Scientific Report

regarding the implementation of the project between January - December 2021 (Stage II)

The overall objective of the proposal is to continue previous research and to find the best way to deliver the irradiated solutions for wound dressings in view of obtaining the best antimicrobial effect. In this respect, it is proposed to use UV photo-crosslinked hydrogels. Within this stage, the activities were: *photoinitiator rate of free radical production during irradiation; *fabrication of film hydrogel by photopolymerization; *characterization of hydrogel: swelling measurements, UV-Vis and FTIR absorption spectroscopy, scanning electron microscopy, LIF; *unirradiated and irradiated CPZ loading into film hydrogels; *characterization of the loaded hydrogel: UV-Vis and FTIR absorption spectroscopy, SEM, LIF, drug release assay.

Within this stage the following were realized:

-The photoinitiator rate of free radical production during irradiation.

-Fabrication of film hydrogel by photopolymerization.

-Characterization of hydrogel: swelling measurements, UV-Vis and FTIR absorption spectroscopy, scanning electron microscopy (SEM), LIF.

-Unirradiated and irradiated CPZ loading into film hydrogels.

-Characterization of the loaded hydrogel: UV-Vis and FTIR absorption spectroscopy, SEM, LIF and LIF lifetime, drug release assay.

Results

The irradiation source was a Continuum Nd: YAG laser (10 Hz, 6 ns FWHM) emitting at 266 nm. Photoinitiator solutions and photoinitiator-polymer mixtures were exposed at 266 nm for 1, 5, 10, 20, and 30 min at 0.25 mJ (I = 6.6 mW/cm^2), 0.45 mJ beam energies (I = 11 mW/cm^2), 0.75 mJ (I = 19.7 mW/cm^2), and 1 mJ (I = 26.3 mW/cm^2). The mold was 3D printed from polylactic acid and has an inside diameter of 0.7 cm and a height of 0.1 cm. The mold had a total volume of 35 µL.

The laser-induced fluorescence (LIF) signal was collected using a optic fiber and recorded using a SpectraPRo SP-2750 (Acton Research) spectrograph. Absorption spectra were recorded with a Perkin Elmer spectrophotometer, Lambda 950 model and FTIR spectra with Nicolet iS50 FTIR spectrometer.

The surface morphology of deposited films was inspected by scanning electron microscopy (SEM) with an FEI Inspect S electron microscope at 20 kV acceleration voltage in high vacuum using top-view and cross-section modes.

Photopolymerization with pulsed laser radiation has various advantages, including a quick crosslinking time due to the ability to vary the beam energy and select the excitation of chromophores due to monochromatic light. Fluorescence emission provides various advantages over conventional crosslink monitoring methods, including fast response time, high sensitivity, and non-invasive in situ research.

Irgacure 2959 (2-hydroxy-1- [4- (2-hydroxyethoxy) phenyl] -2-methyl-1-propanone), riboflavin, methacrylate gelatin, GelMa, (natural polymer), polyethylene glycol diacrylate, PEGDa, (synthetic polymer) and L-arginine were purchased from Sigma-Aldrich. The solvent used was ultrapure water. Concentrations of 0.05%, 0.35% and 0.7% were used for Irgacure 2959. The concentration of 0.7% is the maximum amount that could be dissolved in ultrapure water.

UV-Vis absorption spectroscopy was used to analyse Irgacure 2959 solutions, and the absorption spectra of non-irradiated and irradiated solutions at energies of 0.25 mJ, 0.45 mJ, 0.75 mJ, and 1 mJ are shown in Figure 1.

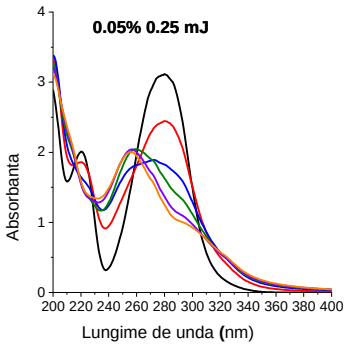

Figure 1. UV-Vis absorption spectra of Irgacure exposed to 266 nm laser pulsed radiation at various energies and concentrations.

Changes in the UV-Vis absorption spectra of Irgacure 2959 suggest photo-decomposition in the 25 benzoyl and hydroxyalkyl radicals.

The loss of the 1663 cm^{-1} band and the emergence of the 1725 cm^{-1} band was the first evidence that Irgacure 2959 had been photo-degraded (Figure 2). Both bands are attributed to the stretching vibration C = O, however the shift of the band to higher wave numbers supports ketone group cleavage. The disappearance of the band with a maximum of 1254 cm^{-1} also confirmed this (vibration of the C-C-C skeleton from the aromatic ketone). Furthermore, the bands responsible for C-H vibration and C-CH3 skeletal vibration in C-CH3, such as 1392, 1276, 1011, 985, and 976 cm^{-1}, promote Irgacure2959 photo-degradation into benzoyl and hydroxyalkyl radicals.

