Effect of the Zn-aminoacid MOF concentration on the in vitro biocompatibility of red and green tomato seed cells over collagen-starch-ZnMOFs hydrogels

Cabrera-Munguía Denis A.1, Palao-Portillo Andrea M.1, León-Campos M. Ileana1, Aguirre-Joya Cristian2, Aguillón-Gutiérrez David R.2 & Claudio-Rizo Jesús A.1*

1Materiales Avanzados, Facultad de Ciencias Químicas, Universidad Autónoma de Coahuila, Saltillo, Coahuila, México.
2Centro de Investigación y Jardín Etnobiológico, Universidad Autónoma de Coahuila, Viesca, Coahuila, México.

*Corresponding Author (Claudio-Rizo Jesús A.) Email: jclaudio@uadec.edu.mx

DOI: http://doi.org/10.38177/AJBSR.2024.6202

Copyright © 2024 Cabrera-Munguía Denis A. et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

ABSTRACT

A balance between macronutrients (N, P, K, Ca, Mg) and micronutrients (Fe, Zn, Mn, Mo, B, Cl) is essential for the thriving of red tomato (Solanum lycopersicum) and green tomato (Physalis philadelphica) plants as well as good water irrigation. Zn is a vital micronutrient in seed development, fruit set, and maturity, while histidine plays a critical role in plant growth and development. Hence, a MOF based on Zn and histidine (ZnHis) was added in different amounts (X=0, 10, 30, and 50 wt. %) into a composite hydrogel of collagen and starch to supply of water and micronutrients necessary for the in vitro proliferation, and cell migration of red tomato and green tomato cells living over these hydrogels. The functional groups and reticulation degree were assessed by ATR-FTIR and the ninhydrin assay. The metabolic activity of red tomato and green tomato cells living on these hydrogels was tested by reaction with MTT. Cell migration of red tomato and green tomato cells was studied by light microscopy. The results evidencing that 10 wt. % and 30 wt.% of ZnHis are the preferable concentrations to promote cell metabolism, and cell migration of red tomato and green tomato cells extracted from seeds.

Keywords: Red tomato (Solanum lycopersicum); Green tomato (Physalis philadelphica); Hydrogel; Collagen; Starch; MOFs; Zinc; Biofertilizers.

1. Introduction

The red tomato plant (Solanum lycopersicum) is a crop that depends on water availability, temperature, and salinity. The water supply affects plant growth, the photosynthesis process, tomato production, and the quality of tomato crop [1]. On the other hand, green tomato plant (Physalis philadelphica) enriches the diversity of the genus Physalis in México, where takes part in the recipes of salads, stews, and salsas [2]. Red tomato requires macronutrients such as nitrogen, phosphorous, potassium, calcium, and magnesium for crop development. But also, micronutrients like iron, zinc, manganese, molybdenum, boron, and chloride which are vital as well as macronutrients for the growth, development, and quality of horticultural crops affecting the yield and plant growth, size, color, and flavor of the fruit, and extended shelf life [3]. In the case of the green tomato (Physalis philadelphica) crop is not as demanding as the red tomato, the issue is to provide the green tomato plant with a sunny place with drained soil.

Nowadays, agriculture consumes more than 70% of freshwater in all regions around the world, which is inconvenient to the present water scarcity that prevails because of the worldwide population increment. Hydrogels composed of biopolymers (e.g. lignin, gelatin, alginate, guar gum, chitosan, and starch) represent an alternative solution that serves as a reservoir of water and nutrients to the plant roots [4,5]. Superabsorbent hydrogels can be made using biopolymers with a chemical structure that contains hydrophilic groups (e.g. -COOH, -OH, -NH2) [6-8], some examples are cellulose and sodium alginate which have already been applied in tomato crops in conditions of water scarcity [9,10].

Starch is a biodegradable polymer suitable for agriculture with outstanding swelling properties obtaining superabsorbent hydrogels, where water is delivered to plants under water stress conditions or is swelled into the
hydrogel network when water is abundant. In a previous work of our research group hydrogels based on collagen where modified with starch [8] obtaining biocompatible hydrogels with controlled release of therapeutic molecules.

