PRECLINICAL RESEARCH COMPENDIUM

2014-2021

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Acknowledgement

We sincerely thank Dr. T. V. Anil Kumar and his team at Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST) for their pioneering research in developing this technology. Their invaluable contributions laid the foundation for the PrisTINE technology used in the manufacturing of CholeDerm®, which was transferred to Alicorn Medical Pvt. Ltd. at the proof-of-concept stage.

Disclaimer

Preclinical Research Compendium

Scientific Insights on CholeDerm®

First Edition, 2025

Comprehensive
Preclinical References
for Medical Professionals

Faster Healing, Better Living

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Introduction

Chronic wounds pose a significant challenge in clinical practice, requiring advanced materials to accelerate healing while minimising complications. CholeDerm®, developed by Alicorn Medical Private Limited, represents a pioneering breakthrough in tissue engineering and wound care. Derived from porcine gall bladder through a patented non-enzymatic and non-detergent process, CholeDerm® preserves the natural extracellular matrix (ECM) essential for supporting cellular growth, angiogenesis, and tissue remodelling.

CholeDerm® has been extensively studied in preclinical models and has consistently demonstrated its efficacy in promoting rapid wound closure, minimising immunogenic responses, and enhancing the quality of tissue regeneration. Its applications extend to diverse wound types, including diabetic ulcers, trauma wounds, surgical wounds, and other chronic skin conditions.

This compilation of scientific studies provides an evidence-based understanding of CholeDerm® and its potential to address unmet needs in wound care. Organised chronologically, the included publications outline the scaffold's unique properties, biocompatibility, and versatility. From its biomolecular composition to comparative studies with commercially available alternatives, CholeDerm® stands out as a safe and effective solution for clinicians.

This book serves as a reference guide for healthcare professionals, offering insights into the scientific foundations of CholeDerm®, frequently asked questions, and contact details for further inquiries. We hope it aids clinicians in making informed decisions to improve patient outcomes.

Overview of CholeDerm®

CholeDerm® is an innovative wound care product derived from the porcine gall bladder, utilising a non-enzymatic, non-detergent process to preserve its natural extracellular matrix (ECM). This advanced tissue-engineered scaffold is designed to accelerate wound healing and tissue regeneration by mimicking the natural ECM environment, which plays a crucial role in cellular activities such as proliferation, migration, and differentiation. The scaffold's unique composition makes it a promising solution for managing various wound types, including chronic, surgical, traumatic, and diabetic ulcers.

The manufacturing process of CholeDerm® ensures that the scaffold retains vital components such as collagen, elastin, glycosaminoglycans, and other proteins, which are critical for effective wound healing. The ECM derived from the porcine gall bladder offers superior structural and biochemical properties that support cellular functions, promote angiogenesis, and reduce scar formation, making CholeDerm® an ideal option for tissue repair and regeneration.

In clinical applications, CholeDerm® has shown promising results, with preclinical studies demonstrating faster healing rates, reduced inflammation, and improved tissue regeneration compared to conventional wound care products. The scaffold has been evaluated for safety, immunogenicity, and biocompatibility, with results indicating that it is well-tolerated in animal models and has a favourable immune response profile.

Overall, CholeDerm® offers an advanced and scientifically validated solution for clinicians seeking an effective, biocompatible option to manage difficult-to-heal wounds. Its ability to enhance tissue repair and improve clinical outcomes positions it as a key player in regenerative medicine and wound care.

CholeDerm® is available in multiple sizes, and is tailored to meet the specific needs of different wound types. It can be used as a temporary wound covering to provide a moist environment that protects the wound site while enhancing cellular migration and tissue formation. The non-enzymatic, non-detergent process ensures that the structural integrity of the ECM is preserved, facilitating natural healing processes without introducing harmful chemical agents or detergents.

CholeDerm® Mechanism of Action

CholeDerm® is an advanced extracellular matrix (ECM) product designed to overcome the challenges of chronic wounds. Developed through patented Pristine Technology, it retains essential healing-associated proteins, creating an optimal wound environment for regeneration and repair.

1. ECM Restoration and Protease Regulation

Stabilizing the Wound Bed
Chronic wounds often have excessive matrix metalloproteinases (MMPs) that degrade ECM. CholeDerm® reduces protease activity, preserving the structural integrity of the wound bed and promoting cell attachment and migration, essential for healing.

Preserving Healing Molecules
Unlike traditional ECM products exposed to enzymes and detergents, CholeDerm® maintains native proteins and macromolecules, ensuring the ECM retains its natural bioactivity for tissue repair.

2. Inflammation Modulation for Optimal Healing

Balancing the Immune Response
CholeDerm® shifts the wound environment from pro-inflammatory to anti-inflammatory, preventing excessive tissue damage and supporting a controlled healing process.

M2 Macrophage Activation
By promoting M2 macrophages, CholeDerm® enhances bacterial clearance while reducing prolonged inflammation. These macrophages release pro-healing cytokines, supporting a smooth transition to the proliferative phase.

3. Supporting Tissue Regeneration and ECM Remodeling

Fibroblast Proliferation
CholeDerm® enhances fibroblast activity, which is crucial for new ECM formation. The newly formed ECM supports keratinocyte migration, leading to improved wound closure.

Collagen Deposition
The Type I collagen in CholeDerm® strengthens the wound, increasing tensile strength and ensuring long-term tissue stability.

4. Moisture Balance for Enhanced Healing

Optimized Hydration
The presence of glycosaminoglycans (GAGs) in CholeDerm® helps maintain a moist wound environment, preventing desiccation while regulating protease activity.

Cellular Function Support
Balanced moisture levels encourage fibroblast and keratinocyte activity, essential for tissue repair.

5. Faster Reepithelialization and Wound Closure

Granulation and Reepithelialization
CholeDerm® accelerates the formation of granulation tissue and enhances reepithelialization, leading to faster wound coverage and complete closure activity.

Angiogenesis Support
Through M2 macrophage-mediated signalling, CholeDerm® stimulates angiogenesis, improving blood supply, oxygenation, and nutrient delivery to the wound.

Conclusion

By stabilising the ECM, regulating inflammation, and supporting tissue regeneration, CholeDerm® effectively addresses key barriers to chronic wound healing. Its unique properties make it a powerful wound care solution, offering faster recovery and improved patient outcomes.

Article JOURNAL OF BIOMEDICAL MATERIALS RESEARCH PART B

Biomaterial properties of cholecyst-derived scaffold recovered by a non-detergent/enzymatic method

Thapasimuthu V. Anilkumar, Vadavanath P. Vineetha, Deepa Revi, Jaseer Muhamed, Akhila Rajan

https://doi.org/10.1002/jbm.b.33131 Year of Publication : **2014**

METHODOLOGY

This study investigates the preparation and characterization of scaffolds derived from the extracellular matrix (ECM) of porcine cholecyst (gall bladder) (CDS), jejunum (JDS), and urinary bladder (UDS). The ECM was isolated by mechanical delamination using a non-detergent/non-enzymatic method, preserving the structural and functional integrity of the biomaterial.

The scaffolds were evaluated for:

- Gross morphology
- Decellularization
- Chemical composition
- Structural properties
- Preclinical safety, including in vitro cytotoxicity and in vivo wound-healing performance in rabbits.

