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Incorporation of Se (IV) Complexes based on Amino Acids in Biomatrixes in Hydrogel State: Effect of the Amino Acid on the Structure and Properties of Biomatrixes for Biomedical Applications

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ABSTRACT

Selenium is a non-metal that shows biological interest since it is responsible for modulating various proteins at the micronutrient level in living beings. In this work, new complexes based on the Se (IV) ion with amino acids such as phenylalanine (Se-F), histidine (Se-H) and tryptophan (Se-T) were hydrothermally synthesized and characterized. These were incorporated into biomatrixes based on semi-interpenetrated polymeric networks (Semi-IPN) of collagen-polyurethane-guar gum (CPGG) by the microemulsion process using a mass ratio of 1 wt.% with respect to collagen. The structural and crystalline characteristics that the selenium-amino acid complexes show a performance in modulating the properties of the biomatrixes under study. The results indicate that the incorporation of the complex decreases the crosslinking of the hydrogel, generating granular surfaces with porosity dependent on the type of amino acid. The CPGG Se-T biomatrix shows a swelling capacity of 10200 ± 1100 higher than the CPGG base matrix; while the CPGG Se-F and CPGG Se-T biomatrixes present slow degradation at both physiological and acidic pH. Interestingly, the matrix that includes the Se-F complex significantly stimulates the metabolic activity of L929 fibroblasts for up to 48 h, stimulating their proliferation. The fibroblasts encapsulated on these novel biomatrixes show recurrent release capacity for up to 7 days, where the structure of the CPGG Se-H biomatrix exhibits greater release from the encapsulated cells. These results demonstrate that these innovative biomatrixes could be used in biomedical applications such as dermal tissue regeneration and cell release for a specific biological fate.

Keywords: Selenium-complex; Biomatrix; Amino acid; Biomedicine; Polymers; Biotechnology.

1. Introduction

Selenium is a non-metal and represents a trace element required by living beings to catalyze their enzymatic processes. In mammals, it has been shown that the redox capacity and ligand exchange that this element has can control physiological processes such as cell reproduction, function hormonal, production of genetic material (DNA synthesis), protection against infections and shows antioxidant properties avoiding damage caused by free radicals [1-3]. From the point of view of the coordination chemistry of this element, the coordination sphere of the Se (IV) ion can accept electron pairs from ligands such as sulfides, cyanide, glutathione, proteins and amino acids, generating trigonal, monoclinic and/or amorphous (reddish brown amorphous selenium) structures; these types of structures are formed by the interaction of selenate (SeO_4^{2-}) and/or selenite (SeO_3^{2-}) species that coordinate with the aforementioned ligands, generating complexes with specific bioactivity [4-5].

Recently, the synthesis of new complexes based on Se(IV) with amino acids such as phenylalanine (Se-F), histidine (Se-H) and tryptophan (Se-T) using trimesic acid as a template to generate coordination polymeric blocks has not been reported, representing novel coordination polymers with selective biological activity. The F, H and T amino acids were chosen for the design of these new complexes based on Se (IV) since they are responsible for modulating processes such as protein metabolism [6], modulating the growth of neurons [7] and the production of agents with biological interest such as melatonin and serotonin [8], for each of the amino acids respectively. However, due to low solubility of this type of complex in water, for biomedical applications it is advisable to have release or dosage

vehicles for these complexes so that they reach their biological fate and they have the expected biochemical effect [9-10]. With this in mind, the use of biomatrixes in the hydrogel state could represent a potential alternative to study the cell-selenium complex interaction, since the three-dimensional (3D) structure of the hydrogel allows the inclusion of a diversity of inorganic components such as silica particles and metal-organic frameworks (MOFs) allowing to know the effect of these encapsulated components on the biological response [11-12]. In our working group, a variety of MOFs have been encapsulated by the microemulsion method in hydrogels of semi-interpenetrated polymeric networks (Semi-IPN) of collagen-polyurethane-guar gum (CPGG), and the effect of the chemical structure of the MOF on its bioactivity, and contaminant capture properties present in wastewater has been studied [12-13]. Based on this, the present work proposes the synthesis of new Se-F, Se-H and Se-T complexes and their structural and chemical characterization, and they will be incorporated into CPGG matrices to evaluate the effect of the amino acid structure that conforms the selenium complex on the physicochemical, structural properties and biological response of the hydrogels under study, thus generating innovative biomatrixes that could be applied in biomedical fields, such as the modulation of cellular metabolism dependent on the chemical composition of the biomatrix (Figure 1).

