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Tailoring Plant Cell Growth with Collagen-Starch Biomatrices with Calcium bioMOFs

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ABSTRACT

Biomatrices designed to stimulate the growth of tomato cells were prepared in this study. These biomatrices were composed of collagen-starch (CA) through a system of semi-interpenetrating polymer networks, allowing the inclusion of calcium metal-organic frameworks (MOFs) to facilitate plant metabolic stimulation. BioCaMOFs were prepared under hydrothermal conditions (100°C, 48 h) using $Ca(NO_3)_2$ and the essential amino acids L-phenylalanine, L-histidine, and L-tryptophan. A 1 mg of each bioCaMOF was dispersed in the collagen-starch matrix, generating the biomatrices CA-MOFCaF, CA-MOFCaH, and CA-MOFCaT, respectively. Infrared spectroscopy was employed to identify the chemical bonds present in each component of the biomatrices. The results indicated that the CA-MOFCaT biomatrix exhibited increased water swelling capacity (2900 ± 180 %) and the highest crosslinking index (74 ± 4 %). Tomato cells derived from sterile seeds (50,000 cells per biomatrix), both red and green varieties, migrated within the biomatrices, enhancing their metabolic activity. The biomatrices containing bioactive calcium MOFs actively stimulated the metabolism of plant cells for up to 48 hours upon contact. Determining that the biomatrix CA-MOFCaF promotes greater stimulation in green tomato cells, while CA-MOFCaT does so with red tomato cells. Such biomatrices hold promise as effective 3D cultures that promote the metabolism of plant cells and could lead to innovative formulations of biofertilizers.

Keywords: Biomatrix; Collagen; Starch; Calcium bioMOF; Hydrogel; Essential amino acids; Metabolism of plant cells; Tomato; Biofertilizer.

1. Introduction

Biofertilizers represent a sustainable and eco-friendly approach to enhancing plant growth and productivity. These biological formulations harness the power of beneficial microorganisms, such as bacteria, fungi, and algae, to modulate plant growth processes and improve nutrient uptake [1]. One of the key benefits of biofertilizers is their ability to establish symbiotic relationships with plants, where microorganisms colonize the rhizosphere or plant tissues and provide various growth-promoting benefits [2]. For instance, nitrogen-fixing bacteria like *Rhizobium* and *Azotobacter* convert atmospheric nitrogen into ammonia, which can be readily absorbed by plants, thus reducing the need for synthetic nitrogen fertilizers [3].

Similarly, *mycorrhizal* fungi form associations with plant roots, extending their reach into the soil and enhancing nutrient uptake, particularly phosphorus and micronutrients [4]. Biofertilizers also play a crucial role in enhancing soil fertility and structure [1-2]. By promoting the decomposition of organic matter and releasing plant-available nutrients, they contribute to soil health and sustainability. Additionally, certain microbial strains have been shown to suppress soil-borne pathogens and enhance plant resilience against diseases, thereby reducing the reliance on chemical pesticides [5].

Moreover, biofertilizers offer environmental benefits by minimizing nutrient leaching and runoff, thus mitigating the risk of water pollution and eutrophication [6]. Their use in agriculture aligns with sustainable farming practices, promoting biodiversity and reducing the ecological footprint of crop production [1-2].

Furthermore, advancements in biotechnology have led to the development of novel biofertilizer formulations tailored to specific crops and environmental conditions [7]. These include bioinoculants containing selected

microbial strains with proven efficacy in enhancing plant growth and stress tolerance. Integrated approaches combining biofertilizers with organic amendments, cover cropping, and precision agriculture techniques further optimize nutrient management strategies and promote sustainable agricultural practices [8].

Collagen-starch hydrogels have emerged as innovative and sustainable biofertilizers, offering unique advantages for promoting plant growth and enhancing soil fertility. These hydrogels, derived from natural sources like collagen and starch, possess excellent biocompatibility and biodegradability, making them environmentally friendly alternatives to traditional chemical fertilizers [9]. The use of collagen-starch hydrogels as biofertilizers is rooted in their ability to serve as effective carriers for nutrients, water, and beneficial microorganisms, facilitating their controlled release into the soil. By encapsulating essential nutrients such as nitrogen, phosphorus, and potassium within the hydrogel matrix, these biofertilizers ensure a steady and prolonged supply of nutrients to plant roots, promoting healthy growth and development [1-2]. Besides, collagen-starch hydrogels have the capacity to retain water and improve soil moisture levels, thereby enhancing drought tolerance and reducing water stress in plants [9]. This water retention capability not only conserves water resources but also contributes to the maintenance of optimal soil conditions for plant growth. Another advantage of collagen-starch hydrogels as biofertilizers lies in their ability to support the growth and proliferation of beneficial microorganisms such as nitrogen-fixing bacteria and *mycorrhizal* fungi [10]. These microorganisms form symbiotic relationships with plant roots, aiding in nutrient uptake, improving soil structure, and enhancing plant resilience against environmental stresses. Furthermore, the biodegradable nature of collagen-starch hydrogels ensures that they break down over time, releasing organic matter and nutrients into the soil, thereby enriching soil fertility and promoting long-term sustainability [11]. Unlike synthetic fertilizers, which can lead to soil degradation and environmental pollution, collagen-starch hydrogels offer a holistic approach to soil management, fostering ecosystem health and biodiversity.

