Strength in numbers: the joint potential of carbon nanostructures and nanocellulose for cancer theranostics

La unión hace la fuerza: el potencial conjunto de las nanoestructuras de carbono y la nanocelulosa para la medicina teragnóstica contra el cáncer

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Abstract

Traditional cancer treatments encompass chemotherapy, radiotherapy and surgery which lead to terrible collateral damage mainly due to a lack of specificity between the treatment and the affected area. To solve this problem, nanotheranostics involves the use of nanotechnology to obtain both better treatments and diagnosis by improving the tumor screening and the targeting in drug release.

In this context, carbon-based nanomaterials (CBNs), such as fullerenes, graphene quantum dots (GQDs), carbon nanotubes (CNTs), and graphene oxide (GO), are promising nanomaterials due to their unique properties. The functionalization of these materials is expected to be a key process to improve their solubility, stability and biocompatibility for their use in several biomedical applications like biosensing, drug delivery or tissue engineering. Moreover, a novel form of cellulose named 'nanocellulose' seems to be a promising tool in cancer therapy as it can be combined with the aforementioned nanomaterials, improving their therapeutic effectiveness against cancer cells.

In summary, carbon-based nanocomposites and nanocellulose offer significant potential to improve cancer treatment through better drug delivery, reduced side effects, and enhanced targeting of cancer cells.

Introduction: cancer and nanotechnology

Nowadays, cancer disease continues being one of the main causes of death in the global population. In this sense, both its diagnosis and treatment stand as crucial topics of study for an early detection and the recuperation of patients. Conventional therapy strategies involve chemotherapy, radiotherapy and surgical interventions leading to severe side effects while sometimes failing in their therapeutic efficacy. Although the tumor can sometimes be removed efficiently from the patient by careful medical practice, the treatment usually results insufficient when the cancer has become metastatic [1].

As a solution, nanotechnology presents a range of appropriate intervention possibilities, from tumor screening to creating targeted drug delivery systems. In addition, nanocomposites are also being used to target new pathways, stimulate T cells through artificial antigen presentation, or elevate levels of immunostimulatory cytokines to alter the tumor microenvironment [2].

The combination of chemotherapeutic drugs and immune adjuvants in biodegradable nanoparticles has demonstrated significant potential in enhancing antitumor effectiveness and inducing strong antitumor immunity (*Figure 1*).

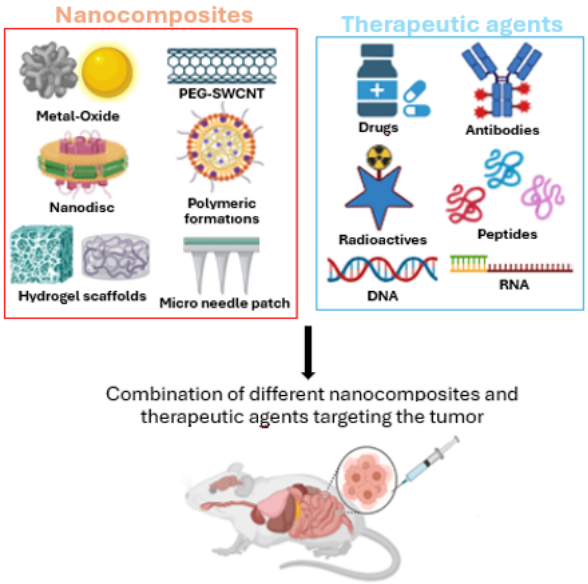

Figure 1. Artificially synthesized nanocomposites can carry a variety of molecules and biomolecules to target the tumor cells. Created with BioRender.com.

In this context, the novel term 'theranostics' (therapy + diagnostics) emerges, combining the molecularly targeted diagnostic imaging with the chemo- or radionuclide therapy [3]. Here, nanoparticles play a fundamental role since they can act as biocompatible and biodegradable targeted nanosystems [4] due to the differentiative properties that present over the bulk materials [1]. These have a small size, large surface area, exceptional optical properties, high electrical and thermal conductivities, and superior mechanical properties that make them extremely suitable for biomedical applications [5] such as tissue engineering, biosensing, drug delivery and diagnosis [6].

