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Study of composition of hydrogels and bioactive zinc (II) MOFs on plant tissue growth

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ABSTRACT

In this work, we report the synthesis of metal-organic frameworks (MOFs) using zinc (II) and the amino acids L-phenylalanine (Phe), L-tryptophan (Trp), and L-histidine (His) as ligands. They were incorporated in hydrogel matrixes comprised of collagen, starch, green and red tomato cells to obtain composite hydrogels.

The cell migration into the polymer matrices, the resistance to degradation and *in vitro* biocompatibility of hydrogels was studied. The cell migration assay showed a high confluence of plant cells, indicating a favorable environment for cell migration and proliferation in the presence of MOF-Zn(His) and MOF-Zn(Phe). During a degradation kinetics in vegetable substrate for 10 days, a rapid loss of water was observed in the first two days, indicating a remarkable water absorption capacity in the hydrogels. The results of metabolic activity in red and green tomato cells revealed a significant increase, exceeding 164±12 %, suggesting promising cell development in this type of composite hydrogels. These results support the potential of hydrogels synthesized with MOFs in agricultural and biotechnological applications, opening perspectives for future research and applications in plant tissue engineering.

Keywords: Metal organic frameworks (MOFs); Zinc (II); Amino acids; L-phenylalanine (Phe); L-tryptophan (Trp); L-histidine (His); Hydrogels.

1. Introduction

The metal–organic frameworks (MOFs) are coordination polymers constituted by a metallic center. The choice of the metal ion and organic ligand determines the properties and the potential applications. Some common metal ions used in MOFs include Zn^{+2}, Al^{+3}, Fe^{+3} and Zr^{+4} and among others and an organic ligand commonly used in MOFs include terephthalic acid, trimesic acid and 2-methylimidazole. These materials are characterized to present high thermal stability and large surface area, because of this, they have been widely applied as adsorbent, catalysts (bifunctional materials with the simultaneous presence of both Lewis acid and base), and recently as biocompatible materials with antimicrobial properties [1].

Recently, some efforts have been made for the development of MOF-based hydrogels represent an exciting advancement in material science; compared to initial MOFs in many ways for example, mechanical strength, absorption capacity and total pore volume [2].

Indeed, hydrogels have shown promising applications in agriculture due to their unique properties, such as water absorption capacity, biocompatibility, biodegradability, and the ability to encapsulate and release active ingredients in a controlled manner. Here are some convincing roles of hydrogels in agriculture. Some roles of hydrogels in agriculture are water reservoirs in soil, seed coating agents, provide drought resistance to crops, act as reservoirs for critical nutrients, function as seed coating agents and improve transplantation success rate [3], [4].

Zinc (Zn) plays crucial roles in the physiology and metabolism of plants, including enzyme function, hormone synthesis, and ion transport. Its involvement in various biochemical processes makes it an essential micronutrient for plant growth and development. In soil-plant systems, the physicochemical interactions of zinc are multifaceted

and can significantly influence its availability to plants. Here are some key aspects of zinc's involvement in soil-plant systems: zinc speciation and complexation, zinc transport and translocation and fertilization and management [5]. Also, biochemical factors, plant protection strategies include physical: shape, surface properties and mechanical [6]. Precisely, the coordination sphere of the zinc (II) ions plays a crucial role in numerous biological processes, showcasing their physiological significance at the cellular level. Over the years, various discoveries have unveiled the intricate functions of zinc ions in living organisms [7].

The starch is a major source of carbohydrate and the most abundant storage polysaccharide in plants. It holds significant potential for various applications in the pharmaceutical, biomedical, and agricultural industries, particularly in the development of hydrogel systems. Here are some key reasons why starch is an excellent candidate for hydrogel systems in agriculture: inherently biocompatible, meaning it is well-tolerated by living organisms, including plants, this polymer is readily biodegradable and Starch-based hydrogels can be engineered to exhibit desirable mechanical properties, including flexibility, strength, and stability [8].