Calcium-based Metal-Organic Frameworks (bioMOFs) have emerged as a promising tool in modern agriculture for enhancing plant growth and promoting sustainable crop production [12]. These innovative materials, composed of calcium ions coordinated with organic ligands, offer unique advantages for nutrient delivery, soil conditioning, and plant health promotion [13]. One of the key benefits of calcium bioMOFs lies in their ability to serve as efficient carriers for essential nutrients, such as nitrogen, phosphorus, potassium, and micronutrients [13]. By encapsulating these nutrients within their porous structure, bioMOFs facilitate controlled release mechanisms, ensuring a steady and prolonged supply of nutrients to plant roots. This controlled nutrient delivery system optimizes nutrient uptake by plants, promoting balanced growth and maximizing crop yield [12-13]. Additionally, calcium bioMOFs exhibit excellent water retention properties due to their high surface area and porosity [12-13]. They can absorb and retain water molecules, thereby improving soil moisture levels and enhancing drought tolerance in plants. This water retention capacity is particularly beneficial in arid and water-stressed environments, where maintaining adequate soil moisture is essential for plant survival and productivity.

In addition to nutrient delivery and water retention, calcium bioMOFs also play a crucial role in soil conditioning and fertility enhancement [14].

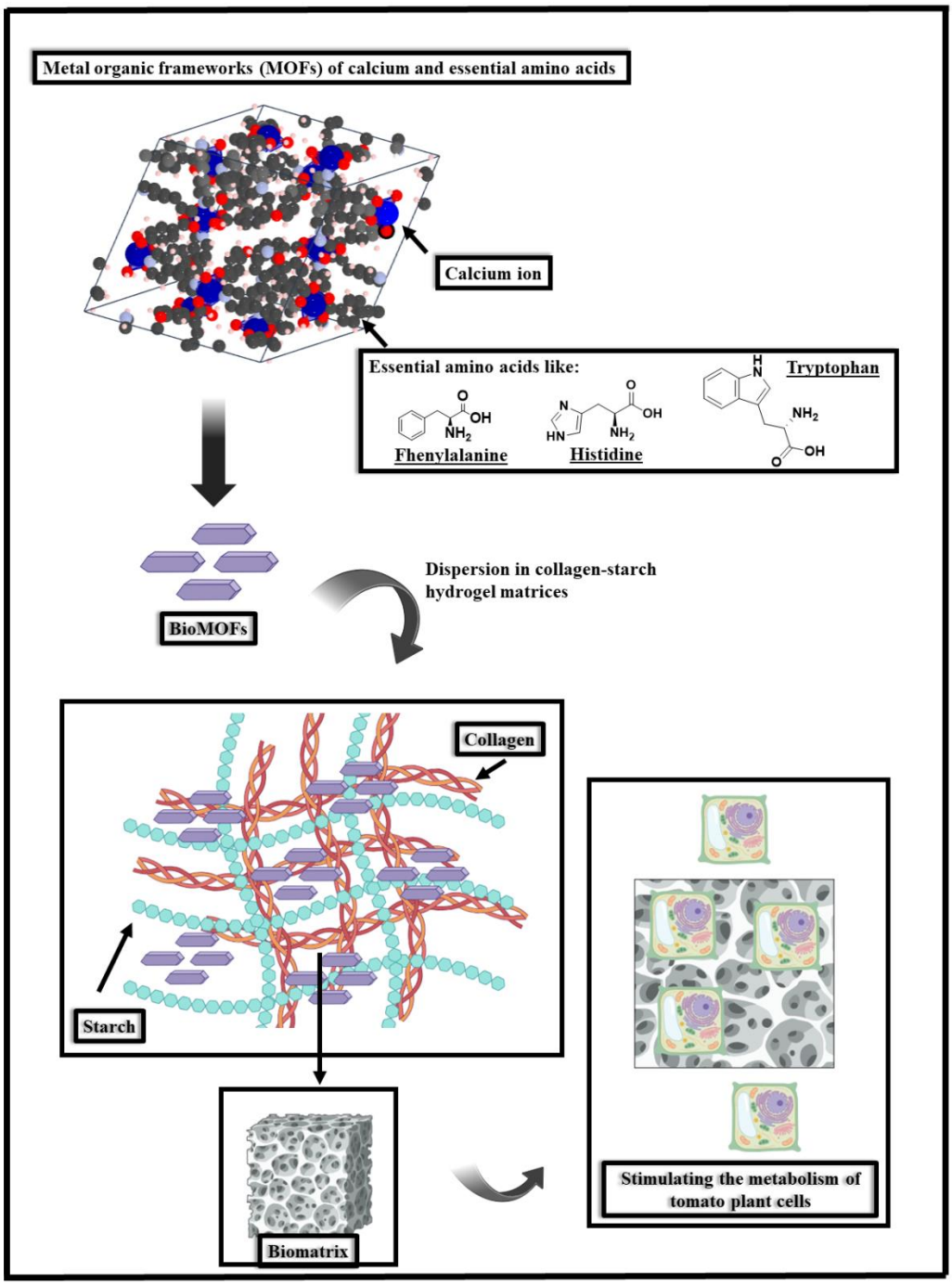

Figure 1. General scheme for obtaining new biofertilizers based on collagen-starch-calcium bioMOFs to modulate the growth of plant cells

