and characterisation of a sterile, hydrogel-based delivery system (APOgel) tailored for phase III clinical evaluation of the apoptotic secretome (APOSEC) in chronic wound therapy. Building on the composition of the reference product Nu-Gel™, APOgel was optimised to withstand terminal steam sterilisation and to provide key characteristics, such as rheological behaviour, storage stability and pH. Autoclaving reduced viscosity due to polymer degradation, resulting in a viscosity profile comparable to Nu-Gel™; subsequent storage stability studies at 4°C confirmed the maintenance of predefined critical quality attributes. A supplier-related variability in sodium alginate was observed, underscoring the need for consistent raw material sourcing. The syringe-based point-of-care mixing system (APOSEC 2.0), in which the hydrogel is combined with a model medium immediately prior to application, was evaluated for its ability to uniformly incorporate and deliver both large and small surrogate molecules. Studies on viscosity, mixing and dosing uniformity, as well as content distribution, confirmed the system's robustness and operator independence. *In vitro* release studies in Franz diffusion cells revealed comparable profiles to Nu-Gel™, while demonstrating an enhanced release performance of APOgel under non-sink conditions. Collectively, the results validate APOgel as a clinically suitable formulation and confirm the feasibility of APOSEC 2.0 as a reliable delivery system.

Zusammenfassung

Diese Masterarbeit beschreibt die Entwicklung und Charakterisierung eines sterilen, hydrogelbasierten Trägersystems (APOgel), das für die klinische Phase-III-Prüfung des apoptotischen Sekretoms (APOSEC) in der Therapie chronischer Wunden vorgesehen ist. Aufbauend auf der Zusammensetzung des Referenzprodukts Nu-Gel™ wurde APOgel so optimiert, dass es der terminalen Dampfsterilisation standhält und gleichzeitig wesentliche Eigenschaften wie rheologisches Verhalten, Lagerstabilität und pH-Wert beibehält. Die Autoklavierung führte infolge einer Polymerdegradation zu einer Viskositätsreduktion, wodurch ein dem Referenzprodukt vergleichbares Profil erzielt wurde. Nachfolgende Stabilitätsstudien bei 4°C bestätigten die Aufrechterhaltung vordefinierter kritischer Qualitätsattribute. Die Variabilität des Natriumalginats zwischen Herstellern unterstrich die Bedeutung einer konsistenten Rohstoffquelle. Das spritzenbasierte Point-of-Care-Mischsystem (APOSEC 2.0), bei dem das Hydrogel unmittelbar vor der Anwendung mit einem Modellmedium kombiniert wird, wurde hinsichtlich seiner Fähigkeit geprüft, sowohl kleine als auch große Surrogatmoleküle gleichmäßig einzubinden und freizusetzen. Untersuchungen zu Viskosität, Misch- und Dosiergenauigkeit sowie Wirkstoffverteilung bestätigten die Robustheit und Anwenderunabhängigkeit des Systems. *In vitro*-Freisetzungsstudien in Franz-Diffusionszellen zeigten vergleichbare Profile zu Nu-Gel™, wobei APOgel unter Nicht-Sink-Bedingungen eine verbesserte Freisetzungsleistung aufwies. Insgesamt validieren die Ergebnisse APOgel als klinisch geeignete Formulierung und bestätigen die Praxistauglichkeit von APOSEC 2.0 als zuverlässiges Freisetzungssystem.

Acknowledgements

I thank Aposcience AG for initiating this project and for their financial and collegial support. I extend my gratitude to the team at the Medical University of Vienna as well as to my colleagues at LKH-University Hospital Graz for their assistance and access to facilities.

I am sincerely thankful to my supervisor, Prof. Lea Ann Dailey, for her expert guidance. My deepest gratitude goes to my co-supervisor, Dr. Gianluca Bello, for his invaluable support throughout this work.

Table of contents

1 Introduction

1.1 Apoptotic secretome (APOSEC) for the treatment of chronic wounds

Chronic non-healing wounds represent one of the most common complications of diabetes and significantly increase the risk of amputation and premature death. [1] The pathophysiology of these ulcers is multifactorial, involving impaired angiogenesis, excessive apoptosis of endothelial cells, and dysfunctional wound healing processes; current therapeutic approaches, from cell-based therapies, cell-free secretomes, skin substitutes and gene therapy, have shown promise in the treatment of such chronic wounds. [1], [2] Amongst the therapies under development, the apoptotic secretome (APOSEC) obtained from stressed peripheral blood mononuclear cells (PBMCs) was previously developed and investigated for its angiogenic and tissue-regenerating activity. [3], [4] APOSEC completed phase I and II clinical studies (MARSYAS) [5], [6], [7] as a non-toxic, cell-free, safe and GMP-compliant topical therapy for ulcers conceived to be delivered via a sterile gel. To establish the delivery system for APOSEC for broader clinical testing and eventual regulatory approval, the current study focuses on the development of a sterile topical gel (APOgel) as well as a unique point-of-care mixing system (referred to as APOSEC 2.0 in this study) for the delivery of APOSEC for phase III trials.

In these clinical studies, lyophilised APOSEC – or the culture medium (AM2 medium) as a placebo – was reconstituted with 200 µL of physiological saline (0.9% NaCl, sodium chloride) and then combined with 800 µL of Nu-Gel™ Hydrogel (Systagenix, Gatwick, West Sussex, UK), a sterile alginate-based gel designed for topical use (Table 1). The resulting mixture, approximately 1 mL in volume, was applied directly to the open wound using a sterile syringe (without a needle), followed by the placement of a fresh wound dressing. Incorporating APOSEC into the hydrogel facilitates its prolonged retention at the wound site. Additionally, the use of hydrogels in diabetic wound care has been linked to therapeutic benefits through two main mechanisms: absorption of wound exudate and maintenance of a moist environment, both of which support the healing process. [8], [9]

A critical step in the production of APOSEC is the γ-irradiation (60 Gy) process of cultured PBMCs extracted from peripheral blood; the irradiation leads to the upregulation of pro-angiogenic factors and the modulation of lipid mediators with known roles in inflammation and tissue regeneration. [4], [5] Such factors and mediators are then separated from the cell's residues, sterilised, virally inactivated and ultimately lyophilised. These procedures have been developed under good manufacturing practices (GMP). [10] APOSEC contains the following key cytokine components: interleukin (IL)-8 (0-5214 pg/mL), epidermal growth factor (EGF; 25-226 pg/mL), and transforming growth factor-β (TGF-β; 2575-21732 pg/mL). [5]

The characterisation of APOSEC reveals a complex composition that includes a diverse array of secreted proteins, lipids, and extracellular vesicles (EVs), which collectively contribute to its bioactivity. Studies have emphasised the importance of both the protein and exosome fractions in enhancing cellular activities, such as mobility and angiogenesis. Specifically, bioinformatics analyses have shown that the genes encoding the proteins released by irradiated PBMCs are mediators of regenerative pathways. Key components include miRNAs, which influence cellular communication and regenerative processes, and various proteins involved in promoting angiogenesis. [3] Moreover, evidence suggests that the complete APOSEC is more effective than isolated EV subfractions in promoting wound healing, as demonstrated in *in vivo* models. APOSEC has been shown to accelerate wound closure in diabetic mouse models, suggesting its potential as a cell-free therapeutic agent. [2], [4] The administration of APOSEC under GMP has further facilitated its transition into clinical applications, underscoring its promise for improving tissue recovery in chronic wound scenarios. Collectively, these findings, together with the phase I and II clinical trials, position APOSEC as a robust candidate for further development in regenerative therapies, highlighting the necessity to utilise its comprehensive secretome composition to maximise therapeutic efficacy.

1.2 APOSEC 2.0

The MARSYAS trials revealed preparation and dosing challenges with APOSEC, prompting the creation of APOSEC 2.0, a closed, rapid-mix system that reduces contamination risk, lowers costs, and adapts dosing to wound size. The complete target product profile (TPP; Figure 1) planned for APOSEC 2.0 is composed of an autoclavable cyclic olefin polymer (COP) syringe filled and sealed with 3g of the sterile hydrogel APOgel, a Luer connector (Combifix® adapter), a glass vial containing the lyophilised APOSEC powder, a vial containing 1 mL sterile saline solution, and a sterile, graduated 5 mL syringe with a needle for the resuspension of the APOSEC powder and subsequent mixing with the APOgel, all sealed in a sterile bag.

A 5 mL graduated syringe is first used to transfer 1 mL of saline into the APOSEC vial for reconstitution. The resulting solution is then drawn back into the same syringe. After removing the needle, the syringe is connected via a Luer connector to a COP syringe pre-filled with APOgel. The gel and reconstituted APOSEC are thoroughly mixed by repeatedly transferring the contents between the two syringes 20 times, ensuring complete homogenisation (Figure 1B). Finally, the entire mixed preparation is collected into one syringe. The resulting gel, now containing solubilised APOSEC, is ready for direct application of the prescribed volume to the wound surface.

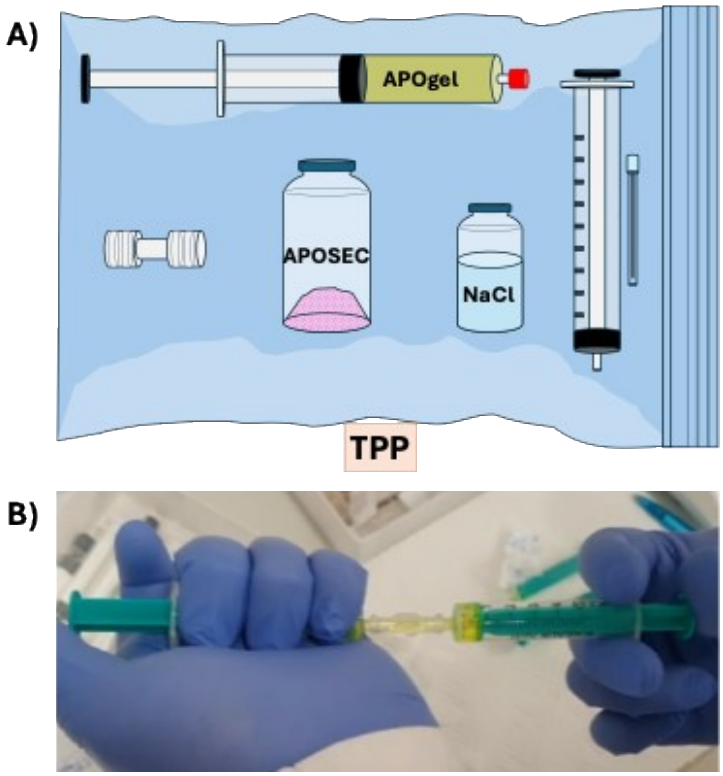

Figure 1: (A) Schematic of the APOSEC 2.0 product kit, constituted of a sterile bag containing 3 g of sterile APOgel pre-filled in a COP syringe, a Luer connector, a vial of lyophilised, sterile APOSEC, a vial containing sterile isotonic saline for injections, a sterile graduated syringe with needle for the resuspension of APOSEC, mixing with the gel and application to patients. (B) Illustration of the syringe-to-syringe mixing procedure of the reconstituted APOSEC fluid (containing fluorescein for better visualisation) using two Omnifix® Luer Lock solo syringes via Combifix® adapter.

