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Hydrogels for biomedical applications from glycol chitosan and PEG diglycidyl ether exhibit pro-angiogenic and antibacterial activity

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ABSTRACT

We aimed at producing a hydrogel from a chitosan (CS) derivative soluble in physiological conditions to avoid any purification step thus allowing to use the materials also as an *in-situ* forming material. So, we crosslinked glycol chitosan (GCS) with poly(ethylene glycol) diglycidyl ether (PEGDE) in water at 37 °C. The scaffolds, referred as GCS-PEG, were specifically designed to be used as wound dressing materials as such (after crosslinking) or as *in-situ* forming materials.

Different amounts of PEGDE were tested. The obtained scaffolds showed macroscopic pores and a tailorable swelling in water by controlling the crosslinking degree. Moreover, GCS-PEG scaffolds displayed a significant antimicrobial activity against *Staphylococcus aureus*. *In-vivo* study using the chick embryo choriallantoic membrane resulted in a highly pronounced pro-angiogenic activity suggesting important tissue regeneration properties. Moreover, the employed materials are commercially available, no organic solvents are required and the scaling up is quite predictable.

1. Introduction

The wound dressing concept *i.e.*, the control of wound bleeding and protection from environmental elements, is a continuously investigated topic in tissue engineering. Nowadays, an optimal material for wound dressing should also contribute to the promotion of the wound healing process and to the protection against bacterial infection.

In 1979, Turner outlined the main parameters that an ideal wound dressing should have (Turner, 1979). In particular, the wound dressing should maintain a high humidity at the wound/dressing interface, absorb exudates and toxic component from the wounds surface, allow gaseous exchanges, protect the wound from bacterial penetration (e.g., by showing antimicrobial activity), and it should be non-toxic and biocompatible.

So far, it seems that no single material can be endowed with all the requirements for the healing process. Thus, in many cases, the materials for wound dressing are made from blends of different components to ensure the presence of the above desirable properties.

Among the materials used to prepare wound dressings, polysaccharides such as hyaluronic acid (Palumbo, Pitarresi, Mandracchia, Tripodo, & Giammona, 2006; Pitarresi, Palumbo, Tripodo, Cavallaro, & Giammona, 2007; Tripodo et al., 2015), or polyesters (Wischke, Tripodo, Choi, & Lendlein, 2011), or chitosan (CS) have attracted the attention of many researchers (Di Gioia et al., 2015; Muzzarelli, 2009). CS, in particular, shows favorable properties including biocompatibility, biodegradability and non-toxicity. Moreover, it is hemostatic, shows hydrating activities and possesses antimicrobial properties against a wide variety of microorganisms such as fungi, algae and bacteria (Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003). However, CS applications are limited by its low solubility in neutral and physiological conditions (Trapani et al., 2014). This is why recent papers showed the preparation of wound dressing based on derivatized CS to make it more soluble in physiological conditions. Moreover, most of the recent literature reports on the preparation of fibers, hydrogels, membranes, scaffolds and sponges for wound dressing containing CS in association with different kind of polymers such as collagen, gelatin,

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PVA, sodium alginate, hyaluronic acid, to improve the wound healing properties (Wang, Zhu, Xue, & Wu, 2012).

In the present study, we aimed to prepare CS -based scaffolds starting from a commercially available CS derivative, as the glycol chitosan (GCS), chemically crosslinked with diepoxy PEG (PEGDE) under mild reaction conditions. GCS is a CS derivative that is water soluble in physiologic environment and possesses potentially useful biological properties for tissue engineering purposes since it is non-toxic and stimulates chondrocyte growth at low concentration (Amsden, Sukarto, Knight, & Shapka, 2007). The obtained hydrogel should possess all the benefits such materials bring with them such as retention of the 3D structure after swelling (Mandracchia, Pitarresi, Palumbo, Carlisi, & Giammona, 2004, 2011), physicochemical stability (Pitarresi, Pierro, Tripodo, Mandracchia, & Giammona, 2005; Tripodo, Pitarresi, Palumbo, Craparo, & Giammona, 2005), possible biodegradation (Pitarresi, Casadei et al., 2007; Pitarresi et al., 2008), responsiveness to external stimuli (Dispenza, Tripodo, LoPresti, Spadaro, & Giammona, 2009; Giammona et al., 2006; Mandracchia et al., 2018), and the possibility to employ different synthetic routes (Pitarresi et al., 2008; Pitarresi, Tripodo, Triolo, Fiorica, & Giammona, 2009). To the best of our knowledge, the chemical modification of GCS with PEGDE proposed here was not previously described in literature. PEGDE was selected because it should be able to play the dual function of water retaining polymer and crosslinker giving rise to hydrogels with different water affinity depending on the amount of PEGDE employed.

