AUTEX Research Journal, DOI 10.2478/aut-2020-0009 © AUTEX

ANTIBACTERIAL EFFECT OF GRAPHENE AND GRAPHENE OXIDE AS A POTENTIAL MATERIAL FOR FIBER FINISHES

Anna Olborska[1], Anna Janas-Naze[1], Łukasz Kaczmarek[2], Tomasz Warga[2]*, Dewi Suriyani Che Halin[3]

1 Department of the Oral Surgery, Central Clinical Hospital, Medical University of Lodz, Pomorska 251 St., 92-213, Łódź, Poland
2 Institute of Materials Science and Engineering, Lodz University of Technology, Stefanowskiego 1/15 St., 90-924, Łódź, Poland
3 Centre of Excellence Geopolymer and Green Technology, School of Materials Engineering, Jalan Kangar-Arau, 02600 Arau, Perlis, Malaysia
*Corresponding author. E-mail: tomasz.warga@p.lodz.pl

Abstract:

The dynamic development of the world economy entails an increasing exchange of goods and population. This means that we are globally struggling with increasing levels of nosocomial infections. The increasing use of antimicrobial agents triggers the microorganisms' immune system, which in turn contributes to the increasing amount of antibiotic-resistant microorganisms, making it necessary to control the development of unwanted microorganisms, including bacteria, especially those carried on the body and clothing.

Currently, there is no unique method to combat the multiplication of microorganisms and eliminate threats to human health and life. For this reason, this article describes the possibilities of using graphene materials as a potential additive materials in fiber finishes as an antibacterial aspect in various areas of life. However, the literature does not explain the mechanisms behind the antibacterial properties of graphene, strongly limiting its textile application. The research is conducted using molecular dynamic simulations of interaction between graphene materials and murein. The obtained results suggest the electrostatic mechanism of blocking the growth and division of bacteria. Due to the physical interaction, bacterial cell becomes "trapped" without changing its growth parameters. This may lead to an increase of internal cell pressure, rupture of its wall and consequently its death.

Keywords:

Graphene; Antimicrobial fibers; Nanofibers.

Introduction

The variety of materials used for the production of fibers (natural and synthetic, including a different chemical structure and a different degree of functionalization[1]–[4]) poses many issues, especially in the area of eliminating the multiplication of bacteria on their surface [5]. Widely used surface modifications must, however, take into account the elimination of skin allergic reactions, as well as the lack of toxicity in contact with live tissue, which requires the development of dedicated modification methods for specific types of materials, and even for specific methods of their production. For this reason, in many scientific centers around the world there is an intensive search for universal methods and techniques that allow to modify the fibers of materials in order to eliminate the growth of bacteria on their surface that threaten the health and life of living organisms. In this area, a representative of 2D materials – graphene, made of carbon atoms with a hexagonal structure, between which there are delocalized bonds – in increasingly being considered [6]–[8] .

Graphene is a flat structure, composed of carbon atoms bonded into hexagons, reminiscent of honeycomb, with atom hybridization of sp^2 [9], [10]. Currently, there are several methods for obtaining graphene: in case of its powder form, the Hummers method [11] together with its varieties [12]–[14] is the most popular. The disadvantage of these methods is the possibility of obtaining graphene with an area of only few mm^2, often with an irregular and deformed structure. It is often the case that the graphite fraction in the graphene suspension can exceed 20%. The advantage, however, of the graphene powder in both oxidized and reduced forms is the technical ease of its application in the form of thin films or dispersion in a liquid medium. On the other hand, regarding the large-surface form, the most popular methods are: Si sublimation from SiC-xH systems [15]–[17], CVD (chemical vapour deposition) methods of vapor deposition on a metallic substrate (copper, nickel) [18]–[22] or the metallurgical method of producing graphene HSMG (High Strength Metallurgical Graphene) from liquid phase [23].

Graphene has strong cytotoxic properties when in contact with bacteria. The mechanisms of its impact are similar to those of other synthetic carbon-based materials, such as fullerenes or carbon nanotubes (CNTs). During direct contact with CNTs, intracellular contents are released from bacteria due to physical damage to bacteria cell membrane by CNTs [24]. Liu et al. [25] in order to study the antibacterial mechanisms, tested four types of graphene-based materials: graphite (Gt), graphite oxide (GtO), graphene oxide (GO) and reduced graphene oxide (rGO) in order to study the interaction with *Escherichia coli*. Based on the conducted experiments, it was demonstrated that

the bacteriological interaction of these materials depends on the material's properties, such as size, solubility, dispersion, duration of interaction and concentration, density of functional groups and the production of cellular oxidative stress. Graphene materials of smaller size and more functional groups react more actively with bacterial cell membrane and penetrate into their cells. Counting the colonies of *Escherichia coli* in an aqueous dispersion to compare the antibacterial activity of Gt, GtO, GO and rGO indicates that the majority of bacteria are rendered harmless in the first hour of incubation, and as the material concentration increases, the cell death rate increases. GO is the most cytotoxic on bacteria, followed by rGO, Gt and GtO. Graphene materials can oxidize bacteria lipids, proteins, DNA, as well as glutathione, which in bacteria is a strong antioxidant and prevents damage caused by oxidative stress. Glutathione is oxidized the most by rGO. Toxic superoxide anions (O^{2-}) which are free radicals are produced by Gt, GtO, GO and rGO. The conducted research indicates that bacterial cell death occurs as a result of membrane stress and cutting of bacterial cell walls by sharp edges of nano sheets, another cause of bacterial cell damage is oxidative stress in the cytoplasm.

Since studying graphene and materials belonging to the graphene family, a lot of research has been done on the impact of these nanomaterials on microorganisms, but there is still a need to study these interactions. Pulingama T. et al. [6] explained the effect of GO on Gram-positive *Staphylococcus aureus* and *Enterococcus faecalis*, Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*. The effect of GO depends on the concentration and duration of GO's interaction, as well as on the morphology of the bacterial cell surface and is different for two types of bacteria. Increasing the GO concentration translates to increasing the loss of membrane integrity. The mechanism of antibacterial interaction with Gram-positive bacteria was cell trapping, and most bacterial inactivation occurs through a bacterial wrapping mechanism. Physical contact of GO with Gram-negative bacteria causes their inactivation by breaking the cell membrane. ATR-FTIR (Attenuated total reflectance-Fourier transform infrared spectroscopy) characterizations of the GO treated bacterial cells showed changes in the fatty acids, amide I and amide II of proteins, peptides and amino acid regions compared to untreated bacterial cells.

Biofilm plays an important role in the infection process. A biofilm is a collection of microorganisms of one or more species or different types of microorganisms adjacent to solid surfaces. Biofilms are resistant to the immune system, antibiotics, disinfectants. Fallatach H. et al. [26] used the bacterium *Pseudomonas putida* KT_{2440} for the tests showing the antibacterial effect of the colloidal GO suspension on the biofilm. It is an active producer of biofilm in solid and aquatic environment. Biofilm produced by this bacterium, that differ in maturity (24, 48 and 72h), were treated with GO (85μg/ml or 8,5μg/ml). The age of the biofilm depended on its sensivity to GO, presumably due to changes in cells at different stages of their maturation. It was investigated that GO did not affect 24- and 72-hour biofilms, but exerted an antibacterial effect on 48-hour biofilms. The effect of GO on biofilm was also dependent on the concentration of GO nanoparticles. Lower GO concentration (8,5 μg/ml) did not have antibacterial effect, nor did they cause a violation of bacterial cell membrane integrity.

