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PHOTOCROSSLINKED KAPPA CARRAGEENAN FOR TISSUE ENGINEERING APPLICATIONS

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KEYWORDS
Methacrylated kappa carrageenan, physical and chemical crosslinking, mechanical properties, cell encapsulation, tissue engineering

ABSTRACT
The ability to encapsulate cells in three-dimensional (3D) environments is potentially of benefit for tissue engineering and regenerative medicine. In this work, we introduce methacylated kappa carrageenan (Me-k-Ca) as a promising hydrogel for creating highly tunable mechanical platforms through modulation of methacrylation degree and gel concentration. Cells encapsulated in Me-k-Ca exhibited high viability. These data suggests that Me-k-Ca hydrogels could be used for tissue engineering applications that require certain mechanical features while preserving cell viability.

INTRODUCTION

Photopolymerization using acrylate and its derivatives to form hydrogels has many advantages over other gelation methods including fast curing rates. Kappa-carrageenan (k-CA), a natural origin polymer, has been recently proposed for tissue engineering applications (Perreira et al, 2009). However, k-CA hydrogels are produced only by means of physical crosslinking, yielding hydrogels with weak mechanical properties. To overcome this disadvantage, we propose a simple method to functionalize k-CA, rendering photocrosslinkable k-CA, to prepare hydrogels using a combination of physical and chemical crosslinking methods. We hypothesized that the physical and mechanical properties of the modified k-CA can be easily tuned by applying different crosslinking mechanisms.

MATERIALS AND METHODS

Methacrylated k-Carrageenan (Me-k-CA) was synthesized by reacting 1% (wt/v) of k-Carrageenan (k-CA, Sigma, Germany) in distillated water (diH_2O) with 4 or 8 mL of methacrylic anhydride (MA) so that methacrylated k-CA with different degrees of substitution could be obtained (Figure 1)

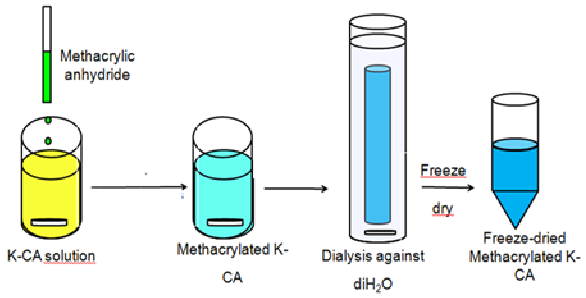

Figure 1. Schematic representation of the synthesis protocol.

The chemical modification of modified material was evaluated by: proton nuclear magnetic resonance (**^{1}H NMR)** and Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (**FTIR-ATR**).
The materials were dissolved in potassium chloride (**KCl**) or 0.5% (w/v) 2-hydroxy-1-[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propanone **Irgacure 2959**) solutions to fabricate hydrogels with different crosslinking mechanisms.

RESULTS AND DISCUSSION

Methacrylate groups were successfully incorporated into the k-CA polymer chains, leading to the production of Me-k-CA (Figure 2A).
The chemical modification as well as the degree of hydroxyl substitution by methacrylate groups were confirmed by ^{1}H NMR (Figure 2B).

The Fourier transform infrared spectroscopy with attenuated total reflection (FTIR-ATR) confirmed the

chemical modification of the k-CA by the presence of the C=C bond peak of methacrylic group appearing around 1400 cm^{-1}, present on both 4:1 and 8:1 formulations (Figure 3). The absorption band present around 1600 cm^{-1} corresponds to the C=O bond of the ester introduced in the chain. The enrichment in the C-H aliphatic groups was confirmed by the appearance of a peak at 2375 cm^{-1}.

