

Physicochemical and Biological Characterization of Composites in Hydrogel State made of Collagen, Guar gum and Calcium/Aminoacid based MOFs

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DOI: <http://doi.org/10.38177/AJBSR.2023.5107>

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Article Received: 15 January 2023

Article Accepted: 24 February 2023

Article Published: 22 March 2023

ABSTRACT

Calcium/aminoacids based MOFs (CaHis, CaPhe, and CaTrp) were synthesized by the hydrothermal method. Then, these materials were applied as a filler in the synthesis of composites in hydrogel state based on the biopolymer matrix of collagen-guar gum (CG). The calcium MOFs materials were characterized by ATR-FTIR and XRD, while the morphology, the degradation profile, crosslinking and swelling degree, proliferation and cell viability of porcine dermis fibroblasts were the characteristics analyzed for the composites in hydrogel state. The results indicated that the incorporation of Ca-MOFs into the CG matrix enhances the physicochemical properties of CG, but without affecting their biocompatibility. In this way, the CG-CaTrp demonstrated to be the material with the best physicochemical and biological properties for potential application in tissue engineering of skin.

Keywords: Collagen; Guar gum; Calcium; Aminoacids; MOFs; Wound dressings.

1. Introduction

The development of composites in hydrogel state for tissue engineering requires the creation of new formulations that enhance the biocompatibility without affecting the physicochemical properties of the hydrogel [1]. In this sense, the application of BioMOFs (Biocompatible Metal-Organic Frameworks) as a filler into biopolymeric matrices to enhance the physicochemical properties without affecting their biocompatibility represents an innovative research area [2].

To synthesize BioMOFs the main idea is the use of biocompatible metals and organic ligands. The most studied biocompatible metals include Zn(II), Fe(II) and Cu(II) [3]. However, there are other biocompatible metals such as Ca(II) which plays a key role in the formation of hydroxyapatite and thus bone regeneration, but also, participates in the regulation of blood clotting, heart rhythms and nerve functions [4]. In the case of organic ligands, carboxylates and imidazolates have been proved as organic ligands due to their excellent Lewis base properties, and to their high biocompatibility of their degradation products which can be easily excreted by the urine and feces [5]. On the other hand, collagen and guar gum are biocompatible polymers. The first one is a protein found in the human body being the major component of bone, skin, muscles, tendons, and cartilage; its fiber-like structure make it an excellent biomaterial for its application as a scaffold for regeneration of cartilage and skin [6]. While guar gum is a galactomannan polysaccharide commonly used in the food and pharmaceutical industry as an emulsifier and a thickener [7].

In our research group it has been deeply studied the biopolymer matrix made of collagen-guar gum, specifically their physicochemical and biological properties [8,9], the previous results indicated that a 30% wt of guar gum in collagen is the indicated to improve the gelling rate and the crosslinking, but also its swelling capacity and storage

modulus. In other previous works, this composite matrix have been modified with MOFs based on other biocompatible metals such as Mg(II), Zn(II) and Ca(II) to enhance their physicochemical structure without affecting their biological properties, but in this case using not biocompatible organic ligands like 2,6-pyridinedicarboxylic acid and 3-amino-1,2,4-triazole [10,11].

Thus, the objective of this work is the synthesis of a bioMOF based on calcium and aminoacids such as histidine (His), phenylalanine (Phe) and tryptophan (Trp); and its application as a filler in the collagen-guar gum composite hydrogel matrix (CG). The bioMOFs were characterized by ATR-FTIR and XRD to confirm the formation of the Ca-O coordination bond. In the case of their composites with CG, it was analyzed their swelling and crosslinking degree, degradation profiles, morphology, proliferation, and cell viability of porcine dermis fibroblast. The main idea is to analyze the effect of the chemical structure of the aminoacids with the physicochemical and biological properties of the composites in hydrogel state for their potential biomedical application in tissue engineering of skin.

2. Experimental

2.1. Chemicals

Calcium nitrate tetrahydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$), 1,3,5-benzenetricarboxylic acid (TMA), L-histidine (His), L-tryptophan (Trp), L-phenylalanine (Phe), guar gum (extracted from *Cyamopsis tetragonoloba*, $M_w = 220$ kDa), and 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were purchased from *Sigma-Aldrich Co.*, and they were used as received. Calcein acetoxymethyl ester (calcein-AM) was purchased from ThermoFischer Scientific. Collagen from porcine dermis was extracted by enzymatic hydrolysis with pepsin as reported elsewhere ($M_n \alpha_1 = 220$ kDa, $\alpha_2 = 110$ kDa) [12]. A polyurethane crosslinker was prepared from 1,6-hexamethylene diisocyanate and glycerol ethoxylate as reported elsewhere [13].

2.2. Synthesis of Calcium/Aminoacid MOFs

Calcium/aminoacid MOFs were synthesized by the hydrothermal method. In a typical synthesis procedure water solutions of 1 mmol of calcium nitrate tetrahydrate, 1 mmol of TMA, and 1 mmol of the suitable amino acid (Trp, Phe, or His) were mixed with magnetic stirring at room temperature adjusting its pH to 4.4 [14,15]. Then, the mixture was transferred to a Teflon-lined autoclave, and the reaction was carried out at 120 °C for 72 h. The white precipitate obtained from the reaction was filtered, rinsed with water, and dried at 60 °C. The obtained calcium coordination polymers were labeled as CaHis, CaPhe, and CaTrp depending on the amino acid used as ligand.

