POLYMER COMPOSITES FILLED WITH DNA-FUNCTIONALIZED GRAPHENE NANOPLATELETS: EFFECTS OF DNA MODIFICATION ON THE CURING BEHAVIOR AND PROPERTIES OF PDMS-BASED MATRICES

M. Gabriella Santonicola[1,*], Elisa Toto[1] and Susanna Laurenzi[2]

[1]Department of Chemical Materials and Environmental Engineering, Sapienza University of Rome, Via del Castro Laurenziano 7, 00161 Rome, Italy
Email: mariagabriella.santonicola@uniroma1.it
[2]Department of Astronautical Electrical and Energy Engineering, Sapienza University of Rome, Via Salaria 851-881, 00138 Rome, Italy Email: susanna.laurenzi@uniroma1.it

*corresponding author: mariagabriella.santonicola@uniroma1.it

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Abstract
In this paper, we report our recent results on the preparation and characterization of biocompatible nanocomposites made of a silicone-based polymer matrix with tunable elasticity (polydimethylsiloxane, PDMS, and its mixtures with hydroxyl-terminated PDMS) and DNA-functionalized graphene nanoplatelets as fillers. The aim is to exploit the biocompatible character and the high flexibility of the PDMS matrix, together with the exceptional mechanical strength, and good thermal and electrical conductivity of the graphene nanofillers (GNP) for applications in biomedical and sensing devices. Thermal analysis by differential scanning calorimetry (DSC) was conducted to study the curing process of the PDMS-based composites containing the DNA-functionalized graphene nanoplatelets. The properties of the final composites after cure were characterized using several techniques, including optical microscopy, surface wettability, and nanoindentation. We show that the elasticity of the polymer matrix can be varied by adding a less viscous component (PDMS-OH), whereas the electrical conductivity of the nanocomposite can be tuned by acting on the concentration and type of graphene-DNA assembly.

1. Introduction

Multifunctional materials designed by integrating a nano-hybrid component with bioactive and electrically conductive properties in a polymeric support matrix have many attractive features for applications in the biomedical and biotechnology fields. For example, it is well known that cells sense the stiffness of their microenvironment [1], and they can regulate their shape and proliferation according to the rigidity of the underlying substrate [2]. Moreover, flexible substrates that are also electrically conductive can be an ideal tool in applications involving electro-responsive cells, such as cardiac and neuronal cells.

In previous work from our group it was established that nanostructured films made by DNA-solubilized carbon nanotubes, retain their electrical conductivity, as indicated by atomic force microscopy experiments combined with electrical measurements on the nanoscale level [3]. Here, we use a similar procedure to solubilize graphene nanoplatelets in DNA solutions and then embed the self-assembled hybrid in a flexible matrix based on silicone polymers. Such type of non-covalent functionalization can lead to DNA-functionalized graphene nanostructures with different electrical

properties depending on the configuration (tilted or flattened) of the DNA molecules near the graphene surface, as revealed by surface-enhanced Raman spectroscopy (SERS) mapping [4]. These findings were useful in the design of nanocomposite surfaces for sensing applications in various fields, including the design of UV-sensitive surfaces with the ability to detect in real-time the degradation effects of ultraviolet radiation at the level of the DNA element [5, 6].

In this work, we illustrate the fabrication of flexible composite materials by integration of the GNP/DNA element with a PDMS-based matrix. In particular, the possibility to embed the hybrid element in a more elastic matrix, which can be obtained by blending the PDMS base with a less viscous component (the hydroxyl-terminated PDMS-OH), is presented. The possibility of reducing the matrix viscosity of nanocomposite prepolymer mixtures has great influence on the dispersion state of the nanofiller and on the outcome of the fabrication process, from spin-coating to liquid composite molding [7-9]. However, the curing behavior of a modified matrix is often characterized by unexpected features [10, 11]. In our case, the behavior of the PDMS matrix is highly affected by the presence of the less viscous PDMS-OH component, with poor cure conditions and phase segregation occurring for PDMS/PDMS-OH mixtures with 50 wt% or higher of PDMS-OH [12]. Here, we focused our investigation on composites with PDMS/PDMS-OH matrices at ratio 75:25 (w/w), which could be successfully cured.