KEY FINDINGS

KEY FINDINGS	CLINICAL IMPLICATION
Gross Morphology & Physical Properties: CDS (Cholecyst Derived Scaffold) was greenish-yellow, with swelling and water uptake similar to the other scaffolds. Dimensions: CDS (7 x 7 cm), JDS (7 x 10 cm), UDS (8 x 8 cm).	**Visual Identification** : CDS is distinct in colour (greenish-yellow) for easy clinical identification.
Decellularization : CDS had minimal residual cell nuclei, indicating effective decellularization. The double-stranded DNA content was lowest in CDS.	**Effective Decellularization** : Minimal cell remnants suggest high quality and minimal immunogenicity of CDS.
Biochemical Composition : CDS had the highest collagen (38.5 mg/100 mg) and sulfated glycosaminoglycan (sGAG) content. No significant differences in elastin levels between CDS and UDS.	**High Collagen Content** : The high collagen content in CDS aids tissue regeneration, making it ideal for wound healing.
Growth Factors : VEGF content (5.05 ng/mg in CDS) and bFGF content (0.92 ng/mg in CDS) were comparable to other scaffolds, supporting angiogenesis and tissue repair.	**Angiogenic Potential** : The presence of VEGF and bFGF in CDS suggests its strong role in promoting angiogenesis, cell proliferation, and tissue regeneration, aiding in faster wound healing.
Microscopic & Structural Observations : SEM and EDS analysis showed additional iron in JDS and calcium in UDS, while CDS showed a mesh-like structure that transforms to a gel-like appearance upon rehydration.	**Scaffold Structure** : The unique mesh-like structure and rehydration behaviour of CDS are beneficial for tissue integration and support.

Preclinical Safety : The scaffolds were non-cytotoxic, non-pyrogenic & biocompatible in rabbit subcutaneous implantation tests.	**Biocompatibility** : CDS is safe for implantation, ensuring minimal adverse effects when used in clinical applications.
Wound Healing : Complete re-epithelialization and granulation tissue formation were observed by day 14, showing comparable healing to commercial skin substitutes (CSIS).differences in elastin levels between CDS and UDS.	**Promising Wound Healing** : CDS demonstrated effective wound healing, comparable to established skin substitutes, supporting its use in clinical settings healing.

RELEVANCE TO CLINICAL PRACTICE

This study highlights the potential of cholecyst-derived extracellular matrix (CDS) as a versatile scaffold for regenerative medicine. CDS scaffolds demonstrated promising results in wound healing, with high collagen and sulfated glycosaminoglycan (sGAG) content supporting tissue regeneration. The scaffolds' biocompatibility and effective decellularization make them suitable for use in clinical applications such as **skin grafting and wound care**. CDS presents a cost-effective, natural alternative to traditional biomaterials for treating full-thickness skin wounds, with further potential for use in complex surgical grafts and post-surgical reconstructions.

IMPLICATIONS FOR CLINICAL PRACTICE

- **Wound Healing Applications**
  The cholecyst ECM scaffold may be an effective option for treating full-thickness skin wounds, promoting tissue regeneration and aiding wound closure.
- **Surgical Grafting**
  Its natural composition and biocompatibility make the CDS scaffold an ideal candidate for complex wound care, including graft-assisted healing and skin regeneration procedures.

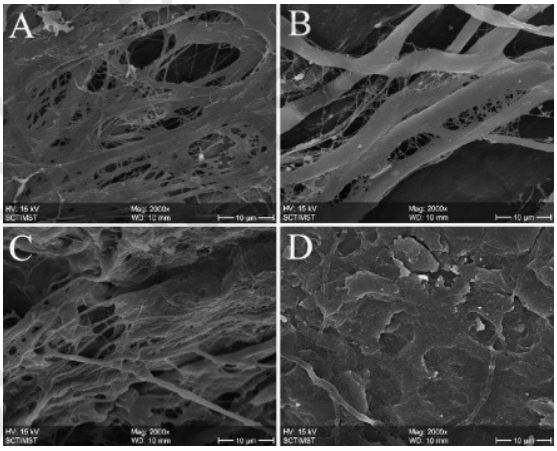

Figure 1 : Scanning electron micrographs of the scaffolds; CSIS (A), CDS (B), JDS (C) and UDS (D).

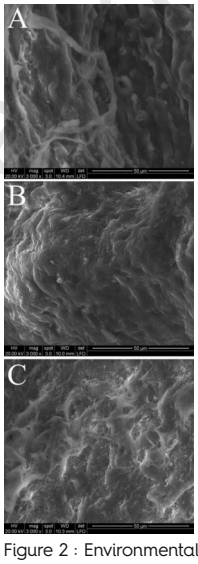

Figure 2 : Environmental scanning electron micrographs.
CDS (A), JDS (B), and UDS (C).

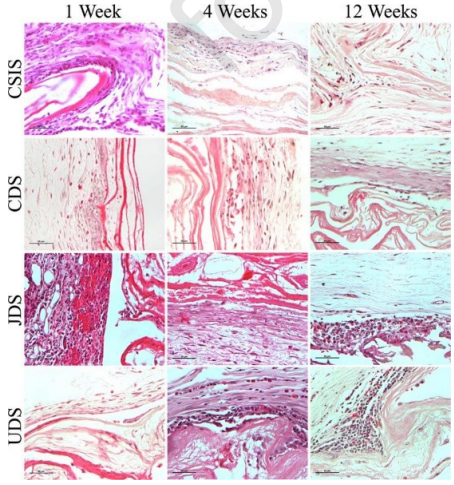

Figure 3 : Photomicrographs showing the nature of local tissue response induced by the scaffold in a rabbit subcutaneous implantation

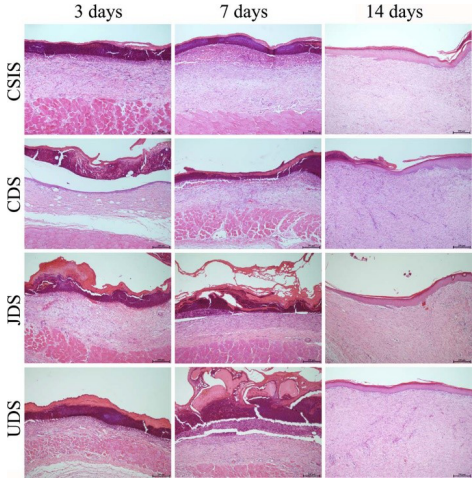

Figure 4 : Histomorphology of the wound-healing response (paraffin sections, Haematoxylin, and eosin stains); rabbit full-thickness skin wound

Article JOURNAL OF TISSUE ENGINEERING

Porcine Cholecyst–Derived Scaffold Promotes Full-Thickness Wound Healing in Rabbit

Deepa Revi, Vadavanath Prabhakaran Vineetha, Jaseer Muhamed, Akhila Rajan and Thapasimuthu Vijayamma Anilkumar

https://doi.org/10.1177/2041731413518060 Year of Publication : **2014**

METHODOLOGY

SCAFFOLD PREPARATION

Extracellular matrix (ECM) scaffolds (CDS) were extracted from porcine gallbladders using an in-house procedure without using detergents or enzymes. The lamina propria was mechanically recovered after 10% NBF treatment, followed by thorough washing, lyophilisation, and sterilisation with ethylene oxide.

PHYSICAL PROPERTY ASSESSMENT

- **Moisture Content** : Determined by drying the samples and calculating moisture loss.
- **Fluid Uptake** : Scaffolds soaked in PBS to measure weight changes over time.
- **Evaporative Water Loss (EWL)** : Measurement of weight loss from soaked scaffolds.
- **Water Vapor Transmission Rate (WVTR)** : Measured by evaluating the water evaporation from sealed scaffolds.
- **Mechanical Testing** : Measured Young's modulus, flexural rigidity, and suture retention strength.

BIOMOLECULE & DNA CONTENT

Collagen, elastin, GAGs, and DNA content were quantified using specific assays.

IN VIVO WOUND HEALING EXPERIMENT

- Full-thickness excision wounds (1 cm^2) were created on 12 rabbits, divided into 4 groups (3 rabbits per group). The wounds were grafted with CDS or SIS scaffolds.
- Samples were collected for histological and morphometric analysis at 3, 7, 14, and 30 days post-surgery.

HISTOLOGY & HISTOMORPHOMETRY

Histological techniques like H&E, picro-sirius red, and Van Gieson staining were used to assess re-epithelialization, collagen deposition and elastin. Immunohistochemistry was performed for markers like PCNA, ASMA, vimentin, and CK-14. Morphometric analysis was conducted to quantify various healing parameters.

