Review

APPLICATIONS OF CHITOSAN–GRAPHENE OXIDE NANOCOMPOSITES IN MEDICAL SCIENCE: A REVIEW

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Abstract

Combinations of biopolymers with nanostructured carbon materials have been the subject of interest of many scientists in recent years. Particularly significant are nanocomposites made of chitosan, which is a linear aminopolysaccharide obtained in the process of deacetylation of chitin, and graphene oxide (GO).

These systems, due to the atypical properties of both components such as non-toxicity, biocompatibility with human tissues and organs as well as bacteriostaticity, are characterised by a wide range of biomedical applications.

They may be used in emergency medicine as dressing materials which accelerate wound healing, as well as carriers of drugs/genes and biological macromolecules, for example proteins, peptides and nucleic acids. In addition, CS-GO systems can potentially be used in regenerative medicine as scaffolds for cell culture.

For this reason, the current publication presents the possibilities of the application of chitosan–graphene oxide nanocomposites in medicine considering the characteristics of the system components.

Keywords: *nanomedicine, biopolymers, chitosan, graphene, graphene oxide*

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1. Introduction

The beginning of the 21st century brought a rapid increase in research in the field of nanotechnology science. The most important were works carried out in the field of nanomedicine focused on the implementation of nanoscale materials in general clinical practice, enabling the development of new diagnostic and medical imaging methods, as well as innovative methods of disease treatment.

Particularly promising is the use of gold and silver nanostructures and carbon nanostructures (graphene and graphene oxide) allowing the delivery of therapeutic substances directly to the disease-affected cells. This solution provides safe therapy, increasing the effectiveness of the administered drugs. Additionally, nanostructured carbon materials can be used in photodynamic therapy or tissue engineering [1-3].

At the same time, it should be noted that there has been great interest among scientists in the last few years in the application of biopolymers in medical sciences, mainly chitin and its derivatives, for example chitosan. This trend results from their high degree of biocompatibility with tissues and organs of the human body.

Due to the unique properties, such as a high degree of biodegradability, affinity for proteins, antibacterial properties and lack of *in vitro* cytotoxicity effects, chitosan is now more widely used. First, it can be used in dressing materials for the treatment of hard-to-heal wounds. In addition, it can be a carrier of active substances - drugs - and may be used as a material for the preparation of bioresorbable cell scaffolds used for the regeneration of damaged tissues [4-6].

The aim of this article is to review potential medical applications of chitosan - graphene oxide systems (CS-GO) considering the characteristics of the above components.

2. Chitosan – the biopolymer of the 21st century

Chitosan (Fig. 1.) is a natural aminopolysaccharide obtained in the process of total or partial N-deacetylation of chitin (Fig. 2.) which is a building component of the outer skeletons of marine crustaceans, such as crabs, crayfishes or shrimps, and is a component of the cellular walls of filamentous fungi belonging to the *Zygomycetes* and *Basidiomycetes* classes [4,7].

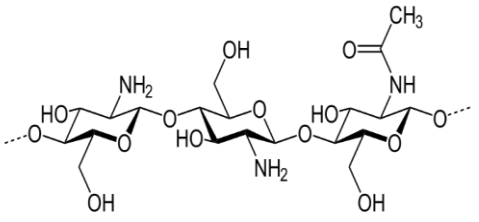

Figure 1. Structural formula of chitosan [8]

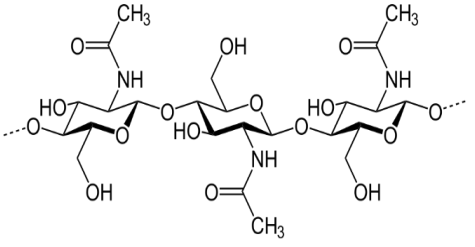

Figure 2. Structural formula of chitin [9]

Chemically, chitosan is built of linearly connected β(1→4)-2-amino-2-deoxy-D-glucopyranoses and β(1→4)-2-acetamido-2-deoxy-D-glucopyranoses. Since there are three types of reactive functional groups present in the structure of the polymers, amine, acetamide and primary and secondary hydroxyl groups, it is characterised by a high ability for chemical modification, which contributes to a wide spectrum of its use [8].

Chitosan is commercially available in the form of a white powder, which is not soluble in water, with simultaneous solubility in diluted organic acids such as acetic, lactic, hydrochloric, formic or succinic acid [8].

2.1. Biological properties of chitosan and directions of biomedical applications

Chitosan, due to its atypical properties, is called the biopolymer of the 21st century. This compound is non-toxic, biocompatible with living tissues, bacteriostatic and biodegradable. In the human body, it decomposes under the influence of lysozyme where the ratio of N-acetyl-D-glucosamine and d-glucosamine residues significantly affects the rate of biodegradation. Apart from that, it has antiviral and antifungal properties and does not cause allergy or skin irritation [4-5, 8].

The above properties mean that chitosan has aroused great and still growing interest in both domestic and foreign medical institutes. A wide range of applications includes cosmetology, pharmacology, implantology and biomedical engineering [5].

This compound, due to its high moisturising properties, is often used to produce skin care cosmetics. It creates a hydrophilic protective film on the skin surface protecting it against harmful external factors and ensuring an appropriate level of skin hydration [5].

