Universidade Estadual de Campinas

Instituto de Biologia

FERNANDA MACHADO CROISFELT

HIDROGÉIS DE *PNIPAAM-CO-AAM* COMO SISTEMAS CARREADORES DE PRODUTOS BIOATIVOS DE INTERESSE FARMACÊUTICO

PNIPAAM-CO-AAM HYDROGELS AS CARRIER SYSTEMS OF BIOACTIVE PRODUCTS OF PHARMACEUTICAL INTEREST

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Orientador/Supervisor: Prof.ª Dr.ª Priscila Gava Mazzola

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Prof (a) Dr (a) Priscila Gava Mazzola

Prof (a) Dr (a) Guilherme Diniz Tavares

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“Man cannot remake himself without suffering,

for he is both the marble and the sculptor”

Alexis Carrel

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RESUMO

Hidrogéis são compostos poliméricos de configuração tridimensional que possuem a capacidade de absorver grandes quantidades de água. É possível encontrar utilização biológica para os hidrogéis em curativos, lentes de contato, e na liberação controlada de fármacos. Hidrogéis de poli (N-isopropilacrilamida) (PNIPAAm) são termoresponsivos e estáveis. Bromelina é o conjunto de proteases obtida principalmente a partir do abacaxi. Pode atuar em processos de cicatrização e debridamento seletivo de queimaduras, além de possuir atividades anti-inflamatória e fibrinolítica. Sendo assim, a junção do hidrogel de PNIPAAm com um ativo como a bromelina pode apontar para uma aplicação na cicatrização de feridas. Este trabalho avaliou a possibilidade de tal associação a partir do desenvolvimento e caracterização do hidrogel. Os resultados obtidos mostram que o comportamento da bromelina incorporada ao hidrogel indica ação considerável e manutenção de sua atividade diante dos testes ao qual o sistema foi exposto, o que implica na possibilidade de uso terapêutico para tal.

Palavras-chave: Desenvolvimento farmacêutico; hidrogel; bromelina; liberação controlada; polímero termossensível.

ABSTRACT

Hydrogels are polymer compounds with three-dimensional configuration that have the ability to absorb large amounts of water. Biological use can be found for hydrogels in dressings, contact lenses, and controlled release of drugs. Poly (N-isopropylacrylamide) (PNIPAAm) hydrogels are thermoresponsive and stable. Bromelain is the set of proteases obtained mainly from pineapple. It can act in processes of cicatrization and selective debridement of burns, besides having anti-inflammatory and fibrinolytic activities. Thus, the junction of the PNIPAAm hydrogel with an active substance such as bromelain can point to an application in wound healing. This work evaluated the possibility of such association from the development and characterization of the hydrogel, as evidenced in chapter II of this work. The results show that the behavior of bromelain incorporated to the hydrogel indicates considerable action and maintenance of its activity in the tests to which the system was exposed, which implies the possibility of therapeutic use for this.

Key-words: Pharmaceutical development; hydrogel; bromelain; controlled release; thermosensitive polymer.

LISTA DE SIGLAS E ABREVIATURAS

PNIPAAm/NPA - Poli (N-isopropilacrilamida) / poly(n-isopropylacrylamide)

TCIS - Temperatura Crítica Inferior de Solução

LCST - Lower Critical Solution Temperature

AAm - Acrilamida/Acrylamide

NaCMC - Carboxymethyl cellulose

PEG- Poly (ethylene glycol)

PHEMA - Poly (2-hydroxyethyl methacrylate)

PLGA - Poly (lactic-coglycolic acid)

PVA - Polyvinyl alcohol

SCLG - Scleroglucan

AMD - Age-related macular degeneration

APMA - 4-vinylpyridine (VP) and N-(3-aminopropyl) methacrylamide

CD - Cyclodextrin

CEX - Cephalexin

BCCs - Carcinomas

SCCs - Squamous cell carcinomas

CMs - Cutaneous malignant melanomas

TAC - Tacrolimus

VEGF - Vascular endothelial growth factor

SSD - Silver sulfadiazine

CLX - Chlorhexidine

CTZ - Clotrimazole

PEO - Poly (ethylene oxide)

PPO - Poly (propylene oxide)