STATISTICAL ANALYSIS

Data were analysed using Student's t-test, with p-values < 0.05 considered statistically significant.

RELEVANCE TO CLINICAL PRACTICE

This study underscores the potential of CDS as an alternative to traditional graft materials like SIS. The cholecyst-derived scaffold has demonstrated promising wound healing capabilities, including enhanced cell proliferation, collagen deposition, and regeneration of tissue. It is particularly relevant for clinicians in wound care and regenerative medicine, where advanced materials are required for full-thickness skin wounds, chronic wounds, or post-surgical tissue repair.

PARAMETER	CDS (CHOLECYST-DERIVED SCAFFOLD)	SIS (SURGICAL INTESTINAL SUBMUCOSA)
Physical Properties	Similar moisture content, fluid uptake, and EWL; lower WVTR	Similar to CDS except for WVTR
Collagen Content	Comparable to SIS	Comparable to CDS
Elastin Content	Higher than SIS	Lower than CDS
GAG Content	Higher than SIS	Lower than CDS
DNA Content	Lower than SIS	Higher than CDS
In Vivo Wound Healing	Similar to SIS: Good re-epithelialization and granulation tissue formation	Comparable healing to CDS
Histological Observations	Complete re-epithelialization by day 14, angiogenesis, collagen deposition, and elastin formation	Similar findings as CDS
Cell Proliferation	Slightly higher on days 3 and 14	Similar to CDS
Collagen Remodeling	Type I collagen increased in the late stages of healing	Similar to CDS

IMPLICATIONS FOR CLINICAL PRACTICE

Clinical Adoption of CDS :

CDS is a viable alternative to SIS for clinical skingrafting, particularly in cases requiring tissue regeneration, such as severe burns or chronic ulcers. Its regenerative potential can significantly improve healing outcomes compared to conventional grafts.

Enhanced Graft Materials :

CDS scaffolds could be incorporated into routine clinical practice for skin grafting, improving patient outcomes in full-thickness skin wounds and cases requiring more robust tissue regeneration.

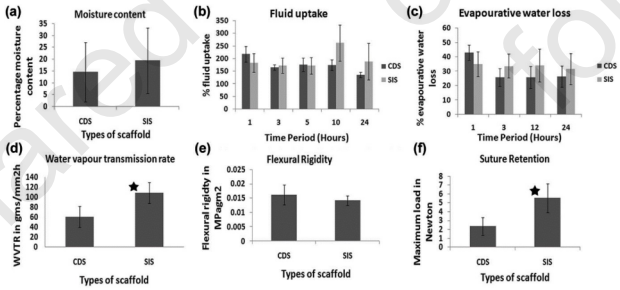

Figure 1; Physical properties of the scaffold: (a) moisture content, (b) fluid uptake, (c) evaporative water loss (EWL), (d) water vapour transmission rate (WVTR), (e) flexural rigidity and (f) suture retention strength. The CDSs have lower WVTR (p value = 0.001) and sutur retention strength (p value = 0.003) than SIS. CDS: cholecyst-derived scaffold; SIS: small intestinal submucosa.

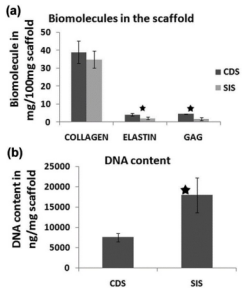

Figure 2 (a) Content of biomolecules in the candidate skin graft: the CDSs have higher elastin (p value = 0.0009) and sulphated GAG (p value = 0.006) than SIS. (b) DNA content in the scaffold: CDSs have lower DNA (p value = 0.03) compared to SIS.

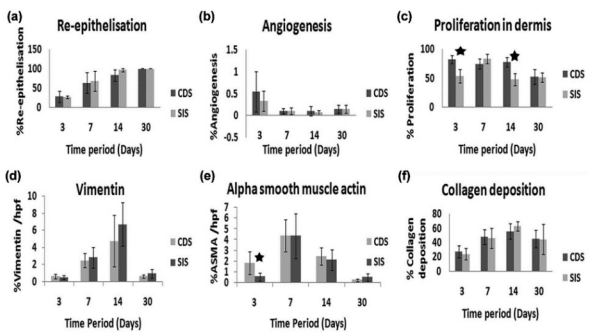

Figure 3; Quantitative histomorphometry for early stage wound-healing parameters: (a) percentage re-epithelisation (b)angiogenesis, (c) cell proliferation in dermis, (d) area occupied by vimentin-positive cells, (e) area occupied by alpha smooth muscle actin positive cells and (f) collagen deposition. The CDS-grafted wound showed higher ASMA-positive area on 3rd day (p value = 0.009) as well as cell proliferation on 3rd (p value = 0.02) and 14th day (p value = 0.008).

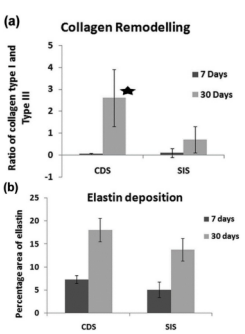

Figure 4 Quantitative data for late-stage wound-healing parameters: (a) ratio of collagen type I to collagen type III ratio, (b)elastin deposition. CDS had higher type 1 to type III collagen ratio compared to SIS on 30th day (p value = 0.005).

Article JOURNAL OF BIOMEDICAL MATERIALS RESEARCH PART B

Comparative local immunogenic potential of scaffolds prepared from porcine cholecyst, jejunum, and urinary bladder in rat subcutaneous model

Jaseer Muhamed, Deepa Revi, Akhila Rajan, Thapasimuthu V. Anilkumar

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METHODOLOGY

SCAFFOLD PREPARATION

ECM was isolated from porcine cholecyst (CDS), jejunum (JDS), and urinary bladder (UDS) using a non-detergent/non-enzymatic method. The organs were incubated in 10% NBF for crosslinking, followed by mechanical delamination. The scaffolds were washed, lyophilised, and stored for later use.

CELLULARITY IN SCAFFOLD

The scaffolds were sectioned, stained with haematoxylin and eosin, and analysed for cellularity by counting nuclei using Image-Pro software.

ANIMAL IMPLANTATION

ECM scaffolds were implanted subcutaneously in Sprague-Dawley rats, with 8 rats per scaffold type. The animals were euthanised at different time points (3, 7, 14, and 28 days), and tissue samples were collected for histology and gene expression studies.

HISTOTECHNOLOGY

Tissue samples were processed for routine histopathology, stained with haematoxylin and eosin, and analysed for macrophage and lymphocyte subpopulations via immunohistochemistry.

REAL-TIME PCR

RNA from implant tissues was extracted, cDNA synthesised, and gene expression of M1/M2 macrophage markers, TH1/TH2 cytokines, and other genes was analysed by RT-PCR.

STATISTICAL ANALYSIS

Data were analysed using one-way ANOVA with the Tukey-Kramer multiple comparisons test, considering differences significant at $p < 0.05$.

KEY FINDINGS

KEY FINDINGS	CLINICAL IMPLICATION
Cellularity of Scaffolds : CDS scaffolds showed fewer nuclei (486+/-84/mm²) compared to JDS (6232+/-321/mm²) and UDS (8889+/-772/mm²), indicating lower cellularity in CDS.	**Clinical Consideration for Scaffold Selection** : Lower cellularity in CDS could reduce the risk of immune rejection, which is desirable for clinical applications, particularly in tissue engineering, where scaffold integration with host tissue is critical.
Implantation & Tissue Response : CDS showed less severe tissue reactions compared to JDS and UDS, with less inflammation and better degradation.DNA content was lowest in CDS.	**Preferred Use of CDS in Clinical Applications** : CDS scaffolds could be considered a better option for clinical use due to their lower inflammatory response, offering better tissue integration and less risk of complications.