2.3. Synthesis of Composite Hydrogels

Composite hydrogels based on collagen, guar gum and calcium/aminoacid MOFs were prepared by the microemulsion method. First, 1 mL of collagen solution (6 mg L^{-1}) was mixed with 15 μL of polyurethane in a culture plate (used as a mold). After that, 120 μL of guar gum solution (0.5 % wt.) and 1% wt of CaHis, CaPhe, or CaTrp 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 CG-CaHis, CG-CaPhe, or CG-CaTrp depending on the MOF used. For the comparison of results, a hydrogel formulation without MOF was prepared (CG).

2.4. Physicochemical characterization

The crystal structure of the composites in hydrogel state was analyzed by WAXS using a SAXSEmc2, Anton Paar diffractometer with a Cu K α X-Ray source ($\lambda=1.54 \text{ \AA}$). The chemical structure of materials was analyzed by ATR-FTIR using a Frontier, Perkin Elmer system, the spectra were recorded on dried hydrogel at 16 cm^{-1} of resolution, in a range from 4000 to 650 cm^{-1} , with an average of 16 scans.

The crosslinking degree of composite hydrogels were analyzed reacting the polymeric matrixes with ninhydrin for 30 min at $90 \text{ }^\circ\text{C}$. The absorbance of the liquid phase obtained after completing 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 1 [16]:

$$\text{crosslinking degree (\%)} = \left(1 - \frac{A_{\text{sample}}}{A_{\text{collagen}}}\right) * 100 \quad (1)$$

Where A_{sample} and A_{collagen} are the absorbances of solutions obtained after ninhydrin reacted with hydrogels or unreticulated collagen, respectively. UV-Vis absorption measurements were performed with a ThermoScientific MultiSkan Sky spectrophotometer.

For the determination of the swelling capacity, the mass of freshly prepared hydrogels 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 2:

$$\text{Swelling degree (\%)} = \frac{m_0 - m_i}{m_i} * 100 \quad (2)$$

Where m_0 is the initial mass of hydrogels and m_i is the mass of the dried hydrogel, respectively.

For the degradation behavior profiles, the hydrogels were immersed in water solutions at pH 1 and 13 at $25 \text{ }^\circ\text{C}$. Then, changes in the hydrogel mass over immersion time were assessed. The mass loss or gain of hydrogels was calculated according to the following Equation 3:

$$\text{Mass, \%} = \frac{M_t}{M_0} * 100 \quad (3)$$

Where M_0 is the initial mass of hydrogel and M_t is the mass of the sample at determined time. Scanning electron microscopy was performed with a TOPCON SM-510 microscope operated at 10 kV .

2.5. Evaluation of In-Vitro Biocompatibility

The effect of the chemical structure of Ca/aminoacid MOFs on the metabolic activity of porcine dermis fibroblasts growing in contact with hydrogels was evaluated by the MTT assay. For this, $150 \text{ }\mu\text{L}$ of cell suspension (30 000 cells/mL) were seeded over hydrogels in polystyrene culture plates and incubated for 24 and 48 h at $37 \text{ }^\circ\text{C}$ (samples were prepared in triplicate). PBS-1X was used as the positive control. At the evaluation time (24 or 48 h), $15 \text{ }\mu\text{L}$ of 3-(4,5-dimethylthiazol-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 $200 \text{ }\mu\text{L}$ were taken from the liquid medium and the absorbance was measured at 560 nm . Cell viability was calculated using Equation 4:

$$\text{Cell viability (\%)} = \frac{A_{\text{sample}}}{A_{\text{control}}} * 100 \quad (4)$$

Where A_{sample} and A_{control} represent the absorbances for each sample or formulation and PBS-1X, respectively.

In addition, the effect of the composition of Ca/aminoacid MOFs on the proliferation of fibroblasts was studied by fluorescence microscopy. For this, 1 mL of fibroblasts culture (30 000 cells/mL) was mixed with 1 mL of leaches extracted from semi-IPN hydrogels 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. Fluorescence microscopy observations were performed using a VELAB VE146YT microscope using a blue LASER as excitation source ($\lambda = 441 \text{ nm}$).

3. Results and Discussion

3.1. Characterization of Calcium/Aminoacid Coordination Polymers

The FTIR spectra of CaHis, CaPhe and CaTrp is shown in Figure 1. In general, it is observed a broad band between 3300 cm^{-1} and 3600 cm^{-1} which is relative to the hydrogen bonded to groups $-\text{NH}_2$ and $-\text{OH}$ stretching bands from the skeleton of aminoacids and TMA [17-19].

