BIOEVALUAREA MATERIALELOR HIBRIDE ARGILE - MEDICAMENTE UTILIZATE ÎN APLICAȚII BIOMEDICALE CA SISTEME CU ELIBERARE CONTROLATĂ – PARTEA A II-A

BIOEVALUATION OF DRUG - MINERAL CLAY HYBRID MATERIALS FOR BIOMEDICAL APPLICATIONS AS DRUG DELIVERY SYSTEMS – PART II

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In this study, the bioevaluation results of drug- clay hybrid materials obtained in Synthesis and characterization of drug - mineral clay hybrid materials for biomedical applications as drug delivery systems – part I [1] are presented, in order to obtaine drug delivery systems used in cancer treatment. The bioevaluated materials were the nine types of hybrid materials, synthesised using three types of mineral phases with different structural characteristics as matrix and epirubicin, fludarabine, gemcitabine as active substances.

For morpo- structural characterization were used the following experimental techniques: X-ray diffraction, thermal analysis, scanning electron microscopy, which offered information about the proper interlayer intercalation of cytostatic, the good adsorption of drug into the matrix, the microstructure. In this study, the characterization was continued with bioevaluation, presenting informations about the kinetics and drug release. Thus, analysis performed was the in vitro cytotoxicity which established the potential use for biomedical applications showing the antitumoral activity.

În acest studiu, sunt prezentate rezultatele bioevaluării unor materiale hibride medicament- argile sintetizate in Synthesis and characterization of drug - mineral clay hybrid materials for biomedical applications as drug delivery systems – part I [1], în vederea obținerii de sisteme cu eliberare controlată folosite în tratamentul cancerului. Materialele supuse bioevaluării au fost cele nouă tipuri,obținute utilizând trei tipuri de faze minerale cu diferite caracteristici structurale ca matrice suport și epirubicină, fludarabină și gemcitabină ca și substanțe active.

În vederea caracterizării structurale și morfologice s-au folosit tehnicile: difracția de raze X, analize termice, microscopie electronică de baleiaj, care au oferit informații despre intercalarea interstrat adecvată a citostaticelor, o adsorbție bună în matrice și microstructură. In acest studiu s-a continuat cu bioevaluarea, prezentând informații despre cinetică și eliberarea medicamentului. Astfel, analizele realizate au fost citotoxicitatea in vitro care a oferit informații despre potențialul de utilizare al materialelor în aplicații biomedicate, reflectând activitatea antitumorală.

Keywords: *epirubicin, fludarabine, gemcitabine, kaolinite, halloysite, montmorillonite, hybrid materials, drug delivery systems*

1. Introduction

In synthesis of drug delivery systems ore often used as support the natural materials, mineral clays. Most important mineral clays are kaolinite ($Al_2O_3 \cdot 2SiO_2 \cdot 2H_2O$), halloysite ($Al_2O_3 \cdot 2SiO_2 \cdot 2H_2O$ + $2H_2O$), montmorillonite ($Al_2O_3 \cdot 4SiO_2 \cdot H_2O$ + H_2O) and ilite.

Kaolinite and halloysite have a similar structure, with the difference of quantity of water, less for. Iillite are a group of minerals with similar structure to montmorillonite, but in comparison, illite have high plasticity. In comparison to kaolinite, for MMT the water is harder to eliminate, but has a higher plasicity [2].Thus, MMT is often used in pharmaceutical field, such as excipient and active substance [3]. Montmorillonite is a medical clay, used in detoxification process, typical symptoms of side effects caused by anticancer drugs [4].

In addition to these conventional pharmaceutical use, the clay mineral can be effectively used in the development of new controlled release systems. Almost all pharmaceutical dosage forms are controlled release systems, because they are used for administration of medicines intended to come within range and to maintain a certain level during the treatment. However, the therapeutic effect of a proper pharmaceutical treatment will depend on a number of factors: some depend only on the drug, some of the patient.

One of the biggest challenges in medicine is the treatment of cancer. Therapy against cancer is one of the three pillars of this type of treatment, along with surgery and radiation therapy. In general, anti-tumor drugs are classified into three categories: cytotoxic agents, biological agents, hormonal agents.

Currently, most antitumor drugs are administered intravenously. Intravenous route is the most direct and variable adsorption methods beyond the gastrointestinal tract. Lead to an immediate and

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complete bioavailability and thus a precise dosage. However, this approach may be hazardous, because they are administered to normal tissue very high concentrations of the drug. Chemotherapy IV is designed to provide the maximum tolerated dose of the cytotoxic agent to kill cancer cells in a short period of treatment followed by a period of several weeks without any further use. In addition to possible side effects, this route requires visits to hospital care and palliative treatment [7-13].

