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Enhanced adhesion and *in situ* photothermal ablation of cancer cells in surface-functionalized electrospun microfiber scaffold with graphene oxide

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ABSTRACT

The physicochemical characteristics of a biomaterial surface highly affect the interaction with living cells. Recently, much attention has been focused on the adhesion properties of functional biomaterials toward cancer cells, since is expected to control metastatic spread of a tumor, which is related to good probability containing the progression of disease burden. Here, we designed an implantable poly(caprolactone)-based electrospun microfiber scaffold, henceforth PCL_{MF}-GO, to simultaneously capture and kill cancer cells by tuning physicochemical features of the hybrid surface through nitrogen plasma activation and hetero-phase graphene oxide (GO) covalent functionalization. The surface immobilization of GO implies enhanced cell adhesion and proliferation, promoting the selective adhesion of cancer cells, even if allowing cancer associated fibroblast (CAFs) capture. We also display that the functionalization with GO, thanks to the high near-infrared (NIR) absorbance, enables the discrete photothermal eradication of the captured cancer cells *in situ* (≈98%).

1. Introduction

It has been postulated that surgical resection and ablation of a primary tumor may result in circulating tumor cells (CTCs) that, once disseminated from solid tumors into the bloodstream, can migrate into healthy tissues giving rise metastases that basically lead to death of patients (Cristofanilli et al., 2004; Nagrath et al., 2007). Hence, the control of metastatic spread of a primary tumor is related to good probability of eluding extravasation of CTCs to target organs, thereby containing the progression of disease burden (Griffith and Gray, 2011).

Migration of CTCs to specific organs is regulated by complex factors driven by cell microenvironment at those sites (Gao and Yuan, 2014). Approaches to recruit CTCs have focused on both mimicking the microenvironment of a target organ (Lee et al., 2012; Moreau et al., 2007) (*i.e.*, bone microenvironment for breast cancer metastasis), through the local immune modulation of implantable scaffolds (Azarin et al., 2015; Erler et al., 2009) and by modulating the cell adhesion properties of biomaterials surface (Gao and Yuan, 2014). Very recently, it has been demonstrated that the immune response upon PLGA-based scaffold implantation implies massive macrophages and neutrophils infiltration, forming a "pre-metastatic niche" inclined to recruit CTCs and tumor-associated cells (Gower et al., 2014; Graham et al., 2013). Azarin et al. have shown that the implantation of engineered scaffolds *in vivo* can reduce colonization of solid organs and tumor burden, with evident therapeutic implications (Moreau et al., 2007). However, using this approach CTCs might persist in the colonized scaffold for long periods permitting cell dissemination into the circulatory system again. Hence, the *in situ* eradication of the captured cancer cells could be an auspicious strategy.

Great attention has been focused on graphene oxide (GO) since it exhibits multifunctional properties that are useful for photothermal therapy applications, including enhancement of guided imaging (Hu et al., 2012), enhanced chemotherapy (Zhang et al., 2011; Fiorica et al., 2017) and low-power efficient photothermal ablation of cancer cells (Chen et al., 2016). In addition, GO coating has been proposed as potential nanostructure with high cell adhesion force for improving the interaction of biomaterials and living cells, highlighting that GO can promote cell adhesion on

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biomaterial surface by means of non-specific mechanisms. Indeed, it was proposed that hydroxyl and carbonyl groups present on the GO surface enable increased interaction with proteins improving cell adhesion (Chang et al., 2016; Chung et al., 2013). Taking into account these current knowledge, we sought to develop an implantable hybrid scaffold, which consists of polyester microfiber and graphene oxide (GO) coating, for promoting cancer cell adhesion and to *in situ* kill captured tumor cells by photothermal ablation.

The biomaterial is an electrospun microfiber scaffold composed of poly(caprolactone) (PCL), a FDA approved material (Koepsell et al., 2011), partially layered with GO nano-sheets at the microfiber surface through low-vacuum nitrogen-plasma activation. We investigated the covalent nature of the interactions taking place between GO and amino functions inserted through plasma exposure. Also, the *in vitro* recruitment of breast cancer cells and normal fibroblasts is studied as a function of GO-mediated recognition, suggesting a potential pivotal role of GO in recruiting cancer cells and cancer associated cells (CAFs) *in vivo* (Jeong et al., 2016). Low-vacuum plasma-assisted GO covalent deposition has the potential to be widely applicable to promote cancer cell adhesion and CTCs photothermal ablation, establishing this technology as potential tool for the production of GO-functionalized biomaterials for the *in vivo* reduction of tumor burden and metastasis outbreak.

2. Materials and methods

2.1. Materials

Poly(caprolactone), dichloromethane (DCM), *N,N*-dimethylformamide (DMF) and Dulbecco's Phosphate Buffer Solution (DPBS) were purchased from Aldrich (Milan, Italy). Graphene oxide nanosheets were purchased from ACS Material, U.S.A. O_2 (99.5%), N_2 (99.8%) were purchased from Air Liquide Italia Service Srl. Dulbecco's Modified Essential Medium (DMEM) was purchased from Euroclone. Celltracker green®, Celltraker Cy5® and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Life Technologies.