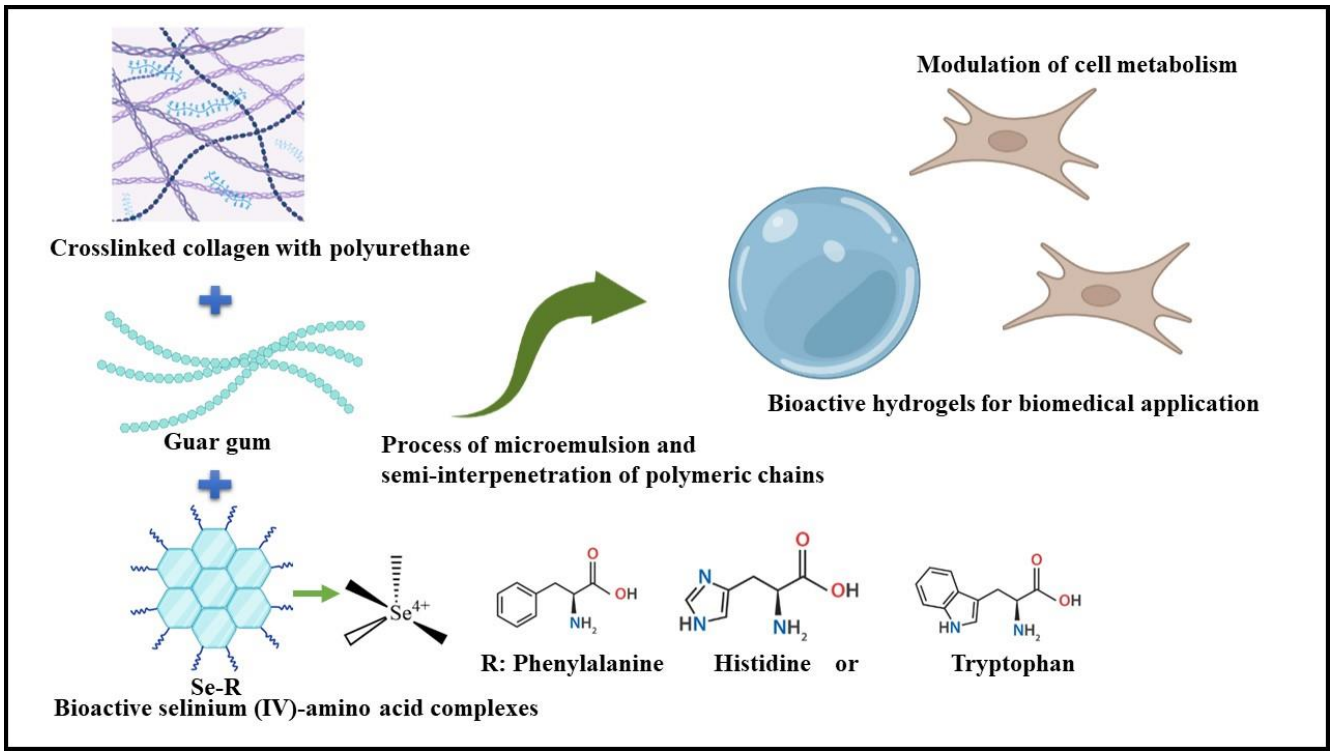

Figure 1. General scheme for obtaining biomatrixes based on collagen-polyurethane-guar gum hydrogels with complexes comprised from Se (IV) with different amino acids for potential biomedical application

2. Experimental Section

2.1. Chemicals

Selenium(IV) chloride ($SeCl_4$), 1,3,5-benzenetricarboxylic acid (BTC), L-histidine (His), L-trypthopan (Trp), L-phenylalanine (Phe), guar gum (extracted from Cyamopsis tetragonoloba, M_w = 220 kDa), and 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich Co., and they were

used as received. Calcein acetoxymethyl ester (calcein-AM) was purchased from Thermo- Fischer Scientific. Collagen form porcine dermis was extracted by enzymatic hydrolysis with pepsin as reported elsewhere (M_n α_1 = 220 kDa, α_2= 110 kDa) [14]. A polyurethane crosslinker was prepared from 1,6-hexamethylene diisocyanate and glycerol ethoxylate as reported elsewhere [15].

2.2. Synthesis of Se(IV)-based complexes

Se(IV)-based complexes were synthesized by the hydrothermal method. For this, 1 mmol of Selenium(IV) chloride, 1 mmol of BTC, and 1 mmol of the suitable amino acid (F, H or T) were mixed with magnetic stirring at room temperature. Then, the mixture was transferred to a Teflon-lined autoclave, and the reaction was carried out at 120°C for 72 h. The colorless precipitate obtained from the reaction was filtered, rinsed with water, and dried at 60°C. The obtained Se(IV)-based complexes were labeled as Se-F, Se-H and Se-T depending on the amino acid used as ligand.

2.3. Preparation of Hydrogel Biomatrixes

Previously, a collagen solution (6 mg mL^{-1}) containing 1 wt.% of Se(IV)-based complexes with respect to collagen, and guar gum solution (0.5 wt.%) were prepared. Biomatrixes were prepared by mixing 1 mL of collagen solution and 15 µL of polyurethane crosslinker in a culture plate (used as a mold). After that, 120 µL of guar gum solution was added (representing 30 wt.% of the mass of the CPGG biomatrix), 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 biomatrixes, which were labeled as CPGG Se-F, CPGG Se-H and CPGG Se-T depending on the Se(IV)-based complex used. For the comparison of results, a biomatrix formulation without Se(IV)-based complex was prepared (CPGG).

2.4. Characterization

FTIR spectra were recorded using a Perkin-Elmer Frontier spectrophotometer equipped with a total attenuated reflectance accessory (ATR) with a spectral resolution of 4 cm^{-1}. WAXS (SAXS-Emc2 Anton Paar diffractometer) was used to determine the crystallinity of complexes. Scanning electron microscopy was performed with a TOPCON SM-510 microscope operated at 10 kV. UV-Vis absorption measurements were performed with a ThermoScientific MultiSkan Sky spectrophotometer. Fluorescence microscopy observations were performed using a VELAB VE146YT microscope using a blue LASER as excitation source (λ = 441 nm).