Chitosan is also used as a component of dressing materials in the treatment of dermatological diseases. In the early stages of wound healing, it contributes to collagen synthesis, stimulates tissue granulation, facilitates epidermisation and supports the regeneration of nerves and vessels in the control of wounds. Dressings made of chitosan do not cause hypertrophic scars and the dressing itself is absorbed by the body after the healing process. There are many anti-haemorrhagic chitosan-based dressings available on the medical market such as Syvek-Patch® (Marine Polymer Technologies), HemCon® (HemCon), Chitoflex® (HemCon), Chito-Seal® (Abbott), Clo-Sur® (Scion Cardiovascular), Trau-maStat® (Ore-Medix), Tromboguard® (Tricomed SA) or Excel Arrest® (Hemostasis LLC Co.) [10-11].

In addition, hydrogel chitosan membranes may be carriers for the controlled release of pharmacological preparations, e.g. antibiotics, thus ensuring the greater effectiveness of therapy [5, 12].

Of particular note is also the possibility of using chitosan in the treatment of hypercholesterolaemia. This compound exhibits selective sorption to cholesterol. Satisfactory results were achieved owing to the use of chitosan hydrogel deposits enriched with Mg (II) ions. A reduction of LDL cholesterol by 25% was achieved while maintaining an unchanged level of HDL [6, 13].

A large part of the research refers also to the use of chitosan in tissue engineering. Thermosensitive chitosan gels are used as scaffolds for the regeneration of damaged tissues and organs. They mimic the biological functions of the extracellular matrix, maintain the structure and functions of the formed tissue structures and contribute to the growth, adhesion and differentiation of cells [6, 14].

3. Nanostructured carbon materials – graphene and graphene oxide

In the last few years, apart from the high interest in biopolymers, there has been a noticeable increase in research in the field of medical applications of nanostructured carbon materials, among which graphene holds a key position.

Graphene is an allotropic variety of carbon of a two-dimensional form, isolated in 2004 from graphite, for which Russian researchers A. Geim and K. Novoselov received the Nobel Prize in physics in 2010. It is built of hexagonally-bonded carbon atoms and its spatial structure resembles a honeycomb [15].

Due to its excellent optical, electronic, thermal and mechanical properties, it can be used in many fields of science, including medicine. Over the last decade, several works have been published which deal with the potential use of graphene in various branches of medicine such as the delivery of drugs and genes, the detection and imaging of tissue, cellular or gene structures and as an anti-cancer therapy [16].

However, the quality of graphene used in biological research is not without significance. The manufacturing method and processing techniques have a pronounced impact on the potential toxicity of the nanomaterial to cells [17]. A lack of surface regularity, sharp edges, cavities and different distances between carbon atoms mean that graphene can lead to the disruption of the lipid bilayer of the cell membrane in the human body, disrupting the transport of electrons in the mitochondrial respiratory chain and, as a result, forcing the cell to activate the apoptosis pathway. In addition, the hydrophobic nature of the nanomaterial, resulting in the lack of solubility in most solvents, significantly limits its use [18].

Therefore, graphene oxide is being used increasingly more often in research for medical applications, as, unlike graphene, it is not toxic and has a hydrophilic character.

3.1. Graphene oxide – characteristics and application in medicine

Graphene oxide (GO) is an oxidised form of graphene that has carboxyl, hydroxyl and epoxy functional groups in its structure. Fig. 3 presents a comparison of the structure of graphene and graphene oxide [19].

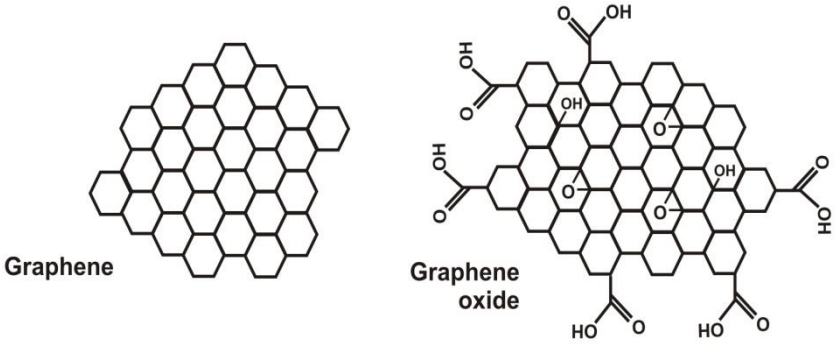

Figure 3. Structure of graphene and graphene oxide (author's own work based on [19])

Graphene oxide is obtained mainly by the Hummers method and modified Hummers technique. During the preparation of GO, the powdered form of graphite is oxidised to graphite oxide by means of reagents such as KNO_3, $KMnO_4$, $NaNO_3$ or H_2SO_4. The next step is exfoliation of the obtained graphite oxide to graphene oxide, which is presented in Fig. 4. [20].

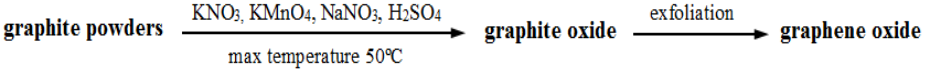

Figure 4. Diagram of graphene oxide synthesis from graphite powders (author's own work based on [20])

Due to the non-typical biological properties and high solubility in physiological solution, known as hydrophilicity, there have been many reports in the literature regarding the possibility of using graphene oxide in medicine.

Bactericidal and disinfecting properties, as well as the presence of oxygen on the GO surface, favour its use as a component of dressing products that accelerate the wound

healing process. The complex built of silver and GO allows the destruction of Gram (+) bacteria cells by about 87% and Gram (-) bacteria by as much as 100% [21].

