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Novel Nanobiocomposite Hydrogels Based on Gelatin/Chitosan and Functionalized Graphene

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Abstract. Composites of gelatin, chitosan and graphene layers functionalized with serinol pyrrole, containing hydroxyl groups, were prepared to create electrically conductive, mechanically robust and biodegradable hydrogels. The incorporation of hydrophilic graphene layers (up to 1% mass in wet condition) into the polymer matrix significantly improved mechanical and electrical properties. These composite hydrogels are promising candidates for use as conducting substrates for the growth of electro-responsive cells in tissue engineering.

Keywords: Gelatin, Chitosan, Graphene Nanoparticles, Electrically Conductive, Biodegradable Hydrogels.

PACS: 81.05.Qk, 81.05.Rm; 87.85.J

INTRODUCTION

Hydrogels are three dimensional polymeric networks made from natural or synthetic polymers by utilizing different types of crosslinking mechanisms. They have been gaining increasing attention for biomedical applications such as tissue engineering, drug encapsulation and controlled release due to their resemblance to native extracellular matrix (ECM), remarkable biocompatibility, controllable biodegradability, and tunable physico-mechanical properties. 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. On the other hand, 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.

Gelatin, denatured and partially hydrolysed native collagen, has been extensively used in the field of tissue engineering due to its various desirable features, including biocompatibility, biodegradability, low cost, and eases of manipulation. Likewise, chitosan exhibits several beneficial properties that make it a good candidate for biomedical applications: it is biocompatible, biodegradable, non-antigenic, non-toxic, antimicrobial and shows haemostatic activity. In addition, its structural similarity to some polysaccharides and ECM constituents and cationic surface charges promote cell attachment and growth [1].

However, hydrogels are typically non-conductive which limits their applications for bioactive scaffolds for the growth of electro-responsive cells in tissue engineering. It would desirable to obtain composite hydrogels with improved mechanical properties and electrical conductivity. In recent times, hydrogels with electroconductive properties have been developed through incorporating different nanomaterials to their network.

Graphene [2], 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 [3]. 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

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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 [4]. 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.

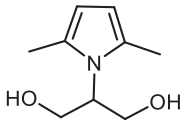

FIGURE 1. 2-(2,5-dimethyl-1H-pyrrol-1-yl)-1,3-propanediol (SP)

In this work, conducting composite hydrogels were prepared by a facile preparation method using gelatin and chitosan as the natural component and poly(ethylene glycol)diglycidyl ether (functionalized PEG) as the cross-linker. 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 gelatin/chitosan and epoxy groups of PEG. Graphene nanoplatelets, functionalized with serinol pyrrole, were added to the hydrogel networks in various amounts to improve the mechanical properties and the electrical conductivity of the hydrogels. Samples were characterized by means of thermogravimetric analysis (TGA), Infrared and Raman spectroscopies, scanning and transmission electron microscopy (SEM, TEM), mechanical (tensile and compressive) and electrical measurements.

EXPERIMENT

Synthesis of Composite Hydrogels

Functionalization of graphene layers was performed as reported in [5-6], by giving thermal energy to the GnP/serinol pyrrole adduct.

The G/PEG hydrogel was prepared in aqueous solution, and the synthetic procedure involved the reaction between gelatine/chitosan amino-groups and the epoxy groups of functionalized PEG. The ratio between natural components (G and CH) and the crosslinking agent was 4.7 and it is maintained constant for all the materials synthesized. Briefly, G (3 g) was completely dissolved in 30 ml distilled water at 40 °C under mild magnetic stirring followed by the addition of established amount of grapheme nanoparticles. After stirring for half an hour, functionalized PEG (1.4 g) and EDA (70 mg) were droprwise added into the mixture. The reaction mixture was gently stirred at 40 °C for 20 min and then added CH solution (2 wt%, 32 g) into the G/PEG/Graphene reaction mixture and continued 45 min magnetic stirring and 20 min sonication for obtaining homogeneous solution. Finally, the reaction mixture was poured into the glass plate for gel formation. Then the gels are cut into rectangular bar and then lyophilized.