Active substances used for preparation of the mineral clay /antitumor drug hybrids were epirubicin, fludarabine, gemcitabine, cytostatic with a large usage spectra in cancer treatment (breast cancer, stomach cancer, lung cancer, ovarian cancer, etc.)

The purpose of this study is to bioevaluate a drug delivery system based on three different types of mineral clays- as matrix and three different antitumoral drugs– as active substances, in order to establish its antitumoral activity and posibilty to be used as drug delivery systems, the materials are characterised.

2. Experimental

2.1. Materials

There are used for this purpose three types of clay minerals and three types of chemotherapy: epirubicin, fludarabine, gemcitabine as presented in *Synthesis and characterization of drug - mineral clay hybrid materials for biomedical applications as drug delivery systems – part I* [1].

The materials used and their preparations have been assigned the following codes shown in Table 1.

2.2. Preparation of the clay hybrid materials

The clay -drug hybrid materials have been obtained by mixing an aqueous solution of the active substance with the swelled clay, obtained by gradually introducing 1 g of the clay in 50 mL distilled water pre-heated to a temperature of 60 ° C, as can see in *Synthesis and characterization of drug - mineral clay hybrid materials for biomedical applications as drug delivery systems – part I* [1].

2.3. Characterization

The materials used in the synthesis and the obtained hybrids were characterized from both chemical and microstructural points of view using different experimental techniques: X-ray diffraction (XRD), thermal analysis (DTA-TG), scanning electron microscopy (SEM), images and conclusions presented in *Synthesis and characterization of drug - mineral clay hybrid materials for biomedical applications as drug delivery systems – part I* [1].

Cytotoxicity tests were realized in order to confirm biocompatibility. Furthermore, the anti-tumoral activity of the designed materials was studied to highlight their efficiency against the proliferation of cancerous cells.

For the in vitro citotoxicity of the synthesized materials, experiments were conducted with the HEp cell line. 20 000 events were acquisitioned in a Beckman Coulter Epics XL flowcytometer. The antiproliferative effect indicated by the percentage of cells in G0/G1, S and G2/M phases of the cell cycle was analyzed using FlowJo software.

3. Results and discussion

3.1. UV-Vis spectrometry

The kinetics and the drug release of cytostatyc from hybrid materials were obtained using UV –Vis spectrometry. Figure 1. presents the release graphs of E, E and G which have the specific curves, as is known from the literature. Analyzing the kinetic release, it can be observed in first five hours the materials present a rapid drug release. For the following period of time a slower release can be seen, until the saturation is established. For al the hybrid materials we observe the highest release is obtained for the clay 2 based material and the lowest fost clay 3 hybrid material. Comparing Figure 1 with the theoretical graphs it can be noticed the obtained hybrid materials can be used as a controlled release system.

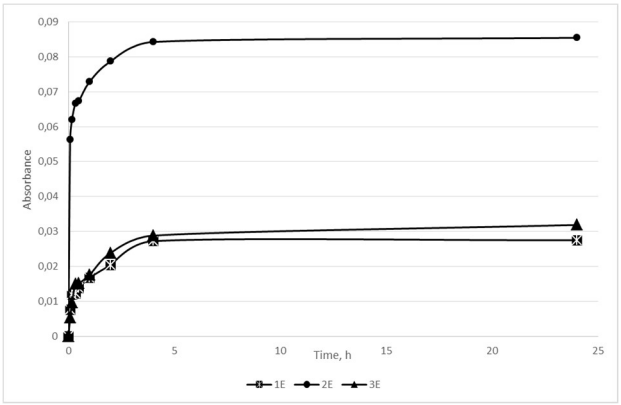
3.2. Citotoxicity

In Figures 2-7 are presented fluorescent microscopy images obtained as follows: cells were maintained in 75-cm^3 plastic tissue culture flasks containing DMEM medium (Gibco, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Invitrogen, USA) and antibiotics. Cells were removed from the surface of

Table 1

Materials code/ *Codurile materialelor*

Drug / Clay	epirubicin	fludarabine	gemcitabine
Clay 1 Hydratated sodium aluminosilicate	1E	1F	1G
Clay 2 Muscovite, Kaolinite	2E	2F	2G
Clay 3 Magnesium and Iron Hidrosilicate, Calcium Aluminosilicate Hydrate	3E	3F	3G



