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MULTIFUNCTIONAL NANOSYSTEMS BASED ON TWO FLUORESCENT DYES, DOXORUBICIN AND CURCUMIN

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The *aim* of the work was to review the literature data regarding the prospects for the creation and use of multifunctional fluorescent two-dye nanosystems, which enable investigating the distribution of fluorescent components with significant acceleration of the study and introduction of nanomedicines into practice. Special attention is paid to the use of two substances with hydrophobic and hydrophilic properties in one nanoparticle (NP), capable of penetrating a living cell.

The *method* of fluorescence confocal microscopy enables observation of the nanoscale dynamics of distribution and stability of drugs over time. The concomitant use of doxorubicin (DOX) and curcumin (CUR) in single nanoparticle causes synergism in the action of medical drugs, and their own fluorescence makes it possible to use them as multifunctional fluorescent nanosystems.

Results. Data from the literature indicate that the use of two or more fluorescent dyes provide an advantage over other, more expensive methods when studying the penetration and distribution of NPs in living samles. The use of nanocarriers is an effective way to significantly increase the bioavailability of those drugs which are poorly soluble in water. A promising direction of nanomedicine is the creation of complex bio-compatible multifunctional nanomaterials based on several active drugs, with the simultaneous use of their enhancers and the strategy of active targeting. Such recent structures enable targeted and controlled penetration of medicinal compounds into the sites of localization of pathological processes, reducing the toxicity of drugs to normal cells.

Conclusions. The use of the fluorescence microscopy method, as exemplified by the two dyes, DOX and CUR, enables to trace the stages of interaction of loaded DOX and CUR nanoparticles with cultured cells, and their release from NPs to determine their amount and localization in organelles cells.

Key words: multifunctional nanosystems, doxorubicin, curcumin, synergism, two fluorescent dyes.

Nanomedicine deals with the design and development of nanoscopic means of delivery and diagnostics [1, 2]. Currently, the multifactorial nature of diseases and the lack of adequate treatment lead to the development of new strategies for the search for potential drugs [3]. Many chronic and acute diseases are associated with inappropriate or exaggerated activation of genes involved in inflammation [4]. Due to the wide use of various methods of diagnosis and treatment, nanomedicine provides an opportunity to improve the treatment of various diseases, including chronic diseases and cancer [1]. Enhancement of drug delivery to tumor sites through passive or active targeting leads to the reduction of adverse side effects on healthy tissues [5]. Understanding the interaction of NPs with living cells is extremely important when these particles are used to transport and deliver water-insoluble and water-soluble drugs to cells. Due to the application of nanotechnology, rapid progress is observed in medicine and biomaterials in recent years [6].

Cancer remains one of the main causes of death worldwide [7–10]. Currently available cancer treatments include surgery, chemotherapy, radiation therapy, or specific combinations thereof. These treatments may be invasive with serious adverse effects [11]. Cancer is a very complex disease that involves several ways of development [1]. Treatment failure is related to the complex and diverse biology of cancer cells [11–13], because a cancer lesion usually consists of a family of genetically/phenotypically different cell types that arise over many years [12, 13]. During treatment, the disease progresses, which is marked by many consecutive mutations in the cell line [1]. Traditionally, cancer treatment is associated with chemotherapy [10]. Chemotherapeutic agents act by suppressing the proliferation of cancer cells [7]. High rates of tumor recurrence and mortality are associated with a small population of cancer stem cells that evade therapy and generate new tumors with drug resistance [8, 9, 14, 15]. Malignant tumor cells and normally functioning cells share many of the same biological characteristics. The main difference of the cancer cells is that they overuse and overexpress some biological characteristics, most of which are aimed at protecting cancer cells [11]. Surviving tumor cells with favorable mutations [1] possess acquired resistance to chemotherapy drugs, which is the main reason for the ineffectiveness of anti-oncology treatment [16]. Cancer chemoprevention can be considered as a strategy designed to minimize the progression or recurrence of cancer [17].

In most cases, chemotherapy drugs do not distinguish between cancer cells and normal cells, thus leading to unwanted serious side effects [10, 18]. Over time, non-selective delivery of those agents damages normal cells [7]. Available anticancer chemotherapy agents demonstrate limited efficacy, oftenly have serious side effects, and are costly [19]. Therefore, new drugs with improved efficacy against tumor cells and less toxicity to normal cells are urgently needed [20].

The resistance of human tumors to many chemotherapeutic agents is referred to as multiple drug resistance (MDR) [13, 21]. Acquired resistance is the main obstacle to successful cancer treatment [20, 22, 23]. In cases of acquired resistance, inhibition of tumor development by a single drug may not be sufficient for complete destruction of cancer lesions [1].

To ensure effective non-invasive treatment, it is desirable to develop target-oriented carriers for delivery of cytotoxic agents directly to cancer cells [7, 11], which improves their antitumor activity [24] through the use of nanotechnology [25]. Targeted delivery improves therapeutic efficacy and reduces toxicity and frequency of drug administration, but co-delivery of chemotherapeutic drugs with different solubility is challenging [10]. Some anticancer drugs have limited water solubility and a short circulation time in the blood and body [10, 24].

Doxorubicin

Doxorubicin (DOX), or Adriamycin, is a well-known antitumor anthracycline antibiotic that has a broad spectrum of antitumor activity [24, 26–29] and a known chemotherapeutic agent [30–33] against various solid human tumors [34] such as cancer of mammary gland [35, 36, 37, 38], lungs [32], ovaries [32, 38], cervix [38] and uterus, hematological malignancies [39], Hodgkin's disease, soft tissue [32] and primary bone sarcoma, multiple myeloma, neuroblastoma [35], prostate [38], leukemia [34, 35], colon cancer [24], as well as against several other cancer types [26]. Conventional chemotherapy uses DOX against invasive B-cell lymphoma [40]. The antitumor effect of DOX is mediated through DNA intercalation and inhibition of the topoisomerase 2α isozyme (Top2α), toward the inhibition of DNA replication, which causes cell death [27, 41].

DOX has obvious advantages due to its significant efficacy and low cost [32]. It has demonstrated potential antitumor activity against various cancer cells [21], but unlike other chemotherapeutic agents such as cisplatin, paclitaxel, docetaxel, etoposide, and oxaliplatin, the efficacy of DOX is limited due to the ability of cancer cells to acquire chemoresistance [21, 27, 28, 32, 42, 43]. Doxorubicin has proven itself as a highly effective antitumor agent used for the treatment of a wide range of oncological diseases [44]. Clinical use of high-dose doxorubicin (DOX) in cancer treatment is limited by its cardiotoxicity [44] and hepatotoxicity [45]. Co-treatment with DOX and pyrrolidine dithiocarbamate (PDTC) attenuates DOX-induced apoptosis in Chang-L liver cells [45] and human hepatocytes [20, 45]. These results suggest that DOX/PDTC co-treatment may provide a safe and efficient therapeutic strategy against malignant hepatoma cells with Bcl-xL [45].

