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#### **Conductive nanostructured materials based on poly-(3,4-ethylenedioxythiophene)**

#### (PEDOT) and starch/κ-carrageenan for biomedical applications

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#### Highlights

- Nanostructured PEDOT were synthesized using starch/κ-carrageenan aerogels as templates.
- $\kappa$  -carrageenan acted as an environmentally-friendly doping agent in the system.
- Nanostructured PEDOT showed good mechanical and electrical properties for biomedical applications.

• Properties of the obtained material are relevant for biomedical applications.

#### ABSTRACT

Smart electroactive biomaterials are sought to allow the direct delivery of electrical, electrochemical and electromechanical signals to biological tissues. Specifically, poly-(3,4-ethylenedioxythiophene) (PEDOT) is a polymer of special interest attending to its biocompatibility, tuneable electrical conductivity and processing versatility. In this work, nanostructured PEDOT was synthesized using starch/ $\kappa$ -carrageenan aerogels as templates.  $\kappa$ -carrageenan biopolymer acted as doping agent of the conductive polymer to enhance the biocompatibility and the electrical response. The physicochemical, morphological, mechanical and electrical properties of the nanostructured PEDOT and templates were characterized. The incorporation of  $\kappa$ -carrageenan to the nanostructured materials resulted in an increase in the compressive strength of *ca*. 40% and a decrease in the electrical impedance of one order-of-magnitude. The synergistic combination of the inherent electrical properties of the aerogel template resulted in electroactive PEDOT nanostructures with relevant properties for biomedical applications.

#### **Keywords:**

aerogel; κ-carrageenan; nanostructuration; supercritical CO<sub>2</sub> drying; PEDOT

#### 1. Introduction

A new generation of smart electroactive biomaterials are actively sought in tissue engineering to allow the direct delivery of electrical, electrochemical and electromechanical stimuli to cells (Mailloux & Katz, 2014). Conductive polymers containing stimuliresponsive delivery systems of neurotrophic factors, cell adhesion molecules or various drugs have been designed for the development of advanced tissue-electrode interfaces (Goding, Gilmour, Martens, Poole-Warren, & Green, 2015a). Poly-(3,4ethylenedioxythiophene) (PEDOT) is a conductive and electrochemically switchable polymer used in the stimulation and the recording of the signalling of central nervous system cells (Harman et al., 2015; Plato, Gustavsson, & Krantz, 1997; Richardson-Burns et al., 2007). Additionally, PEDOT is particularly interesting for tissue engineering purposes attending to its biocompatibility, electrical conductivity and processing versatility (Kaur et al., 2015 ;Chairam & Somsook, 2008).



Scheme 1. Chemical oxidation of the 3,4-ethylenedioxythiophene (EDOT) in the presence of iron (III) salts.

Polymerization of PEDOT takes place by electrochemical deposition or chemical oxidation. Namely, the chemical oxidation route (Scheme 1) is used to control the reaction sites since it depends on the availability of an oxidizing agent. The physicochemical and biological properties of the PEDOT depend on the nature and content of the doping agents. These doping agents are substances that allow the electrical conductivity in the polymer by forming strong charge trapping centres (polaron and bipolaron) (Vega-Rios, Olmedo-

Martínez, Farías-Mancilla, Hernández-Escobar, & Zaragoza-Contreras, 2014). Certain polysaccharides might be added as doping agents to favour the biocompatibility of the material whilst preserving the electrical properties.  $\kappa$ -carrageenan, a linear sulfonate polysaccharide, is particularly promising as a doping agent since the sulfonate groups of  $\kappa$ carrageenan may dope the conductive polymer by the interaction between the sulfonate groups and the said polymer (Pérez-Madrigal, Estrany, Armelin, Díaz, & Alemán, 2016). Moreover,  $\kappa$ -carrageenan may form double helix structures in the presence of cations (K<sup>+</sup>, Ca<sup>2</sup> +, Co<sup>2+</sup> and Fe<sup>3+</sup>) through ionic interactions with the sulfonate groups. The resulting structural arrangement promotes the aggregation to junction areas and the cross-linking of the polysaccharide-cation system with the conductive polymer thus improving the mechanical properties of the material (Fardioui, el kacem Qaiss, & Bouhfid, 2017; Kara, Tamerler, Bermek, & Pekcan, 2003; Li et al., 2016).

