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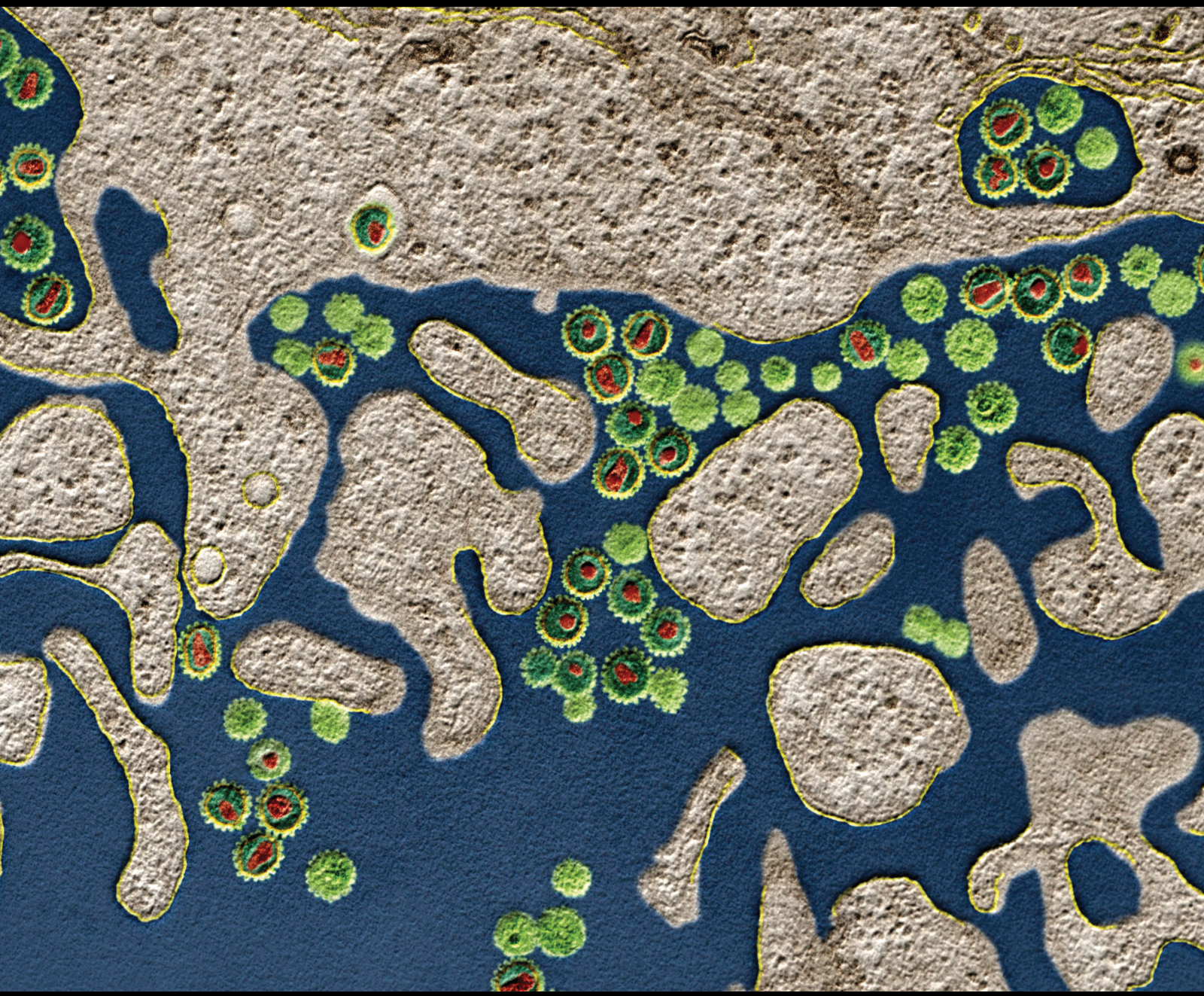

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Review Article

Update on the Use of Nanocarriers and Drug Delivery Systems and Future Directions in Cervical Cancer

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Cervical cancer represents a major health problem among females due to its increased mortality rate. The conventional therapies are very aggressive and unsatisfactory when it comes to survival rate, especially in terminal stages, which requires the development of new treatment alternatives. With the use of nanotechnology, various chemotherapeutic drugs can be transported via nanocarriers directly to cervical cancerous cells, thus skipping the hepatic first-pass effect and decreasing the rate of chemotherapy side effects. This review comprises various drug delivery systems that were applied in cervical cancer, such as lipid-based nanocarriers, polymeric and dendrimeric nanoparticles, carbon-based nanoparticles, metallic nanoparticles, inorganic nanoparticles, micellar nanocarriers, and protein and polysaccharide nanoparticles. Nanoparticles have a great therapeutic potential by increasing the pharmacological activity, drug solubility, and bioavailability. Through their mechanisms, they highly increase the toxicity in the targeted cervical tumor cells or tissues by linking to specific ligands. In addition, a nondifferentiable model is proposed through holographic implementation in the dynamics of drug delivery dynamics. As any hologram functions as a deep learning process, the artificial intelligence can be proposed as a new analyzing method in cervical cancer.

1. Introduction

Cervical cancer (CC) represents nowadays a serious medical challenge despite the early efforts to diagnosis and treatment. One of the most common causes of precancerous cervical lesions represents the persistent infection of the cervix with "high risk" genotypes of *Human papillomavirus* (HPV). If the continuous infection is not early treated in time, it can determine invasive CC [1]. Other factors such as immunosuppression, parity, smoking, and use of oral contraceptives may also contribute to CC promotion [2]. Cervical cancer-related mortality represents worldwide the fourth leading cause and the most often diagnosed cancer in females among 23 countries. In 2020, the global estimated number of new CC cases reached 604,000. On the other hand, 342,000 females died because of CC and most of them represented women from low- and middle-income countries. Still, available large-scale screening methods, the raise of socioeconomic status in different regions, and the reduction of HPV persistent infection risks within population have decreased over the last decades the incidence and mortality rates related to CC [3].

Since it was found that HPV is necessary but not sufficient to develop CC, more than 100 types of HPV have been studied, and a significant number of them have been incriminated to play an important role in the cancer pathogenesis [3]. *Human papillomavirus* is a nonenveloped double-stranded DNA virus, which present a high affinity to the mucosa or the skin. The high-risk HPV (Hr-HPV) genotypes, such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70 are associated with mucosal infection and may slowly progress into intraepithelial neoplasia, and further, into invasive cancer. Contrariwise, the low-risk HPV (Lr-HPV) types such as 6, 11, 42, 43, and 44 often determine benign cutaneous lesions like warts and are not usually associated with malignant lesions. The mature HPV particle has a size of 50-60 nm and consists of around 8000 bp genome that comprises an icosahedral capsid and various core genes involved in replication and transcription (E1, E2), packaging (L1, L2), or which have various roles such as stimulating the cell cycle entrance, viral immunity avoidance or viral release and transmission (E4, E5, E6, E7) [4, 5].

The development of HPV-infected epithelial cells to invasive cancer is a lasting progress linked to the assembling of the DNA changes inside the host cell genes [4]. *Human papillomavirus* cycle starts with the microtraumas of the basal layer and its HPV infection that impair the epithelial barrier. When the epithelial cells start their differentiation, an increased number of viral copies replicates and expresses L1 and L2 core genes, creating new virions that are launched from the epithelial layer. For persistent HPV infection, the virus needs to affect the basal layer cells showing stem cell-like characteristics with capacity of proliferation. *Human papillomavirus* oncoproteins, mainly the E5, E6, and E7 genes, incorporate DNA viral genes inside the host (human), DNA, developing into a malignant form and leading to tumor growth. These proteins increase cell proliferation and decrease apoptosis by corrupting various intracellular signaling pathways such as the degradation of p53 and pRB tumor suppressors, alteration of cell cycle regulation, p16 overexpression, driving the S-phase reentry in the upper epithelial layers or apoptosis resistance. The overexpression of E6/E7 core genes represents an essential key that have an impact on tumor suppressor genes, especially those that regulate the cell cycle [5–7].

Low-risk HPV types do not stimulate cell proliferation and the role of E6 and E7 proteins is uncertain in infected basal layer cells because are not usually related to invasive cancer. In benign HPV infections, the HPV DNA accesses the cellular nucleus but is localized extrachromosomally, compared to invasive cancers where the viral DNA integrates inside the host genome. Regarding Lr-HPV types, the role of wound healing response in determining the initial proliferation of the HPV infected cell is believed to be crucial. Understanding the altered molecular mechanisms, which occur during the development of HPV infection to cervical intraepithelial neoplasia and invasive cervical cancer, offers a better comprehension of the various pathways involved and inspires the new development of targeted treatment via nanotechnology [5, 6, 8, 9].

The implementation of screening programs for cervical cancer among females brought an enormous positive impact in the early diagnosis and treatment. The screening tests comprise cytology test and HPV detection, which are able to detect the premalignant lesions and the serotypes of HPV. Moreover, three anti-HPV vaccines have been produced against some of the HPV types in order to decrease the incidence and prevalence of HPV still, they could not entirely remove the condition. These vaccines create antibodies for HPV serotypes 16 (the monovalent vaccine), 16, 18 and 6, 11, 16, 18, respectively [10].

Since this condition represents a wide health problem due to its increase mortality and morbidity, various treatments have been developed according to the cancer staging. In the initial stage, surgical treatment or radiotherapy is very effective. For terminal stages, guidelines recommend chemotherapy, radiotherapy, immunotherapy, and targeted therapy [11]. Chemotherapeutics drugs such as DNA-interactive agents and antimetabolites are usually combined with various cytotoxic drugs in order to increase the control efficacy. Chemotherapy, frequently associated with other therapies like radiotherapy or surgery, is indicated for curative purpose, in order to extend the patient's life or to alleviate pain symptoms of terminal stage patients. Unfortunately, none of the treatment leads to adequate results and their failure is due to the side effects and resistance mechanisms that most of the anticancer drugs carry. The common limitations of chemotherapies represent low solubility in water due to their hydrophobic character, lack of selectivity of cancerous cells that can induce a huge damage to normal cells also, and the potential to develop multidrug resistance [6, 7, 11]. Conventional chemotherapy used in cervical cancer has its limitations. Most of the agents are extracted and produced artificially from plants, and this causes low water solubility. The need to use different solvents to form the correct dose of chemotherapy enhances their toxicity. Another issue represents the potential for early damage of normal cells, apart

from cancerous cells, due to their low selectivity. Moreover, the multidrug resistance effect can impair the drug delivery outside the cell by increasing the efflux pumps in the cell membrane. However, all these restrictions could be overcome by using nanotechnology via drug delivery systems. Nanotechnology represents the procedure of creating nanomaterials (nanoparticles, nanosheets, nanotubes, or nanorods) at the molecular and atomic stage, with sizes that go from 1 to 100 nm, and constitutes a promising approach to treat different types of cancers [6, 11].

Nanoscale size drugs represent the state of the art in the area of nanoparticle applications because of their amazing capacity to change important properties of drugs. The advantages of nanocarriers in cervical cancer therapy are that they can refine water solubility, agent delivery profile and diffusion, immunogenicity, and bioavailability, making them suitable for two delivery pathways: self-delivery and passive delivery. In the former, the delivery time is very important as the chemotherapeutic agent combines to the structure matter of nanocarriers in order to be easily released; otherwise, the agent will not contact the target and it will be rapidly dissociated from the carrier. In what regards the later, the agent encapsulates inside the nanocarrier due to the hydrophobic effect and will be release to the targeted site [6, 12–15]. Various treatment methods developed through nanotechnology can improve life quality and duration for patients with cervical cancer (Figure 1). Nanomaterials' ability to accumulate at the site of the tumor more than in normal cells, via a passive targeting action called the enhanced permeability and retention (EPR) effect, can improve the efficacy of the drugs, by decreasing the systemic side effects that are conventionally present. Moreover, their protective role of agents that usually degrade inside the body via different biochemical reactions and increase drug bioavailability and life. Another advantage is the possibility to combine different drugs as the nanocarrier can transport several types of agents, enhancing the potential of treatment [6, 13–16].

Among the conventional chemotherapy drugs, cisplatin presents the best response in cervical cancer treatment, being the first choice in therapeutic protocols. Cisplatin determines apoptosis of the cancer cell and deactivates its function when it binds and crosslinks with tumor DNA. However, this drug has its own limitations when side effects (neutropenia, thrombocytopenia, neurotoxicity, nephrotoxicity, or hematological toxicity) or tumor resistance occur. Various nanocarriers reduced the side effects of the drug and increase treatment feasibility and efficacy [18–22]. Paclitaxel is a chemotherapeutic drug that is widely used in human cancers, but just like cisplatin, its clinical applications are limited by drug resistance development due to various factors and dose-limiting toxicity [20, 23]. Both *in vitro* and *in vivo* studies show that curcumin increases paclitaxel-induced cytotoxicity by the downregulation of Akt pathways and nuclear factor-kB (NF-κB) and enhances its efficiency in cervical cancer [23, 24].

Methotrexate (MTX) proved to be efficient in various types of tumors. This drug is an inhibitor of dihydrofolate reductase that lowers the reversal of dihydrofolate into tetrahydrofolate, which is an important key for the synthesis of DNA and RNA. A study has shown that in combination with chitosan or methoxypoly nanoparticles it is more efficient in tumor growth inhibition and proliferation [25].

Different nanoparticles have been developed to carry multiple drugs at the same time in order to increase treatment efficacy. Therefore, an *in vitro* study reveals that d-α-tocopheryl polyethylene glycol 1000 succinate-b-poly(ε-caprolactone-ranglycolide) (TPGS-b-(PCL-ran-PGA)) nanoparticles loaded with docetaxel and endostatin reduce the viability of HeLa cells and hinder the tumor growth in a xenograft model [26]. Various studies demonstrate that nanoparticles have the great potential to simultaneously carry and deliver multiple drugs [27–29], photosensitizing molecules [30–32], genetic molecules [33], and protein, enhancing the efficacy of treatment (Figure 1).

2. Drug Delivery Systems

Drug delivery systems represent the novel pathways of drug administration that target specific situses inside the organism, in order to decrease overall toxicity and enhance bioavailability. Each drug delivery system has unique features such as physical, chemical, and morphological varieties. Moreover, particular chemical or physical interactions make them compatible with varied agent polarities. According to the type of administration, drug delivery systems are systemic and localized. Therefore, the systemic drug delivery pathways employ nanoparticles such as dendrimers, liposomes, and micelles, with specific characteristics on their surfaces that help to localize the desired situs (Figure 2) [6, 34].