In literature some papers report on CS (and not glycol chitosan) crosslinked with an epoxy PEG which required purification steps and strong reaction conditions. Due to the use of CS, the Authors had to dissolve it in 0.4% acetic acid while diepoxyPEG 0.3–1.8 g was dissolved in water and added into the CS solution drop wisely. Then, CS and diepoxyPEG were reacted at 80 °C for 24 h under magnetic stirring (Wang et al., 2012). A similar approach was proposed by Tanuma et al. (2010). Also a review on the topic shows alternative approaches (Casettari et al., 2012). Recently (2017), a paper proposed the crosslinking of carboxymethyl chitosan with a diepoxy PEG but in NaOH solution at 60 °C (Gonzalez, Edlund, Vidaurre, & Ribelles, 2017).

Considering the precedent literature, we aimed at producing a hydrogel from a water soluble (in physiological conditions) CS derivative to avoid any purification thus enabling to use the materials also as in-situ forming material.

GCS-PEG scaffolds, indeed, were prepared by using GCS water dispersions at two different GCS concentrations (1 or 2 wt/vol%) by adding four different amounts of the cross-linker PEGDE in order to verify the influence of the concentration of the crosslinker on the main properties of the scaffolds. The reaction was performed in water at 37 °C. The prepared scaffolds have been characterized by FT-IR spectroscopy, thermal analysis (DSC) and scanning electron microscopy (SEM). Moreover, some technological features have been evaluated such as the mass loss upon washing in water as well as the water uptake of these scaffolds. Interestingly, it was found that the GCS-PEG scaffolds displayed a significant antimicrobial activity against *Staphylococcus aureus* and a pronounced pro-angiogenic activity which suggest together a high potential of application as wound dressing.

2. Materials and methods

All reagents were of analytical grade, unless otherwise stated. Glycol chitosan (GCS) and Poly(ethylene glycol) diglycidyl ether 500 Da (PEGDE) were purchased from Sigma-Aldrich (Milan, Italy). Muller Hinton Broth medium was purchased from Oxoid (Milan, Italy). Microtiter 24 wells Costar® were purchased from Corning Incorporated (New York, USA). *Staphylococcus aureus* ATCC 29213 was purchased from Biogenetics Srl (Italy) Microbiologics St. Cloud (Minnesota, USA).

2.1. Preparation of glycol chitosan-PEG (GCS-PEG) scaffolds

Exactly weighed amounts of GCSwere dissolved in double-distilled water to form homogeneous dispersions at two different concentrations of 10 mg/mL (1 wt/vol%) and 20 mg/mL (2 wt/vol%), the two GCS dispersions named GCS1% or GCS2%. Then, a calculated amount of PEGDE was added to the GCS solutions and vortexed for 1 min to ensure an optimal mixing. To each GCS solution, *i.e.,* 1 or 2 wt/vol%, PEGDE was added to gain different molar ratio PEGDE to GCS corresponding to 8, 16, 33 or 50 mol/mol% (expressed as moles of PEGDE/moles of GCS repetitive units). Depending on the GCS concentration and PEG to GCS ratio, the samples were named as: GCS1%-PEG8%, GCS1%-PEG16%, GCS1%-PEG33%, GCS1%-PEG50%, GCS2%-PEG8%, GCS2%-PEG16%, GCS2%-PEG33% or GCS2%-PEG50%.

The final solutions were placed into glass tubes and left at 37 °C overnight. The obtained GCS-PEG gels were washed with 3×20 ml of water, freeze-dried and characterized.

2.2. FT-IR analysis

ATR-FT-IR spectra were recorded in the range 4000–400 cm^{-1} using a Perkin–Elmer 1600 IR Fourier Transform Spectrophotometer (Monza, Italy). The resolution was 4 cm^{-1}.

2.3. Differential scanning calorimetry (DSC)

DSC studies were performed using a Mettler Toledo DSC 822e STARe 202 System equipped with a DSC Mettler STARe Software. Aliquots of about 2 mg of each sample were placed in an aluminium pan before performing DSC measurements. Then, each calorimetric pan was hermetically sealed and submitted to DSC analysis, consisting of a heating scan from 25 to 250 °C (preliminary studies did not evidence any thermal event below 25 °C), at the rate of 20 °C/min under a nitrogen flow at 20 $cm^3 \cdot min^{-1}$. The calorimetric system was calibrated in transition temperature using indium (purity 99.9%) and following the procedure of the Mettler STARe Software. Reproducibility was checked by running the sample in triplicate.