Granulation & Degradation : CDS exhibited more granulation tissue and faster degradation compared to JDS and UDS, with minimal degradation in the latter.	**Adoption of Decellularization Methods** : The faster degradation and better granulation response of CDS suggest its suitability for regenerative applications, indicating that decellularisation methods are effective in improving scaffold functionality.
Macrophage Response : M2 macrophages were more prevalent in CDS-induced reactions at later stages, whereas JDS and UDS favoured M1 responses early on.	**Further Exploration of Immunogenic Factors** : CDS's M2 macrophage polarisation supports tissue repair and regeneration, making it a better option for grafts. Research into optimising M2 macrophage responses could enhance the safety and efficacy of CDS in clinical settings.
Lymphocyte Response : CDS induced a balanced CD4/CD8 ratio, while JDS and UDS favoured cytotoxic T cells and helper T cells at different stages.	**Clinical Consideration for Scaffold Selection** : The balanced immune response in CDS suggests it may offer better outcomes in preventing chronic inflammation and promoting healing, making it an attractive material for scaffold selection in clinical settings.
Gene Expression : CDS promoted consistent M2 polarisation, whereas JDS showed an initial M1 response that switched to M2 later, and UDS mostly favoured M1 responses.	**Clinical Adoption of CDS as a Scaffold** : The consistent M2 polarisation in CDS promotes tissue repair, supporting its use in clinical applications where regeneration is crucial, such as skin grafting or wound healing.

RELEVANCE TO CLINICAL PRACTICE

This study provides valuable insights into the immunogenic potential of extracellular matrix (ECM) scaffolds derived from porcine cholecyst (CDS) compared to those derived from jejunum (JDS) and urinary bladder (UDS). Understanding the immune response to these scaffolds is crucial for clinicians, especially in regenerative medicine and tissue engineering. The study highlights that CDS induces less immunogenic response, showing favorable characteristics like TH2 polarisation and M2 macrophage activity, which are conducive to graft acceptance and constructive tissue remodeling. This could translate into better clinical outcomes when using CDS-derived scaffolds for regenerative treatments, leading to fewer complications and better integration of the scaffold with host tissue.

IMPLICATIONS FOR CLINICAL PRACTICE

Preferred Use of CDS in Clinical Applications

Given its less immunogenic nature, CDS scaffolds could be a more suitable option for clinical use in regenerative medicine, particularly in tissue engineering and grafting procedures.

Clinical Consideration for Scaffold Selection

Clinicians should consider the immunogenic potential of ECM scaffolds when selecting materials for regenerative treatments. The findings suggest that scaffolds with lower immunogenicity, like CDS, may be preferable to reduce the risk of rejection and improve long-term outcomes.

Article JOURNAL OF BIOMEDICAL MATERIALS RESEARCH PART B

Comparative profiling of extractable proteins in extracellular matrices of porcine cholecyst and jejunum intended for preparation of tissue engineering scaffolds

Jaseer Muhamed, Akhila Rajan, Arun Surendran, Abdul Jaleel, Thapasimuthu V. Anilkumar

https://doi.org/10.1002/jbm.b.33567 Year of Publication : **2015**

METHODOLOGY (BRIEF DESCRIPTION)

ECM ISOLATION

Extracellular matrices (ECMs) were isolated from porcine gall bladder (cholecyst) (CDE) and jejunum (JDE) without cross-linking, stored at -80°C in Dulbecco's modified minimum essential medium with 20% dimethyl sulfoxide.

PROTEIN EXTRACTION

ECMs were minced, homogenised in extraction buffer, and centrifuged. The supernatant was concentrated, and protein content was estimated using the Micro-Lowry method.

SDS-PAGE & IN-GEL DIGESTION

Protein extracts were separated on a 10% SDS-PAGE gel, stained, and excised for in-gel tryptic digestion.

NANOFLOW LIQUID CHROMATOGRAPHY & MASS SPECTROMETRY

Peptides were separated using nanoUPLC with a C18 column and analysed using SYNAPT G2 HDMS in positive mode ESI.

DATA ANALYSIS

LC-MSE data was processed using ProteinLynx Global SERVER (PLGS), and proteins were identified by searching against the Sus scrofa database. Functional protein classifications were determined through UniProt.

KEY FINDINGS

KEY FINDINGS	CLINICAL IMPLICATION
Protein Extraction Efficiency : Proteins extracted with 3 mg/mL concentration.	**Implication for Scaffold Development** : Efficient extraction methods ensure sufficient protein availability for scaffold manufacturing.
SDS-PAGE Analysis : Distinct protein bands in both CDE and JDE.	**Insight into Scaffold Composition** : Identifies protein diversity, aiding in scaffold quality and composition optimisation.
Protein Composition : CDE had higher ECM proteins (8.4%) and lower cellular proteins (79.2%) than JDE.	**Choice of Scaffold Material** : CDE's higher ECM content and lower cellularity make it preferable for tissue regeneration with less immune rejection risk.
Enzymatic Protein Composition : CDE had fewer enzymatic proteins (21.3%) than JDE (27.4%).	**Effect on Wound Healing** : Enzymatic proteins promote healing, but lower enzymatic content in CDE may reduce unwanted inflammatory responses.

Wound Healing Potential : CDE contained immunological proteins like complement component C3 and prolargin.	**Wound Healing Benefits** : CDE's immunological proteins suggest its effectiveness in wound healing and tissue regeneration.
Immunological Proteins : JDE contained MHC and SLA histocompatibility antigens.	**Clinical Relevance for Graft Acceptance** : The lower immunogenicity of CDE reduces the risk of graft rejection, making it a safer option for clinical applications.

RELEVANCE TO CLINICAL PRACTICE

This study emphasises the potential of porcine cholecyst-derived ECM (CDE) as a preferred material for wound healing scaffolds. Due to its lower immunogenicity and rich ECM content, CDE reduces the risk of immune rejection, making it safer and more suitable for use in clinical wound care, particularly for patients at high risk of graft rejection. Moreover, proteins such as fibronectin, nidogen, decorin, and prolargin in CDE support tissue regeneration and wound closure. Therefore, CDE-based scaffolds could promote faster and more effective healing, reducing complications in chronic wound management.

IMPLICATIONS FOR CLINICAL PRACTICE

Use of CDE for Wound Care

Clinicians should consider using CDE-derived ECM scaffolds for patients with a high risk of immune rejection. The lower histocompatibility antigen presence enhances safety.

Application for Chronic and Immunocompromised Wounds

CDE's immunomodulatory proteins like prolargin and clusterin suggest its suitability for chronic and immunocompromised patients.

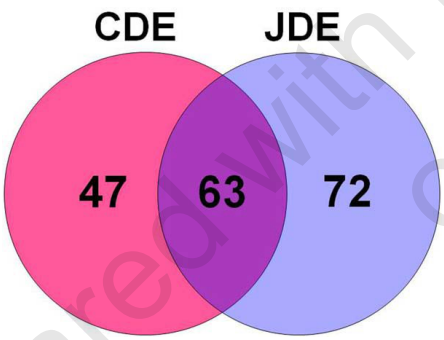


Figure 1; Venn diagram showing the sharing of proteins between the CDE and JDE as identified by mass spectroscopy.

