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Synergistic effect of κ-carrageenan and gelatin blends towards adipose tissue engineering

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ABSTRACT

The current paper focuses on the functionalization of κ-carrageenan and gelatin as extracellular matrix polysaccharide and protein mimic respectively to produce hydrogel films for adipose tissue engineering. More specifically, κ-carrageenan as well as gelatin have been functionalized with methacrylate and methacrylamide moieties respectively to enable subsequent UV-induced crosslinking in the presence of a photo-initiator. The gel fraction, the mass swelling ratio and the mechanical properties of both the one-component hydrogels and the protein/polysaccharide blends have been evaluated. The mechanical and swelling properties of the blends could be tuned by varying the hydrogel composition as well as the crosslinking method applied. The *in vitro* biocompatibility assays indicated a significantly higher cell viability of adipose tissue-derived mesenchymal stem cells seeded onto the blends as compared to the one-component hydrogels. The results show that the blends of gelatin and κ-carrageenan clearly outperform the one-component hydrogels in terms of adipose tissue engineering potential.

1. Introduction

Adipose tissue engineering holds promise for treatments associated with loss of the subcutaneous fat layer due to congenital defects, trauma or surgical resections including third-degree burns and women undergoing lumpectomies after breast cancer treatment (Flynn & Woodhouse, 2008). Current clinical strategies including the usage of soft tissue fillers and lipofilling procedures are associated with several drawbacks. These techniques are not suitable for filling large volumes ranging between 200 and 400 mL, lack the potential for adipose tissue regeneration and have only a temporary character resulting in an imposing need for repeated procedures (Alam et al., 2008). One of the most promising approaches to regenerate adipose tissue is based on the development of a scaffold that mimics the extracellular matrix (ECM) of native adipose tissue. The ideal scaffold should fulfil a number of requirements to function as an optimal candidate for adipose tissue regeneration. The matrix should be characterized by an interconnective porous network to facilitate cell migration and should provide an efficient nutrient/waste exchange (Torgersen et al., 2013). In addition, the materials should be biocompatible and biodegradable (Flynn & Woodhouse, 2008). Furthermore, the scaffold should provide sufficient mechanical support and should stimulate cell adhesion, proliferation and differentiation of the embedded cells into adipose tissue (Nakajima, Yamaguchi, Ozutsumi, & Aso, 1998). Several studies have already shown that the differentiation of adipose tissue-derived stem cells (ASCs) into adipocytes is improved when the stiffness of the scaffold is in the same range as the mechanical properties of native adipose tissue (*e.g.* 2 kPa for breast tissue) (Young & Christman, 2012). Hydrogels (*i.e.* crosslinked hydrophilic polymers) are appealing materials for the fabrication of these scaffolds due to their high water content, excellent biocompatibility and similar structural and mechanical properties to native tissue (Nguyen & West, 2002). Hydrogels can be constituted from synthetic, natural or semi-synthetic materials. Amongst the natural compounds, proteins and polysaccharides can be distinguished.

With respect to the latter material class, κ-carrageenan is an interesting compound that consists of alternating 4-linked 3,6-anhydro-D-

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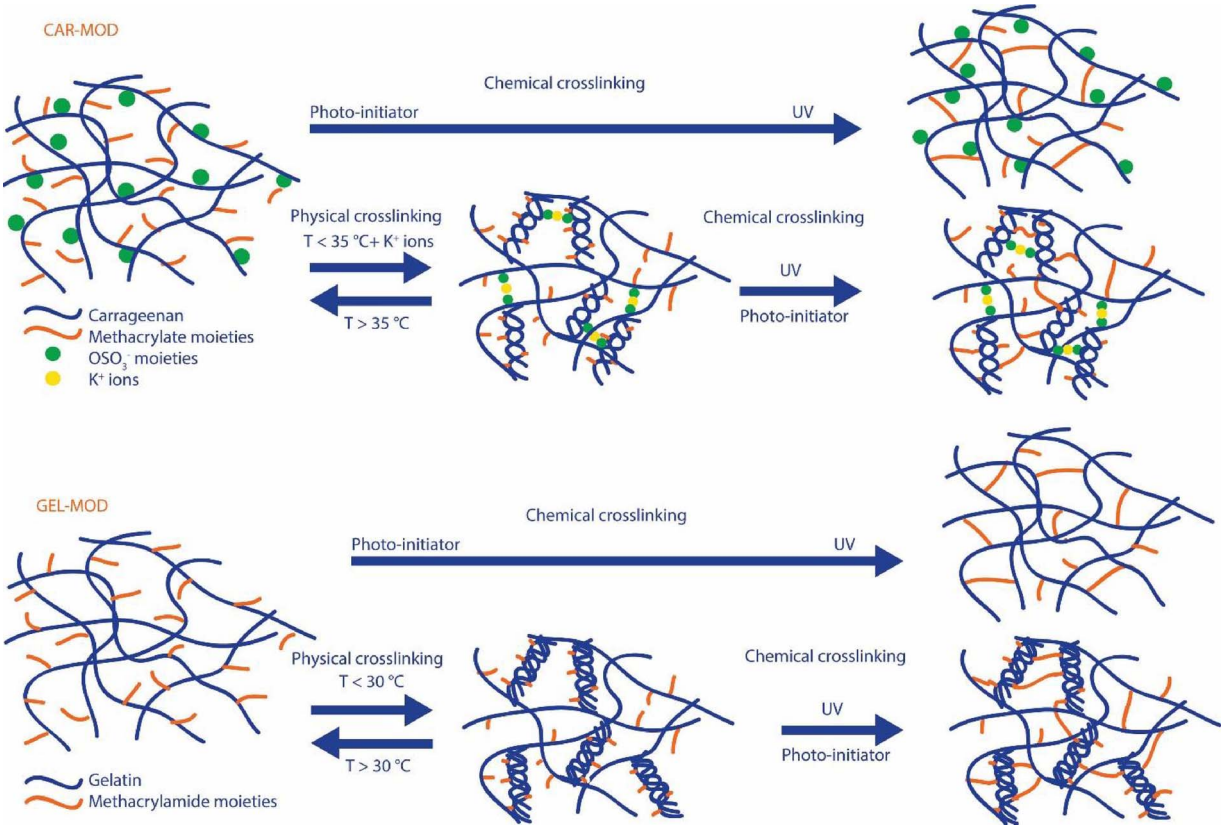