Their porous structure provides habitat and support for beneficial soil microorganisms, such as nitrogen-fixing bacteria and *mycorrhizal* fungi, which contribute to nutrient cycling and soil health. On the other hand, the gradual degradation of bioMOFs releases calcium ions into the soil, promoting soil aggregation, pH buffering, and overall soil fertility improvement. Another notable advantage of calcium bioMOFs is their potential to mitigate environmental stress factors and enhance plant resilience against biotic and abiotic stresses. Studies have shown that bioMOF-treated plants exhibit increased tolerance to various stressors, including salinity, heavy metals, and pathogens, leading to improved crop quality and yield stability [15].

Based on these background findings, the present study proposes the creation of biomatrices to modulate the metabolism of plant cells. To achieve this, a family of calcium bioMOFs was prepared using essential amino acids such as L-phenylalanine (MOFCaF), L-histidine (MOFCaH), and L-tryptophan (MOFCaT). These bioMOFs were then dispersed in collagen-starch hydrogel matrices using a weight ratio of 1% for each calcium bioMOF. The hypothesis of the study suggests that based on the chemical structure of the calcium bioMOF, the physicochemical properties and metabolic activity of tomato cells could be adapted to generate new biomatrices for controlling plant growth and developing sustainable biofertilizers (Figure 1).

1.1. Study Objectives

The following are the main objectives of this study. (i) Synthesize hydrogel-based biomatrices using collagen, starch, and calcium-based metal organic structures to modulate the metabolism of tomato cells. (ii) Evaluate the chemical structure of the biomatrices generated through infrared spectroscopy. (iii) Determine the swelling-crosslinking relationship of the biomatrices. (iv) Study the effect of migration of encapsulated tomato cells in the biomatrices using phase-contrast microscopy. (v) Determine the influence of the chemical composition of the biomatrices on the metabolism of cells derived from red and green tomato seeds.

2. Materials and Methods

2.1. Materials

Porcine tendons, harvested from pig legs at the bustling local market, formed the foundation of our study. These tendons, procured with care, were subjected to a meticulous array of experiments with the purpose of obtaining type I collagen with molecular weights of 110 kDa and 220 kDa. To begin, a selection of essential chemicals including sodium chloride, potassium chloride, monopotassium phosphate, sodium acid phosphate, and sodium hydroxide were sourced from the reputable *CTR Scientific*. 2-propanol, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), glycerol ethoxylate (GE), hexamethylenediisocyanate (HDI), trimesic acid and a host of amino acids such as L-histidine, L-phenylalanine, and L-tryptophan. Additionally, essential reagents including calcium nitrate ($Ca(NO_3)_2$), trimesic acid (TA), and starch (with a molecular weight of 500 KDa) were obtained from the reliable shelves of *Sigma-Aldrich*. Our plant cell culture endeavors were supported by the acquisition Murashige and Skoog culture media from the same *Sigma-Aldrich*. As our research ventured beyond the confines of the laboratory, we sought the nurturing embrace of nature itself. Tomato plants and seeds, vibrant with life, were procured from a local greenhouse, ready to be integrated into our scientific exploration.

2.2. Synthesis of biomatrices

In the pursuit of novel biomaterials, hydrogel biomatrices were meticulously crafted through a series of precisely orchestrated steps. Firstly, Type I collagen, extracted from porcine tendons via enzymatic hydrolysis, was transformed into a foundational material. This collagen, with molecular weights of 220,000 Da and 110,000 Da, underwent a method meticulously detailed in existing literature [16], ensuring the integrity of the derived biomatrix. Parallelly, the polyurethane crosslinker, an integral component of our biomatrices, was synthesized from glycerol ethoxylate (GE) and hexamethylenediisocyanate (HDI). The synthesis protocol, well-documented