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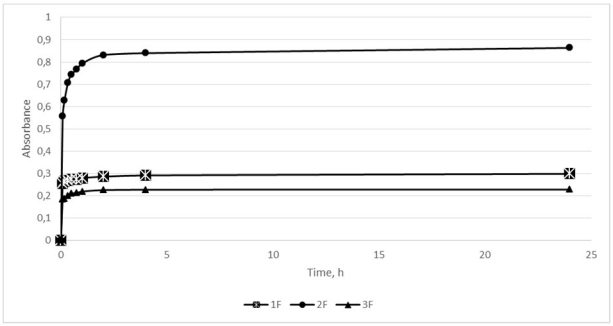

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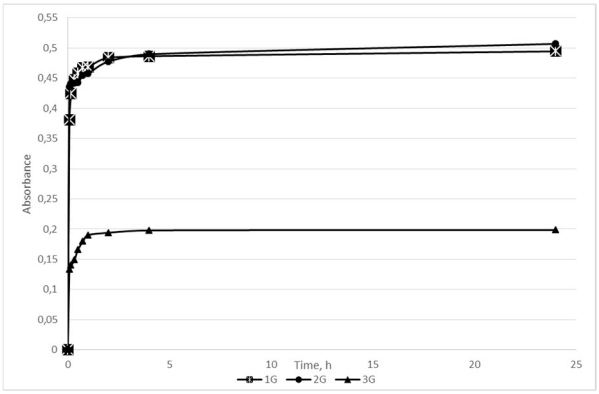


c

Fig. 1. - UV-Vis spectrometry for hybrid materials/ *Spectrometria UV-Vis pentru materialele hibride*.

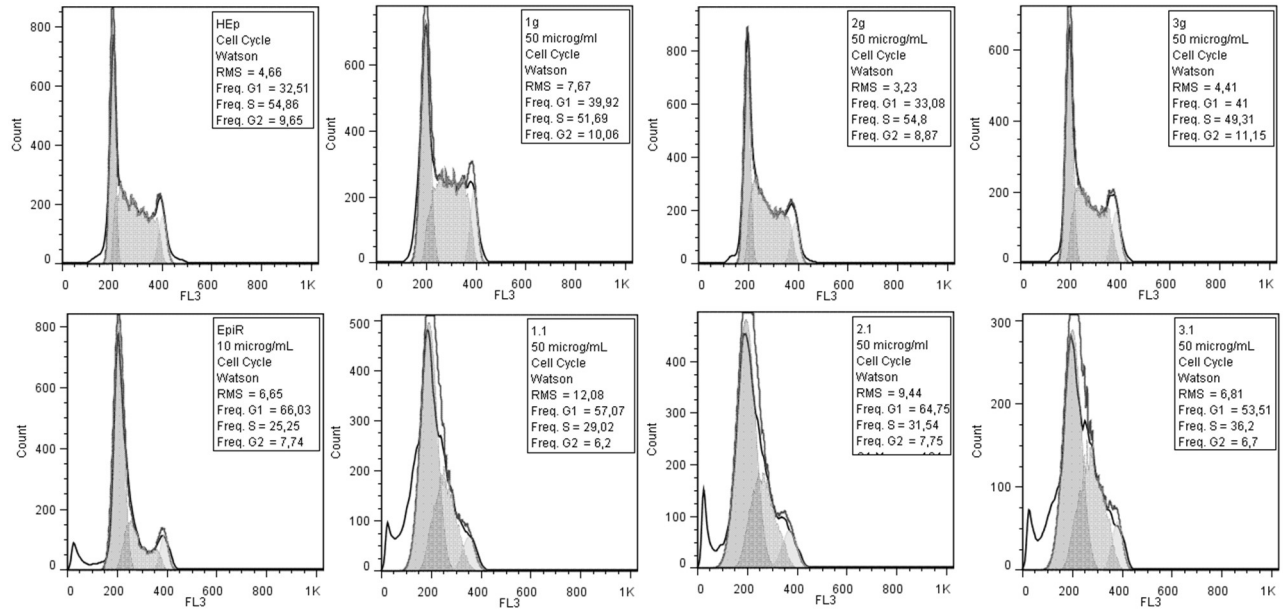


Fig.2. - Cell cycle distribution by DNA flow cytometry of HEp cells (reference, swelled clays -1st row) and HEp cells exposed to hybrid materials (epirubicine reference, hybrid materials – 2nd row) / *Reprezentarea ciclului celular determinat prin citrometrie (control, argila – primul rand și control epirubicina, material hibride- randul 2).*

culture flasks by addition of 0.25% trypsine (Gibco, USA), centrifuged for 5 minutes at 1300 rpm, and counted. 3x105 cells/ml were cultured in petri dishes in complete medium containing 50µg/ml of each synthetized material, and incubated for 20 hours. At the end of this incubation the cells were removed with trypsine, washed with PBS, fixed in 70% could ethanol and stained in 100µg/ml propidium iodide, for 1 h, at 37^{0}C. To identify the underlying of cells in mechanism associated with the anti-proliferative effects of some cytostatic combined with simple clays from montmorillonite class, it was evaluated the effect on cell cycle progression by measuring DNA content by flow cytometry. Pure clays had no noteworthy effects on cell cycle progression. After 20h of treatment, 50µg/ml of cytostatic-clay combinations caused a significant cell cycle arrest in G0/G1 phase and a decrease in the percentage