1.3 Hydrogels as drug delivery systems

Hydrogels have been widely studied in various contexts and used as effective drug delivery vehicles across several therapeutic applications for many decades. [11], [12], [13] Their flexibility and versatility are among their key advantages. Structurally, hydrogels consist of three-dimensional polymer networks capable of entrapping large amounts of water due to their hydrophilic backbones. [14] They can be categorised, among others, according to their source (natural, synthetic), type of cross-linking (physical, chemical), responsiveness (e.g. pH, temperature) or physical appearance. Alginate, a low-cost natural polymer from brown algae, forms ionically crosslinked, pH-responsive gels with good water retention. Similarly, semi-synthetic cellulose derivatives HEC and CMC offer strong water retention, high hydrophilicity, and pH-responsiveness, making all three common gelling agents. [15], [16]

Due to the gels' continuous interlinked polymer network, hydrogels are often described as having effectively infinite molecular weight. [17] Upon contact with water, hydrogels undergo swelling, the extent to which their polymer network absorbs water, causing it to expand and subsequently relax over time. [18] This swelling behaviour is influenced by factors such as crosslinking density, chemical composition, and external stimuli like pH or temperature. [19]

As swelling progresses, the polymer network expands, increasing the mesh size (ξ), defined as the distance between two crosslinks (Figure 2). This is particularly relevant for the diffusion of a drug incorporated into the gel, as a smaller mesh size leads to drug retention, while increased mesh sizes facilitate diffusion. [18] Consequently, formulation strategies aiming for higher drug release rates often reduce crosslinker (e.g. calcium) or polymer (e.g. alginate) concentrations, thereby increasing mesh size. These dynamic structural changes contribute to the non-Fickian diffusion behaviour observed in hydrogels, where drug release is governed not only by passive diffusion along a concentration gradient but also by the time-dependent swelling and relaxation of the matrix. [20] Emerging evidence in the treatment of chronic, non-healing wounds indicates that topical preparations with a more acidic pH can enhance therapeutic outcomes by inhibiting bacterial growth, improving tissue oxygenation, and promoting cellular signalling involved in wound repair. [21] In addition, the pH of the vehicle gel may influence the biological activity of APOSEC components, a factor that has not yet been explored. Understanding these interactions is therefore essential for optimising hydrogel design and therapeutic efficacy.

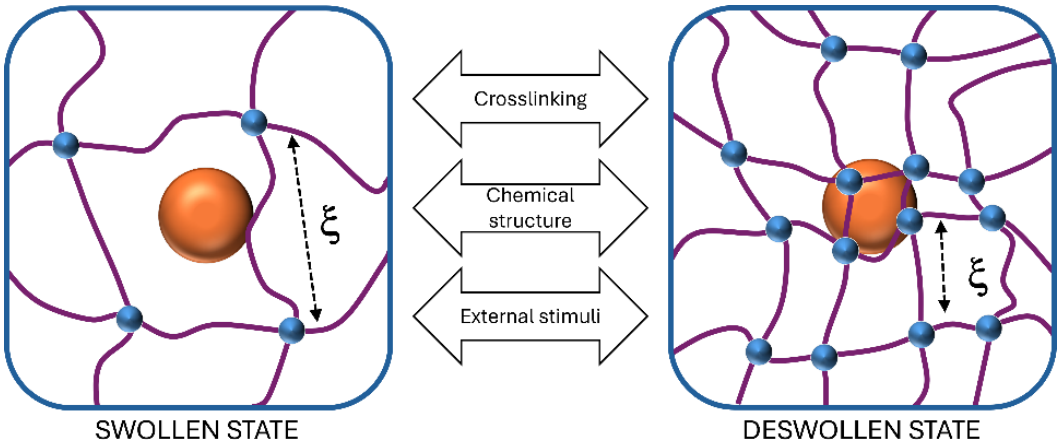

Figure 2: Illustration of mesh size (ξ) in swollen vs. deswollen gels and entrapment of a molecule (adapted from Klinger, 2012 [19])

Changes in viscosity of hydrogels can be influenced by thermal modifications, such as heat sterilisation or cold storage. [22] Various physical and chemical sterilisation methods for hydrogels have been evaluated in the past. While chemical sterilisation with gases or liquids is less common due to toxicity concerns, physical methods, especially γ-irradiation and thermal sterilisation, are widely applied due to their effectiveness and absence of harmful residues. [23], [24], [25] However, both γ-irradiation and thermal sterilisation can cause not only polymer but also drug degradation, which would ultimately result in viscosity alteration. [24] While γ-irradiation is highly effective, thermal sterilisation using saturated steam under pressure

(autoclaving) was chosen in this study not only for its regulatory endorsement but also for its reliability, efficiency, and scalability. [23], [25]

Despite these advantages, the high temperatures can affect the structure of gel-forming polymers and reduce the hydrogels' water content through evaporation. [26] Additionally, water vapour can cause the degradation and hydrolysis of some polymers, altering their gel structure and properties. [14], [27] This effect is well-documented and stems from polymer chain degradation, which reduces molecular weight and thereby viscosity. [15], [22], [28] Specifically, glycosidic bonds of sodium alginate undergo hydrolytic cleavage, while cellulose derivatives (CMC, HEC) may be susceptible to oxidative degradation, potentially due to free radical formation of present hydroperoxides. Notably, the extent of degradation can vary depending on the manufacturer and batch-related impurities, contributing to differences in viscosity loss. [25] Furthermore, an older study suggests that propylene glycol may help stabilise alginate solutions by moderating temperature-dependent viscosity changes. [29]

1.4 Rheometry

Rheometry comprises experimental techniques to measure the flow and deformation of materials (rheology) under stress or strain, providing qualitative and quantitative insights into complex fluids and soft solids. [30] Measurements may assess steady shear viscosity, viscoelastic moduli, or extensional behaviour, which for many materials – including hydrogels – depend on shear rate, frequency, or strain amplitude.

Hydrogels are generally non-Newtonian, with viscosity depending on shear rate. The power-law model describes this behaviour:

Equation 1

$$\eta = K\dot{\gamma}^{n-1}$$

where η is apparent viscosity, K the consistency index, $\dot{\gamma}$ the shear rate, and n the flow index. Materials with n < 1 are shear-thinning, n > 1 shear-thickening, and n = 1 Newtonian. [30] Many hydrogels are also thixotropic, showing reversible, time-dependent viscosity reduction under shear. [31] Shear-thinning facilitates spreading, injection, or extrusion, while shear-thickening occurs in highly crosslinked or particulate systems.

Hydrogels are viscoelastic, exhibiting both elastic (solid-like) and viscous (liquid-like) responses. [32] The complex modulus quantifies these properties:

Equation 2

$$G^* = G' + iG''$$

where G' represents stored elastic energy and G'' dissipated viscous energy. The phase angle is defined as:

Equation 3

$$\delta = \arctan(G''/G')$$

which indicates relative contributions: $\delta < 45°$ is predominantly solid-like, $\delta > 45°$ is more liquid-like. Measurements are usually within the linear viscoelastic region (LVR), where stress and strain are proportional and the structure preserved, and frequency sweeps reveal relaxation behaviour. [32]

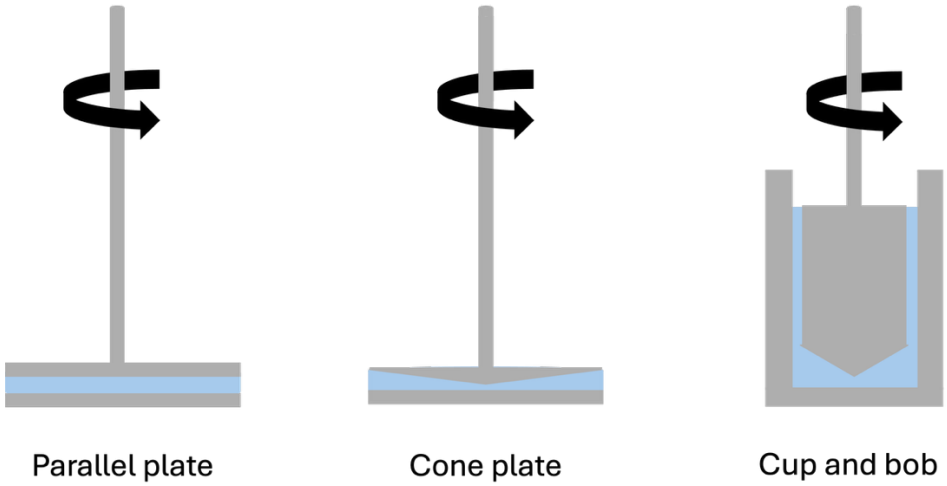

Figure 3: Schematic of parallel-plate, cone-plate and cup-bob geometries (adapted from Herrada-Manchón, 2023 [33])

Rheological characterisation typically employs rotational and oscillatory rheometry. Rotational tests apply steady shear to generate flow curves, determining apparent viscosity, yield stress, and flow indices. Rotational rheometry can also be adapted to measure extensional flow, providing insights into the material's response to stretching. Oscillatory tests use sinusoidal deformation to probe viscoelastic properties (G', G'') without disrupting the sample structure. Standard geometries, as shown in Figure 3, include parallel-plate (heterogeneous or high-viscosity samples), cone-plate (uniform shear, minimal volume) and cup-bob systems (ideal for soft or particle-laden hydrogels). [33]

1.5 Franz-type diffusion cell

Franz-type diffusion cells are widely used as a standard *in vitro* method for simulating topical and transdermal delivery. The system consists of a donor and a receptor compartment separated by a membrane or an excised skin sample (Figure 4). The formulation under investigation is applied to the membrane surface in the donor chamber, while the receptor compartment is filled with an appropriate medium, commonly phosphate-buffered saline, maintained at around 32°C and continuously stirred to ensure homogeneity. Franz cells are available in static and flow-through configurations. Static systems retain a fixed receptor

volume, with samples periodically withdrawn and replaced, while flow-through models continuously circulate fresh receptor fluid, providing reduced boundary layer effects. The diffusion area and receptor volume vary between cells, typically ranging from 1-3 cm^2 and 5-15 mL, respectively, influencing flux normalisation and data comparability. [34], [35]

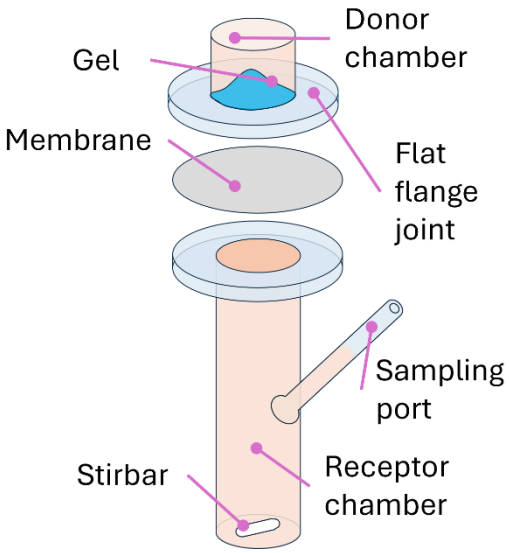

Figure 4: Schematic setup of a Franz cell.