elsewhere [17], yielded a crosslinker within the molecular weight range of 3000–7500 Da, ensuring optimal structural integrity for biomatrices. The centerpiece of our innovation, the calcium-based bioMOFs, emerged from hydrothermal synthesis. Employing $Ca(NO_3)_2$ alongside amino acids L-tryptophan, L-histidine, and L-phenylalanine, and trimesic acid (TA) as a co-ligand, a family of bioMOFs—MOFCaF, MOFCaH, and MOFCaT—came into existence. With equimolar ratios of metal ions and ligands, and a 30:60 ratio for TA:amino acid, the hydrothermal conditions at 100 ºC for 24 hours facilitated the formation of robust coordination polymer networks. Following synthesis, the resultant solid was meticulously filtered, washed with distilled water, and dried at 60 ºC for 24 hours [18]. In the subsequent stages of biomatrix production, dispersions of each Ca-bioMOF were integrated into a collagen solution at a ratio of 1 mg of bioMOF using 24-well culture plates. Augmenting this suspension, 15 wt.% of polyurethane served as the crosslinking agent, bolstering the structural integrity of the biomatrix. To further fortify the matrix, starch, comprising 30 wt.%, was introduced into the mixture, culminating in the formation of semi-IPN systems. Maintaining a pH of 7.4 with a phosphate-buffered solution (PBS 10X), the reaction mixture underwent incubation at 37°C for 24 hours, allowing for the maturation of the biomatrices (Figure 1). Table 1 elucidates the chemical compositions and designations of the synthesized biomatrices, showcasing the culmination of meticulous design and precise execution in the realm of biomaterial innovation.

Table 1. Mass Composition (%) of Biomatrix Components

Biomatrix	Collagen (%)	Starch (%)	Polyurethane Crosslinker (%)	CabioMOF (%)
CA	60	25	15	0
CA-MOFCaF	60	18	15	7
CA-MOFCaH	60	18	15	7
CA-MOFCaH	60	18	15	7

2.3. Physicochemical characterization techniques of biomatrices

To assess the properties of the hydrogel state biomatrix, several analytical techniques were employed. The maximum swelling capacity was determined by comparing the mass of the hydrogel state biomatrix with that of the water-free biomatrix. This calculation provided valuable insights into the biomatrix's ability to absorb and retain water, crucial for its functionality in various applications. The crosslinking index, a key parameter influencing the biomatrix's mechanical and structural properties, was indirectly determined using the ninhydrin test. This test allowed for the quantification of crosslinked amino groups within the biomatrix, offering insights into its crosslinking density and stability.

Further characterization of the hydrogels was conducted using Fourier transform infrared (FTIR) spectroscopy. Employing *PerkinElmer* Frontier equipment, spectra were acquired within the range of 600–3600 cm^{-1} with a resolution of 16 cm^{-1}. This analytical approach enabled the identification of functional groups present in the biomatrix, elucidating its chemical composition and molecular structure. Such insights are crucial for understanding the interactions within the biomatrix and optimizing its performance for specific biomedical applications.

2.4. In vitro biocompatibility tests with tomato plant cells

Cells derived from both red and green tomato seeds were utilized in this study. Initially, 500 mg of seeds were collected and washed in a 2% ethanol solution for 30 minutes to remove any contaminants. Subsequently, the seeds were individually homogenized for 15 minutes and centrifuged at 3000 rpm for 15 minutes to obtain the plant cell pellets. These plant cell pellets were then seeded into culture dishes containing 30 mL of Murashige and Skoog (MS) medium and incubated at 25°C for 48 hours to facilitate proliferation and growth. Cell cultures derived from the seeds, with an initial cell density of 100,000 cells/mL, were examined under a bright-field microscope, and cell counts were performed using a Neubauer Chamber. To assess the migration of plant cells within the biomatrices, during the material preparation process under sterile conditions in a biosafety cabinet, 100 µL of cell suspension was added to each biomatrix formulation. The biomatrices with encapsulated cells were then incubated for 24 hours at 37°C to allow for hydrogel formation; subsequently, the systems were dried under sterile conditions at room temperature. The dried films were inspected under a bright-field inverted microscope (VELAB VE-403) to observe the adherence and migration of plant cells in each type of biomatrix. In other experiment, the metabolic activity of the cells growing on biomatrices was determined, the tetrazolium salt reduction (MTT) assay was employed. This assay provides a measure of cellular viability and proliferation by quantifying the conversion of MTT into formazan crystals, indicative of active mitochondrial function. These *in vitro* experiments aimed to evaluate the compatibility of tomato plant cells with the biomatrices, shedding light on their potential suitability for various biomedical applications.

The experiments were meticulously conducted in triplicate to ensure robustness and reliability of the data. Subsequently, the means and standard deviations were calculated for each dataset, providing a comprehensive understanding of the experimental outcomes. To analyze the variance between the means of different experimental conditions, a One-way Analysis of Variance (ANOVA) was employed. Following this, the statistical significance was determined through a Tukey test, utilizing a confidence limit of 95% (*$p<0.05$*). This stringent methodology ensured accurate assessment and comparison of the experimental results, enabling confident conclusions to be drawn from the research findings.