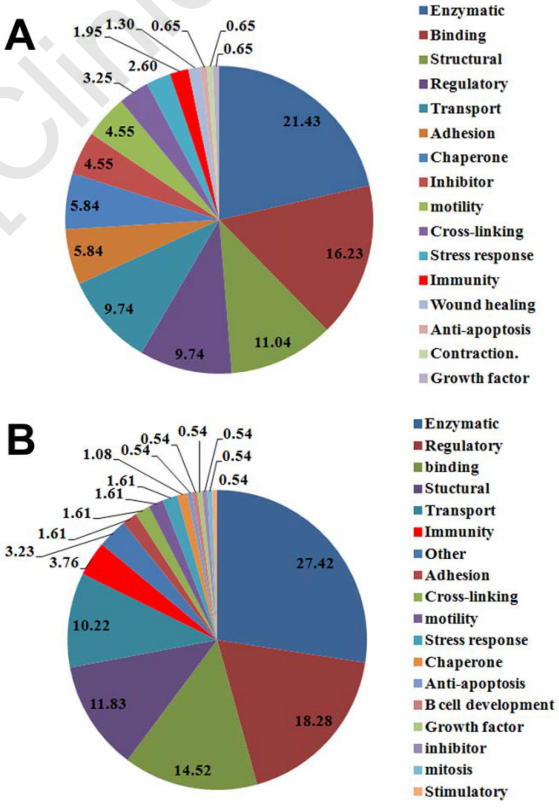


Figure 2; Extractable proteins of CDE (A) and JDE (B) extracellular matrices classified based on their function.

Biocompatibility and Immunophenotypic Characterization of a Porcine Cholecyst–derived Scaffold Implanted in Rats

Jaseer Muhamed, Deepa Revi, Akhila Rajan, Surendran Geetha, Thapasimuthu V. Anilkumar

https://doi.org/10.1177/0192623314550722 Year of Publication : **2015**

METHODOLOGY

PREPARATION OF SCAFFOLD

- Porcine cholecyst-derived scaffolds (CDS) were prepared using a nondetergent/enzymatic procedure.
- Porcine organs were collected, ECM beneath the mucosa was delaminated, and tissue was sterilised and stored.
- Commercial small intestinal submucosa (CSIS) was used for comparison.

ANIMAL IMPLANTATION

- 12 Sprague-Dawley rats were divided into two groups for CDS and CSIS implantation, with 4 subcutaneous implants (1 cm^2) on each animal's dorsum.
- Tissue samples were collected at 1, 4, and 12 weeks for histopathological and gene expression studies.

HISTOPATHOLOGY

- H&E staining and inflammatory response, fibrosis, and neovascularisation were analysed.
- Irritancy scores were calculated, comparing CDS to CSIS.

IMMUNOHISTOCHEMISTRY

- Immunostaining for macrophages (CD80 for M1, CD163 for M2) and T-lymphocytes (CD4 and CD8) was conducted.
- Quantification of immune cell counts and tissue area was performed.

GENE EXPRESSION ANALYSIS

RNA isolation and Real-time PCR were conducted to evaluate gene expression related to macrophage polarisation and T lymphocyte phenotyping.

STATISTICAL ANALYSIS

One-way ANOVA and Tukey-Kramer tests were used, with a p-value of <0.05 indicating statistical significance.

KEY FINDINGS

KEY FINDINGS	CLINICAL IMPLICATION
Histopathological Response : Both CDS and CSIS induced initial acute inflammation, but CDS showed minimal inflammation and more neovascularisation by 12 weeks.	CDS may promote faster healing and reduced inflammation compared to CSIS, indicating a better long-term tissue integration and regenerative potential.
Biocompatibility : Both scaffolds showed similar biocompatibility, with CDS showing a minimal irritancy score.	CDS is a non-irritant material and comparable to CSIS, making it suitable for clinical use, with minimal risk of irritation or adverse effects.

Immunohistochemical Analysis : M2 macrophages predominated in CDS, while the M1/M2 ratio was balanced by 12 weeks. CDS induced consistent TH2 polarisation, while CSIS showed a shift from TH1 to TH2.	CDS's ability to induce consistent TH2 polarisation might make it more favourable for tissue regeneration and integration, promoting immune tolerance and minimising rejection risk.
Gene Expression : CDS promoted M2 macrophage polarisation and TH2 response more strongly than CSIS.	M2 macrophage polarisation suggests that CDS could be particularly beneficial in chronic wounds or conditions requiring prolonged tissue remodelling.

RELEVANCE TO CLINICAL PRACTICE

This study confirms that Cholecyst-Derived Scaffold (CDS) is biocompatible, non-irritant, and promotes favourable immune responses for tissue integration. The consistent TH2 polarisation observed with CDS suggests a pro-regenerative environment, reducing chronic inflammation and foreign body reactions. Compared to the reference material CSIS, CDS demonstrated higher neovascularisation and controlled fibrosis at later time points, which can benefit wound healing applications. The early predominance of M2 macrophages indicates a reduced inflammatory response, potentially leading to better graft acceptance and faster healing. Additionally, CDS showed a lower CD4+ T-cell response than CSIS, suggesting a lower risk of immune-mediated rejection. These findings highlight CDS as a promising scaffold material for clinical applications in wound healing, soft tissue repair, and regenerative medicine, where minimising immune rejection and promoting vascularisation are critical for successful outcomes.

IMPLICATIONS FOR CLINICAL PRACTICE

Wound Healing and Regenerative Applications

CDS induces a controlled inflammatory response with early M2 macrophage predominance, essential for tissue remodelling and graft integration. Its consistent TH2 polarisation helps reduce chronic inflammation and foreign body reactions, promoting better healing outcomes.

Application for Chronic and Immunocompromised Wounds

Compared to CSIS, CDS demonstrated lower CD4+ T-cell infiltration, indicating a lower risk of immune rejection. This makes CDS a favourable choice for clinical settings requiring xenogeneic scaffolds, ensuring better tissue integration and healing with minimal adverse immune responses.

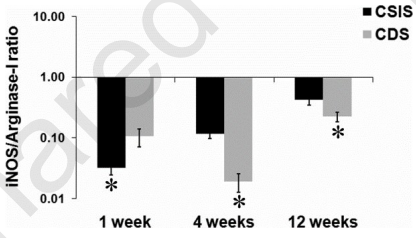


Figure 1 The ratio of number of CD80 M1-positive macrophages to the number of CD163-positive M2 macrophages (mean+SD, n ¼ 4).

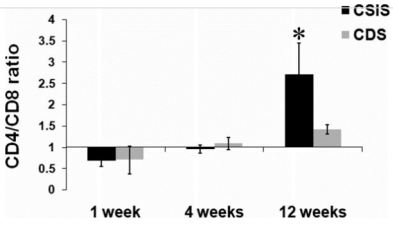


Figure 2 The ratio of number of CD4-positive helper T cells to the number of CD8-positive cytotoxic-T cells (mean+SD, n ¼ 4). * represents significant difference between the groups, p value < .05.

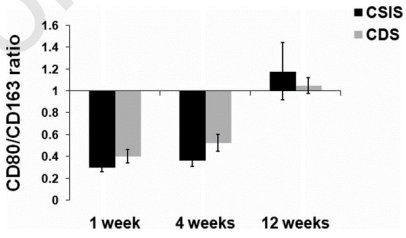


Figure 3 The phenotypic polarisation of macrophages in the tissue reaction demonstrated by the ratio of fold change in mRNA expression of inducible nitric oxide synthase (iNOS) to that of arginase-I (mean + SD, n ¼ 4), which are functional markers for M1 and M2 macrophages, respectively.
* represents significant difference between the groups, p value < .05.

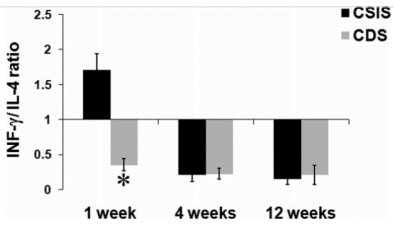


Figure 4 The phenotypic polarisation of T-lymphocytes in the tissue reaction demonstrated by the ratio of fold change in mRNA expression of interferon-gamma (INF-g) to that of interleukin-4 (IL-4), which are functional markers for TH1 and TH2 cells, respectively (mean+SD, n¼4). * represents significant difference between the groups, p value < .05.

Article VETERINARY RESEARCH COMMUNICATIONS # SPRINGER NATURE B.V.