2. Experimental Section

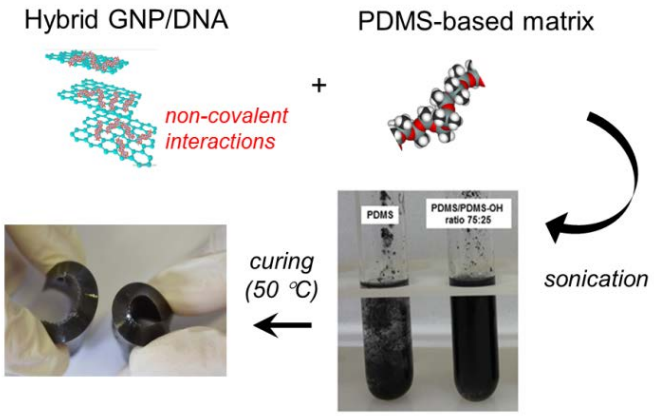
2.1. Materials

Exfoliated graphene nanoplatelets (GNP) were purchased from XG Sciences (USA). Grade C nanoplatelets (GNP-C) with a particle diameter of less than 2 μm and a typical particle thickness of a few nm (average surface area of 750 m^2/g) were used as received. Poly(dimethylsiloxane) elastomer and curing agent (PDMS, Sylgard 184) were obtained from Dow Corning (USA). Double-stranded DNA and hydroxyl-terminated poly(dimethylsiloxane) (PDMS-OH, average Mn ~550) were purchased from Sigma-Aldrich (Germany). DNA solutions and GNP/DNA dispersions were prepared in sterile deionized water (resistivity 18.2 MΩ·cm) produced by a Millipore Direct-Q3 UV water purification system (Millipore, France).

2.2. Preparation of PDMS/GNP/DNA nanocomposites

Composite materials with polymer matrix PDMS/PDMS-OH (75:25 w/w) containing the hybrid element GNP/DNA were prepared by sonication in ultrasound water bath. First, aqueous dispersions with different ratios of GNP and double-stranded DNA by weight were prepared in ultrapure water and sonicated in cold bath to prevent DNA degradation. An optimal condition for the GNP/DNA dispersion was found at ratio 1:1, and this value was used for all subsequent preparation of the nanocomposites. The GNP/DNA dispersions were dried in oven at 50 °C overnight before addition to the PDMS polymer matrix.

The PDMS/GNP/DNA composites were prepared by adding a required weighted amount of dried GNP/DNA to the PDMS base (pure or with diluting agent PDMS-OH) and sonicating the mixture for 1-2 h. Right before curing, the crosslinking agent was added (base/curing agent 10:1 v/v) and the mixture were mechanically mixed for 5-10 min. Next, they were poured in moulds (Corning cell culture plates, well diameter 3.5 cm) and cured in oven at 50 °C for 24 hours. To obtain polymer matrices with different elasticity, the PDMS prepolymer was mixed with the lower molecular weight PDMS-OH (average Mn ~550) at ratio 75:25 w/w before addition of the hybrid GNP/DNA element.



Scheme 1. Preparation of PDMS-based nanocomposites with hybrid GNP/DNA elements and different elastic properties depending on the addition of hydroxyl-terminated component (PDMS-OH).

2.3. Characterization Methods

Differential scanning calorimetry (DSC) measurements were carried out using a Perkin Elmer Pyris 1 instrument (Perkin-Elmer, USA) under nitrogen atmosphere. The DSC instrument was calibrated for temperature and heat flow with indium and tin standards. Nanocomposite mixtures (about 20 mg) were sealed in standard aluminum pans and measured in the temperature range 30 °C – 200 °C at different heating rates (5, 10, 15 and 20 °C/min). A baseline subtraction was applied to correct for slope or variations in heat transfer effects by subtracting the measurement of an empty aluminum pan in both sample and reference holder under identical experimental conditions from the sample runs.