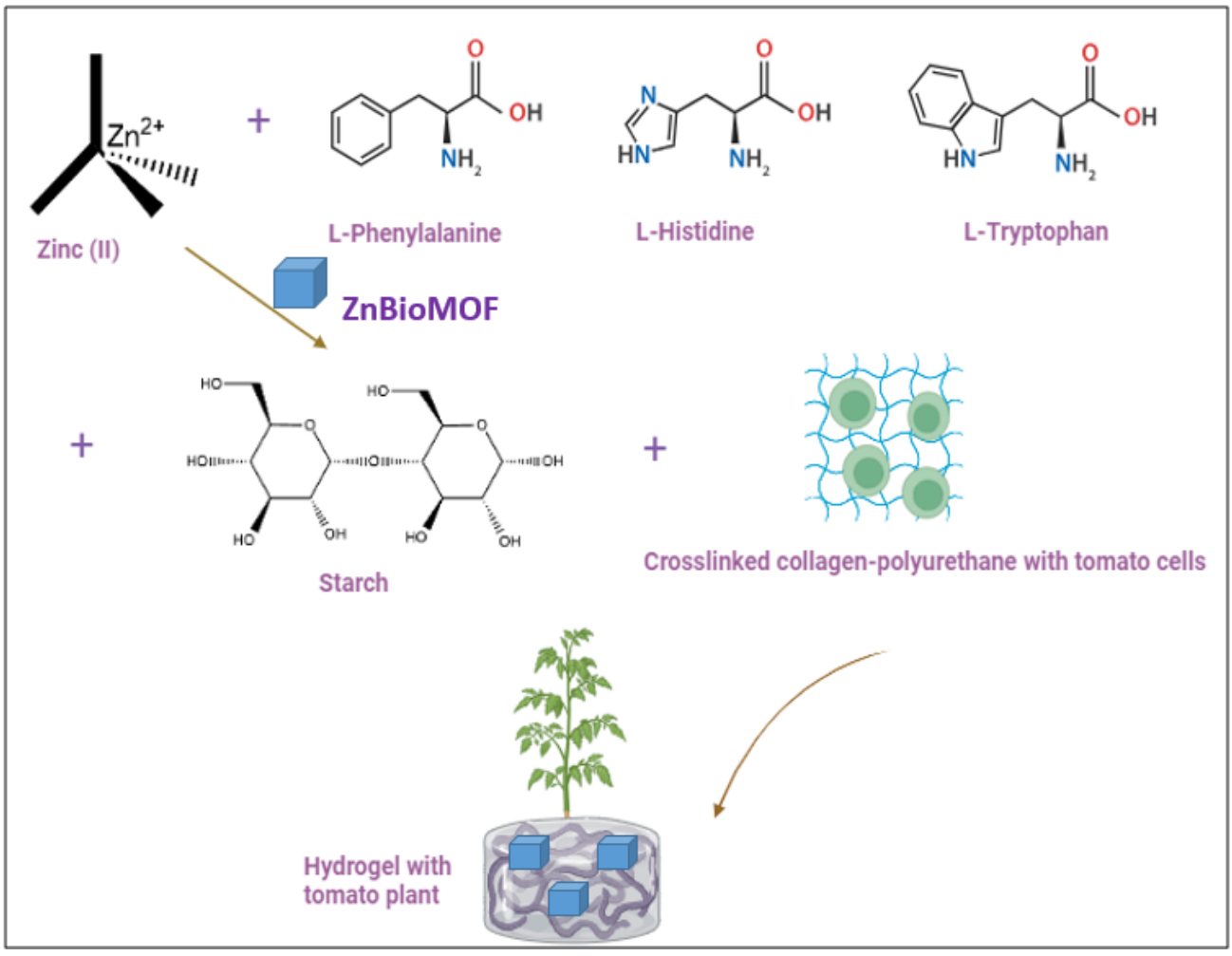

Figure 1. General scheme for obtaining matrixes based on collagen polyurethane-starch hydrogels with complexes comprised form Zn (II) with different amino acid for plant tissue growth

In this work we focus on the use of the amino acids like phenylalanine, tryptophan, and histidine, because these will confer certain characteristics to the hydrogels that will be suitable for agricultural application. With this in

mind, tryptophan serves as a crucial precursor for the biosynthesis of several essential plant growth regulators, including auxins, melatonin, and serotonin. These compounds play significant roles in various physiological processes, such as cell elongation, root development, flowering, and stress responses. Here's how tryptophan contributes to the production of these plant growth regulators [9]. Also, phenylalanine-derived compounds play diverse and essential roles in plant defense, ultraviolet (UV) protection, signaling, and reproduction. These compounds are synthesized through the phenylpropanoid pathway, which branches out to produce various secondary metabolites, including flavonoids, phenolics, anthocyanins, and phenylpropanoid/benzenoid volatiles [10]. Besides, histidine, an essential amino acid, is known for its roles in protein synthesis and as a precursor for the biosynthesis of various important compounds in plants. Recent physiological studies have indeed shed light on novel functions of histidine in plants, particularly in metal ion chelation, ion transport, and reproduction [11].

