Biocompatibility assessment of a novel atelocollagen formulation developed for skin and wound care management in oncology patients

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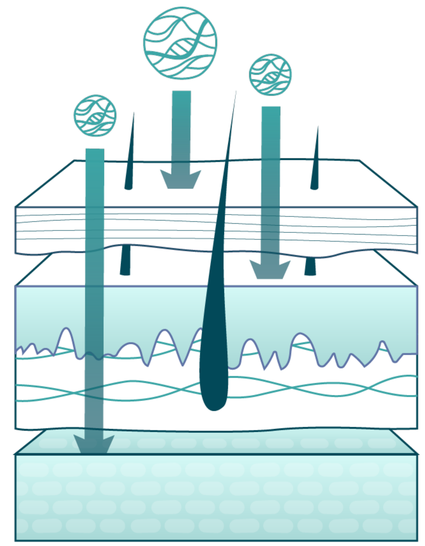


AIM

Standard-of-care treatments for cancer (radiotherapy or chemotherapy) are regularly accompanied by significant dermatological complications. However, the tools available for skin care management today are extremely limited and often ineffective.

To address this unmet need, a unique atelocollagen complex was developed, containing three distinct molecular weight ranges intended to penetrate deep into the skin. The present study aimed to evaluate the biocompatibility and efficacy of this novel formulation.

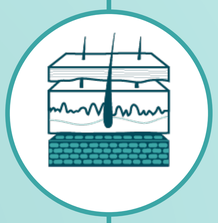
METHOD

Novel collagen synthesis

Triple-helix atelocollagen (CollX^3) was developed through a series of modifications resulting in a protein complex of three distinct MW.

In vitro biocompatibility

In vitro cell viability assessment in a range of human skin cell lines.

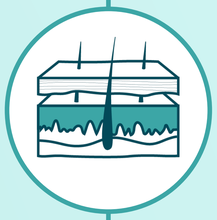


3D-skin assays

In vitro skin irritation test of CollX^3 in reconstructed human skin epidermis and corneal epithelium.

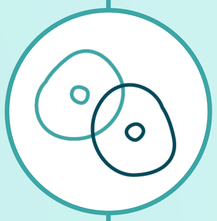
Tumorigenicity

Tumorigenicity safety assessment on colorectal and ovarian cancer cells.



Penetration assessment

Ex vivo penetration assessment on human skin epidermis and dermis.



Assessment of regeneration markers

Expression levels of COL1 and ELN validated via qPCR in dermal human fibroblasts.

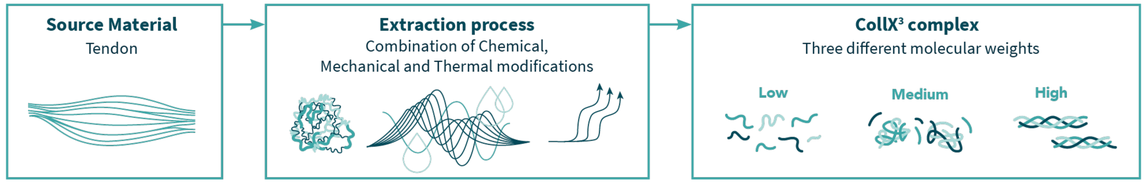
Wound healing

In vitro and *ex vivo* wound healing assessment on human epidermis and dermis tissues.

CHARACTERIZATION OF NOVEL ATELOCOLLAGEN COMPLEX

Unique Method of Production

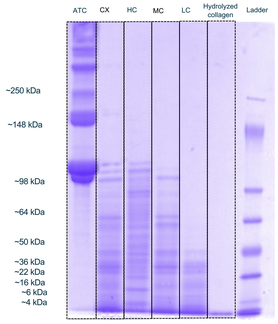
The protein complex is created through a series of chemical, thermal, and mechanical modifications that yield collagen of three distinct molecular weights, with reduced antigenicity and enhanced biocompatibility.

