DLP 3D printable carboxymethylcellulose hydrogels

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Abstract— **Recent progresses in Tissue Engineering are directed towards the development of technologies able to provide patient specific scaffolds. Additive manufacturing (AM) techniques combining with suitable materials are able to create a physiological milieu for cell growth. However, the development of bio-based biocompatible and sustainable photocurable materials for 3D printing is a stringent request to employ these techniques in biomedical applications. In this work, aqueous formulations based on methacrylate carboxymethylcellulose were produced and easily 3D-printed through a Digital Light Processing (DLP) apparatus with the aim to produce 3D shaped hydrogels with excellent swelling ability and mechanical properties. Envisaging the application of the hydrogels in the biomedical field, cytotoxicity is also evaluated, demonstrating the promising use in this filed.**

Keywords—methacrylate cellulose, digital light processing, bio-inks

INTRODUCTION

Additive manufacturing (AM) has gained increasingly interest in biomedical fields thanks to the possibility to produce automatized custom-made objects [1]. Among the different AM technologies light-assisted printing, including stereolithography (SL) and digital light processing (DLP) utilizes photopolymerization promoted by light irradiation to model complex structures with high resolutions [2]. There are extensive efforts towards the development of bio-based photo-curable formulations for light assisted printing technologies, however the number of nature-derived inks is still limited. Tonda-Turo et al. [3] described methacrylated chitosan-based hydrogel as bioink for cell encapsulation and fabrication of well-defined scaffold architectures by AM. In this work a platform of photocurable bio-inks based on carboxymethylcellulose was proposed as promising materials to recreate complex living tissues through DLP techniques.

MATERIALS AND METHODS

A. Materials

Carboxymethyl cellulose sodium salt (CMC) medium viscosity, methacrylic anhydride and lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) were purchased from Sigma Aldrich. For cytotoxicity test, DMEM - Dulbecco's Modified Eagle Medium purchased from Carlo Erba (Italy) was used.

B. Cellulose functionalization

CMC was modified by following a previously reported method[4]. 2.00 g of carboxymethyl cellulose sodium salt was dissolved in 100 mL of water at 50°C. The pH was periodically adjusted to 8.0 with 3N sodium hydroxide (NaOH) for the entire duration of the reaction. After cooling the solution at 4°C, 4 mL of methacrylic anhydride (MA) was added dropwise to the CMC solution and the reaction was continued for 24 h at 4°C. The resulting mixture was precipitated in ethanol and dialysed for 3 days to remove the unreacted methacrylic acid and methacrylic anhydride. After dialysis, the methacrylated cellulose (M-CMC) was freeze dried for two days.

C. Hydrogel preparation and printing

A water solution of M-CMC (20 mg.mL^{-1}) was prepared under magnetic stirring at 50°C for 1 h. Afterwards, the solution was allowed to cool down and 2 %w/w of bis(acyl)phosphane oxi lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) was added.

The solution was placed in the vat of a ASIGA UV-MAX DLP printer with XY pixel resolution of 62 μm using a light-emitting diode light source (385 nm); the layer thickness was fixed to 50 μm and the light intensity to 30 mW/cm^2 while the exposure time was varied for the different prints. A post-curing process performed with a medium-pressure mercury lamp (6 min with a UV lamp provided by Robot Factory; light intensity 12 mW/cm^2) followed the printing process.

D. Characterizations

Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) and ^{1}H NMR nuclear magnetic resonance were used to confirm the successful methacrylation of CMC and then to evaluate the photopolymerization reaction. Real-time photorheological measurements were carried out to investigate the kinetics of photopolymerization of the M-CMC hydrogel using an Anton PAAR Modular Compact Rheometer (Physica MCR 302) in parallel-plate mode equipped with a UV-light source (Hamamatsu LC8 lamp, light intensity 25 mWcm^{-2}). To evaluate the physico-chemical and mechanical characteristics of the different hydrogels: swelling and uniaxial compression test were carried out.

RESULTS AND DISCUSSIONS

Methacrilated CMC (M-CMC) has been synthesized using methacrylic anhydride (MA). Modification of the CMC was confirmed by Fourier-transform infrared spectroscopy (FT-IR) and proton nuclear magnetic resonance (^{1}H NMR). The The degree of substitution was calculated and corresponds to 0.73, which indicates as more than 50% of the carboxylate groups present in the CMC have been effectively grafted with methacrylic groups. The photo-curable properties of M-CMC based formulations were evaluated by photorheology. The kinetics of the photocrosslinking reaction was recorded turning on the light with 60 seconds delay; as visible in Figure 1, the gel point (G'=G'') was reached in 5 seconds and the reaction results almost completed within 60 seconds.