After administration, major portion of DOX is eliminated from the blood circulation and distributed in deep tissues [24]. However, high efficacy of doxorubicin is achieved at very high doses, which are accompanied by serious side effects of DOX on healthy tissues [20, 24, 46, 47], as DOX is a non-selective cytotoxic drug [40]. The use of DOX in chemotherapy is limited mainly due to its wide range toxicity [15], including dose-dependent [47, 48] and irreversible cardiomyopathy [16, 34, 35, 46, 47], renal [20, 39], hepatic [20], pulmonary, hematological and testicular toxicity [26]. Apoptosis and defective autophagy are thought to contribute to DOX-induced cardiotoxicity [35], and the cardiotoxic mechanism of doxorubicin is partially mediated by mitochondrial dysfunction leading to myocyte apoptosis [44].

Prolonged use of Doxorubicin causes neurobiological side effects [49]. Antioxidants protect cells from damage caused by free radicals; they are used for the treatment and prevention of such diseases as cancer, cardiovascular diseases, diabetes, brain stroke, and skin diseases [50]. The antioxidant effect may reduce side effects, including DOX-induced cardiotoxicity [46].

In addition to toxicity, the effectiveness of doxorubicin as antitumor agent is limited by the appearance of MDR phenotype in cancer cells [15, 16, 20, 42, 48]. MDR is recognized as a major obstacle in the effective treatment of many malignant neoplasms [21, 22]. For effective cancer chemotherapy, due to the MDR, increasing the dosage of DOX is not recommended [27, 31, 48].

To date, a large number of molecular pathways and mechanisms that contribute to resistance to DOX have been identified. Resistance to DOX in cell lines is mainly depends on both pumping and non-pumping mechanisms. The pumping mechanisms involve directly MDR1/P-gp or indirectly (c-Jun), which cause DOX efflux [33]. Many of the newer therapeutic drugs are aimed at reducing the activity of drug transporters such as P-gp, increasing the accumulation of DOX in cancer cells [27, 32, 33]. A potential solution to the problem of treating cancer cells with MDR is the use of several drugs encapsulated in nanoparticles [51, 52].

Natural phytochemical compounds enhance antioxidant activity

It is important to develop new chemotherapy drugs or alternative treatment approaches that are safer for the patient, and more efficient in killing the tumor. Natural phytochemicals are attracting increasing interest because they are not associated with toxicity and side effects [4, 8, 9]. The use of phytochemicals may provide a promising strategy for cancer treatment without the harmful side effects commonly seen with chemotherapy and radiotherapy [8].

To achieve the best possible therapeutic effect, new drugs and delivery strategies need to be developed [53, 54]. There is increasing evidence that phytochemicals in food have antitumor effects in various types of cancer. The proapoptotic and antiproliferative effects of phytochemicals [53, 54] indicate their ability to inhibit the growth of several types of blood, skin, brain, colon, ovarian, breast, prostate, and pancreatic cancers [8, 9, 23]. Known antioxidants with outstanding antioxidant activity used in pharmaceuticals and cosmetics from phenolic acids: ferulic acid, caffeic acid [50] and gallic acid have antitumor effects on many types of cancer [55]; polyphenols with such effect include: ellagic acid, curcumin, genistein, hydroxytyrosol and resveratrol [50]. Herbal dietary antioxidant supplements containing indole-3 carbinol and resveratrol have been recognized as antiproliferative agents in cancer [46]. These two compounds have both similar and unique molecular targeting profiles and together show an obvious synergistic effect [46]. In order to intervene in the process of carcinogenesis, molecules with chemopreventive potential were identified, including nonsteroidal anti-inflammatory drugs and phytochemical antioxidants (curcumin, ferulic acid, resveratrol, ellagic acid, epigallocatechin-3-gallate (EGCG), indole-3-carbinol), etc. [12,17], which showed chemopreventive/antitumor activity *in vivo* and *in vitro* [12].

Natural phytochemical compounds demonstrated significant efficacy in the treatment and prevention of many chronic diseases [12], including various cancers [8, 9, 56,] because they simultaneously target multiple pathways involved in disease progression [57]. Herbal natural products cannot replace chemotherapy, but some phytochemicals are widely used to ameliorate the side effects of DOX. For example, to prevent cardiotoxicity [27], herbal agents enhance the activity of antioxidant enzymes such as superoxide dismutase (SOD), increase the level of glutathione (GSH) and decrease the level of malondialdehyde (MDH) [27]. In recent

years, numerous studies have focused on the use of natural products together with chemotherapeutic drugs to increase therapeutic efficacy and reduce their cytotoxicity [15]. Plant products cause marked inhibition of proliferation, motility and invasion of resistant cancer cells [8].

Despite enormous efforts in the preclinical trials, the application of phytochemicals to humans has achieved only limited success [23]. Cell culture studies and various clinical trials have shown limited efficacy of phytochemicals against various types of cancer [12]. The use of individual or combined phytochemicals in cancer models *in vitro* and *in vivo* did not demonstrate complete destruction of cancer cells [8, 9]. The introduction of natural compounds into the clinical practice is largely hindered by their poor solubility [23, 53, 54], rapid metabolisation [23, 53, 54, 56], low stability, poor absorption [54], which ultimately leads to insufficient bioavailability [16 , 56] in oral treatment of carcinogenesis [12]. Compared to chemotherapy drugs, natural products are tolerated by normal cells even at high doses. A growing number of studies have shown the anticancer effects of phytochemicals that can affect molecular pathways [23] and cellular events including apoptosis, cell proliferation, migration and invasion. Nanotechnology for the delivery of natural agents has shown that drugs encapsulated in nanoparticles can be protected from the destructive effects of external environments [53]. The desire to exploit traditional natural compounds for their chemotherapeutic and chemopreventive potential in clinical settings has prompted drug delivery scientists to develop NPs, liposomes, microemulsions, and implants that can bypass their poor oral bioavailability [56]. The use of phytochemicals in combination with nanotechnology enhances the therapeutic effect. Nanocarriers can increase the solubility and stability of phytochemicals, extend their half-life in the blood, and achieve targeted delivery [53, 54, 58].

Chrysin is a natural flavonoid that has several important pharmacological properties. It has antioxidant, antihypertensive, antidiabetogenic, anti-inflammatory, antitumor, antiviral potential [26]. Chrysin attenuated DOX-induced toxicity in kidney and liver tissue and abrogated breast cancer chemotherapy resistance *in vitro* [26].

