Scientific Report

regarding the implementation of the project between September - December 2020 (Stage I)

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 objectives were: *update the concept of the project to last-minute published progress to reach the aims specified in the project proposal; *irradiation of chlorpromazine solutions using 266 nm emitted by Nd:YAG laser at 6.5 mJ.

Within this stage were obtained the following results:

1. Update the concept of the project to last-minute published progress to adapt the research in view of reaching the aims specified in the project proposal.
2. Irradiation of chlorpromazine (CPZ) solutions at 266 nm, the fourth harmonic of the pulsed beam emitted by Nd:YAG laser.

1. Update the concept of the project to last-minute published progress to adapt the research to reach the aims specified in the project proposal.

In the case of chronic wounds, the potential for infection and colonization increases due to the presence of avascular ulcers, which provide an favorable environment for uninhibited growth of microorganisms [1]. The rate of infection depends on the type of wound, its mode of care and the general health of the patient [2]. Thus, products are needed to treat wounds, that are more economical and effective and that provide an optimal healing environment for the wound.

In this sense, hydrogels were created, both natural and artificial, in order to be used in healing wounds. Hydrogels can make the most of the beneficial results of therapy, by improving their effectiveness and reducing the toxicity and doses required; this is because hydrogels can provide spatial and temporal control over the release of therapeutic agents, including small molecule drugs, macromolecular drugs and even cells.

Of all the hydrocolloid dressings, alginate or hydrogels, each with its own advantages and limitations, hydrogels are the best and have all the characteristics necessary for an ideal dressing. Hydrogels are made of crosslinked polymers, natural or synthetic, and are used in a variety of biomedical fields. They are used in the administration of medicines, wound dressings, contact lenses and as electrodes or sensors. [3]. Various polymers with good bio-compatibility are used to form hydrogels. The natural ones are alginate, chitosan, gelatin and collagen, and the synthetic ones are polyurethane, poly (ethylene glycol), polycaprolactone, poly (vinyl pyrrolidone), poly (lactide-co-glycolide), polyacrylonitrile, or poly (amino acids).

Hydrogels can provide spatial and temporal control over the release of the therapeutic agents, including small molecule drugs, macromolecular drugs, and even cells. Conventional drug administration often requires high doses or repeated administration to stimulate a therapeutic effect, which can reduce the overall efficacy and lead to severe side effects, even toxicity. [4], [5]. In this sense, hydrogels can make the most of on the beneficial results of therapy by improving their effectiveness and reducing the toxicity and dose.

Hydrogels can be produced by exposing polymers to UV radiation, the process being a suitable tool due to easier processing compared to chemical or freeze-drying techniques. More, irradiation makes possible hydrogel formation and sterilization in one step. Photopolymerization uses light to dissociate the initiator into free radicals, which react with the double bonds of crosslinking monomers or pre-polymers. [6].

Polymers and initiators selection

To meet the project objectives, the following polymers and initiator were chosen for the production of hydrogels by photopolymerization: natural polymer - methacrylate gelatin (GelMA), synthetic polymer - polyethylene glycol diacrylate (PEGDA), photoinitiator type I - Irgacure 2592 and photoinitiator type II - riboflavin (co-initiator - L-arginine) and a commercial kit for the formation of hydrogels - EncapGel-UV Kit. The polymers, photoinitiators and co-initiator are water-soluble, non-cytotoxic (generate only a minimal immune response) and biocompatible.

Table 1 presents the crosslinking conditions of the polymers, conditions identified in the literature.

Table 1. Photopolimerization conditions identified found in the literature for GelMA and PEGDA using photoinitiators such as Irgacure 2959 or Riboflavin.

