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Engineered Matrices Based on Gellan Gum and Biocompatible Synthetic Polymers for the Release of Molecules with Antioxidant Activity

Saverio Caporalini[a,c,*], Patrizia Cinelli[a], Caterina Cristallini[a,b], Maurizia Seggiani[a], Niccoletta Barbani[a,b]

[a]Department of Civil and Industrial Engineering, University of Pisa, Largo Lucio Lazzarino 1, 56126 Pisa, Italy
[b]CNR - Institute for Chemical and Physical Processes, IPCF section of Pisa, Largo Lucio Lazzarino 1, 56126 Pisa, Italy
[c]Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Via Savi 10, 56126 Pisa, Italy
saverio.caporalini@phd.unipi.it

Over the past few decades, microbial polysaccharides hydrogels have been used in the field of tissue regeneration due to their ability to mimic the physical-chemical and mechanical characteristics of the extracellular matrix. Gellan gum, an anionic heteropolysaccharide that can form hydrogel in presence of di or trivalent cations, has interesting properties in terms of biodegradability, biocompatibility and the ability to integrate with surrounding tissues. However, it also has some limitations such as the lack of specific sites within the polysaccharide chains for cell recognition and adhesion, high hygroscopicity and limited mechanical properties. Gellan gum has also been proposed for application in the field of controlled release of bioactive molecules; the bio-inert nature of gellan gum makes it suitable for the encapsulation of biomolecules, drugs, enzymes and nutraceutical formulations. Furthermore, the anionic nature of this polysaccharide makes it suitable as a pH sensitive drug delivery system. This work deals with the production and characterization of hydrogels based on gellan gum and polyvinyl alcohol (PVA) or polyvinylpyrrolidone (PVP) for the creation of matrices, containing curcumin as an antioxidant agent, having a micro-engineered surface obtained using micro-molded silicone molds. The properties of gellan hydrogels mixed with PVA or PVP containing curcumin, before and after crosslinking with Ca^{2+} ions, were investigated. The highest curcumin solubility was observed in PVP solution (0.0156 mg/mL) as compared to PVA solution (0.0074 mg/mL). The contribution of gellan hydrogels mixed with PVA or PVP to the antioxidant capacity of curcumin, expressed as % inhibition, was 22% and 25%, respectively.

1. Introduction

Hydrogels are used for various applications in biomedical field due to absorption of a large amount of water, making them very similar to living tissues. This capacity of hydrogels derives mainly from the presence of hydrophilic groups in the polymer chains, such as amino, carboxylic and hydroxyl groups. These hydrophilic polymeric networks can be prepared through various methods which affect the properties and the viscoelasticity of the gels. Hydrogels can be produced from water-soluble polymers and can be formulated in various physical forms, including microparticles, nanoparticles, coatings and films. It is also possible to obtain a microstructured surface through microfabricated molds, to favor the cellularization of the devices. Applications in the biomedical field include contact lenses, artificial corneas, wound dressings, suture liners, catheters and electrode sensors (Bharskar 2020).
Among the various polymers used for preparing hydrogels, the natural polymers, and in particular the polysaccharides, have several advantages over synthetic polymers. They are extracted from natural sources, are non-toxic and biodegradable. Polysaccharides can be cross-linked through ionic bonds in order to produce three-dimensional structures. Polysaccharide hydrogels can be modified to favor the interaction with living cells (Vyazmin et al. 2021) and have been frequently used in tissue regeneration in particular in the development of