3. Results and Discussion

3.1. Structural and physicochemical characterization

The chemical structure of the biomatrices was inspected using infrared spectroscopy with an attenuated total reflectance accessory, and the spectra of each biomatrix are presented in Figure 2. The calcium-free bioMOF matrix (CA) exhibits stretching vibrations for amino and hydroxyl bonds around 3400 cm^{-1}, abundant in both the amino acids of collagen and the glucose units comprising starch. The -CH bonds are observed at 2700 cm^{-1}, associated with the organic matter skeletons characterizing the biopolymers forming the semi-IPN matrix. The urea crosslinking bond vibration is visualized at 1720 cm^{-1}, resulting from the addition of the isocyanate groups from the polyurethane crosslinker to the amino groups of collagen [9-17]. Additionally, the vibrational modes of amide I and amide II carbonyl bonds related to fibrillary collagen protein are presented at 1650 cm^{-1} and 1600 cm^{-1}, respectively. The urethane C-N bonds are observed at 1480 cm^{-1}, while vibrations in the plane of C-C bonds are

noted at 1250 cm^{-1}. The glucosidic (C-O-C) bonds, which bind the glucose units of starch, are found in the region around 1100 cm^{-1}.

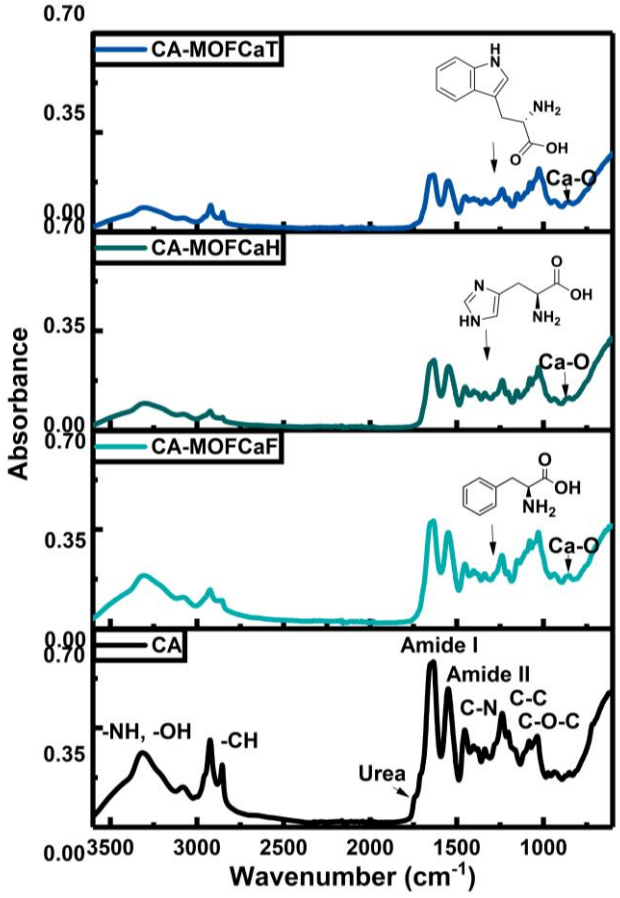

Figure 2. IR spectra of collagen-starch biomatrices coupled to calcium bioMOFs

For the biomatrices containing calcium bioMOFs, variations in the intensities of amino, hydroxyl, methylene, urea, amide I, amide II, and glucosidic bonds can be observed. This variation is associated with each bioMOF experiencing short-range hydrogen bonding interactions to become occluded within the polymeric system in a hydrogel state. The C=C bonds of the aromatic ring of MOFCaF are observed between 1500-1250 cm^{-1}; the -C=N and C=C bonds of the imidazole ring for MOFCaH are shown in the region between 1600-1200 cm^{-1}, while the C=C and C-N bonds for the indole ring of MOFCaT are appreciated between 1590-1250 cm^{-1}, ensuring that the calcium bioMOFs are occluded in the collagen-starch-based polymeric matrix. Additionally, an overtone at 950 cm^{-1} is detected for the biomatrices with calcium bioMOFs, indicating the presence of Ca-O bonds generated by the coordination of the carboxylate groups of the amino acids phenylalanine, histidine, and tryptophan with the coordination sphere of the calcium ion. A greater decrease in absorption signals for the different bonds comprising the biomatrices is observed in CA-MOFCaT, indicating that the indole nitrogen exhibits the capacity to crosslink with the biopolymers and polyurethane.

The crosslinking and swelling capacity of the biomatrices were also studied, and the results are depicted in Figure 3. Crosslinking indices of 47 ± 3%, 58 ± 3%, 60 ± 3%, and 75 ± 4% are recorded for CA, CA-MOFCaF, CA-MOFCaH, and CA-MOFCaT, respectively. Statistically significant differences are determined for the

crosslinking values of the biomatrices with calcium bioMOFs compared to the control. The carboxylate groups present in the bioMOFs can generate amide interactions that regulate the crosslinking of the collagen-starch system. Regarding the chemical structure of the amino acid comprising the calcium bioMOF, it is noted that tryptophan exhibits a basic or nucleophilic character, promoting the addition of indole nitrogen to both the isocyanate groups of the crosslinker and the amino groups of collagen, significantly enhancing its crosslinking, and generating a system with greater semi-interpenetrating capacity, as inspected by IR spectroscopy. The improvement in the crosslinking index may induce significant changes in surface topology and mechanics, altering pore size, water retention capacity, and modulation of cellular metabolism [19].