Quercetin in combination with chemotherapeutic drugs maximizes the effectiveness of those agents in inducing apoptosis in cancer cells and is very effective in eliminating MDR. The combination of nano-Quercetin and doxorubicin enhances the toxic effects of doxorubicin on MCF-7 human breast cancer cells [59]. Co-administration of ellagic acid or rosmarinic acid with DOX significantly reduces adverse neural changes caused by long-term use of DOX, because they have a neuroprotective effect that is related to their antioxidant, anti-inflammatory and anti-apoptotic properties [49].

Natural antioxidants play an important role in the prevention of many diseases, because they are able to block oxidative chain reactions [49]. It has been shown that the use of phytochemicals affects many molecular mechanisms with a lesser toxic effect. Combinations of phytochemicals promote the death of cancer cells, inhibit the proliferation and invasion of cancer cells, sensitize cancer cells, and strengthen the immune system, which makes them a certain alternative in the chemoprevention of some types of cancer [23]. Some results have demonstrated efficacy in causing inhibition of breast cancer cell proliferation by a combination of phytochemicals used at bioavailable concentrations [23]. For example, the combination of gallic acid and CUR stimulated apoptosis in triple-negative breast cancer MDA-MB-231 [55]. Some natural substances that have a significant antioxidant effect are worth further research both experimentally and clinically [4]. In scientific developments aimed at reducing inflammation, CUR, resveratrol and sulforaphane were among the most promising natural molecules for the prevention and treatment of a number of chronic inflammatory and autoimmune disorders [41]. The use of nanoparticles, like the new drug delivery systems for lipophilic compounds, allowed to significantly improve the bioavailability of natural compounds such as curcumin, ellagic acid, green tea polyphenols, and resveratrol [12]. Future experiments should focus on enhancing the delivery of phytochemicals using their various synergistic combinations using *in vitro* and *in vivo* models [41].

Curcumin

Curcumin (CUR) is a low-toxic natural drug derived from polyphenols extracted from *Zingiberaceae* plants. CUR has great prospects in the prevention and treatment of various diseases [60, 61]. Significant prophylactic and/or therapeutic effects of CUR were

observed in experimental animal models of a number of diseases, including atherosclerosis, diabetes, diseases of the eyes, respiratory organs, liver, pancreas, intestines, stomach, and neurodegenerative diseases [4]. The plant-derived CUR with anti-proliferative, anti-inflammatory, anti-angiogenic and apoptosis-inducing properties has great antitumor [62] potential in various types of cancer, including pancreatic cancer [59]. *In vitro* and *in vivo* studies have shown that CUR can induce cancer cell death in numerous animal and human cell lines, including leukemia, melanoma, and carcinomas of the breast, lung, colon, kidney, ovary, and liver [19]. In addition, CUR is able to induce cell death in numerous apoptosis-resistant cell lines, probably by activating cell death mechanisms other than apoptosis [19]. Also, CUR was associated with anti-amyloid, antidepressant, antioxidant, anti-inflammatory [4,], anti-diabetic, anti-rheumatic, antiviral [63] and antibacterial effects [6]. The optimal potential of CUR, when when it is taken orally, is limited by insufficient solubility in the aqueous medium [12, 62, 64], which leads to poor bioavailability of curcumin [12, 19, 62, 63–65]. Poor absorption [19], high rate of metabolisation, and rapid systemic elimination [19, 62] are also the reasons for reduced bioavailability of curcumin. Thus, low concentration in target organs are factors that need to be improved in order to use curcumin as an anticancer drug [19]. The use of nanoparticle compositions to increase the bioavailability of curcumin is a new direction of research [63]. To that end, a large number of nanomaterials have been developed to enhance the effect of CUR *in vitro* and *in vivo* [15, 40, 60, 61].

CUR is capable of inhibiting cancer metastasis and proliferation [8] and influencing cellular molecular pathways [27]. In total, studies suggest that CUR is a potential agent for ameliorating the side effects of chemotherapeutic agents and enhancing their cytotoxicity against cancer cells [27]. Curcumin has been recognized and approved by the FDA for clinical trials as an adjunct to the main chemotherapy regimen [66] because such combinatorial treatment with curcumin sensitizes cancer cell swhich are resistant to existing anticancer drugs [16, 63, 66]. The use of curcumin may be a new method of treatment for various types of cancer [19].

Nanoparticles are particularly efficient in the development of new drugs with curcumin, which has suboptimal physicochemical and pharmacokinetic properties for cancer chemoprevention [12]. Despite the promising results of clinical trials of curcumin against colorectal cancer, pancreatic cancer, and breast cancer, its medical applications are still limited, due to curcumin's low solubility and limited stability [67]. But with the help of delivery platforms, high solubility, long-lasting effect and high loading of cells with curcumin were achieved [67]. The designed formulation of magnetic nanoparticles with curcumin showed strong antitumor properties in a concentration-dependent manner during their uptake by MDA-MB-231 breast cancer cells. Accumulation of nanoparticles in cells occurred by endocytosis. During clinical studies, imaging and magnetic targeting were used [68]. Nanoparticles with encapsulated CUR in PLGA show significant antitumor activity in prostate cancer *in vitro* and *in vivo* [19].

CUR exerts its antitumor effect by inhibiting the initiation, progression, and metastasis of various types of cancer, and suppressing carcinogenesis by interfering with two main processes: angiogenesis and tumor growth [19]. CUR has three main molecular targets: protein kinase C (PKC), mTOR, and epidermal growth factor receptor (EGFR) tyrosine kinase [19], and also suppresses inflammatory mediators in acute and chronic diseases, including NFκB, lipoxygenase (LOX), inducible oxide synthase nitrogen (iNOS) [4] and cyclooxygenase-2 (COX-2) [4, 69]. Acrolein is known to be involved in Alzheimer's disease, as well as in diseases such as diabetes and atherosclerosis [69]. Acrolein may play an important role in the development of atherosclerosis through the inflammatory response involving cyclooxygenase-2 (COX-2) in endothelial cells. Therefore, inhibition of COX-2 by curcumin will be aimed at the treatment of atherosclerosis [69].

The use of CUR provides a possible promising treatment for diseases associated with SARS-CoV-2, because it reduces the oxidative stress associated with the pathogenesis of the viral infection [3]. CUR is able to suppress acute and chronic inflammation and penetrate through the blood-brain barrier [3]. CUR has the ability to alleviate oxidative damage caused by heat stress, reduce ROS production, and induce apoptosis [70]. CUR has an anti-inflammatory effect by suppressing metabolic reactions and proteins that provoke inflammation and oxidation [71].

