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3D PRINTING OF NANOCOMPOSITE HYDROGELS INK

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INTRODUCTION

Recent trends show the increase in scientific research to incorporate different nanoparticles into the conventional hydrogel aiming to combine the advantageous characteristics of the nanoparticles with the biological properties of hydrogels. Nanocomposite hydrogels are defined as polymeric networks and nanoparticles or nanostructures with each other crosslinked (GAHARWAR; PEPPAS; KHADEMHOSSEINI, 2014). The crosslinking can be covalently or physically, depending on the type of application.

Nanocomposite hydrogels can be engineered to present superior physical, chemical, electrical, and biological properties, opening possibilities in developing advanced biomaterials for applications that would not be possible with conventional hydrogels (LEE et al., 2020). An extensive range of nanoparticles can be used with natural or synthetic polymers to take nanocomposite hydrogels as cellulose nanocrystals and Laponite.

Cellulose nanocrystals (CNC), a rod-like nanoparticle with a 5-10 nm width and length up to 300 nm, attract attention, considering they are biocompatible and have high attractive mechanical properties for the mechanical strengthening of hydrogels with potential biomedical applications (TRACHE et al., 2017).

Laponite is a trioctahedral smectite nanoparticle with disk -shaped geometry and size of 30-50 nm in diameter and 1-2 nm in thickness (RUZICKA; ZACCARELLI, 2011; XAVIER et al., 2015; GAHARWAR et al., 2019). Laponite is widely used as a rheological modifier in various products. Depending on Laponite concentration, the internal arrangements of the structure can result in low viscosity dispersions, colloidal gels, or Wigner glasses during the hydration process (RUZICKA; ZACCARELLI, 2011).

The main objective of this work is to combine the use of CNC and Laponite nanoparticles with carboxymethylcellulose (CMC). CMC is a biodegradable polysaccharide widely used as superabsorbent hydrogels with smart behavior in response to the physiological environment (SANNINO; DEMITRI; MADAGHIELE, 2009).

The combination of CNC and Laponite nanoparticles with the CMC polymeric matrix can result in nanocomposite hydrogels with

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physiochemical and enhanced rheological properties, which enable their use for the 3D extrusion-based printing process.

MATERIALS AND METHODS

Materials: CMC with Mw = 250,000 g.mol^{-1} and DS = 0.70 was purchased by Sigma-Aldrich (Saint Louis, MA, USA). The nanoparticles CNC and Laponite XLG in spray-dried powder form were purchased by CelluForce (Montreal, QC, Canadá) and Southern Clay Products, Inc. (USA).

Ink preparation: Two CMC/CNC inks were prepared by adding 6 (I1) and 8 wt.% (I2) of CNC in 1 wt.% of CMC dissolved in deionized (DI) water for 30 min using a vortex agitator at room temperature (23ºC). The powders were added to DI in small amounts to avoid clusters.

CMC and Laponite mixtures were prepared by adding 1 wt.% of CMC concentrations in 3 (I3) and 4.0 wt.% (I4) of Laponite. Both powders mixtures were dissolved in DI water under magnetic stirring followed by vortex mixing until reaching a homogeneous dispersion.

Rheological characterizations: Rheological properties were measured using a Modular Compact Rheometer (MCR-102, Anton Paar, Graz, Austria) equipped with parallel plate geometry of a 50 mm diameter and 1 mm gap. Steady shear viscosity was obtained in the range of 0.01 to 800 s-1. Viscosity curves were fitted to the Ostwald-de Waele viscosity model (Morrison 2001), as follows:

$$n(\gamma) = m\gamma^{n-1} \quad (1)$$

Where m is the consistency index associated with the magnitude of the viscosity and n is the power-law index defines the viscosity behavior.

Frequency sweep tests were performed inside the linear viscoelasticity region (γ_0 =1%) in the frequency range of 0.1 to 240 s^{-1}. Finally, three-interval thixotropy tests (3ITT) were performed to evaluate viscosity recovery's ability after the shear application. A low shear rate of 1s^{-1} was applied for 25 s, followed by a constant shear rate (100 – 700s^{-1}) applied for 50 s and back to 1s^{-1} for 250 s. Viscosity recovery percentage was calculated by:

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$$\eta_{rec} = \frac{\eta_{trec}}{\eta_i} x100$$

Where η_{trec} is the viscosity at a recovery time and η_i is the viscosity at rest interval.

3D Printing tests: All printing analyses were performed on the Educational Starter bioprinter (3D Biotechnologies Solutions, Brazil) using a 22G general-purpose needle tip of 12.22 mm in metal length with a 0.41 mm inner diameter (Nordson EFD, USA).

RESULTS AND DISCUSSION

Rheological studies allowed the analysis of the interaction mechanisms between Laponite platelets, CNC rods, and CMC polymer chains. An increase in viscosity and pronounced pseudoplastic behavior was observed when adding CMC in aqueous dispersions of Laponite and CNC. CMC solutions exhibited shifts from a near- Newtonian behavior to a substantial increase in viscosity and shear-thinning after adding CNC and Laponite particles. Moreover, the presence of CMC inhibited the aging of Laponite and induced physical gelation and thixotropy in the system with CNC and Laponite.

In the CMC/Laponite system, the changes in rheological properties can be associated with hydrogen bonds and electrostatic interactions between polymeric chains and Laponite platelets. The presence of hydroxyl groups (-OH) in CMC and silanol groups (Si-O) in Laponite induce physical interactions that strengthen the network, leading to changes in the viscous, viscoelastic and thixotropic rheological behavior, even adding small amounts of CMC. In the CMC/CNC system polymer–particle interactions can be associated with phase changes due to strong electrostatic repulsion between CNC rod-like and CMC chains, promoting the depletion interactions.

The accentuated shear behavior, rapid viscosity recovery, and solid-like viscoelastic behavior ensured that the inks could be dispensed and subsequently retain the shape of the printed object. Three printing parameters were analyzed: printing speed, extrusion multiplier, and printing distance. Each had different relevance in print depending on the nanoparticle used. Finally, aiming at tissue engineering applications, the cytotoxicity of the printed scaffolds was

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evaluated, showing that none of the two crosslinkers showed cellular toxicity.

FINAL CONSIDERATIONS

This work showed that rheological properties and printability studies are essential in developing inks for 3D extrusion printing. The different analyzes carried out to evaluate the printability of the inks showed that CMC is a versatile polymer that shows good printability when mixed with other nanoparticles. Rheological tests and filament formation, melting, and collapse tests allowed defining an ideal ink. The compositions of the ideal inks in the printing processes presented adequate properties to obtain printed objects with structural consistency, similar to those drawn in the CAD model. Finally, we believe CMC systems have great potential for biomedical applications that require such rheological behavior in highly hydrated systems, such as injection drug delivery systems and extrusion bioprinting.

Future works can complement the studies of crosslinking mechanisms and swelling properties in CMC/Laponite hydrogels and evaluate the printing parameters defined in this research in the fabrication of structures with complex shapes. In addition, the research can focus on printing cell-loaded inks (bioinks) using the CMC and the two different nanoparticles and evaluating cell viability and possible use in tissue engineering.

Keywords: Carboxymethylcellulose, Laponite, Nanocrystal Cellulose, Rheology, Printability.

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