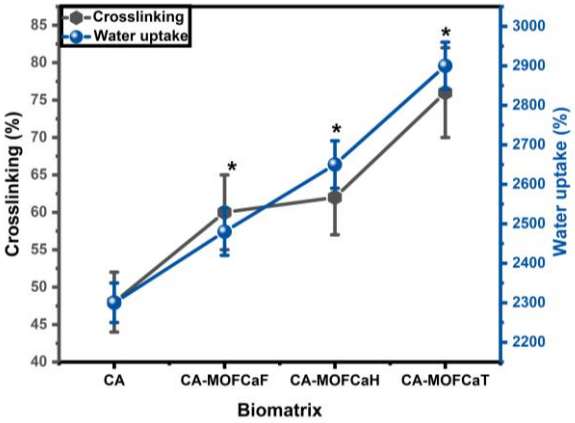

Figure 3. Crosslinking-swelling relationship of collagen-starch biomatrices with calcium bioMOFs

Regarding the maximum swelling capacity exhibited by the biomatrices, values of 2250 ± 120%, 2440 ± 132%, 2650 ± 114%, and 2900 ± 180% are observed for CA, CA-MOFCaF, CA-MOFCaH, and CA-MOFCaT, respectively. Similarly, statistically significant differences are found for the biomatrices with calcium bioMOFs compared to the matrix without MOF (CA). The increase in water absorption capacity is attributed to the high surface area presented by the calcium bioMOFs, coupled with their composition rich in amino and carboxylate groups, which promote greater water uptake in their structure. The polarity of the amino acid in the calcium bioMOF also plays a significant role in the swelling capacity of the biomatrix, determining that the more hydrophobic the amino acid, the lower the water absorption capacity of the matrix. This is evident in the case of CA-MOFCaF, where the aromatic ring of phenylalanine represents a hydrophobic region that limits water uptake. On the other hand, the indole ring in tryptophan exhibits a high capacity to form hydrogen bonds with water molecules, significantly enhancing the swelling of the CA-MOFCaT biomatrix. The biomatrices demonstrate a superabsorbent capacity for water (values exceeding 2000%), which could be harnessed for the controlled supply of water to plant tissues, thereby promoting the growth and proliferation of encapsulated plant cells within such biomatrices [20]. In addition, these biomatrices can be used as substrates for plant cultivation that promote water control for sustainable agricultural strategies [21].

3.2. In vitro biocompatibility studies with tomato cells

Cell inocula derived from tomato growing in MS medium were encapsulated in the biomatrices under study to observe their adhesion and migration into each polymeric matrix during 48 h. Micrographs are shown in Figure 4.

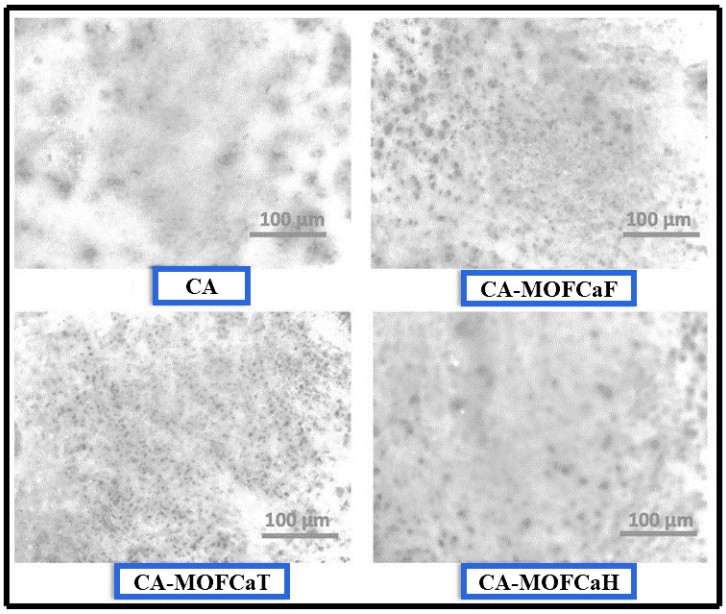

Figure 4. Encapsulation, proliferation and migration of tomato cells in collagen-starch biomatrices with calcium bioMOFs

In all systems under study, dense populations of tomato cells adhered to the surface of each biomatrix, and it was also observed that cells tended to migrate into the interior of each biomatrix. The addition of calcium bioMOFs promoted greater growth of plant cells migrating into these biomatrices. Once inside each biomatrix, cells associated into populations growing on the granular fibrillary clusters of the collagen-starch system. It is evident that the porosity characterized for each surface is important to allow proliferation and migration of tomato cells. A higher presence of plant cell populations is appreciated for the CA-MOFCaT biomatrix, indicating that the high crosslinking and swelling of this system influence plant cell migration. However, significant cell confluence can also occur in the CA-MOFCaF and CA-MOFCaH biomatrices, which is associated with the presence of calcium ions, an important trace element for plant cell metabolism. These results demonstrate the great potential of these matrices for the encapsulation of tomato cells without altering their fundamental functions such as proliferation, adhesion, and migration. The presence of calcium bioMOFs in these biopolymeric matrices could enable the controlled release of encapsulated cells to application sites such as nutrient-deficient soils that do not support plant growth, representing a potential alternative for soil bioremediation in agricultural uses [22].