Synthetic scaffolds from organic polymers (e.g., PEDOT) with meso and macroporous structures and the appropriate surface chemistry are desirable for tissue engineering to support the growth of new tissues and to provide good mechanical properties for handling and for matching the solicitation in the intended implantation site (Goding et al., 2015a; Niño-Medina et al., 2010; Starbird, García-González, Smirnova, Krautschneider, & Bauhofer, 2014). Several methods have been proposed for the processing of scaffolds from organic polymers (e.g., emulsion templating, freeze-casting, emulsion polymerization, salt leaching) (Akay, Birch, & Bokhari, 2004; Kirdponpattara, Khamkeaw, Sanchavanakit, Pavasant, & Phisalaphong, 2015; Xiao, Zhu, Chen, & Zhang, 2017). Polysaccharides in the form of gels and aerogels have been applied as templates for the processing of scaffolds or as scaffolds themselves in tissue engineering as well as delivery carriers of bioactive compounds (Alnaief, Obaidat, & Mashaqbeh, 2018; García-González, et al., 2015; García-

González et al, 2012; Guex et al., 2017; Manzocco et al., 2017; Martins et al., 2015; Ren, Gong et al., 2016; Starbird et al., 2014). The processing of nanoporous PEDOT scaffolds using polysaccharide gels as templates has attractive advantages like easy removal of the template, mild operating conditions, availability of resources or low carbon footprint (Starbird et al., 2014; Xie, Wu, Xu, & Wang, 2015; Zhao et al., 2016).

In this work, the processing of PEDOT nanostructures using starch/ $\kappa$ -carrageenan aerogels as template is reported. The morphological, physicochemical and mechanical properties of the polysaccharide aerogels were characterized to provide a suitable template. PEDOT was then synthesized by the polymerization of the 3,4-ethylenedioxythiophene within the polysaccharide aerogel saturated with iron (III) chloride (oxidizing agent). The  $\kappa$ -carrageenan in the aerogel acted as doping agent. The PEDOT nanostructure was obtained by the removal of the polysaccharide template and the subsequent supercritical drying for the extraction of the solvent. The obtained nanostructured PEDOT was evaluated regarding its electrical and mechanical properties and cytoxicity to be used in tissue regeneration with electrical stimulation.

#### 2. Materials and methods

#### 2.1. Materials

Native corn starch (Starch Amylo N-460; amylose content: 52.6%) was provided by Roquette (Lestrem, France). Ethanol ( $\geq$  99.5 % purity) was obtained from Emparta (Madrid, Spain) and the  $\kappa$ -carrageenan from Ceamsa (Pontevedra, Spain). Iron (III) chloride (98% purity) was supplied by Acros Organic (Barcelona, Spain), 3,4ethylenedioxythiophene (EDOT, 97 % purity) by Sigma Aldrich (Madrid, Spain) and 2propanol by Panreac (Barcelona, Spain). Acetonitrile (HPLC reagent grade) was obtained from Merck (Darmstadt, Germany) and dimethylsulfoxide (DMSO, certified ACS reagent

grade) was purchased from Fisher Scientific (Madrid, Spain). Distilled water was used in all gelation experiments. CO2 (>99.9 mol% purity) was supplied by Praxair (Madrid, Spain). Roswell Park Memorial Institute 1640 Medium (RPMI 1640 Medium) was obtained from ThermoFisher Scientific (Madrid, Spain). Trypan blue solution 0.4% (w/v), retinoic acid and trypsin-EDTA were purchased from Sigma-Aldrich (Madrid, Spain).

#### 2.2 Preparation of cylindrical starch/k-carrageenan alcogels

10 wt. % starch aqueous dispersions with varying concentrations of  $\kappa$ -carrageenan (0-0.75 wt. %) were prepared in distilled water, at room temperature and with magnetic stirring for 5 min. The obtained dispersions were heated to 121 °C and pressurized at 1.1 bar for 20 min in a closed autoclave (Raypa, AES-12 model, Terrassa, Spain). The resulting clear and homogeneous solutions were stirred for 20 s and then immediately transferred to cylindrical polypropylene moulds (length: 40.0 mm; internal diameter: 12.4 mm). The moulds were sealed with Parafilm and, once gelified, stored at 4 °C during 48 h for retrogradation (García-González, Uy, Alnaief, & Smirnova, 2012). Then, the hydrogel cylinders underwent a direct solvent exchange procedure (García-González & Smirnova, 2013; Quignard, Valentin, & Di Renzo, 2008) by soaking the gel directly in ethanol and transferring them to fresh ethanol (second solvent exchange) after 24 h. Gels were denoted as SG-KCX, where X=0 to 0.75 depending on the weight content in  $\kappa$ -carrageenan in the initial dispersion.

#### 2.3 Supercritical drying of starch/ĸ-carrageenan gel cylinders

The starch/ $\kappa$ -carrageenan alcogels were dried by extraction of the solvent with a continuous flow of supercritical carbon dioxide (40 °C, 120 bar) of 5 g·min<sup>-1</sup> during 3.5 h in a 100-mL autoclave (Thar Technologies, Pittsburgh, PA, USA). This drying time corresponds to the optimum duration to achieve the complete solvent removal with a minimum impact on the

textural properties of the starch-carrageenan aerogel backbone (García-González et al., 2012; Goimil et al., 2017). The drying process was monitored by the measurement of the amount of ethanol extracted after certain time periods to ensure the complete drying of the gels (Fig. S1 in Supplementary Information). Aerogels obtained from SG-KCX gels were denoted as S-KCX.