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wound dressing materials thanks to their ability to absorb tissue exudates, prevent wound dehydration, and allow oxygen to permeate the wound (Guastaferro et al. 2021).
Hydrogels are used as vectors for the delivery of drugs and cells and as platforms to protect therapeutic agents and cells from hostile conditions. For hydrogel preparation, a large variety of polysaccharides from different origins, including plants, animals, microorganisms and algae are used (Das and Giri 2020). Among them, gellan gum is one of the most interesting. It is an anionic heteropolysaccharide produced and secreted by *Sphingomonas elodea* (Gram negative bacteria), contains repeating units of glucose, glucuronic acid and rhamnose and acquires great importance in the food, pharmaceutical and biomedical industries thanks to its biocompatibility, non-toxicity, stability and biodegradability (Gering et al. 2021). Anionic polysaccharides such as gellan gum are numerous and are used as food additive after FDA approval. Gellan gum is used for the encapsulation of drugs, enzymes, cells and microorganisms due to its exceptional gelling and hydrophilic properties. Numerous works have been done with gellan gum in the biomedical field for different purposes. Das et al. worked on drug and cell delivery systems to regenerate various tissues using gellan gum hydrogels showing promising results (Das and Giri 2020). Despite its many strengths, gellan gum also has some limitations such as the lack of specific sites within the polysaccharide chains for cell recognition and adhesion, high hygroscopicity and limited mechanical properties. The gellan finds great application in the administration of drugs thanks to the possibility of having a very fine control of the release through swelling. Dey et al. carried out various studies on the administration of drugs through gellan gum hydrogel beads containing quercetin, with antitumor activity, using low concentrations of calcium chloride as a cross-linking agent to obtain encapsulation, swelling and modifiable release profiles (Dey et al. 2020). Abramov et al. introduced a unique dermal molecule release system using a biopolymer film with hydrophobic nanodomains acting as a reservoir to hold solubilized curcumin, showing nanodome carrying capacity of up to 85% by weight (Abramov and Garti 2021).
To overcome the intrinsic no-cellular nature of gellan gum, it is necessary to add molecules that can be recognized by the cells, so that they can proceed with replication. The cells in the presence of antioxidant molecules would be able to prevent the oxidation-reduction reactions that release free radicals, responsible for cell damage causing oxidative stress and then apoptosis. Antioxidant molecules may have an important role both in cell recognition and as therapeutics. Curcumin is a hydrophobic polyphenol, extracted from the Curcuma longa plant, with pharmacological properties thanks to its antioxidant activity. Due to its nature, it is poorly soluble in water and is poorly bioavailable when used on its own.
This work deals with the production and characterization of hydrogels based on gellan gum and polyvinyl alcohol (PVA) or polyvinylpyrrolidone (PVP) to create matrices, containing curcumin as an antioxidant agent, having a micro-engineered surface to induce cell adhesion. PVA and PVP polymers, both widely used in biomedical applications, were added to promote the curcumin solubilization and stability.
The properties of gellan gum hydrogels blended with PVA or PVP containing curcumin were studied in view of a possible use in tissue engineering such as wound dressing. The curcumin antioxidant activity was measured using DPPH assay.

2. Experimental

2.1 Solution preparation

Gellan (GE) Curcumin (CU) polyvinylpyrrolidone (PVP) and polyvinyl alcohol (PVA) were purchased by Sigma (St. Louis, MO, USA). A GE solution in water at 1% (w/v) was obtained under mild stirring at 70°C. To improve CU solubilitation,1% (w/v) PVP and PVA solutions were prepared and an amount of CU (1% in weight) was added to the solutions and kept under mild stirring at 70°C for 3 days. The blends were then centrifuged in order to eliminate the unsolved CU and the quantitative determination of dissolved CU in PVA-CU and PVP-CU solutions was performed by UV-visible spectroscopic analysis at 423 nm. GE/PVP-CU and GE/PVA-CU at different ratios (10/90, 20/80, 30/70, 50/50 v/v) were obtained.

2.2 Microstructured film preparation

Using prepared blends, microstructured film were obtained by soft lithography. A productive drawing obtained by CAD systems was realized in accordance with a predefined geometry. CAD model was applied to soft lithography technique to obtain a silica master and the corresponding soft molds were prepared by polymerization of a vinyl-terminated polydimethylsiloxane (PDMS) oligomer (Sylgard 184 Silicone Elastomer Kit, Dow Corning Corporation, USA) in an under-vacuum oven at 40 °C for 24 h. A predefined volume of GE, GE/PVP-CU and GE/PVA-CU, obtained as previously described, was deposited by using a Gilson syringe on the PDMS mold.
In the case of PVA-based samples, the molds containing the solutions were subjected to thawing freezing cycles in order to induce PVA crosslinking. Subsequently, all the samples were dried in a ventilated oven at 37 °C and cross-linked using a 2% w/v solution of calcium chloride to induce gel formation.

2.3 Quantitative determination of curcumin

To determine the solubility of the curcumin contained in the PVP-CU and PVA-CU solutions, an amount of these solutions was centrifuged to ensure that the excess of curcumin settles at the bottom. The supernatant containing curcumin was collected and used for further analysis based on UV-absorbance at 423 nm. To determine the concentration of dissolved curcumin, a suitable calibration curve was used (Dhingra et al. 2021).

2.4 Optical Microscope (OM) analysis

Optical images were acquired by means of the optical microscope paired with the Fourier transformed infrared (FT-IR) spectroscopy apparatus (Perkin Elmer).