Topography of the surface affects the formation of biofilm, which may also be important for the surface of the fiber, which is subjected to modification by graphene. The process of inhibiting the development of bacterial cultures on the coated surface is different than in suspension. This was confirmed by Yadav N. et al. [27] by examining the anti-bacterial effect of GO coated surfaces prepared by two different methods (Hummers and improved, i.e. GOH and GOI) on bacteria *Escherichia coli* and *Staphylococcus aureus*. A less porous, inhibiting biofilm formation, thinner film was created by producing GOI. In contrast, GOH was characterized by greater surface roughness as well as uneven GO thickness, which supported the adhesion of bacterial cells. GOH and GOI showed a selective action against bacteria. In suspension, GOI inhibits *E.coli* cells. On the other hand, GOH is clearly more selective in relation to *Staphylococcus aureus*. Oxidative stress is lower on the coated surface compared to the suspension, which creates a greater possibility of contact of microorganisms with sharp GO edges. It is thought that GO interferes with bacterial adhesion by affecting fimbriae or pili – structures that allow bacteria to adhere to the surface during biofilm formation. This fact can be an argument in the use of graphene as an additive in fiber production process or at the stage of creating finishes on the surface of fabrics to eliminate the multiplication of bacteria on their surface.

Tissue engineering uses scaffolds that enable the regeneration and reconstruction of lost tissue by creating favorable conditions for the colonization of stem cells and vessels. Marín J. et al. [8] studied the antibacterial activity of porous membranes, based on nanofibrous chitosan (CS), polyvinyl alcohol (PVA) and graphene oxide (GO) against Gram-positive bacteria *Bacillus cereus* and *Staphylococcus aureus* and Gram-negative *Salmonella enterica* and *Escherichia coli*, by contact of the electrospun nanofiber scaffolds above inoculum bacterial in Müeller Hinton agar. the highest antibacterial effectiveness occurs when the GO scaffoldings were increased to 1%. For sites with a potential need for antibacterial activity and the possibility of inflammation, scaffolds with 1% GO should be used, which may also be the basis for the development of modified fibrous materials used in dentistry.

One of the most clinically important pathogens resistant to many drugs are: Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus epidermidis* (MRSE). Lisa Elias et al. [28] investigated the effect of GO and CNF (carbon nanofibers) on these bacteria irradiated with light-emitting diode (LED). The antibacterial properties of GO and CNF against MRSE and MRSA were tested by dispersing nanomaterials in a 0.9% NaCl solution at a concentration of 80μg/ml for 1.5 and 3 hours. GO and CNF combined with LED light for 3 hours resulted in loss of viability of MRSA and MRSE cells. Carbon nanomaterials, GO and CNF, after exposure to LED light show increased effective antibacterial effect on life-threatening microorganisms.

Due to the growing need for environmental protection, it is necessary to eliminate the packaging materials used so far and replace them with biodegradable nanocomposites. Fang Li et al. [29] studied PHBV nanocomposite (poly (3-hydroxybutyrate-*co*-3-hydroxyvalerate) with hybrids graphene oxide/cellulose nanocrystals (CNCs). *Escherichia coli* and *Staphylococcus aureus* strains were used to assess the antibacterial effect of PHBV nanocomposites with nanofiller by agar diffusion method. Pure PHBV did not affect bacteria. Ternary nanocomposite 1% by weight covalently connected CNC-GO showed 100% antibacterial activity. The research creates the possibility of producing nanocomposites for applications not only in medicine but also in textile industry characterized by increased strength properties, in which inhibition of the growth of microorganisms is observed.

Khorrami S. et al. [30] investigated that the order of procedures in the process of producing nanocomposites may affect their properties in the impact on microorganisms. For this purpose, the effect of bacteria on silver oxide and graphene composite (Ag-GO) resulting from two synthesis procedures were compared. By adsorbing silver nitrate on the GO surface, reducing and stabilizing with the extract of green walnut husk, Ag-GO-I was produced. When applying the new procedure, Ag-GO-II was created by exposing GO to the extract and then Adding $AgNO_3$. When using the first method, a large amount of silver nanoparticles has gathered between GO sheets. This resulted in a significant reduction in their agglomeration, reduction of their free movement and release, thus reducing the antibacterial activity. During the second method, GO sheets were completely covered with Ag nanoparticles without aggregation, which resulted in greater antibacterial activity compared to the produced Ag-GO-I. The effect of the synthesized nanocoproteins was tested against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* strains by the agar well diffusion method. These studies confirm the potential in the production of materials as possible finishes for fibrous materials used not only in medicine, but in everyday life.

Bacterial destruction is also possible due to semiconductor catalysis. This was supported by the research of Yang Y. et al. [31] A group of bismuth oxyhalides, BiOX (X= Cl, Br, I) photocatalysts was used to prepare BiOI-GO. BiOI is a stable nanocomposite that has demonstrated a synergistic photocatalytic-photothermal effect to improve the antibacterial properties of *Acinetobacter Baumannii* (ATCC 19606) through higher TOC (total organic carbon) removal efficiency and K^+ ion leakage in comparison to the individual photocatalytic process. The photocatalytic antibacterial effect of the composite is possible due to the large surface of GO, as well as due to GO conductivity, electron transfer occurs. The presence of GO also increases the absorption of light. In BiOI-GO, the temperature around BiOI crystals increases due to the photothermal effect of GO. Synergistic photothermal and photocatalytic processes having an antibacterial effect may be used for self-disinfection of clothing fabrics in the event of microbial contamination.

An attempt to explain the effect of graphene in contact with bacteria was undertaken by the team of Sharma A. et al. [32] who studied crystal structure, morphology and local electronic structure and their effect on the antibacterial properties of GO on *Escherichia coli*. The field emission scanning electron microscopy (FESEM) showed bacterial cracking due to close contact with sharp GO edges. Parallel comparison of the results using scanning transmission-ray microscopy (STXM) and near edge X-ray absorption fine structure (NEXAFS) showed the formation of reactive oxygen species . ROS arose due to the transfer of electrons from microbial membranes to GO. Denaturation of bacterial protein induced by oxidative stress has occurred, which can also occur with the surface of fiber finishes. Therefore the cellular oxidative stress induced molecular denaturation of bacterial protein is likely the cause of bacterial entrapment.

Mei L. et al. [7] indicated the possibility of using carbon nanomaterial as an antibacterial agent for phototherapy. Compared with pure GO, they investigated that by functionalizing GO and obtaining amino-functionalized graphene oxide (GO-NH_2), it is possible to increase antibacterial activity as a result of obtaining very good photothermal efficiency by this complex. Electrostatic attraction facilitates GO-NH_2 to reach the surface of Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria. Irradiation of GO-NH_2 with white light caused 32 times stronger inhibition of bacterial growth through loss of wall and cell membrane integrity and cytoplasm leakage.