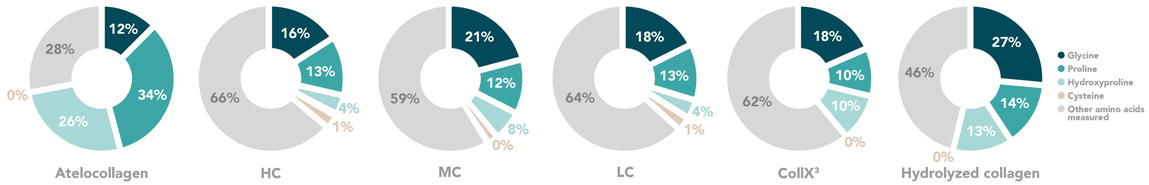


Comprehensive protein analysis profile

The **amino acid profile** of CollX³ closely resembles the natural collagen molecule with significant quantities of glycine which is the most abundant single amino acid in collagen. Moreover, CollX³ is characterized by high concentration of Hydroxyproline, Alanine, Glutamic acid, Proline and Hydroxyl residues. It is worth mentioning that CollX³ contains negligible amounts of Cysteine.

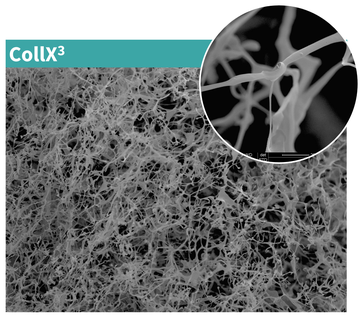
SDS-PAGE protein analysis of CollX³ confirms the presence of three distinctive ranges of molecular weight, characteristic of collagen. CollX³'s amino acid profile provides the essential building blocks for collagen synthesis, supporting the health and integrity of the skin.

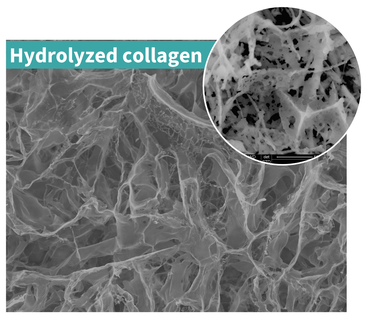


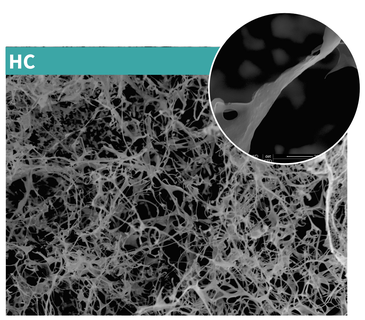


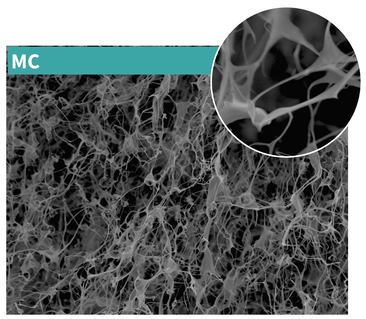
Microstructural Characteristics

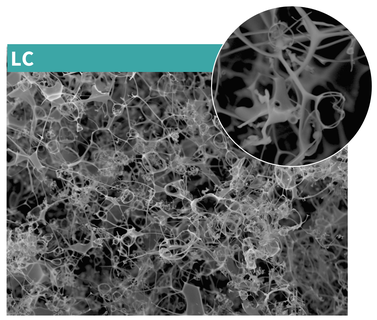
Compared to the widely employed hydrolyzed collagen peptides, CollX³ retains the intact fibrillar and porous architecture of atelocollagen. This distinctive characteristic of CollX³ confers enhanced integration withing the cellular and tissue microenvironment.