PEO - Poly (ethylene oxide)

BIS - N, N'-Methylenebisacrylamide

SEM - Scanning electron microscopy

FTIR - Infrared spectroscopy

MTS - Mitochondrial activity assay

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SUMÁRIO

INTRODUÇÃO

Devido à sua atividade proteolítica, a bromelina possui aplicações nas indústrias farmacêutica e cosmética. A atividade anti-inflamatória da bromelina foi comprovada por Seligman (1), e estudos seguintes sustentam o seu uso em diferentes situações (2-6). Aplicações terapêuticas da bromelina já foram demonstradas *in vivo* e *in vitro* graças às suas atividades fibrinolítica e cicatrizante (7).

Muitas abordagens têm sido investigadas para a entrega de moléculas bioativas (8-10) na última década, sendo os hidrogéis extensivamente apontados como matrizes para liberação controlada (11-13). Hidrogéis termossensíveis são de grande importância, pois tem o potencial de liberar o ativo de interesse em uma determinada faixa de temperatura. O poli (N-isopropilacrilamida) ou PNIPAAm é um polímero caracterizado por apresentar temperatura crítica inferior de solução (TCIS) de aproximadamente 32ºC. Abaixo dessa temperatura o hidrogel absorve grandes quantidades de água e acima dele expele todo o conteúdo aquoso, o que indica uma possível aplicação tópica de liberação controlada, visto que a temperatura do corpo humano se encontra na faixa dos 37ºC (14).

A copolimerização do PNIPAAm com acrilamida (AAm) permite a síntese de hidrogéis com características melhoradas, ampliando assim sua esfera de aplicação. Em artigo publicado em 2015 (15), nosso grupo apontava para a possibilidade de se desenvolver um sistema de liberação controlada de bromelina a partir de hidrogéis de PNIPAAm, visto que esses polímeros se mostraram capazes de reter e liberar a enzima em condições de temperatura próximas a do corpo humano.

Sendo assim, a caracterização de um sistema como este contribui para a criação de uma plataforma de entrega sustentada de macromoléculas, capaz de aumentar a estabilidade e controlar a liberação de uma enzima de uso comprovadamente terapêutico.

OBJETIVOS

OBJETIVO GERAL

Preparar e caracterizar hidrogel de *PNIPAAm-co-AAm* contendo bromelina visando liberação controlada tópica.

OBJETIVOS ESPECÍFICOS

- Sintetizar hidrogel termossensível contendo PNIPAAm
- Estudar o perfil de intumescimento do hidrogel produzido
- Estudar a absorção e liberação da bromelina pelo hidrogel
- Caracterizar morfologicamente o hidrogel
- Caracterizar químico-fisicamente o hidrogel
- Determinar a viabilidade celular do hidrogel

CAPÍTULO I

Hydrogels for controlled release and topical use: a ten-year review

Authors

Fernanda Machado Croisfelt[1], Lucas Militão[2], Janaína Artem Ataide[3], Angela Faustino Jozala[4], Elias Basile Tambourgi[5], Xiaochen Gu[6], Edgar Silveira[7], Priscila Gava Mazzola[2]

Affiliation

[1]Biology Institute, State University of Campinas (UNICAMP), Campinas, SP, Brazil

[2]Faculty of Pharmaceutical Sciences, State University of Campinas (UNICAMP), Campinas, SP, Brazil

3Faculty of Medical Sciences, State University of Campinas (UNICAMP), Campinas, SP, Brazil

[4]Department of Technology & Environmental Process, University of Sorocaba (UNISO), Sorocaba, SP, Brazil

[5] School of Chemical Engineering, State University of Campinas (UNICAMP), Campinas, SP, Brazil

[6] College of Pharmacy, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada

[7]Genetics and Biochemistry Institute, Federal University of Uberlândia (UFU), Uberlândia, MG, Brazil

Key words: hydrogel, topical applications, polymeric materials, drug release.

Abstract: Hydrogels are polymeric compounds with tridimensional structure that are able to load water and other substances. Such characteristic makes hydrogels suitable for innumerous applications in biomedical fields. For almost 60 years hydrogels have been studied and progress have been made in topical use. Natural and synthetic polymers have been applied to develop smart hydrogels that respond to physiological stimuli while releases drugs and bioactives. In view of this, this paper approaches the use of important materials in hydrogel's synthesis as highlights the main sites affected by topical disorders and routes for therapeutic controlled release.