Oxidants and free radicals cause oxidative stress and mitochondrial dysfunction, which are *major* events that occur during neuronal death in Parkinson's disease (PD) and other neurodegenerative disorders, including Alzheimer's disease (AD) [72]. CUR exhibited anti-amyloid-β activity when encapsulated in biodegradable nanoparticles PLGA with diameter of 80 to 120 nm for drug delivery across the blood-brain barrier to neuronal cells in Alzheimer's disease [64]. Therapy should be aimed at preventing and delaying the onset of PD progression; phenolic antioxidants can help in this [72].

Drug delivery systems

To date, the most common method of cancer treatment is chemotherapy, the therapeutic effect of which is far from optimal due to a significant non-specific toxicity of chemotherapeutic agents. Due to incorrect selective recognition of cancer and normal cells in patients, they continue to cause various side effects. Drug delivery systems such as nanoparticles, liposomes, and polymers provide therapeutic concentrations of various potent chemopreventive agents [25, 28, 31–33, 41]. That is why the idea of cancer nanotechnology has been put forward, which provides a unique approach against cancer through the use of nanotechnology [25], where nanoparticles are indispensable for maintaining synergistic drug ratios in combination therapy [1]. In order to overcome the problem of subtherapeutic drug concentrations in the target area and/or to reduce the effect on normal host tissues [10], a diverse assortment of multifunctional delivery systems have been developed, such as polymer-drug conjugates, liposomes [10, 12], micelles, nanogels [73], microemulsions [12] microspheres and nanospheres [10], inorganic [74, 75] and organic nanoparticles [76], albumin [51, 77–79], which significantly increase the prophylactic/therapeutic effectiveness of selected agents [12].

The treatment of cancer for combined and targeted therapy, using NPs as means of drug delivery, provides promising opportunities for prolonging the circulation time of the nanopreparation and directed intracellular delivery of NPs to specific locations [1]. Local DOX delivery platforms have already been developed, which offer numerous prospects for the regional treatment of tumors, especially tumors that rarely metastasize [33].

Nanodrugs can help to overcome the number of limitations by placing several therapeutic drugs with different physico-chemical properties and pharmacological behavior, such as hydrophobic and hydrophilic, in the same NP, increasing their solubility. Nanopreparations mitigate cytotoxicity and improve the pharmacokinetic profiles of drugs [1]. For example, for DOX, drugs are being developed to reduce its side effects [27] with increased efficacy without increasing its dose, as well as reducing DOX resistance of cancer cells [1, 27]. Considerable efforts have been made to incorporate DOX into various nanomaterials: micelles [25], liposomes, polymeric NPs and mesoporous silicon [32], nanodiamonds [25].

Typically, DOX is encapsulated in various nanocarriers for the delivery to cancer cells [30, 32]. The mechanism of action of various NPs is related to the active transport of DOX. NPs are endocytosed and located near the perinuclear membrane, thus weakening the efflux mechanisms of DOX through the cell membrane, and are more efficient compared to free DOX, which passively enters the cell [33]. The efficacy of most nanocarrier-based delivery systems is greater than that of pure DOX [31] but insufficient [80], leading to undesirable systemic toxicity and slow clearance from cancer cells [31, 32]. Improved liposomal carriers of DOX have been developed, which are approved by the FDA and have antitumor efficacy that exceeds that of conventional DOX and are more likely to accumulate in solid tumor tissues [33]. Liposomal delivery of DOX has insufficient efficacy against multidrug-resistant tumors. Therefore, drug delivery systems for DOX, which improve the therapeutic window, efficacy and safety of drugs, are still under the development [33, 37]. For example, the main problems of liposomal vesicle delivery system are stability problem, poor batch-to-batch reproducibility, sterilization difficulties and low drug loading [12]. The advantage of using polymeric NPs is their ability to change the properties of their surface. Various functional groups can be covalently or non-covalently conjugated to polymer chains [81] to increase or decrease the average residence time of NPs in the gastrointestinal mucosa [12]. Active targeting increases the bioavailability of drugs at tumor sites [5].

PEG

In recent years, biodegradable polymeric micelles have attracted increasing attention due to their potential use as delivery vehicles

for chemotherapeutic drugs [10, 81]. Polymeric micelles and other nanocarriers have demonstrated the ability to significantly increase the antitumor efficacy of various chemotherapeutic drugs [10]. Hydrophobic segments of polymer micelles are packed together in aggregates (core), which serve as a storage place for drugs that are poorly soluble in water. Their hydrophilic shell usually consists of poly(ethylene glycol) (PEG), which provides increased solubility in water and prolonged circulation in the blood [10, 81]. Nanoparticles using PEG in combination with DOX-CUR have enhanced drug penetration into tumors, better local drug accumulation, and comparatively lower side effects [81]. The natural affinity of molecules of cationic carriers to the microcirculatory bed of a tumor when cationic liposomes are filled with drugs is known. They cause tumor vascular defects, change the function of vessels and limit the growth of primary tumors and their metastasis [56]. Cationic liposomes can be enhanced by the incorporation of PEG, which provides significant advantages for non-specific and vessel-specific tumor targeting [56].

PLGA, PLG

Poly(lactic co-glycolic acid) (PLGA) (Fig. 1), poly(lactic acid) (PLG) are copolymers used in therapeutic devices approved by the Food and Drug Administration (FDA) due to their biodegradability and biocompatibility. Copolymer with a monomer ratio of 50:50 shows faster degradation in about two months. The flexibility in degradation has made PLGA suitable for the fabrication of many medical devices such as grafts, sutures, implants, prostheses, surgical sealants, to create micro- and NPs based on them [82].

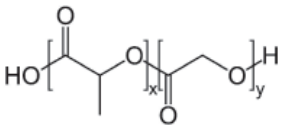

Fig. 1. **Poly(lactic-co-glycolic acid) structure [82]**
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Amphiphilic PEI-PDLLA and DSPE-mPEG copolymers were created by the self-assembly method for the preparation of polymer hybrid nanoparticles for co-delivery of curcumin and small interfering RNA [83].

Albumin

Albumin is the main transport protein of blood circulation [7]. Albumin is a universal protein carrier that is biocompatible and non-toxic [77, 78], non-immunogenic [77, 78] and biodegradable [77] polymer. It is an ideal material for the fabrication of nanoparticles for drug delivery [77] because it has good physicochemical stability, targeting potential, and chemical functionality [78]. There are various drug binding sites in the albumin molecule [77], so albumin nanoparticles have attracted considerable attention due to their high ability to bind various drugs [77, 84]. Thus, albumin is considered as one of the most useful and versatile carrier proteins in the pharmaceutical and clinical fields and the carrier which is safer than many synthetic polymers [78, 85–87].

