FABRICATION AND CHARACTERIZATION OF GRAPHENE-LOADED CHITOSAN HYDROGELS WITH CROSS-LINKING GRADIENT

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[ENGINEERING OF BIOMATERIALS 148 (2018) 40]

Introduction

Hydrogels can be defined as polymeric networks able to absorb large amount of water. Thanks to the aqueous environment and the rubbery nature, hydrogels resemble native cell environment - the extracellular matrix (ECM). However, mimicking the complex, multi-level structure of ECM is still a challenge in tissue engineering. Various techniques have been applied to create hydrogels with gradients of microstructure, mechanical properties and biosignals: 3D printing [1], cross-linking by photopolymerisation using gradient-mask [2] and microfluidic methods [3]. However, most of these processes require use of advanced equipment.

Chitosan (CS) and other natural polysaccharides are extensively tested in tissue engineering [4,5] due to their similarity to natural tissues. CS-based hydrogels possess superior biological properties but their applicability is limited by poor mechanical properties and stability. In mild acidic conditions, the amino groups of CS get protonated and the polymer can be fully dissolved. When increasing the pH, CS solution forms a hydrogel via secondary interactions.

The aim of this study, was to fabricate CS-based hydrogels with gradient properties by gelation method. To enhance the hydrogel properties, various forms of graphene (GO, rGO, GO-PEG) and tannic acid (TAc, cross-linker) were introduced to polymer matrix.

Materials and Methods

CS (High Mw, DD >90%) and sodium tripolyphosphate (TPP) were obtained from Acros Organics, USA. Lactic acid (LAc, 88%), TAc, NaOH, NaCl were purchased from Avantor Performance Materials Poland S.A. Three types of graphene materials: graphene oxide (GO), reduced graphene oxide (rGO) and PEG grafted GO were prepared in ITME, Poland.

CS solution was prepared by dissolving CS (5% w/v) in 5% LAc. Next, stable suspension of GO, rGO or GO-PEG (0.5% to CS weight) and TAc (10% to CS weight) were added to CS solution. The solutions were homogenized by sonication in water bath, transferred into molds and frozen at -20°C for 24 h. Next, they were immersed in gelling solution at 4°C. Types of gelling solutions are summarized in TABLE 1. Finally, the samples were washed with distilled water and stored for 24 h.

TABLE 1. Gelling system compositions.

Lp.	1st bath	Time	2nd bath	Time
1	5% NaCl + 0,5% TPP	24 h	-	-
2	5% TPP	2 h	10% NaOH	10 min
3	5% TPP	4 h	10% NaOH	10 min

XRD, XPS, ATR-FTIR and SEM were used to characterize graphene materials and the obtained hydrogels. Rheological (rotational rheometer with parallel plates), thermal (DSC) and mechanical (compression test) properties of the samples were examined. Also, *in-vitro* degradation (PBS, 37°C) and bioactivity (SBF, 37°C) tests were carried out.

Results and Discussion

Form and properties of the hydrogels can be easily controlled by the time and composition of the gelling solution. In the first gelling system, the gel was formed within the whole volume of the sample. The second composition resulted in the formation of a dense outer shell and a semifluidic center. After increasing time to 4h, CS solution gelled completely and created multilayered hollow rods. FIG. 1 shows gradient microstructures of CS/GO hydrogel after lyophilisation.

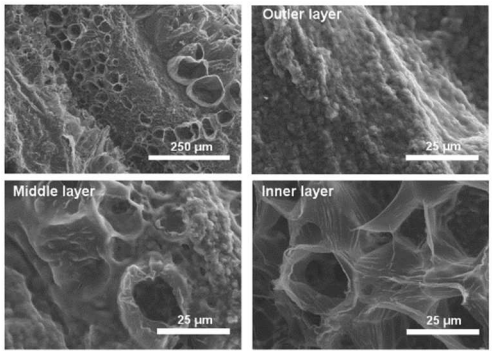

FIG. 1. SEM images of CS/GO hydrogels after gelling in 2nd system (2 h TPP + 10 min NaOH) and freeze-drying.

ATR-FTIR measurements (FIG. 2) confirmed successful cross-linking of CS-based hydrogels by TPP. In addition, nanocomposites modified with different graphene forms exhibited improved stability during PBS incubation test and high *in vitro* bioactivity.

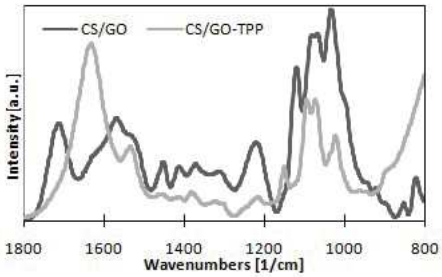

FIG. 2. ATR spectra of CS/GO and CS/GO-TPP.

Conclusions

A novel method was developed to obtain gradient CS hydrogels. Properties of the hydrogels can be controlled by gelling conditions. Gradient nanocomposites loaded with graphene mimic complex structure of ECM and constitute promising materials for tissue engineering.

Acknowledgments

This work was financed by the National Center for Research and Development, Poland under BioMiStem grant No STRATEGMED3/303570/7/NCBR/2017 and supported by the grant No 15.11.160.019 (Faculty of Materials Science and Ceramics, AGH University of Science and Technology).

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