2.4. Mass loss upon washing in water

GCS-PEG samples for mass loss experiment were prepared as indicated before but without washing in water. Lyophilized samples of GCS-PEG were weighed (Wd1) and placed in 50 mL tubes containing 30 mL of double distilled water and left at 37 °C for 24 h by gently stirring in an orbital shaker. The water was changed three times during the 24 h. After this time, the scaffolds were collected, freeze dried and weighed (Wd2). The experiments were performed in triplicate and the data were expressed as retained mass, Eq. (1):

$$\text{Mass retained (\%)} = \text{Wd2/Wd1} \cdot 100 \quad (1)$$

2.5. Water uptake studies

The water uptake studies on GCS-PEG scaffolds were performed in double-distilled water. Firstly, considering the results from mass loss, the lyophilized samples were subjected to extraction in water for 24 h, then lyophilized again. Each dry scaffold was carefully weighed (W_1) and allowed to swell in water for 24 h. After this time, the scaffolds were recovered and the excess of water was gently removed by filter

paper and weighed (W_2). The swelling ratio was calculated as, Eq. (2):

$$\text{Water uptake (\%)} = (W_2\text{-}W_1)/W_1 \quad (2)$$

2.6. Scanning electron microscopy

Morphology of the surface and the cross-section of the lyophilized GCS-PEG samples was studied by a ∑igma Zeiss field emission scanning electron microscope (FE-SEM).

Prior to the analysis, the samples were stuck on stubs with a carbon adhesive disc and coated with a 2 nm palladium layer by an electron beam evaporator.

The SEM probing e-beam was set at an acceleration voltage of 3 kV; 30 μm slit aperture and the in-lens detector were used.

2.7. Antimicrobial activity

Antimicrobial activity tests were performed by recording the number of growing bacteria colonies on petri dishes.

Staphylococcus aureus ATCC 25923 was used as test organisms, which was prepared from fresh colonies on Mueller Hinton Agar (MHA, Oxoid). Bacterial suspension (used for the test) was composed of 2–3 colonies of the strain taken from an MHA plate and dissolved in 2 mL of Muller Hinton Broth (MHB). The resulting suspensions were diluted with 0.85% NaCl solution, then adjusted to 1×10^8 CFU/mL (0.5 Mc Farland) and used for the antibacterial test.

The preparation of the experiment with standard plate count method consisted of addition of standard inocula to 3 mL of MHB in every well of the plate (24 wells) containing the samples (10 mg of lyophilized GCS (used as control) or GCS-PEG).

After the incubation time 24 h or 48 h at 37 °C, 100 μL of the bacterial culture in contact with the samples, was diluted with phosphate buffer diluent (in this way the bacteria are dilute enough to count accurately). A wide series of dilutions (e.g., 10^{-4} to 10^{-10}) is normally plated because the exact number of bacteria is usually unknown. The plates were incubated for 24 h or 48 h at 37 °C, and the number of colonies formed were counted and the growth inhibition rate (IR) was calculated as Eq. (3) (Zheng & Zhu, 2003).

$$IR(\%) = N_0 - N_1/N_0 \times 100 \quad (3)$$

N_0 and N_1 are the average number of colonies of the control group and the experimental groups, respectively.

2.8. Chick embryo choriallantoic membrane (CAM) assay

Fertilized White Leghorn chicken eggs (20 per group) were incubated at 37 °C at constant humidity. On day 3, a square window was opened in the shell, and 2–3 mL of albumen were removed to allow detachment of the developing CAM from the shell. The window was sealed with a glass, and the eggs were returned to the incubator. On day 8, eggs were treated with 1 mm^3 sterilized fragments of GCS1%-PEGxx and GCS2%-PEGxx or a gelatin sponge soaked with 50 ng vascular endothelial growth factor (VEGF), used as a positive control was placed on the top of the growing CAM, as previously described (Ribatti, Nico, Vacca, & Presta, 2006). CAMs were examined daily until day 12 and photographed *in ovo* with a stereomicroscope equipped with a camera system (Olympus Italia, Rozzano, Italy). At day 12, the angiogenic response was evaluated by image analysis. Briefly, microscopic images obtained from the stereomicroscope were converted in gray-scale and analyzed using the Aperio Positive Pixel Count algorithm embedded in the ImageScope (Leica Biosystems, Nussloch, Germany). The algorithm input parameters were initially set to obtain the identification of pixels related to the blood vessels as strong positive and to the background as medium and weak positive and tuned to minimize non-specific pixel recognition as strong positive. The algorithm output is composed of the number of strong positive pixels (Nsp), the number of medium positive pixels (Np), the number of weak positive pixels (Nwp). A morphometric value is then defined and calculated by the algorithm as: *Number of strong positive pixels* (%) = $Nsp/Np + Nwp + NspX100$. Statistical significance between the stimulated, normal and inhibited experimental groups was assessed by one-way Anova followed by Tukey multiple comparison post-test. The statistical analysis and graph plotting were performed with the Graph Pad Prism 5.0 statistical package (GraphPad Software, San Diego, CA, USA).

2.9. Statistics

Data from different experimental groups were compared by a one-way analysis of variance (ANOVA) with $p < 0.05$ at 99% level of confidence (GraphPad Prism v. 4.00 GraphPad Software, Inc., San Diego, CA). Bonferroni post tests were used for post hoc contrast.

