Fabrication and Characterization of Aloe Vera-Based Coatings via MAPLE: An Antimicrobial Strategy for Addressing Antibiotic Resistance through Essential Oil Influence

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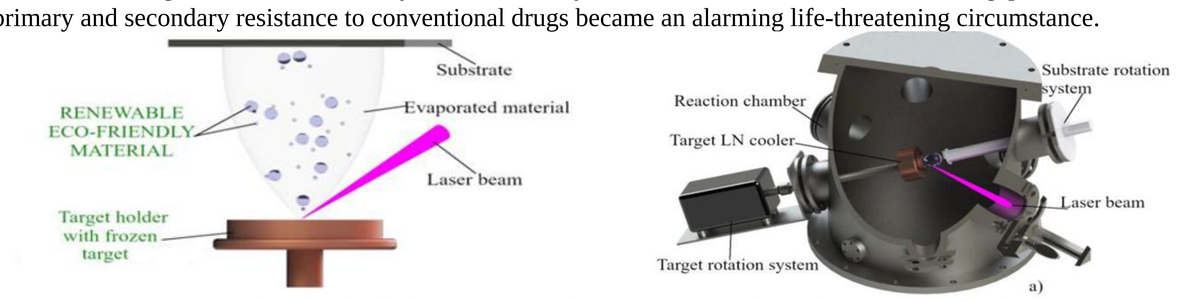
MOTIVATION AND AIMS

This study aims to obtain functionalized implants covered with innovative apatite-lignin-aloe vera (HA/Lig/AV) coatings fabricated by Matrix Assisted Pulsed Laser Evaporation (MAPLE).

The use of NATURAL AND RENEWABLE PRODUCTS **(Lignin and Aloe Vera plant extract)** for infections prevention is a green alternative for synthetic currently-used antibiotics, since the concerning phenomenon of primary and secondary resistance to conventional drugs became an alarming life-threatening circumstance.



Matrix Assisted Pulsed Laser Evaporation (MAPLE) experimental set-up and mechanism.

The use of these natural-derived products involves reduced costs and represents an attractive solution for the fabrication of biodegradable thin films with antibacterial, antioxidant and anti-inflammatory potential

MATERIALS AND METHODS

Study I

HA-Lig (MAPLE) - important source of natural antimicrobial and antifungal compounds.

Study II

HA-Lig-AV (MAPLE)-renewable, eco-friendly biomaterial

Investigated Sample Code

SAMPLE CODE
HA-Lig (DMSO)
HA-Lig-AV-recipe 1(DMSO)
HA-Lig-AV-recipe 2(DMSO)
HA-Lig-AV-recipe 3(DMSO)

KrF* excimer laser source: λ= 248 nm;pulse duration ≤ 25 ns, 10 Hz laser repetition rate; laser fluence of 300 mJ/cm^2; room temperature, a dynamic pressure of 2x10^{-5}mbar, 150 000 laser pulses, target-substrate separation distance 50 mm, spot size was set to 20 mm^2

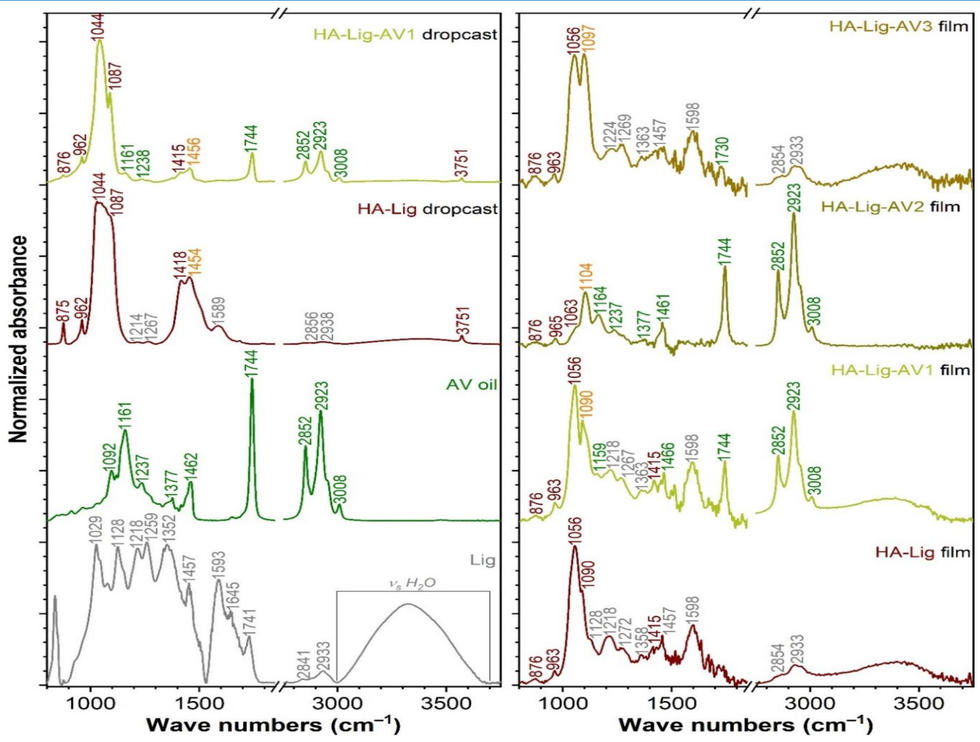
Targets: **HA-Lig** (10% w/v) and **HA-Lig-AV** (3 recipes) in **DMSO**

