Summary: Hydrogels for ophtalmic drug delivery

Due to the low penetration of drugs into the eye due to their morphological barriers and their rapid excretion through the lacrimal apparatus, it is necessary to find another way to instill topical drugs into the eye. One option that has been considered for topical drug delivery is hydrogels.

This project synthesized a hydrogel composed of a polymer: Modified hyaluronic acid, which is interlaced by an enzymatic reaction with natural phenolic compounds.

A gel is a three-dimensional network of flexible chains formed by segments connected in a certain way and inflated by a liquid. If the fluid that penetrates between the chains is organic, it is called the organogel; however, if the liquid is water, the gel is called hydrogel.

The gels can be calcified into two distinct groups determined by the bond between the monomers of the three-dimensional network. Physical gels are those that are part of the three-dimensional network through links that are not fully stable but maintain a continuous reaction of link ⇔ non-bond that can occur in both directions.1

The joints of these types of gels are usually due to Van der Waals forces. On the other hand are the chemical gels, which are covalent bonds, a stronger type of bond that breaks once breaks down the polymer.

On the one hand, a biopolymer with appropriate properties for a hydrogel intended for topical application, such as biocompatibility and lubrication, was used as hyaluronic acid is a polysaccharide belonging to the group of glycosaminoglycans composed of groups of D-glucuronic acid and N-acetyl-D-glucosamine disaccharides bound by beta.

It is mainly produced by mesenchymal cells and can form in other cells. It is abundantly found in the human body, in various tissues and fluids of the body, such as skin, vitreous humor, synovial fluid, bone tissue and several of the body's vital organs. In addition, hyaluronic acid has different physicochemical properties that may be useful for an application in ophthalmic surgery due to its viscoelastic properties.

Therefore, we can count on their biocompatibility in topical uses since it is a polymer that is already present in the human body.

On the other hand, phenolic compounds that have an antioxidant nature have also been used.

Phenolic compounds are those containing in their structure one or more phenolic groups or aromatic rings attached to a hydroxyl. These compounds are organic and come from some higher plants, of which they are secondary metabolites. These are also linked to defense against ultraviolet radiation or the aggression of pathogenic organisms, so phenolic compounds may have the ability to inhibit the metabolism of microorganisms.

The synthesis of hydrogels for this project was performed using the enzyme laccase, which is responsible for generating the covalent bonds that give rise to a hydrogel. Use of this enzyme allows for a controlled reaction under gentle conditions, as it acts as a biological catalyst for the highly specific reaction.

Under these conditions, the laccase, using the oxygen in the medium and generating water as a reaction product, oxidizes the phenolic compounds which then react with the HA thiols to generate the hydrogel. These conditions make the synthesized hydrogels possible drug encapsulation and release platforms.

Therefore, there are several goals to be fulfilled for this project, the most important being to fear the enzymatic synthesis of a hydrogel from the biopolymer of hyaluronic acid and natural phenolic compounds as a platform for ophthalmological applications; and producing a drug release platform using a hydrogel as a transport method. In addition, there are other objectives that we have proposed as secondary, such as the modification and characterization of hyaluronic acid to obtain a polymer with the thiol functional groups that will participate in the hydrogel formation reaction; Preparation of different hydrogels from the polymer modified with various natural phenolic compounds through the action of the enzyme laccase and lastly, the study of the properties of these hydrogels in terms of degradation, antioxidant capacity and biocompatibility.

To achieve these goals, we have used a number of methods that follow the steps required to perform the synthesis of hydrogels with chemical bonds that are needed to establish the drug release platform.

We first modified hyaluronic acid in two steps: in the first, we performed the amination, which is the process by which hyaluronic acid (with a molecular weight of 200kDa) is modified by adding adipic acid dihydrogen (ADH). We then

synthesized the modified hyaluronic acid with thiol groups with the help of Traut's reagent, a process known as thiolation.

Once we obtained the modified hyaluronic acids, we proceeded to characterize them through the method of characterization of the amino groups using the TNBSA compound in the case of ADH-modified acids. On the other hand, we have characterized the acids modified with thiol groups (-SH) through the Ellman procedure. In both cases, the objective is to determine the number of functional groups that have reacted with the acid in the same way that the number of groups that have not reacted is determined.

Subsequently, the hydrogels were synthesized. To synthesize we first prepare hyaluronic acid, which will be the main component of hydrogels. Subsequently, laccase, which is an enzyme that belongs to the group of blue copper oxidases and comes from some plants and fungi. And finally, it will be necessary to add various phenolic materials, which will be oxidized by the action of lacase and serve as a cross-linking agent for the formation of hydrogel, in this way it will be possible to determine if they can contribute properties different from those of a conventional hydrogel.

Once the hydrogels were obtained with phenolic compounds, they separated several samples to characterize their properties for future ophthalmological applications. Once the hydrogel samples are ready, we can begin testing to determine if the phenolic compounds that have been used to form the hydrogels can add new properties to the hydrogels. Among the properties that this phenolic compounds can bring are antimicrobial or antioxidant, among others. .

Of the phenolics we have prepared, we have chosen 3 of them because they have the most interesting properties in terms of structure and biological function. Therefore, we will do the tests mainly with the compounds of catechin, ellagic acid and rutin.

We then measured the antioxidant activity of hydrogels through a DPPH assay, measured how the phenolic compounds degrade within the hydrogel, and measured the antimicrobial activity of these hydrogels by comparing P. growth. aeuroginous on its own, with its growth with exposure to hydrogels with phenolic compounds. Finally, we also measured the viability of some epithelial cells, such as fibroblasts and keratinocytes, by having a medium where there were hydrogels with phenolic compounds, comparing it with the viability of these cells only with

the environment with the intention to test the biocompatibility of epithelial cells with phenolic compounds.
Once the results, which were mostly calculated by colorimetric methods, were discussed and a conclusion was reached for each phenolic compound on each of the characteristics tested.

In conclusion, In this project we have worked with hyaluronic acid and modified this polymer with the thiol functional group and characterized that the reaction level with thiol groups could be observed with a result. of 67 μmol SH / g in the HA-SH polymer Subsequently, several hydrogels could be synthesized from the modified acid mentioned above, with the intervention of the lacase enzyme, combining the resulting hydrogels with various phenolic compounds, from which it has been possible to study the properties that they contributed to the hydrogel, such as their rate of degradation, their biocompatibility and their antioxidant capacity, and the control of the growth of microorganisms.
The testing of the antioxidant property of phenolic compounds, it has been found that the compound with the least activity of this kind is routine as it reaches almost 10% of antioxidant activity, whereas in the case of catechin and ellagic acids, exceed 50%, so the routine would not be a good choice for an antioxidant application. In the case of compound degradation, ellagic acid was 100% degraded within 24 hours, whereas in the case of routine and catechinic acid, phenolic compounds were still present within the hydrogels after 24 hours. . In the case of growth control of microorganisms, in all cases except the routine, the viability of P. aeuroginosa was reduced by at least 60%, so it can be co-coated in that the compounds have antimicrobial capacity, and which can therefore be counted on in the use of chronic wound treatment. On the other hand, in affecting cell viability, in all cases, except for ellagic acid for keratinocytes, it remained above 80%, which allows us to consider phenolic compounds as biocompatible, except in the case of ellagic acid which could be toxic to keratinocytes.
Therefore, one of the major goals of the hydrogel synthesis by substrate oxidation with laccase was to achieve the same as the set secondary targets. On the other hand, the objective of producing the test for this hydrogel as a platform for the delivery of drugs not performed due to lack of time in the laboratory, so that further study is required to be tested in the future. works.