The metabolic activity of red and green tomato cells extracted from seeds grown in MS medium was also evaluated using the tetrazolium salt reduction assay (MT), and the results are shown in Figure 5 for incubation times of 24 h and 48 h. In the case of green tomato cells (Figure 5a), metabolic activity values exceeding 75% are observed, indicating that there are no cytotoxic effects that hinder the metabolic functions of green tomato cells. At 24 h, there is no significant stimulation of metabolic activity observed for tomato cells growing on the biomatrices; only the CA-MOFCaF matrix shows significant variation compared to the control and the matrix without bioMOF (CA). At 48 h of evaluation, it is observed that cells growing in the biomatrices with calcium bioMOFs experience a metabolic hyperstimulation, reaching values exceeding 200%, with statistically significant differences compared to CA and the control. This hyperstimulation is associated with the controlled release of the essential amino acids

phenylalanine, histidine, and tryptophan, as well as calcium ions, which activate the metabolism of green tomato cells after 48 h of contact with the biomatrices. The CA-MOFCaF matrix promotes a greater stimulation of metabolic activity in green tomato cells. The influence of phenylalanine on green tomato growth is a subject of significant interest in agricultural research. Phenylalanine, an essential amino acid, plays a crucial role in plant growth and development as it serves as a precursor for various secondary metabolites, including lignin, flavonoids, and phenolic compounds [23]. These secondary metabolites are involved in numerous physiological processes such as defense against pathogens, regulation of plant responses to environmental stresses, and modulation of root development. Studies have shown that adequate levels of phenylalanine are essential for promoting healthy growth in green tomatoes. Phenylalanine contributes to the synthesis of lignin, a component of plant cell walls that provides structural support and helps plants withstand mechanical stress. Additionally, phenylalanine-derived phenolic compounds act as antioxidants, protecting plant cells from oxidative damage caused by reactive oxygen species [24].

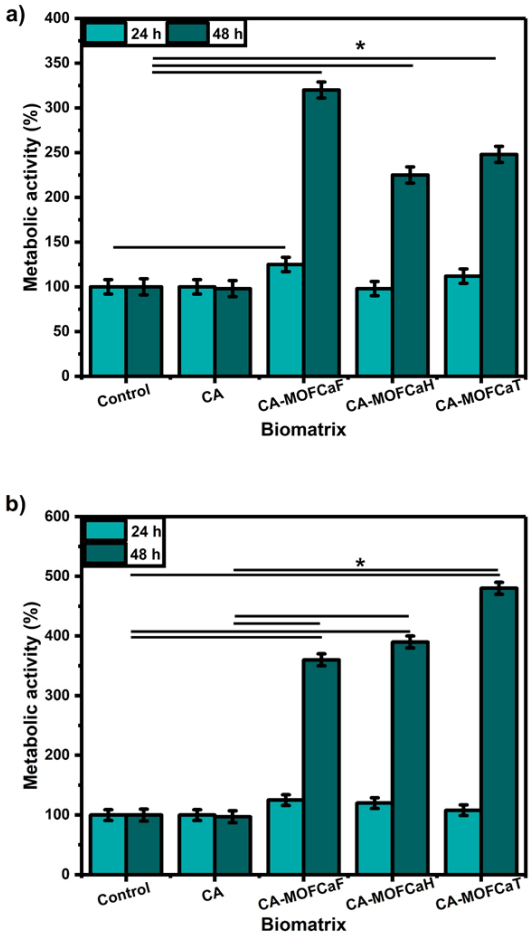

Figure 5. Evaluation of the metabolic activity of a) green and b) red tomato cells by the MTT salt reduction assay

Furthermore, phenylalanine metabolism intersects with various signaling pathways involved in plant growth regulation. It influences hormone biosynthesis and signaling, particularly auxin, which plays a pivotal role in controlling cell division, elongation, and differentiation. Moreover, phenylalanine-derived metabolites can modulate gene expression, affecting the expression of genes involved in growth-related processes [25]. However,

excessive levels of phenylalanine can have detrimental effects on green tomato growth. High concentrations of phenylalanine may disrupt metabolic pathways, leading to metabolic imbalances and inhibiting plant growth. Moreover, phenylalanine can serve as a precursor for allelopathic compounds that inhibit the growth of neighboring plants, highlighting the importance of maintaining optimal phenylalanine levels for promoting green tomato growth [26].