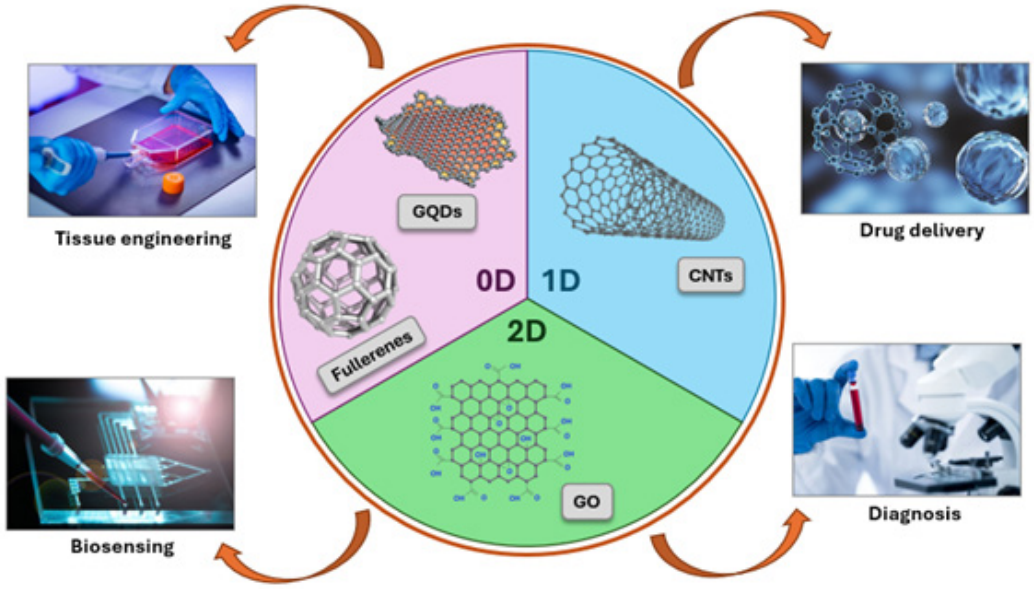

Figure 2. Schematic of the most investigated CBNs in the field of biomedicine and their possible uses in cancer theranostics.

Carbon nanotechnology in the context of cancer theranostics

Specifically, carbon-based nanomaterials (CBNs) have gained interest among the scientific community in the last decades due to their hydrophobic nature that makes them suitable for loading the drug of interest [7–9]. Also, these have been proved to be more biocompatible and safer than metal-based nanomaterials for their use in cancer theranostics [10].

Carbon is one of the most abundant elements and therefore it has diverse allotropes that enable the formation of different types of CBNs with specific characteristics [11]. These can be classified into zero-, one-, two- and three-dimensional graphenic forms. Among them, the most remarkable in the biomedical field (Figure 2) are 0D fullerenes and graphene quantum dots (GQDs), 1D carbon nanotubes (CNTs) and 2D graphene oxide (GO) [11].

Structurally, fullerenes are spherical molecules built up of pentagons and hexagons, with full insolubility in water, but they can be functionalized to change this property [12]. In this sense, biocompatible C60 fullerenes are soluble in water, and they can inactivate free radicals to protect cell membranes from oxidation, so they were used as therapeutic agents for ulcerative colitis [13]. Another 0D CBN are GQDs which present unique fluorescent properties due to their quantum confinement effects, good hydrophilicity, and the possibility to be made up either with semiconductor or insulating behavior [14]. These features, combined with the presence of surface functional groups, enable GQDs to create new chemotherapeutics and radioisotopes that can be used for cancer treatment.

Moreover, 2D GO is made up of a carbon monolayer whose structure is interrupted by a range of oxygen functional groups, providing insulating and reactivity properties to this material [15,16]. In recent times, it has also been considered as an ideal nanomaterial for cancer therapy due to its distinctive physicochemical features, implicit size- and shape-dependent optical properties, extremely large surface to volume ratio and flexible surface properties [16].

On the other hand, CNTs consist of rolled up graphitic sheets which confers a cylindrical shape, and they can be electronically metallic or semiconducting depending on the arrangement of the hexagonal carbon rings along the tubular surface [11]. These have a great appeal for carrying chemotherapeutic drugs, genes and proteins against cancer as well as being efficient photothermal agents due to their strong near-infrared (NIR) light absorption capability [11].

In 2023, a Portuguese group led by S. Abreu [17], demonstrated through an ex vivo study on human colorectal carcinoma cell line (HT-29) that the conjugation of chemotherapy drugs like 5-fluorouacil with functionalized CNTs resulted in a higher effective reduction in tumor growth. This was attributed to an extended half-life and cytotoxicity of the drug, added to a tenfold increase in drug uptake by the tumor due to the conjugated administration, likely through enhanced permeability and retention.