2.5 Infrared Chemical Imaging

Spectral images were acquired in transmission mode using the infrared imaging system Spotlight 300 (Perkin Elmer). The spectral resolution was 4 cm^{-1} and the spatial resolution was 6.25 μm. Background scans were obtained from a region of no sample. IR images were acquired with a liquid nitrogen cooled mercury cadmium telluride line detector composed of 16 pixel elements. The Spotlight software used for the acquisition was also used to pre-process the spectra. IR spectral images were produced by using the absorbance in a given frequency range, 4,000–720 cm^{-1}. Spectra contained in the spectral images were analyzed using a compare correlation image. The obtained correlation map indicates the areas of an image where the spectra are most similar to a reference spectrum.

2.6 DPPH assay curcumin solution process

The test allows the evaluation of antioxidant activity by reacting the film sample to be analyzed with a solution of DPPH [2,2-diphenyl-1-picrylhydrazyl, PM 394.33, $C_{18}H_{12}N_5O_6$] at 0.004 % w/v in ethanol. The samples (20 mg) were incubated in 6 mL of DPPH solution in the dark at room temperature for 30 min and analyzed by UV-absorbance to show the decrease in radical peak at 517 nm. Antioxidant compounds which can transfer a hydrogen atom to the radical cause a discoloration of the solution. The free radical scavenging activity (ESCA) of extracted compound is evaluated by the ESCA of DPPH (inhibition in %) and calculated using the following equation:

$$Inhibition\ (\%) = \frac{A_0 - A_S}{A_0} \cdot 100\ \% \quad (1)$$

where A_0 is the absorbance of initial DPPH solution, and A_S is the absorbance of DPPH solution with compound. The decrease of absorbance (discoloration) is proportional to the antioxidant charge present in the sample.

3. Results and discussion

The quantitative analysis of PVP-CU and PVA-CU blends showed a satisfying CU solubility in both polymeric solutions compared to the concentration of CU dissolved in water (0,0007 mg/mL). The highest CU solubility was observed in PVP solution (0.0156 mg/mL) as compared to PVA solution (0.0074 mg/mL). As expected PVP and PVA are water-soluble polymers suitable to increase the solubility of poorly-soluble drugs as CU.

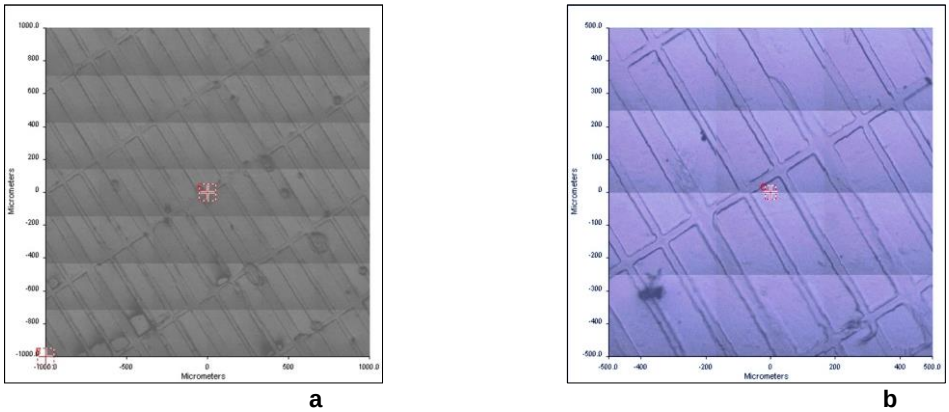

Figure 1: Optical images of the surface of dried (a) and swelled (b) GE matrices

Figure 1 shows the optical images of the dried and swelled micro-structured GE hydrogel. The correct reproduction of the geometry that characterizes the surface of the mold resulted well evident both for dried and hydrated GE samples (the latter stained with blue methylene, Figure 1b). It is important to note that water absorption in the hydrated sample does not change the geometry of the hydrogel surface. The OM analysis was carried out also on dried and swelled micro-structured hydrogels obtained using GE/PVP-CU and GE/PVA-CU; also in this case a good reproducibility of the surface geometry was obtained. Figure 2 shows the images of the surface of swelled GE/PVA-CU and GE/PVP-CU matrices having a 50/50 GE/polymer weight ratio.