#### **2.4 EDOT polymerization**

For the preparation of the PEDOT nanostructures, 150 mg of the starch/  $\kappa$ -carrageenan aerogel template was added to 2.5 mL of FeCl<sub>3</sub> 2.3 M in ethanol. After 24 h of mixing at 750 rpm, the gels acquired a yellowish appearance and then they were washed with 2.5 mL of ethanol. Subsequently, 2.5 ml of EDOT in 2-propanol (0.5 M) was added to the alcogels. After 24 h of reaction, a homogenous blue colour was obtained. The resulting gel was washed five times with 2.5 mL of 2-propanol. DMSO washes were performed to remove the template until no white precipitate from starch was observed after addition of few drops of acetonitrile to the DMSO solution. Finally, the PEDOT gel was washed twice in acetonitrile. PEDOT nanostructures were obtained by extraction of the acetonitrile through supercritical drying (*cf.* Section 2.3). The PEDOT nanostructures were denoted as P-0 and P-0.25 depending on the  $\kappa$ -carrageenan-starch aerogel (S-KC0 or S-KC0.25) used as template. PEDOT nanostructures without template removal were also obtained for the sake of comparison and denoted as P-SKC0 and P-SKC0.25.

#### 2.5 Materials characterization

Changes in volume (i.e. shrinkage) of the wet gels after solvent exchange and supercritical drying were studied by measuring the dimensions of the hydrogels, alcogels and aerogels in triplicate with a caliper (0.1 mm accuracy).

Textural properties of the starch/ $\kappa$ -carrageenan aerogels were determined by lowtemperature N<sub>2</sub> adsorption–desorption analysis (ASAP 2000 Micromeritics Inc.; Norcross, GA, USA). Before the measurements, samples were dried under vacuum (<1 mPa) at 60 °C for 20 h. Specific surface areas (A<sub>BET</sub>) of the aerogels were determined by applying the BET (Brunauer–Emmett–Teller) method. Specific pore volume (V<sub>p,BJH</sub>) and mean pore diameter (d<sub>p,BJH</sub>) were estimated using the BJH (Barrett–Joyner–Halenda) method.

The bulk density ( $\rho_{bulk}$ ) of the prepared cylindrical aerogels was determined by measuring their dimensions and weight. The skeletal density ( $\rho_{skel}$ ) of the aerogels were measured using a helium-pycnometer (Quantachrome MPY-2; Boynton Beach, FL, USA) operating at room temperature and 1.03 bar. Values were determined from five replicates. The resulting overall porosity ( $\epsilon$ ) was calculated from Eq (1).

$$\varepsilon = \left(1 - \frac{\rho_{bulk}}{\rho_{skel}}\right) \times 100 \tag{1}$$

Micrographs of the prepared starch/κ-carrageenan aerogels and PEDOT nanostructures were recorded by scanning electron microscopy (SEM, Zeiss FESEM ULTRA PLUS, Oberkochen, Germany). Samples were iridium-sputtered (10 nm thickness) prior to imaging in order to minimize charging and to improve the contrast of the images. Energy-dispersive X-ray spectra (EDX) of PEDOT-containing materials were obtained using an x-act Silicon Drift Detector (Oxford Instrument, Abingdon, Oxfordshire, England), connected to the SEM microscope and operating at a voltage of 20 kV and a working distance of 8.5 mm.

The compressive strength of the aerogel specimens was evaluated with a universal testing machine (Bionix MTS, Model 370.02, Tulsa, OK, USA) at the cross-head speed of 0.2  $\text{mm}\cdot\text{min}^{-1}$ . The recorded force was divided by the initial length of the specimen to

determine the compressive strain at 10 % deformation. The elastic moduli were obtained from the slope of the stress-strain curves. Compression tests were conducted at room temperature and five replicates of each specimen were used.

Thermogravimetric analysis (TG) of the aerogels was carried out with a TGA/DSC equipment (TA Instrument, model SDT Q600, New Castle, DE, USA). 10 mg of each aerogel was placed in the crucible and heated from room temperature to 700 °C, at a rate of  $10 \text{ }^{\circ}\text{C} \cdot \min^{-1}$  and under a nitrogen atmosphere.