The choice of membrane strongly affects permeation. [36] Synthetic membranes (e.g. polycarbonate, silicone, cellulose acetate, or polydimethylsiloxane) are used for reproducible screening, while excised human or porcine skin is more physiologically relevant but more variable and less stable. Drug permeation across a membrane is governed by Fick's first law of diffusion (modern notation), which states that the flux (J) is proportional to the concentration gradient across the membrane [37]:

Equation 4

$$J = -D\frac{dC}{dx}$$

D is the diffusion coefficient and $\frac{dC}{dx}$ the concentration gradient; the negative sign indicates diffusion from high to low concentration.

Under steady-state conditions, the concentration gradient remains constant, and the relationship can be expressed as:

Equation 5

$$J_{ss} = \frac{D \cdot K \cdot \Delta C}{h}$$

where K is the drug partition coefficient, ΔC the difference in concentration of the drug on both sides of the membrane and h the membrane thickness. [38] The lag time (t_{lag}) is another permeability parameter that corresponds to the time required to reach a steady state and

provides information about the diffusional resistance and thickness of the membrane. It can be determined from the intercept of the linear region of the cumulative permeation curve with the time axis.

Steady state conditions are maintained experimentally when the concentration of the permeant in the receptor phase remains below approximately 20% of its saturation solubility, ensuring that ΔC remains constant throughout the experiment. The term “perfect sink conditions” typically applies when the drug concentration stays below 10% of its solubility. Deviation from this state leads to non-sink conditions, in which the decreasing concentration gradient causes apparent reductions in flux and permeability. This can be controlled by continuous stirring, increasing the volume of the receptor medium or by its frequent replacement. [39]

1.6 Aims and study hypotheses

Since a point-of-care syringe-mixing system like APOSEC 2.0 has not yet been systematically investigated, this thesis is centred on its evaluation alongside the production and characterisation of an in-house hydrogel (APOgel) designed for this system. The work pursued two objectives. First, to develop a terminally sterilised hydrogel suitable for clinical trial applications, with a formulation loosely based on Nu-Gel™ and viscosity as the primary quality attribute assessed under various conditions. Second, to evaluate the syringe-mixing system APOSEC 2.0 using a model medium (AM2), focusing on mixture viscosity, uniformity, operator-dependent variability, and release behaviour. It was hypothesised that both the hydrogel and the APOSEC 2.0 system would meet their predefined quality attributes, demonstrating that a clinically suitable hydrogel can be reliably handled within the mixing system.

2 Methods

2.1 Materials

Pharmaceutical grade carboxymethyl cellulose sodium salt (CMC), hydroxyethyl cellulose (HEC; 250HX; 10,000 mPa·s), propylene glycol (PG; PHE 11.0) and sodium alginate (NaAlg; PHE 11.0) were purchased from Gatt-Koller (Absam, Austria). An additional APOgel batch was produced with Vivapharm® sodium alginate (PH 176) from JRS Pharma (Rosenberg, Germany). Sodium chloride (NaCl, purity ≥99 %) was provided by Carl Roth (Karlsruhe, Germany). A custom-formulated cell culture medium (AM2), containing Ringer’s lactate solution (838.75 mL/L), albumin (8 g/L, or 0.120 mM) and recombinant human insulin (125U/L, or 0.075 mM) for a ratio-weighted, total protein concentration of 0.195 mM [40], was purchased from PAN Biotech (Aidenbach, Germany). Fluorescein sodium salt (SF) and phosphate-buffered saline (PBS) were obtained from Sigma Aldrich Ltd. (Dorset, UK). Water for injections

to produce APOgel was provided by LKH-University Hospital Graz. Pierce™ Bradford Protein Assay Kit was purchased from Thermo Fisher Scientific (Massachusetts, USA). ClearJect® Luer Lock 5 mL cyclic olefin polymer (COP) syringes, suitable for autoclaving [41], were provided as a complete system with plunger stoppers, tip caps and plunger rods by Gerresheimer (Düsseldorf, Germany). AM2 medium was filled into Omnifix® Luer Lock Solo 5 mL 3-piece syringes, which were obtained, along with the corresponding Combifix® adapter (female to female), from B. Braun (Melsungen, Germany). Unjacketed Franz-type diffusion cells (orifice: 11.28 mm, receptor volume: 2 mL, with flange joint) were provided by PermeGear (Pennsylvania, USA), including magnetic stir bars and gaskets. Hydrophilic Nuclepore™ polycarbonate membranes (pore size: 0.2 µm, diameter: 25 mm) were supplied by Cytiva (Massachusetts, USA). Parafilm® was purchased from Amcor Limited (Zürich, Switzerland). Marketed gels used (section 3.1) were Nu-Gel™ from 3M (Minnesota, USA), Purilon® Gel from Coloplast (Humlebæk, Denmark), Cutimed® Gel from BSN medical (Hamburg, Germany), DracoHydrogel from Dr. Ausbüttel & Co. GmbH (Dortmund, Germany), VARIHESIVE® Hydrogel from Convatec plc. (London, United Kingdom), Hydrosorb® Gel from Paul Hartmann AG (Heidenheim an der Brenz, Germany) and INTRASITE Gel from Smith & Nephew plc (London, United Kingdom).

2.2 APOgel formulation and characterisation

Although Nu-Gel™ had proven suitable as a delivery vehicle and facilitated successful topical administration in previous APOSEC trials [5], [42], the choice was made to develop a custom hydrogel due to factors such as intellectual property, sourcing, cost, and ease of manufacturing. APOgel was developed from the published composition [43] of Nu-Gel™ (3M) with adjustments during optimisation, as Nu-Gel™'s excipient properties and sterilisation method are unreported. Both compositions are listed in Table 1.

Table 1: Reported composition of Nu-Gel™ and the proposed composition of APOgel.

Component	**Nu-Gel™ composition [43] (%)**	**APOgel composition (%)**
Water	70.8	68.9
Propylene glycol (PG)	25.0	25.0
Sodium alginate (NaAlg)	3.0	3.0
Carboxymethylcellulose (CMC)	1.0	1.0
Hydroxyethylcellulose (HEC)	0.1	2.0
Sodium chloride (NaCl)	0.1	0.1

2.2.1 Rheological properties

The dynamic viscosity of gels was measured using a modular compact rheometer (MCR 302, Anton Paar AG, Graz, Austria) with a 25 mm diameter cone-plate measuring system maintained at 23°C via a Peltier heater and a gap distance of 0.1 mm. Zero-gap calibration was performed beforehand to ensure correct settings. A finite amount of hydrogel (400-600 mg), equilibrated to room temperature, was dispensed onto the lower plate. Dynamic viscosity was determined by applying increasing and then decreasing shear rates to assess rheological behaviour. [44] The data obtained from the rheometer was then plotted as viscosity (Pa·s) vs shear rates (s^{-1}). For comparison between different batches, the viscosity of selected shear rates (1, 10 and 100 s^{-1}) was looked at more closely.

2.2.2 APOgel production and sterilisation

Steam sterilisation is known to reduce the viscosity and mechanical stability of hydrogels with cellulose ethers and alginate. [23] To compensate, APOgel's pre-sterilisation viscosity was raised above 700 Pa·s (1 s^{-1}) in small-scale pilot batches, achieving a post-sterilisation viscosity comparable to Nu-Gel™ (~340 Pa·s at 1 s^{-1}). This was accomplished by increasing HEC from 0.1% to 2% (Table 1).

APOgel was produced in the cleanroom class D of the hospital pharmacy at LKH-University Hospital Graz under GMP conditions [45]. Three replicate 3 kg batches were prepared, with ± 1% weighing error for all components. The dry components and PG were manually mixed before being combined with NaCl dissolved in water for injections. The ingredients were blended for 1 min 30 s at 750 rpm using a jacketed, vertical mixer (Stephan Mixer UM-12, ProXES GmbH, Hameln, Germany) under a vacuum of 0.8 bar below atmospheric pressure. This was followed by an additional 1-min mixing step at 1500 rpm. Finally, the set vacuum was maintained for 1 min without rotation. The gel was thereafter filled into polypropylene wide-neck jars and stored at room temperature until further use. For quality assurance, all fill weights and detailed procedures were documented.

Sterilisation of syringes containing APOgel was performed at a separate location under non-GMP conditions. APOgel was manually loaded into COP syringes (3 g ± 1%), then terminally sterilised using a vertical autoclave (Classic-Line HV-series, HMC Europe, Tüssling, Germany) set to the liquid program (121°C, 2 bar, 15 min). After the autoclaving cycle, the syringes were allowed to cool to room temperature (23°C, approximately 1 h) followed by storage at 4°C in darkness for a minimum of 24 h prior to further characterisation.

2.2.3 pH measurements

The relative pH of APOgel was determined before and after steam heat sterilisation. APOgel samples were diluted to a final concentration of 10 mg/mL in distilled water (the pH of which was measured separately before dilution) to assess the influence of the gel on the water's pH. Nu-Gel™ was used as a positive reference. The pH was measured using a calibrated pH meter (Seven Compact S220, Mettler Toledo, USA) equipped with an InLab Micro electrode (Mettler Toledo). pH values were recorded immediately after dilution.

2.3 Characterisation of the APOSEC 2.0 application system

After finalising the optimised sterile APOgel formulation, the study examined how the syringe-mixed system APOSEC 2.0 influences key quality attributes of the resulting gel mixture.

2.3.1 APOSEC 2.0 preparation and analysis

To minimise costs, the custom-formulated cell culture medium AM2, which is used to culture PBMCs during APOSEC production, served as a substitute for APOSEC. For all APOSEC 2.0 experiments, the mixture was prepared as follows: Sterile APOgel (3 g ± 1%) was filled into a COP syringe, then connected via a Luer adapter to an Omnifix® syringe prefilled with 1 g ± 1% AM2 medium. The contents were mixed by transferring them back and forth between the two syringes, concluding 20 total passes, which equals one complete syringe-to-syringe mixing cycle (Figure 1B). The final sample was retained in the graduated syringe for easier dosing. A mixture of Nu-Gel™ (positive control) was prepared using the same procedure. The following list of experiments is organised in line with the CQA Table 3.

VISCOSITY

The rheological behaviour of the mixture was characterised according to section 2.2.1 for all APOgel batches produced.

MIXING UNIFORMITY

The APOSEC 2.0 mixing method is a manual process; hence, operator variability may influence properties such as viscosity. To explore this, three levels were defined based on the number of mixing operations each volunteer had previously performed (no experience = 0, some experience = 3–5, and experienced = >20). Each group consisted of at least three volunteers ($n \geq 3$), all of whom received oral instructions only. The rheological properties of the resulting mixtures were then characterised.

DOSING UNIFORMITY

The reproducibility of manual dosing was assessed by having three volunteers dispense three approximately 1 g doses from the mixture. Each dose was administered according to the syringe graduation marks and subsequently weighed.

CONTENT UNIFORMITY

To gain insight into the intra-syringe distribution of APOSEC components in the mixture, three ~100 mg samples were taken from the top, middle, and end of the syringe, discarding ~1.85 g of gel between each sample. The mass of each sample was noted and then diluted 1:100 with PBS. The AM2 medium (detailed composition listed in section 2.1) was pre-spiked with sodium fluorescein (SF; 0.025% w/w) before being combined with APOgel to track low-molecular-weight, soluble APOSEC components, while total protein was measured to represent high-molecular-weight components. Fluorescence (excitation/emission: 485/535 nm) and absorbance (595 nm) were measured using a Tecan™ Infinite 200 microplate reader (Tecan Ltd., Männedorf, Switzerland). SF and total protein concentrations were calculated using linear calibration curves prepared with SF (0.0156-1 µg/mL; R^2 = 0.993, LOD = 0.00518 µg/mL, LOQ = 0.0157 µg/mL) and bovine serum albumin BSA (2.5-25 µg/mL; R^2 = 0.992, LOD = 1.158 µg/mL, LOQ = 3.509 µg/mL) as standards. Total protein content was determined via Bradford assay according to its manual [46].