Polymer/ conc.	**photiniţiator/ conc.**	**Co-initiator/ conc.**	**$\lambda_{xcitation}$ (nm)**	**Intensity/ fluence**	**Exposure time**	**Mold**	**Observations / Reference**
GelMa C=2.5-20 % w/v	Irgacure 2959 C=0.05%, 0.1%, 0.5%, 1%	-	365±20	2-150 mW/cm^2	290 s- 4 s	NA	The total energy flow for crosslinking the GelMa solution can be minimized by using low intensity radiation coupled with a high concentration of photoinitiator [7].
GelMa + PEGDA C=10%+5% C=20%+5% C=30%+5% w/v	Irgacure 2959 C=0.1% w/v	-	365	90 mW/cm^2	10 min	NA	Polymers and photoinitiator were dissolved in PBS The hydrogels were dried by lyophilization [8]
PEGDA C=98% w/w	Irgacure 2959 C=2% w/w	-	365	1273 mW/cm^2	15 min	- two glass slides with a controlled distance	fCNTs were added prior to polymerization (10% of PEGDA volume) [9]
PEGDA C=20-100% wt	2-hydroxy-2-methyl-propiophenone C= 0.5-5% wt%	-	365	0.5-5 mW/cm^2	1-20 min	-made from aluminum -16x8x2 mm	For 5 mW/cm^2 the hydrogel degrades. A smaller amount of photoinitiator requires a longer irradiation time [10].
GelMA / PEGDA C= 7.5% w/v C= 12.5% w/v C=3.75%+3.75%	Irgacure 2959 C=0.5% w/v	-	365	1.88 J/cm^2 3 J/cm^2	NA	-diameter:6.5 mm / 10 mm -height: 1 mm	The polymers were dissolved in DMEM culture medium with 1% penicillin-streptomycin at 65 ºC under stirring conditions. [11]
PEGDA C=20% C=40% w/v	Irgacure 2959 C=0.05% w/v C=0.1% w/v	-	365	NA	30 min	two microscope slides separated by spacers of 0.3 mm and 0.6 mm	Studies on the encapsulation and release of theophylline. Rhodamine B permeability studies using a laboratory-made device. [12]
GelMa C=20% w/v	Irgacure 2959 C=0.5% w/v	-	365	2.6 mW/cm^2	30 min	-PTFE -diameter: 5mm -height: 1.8 mm -volume:35 µL	Incorporation of Abraxane into hydrogels [13]
GelMa C=20% w/v	Irgacure 2959 C=0.5% w/v	-	365	3.5 mW/cm^2	5 min	-PTFE -diameter: 8 mm -height: 1 mm	[14]
Dextran methacrylate C=25% w/v	Riboflavin C=0.01%-20% wt	L-arginine C=0.1-100% wt	Ecolux, FI718-SP-35-ECO	NA	15-40 min	-Teflon -height: 1 mm	Hydrogel lamp-precursor distance = 15 cm. The optimal concentration of α-arginine was between 5 and 10 % (wt) [15].

Note: NA-information were not provided

2. Irradiation of chlorpromazine solutions using 266 nm from Nd:YAG at 6.5 mJ.

CPZ solutions at a concentration of 2 mg/mL and a volume of 2 mL were irradiated with a laser beam emitted at 266 nm as the fourth harmonic of the Nd: YAG laser (10 Hz, 6 ns FWHM). Laser radiation exposure times were 1, 5, 15, 30 and 60 minutes. The samples were investigated by UV-Vis and FTIR absorption spectroscopy and laser-induced fluorescence.

The UV-Vis absorption spectrum of the unirradiated CPZ in Figure 1a is characterized by two absorption bands with maxima at 254 nm and 307 nm. Following irradiation, the intensity of the band at 254 nm shows a hypochromic shift until the end of irradiation. The band at 315 nm suffers a 20 nm bathochromic shift and is companied by its broadening.

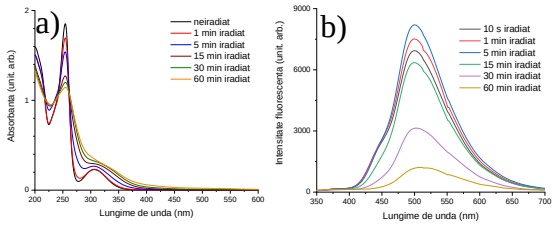

Figure 1. a) Absorbance spectra of unirradiated CPZ and irradiated CPZ solutions for 1, 5, 15, 30, 60 min.
b) Fluorescence spectra of unirradiated COZ and irradiated CPZ solutions for 1, 5, 15, 30, 60 min.

The LIF spectrum of the CPZ solution, with a concentration of 2 mg/mL, irradiated for 60 minutes is characterized by the presence of a single band with a maximum at 499 nm (Figure 1b). The fluorescence intensity increases during the first 5 minutes of irradiation and then begins to decrease.

The IR spectra of unirradiated and irradiated CPZ solutions are shown in Figure 2.

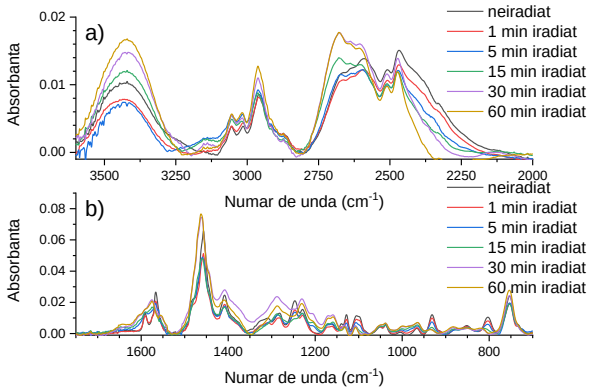

Figure 2. FTIR spectra of unirradiated CPZ and irradiated CPZ for 1, 5, 15, 30, 60 min in the domain: a) 3600-2000 cm-1 and b) 1750-700 cm-1.

The appearance of the two bands at 1205 cm^{-1} și la 1086 cm^{-1} indicates the attachment of phenol groups to the molecular structure of CPZ and the generation of oxidative forms.

Conclusions

During this stage, a documentation was made on project concept, establishing the following: the polymers and photoinitiators to be used, the photopolymerization protocol and the analysis methods of the hydrogels formed. CPZ solutions were also irradiated and analyzed by UV-Vis and FTIR absorption spectroscopy and laser-induced fluorescence.