Albumin-based NPs (ANPs) are widely used as drug carriers due to their great storage stability. Co-encapsulation of curcumin and doxorubicin in albumin nanoparticles resulted in efficient killing of MCF-7 breast cancer cells [51]. Double-layered microbubbles in which albumin was coated with a layer of silicone oil and filled with doxorubicin and curcumin, penetrated MDA-MB-231 cancer cells. The combination of these drugs produced a synergistic effect on inhibition of cell proliferation [79]. NPs based on albumin do not cause any serious side effects in the body and have better properties of controlled release than liposomes [77].

Nanovectors

Exosomes, or extracellular vesicles (EVs), are nanoscale bilayered natural structures produced by almost all types of cells [88–90] and continuously circulate in the peripheral blood, serving as a transport shuttle for the delivery of various molecules [88]. They are the main mediators in intercellular communication [88] through the transfer of molecular components (proteins, RNA, DNA and lipids) to recipient cells, for a possible change in their behavior [90]. EVs are promising nanocarriers which can transfer biological information between cells; however, they are limited in their ability to target specific recipient cell types [89] because the tumor-specific delivery of therapeutics is challenging [88]. The adaptation of EVs as a targeted drug delivery system to increase therapeutic efficacy has shown their advantages over liposomal therapy [90]. The therapeutic efficacy of grapefruit-derived nanovectors (GNVs) for the delivery of doxorubicin or paclitaxel in lung metastases has been demonstrated [88].

Modern treatments use actively directed drug delivery systems to specific receptors [5]. Targeted modified exosomal membranes (A15-Exo) that were co-loaded with DOX and cholesterol-modified miRNA 159 induced synergistic therapeutic effects in triple-negative breast cancer MDA-MB-231 cells both *in vitro* and *in vivo* [89]. Modification of the surface of nanoparticles with the help of targeting ligands leaded to efficient navigation in the complex *in vivo* environment, increased metabolism and prolonged intracellular release of the drug payload. These advantages make nanoparticles a treatment modality potentially superior to conventional cancer therapy [1].

Nanoparticles using folic acid (FA) receptors as targets for drug delivery systems are a promising system [5]. The folate receptor is specific for some cancer cells, including breast cancer cells. They have obvious tumor targeting, reduce drug accumulation in important organs, and show improved antitumor efficacy among others [5].

The development of drugs for intranasal delivery may provide a non-invasive therapeutic approach for the treatment of diseases related to the central nervous system [91]. The developed hybrid nanovector derived from grapefruit and polyethylenimine (PEI), coated with folic acid, intranasally delivered microRNA — miR17 into folate receptor-positive GL-26 brain tumor [91].

Increased expression of human epidermal growth factor receptor 2 (HER2) promotes the growth of cancer cells and makes them particularly aggressive and is a hallmark of HER2+ breast cancer [92]. GNVs were loaded with DOX and conjugated with a HA1 aptamer (GNVs-DOX-HA1) specific to HER2+ breast cancer cells [92]. *In vitro* and *in vivo* experiments showed that it significantly promoted the targeted delivery of GNVs-DOX to MDA-MB-453 breast cancer cells and showed higher internalization into HER2+ cancer cells and tumor spheroids and more cancer suppression *in vitro* than free DOX and GNVs-DOX [92].

Inflammation is a sign of cancer and activated immune cells are inherently capable of accumulating at sites of inflammation [93]. GNVs coated with membranes of activated leukocytes (IGNVs) are capable of enhanced homing to inflammatory tumor tissues, resulting in growth inhibition in two tumor models [93]. One of the main obstacles to the successful delivery of targeted agents with the help of nanovectors is the filtering role of the liver in the rapid removal of nanovectors from the blood circulation [88]. Functionalization of extracellular vesicles derived from non-tumor or immune cells with poly(ethylene glycol) PEG molecules leads to protection of nanoparticles from nonspecific uptake in the systemic circulation and increases their tumor targeting potential [90]. For example, pegylated extracellular vesicles passively loaded with DOX showed better antitumor activity in B16. F10 murine melanoma models *in vivo*, compared to clinical application of liposomal DOX in the same tumor model [90].

Curcumin and protection against DOX-mediated toxicity

Clinical use of DOX is often associated with severe side effects, namely dose-dependent cardiotoxicity [20, 21, 32, 41] hepatotoxicity [20] and nephrotoxicity [20, 27, 31, 33, 80, 94]. DOX-mediated cardiomyopathy causes oxidative stress, iron overload, calcium dysregulation, inflammation, and apoptosis [27]. DOX chemotherapy has been reported to induce inflammation, which is associated with DOX disruption of the intestinal flora, leading to the release and accumulation of endotoxins. They lead to systemic inflammation and damage to several organs [41]. For successful chemotherapy, it is necessary to introduction of auxiliary means capable of reducing the toxicity caused by doxorubicin without impairing its antitumor effect [95]. According to research, phytochemicals with antioxidant effects, which have cardioprotective effects, moderate inflammation and apoptosis. Medicinal plants and bioactive phytochemicals can serve as effective adjuncts to reduce the cardiotoxic effects of doxorubicin in vitro and in vivo [95]. Turmeric and curcumin obtained from it have various pharmacological effects, namely: antioxidant, hepatoprotective, anti-inflammatory, antithrombotic, antiapoptotic [96]. Curcumin inhibits the formation of reactive oxygen species in fibroblast cells [97] and H9c2 heart cells [50]. CUR has cytoprotective effects against doxorubicin-induced cytotoxicity [98] in heart, kidney, liver and blood components [36]. CUR protects against cardiotoxicity, which is manifested by a significant decrease in the level of myocardial enzymes in blood serum and an improvement in antioxidant capacity. CUR inhibits autophagy, promotes cardiomyocyte survival during DOX-induced oxidative stress, and inhibits cardiomyocyte-induced pyroptosis [35]. Curcumin can modify several cellular signaling pathways and activate numerous antioxidant and detoxification enzymes to regulate

oxidative stress in the cell [3]. Research on H9c6 heart cells, after oral administration of curcumin, showed suppression of oxidant stress parameters [99], inhibition of fibrosis, hypertrophy, and tissue apoptosis [50]. Experiments in an animal model showed a reduction in infarct size and myocardial apoptosis after administration of curcumin [99].

CUR is also an effective sensitizer that limits the activity of some proteins associated with MDR and also inhibits the proliferation of various types of cancer cells when DOX and CUR are used simultaneously, which enhances the therapeutic efficacy of DOX [21]. Due to the antioxidant and anti-inflammatory activity, CUR and other herbal agents [41] can effectively mitigate DOX-mediated cardiotoxicity [44, 96].