2.3.2 APOSEC 2.0 release profiles

Release studies of analytes (sodium fluorescein and total protein) incorporated into APOgel were conducted using Franz-type diffusion cells [47]. Two study protocols were compared: (1) a 6-h study to assess initial release rates under sink conditions [48], and (2) a 72-h study to evaluate the partitioning of components under non-sink conditions, reflecting a therapeutically relevant dosing frequency of once every three days.

AM2:APOgel mixtures were prepared in syringes as previously described (section 2.3.1). The control was a solution without gelling agents (NaCl 0.1% w/w, PG 25%, water for injections 68.9%), chosen to represent maximum un-retarded release. Tests were carried out in triplicate. Diffusion cells were assembled as shown in Figure 4 and secured via a horseshoe clamp. To prevent leakage, a gasket was placed between the receptor chamber and the hydrophilic polycarbonate membrane (pore size: 0.2 µm, diameter: 25 mm). The receptor chamber was filled with 2 mL of PBS and continuously stirred at 400 rpm via a magnetic stir bar. A finite dose of each sample (500 mg ± 100 mg) was placed onto the membrane with a permeation area of 0.99 cm^2. To prevent evaporation during the diffusion process, the sampling port was covered with aluminium foil, whereas the donor chamber was occluded with Parafilm® and aluminium foil. All cells were placed in a thermostatically controlled water bath (model 1092 from GFL;

Burgwedel, Germany) at skin surface temperature (32±0.5°C). For the 6-h experiment, samples (1 mL) were taken every hour and replaced with an equal volume of PBS. For the 72-h experiment, the entire volume of the receptor chamber (2 mL) was replaced every 24 h.

SF and total protein were quantified as described in section 2.3.1 using the following calibration curves: SF, 0.0156-1 µg/mL (6-h study: $R^2 = 0.993$, LOD = 0.00518 µg/mL, LOQ = 0.0157 µg/mL; 72-h study: $R^2 = 0.993$, LOD = 0.00027 µg/mL, LOQ = 0.00083 µg/mL) and BSA, 2.5-25 µg/mL (6-h study: $R^2 = 0.992$, LOD = 1.158 µg/mL, LOQ = 3.509 µg/mL; 72-h study: $R^2 = 0.989$, LOD = 1.086 µg/mL, LOQ = 3.29 µg/mL).

Their permeation profiles were then visualised by plotting the cumulative amount per surface area (µg/cm^2) against time (h). The cumulative amount for each replicate cell was determined according to Equation 6, accounting for dilution factors [49]:

Equation 6

$$Qn = \frac{Cn \times Vc + \sum_{i=1}^{n-1}(Ci \times Vs)}{A}$$

Qn is the cumulative amount (µg/cm^2) of the target compound permeated at time point n, Cn is the concentration of the target compound in the sample at time point n (µg/mL), Vc is the volume of the receptor medium (mL), $\sum_{i=1}^{n-1} Ci$ is the sum of the concentrations of the target compound (µg/mL) assessed at sampling intervals 1 through $n - 1$, Vs is the volume of the sample (mL) and A the surface area (cm^2).

The cumulative release of SF and total protein into the acceptor compartment was analysed for both the 6-h and 72-h studies using an exponential kinetic model of drug release from swollen gels under non-sink conditions. [48] The 6-h release was likely under sink conditions due to the hourly sampling, whereas the 72-h experiments most likely displayed non-sink conditions due to possible saturation of the receptor medium between samplings. These conditions, therefore, demand kinetic modelling that goes beyond simple zero- or first-order models. To unify the fitting approach, the non-sink exponential release model was applied for both time points.

Briefly, the relationship between drug concentration at time t ($C(t)$) is proportional to the maximum predicted released concentration of drug at infinite time (C_∞), which in the model fitting is expressed by the asymptote of the fitting curve. C_∞ is reduced over time by the reabsorption, more precisely the back-diffusion, of the drug by the swelling gel from the bulk, a process described by the reabsorption kinetic constant ($K(-r)$). This can occur particularly under non-sink conditions, where the concentration gradient is reduced, slowing down further release. Hence, the exponential equation used to fit the release kinetics is:

Equation 7

$$C(t) = C_\infty \times \left(1 - exp^{-K(-r) \times t}\right)$$

To understand the release kinetics from the gel to the bulk ($K(r)$), at equilibrium the kinetic constant of release $K(r)$ can be calculated by Equation 8:

Equation 8

$$Keq = \frac{C_{\infty}}{C_f^{\infty}} = \frac{K(r)}{K(-r)}$$

Where C_f^{∞} refers to the concentration of drug left in the gel at the equilibrium, a value that can be back-calculated from the experimental measurements.

A separate diffusion test, dedicated to assessing water uptake, was conducted by weighing the entire loaded Franz cell assembly at 0, 24, 48, and 72 h. Before each measurement, the acceptor medium was fully replaced with fresh medium to assess fluid uptake by the mixture at each time point.

2.4 Quality by Design approach of APOgel and APOSEC 2.0

Table 2: Quality target product profile with CQAs and target specifications for the terminal sterilisation of APOgel in pre-filled COP syringes.

Terminally sterilised APOgel pre-filled in COP syringes		
CQA	**Test method**	**Specification**
Viscosity	Dynamic viscosity	Within ± 15% of reference product (from phase I/II studies) at different shear rates
Storage stability	Dynamic viscosity	Within ±15% from initial viscosity at defined time points
pH	pH-meter	pH of dilution below control

Table 3: Quality TPP with CQAs and target specifications for the APOSEC 2.0 product, which is defined as the product applied to the wound following point-of-care mixing of reconstituted APOSEC with the sterile APOgel.

APOSEC 2.0 = APOSEC:APOgel application mixture		
CQA	**Test method**	**Specification**
Viscosity	Dynamic viscosity	<10% difference to APOSEC:Nu-Gel™ mixtures
Mixing uniformity	Dynamic viscosity following mixture by multiple operators	Coefficient of variation < 10%
Dosing uniformity	Weight measurement	Within ±15% of the target dose of 1 g
Content uniformity in syringe	Quantification of APOSEC components in multiple doses from the same mixture	Within ± 15% of the mean content in each syringe
Release profile	Franz cell diffusion studies	$K(r)$ within ±15% of Nu-Gel™

Quality requirements for semi-solid drug products applied topically to open wounds were derived from European Medicines Agency guidance. [50] CQAs defined for sterile APOgel in pre-filled syringes as well as for the APOSEC 2.0 product are listed in Table 2 and Table 3.

The development of APOgel and APOSEC 2.0 followed a Quality by Design (QbD) framework, a systematic approach building quality into pharmaceutical products through strategic design rather than relying on final-product testing. This begins with defining a Target Product Profile (TPP; Figure 1), which is translated into a Quality Target Product Profile (QTPP) with Critical Quality Attributes (CQAs) – the parameters critical to product safety and efficacy. [51], [52]

It should be noted that chemical stability, sterility and biological activity remain critical attributes, though they are not addressed in this study.

2.5 Data and image processing

Data obtained from the instruments were analysed using Microsoft Excel (16.100.1), visualised with Graphpad Prism (10.01), and images were created with Microsoft PowerPoint (16.100.1).

3 Results and Discussion

3.1 Rheological profiling of commercial hydrogels

To position APOgel within the broader market context, several commercially available hydrogels for wound care were examined for their dynamic viscosities (Figure 5).

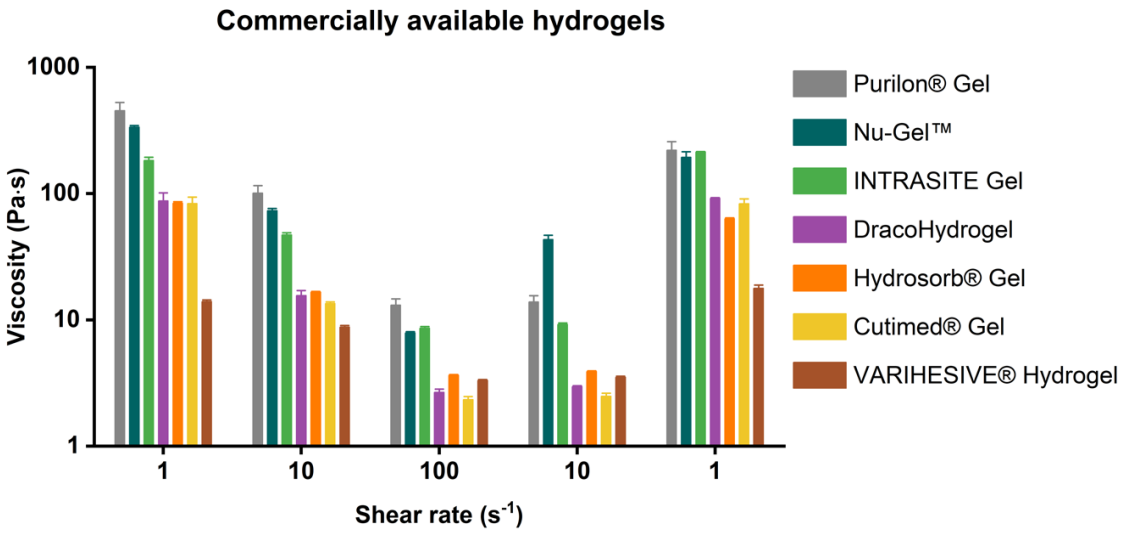

Figure 5: Selected dynamic viscosity (Pa·s) values of commercially available hydrogels. Results represent the mean ± standard deviation of n=3 replicates.

VARIHESIVE® Hydrogel exhibited the lowest viscosity of all (~14 Pa·s at 1 s^{-1}), while Cutimed® Gel, Hydrosorb® Gel, and DracoHydrogel clustered at similar levels (~80-90 Pa·s at 1 s^{-1}), all showing typical shear-thinning with near-complete recovery. INTRASITE® Gel (~180 Pa·s at 1 s^{-1}) displayed similar shear-thinning, but its viscosity slightly exceeded the initial value at the final low-shear measurement, reflecting a mild thixotropic overshoot observed in VARIHESIVE® Hydrogel and DracoHydrogel as well. Purilon® Gel and Nu-Gel™

exhibited the highest viscosities (~460 Pa·s and ~340 Pa·s at 1 s^{-1}, respectively). APOgel, whose viscosity is presented in the following section, was loosely modelled on Nu-Gel™ and is therefore expected to fall within the upper-viscosity segment of the market.

3.2 APOgel characterisation

VISCOSITY

APOgel was developed to closely match Nu-Gel™'s composition (avoiding new excipients) and to allow terminal sterilisation in pre-filled syringes. The sterile gel's viscosity was targeted to remain within ±15% of Nu-Gel™ across 1-100 s^{-1} shear rates (Figure 6). Increasing HEC to 2% raised the pre-sterilisation viscosity (~710 Pa·s at 1 s^{-1}), which dropped to ~340 Pa·s (1 s^{-1}) after autoclaving, yielding a post-sterilisation profile comparable to Nu-Gel™. This adjustment introduced mild thixotropy, deemed unlikely to affect therapeutic performance. Throughout this chapter, all post-sterilisation values – including the value above – correspond to autoclaved samples analysed after one day of storage at 4°C.