Substrates: Ti substrates,<111> single-crystalline Si wafers;glass

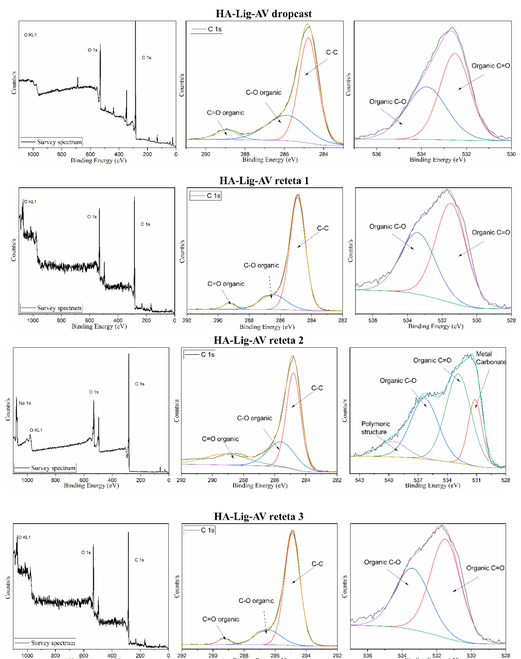
Adherence average values of investigated samples

Sample Code	Adherence average values ±SD (MPa)
HA-Lig-AV-recipe 1	19.5±4
HA-Lig-AV-recipe 2	29 ±0.5
HA-Lig-AV-recipe 3	28±0.5

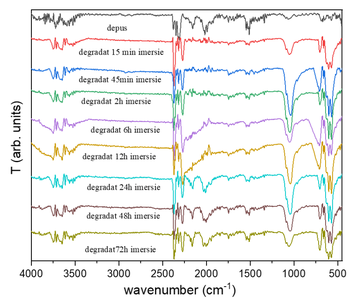
RESULTS AND DISCUSSIONS



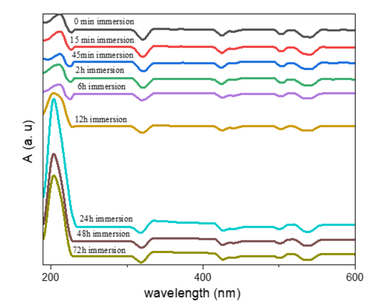
FTIR spectra of the investigated materials: Lignin powder; Aloe Vera essential oil, HA-Lig (dropcast and thin film); composite films: HA-Lig-AV1 (dropcast and thin film); HA-Lig-AV2 thin film; HA-Lig-AV3 thin film.