On the other hand, zinc plays a key role as a micronutrient in tomato plant growth while histidine is a source of nitrogen and is vital for the growth and development of tomato plants [3,11]. Both components can be combined in the form of MOFs where Zn is the metal center and histidine the organic ligand. In this proposed study, hydrogels synthesized from collagen and starch were modified with the addition of a MOF composed of zinc as a metal ion and histidine as an organic ligand (ZnHis). Hydrogels were characterized by ATR-FTIR and the ninhydrin assay to know the physicochemical interactions between the components. Also, the effect of the concentration of ZnHis in the collagen-starch hydrogel was studied and related to their in vitro biocompatibility with the cell metabolism, and cell migration of red and green tomato seed cells.

Figure 1. Synthesis of collagen-starch-ZnHis(X) hydrogels for in vitro biocompatibility of red and green tomato cells

1.1. Study objectives

The following are the main objectives of this study. (i) The preparation of composite hydrogels based on collagen, with a network modified with starch and ZnHis MOF, varying the concentration of this latter. (ii) Investigate the functional groups present in composite hydrogels by ATR-FTIR to correlate it with the physicochemical interactions between collagen, starch and ZnHis MOF. (iii) Elucidate the effect of ZnHis MOF concentration into the reticulation index of hydrogels, as well as, categorized the networks into chemical hydrogels (IPN: interpenetrated networks) or physical hydrogels (semi-IPN). (iv) Analyze the effect of ZnHis MOF concentration in the in vitro biocompatibility in red and green tomato seed cells. (v) Investigate the consequences of varying ZnHis MOF concentration in the cell migration of red and green tomato seed cells.
2. Materials and Methods

2.1. Chemicals

L-histidine (His), zinc nitrate hexahydrate (Zn(NO$_3$)$_2$•6H$_2$O), 1,3,5-benzenetricarboxylic acid (TMA), starch (soluble, extracted from potato, Mn 300 g mol$^{-1}$), 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and Murashige and Skoog (MS) culture were purchased from Sigma Aldrich Co., and they were used as received. The polyurethane crosslinker (Mn 3000 to 7500 g mol$^{-1}$) was elaborated from glycerol ethoxylate and 1,6-hexamethylene diisocyanate and glycerol ethoxylate using the procedure reported elsewhere [12]. Collagen type I was extracted from the porcine dermis was extracted by enzymatic hydrolysis with pepsin as reported elsewhere (Mn $\alpha_1=220$ 000 g mol$^{-1}$, $\alpha_2=110$ 000 300 g mol$^{-1}$) [13]. Red tomato (Solanum lycopersicum) and green tomato (Physalis philadelphica) seeds were bought from a local greenhouse.

2.2. Synthesis of Zn-histidine-MOF

Zinc-histidine MOFs was synthesized by the hydrothermal method. The synthesis involves creating an ethanol solution of 1 mmol of zinc nitrate hexahydrate, 1 mmol of TMA, and 1 mmol of histidine were mixed with magnetic stirring at room temperature [14]. Then, the mixture was transferred to a Teflon-lined autoclave, and the reaction was carried out at 120 °C for 12 h. The white precipitate obtained from the reaction was filtered, rinsed with water, and dried at 60 °C. The obtained zinc coordination polymer was labeled as ZnHis.

2.3. Synthesis of collagen-starch-ZnHis(X) hydrogels

The hydrogels based on collagen, starch and ZnHis MOFs were prepared by the microemulsion method. A stock solution of starch (0.5 % wt) was made, also a certain amount of ZnHis (X=0, 10, 30 and 50 wt. %) was dispersed in 10 mL of collagen solution (6 mg L$^{-1}$). The culture plates of 24 wells were used as molds for hydrogels, in each well was mixed 1 mL of ZnHis-collagen solution, 100 μL of starch solution, 20 μL of polyurethane, and 200 μL of phosphate-buffered saline solution (PBS-10X) to adjust the pH to 7, this step was made keeping the temperature from 4 to 5 ºC using an ice bath. Then, the reticulation was carried out by heating in a lab stove at 37ºC for 4 h to obtain the hydrogels, which were identified as CS-ZnHis(0), CS-ZnHis(10), CS-ZnHis(30) and CS-ZnHis(50) depending on the ZnHis concentration.