3. Result and discussion

Here, we showed an effectively simple way to yield a chemical crosslinking of glycol-chitosan (GCS), by applying a one-step procedure, in water, at 37 °C which, predictably, does not require a post-production purification. Even the used crosslinker, the Poly(ethylene glycol) diglycidyl ether, is one of the most accepted polymer for biomedical applications and as such, considered highly biocompatible.

With respect to the recent literature, mostly focusing on CS crosslinking, or more recently carboxymethyl chitosan, we do not use any solvent other than water and the pH is not adjusted by using either acid or base (Gonzalez et al., 2017; Tanuma et al., 2010; Wang et al., 2012). Furthermore, we were able to cure the hydrogel at 37 °C which is the physiological temperature.

GCS-PEG scaffolds were prepared by using GCS water dispersions at two different GCS concentration (1 or 2 wt/vol%) by adding four different amounts of the cross-linker PEGDE. The preparation was effective and reproducible, so that, starting from an aqueous dispersion of GCS plus different amounts of PEGDE at 37 °C, transparent hydrogels were obtained, Fig. 1a (for additional pictures please see SI1-3).

The GCS-PEG hydrogels were purified in water and, then, were lyophilized to give sponge-like structures, Fig. 1b. The lyophilized materials are resistant to manipulation with a typical monolithic structure. After re-hydration, the materials showed a coherent translucent aspect, Fig. 1c.

The synthesis of GCS-PEG hydrogels takes place as result of the nucleophilic attack of GCS amine groups to epoxy group of PEGDE, Fig. 1.

To better assess the main physicochemical behaviours of GCS-PEG hydrogels, thermal studies were performed to see whether the obtained hydrogels could show some crystallinity or Tg. Really, the DSC data gained from the tested samples, do not follow a predictable trend. It would depend from the degree of hydration of the polymers, on the other side, while we found a dehydration and melting temperature for GCS, respectively as such from vendor and after freeze drying, that were not found in other papers, Table 1 (Singh et al., 2018).

The thermogram of non-crosslinked GCS, after freeze drying, showed a melting peak at around 142.1 °C. The same melting peak was found in the crosslinked samples but at higher temperature than native GCS indicating a higher degree of molecular stability for the networks. Only three samples, namely, GCS1%-PEG50%, GCS2%-PEG16% and GCS2%-PEG33%, did not show thermal transitions attributable to a melting point.

Therefore, as seen, the freeze drying induced some modification in the thermal profile of non-crosslinked GCS. The polymer, when analysed as just received from the vendor, showed a broad loss of wa-