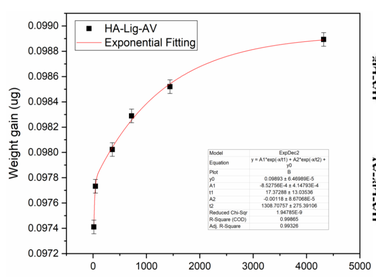
XPS spectra of the investigated materials



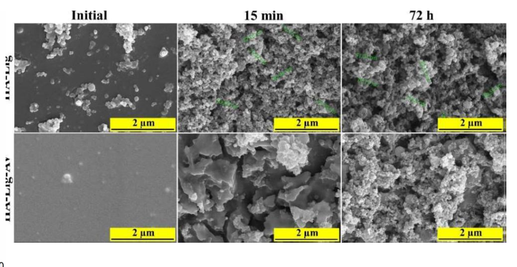
Time evolution of the absorbance of the solution



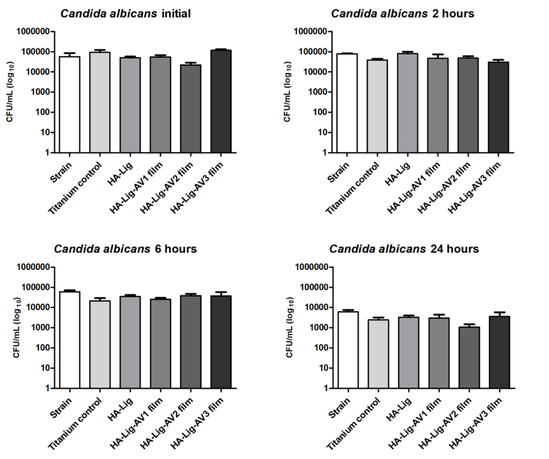
FTIR of degraded materials



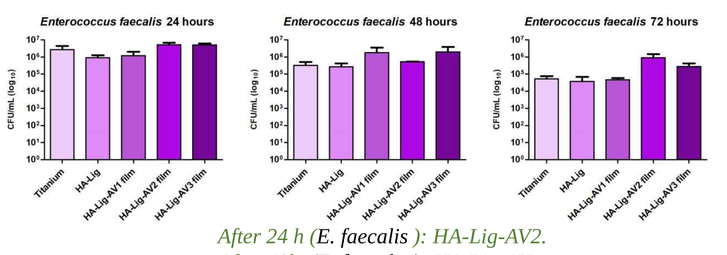
Weight gain, Δm, as a function of immersion time in SBF(15 min, 45min, 2h, 6h, 12h, 72h).



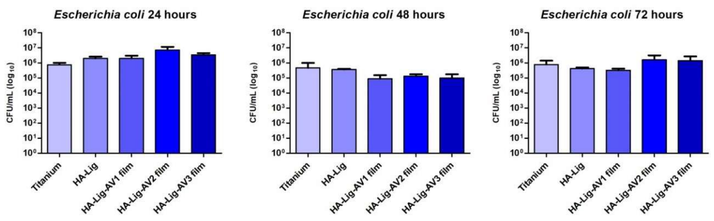
SEM micrographs of HA-Lig and HA-Lig-AV2 samples, deposited initially and at different degradation time intervals (15 min and 72h).



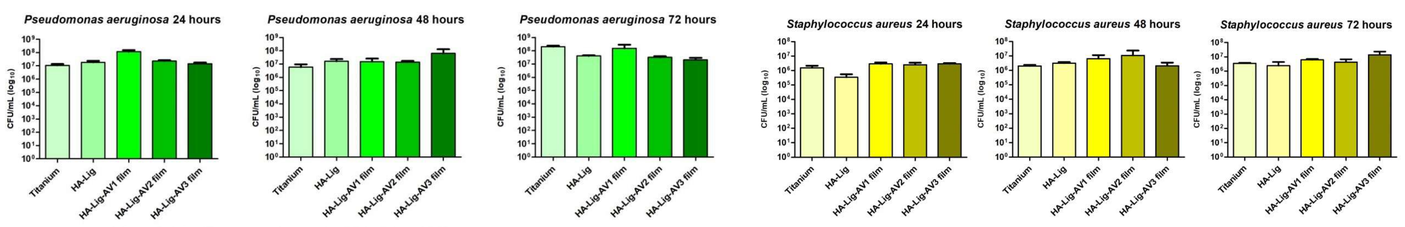
After 24 h (Candida albicans): HA-Lig-AV2.