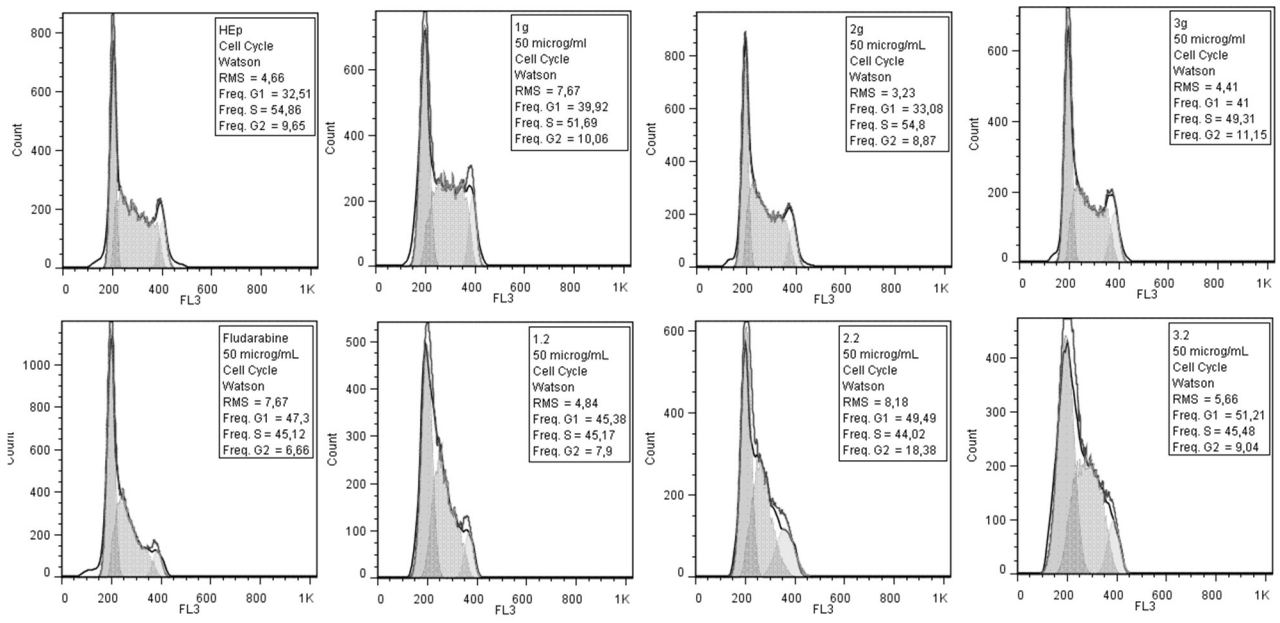


Fig.3 - Cell cycle distribution by DNA flow cytometry of HEp cells (reference, swelled clays -1st row) and HEp cells exposed to hybrid materials (fludarabine reference, hybrid materials – 2nd row) / *Reprezentarea ciclului celular determinat prin citrometrie (control, argila – primul rand și control fludarabina, material hibride- randul 2) .*

