Hybrid hydrogels based on gelatin, chitosan and functionalized graphene layers

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***Abstract*—Hybrid hydrogels were prepared based on gelatin, polyethylene glycol diglycidyl ether, chitosan and graphene layers functionalized with serinol pyrrole, bearing hydroxyl groups. Large water uptake was observed. Very good mechanical properties and electrical properties were obtained by using a low amount of graphene layers (up to 1% mass in wet condition).**

***Keywords*— biodegradable hydrogels, chitosan, gelatin, graphene layers, serinol pyrrole, stress-strain curve**

I. INTRODUCTION

Hydrogels are receiving increasing attention for biomedical applications such as tissue engineering, drug encapsulation and controlled release. Indeed, hydrogels absorb large amount of water, similar to that of biological tissues, can be shaped in any form and size, under cyto-compatible conditions, are characterized by flexibility, softness, biocompatibility, biodegradability.

Naturally-derived hydrogels are inherently biocompatible, bioactive, biodegradable and promote many cellular functions. However, they have poor mechanical properties, poor control of degradation, difficult sterilization and purification. Synthetic hydrogels are highly reproducible, have highly controllable degradation rate and are generally characterized by high mechanical properties. However, they find it difficult to interact with cells and proteins. Hybrid hydrogels are thus investigated, to combine the benefits of natural and synthetic polymers. Examples of hybrid hydrogels are those based on gelatin, polyethylene glycol diglycidyl ether and chitosan. They show robust and tuneable mechanical properties, they allow cell adhesion and proliferation, their degradation can be controlled through their chemical composition and cross-linking density. They might have a great potential for biomedical applications. It would desirable to obtain hybrid hydrogels with improved mechanical properties and electrical conductivity.

Graphene [1]-[3], the thinnest material on earth, has outstanding chemical, mechanical and thermal properties. Graphene and graphene related materials (GRM) are thus ideal materials for pursuing outstanding properties for hybrid hydrogels [4]. Ultimate dispersion of graphene layers in the hydrogel should be achieved, to fully exploit graphene and GRM properties. It is evident that the solubility parameter of graphene layers has to be modified to have compatibility with an hydrophilic environment. A facile and sustainable functionalization of graphene layers was performed with pyrrole compounds. It was shown that, by properly selecting the pyrrole compound, compatibility and stable dispersions of graphene layers were obtained in surroundings having different solubility parameters. [5]-[8] In particular, by using 2-(2,5-dimethyl-1*H*-pyrrol-1-yl)-1,3-propanediol (serinol pyrrole, SP) whose structure is in Figure 1, stable water dispersion were prepared.

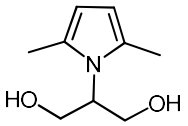

Fig. 1: 2-(2,5-dimethyl-1*H*-pyrrol-1-yl)-1,3-propanediol (SP)

In this work, hybrid hydrogels were prepared, based on gelatin, polyethylene glycol with epoxy groups as chain ends, chitosan and graphene layers functionalized with serinol pyrrole. Graphene nanoplatelets were added to the hydrogel in various amounts. The hydrogels were prepared in water, in the absence of organic solvents, chemical foaming agents or surfactancts, relying on crosslinking promoted by the reaction of amino groups of chitosan and epoxy groups of PEG. Samples were characterized by means of thermogravimetric analysis (TGA), Infrared and Raman spectroscopies, scanning and transmission electron microscopy (SEM, TEM), mechanical and electrical measurements.

II. EXPERIMENTAL PART

Functionalization of graphene layers was performed as reported in [7],[8], by giving thermal energy to the GnP/serinol pyrrole adduct.

In brief, hybrid hydrogels were prepared following a procedure reported in [9]. The ratio between natural components (G and CH) and the crosslinking agent was 4.74 and it is maintained constant for all the materials tested. Three different amounts of graphene nanoplatelets were added and the exact amounts were evaluated by TGA. In the following and in particular in Figures and Tables, hybrids hydrogels are indicated as Hybrid 1-4. Hybrid 1 is G/PEG/CH. Hybrid 2-4 are G/PEG/CH with increasing amount of graphene nanoplatelets (see Table 1 below).

Tensile tests were carried out on bars cut from hydrogels, whereas compression tests were carried out on cubic specimens. Mechanical tests were carried out by an Instron series 3366 testing machine, equipped with a 50 N load cell.

III. RESULTS AND DISCUSSION

TGA was used to evaluate the actual amount of graphene layers in the dry samples: data are in Table 1.

TABLE I
GRAPHENE CONTENT OF THE HYBRID HYDROGELS IN INITIAL CONDITION

Sample code	*Graphene content (mass %)*
Hybrid 1	/
Hybrid 2	4.6
Hybrid 3	7.2
Hybrid 4	7.4

The water uptake was calculated as in Eq. (1):

$$\text{Water uptake (\%)} = (M_s\text{-}M_d)/M_d \cdot 100 \qquad (1)$$

where M_s and M_d denote the swollen and initial dry mass of the samples, respectively.

Figure 2 shows the water uptake results.

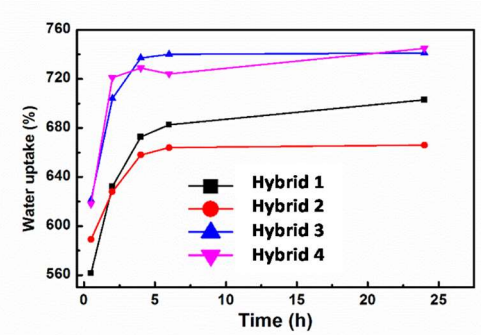

Fig 2. Water uptake of the hydrogels as a function of soaking time.