Their intended role is to decrease the frequency and dosage of the agent, the systemic side effects due to their specific target, and the oscillations of drug concentration inside the body. The target specificity is achieved by nanocarrier conjugation to a variety of ligands with great affinity for the damaged cell sites such as tumor cells. Hence, the nanoparticles can encapsulate the drugs or molecules inside their structure and/or can engross the drug or molecule in the external surface. On the other side, the localized delivery pathways release outright the drug to the tumor site, limiting the drug systemic toxicity (Figure 3). The delivery system is placed near the cancer site or directly on the tumor, which is suitable for cervical cancer treatment [35–37].

3. Nanoparticles Used for Drug Delivery Systems

Nanoparticles applied in drug delivery systems present various advantages compared to the conventional therapy for cervical cancer. Their size is below 1000 nm so they can go through the tiniest vessels and bypass the phagocytes' rapid clearance in order to last longer in the bloodstream. In addition, they easily enter cells or tissues to reach targeted organs such as the cervix, liver, spleen, or others. Because of their biodegradability, heat sensitivity of structures, and pH, they possess controlled-release attributes which make them capable for drug or molecule delivery [6].

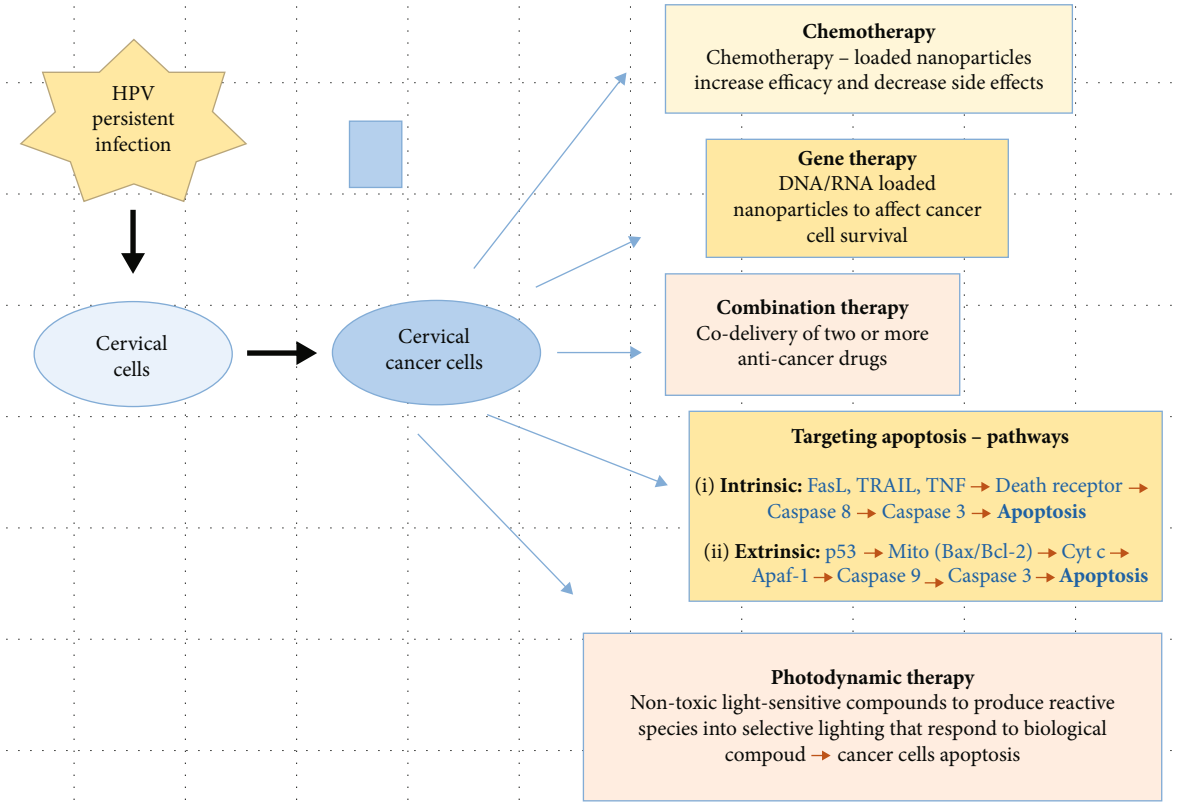

Figure 1: Nanotechnology application in the treatment of cervical cancer (modified after Chen et al. [17]). HPV: human papilloma virus.

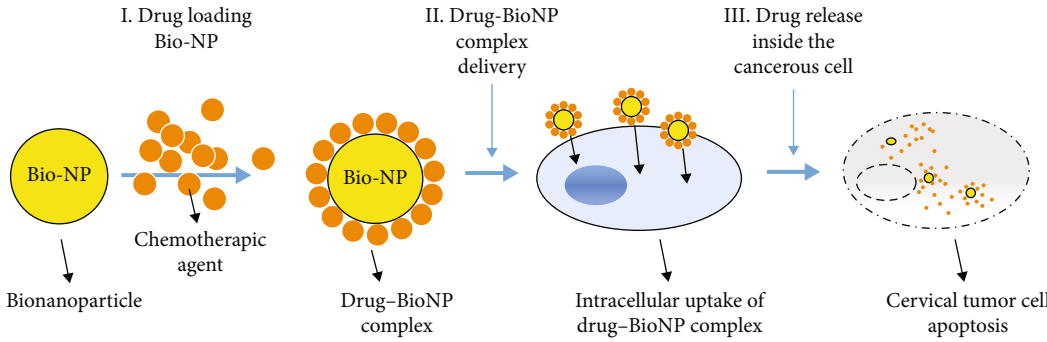

Figure 2: Systemic drug delivery system in cervical cancer; Bio-NP: bionanoparticles.

3.1. Lipid-Based Nanocarriers. Lipid nanocarriers are usually synthetized from phospholipids, triglycerides, or cholesterol, and they enhance drug solubility, encapsulation, and delivery, thus raising chemotherapy absorption. Beside lipid nanocarriers, other organic nanoparticles are liposomes, solid lipid nanoparticles (SLN), and nanostructured lipid carriers (NLC) [38, 39]. Liposomes were firstly found in 1960 by Alec Bangham and are probably the most studied among nanoparticles [40, 41]. A main advantage of liposomes is that they are able to deliver both hydrophilic and hydrophobic drugs, such as phytochemicals, chemotherapeutic particles, and immune-cytokines in order to reach the cancer cells. Recent studies, have shown their efficient applicability in cervical cancer when combined with cisplatin (lipoplatin), paclitaxel, interleukin 2 (IL-2), and curcumin. When loaded with liposomes nanoparticles increase drug stability, bioavailability, and tumor cell absorption [18, 24, 42, 43].

Solid lipid nanoparticles are advantageous because they raise drug solubility and reduce the dose of drug. Moreover, SLNs enhance drug stability by its lipid matrix that has the role to secure the components that are chemically instable and supply the attachment and incorporation inside the cancerous cell [39]. Nevertheless, SLN presents a decreased loading volume of the drug and rapid expulsion of the drug through its depositing [44]. Colloidal drug carrier systems as nanostructured lipid carriers are formed by a mix of liquid and solid lipids which makes them good candidates for drug delivery. They potentiate the bioavailability of compounds with low solubility, protect susceptible active agents, and

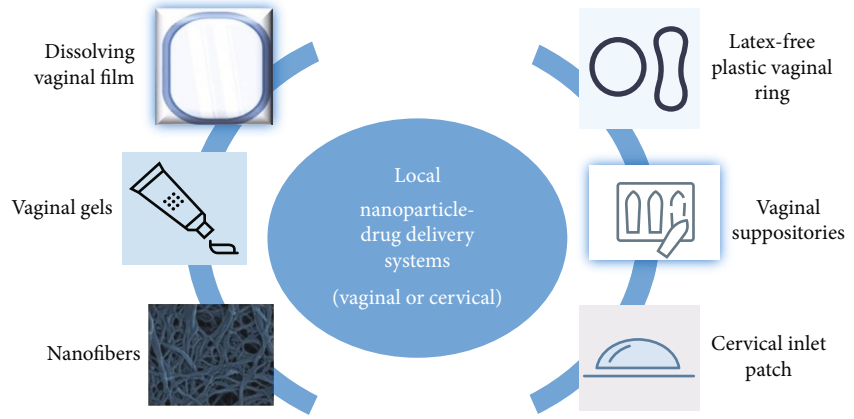

Figure 3: Localized drug delivery systems in cervical cancer.

ease the directed delivery of the drug [45, 46]. Zhang et al. used both in HeLa cells and mouse cervical cancer models, folic acid (FA) modified, cisplatin- (CIS-) loaded nanostructured lipid carriers (NLCs) for cervical cancer chemotherapy, showing its efficacy in selective release in tumor cells that highly express FA receptors. Moreover, the FA-CIS-NLC-targeted transfer CIS to cancer cell increases its antitumor power [47].

3.2. Polymeric and Dendrimeric Nanoparticles. Polymeric nanoparticles are biocompatible structures that have a great preservation for chemical or enzyme-catalyzed degradation, penetration capacity, and controlled release inside the cancerous cell. They are capable to load antibodies, DNA or RNA, allowing particular interaction in the individual targets [48–50]. The drug delivery is easily directed by the degradation rate of nanopolymers, making it simpler to control. The degradation products have no toxicity, and absorbable parts metabolize with no need of surgical removal intervention, once the agent delivery is depleted [51]. Various derivatives of poly(lactide-*co*-glycolide) (PGLA) loaded with docetaxel, in both *in vitro* and *in vivo* cervical cancer treatment, showed good delivery control and sustainability and revealed a higher efficiency of cellular uptake and antitumor ability [26, 52, 53]. When combined with polymeric nanoparticle and drug, the folate receptor acts as a feasible target on cervical cancer cells, due to its high expression [54, 55]. Studies showed that FA conjugated with chitosan [56], chitosan-coated PLGA nanocarriers [57], gelatin [54], and L-tyrosine-polyphosphate [58] and filled with selenocystine, carboplatin, cisplatin, and silver carbene complex present a 10-fold higher specificity of drug compared with the control.

The structure of dendrimers enables the fastening and presentation of antigen molecules on their extremity, making them multifunctional. Merkuria et al. show that doxorubicin-loaded dendrimers garnished with IL-6 antibodies display greater cellular incorporation and decrease the value of IC50. Moreover, it raises the drug loading rate and drug discharge rate and has a higher cytotoxicity when compared with RGD (arginyl-glycyl-aspartic acid) peptide-conjugated in HeLa cervical cancer cells [59].

3.3. Carbon-Based Nanoparticles. Carbon-based nanotubes have been extensively studied since 1990 and are attractive nanoparticles in increasing the pharmacological profile of various diagnosis and therapeutic agents. Nowadays, they divide into single-walled carbon nanotubes (SWCNT) and multiple-walled carbon nanotubes (MWCNT), each with different characteristics. They brought great contribution in imaging and drug delivery, due to their thermal, mechanical, and electrical features [60, 61]. The photothermic treatment of solid tumors using SWCNT enhanced by near-infrared light (NIR) determine a noninvasive cell death, without noxious side effect. The efficiency of SWCNT and MWCNT was proven in the treatment and early diagnosis of cervical cancer, but even if they are very promising, there are still some issues regarding toxicity and biocompatibility due to the lack of selectivity for these treatments [59, 62].

3.4. Metallic Nanoparticles (MNP). The synthesis of MNP (gold, silver, iron oxide, and silica) is achieved by chemical and physical procedures. Compared to other nanoparticles, gold and silver nanocarriers possess a particular feature, called the surface plasmon resonance (SPR), which makes the cellular surface functionality more versatile and biocompatible. There are still doubts regarding their toxicity related to the ionized or the particulate structure. Two mechanisms were proposed, the transcytosis and paracellular conveyance, but the *in vivo* carriage and the absorption process are still unclear [63, 64]. However, gold nanoparticle- (AuNP-) loaded gallic acid (GA) slows down tumor cells proliferation by causing cellular apoptosis in CaSki or HeLa cell cultures, compared to free GA. Surprisingly, a high dose of AuNPs-GA (150 μM) complex did not affect the normal cervical cells, compared to the GA group. Therefore, the study revealed that even if AuNPs-GA efficiency is lesser than GA alone, no cellular toxicity was reported in the normal cervical cells group when AuNPs-GA was applied [65]. Another study shows that AuNP-conjugated doxorubicin presents a higher anticancer activity in human cervical cancer cells, compared to free drugs [66]. The AuNP conjugation with bioactive molecules reduces overall toxicity and increases mitochondria targeting in cancer cells. Phloroglucinol conjugated with AuNPs determines apoptosis in HeLa

cancer cells by increasing the permeability of the mitochondrial membrane [67]. Another study showed that if loaded with *Podophyllum hexandrum* plant extract, the AuNPs determine DNA impairment and cellular cycle block at G2/M phase in HeLa cells [68].

Bionanotechnology via green synthesis is safer and less expensive. The photosynthesized *Catharanthus roseus* (CR) AuNPs enhance mitochondrial-mediated apoptotic signaling pathway through reactive oxygen species (ROS), causing high toxicity in HeLa cell cultures [69]. Silver nanoparticles (AgNPs) have an extensive use in the health care industry due to their particular features, being an anti-inflammatory, antibacterial, antiviral, antiangiogenic, or anticancer product. If silver is used in low quantities, it causes no damage in animal cells, compared to increased toxicity against bacteria or cancerous cells. In cervical cancer treatment, few studies are available for AgNPs obtained by chemical synthesis like ultraviolet radiation, photochemical reduction, laser ablation, or aerosol technologies [70–72]. Just like in the case of AuNPs, green synthesis of AgNPs is mostly used due to ecofriendly production. Yuan et al. reported that AgNPs conjugated with camptothecin (CPT) showed cell proliferation inhibition and enhanced cytotoxicity and apoptosis, through varied mechanisms such as raising the levels of oxidative stress markers and accelerating multiple proapoptotic gene expression [73]. Varied medicinal plants with antioxidant properties such as *Piper longum* [74] and *Cleistanthus collinus* [75] have been used for AgNP synthesis, showing favorable responses in anticancer treatment.

Iron oxide nanoparticles have been extensively studied due to their amazing capacity of combining drug delivery systems, imaging, and treatment characteristics. The effect of supermagnetic DMSO@ γ-Fe_2O_3, combined with chemotherapy agent carmustine on cervical cancer under a variable magnetic field showed an increased toxic effect, enhanced by nanomagnetic fluid thermotherapy used on cervical cells. Superparamagnetic iron oxide nanoparticles (SPIONs) are great for their noninvasive diagnosis and therapeutic use but there is still a slowly progress into clinical application [76, 77].

