CRS Controlled Release Society

كلية العلوم والتقنيات فاس
FACULTÉ DES SCIENCES ET TECHNIQUES DE FÈS

3D-Printed Antimicrobial Wound Dressing with Argan Nutshell Lignin & Essential Oils for Advanced Healing

Khaoula Sebbar (1), Masoud Adhami (2), Eneko Larraneta (2)*, Nada Al Moudani (1), Lotfi Aarab(1), Saad Koraichi Ibnsouda (1), Soumya Elabed (1)**

(1) Laboratory of Microbial Biotechnology and Bioactive Molecules, Faculty of Sciences and Technologies, Sidi Mohamed Ben Abdellah University of Fez BP 2202, Morocco.
(2) Belfast School of Pharmacy, Queen's University, 97 Lisburn Road, Belfast BT9 7BL, United Kingdom

INTRODUCTION

Problematic:

Modern wound care still heavily relies on **synthetic antibiotics and non-biodegradable materials**, which often fail to prevent infections caused by **antibiotic-resistant bacteria** and contribute to environmental and clinical complications.

Gaps in Current Solutions:

- Most available dressings are **expensive**, **non-renewable**, or **lacking biocompatibility**.

- **Essential oils**, despite their powerful antimicrobial and antioxidant properties, remain **underutilized** due to their volatility and instability.

- **Natural resources** and **Agro-waste** in developing countries like Morocco are **poorly valorized** for biomedical purposes.

- **This Work Pioneers a Low-cost, Bioactive, and Eco-responsible Wound Healing Solution, Made by Transforming Local Waste into High-value Medical Innovation.**

OBJECTIVES

1. Valorization of Moroccan Agro-Waste = Argan Nutshells :
Transform ***Argania spinosa* (Argan)** Nutshells, a locally abundant byproduct of Moroccan agriculture, into high-value **Lignin (LIG)** biopolymers, promoting circular economy principles and sustainable material sourcing.

2. Design of Cost-Effective, Tailor-Made Meshes via 3D Printing
Utilize **3D bioprinting technology** to fabricate custom porous wound dressings that ensure flexibility, high surface area, and low-temperature encapsulation, optimized for natural bioactive compound preservation and scalability.

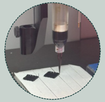
3. Replacement of Synthetic Antibiotics with Natural Agents :
Incorporate ***Mentha pulegium* (MPEO)** and ***Rosmarinus officinalis* (ROEO),** two Essential Oils (EOs) as potent antimicrobial and antioxidant alternatives, offering a greener strategy to combat infection and antibiotic resistance, while supporting fibroblast viability and tissue regeneration.

4. Integration of Eudragit as a Smart Polymeric Matrix :
Employ **Eudragit (EUD)** for its controlled-release properties, structural integrity, and biocompatibility, serving as a versatile encapsulating polymer for both LIG and EOs in biomedical applications.

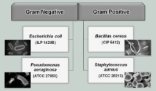
MATERIALS & METHODS

→ Materials :

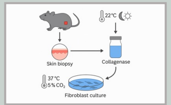
- ***Antimicrobial Agents:*** MPEO and ROEO,
- ***Biopolymer:*** Lignin Extracted from Agan,
- ***Synthetic polymer:*** Eudragit RS PO,
- ***Solvant***
- ***Plasticiser***
- ***Solubilizer***



→ Microorganisms :



→ Cell Culture :

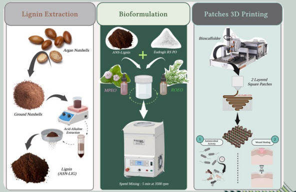


→ 3D Printing Formulations:

Table 1: Composition of the formulations that go into making each group of the 3D printed wound dressings.

Formulations	Composition (%)						
	Polymers				Solvant	Essential Oils	
	ANS-LIG	EUD RS PO	PEG	Soluplus	Acetone	MPEO	ROEO
Pure EUD	-	85%	5%	10%	35%	-	-
EUD+MPEO	-	75%	5%	10%	35%	10%	-
EUD+ROEO	-	75%	5%	10%	35%	-	10%
LIG/EUD	40%	35%	5%	10%	35%	-	-
LIG/EUD+MPEO	40%	35%	5%	10%	35%	10%	-
LIG/EUD+ROEO	40%	35%	5%	10%	35%	-	10%

→ Step by Step Process :



→ Characterizations of the 3D printed Wound Dressings :

1. *Morphological Characterizations*
2. *Chemical Characterization*
3. *Mechanical Characterization*
4. *Thermal Characterizations*
5. *Contact Angle Measurement*

6. *Antioxidant Activity*
7. *Swelling and Degradation Behaviors*
8. *Antibacterial Properties*
9. *Haemocompatibility Test*
10. *In Vitro Cytotoxicity Test*

RESULTS & DISCUSSIONS

1 Morphological Characterisations

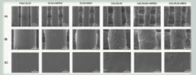


Figure 1: Scanning Electronic Microscope Surface Characterization of 3D-Printed Wound Dressings with Various Formulations: **(A)** SEM Images at Initial Magnification, **(B)** SEM Images at Increased Magnification, and **(C)** SEM Images at Highest Magnification. Scale Bars: 500 µm, 100 µm, and 10 µm, respectively.