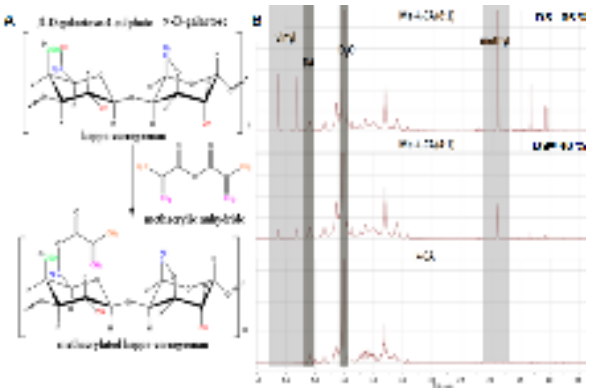

Figure 2. *Schematic illustration of the pH controlled synthesis (pH>8) of methacrylated k-CA (Me-k-CA) by reacting kappa carragenenan (k-CA) with methacrylic anhydride (MA). The esterification process consists in the subtitution of the side hydroxyl groups with photocrosslinkable metahcrylate groups (**A**). The ^{1}H NMR spectra (T=50ºC) of k-CA, Me-k-CA 4:1 and 8:1 recorded in deuterated water (D_2O). α-anomeric protons of the k-CA (DA) were located at δ 5.11 ppm and vinyl groups of the methacrylate were found around δ 5.7-5.3 ppm, while the methyl group of MA was located at δ 1.8 ppm. The D_2O peak at δ 4.3 ppm was used as internal reference. The degree of substituition (DS) was calculated as percentage (%) of the monomer hydroxyl groups substitution with the metahcrylate groups (**B**).*

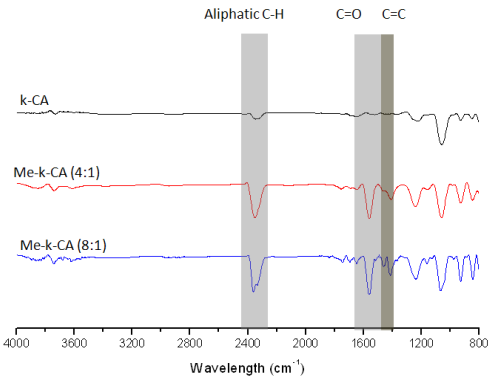

Figure 3. *FTIR-ATR spectra of k-CA, Me-k-CA (4) and Me-k-CA (8). All spectra were obtained at a resolution of 4 cm^{-1} in the range 4400–800 cm^{-1} for an average of 32 scans.*

K-CA is capable of reversible physical gelation from random coils to double helices when the temperature decreases. The gelation process was strongly affected by the presence of monovalent ions (eg. K^{+}). It was observed that the 4:1 and 8:1 Me-k-CA formulations were still able to form a hydrogel in the presence of cations, but not when the temperature was decreased. This might be due to the methacrylated groups that do not allow the formation of double helices and hydrogen bonds to confer a 3D structure. Besides that, the methacrylated k-CA, was also chemically crosslinked by UV curing. **Fig 4.** represents a schematic representation of these process.

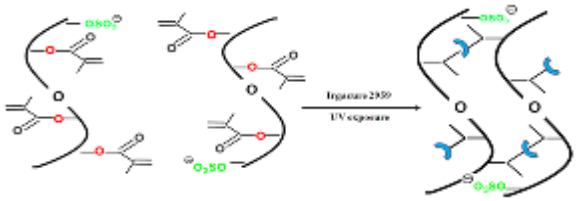

Figure 4*. Schematic representation of the chemical crosslinking of the methacrylated k-CA. To create a hydrogel network, the methacrylated k-CA was crosslinked using UV exposure in the presence of Irgacure 2959, as photoinitiator*

CONCLUSIONS

Herein, we demonstrated that k-CA can be chemically modified by the addition of methacrylate groups to the polymer chain. The methacrylation degree can be easily tuned, yielding polymers with variable physical- and photo- crosslinking abilities and thus tailored to specific functionalities in regenerative medicine applications.

REFERENCES:

Pereira, RC et al., J Tissue Eng Regen Med, 2009. **3**(2): p. 97-106.

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AUTHORS' BIOGRAPHIES

Silvia M. Mihaila was born in Brasov, Romania and has a bachelor degree in Chemistry and Physics from Materials Engineering Faculty (Transilvania University, Brasov). Under the scope of Erasmus Program, she spent 6 months at Coimbra University developing PVA membrane for encapsulation and release of porphyrinic compounds. She has worked as a researcher under PROf Ana Aguiar Ricardo (FCT, UNL) for the development of on/off polymeric system bu using supercritical fluid technology. Currently, Silvia M. Mihaila is enrolled in the MIT-Portugal Program and is a student at 3'Bs Research Group, University of Minho.