Functionalized CNTs are typically engaged by cells though an active process of endocytosis or they can passively diffuse into cells. The method of cellular internalization is determined by various factors, including the size, shape, surface charge, hydrophobicity of the nanoparticles, and the type of cancer cell [17].

It is important to highlight that the surface of carbon nanomaterials must have a correct balance between hydrophobicity and hydrophilicity to enhance affinity and promote their cellular adsorption, together with ensuring that drugs are correctly adsorbed on their surface. To be introduced into the system, they must exhibit high biocompatibility, cell specificity and an appropriate dimension, in order to overcome the biological, physicochemical, physiological and

mechanical barriers from cell membranes and enzymes. Nevertheless, multidrug resistance (MDR) is the key factor contributing to the unsuccessful outcomes of chemotherapy in many cancers, so other solutions must be sought [18].

Subsequently, various trials have been conducted to address and overcome this resistance that some metastatic tumors and tumor stem cells exhibit towards multiple chemotherapy drugs. The main cause of MDR in colon cancer patients, and in others like leukemia, is the active expulsion of drugs, due to the overexpression of P-glycoprotein (P-gp) in the cancerous tissues. Even though there have been numerous attempts to counteract this, the significant toxicity of these protein inhibitors has posed a substantial challenge in their clinical application [19]. A trial was conducted by the National Chromatographic R&A Center in China [19], where water soluble single-walled carbon nanotubes were functionalized with antibodies against P-gp, in conjunction with the chemotherapeutic drug doxorubicin, leading to higher specific recognition of the multi-drug resistant human leukemia cells (K562), while improving at the same time drug loading, cytotoxicity and controlled pharmacological release.

Underlyingly to these trials there is an open question regarding if these nanomaterials are intrinsically harmful or not. Numerous studies have been conducted on the safety and toxicity of these nanoparticles. A research from 2006 by Sayes and co-workers [20] indicated that chemically functionalized CNTs were less cytotoxic compared to those that were not. Therefore, the functionalization and structure alteration of these CNTs promote their dispersion, potentially reducing their size, and concomitantly modifying their surfaces. In this way, biocompatible groups can be introduced, thereby decreasing their toxicity. However, extremely small sizes can also result in toxicity due to their large surface area. In other words, small nanoparticles possess a high surface area relative to their total mass, which increases the chance to interact with surrounding biomolecules and, as a consequence, to trigger adverse responses.

Thus, the exploitation of CNTs comes with certain obstacles, including their low solubility, the release of potential toxic ions during synthesis, a strong hydrophobic nature leading to clumping and precipitation in water-based solutions, and a heightened viscosity. To address these issues, two potential approaches can be adopted: CNTs can be functionalized, which enhances, as we have described above, their solubility, stability, and functionality, or CNTs can be turned into different nanocomposites such as with gold or cellulose [6].

A new team player: Nanocellulose

Nanocellulose is composed of various nanofibers made of polymerized β-1,4-D (+) glucose units, which are elongated chains of cellulose with a significant length to diameter ratio. Their crystalline segments contribute to their robust mechanical strength, while the amorphous parts are responsible of their flexibility. The diameter of these nanofibers ranges usually from ten to twenty nanometers, and their length is at least ten times their diameter. This geometric property, coupled to its polymeric nature, makes it highly useful in various application fields [21, 22].

Three types of nanocellulose can be differentiated: microfibrillated cellulose (MFC), bacterial cellulose (BNC), and nanocrystalline cellulose (CNC). Specific bacteria can produce BNC by a bottom-up methodology and this one does not contain any trace of lignin or hemicellulose, which is beneficial as it eliminates the necessity for chemical isolation treatments. Furthermore, it is structured by a wide three-dimensional network of nanofibers with nanoscale diameters. The structure of BNC is built from both intermolecular and intramolecular hydrogen bonds among these nanofibers, enabling the creation of structures with an extensive surface area, open porosity, and strong tensile strength. As a result, and in addition to its excellent permeability and low toxicity, it has found broad applications in wound healing (e. g. treating skin burns) and tissue engineering (such as in the creation of artificial blood vessels) [21].