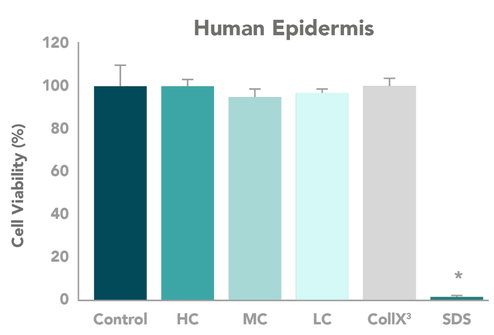


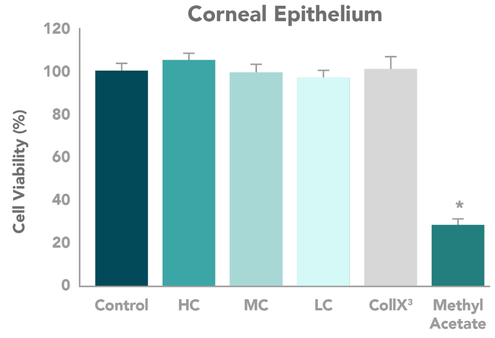


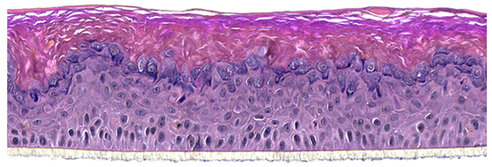
BIOCOMPATIBILITY ASSESSMENT

3D-skin irritation assays

3D skin models have emerged as a promising alternative to *in vivo* testing on animals for cosmeceuticals products, providing a reliable and ethical solution in the post-animal testing era. Here, skin and eye irritation potential was measured using a reconstructed human epidermis (SkinEthic™ RhE) and reconstructed corneal epithelium (SkinEthic™ HCE) following the Organization for Economic Co-operation and Development (OECD) Test Guidelines 439 and 492 respectively. CollX³ was classified as non-irritant after exposure to 3D-skin and corneal models according to OECD Test Guidelines 492.







SkinEthic RHE / Human Epidermis

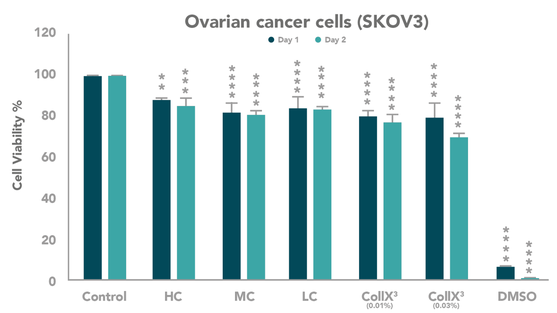
Reconstructed Human Epidermis (SkinEthic™ RHE) from normal human keratocytes was purchased from Episkin Ltd (France). The reconstructed model is similar to the in vivo human epidermis.

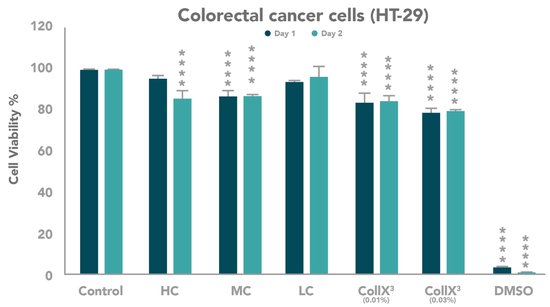
SkinEthic HCE / Corneal Epithelium

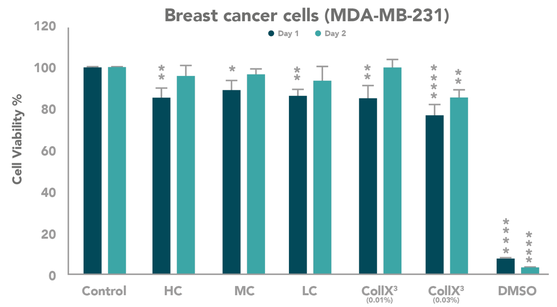
Reconstructed Human Corneal Epithelium (SkinEthic™ HCE) is composed from human corneal keratocytes and was purchased from Episkin Ltd (France). The reconstructed model is similar to the human cornea with presence of basal, wing and mucus production cells.

Tumorigenicity

Potential tumorigenicity of CollX³ and its constituents was assessed *in vitro* via MTT assay on three different cancer cell lines: Ovarian cancer cells (SKOV3), Breast cancer cells (MDA-MB-231) and Colorectal cancer cells (HT-29).The tumorigenicity assessment revealed that CollX³ has anti-proliferative activity against cancer cells. CollX³ (0.03%) showed the most potent anti-proliferative effect against ovarian and colorectal cancer cells.