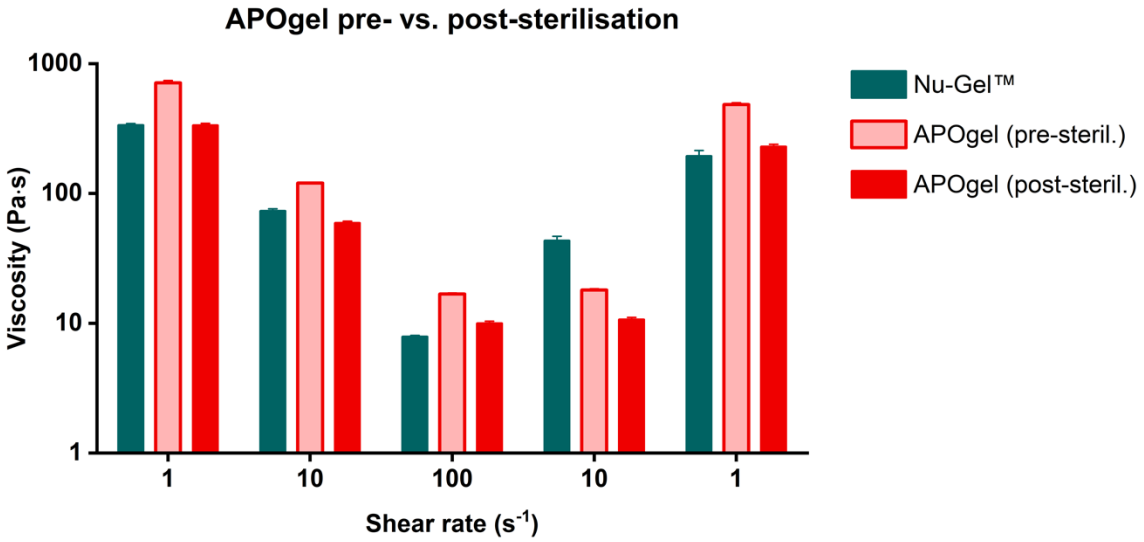

Figure 6: Selected dynamic viscosity (Pa·s) values of Nu-Gel™ and APOgel tested before and after autoclaving. Results represent the mean ± standard deviation of n=3 batches.

The APOgel batches analysed thus far were produced with NaAlg powder supplied by Gatt-Koller (Absam, Austria; abbreviated as GK). To further investigate whether the choice of gelling agent supplier influences viscosity, an additional APOgel batch was produced using NaAlg powder from JRS Pharma (Rosenberg, Germany; abbreviated as JRS). With JRS-APOgel starting at a higher initial viscosity (~970 Pa·s at 1 s^{-1}) compared to GK-APOgel (~710 Pa·s at 1 s^{-1}), both gels decreased after autoclaving (~470 Pa·s vs. ~340 Pa·s at 1 s^{-1}), with only the post-autoclaving values shown in Figure 7. Notably, JRS-APOgel showed a much greater recovery during storage, increasing to ~550 Pa·s over 90 days, whereas GK-APOgel rose only to ~380 Pa·s (1 s^{-1}). In summary, gels produced with NaAlg from JRS Pharma not only

exhibited higher viscosity at the outset but also responded more strongly to processing and storage than those produced with NaAlg from Gatt-Koller. Source-related variability has already been reported in the literature, underscoring the importance of careful raw material selection for pharmaceutical manufacturing. [53], [54]

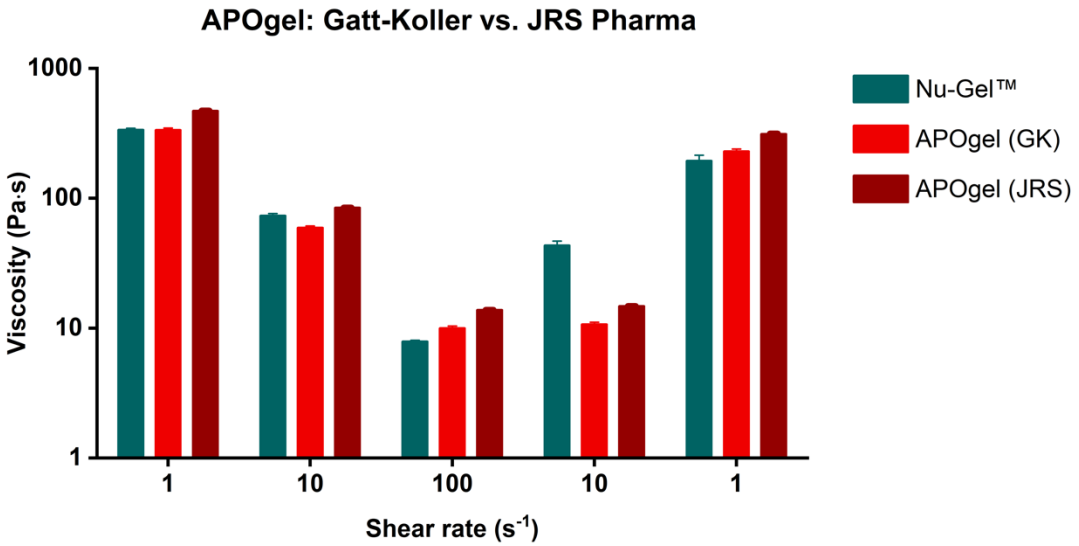

Figure 7: Selected dynamic viscosity (Pa·s) values of Nu-Gel™ and two sterile APOgel batches produced with NaAlg powder from Gatt-Koller (GK) and JRS Pharma (JRS). Results represent the mean ± standard deviation of n=3 replicates.

STORAGE STABILITY

Autoclaved APOgel showed a gradual increase in viscosity over time, reaching 11% at 1 s^{-1} above the initial value after 90 days, yet remaining within the ±15% specification (Figure 8). While the thermosensitivity of gelling agents has been discussed earlier, detailed studies on long-term cold storage of hydrogels are still scarce.

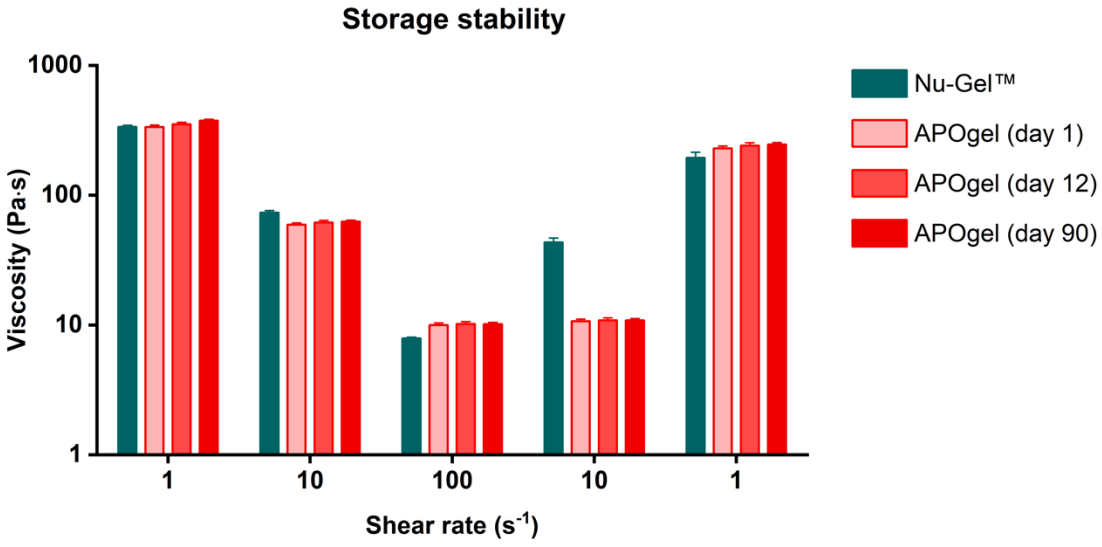

Figure 8: Selected dynamic viscosity (Pa·s) values of Nu-Gel™ and sterile APOgel tested after storage at 4°C for 1, 12 and 90 days. Results represent the mean ± standard deviation of n=3 batches.

pH

Following the rheological characterisation, the pH of APOgel was examined. APOgel diluted in water (pH 9.54 ± 0.06) lowered the pH by at least 0.3 units, with measurements of 8.73 ± 0.44 before sterilisation and 8.54 ± 0.28 after autoclaving, indicating that terminal sterilisation does not compromise this property. The positive control Nu-Gel™ showed a comparable effect (8.74 ± 0.20). Such a decrease is beneficial, as more acidic topical formulations are known to inhibit bacterial growth, improve oxygenation, and promote cellular signalling that supports wound healing. [21]

3.3 APOSEC 2.0 product characterisation

3.3.1 Viscosity and uniformity studies

VISCOSITY

Pilot studies were conducted to assess how the proposed APOSEC:APOgel mixing procedure affects the final APOSEC 2.0 product. AM2 medium, a cost-effective surrogate with similar viscosity to reconstituted APOSEC, was used. In preliminary attempts, it was found that optimal mixing of 1 g (=1 mL) AM2 with 3 g APOgel was achieved by passing the mixture 20 times between the syringes, resulting in a mixture which reduced the viscosity of both APOgel and the reference Nu-Gel™ to approximately 110 Pa·s and 140 Pa·s at 1 s^{-1}, respectively. While relevant studies [55], [56] indicate variability in gel properties due to differences in mixing techniques, the literature on the effects of manual hydrogel mixing in syringes remains limited.

MIXING UNIFORMITY

In preliminary studies, it was observed that participants tend to mix more carefully when doing so for the first time. Therefore, it was hypothesised that with more practice, participants would apply higher shear forces, enhancing gel breakdown and viscosity reduction. [57] Interestingly, in our follow-up study, operators with varying experience levels (no experience = 0, some experience = 3–5, and experienced = >20) produced mixtures with comparable viscosities. Coefficients of variation for viscosity measurements at each selected shear rate were below 6%, suggesting no meaningful change in viscosity through repeated practice, at least in this context (Figure 9).

DOSING UNIFORMITY

The consistency of delivered doses from a syringe filled with 4 g of the mixture was assessed by weighing approximately 1 g aliquots. Across the first three doses dispensed, all volunteers achieved relatively consistent amounts (volunteer 1: 9.40 ± 0.39 g, volunteer 2: 9.53 ± 0.09 g, volunteer 3: 1.03 ± 0.11 g), indicating low variability within the ±15% specification. As expected,

the mean amount of the fourth dose across all volunteers was significantly lower (7.58 ± 0.14 g), likely due to residual material remaining in the syringes and the Combifix® adapter.

