Research Article

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In Vitro Evaluation of a Collagen–Chitosan Wound Dressing (COLSORB®) as a Bilayer Scaffold for Human Skin Cell Co-Culture

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ABSTRACT

Background: Collagen–chitosan dressings are widely used to promote cutaneous wound repair, yet their ability to support coordinated dermal–epidermal cell organization under prolonged culture stress has been less explored.

Objective: To assess cell adhesion, migration and spatial organization of primary human dermal fibroblasts and keratinocytes cultured on a collagen–chitosan sheet (COLSORB®, Medbiotec Inc., Türkiye) in a bilayer configuration mimicking human skin.

Methods: Primary fibroblasts and keratinocytes were isolated from human skin obtained under institutional ethics approval. Fibroblasts were first expanded and seeded on one side of COLSORB sheets and cultured for 10 days. Keratinocytes were then seeded on the opposite surface using an insert system and co-cultured for an additional 20 days. Constructs were processed for paraffin embedding, hematoxylin–eosin (H&E) staining and immunohistochemistry (IHC) for keratinocyte cytokeratins.

Results: COLSORB maintained structural integrity throughout 30 days of co-culture. H&E sections showed abundant spindle-shaped cells distributed within and along the collagen–chitosan matrix, consistent with fibroblast colonization. Cytokeratin IHC demonstrated strongly positive epithelial cell clusters lining the scaffold surface and extending along collagen strands, confirming keratinocyte engraftment. Areas of multi-layered keratinocyte growth overlying fibroblast-populated regions recapitulated an early dermal–epidermal organization.

Conclusion: Under prolonged co-culture conditions, the COLSORB collagen–chitosan dressing supports attachment and migration of human dermal fibroblasts and keratinocytes and allows partial reconstruction of a bilayered skin-like structure. COLSORB may serve not only as a wound dressing but also as a simple, clinically relevant scaffold for human skin equivalent models.

Keywords: Collagen–Chitosan Scaffold; COLSORB; Fibroblast; Keratinocyte; Co-Culture; Skin Equivalent; Wound Dressing

Abbreviations: H&E: Hematoxylin–Eosin; IHC: Immunohistochemistry; PBS: Phosphate-Buffered Saline; FBS: Fetal Bovine Serum

Introduction

Full-thickness skin loss caused by trauma, burns or chronic ulcers remains a major clinical problem associated with infection, scarring, functional impairment and high treatment costs. Conventional wound management often fails to restore normal skin architecture, particularly in complex or chronic wounds. Tissue-engineered skin substitutes and advanced wound dressings aim to provide temporary coverage while also modulating inflammation and guiding regeneration of the dermal and epidermal compartments. Type I collagen is the principal structural component of dermal extracellular matrix and has long been used in wound dressings and scaffolds because of its biocompatibility, biodegradability and ability to support dermal fibroblast attachment and migration. Chitosan, a partially deacetylated derivative of chitin, adds hemostatic, antimicrobial and immunomodulatory properties and has been reported to enhance fibroblast and keratinocyte proliferation and collagen deposition [1]. Collagen–chitosan composite matrices therefore represent attractive biomaterials for wound healing and skin tissue engineering, and a number of experimental and clinical studies have demonstrated accelerated wound closure and improved quality of repair with such dressings. Beyond their use as passive dressings, collagen–chitosan scaffolds can be colonized with dermal fibroblasts and epidermal keratinocytes to create living skin equivalents.

Co-culture of these cell types on bilayer scaffolds supports keratinocyte stratification and extracellular matrix synthesis and offers platforms for autologous or allogeneic grafts and for in vitro testing of biomaterials and drugs [2]. However, many reported scaffolds are laboratory-fabricated prototypes that may not be directly translatable to routine clinical use. COLSORB® (Medbiotec Inc., Türkiye) is a clinically used wound dressing composed of 99% collagen and 1% chitosan, designed to enhance granulation and epithelialization in acute and chronic wounds. While its clinical application focuses on coverage and modulation of the wound bed, its performance as a three-dimensional scaffold for coordinated dermal–epidermal cell growth under long-term culture conditions has not been characterized. The aim of this pilot study was to evaluate whether COLSORB can serve as a bilayer scaffold for primary human dermal fibroblasts and keratinocytes in vitro. We established a simple co-culture model in which fibroblasts were first allowed to colonize the scaffold, followed by seeding of keratinocytes on the opposite surface using an insert-based system that approximates the organization of dermis and epidermis. Cell attachment, distribution and morphology after a total of 30 days of culture were examined by H&E staining and keratinocyte-specific immunohistochemistry.