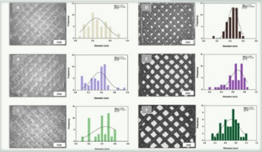


Figure 2: Light Microscope Images and Pore Diameter Distributions of 3D-Printed Wound Dressings: **(A)** Pure EUD, **(B)** EUD+MPEO, **(C)** EUD+ROEO, **(D)** LIG/EUD, **(E)** LIG/EUD+MPEO, and **(F)** LIG/EUD+ROEO.

2 Chemical Characterization

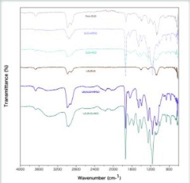


Figure 3: ATR-FTIR spectra of EUD powder with or w/o LIG, 3D-printed wound dressings containing MPEO or ROEO.

3 Mechanical Characterization

3DP Mesh	Mechanical Properties		
	Tensile strength (MPa)	Strain at break (%)	Young's Modulus (MPa)
Neat EUD	0,29 ± 0,26	1,12 ± 0,66	26.02 ± 25.8
EUD+MPEO	0,85 ± 0,25	7,73 ± 2,87	11.03 ± 5.2
EUD+ROEO	1,04 ± 0,32	17,26 ± 4,71	6.03 ± 2.5
LIG/EUD	6,39 ± 3,69	1,65 ± 0,63	387 ± 268
LIG/EUD+MPEO	36,81 ± 4,71	6,3 ± 0,62	584 ± 94
LIG/EUD+ROEO	23,06 ± 1,14	8,61 ± 4,14	268 ± 129

Table 2: The tensile and flexural mechanical characteristics involving the different tested wound dressings.

4 Thermal Characterizations

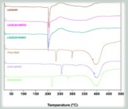


Figure 4: DSC thermograms of pure EUD, MPEO, and ROEO essential oils, as well as the ensuing 3DP samples of EUD control, LIG/EUD, EUD+EOs, and LIG/EUD+EOs.

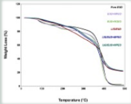


Figure 5: TGA thermograms of pure LIG, pure EUD, MPEO, and ROEO essential oils, as well as the ensuing 3DP samples of EUD control, LIG/EUD, EUD+EOs, and LIG/EUD+EOs.

5 Contact Angle Measurement

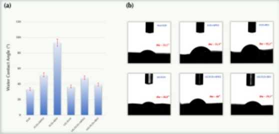


Figure 6: Assessment of Eudragit RS PO and Argan Shell Lignin Composite Wound Dressings, with and without MPEO or ROEO: (a) Contact Angle Visualization and (b) Quantitative Contact Angle Measurement.

6 Swelling & Degradation

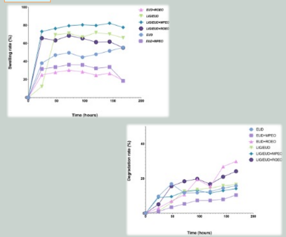


Figure 7: Swelling Kinetics and Degradation Behavior of the 3D-Printed Wound Dressings.

7 Antioxidant Activity

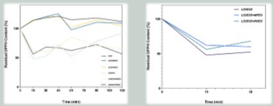


Figure 8: Time-Dependent DPPH Levels in LIG/EUD 3D-Printed Wound Dressings Infused with MPEO or ROEO, Starting from a 50 mg/L DPPH Concentration.

8 Antibacterial Properties

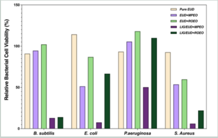


Figure 9: Evaluating the Antibacterial Power of 3D-Printed Wound Dressings: Pure EUD, EUD+MPEO, EUD+REO, LIG/EUD, LIG/EUD+MPEO, and LIG/EUD+REO against *B. subtilis* (a), *E. coli* (b), S. *aureus* (c), and *P. aeruginosa* (d) through Optical Density (OD) Assay – Results Averaged from Three Independent Trials.

9 Biocompatibility (MTT Assay)

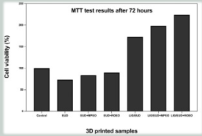


Figure 10: MTT-Based Mouse Dermal Fibroblast Cell Viability of Control and 3D-Printed EUD-Based Scaffolds Enriched with Lignin and Essential Oils After 72 Hours.

CONCLUSIONS

- **First-time use of Moroccan Argan Nutshell Lignin (ANS-LIG) was Successful**, by valorizing local agro-waste as a 3D-printed biopolymer for biomedical use.

- **Strong Antibacterial Performance using Local Essential Oils,** especially the LIG/EUD+MPEO Wound Dressing, which drastically reduced *S. aureus* (6%) and *E. coli* (7.5%) viability, proving its potency against wound pathogens.

- **Excellent Biocompatibility and Dermal Cell Proliferation, since all of the 3D-printed Patches** enhanced fibroblast viability, especially LIG/EUD+ROEO with up to 225%, confirming safe and potential regenerative interaction with skin cells.

AKNOWLEGEMENTS

| We gratefully acknowledge Professor *Larraneta E.* and his team at the School of Pharmacy QUB, for their valuable support & collaboration.

| We sincerely thank the Moroccan Ministry of Higher Education and the OCP Foundation for supporting this work through the APRD program.

CONTACT

| Khaoula SEBBAR
| PhD Student
| FST-Fez, MOROCCO
| Khaoula.sebbar@usmba.ac.ma