Fig. 1. Overview of the different crosslinking mechanisms for κ-carrageenan and gelatin.

galactose and 4-linked β-D-galactose-4-sulphate units. It is a suitable candidate for tissue engineering applications due to its gelation properties and its close resemblance to glycosaminoglycans which are present in the ECM of native adipose tissue (Rocha, Santo, Gomes, Reis, & Mano, 2011). κ-carrageenan forms a gel upon cooling (cfr. upper critical solution temperature (UCST) behavior with a transition temperature of 35 °C) in the presence of the appropriate potassium salt conditions as a result of hydrogen bonds and the occurrence of ionic interactions between the potassium ions and the sulphate moieties (Fig. 1) (Mihaila et al., 2013).

However, when cell-interactivity is a necessity, a frequently studied material for tissue engineering applications is gelatin. Gelatin is a single-stranded protein extracted from collagen which is the main component of the ECM (Graulus et al., 2015; Mariman & Wang, 2010). Gelatin exhibits cell-interactive properties due to the presence of arginine, glycine and aspartic acid (RGD) tripeptides within its backbone (Mariman & Wang, 2010; Van Nieuwenhove et al., 2016). Furthermore, the physical gelation of gelatin occurs when the temperature is below its UCST (*i.e.* 37 °C) by the formation of triple helices (Fig. 1) (Van Den Bulcke et al., 2000).

Both carrageenan and gelatin hydrogels have to be chemically crosslinked to avoid dissolution at body temperature. Different crosslinkers have already been described earlier in literature to create carrageenan and gelatin hydrogel blends. Padhi et al. (2016) used glutaraldehyde as crosslinker to produce hydrogel blends which are suitable for biomedical applications. Furthermore, Sharma, Bhat, Vishnoi, Nayak, and Kumar, 2013) investigated two different crosslinkers including glutaraldehyde and 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride-N-hydroxysuccinimide (EDC-NHS) to produce cryogel blends of gelatin and carrageenan for tissue engineering applications.

In the present work, we investigate the potential of photo-crosslinkable moieties which are incorporated along the backbone of both κ-carrageenan as well as gelatin to avoid the usage of potentially toxic crosslinkers such as glutaraldehyde and allow cell encapsulation in future experiments. More specifically, we have modified κ-carrageenan with methacrylate groups and gelatin with methacrylamide functionalities which can crosslink upon UV-irradiation in the presence of a suitable photo-initiator (Fig. 1). The physical and chemical crosslinking mechanisms can also be combined to obtain dual crosslinked hydrogels (Fig. 1) (Billiet et al., 2013; Mihaila et al., 2013).

The aim of the present study is to crosslink methacrylated κ-carrageenan and methacrylamide-modified gelatin as well as blends into hydrogel films which will be seeded with ASCs. We hypothesize that a combination of a sulphated polysaccharide and a protein will lead to a superior mimic of the ECM thereby optimizing the material biocompatibility and interaction with the ASCs. Furthermore, the effect of the biopolymer concentration and the crosslinking method on the crosslinking efficiency, the mechanical properties, the water uptake capacity and the crosslink density of the hydrogels developed will also be evaluated.

2. Materials and methods

2.1. Materials

κ-Carrageenan, extracted from red algae, was purchased from Sigma-Aldrich (Diegem, Belgium). Gelatin type B, isolated from bovine hides *via* an alkaline process, was kindly supplied by Rousselot (Ghent, Belgium). Methacrylic anhydride, sodium hydroxide (NaOH) and potassium chloride (KCl) were obtained from Sigma-Aldrich (Diegem, Belgium). Dimethyl sulfoxide (DMSO) (99.85%), potassium phosphate monobasic (KH_2PO_4) and sodium phosphate dibasic (Na_2HPO_4) were obtained from Acros Organics (Geel, Belgium). All ^1H NMR spectra were recorded in deuterium oxide (D_2O) purchased from Euriso-top (Saint-Aubin Cedex, France). The photo-initiator Irgacure 2959® (1-[4-