Introduction

Hydrogels are tridimensional polymeric networks able to absorb large amounts of water (16). Since its first synthesis, by Wichterle and Lim in 1960 (17), these materials have been explored in medical and pharmaceutical fields (18).

The main characteristic of hydrogels is associated to the presence of hydrophilic and hydrophobic groups in the polymeric chain (19). The balance between these groups make water absorption possible at levels from 10% to 99% (20). At the same time, crosslinks have to be present in order to avoid dissolution in aqueous environment (21).

Crosslinks are responsible for connecting the networks, which determines if the polymeric chain shows viscous-elastic or elastic behavior (21). The volume of water absorbed by a hydrogel is limited by the elasticity of the polymer networks, that defines its swelling capacity (22). When swollen, a hydrogel can absorb such amount of water that the mass of liquid present in its polymer networks becomes greater than the mass of the polymer that composes it (20).

For many applications is important for hydrogels to be biodegradable, and for that, unstable bonds are frequently introduced into the hydrogel's matrix. These bonds either can be present in the polymer backbone or in the crosslinks used to prepare the hydrogel. The bonds can be broken under physiological conditions, either enzymatically or chemically. Therefore, a great variety of methods to establish crosslinking have been used in hydrogel preparation (21). Physical and chemical methods have been applied in order to develop hydrogels. In physically crosslinked gels, dissolution is prevented by physical interactions that exist between polymeric chains (21). Physical crosslinks are based on hydrogen bonds, ionic bonds, van der Waals interactions or hydrophobic connections (23). In chemically crosslinked hydrogels, covalent bonds are present between the polymeric chains (19). Chemical crosslinks need a mediator agent for the reaction to occur, but the formed gels present more mechanical stability, since covalent bonds are stronger (20).

The water content of a hydrogel determines its physical and chemical characteristics, which guarantees unique properties to this structure. Compared to other classes of biomaterials, hydrogels have the advantages of being able to present biodegradability, biocompatibility, suitable mechanical strength and porous structure (24). In comparison to other variety of biomaterials, hydrogels have more properties common to living tissues and elastic low surface tension with water and biological fluids (16).

Natural hydrogels are composed by biopolymers, polymers that occur in nature and are mainly biodegradable (25). Even though these materials are compatible with living tissues and present low or none toxicity, in order to synthesize them chemical modifications must be performed (16). Such types of hydrogel are being gradually replaced by synthetic ones, aiming to achieve better service life, higher capacity of water absorption and superior gel strength (26).

Therefore, several synthetic strategies are being applied to create hydrogels with defined network structures, desirable chemical compositions, and tunable mechanical strength. These hydrogels can be prepared from completely synthetic components and show incredible stability even under stressful environmental conditions (27). By modifying the polymeric chains with stimuli-responsive functional groups, the hydrogels can answer to different stimuli, such as heat, pH, light, chemical agents and magnetic fields (28).

Materials for hydrogel preparation

ALGINATE

Alginate is co-polymer produced by brown algae (29) and bacteria, such as *Azotobacte*r and *Pseudomonas*. Structurally, alginate is composed of two uronic acids: d-mannuronic acid and l-guluronic acid. To form hydrogels of this material it is necessary that the crosslinking reaction occurs by the substitution of sodium ions by calcium ions, as the following reaction (30):

$$2Na\ (alginate) + Ca^{2+} \rightarrow Ca\ (alginate)^2 + 2Na^+$$

While the synthesis happens, during gelation, proteins, cells and DNA can be retained with full biological activity in the hydrogel matrix. At the same time that such molecules are entrapped, they are still free to migrate, which is very important in many applications (30).

Alginate is pointed for wound healing application and encapsulation of therapeutic agents, especially because of its biocompatibility, biodegradability and availability (31). Hydrogels made of alginate have been considered in the treatment of several kinds of wounds, since its high water content, elasticity, permeability and ability to create a moist environment in the wound bed, make it more feasible (32).