The problem of water treatment and disinfection is being considered all over the world. Filina A. et al. [33] demonstrated the antibacterial activity of the functionalized, porous GO sponge (sp-GO) in continuous and periodic flow tests. Sp-GO was functionalized with natural antibacterial agents such as enzyme [lysozyme (LYS)], peptide [nisin (NIS)] and polyamide [ε-poli-L-Lysine (PLL)]. The resulting functionalized nanocarbons (GO/NIS, GO/LYS, GO/PLL) were tested for antibacterial effects, in terms of cell growth and cell membrane integrity. Two strains were selected: Gram-positive *Bacillus subtilis* 6633 and Gram-negative *Escherichia coli* K12. Inhibitory effects on *Escherichia coli* have not been shown by unbound NIS and LYS. This is due to the cell structure of Gram-negative bacteria. In these bacteria, the effect of lysozyme on peptidoglycan, the major component of cell walls, is restricted by the outer cell membrane. Similarly, the outer cell membrane prevents NIS access to the cell of Gram-negative bacteria. for this reason, NIS and LYS were not tested after immobilization on GO. Complete inhibition of *E. coli* K12 growth was obtained only with PLL. Gram-positive *B. subtilis* was inhibited by all antibacterial agents (NIS, LIS, PLL) for which the minimum inhibitory concentration (MIC) was determined. The functionalized sponge improved bacterial retention of both strains in continuous filtration, probably due to electrostatic interactions. This fact will be used to create a new form of fibrous filters, thanks to which it will be easy to shape them without the need to use appropriate material forms, as is the case with porous materials.

Bone implants are increasingly used in treatment. Materials used for this purpose should exhibit antibacterial properties to prevent the development of bacterial infection and the associated possibility of osteomyelitis. It is advisable to limit the

use of antibiotics for this purpose to prevent the development of bacterial resistance to antibiotics. The second feature that should characterize bone implants is good osseointegration. In order to combine these two properties and to find materials that are double functional coatings on bone implants, Li M. et al. [34] created ultra-thin multilayer films of {$(GO/Lysozyme)_n$ $(GO/Lys)_n$} using layer-by-layer (LBL) assembly method with alternative GO and lysozyme deposition. These ultra-thin films show very high efficiency in destroying Gram-positive *Staphylococcus aureus* (ATCC #6538) and Gram-negative *Escherichia coli* (ATCC #8739) bacteria., as well as increased osteogenic differentiation. With the most advanced layer, lysozyme, the $(GO/Lys)_n$ films revealed stronger antibacterial properties of GO, while LBL technique allowed the creation of thin layers that reduced its cytotoxicity. Instead of foil, it is also possible to produce fibrous materials with suitably modified graphene surface.

Jira J. et al. [35] studied the impact of two standardized media recommended for determining bacterial sensitivity to antibiotics on the process of inhibiting bacterial growth by two carbon-based nanomaterials. The effect of nanodiamond and graphene oxide were compared in both annealed (oxidized) and reduced (hydrogenated) forms in two types of cultivation media Luria-Bertani (LB) and Mueller-Hinton (MH) broths. Studies have shown that nanomaterials significantly reduced (45%) the number of *E. coli* colony forming units in LB medium for at least 24 hours relative to control. Researchers suggest that the interaction of ND and GO particularly increases oxidative stress in bacterial cells. No phenomenon of increased cell membrane disruption or differences in ion concentrations was observed, so salty agars did not increase the bactericidal effect of the used nanomaterials. Liang Y. at all [36] created a nanoplatform with antibacterial activity on the basis of a hybrid hydrogelCS via simple electrostatic interaction embedded with thin-layer graphene oxide sheets modified with zinc oxide quantum dots (ZnOQDs). A biocompatible ZnOQDs@GO-CShydrogel was created, which showed very high effectiveness of bacterial destruction (98,9% and 99,5% against *S. aureus* and *E. coli*, respectively). The high effectiveness of the platform is due to several synergistic effects. The 808nm laser radiation used for GO irradiation triggers the hyperthermia effect of GO sheets that can kill bacteria. This effect is supported by ZnOQDs through the possibility of immediate production in the ROS nanoplatform and the release of Zn^{2+} from ZnOQDs in an acidic intracellural environment. In addition, there is an antibacterial effect of the hydrogel. The potential for bacterial killing by hybrid hydrogels shown here is very important in assisting wound heling in vivo. The carrier can also be a fibrous material on the surface of which a modified graphene oxide (GO) will be inculated with modified zinc oxide.

In order to produce an antibacterial fabric containing GO for medical applications, Yaghoubidoust, F. & Salimi, E. [37] deposited GO nanoparticles on cotton surface using dip-coating procedure. GO could uniformly cover the cotton surface and the modified fabric neutralized almost 70% of Gram-negative *E. coli* and 93% of Gram-positive *S. iniae* bacteria. The developed method is characterized by low coating costs, is simple and efficient which gives hope for the possibility of its wider application to obtain antibacterial fabrics.

Jia L. et al. [38] using electrospinning process by introducing TiO_2 nanofibers and graphene oxide (GO) sheets into cellulose acetate (CA) nanofibers created nanofibers TiO_2/GO/CA@ cotton. The resulting material showed high antibacterial efficacy against *B. subtilis* and *B. cereus* bacteria, with inhibition rate above 95%. The addition of TiO_2 and GO resulted in an improvement in hydrophilicity. Better antibacterial activity and better hydrophilicity allows the use of these composites for the production of antibacterial nanofiber/cotton hybrids.

Graphene oxide (GO) nanosheets were introduced onto cotton fabric by radiation-induced cross-linking under the domestic microwave. In order to modify the GO surface, cotton fabrics have undergone ion implantation using different doses of Fe^{3+} ions [39]. The resulting material showed very good washing, mechanical, thermal and antibacterial properties. The ion-implanted GO-cotton showed higher antibacterial activity. An increase in the number of Fe ions increased bacterial activity. The interaction of GO in combination with cotton and Fe ions has been shown by antibacterial tests against *Escherichia coli* and *Staphylococcus aureus*. It has been assumed that the mechanism of antibacterial action depends on the size and geometry of GO.

Sun S. et al. [40] demonstrated the possibility of producing multi-functional three-dimensional porous materials for potential applications in removing oil leaks and disinfecting water. By nature porous, the commercial melamine sponge (MS) has been functionalized by RGO and by Ag/RGO. RGO-MS and Ag/RGO-MS showed high oil-water separation capacity. RGO-MS showed a high absorption capacity from 41 to 91 times higher than its various organic solvents and oils. The RGO-MS absorption capacity was higher than that of the Ag/RGO-MS. Moreover, Ag/RGO-MS in addition to the ability to absorb oils and organic solvents, as well as to recycle, showed antibacterial activity against bacteria *Staphylococcus sciuri*, Shewanella MR-1, *Pseudoalteromonas lipolytica* and *Vibrio natriegens*. However, it is also possible to use fibrous materials with a nanoporous structure, which will further increase the absorbency of such structures.

To sum up, the available literature has proven numerous times the antibacterial properties of the graphene and its derivatives. Understandably, this has brought an enormous attention to apply the material in solutions aiming at limiting bacterial growth or directly causing their death. However, the literature lacks an analysis of the phenomenon happening at molecular level, which are responsible for blocking the cell division of bacteria as a result of its interaction with graphene, including its oxidized form. The lack of systematics of conducted analyzes effectively limits the real use of this phenomenon in medicine, e.g. for the finishes of dressing materials, because it is not known what mechanisms are responsible for the actual death of bacterial cells. Therefore, this article attempts to clarify the bactericidal properties of graphene oxide using molecular modelling. The analysis of the mechanisms at the level molecular interactions

between the mentioned phases will be the basis for explaining the reason for the occurrence of this phenomenon.