The electrical behaviour of the conductive materials was evaluated for electrical resistance, and impedance. A voltage sweep was applied to the conductive materials located in the compression equipment at 2 % strain during the mechanical tests and the current value was recorded using a simple four-point probe (AUTOLAB, PGSTAT-302 model, Utrecht, Netherlands). The resistivity of each probe was obtained from the current-voltage curves corrected by the area and length. The conductivity is the reciprocal of the resistivity, and it was reported for the conductive nanostructures. Meanwhile, the impedance analysis was performed at 50 mV, in a frequency range of 0.1 to 10<sup>5</sup> Hz (AUTOLAB, PGSTAT-302 model, Utrecht, Netherlands). The Kramers-Kronig test and the data fitting were performed using the NOVA v.2.1 software (Utrecht, Netherlands). An equivalent model was built by a combination of resistors (R), capacitors and constant-phase-elements (CPE). The CPE pseudocapacitance was estimated by the NOVA v.2.1 software (Hirschorn et al., 2010).

#### 2.6. In vitro cytotoxicity tests

Human neuroblastoma SH-SY5Y cells (BRAINco Biopharma S.L.) were grown in RPMI 1640 medium with GlutaMAX<sup>TM</sup> supplemented with 10% fetal bovine serum, 100 U·mL<sup>-1</sup> penicillin, 0.1 mg·mL<sup>-1</sup> streptomycin, and 50  $\mu$ g·mL<sup>-1</sup> gentamicin, and they were maintained in standard conditions (37 °C, 95% humidity, 5% CO<sub>2</sub>). Cells were cultured

until 80% confluence and then seeded in duplicate in a 24-well plate pretreated with poly-D-lysine (400000 cells per well) and incubated at standard conditions. PEDOT nanostructured material (8 mg) was placed in each well with 400  $\mu$ L of medium and incubated at standard conditions for 2 hours before the cell seeding. After 24 hours, neuronal differentiation was induced by treatment with 10  $\mu$ M retinoic acid in culture medium supplemented with 1% fetal bovine serum, 100 U·mL<sup>-1</sup> penicillin, 0.1 mg·mL<sup>-1</sup> streptomycin, and 50  $\mu$ g·mL<sup>-1</sup> gentamicin for 3 days. Then, the PEDOT-based material was transferred to a 1.5-mL Eppendorf tube and washed with phosphate-buffered saline solution. Cells were detached from the material with trypsin-EDTA and 0.4% (w/v) trypan blue solution was added. The viability of the cells detached from the PEDOT-based material was calculated from the blue-stained cells and the dye-excluding viable cells counted with an optical microscope in a hemocytometer chamber.

#### **2.7. Statistical analysis**

Results were expressed as mean  $\pm$  standard deviation. One-Way ANOVA test and Two-Sample t-tests (statistical significance p < 0.05) were used to evaluate the effect of  $\kappa$ -carrageenan in the aerogels on the bulk density and the overall porosity of the aerogels (Minitab® 17.1.0, Erie, PA, USA).

#### 3. Results and discussion

# **3.1** Physicochemical and morphological characterization of mesoporous starch/κcarrageenan aerogels

Starch/ $\kappa$ -carrageenan aerogels were obtained in a cylindrical shape, they were mechanically stable and had a whitish colour, regardless of the  $\kappa$ -carrageenan content used. The overall volume shrinkages ranged from *ca*. 45% for S-KC0 and S-KC0.25 aerogels to *ca*. 70% for S-KC0.75 aerogels with respect to the original hydrogel volume (Fig 1). The obtained

shrinkage values were lower or similar to those reported in other studies for starch and  $\kappa$ carrageenan aerogels with values up to 70% (García-González & Smirnova, 2013; Ganesan & Ratke, 2014; Mehling et al., 2009)

Bulk densities of S-KC0 and S-KC0.25 aerogels were between 0.18 and 0.21 g·cm<sup>-3</sup> and had the highest overall porosity (87%) among all the tested formulations (Table 1). Mesoporous materials with similar bulk densities (0.07-0.46 g·cm<sup>-3</sup>) and overall porosities (*ca.* 90%) have been denoted as aerogels (García-González et at.,2011; García-González & Smirnova, 2013).



Figure 1. Overall volume shrinkage of starch/ $\kappa$ -carrageenan aerogel cylindrical monoliths at different  $\kappa$ -carrageenan contents.

Table 1. Physicochemical characterization of starch/ $\kappa$ -carrageenan aerogels. Values are expressed as mean  $\pm$  standard deviation (*n*=3).