Mesoporous silica nanoparticles (MSNs) present great features such as tunable proportion, high load volume, morphology, stability, and simply possibility to modify internal and external surfaces of the NP and this make them very attractive for the cervical cancer diagnosis, treatment, and promising in cancer theragnostics [78]. Using MSNs, Franco et al. showed increased cellular link-up and nanomaterial transfer among immune cells and augmentation of interaction between MSNs and macrophages to coordinate an immune response in cervical cancer [79].

Selenium nanoparticles (Se NP) have demonstrated their use in cervical cancer, and their antitumor outcome is due to the inhibition of migration and invasion activity which offer an antimetastatic effect [29]. Rajkumar et al. analyzed the anticancer properties of green synthesis Se NP from *Pseudomonas stutzeri* (MH191156) as an efficient source of Se NP and its antitumor and antiangiogenic characteristics against cervical cancer cells [80]. Other authors proved its efficacy in cervical cancer by using it as a drug delivery system combined with doxorubicin [81], or by targeted siRNA delivery silence Derlin1, enhancing anticancer effect [82].

3.5. Inorganic Nanoparticles. The cooper oxide nanoparticles revealed amazing cytotoxicity results when they were tested against different cancer cells like human cervical (HeLa cells), breast (MCF-7 cells), lung (A549 cells), and epithelioma (Hep-2 cells) [83]. Although zinc oxide nanoparticles have been successfully used in the cosmetics industry due to their photocatalytic action, it was shown that if used among paclitaxel and cisplatin determine selective cancerous cell death *in vitro* human squamous cell carcinoma [84]. Barium carbonate like AuNP is used through the green synthesis of NP and can generate tumor cell apoptosis, by affecting the size and surface activity of the cell and increasing the production of reactive oxygen species (ROS) [85, 86]. Magnetic nanoparticles are made of a nanomagnetic material which has magnetic response and high paramagnetism. The magnetic nanoparticles that are usually used are magnetite and maghemite. Due to their properties, they can be placed under a magnetic field in order to deliver a targeted drug or as a magnetic resonance imaging contrast agent [87].

3.6. Micellar Nanocarriers. Micellar nanocarriers represent colloidal particles formed by amphiphilic block copolymers, which can combine among them. *In vivo*, they are very stable, with the capacity to solubilize water-hydrophobic drugs and increase the blood-circulating period due to their small sizes that vary between 10 and 100 nm [88]. When their surface is PEGylated, they can cross via passive transport through inflammatory tissues and tumor vessels and maintain a higher treatment concentration in the tumor site. There are three conjugation methods between drugs and copolymers by which micelles forms. The direct dissolution process employs the water environment to load the drug with the polymeric micelles. The solvent evaporation method uses a volatile organic solvent to disband the desired drug and copolymer. The third process is dialysis, in which through a dialysis bag the agent, washed up in solution, and the copolymer soaked in organic solvent, are combined. The micelles form subsequent dialysis process of the two components [89–91].

3.7. Protein and Polysaccharide Nanocarriers. Proteins are natural biomolecules intensely used in nanotechnology with single or multiple functions. In order to increase the targeting process, the protein nanocarrier is damaged by chemical alteration and after that it is conjugated with the targeting ligand, which will amplify the exact delivery toward cells or tissues. Albumin is a multifunctional protein that contains some hydrophobic pockets, which ease the link between the drug and amphiphilic or hydrophobic molecules. Li et al. used in a phase 2 study nanoparticle albumin-bound paclitaxel (nab-paclixatel) and nedaplatin (NDP) for patients with advanced, recurrent, and metastatic cervical cancer with good activity and tolerable results, [92]. Alberts et al. showed similar results in a phase 2 trial, using albumin-bound nab-paclitaxel in the treatment for recurrent and metastatic cervical cancer [93].

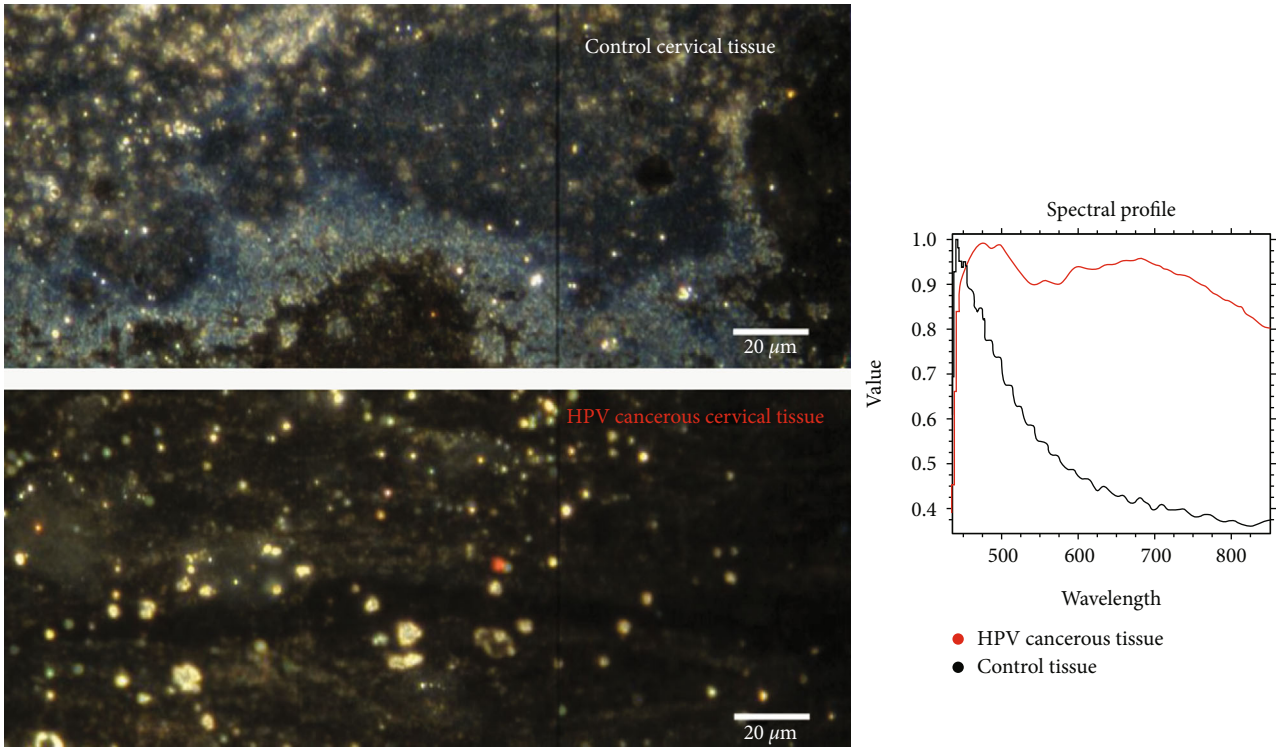

Figure 4: Enhanced dark field hyperspectral microscopy-control cervical tissue versus HPV 16 cervical tissue.

Gelatin nanoparticles (GNP) have a large applicability to target damaged tissue such as cancer, tuberculosis, vasospasm, or HIV [94]. The polysaccharides are much the same as proteins, being composed of monosaccharides clusters connected by O-glicosidic bonds. They are advantageous because they are very versatile and have specific attributes. Due to their similar structure with extracellular matrix they can bypass various immunological reactions, making them suitable candidates for drug delivery systems [95]. Still, the polysaccharides can easily disintegrate (oxidation process) if melting temperature is used for their achievement. Moreover, features such as water solubility put limits on some applications areas [96].

4. Future Perspectives

Regarding the theoretical models of controlled drug release, in addition to the classical ones, which invoke diffusion equations by Fickian, and non-Fickian processes, a new class of model is based on the description of drug release processes by continuous and indistinguishable curves (multifractals curves). As the use of such curves implies the property of self-similarity in any release points of the matrix (i.e., the part reflects the whole, the whole reflects the part, i.e.,the holographic principle), it follows that drug delivery mechanisms can be assimilated to holographic implementations of release dynamics. Following this, the class of holographic mechanism of controlled drug release it may be proposed [97–104].

For a deep understanding of phenomena, which happened in the human organism, in cases with human papilloma virus infection, we decided to analyze by comparison of two samples (HPV 16 and control) on the micronic scale. Moreover, after this achievement of obtaining the nanoscale optical imaging of the samples on 20 μm through the enhanced darkfield hyperspectral microscopy, we realized a spectral analysis (see Figure 4). The interpretation of the obtained spectral profile showed that there is a clear difference between the two samples. Thus, the graph corresponding to the control sample is defined by a decreasing nondifferential curve that shows an exponential decrease. In contrast, the graph corresponding to the HPV sample is represented by an increasing nondifferential curve showing a saturation level. In addition, for any of the nondifferential curves presented above, fractal dimensions can be calculated, as well as their succulence and lacunarity.

With this occasion, in cases of patients with HPV infection, more perspectives can be opened on the main directions within artificial intelligence that can become a primary step for much faster identification and diagnosis compared to Pap smear tests or DNA HPV test. This new technique will have at least the same accuracy.

The dimension, configuration, and some particular chemical and biophysical features concur to nanoparticle efficiency. Moreover, the specific biochemical and biophysical characteristics that a drug possess bring a huge contribution to the perfect nanoparticle-drug delivery complex. Further modulation regarding the dimension, shape, surface feature, and aqueous-solubility may augment nanoparticles bioavailability and bioactivity. Even though the nanotechnology is continuously changing nowadays, there are still some doubts regarding practical applications of nanoagents. Several questions regarding their safety, toxicity, and effective regulation need to be answered. Because of the toxic

reactions that nanoparticles may have in normal cells, recently, there have been attempts to conjugate nanoparticles with natural compounds via the green chemistry pathway. Various studies showed that biosynthetic processes through bionanotechnology reduce the toxicity dilemma and lower the side effects that most of the conventional nanoparticles have [105]. For this reason, hybrid nanocarriers represent the most encouraging application for nanomedicine, giving the heterogeneous properties of various compounds in a singular delivery system.

5. Conclusions

It is clear that nanoparticles represent an important key for the progress of drug delivery systems in cervical cancer, having an extensive use in prevention, screening, diagnostic, management, and treatment compared to other methods. All the assembled data from the literature show that chemotherapy drug-loaded nanocarriers provide a pertinent therapeutic strategy against cervical cancer, and the ongoing perfection of drug delivery systems will further integrate nanotechnology into clinical practice. As any hologram function as a deep learning process, the artificial intelligence can be proposed as a new analysis method of cervical cancer.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare no conflict of interests.

Authors' Contributions

Loredana Maria Himiniuc, Bogdan Florin Toma, Razvan Popovici, Ana Maria Grigore, Alexandru Hamod, Constantin Volovat, Simona Volovat, Irina Nica, Decebal Vasincu, Maricel Agop, Mihaela Tirnovanu, Lacramioara Ochiuz, Anca Negura, and Mihaela Grigore contributed equally to this work.

References

[1] *WHO guideline for screening and treatment of cervical precancer lesions for cervical cancer prevention*, 2nd edition, , 2021https://www.who.int/publications/i/item/9789240030824.

[2] R. Herrero and R. Murillo, "Cervical cancer," in *Cancer Epidemiology and Prevention*, M. J. Thun, M. S. Linet, J. R. Cerhan, C. A. Haiman, and D. Schottenfeld, Eds., pp. 925–946, Oxford University Press, New York, NY, USA, 4th edition, 2018.

[3] H. Sung, J. Ferlay, R. L. Siegel et al., "Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries," *CA: a Cancer Journal for Clinicians*, vol. 71, no. 3, pp. 209–249, 2021.

[4] S. D. Balasubramaniam, V. Balakrishnan, C. E. Oon, and G. Kaur, "Key molecular events in cervical cancer development," *Medicina*, vol. 55, no. 7, p. 384, 2019.

[5] J. Doorbar, N. Egawa, H. Griffin, C. Kranjec, and I. Murakami, "Human papillomavirus molecular biology and disease association," *Reviews in Medical Virology*, vol. 25, Suppl.1, pp. 2–23, 2015.

[6] J. Shi, P. W. Kantoff, R. Wooster, and O. C. Farokhzad, "Cancer nanomedicine: progress, challenges and opportunities," *Nature Reviews Cancer*, vol. 17, pp. 20–37, 2017.

[7] S. Gupta and M. K. Gupta, "Possible role of nanocarriers in drug delivery against cervical cancer," *Nano Reviews & Experiments*, vol. 8, no. 1, article 1335567, 2017.

[8] S. de Sanjose, M. Brotons, and M. A. Pavon, "The natural history of human papillomavirus infection," *Clinical Obstetrics & Gynaecology*, vol. 47, pp. 2–13, 2018.

[9] N. Egawa and J. Doorbar, "The low-risk papillomaviruses," *Virus Research*, vol. 231, pp. 119–127, 2017.

[10] B. F. Lees, B. K. Erickson, and W. K. Huh, "Cervical cancer screening: evidence behind the guidelines," *American Journal of Obstetrics and Gynecology*, vol. 214, no. 4, pp. 438–443, 2016.

[11] S. Brucker and U. A. Ulrich, "Surgical treatment of early-stage cervical cancer," *Oncology Research and Treatment*, vol. 39, no. 9, pp. 508–514, 2016.

[12] A. Z. Mirza and F. A. Siddiqui, "Nanomedicine and drug delivery: a mini review," *International Nano Letters*, vol. 4, no. 1, 2014.

[13] H. Lu, J. Wang, T. Wang, J. Zhong, Y. Bao, and H. Hao, "Recent progress on nanostructures for drug delivery applications," *Journal of Nanomaterials*, vol. 2016, Article ID 5762431, 12 pages, 2016.

[14] M. Ferrari, "Cancer nanotechnology: opportunities and challenges," *Nature Reviews. Cancer*, vol. 5, no. 3, pp. 161–171, 2005.

[15] F. Danhier, "To exploit the tumor microenvironment: since the EPR effect fails in the clinic, what is the future of nanomedicine?," *Journal of Controlled Release*, vol. 244, Part A, pp. 108–121, 2016.

[16] S. M. Moghimi and A. C. Hunter, "Capture of stealth nanoparticles by the body's defences," *Critical Reviews in Therapeutic Drug Carrier Systems*, vol. 18, no. 6, pp. 24–550, 2001.

[17] J. Chen, W. Gu, L. Yang et al., "Nanotechnology in the management of cervical cancer," *Reviews in Medical Virology*, vol. 25, Supplement 1, pp. 72–83, 2015.

[18] N. Casagrande, M. de Paoli, M. Celegato et al., "Preclinical evaluation of a new liposomal formulation of cisplatin, lipoplatin, to treat cisplatin-resistant cervical cancer," *Gynecologic Oncology*, vol. 131, no. 3, pp. 744–752, 2013.