Materials and Methods

Study Design and Ethics

Primary human skin cells were obtained from surplus skin tissue collected under an existing protocol approved by the Ethics Committee of Selçuk University. The current work used anonymized cells derived from that project with permission of the principal investigator. All procedures complied with the Declaration of Helsinki and institutional regulations.

Collagen–Chitosan Dressing (COLSORB)

COLSORB® sheets (Medbiotec Inc./Corp., Türkiye) consist of a lyophilized porous matrix containing 99% type I collagen and 1% chitosan, supplied terminally sterilized for clinical use as a wound dressing. Rectangular sheets were aseptically cut into circular discs sized to fit standard 24-well culture plates (approximately 15 mm in diameter). Discs were pre-wetted in sterile phosphate-buffered saline (PBS) and equilibrated for 30 minutes in fibroblast culture medium before cell seeding (Figure 1).

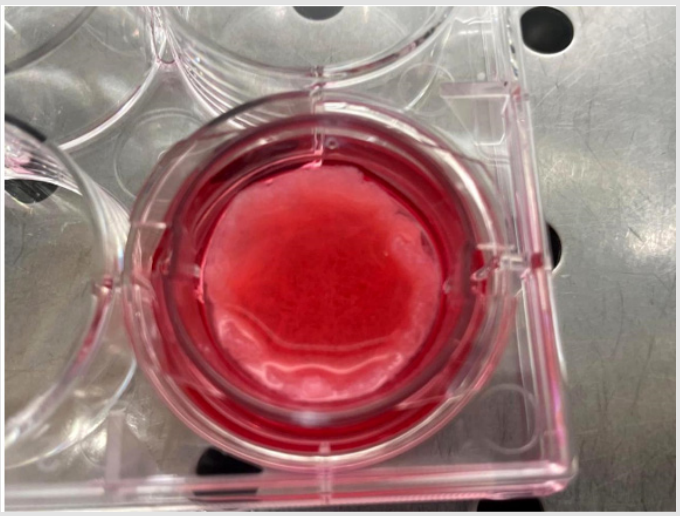

Figure 1: Macroscopic view of COLSORB collagen–chitosan sheet after 30 days of co-culture with fibroblasts and keratinocytes, showing preserved integrity in the culture well.

Cell Isolation and Primary Culture

Dermal fibroblasts and epidermal keratinocytes were isolated from human skin using standard enzymatic separation. Briefly, skin was rinsed in PBS containing antibiotics, subcutaneous fat was removed, and specimens were incubated overnight at 4°C in dispase solution to separate epidermis from dermis. The epidermis was digested with trypsin to obtain a keratinocyte suspension, whereas the dermis was minced and digested with collagenase to release fibroblasts (Figure 2). Cells were expanded in monolayer culture. Fibroblasts were maintained in Dulbecco's modified Eagle medium (DMEM, high glucose) supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine and 1% penicillin–streptomycin at 37°C in a humidified atmosphere with 5% CO_2. Keratinocytes were cultured in a defined keratinocyte growth medium containing low calcium, growth supplements and antibiotics under the same incubation conditions. Subculture was performed at 70–80% confluence using 0.05% trypsin–EDTA, and early-passage cells were used for scaffold seeding.

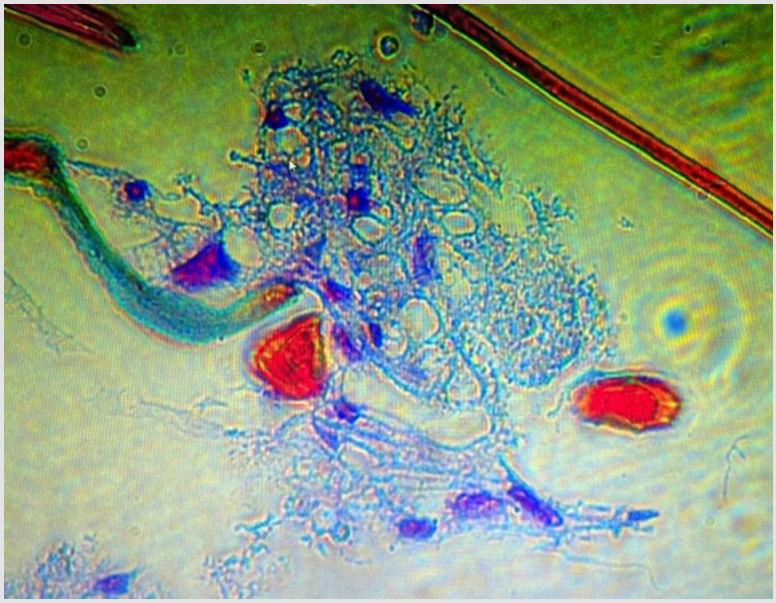

Figure 2: Hematoxylin–eosin staining of COLSORB section after 30-day culture demonstrating spindle-shaped fibroblast-like cells infiltrating the collagen–chitosan matrix.