The present work focused on designing collagen hydrogels with MOFs L-tryptophan (Trp), L-phenylalanine (Phe) and L-histidine (His) based on Zn (II). The purpose of this project was to evaluate the possible application of collagen-starch hydrogels with ZnbioMOFs for plant cultivation. For this, green tomato, and red tomato (*solanum lycopersicum*) cells were used. These matrixes were characterized by cell migration assay to observe the confluence of plant cells. Degradation kinetics were carried out in vegetable substrate, indicating materials with properties for water management in agricultural cultivation techniques. The biological response was measured by cell viability of vegetal cells green tomato and red tomato. The main idea is to analyze the effect of the chemical structure of the amino acids of ZnMOFs with the biological properties of the composites in hydrogel state for their potential agriculture application. In the (Figure 1) show the general scheme of this matrixes.

1.1. Study Objectives

The following are the main objectives of this study. (i) Considering the above, herein, hydrogel based on collagen-polyurethane combined with Zn (II)-based metal-organic frameworks have been prepared. The aim of this work also involves the use of different types of amino acids (AA) as ligands (L-tryptophan (Trp), L-phenylalanine (Phe), and L-histidine (His)) during the preparation of MOFs. (ii) Evaluate the physicochemical behavior of hydrogels using SEM. (iii) Evaluation of the *in vitro* biocompatibility using MTT. (iv) Evaluate of substrate degradation kinetics. (v) Evaluate cell migration assay in these hydrogels.

2. Materials and methods

2.1. Materials

Collagen (C) type I was obtained from porcine dermis (6 mg/mL) through an enzymatic procedure. Glycerol ethoxylate (1000 g mol^{-1}), polyurethane crosslinker was prepared form 1,6- hexamethylene diisocyanate (HDI) as reported elsewhere [12], 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), L-tryptophan (Trp), L-phenylalanine (Phe) and L-histidine (His), phosphate buffer solution (PBS), 1,3,5-benzenetricarboxylic acid (BTC), starch (100,000 Da) were purchased from Merck/Aldrich and used as received [13]. For the cell migration assay, primary tomato plant cultures were used growing in Murashige and Skoog culture media were all obtained from Sigma-Aldrich, with a cell population of 100,000 cells per mL. Tomato (*Solanum lycopersicum*) plants and seeds were purchased from a local greenhouse.

2.2. Methods

2.2.1. Synthesis of Zn (II)-based MOFs

The Zn-based MOFs were prepared in a one-pot procedure by a hydrothermal method. For this, 1 mmol of Zinc (II), 1 mmol of BTC, and 1 mmol of the suitable amino acid (Phe, His or Trp) were mixed with magnetic stirring at room temperature [14]. In brief, each component was dissolved in ethanol and sonicated. Then, the three solutions were placed together in an Erlenmeyer flask and mixed under constant stirring. Next, the new homogeneous solution was transferred to a Teflon-lined autoclave, and the reaction was carried out at 120 °C for 12 h. Lastly, the final product was recovered by filtration, washed several times with water, and dried in an oven at 60 °C. The formed Zn-based MOFs were obtained as a white solid in all cases, regardless of the type of amino acid used as ligand (Trp, Phe, or His), where each product was labeled as MOF-Zn(Trp), MOF-Zn(Phe) and MOF-Zn(His), respectively [15].

2.2.2. Synthesis of composite hydrogel

Composite hydrogels based on collagen, starch and zinc/amino acid MOFs were prepared by the microemulsion method [16]. First, 10 mL of collagen solution (6 mg L^{-1}) was mixed with 20 µL of polyurethane in a culture plate (used as a mold), 500 mg of starch. After that, and 7% wt of Zn(His), Zn(Phe), or Zn(Trp) was added, and after mixing the pH was adjusted to 7 by adding 300 µL of phosphate buffered saline (PBS-10X). The reticulation reaction was carried out by heating at 37 °C for 4 h to obtain the hydrogels, which were labeled as MOF-Zn(His), MOF-Zn(Phe), or MOF-Zn(Trp) depending on the MOF used. For the comparison of results, a hydrogel formulation without MOF was prepared [14].