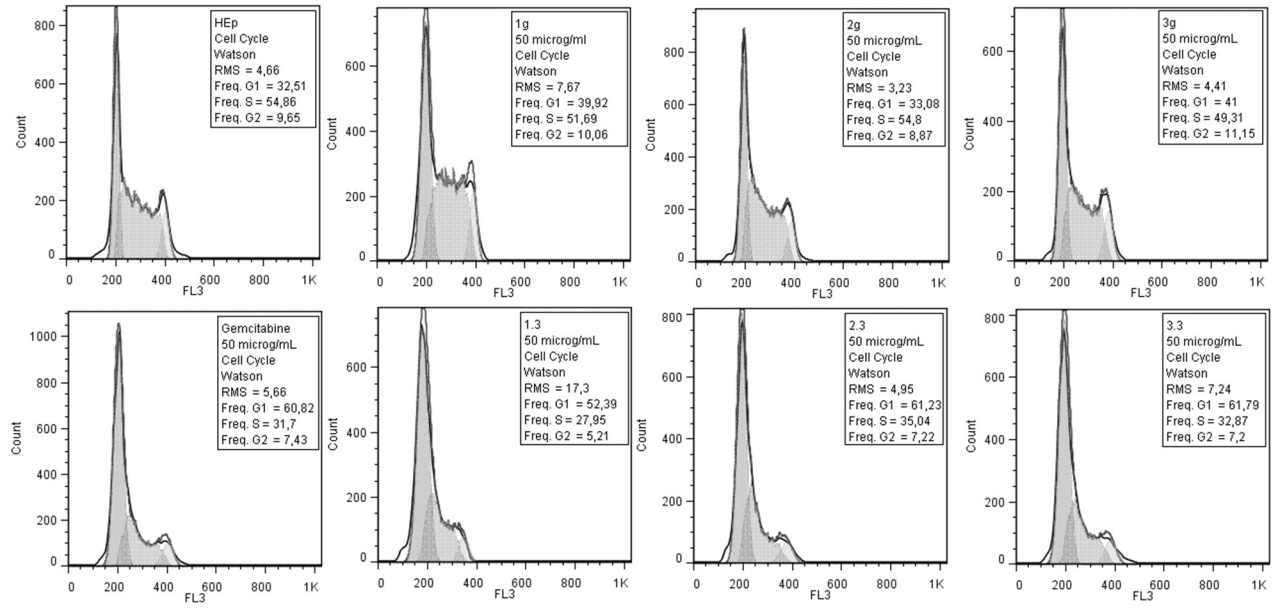


Fig.4 - Cell cycle distribution by DNA flow cytometry of HEp cells (reference, swelled clays -1st row) and HEp cells exposed to hybrid materials (gemcitabine reference, hybrid materials – 2nd row) / *Reprezentarea ciclului celular determinat prin citrometrie (control, argila – primul rand și control gemcitabina, material hibride- randul 2) .*

of cells in S and G2/M cell cycle phases. Epirubicin dominantly caused cell accumulation at G1 phase and subG0 peack apearance comparing to the control. It was found that the combination of epirubicin with all montmorillonite clays exhibited higher G1 accumulation than treatment of other combinations.