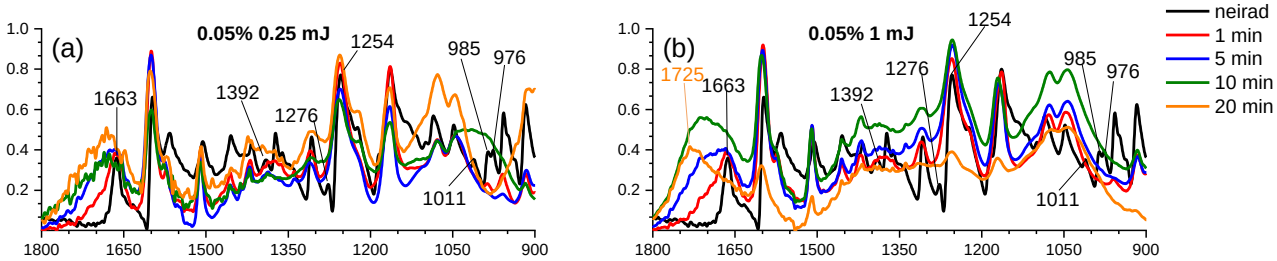

Figure 2. FTIR spectra of unirradiated and irradiated Irgacure 2959 solutions: (a) concentration 0.05% and energy 0.25 mJ; (b) 0.05% concentration and 1 mJ energy;

During irradiation, laser-induced fluorescence (LIF) was measured in real time. This method has the advantage of monitoring the photo-degradation of the Irgacure 2959 molecule in real time. Every 5 seconds, the LIF spectrum was collected. This approach offers the irradiated solution's fluorescence spectrum as well as its fluorescence kinetics profile (Figure 3).

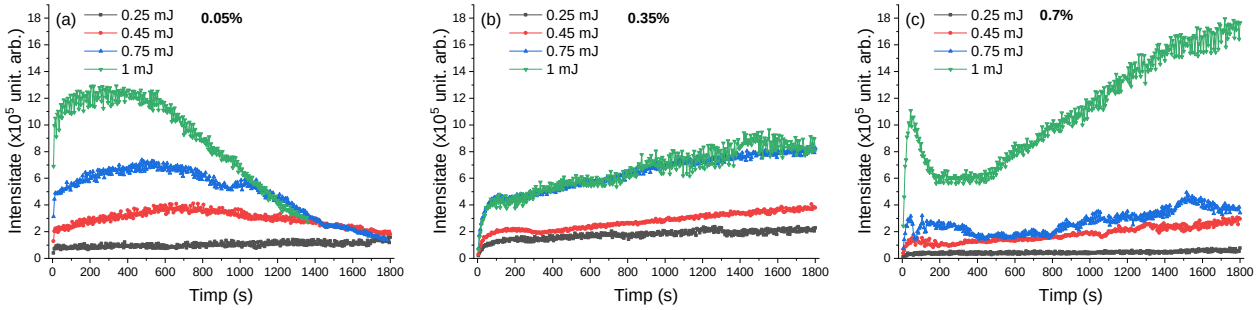

Figure 3. LIF kinetics profile for Irgacure 2959 irradiated solutions at energies of 0.25, 0.45, 0.75 and 1 mJ at concentrations: (a) 0.05%, (b) 0, 35%, (c) 0.7%.

To allow proper dissolution of each component, the polymer-photoinitiator solutions were stirred with a magnetic stirrer at 200 rpm for 30 min at 70 ° C. A 35 µL volume of the polymer-photoinitiator mixture solution was placed in the mold and exposed to pulsed laser radiation emitted at 266 nm under the same conditions as the simple photoinitiator solution, with exposure times of 1, 5, 10, 20, and 30 min and energies of 0.25 mJ, 0.45 mJ, 0.75 mJ, and 1 mJ.

Hydrogels were removed from the mold and immersed in ultrapure water (3 mL) at room temperature for 24 h to eliminate precursor residues and reach absorption equilibrium (detection of non-reactive compounds). They were dried in a desiccator for 24 h before being stored in a dark desiccator at 4^{o}C.

The following samples were prepared: Irgacure 2959 (0.05%, 0.35% and 0.7%) + GelMa (10%), Irgacure 2959 (0.7%) + GelMa (15%), Riboflavin (0, 05%, 0.7%) + L-arginine (0.1%) + PEGDa (10%), Irgacure 2959 (0.05%, 0.35% and 0.7%) + PEGDa (10%). These were subjected to beam intensities of 0.25, 0.44, 0.75, and 1 mJ at time periods of 1, 5, 10, 20, and 30 minutes. Irgacure 2959 (0.05 %) + GelMa were the only suitable hydrogel formed. Even after 60 min of 1 mJ exposure, no hydrogels were generated for Riboflavin + L + arginine-PEGDa. For the remaining combinations, hydrogels in the form of thin films were produced.

