ACTIVITY REPORT

NEW NANOCOMPOSITES BASED ON BIOCOMPATIBLE POLYMERS AND GRAPHENE FOR DENTAL APPLICATIONS - BIOGRAF (230/2014)

Project Coordinator

Dr. STELA MARIA PRUNEANU

Work package 1 (2014) Preliminary studies on the elaboration and implementation of new nanocomposites used in dental restorations.

A.1.1 Preliminary research on the preparation of graphene nanofillers (CO-INCDTIM)

A.1.2 Testing the reaction conditions for graphene preparation- Part I (CO-INCDTIM)

According to the literatura, graphene flakes can be prepared by various methods, including: (i) catalytic Chemical Vapor Deposition; (ii) chemical route. Both methods were tested, as following described.

(i) catalytic Chemical Vapor Deposition with induction heating (CCVD-IH)

Graphene flakes were prepared by catalytic Chemical Vapor Deposition with Induction Heating, using MgO as catalyst. Briefly, the catalyst was put into the reaction chamber and the temperature was slowly increased to 1000° C. Methane gas was used as carbon source. After about 30 minutes, the synthesis was stopped and the reaction product was purified in HCl. Finally, the reaction product was washed with distilled water and dried for 24 h. The

morphological and structural characterization of graphene flakes was performed by TEM and Raman Spectroscopy.

(ii) *chemical route*

Graphene oxide (GO) was prepared in two steps. In the first step, the pre-oxidisation of graphite was performed in a warm solution of concentrated sulphuric acid, potassium persulphate and phosphorus pentoxide. The resultant mixture was heated to 100^{o}C and then left to slowly cool. After several hours, the mixture was carefully diluted with water, filtrated and washed until the pH water was neutral. The solid was dried at room temperature overnight. In the second step, a suspension of the pre-oxidized graphite powder mixed with sodium nitrite in sulphuric acid was cooled in an ice-water bath. Potassium permanganate was slowly added to the mixture so the temperature would not reach 20^{o}C. After more than 10 h, it was slowly warmed to 35-37^{o}C, for 2 hours. The reaction was stopped by adding hydrogen peroxide, until no more gas development was observed. The GO product was then suspended in distilled water and dialyzed for 5 days. The solid GO was obtained by liophilization.

Chemically reduced graphene (rGO) was obtained by reducing GO with a reducing agent.

N-doped graphene (N-rGO) was prepared by dispersing GO powder in water for several minutes (by ultrasound). Next, urea was added and the resulted homogeneous solution was stirred at high temperature (160-170^{o} C), for few hours. The resulting black suspension was filtered and washed with water and then thermally treated under argon at around 600^{o} C.

The morphological and structural characterization of GO, rGO and N-rGO were investigated by TEM, FTIR Spectroscopy and X-Ray powder Diffraction.

A.1.3. Preliminary studies on the synthesis of the bioactive nanofillers and of dimethacrylate polymers, used in the preparation of nanocomposites (P1-UBB)

Various types of nanofillers were synthesized: hydroxylapatite (HA), hydroxylapatite with graphene and gold nanoparticles (HA-Gr-Au), hydroxylapatite with graphene and silver nanoparticles (HA-Gr-Ag) and hydroxylapatite with zirconium (HA-Zr). The nanofillers were characterized by TEM, X-Ray powder Diffraction and Thermogravimetric Analysis. Besides nanofillers, the composites also contained polymers with high viscosity and high molecular mass.

A.1.4. Experimentation of lab technology for the preparation of new nanocomposites, used in dental restorations- Part I (P1-UBB)

Preliminary experiments regarding the influence of the nanofiller (graphene) on the physico-chemical and mechanical properties of the composites were performed. The experiments proved that the samples with graphene had a higher Young modulus (around 15 GPa), higher hardness and higher bending resistance, compared with the samples without graphene. In addition, the surface homogeneity of the sample prepared with the optimum composition was investigated by Atomic Force Microscopy.

A.1.8. Experimental model -Part I (P3-Apel Laser SRL)

The influence of laser radiation upon the polymerization process of dimethacrylic monomers was studied. Some important parameters for the polymerization reaction were determined, such as the optimum concentration of the reactants (monomer, graphene, bioactive nanofiller), the polymerization time as well as the esthetical characteristics of the final nanocomposite material. The polymerization reaction took place under irradiation with 400-480 nm wavelengths, for short periods of time (around 60 sec).

