PROJECT TITLE

NEW NANOPARTICLES BASED ON APTAMER-FUNCTIONALIZED CHITOSAN FOR TARGETING TUMOR CELLS (NANOROWAL)

Project manager: prof.*em*.dr.ing.*Dr.H.C.* Marcel POPA

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ABSTRACT

The project will be developed on the basis of a partnership between the "Gheorghe Asachi" Technical University of Iasi, "Apollonia" University of Iasi and the Center for Education and Research in Macromolecules, University of Liège, the partnership being governed by a partnership agreement. The aim of this project is to develop new chitosan based nanocapsules with an aptamer, ionic and covalent crosslinking, to target tumors and deliver an antitumor drug in a sustained way. To increase solubility in aqueous medium to neutral pH, in a first step the Belgian partner will achieve the production of water-soluble chitosan by carboxyl group grafting, and in the second step the previously obtained carboxymethyl chitosan will be modified with unsaturated methacrylate groups. These double bonds will serve for grafting the AS1411-SH aptamer through a click reaction. The Romanian partners will obtain nanoparticles based on this functionalized chitosan which they will characterize physico-chemicaly and morphologically, and the samples will also be analyze for their biomaterial properties. The ability of these particles to preferentially target tumor cells will be studied on cell cultures. The two partners will participate in the characterization in terms of antitumor activity of the most promising drug-loaded nanoparticles based on chitosan functionalized with aptamer.

PARTNERS

Technical University "Gheorghe Asachi" from Iasi
Legal Representative (Rector / Director): Ion GIURMA
Chief Financial Officer / Chief Accountant: Mariana CRIVOI
Legal Advisor: Mirela TROIA
Project Director (CO): Marcel POPA

APOLLONIA University in Iasi
Legal Representative (Rector / Director): Rodica GHIURU
Economic Director / Chief Accountant: Raluca POPOVICI
Legal Advisor: Alex TOMA
Project Leader (P1): Leonard Ionut ATANASE

Université de Liège
Legal Representative (Rector / Director): Albert CORHAY
Project Manager (P2): Christine JEROME

PROJECT TEAM

Coordinator (CO): Technical University “Gheorghe Asachi” Iasi
POPA MARCEL – project manager
PEPTU CATALINA ANISOARA– researcher
STAN CORNEL SERGIU – researcher
SAVIN CORINA LENUTA – researcher
TINCU CAMELIA ELENA – researcher

Partner 1 (P1): “Apollonia” University from Iasi
ATANASE LEONARD IONUT – partner responsible

Partner 2 (P2) : Université de Liège
CHRISTINE JEROME – partner responsible
RAPHAËL RIVA – researcher
LECOMPTE PHILIPPE - researcher

GENERAL OBJECTIVE

Obtaining chitosan-based innovative nanocarriers functionalized with aptamer for the active target of tumor cells

SPECIFIC OBJECTIVES

1. Synthesis of the carboxylate chitosan derivative with high solubility in water.
2. Synthesis of a carboxylate chitosan derivative functionalized with the aptamer AS1411-SH.
3. Physical and chemical characterization of new functionalized surface nanoparticles with AS1411-SH aptamer, with predefined characteristics: diameter, surface characteristics, dimensional polydispersity.
4. Evaluation of behavior in different physiological environments and the ability to encapsulate an antitumoral drug (5-fluorouracil).
5. Testing of biological tolerance (toxicity, biocompatibility, biodegradability, etc.) for selected samples
6. Evaluation of in vitro release kinetics of an antitumoral drug (5-fluorouracil)

RESULTS

Carboxymethyl chitosan (CCs) was synthesized by reacting chitosan with acetic acid monochloride in a concentrated NaOH solution under heterogeneous conditions. This basic treatment was aimed at a hydrogen partial substitution of the primary hydroxyl groups of the chitosan, allowing monochloro acetic acid grafting corresponding to a SN2 mechanism, which has led to increase its solubility in water. The products obtained were characterized physico-chemical. Obtaining the desired product was evidenced by spectral analyzes (FTIR, ^{1}H-NMR).

Cs was used for the introduction of crosslinkable methacrylate side groups by his reaction with glycidyl methacrylate, in water, on the basis of a transesterification mechanism, spectral analysis (FTIR, ^{1}H-NMR) showing the preparation of the desired product.