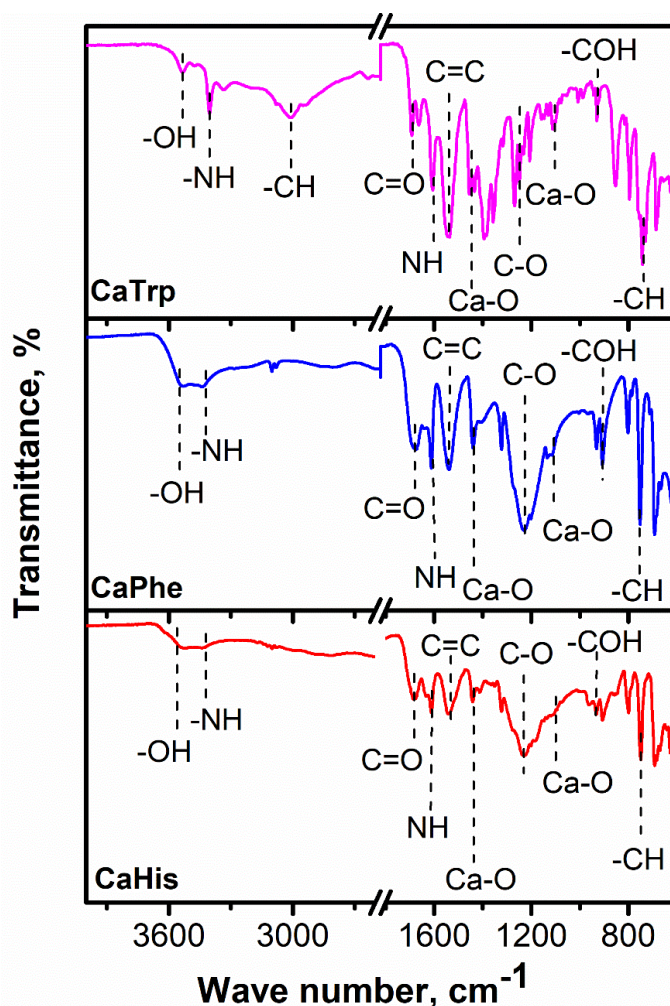


Figure 1. FTIR spectra of Ca/aminoacid coordination polymers

In the case of CaTrp there is a well-defined band at 3406 cm^{-1} related to the N-H stretching vibration that belongs to the indole ring from tryptophan this obeys to the aromatic stability of nitrogen, thus this aromatic nitrogen is not involved in the coordination with calcium. The opposite occurred with the high basicity of the nitrogen from the imidazole ring in histidine that possibly is also involved in the coordination bond with calcium. Also, there is band at 750 cm^{-1} which is ascribed to the aromatic C-H bending, in the case of CaTrp there is an additional band at 3013 cm^{-1} which is also assigned to the aromatic C-H stretching vibration [18]. Typically, in aminoacids the -COO antisymmetric stretch is at 1580 cm^{-1} while the $\text{H}_2\text{N-H}$ antisymmetric bending modes are at 1606 cm^{-1} [17]. However, the spectra of calcium coordination polymers indicate three bands at $\sim 1685\text{ cm}^{-1}$, $\sim 1614\text{ cm}^{-1}$ and $\sim 1540\text{ cm}^{-1}$, related to the C=O groups from aminoacids and TMA, the N-H antisymmetric bending mode of -NH_2 from aminoacids, and the C=C bending vibrations from the aromatic moieties of TMA and aminoacids. The spectra also exhibit a band at 1326 cm^{-1} and 1235 cm^{-1} associated to the C-N and C-O bonds of esters that along with the disappearance of the absorption band of -COH from carboxylic groups at 930 cm^{-1} denotes that the main metal-organic bond in these materials is related to carboxyl groups and calcium [19]. In addition, the asymmetric vibration of Ca-O bond is associated to the bands at 1444 cm^{-1} and a broad band at 1093 cm^{-1} [20].