Sample	A <sub>BET</sub>	V <sub>p,BJH</sub>	d <sub>p</sub> , <sub>BJH</sub>	Pbulk	$\rho_{skel}$	3
	$(m^2 \cdot g^{-1})$	$(cm^{3} \cdot g^{-1})$	(nm)	$(g \cdot cm^{-3})^*$	$(g \cdot cm^{-3})^*$	$(\%)^*$
S-KC0	235±12	1.12±0.06	17.5±0.9	$0.182\pm0.002^a$	$1.40\pm0.03^{a}$	$87.0\pm0.1^{a}$
S-KC0.25	226±11	0.88±0.04	13.1±0.7	$0.207 \pm 0.005^{b}$	$1.44\pm0.04^{a}$	$85.6\pm0.1^{b}$
S-KC0.35	219±11	1.10±0.06	17.7±0.9	$0.245 \pm 0.009^{c}$	$1.46\pm0.03^{a}$	$83.2\pm0.1^{\circ}$

S-KC0.50	231±12	1.25±0.06	17.9±0.9	$0.240 \pm 0.004^{\circ}$	$1.42\pm0.02^{a}$	$83.2\pm0.1^{\circ}$
S-KC0.65	214±11	1.15±0.06	19.2±1.0	$0.287\pm0.014^d$	$1.42\pm0.02^{a}$	$79.7\pm0.1^{d}$
S-KC0.75	194±10	0.79±0.04	14.7±0.7	$0.314\pm0.048^{\text{d}}$	$1.42\pm0.01^{\text{b}}$	$78.0\pm0.2^{\text{e}}$
P-SKC0.25	27±1	0.09±0.01	9.8±0.5	$0.200\pm0.022$	$1.24\pm0.03$	83.9 ± 0.1
P-0.25	8±1	0.02±0.01	7.9±0.4	$0.263\pm0.018$	$1.01\pm0.01$	$74.0 \pm 0.1$

\*Results were compared and equal letter (superscript) in the same column denotes statistically homogeneous groups (p<0.05).

Nitrogen adsorption-desorption isotherms of all the aerogel templates were type IV according to the IUPAC classification, which is typical of mesoporous materials (inset in Fig. 2). The specific surface areas ( $A_{BET}$ ) and mean pore sizes ( $d_{p,BJH}$ ) of the aerogels were almost independent of the concentration of  $\kappa$ -carrageenan, holding a value of 194-235 m<sup>2</sup>·g<sup>-1</sup> and 13-19 nm, respectively (Table 1). A certain trend towards lower  $A_{BET}$  values seems to take place with higher  $\kappa$ -carrageenan contents in the aerogel. The pore size distribution of all the aerogels were unimodal with a maximum in the 20-40 nm range (Fig. 2).



Figure 2. Pore size distribution of starch/ $\kappa$ -carrageenan cylindrical monoliths at different  $\kappa$ carrageenan contents from BJH-desorption measurements. Inset: Nitrogen adsorption– desorption isotherm obtained from S-KC0.25 aerogel, representative of all the S-KCX aerogels. Legend: S-KC0 (black squares), S-KC0.25 (white diamonds), S-KC0.35 (white triangles), S-KC0.5 (black triangles), S-KC0.65 (white squares) and S-KC0.75 (black diamonds).

The microstructure of the aerogel templates was analysed by scanning electron microscopy (SEM) (Fig. 3a,b). A three-dimensional (3D) porous structure formed by a mesh of fibrous strands was observed for S-KC0 and S-KC0.25 aerogels. Pores were interconnected throughout the aerogel structure in both cases. The main contribution of starch in the aerogel templates seems to have a strong influence in the structural organization of the biomaterial and resembled the network structures of starch aerogels (García-González & Smirnova, 2013; Goimil et al., 2017). The presence of  $\kappa$ -carrageenan in the aerogel formulation did not have a significant impact on the porous morphology although the fibrous network seemed to be denser, likely due to the formation of junction zones between the fibres promoted by  $\kappa$ -carrageenan. SEM images showed an attractive dual porosity for tissue engineering purposes with macropores and mesopores in a 77:23 and 79:21 ratio for S-KC0 and S-KC0.25 aerogels, respectively.



Figure 3. SEM images of the textural appearance of (a) S-KC0 (b) S-KC0.25, (c) P-SKC0, (d) P-SKC0.25 and (e) P-0.25 nanostructures.

# **3.2 PEDOT** nanostructuration from starch/κ-carrageenan aerogel templates and morphological characterization

The nanostructuration of PEDOT was carried out using starch/ $\kappa$ -carrageenan aerogels as templates and FeCl<sub>3</sub> as oxidizing agent. The effect of several oxidizing agents, particularly the use of iron (III) salts, for the synthesis of PEDOT (Scheme 1) has been previously studied (Elschner et al., 2010; Zhang & Suslick, 2015). The interaction between the polysaccharide template and the oxidizing agent during the EDOT oxidation provides the porosity of the nanostructured PEDOT (Starbird et al., 2014). Moreover,  $\kappa$ -carrageenan presents sulfonate groups that have been reported as doping agents in PEDOT films (Pérez-

Madrigal et al., 2016).