Research methodology

In order to determine the mechanisms of blocking the cell division of bacteria in contact with graphene oxide, SCIGRESS v.FJ 2.7 software was used to create a model system - a graphene structure consisting of 150 carbon atoms and 42 hydrogen atoms at the edge to saturate the bonds located there. This structure represents the actual molecular systems from the point of the structure of graphene and murein, which is the building block of the bacterial cell wall. Then the created system was optimized to achieve the minimum energy. After optimization of the analyzed graphene model, the C-C bond length was 1,42Å, while the torsion angle C-C-C 120°. For such an energy-optimized structure, computer simulations were carried out using molecular mechanics.

Then, modeling of molecular systems was performed, in which, as a function of changing the amount of generated carboxyl, hydroxyl and epoxy groups, with carbon hybridization of sp^3, the thermodynamic stability of the system was analyzed along with charge distribution map. These groups are typical for oxidized graphene structures, according to research by Aliyev et al. [41]. The above calculations were made for graphene with the following oxidation degree: 0%, 2.6%, 5.2% and 10.5%. It was assumed that oxygen groups are located on the edges of flake, which is in accordance with the process of graphite exfoliation using the Hummers' method.

The analysis took into account only the outer material of the material cell, which is the basic building block of the cell wall, namely murein. Graphene, regardless of whether in pristine or oxidized form, interacts only with the cell wall. For this reason, in described molecular mechanics analyzes, other materials that make up the bacterial cell are omitted. Therefore, the spatial optimization process of the murein fragment made of 283 atoms (89 carbon, 26 nitrogen, 19 oxygen and 149 hydrogen atoms) was carried out in order to achieve the minimum energy. Then, using molecular mechanics for the system created in this way, computer simulations of charge distribution were carried out.

In the last stage of research, the molecular interactions of murein at the phase boundary with pristine graphene, as well as its oxidized form, were analyzed. Based on the analysis of molecular interactions occurring at the murein/graphene and murein/graphene oxide boundary, an interface was made regarding the mechanisms of blocking bacterial cell division.

Results

First, molecular analyzes were carried out for graphene with one type of oxygen groups, respectively: -COOH, -COH, and -COC- while maintaining a variable proportion of oxygen relative to graphene: 0, 2.6, 5.2 and 10.2% at. of oxygen. The analyzes of oxidized graphene structures carried out using molecular mechanics prove that depending on the type of oxygen group present, the thermal energy of the systems as a function of changing the degree of oxidation:

(a) slightly increases in the case of the -COC- group (from E~436kcal/mol for 0% to 490kcal/mol for 10.2%);

(b) decreases, with the decrease from E~436kcal/mol at 0% up to -190kcal/mol for 10.2%, in the case of -COH group;

(c) clearly decreases from E~436kcal/mol for 0% to -715kcal/mol for 10.2% in the case of -COOH groups.

For this reason, oxidized graphene was selected for further analysis, on the edges of which there are only -COOH groups due to the highest thermodynamic stability among the analyzed oxygen groups.

The analysis of molecular electrostatic potential is a useful tool for assessing the distribution of charges at atomic level. In addition, it helps to predict the chemical reactivity of the compounds tested along with the assessment of the type of reaction: electrophilic or nucleophilic substitution. Color red on electrostatic distribution maps means electron-rich regions (negative). In contrast, color blue marks the electron-poor regions (positive). Green areas are neutral – the electrostatic potential is zero. Areas rich in electrons are prone to initiate chemical reactions with electrophilic groups. Regarding the electron-poor regions, these areas will prefer to initiate chemical reactions with nucleophilic groups.

Taking into account only the carboxyl groups in graphene, it can be seen that with an increase in the number of oxygen groups a characteristic disturbance of charge distribution on the surface of the GO flake is observed (Fig.1). In addition, areas with negative charge are clearly marked in the area of the -COOH groups. At the same time, the graphene oxidation level increased in the range of 0÷10,5% at. of oxygen causes the structure to lose its flatness. This character is more visible in the case of higher oxidation values. This is the effect of compensation of the electron clouds interaction between the -COOH groups and graphene.

When examining a fragment of murein, it can be seen that the distribution of charge is not affected by nitrogen groups. In the case of an isolated system, the analyzed spatial structure of murein is clearly polarized (Fig.2). However, in contact with graphene or graphene oxide, synergism of the interaction of both molecules, both murein and GO is observed. At the same time, the nature of polarization of the system is clearly visible in the case of ever higher values of oxidized graphene (Fig.1).

In addition, in the case of even slight oxidation of graphene, the polarization of its structure with a negative center located in the oxygen group is clearly visible. This fact may indicate a tendency to create chemical bonds with the amine groups of murein, which in turn will allow achieving high thermodynamic stability.