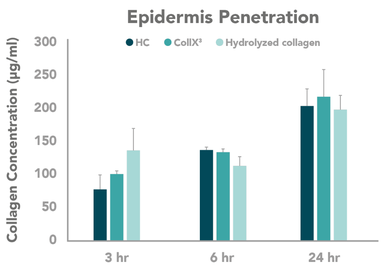


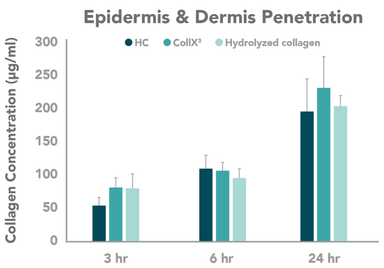




Skin penetration assessment

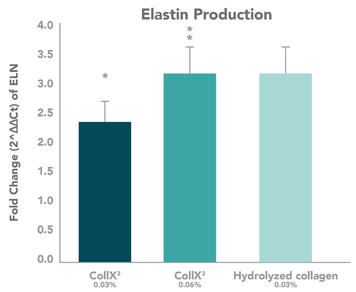
Ex vivo human skin permeation experiment was utilized to evaluate skin permeability properties of CollX³. The innovative CollX³ formula showed increased permeation through single (Epidermis) and double (epidermis & dermis) skin layer compared to hydrolyzed collagen.

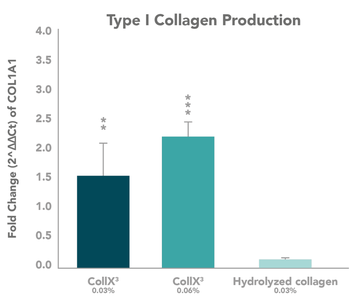




Assessment of wound regeneration markers

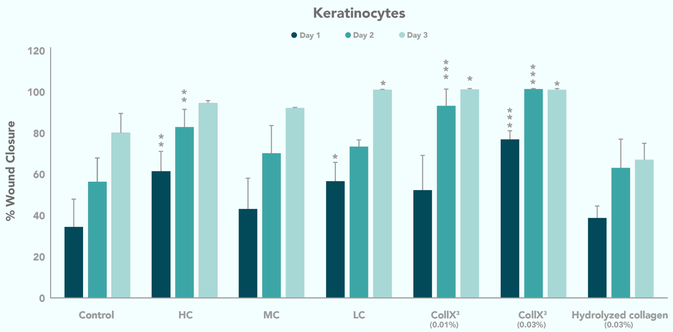
CollX³ significantly promoted the synthesis of Type I collagen (helps to strengthen the tissue and promote wound closure) and Elastin (provides elasticity and resilience to the tissue and helps to restore its normal function) in dermal fibroblast human cells enhancing skin elasticity and healing properties.