For joint encapsulation of DOX and CUR, polymers are used that self-assemble into micelles in water, have a hydrophobic core and a hydrophilic shell, and are therefore able to capture both lipophilic and hydrophobic compounds. [27, 100]. For example, a polydofamine-based nanoplatform was developed to load DOX) and CUR for near-infrared photochemotherapy of primary colon tumors [101]. A magnetic nanocomposite for co-delivery of DOX and CUR was coated with hydroxyapatite and cross-linked with b-cyclodextrin [102]. According to the results of confocal microscopy, the magnetic nanocomposite efficiently delivers DOX and CUR inside DOX-resistant MCF-7 (MCF-7/adr) human breast carcinoma cells [102].

Curcumin-loaded mesoporous nanoparticles show a good cardioprotective effect, which can be explained by the increased bioavailability of curcumin, which leads to good antioxidant and anti-inflammatory activity and significantly protects the myocardium from the toxic effects of DOX [96]. Administration of CUR protects heart tissue and reduces oxidative damage to the kidneys by stimulating the activity of antioxidant enzymes such as SOD (superoxide dismutase), CAT (catalase) and increasing the reduced level of reduced glutathione GSH [27, 71, 96] and by significantly reducing the increased level of malonate of dialdehyde [96].

Active targeting strategy

There is a great demand for the development of nanosystems with more precise targeting to the tumor microenvironment to enhance drug accumulation and improve transport parameters and thus therapeutic efficacy [103]. In order to create more specific targeted nanopreparations, an active targeting strategy is used. The surface of NPs can be modified by receptors and ligands to promote accumulation in cancer cells [27]. A multifunctional synergistic nanosystem consisting of gold nanoparticles with a pH-sensitive hydrazone surface bond was created. After being loaded onto the surface, doxorubicin is efficiently released into the acidic tumor microenvironment. For more specific binding to tumor cells, the folate receptor recognition factor avb3 was used, thanks to which NPs could accumulate precisely at the tumor site [103]. An active targeting strategy enables DOX to reach cancer cells using ligands or antibodies against selected targets on the tumor [37]. Resistance to DOX can be overcome or reduced by using NPs that are not recognized by P-glycoprotein, one of the main mediators of resistance to MDR [37].

A preparation of DOX-loaded PEG micelles with incorporated folic acid was developed, which significantly promotes the intracellular uptake of DOX compared to free DOX and PEGylated DOX liposomes (Doxil) in 4T1.2 breast cancer cells and the multidrug-resistant ovarian cancer cell line NCI-ADR-RES . The maximum allowable dose of DOX-loaded micelles was 1.5 times higher than that of free DOX and significantly inhibited the function of the P-gp efflux pump, and the antitumor activity was further enhanced after folic acid decoration [31]. Being loaded with DOX, such NPs showed low toxicity compared to free DOX [33]. Modified NPs produce a synergistic effect with the drug through binding to specific receptors on tumor cells. Therefore, the use of NPs as drug carriers in cancer treatment is a promising form [37].

Fluorescence microscopy on DOX and CUR models, molecular imaging

Fluorescence-based methods such as fluorescence microscopy, confocal laser scanning microscopy, and flow cytometry are widely used in many scientific experiments [104]. Using confocal microscopy, thanks to the fluorescence of individual substances or fluorescent nanoparticles, it is possible to find out unique molecular mechanisms that, for example, contribute to the synergistic effect of phytochemicals in their combination. Such studies pave the way for evaluating their effectiveness in vitro and in vivo [46]. To find out the mechanism of action of nanoparticles

based on multidrugs, it is necessary to conduct a large number of studies, and the standard methods of monitoring apoptosis are fluorescence microscopy and flow cytometry [46, 104].

Currently, the field of nanomedicine is experiencing a noticeable boom, dozens of products are undergoing clinical trials. This is mainly due to the significant potential of nanomedicine to address the need for the treatment of life-threatening diseases. Nanomedicine pays special attention to drug delivery and molecular imaging [32, 37, 103, 105]. Polymer therapeutic drugs can be singled out as one of the most successful fields that are regularly used in clinical practice [105]. Numerous therapeutic systems based on NPs, which have been developed in recent years, have shown low toxicity, sustained drug release, molecular targeting, and imaging functions [37]. Polymeric hybrid nanoparticles based on amphiphilic copolymers, thanks to multicolor fluorescence, can be used as a theranostic nanomedicine for simultaneous therapy and imaging both in vitro and in vivo [83]. For example, in addition to therapeutic applications, DOX molecules exhibit significant fluorescence [29]. Therefore, there is great interest in using DOX as a multifunctional tool for cancer therapy and diagnosis [32]. The fluorescent properties of DOX endow NPs with imaging capabilities [106], which makes them a multifunctional system for diagnosis and treatment [32]. For example, when incubated with cancer cells, DOX is used as a fluorescent probe, when its fluorescence signals are clearly observed in the nuclei during excitation with 488 nm light [32].

Despite the rapid destruction of CUR under the influence of light, it is widely used in fluorescence microscopy [58]. Thus, the cellular internalization of curcumin* PLGA nanoparticles was confirmed by fluorescence and confocal microscopy with a wide distribution of fluorescence in the cytoplasm and in the nucleus [64]. For example, *ex-vivo* fluorescence imaging demonstrates that the combined action of curcumin and doxorubicin is effective in the prevention and treatment of hepatocellular carcinoma (HCC) [22]. Thus, when studying the potential of systems that deliver curcumin to various tissues for the purpose of cancer chemoprevention and treatment of chronic diseases, curcumin is often used as a fluorescent model agent for confocal microscopy [12].

In order to study the interaction of various NPs with the cell, the DOX and CUR model can be applied. In this combination, CUR is used as a sensitizer for chemotherapeutic agents, and DOX as a model drug. DOX's own fluorescence was used for microscopic studies [43]. The native fluorescence of DOX and CUR and their resolution was enhanced by introducing mixed micelles of Triton X-100 and SDS. [107]. Studying the movement of DOX, its localization and retention is necessary for further understanding of the mechanisms of toxicity and resistance to DOX, studying and improving the action of nanopreparations, with the aim of establishing a better treatment protocol in clinical conditions [108]. Fluorescence is used to obtain additional spatial and temporal information in order to visualize the processes that occur in nanopreparations in vivo and in vitro, as well as to determine the relative concentration of drugs, the strength of the effect of medical drugs, and to confirm the effectiveness of high-tech systems [108].