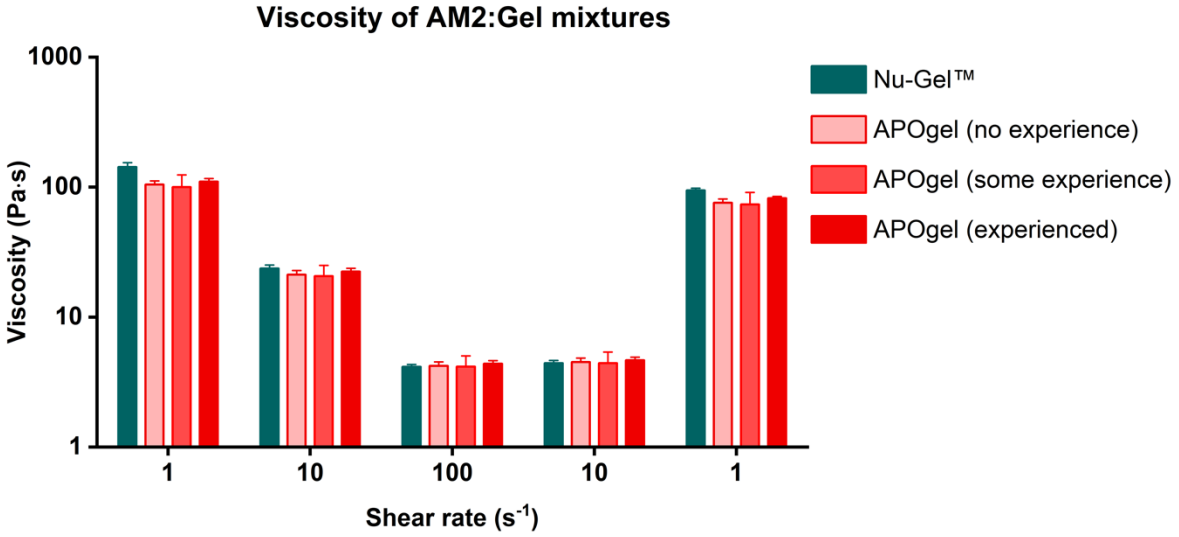

Figure 9: Selected dynamic viscosity (Pa·s) values of AM2 mixtures with Nu-Gel and sterile APOgel (1:3). AM2:APOgel mixture preparations were grouped based on the experience level of the operator. Results represent the mean ± standard deviation of n=3 operators per group.

CONTENT UNIFORMITY

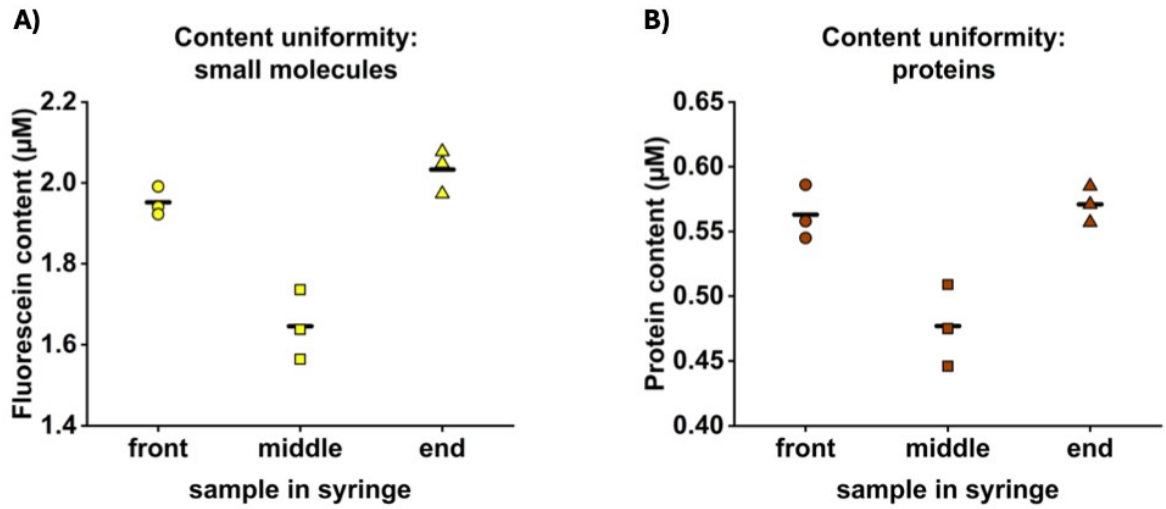

Figure 10: Uniformity of content of a small molecule marker, SF (A), and total proteins (B) in three sequential samples from three different syringes.

To evaluate whether the mixing approach achieves a homogenous mixture of AM2 components, three equal volumes were sequentially dispensed from each syringe into pre-weighed vials, and both a small-molecule marker (SF) and total protein content were measured according to section 2.3.1. Surprisingly, in all three tested syringes, the concentrations of both SF and total protein in the front and end fractions were higher than in the middle fraction, indicating a slight concentration gradient towards both ends of the syringe (Figure 10).

Notably, the concentration was marginally higher at the end of the syringe, closest to the flange. A recent study by Dani *et al.* (2021) [58] highlighted the challenges of producing a homogeneous mixture via syringe-to-syringe mixing of viscous biomaterials, applying a similar method of sampling and dispensing as described in section 2.3.1. Although this study focused on the effects of different geometries of the mixing units on viscosity, it provided evidence that discrepancies can occur between the front and the end of the syringe. The importance of analysing concentration gradients is further emphasised in the work by Xing *et al.* (2019) [59], where fluid dynamics within syringes were shown to cause uneven protein distribution. To conclude, all fractions of the tested APOgel syringes remained within ±25% of each syringe's mean, with at least two-thirds within ±15%, though future optimisation of the mixing protocol may be necessary to improve dose uniformity for the APOSEC 2.0 system.

3.3.2 *In vitro* release studies

APOSEC release behaviour was emulated using AM2:gel mixtures in Franz-type diffusion cells [47], monitoring SF and total protein (insulin and albumin) over 6 h under sink conditions and 72 h under non-sink, therapeutically relevant conditions. A low-viscosity control was included to assess the effect of gel viscosity on release. Full experimental details are provided in section 2.3.2. Throughout this chapter, references to APOgel and Nu-Gel™ pertain specifically to their mixtures with AM2.

Table 4: Release rate constant ($K(r)$; 10^{-3} h^{-1}) and predicted maximum release (C_∞; µM) of SF and proteins from samples mixed with AM2, obtained by fitting the release profiles to Equation 7.

	AM2:PPG/water		**AM2:Nu-Gel™**		**AM2:APOgel**	
	$K(r)$ **(10^{-3} h^{-1})**	C_∞ **(µM)**	$K(r)$ **(10^{-3} h^{-1})**	C_∞ **(µM)**	$K(r)$ **(10^{-3} h^{-1})**	C_∞ **(µM)**
SF (6 h)	208.1 ± 47.5	63.5 ± 0.2	35.3 ± 5.9	33.4 ± 1.6	35.45 ± 4.03	45.3 ± 2.2
Protein (6 h)	39.2 ± 7.8	21.0 ± 0.6	7.4 ± 0.4	2.41 ± 0.06	10.5 ± 3.6	11.7 ± 2.6
SF (72 h)	20.9 ± 0.7	44.20 ± 0.05	11.2 ± 1.2	38.2 ± 1.3	15.1 ± 1.9	65.1 ± 1.3
Protein (72 h)	2.9 ± 0.4	4.4 ± 0.3	1.1 ± 0.4	2.3 ± 0.4	2.0 ± 0.5	11.3 ± 1.6

Under sink conditions (6 h; Figure 11A and C), the release profiles of both total protein and SF differed substantially between the gels and the control without gelation agents, confirming that the gels slow the release of both proteins and small molecules. [18] For both analytes, the release rate $K(r)$ of the control was higher than that of Nu-Gel™ and APOgel, which clustered

together (Table 4). This difference was also noticeable when comparing the maximum predicted released concentration of the analyte at infinite time (C_∞; equal to the asymptote).

Looking at the 6-h SF release of APOgel and Nu-Gel™, $K(r)$ values were almost identical: within the protein release group, APOgel was slightly higher than Nu-Gel™. Consistent with expectations, SF permeated faster and in greater amounts than proteins, reflecting size-dependent diffusion, as larger proteins are sterically hindered within the gel matrix. [18]

When examining cumulative release over 72 h, a more complex pattern emerged (Figure 11B and D). The control consistently showed the fastest release (highest $K(r)$; Table 4), whereas Nu-Gel™ levelled off earlier, and APOgel exhibited a more sustained and extensive release of both SF and total proteins. C_∞ values indicated that APOgel had the highest overall release within 72 h, whereas Nu-Gel™ released more slowly and to a lesser extent.

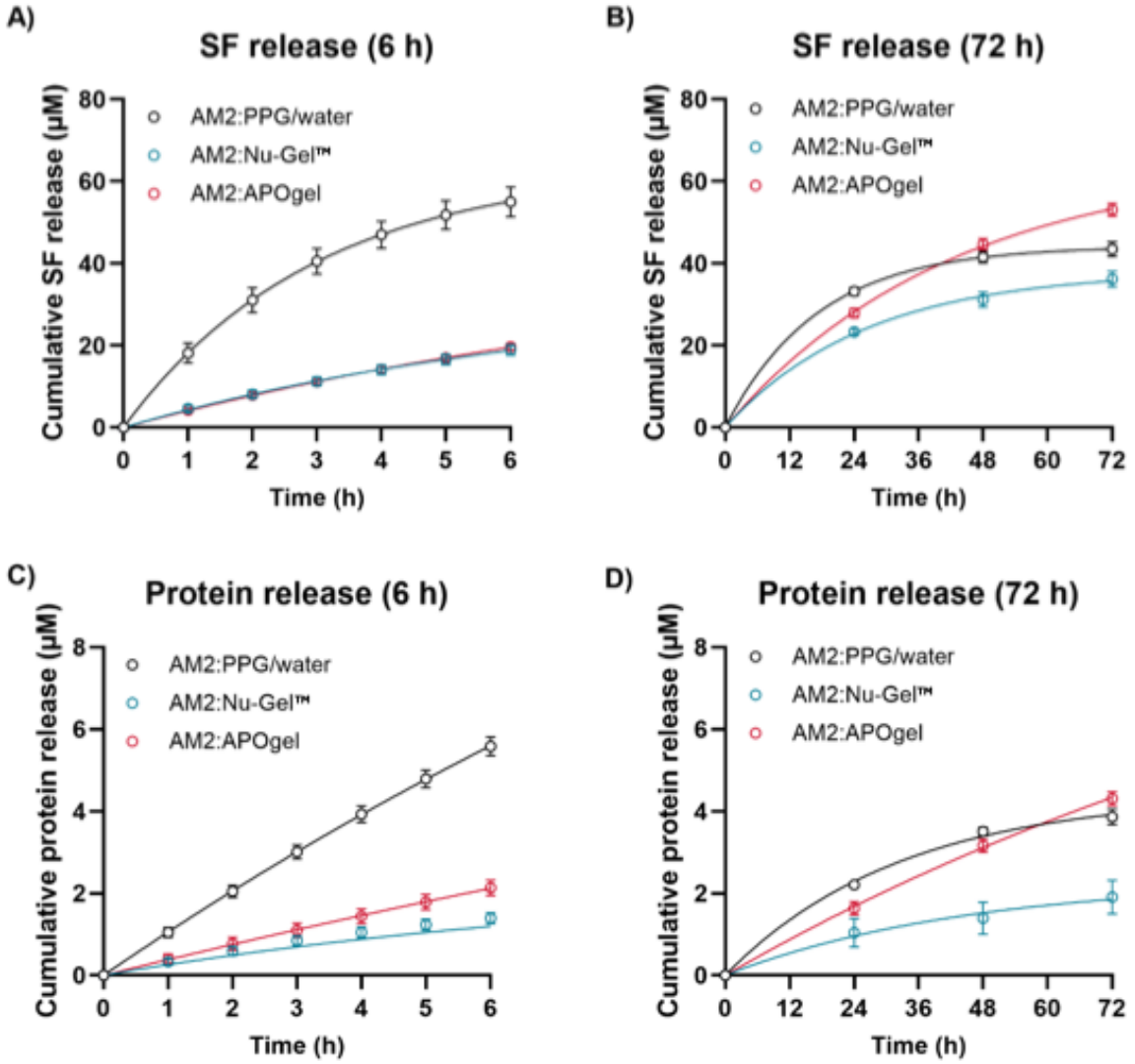

Figure 11: In vitro release profiles of SF over 6 h (A) and 72 h (B), compared to release profiles of total proteins over 6 h (C) and 72 h (D) from AM2:gel mixtures prepared according to the TPP. A low viscosity control mixture (AM2:PPG/water) was included to highlight the effects of the gel component of the mixture on release profiles. Coloured lines represent the fitting of the curves to Equation 7. The adjusted coefficient of determination values (adjusted R^2) for all fitted curves were > 0.998. Data points represent the mean ± standard deviation of n=3 batches.