[19] X. Xue, M. D. Hall, Q. Zhang, P. C. Wang, M. M. Gottesman, and X. J. Liang, "Nanoscale drug delivery platforms overcome platinum-based resistance in cancer cells due to abnormal membrane protein trafficking," *American Chemical Society Nano*, vol. 7, no. 12, pp. 10452–10464, 2013.

[20] A. Kumari, S. K. Yadav, and S. C. Yadav, "Biodegradable polymeric nanoparticles based drug delivery systems," *Colloids and Surfaces B: Biointerfaces*, vol. 75, no. 1, pp. 1–18, 2010.

[21] X. Zhen, X. Wang, C. Xie, W. Wu, and X. Jiang, "Cellular uptake, antitumor response and tumor penetration of

cisplatin-loaded milk protein nanoparticles," *Biomaterials*, vol. 34, no. 4, pp. 1372–1382, 2013.

[22] S. Guo, L. Miao, Y. Wang, and L. Huang, "Unmodified drug used as a material to construct nanoparticles: delivery of cisplatin for enhanced anti-cancer therapy," *Journal of Controlled Release*, vol. 174, pp. 137–142, 2014.

[23] N. I. Marupudi, J. E. Han, K. W. Li, V. M. Renard, B. M. Tyler, and H. Brem, "Paclitaxel: a review of adverse toxicities and novel delivery strategies," *Expert Opinion on Drug Safety*, vol. 6, no. 5, pp. 609–621, 2007.

[24] C. N. Sreekanth, S. V. Bava, E. Sreekumar, and R. J. Anto, "Molecular evidences for the chemosensitizing efficacy of liposomal curcumin in paclitaxel chemotherapy in mouse models of cervical cancer," *Oncogene*, vol. 30, no. 28, pp. 3139–3152, 2011.

[25] J. Chen, L. Huang, H. Lai et al., "Methotrexate-loaded PEGylated chitosan nanoparticles: synthesis, characterization, and *in vitro* and *in vivo* antitumoral activity," *Molecular Pharmaceutics*, vol. 11, no. 7, pp. 2213–2223, 2014.

[26] B. Qiu, M. Ji, X. Song et al., "Co-delivery of docetaxel and endostatin by a biodegradable nanoparticle for the synergistic treatment of cervical cancer," *Nanoscale Research Letters*, vol. 7, no. 1, p. 666, 2012.

[27] X. Xu, K. Xie, X. Q. Zhang et al., "Enhancing tumor cell response to chemotherapy through nanoparticle-mediated codelivery of siRNA and cisplatin prodrug," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 46, pp. 18638–18643, 2013.

[28] S. Balasubramanian, A. R. Girija, Y. Nagaoka et al., "Curcumin and 5-fluorouracil-loaded, folate- and transferrin-decorated polymeric magnetic nanoformulation: a synergistic cancer therapeutic approach, accelerated by magnetic hyperthermia," *International Journal of Nanomedicine*, vol. 9, pp. 437–459, 2014.

[29] F. Martínez-Esquivias, M. Gutiérrez-Angulo, A. Pérez-Larios, J. Sánchez-Burgos, J. Becerra-Ruiz, and J. M. Guzmán-Flores, "Anticancer activity of selenium nanoparticles in vitro studies," *Anti-Cancer Agents in Medicinal Chemistry*, vol. 21, 2021.

[30] A. Kubin, H. G. Loew, U. Burner, G. Jessner, H. Kolbabek, and F. Wierrani, "How to make hypericin water-soluble," *Pharmazie*, vol. 63, no. 4, pp. 263–269, 2008.

[31] H. Eshghi, A. Sazgarnia, M. Rahimizadeh, N. Attaran, M. Bakavoli, and S. Soudmand, "Protoporphyrin IX-gold nanoparticle conjugates as an efficient photosensitizer in cervical cancer therapy," *Photodiagnosis Photodynamic Therapy*, vol. 10, no. 3, pp. 304–312, 2013.

[32] M. Benito, V. Martín, M. D. Blanco, J. M. Teijón, and C. Gómez, "Cooperative effect of 5-aminolevulinic acid and gold nanoparticles for photodynamic therapy of cancer," *Journal of Pharmaceutical Sciences*, vol. 102, no. 8, pp. 2760–2769, 2013.

[33] B. Liu, S.-M. Han, X.-Y. Tang, L. Han, and C. Z. Li, "Cervical cancer gene therapy by gene loaded PEG-PLA nanomedicine," *Asian Pacific Journal of Cancer Prevention*, vol. 15, no. 12, pp. 4915–4918, 2014.

[34] S. Mignani, S. el Kazzouli, M. Bousmina, and J. P. Majoral, "Expand classical drug administration ways by emerging routes using dendrimer drug delivery systems: A concise overview," *Advanced Drug Delivery Reviews*, vol. 65, no. 10, pp. 1316–1330, 2013.

[35] J. B. Wolinsky, Y. L. Colson, and M. W. Grinstaff, "Local drug delivery strategies for cancer treatment: gels, nanoparticles, polymeric films, rods, and wafers," *Journal of Controlled Release*, vol. 159, no. 1, pp. 14–26, 2012.

[36] C. McConville, "The use of localised vaginal drug delivery as part of a neoadjuvant chemotherapy strategy in the treatment of cervical cancer," *Gynecology and Obstetrics Research - Open Journal*, vol. 2, no. 1, pp. 26–28, 2015.

[37] T. Jung, W. Kamm, A. Breitenbach, E. Kaiserling, J. X. Xiao, and T. Kissel, "Biodegradable nanoparticles for oral delivery of peptides: is there a role for polymers to affect mucosal uptake?," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 50, no. 1, pp. 147–160, 2000.

[38] S. Khan, S. Baboota, J. Ali, S. Khan, R. S. Narang, and J. K. Narang, "Nanostructured lipid carriers: an emerging platform for improving oral bioavailability of lipophilic drugs," *International Journal of Pharmaceutical Investigation*, vol. 5, no. 4, pp. 182–191, 2015.

[39] S. V. Talluri, G. Kuppusamy, V. V. S. R. Karri, S. Tummala, and S. R. V. Madhunapantula, "Lipid-based nanocarriers for breast cancer treatment - comprehensive review," *Drug Delivery*, vol. 23, no. 4, pp. 1291–1305, 2016.

[40] F. Movahedi, R. G. Hu, D. L. Becker, and C. Xu, "Stimuli-responsive liposomes for the delivery of nucleic acid therapeutics," *Nanomedicine*, vol. 11, no. 6, pp. 1575–1584, 2015.

[41] G. Bozzuto and A. Molinari, "Liposomes as nanomedical devices," *International Journal of Nanomedicine*, vol. 10, pp. 975–999, 2015.

[42] N. Saengkrit, S. Saesoo, W. Srinuanchai, S. Phunpee, and U. R. Ruktanonchai, "Influence of curcumin-loaded cationic liposome on anticancer activity for cervical cancer therapy," *Colloids and Surfaces B: Biointerfaces*, vol. 114, pp. 349–356, 2014.

[43] R. Rangel-Corona, T. Corona-Ortega, I. del Río-Ortiz et al., "Cationic liposomes bearing IL-2 on their external surface induced mice leukocytes to kill human cervical cancer cells in vitro, and significantly reduced tumor burden in immunodepressed mice," *Journal of Drug Targeting*, vol. 19, no. 2, pp. 79–85, 2011.

[44] A. J. Almeida and E. Souto, "Solid lipid nanoparticles as a drug delivery system for peptides and proteins," *Advanced Drug Delivery Reviews*, vol. 59, no. 6, pp. 478–490, 2007.

[45] C. W. How, R. Abdullah, and R. Abbasalipourkabir, "Physicochemical properties of nanostructured lipid carriers as colloidal carrier system stabilized with polysorbate 20 and polysorbate 80," *African Journal of Biotechnology*, vol. 10, no. 9, pp. 1684–1689, 2011.

[46] R. H. Müller, K. Mäder, and S. Gohla, "Solid lipid nanoparticles (SLN) for controlled drug delivery - a review of the state of the art," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 50, no. 1, pp. 161–177, 2000.

[47] G. Zhang, F. Liu, E. Jia, L. Jia, and Y. Zhang, "Folate-modified, cisplatin-loaded lipid carriers for cervical cancer chemotherapy," *Drug Delivery*, vol. 23, no. 4, pp. 1393–1397, 2016.

[48] Y. Yuan and B. Liu, "Self-Assembled nanoparticles based on PEGylated conjugated polyelectrolyte and drug molecules for image-guided drug delivery and photodynamic therapy," *ACS Applied Materials & Interfaces*, vol. 6, no. 17, pp. 14903–14910, 2014.

[49] J. Yang, S. Li, F. Guo, W. Zhang, Y. Wang, and Y. Pan, "Induction of apoptosis by chitosan/HPV16 E7 siRNA

complexes in cervical cancer cells," *Molecular Medicine Reports*, vol. 7, no. 3, pp. 998–1002, 2013.

[50] G. J. Rad and D. W. Hoskin, "Delivery of apoptosis-inducing piperine to triple-negative breast cancer cells via co-polymeric nanoparticles," *Anticancer Research*, vol. 40, no. 2, pp. 689–694, 2020.

[51] J. R. Joshi and R. P. Patel, "Role of biodegradable polymers in drug delivery," *International Journal of Pharmaceutical Science and Research*, vol. 4, no. 4, pp. 74–81, 2012.

[52] Y. Ma, L. Huang, C. Song, X. Zeng, G. Liu, and L. Mei, "Nanoparticle formulation of poly(ε-caprolactone-co-lactide)-d-α-tocopheryl polyethylene glycol 1000 succinate random copolymer for cervical cancer treatment," *Polymer*, vol. 51, no. 25, pp. 5952–5959, 2010.

[53] X. Zeng, W. Tao, L. Mei, L. Huang, C. Tan, and S. S. Feng, "Cholic acid-functionalized nanoparticles of star-shaped PLGA-vitamin E TPGS copolymer for docetaxel delivery to cervical cancer," *Biomaterials*, vol. 34, no. 25, pp. 6058–6067, 2013.

[54] N. Dixit, K. Vaibhav, R. S. Pandey et al., "Improved cisplatin delivery in cervical cancer cells by utilizing folate- grafted non-aggregated gelatin nanoparticles," *Biomedicine & Pharmacotherapy*, vol. 69, pp. 1–10, 2015.

[55] C. Zhang, Z. Zhang, and L. Zhao, "Folate-decorated poly(3-hydroxybutyrate-co-3-hydroxyoctanoate) nanoparticles for targeting delivery: optimization andin vivoantitumor activity," *Drug Delivery*, vol. 23, no. 5, pp. 1830–1837, 2016.

[56] B. Yu, H. Li, J. Zhang, W. Zheng, and T. Chen, "Rational design and fabrication of a cancer-targeted chitosan nanocarrier to enhance selective cellular uptake and anticancer efficacy of selenocystine," *Journal of Materials Chemistry B*, vol. 3, no. 12, pp. 2497–2504, 2015.

[57] J. Ji, P. Zuo, and Y.-L. Wang, "Enhanced antiproliferative effect of carboplatin in cervical cancer cells utilizing folate-grafted polymeric nanoparticles," *Nanoscale Research Letters*, vol. 10, no. 1, p. 453, 2015.

[58] A. J. Ditto, K. N. Shah, N. K. Robishaw, M. J. Panzner, W. J. Youngs, and Y. H. Yun, "The interactions between L-tyrosine based nanoparticles decorated with folic acid and cervical cancer cells under physiological flow," *Molecular Pharmaceutics*, vol. 9, no. 11, pp. 3089–3098, 2012.

[59] S. L. Mekuria, T. A. Debele, H. Y. Chou, and H. C. Tsai, "Correction to "IL-6 antibody and RGD peptide conjugated poly(-amidoamine) dendrimer for targeted drug delivery of HeLa cells"," *The Journal of Physical Chemistry B*, vol. 120, no. 25, p. 5786, 2016.

[60] H. Gong, R. Peng, and Z. Liu, "Carbon nanotubes for biomedical imaging: the recent advances," *Advanced Drug Delivey Reviews*, vol. 65, no. 15, pp. 1951–1963, 2013.

[61] R. Li, R. Wu, L. Zhao et al., "Folate and iron difunctionalized multiwall carbon nanotubes as dual-targeted drug nanocarrier to cancer cells," *Carbon*, vol. 49, no. 5, pp. 1797–1805, 2011.

[62] H. K. Moon, S. H. Lee, and H. C. Choi, "In vivo near-infrared mediated tumor destruction by photothermal effect of carbon nanotubes," *American Chemistry Society Nano*, vol. 3, no. 11, pp. 3707–3713, 2009.

[63] S. J. Choi, J. K. Lee, J. Jeong, and J. H. Choy, "Toxicity evaluation of inorganic nanoparticles: considerations and challenges," *Molecular & Cellular Toxicology*, vol. 9, no. 3, pp. 205–210, 2013.

[64] R. Hu, M. Zheng, J. Wu et al., "Core-shell magnetic gold nanoparticles for magnetic field-enhanced radiophotothermal therapy in cervical cancer," *Nanomaterials*, vol. 7, no. 5, p. 111, 2017.

[65] J. Daduang, A. Palasap, S. Daduang, P. Boonsiri, P. Suwannalert, and T. Limpaiboon, "Gallic acid enhancement of gold nanoparticle anticancer activity in cervical cancer cells," *Asian Pacific Journal of Cancer Prevention*, vol. 16, no. 1, pp. 169–174, 2015.

[66] R. Sinha, G. J. Kim, S. Nie, and D. M. Shin, "Nanotechnology in cancer therapeutics: bioconjugated nanoparticles for drug delivery," *Molecular Cancer Therapeutics*, vol. 5, no. 8, pp. 1909–1917, 2006.

[67] M. Mahalakshmi and P. Kumar, "Phloroglucinol-conjugated gold nanoparticles targeting mitochondrial membrane potential of human cervical (HeLa) cancer cell lines," *Spectrochimica Acta Part A Molecular and Biomolecular Spectroscopy*, vol. 219, pp. 450–456, 2019.

[68] M. Jeyaraj, R. Arun, G. Sathishkumar et al., "An evidence on G2/M arrest, DNA damage and caspase mediated apoptotic effect of biosynthesized gold nanoparticles on human cervical carcinoma cells (HeLa)," *Materials Research Bulletin*, vol. 52, pp. 15–24, 2014.

[69] Y. Ke, M. S. Al Aboody, W. Alturaiki et al., "Photosynthesized gold nanoparticles from Catharanthus roseus induces caspase-mediated apoptosis in cervical cancer cells (HeLa)," *Artificial Cells, Nanomedicine, and Biotechnology*, vol. 47, no. 1, pp. 1938–1946, 2019.