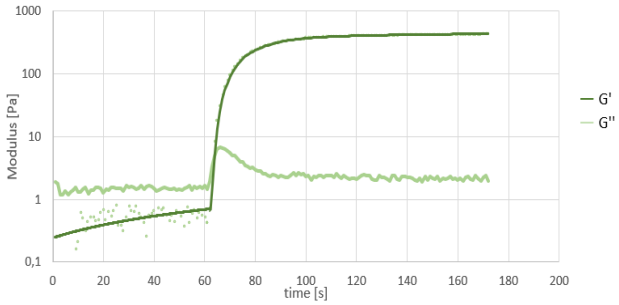

Figure 1. Photorheology tests performed on the water solution (20mg ml^{-1}) of M-CMC in the presence of LAP photoinitiator. Time sweep1Hz and 1% amplitude.

The reactivity and the final eleastic modulus (>500 Pa) of the cured hydrogel result suitable for the use of the material as DLP printable ink.

Thus, through DLP 3D printer, simple structures like cylinders (Figure 2 A, B) , parallelepipeds as well as more complex geometries were successfully fabricated both with water based solutions (Figure 2 C) and culture medium-based (Figure 2 D). In this latter case the dye present in the medium allows to obtain a better resolution competing with the photoinitiator in absorbing the light and avoiding thus the overcuring of the material.

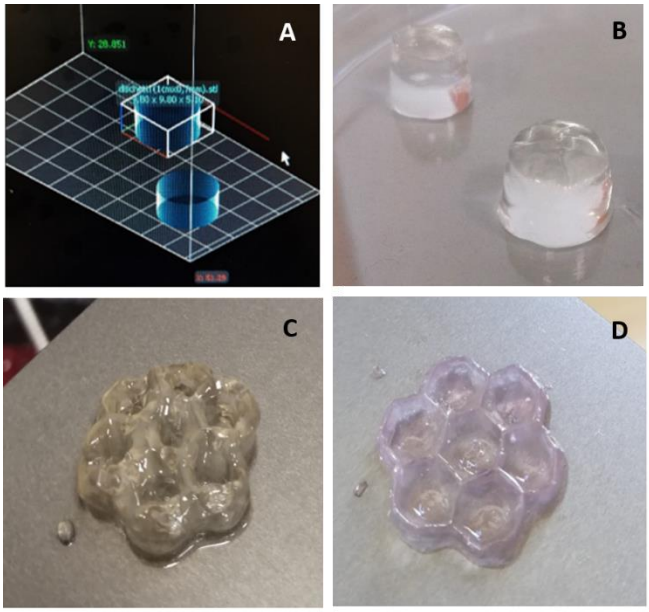

Figure 2. CAD design (A) and 3D printed cylinders (B) Honeycomb structure printed from a water based M-CMC solution (C) and from a culture medium based solution (D).

The 3D printed hydrogels appeared mechanically stable and flexible. The determination of the crosslink degree of the hydrogel was evaluated by extracting the soluble unreacted M-CMC from the insoluble fraction dipping the samples in water for 24h at 37°C. The crosslinked M-CMC fraction was calculated to 87 wt%. Compressive elastic modulus of cylindrical samples was measured and the compressive modulus was evaluated as the slope of the first linear region and calculated to be of 76 KPa. The swelling ability of DLP 3D printed M-CMC hydrogel was also evaluated. The possibility to further incorporate water in the hydrated state after printing, without breaking or deforming was assed showing the great potential of this material. The printed hydrogels were able to absorb up to 650 % water compared to the initial weight.

Finally, the 3D printed hydrogels solubilized in the culture medium underwent preliminary cytotoxicity testing. This confirmed the biocompatibility of the developed formulation as the cell viability of NIH/3T3 fibroblasts was 96.7 ± 5.2 % compared to CTRL.

CONCLUSIONS

The need of investigating the bio-based materials universe to make them suitable for 3D printing is a stringent request for the expansion of this brilliant production technique and, in the present case, the goal of producing a new ink for DLP printing starting from lignocellulosic based materials was successfully pursued. A metacrylated-CMC water based formulation was 3D printed and the resulting hydrogel presented extremely promising mechanical and swelling properties. Furthermore, the preliminary cytotoxicity tests confirmed its potential in the biomedical application field. This pave the way for future studies on 3D shaped scaffolds for *in vitro* studies or for tissue engineering applications. The further possibility to use cellulose-based fillers (e.g cellulose nanocrystals) could allow to tune the mechanical properties of the hydrogels that will be able to mimic the properties of different tissues.

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