Differences between Nu-Gel™ and APOgel likely arise from formulation factors, particularly the impact of sterilisation on the APOgel network structure and crosslinking density, which could have influenced the release profiles. [15] By contrast, the control demonstrated an initial burst followed by slower release, highlighting the effect of the gel matrix on modulating diffusion.

These observations are consistent with therapeutic considerations: the sustained and higher release observed for APOgel may be advantageous when prolonged drug availability is desired. [20] However, potential experimental artefacts, such as the formation of small bubbles beneath the membrane, may have influenced diffusion over time; therefore, the results should be interpreted with this consideration in mind.

In a final Franz cell analysis, water uptake was assessed at 0, 24, 48, and 72 h, with the acceptor medium replaced before each measurement. Both Nu-Gel™ and APOgel displayed similar fluid uptake from the donor chamber (Figure 12), reflecting their swelling properties, whereas the control showed no detectable uptake. This characteristic of the gels is considered therapeutically advantageous, as the gels can absorb wound exudate and maintain a moist environment conducive to wound healing. [60]

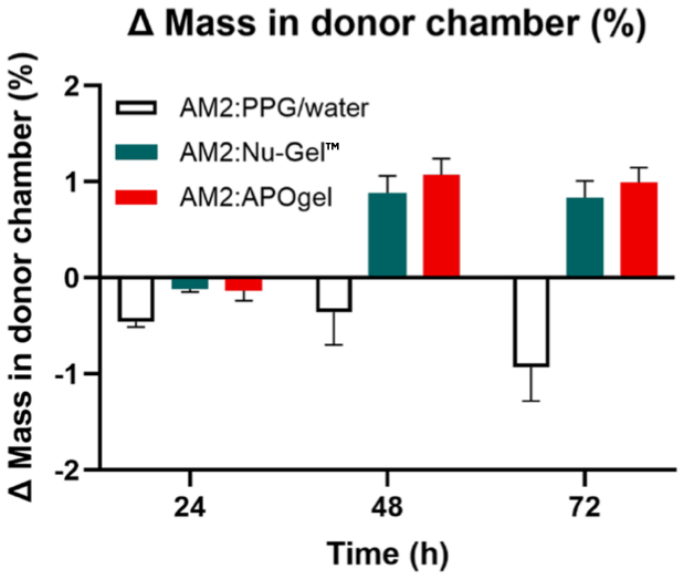

Figure 12: Change in sample mass within the donor chamber over 72 h relative to time point 0 h. Values represent the mean ± standard deviation of n=3 batches.

4 Conclusion

This thesis demonstrates the successful development of APOgel, a sterile hydrogel formulation designed as the delivery vehicle for the apoptotic secretome in phase III clinical trials. The formulation met the predefined critical quality attributes of viscosity, pH, and storage stability. Autoclaving caused predictable viscosity reduction, which was compensated for by formulation adjustments and gradual recovery during storage.

Evaluation of the point-of-care syringe-mixing system (APOSEC 2.0) confirmed its suitability for clinical use. Mixing and dosing uniformity met the specifications across different operator experience levels, and content distribution was largely homogeneous. *In vitro* release studies showed comparable behaviour to the reference gel Nu-Gel™ at early time points and revealed an enhanced release profile for APOgel under prolonged conditions, which may offer therapeutic benefits. Although the $K(r)$ values for the 72-h release, as well as the viscosity of APOSEC:APOgel mixtures, deviated slightly from their specifications compared to the Nu-Gel™ reference, this deviation was deemed non-detrimental to product performance; all other critical quality attributes were fulfilled, and overall system performance was consistent and reliable.

To build upon this robust physicochemical foundation, the critical next steps involve confirming the sterility and chemical stability of APOgel and validating the retention of biological activity after the APOSEC 2.0 mixing process.

Altogether, APOgel and APOSEC 2.0 provide a scalable and user-friendly drug delivery system, establishing a solid foundation for their implementation in upcoming phase III clinical trials and supporting the clinical translation of APOSEC as a novel therapy for chronic wounds.

List of figures

List of tables

References

[1] M. G. Monaghan, R. Borah, C. Thomsen, and S. Browne, 'Thou shall not heal: Overcoming the non-healing behaviour of diabetic foot ulcers by engineering the inflammatory microenvironment', *Advanced Drug Delivery Reviews*, vol. 203, p. 115120, Dec. 2023, doi: 10.1016/j.addr.2023.115120.

[2] D. Bormann, A. Gugerell, H. J. Ankersmit, and M. Mildner, 'Therapeutic Application of Cell Secretomes in Cutaneous Wound Healing', *Journal of Investigative Dermatology*, vol. 143, no. 6, pp. 893–912, June 2023, doi: 10.1016/j.jid.2023.02.019.

[3] L. Beer *et al.*, 'Analysis of the Secretome of Apoptotic Peripheral Blood Mononuclear Cells: Impact of Released Proteins and Exosomes for Tissue Regeneration', *Sci Rep*, vol. 5, no. 1, Art. no. 1, Nov. 2015, doi: 10.1038/srep16662.

[4] T. Wagner *et al.*, 'Different pro-angiogenic potential of γ-irradiated PBMC-derived secretome and its subfractions', *Sci Rep*, vol. 8, p. 18016, Dec. 2018, doi: 10.1038/s41598-018-36928-6.

[5] E. Simader *et al.*, 'Safety and tolerability of topically administered autologous, apoptotic PBMC secretome (APOSEC) in dermal wounds: a randomized Phase 1 trial (MARSYAS I)', *Scientific Reports*, vol. 7, p. 6216, July 2017, doi: 10.1038/s41598-017-06223-x.

[6] S. Wuschko *et al.*, 'Toxicological testing of allogeneic secretome derived from peripheral mononuclear cells (APOSEC): a novel cell-free therapeutic agent in skin disease', *Sci Rep*, vol. 9, no. 1, Art. no. 1, Apr. 2019, doi: 10.1038/s41598-019-42057-5.

[7] A. Gugerell *et al.*, 'Safety and clinical efficacy of the secretome of stressed peripheral blood mononuclear cells in patients with diabetic foot ulcer—study protocol of the randomized, placebo-controlled, double-blind, multicenter, international phase II clinical trial MARSYAS II', *Trials*, vol. 22, p. 10, Jan. 2021, doi: 10.1186/s13063-020-04948-1.

[8] J. C. Dumville, S. O'Meara, S. Deshpande, and K. Speak, 'Alginate dressings for healing diabetic foot ulcers', *Cochrane Database of Systematic Reviews*, vol. 2015, no. 3, June 2013, doi: 10.1002/14651858.CD009110.pub3.

[9] 'A Randomized Controlled Trial: Effects of Alginate Gel in the Treatment of Diabetic Foot Ulcers', *J Surg*, vol. 10, no. 6, May 2025, doi: 10.29011/2575-9760.011316.

[10] V. Vorstandlechner *et al.*, 'The Secretome of Irradiated Peripheral Mononuclear Cells Attenuates Hypertrophic Skin Scarring', *Pharmaceutics*, vol. 15, no. 4, p. 1065, Mar. 2023, doi: 10.3390/pharmaceutics15041065.

[11] S. Woerly, 'Restorative surgery of the central nervous system by means of tissue engineering using NeuroGel implants', 2000.

[12] Q. Lin, J. Y. C. Lim, K. Xue, X. Su, and X. J. Loh, 'Polymeric hydrogels as a vitreous replacement strategy in the eye', *Biomaterials*, vol. 268, p. 120547, Jan. 2021, doi: 10.1016/j.biomaterials.2020.120547.

[13] M. Goh, M. Du, and W. R. Peng, 'Advancing burn wound treatment: exploring hydrogel as a transdermal drug delivery system', 2024.

[14] E. M. Ahmed, 'Hydrogel: Preparation, characterization, and applications: A review', *J Adv Res*, vol. 6, no. 2, pp. 105–121, Mar. 2015, doi: 10.1016/j.jare.2013.07.006.

[15] W. L. Stoppel, J. C. White, S. D. Horava, A. C. Henry, S. C. Roberts, and S. R. Bhatia, 'Terminal sterilization of alginate hydrogels: Efficacy and impact on mechanical properties', *J Biomed Mater Res*, vol. 102, no. 4, pp. 877–884, May 2014, doi: 10.1002/jbm.b.33070.

[16] X. Wen, D. Bao, M. Chen, A. Zhang, C. Liu, and R. Sun, 'Preparation of CMC/HEC Crosslinked Hydrogels for Drug Delivery', *BioResources*, vol. 10, no. 4, pp. 8339–8351, Oct. 2015, doi: 10.15376/biores.10.4.8339-8351.

[17] J. M. Rosiak, P. Ulański, and A. Rzeźnicki, 'Hydrogels for biomedical purposes', *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*, vol. 105, no. 1–4, pp. 335–339, Nov. 1995, doi: 10.1016/0168-583x(95)00550-1.