For instance, calcium alginate hydrogels have also been investigated to load hydrophobic drugs. The development of such combination can rely on alginate's hydrophilic crosslinked matrix in order to increase the solubility of such drugs. In that matter, the hydrogels may be used as delivery vehicle for sustained release (33).

CARBOXYMETHYL CELLULOSE (NaCMC)

Sodium carboxymethyl cellulose (NaCMC) is a water soluble adhesive polymer that occurs naturally (34). Hydroxyl and carboxyl groups are present in its structure, which makes it suitable for chemical modifications (35).

NaCMC has been used as a viscosity inducer in ocular preparations, like eyes drops and artificial substitutes for tears. When presented alone, NaCMC can form a transparent solution or a membrane with low mechanical strength, which limits its application as an ocular biomaterial. In order to overcome this, it is possible to blend NaCMC with a rigid polymer, like PVA (polyvinyl alcohol). This can improve the transmittance of the inserts and mechanical properties, while it keeps the biodegradable and adhesive characteristics of NaCMC. (34).

The water absorbent properties of NaCMC are another valid point of this polymer, as its excellent skin and mucous membrane compatibility. NaCMC is able to maintain an optimal moist environment in wound region for extracellular matrix formation and re-epithelialization, being proper as a dressing for treatment of burn wounds (36).

NaCMC can also be used as a hydrophilic polymer and a pH responsive component to improve the swelling ratio of PNIPAAM [poly (n-isopropylacrylamide)] hydrogels. Such association can produce pH/temperature responsive hydrogels (37), that ar suitable for the controlled release of drugs and bioactives (36).

CHITOSAN

Chitosan is a semi-crystalline, biocompatible and biodegradable amino polysaccharide. It is obtained from the exoskeletons of crustaceans in a process that evolves demineralization, deproteinization, deacetylation of chitin, extraction of chitosan and precipitation (38, 39). Chitosan is also non-toxic and can be used to produce gels membranes, coatings and fibers (40).

Hydrogels development with chitosan is very suitable, being that the presence of several hydroxyl groups makes the polymeric chain to swell rapidly, as it maintains its original shape. These hydrogels present phase transition in face of environmental stimuli, like pH, temperature and ionic strength (38).

Chitosan is one of the main choices for drug delivery. It’s matrix have been used to encapsulate drugs, cells and growth factors, among other therapeutic agents (39). Hydrogels of chitosan can be used in epidermal and intracorporal implants, since they can maintain constant drug concentration for long periods of time (38).

Hydrogels based on natural polymers are often chosen for controlled release of bioactive molecules (41). Hydrogels crosslinked with dialdehydes and dicarboxylic acids have been developed, in order to create drug delivery systems that present less adverse effects than the synthetic ones. However, that are few *in vivo* tests regarding this application. In that sense, chitosan hydrogels have been prepared by crosslinking with glutaraldehyde and glutaric acid, in order to obtain compatible and biodegradable materials for topical use (38). At the same time, studies to synthesize modified chitosan hydrogels to improve its adhesiveness and evaluate its suitability for topical application, have been performed (42).

CYCLODEXTRINS

Cyclodextrins are cyclic oligosaccharides that can form tridimensional compositions. Their structure present hydroxyl groups, which makes the interior hydrophobic while the exterior is water soluble. There are three exiting forms of cyclodextrins: alpha-, beta- and gamma-cyclodextrins (43, 44).

The behavior showed by the cyclodextrin molecules makes them suitable for developing hydrogels, since the hydrophobic interior is able to load substances. This occurrence turns possible the formation of noncovalent complexes with drugs, as the polymer's solubility, diffusivity and stability can be changed (45).

Co-polymerization with cyclodextrin can provide the formation of hydrogels with novel mechanisms of drug uptake/retention (46). Drugs can be trapped in the cyclodextrin cavities as the loading is efficient in the hydrophilic network of the associated polymer and the polymeric networks remain together when the hydrogel enters into contact with physiological fluids. However, co-polymerization can significantly change physicochemical properties of the hydrogel, especially swelling and viscoelastic properties (47).

DEXTRAN

Dextran is a biopolymer composed of polysaccharides that presents biocompatibility and biodegradability besides being non-immunogenic and non-antigenic. Such material has been widely applied in various biomedical applications, such as drug delivery and tissue engineering. Scaffolds made of dextran are soft and flexible (48), which favors to handle wound treatment, since it can be used for tissue re-epithelialization (49).