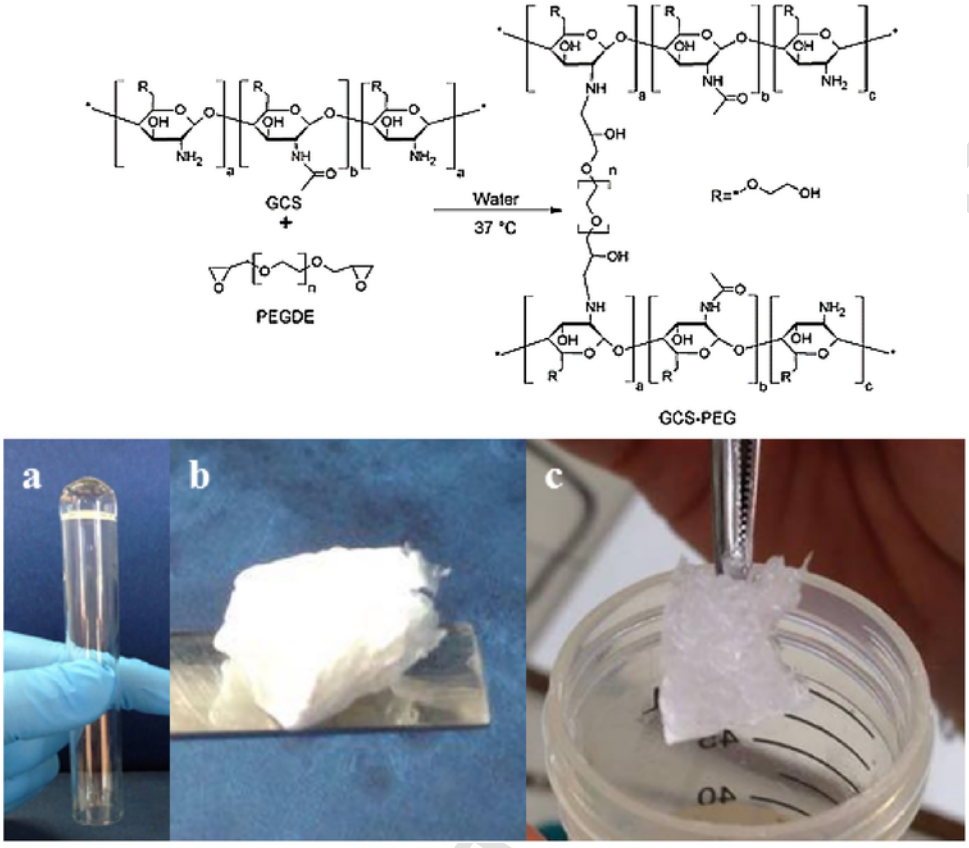

Fig. 1. Schematic representation of the chemical crosslinking of GCS with PEGDE in water at 37 °C and optical images of GCS-PEG 1% (w/v) crosslinked with PEGDE 0.08% (mol/mol). In particular: a) just prepared GCS-PEG hydrogel; b) freeze dried GCS-PEG; c) swollen after freeze drying.

Table 1
DSC main thermal behaviors of GCS-PEG materials (n = 3).

Sample	Dehydration °C midpoint	T_m °C peak
GCS as such	**112 ± 0.2**	NA
GCS Lyo	NA	**142.1 ± 0.4**
GCS1%-PEG8%	NA	**160.2 ± 1.2**
GCS1%-PEG16%	NA	**160.3 ± 0.8**
GCS1%-PEG33%	NA	**168.6 ± 0.7**
GCS1%-PEG50%	**98.5 ± 1.4**	NA
GCS2%-PEG8%	NA	**154.3 ± 1.3**
GCS2%-PEG16%	**125.6 ± 1.0**	NA
GCS2%-PEG33%	**112.4 ± 0.8**	NA
GCS2%-PEG50%	NA	**166.3 ± 0.5**

ter at around 100 °C, after freeze drying, a melting effect at 142.1 °C was observed. The most reliable explanation would be that the hydration water, before elimination by freeze drying, separated and softened the polymer chains avoiding a molecular rearrangement toward an ordered stacking, thus preventing crystallization Fig. 2.

This hypothesis is supported also by the thermal events found in the crosslinked systems. In fact, some of them showed a melting temperature, while others did not. It indicates that the exposure to the air, even for short periods, can hydrate the samples leading to amorphous products.

The FTIR spectra showed the peak at around 1100 cm^{-1}, attributable to the stretching of C—O bond from ether groups belonging to PEG (Fig. SI1) confirming the presence of PEG within the GCS chemical structure. Moreover, the absence of the characteristic peaks at ≈ 911 and 839 cm^{-1} attributable to the epoxy ring, further support that they are consumed or hydrolyzed during the crosslinking process (Fig. 3).

Fig. 4a shows the mass loss of GCS-PEG scaffolds after contact with water for 24 h. Notably, by increasing the amount of PEGDE for both GCS1% or GCS2%, the mass loss increases. This trend was maintained for all the samples except for GCS1%-PEG16% for which mass loss was slightly lower than GCS1%-PEG8%.

Water uptake properties are critical for scaffolds used as wound dressing because they must absorb a large amount of wound exudates also preventing bacterial invasion of the wounds.

Lyophilized scaffolds were characterized for their water uptake ability after 24 h of incubation at 37 °C. As reported in Fig. 4b, the water uptake is higher for GCS1%-PEG8% scaffolds reaching 30 times the original (dry) weight. On the other side, GCS2%-PEG8% scaffolds showed a 15 times higher weight with respect to the dry state.

In this way, depending on the application, the water uptake ability of a single material could be tailored by controlling the amount of crosslinker.

The SEM images of the scaffolds GCS-PEG at two different concentrations and with a PEGDE contents of 50 mol/mol% are shown in Fig. 5.

The upper surface morphology of both samples appears smooth and without any evident discontinuity. On the other side, the cross section showed stratified foil-like structures with pores and connections. However, the thickness of the "foil" forming the different layer of the material was measured, showing that the materials at lower concentration in PEG but at the same concentration in GCS, have different thicknesses, i.e., ≈ 0.7 μm for GCS1%-PEG8% and ≈ 2 μm for GCS1%-PEG33%, see SI4. Obviously these data are not statistically significant but could give a qualitative information on the final materials.