The aerogel templates (S-KC0 and S-KC0.25) and the conductive PEDOT nanostructures (P-0 and P-0.25) presented uniform whitish and blackish colorations, respectively (Fig. S2). EDX spectra (Fig. S3) confirmed that the PEDOT synthesis was effective since the relative percentages of sulphur (mainly from PEDOT) were closely related to the percentages of the added monomer content. The highest sulphur content was observed in the P-0.25 sample with an increasing signal ratio in the measurements, as a result of the removal of the starch template that contained less sulphur than the remaining PEDOT nanostructure.

SEM pictures showed that PEDOT polymerization preferentially took place in the junction areas of the fibrillar 3D network resulting in a less homogeneous structure (Fig. 3c,d). After the removal of the template, the resulting PEDOT nanostructure had a globular appearance forming aggregates (Fig. 3e). The PEDOT nanostructures (P-SKC0.25 and P-0.25 in Table 1) maintained the high porosity of the templates, although with a higher macropore-to-mesopore contribution (98:2 and 99:1, respectively). Two pore families were mainly obtained: the internal mesoporosity from the aggregates and the macroporosity between the aggregates. An open porosity was observed indicating interconnectivity of the pores. Even though nanoporous structures may not allow cellular in-growth due to the cell size, alternative cell growth strategies with mesoporous structures have been described for the improvement of long-term functions in osteoblasts (Low, Williams, Canham, & Voelcker, 2006; Webster, 2000). The obtained nanostructure for the PEDOT-based material can also be of interest for biomedical applications to improve cellular interaction, protein adhesion and drug delivery (Maleki et al., 2016; Ulker & Erkey, 2014).

#### 3.3 Mechanical properties of the aerogel templates and PEDOT nanostructures

Compression performance tests were used to evaluate the mechanical properties of the aerogels as a function of the  $\kappa$ -carrageenan content (Fig. 4). The highest value for the elastic modulus was obtained for the P-SKC0.25 aerogels (0.066 MPa) and the lowest was for the S-KC0 aerogels (0.034 MPa). The macroporosity and bulk density are important parameters in determining the collapse of the structure of the porous materials by compressive stresses (Schestakow, Karadagli, & Ratke, 2016). The elastic behaviour is dominated by the elastic deformation of the porous structure and the macropores freely deform by bending into their open space (Ganesan, Dennstedt, Barowski, & Ratke, 2016). The highest contribution of macropores was for the P-SKC0.25 aerogel (97.7%) and the lowest one was for the S-KC0 aerogel (76.6%). Accordingly, the incorporation of κcarrageenan in the aerogel caused an increase in the elastic modulus of *ca.* 40% (Fig. 4a). The polymerization of the PEDOT into the aerogel templates resulted in an enhancement of the mechanical properties, regardless of the  $\kappa$ -carrageenan content. The stress at 10% deformation for the P-SKC0.25 aerogel (0.74 MPa) confirmed that the addition of кcarrageenan to the formulation increased significantly (p < 0.05) the mechanical response by ca. 30% (Fig. 4b). Similar mechanical properties but with higher  $\kappa$ -carrageenan contents were denoted as structurally stable materials with high mechanical strength (Manzocco et al., 2017). It can be concluded that the mechanical properties were improved by the incorporation of the doping agent. Moreover, the improvement of the mechanical properties was directly related to the pore size and the bulk density.



Figure 4. Effect of  $\kappa$ -carrageenan on the starch/ $\kappa$ -carrageenan aerogel templates: (a) Elastic moduli and (b) stress at 10% deformation of S-KC0, S-KC0.25, P-SKC0 and P-KC0.25 aerogels.

#### **3.4** Thermal properties of the aerogel templates and PEDOT nanostructures

The thermal stability of the nanostructured materials was evaluated by thermogravimetric analysis (TGA) (Fig. 5). The initial weight loss at around 100 °C was associated with the dehydration of adsorbed and bound moisture in the aerogel network and it was evident in all the samples. Conductive materials (P-0, P-0.25, P-SKC0 and P-SKC0.25) showed a thermal loss at 222 °C related to the PEDOT degradation. This temperature of degradation is lower than the values in other studies (Khalil, Homaeigohar, Häußler, & Elbahri, 2016; Starbird et al., 2014), likely due to PEDOT-polysaccharide interactions. The PEDOT content in the P-SKC0 and P-SKC0.25 samples was accordingly estimated at *ca*. 28 wt. %. All the aerogels showed a degradation stage at 277 °C, related to the polysaccharide degradation (Starbird et al., 2014). The weight residues at 700 °C were higher in the conductive samples (P-0, P-0.25, P-SKC0 and P-SKC0.25) with regards to the aerogel templates (S-KC0 and S-KC0.25) and they may be related to the ions used in the

polymerization.