Dextran is soluble in water and organic solvents, which makes it feasible for bioapplications. Because dextran can be co-polymerized with other polymers, its physical and biological properties can be manipulated according to the application required (49).

Hydrogels made of dextran have been used as a platform to incorporate drug/growth factor loaded particles in skin regeneration (50). These hydrogels can also enhance the drug penetration by improving its pharmacological effects (51).

POLY (ETHYLENE GLYCOL) (PEG)

Poly (ethylene glycol) is a polymer of ethylene oxide and can have a molecular weight inferior to 100,000. PEGs with molecular weights under 1000 tend to be viscous and colorless, while the ones with higher molecular weights are waxy and white. PEGs are amphiphilic and soluble in aqueous environments as well as in organic solvents such as ethanol, acetone, and chloroform (52).

Systems of insoluble networks can be formed with PEG polymer alone, however, to reach stronger crosslinks, additional groups must be added to the polymeric chain. Groups such as acrylate, amine and carboxyl can form a biomaterial with good mechanical strength and more resilient networks, while make the polymer more degradable (53).

PEGs present considerable biocompatibility and non-immunogenicity, which are important features for these materials to be released by the Food and Drug Administration (FDA), for biomedical use. Therefore, many studies have been conducted with PEG in tissue engineering and wound healing (53).

In drug delivery, PEG is one of the most popular polymers used. When synthesized as a hydrogel, PEG have actions in directing cell proliferation and differentiation (54). In addition, it can provide focused release of drug, with therapeutic range, while understates adverse reactions and preserves therapeutic bioactivity (55).

POLY (2-HYDROXYETHYL METHACRYLATE) (PHEMA)

Poly (hydroxyethyl methacrylate) is one of the first developed synthetic polymers (56) being introduced by Wichterle and Lim in 1960 (17). Since then, it has been applied in medical and pharmaceutical areas especially for its non-toxicity and biocompatible properties (56).

PHEMA hydrogels have high water absorbing capacity and for that are widely applied in drug delivery (57). Its mechanical and swelling properties facilitates the delivery of drugs and bioactive agents. Such properties depend on the crosslinking agents used in the hydrogel synthesis (58). The high-water content of PHEMA hydrogels enable the uptake of some drugs by the simple immersion in concentrated solution. Especially used for the development of contact lenses, PHEMA hydrogels diffuse the drug between the lens and the cornea, increasing the drug permanence significantly, so ocular bioavailability can be enhanced (59).

POLY (LACTIC-COGLYCOLIC ACID) (PLGA)

Poly (lactic-co-glycolic acid) is one of the most used polymers in biomedical applications. PLGA can be hydrolyzed into lactic acid and glycolic acid, which can be easily metabolized by the human body, via the citric acid cycle. Therefore, PLGA is biodegradable and approved by the FDA for use in humans, especially for drug delivery systems. For that matter, PLGA-based materials have been extensively investigated (60).

The chemical degradation of PLGA is well characterized and controllable (60, 61). Thus, PLGA can deliver drugs to a target site from is matrix, notably for topical administration (62).

PLGA's stability is affected by factors that include polymer molecular weight, ratio of lactic to glycolic acid in the co-polymers, polymer-drug ratio, preparation process and environmental conditions such as pH and temperature (63). These factors also affect the ability of PLGA to form a drug delivery system and may control its hydrolysis and degradation. The type of drug is responsible for setting the release rate, as well as the mechanical strength and swelling behavior (64).

POLY (N-ISOPROPYLACRYLAMIDE) (PNIPAAm)

Poly (N-isopropylacrylamide) is a known synthetic polymer. PNIPAAM is considered thermoresponsive, since it reacts to temperature stimuli, and so, has a defined LCST. Lower critical solution temperature (LCST) is a temperature in which the polymer is presented in different conditions below and above it (12). For hydrogels of PNIPAAM the LCST is around 32ºC (65).

