CHARACTERIZATION OF GRAPHENE OXIDE-LOADED CHITOSAN HYDROGELS AND THEIR APPLICATION FOR 3D PRINTING

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[ENGINEERING OF BIOMATERIALS 143 (2017) 83]

Introduction

Nowadays, additive manufacturing methods, and 3D printing especially, are widely applied in numerous fields from automotive and aerospace to biomedicine and tissue engineering[1]. In the latter, this technique is used e.g. for fabrication of TE scaffolds from variety of biodegradable polymers. Among them, hydrogels seem to be one of the most promising due to their high water content and ability to mimic microenvironment of a natural extracellular matrix (ECM)[2]. Hydrogels possess excellent biological properties but their printability might be challenging, as – when printed - those relatively soft structures tend to collapse under the weight of subsequent layers. To print hydrogel scaffolds in a highly precise manner, it is necessary to have appropriate gel ink viscosity and homogeneity. From the biological perspective, the most important is the biocompatibility of the scaffold material. From the variety of materials of natural origin, chitosan is of particular interest thanks to its antibacterial, mucoadhesive, hemostatic, analgesic, and antioxidant properties, as well as excellent biocompatibility and biodegradability.

In this work, chitosan and chitosan/graphene oxide hydrogels were designed and optimized for 3D printing. Investigated parameters included solvent type and solution concentration, gel viscosity, printing pressure, feed rate, needle gauge, and post-treatment with e.g. sodium hydroxide.

Materials and Methods

Chitosan (CS, High Mw, Sigma-Aldrich) and chitosan/GO inks for printing were prepared by dissolving the chitosan (5% w/v) in 10% lactic acid or mixture of lactic acid and gallic acid. Next, different amounts of stable GO suspension in distilled water were added to CS solution (0%, 0.5% and 1% to CS weight). At each step, the solutions were homogenized thoroughly by vortexing and sonication in a water bath. The prepared inks were transferred to a special syringe and centrifuged to remove remaining air bubbles. Hydrogel inks were printed using 3D-Bioplotter® (Envisiontec, Germany) (STL files created in SketchUp 2016 3D modelling software). After drying, some of the scaffolds were post-treated by neutralization in 1M NaOH solution.

Rheological properties of the hydrogel inks were examined using rotational Kinexus rheometer with a parallel plate geometry (Malvern Instruments Ltd, UK). Also, ATR-FTIR Nicolet™ iS™, Thermo Fisher Scientific) spectroscopy and optical microscopy were used to characterize the samples. Fibroblasts viability was assessed with Alamar Blue assay.

Results and Discussion

Rheological characterization confirmed that chitosan-based hydrogel inks are non-newtonian fluids and exhibit shear-thinning behavior – with increasing shear stress, the fluid viscosity decreased (FIG. 1). Rheological properties of chitosan depend on the solution concentration. Addition of graphene oxide to the chitosan matrix resulted in the increase of the gel viscosity.

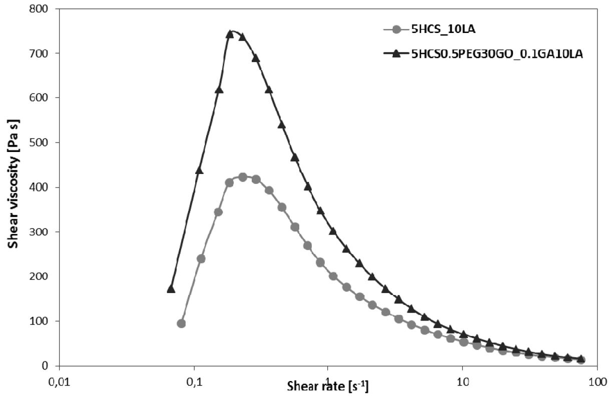

FIG. 1. Viscosity vs. shear rate for chitosan (dots), and chitosan/GO (triangles) inks.

The best printing resolution was achieved with 5% (w/v) chitosan solution modified with graphene oxide. Optimized printing parameters were as follows: 25 gauge dispense tip (Nordson EFD), pressure 5 bar, speed 1.5-2 mm/s, at room temperature. Thanks to relatively high concentration of the gel ink and the low feed rate, remaining solvent could partially evaporate, hardening the printed layer and securing enough structural support to avoid collapsing of the hydrogel struts under the weight of another layer. Printed scaffold could be easily manipulated using tweezers. Some drying-induced shrinkage was observed, however in general, scaffolds remained their designed morphology. When immersed in water, dried chitosan scaffolds swelled and dissolved rapidly; dried and then neutralized scaffolds, after some initial swelling, remained stable. Interestingly, also dried-only CS/GO composite scaffolds were stable in the aqueous environment. It is possible that graphene oxide sheets crosslinked chitosan network, increasing its stability.

Conclusions

Chitosan and chitosan/GO composite hydrogels can be 3D printed creating scaffolds with predefined architecture. Appropriate ink preparation is crucial for successful printing. Higher gel viscosity results in better printing resolution.

Acknowledgments

Dr. Joseph A.M. Steele at Kohn Lab is gratefully acknowledged for his mentoring and assistance with 3D printing. This research was supported by the New Jersey Center for Biomaterials, Rutgers University (through ICF-BSE International Summer Exchange Program), and by the grant No 15.11.160.019 (Faculty of Materials Science and Ceramics, AGH University of Science and Technology).

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