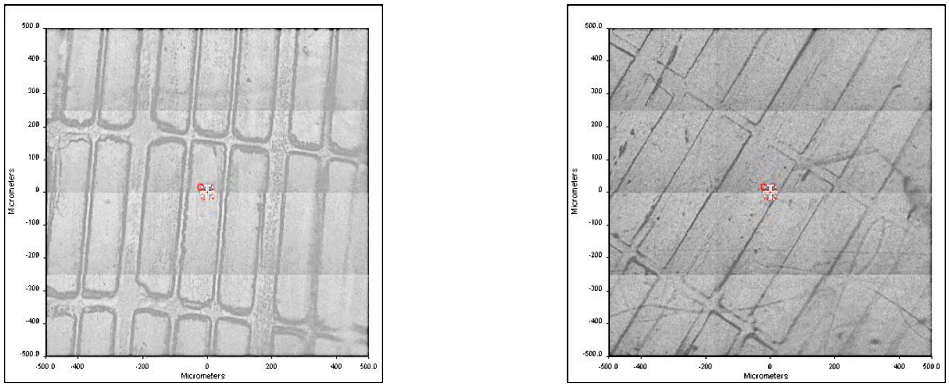

Figure 2: Optical images of GE/PVA-CU (a) and GE /PVP-CU surfaces (b)

Spectral images were acquired in transmission mode using the Infrared Imaging System Spotlight 300. Figure 3 shows 2D and 3D chemical maps and representative spectrum (reference spectrum, Figure 3c) of GE/PVP-CU film. The rectangular profiles are well defined and the corresponding spectrum shows the characteristic peaks of both PVP (1650 cm^{-1}) and GE (1064 cm^{-1}).

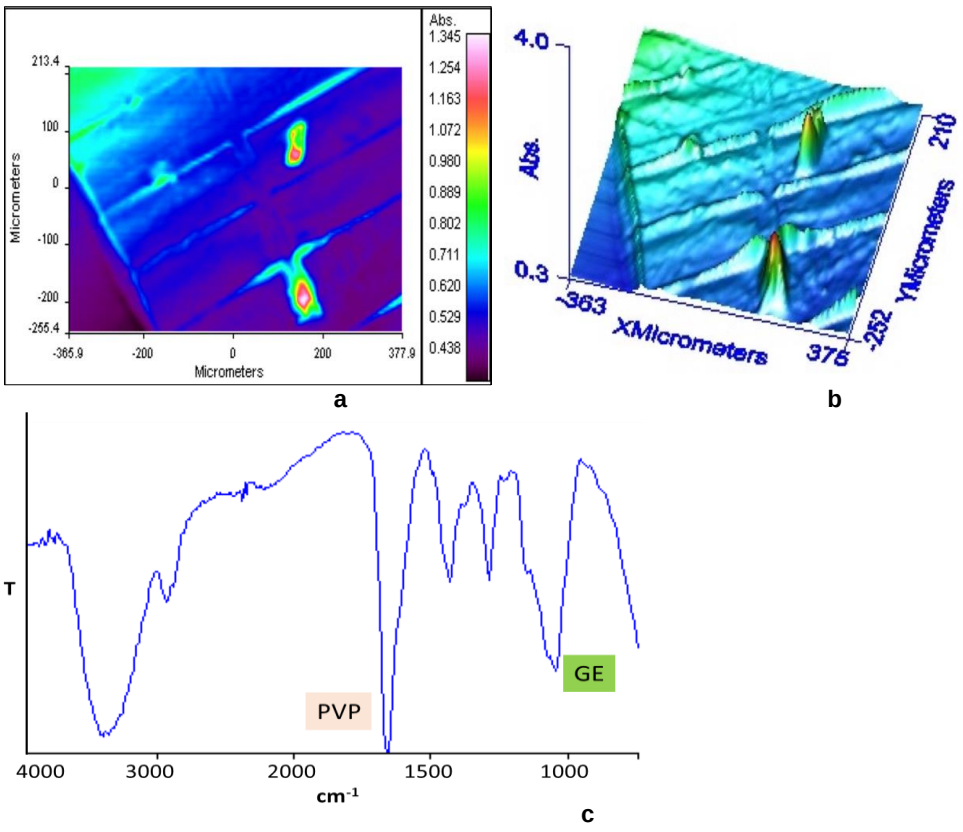

Figure 3: 3D and 2D chemical maps of the surface of GE/PVP matrix (a-b), reference spectrum (c)

In Figure 4, FTIR results obtained for GE/PVA-CU matrix is reported. Even in this samples, a regular surface microstructure is evident and the corresponding spectrum (Figure 4c) presents the absorption bands attributable to components, GE and PVA.