The grafting of the aptamer AS1411-SH to the CCs-M chain was performed by addition of thiol group to the unsaturated methacrylic group according to a "click" thiol-ene reaction, as shown below (Fig.1).

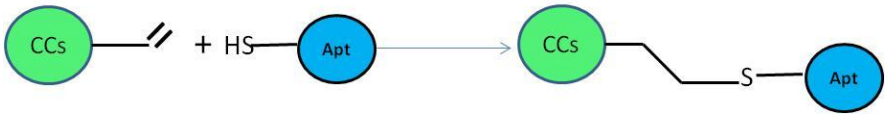

Fig.1. Preparation mechanism of CCs-Apt

Based on CCs-M, nanoparticles (NPs) were subsequently obtained by double crosslinking of the modified polysaccharide in the reverse emulsion using non-toxic ionic crosslinkers (sodium sulfate, sodium tripolyphosphate). The covalent crosslinking, required to provide structural stability of the nanoparticles, was accomplished with glutaraldehyde at the -NH_2 groups of chitosan.

Fig. 2 shows the electron microscopy photograph of some nanoparticles, highlighting their morphology and the diameter, which is in the order of hundreds of nm.

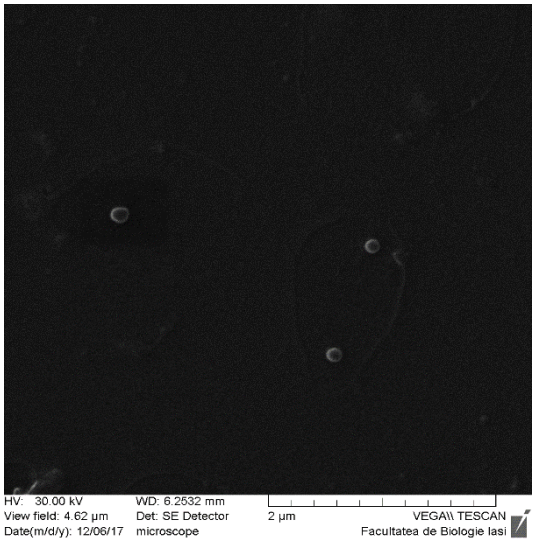

Fig. 2. Electronic microscopy of some nanoparticles based on CCs-M.

Were also prepared nanocapsules based on a mixture of aptamer-modified and unmodified chitosan by an interfacial condensation reaction with poly(itaconic alhydride-alt-N-vinyl pyrrolidone) (poly-NVAPAI)). The higher capability of encapsulation of drugs by such nanocarriers has led us to make further studies on this type of nanocapsules.

The degree of swelling (Q) of aptamer-functionalized nanocapsules (NCA) in a slightly basic aqueous medium (pH = 7.4) - an important property that decides the amount of encapsulated drug - is between 1240-1680% and decreases with the increase in the ratio between Cs and NVPAI, implicitly increasing the amount of chitosan in the system. In a poorly basic environment, the carboxylic groups pass into the carboxylate anion. These cause electrostatic rejection, so increasing the amount of chitosan carboxylate in the nanocapsule composition causes an increase in swelling.

Nanocapsules pre-sterilized by UV irradiation, placed on cellular fibroblast cultures, retain high cell viability. It is found that both drug-free and drug-loaded nanoparticles show no cytotoxic effects at high concentrations and do not inhibit proliferation. The obtained results reveal that cell viability decreases as the amount of nanocapsules exposed to fibroblast cells increases, and increases with increasing Cs/NVPAI ratio, implicitly increasing the amount of chitosan/mixture of chitosan and chitosan carboxylate functionalized with aptamer from the initial polymer system.

The 5-FU encapsulation efficiency in the synthesized nanocapsules varies between 28% and 40% and the 5-FU release process from the aptamer functionalized nanocapsules in phosphate buffer solution (pH = 7.4) at 32 ± 0, 5 ° C is illustrated in Fig. 3. The release efficiency is between 42% and 55% depending on the type of nanocapsule.