For the determination of the swelling capacity, the mass of freshly prepared biomatrixes was registered. Then, samples were dried at room temperature until obtain a constant mass, measurements were performed in triplicate. The swelling degree was calculated using Equation 1 (Eq.1):

$$Swelling\ degree\ (\%) = \frac{m_0 - m_i}{m_i} * 100 \qquad \text{(Eq.1)}$$

Where m_0 is the initial mass of swollen biomatrixes and m_i is the mass of the dried biomatrix, respectively.

The reticulation of semi-IPN biomatrixes was analyzed reacting the polymeric matrixes with ninhydrin for 30 min at 90°C. The absorbance of the liquid phase obtained after the reaction was measured by spectrophotometry at 567

nm (samples were prepared in triplicate). Results were compared with unreticulated collagen, and the crosslinking degree was calculated with Equation 2 (Eq.2):

$$Crosslinking\ degree\ (\%) = \left(1 - \frac{A_{sample}}{A_{collagen}}\right) * 100 \qquad \text{(Eq.2)}$$

Where A_{sample} and $A_{collagen}$ are the absorbances of solutions obtained after ninhydrin reacted with biomatrixes or unreticulated collagen, respectively.

2.5. Evaluation of the in vitro biological response

The effect of the chemical structure of Se(IV)-based complexes on the metabolic activity of L929 fibroblasts growing in contact with biomatrixes was evaluated by the MTT assay. For this, 150 µL of cell suspension (30 000 cells/mL) were seeded over biomatrixes 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. 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. Metabolic activity of fibroblasts was calculated using Equation 3 (Eq.3):

$$Metabolic\ activity\ of\ fibroblasts(\%) = \frac{A_{sample}}{A_{control}} * 100 \qquad \text{(Eq.3)}$$

Where A_{sample} and $A_{control}$ represent the absorbances for each sample or formulation and PBS-1X, respectively. Values less than 60% cell viability are considered cytotoxic.

The effect of the composition of Se(IV)-based complexes on the proliferation of fibroblasts was studied by fluorescence microscopy. For this, 1 mL of fibroblasts culture (30000 cells/mL) was mixed with 1 mL of leaches extracted from semi-IPN biomatrixes and incubated at 37°C for 48 h. After incubation, cells were stained with calcein-AM, following the instructions provided by the supplier, PBS-1X was used as the control. Stained cells were transferred to a slide and were inspected with a fluorescence microscope.

2.6. Encapsulation and Release of fibroblasts

For the encapsulation of L929 fibroblasts, biomatrixes were prepared as described previously, but 200 µL of cell culture (30 000 cells/mL) was added to each hydrogel before carrying out the gelation. Then, biomatrixes were placed in PBS-1X as releasing medium (10 mL per hydrogel), and then they were incubated at 37 °C without stirring. Aliquots of the releasing medium were taken at different time intervals and the absorbance was measured at 446 nm which is related to cell turbidity. The experiment was realized in triplicate.

2.7. Statistical Data Analysis

All experiments were independently carried out at least three times. Mean and standard deviation (SD) are presented for each data set. Data sets were compared using analysis of variance (ANOVA). The difference in the means was checked with a Tukey test and was considered statistically significant at level $p < 0.05$.

3. Results and Discussions

3.1. Chemical and structural characterization of the Se(IV) complexes

The coordination complexes of Se (IV) with the F, H and T amino acids were characterized by IR spectroscopy, wide-angle X-ray scattering (WAXS) and superficially by scanning electron microscopy (SEM); the results are presented in Figure 2.