2.2.3. Cell migration assay

Composite hydrogels based on collagen, starch and zinc/amino acid MOFs were prepared by the microemulsion method and were added primary plant cultures were used growing in Murashige and Skoog medium, with a cell population of 100,000 cells per mL [17]. They were them left to dry and the micrographs were obtained with a VELAB VE-403 inversion microscope using a 100X objective [18].

2.2.4. Substrate degradation kinetics

The resistance to degradation of the hydrogels under study was carried out in the presence of commercial, non-sterilized vegetable substrate. This type of experiment aims to determine what happens to the integrity of the hydrogel as it has a period of contact with the vegetable substrate.

The mass of the hydrogels was monitored to determine degradation by comparison with the initial mass. The experiment was carried out in triplicate.

2.3. Evaluation of the in vitro biocompatibility

The effect of the chemical structure of Zn/amino acid MOFs on the metabolic activity of green tomato and red tomato cells growing in contact with hydrogels was evaluated by the MTT assay. For this, 1 mL of cell suspension (200 000 cells/mL) were 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 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 and incubated for 2 h more [19]. After that, 1 mL of methanol 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 570 nm. Cell viability was calculated using Equation 1:

$$cell\ viability\ \% = \frac{A_{sample}}{A_{control}}\ x\ 100 \quad (E.c\ 1)$$

Where A_{Sample} and $A_{control}$ represent that absorbances for each sample of formulation [20].

2.4. Statistical analysis

The experiments were done in triplicate. The means and standard deviations are considered for data set. The mean of each experimental condition was compared by One-way Analysis of Variance (ANOVA). The statistical significance between the compared means was established by applying a Tukey test with a confidence limit of 95% (*p<0.05).

3. Results and discussion

3.1. Cell migration assay

Cell migration requires the coordination of numerous cellular processes including polarization, actin cytoskeletal reorganization, membrane cycling, and adhesion. One approach to studying this complex phenomenon has come through the use of small molecules which can be used to temporally and spatially regulate individual proteins and processes. Numerous small molecules that are known to inhibit cell migration exist and have been used extensively; however, our ability to perturb specific protein function is limited by the compounds available [21].

Cell migration is an important aspect to study it can also be applied to developmental, immunological, and wound healing studies and others.

The invasion assay provides thorough analysis of the ability of cells to sense a particular chemo-attractant and migrate through a physical barrier toward it. This test can be further used to investigate cell invasion by adding a layer of extracellular matrix or a layer of endothelial cells on top of the transwell membrane to mimic the process of the extracellular matrix (ECM) invasion and extravasation [22], [23].

The cell migration assay is observed in (Figure 2a and b), the confluence of plant cells in dark regions, indicating that the cells migrated into the polymer matrices that include the MOF-Zn(His) and MOF-Zn(Phe) to a greater extent, inducing greater activity metabolic, increasing its population.

It is appreciated that all the matrices that make up the hydrogels under study allow the migration and internalization of cells in their structures, which is associated with their granular fibrillar morphology with interconnected porosity. Red and green tomato cells migrate and are homogeneously distributed on the surfaces under study. Upon reaching the matrices, the cells adhere and begin to invade the interior of the matrix; this is promoted by the high biocompatibility of the polymeric components that make up the hydrogels.