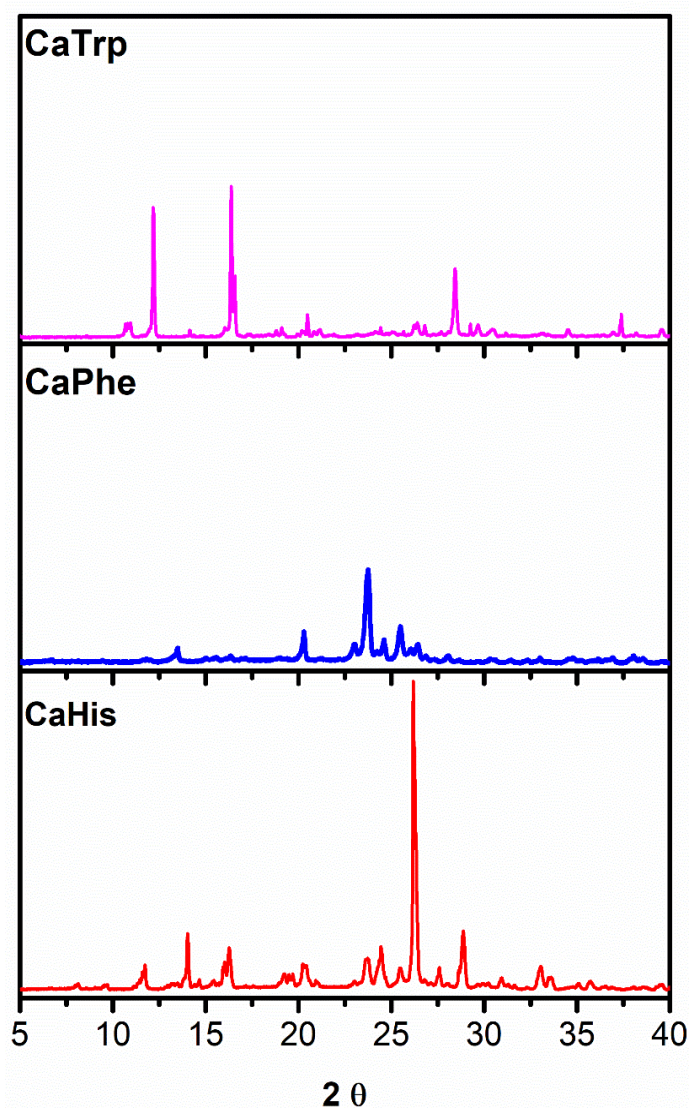


Figure 2. XRD diffractogram of Ca/aminoacid coordination polymers

The XRD diffractograms of CaHis, CaPhe and CaTrp are exhibited in Figure 2, indicating sharp and intense diffraction peaks typical of crystalline structures which is a desirable characteristic for these materials [14, 15]. It is also noticed that CaHis shows the greatest crystallinity which is ascribed to a greater packing and molecular ordering than CaTrp and CaPhe, respectively, this is due to the major coordination between Ca and carbonyl groups and the disappearance of the intensity of $-COH$ at 930 cm^{-1} , which is more pronounced in CaHis sample.

3.2. Physicochemical characterization of composites in hydrogel state

Figure 3(a) exhibits the crosslinking degree obtained by ninhydrin assay of hydrogels based on collagen, guar gum, and calcium/aminoacid based MOFs. Except for CG-CaPhe composite, all the materials show an entanglement percentage greater than 60% indicating a chemical crosslinked hydrogel. The incorporation of CaHis and CaTrp into the CG matrix generates reticulation points increasing the crosslinking degree in a 19% and 7% in comparison with CG composite. These results are in line with the packing and molecular ordering found in the XRD results, indicating that a material with better crystallinity operates as a reticulation point where the negative charge of carboxylic groups from aminoacid moieties of MOFs interact with amine groups of collagen forming covalently crosslinked hydrogels. These results are also related to the best dispersion of CaHis in water than CaTrp and CaPhe, which is due to the polarity of the aromatic ring in each aminoacid.

Figure 3(b) indicates the swelling degree of each sample. The swelling of a hydrogel is ascribed to the amount of the free hydrophilic groups such as $-OH$ and $-NH_2$ in this case.

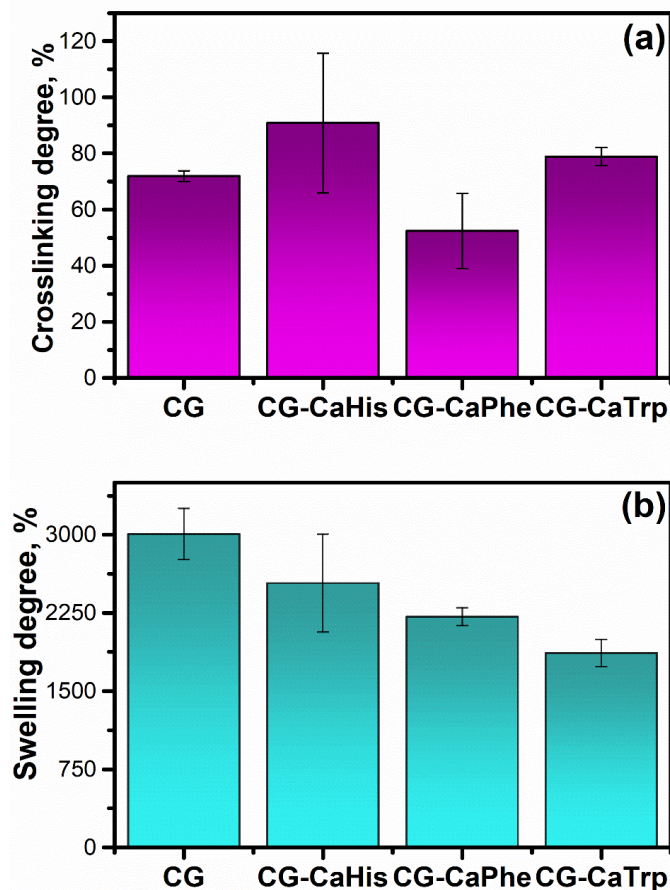


Figure 3. (a) Crosslinking and (b) Swelling degree of composites in hydrogel state

It is observed that the CG matrix losses hydrophilic groups when calcium/aminoacid based MOFs are incorporated. In addition, the loss of the swelling capacity is also related to the rigidity of the aromatic ring associated to each aminoacid, this explains the lowest swelling capacity of CG-CaTrp.