In addition, graphene oxide can be potentially used as a drug carrier, among others, in anti-cancer therapy. The results of the use of doxorubicin, DOX (an antibiotic with cytostatic effect), applied to the GO surface were published, based on which a beneficial relationship was observed between the degree of release of the chemotherapeutic agent and the pH value of the environment in which the drug was released. For more acidic and alkaline reactions, a greater degree of activation of the preparation was noted, which is important in anti-cancer therapy. The reaction of cancer cells and the microenvironment of the tumour is slightly acidic, which may cause intense decay of the GO-DOX complex and contribute to more effective chemotherapy [22]. Interesting research was also carried out to obtain a combination of graphene oxide functionalised with polyacrylic acid with carmustine used in the oncological treatment of brain tumours [23].

The potential direction of biomedical applications of graphene oxide is also its use as a substrate for the immobilisation of enzymes. The immobilisation of enzymes on the surface of the GO sheet may occur without additional cross-linking substances and structure modification, and the spatial arrangement of atoms located in the immobilised enzyme molecule depends primarily on the interaction of enzyme molecules with GO functional groups [24].

4. Biomedical applications of chitosan – graphene oxide nanocomposites

In the last decade, there has been a significant development in research in the field of application of biopolymers enriched with nanoparticles, which was confirmed by numerous publications on the potential medical applications of chitosan-graphene oxide systems.

In the following part of the article a short review of the latest works devoted to this subject was performed, focusing mainly on presenting the leading directions of applications such as materials with antibacterial properties, drug and gene carriers, scaffolds for use in tissue engineering and immunosensors.

4.1. Antibacterial activity

Due to the antibacterial and disinfecting properties of chitosan and graphene oxide, systems containing both components constitute an interesting antimicrobial material for use in emergency medicine.

Sundar et al. [25], in 2014, was one of the first groups to synthesise the CS-GO nanocomposite. Antibacterial tests were performed against bacteria of the strain *Escherichia coli* (gram-negative) and *Bacillus subtilis* (gram-positive), which showed that the tested nanocomposite with a content of 2% GO by weight was characterised by a significantly greater antibacterial activity compared to the individual activity of graphene oxide and chitosan. Furthermore, based on SEM images, the researchers observed that the structure of the system was characterised by a much higher degree of roughness than the surface of GO itself. This confirmed the validity of the thesis that the nanocomposite can be used in medicine.

The antibacterial activity of systems containing chitosan and graphene oxide was also investigated by Marta et al. [26]. The researchers developed a ternary hybrid CS-GO nanocomposite which additionally contains silver nanoparticles that exhibit anti-bacterial properties against *Staphylococcus aureus*. They carried out microbiological tests on two representative bacterial strains characterised by resistance to methicillin (UCLA 8076 -

containing cells susceptible to low drug concentrations, and UCLA 1190R - showing resistance to high methicillin concentrations) and with respect to system components (GO, CS-GO and AGNPs -GO). Based on the research, the authors stated that the ternary nanocomposite CS-AgNPs-GO is characterised by higher antibacterial activity than most materials containing selected components of the system: AgNPs and CS-AgNPs. The hybrid system CS-AgNPs-GO, in which the ratio of chitosan to AgNPs-GO was 1:4, was recognised as the material with the highest antibacterial activity.

Work on the formation of ternary systems was also carried out by Mahmoudi et al., Konwar et al. and Li et al.

Mahmoudi et al. [27] carried out microbiological tests of a system composed of chitosan, polyvinylpyrrolidone and graphene oxide with a concentration of 1 to 3wt%. The tests were carried out against two bacterial strains: *Staphylococcus aureus* and *Escherichia coli.* In results of the work, the researchers noticed that the CS-PVP-GO nanocomposite showed a stronger antibacterial effect against *S. aureus* than *E. coli.* This was due to the electron affinity between opposite charges present on the bacterial cell wall and the graphene oxide sheet.

The second research group [28] developed nanocomposite hydrogel chitosan films containing graphene oxide coated with iron oxide using the co-precipitation method to obtain the CS-GIO system (Fig. 5.).