2.4. FTIR and reticulation index

The ATR-FTIR spectra of dried hydrogels (xerogels) were acquired in a range from 4000 to 650 cm$^{-1}$ at 16 cm$^{-1}$ of resolution and with an average of 16 scans. using a Frontier, Perkin Elmer system. The ninhydrin assay ((1 ml, 1 wt.%, citrate buffer, pH 5.0) was applied to analyze the crosslinking percentage of composite hydrogels (samples were prepared by triplicate), allowing the Shift base to react with the primary amine groups of collagen for 2 h at 90°C obtaining a purple color. The absorbance of each sample was analyzed using a ThermoScientific MultiSkan Sky spectrophotometer at 567 nm. The reticulation index was obtained with the Equation (1) [15]:

\[
\text{Crosslinking degree, } \% = \left(1 - \frac{A_{\text{sample}}}{A_{\text{collagen}}} \right) \times 100
\]

Where $A_{\text{sample}}$ and $A_{\text{collagen}}$ are the absorbances of solutions obtained after ninhydrin reacted with hydrogels and unreticulated collagen, respectively.
2.5. MTT assay, cell migration and proliferation

Red and green tomato seed cells were isolated and cultured in MS medium. The effect of the concentration of ZnHis that contains the collagen-starch hydrogel on the metabolic activity of vegetable cells growing in contact with hydrogels was evaluated by the MTT assay. For this, 1 mL of cell suspension (100 000 cells/mL) was seeded over hydrogels in polystyrene culture plates and incubated for 24 and 48 h at 37 °C (samples were prepared in triplicate). PBS-1X was mixed with 1 mL of cell suspension and it was used as the positive control. At the evaluation time (24 or 48 h), 15 μL of 3-(4,5-dimetilthiazol-2-yl)-2,5-diphenyltetrazolium solution (1% wt. in sterilized PBS-1X) was added in each well and incubated for 2 h more. After that, 1 mL of propan-2-ol was added to dissolve the resulting blue formazan crystals. Aliquots of 200 μL were taken from the liquid medium and the absorbance was measured at 560 nm. Cell viability was calculated using Equation 2:

\[
\text{Cell viability, } \% = \frac{A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (2)
\]

Where \( A_{\text{sample}} \) and \( A_{\text{control}} \) are the absorbances of solutions obtained after MTT reacted with the sample and the control, respectively.

Cell migration micrographs were taken and evaluated test using a VELAB VE-403 inversed microscope. In this case, hydrogels were synthesized by the procedure mentioned above, however, after the collagen-MoMOFs solution was added to each well, 1 mL of vegetable cells was added, and then the other components such as starch, crosslinking agent, and PBS-10X were added as mentioned before. After incubation at 37°C during 4 h, the hydrogels were dried to obtain the xerogels, which were observed using the microscope.

3. Results and Discussion

3.1. FTIR

The ATR-FTIR spectra are shown in Figure 2, at around 3300 cm\(^{-1}\) its observed a broad band assigned to amine and hydroxyl groups related to functional groups of collagen and starch, respectively.

![Image of ATR-FTIR spectra](attachment:image.png)

**Figure 2.** ATR-FTIR spectra of CS-ZnHis(X) xerogels
Two bands at around 2900 cm\(^{-1}\) correspond to symmetric and asymmetric elongations from C-H bonds that belong to collagen and starch. The band located at 1600 cm\(^{-1}\) represents the elongation of the C=O bond, better known as Amide I, while at 1450 cm\(^{-1}\) there is a band that belongs to the N-H bond, known as Amide II, typically found in the spectrum of polypeptides such as collagen. The band around 1700 cm\(^{-1}\) is related to the reticulation degree, this obeys to the reaction between amine groups of collagen and isocyanate groups of polyurethane. Finally, the band associated with the bond Zn-O is found at 580 cm\(^{-1}\), for that reason, there is no appreciation in the range used to acquire the spectra.