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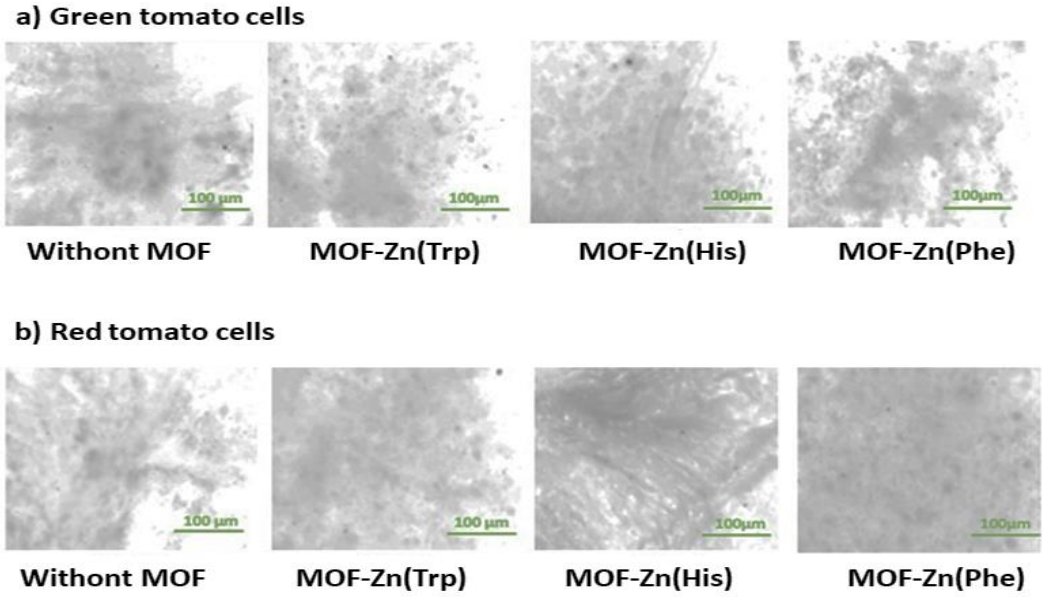

Figure 2. Micrograph of migration of green tomato cells in the different hydrogels with bioactive Zn (II) MOFs a) in the figure b) micrograph of red tomato cells in the different MOFs using and Inverted Microscope (100x)

3.2. Substrate degradation kinetics

The degradation profiles under these conditions (Figure 3) show that all matrices lose around 94 % and 98 % of their initial mass in the first two days.

In the literature it is mentioned that these degradation profiles of the hydrogels they are related with polymers that are covalently linked to degradable monomers [24].

All hydrogels of MOF-Zn(Trp), MOF-Zn(His) and MOF-Zn(Phe) maintained their dry mass corresponding to 2 %, indicating that the composite matrix does not suffer degradation effects regardless of the type of bioactive Zn(II) MOF. This water dosing process is controlled (linear trend).

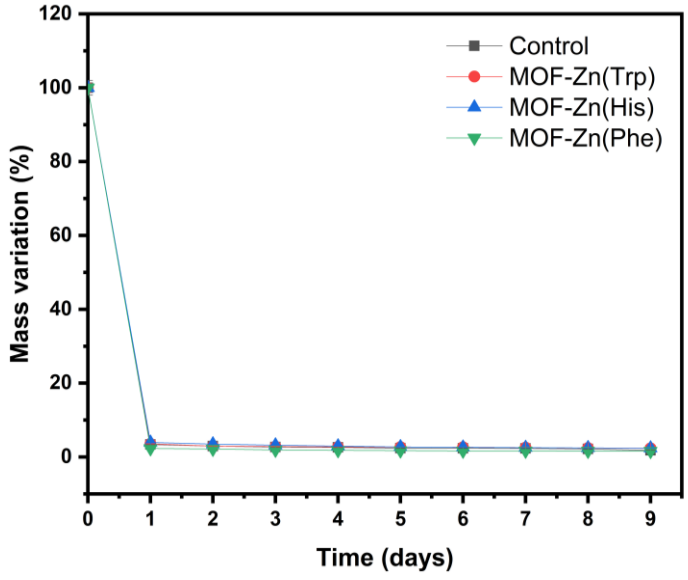

Figure 3. Mass variation profiles in contact with vegetable substrate

Interestingly, hydrogels have the capacity to diffuse water and are structurally stable for long periods of contact with the substrate. This can be used for plant cultivation strategies where the substrate is irrigated with water, the hydrogel swells again and supplies controlled way the water to the plant tissue, guaranteeing its use as a versatile growth scaffold.

In this biomatrices no significant variations in the masses are observed, regardless of the chemical nature of the MOF-Zn, indicating that there is no chemical, physical, and biological degradation processes associated with the substrate components. There are reports that indicate that MOFs have metal ions that exhibit antimicrobial properties, and this can be exploited to prevent the biodegradation of the matrix associated with pathogenic microorganisms in both animal and vegetable tissues [25].

3.3. Evaluation of the *in vitro* biocompatibility

For the cell viability experiment, a microculture tetrazolium (MTT) (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) colorimetric assay was performed to determine the cytotoxicity.