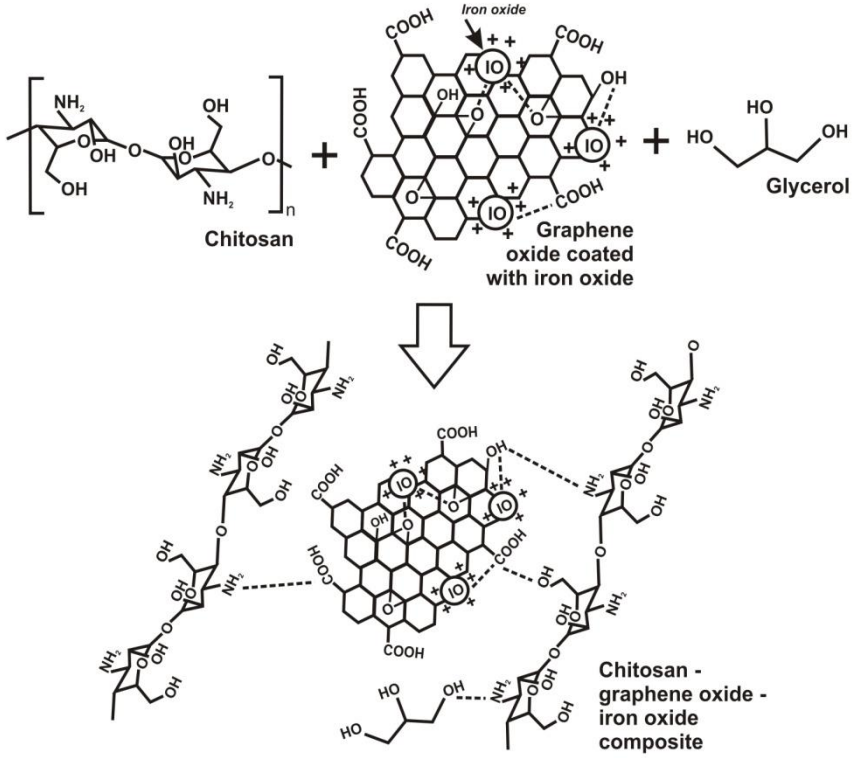

Figure 5. Diagram of CS - GIO composite formation (author's own work based on [28])

Scientists tested antibacterial activity against *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative) bacteria and an opportunistic skin pathogen - *Candida albicans*. The generated chitosan-GIO system was tested by the diffusion method in agar

medium and using a test of direct contact between the analysed nanocomposite and microorganisms. Based on the performed studies, the authors observed a high degree of antimicrobial activity of the obtained system, which was confirmed by SEM images showing pronounced damage to bacterial membranes with the release of intracellular materials.

Li et al. [29] attempted to make an anti-microbial assessment of graphene oxide composite functionalised with chitosan and guanidine hydrochloride (GO-CS-PHGC), obtained by combining CS-PHGC complexes on the surface of GO sheets (Fig. 6).

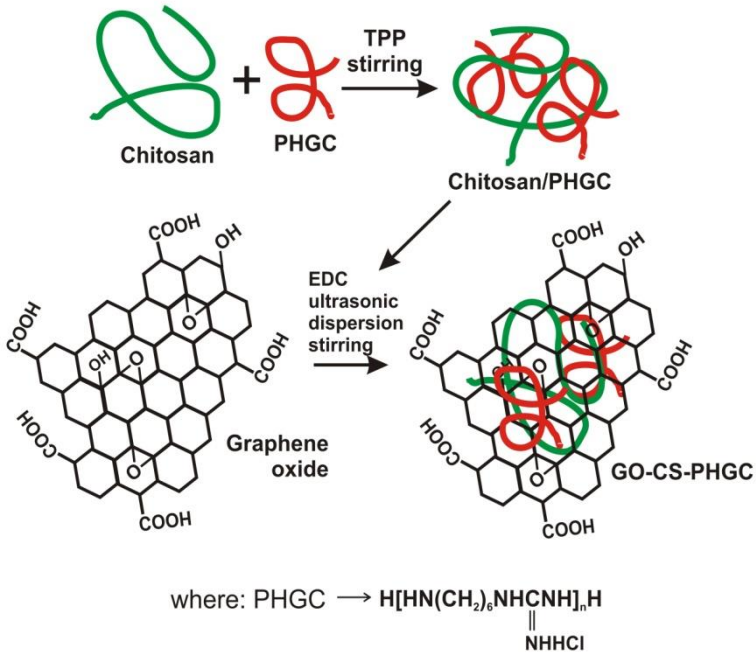

Figure 6. Diagram of obtaining the GO-CS-PHGC composite (author's own work based on [29])

Based on microbiological tests carried out against *Escherichia coli* and *Staphylococcus aureus*, the authors observed much higher antibacterial activity of the GO-CS-PHGC system than in the case of individual components. The minimum inhibitory concentration (MIC) value of system containing all components against *E. coli* bacteria was 32 μg/ml. This proved that the nanocomposite produced may be an interesting material for the preparation of disinfectants that effectively inhibit the growth and multiplication of bacteria.

The studies carried out by Chowdhuri et al. are also significant [30]. Researchers developed antibacterial CS-GO nanocomposites of new generations with zinc oxide (ZnO) applied to the surface. Based on the microbiological tests against *E. coli* and *S. aureus*, the authors observed that the generated system caused a significant combating pathogen by inducing large amounts of active superoxide ions (O_2^-), leading to oxidative damage.

The minimum inhibitory concentration value against *E. coli* was 2.5 μg/ml, and in the case of *S. aureus* bacteria was 5 μg/ml. Therefore, the use of the GO-CS/ZnO

nanocomponent is a very promising material to produce antibacterial agents which effectively inhibit the development of pathogens.

4.2. Drug delivery

The second but extremely important area of biomedical applications of nanocomposites based on chitosan and graphene oxide is their use as potential carriers for the controlled delivery of drugs and biological macromolecules such as proteins, peptides or nucleic acids. Such systems allow the delivery of pharmaceuticals to precisely defined places in the human body e.g. cancer cells, thus contributing to increase the effectiveness of the applied therapy. This has been confirmed by numerous scientific publications devoted to research into the possibility of using CS-GO systems as effective drug carriers.