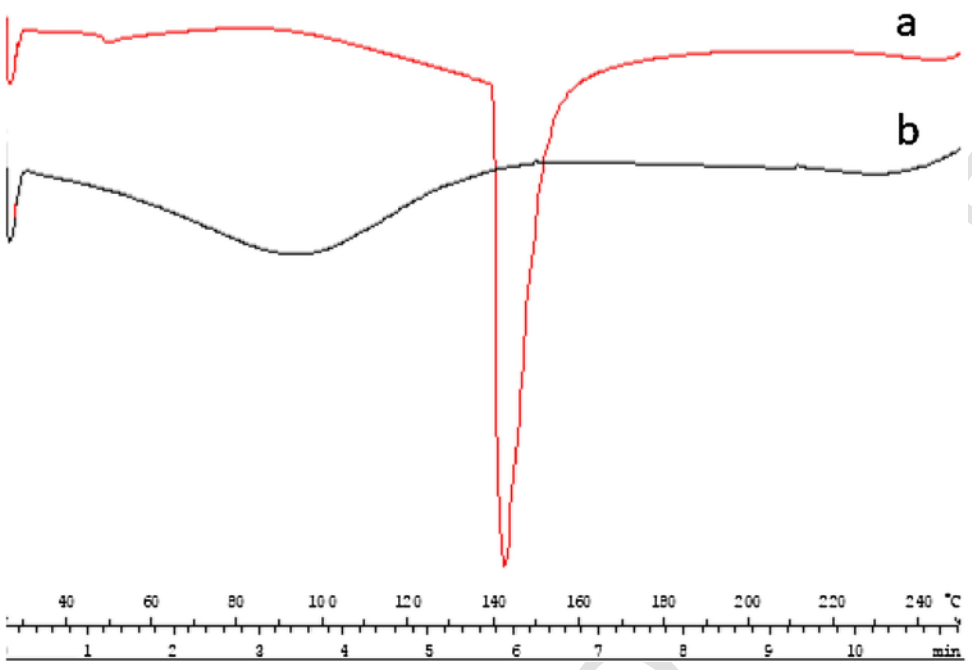

Fig. 2. Thermograms of a) GCS after freez drying, b) GCS before freez drying.

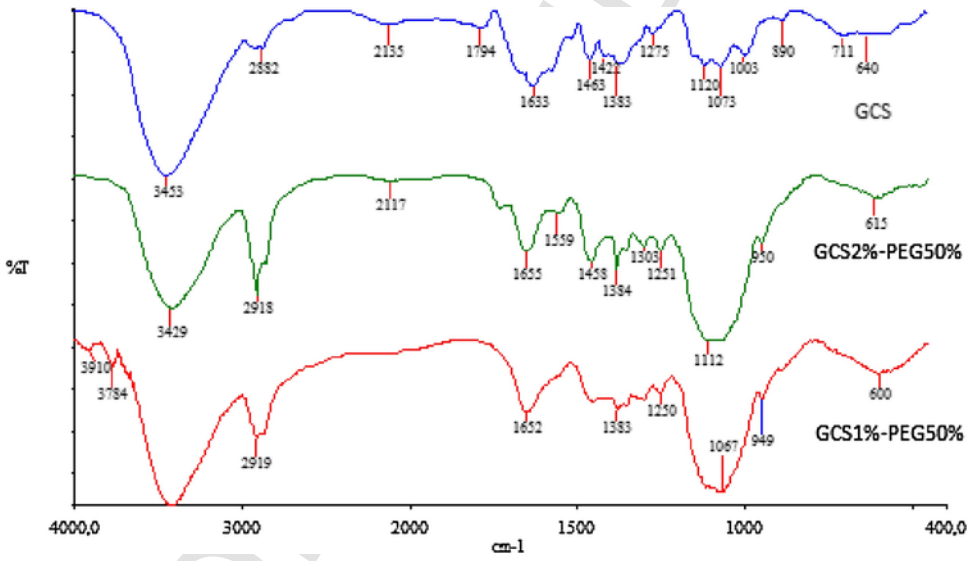

Fig. 3. FTIR spectra of GCS, and two representative samples of GCS-PEG scaffolds; GCS 2% dispersion crosslinked with 50 mol/mol% of PEGDE (GCS2%-PEG50%) and GCS 1% dispersion crosslinked with 50 mol/mol% of PEGDE (GCS1%-PEG50%).

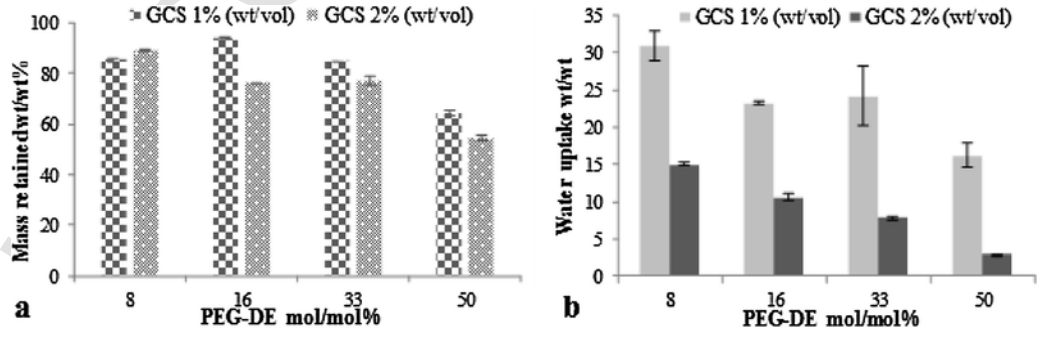

Fig. 4. a) Mass loss experiment on GCS-PEG scaffolds in bidistilled water (expressed as retained mass), n = 3; and b) water uptake for GCS-PEG scaffolds, n = 3.