The use of fluorescence microscopy reveals increased cell permeability, localization of NPs and drugs, determines time processes and reveals enhanced cytotoxicity [110]. Confocal laser scanning microscopy images showed that the simultaneous use of DOX and CUR led to vesicle swelling and drug release into the cytosol. Once in the cytosol, CUR blocked the transport of DOX from cells by inhibiting P-gp expression [51]. The use of fluorescence makes it possible to determine the penetration time and relative amounts of drugs directly in the cells, and provides insight into the process of transferring the relevant substances into the cell [40]. The fluorescence intensity of DOX [109] and CUR [107] demonstrates the distribution of drugs in different areas of the cell [40]. Through microscopy, it is possible to show the effects of drug redistribution and the efficiency of internalization [10]. Using a confocal microscope, it is possible to observe the internal concentration of DOX and CUR vapor-loaded liposomes that are labeled with rhodamine B in HeLa cells for up to 12 hours [110]. Under fluorescence microscope observation, it was confirmed that the uptake of DOX in tumor cells increased in a time-dependent manner, and the cellular uptake of the micelles was most likely via endocytosis or potentially pinocytosis [10].

Optimizing methods in medicine are constantly evolving, so a non-invasive, objective and reproducible method of assessing apoptosis depending on time was developed. Apoptosis induced by doxorubicin in breast cancer cell culture was measured Real-Time

Detection Using the Back Reflection Spectroscopy [104].

Nowadays, FRET is being promoted as a routine tool for use in nanomedicine research. Nanobio interactions are dynamic, interrelated, so a clear understanding of such processes primarily affects the effectiveness of nanomedicine, reveals the functional integrity of nanobio interactions [108]. Generally, FRET has been successfully applied in vivo to monitor the release, clearance, and circulation of drugs, as well as to study intertissue transport and cellular uptake. The imaging requirements of in vivo FRET are more stringent than those for conventional fluorescence microscopy, therefore, it is not often used for in vivo imaging in mouse or patient models. If the conditions are met, FRET becomes an extremely sensitive tool for visualizing the behavior of NPs in a biological context [108].

The problem of cancer chemoprevention is related to the need to inhibit or activate many biological processes in cells and organs [17]. Most of the known active substances alone do not cope with the set goal due to problems with the delivery of drugs in the required amount to the target cells [17]. Nanosystems of drug delivery contribute to the accelerated study of absorption and transport of active substances in vitro and in vivo [17], especially fluorescent ones. During the use of several fluorescent dyes, the study of the processes of nanoparticle production, absorption and transport of drugs by nanoparticles is accelerated [40, 81, 111], and the level of their synergistic cytotoxicity is estimated [112].

Unfortunately, chemoprophylaxis research is at an initial stage, due to the need to conduct a large amount of research [17]. Various methods, including fluorescence microscopy, were used to confirm the successful fabrication of prodrug nanoparticles [81]. Nanoparticle generation and intracellular distribution of nanoparticles and DOX and CUR chemopreparations directly into different areas of the cell [40] is visualized using confocal fluorescence laser scanning microscopy images [111]. Fluorescence microscopy showed that after adding curcumin nanoparticles encapsulated in cross-linked cyclodextrin to HeLa cells, more than 90% of the cells died after 20 hours [67]. Using DOX and CUR, fluorescent microscopic observation of cells without fluorescent labels can be performed in real time, which is a valuable tool for evaluating the optimal conditions for the antitumor activity of drugs [67].

With the help of optical microscopy, the fluorescence intensity of DOX and CUR in excised organs and tumors was used to determine their localization and amount in tumor tissues [81]. Fluorescence lifetime imaging microscopy (FLIM) is a powerful technique for distinguishing the unique molecular environment of fluorophores and is sensitive to many biomedical processes, including disease progression and drug efficacy [113]. FLIM as a non-destructive method is widely used in autofluorescence molecular imaging, to study the monitoring of cellular metabolism and protein-enzyme interactions with endogenous fluorophores NAD(P)H and FAD, to study metabolism in cancer and stem cells, immune cells, and the brain [113].

A multifunctional system of nanopreparations based on Fe_3O_4/o-Carboxymethyl Chitosan/Curcumin was developed for chemotherapy and fluorescence imaging in the HT29 cancer cell line [114]. The nanodrug system successfully delivers curcumin to HT29 cells and can be used as a tool to monitor drug circulation using a fluorescence technique [114]. A novel nanocomposite of silver nanoparticles coated with albumin was developed for specific targeting against MDA-MB 231 human breast tumor cells [115]. Their anticancer effect, which occurred through the mechanism of apoptosis, was assessed by morphological changes in cancer cells using inverted fluorescence microscopy. Thanks to albumin, which covers silver nanoparticles, their absorption by tumor cells occurs [115].

The aim of the work was to generalize literature data regarding the prospect of joint use of hydrophobic and hydrophilic drugs in therapy. Special attention is directed to the use of hydrophobic CUR as a complex drug with antitumor and physiological activity in the composition of nanomaterials together with water-soluble DOX. Such a double structure has synergistic properties, and double fluorescence in different parts of the visible spectrum makes it possible to follow the processes that occur during the penetration of the nanostructure into the cell and its interaction with the components of cellular organelles. The synergistic effect of CUR is associated with a reduction in side effects from the action of DOX.

CUR is not toxic and has many beneficial effects, so it is considered as a medicine or pharmaceutical agent. It has been shown that the use of various methods to improve the solubility of hydrophobic CUR in an aqueous environment and reduce the rate of its metabolism in the body are effective for the treatment of chronic diseases. The use of block

polymers, albumin, polymer micelles, and nanovectors makes it possible to include and transport both hydrophobic and hydrophilic molecules and particles. Based on them, it is possible to create multicomponent nanocarriers, for example, with DOX and CUR, with scientific and medical applications. The current combination strategy is promising, but further research is needed to establish better methods.

Multifunctional nanosystems enter cells in a targeted and controlled manner and are used to reduce the toxicity of drugs relative to normal cells. The effective use of the capabilities of multifunctional nanosystems is possible on the condition of scientific substantiation of the features of the combined use of components and an in-depth study of the properties of these substances. Based on CUR and DOX, combinations with synergistic enhancement have already been developed. The created multifunctional therapeutic nanosystems will be widely used in medicine in the future.

Modern nanomedicine uses nanocarriers loaded with several drugs that have both hydrophobic and hydrophilic properties. Currently, there are a number of bioactive polymer conjugates and polymer formulations in clinical development, such as micelles, hydrogels, and polymer-coated NPs, which can deliver multiple drugs. Nanoscale multifunctional systems have been created, which consist of several substances, which have an advantage over single-component ones due to the synergistic action of the components. The results show that the joint encapsulation of drugs affects their physicochemical properties and can have a synergistic effect.