The dry hydrogels were incubated for 24 h in one mL of unirradiated CPZ. After that, the hydrogels were removed, placed on a vessel, and dehydrated. These hydrogels were subjected to the following testing methods: swelling behaviour, UV-Vis and FTIR absorption spectroscopy, scanning electron microscopy (SEM), and laser induced fluorescence (LIF).

The irradiated samples 1 min had the greatest degree of swelling, depending on the irradiation time. After 1 min, the light curing process produces longer polymer chains, causing the hydrogels to stiffen and absorb less water. The best swelling ratio was observed when I 0.05 % + 10% GelMa was irradiated for 1 min at 0.75 mJ. The hydrogels turned yellow when exposed above 5-10 min, hence these conditions were deemed unsuitable.

Before usage, the chlorpromazine (CPZ) loaded hydrogels were dehydrated. They were immersed in 1 mL of Mueller-Hinton culture medium and phosphate buffered saline and incubated at 37 ° C for varying times (2, 4, 8, 24 and 48 h). Figure 4 presents the CPZ release in Mueller-Hinton culture medium

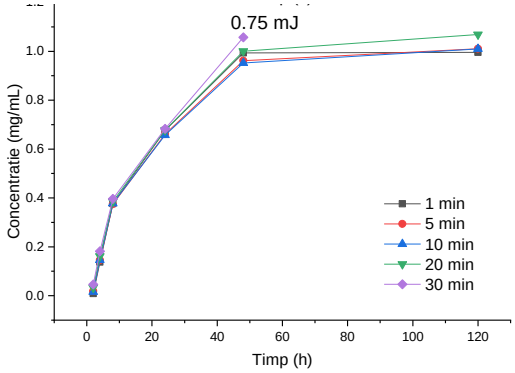

Figure 4. CPZ release in Mueller-Hinton culture medium

For all energies employed, a continuous release of CPZ in MH and PBS was seen during a 48-hour period. Hydrogels formed at energies of 0.25 mJ in both MH and PBS had the lowest released concentration.

In the IR spectra of dry hydrogels, only the presence of GelMA is observed, with no changes between the hydrogels resulting from different irradiation periods. As a result, the residues of the precursors were eliminated from the hydrogels. In addition, after the CPZ release studies, the IR spectra of the hydrogels were examined, and no CPZ was found in the dried hydrogels. At the same time, the FTIR spectrum of the hydrogels revealed no substantial changes in the polymer structure during the photopolymerization process.

For the mixture of Irgacure 2959 (0.7%) and GelMa (10%) solutions, the changes induced in the fluorescence kinetics profiles are different from those resulting from the irradiation of the Irgacure 2595-GelMa mixture where the photoinitiator concentration was 0.05%. And in this case, for the samples containing GelMa, a significant decrease of the fluorescence intensity was observed compared to the one resulting from the irradiation only of the Irgacure 2959 solution, suggesting the formation of the hydrogel. As in the case of using only Irgacure 2959 solution at a concentration of 0.7%, water evaporation was observed.

After immersion of the CPZ unirradiated and irradiated loaded hydrogels in one mL of bacterial suspension of S. aureus ATCC 29523, the number of colonies was expressed in CFU/mL. It was observed that both CPZ unirradiated-loaded hydrogel and CPZ irradiated -loaded hydrogel, completely prevented the adhesion of bacterial colonies and the formation of biofilms on the surface of the samples.

In this way the objectives of the project for this stage were met and the estimated results were obtained.

Dissemination:

International conference:

1. T. Tozar; M. Boni; S. Nistorescu; ML. Pascu; A. Staicu; Photodegradation study of Irgacure 2959 during hydrogel formation via 266 nm pulsed laser radiation; OSA Biophotonics Congress: Optics in the Life Sciences, virtual conference, 12–16 April 2021, USA - poster presentation.

2. T. Tozar; M. Boni; S. Nistorescu; ML. Pascu; A. Staicu; Hydrogels photo-crosslinking by 266 nm pulsed laser radiation; 9th International Conference on Radiation in Various Fields of Research, 14-18 June 2021, Montenegro - poster presentation

3. T. Tozar; M. Boni; S. Nistorescu; ML. Pascu; A. Staicu; Irgacure 2959 photodegradation study during hydrogel synthesis using 266 nm pulsed laser light; 23rd International Conference Materials, Methods & Technology, 19-22 August 2021, Bulgaria - oral presentation

Articles:

1. T. Tozar, M. Boni, I. R. Andrei, M. L. Pascu, A. Staicu, "High performance thin layer chromatography-densitometry method based on picosecond laser-induced fluorescence for the analysis of thioridazine and its photoproducts", JOURNAL OF CHROMATOGRAPHY A; 1655, 462488 (2021). Rank according to Web of Science, year of publication: Q1; IF 4.759 / AIS 0.631.
2. T. Tozar, M. Boni, A. Staicu, M. L. Pascu, "Optical characterization of ciprofloxacin photolytic degradation by UV-pulsed laser radiation", MOLECULES 26, 2324 (2021). Rank according to Web of Science, year of publication: Q2; IF 4.411 / AIS 0.694

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