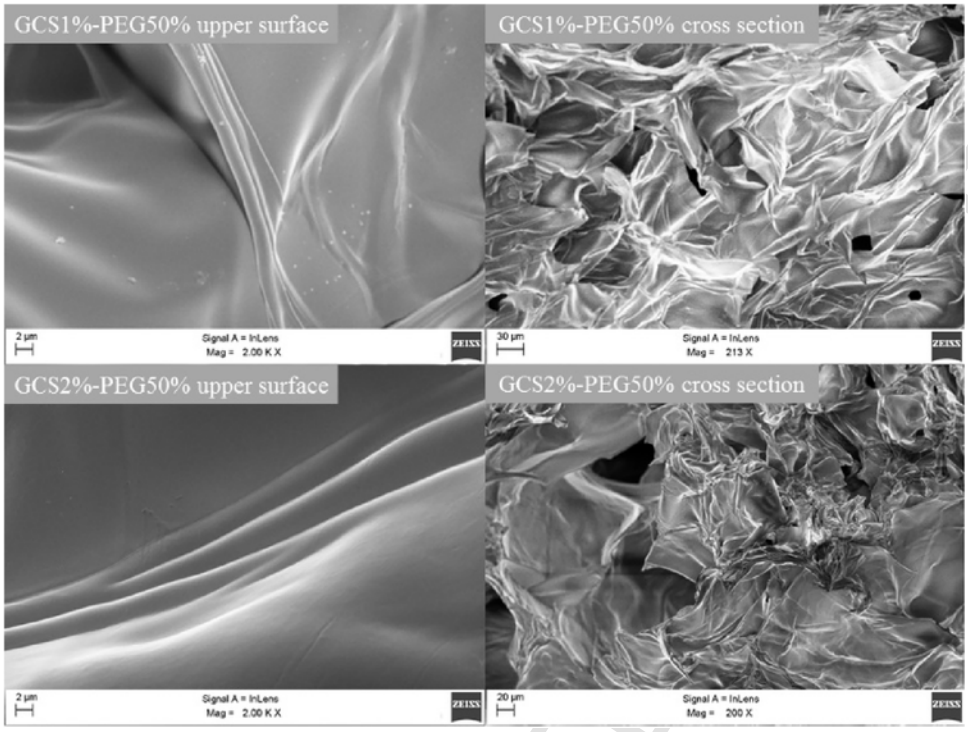

Fig. 5. SEM microphotograph of GCS-PEG scaffolds at the dry state. The pictures represent GCS1%-PEG50% and GCS2%-PEG50%. Upper surface and cross sections are both represented for each sample with a magnification of 2.00 KX and 200 X respectively.

As known, CS, CS -PEG, and GCS show an effective antibacterial activity (Dilamian, Montazer, & Masoumi, 2013; Sinha, Banik, Haldar, & Maiti, 2013). To assess whether GCS-PEG scaffolds here developed retain the native GCS antibacterial activity even after crosslinking, the inhibition rate of the GCS-PEG scaffolds toward *S. aureus* vs the control (CTRL) containing only GCS 1 and 2 wt/vol% was assessed. The experiment was carried out for 24 h or 48 h.

As from Fig. 6, the inhibition rate of all prepared scaffolds is close to 90%, and is comparable to the values obtained for the control, i.e., native GCS. These results evidenced that the presence of the cross-linker PEGDE do not modify the antibacterial activity of the materials which retain a remarkable inhibition rate against *S. aureus*.

The inhibition rate against *S. aureus* of GCS-PEG scaffolds after 48 h (data not shown) was the same than that at 24 h, this is an important result because as it demonstrates that the GCS-PEG-based materials has a prolonged and persistent activity against *S. aureus*. The cell count further demonstrated the absence of growth.

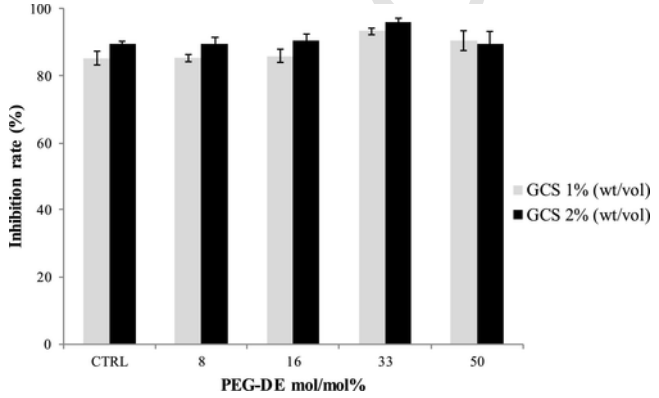

Fig. 6. Inhibition rate of GCS-PEG scaffolds on *Staphylococcus Aureus ATCC* 29213. The CTRL is native GCS (not crosslinked) and the results are referred to the 24 h experiment.