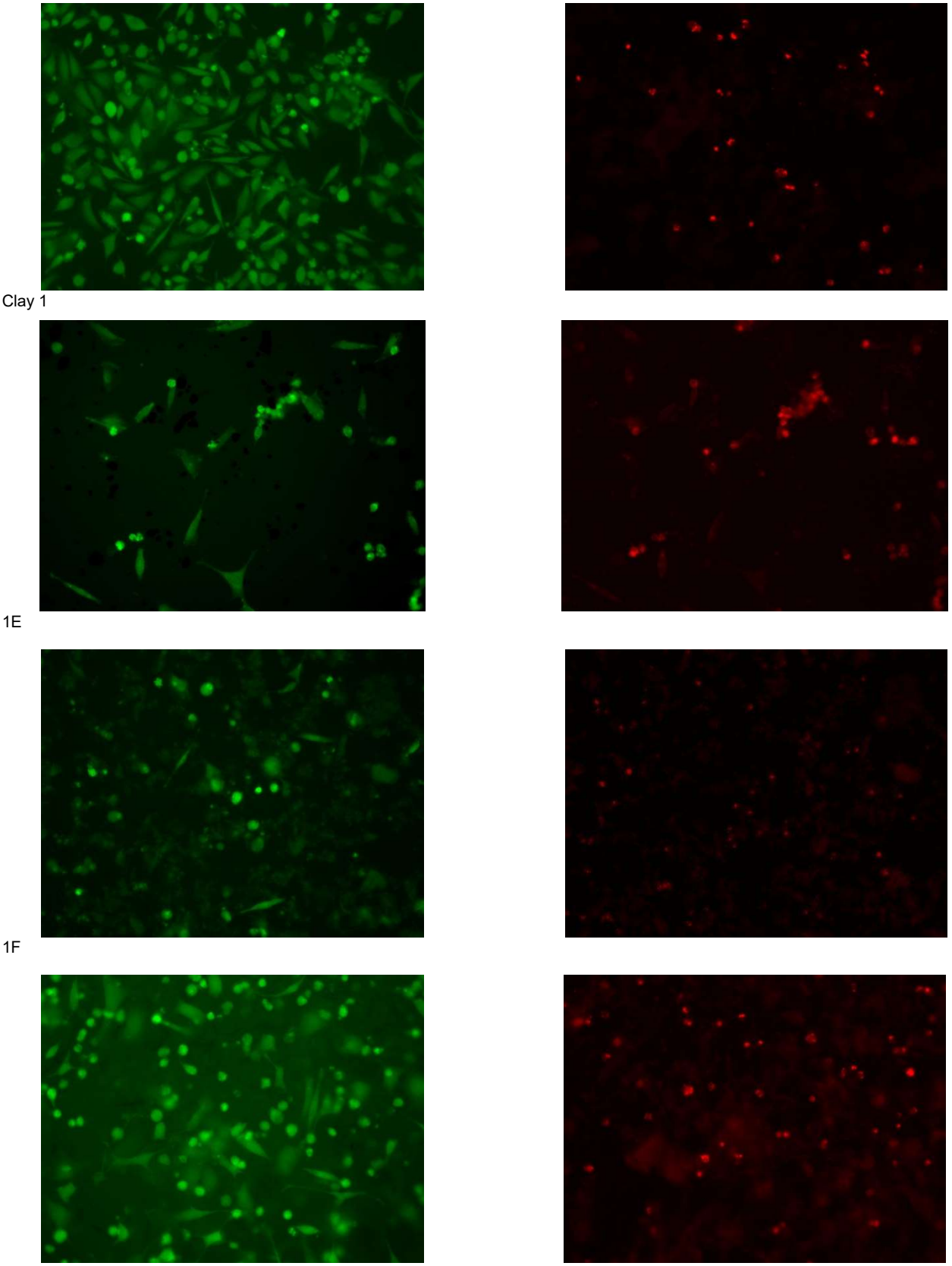
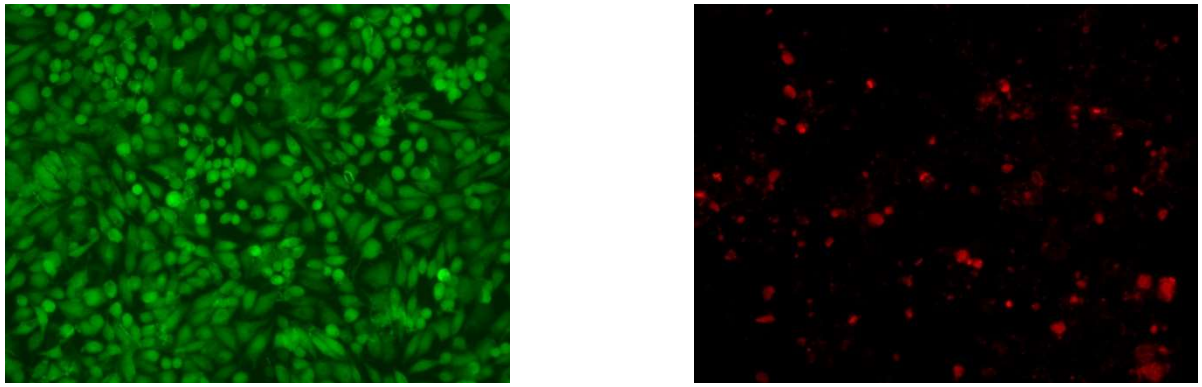
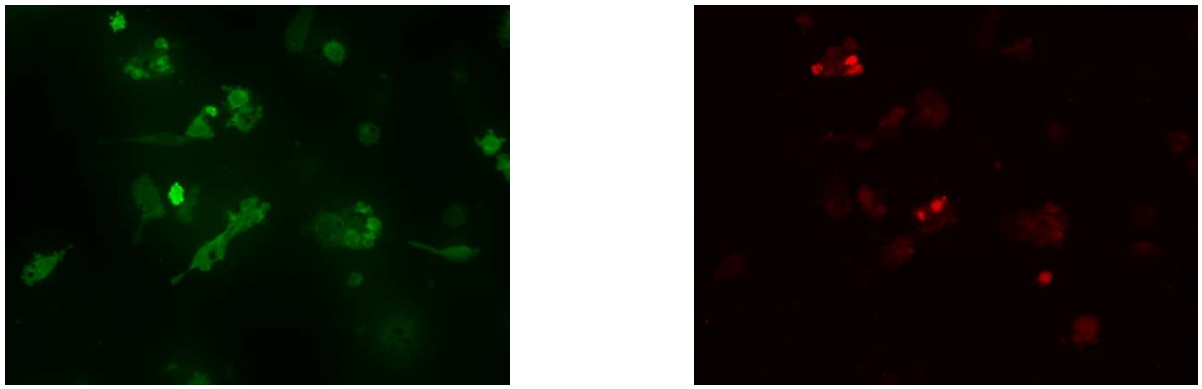