[70] K. Okitsu, A. Yue, S. Tanabe, H. Matsumoto, and Y. Yobiko, "Formation of colloidal gold nanoparticles in an ultrasonic field: control of rate of gold (III) reduction and size of formed gold particles," *Langmuir*, vol. 17, no. 25, pp. 7717–7720, 2001.

[71] R. R. Naik, S. J. Stringer, G. Agarwal, S. E. Jones, and M. O. Stone, "Biomimetic synthesis and patterning of silver nanoparticles," *Nature Materials*, vol. 1, no. 3, pp. 169–172, 2002.

[72] K. B. Narayanan and N. Sakthivel, "Biological synthesis of metal nanoparticles by microbes," *Advances in Colloid and Interface Science*, vol. 156, no. 1-2, pp. 1–13, 2010.

[73] Y. G. Yuan, S. Zhang, J. Y. Hwang, and I. K. Kong, "Silver nanoparticles potentiates cytotoxicity and apoptotic potential of camptothecin in human cervical cancer cells," *Oxidative Medicine and Cellular Longevity*, vol. 2018, Article ID 6121328, 21 pages, 2018.

[74] N. J. Reddy, V. D. Nagoor, M. Rani, and S. S. Rani, "Evaluation of antioxidant, antibacterial and cytotoxic effects of green synthesized silver nanoparticles by _Piper longum_ fruit," *Materials Science & Engineering C, Materials for Biological Applications*, vol. 34, pp. 115–122, 2014.

[75] N. Kanipandian, S. Kannan, R. Ramesh, P. Subramanian, and R. Thirumurugan, "Characterization, antioxidant and cytotoxicity evaluation of green synthesized silver nanoparticles using *Cleistanthus collinus* extract as surface modifier," *Materials Research Bulletin*, vol. 49, no. 1, pp. 494–502, 2014.

[76] S. Wen, W. Xing, L. Gao, and S. Zhao, "Effect of superparamagnetic DMSO@γ-Fe2O3Combined with Carmustine on cervical cancer," *Journal of Nanoscience and Nanotechnology*, vol. 21, no. 12, pp. 6196–6204, 2021.

[77] S. Talluri and R. R. Malla, "Superparamagnetic iron oxide nanoparticles (SPIONs) for diagnosis and treatment of breast, ovarian and cervical Cancers," *Current Drug Metabolism*, vol. 20, no. 12, pp. 942–945, 2019.

[78] A. Gupta, S. S. Kushwaha, and A. Mishra, "A review on recent technologies and patents on silica nanoparticles for cancer Treatment and diagnosis," *Recent Patents on Drug Delivery & Formulation*, vol. 14, no. 2, pp. 126–144, 2020.

[79] S. Franco, A. Noureddine, J. Guo et al., "Direct transfer of mesoporous silica nanoparticles between macrophages and cancer cells," *Cancers*, vol. 12, no. 10, p. 2892, 2020.

[80] K. Rajkumar, S. Mvs, S. Koganti, and S. Burgula, "Selenium nanoparticles synthesized using *Pseudomonas stutzeri* (MH191156) show antiproliferative and anti-angiogenic activity against cervical cancer Cells," *International Journal of Nanomedicine*, vol. 15, pp. 4523–4540, 2020.

[81] Y. Xia, M. Xiao, M. Zhao et al., "Doxorubicin-loaded functionalized selenium nanoparticles for enhanced antitumor efficacy in cervical carcinoma therapy," *Materials Science & Engineering. C, Materials for Biological Applications*, vol. 106, article 110100, 2020.

[82] Y. Xia, G. Tang, C. Wang et al., "Functionalized selenium nanoparticles for targeted siRNA delivery silence Derlin1 and promote antitumor efficacy against cervical cancer," *Drug Delivery*, vol. 27, no. 1, pp. 15–25, 2020.

[83] D. Rehana, D. Mahendiran, R. S. Kumar, and A. K. Rahiman, "Evaluation of antioxidant and anticancer activity of copper oxide nanoparticles synthesized using medicinally important plant extracts," *Biomedicine & Pharmacotherapy*, vol. 89, pp. 1067–1077, 2017.

[84] S. Hackenberg, A. Scherzed, W. Harnisch et al., "Antitumor activity of photo-stimulated zinc oxide nanoparticles combined with paclitaxel or cisplatin in HNSCC cell lines," *Journal of Photochemistry and Photobiology B: Biology*, vol. 114, pp. 87–93, 2012.

[85] Y. N. Wu, D. H. Chen, X. Y. Shi et al., "Cancer-cell-specific cytotoxicity of non-oxidized iron elements in iron core- gold shell NPs," *Nanomedicine: Nanotechnology, Biology and Medicine*, vol. 7, no. 4, pp. 420–427, 2011.

[86] I. Lynch, K. A. Dawson, and S. Linse, "Detecting cryptic epitopes created by nanoparticles," *Science's STKE*, vol. 2006, no. 327, 2006.

[87] M. Dan, Y. Bae, T. A. Pittman, and R. A. Yokel, "Alternating magnetic field-induced hyperthermia increases iron oxide nanoparticle cell association/uptake and flux in blood-brain barrier models," *Pharmaceutical Research*, vol. 32, no. 5, pp. 1615–1625, 2015.

[88] D. Sutton, N. Nasongkla, E. Blanco, and J. Gao, "Functionalized micellar systems for cancer targeted drug delivery," *Pharmaceutical Research*, vol. 24, no. 6, pp. 1029–1046, 2007.

[89] Mourya, "Polymeric micelles: general considerations and their applications," *Indian Journal of Pharmaceutical Research and Education*, vol. 45, no. 2, pp. 128–138, 2011.

[90] G. Pertici, "Introduction to bioresorbable polymers for biomedical applications," in *Bioresorbable Polymers for Biomedical Applications*, G. Perale and J. Hilborn, Eds., pp. 3–29, Woodhead Publishing, 2017.

[91] Y. Zhang, Y. Huang, and L. Song, "Polymeric micelles: nanocarriers for cancer-targeted drug delivery," *AAPS PharmSciTech*, vol. 15, no. 4, pp. 862–871, 2014.

[92] Y. Li, J. Zeng, M. Huang et al., "A phase 2 study of nanoparticle albumin-bound paclitaxel plus nedaplatin for patients with advanced, recurrent, or metastatic cervical carcinoma," *Cancer*, vol. 123, no. 3, pp. 420–425, 2017.

[93] D. S. Alberts, J. A. Blessing, L. M. Landrum et al., "Phase II trial of nab-paclitaxel in the treatment of recurrent or persistent advanced cervix cancer: a gynecologic oncology group study," *Gynecologic Oncology*, vol. 127, no. 3, pp. 451–455, 2012.

[94] J. R. Lakkakula, P. Gujarathi, P. Pansare, and S. Tripathi, "A comprehensive review on alginate-based delivery systems for the delivery of chemotherapeutic agent: aoxorubicin," *Carbohydrate Polymers*, vol. 259, article 117696, 2021.

[95] M. Bassas-Galia, S. Follonier, M. Pusnik, and M. Zinn, "Natural polymers," in *Bioresorbable Polymers for Biomedical Applications*, pp. 31–64, Elsevier, 2017.

[96] L. A. Poole-Warren and A. J. Patton, "Introduction to biomedical polymers and biocompatibility," in *Biosynthetic Polymers for Medical Applications*, pp. 3–31, Elsevier, 2016.

[97] M. Agop, P. E. Nica, P. D. Ioannou, A. Antici, and V. P. Paun, "Fractal model of the atom and some properties of the matter through an extended model of scale relativity," *The European Physical Journal D*, vol. 49, no. 2, pp. 239–248, 2008.

[98] C. Nejneru, A. Nicuță, B. Constantin, L. R. Manea, M. Teodorescu, and M. Agop, "Dynamics control of the complex systems via nondifferentiability," *Journal of Applied Mathematics*, vol. 2013, Article ID 137056, 12 pages, 2013.

[99] L. Himiniuc, M. Agop, V. Ghizdovat et al., "A drug release mechanism controlled by hydrophobic/hydrophilic balance of the matrix. Theoretical and experimental perspectives," *Materiale Plastice*, vol. 57, no. 4, pp. 155–165, 2021.

[100] M. M. Iftime, S. A. Irimiciuc, M. Agop, M. Angheloiu, L. Ochiuz, and D. Vasincu, "A theoretical multifractal model for assessing urea release from chitosan based formulations," *Polymers*, vol. 12, no. 6, p. 1264, 2020.

[101] I. Nica, V. Rusu, M. A. Paun, C. Stefanescu, P. Vizureanu, and A. Aluculesei, "Thermal properties of nanofilled and microfilled restorative composites," *Materiale Plastice*, vol. 46, no. 4, pp. 431–434, 2009.

[102] G. Iovan, S. Stoleriu, I. Nica, S. Solomon, A. Muneanu, and S. Adrian, "Surface characteristics of restorative composite resins after polishing with Profine Lamineer tips," *Materiale Plastice*, vol. 53, no. 4, pp. 755–758, 2016.

[103] M. M. Iftime, D. L. Dobreci, S. A. Irimiciuc, M. Agop, T. Petrescu, and B. Doroftei, "A theoretical mathematical model for assessing diclofenac release from chitosan-based formulations," *Drug Deliver*, vol. 27, no. 1, pp. 1125–1133, 2020.

[104] B. M. Cobzeanu, S. A. Irimiciuc, D. Vaideanu, A. Grigorovici, and O. Popa, "Possible dynamics of polymer chains by means of a Ricatti s procedure - an exploitation for drug release at large time intervals," *Materiale Plastice*, vol. 54, no. 3, pp. 531–534, 2017.

[105] P.-L. Lam, W.-Y. Wong, Z. Bian, C.-H. Chui, and R. Gambari, "Recent advances in green nanoparticulate systems for drug delivery: efficient delivery and safety concern," *Nanomedicine*, vol. 12, no. 4, pp. 357–385, 2017.

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Research Article

Prospects and Challenges of the Drug Delivery Systems in Endometriosis Pain Management: Experimental and Theoretical Aspects

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Endometriosis is considered a serious public health issue because of the large number of females affected by this illness. Chronic pain management in patients with endometriosis demands new strategies to increase the life quality of these patients. The development of drug delivery systems represents a new approach in pain treatment among endometriosis patients. Diclofenac sodium, one of the most utilized nonsteroidal anti-inflammatory drugs (NSAID), has its own limitations when being used in formulas such as oral, parental, or local applications. In this paper, a series of four drug release formulations based on chitosan, 2-hydroxy-5-nitrobenzaldehyde, and diclofenac sodium salt were prepared in view of the investigation of the drug release ability. The formulations were analyzed from a morphological and supramolecular point of view by scanning electron microscopy and polarized light microscopy. The in vitro drug release ability was investigated by mimicking a physiologic environment. A mathematical model, using the fractal paradigm of motion, is utilized to explain the behaviors of the drug delivery system presented in this paper. These results suggest a great potential of the proposed drug delivery system, based on chitosan and 2-hydroxy-5-nitrobenzaldehyde to improve the diclofenac sodium salt bioavailability, and it may represent a future treatment formula for endometriosis pain.

1. Introduction

Endometriosis is a wide benign, chronic, and inflammatory pathology among fertile women that is characterized by painful symptomatology and infertility. The distinctive mark of endometriosis diagnosis is represented by the presence of stromal and glandular endometrial tissues outside the uterus. The symptoms related endometriosis comprises dysmenorrhea,

dyspareunia, and pelvic or lower abdominal pain that frequently has a negative impact on the patient's life quality, career, daily activities, relationships, and fertility. Sometimes, patients may accuse cyclical pain in other areas correlated with endometriosis [1]. Even if endometriosis is a very popular condition, the diagnosis can be difficult, especially in the less severe stages (stages I-II), and at this moment, laparoscopy is considered the "gold standard" for diagnosis [2].

The mechanisms of endometriosis are not entirely understood. It is believed to be an inflammatory condition that involves various endocrine, genetic, immunological, and environmental interplays with great impact in the initiation and progression of the pathology. The disfunction of the immunological system plays a critical role for the development and persistence of endometrial implants inside the peritoneal cavity. Peritoneal fluid represents an important immunological barrier system that contains different immune cells such as mesothelial cells, macrophages, natural killer (NK) cells, T and B lymphocytes, and monocytes. Immunoinflammatory factors, angiogenic factors, and endocrine pathways establish specific and dynamic circumstances that are necessary to create and grow endometriotic implants. The macrophage population is higher within peritoneal fluid and endometriotic implants and contributes to the inflammatory environment but, compared with nonendometriozic patients, presents a decreased phagocytic function and low expression of B scavenger receptor CD36. The ratio between M2 anti-inflammatory macrophages and M1 proinflammatory is inverted in endometriosis patients. An increase level of M1 macrophages found in endometriosis tissue contributes to profibrotic activity, survival, and progression of ectopic implants by angiogenesis and immune tolerance induction [3, 4]. Moreover, oestrogen receptors (ER) may play an important role in macrophage regulation, suggesting a correlation between immunological response and oestrogens. In endometriosis patients, it was shown that ER-α expression is positively linked to proinflammatory cytokine expression in macrophages and ER-β presents anti-inflammatory function [5]. An increased number of proinflammatory cytokines were found within endometrial implants. Therefore, ectopic implants showed a higher expression of transcription factor, nuclear factor-kB (NF-kB), along with fibronectin, intercellular adhesion molecule 1 (ICAM1), insulin-like growth factor I (IGFI), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-8 (IL-8) which enhances growth function within the ectopic implant by promoting the proinflammatory environment [3]. These cytokines launch and enhance the inflammatory response, targeting the recruitment of various proinflammatory cells and mediators. Tumor necrosis factor and its receptors, TNFR1 and TNFR2, represent an extrinsic apoptosis pathway involved in endometriosis genesis, being implicated in inflammatory and endometrial repair [6, 7].

Natural killer (NK) cells are normally abolished by the peritoneal barrier environment, but within endometriosis patients, an overexpression of different surface receptors that can activate or suppress their function was found. They represent cytotoxic effector lymphocytes that do not need a major histocompatibility complex or previous exposure to the antigen to lyse the target cells. Lately, research has been focused to identify various factors, which may suppress NK cell cytolytic function such as IL-6, IL-15, and TGF-β1 [8–11]. The endocrine premature dendritic cells reach maturity and are carried through the lymphatic vessels in response to foreign antigens or various antigens on top of T cells from inflammatory targets. In endometriozic tissues, this physiological process is being modified and the population of CD83+ dendritic cells is significantly decreased, leading to endometrial antigen misrecognition by the circulating antiendometrial stromal cells [7].