Three different amounts of graphene nanoplatelets were added and the exact amounts were evaluated by TGA. The composition of the four hydrogels was reported in Table 1.

Mechanical tests were carried out by an Instron series 3366 testing machine, equipped with a 50 N load cell. Tensile tests were carried out on bars cut from hydrogels, whereas compression tests were carried out on cubic specimens. For the compression test, samples were cut into cubic specimens and compressed by two parallel metal platens connected to the load cell, at a rate of 10% strain per min at room temperature. The specimens were tested in wet condition. Engineering stress was calculated by dividing the recorded force by the initial cross-sectional area. Engineering strain was calculated as the ratio of cross-head displacement to original length/thickness. Initial elastic modulus (stiffness) was determined from the slope of the initial linear segment of stress-strain curves. Cyclic loading-unloading experiments were performed for up to 10 cycles with 80% maximum strain without relaxation time in order to evaluate the reversible behavior of hydrogels.

RESULTS AND DISCUSSION

Four different composite hydrogels namely G/PEG/CH, G/PEG/CH (GO-1), G/PEG/CH (GO-2) and G/PEG/CH (GO-3) were synthesized using a simple and easy synthetic method under aqueous condition without using any solvents, chemical reagents such as, catalysts, coupling agents and so on. Functionalized PEG was used as a crosslinking agent, and the cross-linking process involved mainly between end epoxide groups of PEG and amino

group of gelatin/chitosan chains. TGA was used to evaluate the actual amount of graphene layers in the dry samples and the data are shown in Table 1.

TABLE 1. Composition, tensile and compressive properties of the neat and nanofilled wet hydrogels.

Sample Code	G* (%)	G** (%)	Tensile Properties			Compressive Properties	
			Initial Modulus (MPa)	Strength (MPa)	Starin at Max. Stress (%)	Elastic Modulus (MPa)	Strength at 50% Strain (MPa)
G/PEG/CH	-	0	0.024± 0.12	0.0096 ±0.003	39± 11	0.037± 0.003	0.042 ±0.01
G/PEG/CH (GO-1)	4.6	0.6	0.162 ± 0.01	0.027 ± 0.01	18 ± 4	0.073 ± 0.001	0.059 ± 0.02
G/PEG/CH (GO-2)	7.2	0.8	0.259 ± 0.07	0.046 ± 0.02	22 ± 6	0.079 ± 0.01	0.081 ± 0.01
G/PEG/CH (GO-3)	7.4	0.9	0.353 ± 0.06	0.053 ± 0.03	22 ± 8	0.057 ± 0.001	0.047 ± 0.01

G=graphene nanoplatelet content in dry (*) and wet (**) sample

Figure 2 shows the water uptake results. Water uptake of composite hydrogels 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.

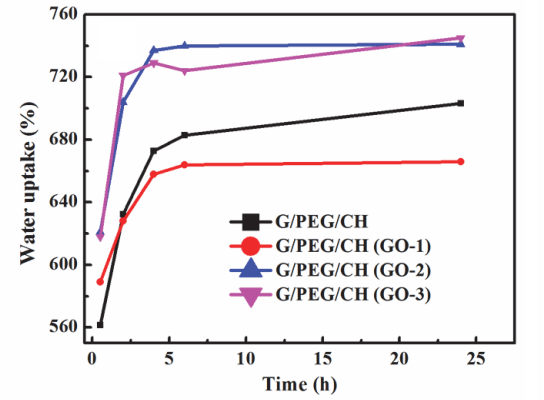

FIGURE 2. Water uptake of the hydrogels as a function of soaking time.