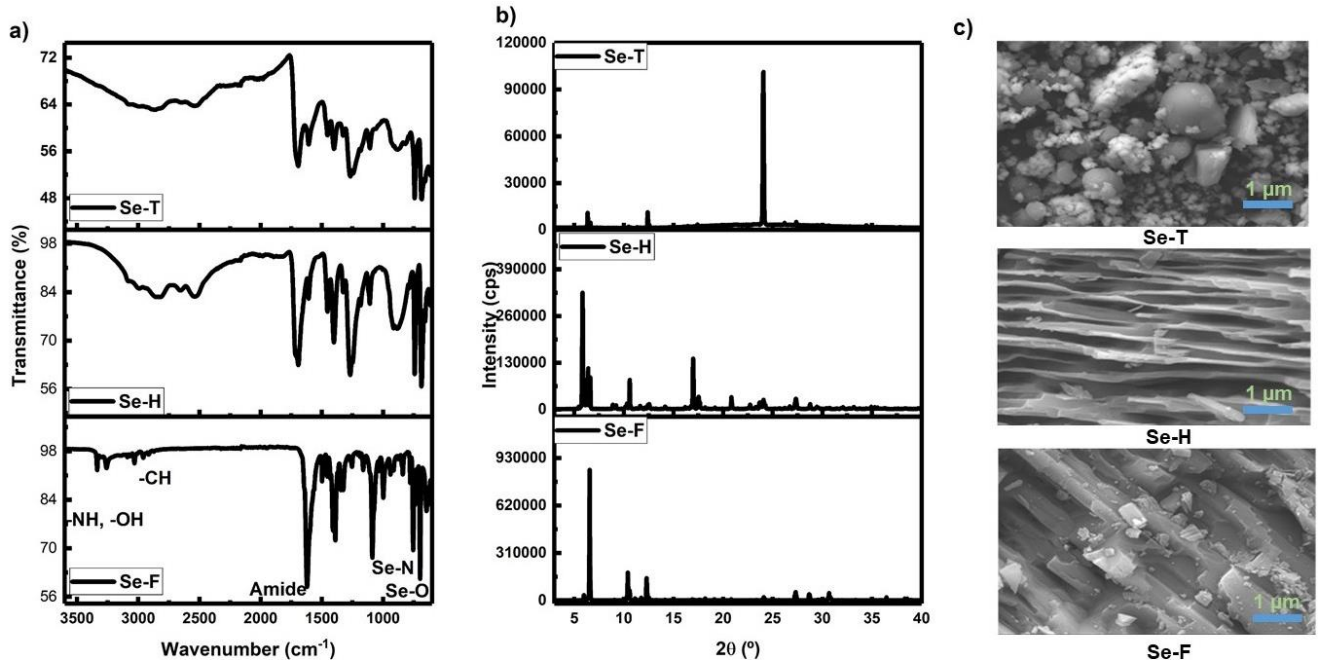

Figure 2. Physicochemical characterization of the Se (IV) complexes with the F, H and T amino acids: a) Evaluation of the chemical structure by FTIR, b) determination of crystallinity by WAXS and c) surface inspection by SEM

In the evaluation of the chemical structure by FTIR (Figure 2a) the stretching bands of the –NH, -OH, -CH, amide carbonyl, Se-N and Se-O bonds at wavenumbers of 3300, 3250, 2700, 1630, 770 and 720 cm^{-1}, are appreciated, respectively. The spectrum for the Se-F complex presents finer and more intense vibration bands, indicating that the hydrophobic character of the aromatic ring in the F amino acid benefits the coordination of the amino acid in the Se(IV) coordination sphere, generating cloisters of large dimension made up of tetragonal complexes that selenate can form when coordinated with this amino acid and trimesic acid.

The presence of the Se-N and Se-O bond bands confirm that amino acids can form coordination bonds with both the amino and carboxylate groups, respectively. In the case of the Se-H and Se-T complexes, both histidine and tryptophan are less hydrophobic than phenylalanine, and the free electron pairs of the imidazole rings tend to generate greater disorder in the form of complexation with the Se(IV) ion, which is associated with the shape of the bands between 3600-2700 cm^{-1} where the –NH, OH and –CH bonds are present; in addition, it is also visualized that in the amide carbonyl region, another vibration of this bond is exhibited at 1600 cm^{-1}, indicating that these amino acids have a greater degree of freedom to generate this type of vibration; besides, the narrower bands that Se-H and Se-T present indicate that these complexes generate complexation species with less organization when Se (IV) is complexed in the presence of trimesic acid template agent; all associated with phenomena of polarity.

The crystal arrangement of the Se-F, Se-H and Se-T complexes was inspected by WAXS (Figure 2b). The diffractogram for Se-F detects diffraction peaks at 2ɵ of 6, 7, 11, 13, 27, 29 and 31º; for Se-H signals are recorded at 2ɵ of 6, 7, 11, 16.5, 17.5, 22, 27 and 29º, and for Se-T there are peaks at 2ɵ of 7, 14, 23 and 27º. The signals at 22, 27 and 29º are associated with rhombohedral or trigonal selenate structures [16]; indicating that the three complexes under study present a crystalline arrangement with this unitary crystalline cell as the main characteristic. The highest intensity in the diffraction peaks can be seen in Se-F, indicating that there is a greater interaction between the components promoted by hydrophobic effects that allows generating surfaces with higher crystallinity, as was observed by the FTIR technique. Interestingly, in Se-T a significant increase in the peak at 23º is observed, which could be related to the fact that there are fewer interconnections of the unitary rhombohedral crystalline cells, generating complexes with variable particle size [17]. Each complex presents characteristic signals, and these are associated with the molecular arrangement that each amino acid has in the coordination sphere of the Se (IV) ion; however, amorphous halos are not seen in the diffractograms, which indicates that these molecular species are highly crystalline. From a biomedical point of view, crystallinity plays a fundamental role in the control of biological responses, such as cell adhesion, cell metabolism, and cell proliferation [18].

The surface morphology was evaluated by SEM (Figure 3c). The micrographs indicate surface morphologies that depend on the type of amino acid used. For Se-F, a stacked rods morphology is seen, resulting from the molecular packing of the rhombohedrons of Se (IV) coordinated with phenylalanine; in the case of Se-H, a superficial morphology characterized by sheets can be seen. These sheets are formed by the repetitive union of trigonal and rhombohedral unit cells that selenate generates by coordinating with histidine; indicating that the lone pairs of electrons from the imidazolic ring are involved in this surface arrangement; and finally for Se-T it is visualized that there is no homogeneous surface, and an aggregation of rhombohedral, tetragonal and quasispherical conformations are appreciated; this type of surface arrangement is the result of the polarity of the indole ring present in tryptophan; the electronic pairs of indole nitrogen can coordinate with the coordination sphere of Se (IV), and in turn the hydrophobicity of the aromatic ring generates molecular disorder that generates this type of irregular morphology. The crystallinity results studied by WAXS are interconnected with those of SEM, where the increase in the signal at 2ɵ of 26º is related to free particles of different molecular arrangement. The structure has a fundamental performance in the surface-cell interaction [19]; the following sections will demonstrate this hypothesis in order to know what type of complex shows to improve both the physicochemical properties and biological response, when these complexes are encapsulated in collagen-polyurethane-guar gum biomatrixes.