PNIPAAM is one of the most extensively studied polymers, since it has water solubility at room temperature. Above the LCST, the polymer solution transforms into an opaque gel, which is caused by hydrophobic interactions (66). At temperatures below the LCST, hydrogen bonds are formed and water molecules occur. When in condition of heating, hydrophobic interactions between the polymer chains become predominant, if the temperature is higher than the LCST (67).

The uptake and release profiles of PNIPAAM hydrogels are mainly in response of the polymer's physicochemical properties. (68). In controlled release systems, hydrophobic drugs can decrease the swelling, at the same time that the opposite happens to hydrophilic drugs (69).

POLYVINYL ALCOHOL (PVA)

Polyvinyl alcohol is synthetic polymer known for its biodegradable and biocompatible properties (70). Considered one of the oldest materials for hydrogels synthesis, PVA has been applied in several biomedical applications, where it's possible to highlight wound management, drug delivery systems and contact lenses (71).

Though PVA presents important applicability, hydrogels of such material have insufficient elasticity and very limited hydrophilic characteristics. As for wound dressing use, for example, PVA hydrogels usually have to be blended with other polymers, either natural or synthetic (72) In that matter, the additional component causes an impact in PVA in water swelling ability and wound moisture (71) .

Following that direction, chemical modifications ought to be done in order to associate wound management to drug delivery systems. The uptake and release of drugs can be achieved in better levels with the join of PVA and another polymer that can work improving the hydrogel's flexibility, adhesiveness and permeability (73).

SCLEROGLUCAN

Scleroglucan (SCLG) is a natural polysaccharide produced by *Sclerotium* fungi. Its structure have properties that points to the development of hydrogels for topical use (74). SCLG is basically constituted of a linear backbone of (1,3)--linked d-glucopyranosyl residues bearing a single (1,6)--linked d-glucopyranosyl unit every three sugar residues of the main chain (75). In aqueous medium SCLG shows triple helix conformation and pseudo plastic behavior, which evidences its transition from sol state to gel (76, 77).

SCLG can be applied in the preparation of modified-release dosage forms for topical delivery and may be altered with pH-responsive groups, which have considerable effect on the polymer's characteristics (78, 79). For instance, a carboxymethyl derivative of scleroglucan (SCLG-CM) is obtained by the reaction with chloroacetic acid in basic medium, which influences the gel properties. This modification turns SCL-CM capable of forming hydrogels without the addition of any salt (80).

Applications

EYES

Eye drops comprise about 90% of the ophthalmic formulations available (81). Even so, only 1% to 7% of the drug is absorbed by the cornea. Lacrimation, protective mechanisms,

nasolacrimal drainage and metabolism degradation are responsible for lower systemic drug concentrations. The solution to improve bioavailability is to increase concentrations in the pharmaceutic preparation and frequency of use. However, this can cause toxic outcomes and lead to side effects, which includes vision loss (82).

To overcome toxicity and promote better use, viscosity enhancers can be used, but this can be irritating and onerous to apply. Ocular inserts are also an alternative, but costs and occasional expelling from the eye are issues (82).

Nowadays researchers have been proposing contact lenses as an alternative to eye drug delivery. Compared to eye drops, contact lenses can present more residence time, which provides more drug flux through the cornea. (81).

Wichterle and Lim were the firsts to describe a biocompatible synthetic material for contact lens applications (83, 84). In their pioneer work, they developed a hydrogel based on poly-2-hydroxyethylmethacrylate (PHEMA) (17) which later came to be optimized by Bausch & Lomb and then approved by the FDA in 1971 (84).

Contact lenses can be classified as hard or soft. Hard lenses are based on hydrophobic materials such as poly (methyl methacrylate) (PMMA) or poly (hexa-fluoroisopropyl methacrylate) (HFIM). Soft lenses are usually made of hydrogels (84).

Hydrogel contact lenses can be a more convenient way to transport drug trough the eye. They show good transmission, chemical and mechanical stability, reasonable cost, high oxygen permeability and biocompatibility. Hydrogels also have the ability to control diffusion behavior through them, which points to their applicability in drug delivery (82).