A porcine-cholecyst-derived scaffold for treating full-thickness lacerated skin wounds in dogs

Satheesan Karthika, Sainulabdeen Anoop, C. B. Devanand, M. K. Narayanan, Madhavan Unni, Saji Eassow, Thapasimuthu Anilkumar

https://doi.org/10.1007/s11259-018-9731-3 Year of Publication : **2018**

METHODOLOGY

The study was conducted on eight dogs with full-thickness lacerated skin wounds, divided into two groups of four animals each.

- Group-I received Cholecyst Derive Scaffold (CDS) grafts prepared by a non-detergent/enzymatic method.
- Group-II received Xenoderm, a commercially available bovine dermal collagen matrix used routinely for wound healing.

The surgical procedure involved irrigating wounds with normal saline and grafting the respective scaffold using polyglactin 910 sutures under general anaesthesia (induced with ketamine hydrochloride and maintained with isoflurane).

Post-surgery, animals received Meloxicam for pain management and antibiotics and were kept under an Elizabethan collar. Wound healing was assessed on days 0, 7, 14, and 28 using the Bates Jensen Wound Assessment Tool (BJWT) to measure tissue edema, necrotic tissue, granulation tissue, and re-epithelialization. Wound surface area was calculated using a graphical method, and the healing rate was calculated based on the percentage reduction in wound size. Biopsies were taken on day 28 for histopathology and scanning electron microscopy. Statistical analysis was performed using Student's t-test to compare wound surface area, healing rates, and haematological/biochemical parameters between the groups.

KEY FINDINGS

KEY FINDINGS	CLINICAL IMPLICATION
Wound Depth and Healing : On day 7, CDS-treated wounds had significantly shallower depths compared to BDC. By day 14 and 28, no significant differences were found.	**Faster Wound Healing** : CDS treatment leads to shallower wounds early, indicating faster initial healing.
Granulation Tissue : CDS-treated wounds showed more rapid formation, covering over 75% of the wound area by day 7. BDC-treated wounds covered only 25% at the same time point. By day 28, both groups had similar granulation tissue levels.	**Enhanced Tissue Regeneration** : CDS fosters faster granulation tissue formation, contributing to quicker wound closure.
Epithelialisation : CDS-treated wounds exhibited higher levels of epithelialisation on days 7 and 14, showing faster healing than BDC-treated wounds.	**Improved Skin Regeneration** : CDS supports faster epithelialisation, which can lead to quicker recovery in clinical settings.

Necrotic Tissue : No necrotic tissue was observed in CDS-treated wounds, whereas BDC-treated wounds showed purulent necrotic tissue on day 7.	**Reduced Infection & Inflammation** : The absence of necrotic tissue in CDS-treated wounds suggests less inflammatory response & better overall healing.
Wound Surface and Healing Rate : CDS-treated wounds had smaller wound surface areas and a higher rate of healing as early as day 7.	**Better Healing Outcomes** : CDS leads to quicker wound healing with reduced surface area, showing a higher efficacy in wound management.
Histopathology and Electron Microscopy : CDS-treated wounds showed complete re-epithelialization and collagen deposition in the superficial dermis. Electron microscopy revealed less chronic inflammation in CDS-treated wounds.	**Histological Evidence of Healing** : CDS-treated wounds show superior tissue regeneration, with lower inflammatory responses compared to BDC-treated wounds.

RELEVANCE TO CLINICAL PRACTICE

This study demonstrates the potential of cholecystic extracellular matrix (CDS) as a promising bioscaffold for treating full-thickness skin wounds in dogs. CDS-treated wounds exhibited faster healing, less necrotic tissue, and better granulation and epithelialisation than BDC-treated wounds. These findings suggest that CDS may be a better alternative to traditional grafts like decellularised bovine dermis (BDC) for veterinary wound healing. Clinicians in veterinary practice could consider incorporating CDS into wound management protocols, especially for large or critical-sized wounds.

IMPLICATIONS FOR CLINICAL PRACTICE

Use of CDS as a Wound Healing Matrix

CDS could be recommended for use in faster wound healing, especially in cases of road accident-related lacerations requiring graft-assisted healing.

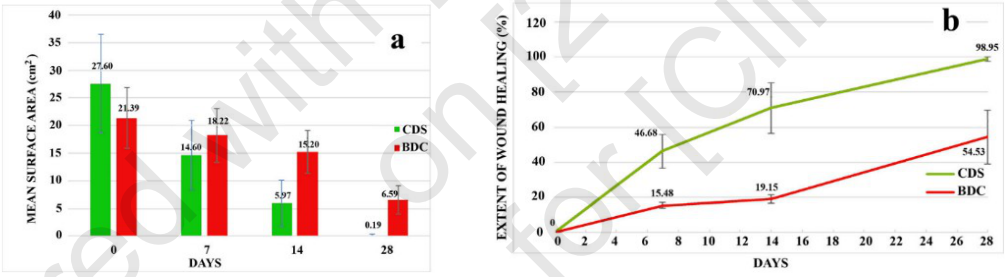


Figure 1 Bar diagram (a) and line graph (b) showing comparison of calculated mean surface area (± S.E) and healing rates (± S.E) respectively of wounds of animals in Groups I (CDS) and II (BDC). CDS- Cholecyst Derived Scaffold. BDC - Bovine Dermal Collagen

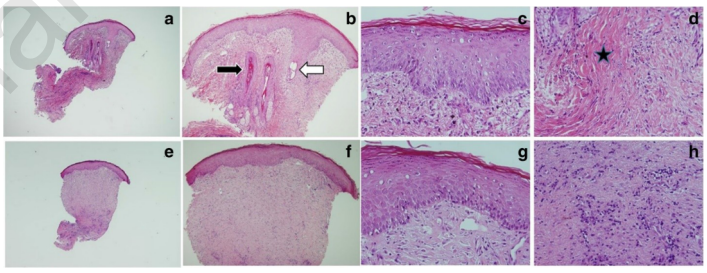


Figure 2 Histomorphology (haematoxilin and eosin staining) of punch biopsy specimens collected from healing wounds of dogs identified as A1 (a, b, c and d) and B2 (e, f, g and h), following graft-assisted healing (28 days) with a porcine-cholecyst-derived scaffold and a commercial grade bovine dermal collagen respectively. At a magnification of 4× (a and e), the entire field of evaluation was apparent. Complete reepithelialisation of the dermis (b, c, f and g) and progressive stage of healing by granulation tissue of the underlying dermis (b, d, fF and h) were evident at higher magnification 10 x (b and f) or 40 x (c, d, g and h). The epidermal histomorphology was similar in dogs grafted with CDS (b and c) and BDC (f and g), but in the dermis hair follicle (black arrow), sebaceous gland (white arrow) and mature collagen (*) were prominent in the former (b, c and d) compared to the later (f, g and h). Mild (d) and moderate (h) chronic inflammation was apparent in the deep dermis

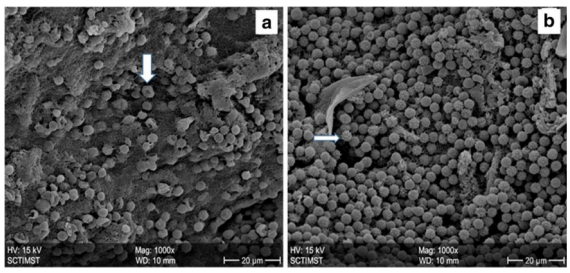


Figure 3 Representative scanning electron micrographs of randomly collected tissue samples from the middle of the healing wounds showing the nature of cell infiltration in CDS-treated (a) and BCD-treated (b) wounds. There was an abundance of cells with smooth surfaces and spiked surfaces, and they were probably macrophages and lymphocytes (indicated by a thin arrow). A thick arrow indicates RBCs. Note the density of cell infiltration was much less in CDS-treated wounds than in BCD-treated wounds

Article ACS APPLIED BIO MATERIALS

Gelatin-Modified Cholecyst-Derived Scaffold Promotes Angiogenesis and Faster Healing of Diabetic Wounds