On the other hand, CNC is produced by subjecting the lignocellulosic biomass to acid hydrolysis (top-down procedure). The process selectively eliminates the amorphous phase and isolates the nanometric crystalline domains within its structure [22]. The resulting CNC has a needle-like or rod-like shape, with diameters ranging from three to ten nanometers, and impressive properties such as high tensile strength, mechanical moduli up to 130-140 GPa, a high surface area and high degree of versatility in surface functionalization. The hydroxyl groups on its surface provide an easy platform for chemical functionalization with amino, carboxyl, aldehyde and thiol groups, throughout grafting or oxidation. These modifications enable the attachment of tiny chemical entities, like fluorophores, metal nanoparticles, biomarkers, or larger protein/polymer macromolecules. It has also been explored for the delivery of suicidal genes and small interfering RNAs to tumors, allowing the silence of specific genes and the induction of apoptosis in cancer cells [23].

Considering the direction and alignment of cellulose chains [24], it can generate six distinct polymorphs. The most prevalent allomorphs are the type I, where the chains are parallel, and the type II, where the chains are antiparallel. Type I CNCs are needle-like crystals measuring 200-300 nm in length and 5-10 nm in width. In contrast, type II CNCs are shorter (50-100 nm) and broader (15-20 nm), exhibiting a more twisted and ribbon-like appearance. These morphological variations impact the final properties of CNCs and their interactions with other entities or materials. An instance of this is the differing rates of enzymatic degradation between types I and II of CNCs [25].

Both nanomaterials (BNC and CNC) have been

termed “generally regarded as safe (GRAS)” by the American Food and Drug Administration (FDA) [26]. This recognition assures a promising future in the development of multifunctional materials, sensors and structures, and it is expected to achieve the same level of success compared to other nanomaterials, with better prospects for sustainability and cost-effectiveness.

To sum up, nanocellulose with its unique properties, holds a great potential for a variety of applications, highlighting its aptitude for modulating and controlling the release of substances and drugs, particularly in targeted cancer therapy. Its adjustable surface chemistry plays a crucial role in controlling the loading and release of active substances in drug delivery systems. Numerous preclinical studies have demonstrated robust evidence of nanocellulose’s character as a distributor of bioactive compounds sensitive to various controlled stimuli, showing significant effectiveness in cancer treatments, with an added focus on targeted therapy and minimized side effects. The hybridization with capecitabine, folate chemical groups, or doxorubicin is commonly found among multifunctional platforms to actively fight against tumor cells [27].

Forefront of nanocellulose for cancer theranostics

A study from Spain’s Instituto de Carboquímica (ICB-CSIC) employed functionalized single-walled carbon nanotubes (SWCNTs) as a nanohybrid platform to target cancer, in conjunction with type II CNCs [28]. The functionalized SWCNT/II-CNC hybrids showed greater activity than the benchmark drug capecitabine against the Caco2 colon cancer cell line. Besides, the authors noted that the effect appears to be inherently linked to the SWCNT/II-CNC complex, particularly amplified by fluorescein and folic acid, as the absence of capecitabine yielded similar outcomes. Later research works from Asia have also confirmed this specificity of functionalized CNC for folate receptors and malignant colon cells. In detail, the spindle-like shape, as compared to others sphere-like nanoparticles, provides a high aspect ratio which causes a lengthened fluid circulation, an improved cellular uptake and binding, a faster internalization, and higher accumulation in the targeted tumors [29].

Eventually, it is important to underscore the function of nanocellulose (Figure 3) not just as a weapon against cancer, but also as a diagnostic tool (which is still greatly needed in developing countries). Given that the processes of carcinogens, cancer invasion and metastasis are still not fully understood, there is an urgent need to devise new strategies for a real-time non-invasive monitoring of cancer onset and progression, as well as swift diagnosis for making timely decisions about specialized treatments. The high surface area and electrical conductivity of CBNs, coupled to the biocompatibility of nanocellulose, create highly sensitive biosensors capable of detecting disease biomarkers at minimum concentrations. A Chinese group [23] recently applied CNCs for therapeutic tumor bioimaging, through acoustic signals or fluorescent agents like fluorescein isothiocyanate from nanoparticles that infiltrate tumors, to examine and visualize the presence and development of cancerous cells. Photoacoustic imaging of mouse cancer models has been already performed using non-conjugated CNCs.