To measure the *in vitro* biocompatibility of composites in hydrogel state, it was evaluated the metabolic activity of green tomato cells and red tomato cells by the MTT assay (Figure 4a and 4b) exhibits that all materials show a percentage cell viability upper to the 60 % after 24 h and 48 h, indicating that are a non-cytotoxic material.

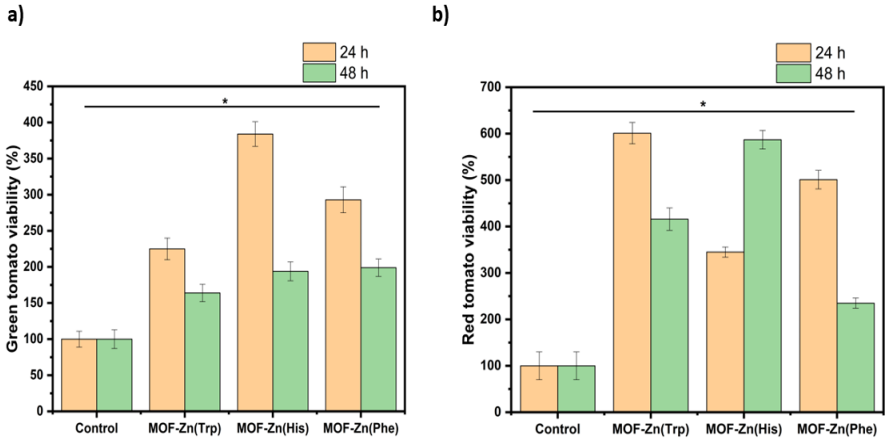

Figure 4. Metabolic activity assay MTT in green tomato cells a) and b) red tomato cells (*Solanum lycopersicum*) *(p <0.05, n=3)*

In the metabolic activity assay, an increase in the percentage of activity is observed, having a minimum metabolic activity percentage of 164 % in green tomato cells, which in comparison with what was reported in literature [26], which claims to have a viability greater than 150 %, indicates that there is the possibility of development of plant cells within the hydrogel, that is, growth in their population.

Red tomato cells show an acceleration of their metabolism in the hydrogel with MOF-Zn(His) at 48 h with activity percentage of 587 %, while in green tomato cells this phenomenon is seen when using the hydrogel with MOF-Zn(Phe) with activity percentage of 200 %. The high biocompatibility and biodegradation of the hydrogel components ensure their good functionality to control the metabolism of plant tissues, taking advantage of this advantage for the generation of biofertilizers in a hydrogel state.

Statistically significant differences for the metabolic activity of cells growing in all biomatrices compared to the control.

4. Conclusions

This project highlights novel results of the hydrogels that were synthesized with MOFs of MOFs-Zn (Trp), MOFs-Zn(His) and MOFs-Zn(Phe) into semi-IPN hydrogels composed of collagen and starch, with the objective to study plant growth.

The rapid loss of water in the first two days, with significant reductions in size and thickness in each measurement, evidenced the outstanding water absorption capacity of the synthesized hydrogels. This capacity, reflected in losses of between 94 % and 98 %, suggests a potential use to retain and gradually release water in agricultural applications.

In the metabolic activity assay, very high values were observed using green and red tomato cells, which is very good for significant cell development within hydrogels with this composition and, therefore, potential growth of the cell population vegetables.

It is clear that the results reveal the efficiency and potential of hydrogels synthesized with bioactive Zn(II) MOFs in applications related to the growth and development of plant tissue, highlighting their usefulness in the agricultural and biotechnological field. These findings open the door to future research and practical applications in the field of plant tissue engineering.

5. Future suggestions

The following are some future suggestions. (i) Incorporate different types of cells such as melon, watermelon, banana, among others, into the hydrogel matrices to test what new characteristics it gives to the hydrogel. (ii) Evaluate physicochemical behavior using FTIR, TGA and DSC. (iii) Perform cell proliferation tests using porcine fibroblast and monocytes cells. (iv) Evaluate the mechanical behavior of these new hydrogels.

Declarations

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Competing Interests Statement

The authors have declared that no competing financial, professional or personal interests exist.

Consent for Publication

All authors contributed to the manuscript and consented to the publication of this research work.

Authors' Contributions

All authors made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data.

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