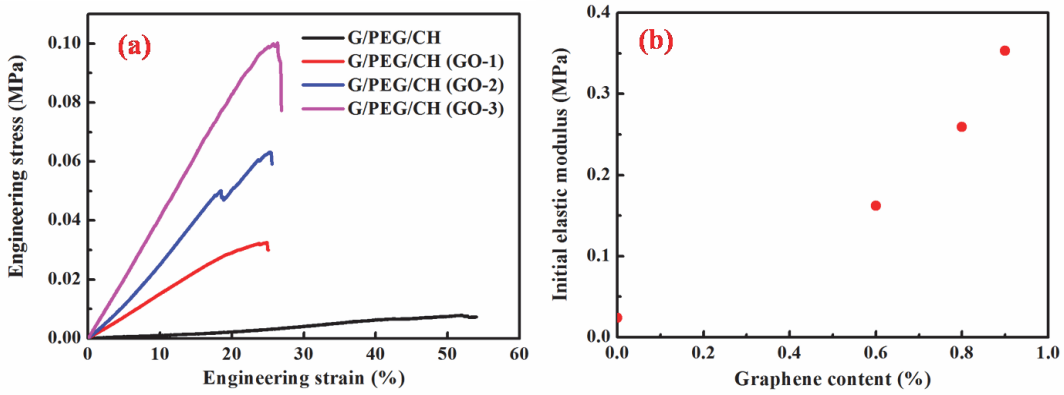

FIGURE 3. The representative tensile stress-strain curves of the composite hydrogels (a) and the initial elastic modulus (tensile) as a function of graphene content in wet samples (b).

The graph in Figure 3(a) shows the representative stress-strain curves obtained from tensile tests. Data of initial elastic modulus and ultimate properties are reported in Table 1. The graph in Figure 3(b) shows the correlation between the elastic modulus and the graphene 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 the graphene reinforced hydrogels. Compressive tests up to 80% of strain were made on wet cubic specimens to test their reversible behavior, and the values of elastic modulus and strength at 50% strain were reported in Table 1. 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 (Figure 4) 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.

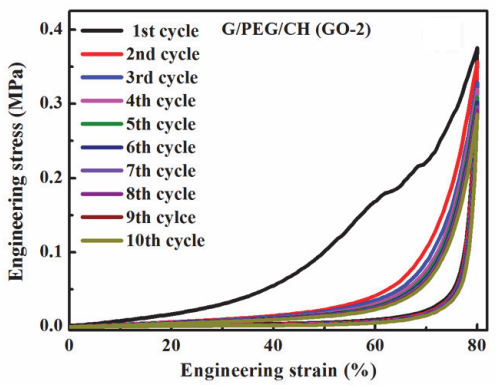

FIGURE 4. Engineering stress *vs* engineering strain obtained from compressive cyclic tests carried out on G/PEG/CH(GO-2)

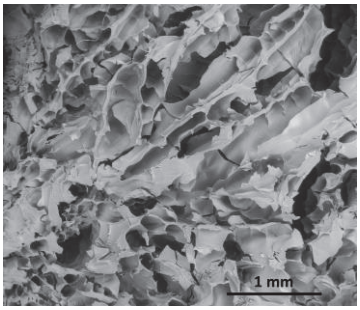

FIGURE 5. SEM images of neat hydrogel.

Hydrogel morphology was analyzed by SEM. The image reported in Figure 4 shows a microporous network with channels and interstices of different size, well interconnected and homogeneously distributed.

Preliminary impedance measurements, carried out on the nanofilled hydrogels both in dry and wet condition, reported optimally tunable electrical properties with characteristic frequency of transition between resistive- and capacitive-like behavior, depending from graphene nanoplatelet content. Such a result appears to be promising in view of the application of electrical stimuli. This work is in progress.

In conclusion, Graphene nanoplatelets were added to hydrogels based on gelatine and chitosan. High water uptake was maintained and increase of mechanical properties, stiffness and strength, was obtained. 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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