Contact angles were measured by the sessile drop method using a DataPhysics OCA15Pro analyzer (DataPhysics Instruments, Germany) with ultrapure water as testing liquid. A minimum of five different drops (volume 3 μl) were analyzed for each material. The same instrument was also used to acquire optical images of the PDMS/GNP/DNA nanocomposites in transmitted light in order to assess the homogeneity of the samples and to determine the sample thickness.

Nanoindentation tests were performed using a NanoTest Platform instrument (Micro Materials Ltd., UK) with several indentations made in random positions on each specimen in order to obtain statistical data. Experiments were performed with a diamond Berkovic tip, applying the load at a rate of 0.5 mN/s and holding the maximum load for 20 s. Hardness and reduced elastic modulus were calculated from the load-displacement curves [13].

3. Results and Discussion

Polymer matrices made of blends of PDMS and PDMS-OH at different weight ratios were investigated to tune the flexibility of the nanocomposite material and improve the dispersion of the GNP/DNA filler in the matrix by reducing the viscosity of the pure PDMS prepolymer. We found a good balance between viscosity reduction and curing time of the mixture PDMS/PDMS-OH at the ratio 75:25 (w/w).

The morphology of the PDMS-based composite materials, in particular the dispersion degree of the GNP/DNA filler in the PDMS polymer matrices, was investigated by optical microscopy. Disks of composite materials (average sample thickness 0.8 ± 0.1 mm) were molded using multiwell cell culture plates and imaged after cure by optical microscopy in transmitted light. Fig. 1 shows the

dispersion state of GNP-C/DNA aggregates as a function of the filler concentration in the PDMS/PDMS-OH matrix. In general, the degree of dispersion of carbon-based fillers in such matrix is higher than for the pure PDMS prepolymer, which is characterized by higher viscosity. A percolation threshold can be individuated at filler concentration between 3 wt% and 5 wt%.

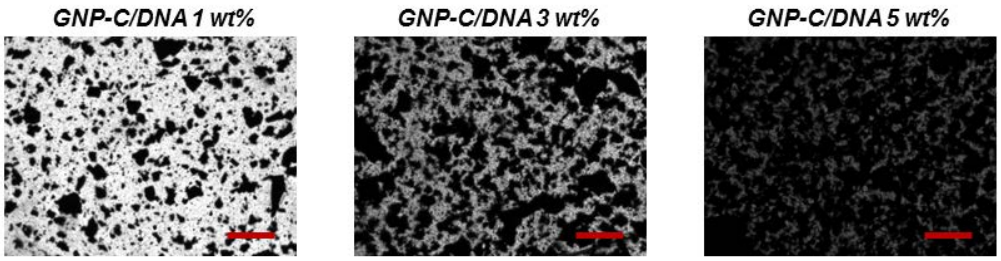


Figure 1. Optical images in transmitted light of PDMS-based composite samples with different concentrations of DNA-functionalized GNP-C embedded in PDMS/PDMS-OH (ratio 75:25 w/w). Scale bar = 1 mm.

The curing behavior of different PDMS/PDMS-OH matrices filled with the GNP/DNA assembly was investigated by DSC. Dynamic curing was carried out at heating rates of 5, 10, 15 and 20 °C/min. The process was analyzed by determining the onset and peak temperatures of the observed exothermic peak caused by the crosslinking reaction of the PDMS thermoset matrix. Fig. 2 shows typical DSC thermograms recorded for a PDMS/PDMS-OH matrix containing 1 wt% of GNP-C/DNA at different heating rates. The exothermic peak temperature clearly varies with the heating rate, hence the maximum reaction rate method proposed by Kissinger can be applied to determine the reaction kinetics parameters, such as the activation energy (E_a) of the curing reaction [14, 15].