Water uptake of hydrogel composites is in the range 650 - 750 %. The graphene layers do not remarkably modify the swelling ability of hydrogels, which is high for all the samples. It could be observed that the water absorption slightly increases with the nanoplatelets content. Such a high water absorption appears suitable for tissue engineering applications.

Hydrogel morphology was analyzed by SEM. SEM image is in Figure 3.

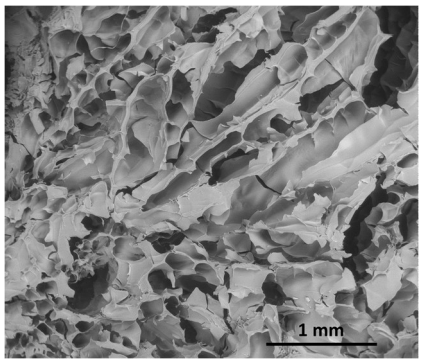

Fig 3. SEM image of the neat hydrogel

Picture in Figure 3 shows a macroporous network with channels and interstices of different size, well interconnected and homogeneously distributed.

The graph in Figure 4 shows representative stress-strain curves obtained from tensile tests. Data of initial elastic modulus and ultimate properties are reported in Table 2.

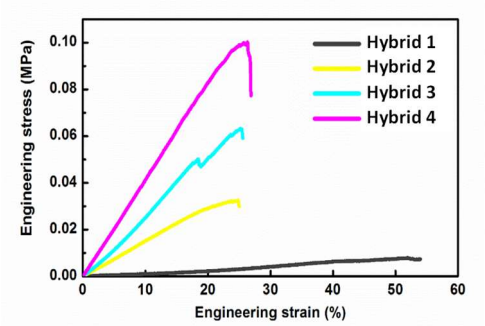

Fig 4. Representative tensile stress-strain curves of wet hydrogels.

TABLE II
TENSILE PROPERTIES OF NEAT AND NANOFILLED WET HYDROGELS

Sample	*G* (%)*	*Tensile Properties*		
		Initial modulus (MPa)	Strength (MPa)	Strain at max stress (%)
Hybrid 1	0	0.024± 0.12	0.0096 ±0.003	39± 11
Hybrid 2	0.6	0.162 ± 0.01	0.027 ± 0.01	18 ± 4
Hybrid 3	0.8	0.259 ± 0.07	0.046 ± 0.02	22 ± 6
Hybrid 4	0.9	0.353 ± 0.06	0.053 ± 0.03	22 ± 8

* Content of graphene nanoplatelet in wet sample

The graph in Figure 5 shows the correlation between the elastic modulus and the GRM content in the wet samples.

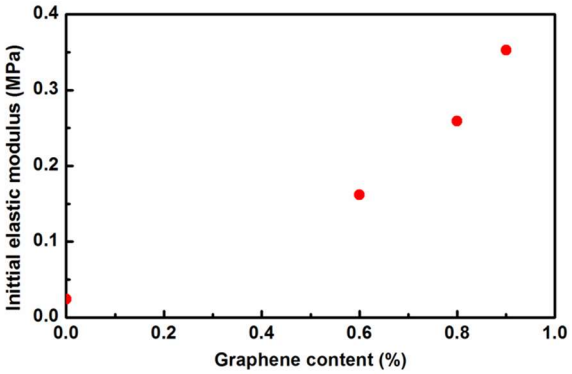

Fig 5. Initial elastic modulus versus GRM content in the wet samples.

The graphene layers, though in such a low amount (always lower than 1% by mass in the wet material), cause a large increase of strength and stiffness, of one order of magnitude. This robust increase of mechanical properties is not associated with a dramatic reduction of strain at break: from about 40% for the neat hydrogel to about 20% for hybrid hydrogels.

Compressive cyclic tests up to 80% of strain were made on wet cubic specimens to test their fatigue resistance. Figure 6 shows an example of stress-strain curve, of Hybrid Sample n. 3.

During the first cycles, the materials show a Mullins effect, with the reduction of stiffness and stress at every cycle. After few cycles the material behavior is stabilized and the hydrogel is able to sustain a compressive strain of 80% with full strain

recovery. All the materials maintained their original shape. and their load bearing capability up to such high level of deformation.

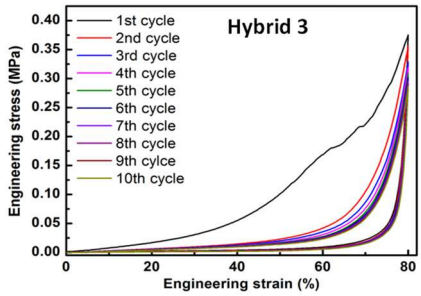

Fig 6. Engineering stress *vs* engineering strain obtained from compressive cyclic tests carried out on Hybrid 3.

Impedance measurements, carried out on the nanofilled hydrogels both in dry and wet condition, show a good electrical behavior. Such a result appears to be promising in view of the application of electrical stimuli. This work is in progress.

IV. Conclusion

Graphene nanoplatelets were added to hybrid hydrogels based on gelatin, chitosan and polyethylene glycol diglycidyl ether. High water uptake was maintained and increase of mechanical properties, stiffness and strength, was obtained without significant embrittlement. Promising electrical behavior was observed. All these properties appear important for tissue engineering applications. In particular, the use of graphene layers could bring the electrical conductivity suitable for cell growth with electro-mechanical stimuli.

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