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Hydroxyapatite and graphene oxide on nanocellulose-alginate ink for 3D bioprinting and bone regeneration

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1. Introduction

The incidence of bone-related diseases has been increasing due to aging of the population. Up to now, therapeutic approaches have been focused on graft and titanium implants, which have been associated with infection, pain and implant rejection [1]. Tissue engineering has become a promising treatment due to the use of scaffolds, which are designed to imitate as closely as possible the native tissue. In addition, three-dimensional (3D) bioprinting has become an emerging additive manufacturing technology in tissue engineering because of its rapid prototyping capacity and the possibility of creating complex structures [2].

This study is focused on the development of nanocellulose-alginate (NC-Alg) based bioinks for 3D bioprinting for bone regeneration. Furthermore, hydroxyapatite (HAP) and graphene oxide (GO) were incorporate as they are biocompatible, bioactive and osteoconductive materials [3].

2. Materials and methods

2.1. Bioink fabrication

To prepare NC-Alg-HAP bioink, Alg and HAP were dissolved in a D-mannitol solution to make an initial 10 % (w/v) and 5 % (w/v) solution, respectively. Then, NC was added. The final bioink proportion of NC:Alg-HAP was 80:20 (v/v). In order to prepare the NC-Alg-GO bioink, Alg and GO were dissolved in a D-mannitol solution to make an initial 10 % (w/v) and 250 μg/mL solution, respectively. Then, NC was added, being the final bioink proportion of NC:Alg-GO 80:20 (v/v).

2.1. Bioink characterization: Rheology

Two rheological procedures were carried out. Oscillation frequency sweeps from 0.1 to 100 Hz were established to study the bioink storage modulus (G') and the loss modulus (G''). The viscosity value was evaluated through shear rate sweep from 0.1 to 100 s-1 followed by a second sweep from 100 to 0.1 s-1.

2.3. 3D Bioprinting

Circular grid-like scaffolds were bioprinted using a 27 G conical nozzle at 30-32 kPa extrusion pressure and 4 mm/s printing speed. After printing, scaffolds were cross-linked with 100 mM CaCl2 solution.

2.4. Scaffold characterization

Scaffolds superficial and inner structures were examined by electron microscope. Then, swelling and degradation studies were performed.

2.5. Biological analysis

Cytotoxicity study was assayed using mouse L929 fibroblasts according to ISO 10993-5-2009. Murine D1 mesenchymal stem cells (D1-MSCs) were embedded in bioinks to evaluate cell viability and metabolic activity after bioprinting.

3. Results and Discussion

NC-Alg-HAP and NC-Alg-GO bioinks rheological frequency sweep measurements showed a higher storage modulus (G') than the loss modulus (G''), indicating an elastic solid- like behaviour. The viscosity curves showed a viscosity decrease under shear rate and an increase of viscosity when shear rate returned to initial values, indicating a shear thinning behaviour bioinks with thixotropic properties (Fig 1). These rheological properties suggested good printability through the extrusion bioprinter. No rheological differences were observed comparing to the control bioink (NC-Alg bioink).

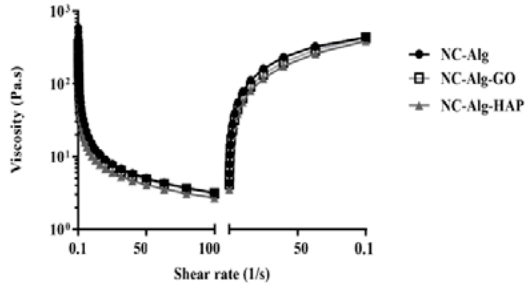

Fig. 1. Rheological properties. Viscosity curves of NC-Alg-HAP and NC-Alg-GO bioinks. NC-Alg bioink was used as a control.

The study on the scaffolds surface and structure demonstrated a good resolution and porous inner structure. In the swelling study water uptake by both printed scaffolds increased over the time until they reached the equilibrium, whereas degradation profiles showed little scaffolds area loss during the 10 days of study. Finally, cytotoxicity study demonstrated that bioinks were no cytotoxic as cell viability was above 70 % in all the assays (Fig.2). In addition, scaffolds with embedded cells showed metabolic activity and high viability after bioprinting.

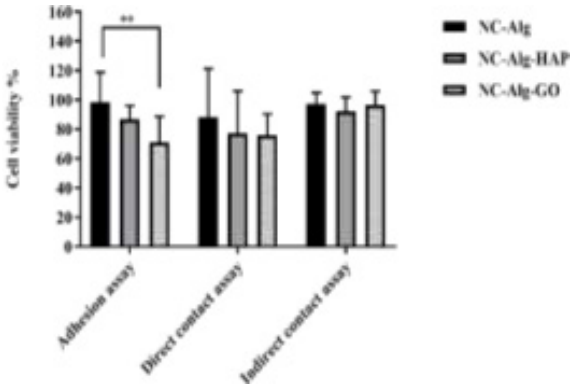

Fig. 2. Cytotoxicity analysis of NC-Alg-HAP and NC-Alg-GO bioinks in adhesion, direct contact and indirect contact assays. NC-Alg bioink was used as a control. Values represent mean ± SD. **: $p<0.01$.

4. Conclusions

Developed NC-Alg-HAP and NC-Alg-GO bioinks showed good properties for bioprinting through extrusion. Moreover, cell viability and metabolism of embedded D1-MSC cells have shown that bioprinted scaffolds may become a feasible tissue engineering approach for bone regeration.

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References

1. Zhang B, Hongyu L, Brochlausen C, He L, et al. Surface-decorated hydroxyapatite scaffold with on-demand delivery of dexamethasone and stromal cell derived factor-1 for enhanced osteogenesis. Materials Science & Engineering C. 2018;89:355-70.

2. Derakhshanfar S, Mbeleck R, Xu K, et al. 3D bioprinting for biomedical devices and tissue engineering: A review of recent trends and advances, Bioactive Materials. 2018;3:144-56.

3. Li M, Xiong P, et al. An overview of graphene-based hydroxyapatite composites for orthopedic applications. Bioactive Materials. 2018;3:1-18.

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