A.1.5 Development of in vitro protocols to study cell viability: cytotoxycity and cell apoptosis studies with graphene (P2-UMF)

A.1.6. In vitro biocompatibility studies of graphene samples –Part I (P2-UMF)

The cytotoxicity and biocompatibility of graphene samples, prepared by catalytic Chemical Vapor Deposition with Induction Heating or by chemical route, were investigated. Cell viability tests and toxicity tests were performed according to ISO10993-5 and ISO10993-6/2009. Since the toxicity of graphene nanostructures is not well known, the tests were performed using various concentrations of graphene. New protocols, for *in vitro* testing of graphene were developed. All the biological and cytotoxicity tests were performed in triplicate, using different exposures and time landmarks, to allow a better understanding of the mechanisms. The experimental approaches and the results were disseminated among the PhD and PostDoc students.

A.1.7. Technical and economical study for marketing the new materials (P3-Apel Laser SRL)

Partner P3 has tested the market, regarding the applicability of the composites with graphene, in *dental restorations*. The market study was encouraging and demonstrated the need to develop novel materials with better mechanical and anti-bacterial properties.

A.1.9 Disemination of the research results to PhD and PostDoc (P1-UBB)

The PhD students and PostDoc were actively involved in most of the research activities.

General Conclusions

All the activities foreseen within this work-package were accomplished:

- *D1-Synthesis and characterization of bioactive nanofiller, graphene and monomers (FM-12/2014);*
- *D2 – In vitro testing of graphene (FM -12/2014);*
- *D3 – Technical and economical study for marketing the new composites (FM-12/2014)*
- *3 ISI papers were submitted for publication*
- *4 papers were presented at International Conference.*

ISI Papers:

1. Sava Sorina, MOLDOVAN Marioara, Sarosi Codruta, Mesaros Anca, Dudea Diana, Alb Camelia Ioana, **Effects of graphene addition on the mechanical properties of composites for dental restoration**, submitted to Materiale Plastice **(P1, P2)**
2. Sarosi Codruta, Biris Alexandru Radu, Sava Sorina, Antoniac Aurora, Moldovan Marioara, **The nanofiller effect on properties of experimental graphene dental nanocomposites**, submitted to JOURNAL OF ADHESION SCIENCE AND TECHNOLOGY **(P1, CO)**
3. Florina Pogacean, Crina Socaci, Stela Pruneanu, Maria Coros, Alexandru R. Biris, Lidia Magerusan, Gabriel Katona, Rodica Turcu, George Borodi, **Graphene based nanomaterials as chemical sensors for hydrogen peroxide - a comparison study of their intrinsic peroxidase catalytic behavior**, submitted to Sensors and Actuators B: Chemical **(CO)**

Papers presented at International Conference:

1. Sorina Sava, Marioara Moldovan, Codruta Sarosi, Anca Mesaros, Camelia Alb, Diana Dudea, **"Effects of graphene addition on the mechanical properties of composites for dental restoration"**, 6th International Conference "Biomaterials, Tissue Engineering & Medical Devices" BiomMedD' 2014 – Constanta, Romania- poster **(P1, P2)**
2. Codruta Sarosi, Stela Pruneanu, Viorica Simon, Doina Prodan, Stanca Boboia, Camelia Alb, Sorina Sava, Aurora Antoniac, Marioara Moldovan **"The nanofiller effect on properties of experimental graphene dental nanocomposites"**, 6th International Conference "Biomaterials,

Tissue Engineering & Medical Devices" BiomMedD' 2014- Constanta, Romania- oral presentation **(P1, P2, CO)**

3. Alb S, Dudea D, Mesaros A, Grecu A, Manole M, Alb C, **Use of Modern Communication Methods by Central European Dentists**, J. Dent Res 93 (Spec Iss C), 2014 (www.dentalresearch.org), IADR/PER Congress 2014, 10-13 September 2014, Dubrovnik, Croatia- poster presentation **(P2)**
4. Alb C, Mesaros A, Dudea D, Buiga P, Gasparik C, Alb S, **Analysis of Maxilary anterior teeth in a young population**, J. Dent Res 93 (Spec Iss C), 2014 (www.dentalresearch.org), IADR/PER Congress 2014, 10-13 September 2014, Dubrovnik, Croatia- poster presentation **(P2)**