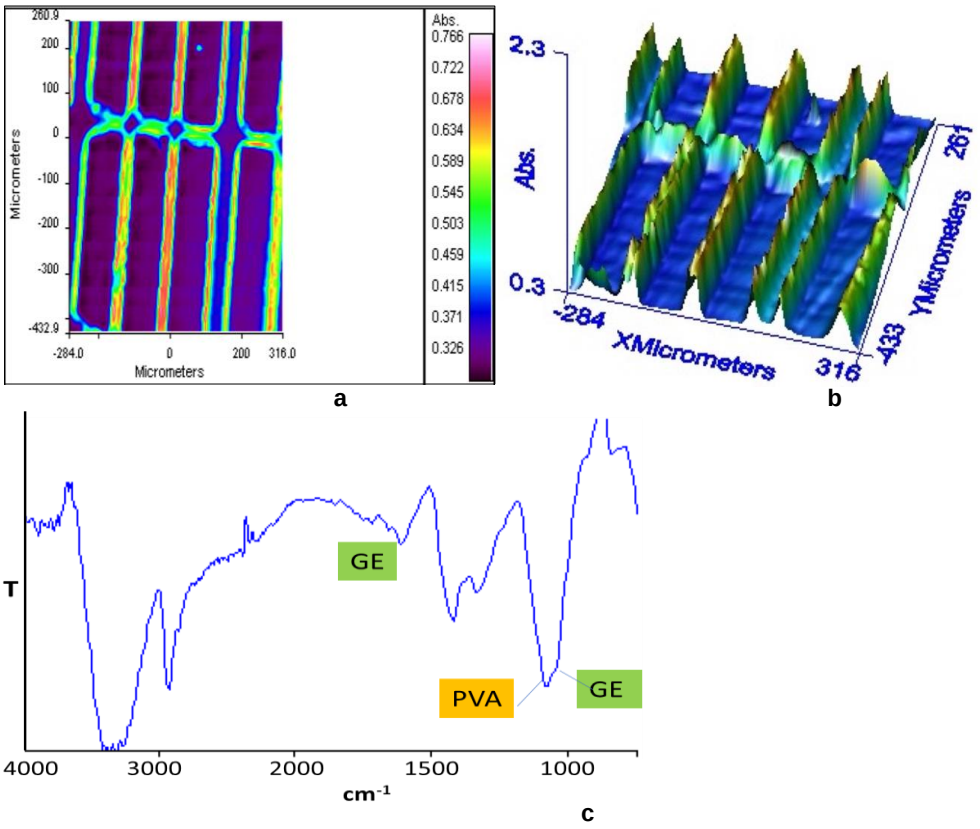

Figure 4: 3D and 2D chemical maps of the surface of GE-PVA matrix (a-b), reference spectrum (c)

The ESCA activity of extracted CU was assessed by the DPPH assay and calculated using the Eq(1), obtaining the inhibition (%) graph (Figure 5).

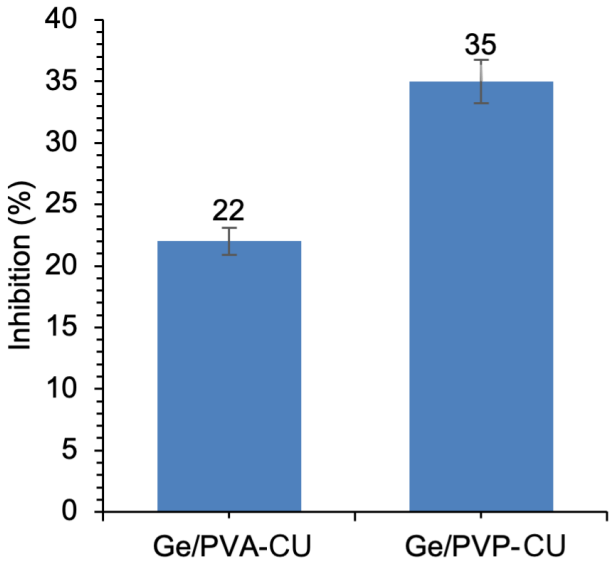

Figure 5: ESCA activity of extracted curcumin

The DPPH test was carried out to demonstrate that the preparation steps of the samples did not interfere with the antioxidant properties of the CU. Figure 5 shows the inhibition values for both systems, however, the percentage of inhibition is higher in the GE/PVP-CU blend probably due to their higher amount of CU incorporated in this sample. No free radical scavenging activity was observed for GE/PVA and GE/PVP samples. The results indicated that the conditions used are suitable for preserving the CU antioxidant properties.

4. Conclusions

The study of hydrogels based on blends of gellan gum and PVA or PVP containing curcumin was carried out in view of a possible use in skin tissue engineering. The addition of water-soluble and biocompatible polymers allowed an enhancement of dissolution in water of curcumin and consequently improved its incorporation into the hydrogels as well the maintenance of the bioactivity. The physico-chemical properties of GE/PVA-CU and GE/PVP-CU hydrogels are resulted optimal for obtaining materials with a microstructured surface able to reproduce with high accuracy the predefined geometry. *In vitro* cellular tests on these matrices are currently under investigation to assess their potential as support for cell adhesion and proliferation.

Acknowledgments

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