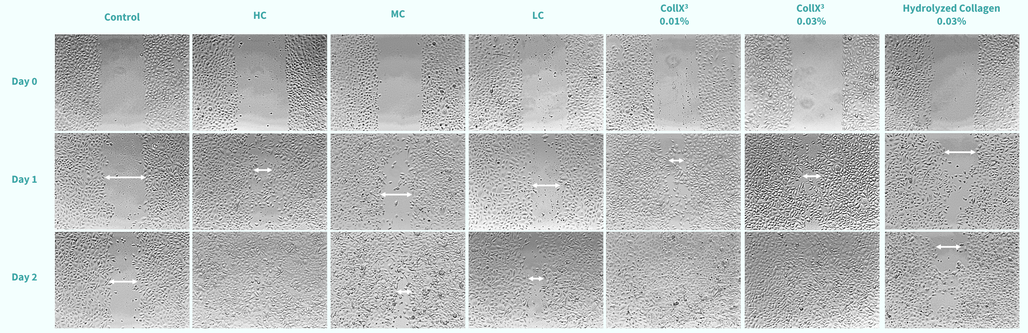




In vitro wound healing assessment

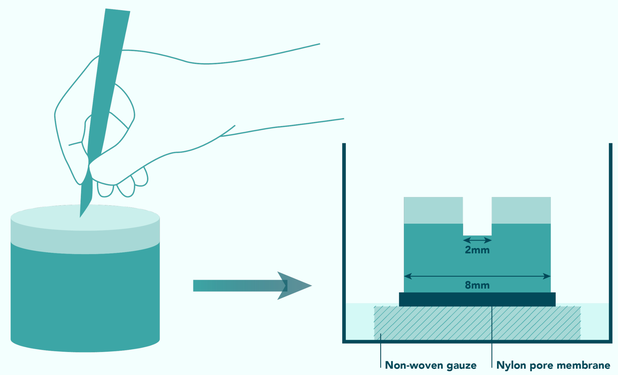
Wound healing properties of CollX³ were assessed *in vitro* via a cell scratch assay. CollX³ and its constituents (HC, MC and LC) significantly accelerated wound closure compared to negative control and hydrolyzed collagen peptides. Complete closure of the wound was observed by day 2 upon treatment with CollX³ (0.03%) with a significantly increased wound healing response compared to the other treatments.



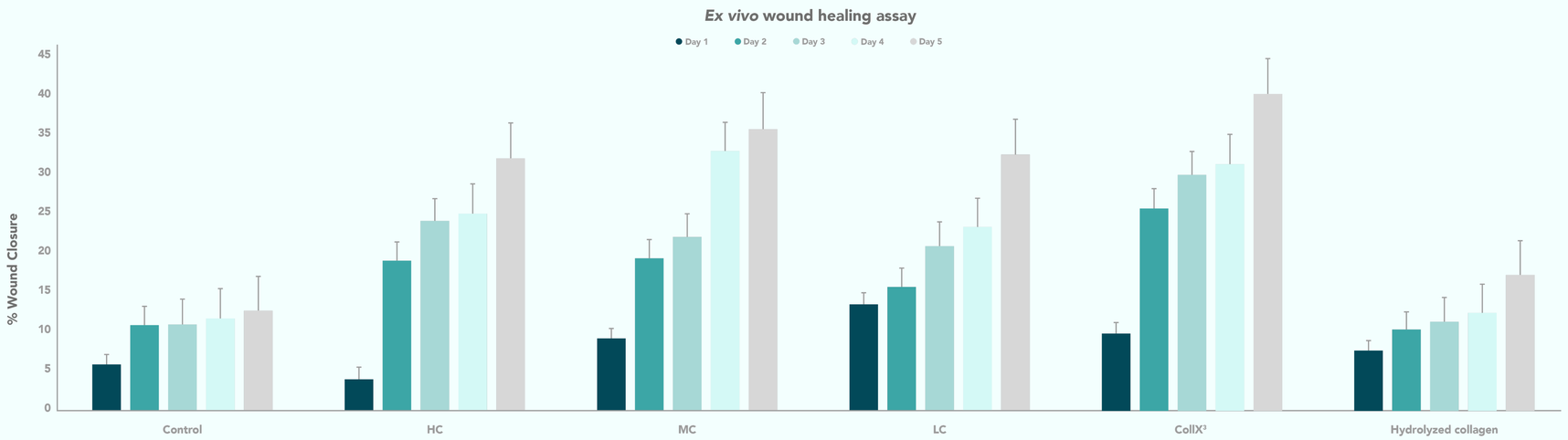


Ex vivo wound healing assessment

Wound healing efficacy of novel CollX³ formulation was evaluated in an *ex vivo* skin wound model. Treatment with CollX³ accelerated wound healing dynamics compared to control and hydrolyzed collagen. The observed acceleration confirms the efficacy of CollX³ for tissue regeneration. In this study, full thickness 8 mm skin biopsies were wounded in the centre with 4 mm biopsies, and then introduced into well chamber inserts and maintained with supplemented growth media. Various collagen treatments, including CollX3, HC, MC, LC, and hydrolyzed collagen, were topically applied to the wound. Images were captured every 24 hrs for 5 days and the percentage of wound closure was calculated.