Inflammation represents an important key in endometriosis pathogenesis, and further studies focused on the intracellular signaling mechanisms will contribute to understand better the inflammatory pathogenesis of endometriosis to develop future therapeutic strategies.

The treatment of symptoms is very wide, having various options, but the underlying pathology frequently demands repeated medical and surgical interventions. The possibilities of medical treatment include oral contraceptives, testosterone derivatives, progestogens, and gonadotropin-releasing hormone (GnRH) agonists. Regarding the surgical approach, there are two modalities used for endometriosis treatment such as ablative techniques and excision [12, 13]. In the management of pain-related endometriosis, they are utilized as first-line therapy nonsteroidal anti-inflammatory drugs (NSAIDs) which represent a group of analgesic drugs. This drug class inhibits the cyclooxygenase- (COX-) 1 and COX-2 enzymes. The COX-2 enzyme is responsible for prostaglandin formation, an important key in inflammatory response initiation, and its inhibition determines therapeutic anti-inflammatory effects. Diclofenac sodium is a traditional NSAID that inhibits both COX-1 and COX-2 with greater impact on COX-2, being comparable to celecoxib, a first generation of the COX-2 inhibitor [14]. Depending on the dose that is used and the time between administrations, diclofenac like other COX-1 and COX-2 inhibitors, associates an increased risk of gastrointestinal, cardiovascular, and renal complications. To reduce the side effects and to improve the variability of diclofenac indications, the pharmaceutical industry developed different formulas with large approaches such as oral, parental, and local applications.

Drug delivery is a research direction of high contemporary interest, meant to improve the bioavailability of therapeutic drugs, to overcome impairments such as limited drug solubility or tendency of aggregation and to limit their side effects by targeted delivery. In time, many types of drug carriers were proposed to fulfill the requirements of in vivo drug release, such as liposomes, hydrogels, nanogels, and micelles [15–18]. Among them, the hydrogels present the advantage of high similarity with human tissues, while those based on natural or derivatives of natural have good biocompatibility and biodegradability. Along this line of thought, chitosan-based hydrogels proved the potential to skip the barrier towards real-world applications, because besides biocompatibility and biodegradation, it has also a large realm of biologic properties [19]. Recent research in the area of chitosan hydrogels revealed a new crosslinking method with monoaldehydes, based on a combined physicochemical

method consisting in the self-assembling of the newly formed imine units into ordered clusters which play the role of crosslinking nodes [20–23]. This nontraditional hydrogelation method proved a great potential for the design of drug delivery formulations, bringing the advantage of the use of biocompatible natural aldehydes with synergic biologic properties [24–26]. In this context, hydrogels prepared from chitosan and a vanillin derivative, 2-hydroxy-5-nitrobenzaldehyde, showed thixotropic behavior [24] and antimicrobial activity [27], promising to be an excellent matrix for the local delivery of diclofenac for the treatment of endometriosis. To further understand the mechanism behind the slow release of drugs from these chitosan-based hydrogels, a multifractal mathematic model is proposed to explain the drug delivery complex mechanisms.

2. Materials and Methods

2.1. Materials. Chitosan of low molecular weight (217.74 kDa, DA: 85%), 2-hydroxy-5-nitrobenzaldehyde (98%), diclofenac sodium salt (DCF) (99%), and phosphate buffer solution from Aldrich were used as received.

2.2. Preparation of the Formulations. A series of four drug delivery formulations were prepared by in situ crosslinking of chitosan with 2-hydroxy-5-nitrobenzaldehyde in the presence of diclofenac sodium salt, according to reference [16]. Shortly, (i) a chitosan solution was prepared by dissolving it in 0.7% acetic acid to give a 2% solution, (ii) a 1% solution of 2-hydroxy-5-nitrobenzaldehyde in ethanol was mixed with DCF, and then, (iii) it was slowly poured into the chitosan solution under vigorous magnetic stirring. The quantities of chitosan and 2-hydroxy-5-nitrobenzaldehyde were calculated to reach four different crosslinking degrees in the final formulations, corresponding to four different ratios of the amine and aldehyde functional groups: 5/1, 4/1, 3/1, and 2/1. The diclofenac amount was kept constant, consistent with the accepted dose (g/kg). The formulation codes were formed from the number corresponding to the ratio of functional groups and the letter D of DCF: 5D, 4D, 3D, and 2D.

2.3. Methods and Equipment. The formulations were frozen in liquid nitrogen and then lyophilized using a Labconco-FreeZone Freeze Dry System equipment for 24 h at −54°C and 1.512 mbar, to obtain the corresponding solid state as xerogels.

The morphology of the formulations was investigated on the corresponding xerogels, using a field emission scanning electron microscope (SEM) EDAX—Quanta 200 at an accelerated electron energy of 20 KeV.

The supramolecular architecture of the formulations was observed by polarized light microscopy (POM) with a Leica DM 2500 microscope, on slim slices of xerogels placed between two lamellae.

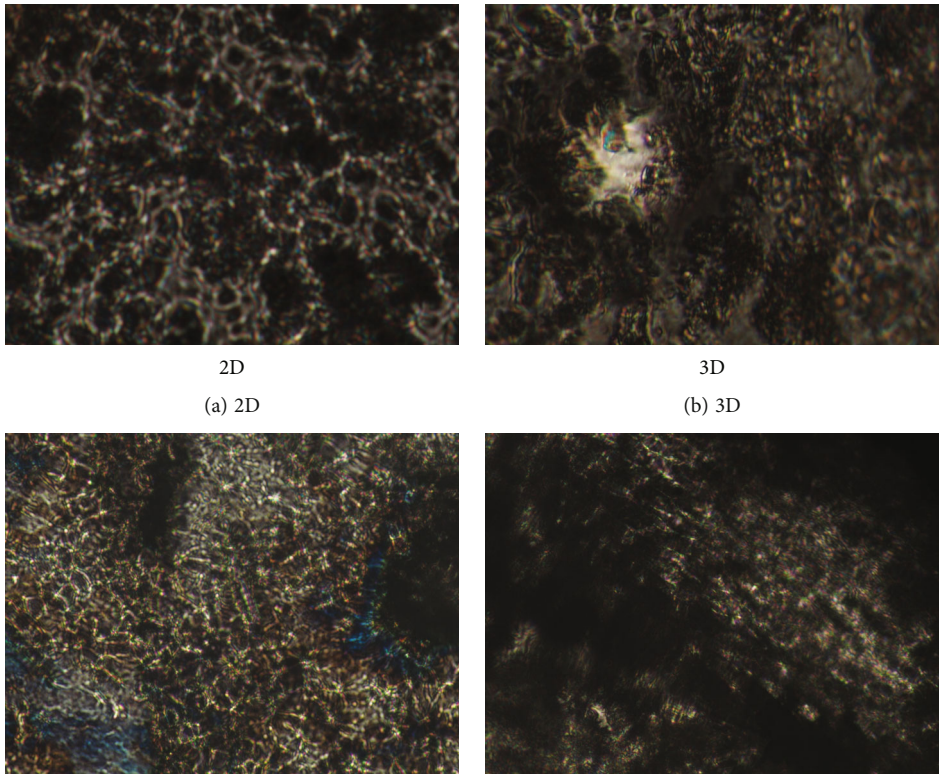
In vitro investigation of the DCF release from formulations was investigated applying a standard procedure [28]. Briefly, the formulation samples were immersed into vials containing 10 mL of phosphate buffer and maintained at 37°C. At certain moments, 2 mL of the supernatant was withdrawn and replenished with fresh buffer solution. The concentration of DCF released into the supernatant was assessed by measuring the specific DCF absorbance and its fitting to a calibration curve. The experiments were performed in triplicate. The absorbance spectroscopy was done on a HORIBA spectrophotometer.

3. Results and Discussions

A series of four formulations were prepared by chitosan hydrogelation with 2-hydroxy-5-nitrobenzaldehyde in the presence of diclofenac sodium salt. The designing of these formulations considered the properties of the components and the intermolecular forces which can be developed between them. Thus, chitosan is a well-known biopolymer with excellent biocompatibility and biodegradability and valuable biologic properties such as antimicrobial activity and blood clotting, hypocholesterolemic, or immunoenhancing effects. 2-Hydroxy-5-nitrobenzaldehyde has been chosen as a chitosan crosslinker, due to the fact that it is a vanillin derivative, nontoxic for the human body, and with good antimicrobial properties [27, 29], having promising synergistic effect with diclofenac drug. The chemical structure of the three components displays polar groups such as –Cl, –OH, –COO–, and $–NO_2$, which promotes intermolecular forces among the three components (such as H-bonds and polar forces) creating the possibility of a prolonged release of the drug and thus a prolonged bioavailability. To appreciate the influence of the matrix on the release kinetic of the drug, four formulations were prepared by varying the ratio between the amine and aldehyde groups and consequently the crosslinking density.

Polarized light microscopy was used to assess the encapsulation of the drug into the matrix (Figure 1). The formulations revealed birefringent banded textures, signatures of the layered phases [20, 24, 30], confirming thus that the self-assembling of the imine units formed between chitosan and 2-hydroxy-5-nitrobenzaldehyde was the main promotor of formulation hydrogelation [20–24]. This texture pattern was evident for all four formulations, signifying that the DCF presence did not hamper the hydrogelation for any of them. Besides, the texture was continuous, without crystals, suggesting that DCF molecules were dispersed into the hydrogel matrix at least at the submicrometric level, under the evaluation limits of the POM [28].

As the formulation morphology is in an important factor affecting the drug kinetics release, scanning electron microscopy was performed to have a better understanding of it. Figure 2 shows that the microstructure of the formulations was not significantly affected by the crosslinking degree. Except for the 2D formulation, which showed a more compressed structure with no clear pores, the other samples revealed a porous morphology, with well-delimited interconnected pores with a diameter around 50 μm. Compared to the neat hydrogels without drug, their pore walls were thick, indicating the encapsulation of DCF into them [24]. This hypothesis is supported by the strong interactions which can develop between the DCF and the hydrogel matrix, which clearly prompted the drug anchoring into



(a) 2D (b) 3D

(c) 4D (d) 5D

Figure 1: Birefringent textures of the 2D–5D formulations evidenced by POM.

matrix. In this view, it can be expected that the diffusion of the DCF molecules through the matrix will be retarded, promoting its prolonged release [31].

The *in vitro* release of DCF from the formulations was monitored by applying conditions which mimic the physiologic environment. As can be seen in Figure 3, the DCF was released in a pulsatile manner, no matter what was the crosslinking degree of the matrix [32]. Taking into consideration the influence of the drug size on the dissolution rate, this behavior can be correlated with the encapsulation of the DCF into the formulations as submicrometric crystals of different sizes [24]. Furthermore, no clear correlation of the release profile to the crosslinking degree was distinguished. The hydrogel matrix with the lowest crosslinking degree (5D) was favorable towards a fast release of almost all DCF amounts over 9 days. On the contrary, the formulation with the highest crosslinking degree (2D) presented a more rapid release compared to those with a medium crosslinking degree (3D and 4D), attaining more than 80% DCF release compared to less than 70%. Nevertheless, the exponential trend line showed a continuous release of the drug for the entire investigation period (Figure 3). This release behavior, which did not match to a clear rule, has been correlated with the dissimilar viscosity of the hydrogelation system, influencing the DCF crystallization, i.e., the growing of crystals of different sizes.

4. Theoretical Model

Taking into account the complexity of the phenomena that occur in release processes (drug diffusion, erosion of polymer matrix, drug solubility, etc.), it is admitted (evidently, as a work hypothesis) that this "complexity" can be "covered" by multifractality. In other words, the polymer-drug complex system release dynamics will be described through continuous and nondifferential curves (multifractal curves and not monofractal curves, i.e., of a single fractal dimension D_F, as is the usual case in [33]). Then, the multifractal theory of motion in its hydrodynamic form becomes functional through the following equations [34, 35]:

$$\partial_t V_D^i + V^l \partial_l V_D^i = -\partial^i Q, \tag{1}$$

$$\partial_t \rho + \partial^l \left(\rho V_D^l \right) = 0, \tag{2}$$

$$Q = 2\lambda^2 (dt)^{[2/f(\alpha)]-1} \frac{\partial_l \partial^l \sqrt{\rho}}{\sqrt{\rho}}, \tag{3}$$

$$\partial_t = \frac{\partial}{\partial t}, \partial_l = \frac{\partial}{\partial X^l}, \partial_l \partial^l = \frac{\partial}{\partial X^l} \left(\frac{\partial}{\partial X^l} \right), \quad i, l = 1, 2, 3. \tag{4}$$

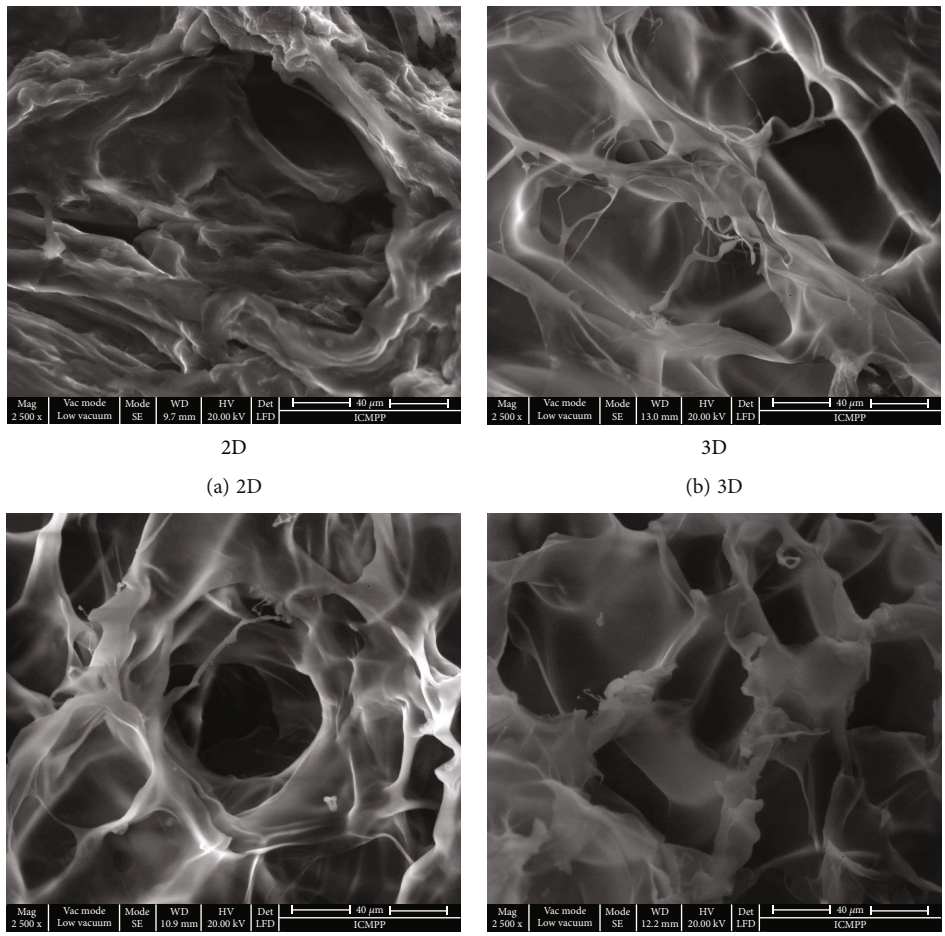


Figure 2: Microstructure of the studied formulations visualized by SEM.