Conclusion

In this review we have highlighted the potential of nanomaterials in targeted drug delivery, immunotherapy, and cancer screening. The use of functionalized carbon nanotubes (CNTs) and nanocellulose in conjunction with chemotherapeutic drugs and immune adjuvants has shown promising results in enhancing antitumor effectiveness, inducing a stronger and more specific response. Thus, CBNs can be used to accumulate in a selective and quick manner in areas of interest while reducing side effects by avoiding undamaged organs [32], being able to overcome multidrug resistance, improve drug loading, cytotoxicity, and controlled pharmacology release. This can be achieved by conjugating them with several targeting ligands that restrictively bind

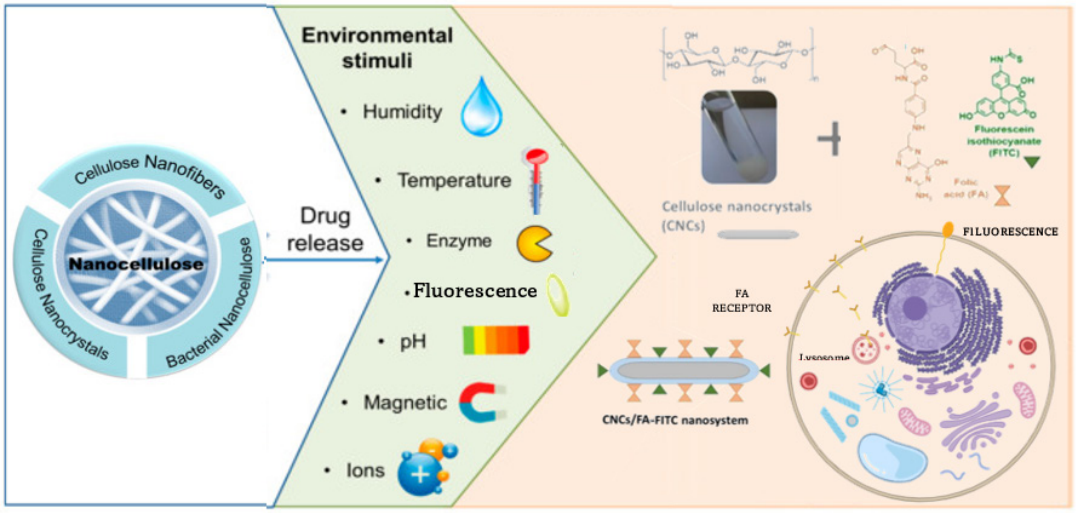

Figure 3. Summary of cellulose nanocrystals potential in cancer diagnosis and treatment. Adapted from references 30 and 31, with full reproduction rights under a creative commons license.

to overexpressed receptors on tumor cells. The used ligands depend both on the CNBs and the tumor model of interest and can be molecules with different nature such as antibodies, lectins or peptides, among others [33].

On the other hand, nanocellulose holds great potential for a variety of applications, with a particular focus on its ability to modulate and control the release of substances and drugs, especially in targeted cancer therapy. Its unique properties make it a promising material for the development of multifunctional materials, with better prospectives for sustainability and cost-effectiveness compared to others.

In summary, the unique properties of nanocellulose and CBNs complement each other, resulting in innovative solutions that are more effective, precise, and adaptable than current technologies. Nanocellulose improves the biocompatibility, reducing the risk of immune responses, ensuring safe interactions with biological systems and a controlled drug release, while CBNs, provide mechanical strength, high surface area, stability and electrical properties, enhancing the overall performance and life expectancy of these composites.

Overall, nanotheranostics can optimize drug delivery systems in different aspects like the biodistribution of the drug as well as it enables a noninvasive pharmacokinetics evaluation, and we can take advantage of this to predict treatment responses promoting personalized medicine [32].

References

[1] Saleem J, Wang L, Chen C. Carbon-Based Nanomaterials for Cancer Therapy via Targeting Tumor Microenvironment. Advanced Healthcare Materials. Wiley-VCH Verlag 2018; 7(20).

[2] Sharma P, Otto M. Multifunctional nanocomposites modulating the tumor microenvironment for enhanced cancer immunotherapy. Vol. 31, Bioactive Materials. KeAi Communications Co.; 2024. p. 440–62.

[3] Zoi V, Giannakopoulou M, Alexiou GA, Bouziotis P, Thalasselis S, Tzakos AG, et al. Nuclear Medicine and Cancer Theragnostics: Basic Concepts. Diagnostics. Multidisciplinary Digital Publishing Institute (MDPI) 2023; 13(19):3064.

[4] Jokerst J V, Gambhir SS. Molecular imaging with theranostic nanoparticles. Acc Chem Res. 2011 Oct 18; 44(10):1050–60.