Thus, the combined approach to treatment consists of co-encapsulation of several medicinal compounds in multifunctional nanostructures, which leads to long-term therapeutic efficacy, reducing side effects. The development of multifunctional, more specific and effective carriers for therapy requires accurate methods and simple methods, which are primarily directed to research on control and study of their various characteristics in order to obtain the necessary parameters. The creation of optimal drug delivery systems requires such research systems that would provide an opportunity to quickly obtain the necessary information, including optical methods.

The use of self-assembling structures is a modern strategy for the creation of nanostructures for the purpose of their transfer through the cell membrane. Special attention is paid to the non-toxicity of the polymers used for self-assembly. Among the macromolecular systems useful for targeted drug delivery are multifunctional amphiphilic Pluronic® triblock polymers, which have studied characteristics.

Pluronic® F-127 provides an attractive route for the encapsulation and delivery of hydrophobic compounds or ingredients. Its micelles with loaded carriers penetrate cells better than the drugs themselves. Some technical and scientific problems can be solved under the condition of using several fluorescent components with the participation of both hydrophobic and hydrophilic drugs. For example, it is possible to solve the question - how does the penetration of a complex nanoplatform through the cell wall take place, how does the hydrophobic part of the nanocontainer behave - does it remain in the hydrophobic membrane in greater quantity, or does it penetrate into the cytoplasm in greater quantity? Developments on targeted transport of nanocomplexes from several drugs continue, with special attention to synergistic and polyfunctional structures. The modern direction is the development of methods for creating hydrophilic structures with a hydrophobic core in the composition of CUR, which are aimed at increasing the amount and time of the drug's action. The greatest interest is caused by monodisperse nanocarriers with a hydrophobic core without traces of water with well-known characteristics, biodegradable and with minimal toxic effects, which includes Pluronic®.

The use of two fluorescent dyes based on the non-toxic Pluronic® polymer with opposite hydrophobicity properties has great potential for studying the interaction of multifunctional nanostructures with cellular ones. Tasks of a practical nature are aimed at the creation and in-depth study of various functional properties of such combined nanomaterials to improve the therapeutic effect of drugs. New tasks are related to multifunctional nanocarriers (nanocontainers), which carry a combination of already known medical or natural drugs. The creation of multifunctional structures provides an opportunity to add different components, and fluorescence provides an opportunity to follow the processes that occur in the cell and trace the time and localization of drug release. Using the method of fluorescence with several components based on non-toxic polymer multicomponent NPs is a way to

quickly develop new drugs with the participation of both hydrophobic and hydrophilic drugs. Scientific studies based on fluorescence clearly reveal the mechanism of interaction of drugs through visualization in order to improve the transport of the medicinal substance in living organisms and create more effective drugs with reduced side effects.

It is promising to create more specifically targeted drugs from complex biocompatible nanomaterials based on CUR, DOX, and enhancers of their effects based on known medicinal and natural drugs. Multifunctional NPs, with the use of coating with receptor structures, significantly enhance the targeted delivery of active medicinal compounds to the localization of pathological processes, reducing the negative effect of drugs.

In order to investigate the processes of drug passage through the plasma membrane and/or exit from the lysosomal compartments of cancer cells, the transport of drugs from cancer cells due to the activation of multidrug resistance, precise methods are needed, among which fluorescence is used.

Further development of natural compounds with chemopreventive/chemotherapeutic potential is related to the development of multifunctional formulations.

To create such nanodrugs, a detailed understanding of the processes involved in the development and implementation of advanced drug delivery systems is required, which will be accelerated by precise methods that include fluorescence studies: confocal microscopy, fluorescence spectroscopy, and flow spectroscopy.

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МУЛЬТИФУНКЦІОНАЛЬНІ НАНОСИСТЕМИ НА ПРИКЛАДІ ДВОХ ФЛУОРЕСЦЕНТНИХ БАРВНИКІВ, ДОКСОРУБІЦИНУ ТА КУРКУМІНУ

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Використання багатофункціональних наносистем у медицині та наукових дослідженнях є актуальним. *Метою роботи* було узагальнити дані літератури щодо перспектив створення та використання флуоресцентних двобарвникових наноконтейнерів, що дає змогу досліджувати розподіл флуоресцентних і нефлуоресцентних компонентів, значно прискорюючи вивчення та впровадження лікарських засобів на практиці. Особлива увага приділяється використанню в одній наночастці (НЧ) двох протилежних за якістю речовин, а саме, що мають гідрофобні та гідрофільні властивості, здатними проникати в живу клітину. Розуміння взаємодії НЧ з живими клітинами надзвичайно важливо, коли ці частинки використовуються для транспортування та доставлення до клітин нерозчинних і розчинних у водному середовищі ліків.

Доксорубіцин (DOX) — це водорозчинний препарат, який використовується в хіміотерапії раку. Повторне застосування DOX обмежене через виникнення звикання до препарату раковими клітинами (MDR). Куркумін (CUR) є одним із препаратів з різноманітними та дуже перспективними фармацевтичними ефектами, слаборозчинний у водному середовищі, а використання наноносіїв є ефективним способом значного підвищення його біодоступності.

Комбіноване застосування DOX и CUR викликає синергізм у дії лікарських засобів. DOX і CUR мають власну флуоресценцію, що дозволяє використовувати їх як багатофункціональні флуоресцентні наносистеми, наприклад, із міцелами Pluronic®, які мають гідрофобне ядро і добре зберігають CUR. Міцели Pluronic® дозволяють використати інші малорозчинні у воді лікарські препарати. Застосування флуоресцентного методу дає змогу спостерігати в часі нанорозмірну динаміку розподілу та стабільності лікарських засобів.

Висновки. Використовуючі флуоресцентний метод на прикладі двох барвників — DOX і CUR можна простежити етапи взаємодії навантажених DOX і CUR наночастинок з культивованими клітинами та вивільнення DOX і CUR з міцел або наночастинок, визначити їх кількість і локалізацію в клітинних органелах або НЧ. Два або більше флуоресцентних барвників мають перевагу перед більш дорогими методами при вивченні проникнення та розподілу НЧ у живих препаратах. Перспективним напрямом у наномедицині є створення комплексних біосумісних багатофункціональних наноматеріалів на основі кількох активних препаратів з одночасним використанням їх підсилювачів та стратегією активного націлювання. Такі сучасні структури дозволяють цілеспрямовано та контрольовано проникати в клітини. Вони значно посилять цілеспрямоване доставляння активних лікарських сполук до осередків патологічних процесів, зменшуючи токсичність ліків відносно нормальних клітин.

Ключові слова: багатофункціональні наносистеми, доксорубіцин, куркумін, синергізм, два флуоресцентних барвника.