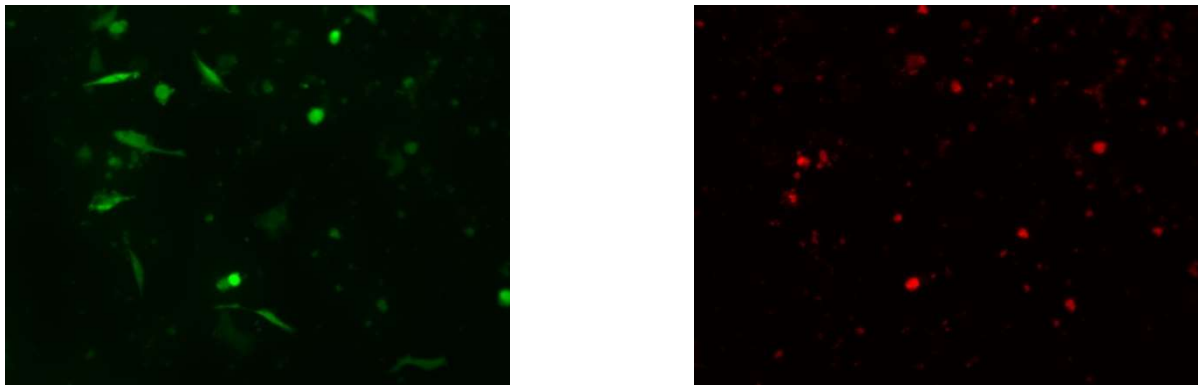
Fig. 5 - Fluorescent microscopy images of clay 1 and hybrid materials 1E,1F,1G (IF 200x), - 100µg/ml / *Microscopie de fluorescență pentru argila 1 și materialele hibide 1E,1F,1G.*



Clay 2



2E



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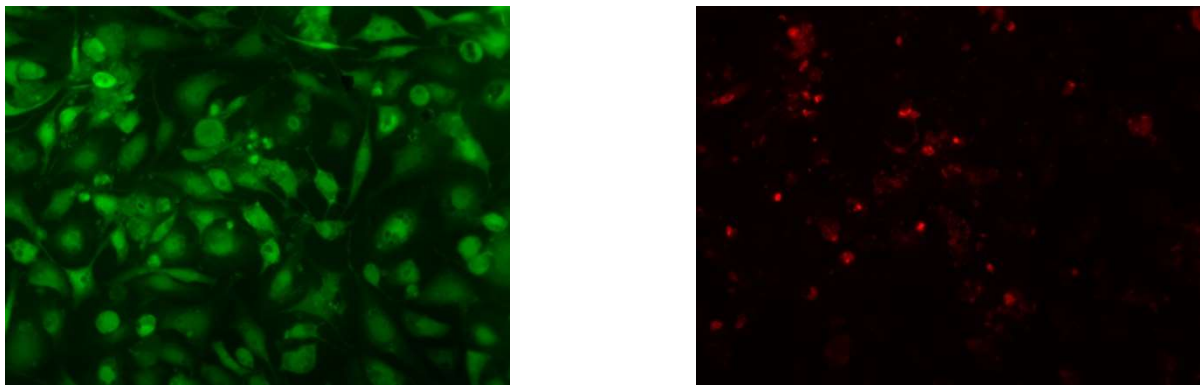

2G

Fig. 6 - Fluorescent microscopy images of clay 2 and hybrid materials 2E,2F,2G (IF 200x), - 100µg/ml / *Microscopie de fluorescență pentru argila 2 și materialele hibide 2E, 2F, 2G.*