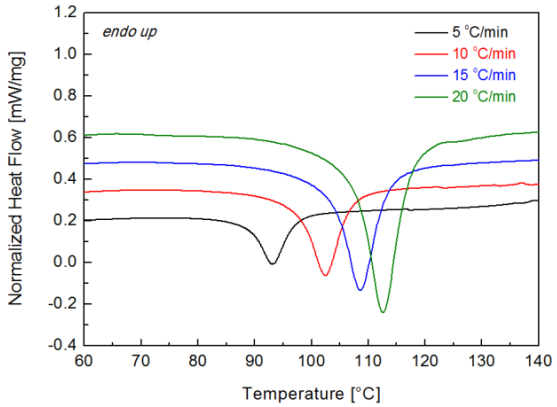


Figure 2. DSC thermograms of PDMS/PDMS-OH prepolymer mixtures (ratio 75:25 w/w) filled with 1 wt% of GNP-C/DNA at different heating rates.

Table 1 summarizes the results for the mixed matrix PDMS/PDMS-OH (ratio 75:25 w/w) with DNA-functionalized GNP-C considering various filler concentrations. In this case, the onset and peak temperatures increase with the concentration of GNP/DNA in the matrix. This situation differs from the case of composites with pure PDMS matrix, where the peak and onset temperatures remain unchanged with GNP/DNA concentrations up to 5 wt%.

Table 1. DSC thermal analysis of the cure kinetics for PDMS/PDMS-OH matrices (weight ratio 75:25) filled with GNP-C functionalized with DNA.

Heating rate (°C/min)	PDMS/PDMS-OH ratio 75:25		PDMS/PDMS-OH ratio 75:25 GNPc-DNA 1wt%		PDMS/PDMS-OH ratio 75:25 GNPc-DNA 3wt%		PDMS/PDMS-OH ratio 75:25 GNPc-DNA 5wt%	
	T_{onset}	T_{peak}	T_{onset}	T_{peak}	T_{onset}	T_{peak}	T_{onset}	T_{peak}
0 (extrapolated)	**81.9**	**87.0**	**83.6**	**88.1**	**87.3**	**93.8**	**89.0**	**94.4**
5	86.9	92.0	88.6	93.2	90.8	99.0	92.3	99.7
10	96.2	101.1	97.2	102.5	100.2	108.0	101.0	108.7
15	102.2	107.1	103.2	108.6	102.7	113.8	106.1	114.8
20	106.1	111.3	107.3	112.7	107.0	118.4	107.8	119.3

T_{onset} = onset temperature (°C) T_{peak} = peak temperature (°C)

The activation energy (E_a) of the curing reaction for each type of nanocomposite with PDMS/PDMS-OH matrix was determined taking into consideration all four heating rates, following the Kissinger equation [14]:

$$\ln\left(\frac{\beta}{T_p^2}\right) = \ln\left(\frac{AR}{E_a}\right) - \frac{E_a}{RT_p} \quad (1)$$

where β is the scan rate, T_p the peak temperature in the DSC trace, A the pre-exponential factor, and R the universal gas constant. From plots of $\ln(\beta/T_p^2)$ versus $1/T_p$, the values of E_a were determined to be 77.5 kJ/mol for the PDMS/PDMS-OH curing system, and 77.0 kJ/mol, 80.6 kJ/mol, and 79.7 kJ/mol for the nanocomposite mixtures with the GNP-C/DNA filler at concentrations of 1 wt%, 3 wt% and 5 wt%, respectively. The regression coefficients for this analysis were in the range 0.9989 – 0.9997 for all systems under investigation. Our results show that the presence of the DNA-functionalized filler in the nanocomposites at 3 and 5 wt% determines an increases of the activation energy of the curing process to an extent less than 4%. When considering PDMS/PDMS-OH matrices prepared with non-functionalized graphene (GNP-C), we observed an increase of the activation energy with the respect to the GNP-C/DNA filler, indicating a favorable contribution of the DNA element on the curing behavior of the nanocomposites. Composite materials containing only pristine GNP-C, when cured using the same process conditions (50 °C for 24 hours), showed poor mechanical properties due to insufficient matrix crosslinking already at 1 wt% of GNP-C concentration.