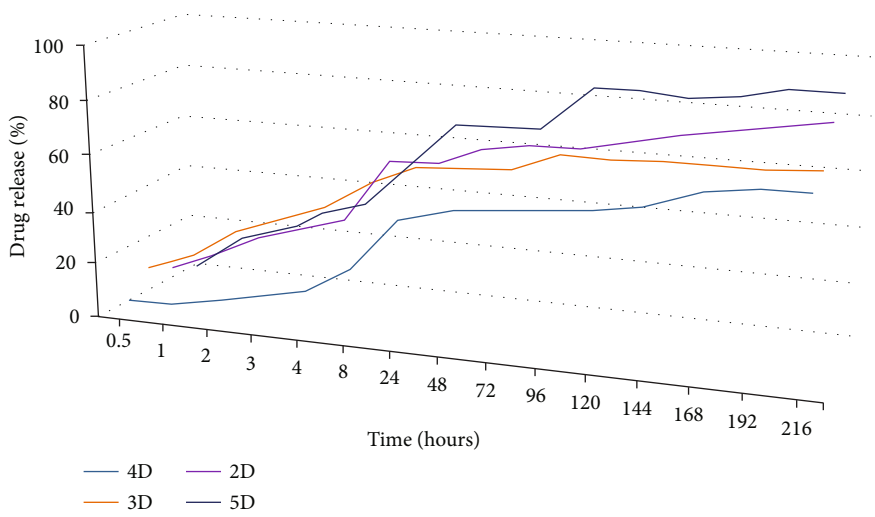


Figure 3: Drug release profile of DCF from formulations and the corresponding exponential trend line.

In relations (1)–(4), the terms have the following meanings:

(i) t is the nonfractal time having the role of an affine parameter of the release curves

(ii) X^l is the multifractal spatial coordinate

(iii) V_D^i is the "multifractal fluid" velocity on a differentiable scale resolution (the polymer-drug complex system is assimilated to a "multifractal fluid"; for details on the "behavior" of such a "physical object," see [33–35])

(iv) ρ is the state density of the "multifractal fluid"

(v) λ is the structural constant specific to the release process associated to the multifractal—nonmultifractal transition

(vi) dt is the scale resolution

(vii) $f(\alpha)$ is the singularity spectrum of order α dependent on the fractal dimension D_F [36, 37]

Operating with multifractal "manifolds" instead of monofractal ones (in the case of dynamic release systems) has some advantages:

(i) Areas of the polymer-drug complex system of a certain fractal dimension may be identified and can be characterized from a release dynamic viewpoint. From here, the number of zones of the polymer-drug complex system which have their fractal dimension in a certain interval of values may be identified

(ii) Universality classes can be identified in the domain of dynamic release systems, even when the attractors have different aspects

Equation (1) corresponds to the multifractal law of specific momentum conservation and equation (2) corresponds to the multifractal conservation law of state density, while equation (3) corresponds to the multifractal specific scalar potential as a measure of the multifractalization degree of the release curves.

Introducing the fractal state function of the form

$$\psi = \sqrt{\rho}\ \exp\ (\mathrm{is}), \quad i = \sqrt{-1}, \tag{5}$$

where $\sqrt{\rho}$ is an amplitude and s is a phase, then, two types of velocities can be defined:

(i) V_D^i velocity at differentiable scale resolution

$$V_D^i = 2\lambda(dt)^{[2/f(\alpha)]-1}\partial^i s \tag{6}$$

(ii) V_F^i velocity at nondifferentiable scale resolution

$$V_F^i = (dt)^{[2/f(\alpha)]-1}\partial^i \ln \rho \tag{7}$$

Now, the synchronization of the dynamics at the two scale resolutions, equivalent to the controlled drug release process, implies the operation with the following constraint:

$$V_D^i = -V_F^i. \tag{8}$$

In this condition, the multifractal conservation law of state density transforms into a diffusion equation of multifractal type:

$$\partial_t \rho = \lambda(dt)^{[2/f(\alpha)]-1}\partial_l\partial^l\rho = \sigma\partial_l\partial^l\rho. \tag{9}$$

It results that these "mechanisms" "manifest"/are "perceived" as diffusions at various scale resolutions in a multifractal space (Fickian-type diffusion, non-Fickian-type diffusion, etc.). To explain this situation it should be considered the one-dimensional drug diffusion of multifractal type from a controlled-release polymeric system with the form of a plane shut, of thickness δ. If drug release of the multifractal type occurs under perfect sink condition, the following initial and boundary conditions can be assumed:

$$\begin{gathered} t = 0, \\ -\frac{\alpha}{2} < x < \frac{\alpha}{2}, \\ \rho = \rho_0 \\ t > 0, \\ x = \pm\frac{\alpha}{2}, \\ \rho = \rho_1, \end{gathered} \tag{10}$$

where ρ_0 is the initial drug state density of the multifractal type in the "device" of the multifractal type and ρ_1 is the drug state density at the "polymer-fluid" interface of the multifractal type. This solution equation under these conditions can take the following form (for details in the classical case, see [38, 39]). In Figure 4 shows the multifractal function representation utilized to analyze the drug release

$$f = \frac{\rho_t}{\rho_\infty} = 2\left(\frac{\sigma t}{\delta^2}\right)^{1/2} = \left\{\pi^{-1/2} + \sum_{n=1}^{\infty}(-1)^n\ \mathrm{erfc}\left[\frac{n\delta}{2(\sigma t)^{1/2}}\right]\right\}. \tag{11}$$

An accurate expression can be obtained for small values of t since the second term of (11) disappears, and then, it becomes:

$$\frac{\rho_t}{\rho_\infty} = 2\left(\frac{\sigma t}{\delta^2}\right)^{1/2} = \mathrm{const}(t)^{1/2}. \tag{12}$$

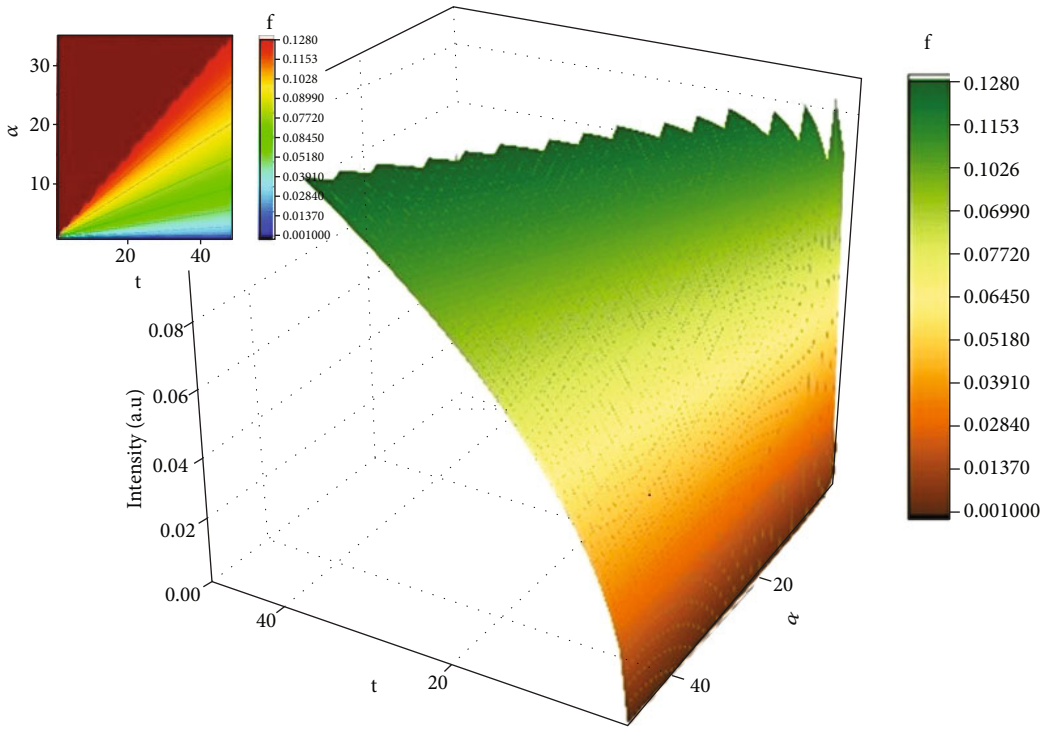


Figure 4: 3D (left side) and contour plot (right side) representations of the multifractal function used for drug release mechanism analysis.

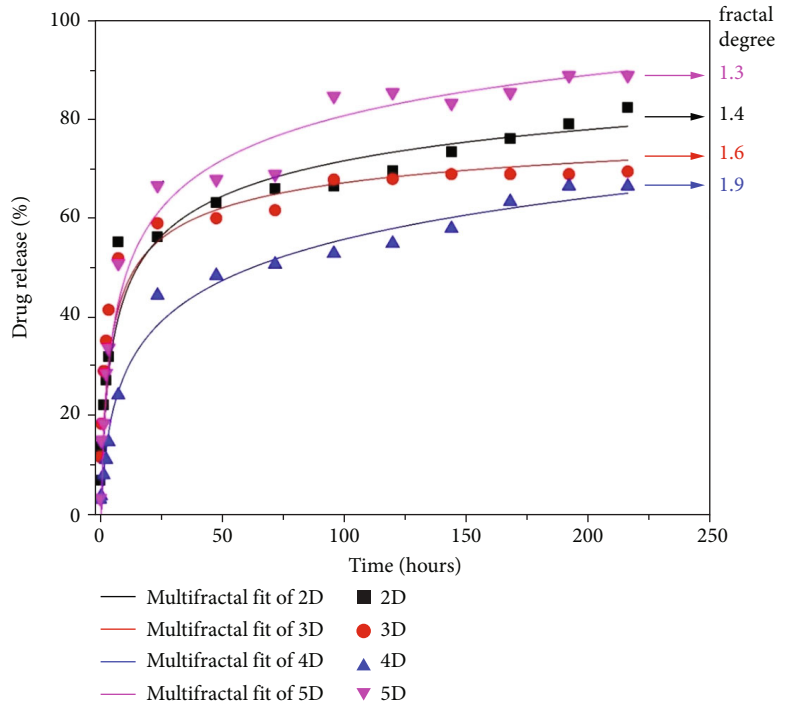


Figure 5: Experimental showcase of the DCF release from formulations fitted by the multifractal theoretical model.

In such a context, ρ_t/ρ_∞ can be assimilated to the fraction of dissolved drug, i.e., $M_t/M_\infty \equiv \rho_t/\rho_\infty$, where M_t is the amount of drug dissolved in time t and M_∞ is the total amount of time dissolved when the pharmaceutical dosage form is exhausted [40, 41]. The confirmation of the model is presented in Figure 5, for the release of DCF from the chitosan-based matrix. The empirical data was fitted with the multifractal function. The model is well equipped to predict the drug release dynamics [38]. The use of any classical model to fit the in vitro release will

not offer any information regarding the mechanism of the drug release, as there are a wide span of factors influencing the release process. Concerning the theoretic model developed in the multifractal paradigm, this can be validated through an adequate calibration on the empirical data, by choosing the constants according to the particularities of our polymer-drug system followed by a normalization of the data. The calibration process is not a trivial one as it strictly depends on the nature of the phenomena investigated; the method was previously tested for other physical phenomena with promising results [42–63]. We can observe that the model fits well all data sets. The saturation is usually reached at around 24–28 hours depending on the formulation and its corresponding fractal degree. This is also due to the morphology of the formulation which has a more organized structure enhancing the release; thus, a link can be made between the differential parameters defining the morphology of the polymer and the fractal degree defining the collective movement of the drug release scenario in a multifractal model. When we further analyze these results in the fractal paradigm, it results that a nonfractal morphology will lead to a higher fractality of the release drug geodesics as it enhances the interactions between the drug and the release media. As the morphology of the polymer formulations becomes fractalized, the release is reduced and the overall fractalization degree of the drug release is reduced.

5. Conclusions

A series of four drug release formulations were prepared by in situ hydrogelation of chitosan by with 2-hydroxy-5-nitrobenzaldehyde in the presence of diclofenac sodium salt as a drug model. The POM and SEM measurements emerged to the conclusion that the formulations have a homogenous dispersion of the drug into the pore walls at the submicrometric level. The size of DCF crystals appeared to vary depending on the system viscosity during the hydrogelation. This favored a pulsatile prolonged release of the drug over 9 days. The mathematical model was performed in the framework of the scale relativity theory and validated by our analysis and experimental data.

Because pain is the most common unpleasant symptom associated with endometriosis or deep infiltrative endometriosis, the current research on NSAIDs and the development of drug delivery systems can open new future perspectives on management of this category of patients. Drug delivery systems already play an important role in reducing symptoms related endometriosis, showing great improvement in the management of this debilitating condition.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

All authors declare no conflict of interest.

Authors' Contributions

All authors contributed equally.

References

[1] A. Prentice, A. J. Deary, and E. Bland, "Progestogens and antiprogestogens for pain associated with endometriosis," *Cochrane Database of Systematic Reviews*, vol. 2, no. CD002122, 2000.

[2] T. P. Canavan and L. Radosh, "Managing endometriosis. Strategies to minimize pain and damage," *Postgraduate Medicine*, vol. 107, no. 3, pp. 213–224, 2000.

[3] L. K. Symons, J. E. Miller, V. R. Kay et al., "The immunopathophysiology of endometriosis," *Trends in Molecular Medicine*, vol. 24, no. 9, pp. 748–762, 2018.

[4] A. S. Laganà, F. M. Salmeri, H. B. Frangež, F. Ghezzi, E. Vrtačnik-Bokal, and R. Granese, "Evaluation of M1 and M2 macrophages in ovarian endometriomas from women affected byendometriosis at different stages of the disease," *Gynecological Endocrinology*, vol. 36, 2020.