Thus, poly-hydro-xyethylmethacrylate (PHEMA), is pointed as an effective biomaterial for the delivery of several ophthalmic drugs especially when associated to 4-vinylpyridine (VP) and N-(3-aminopropyl) methacrylamide (APMA). This combination was tested in contact lenses for the delivery of ibuprofen and diclofenac. In this case, the high polymeric density and the hydrophobic interactions of the PHEMA hydrogels were able to sustain the release of ibuprofen for 24h and diclofenac for almost one week (81, 85).

Diseases such as diabetic retinopathy, glaucoma and age-related macular degeneration (AMD) affect the posterior eye segment and can cause permanent vision loss when not treated in time. The treatment for these diseases usually implies in intravitreally administration of the therapeutic agents (86) **.**

However, these agents have short half-life and limited tissue permeation, which associated to the chronicity of the diseases demands constant administration in order to avoid

disease progression (87). Therefore, new approaches have been investigated in order to develop new and better options that are able to assure and maintain drugs therapeutic doses (88).

Hydrogels in that matter have received some attention, especially nanogels. Nanogels are hydrogels made in nanoscale. They work with the direct load of drugs, both hydrophilic and hydrophobic. Drug release kinetics can be controlled through degradation rate of crosslinks or face of stimuli such as pH and temperature (89).

Described hydrogels (N-isopropylacrylamide and 2-hydroxyl methacrylate-lactide-dextran macromer) were able to bypass ocular biological barriers, being good options to intraocular retinal drug delivery (90). For that, nanogels present promising properties for ocular drug delivery, in alternative to the eye drops conventionally used. (88).

Tests in rats and rabbits were reported (87, 91) and the use of cyclodextrin-based formulations for topical drug delivery to the eye showed important results. Cyclodextrin (CD) hydrogel implants were able to lower the potential of the drug to exit the tear fluid and enter the cornea. Therefore, the drug molecules act at their higher potential and increased activity. With that, it is possible to overcome the main problems regarding eye delivery: drug solubility in tear fluid and permeation through the cornea (92).

SKIN

1. CANCER

Skin cancer is the most common malignance that affects the population, with over a million cases registered each year (93, 94). This type of cancer is named according to the cell it arises from and clinic behavior presented. In that sense, that are three types: basal cell carcinomas (BCCs), squamous cell carcinomas (SCCs) and cutaneous malignant melanomas (CMs) (95).

Skin cancer incidence increases with age, and the association with UV exposure reflects directly in latency until cancer establishment (96). Both types of no-melanomatous cancer occurs with much frequency than melanomas, but are easier to treat and have better prognosis. These forms are derived from epidermal keratinocytes and less deadly than the melanomas, which is due to not leaving their primary site. Therefore, their management is more direct (97).

Malignant melanoma is, among the cited types of cancer, the deadliest one. It arises from epidermal melanocytes and is prone to metastasis (95). When early detected, melanomas can be removed surgically with relative success, however, CMs are fast to invade and spread, which turns long-term survival unreachable (96).

Management of skin cancer starts with excision biopsy and the choice of treatment depends on location, progression, margins and dimensions of the tumor, being that surgical removal is the main option. The focus of treatment lies in eradicate the cancer while preserves normal tissue function. (98, 99).

For both types of carcinoma, once not easily treated with elliptical excision, other options include liquid nitrogen, curettage, diathermy imiquimod, 5-fluouracil (5-FU) or radiotherapy. Liquid nitrogen and imiquimod are more indicated for superficial lesions. Topical approaches can cause intense inflammatory response and though radiotherapy is usually indicated for the elderly, good results can be achieved where lesions are difficult to remove (98, 99).

Although surgery remains a consensus among treatments for non-melanomatous skin cancers, new approaches have been presented over the years. Options like the application of nonsurgical agents and targeting of cellular receptors have been pointed in order to reduce morbidity and mortality while enhances patient's quality of life. (96).

For melanomas, radiation is of less use and appears as a second-line therapy. (100). In different stages of melanoma, a unique approach is used. With tumors that present more than 1 mm in thickness, dissection of the sentinel lymphnode is applied while IFN- is used as adjuvant therapy for stages 2 and 3. When surgery is not indicated, systemic chemotherapy is applied (101).