[18]C. Owh *et al.*, 'Bottom-up design of hydrogels for programmable drug release', *Biomaterials Advances*, vol. 141, p. 213100, Oct. 2022, doi: 10.1016/j.bioadv.2022.213100.
[19]D. Klinger and K. Landfester, 'Stimuli-responsive microgels for the loading and release of functional compounds: Fundamental concepts and applications', *Polymer*, vol. 53, no. 23, pp. 5209–5231, Oct. 2012, doi: 10.1016/j.polymer.2012.08.053.
[20]P. I. Lee, 'KINETICS OF DRUG RELEASE FROM HYDROGEL MATRICES', 1985.
[21]P. Sim, X. L. Strudwick, Y. Song, A. J. Cowin, and S. Garg, 'Influence of Acidic pH on Wound Healing In Vivo: A Novel Perspective for Wound Treatment', *IJMS*, vol. 23, no. 21, p. 13655, Nov. 2022, doi: 10.3390/ijms232113655.
[22]I. Ferreira, A. C. Marques, P. C. Costa, and M. H. Amaral, 'Effects of Steam Sterilization on the Properties of Stimuli-Responsive Polymer-Based Hydrogels', *Gels*, vol. 9, no. 5, p. 385, May 2023, doi: 10.3390/gels9050385.
[23]C. S. A. Bento, M. C. Gaspar, P. Coimbra, H. C. De Sousa, and M. E. M. Braga, 'A review of conventional and emerging technologies for hydrogels sterilization', *International Journal of Pharmaceutics*, vol. 634, p. 122671, Mar. 2023, doi: 10.1016/j.ijpharm.2023.122671.
[24]R. Galante, T. J. A. Pinto, R. Colaço, and A. P. Serro, 'Sterilization of hydrogels for biomedical applications: A review', *J Biomed Mater Res*, vol. 106, no. 6, pp. 2472–2492, Aug. 2018, doi: 10.1002/jbm.b.34048.
[25]E. O. Bakhrushina *et al.*, 'Role of Sterilization on In Situ Gel-Forming Polymer Stability', *Polymers*, vol. 16, no. 20, p. 2943, Oct. 2024, doi: 10.3390/polym16202943.
[26]M. C. Beard *et al.*, 'Autoclaving of Poloxamer 407 hydrogel and its use as a drug delivery vehicle', *J Biomed Mater Res*, vol. 109, no. 3, pp. 338–347, Mar. 2021, doi: 10.1002/jbm.b.34703.
[27]Z. Dai, J. Ronholm, Y. Tian, B. Sethi, and X. Cao, 'Sterilization techniques for biodegradable scaffolds in tissue engineering applications', *J Tissue Eng*, vol. 7, p. 2041731416648810, Jan. 2016, doi: 10.1177/2041731416648810.
[28]S. Alipour, N. Negahban, F. Ahmadi, and E. Parhizkar, 'The Effects of Moist Heat Sterilization Process on Rheological Properties of Hydrophilic gels containing drug model', *Trends in Pharmaceutical Sciences*, vol. 8, no. 3, Sept. 2022, doi: 10.30476/tips.2022.94633.1138.
[29]T. W. Schwarz and G. Levy, 'Viscosity Changes of Sodium Alginate Solutions after Freezing and Thawing**University of California School of Pharmacy, San Francisco', *Journal of the American Pharmaceutical Association (Scientific ed.)*, vol. 46, no. 9, pp. 562–563, Sept. 1957, doi: 10.1002/jps.3030460914.
[30]A. Ghanbari, Z. Mousavi, M. Heuzey, G. S. Patience, and P. J. Carreau, 'Experimental methods in chemical engineering: Rheometry', *Can J Chem Eng*, vol. 98, no. 7, pp. 1456–1470, July 2020, doi: 10.1002/cjce.23749.
[31]H. A. Barnes, 'Thixotropy—a review', *Journal of Non-Newtonian Fluid Mechanics*, vol. 70, no. 1–2, pp. 1–33, May 1997, doi: 10.1016/S0377-0257(97)00004-9.
[32]G. Stojkov, Z. Niyazov, F. Picchioni, and R. K. Bose, 'Relationship between Structure and Rheology of Hydrogels for Various Applications', *Gels*, vol. 7, no. 4, p. 255, Dec. 2021, doi: 10.3390/gels7040255.
[33]H. Herrada-Manchón, M. A. Fernández, and E. Aguilar, 'Essential Guide to Hydrogel Rheology in Extrusion 3D Printing: How to Measure It and Why It Matters?', *Gels*, vol. 9, no. 7, p. 517, June 2023, doi: 10.3390/gels9070517.
[34]W. J. Addicks, G. L. Flynn, and N. Weiner, 'Validation of a Flow-Through Diffusion Cell for Use in Transdermal Research', *Pharm Res*, vol. 4, no. 4, pp. 337–341, Aug. 1987, doi: 10.1023/A:1016405506028.
[35]S.-F. Ng, J. J. Rouse, F. D. Sanderson, V. Meidan, and G. M. Eccleston, 'Validation of a Static Franz Diffusion Cell System for In Vitro Permeation Studies', *AAPS PharmSciTech*, vol. 11, no. 3, pp. 1432–1441, Sept. 2010, doi: 10.1208/s12249-010-9522-9.
[36]A. Haq, M. Dorrani, B. Goodyear, V. Joshi, and B. Michniak-Kohn, 'Membrane properties for permeability testing: Skin versus synthetic membranes', *International Journal of*

Pharmaceutics, vol. 539, no. 1–2, pp. 58–64, Mar. 2018, doi: 10.1016/j.ijpharm.2018.01.029.
[37]A. V. Fick, 'On Liquid Diffusion', *Philosophical Magazine*, vol. 10, pp. 30–39, 1855, doi: 10.1080/14786445508641925.
[38]Y. K. Agrawal and H. M. Tank, 'Enhanced Transmembrane Permeation by Molecular Modification', *Indian Journal of Pharmaceutical Sciences*, vol. 60, no. 4, pp. 222–224, 1998.
[39]J. Siepmann and F. Siepmann, 'Sink conditions do not guarantee the absence of saturation effects', *International Journal of Pharmaceutics*, vol. 577, p. 119009, Mar. 2020, doi: 10.1016/j.ijpharm.2019.119009.
[40]J. L. Knopp, L. Holder-Pearson, and J. G. Chase, 'Insulin Units and Conversion Factors: A Story of Truth, Boots, and Faster Half-Truths', *J Diabetes Sci Technol*, vol. 13, no. 3, pp. 597–600, Oct. 2018, doi: 10.1177/1932296818805074.
[41]K. Larmené-Beld *et al.*, 'Science- and risk-based strategy to qualify prefillable autoclavable syringes as primary packaging material', *Eur J Hosp Pharm*, vol. 29, no. 5, pp. 248–254, Sept. 2022, doi: 10.1136/ejhpharm-2020-002333.
[42]A. Gugerell *et al.*, 'Safety and clinical efficacy of the secretome of stressed peripheral blood mononuclear cells in patients with diabetic foot ulcer—study protocol of the randomized, placebo-controlled, double-blind, multicenter, international phase II clinical trial MARSYAS II', *Trials*, vol. 22, no. 1, p. 10, Jan. 2021, doi: 10.1186/s13063-020-04948-1.
[43]Hardy, Craig Julian and Findlay, Charlotte Maria, 'Sterile gel compositions for wound treatment', EP0693292A1, 01 1996
[44]C. De La Guardia, A. Virno, M. Musumeci, A. Bernardin, and M. B. Silberberg, 'Rheologic and Physicochemical Characteristics of Hyaluronic Acid Fillers: Overview and Relationship to Product Performance', *Facial Plast Surg*, vol. 38, no. 02, pp. 116–123, Apr. 2022, doi: 10.1055/s-0041-1741560.
[45]J. Abraham, 'International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use', in *Handbook of Transnational Economic Governance Regimes*, C. Tietje and A. Brouder, Eds, Brill | Nijhoff, 2010, pp. 1041–1053. doi: 10.1163/ej.9789004163300.i-1081.897.
[46]Thermo Fisher Scientific, 'Pierce™ Detergent Compatible Bradford Assay Kit User Guide', Thermo Fisher Scientific, MAN0016018, 2016. Accessed: May 27, 2025. [Online]. Available: https://assets.thermofisher.com/TFS-Assets/LSG/manuals/23246_23246S_deter_compat_bradford_UG.pdf
[47]T. J. Franz, 'Percutaneous Absorption. On the Relevance of in Vitro Data', *Journal of Investigative Dermatology*, vol. 64, no. 3, pp. 190–195, Mar. 1975, doi: 10.1111/1523-1747.ep12533356.
[48]D. L. Bernik, D. Zubiri, M. E. Monge, and R. M. Negri, 'New kinetic model of drug release from swollen gels under non-sink conditions', *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, vol. 273, no. 1, pp. 165–173, Feb. 2006, doi: 10.1016/j.colsurfa.2005.08.018.
[49]L. Coderch, I. Collini, V. Carrer, C. Barba, and C. Alonso, 'Assessment of Finite and Infinite Dose In Vitro Experiments in Transdermal Drug Delivery', *Pharmaceutics*, vol. 13, no. 3, p. 364, Mar. 2021, doi: 10.3390/pharmaceutics13030364.
[50]European Medicines Agency, 'Guideline on quality and equivalence of locally applied locally acting cutaneous products', EMA/CHMP/703337/2018, 2018. Accessed: Aug. 10, 2025. [Online]. Available: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-quality-equivalence-locally-applied-locally-acting-cutaneous-products_en.pdf
[51]L. A. Dailey, 'Pharmaceutical quality by design in academic nanomedicine research: stifling innovation or creativity through constraint?', *Journal of Interdisciplinary Nanomedicine*, vol. 3, no. 4, pp. 175–182, 2018, doi: 10.1002/jin2.52.
[52]G. Troiano, J. Nolan, D. Parsons, C. Van Geen Hoven, and S. Zale, 'A Quality by Design Approach to Developing and Manufacturing Polymeric Nanoparticle Drug Products', *AAPS J*, vol. 18, no. 6, pp. 1354–1365, Nov. 2016, doi: 10.1208/s12248-016-9969-z.

[53]J. E. Rees and P. J. Rue, ‘Interbatch Variation in Compression Properties of Elcema G250’, *Journal of Pharmacy and Pharmacology*, vol. 30, no. Supplement_1, pp. 25P-25P, Dec. 1978, doi: 10.1111/j.2042-7158.1978.tb10732.x.
[54]V. S. Dave, S. D. Saoji, N. A. Raut, and R. V. Haware, ‘Excipient Variability and Its Impact on Dosage Form Functionality’, *Journal of Pharmaceutical Sciences*, vol. 104, no. 3, pp. 906–915, Mar. 2015, doi: 10.1002/jps.24299.
[55]K. Inoue, Y. X. Song, O. KamiunteN, J. Oku, T. Terao, and K. FujiI, ‘Effect of mixing method on rheological properties of alginate impression materials’, *J of Oral Rehabilitation*, vol. 29, no. 7, pp. 615–619, July 2002, doi: 10.1046/j.1365-2842.2002.00726.x.
[56]J. Li, T. Shelby, H. V. Alizadeh, H. Shelby, and Y. P. Yang, ‘Development and characterization of an automated active mixing platform for hydrogel bioink preparation’, *IJB*, vol. 9, no. 4, p. 705, Aug. 2024, doi: 10.18063/ijb.705.
[57]S. Uman, A. Dhand, and J. A. Burdick, ‘Recent advances in shear-thinning and self-healing hydrogels for biomedical applications’, *J of Applied Polymer Sci*, vol. 137, no. 25, p. 48668, July 2020, doi: 10.1002/app.48668.
[58]S. Dani *et al.*, ‘Homogeneous and Reproducible Mixing of Highly Viscous Biomaterial Inks and Cell Suspensions to Create Bioinks’, *Gels*, vol. 7, no. 4, p. 227, Nov. 2021, doi: 10.3390/gels7040227.
[59]L. Xing, Y. Li, and T. Li, ‘Local Concentrating, Not Shear Stress, That May Lead to Possible Instability of Protein Molecules During Syringe Injection: A Fluid Dynamic Study with Two-Phase Flow Model’, *PDA Journal of Pharmaceutical Science and Technology*, vol. 73, no. 3, pp. 260–275, 2019, doi: 10.5731/pdajpst.2018.009357.
[60]S. Tavakoli and A. S. Klar, ‘Advanced Hydrogels as Wound Dressings’, *Biomolecules*, vol. 10, no. 8, p. 1169, Aug. 2020, doi: 10.3390/biom10081169.