The nanostructured materials were analysed to determine their thermal properties by enthalpic studies (DSC). In all cases (Fig. 5b), there was an initial endothermic stage up to 120 °C and related to the water content. The conductive materials (P-0, P-0.25, P-SKC0 and P-SKC0.25) evidenced an endothermic process at 220 °C related to the PEDOT degradation, followed by an exothermic process. The templates (S-KC0 and S-KC0.25) evidenced an endothermic process at around 277 °C associated to the polysaccharide degradation.



Figure 5. Thermal properties of the nanostructured materials by (a) TGA, and (b) DSC analyses. Legend: S-KC0 (Black-dot), S-KC0.25 (Red-dash), P-SKC0 (Blue-solid), P-SKC0.25 (Green-solid), P-0 (Purple-solid) and P-0.25 (Cyan-solid).

#### **3.5 Electrical characterization of the PEDOT nanostructures**

The electrical conductivity is a critical parameter regarding the properties of nanostructured conductive materials. The electrical conductivity of the samples was determined during the mechanical tests using a four points probe at room temperature. The electrical conductivity

of the P-SKC0 and P-SKC0.25 samples were 0.29 and 0.35  $\mu$ S/cm, respectively. P-SKC0.25 sample showed a higher conductivity than the P-SKC0 sample and it is associated to the presence of  $\kappa$ -carrageenan in the formulation of the former sample. The conductivity values were lower than those reported for PEDOT thin films obtained from commercial formulations (10  $\mu$ S/cm to 120 S/cm) (Lövenich, 2014). The differences in conductivity may be explained by the nature of the doping agent, the material processing method and the lack of a compensatory parameter for the material porosity in the conductivity measurements (obtained from a current-voltage curve).

Electrical impedance spectroscopy analyses (EIS) of the nanostructure were performed to provide a comprehensive electrical study of the electrochemical system (Liu et al., 2007), including effects of the distribution of energy states, the porosity of the materials, mass transfer phenomena and the conductivity in the nanomaterial. The complex of the imaginary impedance (Z'') and the real impedance (Z') (Fig. 6a) showed an improvement in the impedance of ten times (green dots) when  $\kappa$ -carrageenan was added to the formulation. In accordance with the conductivity measurements, the impedance magnitude of P-SKC0.25 nanostructures (green dots in Fig. 6b) at all the frequency range was lower than that for P-SKC0 (blue dots in Fig. 6b).



Figure 6. Comparative impedance measurements at 50 mV (a) Nyquist and (b) Bode plots for the nanostructured conductive materials. Legend: P-SKC0 (blue squares) and P-SKC0.25 (green diamonds).

Nyquist plots for P-SKC0 (Fig. 7a) and P-SKC0.25 (Fig. 7b) nanostructures unveiled three events: (i) the contact resistance (x-intercept at the highest frequency region), (ii) the impedance that is linked with intraparticle porous material and (iii) the impedance related to mass transfer region (spikes at the low frequency region).



Figure 7. Nyquist plot determined from electrochemical impedance spectroscopy (a) P-SKC0 (blue squares) and (b) P-SKC0.25 (green diamonds) nanostructures at 50 mV. Arbitrary frequencies are remarked in red.



Figure 8. PEDOT nanostructure-equivalent circuit consisting of a series connection of two parallel R/CPE circuits.

The impedance response of the conductive materials was represented with a model (Fig. 8) consisting of an equivalent electrical circuit comprising a series connection of two parallel circuits. This model is usually associated with a device containing two layers or two regions with different electrical characteristics (Chen et al., 2010; Yuan, Song, Wang, & J., 2010). The parameters obtained from the modelling of the impedance data in the circuit model are reported in the Table 2. The CPE<sub>2</sub> and CPE<sub>3</sub> components denoted the capacitance of the material bulk. The  $\alpha$ -value describes the deformation of the ideal semicircle in the complex-plane plot attributed to the surface heterogeneity or to continuously distributed time constants for charge-transfer reactions (Barsoukov & Macdonald, 2005; Park & Yoo, 2003). The pseudocapacitance of the CPE ( $C_2$  and  $C_3$ ) in Table 2 were estimated at  $\alpha$ =0.8. This a-value was reported to describe the porosity of the materials (Barsoukov & Macdonald, 2005), the effects of the distribution of energy states and the spatially distributed conductivity in conductive polymers (Bisquert et al., 2000; Chen et al., 2010). A CPE component (W) ( $\alpha$ =0.5) was also added to describe the behaviour at low frequencies likely due to mass transfer processes in the conductive system (Yuan et al., 2010). The resistance of contact R<sub>c</sub> was similar in both systems, meanwhile the resistances R<sub>2</sub> and R<sub>3</sub> were one order-of-magnitude lower for the P-SKC0.25 sample. The interface pseudocapacitance  $(C_2)$  are similar in magnitude for both nanostructured systems and aligned with the typical bulk capacitance values of solid materials (10<sup>-12</sup> F) (Irvine, Sinclair, & West, 1990). The conductive nanostructure containing κ-carrageenan (P-SKC0.25) evidenced at least two orders-of-magnitude higher charge  $(C_3)$  than starch aerogels (P-SKC0) due to a higher doping level because of the presence of  $\kappa$ -carrageenan that enhanced the conductivity in the carrier-transport layer (Chen et al., 2010; Salzmann et al., 2016).