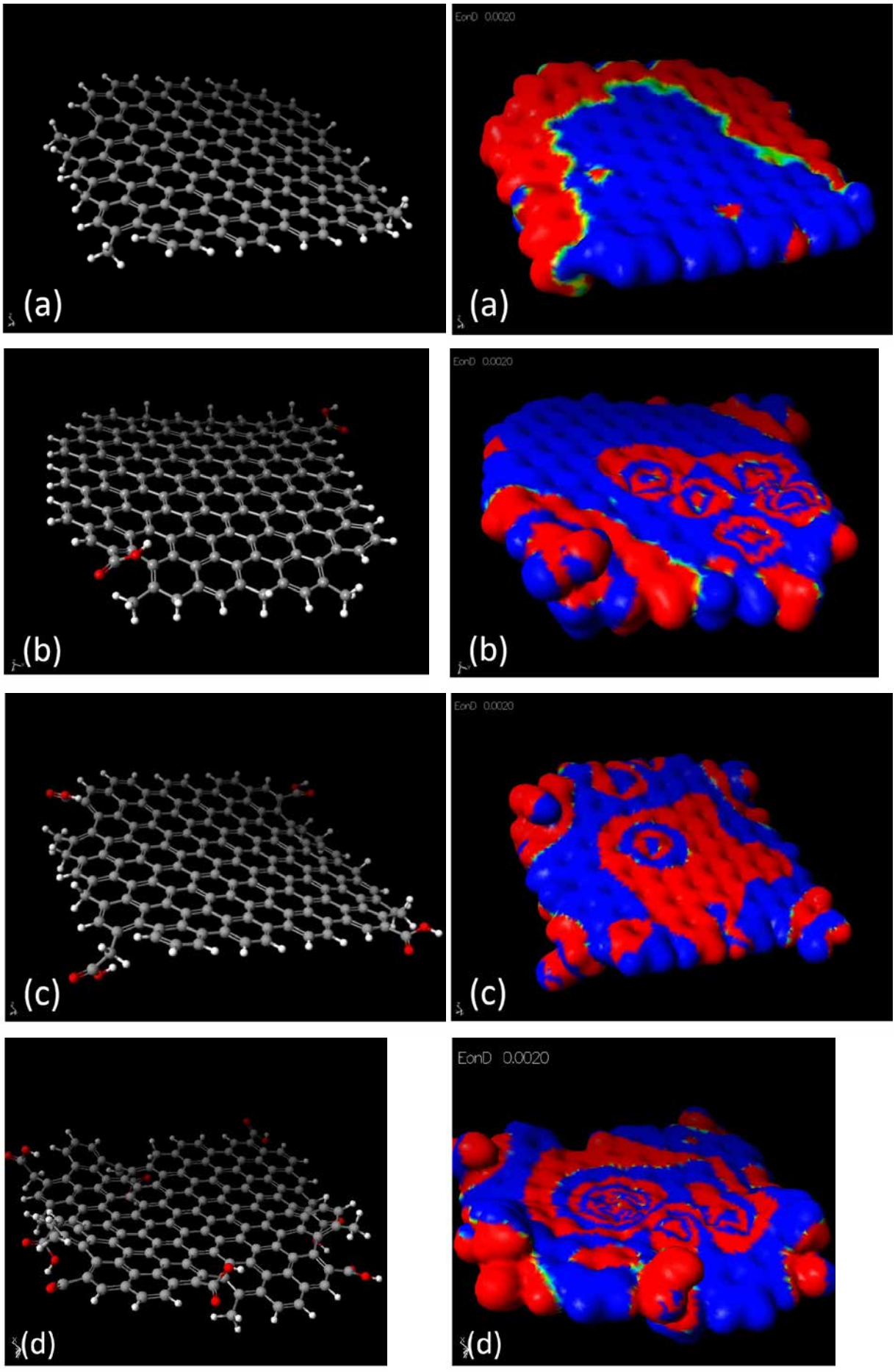

Figure 1. Molecular structure of flake graphene (a) and oxidized graphene at 2,6 % at. O (b); 5,2 % at. O (c) oraz 10,5 % at. O (d) with their respective charge distribution maps.

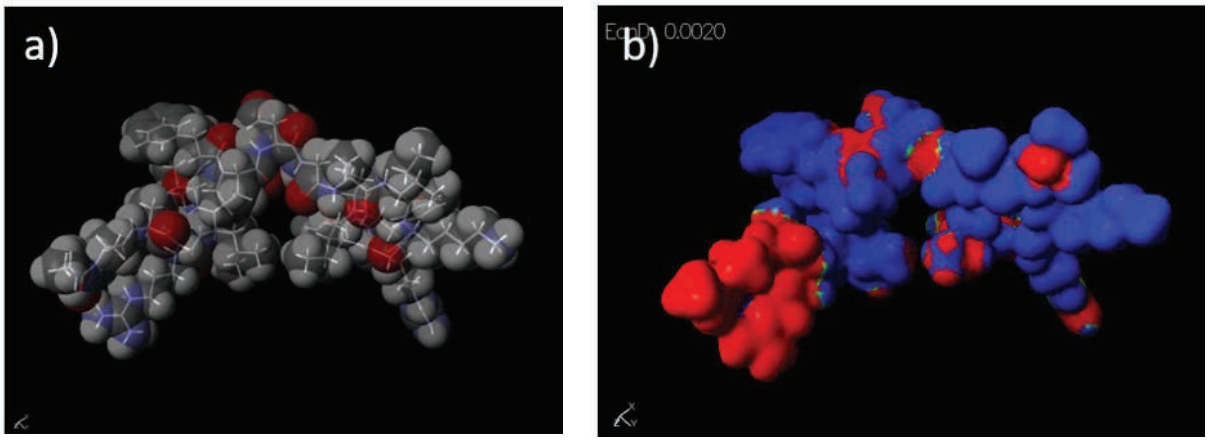

Figure 2. Molecular structure of the murein fragment (a) with the corresponding charge distribution map (b).

Analysis of thermal energy (Fig.3): graphene, oxidized graphene, fragment of murein and systems: graphene/murein, as well as oxidized graphene/murein shows that as the oxidation of graphene increases, its heat energy decreases. With an increase in the degree of oxidation of graphene (0÷10,5% at. O), thermal energy decreases from +435kcal/mol to almost -190kcal/mol. In addition, as the graphene oxidation increases, the heat energy of oxidized graphene/murein system decreases from -90kcal/mol for 0%at. to -500kcal/mol for 10.5%. It is worth noting that the thermal energy of the fragment of the analyzed murein molecule is close to -430kcal/mol. Therefore, it is clear that murein in contact with graphene in the oxidized state above 7% achieves lower thermal energy than murein or graphene alone.

This may indicate physicochemical stabilization of the system, which is the result of an increase in electrostatic interactions at the phase boundary of the analyzed molecular structures (Fig.5). Then, the interaction forces between the nitrogen-containing groups (derived from murein) and oxygen groups (derived from the oxidized form of graphene) increase. In addition, the increasing forces of electrostatic interaction at the murein/graphene boundary as a function of oxygen group content may also contribute to the formation of chemical bonds

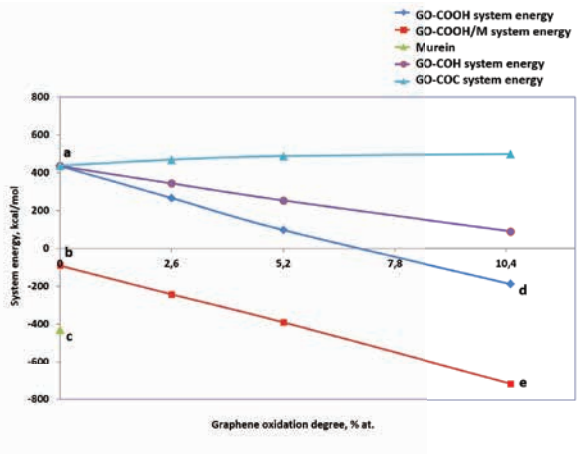

Figure 3. Change in the energy of the graphene/murein and oxidized graphene/murein systems as a function of the increase in the graphene oxidation degree 0-10,5% at. O. Exemplary corresponding structures has been presented in Fig. 4.

between -NH_2 in murein and -COOH in graphene as a result of which, the chemical bond -CONH- and low-molecular product in the form of H_2O are formed. As a result of this phenomenon, the bacterial cell will not be able to perform a cell division due to

Table 1. Summary of HOMO-LUMO and heat of formation values for the analyzed graphene with the oxidation degree in the range of 0÷10,5% at. O, murein and systems based on them (GO/murein).