[5] Z. Liu, "Inflammation and endometriosis," *Frontiers in Bioscience*, vol. 21, no. 5, pp. 941–948, 2016.

[6] F. M. Salmeri, A. S. Laganà, V. Sofo et al., "Behavior of tumor necrosis factor-α and tumor necrosis factor receptor 1/tumor necrosis factor receptor 2 system in mononuclear cells recovered from peritoneal fluid of women with endometriosis at different stages," *Reproductive Science*, vol. 22, no. 2, pp. 165–172, 2015.

[7] P. C. A. Crispim, M. P. Jammal, E. F. C. Murta, and R. S. Nomelini, "Endometriosis: what is the influence of immune cells?," *Immunological Investigations*, vol. 50, no. 4, pp. 372–388, 2021.

[8] S. W. Guo, Y. Du, and X. Liu, "Platelet-derived TGF-β1 mediates the down-modulation of NKG2D expression and may be responsible for impaired natural killer (NK) cytotoxicity in women with endometriosis," *Human Reproduction*, vol. 31, no. 7, pp. 1462–1474, 2016.

[9] Y.-J. Kang, I. C. Jeung, A. Park et al., "An increased level of IL6 suppresses NK cell activity in peritoneal fluid of patients with endometriosis via regulation of SHP2 expression," *Human Reproduction*, vol. 29, no. 10, pp. 2176–2189, 2014.

[10] J.-J. Yu, H.-T. Sun, Z.-F. Zhang et al., "IL15 promotes growth and invasion of endometrial stromal cells and inhibits killing activity of NK cells in endometriosis," *Reproduction*, vol. 152, no. 2, pp. 151–160, 2016.

[11] H.-L. Yang, W.-J. Zhou, K.-K. Chang et al., "the crosstalk between endometrial stromal cells and macrophages impairs cytotoxicity of NK cells in endometriosis by secreting IL-10 and TGF-β," *Reproduction*, vol. 154, no. 6, pp. 815–825, 2017.

[12] V. M. Rice, "Conventional medical therapies for endometriosis," *Annals of the New York Academy of Sciences*, vol. 955, no. 1, pp. 343–352, 2002.

[13] R. L. Barbieri, "Endometriosis 1990. Current treatment approaches," *Drugs*, vol. 39, no. 4, pp. 502–510, 1990.

[14] T. Grosser, S. Fries, and G. A. FitzGerald, "Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities," *The Journal of Clinical Investigation*, vol. 116, no. 1, pp. 4–15, 2006.

[15] K. V. Kozhikhova, M. N. Ivantsova, M. I. Tokareva et al., "Preparation of chitosan-coated liposomes as a novel carrier

system for the antiviral drug Triazavirin," *Pharmaceutical Development and Technology*, vol. 23, no. 4, pp. 334–342, 2018.

[16] Y. Wu, A. Rashidpour, M. P. Almajano, and I. Metón, "Chitosan-based drug delivery system: applications in fish biotechnology," *Polymers (Basel)*, vol. 12, no. 5, p. 1177, 2020.

[17] M. Suhail, J. M. Rosenholm, M. U. Minhas et al., "Nanogels as drug-delivery systems: a comprehensive overview," *Therapeutic Delivery*, vol. 10, no. 11, pp. 697–717, 2019.

[18] S. Cibotaru, A. I. Sandu, D. Belei, and L. Marin, "Water soluble PEGylated phenothiazines as valuable building blocks for biomaterials," *Materials Science and Engineering: C, Materials for biological applications*, vol. 116, 2020.

[19] H. Hamedi, S. Moradi, S. M. Hudson, and A. E. Tonelli, "Chitosan based hydrogels and their applications for drug delivery in wound dressings: a review," *Carbohydrate Polymers*, vol. 199, pp. 445–460, 2018.

[20] M. M. Iftime, S. Morariu, and L. Marin, "Salicyl-imine-chitosan hydrogels: supramolecular architecturing as a crosslinking method toward multifunctional hydrogels," *Carbohydrate Polymers*, vol. 165, pp. 39–50, 2017.

[21] A. M. Olaru, L. Marin, S. Morariu, G. Pricope, M. Pinteala, and L. Tartau-Mititelu, "Biocompatible chitosan based hydrogels for potential application in local tumour therapy," *Carbohydrate Polymers*, vol. 179, pp. 59–70, 2018.

[22] D. Ailincai, L. Marin, S. Morariu et al., "Dual crosslinked iminoboronate-chitosan hydrogels with strong antifungal activity against *Candida* planktonic yeasts and biofilms," *Carbohydrate Polymers*, vol. 152, pp. 306–316, 2016.

[23] A. Bejan, D. Ailincai, B. C. Simionescu, and L. Marin, "Chitosan hydrogelation with a phenothiazine based aldehyde: a synthetic approach toward highly luminescent biomaterials," *Polymer Chemestry*, vol. 9, no. 18, pp. 2359–2369, 2018.

[24] A. M. Craciun, L. Mititelu Tartau, and M. Pinteala, "Nitrosalicyl-imine-chitosan hydrogels based drug delivery systems for long term sustained release in local therapy," *Journal of Colloid and Interface Science*, vol. 536, pp. 196–207, 2019.

[25] M. Iftime, L. Mititelul Tartau, and L. Marin, "New formulations based on salicyl-imine-chitosan hydrogels for prolonged drug release," *International Journal of Biological Macromolecules*, vol. 160, pp. 398–408, 2020.

[26] D. Ailincai, W. Porzio, and L. Marin, "Hydrogels based on imino-chitosan amphiphiles as a matrix for drug delivery systems," *Polymers*, vol. 12, no. 11, p. 2687, 2020.

[27] L. Marin, D. Ailincai, M. Mares et al., "Imino-chitosan biopolymeric films. Obtaining, self-assembling, surface and antimicrobial properties," *Carbohydrate Polymers*, vol. 117, pp. 762–770, 2015.

[28] L. Marin, M. C. Popescu, A. Zabulica, H. Uji-I, and E. Fron, "Chitosan as matrix for bio-polymer dispersed liquid crystal systems," *Carbohydrate Polymers*, vol. 95, no. 1, pp. 16–24, 2013.

[29] M. Kaushal, S. Indoria, T. S. Lobana et al., "Synthesis, structures and antimicrobial activity of 5-nitro-salicylaldehyde-thiosemicarbazonates of zinc(II) coordinated to substituted bipyridines/phenanthrolines," *Polyhedron*, vol. 148, pp. 9–21, 2018.

[30] N. Kasch, I. Dierking, M. Turner, P. Romero-Hasler, and E. A. Soto-Bustamante, "Liquid crystalline textures and polymer morphologies resulting from electropolymerisation in liquid crystal phases," *Journal of Materials Chemistry C*, vol. 3, no. 31, pp. 8018–8023, 2015.

[31] J. S. Varghese, N. Chellappa, and N. N. Fathima, "Gelatin-carrageenan hydrogels: role of pore size distribution on drug delivery process," *Colloids and Surfaces B: Biointerfaces*, vol. 113, pp. 346–351, 2014.

[32] D. Jain, R. Raturi, V. Jain, P. Bansal, and R. Singh, "Recent technologies in pulsatile drug delivery systems," *Biomatter*, vol. 1, no. 1, pp. 57–65, 2011.

[33] L. Nottale, *Scale Relativity and Fractal Space-Time: A New Approach to Unifying Relativity and Quantum Mechanics*, World Scientific Publishing Co. Pte. Ltd., London, 2011.

[34] I. Merches and M. Agop, *Differentiability and Fractality in Dynamics of Physical Systems*, World Scientific, New Jersey, 2016.

[35] M. Agop and V. P. Paun, *On the New Perspectives of Fractal Theory. Fundaments and Applications*, Romanian Academy Publishing House, Bucharest, 2017.

[36] E. A. Jackson, *Perspectives of Nonlinear Dynamics*, vol. 1, Cambridge University Press, New York, 1993.

[37] C. P. Cristescu, *Nonlinear Dynamics and Chaos. Theoretical Fundaments and Applications*, Romanian Academy Publishing House, Bucharest, 2008.

[38] G. Tiwari, R. Tiwari, S. K. Bannerjee et al., "Drug delivery systems: an updated review," *International Journal of Pharmaceutical Investigation*, vol. 2, no. 1, pp. 2–11, 2012.

[39] J. K. Patra, G. Das, L. F. Fraceto et al., "Nano based drug delivery systems: recent developments and future prospects," *Journal of Nanobiotechnology*, vol. 16, no. 1, p. 71, 2018.

[40] O. Z. Fisher, A. Khademhosseini, and N. A. Pepas, "Drug delivery: nanoscale devices," in *Encyclopedia of Materials: Science and Technology*, pp. 1–9, Elsevier, 2010.

[41] K. Kosmidis, P. Argyrakis, and P. Macheras, "Fractal kinetics in drug release from finite fractal matrices," *The Journal of Chemical Physics*, vol. 119, no. 12, pp. 6373–6377, 2003.

[42] M. Agop, P. E. Nica, S. Gurlui, C. Focsa, V. P. Paun, and M. Colotin, "Implications of an extended fractal hydrodynamic model," *The European Physical Journal D*, vol. 56, no. 3, pp. 405–419, 2010.

[43] M. Agop, V. P. Paun, and A. Harabagiu, "El Naschie's $\varepsilon^{(\infty)}$ theory and effects of nanoparticle clustering on the heat transport in nanofluids," *Chaos Solitons & Fractals*, vol. 37, no. 5, pp. 1269–1278, 2008.

[44] M. Agop, P. E. Nica, P. D. Ioannou, A. Antici, and V. P. Paun, "Fractal model of the atom and some properties of the matter through an extended model of scale relativity," *The European Physical Journal D*, vol. 49, no. 2, pp. 239–248, 2008.

[45] M. Agop and C. Murgulet, "El Naschie's $\varepsilon^{(\infty)}$ space-time and scale relativity theory in the topological dimension $D = 4$," *Chaos, Solitons & Fractals*, vol. 32, no. 3, pp. 1231–1240, 2007.

[46] S. Gurlui, M. Agop, M. Strat, G. Strat, S. Bacaita, and A. Cerepaniuc, "Some experimental and theoretical results on the anodic patterns in plasma discharge," *Physics of Plasmas*, vol. 13, no. 6, pp. 063503–06350310, 2006.

[47] I. Gottlieb, M. Agop, and M. Jarcau, "El Naschie's Cantorian space-time and general relativity by means of Barbilian's group.: a Cantorian fractal axiomatic model of space-time," *Chaos, Solitons & Fractals*, vol. 19, no. 4, pp. 705–730, 2004.

[48] C. Nejneru, A. Nicuta, B. Constantin, L. R. Manea, M. Teodorescu, and M. Agop, "Dynamics control of the complex systems via nondifferentiability," *Journal of Applied. Mathematics*, vol. 2013, article 137056, pp. 1–12, 2013.

[49] I. Casian-Botez, M. Agop, P. Nica, V. P. Paun, and G. V. Munceleanu, "Conductive and convective types behaviors at nanotime scales," *Journal of Computational and Theoretical Nanoscience*, vol. 7, no. 11, pp. 2271–2280, 2010.

[50] O. Niculescu, D. G. Dimitriu, V. P. Paun, P. D. Matasaru, D. Scurtu, and M. Agop, "Experimental and theoretical investigations of a plasma fireball dynamics," *Physics of Plasmas*, vol. 17, no. 4, p. 2305, 2010.

[51] M. Colotin, G. O. Pompilian, P. Nica, S. Gurlui, V. Paun, and M. Agop, "Fractal transport phenomena through the scale relativity model," *Acta Physica Polonica A*, vol. 116, no. 2, pp. 157–164, 2009.

[52] M. Agop, P. Nica, and M. Girtu, "On the vacuum status in Weyl-Dirac theory," *General Relativity and Gravitation*, vol. 40, no. 1, pp. 35–55, 2008.

[53] I. Gottlieb, M. Agop, G. Ciobanu, and A. Stroe, "El Naschie's $\varepsilon^{(\infty)}$ space-time, hydrodynamic model of scale relativity theory," *Chaos, Solitons & Fractals*, vol. 34, no. 5, pp. 1704–1723, 2007.

[54] I. Gottlieb, M. Agop, G. Ciobanu, and A. Stroe, "El Naschie's $\varepsilon^{(\infty)}$ space-time and new results in scale relativity theories," *Chaos Solitons & Fractals*, vol. 30, no. 2, pp. 380–398, 2006.

[55] S. A. Irimiciuc, S. Gurlui, and M. Agop, "Particle distribution in transient plasmas generated by ns-laser ablation on ternary metallic alloys," *Applied Physics B*, vol. 125, no. 10, pp. 1–11, 2019.

[56] R. W. Schrittwieser, C. Ionita, C. T. Teodorescu-Soare et al., "Spectral and electrical diagnosis of complex space-charge structures excited by a spherical grid cathode with orifice," *Physica Scripta*, vol. 92, no. 4, p. 44001, 2017.

[57] D. Ailincai, A. M. Dorobanțu, B. Dima et al., "Poly(vinyl alcohol boric acid)-diclofenac sodium salt drug delivery systems: experimental and theoretical studies," *Journal of Immunology Research*, vol. 2020, Article ID 3124304, 14 pages, 2020.

[58] S. A. Irimiciuc, B. C. Hodoroaba, G. Bulai, S. Gurlui, and V. Craciun, "Multiple structure formation and molecule dynamics in transient plasmas generated by laser ablation of graphite," *SpectrochimicaActa Part B: Atomic Spectroscopy*, vol. 165, article 105774, 2020.

[59] M. M. Iftime, D. L. Dobreci, S. A. Irimiciuc, M. Agop, T. Petrescu, and B. Doroftei, "A theoretical mathematical model for assessing diclofenac release from chitosan-based formulations," *Drug Delivery*, vol. 27, no. 1, pp. 1125–1133, 2020.

[60] V. Nedeff, E. Moșneguțu, M. Panainte et al., "Dynamics in the boundary layer of a flat particle," *Powder Technology*, vol. 221, pp. 312–317, 2012.

[61] G. V. Munceleanu, V. P. Paun, I. Casian-Botez, and M. Agop, "The microscopic-macroscopic scale transformation through a chaos scenario in the fractal space-time theory," *International Journal of Bifurcation and Chaos*, vol. 21, no. 2, pp. 603–618, 2011.

[62] M. Agop, V. Griga, B. Ciobanu et al., "Gravity and Cantorian space-time," *Chaos Solitons & Fractals*, vol. 9, no. 7, pp. 1143–1181, 1998.

[63] C. Ciubotariu and M. Agop, "Absence of a gravitational analog to the Meissner effect," *General Relativity and Graviattion*, vol. 28, no. 4, pp. 405–412, 1996.