These results confirmed that the incorporation of  $\kappa$ -carrageenan in the PEDOT nanostructuration process improved the electrical behaviour of the PEDOT in the material. Table 2. Fitting parameters obtained from the modelling of the Nyquist plots of PEDOT nanostructures at 50 mV, following the equivalent circuit approach of Fig. 8.

	P-SKC0	P-SKC0.25
$(\Omega)$	$2.15 \cdot 10^4$	$6.40 \cdot 10^4$
$(\Omega)$	$5.96 \cdot 10^{6}$	$6.64 \cdot 10^5$
(mho)	9.68.10-11	$2.07 \cdot 10^{-10}$
(F)	$1.44 \cdot 10^{-14}$	$7.01 \cdot 10^{-14}$
(mho)	5.39·10 <sup>-10</sup>	$2.02 \cdot 10^{-8}$
(F)	$1.01 \cdot 10^{-13}$	$1.94 \cdot 10^{-11}$
$(\Omega)$	$2.26 \cdot 10^{6}$	$4.22 \cdot 10^5$
(mho)	$4.97 \cdot 10^{-7}$	$6.94 \cdot 10^{-6}$
	(Ω) (Ω) (mho) (F) (mho) (F) (Ω) (mho)	P-SKC0(Ω) $2.15 \cdot 10^4$ (Ω) $5.96 \cdot 10^6$ (mho) $9.68 \cdot 10^{-11}$ (F) $1.44 \cdot 10^{-14}$ (mho) $5.39 \cdot 10^{-10}$ (F) $1.01 \cdot 10^{-13}$ (Ω) $2.26 \cdot 10^6$ (mho) $4.97 \cdot 10^{-7}$

#### 3.6 Cytotoxicity test of PEDOT nanostructures

Conductive polymers have been proposed for several biomedical applications, being neuronal stimulation among them (Goding et al., 2015). Regarding this target application, the neuronal tissue growth should be promoted through 3D-matrices simulating the biological growth conditions as well as through electrical stimulation (Fattahi et al., 2014). Therefore, in this work, the cytotoxicity of PEDOT nanostructures (P-0.25) has been evaluated with the SH-SY5Y human neuroblastoma cell line, a well-established *in vitro* model for neurological studies including neurotoxicity (Tonazzini et al., 2014), differentiated to a neuronal phenotype and thus of relevance for the intended application. Viable cells (14805±5327 cells per mg of PEDOT nanostructure) with a cell viability of 88.3±8.9 % were obtained on the PEDOT-based material after 3 days of cell differentiation according to the viability assay. Moreover, the presence of the PEDOT in the cell growth

medium did not suppress neurite outgrowth during the differentiation process, since the presence of neurites was observed by optical microscopy (Fig. S4).

#### 4. Conclusions

Nanoporous PEDOT-based materials were prepared through a nanostructured templateassisted processing approach using starch/ $\kappa$ -carrageenan aerogels as templates. Starch/ $\kappa$ carrageenan aerogels had high specific surface areas (ca. 200 m<sup>2</sup>·g<sup>-1</sup>) and porosities (ca. 80-85%) and presented an attractive combination of mesoporosity and macroporosity. These templates were effective for the nanostructuration of the conductive polymer resulting in homogeneous and mechanically stable PEDOT nanostructures. Morphological properties of the resulting conductive materials are promising for tissue engineering due to their dual porosity and full pore interconnectivity. The resulting PEDOT nanostructures were compatible with the viability of SH-SY5Y human neuroblastoma cells differentiated to a neuronal phenotype seeded on the polymeric materials. The biopolymer  $\kappa$ -carrageenan contained in the template acted as a doping agent in the PEDOT-based material and notably improved the electrical and mechanical properties of the resulting nanostructured material. The presence of  $\kappa$ -carrageenan in the PEDOT nanostructures reduced the impedance ten times and increased by two orders-of-magnitude the capacitance. The unique combination of properties obtained for this conductive nanoporous material is of high relevance in the biomedical field as an electroactive extracellular matrix for cell stimulation and it will be the subject of further studies.

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