The composites in hydrogel state with Ca-MOFs were degraded in an acid and basic medium (Figure 4). At pH=1, it is evident that the incorporation of the Ca-MOFs enhanced the chemical stability in comparison with the CG matrix, this obeys to the strong chemical interaction between the carbonyl groups from aminoacids with the amine groups of collagen retarding the degradation process and obtaining a residual mass of 60%. When the pH is increased to 13, the incorporation of CaPhe and CaTrp increase the degradation process in basic medium in comparison with the CG polymer matrix. However, the best MOF to retard the degradation of the polymer matrix is CaHis, this result is associated to the more hydrophilic and basic character of histidine with an isoelectric point close to neutrality, slowing down the basic hydrolysis of this material obtaining a residual mass of 60%. Thus, the material with the best degradation profiles at acid and basic medium is CG-CaHis.

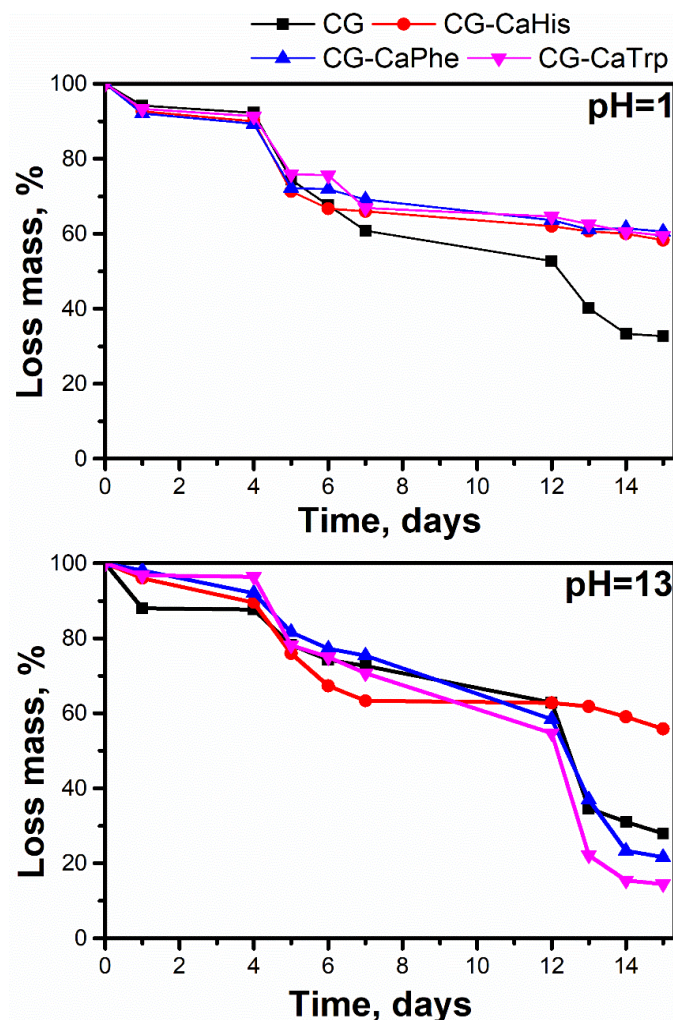


Figure 4. Degradation profiles on acid (pH=1) and basic medium (pH=13)

The morphology of the xerogel composites was analyzed by Scanning Electron Microscopy (Figure 5). The micrographs shown for CG a porous structure with granular agglomerates [11] obtained by the interpenetration of the collagen-polyurethane matrix with guar gum (Figure 5(a)). The chemical structure of the calcium-based MOFs

generates homogeneous surfaces of high interconnected porosity without agglomerates, except for the xerogel of CG-CaHis with some disperse agglomerates as the CG polymer matrix. These kind of flat microstructures with high porosity serves as a scaffold where cells such as fibroblasts can be adhered and repair chronic wounds in skin.