3.2. Influence of the structure of the Se(IV) Complex on the physicochemical properties and morphology of biomatrixes based on CPGG

For an effective application of the Se(IV) complexes, it is necessary that they be encapsulated in a bioactive biomatrix that serves as a support to analyze the effect that the structure of the complex has on the physicochemical and biological properties of this biomatrix, in order to assess whether they will have or not an application potential in biomedical areas. For this, the complexes were encapsulated in collagen-polyurethane-guar gum biomatrixes and different studies were carried out. Figure 3 shows the effect of the structure and composition of the Se(IV) complex on fundamental properties of biomatrixes in the hydrogel state, such as crosslinking and swelling.

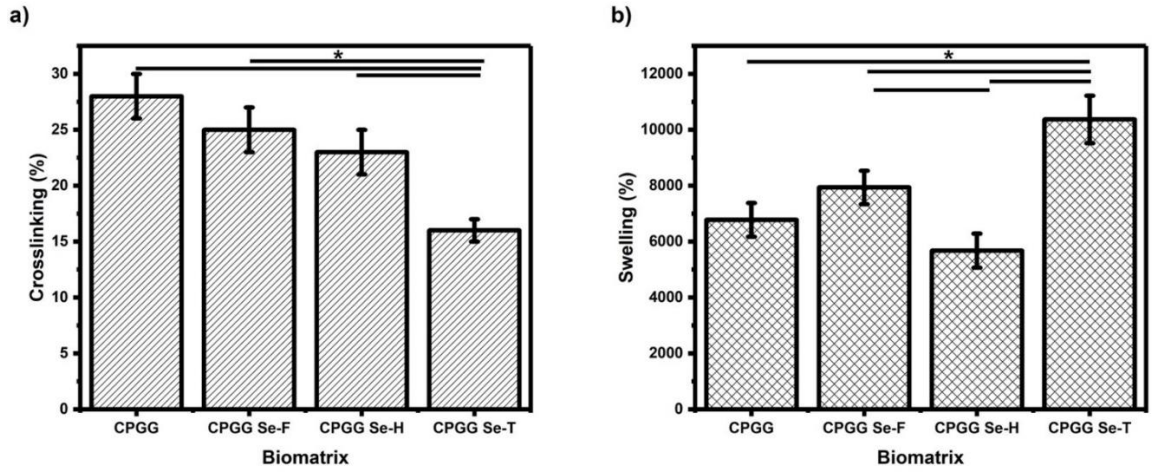

Figure 3. Behavior of a) Crosslinking and b) Swelling of CPGG biomatrixes coupled with new complexes based on Se (IV) and F, H and T amino acids

Crosslinking is related to the physicochemical entanglements that the chains that make up the biomatrix experience; this can be altered by the presence of exogenous agents such as the case of Se (IV) complexes. Crosslinking directly affects properties such as swelling, storage modulus, and release of molecules or cells that are encapsulated in biomatrixes in the hydrogel state [20]. Crosslinking indices of 27 ± 3 %, 25 ± 3 %, 23 ± 2 % and 16 ± 3 % are found for CPGG, CPGG Se-F, CPGG Se-H and CPGG Se-T, respectively, statistically significant differences are determined when comparing the biomatrix with the Se-T complex with respect to the others. The addition of the complex in the biomatrix decreases the crosslinking of the CPGG base biomatrix; this is associated with electronic repulsion events and steric hindrances that the structures of the complexes under study experience with the biopolymer chains (collagen, polyurethane and guar gum). When using the complexes with the highest structural conformation, that is, they have an organized structure such as Se-F and Se-H, there is no significant decrease in the crosslinking of the biomatrix; however, by using the complex that has surface heterogeneity and varied particle size, repulsion events that decrease crosslinking are maximized.

Another physicochemical property that characterizes a biomatrix in a hydrogel state is maximum swelling, which is associated with the molecular relaxation experienced by the chains that make up the biomatrix to allow a balance of water diffusion in its structure. Swelling is of vital importance in biomedical applications, since the water content has an impact on the biological response [21]. Maximum swellings of 6950 ± 145%, 8000 ± 264 %, 5860 ± 450% and 10200 ± 630% are recorded for CPGG, CPGG Se-F, CPGG Se-H and CPGG Se-T, respectively. Statically significant differences are found when comparing the swelling value of the biomatrix containing Se-T with respect to the others. In the first instance, it is observed that the complex with a sheet structure tends to decrease the swelling of the CPGG matrix; and secondly, the complex with the least structural organization is the one that shows the highest swelling of the biomatrix. From the physicochemical point of view, the sheet structure limits the entry of water into the biomatrix, and due to hydrophobicity effects related to the imidazolic ring, it is repelled to the outside; while at the other extreme, high swelling is promoted when particles of smaller size and lower molecular organization are entangled by the polymer chains that make up the biomatrix, allowing higher intra- and intermolecular space to exist so that greater water content can diffuse within of the CPGG Se-T matrix.