References

[1] E. Greenfield and A. T. McManus, "Infectious complications: prevention and strategies for their control," *Nurs. Clin. North Am.*, vol. 32, no. 2, pp. 297–309, 1997.

[2] J. B. Wright, K. Lam, and R. E. Burrell, "Wound management in an era of increasing bacterial antibiotic resistance: A role for topical silver treatment," *Am. J. Infect. Control*, vol. 26, no. 6, pp. 572–577, 1998, doi: 10.1053/ic.1998.v26.a93527.

[3] B. Gupta, R. Agarwal, and M. S. Alam, "Hydrogels for wound healing applications," in *Biomedical Hydrogels*, Elsevier, 2011, pp. 184–227.

[4] R. Langer, "Drug delivery and targeting," *Nature*, vol. 392, no. 6679 Suppl, pp. 5–10, 1988.

[5] W. B. Liechty, D. R. Kryscio, B. V. Slaughter, and N. A. Peppas, "Polymers for Drug Delivery Systems," *Annu. Rev. Chem. Biomol. Eng.*, vol. 1, no. 1, pp. 149–173, 2010, doi: 10.1146/annurev-chembioeng-073009-100847.

[6] E. A. Kamoun, A. Winkel, M. Eisenburger, and H. Menzel, "Carboxylated camphorquinone as visible-light photoinitiator for biomedical application: Synthesis, characterization, and application," *Arab. J. Chem.*, vol. 9, no. 5, pp. 745–754, 2016, doi: 10.1016/j.arabjc.2014.03.008.

[7] C. D. O'Connell *et al.*, "Tailoring the mechanical properties of gelatin methacryloyl hydrogels through manipulation of the photocrosslinking conditions," *Soft Matter*, vol. 14, no. 11, pp. 2142–2151, 2018, doi: 10.1039/C7SM02187A.

[8] Y. Wang *et al.*, "Development of a Photo-Crosslinking, Biodegradable GelMA/PEGDA Hydrogel for Guided Bone Regeneration Materials," *Materials*, vol. 11, no. 8, p. 1345, 2018, doi: 10.3390/ma11081345.

[9] P. Loginos, A. Patsidis, and V. Georgakilas, "UV-cured poly(ethylene glycol) diacrylate/carbon nanostructure thin films. Preparation, characterization, and electrical properties," *J. Compos. Sci.*, vol. 4, no. 1, p. 4, 2020, doi: 10.3390/jcs4010004.

[10] Z. F. Zhang, X. Ma, H. Wang, and F. Ye, "Influence of polymerization conditions on the refractive index of poly(ethylene glycol) diacrylate (PEGDA) hydrogels," *Appl. Phys. A*, vol. 124, no. 4, p. 283, Apr. 2018, doi: 10.1007/s00339-018-1713-3.

[11] A. Vila *et al.*, "Hydrogel co-networks of gelatine methacrylate and poly(ethylene glycol) diacrylate sustain 3D functional *in vitro* models of intestinal mucosa," *Biofabrication*, vol. 12, no. 2, p. 025008, 2020, doi: 10.1088/1758-5090/ab5f50.

[12] K. O'Donnell, A. Boyd, and B. J. Meenan, "Controlling fluid diffusion and release through mixed-molecular-weight poly(ethylene) glycol diacrylate (PEGDA) hydrogels," *Materials*, vol. 12, no. 20, p. 3381, 2019, doi: 10.3390/ma12203381.

[13] M. Vigata, C. Meinert, S. Pahoff, N. Bock, and D. W. Hutmacher, "Gelatin Methacryloyl hydrogels control the localized delivery of albumin-bound paclitaxel," *Polymers*, vol. 12, no. 2, p. 501, 2020, doi: 10.3390/polym12020501.

[14] M. Zhu, Y. Wang, G. Ferracci, J. Zheng, N.-J. Cho, and B. H. Lee, "Gelatin methacryloyl and its hydrogels with an exceptional degree of controllability and batch-to-batch consistency," *Sci. Rep.*, vol. 9, no. 1, p. 6863, 2019, doi: 10.1038/s41598-019-42186-x.

[15] S. Kim and C.-C. Chu, "Visible light induced dextran-methacrylate hydrogel formation using (−)-riboflavin vitamin B2 as a photoinitiator and L-arginine as a co-initiator," *Fibers Polym.*, vol. 10, no. 1, pp. 14–20, 2009, doi: 10.1007/s12221-009-0014-z.

Dissemination:

International conference:

1. I. Negut, C. Ristoscu, T. Tozar, V. Grumezescu, C. Hapenciuc, C. Mihailescu, L. Floroian, I.N. Mihailescu; MAPLE double structures bioglass–PMMA as potential anticorrosive, antimicrobial and drug delivery platforms; "Tehnologii Emergente în Ingineria Materialelor – EmergeMAT", 29-30.10.2020, Bucuresti, Romania.

Principal Investigator,

Tozar Tatiana