In 2010, Rena et al. [31], due to the lack of previous work on the use of GO sheets for the controlled release of drugs containing one aromatic group in their structure, attempted to synthesise graphene oxide coatings functionalised with chitosan. In their studies they used an anti-inflammatory drug, ibuprofen, and an anti-cancer drug, 5-fluorouracil, as model pharmaceuticals. Based on the experiments, the researchers observed that ibuprofen was successfully combined with a nanocomposite by simple physisorption, whereas 5-fluorouracil was dissolved in hexane and water with FGOCs solution. Furthermore, ibuprofen, due to its hydrophobic nature and strong π-π-type bonds with FGOCs, was characterised by the greater possibility of controlled release than in the case of 5-fluorouracil. The authors noted that the FGOCs system with both types of drugs provided cell viability at the level of 93.4% to 30.1% at a concentration of 10-400 μg/ml. These results proved that GO sheets functionalised with chitosan represent a promising material for biomedical applications.

Justin et al. [32] developed a chitosan-graphene oxide nanocomposite for use as a carrier of drugs administered transdermally, using sodium fluorescein (FL) as a model preparation. The composite containing in its composition 2wt% GO was considered the most optimal system. It showed the most advantageous combination of mechanical properties and pharmaceutical loading capacity. The developed material was characterised by Young's modulus at the level of 1-1.3 GPa and a tensile strength from 24 to 34 MPa. It provided much faster release of the drug, with - at the same – an extended time of biodegradation, in comparison with the case when only chitosan was used, which was caused by the presence of oxidised functional groups, the hydrophilic character and large surface area of the GO sheets. Apart from that, the authors observed that the profile of pharmaceutical release from nanocomposites depends on the ratio of drug administered to the amount of GO and on the pH of the environment in which the preparation is released.

The same research group [33] also attempted to use a chitosan nanocomposite and reduced graphene oxide (rGO) as carriers of transdermally delivered drugs. The 1wt% or 2wt% rGO applied in the research ensured the improvement of the electrical conductivity of chitosan and enabled the use of composites for the delivery of pharmaceuticals by means of electroporation and ionophoresis, confirming the thesis that a system made of chitosan and graphene oxide can be effectively used in medicine.

Interesting research was also carried out by Li et al. [34]. The authors developed nanocapsules, $(GO/Cs)_x$ and $G(GO/Cs)_x$, where x is the number of GO/Cs bilayers which are drug transport systems, using an anti-inflammatory drug (ibuprofen) as a model compound. Based on the analysis, the researchers observed that the use of the genipin as a cross-linking agent enables an improvement in the loading of the system with pharmaceuticals and has a positive effect on its release time. The nanocomposite

$G(GO/Cs)_5$ was considered the most optimal system for which the release time of the active substance was about 100 hours.

Many publications have been devoted to the use of systems containing chitosan derivatives and graphene oxide in their structure as drug carriers, which is confirmed, for example, by Chen et al. and Shi et al.

Chen et al. [35] developed microgranules containing chitosan derivatives, reduced graphene oxide and alginates, acting as carriers of small-molecule drugs. In the studies, the authors used three types of polymer derivatives: carboxymethyl chitosan (CMC), quaternised chitosan (QCS) and quaternised carboxymethyl chitosan (QCMS). The obtained systems were characterised by a high pharmaceutical loading efficiency of nearly 83% and moderate release in physiological environments, amounting to 71.6% after 150 h. Rapid release (82.4% drug content) was noted after 20 h in an acidic environment. In addition, cytotoxicity tests indicated a slight interaction in the stellate cells of the liver, which indicates the possibility of using microgranules as effective drug carriers.

Shi et al. [36] prepared, using the electrostatic drop generation method, hybrid particles containing carboxymethyl chitosan (CMCS) and graphene oxide (GO). Based on the experiments, the authors showed that the obtained nanocomposite was characterised by a high degree of lysozyme adsorption, bovine serum albumin or gatifloxacin which was chosen as a model drug. For the CMCS-GO system with a ratio of 40:2, the highest drug loading capacity was achieved which amounted to 0.45 ± 0.19 mg/mg and its prolonged release.

Particularly noteworthy are publications dealing with the application of chitosan-graphene oxide systems in anti-cancer therapy as complexes that ensure the greater effectiveness of doxorubicin (DOX) commonly used in chemotherapy, resulting in a reduction in the drug resistance effect.

Research into the development of nanofibrous scaffolds produced by electro-spinning used to deliver and release DOX was carried out by Ardeshirzadeh et al. and Samadi et al.

In 2014, Ardeshirzadeh et al. [37] attempted to make a system composed of polyethylene oxide (PEO), chitosan (CS) and graphene oxide (GO) with a content of 0.1 to 0.7wt%. As a result of the work, the researchers achieved the successful closure of doxorubicin hydrochloride in the scaffold structure and observed that the release of the drug depends on the pH of the environment in which it occurs. The most intense release of DOX was noted at pH = 5.3, which was due to the reduced interaction between the pharmaceutical and the PEO/CS/GO system.

The second research group [38] synthesised the TiO_2/DOX/GO composite with nanofibres of polylactic acid and chitosan. The developed system was used to release the chemotherapeutic agent for 2 weeks. The drug loading efficiency in the amount of 10, 25 and 50 mg was 97%, 94% and 92%, respectively. This indicated the possibility of using the TiO_2/DOX/GO system in anti-cancer therapy.

The works of Wang et al. and Zhao et al. are also noteworthy for the use of chitosan and graphene oxide in oncology.