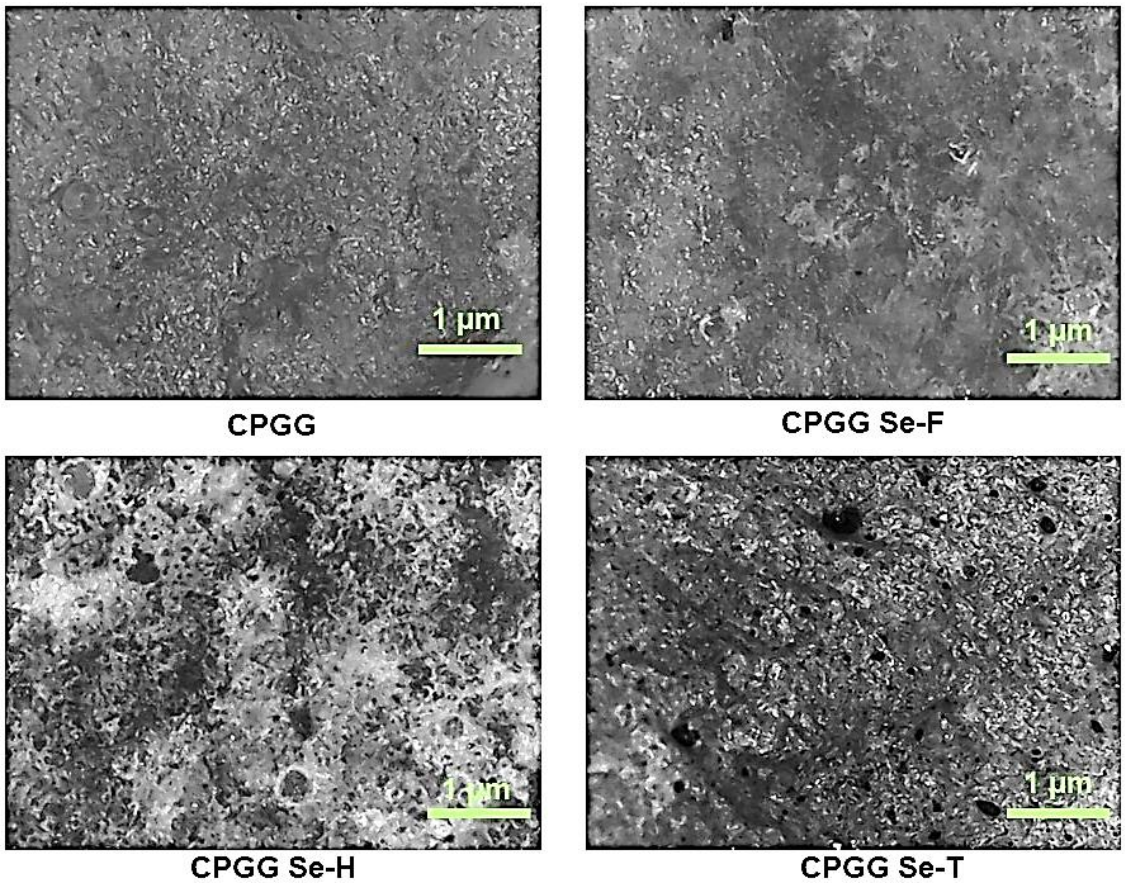

Figure 4. Surface microstructure of CPGG biomatrixes encapsulated with complexes of Se (IV) and F, H and T amino acids

The chemical structure of Se (IV) complexes with amino acids such as F, H, and T shows to affect the surface topology of the CPGG biomatrix; since the entanglement of the polymeric chains during the semi-interpenetration process is influenced by the nature of the Se(IV) complex. Superficial SEM micrographs of the biomatrix under study are presented in Figure 4. The CPGG biomatrix presents a relief characterized by an interconnected fibrillar surface with granulations attributed to guar gum polysaccharide. Depending on the amino acid used in the Se (IV) complexes, the change in the surface morphology of the base biomatrix is notorious. In the case of the CPGG Se-F biomatrix, agglomerations of rods are observed within the biomatrix in a uniform manner throughout the entire surface, presenting microporosity in specific regions, without significantly altering the crosslinking. For CPGG Se-H, a surface with agglomerated regions of sheets of the complex is presented on the surface of the biomatrix, indicating that these sheets alter the semi-interpenetration process between the biopolymers to generate heterogeneous regions with diverse porosity, and finally for the CPGG Se-T biomatrix shows a homogeneous surface with rigidity and higher porosity, which is associated with the entanglement events that the Se (IV) ion and tryptophan regulate during the process of polymeric semi-interpenetration, since in this case the particle size of the complex is smaller and irregular in shape, the microstructure tends to be more homogeneous, allowing better dispersion of the complex over the entire surface, thus improving water capture. The shape of the surface and its polarity properties are important factors that influence basic biological functions such as cell growth, diffusion of nutrients, waste products, and promote specific cell interaction regulated by the composition of the surface [22].

3.3. Effect of the structure of the Se(IV) Complex on the hydrolytic degradation of the biomatrix

The control of the rate of degradation of biomatrixes that modulate the biological performance in applications such as wound healing dressings, tissue regeneration scaffolds, as well as in the release of encapsulated both drugs and/or cells represents a challenge in the design of these innovators biomaterials [23]. The influence of the chemical composition of the complexes based on Se(IV) on the degradation of the CPGG biomatrix was studied by varying the pH (7,4 and 5,0). The degradation profiles that indicate the variation in mass with respect to time of the biomatrixes under these hydrolytic conditions are shown in figure 5.