In the case of red tomato cells (Figure 5b), it is observed that within the initial 24 hours of contact with the biomatrices, there is no significant increase in their metabolic activity, indicating that the composition of these biomatrices is not cytotoxic to this type of cell. After 48 hours of contact with the biomatrices, those containing calcium bioMOFs exhibit a hyperstimulation of their metabolic activity, with significant differences compared to CA and the control. In this scenario, the CA-MOFCaT biomatrix records the highest metabolic activity values. Similarly, the controlled release of essential amino acids and calcium ions from these biomatrices allows for the stimulation of red tomato cell metabolism. The influence of tryptophan on green tomato growth is a subject of considerable interest in agricultural research. Tryptophan, an essential amino acid, serves as a precursor for various important molecules and compounds involved in plant growth and development [27]. One of the key roles of tryptophan in red tomato growth is its involvement in the biosynthesis of auxin, particularly indole-3-acetic acid (IAA) [28]. Auxins are phytohormones that regulate numerous aspects of plant growth, including cell elongation, root development, and fruit formation [29]. Tryptophan acts as a precursor for the synthesis of IAA, which plays a crucial role in promoting cell division and elongation, leading to overall plant growth and development [27]. Additionally, tryptophan-derived metabolites such as serotonin and melatonin also contribute to various physiological processes in plants. Serotonin has been implicated in stress responses, modulation of stomatal opening, and regulation of plant growth, while melatonin is involved in antioxidant defense mechanisms and modulation of circadian rhythms [30]. These molecules can influence green tomato growth by regulating stress tolerance, photosynthesis, and overall plant health. Moreover, tryptophan participates in the synthesis of secondary metabolites such as alkaloids and flavonoids, which play roles in plant defense against pathogens and herbivores. These compounds can enhance the resilience of red tomato plants by deterring pests and pathogens, thereby promoting healthier and more vigorous growth. Furthermore, tryptophan metabolism intersects with various signaling pathways involved in plant responses to environmental cues such as light, temperature, and nutrient availability [30]. By modulating gene expression and signaling cascades, tryptophan can influence the plant's ability to adapt and thrive in changing environmental conditions, ultimately impacting green tomato growth and productivity. However, it's important to note that excessive levels of tryptophan or its metabolites can potentially have adverse effects on plant growth. High concentrations of tryptophan may disrupt metabolic pathways or lead to the accumulation of toxic intermediates, thereby inhibiting normal plant growth processes [31].

The composition of these collagen-starch biomatrices and their selective and controlled ability to provide essential nutrients such as amino acids and calcium from the bioMOFs to tomato cells are features that can be leveraged for the design of sustainable biofertilizers with potential agricultural applications. As the hydrogels regulate water supply, the growth of tomato plants can be tailored to controlled water requirements, thereby avoiding the use of fertilizers that harm aquifers. The chemical composition of these biomatrices would provide important nutritional

requirements to stimulate metabolic activity and growth of tomato plants. Finally, after their useful life, the biomatrices can be easily degraded by soil enzymes or microorganisms, thus representing hydrogels for agriculture with a highly sustainable focus.

4. Conclusion

In conclusion, this study successfully prepared biomatrices designed to stimulate the growth of tomato cells. These biomatrices, composed of collagen-starch (CA) with the incorporation of calcium metal-organic frameworks (MOFs), were developed through a system of semi-interpenetrating polymer networks. The bioCaMOFs, synthesized under hydrothermal conditions using $Ca(NO_3)_2$ and essential amino acids (L-phenylalanine, L-histidine, and L-tryptophan), were dispersed within the collagen-starch matrix to create biomatrices designated as CA-MOFCaF, CA-MOFCaH, and CA-MOFCaT, respectively. Through infrared spectroscopy, the chemical bonds present in each component of the biomatrices were identified. Results revealed that the CA-MOFCaT biomatrix exhibited superior water swelling capacity (2900 ± 180%) and the highest crosslinking index (74 ± 4%). Tomato cells, derived from sterile seeds, including both red and green varieties, demonstrated migration within the biomatrices, enhancing their metabolic activity. Remarkably, the biomatrices containing bioactive calcium MOFs actively stimulated plant cell metabolism for up to 48 hours upon contact. Specifically, CA-MOFCaF displayed greater stimulation in green tomato cells, while CA-MOFCaT exhibited similar effects with red tomato cells. These findings suggest the potential of such biomatrices as effective 3D cultures that promote plant cell metabolism and pave the way for innovative formulations of biofertilizers.

5. Future suggestions

The following are some future suggestions. (i) Vary the content of calcium bioMOF in the biomatrix to enhance the migration and metabolism of red and green tomato cells. (ii) Study the degradation profiles of the hydrogel-state biomatrices in the presence of commercial substrate for vegetables. (iii) Evaluate the germination process of red and green tomato plant seeds in the presence of the developed hydrogels. (iv) Conduct release studies of the amino acids composing the biomatrices to investigate their bioavailability in plant cells. (v) Utilize the biomatrices as biofertilizers in agricultural strategies for tomato cultivation.

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 the 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.

Availability of data and material

Supplementary information is available from the authors upon reasonable request.

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