Wang et al. [39] developed a system for the therapeutic treatment of cancer, made of galactosylated chitosan (GC), graphene oxide (GO) and doxorubicin (DOX). In the studies, scientists achieved the ability to load a chemotherapeutic agent amounting to 1.08 mg drug/mg of polymer, and the use of nanoparticles allowed the release of the drug in a low pH medium – the so-called cancer environment. The GC-GO-DOX system exhibited higher cytotoxicity to HepG2 and SMMC-7721 cells (human liver cancer cells) and a greater intensity of tumour growth inhibition than composites containing pure polymer. The above results proved that the developed GC-GO-DOX system may be

an effective carrier for the delivery of anti-cancer drugs in the future and contribute to more effective therapy for the treatment of liver cancer.

Zhao et al. [40] created a hybrid system of graphene oxide, chitosan and chitosan modified with dimethyl anhydride (GON/CS/CS-DMMA), used for the extracellular and intracellular delivery of doxorubicin. The developed carrier showed an excellent encapsulation efficiency and prolonged circulation time of the chemotherapeutic agent in the blood. The system provided enhanced capture of HepG2 cancer cells and could potentially be a promising drug carrier for oncological treatment.

Apart from the use of the chitosan–graphene oxide system as a carrier of pharmaceutical substances, the nanocomposite may be a platform for biological macromolecules, e.g. proteins, and can be used as an adjuvant to stimulate the immune response.

Emadi et al. [41] developed a CS-GO system to prevent the proteolysis of proteins while maintaining their activity. The nanocomposite was combined with bovine serum albumin (BSA) and collagenase to assess the degree of protein stability and activation. Based on the tests carried out for the GO-BSA and GO-CS-BSA systems, the authors did not notice any significant changes in the structure of proteins after a period of 30 and 60 minutes of exposure to protease. However, the bovine serum albumin itself was completely digested after 1 hour.

Yan et al. [42] discovered the adjuvative activity of a chitosan-graphene oxide system. In the studies, the researchers performed functionalisation of the GO surface to improve biocompatibility and increase adjuvant activity. The obtained nanocomposite was characterised by thermal stability and a positive surface charge. It ensured the reduction of non-specific protein adsorption and stimulated mouse macrophage cell lines RAW 264.7 and a greater number of cytokines to mediate the immune response of cells. The results of the research proved that the CS-GO system is a safe nanoadjuvant for use in immunotherapy and vaccines.

4.3. Gene delivery

Gene therapy is an innovative method of treatment of diseases with a genetic basis, such as cystic fibrosis, Parkinson's disease or cancer. Its main goal is to eliminate malfunctioning cells and introduce changes to their phenotype and physiology. Effective gene therapy requires the use of a gene vector that protects DNA against destruction and facilitates cellular DNA capture with high transfection [43-44].

Bao et al. [45] investigated the possibility of drug and gene delivery by means of a system containing graphene oxide and chitosan, obtained through the amidation process. GO coupled with polymer (64wt% chitosan) enabled the effective attachment of the anti-cancer drug camptothecin (CPT) as a result of the π-π type interaction and hydrophobic interaction. Based on *in vitro* tests, researchers noted the ability to release drugs from the nanocomplex at the level of 17.5% within 72 hours. The system was characterised by a high degree of cytotoxicity against cancer cells. Moreover, the graphene oxide and chitosan complex enabled the condensation of plasmid DNA on the surface of the GO sheet by electrostatic interaction with the cationic nanocarrier and ensured the high efficiency of gene transfection into HeLa cancer cells.

Hu et al. [46] developed a nanocomplex containing graphene oxide and trimethyl chitosan coupled with folic acid used as a carrier of the anti-cancer drugs doxorubicin and plasmid DNA (pDNA). The system, coupled with folic acid, was characterised by a higher degree of cytotoxicity to HeLa cancer cells than a nanocomposite without $C_{19}H_{19}N_7O_6$. In addition, the complex enabled the effective loading of DOX (at the level of about 31%) and pDNA, which indicated the possibility of using the system as a potential carrier for both drugs and genes.

Interesting research was also carried out by Sobolewski et al. [47]. Researchers developed a chitosan-catechol platform containing reduced graphene oxide (rGO) doped with platinum nanoparticles, intended for use as an ink for piezoelectric contactless printing that allows the development of modular biosensors. The obtained systems were subjected to biofunctionalisation with DNA oligonucleotide probes for *Streptococcus agalactiae* (Group B Streptococcus - GBS) using glutaraldehyde as a linker. Additionally, analyses were performed using confocal microscopy, which proved that the nanoplatform showed successful hybridisation of the complementary polymerase chain reaction and small nonspecific binding. This indicated the potential possibility of using chitosan-catechol systems with rGO-Pt as platforms, enabling the easier and more efficient attachment of biorecognition elements for biosensors.

4.4. Tissue engineering and regenerative medicine

Tissue engineering is a new but intensively developing field of science, combining knowledge in the field of medicine, biology and engineering to develop alternative solutions that change the approach to conventional methods of treatment with transplants and replenishing organ and tissue losses, as well as creating new diagnostic solutions [48-51].

The key solution for tissue engineering is the use of biomaterials that enable the induction of specific cellular functions, cell differentiation and modelling of interactions between cells. However, individual biomaterials frequently do not allow the full imitation of cell properties, so the introduction of hybrid systems is becoming increasingly more common [52-53].

Particularly promising systems that provide potential in tissue engineering and regenerative medicine include nanocomposites based on chitosan and graphene oxide.