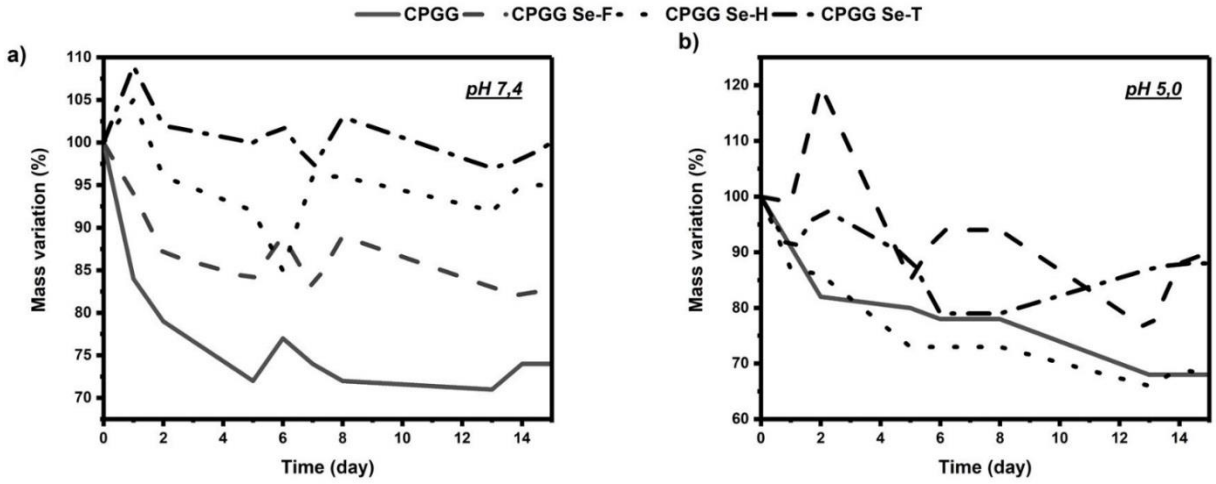

Figure 5. Degradation profiles of biomatrixes containing complexes of Se (IV) and F, H and T amino acids

At physiological pH (7,4) it can be seen that the incorporation of Se (IV) complexes encourages mass loss of all biomatrixes; recording residual masses of 73 ± 5%, 86 ± 8%, 97 ± 6% and 102 ± 9% for CPGG, CPGG Se-F, CPGG Se-H and CPGG Se-T, respectively. Statistically significant differences are determined in the mass variation of the matrix containing Se-T with respect to the matrix without complex. From the superficial and morphological point of view, the formation of aggregates of sheets or rods of the Se-H and Se-F complexes, respectively, encourages the hydrolysis reaction of the biomatrix, allowing to control the rate of degradation; interestingly, the biomatrix with the Se-T complex shows the highest resistance to acid degradation, showing that its mass does not vary during the study time, which is associated with the nucleation centers that promote the trigonal and rhombohedral structures of this complex with the polymeric chains, these centers possess intermolecular forces that show resistance to acid hydrolysis. These results allow to infer that the biomatrixes under study would control the rate of degradation in biomedical strategies that require physiological pH.

Studying the degradation behavior at acidic pH is essential since the soft tissue pH is between 4.5 and 5.0, as well as the variation in ionic strength that this pH provides allows the relaxation of the polymeric chains to be modulated for the release of encapsulated agents [24]. Figure 5b shows the results obtained, in the first 5 days of incubation the biomatrix with Se-F shows a swelling behavior registering a mass of 118 ± 12%, which is associated with the relaxation processes of the polymeric chains promoted by the hydrophobicity of the aromatic ring of phenylalanine allowing the uptake of water. The rest of the biomatrixes do not show a swelling behavior, and at 30 days of incubation, mass variations of 70 ± 5%, 90 ± 8%, 72 ± 6% and 89 ± 8% for CPGG, CPGG Se-F, CPGG Se-H and

CPGG Se-T, respectively. Significant differences in the mass variation at acidic pH of the biomatrixes with Se-F and Se-T are found compared with the rest of the biomaterials. The results indicate that the laminar sheet aggregates promoted by the Se-H complex present susceptibility to acid hydrolysis, obtaining a degradation profile similar to that of the CPGG matrix alone; on the other hand, the aggregates of rods in the biomatrix with Se-F, and the intermolecular forces that promote the structural geometries of Se-T in the associations of the polymeric chains during the process of polymeric semi-interpenetration are responsible for resisting the acid hydrolysis of the CPGG Se-F and CPGG Se-T biomatrixes, respectively. The biomatrixes show a degradation modulated by the structure of the Se(IV) complex and this could be exploited for different biomedical strategies.

3.4. Influence of the structure of the Se (IV) Complex on the in vitro biological response

For applications in biomedicine, biomatrixes should stimulate the metabolism of important cells in regenerative processes, such as fibroblasts; these cells specialize in the generation of new extracellular matrix of soft and hard tissue [25], representing an important cell line to evaluate the variation of their metabolism in the presence of the biomatrixes under study. Figure 6a shows the results of the metabolic activity of fibroblasts evaluated at 24 h and 48 h; the metabolic activity evaluated is related to the activity of mitochondrial dehydrogenases of the cells to allow the reduction of MTT salts to formazan.