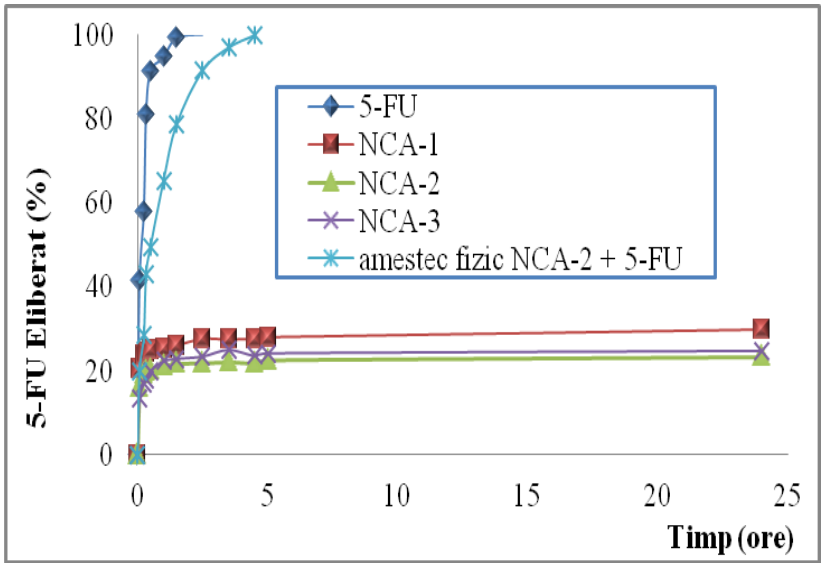

Fig. 3. Time evolution of in vitro release of 5-FU from nanocapsules in phosphate buffer solution (pH = 7.4)

Increasing the amount of chitosan, but also conjugating aptamer to nanocapsules led to a decrease in the percentage of drug released in the studied aqueous medium.

During the project implementation the proposed objectives were met. All scientific objectives have been fully met. Studies of *in vivo* and ex vivo release of the antitumor drug are underway and will be completed by the end of this year.

The proposed mobility was not fully met due to the reduction in funding, both from the Romanian and Belgian side.

Thus, the Belgian partner made four mobilities in Romania, namely:
- Prof. Jérôme Christine made two mobilities in Romania, when he also made trips to the "Stefan cel Mare" University of Suceava in order to perform some analyzes of the products obtained in his laboratory.
- Dr. Raphäel Riva made a mobility in Romania, and he also traveled to "Stefan cel Mare" University of Suceava to perform some analysis of the products obtained in his laboratory.
- Dr. Philippe Lecomte has made a mobility in Romania

Four members of the Romanian team made mobilities at the University of Liège / Belgium as follows:
- Prof. Marcel Popa carried out 2 mobilities
- PhD student Corina Savin has made a mobility
- Prof. Leonard Atanase has made a mobility
- PhD Camelia Elena Iurciuc (Tincu) made a mobility

Prof. Marcel Popa made 3 mobilities at the "Stefan cel Mare" University of Suceava, in which the Belgian partners were involved in conducting analyzes of the reaction products they had obtained.

Within these mobilities, research has been carried out in the Belgian partner's laboratory, as well as physico-chemical analysis of some products obtained in Romania.

DISSEMINATION

1. D.M. Rata, A.N. Cadinoiu, L. Atanase, C. Tincu, M. Popa „**Poli(N-vinil pirolidona-*alt*-amhidrida itaconica) – precursor pentru nanoparticule purtatoare de medicamente**”, 2017 *Sesiunea Ştiinţifică Anuală* a Institutului de Cercetări „Acad. Ioan Hăulică” al Universității „Apollonia”, 27 octombrie, Iasi

2. L.I. Atanase, A. Cadinoiu, D.M. Rata, M. Popa “**Functionalisation of chitosan with aptamer for targeted drug delivery**” *International Conference on Nanomaterials: Synthesis, Characterization and Applications (ICN 2018)***,** 11- 13 May 2018, Kottayam, Kerala, India

3. C. Mihalache, D.M. Rata, A.N. Cadinoiu, L.I. Atanase, M. Popa “**Nanoparticule functionalizate cu aptamer pentru terapia tintitat avand potentiale aplicatii in nanomedicina**” *Sesiunea stiintifica anuala a Institutului de Cercetari “Acad Ioan Haulica”al Universitatii “Apollonia” din Iasi,* 26 octombrie 2018

4. O.M. Daraba, D.M. Rata, A.N. Cadinoiu, L.I. Atanase, M. Popa, L. Ichim, C. Stadoleanu, V. Burlui “**Evaluarea citotoxicitatii unor nanoparticule functionalizate cu aptamer (cu si fara medicament**” *Sesiunea stiintifica anuala a Institutului de Cercetari “Acad Ioan Haulica”al Universitatii “Apollonia” din Iasi,* 26 octombrie 2018