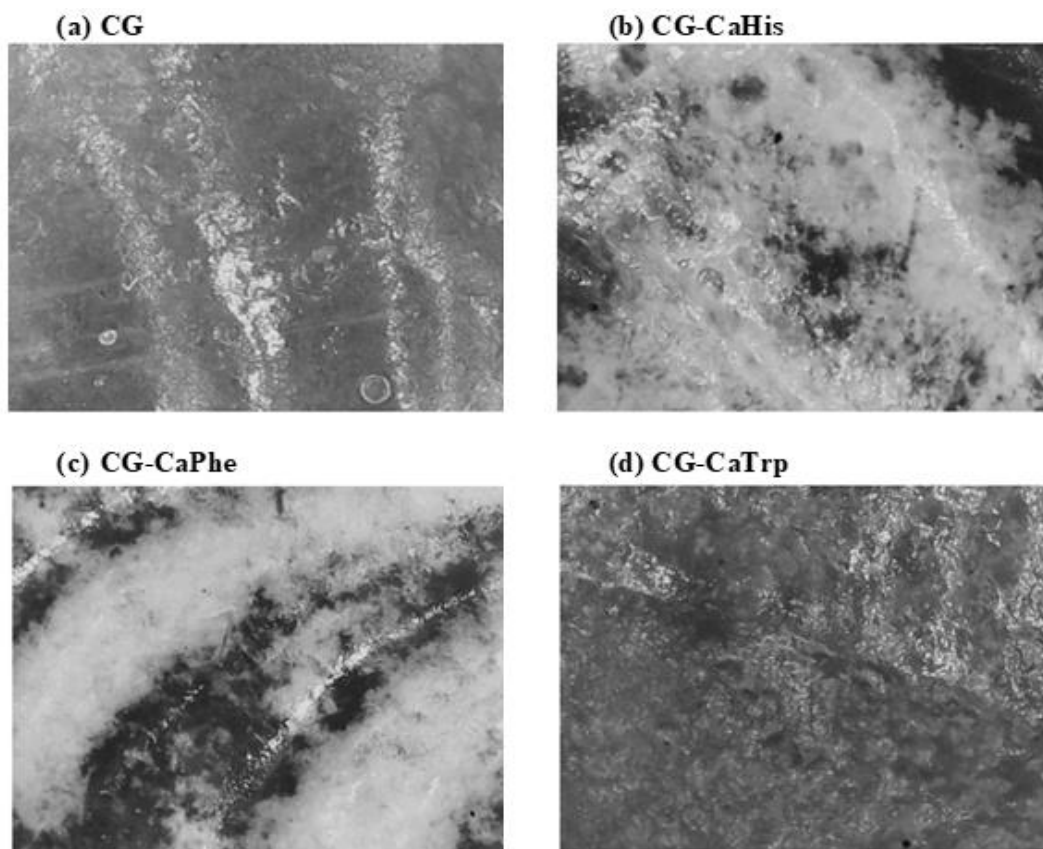


Figure 5. Micrographs of xerogels based on collagen-guar gum-Ca/aminoacid coordination polymers

3.3. Biocompatibility of Collagen-Guar Gum-Calcium/Aminoacid Coordination Polymers

To measure the *in vitro* biocompatibility of composites in hydrogel state, it was evaluated the metabolic activity of porcine dermis fibroblast by the MTT assay. Figure 6 exhibits that all materials show a percentage cell viability upper to the 60% after 24 h, indicating that are a non-cytotoxic material [11]. The incorporation of Ca-MOFs to the CG matrix reduces the fibroblast viability, followed closely by the CG-CaTrp and CG-CaPhe which is attributed to their homogeneous and porous morphology. After 48 h, the metabolism of fibroblasts is well adapted, being the CG-CaPhe the material with best cell viability (101%) followed closely by the CG polymer matrix.

In addition, the cell proliferation of porcine dermis fibroblast in contact with the composites in hydrogel state based on CG and Ca-MOFs was carried out using the LIVE/DEAD™ kit (Figure 7). The green colonies of fibroblasts grow up during 24 h, as it is observed in this case the material with the best fibroblast proliferation is CG-CaTrp, while the CG matrix is the hydrogel with the lowest proliferation in contrast with the results found in the cell viability with MTT. It is important to notice that the MTT assays is associated with the mitochondrial function of fibroblasts in the presence of the biopolymer matrix not with live or dead fibroblasts. Thus, the reduction in the cell viability is associated to the cell's energy, whereas the proliferation test indicates how the cells interacts with the

composite in hydrogel state and how their metabolism is adapted with the hydrogel's morphology leading to the proliferation of live fibroblasts.