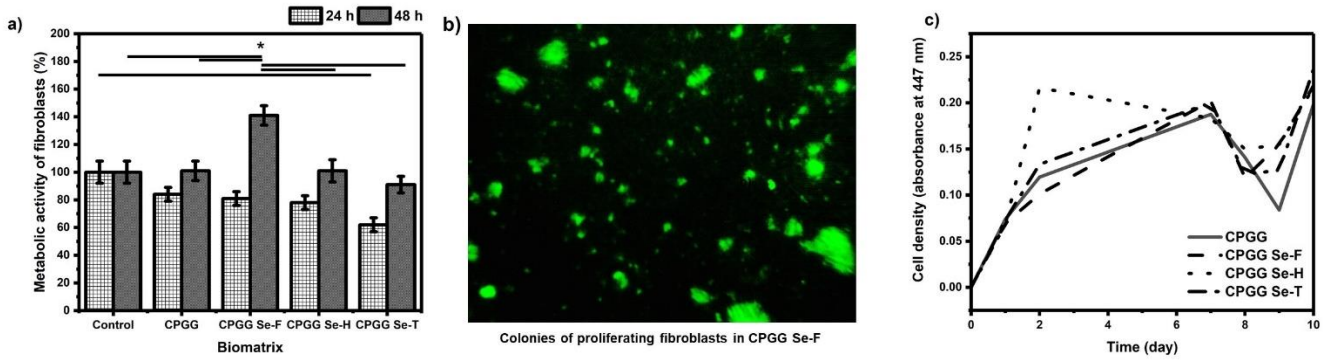

Figure 6. Evaluation of the biological response in vitro: a) Cell metabolism by MTT assay, b) Cell proliferation measured by fluorescent microscopy and c) Release profiles of encapsulated fibroblasts on the biomatrixes

After 24 h of incubation, the cells show metabolic activities greater than 60%, indicating that there is no important cytotoxic character that limits this mitochondrial function; however, after 48 h of culture, the fibroblasts growing on the biomatrixes significantly increase their metabolic activity, determining statistically significant differences for the cells growing on CPGG Se-F with respect to the control and the other biomatrixes.

The composition and structure of the Se-F complex stimulates its enzymatic activity, which is related to the fact that the agglomerations of rods on the semi-IPN matrix produce a surface that improves the metabolic activity of fibroblasts. For the CPGG Se-H and CPGG Se-T biomatrixes, there is no noticeable variation with respect to the control (cells growing in PBS 1X), which indicates that the composition and structure that these complexes generate on the surface of the biomatrix does not have a significant stimulation in the metabolic activity of these tissue-building cells. In Figure 6b, a fluorescence micrograph of fibroblasts growing and proliferating in contact with the biomatrix containing the Se-F complex is presented; dense cell populations can be observed, which are

stained with highly fluorescent calcein produced by the active esterases of living cells, thus verifying that these biomatrixes allow the growth and proliferation of this type of cells, and ensuring that there is no cytotoxicity of the degradation byproducts of biomatrixes incorporated with Se (IV) complexes, allowing basic biological functions such as metabolism, growth and proliferation. With this in mind, it is inferred that these novel biomatrixes could be used in biomedical strategies such as scaffolds for tissue regeneration.

Biomatrixes for modulated biological response must be capable of delivering cells to specific sites of action in order to potentiate the biomedical effect [26]. In this sense, release profiles of encapsulated fibroblasts were carried out on the biomatrixes under study (Figure 6c), and to study the effect of the Se(IV) complex on the release capacity of these encapsulated cells. The results indicate that after the first 7 days the cells begin to be released from the biomatrixes, being the CPGG Se-H biomatrix the one that shows the greatest capacity for cell release, this is associated with the sheet agglomerates that form the Se-H complex on the semi-IPN matrix allow lower inter and intramolecular forces so that the fibroblasts are released more quickly when they are encapsulated in this biomatrix. The rest of the biomatrixes under study show a cell release profile similar to that of the CPGG base matrix, indicating that there is resistance to the release of more cell content because the surface structure presents higher intermolecular interactions. After 7 days, the fibroblasts diffuse into the biomatrixes, and are released again after 10 days of study, which represents a non-sustained release of the cells encapsulated in these biomatrixes. These results could represent biomaterials with potential and biomedical selectivity, since they have the ability to release cells in sites required for a successful biomedical application.

4. Conclusion

Novel Se (IV)-based complexes with the amino acids phenylalanine, histidine and tryptophan were hydrothermally synthesized and physicochemically characterized. The complexes show a direct coordination with the amino and carboxylate groups in the coordination sphere of selenate ions, generating crystalline surfaces with morphology dependent on the chemical structure of the amino acid. The Se-F complex shows a structure of rods that group together to generate highly organized structures, the Se-H complex shows a surface characterized by sheets resulting from the stacking of tetragonal and orthorhombic forms of the complex, while the Se-T coordination compound presents tetragonal, orthorhombic and spheroidal shapes that are not organized in an orderly manner, not generating structures with a defined morphology. These complexes were incorporated into biomatrixes in hydrogel state based on semi-interpenetrating polymeric networks of collagen-polyurethane-guar gum (CPGG) in order to evaluate the influence of the structure and chemical composition of the Se (IV) complex on the physicochemical properties and biological response of these innovative biomatrixes. The results indicate that the incorporation of the complex decreases the crosslinking of the hydrogel, generating granular surfaces with porosity dependent on the type of amino acid. The CPGG Se-T biomatrix shows a swelling capacity of 10200 ± 1100 higher than the CPGG base matrix; while the CPGG Se-F and CPGG Se-T biomatrixes present slow degradation at both physiological and acidic pH. Interestingly, the matrix that includes the Se-F complex significantly stimulates the metabolic activity of L929 fibroblasts for up to 48 h, stimulating their proliferation. The fibroblasts encapsulated on these novel biomatrixes show recurrent release capacity for up to 7 days, where the structure of the CPGG Se-H biomatrix exhibits greater release from the encapsulated cells. These results demonstrate that these innovative biomatrixes

could be used in biomedical applications such as dermal tissue regeneration and cell release for a specific biological fate.

Declarations

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Competing Interests Statement

The authors declare no competing financial, professional, or personal interests.

Consent for publication

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

Authors' Contributions

All authors equally contributed to research and paper drafting.

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