In the process of tissue regeneration, angiogenesis plays a fundamental role in providing the injured tissue with new vessels that will prompt cell (re)growth (Wray, Tsioris, Gil, Omenetto, & Kaplan, 2013). On the other side, one of the most explored strategy to promote angiogenesis is loading a biomaterial with selected growth factors (Wang et al., 2018). Even if the incorporation of angiogenic factors such as VEGF into scaffolds often promotes local angiogenesis, a suitable angiogenic effect could not be achieved, in-vivo, due to the predictable physicochemical instability of VEGF which could reduce its biological activity. In this way an optimal biomaterial should, hopefully, possess a suitable and native angiogenic activity. Thus, we used VEGF as a positive control to compare the pro-angiogenic activity of our materials toward the CAM, an in-vivo assay is a well-established technique to evaluate the pro/anti angiogenic potential of substances and materials (Mandracchia et al., 2016; Ribatti et al., 2006), Fig. 7.

In Fig. 7 it is clearly shown that the pro-angiogenic effect of GCS-PEGDE hydrogels do not significantly differ from the VEGF used as positive control (A in Fig. 7).

These data showed that, independently from the tested material (different GCS or PEG concentrations), the pro-angiogenic effect was always comparable with that of a well-known angiogenic cytokine, namely VEGF. This is a really important feature for a biomaterial intended for wound dressing because it, not only could preserve from bacterial infections due to its intrinsic anti-bacterial behaviors, but also stimulating vascular neo-formation and, possibly, the regeneration of the injured tissue. It wasn't possible to test at the same manner GCS alone nor PEG because being them soluble would spread on the surface of the chick embryo CAM not allowing a reproducible test.

4. Conlcusions

In conclusion, GCS-PEG crosslinked hydrogels were synthetized by a simple chemistry in physiological conditions. The scaffolds were specifically thought for wound dressing applications.

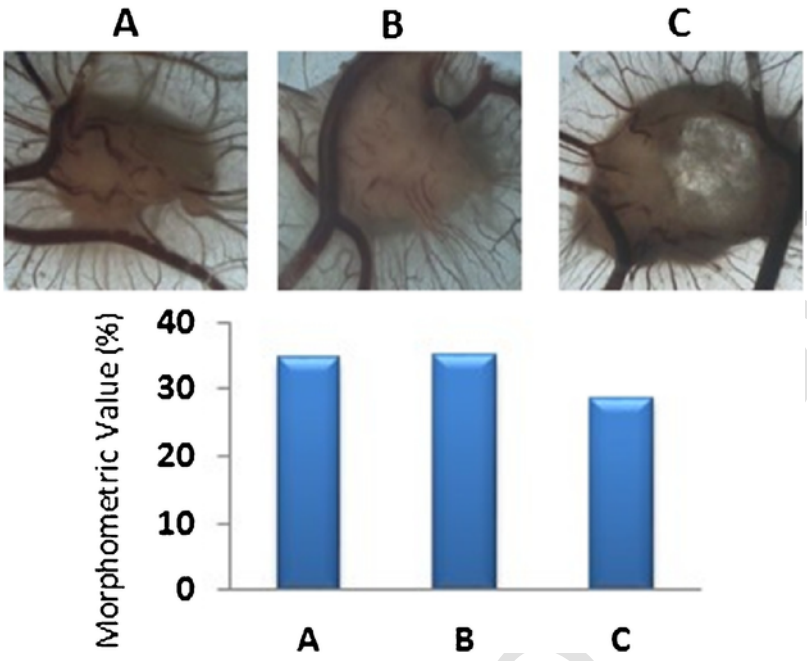

Fig. 7. Angiogenesis activity of GCS-PEG materials and VEGF used as positive control (pro-angiogenic) tested in the in vivo CAM assay. (A) VEGF, (B) GCS1%-PEGxx and (C) GCS2%-PEGxx. The histogram shows the angiogenic response estimated as morphometric value in term of number of strong positive pixels (%). The angiogenic effects are overlapping and the differences between the values are not significant.

The so obtained materials can be molded in different physical forms, *e.g.*, disks or sheets also by varying their thickness. The main physicochemical properties of these GCS-PEG materials could be tailored by varying the degree of crosslinking. Finally, the PEG-crosslinked glycol CS hydrogels retain the pronounced antibacterial activity of the parent CS polymer. Last but not least, this GCS based hydrogels show a pro-angiogenic activity comparable with that of VEGF making these systems ideal candidates as wound dressing materials.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.carbpol.2018.06.061.

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