The concept of the proposed project is to find the modification of graphene oxide (GO) or its reduced form (RGO), which provides the generation of the strong cytotoxic compounds only for cancer cells.

The project will be consisted of two basic steps. The first one will be synthesis and physicochemical characterization of graphene oxide (GO), its reduced form (RGO) and their modified derivatives. Graphene oxide used in the project will be obtained by oxidizing different graphite powders with Staudenmaier's method, Hummers' method, method proposed by prof. Tour's group and own methods based on low-temperature plasma or ozone treatment of the graphite powder. The reduced graphene oxide will be prepared by an intense reduction of graphene oxide (GO) with strong reducing agents. Modifications of graphene oxide and its reduced form will be consisted of covalent or non-covalent linking of different chemical compounds. These compounds will be selected by the least toxicity to healthy cells of the human body.

The second stage will be study about cytotoxicity of the obtained materials. This phase will be conducted on the two types of cultures: two-dimensional (2D) and three dimensional (3D) for four cell lines: HepG2 (liver cancer cells), HT-29 (colon cancer cells) and the corresponding normal cell lines. **2D culture** will be carried out on standard culture plates (culture as a monolayer). This stage of research will be to determination of the most toxic synthesized form of graphene, its concentration and time of incubation, which while not adversely affected on healthy cells from the same organ of the body. **3D culture** will be conducted in the form of cells spheroids, which are the most common type of the spatial culture, which narrow the barrier that exists between the *in vitro* (laboratory conditions) and *in vivo* (natural conditions) conditions. It has been demonstrated that cells growing in the form of spheroids are more resistant to the tested substances than monolayer culture. This stage of the research will be carried out in a pre-designed *Lab-on-a-chip* microsystem. These microsystem through the material which are made of (poly(dimethylsiloxane), PDMS) and due to the special design, enables to carry out in it a long-term spheroid culture. It also allow to observation of changes in a single spheroid. First, spheroid composed of the tested cell lines will be subjected to the impact of graphene oxide, its reduced form, and their modified forms with the parameters set out in the first stage of research. In the case of absence of the cytotoxic effect, concentration and time of incubation will be changed. Innovation in planned research will be further determination of action of graphene derivatives during long-term spheroids culture

Analysis of the effect of the synthesized form of graphene on cancer and healthy *in vitro* culture in both the monolayer and spheroids culture will be based on microscopic observation (morphological changes of cells). Furthermore, cytotoxicity of samples will be determined using commercially available tests and flow cytometry.

For analysis of the effect of the synthesized forms of graphene on cancer and healthy *in vitro* in both the monolayer and spheroids culture is planned to use a variety of microscopic techniques combined with the methods of staining cells, particularly confocal microscopy (changes in cells morphology). Furthermore, cytotoxicity and mechanism of graphene action will be determined using commercially available tests and flow cytometry.

The team takes this subject of research, because graphene oxide and its reduced form have got interesting biological properties. The idea of using these forms of graphene in anticancer therapy is associated with their low toxicity (carbon construction), local action, little ability to move, good thermal conductivity and the possibility of inducing apoptosis - the natural cell death. Moreover, easy functionalization of these forms, expanding the range of their applications in anticancer research. In view of the increasing number of cancer cases, these materials can bring new opportunities for the diagnosis and therapy of such diseases.