Fibroblast Seeding on COLSORB

Pre-equilibrated COLSORB discs were placed in individual wells of 24-well plates. Fibroblasts were resuspended in fibroblast medium and seeded onto the upper surface of each disc at a density appropriate for three-dimensional culture. Plates were incubated for 2 hours to allow initial attachment before adding sufficient medium to fully cover the constructs. Fibroblast–COLSORB constructs were cultured for 10 days with medium changes every 2 days using DMEM supplemented with 10% FBS.

Keratinocyte Seeding and Co-Culture

After 10 days, COLSORB discs populated with fibroblasts were transferred into cell-culture inserts compatible with 24-well plates (pore size 0.4 μm). Keratinocytes were harvested, resuspended in keratinocyte growth medium and seeded onto the opposite surface of the scaffold at an appropriate density. Following a 2-hour attachment period, additional keratinocyte medium was added into the insert, while fibroblast medium or a 1:1 mixture of fibroblast and keratinocyte media was placed in the lower compartment (Figure 3). Co-cultures were maintained for 20 days with medium changes every 3 days. Constructs were inspected macroscopically throughout the culture period to monitor scaffold integrity.

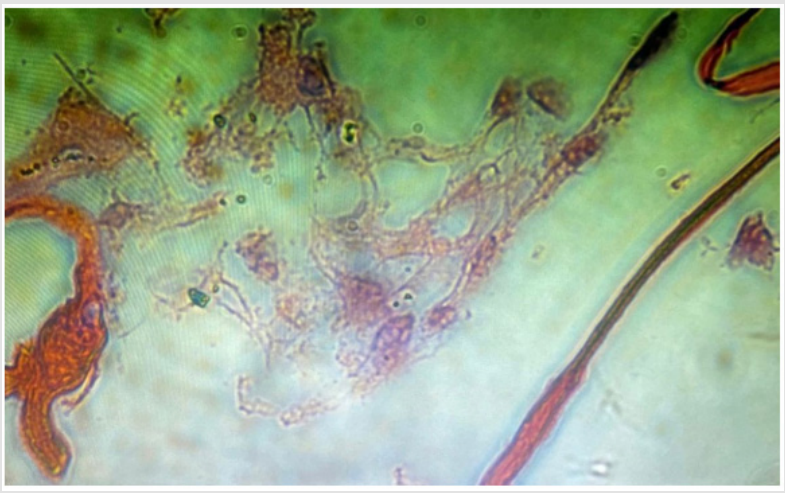

Figure 3: Cytokeratin immunohistochemistry highlighting keratinocyte-positive epithelial cells attached to the scaffold surface and extending along collagen strands.

Histology and Immunohistochemistry

At the end of the 30-day culture period, COLSORB constructs were washed with PBS and fixed in 10% neutral buffered formalin for at least 24 hours. Fixed samples were processed routinely, embedded in paraffin and sectioned at 4–5 μm thickness. For general morphology, sections were stained with hematoxylin–eosin (H&E). For identification of keratinocytes, adjacent sections were subjected to immunohistochemistry using a pan-cytokeratin antibody routinely employed in the pathology laboratory. Following deparaffinization and rehydration, antigen retrieval was performed in citrate buffer (pH 6.0) by heat-induced epitope retrieval. Endogenous peroxidase activity was blocked with hydrogen peroxide, and nonspecific binding was prevented by appropriate blocking solutions. Sections were incubated with the primary antibody at the manufacturer-recommended dilution, followed by a horseradish peroxidase-conjugated detection system and visualization with diaminobenzidine (Figure 4). Slides were counterstained with hematoxylin, dehydrated and mounted.