3.2. Reticulation index

The crosslinking degree is depicted in Figure 3, it was evaluated by the ninhydrin assay, in this test the free amine groups from collagen that are not crosslinked with polyurethane, react with the Schiff base obtaining purple imines. The results evidence that all the hydrogels form semi-interpenetrating networks (<60%), indeed, the interactions between collagen, starch, and ZnHis MOF are related to hydrogen bonds between the amine groups of collagen and histidine, as well as the hydroxyl groups of starch. The CS-ZnHis(10) showed the major reticulation index, while CS-ZnHis(30) and CS-ZnHis(50) are statistically equal (Tukey test with \(\alpha=0.05\)).

![Figure 3. Reticulation percentage of CS-ZnHis(X) hydrogels](image)

3.3. In vitro cell viability

The in vitro metabolic activity of red cell tomato is depicted in Figure 4(a), in all cases the metabolic activity is greater than 60% which is indicative of a non-cytotoxic effect. In the first 24 h, there is not a significant difference between the hydrogels. However, after 48 h the hydrogels, CS-ZnHis(10) and CS-ZnHis(30) are statistically equal (Tukey test with \(\alpha=0.05\)) and show the major metabolic activity, this indicates that at 48 h red tomato cells are well adapted to the surface of the hydrogels.

In the case of green tomato cells (Figure 4(b)) all the materials showed no cytotoxic effects on vegetable cells. It is observed that the incorporation of ZnHis MOF increases the in vitro metabolic activity during the first 24 h. However, after 48 h the cell metabolic activity over CS-ZnHis(50) tends to decrease, this obeys to the lethargy of green tomato cell. While in the case of CS-ZnHis(10) and CS-ZnHis(30), they increase their metabolic activity after 48 h.
3.4. Cell migration

In general, the black spots in the micrographs are indicative of the presence of vegetable cells (Figure 5). The images of red tomato cells that all samples show black spots, however, the sample with most cell population belongs to the material CS-ZnHis(0) without ZnHis MOF.

Figure 5. Red and green tomato cells into xerogels of CS-ZnHis(X)
The micrographs of green tomato cells evidence that the major cell population is located in the material CS-ZnHis(10). Thus, the MOF does not have a positive effect on the cell migration of red tomato cells. But for green tomato cells, the MOF presence has a positive effect on cell migration being the CS-ZnHis(10) the best material.

4. Conclusions

The cross-linking degree and the ATR-FTIR spectra demonstrated that the main interactions between collagen, starch, and ZnHis MOF are related to hydrogen bonds of amine and hydroxyl groups found in collagen, starch, and ZnHis MOF. However, the material CS-ZnHis(10) obtained the major reticulation index leading to the formation of semi-interpenetrating networks with the highest cross-linking interactions in the hydrogel state. In addition, it was found that the materials with the best properties for in vitro cell migration and metabolic activity of red and green tomato cells are CS-ZnHis(10), and CS-ZnHis(30), histidine is an essential amino, and it plays an important role in various biological processes, in tomatoes, histidine contributes to several aspects of the plant’s physiology and health demonstrating that high levels of this amino acid are not necessary to modulate the metabolic activity and migration of tomato cells in the materials studied.

Some prospects of future work are the statistical study of the germination of seeds from red and green tomatoes using as a substrate the composite hydrogels (varying the ZnHis MOF) and a control (model soil). Afterward, it is also suggested to analyze the content of macronutrients and micronutrients of real soil to calculate the amount of hydrogels necessary for the germination and plant growth of red and green tomatoes. As well as, the statistical study of the amount of leaves and the measure of the plant height at a certain time to evaluate the feasibility of using collagen-starch-ZnHis hydrogels as a biofertilizer.

Declarations

Source of Funding

Cabrera-Munguia Denis A. & Claudio-Rizo Jesús A. want to thank CONAHCYT for the financial support obtained with the approved projects CF-2023-G-1348 and FORDECYT/PRONACES/6660/2020 approved in 2023 and 2020, respectively.

Competing Interests Statement

The authors declare that they have no conflict of interest.

Consent for Publication

The authors declare that they consented to the publication of this study.

Authors’ Contributions

All the authors took part in literature review, research, and manuscript writing equally.

Availability of data and material

Supplementary information is available from the authors upon reasonable request.
References