The biocompatibility of the flexible nanocomposite substrates containing the hybrid GNP/DNA complex was assessed by water contact angle measurements at room temperature. Fig. 3 shows optical images of water droplets on composites with 5 wt% of GNP/DNA and polymer matrix consisting of pure PDMS or the mixture PDMS/PDMS-OH (ratio of 75:25 w/w). Addition of the less viscous PDMS-OH component to the silicon matrix significantly lowers its hydrophobicity due to the presence of the hydroxyl functional groups.

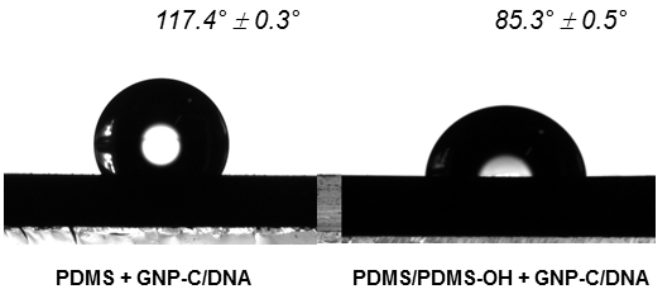


Figure 3. Comparison of surface hydrophobicity of pure PDMS (left) and PDMS/PDMS-OH (right) matrices containing 5 wt% of GNP-C/DNA as measured by sessile drop method with ultrapure water.

Finally, the mechanical properties of the flexible silicone-based nanocomposite samples were measured as a function of the concentration of the hybrid GNP-C/DNA using nanoindentation. Table 2 summarizes the hardness and reduced modulus values for all the samples investigated. As a comparison, the properties of the substrate made of neat PDMS and cured at the same conditions (50 °C for 24 hours) are also reported. Results clearly indicate the increase of the reduced modulus of the nanocomposites as a function of the filler concentration.

Table 2. Mechanical properties of PDMS substrate and PDMS/PDMS-OH based nanocomposites with GNP-C/DNA as filler from nanoindentation tests. Curing process at 50 °C for 24 hours.

Specimen Type	Hardness (MPa)	Reduced Modulus (MPa)
PDMS	5.94	17.10
PDMS/PDMS-OH 75:25	1.10	3.13
PDMS/PDMS-OH 75:25 + GNP-C/DNA 1 wt%	1.29	3.86
PDMS/PDMS-OH 75:25 + GNP-C/DNA 3 wt%	2.40	6.90
PDMS/PDMS-OH 75:25 + GNP-C/DNA 5 wt%	2.70	10.62

4. Conclusions

In this paper, we investigated the fabrication and properties of hybrid nanocomposites prepared by sonication-driven assembly of graphene and DNA in aqueous solution, followed by integration with a silicone PDMS-based matrix in order to create biocompatible substrates with tailored conductive and elastic properties for use in biomedical applications. The flexibility of the polymer matrix was varied by adding a less viscous silicone component, the hydroxylated PDMS-OH, whereas the electrical conductivity of the bioactive material can be tuned by acting on the concentration and type of the graphene-DNA hybrid assembly, in particular at the level of the graphene nanoplatelets size and aspect ratio. Our results show that the presence of the DNA element in the nanofiller enhances the curing process of the PDMS/PDMS-OH matrices with respect to pristine graphene filler. In addition, from

water contact angle measurements we determined that the hydrophobicity of the PDMS/PDMS-OH nanocomposite substrates is significantly lower than that of the same nanocomposites made of neat PDMS, which is an important factor in the design of composite materials for applications in aqueous environment, tipically in the biomedical and biotechnology fields.

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