Li et al. [54] analysed nanocomposites containing chitosan, graphene oxide and hydroxyapatite. The obtained system was characterised by a greater modulus of elasticity and greater hardness than hydroxyapatite alone. Biological tests (cytotoxicity assessment) were performed on the L-929 mouse fibroblast cell lines and the cell lines of osteoblast-like MG-63 cells. In the discussed CS-GO-HA system, significantly higher cell viability and alkaline phosphatase activity were observed than in the case of GO-HA composite. This confirmed the thesis on the possibility of using a combination of chitosan with GO for the regeneration of bone tissue.

In 2014, Depan et al. [55] investigated the mineralisation of the system composed of chitosan and graphene oxide in simulated body fluid, using hydroxyapatite (HAP) as a biomimetic mineralisation model (Fig. 7).

The authors achieved beneficial cellular activity - osteoblast functions: expression of actin, fibronectin, vinculin and proliferation. They found that the application of HAP in the HAP-CS-GO composite resulted in a uniform, spatial growth of osteoblast cells.

In the same year, Dinescu et al. [56] attempted to develop freeze-dried structures containing graphene oxide and chitosan acetate to be used in osteoblast cultures for bone tissue regeneration. The prepared scaffolds were characterised by a developed spatial structure with clearly visible pores. Moreover, the scaffolds did not exhibit cytotoxicity effects and the system containing 3wt% GO was considered the most optimal, enabling an improvement in the mechanical properties and biological activity.

The same group of scientists [57] also compared the possibility of the application of biomaterials containing chitosan alone and systems enriched with graphene oxide. The authors observed that the addition of GO promotes cell proliferation and adhesion as well as differentiation. They noted that the system containing 1wt% graphene oxide was characterised by more favourable properties after osteogenesis than the system

composed of pure polymer. This proved that the use of GO enables improvements and accelerates the osteogenic process.

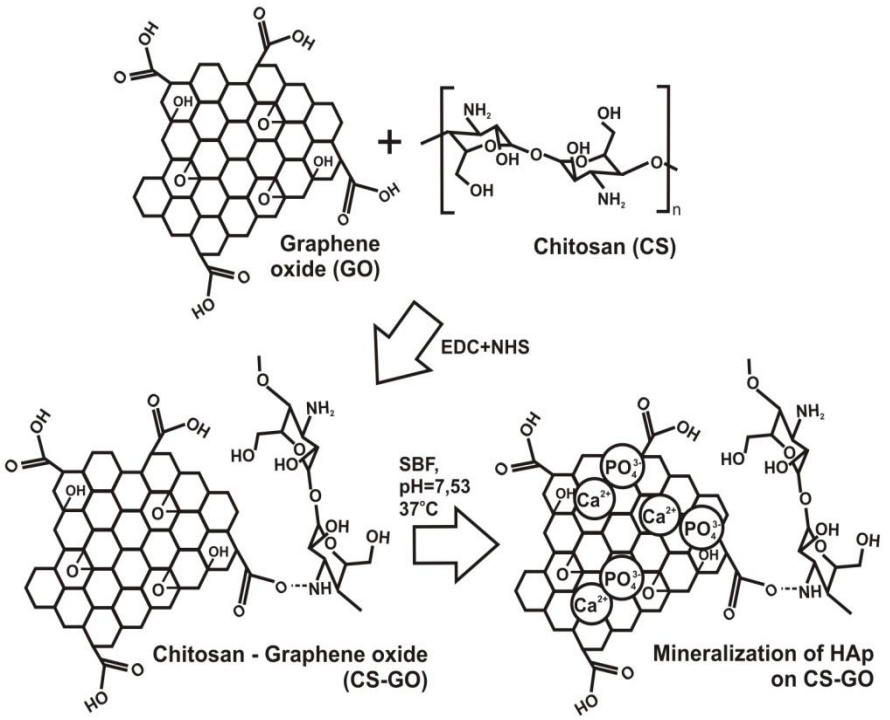

Figure 7. Diagram of obtaining CS-GO system and HAP-CS-GO composite (author's own work based on [55])

Saravanan et al. [58] analysed, in terms of medical applications, lyophilised nanocomposites made of chitosan, gelatine and graphene oxide. The use of GO improved the physicochemical properties of the system. Scaffolds enabled the culture of rat osteoprogenitor cells and the differentiation of mesenchymal mouse stem cells into osteoblasts. In addition, the system caused the faster bridging of losses in the rat tibia and the depositing of larger amounts of collagen.

Research into the application of the systems based on chitosan and graphene oxide in tissue engineering was also carried out by Hermenean et al. [59]. Based on the histomorphometric analysis, the researchers noticed that scaffolds enriched with GO were characterised by much more intense activity of alkaline phosphatase than the systems made from polymer alone. A greater expansion of bone morphogenetic protein (BMP) and Runx-2 was observed, as well as the improved differentiation of osteoprogenitor cells. Thus, the research results indicated the use of scaffolds made of chitosan and graphene oxide as a promising tool for the regeneration of bone defects without the use of exogenous living cells and growth factors.

However, Pandele et al. [60] developed scaffolds composed of a combination of chitosan with vinyl polyalcohol and graphene oxide. The structural analysis of the system showed that the composite was characterised by high porosity and the presence of GO resulted in a more regular distribution of pores, which can significantly affect the application of the system in tissue engineering.