Structure	HOMO [eV]	LUMO [eV]	HOMO-LUMO Energy gap [eV]	Heat of formation [kcal/mol]
Murein				-430,0
GO0% at. O	-6,30	-2,76	3,54	435,0
GO_{COOH}2,6% at. O	-6,48	-2,94	3,54	266,0
GO_{COOH}5,2% at. O	-6,56	-2,98	3,58	97,0
GO_{COOH}10,5% at. O	-7,10	-3,61	3,49	-189,0
GO0% at. O/M	-9,32	-5,93	3,39	-91,0
GO_{COOH}2,6% at. O/M	-9,70	-6,13	3,57	-243,0
GO_{COOH}5,2% at. O/M	-9,68	-6,08	3,6	-391,0
GO_{COOH}10,5% at. O/M	-10,11	-7,77	2,34	-715,0

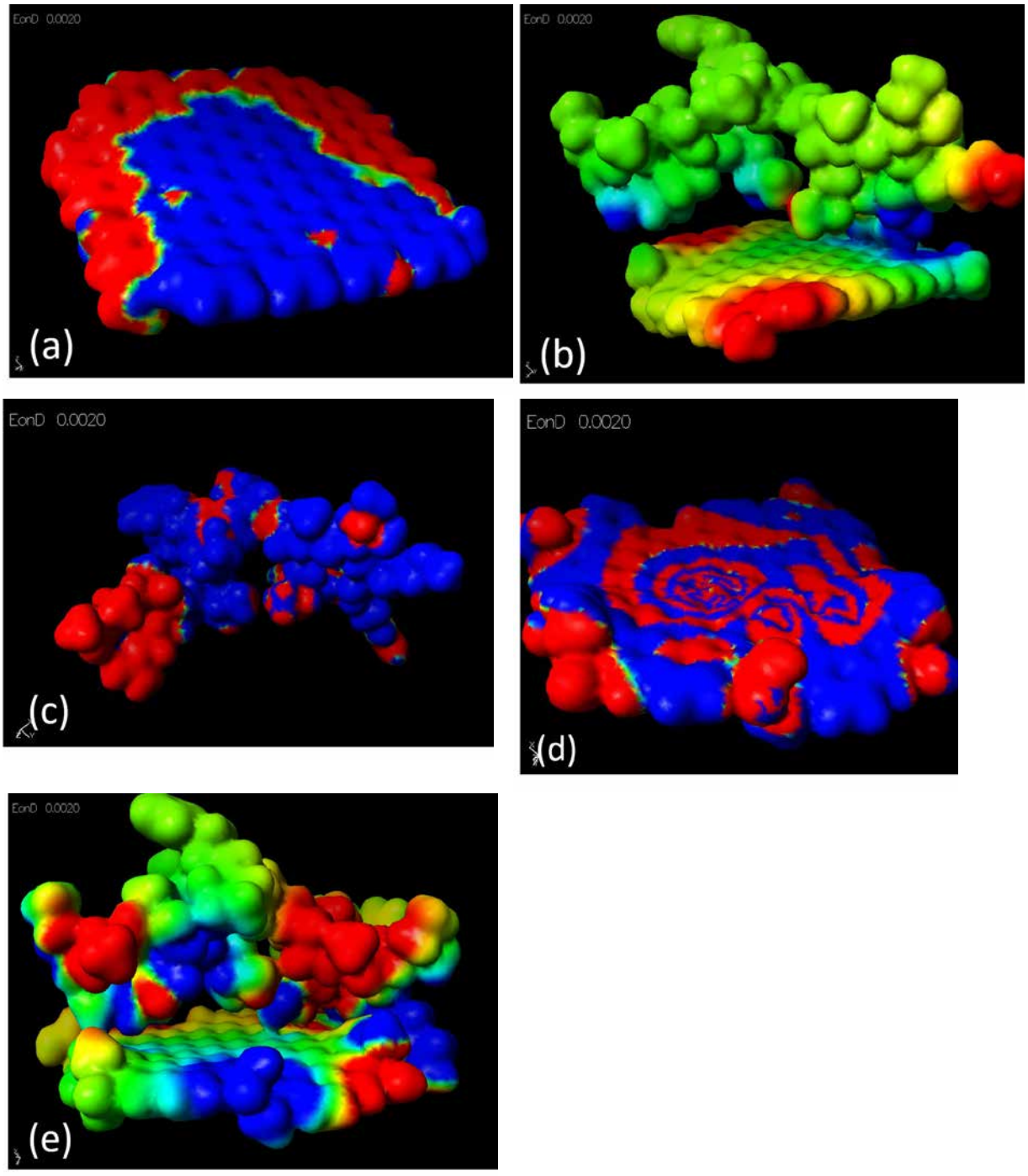

Figure 4. Exemplary structures as shown and marked in Fig. 3.

the phenomenon of "anchoring" its wall or fragments of murein on the surface of oxidized graphene.

In addition, the theory of limit orbitals was used, thanks to which the reactivity of the tested systems was determined. Analysis of the HOMO-LUMO and heat of formation values for the analyzed graphene proves that the increase in the oxidation state in the range of 0÷10,5% at. O does not change the value of the energy gap, which in this range is about 3.55 (Table 1.). A similar value of the energy gap is observed for GO/murein systems with one exception: for the oxidation state of 10.5%, the HOMO-LUMO difference value is -2.34. This may indicate high reactivity of the system. This fact clearly suggests that chemical reactions may occur and, as a consequence, new -CONH- groups may be formed, in which the driving force is a high degree of electrostatic interactions between the substrate. This fact clearly suggests the mechanism of bacterial cell death in contact with the oxidized form of graphene.

Conclusion

Based on the carried out analyzes, it is found that blocking the cellular division of bacteria occurs as a result of pseudo-crosslinking between the nitrogen groups of murein and oxygen groups present on the surface of graphene oxide (GO). The presence of strong interactions at the murein/GO boundary blocks bacterial growth and division. The presence of

