Graphene oxide-protein interactions for the early detection of pancreatic cancer: A bio-nano interface perspective

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To date, pancreatic ductal adenocarcinoma (PDAC) carries a poor prognosis, which is related to both tumour biology and advanced stage at the moment of detection. Thus, PDAC early diagnosis represents a crucial aspect and new, cheap, user-friendly techniques are needed. Indeed, early disease detection is a matter of extreme importance and despite the available diagnostic technologies are promising, they are generally expensive, time-consuming and unlike to be competitive when big data are needed. In this study, we present a nanoparticle-based blood test for the early detection of pancreatic cancer, by characterizing the biomolecular corona (BC) that forms around graphene oxide (GO) nanoflakes. Once embedded in blood, GO nanoflakes (like all kinds of nanoparticles) get covered by a protein layer, whose features and composition depend on both GO physical-chemical properties and molecular source. Subsequently, the identification of specific corona proteins that are related to pathological conditions could provide an effective way to detect tumours by the study of BC composition. Previous findings supported this idea and suggested that a straightforward combination of gel electrophoresis, image processing and statistical data analysis (Fig.1) shall be employed to develop novel diagnostic tools with high accuracy.

GO is a two-dimensional carbon nanomaterial derived from oxidation and exfoliation of graphite, it has low-cost production, hydrophilic nature, high surface area and low curvature. Several studies analyzed GO interaction and functionalization with plasma proteins by which is surrounded when exposed to blood sample. Further, GO binds low amounts of albumin, the most abundant protein in blood, often found in the PC of many nanomaterials. This is a key issue as albumin adsorption in BC may limit the adhesion of other proteins, which are typically present at low concentration in human plasma. For these unique advantages, here we used GO nanometric flakes (lateral size 500 nm) to study BCs from 50 subjects, half of them diagnosed with pancreatic cancer and half of them being healthy volunteers. Cyto-histologically proved PDAC subjects, clinically staged according to the UICC TNM staging system were considered eligible for the analysis (Ethical Committee of University Campus Bio-Medico di Roma approved this study). After exposure of GO to human plasma, we evaluated the protein patterns of the resulting BCs by gel electrophoresis (1D SDS-PAGE). Gel images were acquired by a ChemiDoc (BioRAD) and processed by custom Matlab scripts aiming to distinguish healthy and PDAC samples by the molecular weight distributions of the corresponding BCs (Fig. 2). Finally, statistical data analysis and receiver operating characteristic (ROC) curves were computed (Fig. 3) to quantify sensitivity and specificity of the proposed test. By this approach, we were able to classify correctly cancer patient and healthy subjects in 92% of the analysed samples.

In conclusion, here we demonstrate that the study of BCs formed on GO nanoflakes by 1D SDS-PAGE, coupled with multivariate ROC analysis on the corresponding protein patterns allows discriminating pancreatic cancer patients from healthy subjects with high predicted probability. Furthermore, we predict that a systematic investigation of GO-BC may improve our knowledge of PDAC biology and offer novel opportunities for the development of non-invasive, inexpensive and highly reproducible diagnostic tools.

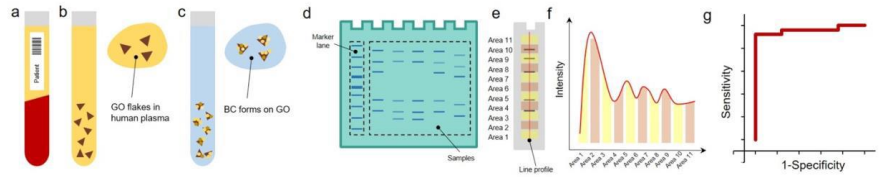

Figure 1. Biomolecular corona analysis of graphene oxide nanoflakes. (a) Blood plasma is separated from donor's blood and incubated with GO nanoflakes. (b). Centrifugation and washing (c) allow the separation of bio-coronated GO, which is analyzed with 1D-electrophoresys (d) using line profiles of the gel bands (e). Intensity profiles are divided in 11 areas (f) to perform ROC analysis (g).

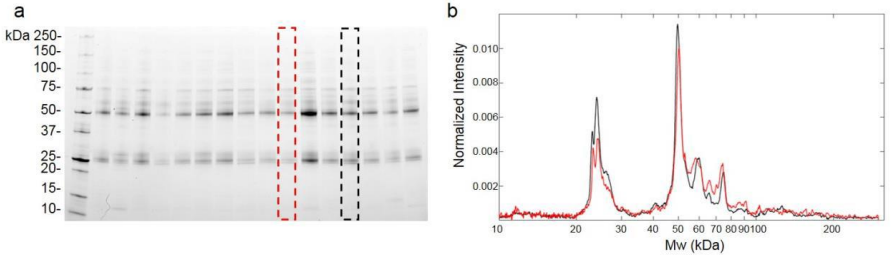

Figure 2. Biomolecular corona characterization by gel electrophoresis. Lanes of an uncut gel (a, dashed lines) and corresponding line profiles (b, solid lines) of a PC patient (red) and a healthy control (black). Intensity profiles reported in panel b.

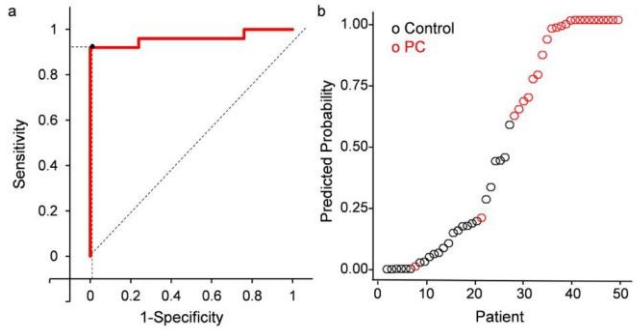

Figure 3. Prediction ability of the nanoparticle-enabled blood test. (a) ROC curve obtained by electrophoretic areas combination and (b) predicted probability graph for control (i.e. healthy subjects) and pancreatic cancer patients.