[5] Díez-Pascual AM. Carbon-based nanomaterials. International Journal of Molecular Sciences. MDPI AG. 2021; 22(14):7726.

[6] Simon J, Flahaut E, Golzio M. Overview of carbon nanotubes for biomedical applications. Materials. MDPI AG. 2019, 12(4):624.

[7] Wong BS, Yoong SL, Jagusiak A, Panczyk T, Ho HK, Ang WH, et al. Carbon nanotubes for delivery of small molecule drugs. Adv Drug Deliv Rev. 2013 Dec; 65(15):1964–2015.

[8] Yang K, Feng L, Liu Z. The advancing uses of nano-graphene in drug delivery. Expert Opin Drug Deliv. 2015 Apr 3; 12(4): 601–12.

[9] Cui Q, Yang X, Ebrahimi A, Li J. Fullerene-biomolecule conjugates and their biomedicinal applications. Int J Nanomedicine. 2013 Dec; 9:77.

[10] Gong H, Peng R, Liu Z. Carbon nanotubes for biomedical imaging: The recent advances. Adv Drug Deliv Rev. 2013 Dec; 65(15):1951–63.

[11] Maiti D, Tong X, Mou X, Yang K. Carbon-Based Nanomaterials for Biomedical Applications: A Recent Study. Frontiers in Pharmacology. Frontiers Media S.A. 2019; 9.

[12] Da Ros T, Prato M. Medicinal chemistry with fullerenes and fullerene derivatives. Chem Commun. 1999; (8):663–9.

[13] Byelinska IV , Kuznietsova HM, Dziubenko NV, Lynchak OV, Rybalchenko TV, Prylutskyy YI, Kyzyma OA, Ivankov O, Rybalchenko VK, Ritter U. Effect of C60 fullerenes on the intensity of colon damage and hematological signs of ulcerative colitis in rats. Mater Sci Eng C. 2018 Dec 1, 93:505-517

[14] Tian P, Tang L, Teng KS, Lau SP. Graphene quantum dots from chemistry to applications. Mater Today Chem. 2018 Dec; 10:221–58.

[15] Dreyer DR, Todd AD, Bielawski CW. Harnessing the chemistry of graphene oxide. Vol. 43, Chemical Society Reviews. Royal Society of Chemistry; 2014. p. 5288–301.

[16] Brisebois, P. P., & Siaj, M. Harvesting graphene oxide – years 1859 to 2019: a review of its structure, synthesis, properties and exfoliation. Journal of Materials Chemistry. C, Materials for Optical and Electronic Devices 2020; 8(5), 1517–1547.

[17] Abreu S, Vale N, Soares OSGP. Combination of CNTs with Classical Drugs for Treatment in Human Colorectal Adenocarcinoma (HT-29) Cell Line. Nanomaterials. 2023 Jul 1;13(13).

[18] Ashique S, Bhowmick M, Pal R, Khatoon H, Kumar P, Sharma H, et al. Multi drug resistance in Colorectal Cancer- approaches to overcome, advancements and future success. Advances in Cancer Biology - Metastasis. 2024 Jul; 10:100114.

[19] Li R, Wu R, Zhao L, Wu M, Yang L, Zou H. P-Glycoprotein Antibody Functionalized Carbon Nanotube Overcomes the Multidrug Resistance of Human Leukemia Cells. ACS Nano. 2010 Mar 23; 4(3):1399–408.

[20] Sayes CM, Liang F, Hudson JL, Mendez J, Guo W, Beach JM, et al. Functionalization density dependence of single-walled carbon nanotubes cytotoxicity in vitro. Toxicol Lett. 2006 Feb; 161(2):135–42.

[21] Chopra H, Gandhi S, Gautam RK, Kamal MA. Bacterial Nanocellulose based Wound Dressings: Current and Future Prospects. Curr Pharm Des. 2022 Feb; 28(7):570–80.

[22] González-Domínguez JM, Ansón-Casaos A, Grasa L, Abenia L, Salvador A, Colom E, et al. Unique Properties and Behavior of Nonmercerized Type-II Cellulose Nanocrystals as Carbon Nanotube Biocompatible Dispersants. Biomacromolecules. 2019 Aug 12; 20(8):3147–60.

[23] Lugoloobi I, Maniriho H, Jia L, Namulinda T, Shi X, Zhao Y. Cellulose nanocrystals in cancer diagnostics and treatment. Journal of Controlled Release. 2021 Aug; 336:207–32.