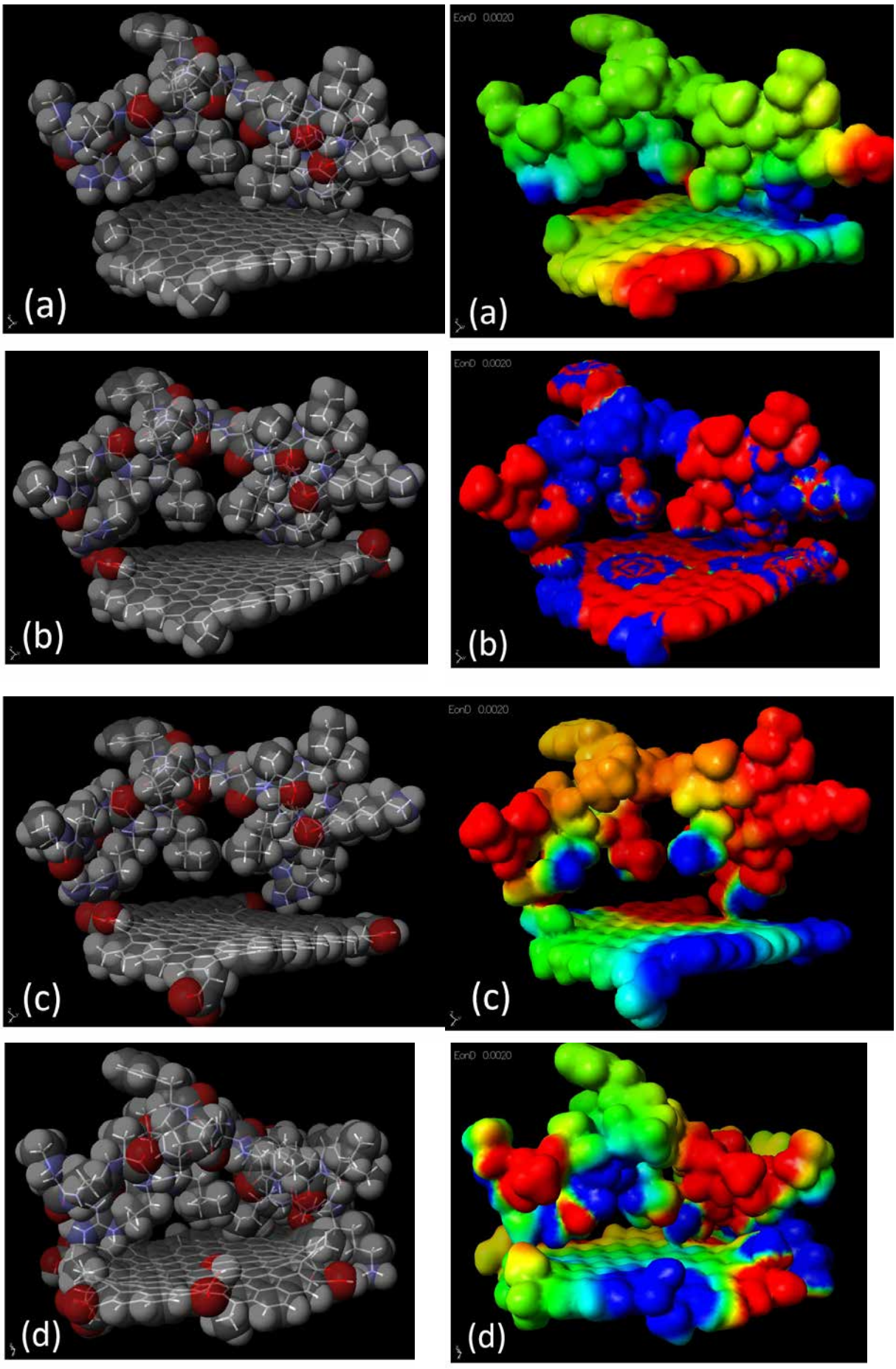

Figure 5.. Molecular interactions of murein at the phase boundary with (a) graphene and graphene oxide with oxidation degree of 2,6% (b); 5,6% (c) and 10,5% (d) with their respective charge distribution maps.

this phenomenon effectively inhibits the formation of so-called septum at the stage of cell division.

Bacterial death may be directly related to this. This is due to the fact that part of nitrogen bridges found in murein, giving the cell wall adequate mechanical strength, is involved in the formation of bonds with oxidized graphene. At the same time, the following are observed: (a) loosening of murein structure, (b) electrostatic and/or chemical "anchoring" of murein on the surface of oxidized graphene with (c) division processes occurring independently inside the bacteria. There may then be an increase in pressure inside the cell and, in the light of mechanical weakening of the wall, its rupture and death.

The conducted molecular analysis clearly point out how a verification of the identified mechanisms of bacteria death in contact with graphene oxide should be conducted. It is crucial to synthesize graphene flakes with varying share of oxygen groups and fixed carbon-to-oxygen ratio. For the extracted, specific flakes it will be possible to conduct proper surface observations regarding their interaction with bacterial cells. It should be noted that to properly verify the mechanisms, interferences between each graphene flakes should be eliminated. This will lead to proper analysis and confirmation of the presented mechanism of bacterial cell death in contact with graphene surface.

In summary, the latest research confirms the beneficial antibacterial effect of graphene, graphene oxide and reduced graphene oxide. The technological ease of modification of above-mentioned forms also allows its transfer to fibrous material. The potential of this type of hybrid systems can contribute to the production of a wide range of materials with unique, antibacterial properties for a wide range of applications, including clothing, medicine, water filtration technologies and functional materials, e.g. to neutralize environmental contamination. However, the problem may be the need to develop methods and techniques for effectively covering the surface of fibers. In this case, the adhesion of graphene-based finishes to a wide range of polymers (polar and non-polar) will determine the bactericidal performance of fibrous materials.

References

[1] *Q. Tarrés, E. Espinosa, J. Domínguez-Robles, A. Rodríguez, P. Mutjé, and M. Delgado-Aguilar, "The suitability of banana leaf residue as raw material for the production of high lignin content micro/nano fibers: From residue to value-added products," Ind. Crops Prod., vol. 99, pp. 27–33, May 2017.*

[2] *Z. Kassab et al., "Identifying Juncus plant as viable source for the production of micro- and nano-cellulose fibers: Application for PVA composite materials development," Ind. Crops Prod., vol. 144, p. 112035, Feb. 2020.*

[3] *D. Flores, J. Villarreal, J. Lopez, and M. Alcoutlabi, "Production of carbon fibers through Forcespinning® for use as anode materials in sodium ion batteries," Mater. Sci. Eng. B, vol. 236–237, pp. 70–75, Oct. 2018.*

[4] *S. Jang, S. G. Bae, D.-G. Shin, K. Cho, Y. Lee, and Y. Lee, "Characteristics of polycarbosilanes produced under different synthetic conditions and their influence on SiC fibers: Part I," Ceram. Int., Nov. 2019.*

[5] *Y. Zhang et al., "Functional & enhanced graphene/polyamide 6 composite fiber constructed by a facile and universal method," Compos. Part A Appl. Sci. Manuf., vol. 123, pp. 149–157, Aug. 2019.*

[6] *T. Pulingam et al., "Graphene oxide exhibits differential mechanistic action towards Gram-positive and Gram-negative bacteria," Colloids Surfaces B Biointerfaces, vol. 181, pp. 6–15, Sep. 2019.*

[7] *L. Mei et al., "Amino-Functionalized Graphene Oxide for the Capture and Photothermal Inhibition of Bacteria," ACS Appl. Nano Mater., vol. 2, no. 5, pp. 2902–2908, May 2019.*

[8] *J. A. Tamayo Marín et al., "Biocompatible and Antimicrobial Electrospun Membranes Based on Nanocomposites of Chitosan/Poly (Vinyl Alcohol)/Graphene Oxide," Int. J. Mol. Sci., vol. 20, no. 12, p. 2987, Jun. 2019.*

[9] *F. Schedin et al., "Detection of individual gas molecules adsorbed on graphene," Nat. Mater., vol. 6, p. 652, Jul. 2007.*

[10] *F. Bonaccorso et al., "2D materials. Graphene, related two-dimensional crystals, and hybrid systems for energy conversion and storage.," Science (80-.)., vol. 347, p. 6217, 2015.*

[11] *Y. Hou, S. Lv, L. Liu, and X. Liu, "High-quality preparation of graphene oxide via the Hummers' method: Understanding the roles of the intercalator, oxidant, and graphite particle size," Ceram. Int., vol. 46, no. 2, pp. 2392–2402, Feb. 2020.*

[12] *N. I. Zaaba, K. L. Foo, U. Hashim, S. J. Tan, W.-W. Liu, and C. H. Voon, "Synthesis of Graphene Oxide using Modified Hummers Method: Solvent Influence," Procedia Eng., vol. 184, pp. 469–477, 2017.*

[13] *N. Yadav and B. Lochab, "A comparative study of graphene oxide: Hummers, intermediate and improved method," FlatChem, vol. 13, pp. 40–49, Jan. 2019.*

[14] *M. H. A. Kudus, M. R. Zakaria, H. M. Akil, F. Ullah, and F. Javed, "Oxidation of graphene via a simplified Hummers' method for graphene-diamine colloid production," J. King Saud Univ. - Sci., vol. 32, no. 1, pp. 910–913, Jan. 2020.*