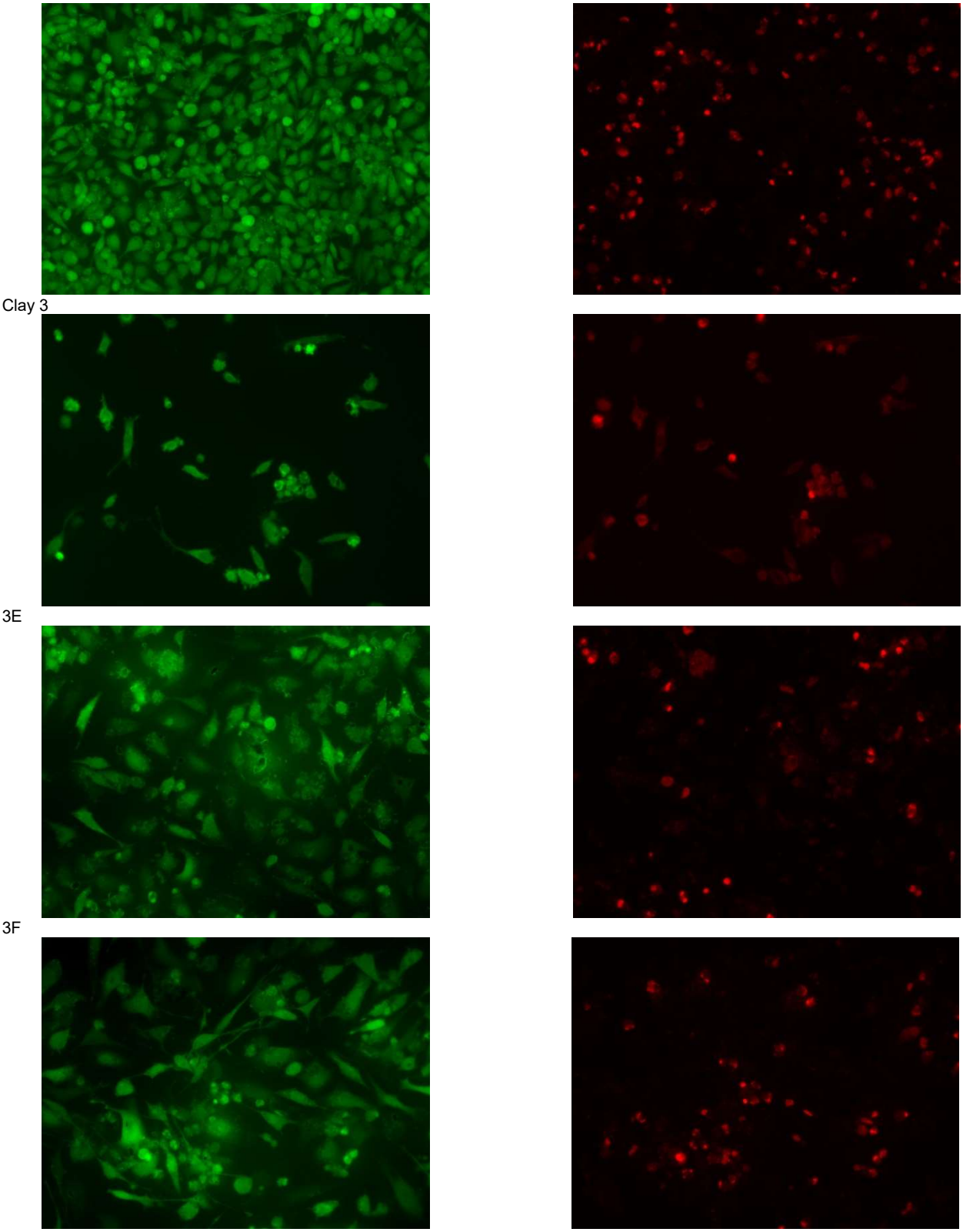

Fig. 7 - Fluorescent microscopy images of clay 3 and hybrid materials 3E,3F,3G (IF 200x), - 100µg/ml / *Microscopie de fluorescență pentru argila 3 și materialele hibide 3E, 3F, 3G.*

3. Conclusions

Recent studies show the possibility of using mineral clay as drug delivery system [1-3]. Thus, the main focus of the present study is the bioevaluation of clay/antitumor drug hybrid materials obtained in *Synthesis and characterization of drug - mineral clay hybrid materials for biomedical applications as drug delivery systems – part I* [1] . With potential applications in drug delivery systems, the hybrid materials were obtained starting from three types of mineral phases with different structural characteristics as matrix and three types of

cytostatic (epirubicin, fludarabin, gemcitabine) as active substances [2].

The materials used in the synthesis and the obtained hybrids were characterized from both chemical and microstructural points of view using different experimental techniques: X-ray diffraction (XRD), thermal analysis (DTA-TG), scanning electron microscopy (SEM). From the XRD patterns and DTA-TG analysis it is noted that the cytostatic has breached the gallery of the clays (diffraction peaks from plane family [0 0 1] shift left on hybrid, incrementing the interplanar c-spacing by ~0,4Å) creating a good interaction with it (major weight loss shifts from 30^0C-150^0C – physical water loss – to 150^0C-500^0C – cytostatic loss). The influence of epirubicine upon mineral morphology was highlighted by the appearance of conglomerates on the clay surface in SEM.

Cytotoxicity tests were carried out in order to confirm biocompatibility. Furthermore, the anti-tumoral activity of the designed materials was studied to highlight their efficiency against the proliferation of cancerous cells. The cell lines experiments showed that epirubicine exerts its antitumor effects by interference with the synthesis and function of DNA and it is most active during the S phase of the cell cycle, making these materials potential candidates for drug delivery systems.

The presented results show that the hybrid materials form this study can be used as potential drug delivery systems, but as perspective UV- Vis spectrometry will be used to identify a kinetic profile for the syntetized hybrid materials.

Acknowledgements

The work has been funded by the Sectoral Operational Programme Human Resources Development 2007-2013 of the Ministry of European Funds through the Financial Agreement POSDRU/159/1.5/S/132397.

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