Manjula P. Mony, Sachin J. Shenoy, Reshmi Raj, Chandrika S. Geetha, Reshma S. Nair, Kanakarajan V. Pratheesh, Chandramohanan Purnima, and Thapasimuthu V. Anilkumar*

https://doi.org/10.1021/acsabm.0c01648 Year of Publication : **2021**

METHODOLOGY

The study focused on the preparation and modification of **Cholecyst-Derived Scaffolds (CDS)** from **porcine gallbladders** using a **non-detergent, non-enzymatic method** to preserve its bioactivity. The process included:

- **Mechanical delamination**, cross-linking, **lyophilisation**, and sterilisation.
- **In vitro angiogenesis assays** using **HUVEC cells** (alamarBlue, transwell migration, scratch, and tube formation assays).
- **Gelatin modification** of CDS using **EDC-NHS chemistry**, generating three variants **(CDS-G1, CDS-G2, CDS-G4)** with increasing gelatin content.
- **Characterization of modified CDS** using TNBS assay (free amino groups), degradation studies, FTIR analysis, and SEM imaging.
- In vivo studies included **a chick chorioallantoic membrane (CAM) assay and a diabetic rat wound model**, which evaluated wound healing and vascularisation through histological and immunohistochemical staining.
- **Statistical analysis** was performed using **ANOVA or Student's t-test** to determine significant differences **($p < 0.05$).**

KEY FINDINGS

KEY FINDINGS	CLINICAL IMPLICATION
Angiogenic Potential : CDS and gelatin-modified CDS promoted angiogenesis in vitro and in vivo.	**Gelatin enhances blood vessel formation**, which is crucial for diabetic wound healing.
Gelatin Modification : The CDS-G4 variant showed the highest angiogenic potential, improving endothelial cell viability and biomolecule retention.	CDS-G4 could be more effective for wound healing applications.
Faster Wound Healing : CDS-G4-treated diabetic wounds healed completely by day 16, significantly faster than untreated wounds.	A promising option for chronic diabetic wounds that typically heal slowly.
Higher M2 Macrophages : M2 macrophages (pro-healing phenotype) were significantly higher in CDS-G4-treated wounds.	Enhanced tissue remodelling and inflammation resolution are critical for chronic wound healing.

Enhanced Capillary Formation : CDS-G4-treated wounds had more functional blood vessels than untreated wounds.	Supports the use of CDS-G4 for ischemic wound treatment.

RELEVANCE TO CLINICAL PRACTICE

The study demonstrates that gelatin-modified cholecyst-derived scaffolds (CDS) significantly improve angiogenesis, tissue regeneration, and wound closure, making them particularly beneficial for diabetic wound care. Given that diabetic patients face delayed healing due to poor vascularisation, CDS-G4 provides a viable solution for clinicians treating chronic wounds.

IMPLICATIONS FOR CLINICAL PRACTICE

Clinical Use of CDS and Gelatin-Modified CDS (CDS-G4)

CDS is an effective scaffold for promoting angiogenesis, tissue remodelling, and wound healing. However, gelatin modification (CDS-G4) further enhances these properties by improving endothelial cell viability and blood vessel formation.

Optimized Healing for Diabetic Wounds

Both CDS and CDS-G4 support wound healing, but CDS-G4 demonstrates a slightly faster closure rate and improved vascularisation. Clinicians can confidently use CDS for chronic wounds, with CDS-G4 offering an additional advantage in cases where enhanced angiogenesis is particularly beneficial, such as diabetic wound care.

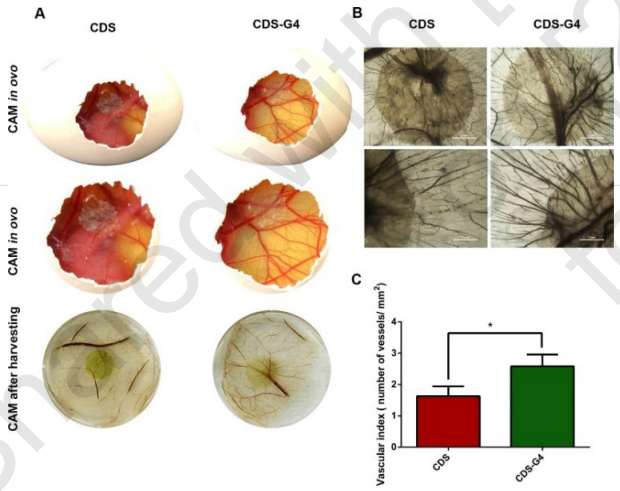


Figure 1 Results of in ovo experiments. Gross appearances of blood vessels in the chorioallantoic membrane (CAM) surrounding the CDS and CDSG4 in ovo (first two rows) and ex ovo after harvesting (third row) (A). Stereomicroscopic images showing the blood vessels surrounding the scaffolds after harvesting the CAM along with the scaffold (B). A bar diagram depicting the "vascular index" suggests the enhanced angiogenic potential of the CDS-G4 in comparison with the bare CDS (C). *p value < 0.05.
CDS – Cholecyst derived scaffold. CDS-G4 – CDS with 4 mg gelatin/cm2.

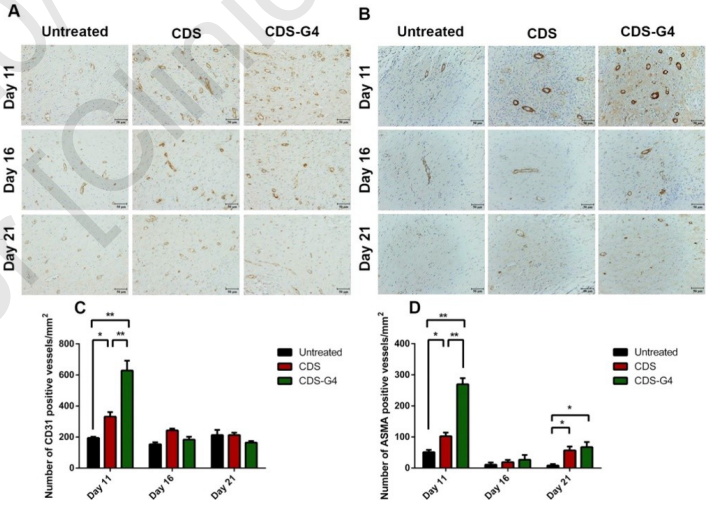


Figure 2 Light microscopy images of immunohistochemistry-stained sections for CD31 showing the capillary vessels (A) and ASMA showing the mature vessels (B) of untreated control and wounds grafted with the unmodified scaffold (CDS) and the gelatin-modified scaffold (CDS-G4) on days 11, 16, and 21. Bar graphs showing the number of CD31-positive capillaries (C) and ASMA-positive mature vessels (D). *p value < 0.05 and **p value < 0.01.

Frequently Asked Questions (FAQ) about CholeDerm®

1 What is CholeDerm®?

CholeDerm® is a novel, porcine cholecyst-derived extracellular matrix (ECM) scaffold designed for wound healing. It is a biocompatible material that promotes tissue regeneration and supports wound healing by providing a natural scaffold for cell migration, proliferation, and differentiation. CholeDerm® is derived from the inner mucosa and outer serosa of the porcine gall bladder using a non-enzymatic, non-detergent process to preserve its natural ECM properties.