Human breast cancer cells (MCF-7) (obtained from Istituto Zooprofilattico Sperimentale della Lombardia e dell' Emilia Romagna, Italy) and human dermal fibroblast cells (HDFa) (obtained from Life Technologies) were maintained in DMEM, containing 10% (v/v) fetal bovine serum (FBS, Euroclone), 100 units per ml penicillin G, 100 μg mL^{-1} streptomycin (Euroclone), and 2 mM L-glutamine (Euroclone, Celbar) at 37 °C, in a humidified atmosphere of 5% CO_2.

2.2. Scaffold preparation

PCL microfiber scaffolds (PCL_{MF}) were prepared as previously described using a widespread electrospinning technique (Gualandi et al., 2016; Pitarresi et al., 2010). PCL_{MF} (300 mg, ≈20 cm^2, ≈540 μm thickness) was then placed in a low pressure plasma reactor (FEMTO, Diener Electronic Plasma-Surface-Technology, GmbH) and treated for 10 min: generator, 40 kHz; potency, 1 W; gas flow, 15 sccm; chamber pressure, 1.0 mbar (Supplementary Fig. S1). The activation was carried out using either O_2, N_2 or air plasma. Next, the activated scaffold (*PCL_{MF}) was immediately soaked in a sealed Petri dish containing an aqueous solution of graphene oxide nano-sheets pH 10 (0.5 mg mL^{-1}). The reaction was maintained at 37 °C for 48 h under continuous stirring (150 rpm) in a Benchtop 808 C Incubator Orbital Shaker model 420. The scaffolds were then energetically washed-up using MilliQ water (5 × 200 mL), washed with water over night (200 mL), and finally dried (0.1 Torr).

2.3. Infrared spectroscopy (FTIR-ATR)

The Fourier transform infrared spectra (FTIR) were recorded in the range of 400–4000 cm^{-1} with 24 scans and 4 cm^{-1} resolution using an attenuated total reflectance accessory in a Bruker Alpha FTIR spectrometer.

2.4. X-ray photoelectron spectroscopy (XPS)

The XPS spectra of PCL_{MF} and PCL_{MF}-GO scaffolds were recorded on a PHI 5000 VersaProbeII (ULVAC-PHI, Inc.); source: Kα, 1486.6 eV; beam: 200 μm, 50 W; time per step: 10 ms; energy step: 0.10 eV; pass energy: 23.50 eV; analyzer mode: FAT. Both samples were dried under vacuum (0.1 Torr) for 24 h before performing the analyses. The carbon (C 1s) line at 284.8 eV was used as reference energy.

2.5. Differential scanning calorimetry (DSC)

DSC analyses were performed using a DSC 131 EVO (by SETARAM Instruments). DSC measurements were carried out on PCL_{MF}, PCL_{MF}-GO and GO under nitrogen atmosphere (flow rate 10 mL min^{-1}), using about 5 mg of sample in a closed aluminum crucible. The heating rate applied was: 10 °C min^{-1} in the range 40–350 °C. Thermograms were normalized to a unit weight to bring all of the variables into proportion with one another.

2.6. Scanning electron microscopy (SEM)

Morphological characteristics of scaffolds were first investigated with a scanning electron microscope (ESEM Philips XL30) operating at 5 kV. The sample was deposited onto a carbon-coated steel stub, dried under vacuum (0.1 Torr), and sputter-coated with gold (15 nm thickness) prior to microscopy examination.

2.7. Atomic force microscopy (AFM)

AFM measurements were performed in tapping mode in air by a Bruker Dimension FastScan microscope equipped with closed-loop scanners. A pyramidal FastScan A probe (resonance frequency = 1400 kHz, tip radius = 5 nm) was employed. PCL_{MF}-GO scaffold was fixed to a steel stub and dried overnight before direct observation.

2.8. Water uptake

Percentage water uptake of scaffolds was determined in water on scaffolds 550 μm in thickness and with a base diameter of 50 mm (Mauro et al., 2013). All samples were dried to constant weight at 0.1 Torr before putting into a test tube containing 50 mL of MilliQ water maintained at 25 °C. The disk was retrieved, gently wiped with soft filter paper, and weighed. Maximum water uptake was observed after about 5 h. The reported values were the average of 6 experiments with a percent mean variation coefficient, CV%, lower than 5%.

2.9. Degradation time

Degradation was observed by following the weight loss of the scaffolds maintained at 37 °C in a volume of 50 mL PBS pH 7.4 up to 12 months from incubation (Tonna et al., 2014; Ferruti et al., 2014).

All samples were dried to constant weight and placed in a test tube containing the degradation medium. The degradation time was established following the scaffold disappearance from the test tube. All experiments were performed in triplicate (n = 9, 3 independent replicates). In all tests the mean CV% was <5%.