[15] *A. Ouerghi et al., "Large-Area and High-Quality Epitaxial Graphene on Off-Axis SiC Wafers," ACS Nano, vol. 6, no. 7, pp. 6075–6082, Jul. 2012.*

[16] *P. Kula et al., "Functionality of graphene as a result of its heterogenic growth on SiC nanoparticles on the basis of reversible hydrogen storage," Int. J. Hydrogen Energy, vol. 39, no. 34, pp. 19662–19671, 2014.*

[17] *Z. Yang et al., "A new direct growth method of graphene on Si-face of 6H-SiC by synergy of the inner and external carbon sources," Appl. Surf. Sci., vol. 436, pp. 511–518, Apr. 2018.*

[18] *P. Trinsoutrot, H. Vergnes, and B. Caussat, "Three dimensional graphene synthesis on nickel foam by chemical vapor deposition from ethylene," Mater. Sci. Eng. B Solid-State Mater. Adv. Technol., vol. 179, no. 1, pp. 12–16, 2014.*

[19] *M. S. Yazici, M. A. Azder, and O. Salihoglu, "CVD grown graphene as catalyst for acid electrolytes," Int. J. Hydrogen Energy, vol. 43, no. 23, pp. 10710–10716, 2018.*

[20] M. P. Lavin-Lopez, J. L. Valverde, S. Ordoñez-Lozoya, A. Paton-Carrero, and A. Romero, "Role of inert gas in the Cvd-graphene synthesis over polycrystalline nickel foils," Mater. Chem. Phys., vol. 222, no. September 2018, pp. 173–180, 2019.

[21] P. Zeller et al., "Detachment of CVD-grown graphene from single crystalline Ni films by a pure gas phase reaction," Surf. Sci., vol. 653, pp. 143–152, Nov. 2016.

[22] T. Kinoshita, S. Maruyama, and Y. Matsumoto, "Ionic liquid wettability of CVD-grown graphene on Cu/α-Al2O3(0 0 0 1) characterized by in situ contact angle measurement in a vacuum," Chem. Phys. Lett., vol. 735, p. 136781, Nov. 2019.

[23] P. Kula et al., "High strength metallurgical graphene for hydrogen storage nanocomposites," Vacuum, vol. 129, pp. 79–85, 2016.

[24] C. Liu, Y. Tang, P. Huo, and F. Chen, "Novel AgCl/CNTs/g-C3N4 nanocomposite with high photocatalytic and antibacterial activity," Mater. Lett., vol. 257, p. 126708, Dec. 2019.

[25] S. Liu et al., "Antibacterial Activity of Graphite, Graphite Oxide, Graphene Oxide, and Reduced Graphene Oxide: Membrane and Oxidative Stress," ACS Nano, vol. 5, no. 9, pp. 6971–6980, Sep. 2011.

[26] H. Fallatah, M. Elhaneid, H. Ali-Boucetta, T. W. Overton, H. El Kadri, and K. Gkatzionis, "Antibacterial effect of graphene oxide (GO) nano-particles against Pseudomonas putida biofilm of variable age," Environ. Sci. Pollut. Res., vol. 26, no. 24, pp. 25057–25070, Aug. 2019.

[27] N. Yadav et al., "Graphene Oxide-Coated Surface: Inhibition of Bacterial Biofilm Formation due to Specific Surface–Interface Interactions," ACS Omega, vol. 2, no. 7, pp. 3070–3082, Jul. 2017.

[28] L. Elias, R. Taengua, B. Frígols, B. Salesa, and Á. Serrano-Aroca, "Carbon Nanomaterials and LED Irradiation as Antibacterial Strategies against Gram-Positive Multidrug-Resistant Pathogens," Int. J. Mol. Sci., vol. 20, no. 14, p. 3603, Jul. 2019.

[29] F. Li et al., "Natural Biodegradable Poly(3-hydroxybutyrate-co -3-hydroxyvalerate) Nanocomposites with Multifunctional Cellulose Nanocrystals/Graphene Oxide Hybrids for High-Performance Food Packaging," J. Agric. Food Chem., vol. 67, no. 39, pp. 10954–10967, Oct. 2019.

[30] S. Khorrami, Z. Abdollahi, G. Eshaghi, A. Khosravi, E. Bidram, and A. Zarrabi, "An Improved Method for Fabrication of Ag-GO Nanocomposite with Controlled Anti-Cancer and Anti-bacterial Behavior; A Comparative Study," Sci. Rep., vol. 9, no. 1, p. 9167, Dec. 2019.

[31] Y. Yang et al., "Synergistic Photocatalytic-Photothermal Contribution to Antibacterial Activity in BiOI-Graphene Oxide Nanocomposites," ACS Appl. Bio Mater., vol. 1, no. 6, pp. 2141–2152, Dec. 2018.

[32] A. Sharma et al., "Structural, electronic structure and antibacterial properties of graphene-oxide nano-sheets," Chem. Phys. Lett., vol. 698, pp. 85–92, Apr. 2018.

[33] A. Filina, N. Yousefi, M. Okshevsky, and N. Tufenkji, "Antimicrobial Hierarchically Porous Graphene Oxide Sponges for Water Treatment," ACS Appl. Bio Mater., vol. 2, no. 4, pp. 1578–1590, Apr. 2019.

[34] M. Li et al., "Graphene Oxide and Lysozyme Ultrathin Films with Strong Antibacterial and Enhanced Osteogenesis," Langmuir, p. acs.langmuir.9b00035, May 2019.

[35] J. Jira et al., "Inhibition of E. coli Growth by Nanodiamond and Graphene Oxide Enhanced by Luria-Bertani Medium," Nanomaterials, vol. 8, no. 3, p. 140, Mar. 2018.

[36] Y. Liang et al., "Facile synthesis of ZnO QDs@GO-CS hydrogel for synergetic antibacterial applications and enhanced wound healing," Chem. Eng. J., vol. 378, p. 122043, Dec. 2019.

[37] F. Yaghoubidoust and E. Salimi, "A Simple Method for the Preparation of Antibacterial Cotton Fabrics by Coating Graphene Oxide Nanosheets," Fibers Polym., vol. 20, no. 6, pp. 1155–1160, Jun. 2019.

[38] L. Jia, X. Huang, H. Liang, and Q. Tao, "Enhanced hydrophilic and antibacterial efficiencies by the synergetic effect TiO2 nanofiber and graphene oxide in cellulose acetate nanofibers," Int. J. Biol. Macromol., vol. 132, pp. 1039–1043, Jul. 2019.

[39] J. Hu, J. Liu, L. Gan, and M. Long, "Surface-Modified Graphene Oxide-Based Cotton Fabric by Ion Implantation for Enhancing Antibacterial Activity," ACS Sustain. Chem. Eng., vol. 7, no. 8, pp. 7686–7692, Apr. 2019.

[40] S. Sun et al., "A bifunctional melamine sponge decorated with silver-reduced graphene oxide nanocomposite for oil-water separation and antibacterial applications," Appl. Surf. Sci., vol. 473, pp. 1049–1061, Apr. 2019.

[41] E. Aliyev et al., " Structural Characterization of Graphene Oxide: Surface Functional Groups and Fractionated Oxidative Debris," Nanomaterials, vol. 9 Aug. 2019.