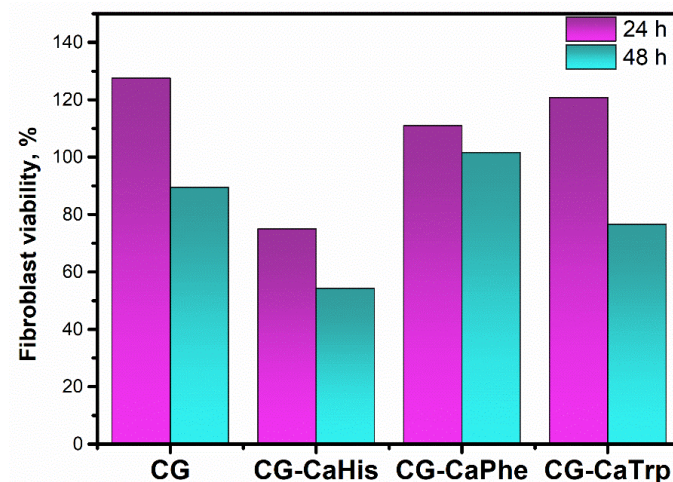


Figure 6. Porcine Dermis Fibroblasts viability on composites in hydrogel state

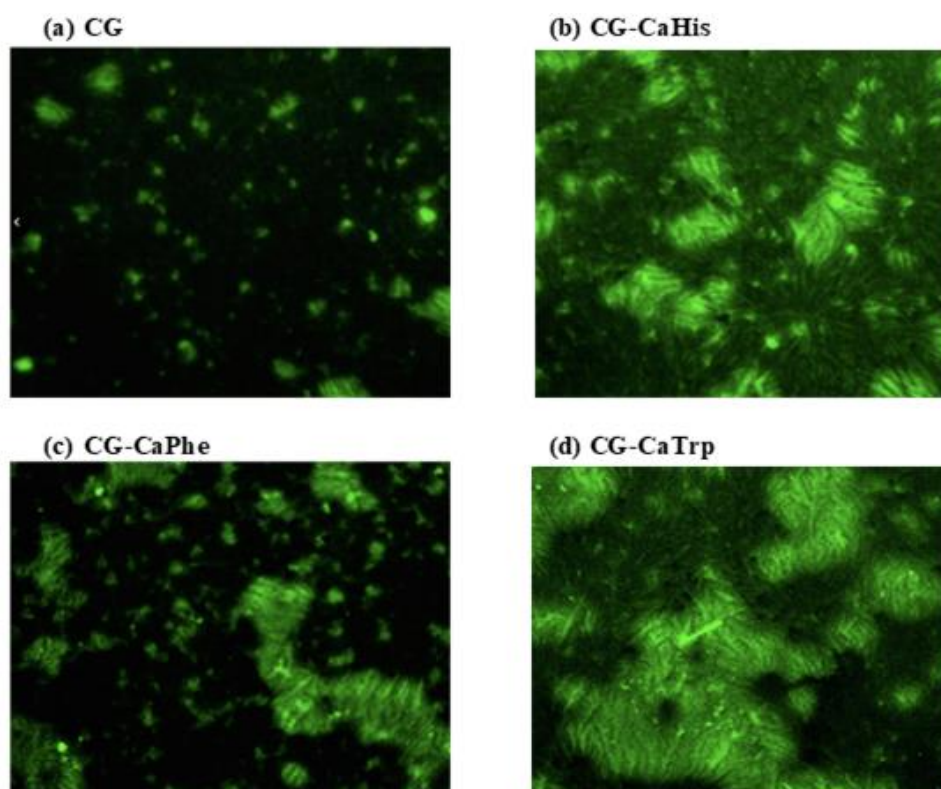


Figure 7. Proliferation of porcine dermis fibroblast growing on composites in hydrogel state (scale at 1 μ m)

4. Conclusions

The characterization of FTIR and XRD of the new calcium/aminoacid based MOFs reveals the formation of materials with packing and molecular ordering which is related to the new coordination bond formed of Ca-O. The crystallinity of these materials was related to the chemical structure of the aminoacid, indicating that the aminoacid His presents the best crystallinity structure which was correlated with its best solubility on water or in other words to the hydrophilic properties of the aromatic ring moieties from each aminoacid.

In general, the incorporation of Ca-MOFs into the CG matrix increases the entanglement of the CG polymer obtaining composites in hydrogel state with a crosslinking degree greater than 60% and thus, leading to covalent-crosslinked hydrogels, except for CG-CaPhe which is attributed to the hydrophobic nature of the benzene ring. These chemical crosslinked hydrogels act as crosslinking points due to the acid-base interaction of carbonyl moieties of aminoacids with the amine moieties of collagen, then reducing the free hydrophilic groups (-OH, -NH₂) of CG and decreasing their swelling capacity in comparison with CG matrix. Moreover, the degradation profiles at acid and basic medium have shown that CG-CaHis presents a high chemical stability in acid or basic medium, that is attributed to its isoelectric point close to the neutrality.

On the other hand, the *in vitro* viability of porcine dermis fibroblasts exhibits that all the composites in hydrogel state are non-citotoxic. The incorporation of Ca-MOFs to CG matrix reduces the fibroblast viability of the CG matrix. However, the study of fibroblast proliferation after 24 h is more conclusive indicating that the composite CG-CaTrp shows the best cell proliferation which is associates to their most homogeneous and porous morphology than the other composites, being an attractive and greener formulation for composites in hydrogel state to be applied in tissue engineering of skin.

Declarations

Source of Funding

Consejo Nacional de Ciencia y Tecnología (CONACyT) supported this study with the grant FORDECYT/PRO NACES/6660/2020.

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.

Acknowledgement

Jesús A. Claudio-Rizo wants to thank Consejo Nacional de Ciencia y Tecnología (CONACyT) for the grant FORDECYT/PRONACES/6660/2020.

References

- [1] S. Utech, A.R. Boccaccini (2016). A review of hydrogel-based composites for biomedical applications: enhancement of hydrogel properties by addition of rigid inorganic fillers. *Journal of Mat Science*, 51: 271–310.
- [2] H. Cai, Y-L. Huang, D. Li (2019). Biological metal–organic frameworks: Structures, host–guest chemistry and bio-applications. *Coordination Chemistry Reviews*, 378: 207–221.