	Control	HC	MC	LC	CollX³	Hydrolyzed collagen
Day 0						
Day 5						

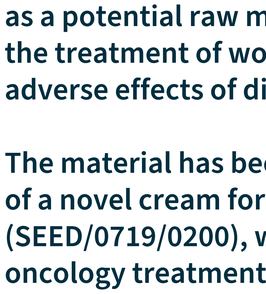


Discussion

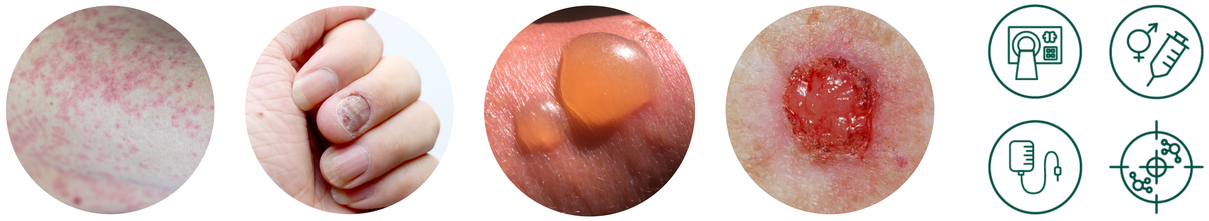
This CollX^3 is a novel atelocollagen biomaterial that has been developed as a potential raw material for various dermaceutical products to aid in the treatment of wounds. Its primary objective is to alleviate the adverse effects of different cancer treatments on the skin.

The material has been used as an active ingredient in the formulation of a novel cream formulation subject to the Mediskin research program (SEED/0719/0200), which is intended for use in patients undergoing oncology treatment and experiencing side effects during radiotherapy and chemotherapy. Preliminary results suggest that Mediskin cream promotes wound healing.

However, further investigation, including clinical trials, is needed to determine the full potential of CollX^3 in healing wounds in humans. A multicenter clinical trial is currently recruiting participants in Cyprus and Greece (NCT05588973) to study the effectiveness of CollX^3 in wound healing.

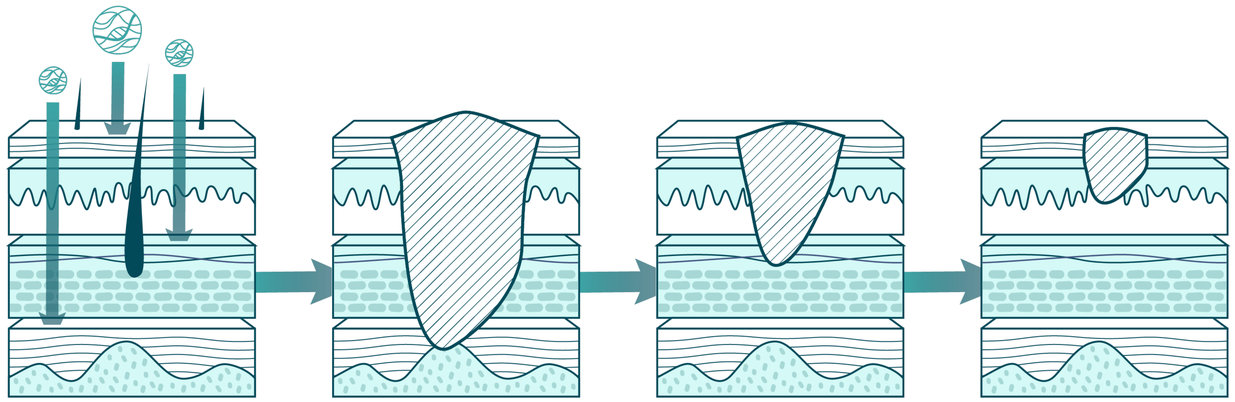


Conclusion



In conclusion, the findings of this study suggest that the novel atelocollagen complex is a safe and effective solution for skin care and wound. The results of the wound healing assay and the assessment using reconstructed skin models demonstrate the ability of the complex to accelerate wound closure while confirming its safety. The validation of COL1 and ELN expression levels through qPCR provides further evidence of the biocompatibility and efficacy of the complex.

Overall, this research offers a promising option for the treatment of skin wounds in individuals who are immunocompromised due to cancer, highlighting the potential for continued development and use of atelocollagen complex in the field of medical skincare.



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