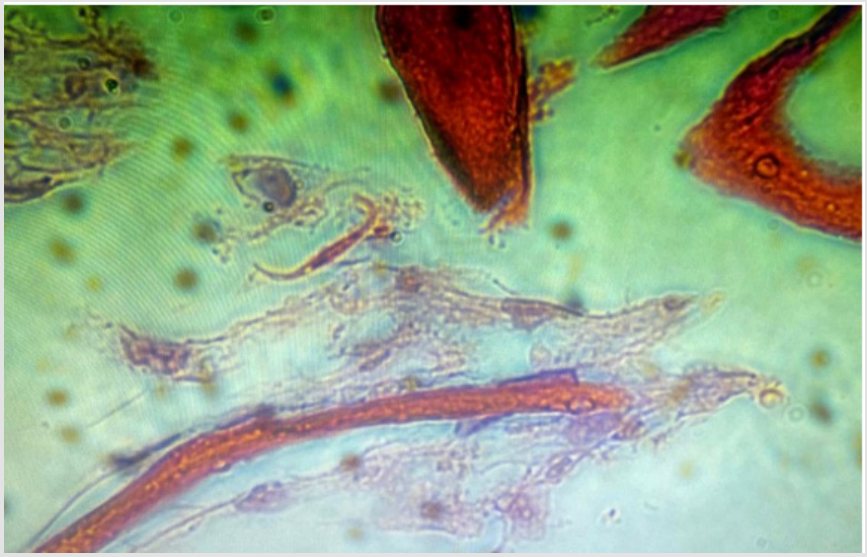

Figure 4: Higher-magnification view of keratinocyte clusters forming multi-layered epithelial-like structures on the COLSORB surface overlying fibroblast-populated regions.

Microscopic Evaluation

Slides were examined using bright-field microscopy at low and high magnification. Qualitative assessment focused on scaffold integrity and porosity, presence and morphology of fibroblast-like cells within the matrix, presence and organization of keratinocyte-like cells along scaffold surfaces, and the pattern of cytokeratin immunoreactivity. Representative images were captured for documentation.

Results

Hydrated COLSORB discs equilibrated rapidly in culture medium and maintained their shape and consistency throughout the 30-day co-culture period. No macroscopic evidence of disintegration, severe contraction or surface peeling was observed, and the scaffolds remained sufficiently coherent to withstand transfer for histological processing. H&E-stained sections revealed a porous collagenous matrix with multiple cell nuclei distributed along and within the scaffold. Cells showed elongated, spindle-shaped morphology aligned with collagen fibers, compatible with dermal fibroblasts. Many cells were located not only on the surface but also in deeper regions of the matrix, indicating active migration and infiltration during the initial 10-day culture period. Immunohistochemistry for cytokeratin highlighted discrete to confluent clusters of epithelial cells at the scaffold interface. Keratinocyte-positive cells formed continuous layers on parts of the COLSORB surface and extended along collagen strands, suggesting robust attachment. In several regions, two to three superimposed cell layers were visible, reminiscent of an early stratified epidermis rather than a single monolayer. Underlying fibroblast-populated zones were visible beneath these keratinocyte-rich regions, mimicking a dermal–epidermal arrangement.

Although specific viability assays were not performed, cellular morphology suggested preserved viability: nuclei appeared intact without widespread pyknosis or karyorrhexis, and the cytoplasm of both fibroblast-like and keratinocyte-like cells was well preserved. There was no histological evidence of a pronounced foreign-body reaction or large areas of necrosis within the scaffold.

Discussion

This pilot study demonstrates that a clinically used collagen–chitosan wound dressing can support co-culture of primary human dermal fibroblasts and keratinocytes and can partially reproduce the bilayer organization of human skin in vitro. After 30 days of culture, fibroblasts had migrated into the interior of COLSORB, while cytokeratin-positive keratinocytes formed epithelial layers on the scaffold surface, indicating effective cell attachment and survival under relatively demanding culture conditions. The experimental approach mirrors established protocols for human skin equivalents, in which a fibroblast-populated dermal matrix is first generated and subsequently overlaid with keratinocytes to form the epidermis. Previous studies using collagen- or collagen–chitosan-based scaffolds have shown that such co-culture strategies promote keratinocyte stratification and extracellular matrix synthesis and result in organotypic constructs with histological similarity to native skin [3-9]. The present work extends those findings to a commercially available collagen–chitosan dressing already used in patients, which may simplify translation of skin-equivalent technologies into clinical practice. The macroscopic stability of COLSORB over 30 days of culture is encouraging, as some collagen-based scaffolds undergo rapid contraction or enzymatic degradation that complicates handling. The small proportion of chitosan in COLSORB may contribute to improved mechanical stability and controlled biodegradation, while also conferring hemostatic and antimicrobial properties that are advantageous in the wound environment.