2 What types of wounds can CholeDerm® be used for?

CholeDerm® is indicated for the treatment of a wide range of chronic and acute wounds, including:

- Partial and full-thickness wounds
- Pressure ulcers
- Venous ulcers
- Diabetic ulcers
- Chronic vascular ulcers
- Trauma wounds (abrasions, lacerations, skin tears)
- Second-degree burns
- Draining wounds
- Surgical wounds (donor sites, post-surgical wounds)

3 What sizes are available for CholeDerm?

CholeDerm® Available in the following Sizes and Packaging Options

Sl No:	Model No:	Size (LxB) cm	Pack Size
1	CD-P7646-5S	7.6x4.6	5 Sheets
2	CD-P7452-5S	7.4x5.2	5 Sheets
3	CD-P6646-5S	6.6x4.6	5 Sheets
4	CD-P5640-5S	5.6x4.0	5 Sheets
5	CD-P5237-5S	5.2x3.7	5 Sheets
6	CD-P4636-5S	4.6x3.6	5 Sheets
7	CD-P3630-5S	3.6x3.0	5 Sheets

Sl No:	Model No:	Size (LxB) cm	Pack Size
8	CD-P7646-1S	7.6x4.6	1 Sheet
9	CD-P7452-1S	7.4x5.2	1 Sheet
10	CD-P6646-1S	6.6x4.6	1 Sheet
11	CD-P5640-1S	5.6x4.0	1 Sheet
12	CD-P5237-1S	5.2x3.7	1 Sheet
13	CD-P4636-1S	4.6x3.6	1 Sheet
14	CD-P3630-1S	3.6x3.0	1 Sheet

4 How does CholeDerm® aid in wound healing?

CholeDerm® works by providing a natural extracellular matrix (ECM) that supports the migration of cells such as fibroblasts, endothelial cells, and keratinocytes, which are essential for wound healing. Its unique composition enhances collagen deposition and tissue remodeling while promoting angiogenesis (formation of new blood vessels). This results in accelerated wound closure and improved healing outcomes, especially for chronic wounds and wounds in diabetic patients.

5 How is CholeDerm® different from other wound care products?

CholeDerm® stands out due to its source from porcine gall bladder, a rich source of ECM proteins such as elastin, collagen & glycosaminoglycans, which are essential for tissue repair. Additionally, it is produced using a non-enzymatic, non-detergent method, preserving its natural biochemical structure. This process ensures that the scaffold retains its bioactivity, making it an effective option for chronic & difficult-to-heal wounds.

6 What are the advantages of using CholeDerm®?

- **Biocompatibility** : CholeDerm® is highly biocompatible and elicits minimal immune response when used as a xenograft.
- **Promotes Faster Healing** : The ECM in CholeDerm® supports faster tissue regeneration, promoting quicker wound closure.
- **Rich in Biomolecules** : The scaffold contains 154 proteins that are critical for cell signaling, tissue repair, and wound healing.

- **Versatile** : CholeDerm® can be used in various types of wounds, including diabetic ulcers, surgical wounds, and pressure ulcers.
- **Non-enzymatic Production** : The unique non-enzymatic production process preserves the structural integrity of the ECM, ensuring its effectiveness.

7 Is CholeDerm® safe to use in clinical practice?

Yes, CholeDerm® has undergone rigorous safety and biocompatibility evaluations to ensure its suitability for clinical use. It has been tested for sterility, cytotoxicity, bioburden, bacterial endotoxin levels, EO residue, skin sensitization, irritation, acute systemic toxicity, material-mediated pyrogenicity, and TSE-BSE risks. These studies confirm that CholeDerm® is free from harmful contaminants and does not induce adverse reactions.

Additionally, CholeDerm® is terminally sterilized using ethylene oxide (EtO) and manufactured under Good Manufacturing Practices (GMP) in a facility compliant with ISO 13485 and USFDA regulations.

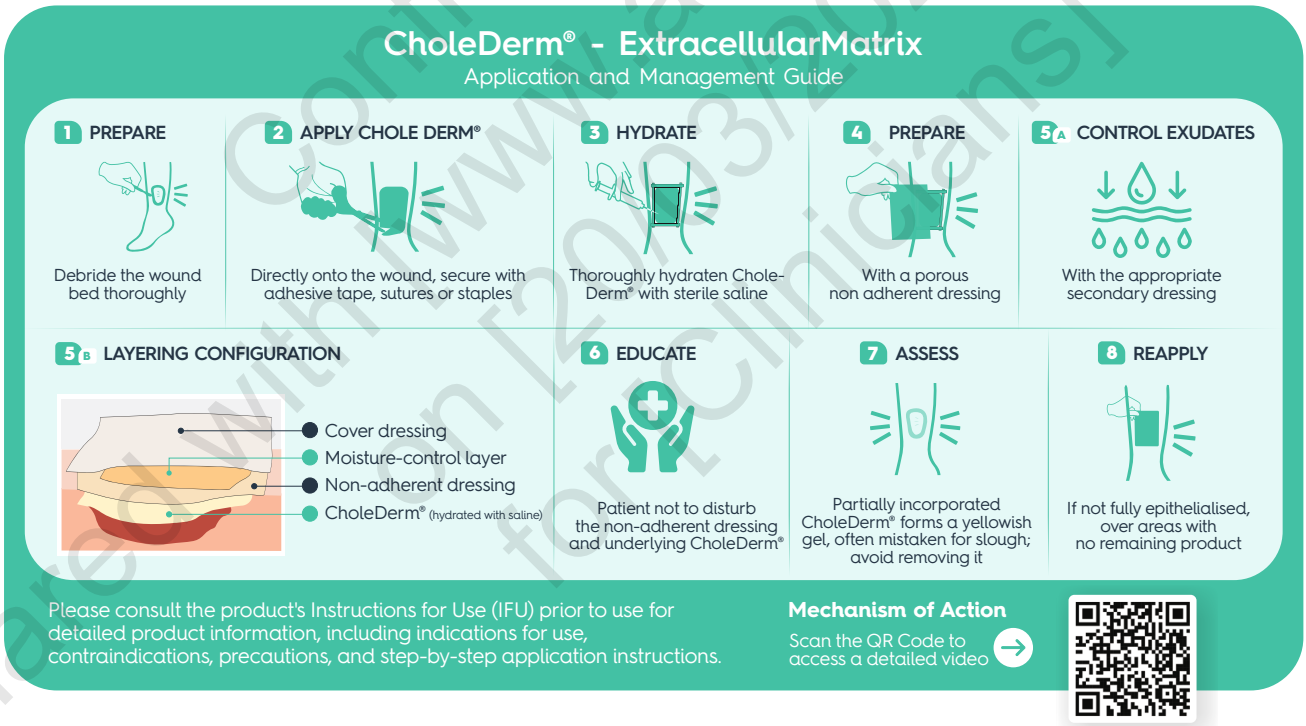
The product holds a Class D Medical Device Manufacturing License from CDSCO, ensuring adherence to stringent safety and quality standards.

While no adverse effects have been reported with similar products, CholeDerm® should be avoided in patients with known porcine tissue allergies, and any existing infections should be managed before application.

8 Can CholeDerm® be used for diabetic wounds?

Yes, CholeDerm® is particularly beneficial for diabetic wounds. Its ability to promote angiogenesis and accelerate healing makes it an excellent choice for patients with diabetic foot ulcers, which are often slow to heal due to impaired circulation and cell function. Studies have shown that CholeDerm® can enhance wound closure in diabetic wounds more effectively than other treatments.

9 How is CholeDerm® applied to wounds?



10 Are there any side effects or contraindications with CholeDerm®?

CholeDerm® is generally well-tolerated, with no reported adverse effects associated with similar products. However, patients with known allergies to porcine tissue should avoid using CholeDerm®. If there are concerns, a patch test or medical consultation should be performed before use. It is also essential to ensure that patients do not have active infections before applying the product.

11 Where can I get more information about CholeDerm®?

For more information on CholeDerm®, including clinical data, case studies, and usage guidelines, don't hesitate to contact Alicorn Medical Private Limited or visit our website at www.alicornmed.com. Contact our sales team or customer support for any specific inquiries or product demonstrations.

Contact Information

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Thiruvananthapuram – 695012, India.

We are committed to supporting clinicians with high-quality wound care solutions. For any inquiries, please reach out to us:

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We're here to assist you with all your needs

Alicorn
Medical Private Limited

CholeDerm is available for purchase through the Government e-Marketplace

GeM Government e Marketplace

MAKE IN INDIA