After 24 h (E. faecalis): HA-Lig-AV2.
After 48h (E. faecalis): HA-Lig-AV1
After 72h (E. faecalis): HA-Lig-AV2



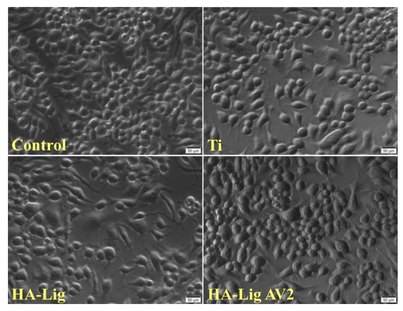
After 24 h (E. coli): HA-Lig-AV3.
After 48h (E. coli): HA-Lig-AV1
After 72h (E. coli): HA-Lig-AV1



After 24 h (P. aeruginosa): HA-Lig-AV3.
After 48h (P. aeruginosa): HA-Lig-AV2
After 72h (P. aeruginosa): HA-Lig-AV2

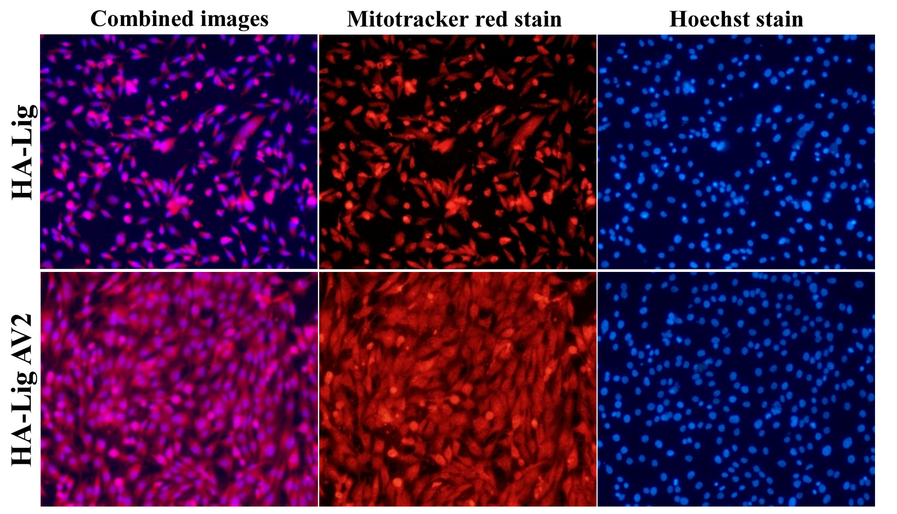
After 24 h (S. aureus): HA-Lig-AV2.
After 48h (S. aureus): HA-Lig-AV3
After 72h (S. aureus): HA-Lig-AV1

Evaluation of the dynamics of *Escherichia coli, Pseudomonas aeruginosa, S.Aureus, E.Faecalis* and *Candida albicans* viability in the presence of tested materials .



Analysis of cell morphology in the presence of AV2 lignin discs

Biocompatibility of investigated materials



Fluorescence microscopy images showing the morphology of MG63 cells maintained on the disk for 48 h. (staining ie mitotracker red – Hoechst, objective 20x)

CONCLUSIONS AND PERSPECTIVES

- Apatite-lignin-aloe vera (HA-Lig-AV) thin films were synthesized by a matrix-assisted pulsed laser evaporation technique.
- FT-IR, XPS revealed the presence of organic materials and proved the integrity of the chemical functions, and the stoichiometry of the unaltered deposited material.
- Tested materials lead to improved cell viability when co-cultured with osteoblasts, and proved better compared to the Ti control.
- Depending on the intended application, the optimal compromise can be made by modifying the recipe, for now, it has been proven that the HA-Lig-AV2 coating has an inhibitory effect in most case-studied strains and fungi.
- The hypothesis of biomineralization (i.e. layer formation of calcium phosphate biomimetic on the surface of the coatings) is supported by the SEM images and FT-IR, and UV-VIS analyses of the degraded films, thus changes in the initial surface morphology were evident after only 15 minutes of immersion in the SBF environment (agglomerations of particles with sizes varying in the range (30-70) nm). The process is intensified after 72h of degradation, observing how the films degraded by HA-Lig- AV2 are covered with an apparently thicker biomimetic layer of calcium phosphate. HA-Lig-AV2 has the maximum intensity after 24h of immersion, and then the absorbance intensity decreases, which can be explained by an HA deposition on the surface of the sample.

Perspectives: ***Introduction of active therapeutic compounds (anti-inflammatory) with different release kinetics!***

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