The studies of Sivashankari et al. are also significant [61]. The research group attempted to develop freeze-dried three-dimensional scaffolds composed of hydroxypropyl chitosan and graphene oxide, with glutaraldehyde used as a cross-linking agent. The obtained nanocomposites were characterised by homogeneous porosity, and the pore size decreased with the increase in GO content. In addition, scaffolds containing graphene oxide were characterised by greater mechanical strength and a controlled degradation rate. A system containing 1% GO by weight has a maximum mechanical strength of about 192 kPa. Apart from that, the developed scaffolds did not induce cytotoxicity effect on cells.

4.5. Immunosensors

Due to the excellent electrical properties of graphene and graphene oxide, a large-scale research into their application in production of immunosensors was carried out. Immunosensors are a type of biosensor which are capable of detecting the presence of antigens and antibodies in the analysed sample. Research is most often performed directly for body fluids, which makes it possible to determine the immune response in the body [62-63].

Immunosensors (Fig. 8) consist of two basic elements: an analytically active layer and a transducer. The analytically active layer enables the formation of a receptor-analyte connection and the generation of physicochemical or chemical signals proportional to the concentration of the analysed substance in the sample. This layer in biosensors is made of biological material immobilised on the supporting layer, which may be prepared from precious metals, or glass; increasingly more often, allotropic varieties of carbon are used, such as diamond, graphite, graphene and graphene oxide. The transducer element converts the physicochemical signal to an electrical signal which, after amplification, can be analysed [64-65].

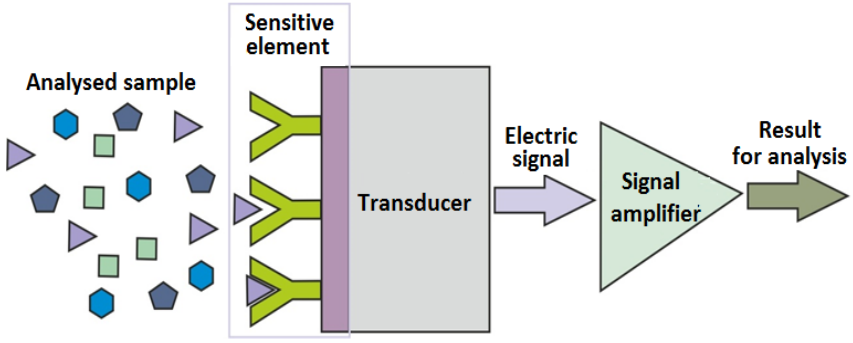

Figure 8. The principle of operation of the immunosensor

The accumulation of biologically active substances in the environment, and especially in ground and sea water, caused increasing interest in scientists in immunosensors to detect and monitor the concentrations of substances directly in ecosystems. Studies have been carried out on immunosensors containing graphene and graphene oxide, which were predisposed to determine the concentration of diclofenac in the water, which belongs to the anti-inflammatory drug group [66], or toxins such as okadaic acid, which are produced by marine algae and classified as DSP (Diarrhoetic Shellfish Poisoning) [67].

Many studies have also been carried out on systems enabling the determination of hormone concentrations directly in body fluids, e.g. progesterone [68], cortisol [69], and oestradiol [70]. Significant potential is seen in the application of immunosensors for the detection of tumour markers [71-76], virus antigens [77] and in studies on the immune responses in patients after organ transplants [78].

The first mention of the use of a substrate consisting of graphene and chitosan comes from 2015. Kavosi et al. [79] presented research on an ultrasensitive electrochemical immunosensor for the detection of the PSA biomarker - a prostate specific antigen that allows the early detection of prostate cancer. The immunosensor was made of a graphene-chitosan layer placed on a carbon electrode. The PSA aptamer was immobilised on this layer. To improve the sensitivity of the sensor, the system was modified by the formation of polyamidoamine incorporated with gold nanoparticles on the surface of dendrimers. The obtained sensor was characterised by a very low detection threshold at the level of 10 fg ml^{-1} with a wide range of concentrations (0.1 pg ml^{-1} to 90 ng ml^{-1}).

In 2017, Afkhami et al. [80] reported the formation of an immunosensor in which a matrix containing gold nanoparticles, graphene and chitosan was used to amplify the signal. The sensor, whose analytically active layer was the BoNT/A antigen immobilised on a modified carbon electrode, allowed the detection and measurement of the concentration of botulinum neurotoxin of serotype A in PBS fluid, milk and human serum in the concentration range of 0.27–268 pg ml^{-1}.

5. Concluding remarks and future outlook

As in many fields of science, research into the use of nanocomposites made of chitosan and graphene oxide in medicine has been characterised by a dynamic growth in recent years.

It is estimated that CS-GO systems hold a breakthrough position among potential dressing and disinfection materials as well as drug carriers, especially anti-cancer and biological macromolecules (proteins, peptides and nucleic acids).

Owing to the use of the discussed nanocomposites, it is possible to break many barriers that inhibit the proper course of conventional chemotherapy, such as drug resistance resulting from the microenvironment of solid tumours characterised by hypoxia and abnormal tumour vasculature, as well as a lack of specificity and bioavailability of chemotherapeutics, leading to the destruction of both cancer and normal cells.

In addition, CS-GO systems present significant potential for applications in tissue engineering and regenerative medicine as scaffolds enabling the regeneration of damaged tissues and organs in the human body.

However, it should be remembered that prior to the implementation of nanocomposites to common clinical practice, it is important to carry out detailed studies for potential cytotoxicity. Possible toxicity and the ability of the system to cross the blood - brain barrier may be one of the main reasons limiting their application in medical sciences.

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