Graphene: The key for pHEMA application as blood contacting devices

Andreia T Pereira[1,2]
Patrícia C. Henriques[1,2,3], K. Schneider[4], Ana L. Pires[5], André Pereira[5], Maria Cristina L. Martins[1,2], Fernão D. Magalhães[3], H. Bergmeister[4], Inês C. Gonçalves[1,2]
[1]i3S - Instituto de Inovação e Investigação em Saúde, Universidade do Porto, Portugal,
[2]INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Portugal,
[3]LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Portugal,
[4]Center for Biomedical Research Medical University of Vienna, Vienna, Austria;
[5]IFIMUP – Instituto de Fisica de Materiais Avançados, Nanotecnologias e Fotonics, Universidade do Porto, Portugal;
Andreia.pereira@i3s.up.pt

Thrombosis and infection are the leading failure causes of blood-contacting devices (BDC), mainly due to poor performance of existing biomaterials[1]. Poly(2-hydroxyethyl methacrylate) (pHEMA) has excellent hemocompatibility, but the weak mechanical properties impair its use as bulk material, being solely explored as a coating. However, instability and difficulty of attachment to the underlying polymer compromise the coating success. This work unravels the use of nanomaterials, namely graphene-based materials (GBM), to reinforce pHEMA, creating composite hydrogels that meet the biological and mechanical requirements to stand-alone as BCD.
GBMs with different exfoliation, oxidation degree and lateral size were used, namely graphene nanoplatelets (GNP M5 and M15), oxidized GNP (GNP M5ox and M15ox) and graphene oxide (GO). GBM were either purchased or produced by modified Hummers' method and characterized by TEM and XPS. pHEMA/GBM composites were produced by *in situ* polymerization of 2-hydroxyethyl methacrylate and tetraethylene glycol dimethacrylate in the presence of the different GBM[1]. Resulting composites were evaluated regarding GBM dispersion and surface topography (SEM), wettability (contact angle), swelling capacity (gravimetry) and mechanical properties (tensile tests). Biocompatibility of pHEMA/GBM was evaluated by assessing the medium extracts cytotoxicity towards HUVECs cells. Non-fouling properties of pHEMA/GBMs were evaluated by assessing their capacity to adhere human platelets (SEM), HUVECs cells (fluorescence microscopy) and bacteria (high-throughput microscopy). A prototype conduit of pHEMA/GO (ID: 4 mm; OD: 6mm) was developed to evaluate its blood compatibility *in vivo* using non-heparinized pigs as animal model. Conduits were connected to pig carotid arteries (A-V shunt), being in contact with circulating blood for 30 min, and materials surface lumen evaluated by SEM.
The lateral size of GBMs ranges from 1.5 to 15 µm, being GO the smallest material. Oxidized forms of GBMs show similar oxidation degree (34%O) and non-oxidized GBMs have low oxygen content (3%O). GO has the highest exfoliation degree. Incorporation of GBMs in pHEMA increases its surface roughness but keeps its surface wettability (contact angle of 25º) and swelling capacity (50%). Among the tested GBMs, GO was the most efficient filler, increasing pHEMA's stiffness and tensile resistance, reaching 2 MPa and 0.7 MPa, respectively. These values empower the application of pHEMA/GO composites in development of blood contacting devices, including small diameter vascular grafts.
Regarding the biological properties, pHEMA/GBMs extracts are not cytotoxic and pHEMA/oxidized GBMs maintain the non-fouling properties of pHEMA, exhibiting negligible levels of adherent cells, blood platelets and bacteria. In vivo hemocompatibility studies show that pHEMA/GO conduits withstand the blood pressure and exhibit negligible adhesion of blood components, revealing better hemocompatibility than ePTFE, a commercial material for vascular assessment.
Our findings reveal pHEMA/GO, a synthetic and off the shelf hydrogel, as a preeminent material for the design of blood-contacting devices that prevent thrombosis and bacterial adhesion.
Funding: PTDC/CTM-COM/32431/ 2017

REFERENCES

[1] Irini Sotiri, Matthew Robichaud, David Lee, Steffen Braune, Maud Gorbet, Buddy D Ratner, John L Brash, Robert A Latour, Ilya Reviakine BloodSurf 2017: News from the blood-biomaterial frontier, Acta Biomaterialia, 87, 55-60.
[2] A.T. Pereira, P.C. Henriques, P.C. Costa, M.C.L. Martins, F.D. Magalhães, I.C. Gonçalves, Graphene oxide-reinforced poly(2-hydroxyethyl methacrylate) hydrogels with extreme stiffness and high-strength, Compos Sci Technol 184 (2019) 107819.