- [3] S. Rojas, A. Arenas-Vivo, P. Horcajada (2019). Metal-organic frameworks: A novel platform for combined advanced therapies. *Coordination Chemistry Reviews*, 388: 202–226.
- [4] P. Pravina, D. Sayaji, M. Avinash (2013). Calcium and its role in human body. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 4(2): 659–668.
- [5] A. Pandey, N. Dhas, P. Deshmukh, C. Caro, P. Patil, M. L. García-Martín, B. Padya, A. Nikam, T. Mehta, S. Mutalik (2020). Heterogeneous surface architected metal-organic frameworks for cancer therapy, imaging, and biosensing: A state-of-the-art review. *Coordination Chemistry Reviews*, 409: 213212.
- [6] C. Helary, I. Bataille, A. Abed, C. Illoul, A. Anglo, L. Louedec, D. Letourneur, A. Meddahi-Pellé, M. M. Giraud-Guille (2010). Concentrated collagen hydrogels as dermal substitutes. *Biomaterials*, 31(3): 481–490.
- [7] A. Indurkar, P. Bangde, M. Gore, A. K. Agrawal, R. Jain, P. Dandekar (2020). Fabrication of guar gum-gelatin scaffold for soft tissue engineering. *Carbohydrate Polymer Technologies and Applications*, 1: 100006.
- [8] E.E. López-Martínez, J.A. Claudio-Rizo, M. Caldera-Villalobos, J.J. Becerra-Rodríguez, D.A. Cabrera-Munguia, L.F. Cano-Salazar, R. Betancourt-Galindo (2022). Hydrogels for Biomedicine Based on Semi-Interpenetrating Polymeric Networks of Collagen/Guar Gum: Synthesis and Physicochemical Characterization. *Macromolecular Research*, 30: 375–383.
- [9] E.E. López-Martínez, J.A. Claudio-Rizo, M. Caldera-Villalobos, J.J. Becerra-Rodríguez, D.A. Cabrera-Munguia, L.F. Cano-Salazar, R. Betancourt-Galindo (2022). Hydrogels for Biomedicine Based on Semi-Interpenetrating Polymeric Networks of Collagen/Guar Gum: Applications in Biomedical Field and Biocompatibility. *Macromolecular Research*, 30: 384–390.
- [10] M. Caldera-Villalobos, D.A. Cabrera-Munguía, T.E. Flores-Guía, G. Viramontes-Gamboa, J.A. Vargas-Correa, L.F. Cano-Salazar, J.A. Claudio-Rizo (2021). Removal of water pollutants using composite hydrogels comprised of collagen, guar gum, and metal-organic frameworks. *Journal of Polymer Research*, 28: 395.
- [11] M. Caldera-Villalobos, D.A. Cabrera-Munguia, J.J. Becerra-Rodríguez, J.A. Claudio-Rizo (2022). Tailoring biocompatibility of composite scaffolds of collagen/guar gum with metal-organic frameworks. *RSC Advances*, 12: 3672.
- [12] J.A. Claudio-Rizo, M. Rangel-Argote, L.E. Castellano, J. Delgado, J.L. Mata-Mata, B. Mendoza-Novelo (2017). Influence of residual composition on the structure and properties of extracellular matrix derived hydrogels. *Materials Science and Engineering C*, 79: 793–801.
- [13] B. Mendoza-Novelo, J.L. Mata-Mata, A. Vega-González, J.V. Caich-Rodríguez, A. Marcos-Fernández (2014). Synthesis and characterization of protected oligourethanes as crosslinkers of collagen-based scaffolds. *Journal of Materials Chemistry B*, 2: 2874–2882.
- [14] J. He, G. Zhang, D. Xiao, H. Chen, S. Yan, X. Wang, J. Yang, E. Wang (2012). Helicity controlled by the chirality of amino acid: two novel enantiopure chiral 3D architectures containing fivefold interwoven helices. *Crystal Engineering Communications*, 14: 3609–3614.

- [15] J-H Zhang, R-Y Nong, S-M Xie, B-J Wang, P. Ai, L-M Yuan (2017). Homochiral metal-organic frameworks based on amino acid ligands for HPLC separation of enantiomers. *Electrophoresis*, 38(19): 2513–2520.
- [16] M. Nair, R. K. Johal, S. W. Hamaia, S. M. Best, R. E. Cameron (2020). Tunable bioactivity and mechanics of collagen-based tissue engineering constructs: A comparison of EDC-NHS, genipin and TG2 crosslinkers. *Biomaterials*, 254: 120109.
- [17] Z. Liu, Y. Zu, Y. Fu, R. Meng, S. Guo, Z. Xing, S. Tan (2010). Hydrothermal synthesis of histidine-functionalized single-crystalline gold nanoparticles and their pH-dependent UV absorption characteristic. *Colloids and Surfaces B: Biointerfaces*, 76(1): 311–316.
- [18] R. Palomino Merino, R. Gutiérrez Pérez, P. Trejo García, L. Chaltel Lima, O. Portillo Moreno, M. E. Araiza García, A. Moreno Rodriguez, E. Rubio Rosas (2018). Influence of L-Tryptophan on Growth and Optical Properties of PbS Nanocrystalline Thin Films. *Journal of Nanomaterials*, Pages 3431942.
- [19] X. Zheng, K. Xu, Y. Wang, R. Shen, Q. Wang (2018). Study of hydrogen explosion control measures by using L-phenylalanine for aluminum wet dust removal systems. *RSC Advances*, 8: 41308–41316.
- [20] J. Jitjamnong, A. Luengnaruemitchai, N. Samanwong, N. Chuaykarn (2019). Biodiesel production from canola oil and methanol using Ba impregnated calcium oxide with microwave irradiation-assistance. *Chiang Mai Journal of Science*, 46(5): 987–1000.