The observed infiltration of fibroblast-like cells throughout the scaffold is consistent with previous reports that collagen–chitosan matrices support fibroblast proliferation, migration and matrix deposition. The formation of multi-layered cytokeratin-positive cell clusters at the scaffold surface suggests that the combination of a fibroblast-populated dermal compartment and the collagen–chitosan matrix provides a permissive microenvironment for early epidermal organization. In vivo, dermal fibroblasts and epidermal keratinocytes engage in reciprocal paracrine signaling to coordinate wound closure and remodeling. A clinically approved collagen–chitosan dressing capable of hosting both cell types may therefore serve as a platform not only for coverage but also for delivery of autologous or allogeneic cells in difficult-to-heal wounds. Several limitations of this study should be acknowledged. The analysis was primarily qualitative and based on a limited number of constructs; no quantitative assessments of cell proliferation, extracellular matrix deposition or barrier function were performed. The distribution of fibroblasts versus keratinocytes was inferred from morphology and cytokeratin staining; inclusion of additional markers such as vimentin, Ki-67 or differentiation markers (e.g. involucrin, specific cytokeratin subtypes) would provide a more detailed picture of tissue organization.

Furthermore, we did not compare COLSORB with other scaffolds or with cultures lacking a scaffold, so relative performance cannot be determined. Finally, the in vitro environment does not fully recapitulate the complexity of an in vivo wound bed, including vascularization, immune cells and mechanical forces. Future work should therefore focus on quantitative image analysis and functional assays, such as metabolic activity tests, gene expression profiling and assessment of epidermal barrier formation. Comparative studies with other clinically available dressings and with advanced full-thickness skin models will help position COLSORB within the existing spectrum of skin-engineering strategies. Ultimately, ex vivo wound models or animal studies using COLSORB loaded with autologous cells may clarify whether the in vitro organization observed here translates into improved healing outcomes in vivo.

Conclusion

The COLSORB collagen–chitosan wound dressing functioned as a supportive three-dimensional matrix for human dermal fibroblasts and keratinocytes in a bilayer co-culture system. Under prolonged culture stress, the scaffold maintained structural integrity and permitted fibroblast infiltration and keratinocyte attachment with partial formation of an epidermis-like layer over a fibroblast-populated dermal compartment. These findings suggest that COLSORB may have utility not only as a passive wound dressing but also as a simple, clinically relevant scaffold for the development of human skin equivalents and cell-based wound therapies.

Acknowledgement

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Conflict of Interest

The authors declare that they have no competing financial or non-financial interests. Although the study was funded by Medbiotec Inc./Corp., none of the authors hold shares in or receive personal payments from the company.

Ethical Statement

Human skin tissues used for derivation of primary fibroblast and keratinocyte cultures were obtained under the approval of the Selçuk University Ethics Committee as part of a separate study. All samples were de-identified before use in the present work, and no additional interventions were performed on donors.

References

1. Rodríguez Vázquez M, Vega Ruiz B, Ramos Zúñiga R, Saldaña Koppel DA, Quiñones Olvera LF (2015) Chitosan and its potential use as a scaffold for tissue engineering in regenerative medicine. BioMed Research International 2015: 821279.
2. Shariati SRP, Shokrgozar MA, Vossoughi M, Eslamifar A (2009) *In vitro* co-culture of human skin keratinocytes and fibroblasts on a biocompatible and biodegradable scaffold. Iranian Biomedical Journal 13(3): 169-177.
3. Iyer K, Chen Z, Ganapa TR, Wu BM, Tawil B (2018) Keratinocyte migration in a three-dimensional *in vitro* wound healing model co-cultured with fibroblasts. Tissue Engineering and Regenerative Medicine 15(6): 721-733.
4. Russo B, Brembilla NC, Chizzolini C (2020) Interplay between keratinocytes and fibroblasts: a systematic review providing a new angle for understanding skin fibrotic disorders. Frontiers in Immunology 11: 648.
5. Nayak S, Dey S, Kundu SC (2013) Skin equivalent tissue-engineered construct: co-cultured fibroblasts/keratinocytes on 3D matrices of sericin hope cocoons. PLoS One 8(9): e74779.
6. Szymański Ł, et al. (2020) A simple method for the production of human skin equivalents *in vitro*. Cells 9(6): 1433.
7. Moon S, Dong Hyun Kim, Jung U Shin (2021) *In vitro* models mimicking immune response in the skin. Yonsei Medical Journal 62(11): 969-981.
8. Weinmüllner R, Barbara Zbiral, Adnan Becirovic, Elena Maria Stelzer, Fabian Nagelreiter, et al. (2020) Organotypic human skin culture models constructed with senescent fibroblasts show hallmarks of skin aging. npj Aging and Mechanisms of Disease 6: 4.
9. Wang L, et al. (2010) Thermogelling chitosan and collagen composite hydrogels for tissue engineering. Journal of Biomedical Materials Research Part A 93(4): 1091-1103.

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