Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/acme

Original Research Article

Graphene based porous coatings with antibacterial and antithrombogenous function—Materials and design

R. Major [a,*], M. Sanak [b], A. Mzyk [a], L. Lipinska [c], M. Kot [d], P. Lacki [e], F. Bruckert [f], B. Major [a]

[a] Institute of Metallurgy and Materials Science Polish Academy of Sciences, 25 Reymonta Street, 30-059 Cracow, Poland

[b] Department of Medicine, Jagiellonian University Medical College, 8 Skawinska Street, 31-066 Cracow, Poland

[c] Institute of Electronic Materials Technology, 133 Wolczynska Street, 01-919 Warsaw, Poland

[d] AGH University of Science and Technology, Faculty of Mechanical Engineering and Robotics, Al. Mickiewicza 30, 30-059 Cracow, Poland

[e] Czestochowa University of Technology, Faculty of Civil Engineering, 69 Dabrowskiego Street, 42-201 Czestochowa, Poland

[f] Laboratoire des Matériaux et du Génie Physique Grenoble Institute of Technology – Minatec 3, Parvis Louis Néel, BP 257 38016 Grenoble Cedex 1, France

ARTICLE INFO

Article history:
Received 10 July 2013
Accepted 26 April 2014
Available online 2 June 2014

Keywords:
Graphene
Porous coatings
Blood
Surface modification
Confocal microscopy

ABSTRACT

The studies considered graphene-based biomaterials dedicated for cardiovascular therapy. Reduced graphene oxide flakes were introduced into the porous structure of the polyelectrolyte based coatings. TEM analysis showed the presence of graphene flakes arranged parallel to the substrate surface, firmly connected to the porous coating. Biomaterials were subjected to a comprehensive diagnosis of the biological and material properties. The material behavior was simulated using finite element method. The coatings were deposited using layer by layer method. Mechanical analysis was done using Berkovich indenter geometry.

They confirmed theoretical FEA based calculations, it was observed the coating stiffness incensement under the influence of introduced particles of graphene.

The endanger of the bacteriology film formation was verified based on the E-coli and Staphylo coccus bacteria. Blood–material interaction was examined in the dynamic flow conditions. Bacteriological analysis showed reduced presence of bacteria after contact with the surface with introduced graphene flakes. Dynamic analyzes on blood showed high activation of the coagulation, strong platelets consumption and a strong immune response. It is caused by sharp edge of the single plane of the graphene flake.

* *Corresponding author*. Tel.: +48 606206255.
E-mail address: r.major@imim.pl (R. Major).

http://dx.doi.org/10.1016/j.acme.2014.04.012

1. Introduction

The main topic in novel biomaterial science is the replacement of injured or diseased tissue by artificial materials. Their properties must be designed to have the minimized influence to the human organism. The biological environment is harsh and can lead to rapid or gradual breakdown of many materials. No polymer is totally impervious to the chemical processes and the mechanical action of the body. The research activity in the frame of the work was focused on: function, durability, biocompatibility and application. In order to the function, the implant has to reveal appropriate properties such as mechanical strength and permeability.

For blood contact, the biocompatibility requirements are on the highest level of all biomaterial applications, which is due to the continuous blood flow and the high reactivity of blood molecules and cells. Blood contacting materials could initiate several life-endangering processes, such as the formation of thrombo-emboli, even in the presence of anti-clotting agents. Thus, the goal of the work is to significantly reduce the likelihood of thrombo-emboli formation.

Recent advancement in nanoscience opens a wide range of applications of nanoparticles toward industrial level as well as in medicine [1]. Nanoparticles exhibit extraordinary physiochemical properties and reflect their unique electrical, thermal, and mechanical properties which further bring along a concern about their toxic behavior when exposed to the environment and living system. In the recent years government and scientific communities put forward more attention toward the bio-safety aspect of the nanomaterials. The main agenda in the field of nanotoxicology is to understand the exposure and assessment of nanoparticles, environmental and biological fate, transport, persistence, transformation, recyclability and overall stability of the nanoparticles. Graphene, as a two-dimensional lattice of single-layered graphite, has recently attracted increasing attentions, due to its unique properties which promise fabrication of exciting nanotechnology-based devices in a broad range of potential applications [2]. Graphene-based materials offer highly promising progress in biological and medical areas such as, antiviral [3], bactericidal [4–8] and nematocidal [9] agents, disease diagnosis [10], biosensing [11,12], cancer targeting [13–15] and photothermal therapy [16–18], drug delivery [19–21], and tissue engineering [22–24].

2. Experimental procedures

2.1. Material numerical design

The finite-element method has gained a growing popularity among the numerical techniques in engineering [25]. This is firstly because the engineering design of modern products requires an engineer to predict accurately the performance and produce the optimal object, which requires an integrated use of the finite-element analysis software in CAD. Secondly, fast progress in hardware performance and a great decrease in the price of computers offer the possibility of using finite-element analysis software. Another important factor is that the analysis functions of the finite-elements program themselves develop rapidly, offering a user-friendly interface and CAD software transor [26]. In recent years, nanoidentation techniques have been used to determine the hardness and Young's modulus of thin films [27]. Indentation hardness measurements are now extensively used for characterization and ranking of coated systems for mechanical applications because they are simple, cheap and reproducible [28]. However, the presence of the underlying substrate – including the interface – of material, may complicate the interpretation of measurement results.

2.2. Preparation of the surfaces

2.2.1. Porous coatings

Porous coatings were deposited using the ''layer by layer'' method using oppositely charged polyelectrolytes [29,30]. A thin, inorganic coating provided the optimal surface charge to begin polyelectrolyte deposition, and surface pre-treatment was accomplished using physical vapor surface nanofabrication to introduce the optimal surface charge. The final scaffold was prepared with the ''layer by layer'' method, allowing the construction of porous coatings.

The biomimetic surface structure was prepared using the construction of a porous coating, consisting of 12 bi-layers of polyelectrolyte poly-L-lysine and hyaluronic acid (PLL/HA), stabilized with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysulphosuccinimide (NHS) [23]. An EDC/sulpho-NHS mixture (EDC at 260 mM and NHS at 100 mM were mixed, v/v) was freshly prepared in a 0.15 M NaCl solution at pH 5.5. The coupling chemistry is based on the reaction of activated carboxylic sites with primary amine groups. The film-coated substrate was put in contact with the mixed EDC/NHS solution for 12 h. The final coating consisted of PLL. The above described layer assembly was designer for the reference coating, without reduced graphene oxide introduced into the structure. The samples were modified with reduced graphene oxide flakes. Reduced graphene oxide flakes were mixed with a polyelectrolyte of the same charge to provide adequate, proper mixing. The reduced graphene oxide flakes were associated in the set up in accordance with the information provided in Table 1.

The new material needed to be biocompatible and biologically stable. Reduced graphene oxide flakes were mixed with

Table 1 – Reduced graphene oxide assembly with polyelectrolyte thin films.

Serial no	Reduced graphene oxide assembly with polyelectrolytes	Reduced graphene oxide concentration, %
1	Reference: 12xPLL/HA cross link + PLL	0
2	12xPLL/(HA + rGO) cross link + PLL	1 in HA
3	12x PLL/(HA + rGO) cross link + PLL	10 in HA
4	12x(PLL/HA) + PLL + rGO + 12x(PLL/HA) cross link + PLL	100 in the single layer