Studies have arisen in attempt to improve melanoma's treatment. Recently, Abkin et al designed a hydrogel containing recombinant HsP70. Chaperones HsP70 are proteins found in the human body that present immunomodulatory activity. In such work, a recombinant HsP70 was applied topically on the melanoma. Results showed that HsP70 can be diffused inside the tumor, through the skin, from the hydrogel. At the same time, the hydrogel [carbopol (1 %), glycerol (1 %), and dimethylsulfoxide (10 %)] was able to reduce the tumor growth and prolong life of the tested animals. Thus, the results reinforced the antineoplastic effect Hsp70 while showed a new non-invasive therapy (102).

Some works over the years have highlighted how effective devices for tumor drug delivery are not currently available. At the same time, other have been attempting to construct systems that allow at least better monitoring and targeting for other therapies. Following that, Nair et al developed poly-N-isopropylacrylamide hydrogel nanoparticles embedded with quantum dots. Quantum dots have poor circulatory behavior but are more readily taken when encapsulated in the hydrogel. In that sense, by using a melanoma model, the researchers showed

that the encapsulated quantum dots accumulated more in the tumor tissue rather than the normal tissue. *In vivo* and *in vitro* tests demonstrated that the association nanoparticles-quantum dots enhanced cancer imaging and targeting (103)

2. PSORIASIS

Psoriasis is a chronic immune skin disease that affects 1% to 3% of people worldwide. It is characterized by the presence of highly inflamed and erythematous scales.(104). The skin scale is caused mainly by hyper-proliferation of the epidermis, with dysregulated keratinocyte differentiation and increased vascularization (105)

The occurrence of psoriasis is a combination of genetic factors, that triggers the immuno-histological changed in the skin, and environmental causes (106). In that sense, psoriasis management usually involves topical and systemic treatments. Since this disease can have significant impacts on quality of life, a systemic approach is more valid, though topical treatment is the first line (107).

Systemic medication involves immunosuppressive and/or antiproliferative drugs, as well as attack of the TNF pathway, (108). It is common to associate systemic therapy with topical treatment; however, one can cause undesirable effects and the other has limited patient adherence. (109, 110).

In that sense, a nanocarrier composite hydrogel was developed by Gabriel et al. Their work describes a formulation to improve patient adherence and delivery of a psoriasis drug locally. The nanocarriers were based on methoxy – poly (ethylene glycol) – hexyl substituted poly (lactic acid) (mPEGhexPLA) and contained Tacrolimus (TAC) (107). TAC is potent immunosuppressive topic drug, approved for the treatment of dermatitis (111) and recommended for facial psoriasis (112).

Since the formulations showed to be safe and efficient in the delivery of TAC, and the release increased drug levels into inflamed skin, the use of reduced drug dose could be possible. By the development of a hydrogel formulation containing TAC, a new path for psoriasis treatment was made in order to improve patient acceptability, at the same time that bypass the mains advantages found in the currently available formulation (107).

3. WOUNDS

Cutaneous wound healing is a very complex process, in which the skin must regenerate in stages. The repair starts with hemostasis, followed by inflammatory response, proliferation and remodeling of tissues (113). These stages are common to every wound regardless type. In

that sense, wounds can be considered incision, excision, punctures, burns, abrasion, or blunt wound (114).

In wounds such second and third degrees and chronic wounds, healing is much slow and complete restoration of tissue function is hardly reached. (50). In most cases the healing failing is result of resistant infections, insufficient blood flow and edema. (115).

Burns are considered one of the most serious wounds, and for such, management must aim the prevention of infections, fast healing and epithelization (116). Topical therapy is essential and primordial for the survival of patients with more serious burns. The prevention of sepsis is a major concern. (117).

Chronic wounds are also a challenge regarding management and healing, and represent a substantial problem in healthcare systems (118). It is estimated that 1% to 2% of the world population will experience such condition in some period of their lifetime (119).

A wound is considered chronic when healing takes more to 8 weeks and can be classified into venous, arterial, pressure or diabetic (120). This occurrence is characterized by longstanding inflammation, infections and formation of resistant microbial biofilms (121).

Infection is a significant concern in patients who have to deal with burns or chronic wounds. Such complication causes major impacts in recovery and health costs (118, 122) Patients affected by these conditions are susceptible to infections due to the removal of the skin's protective barrier combined with the presence of endogenous microflora and prolonged hospital stays (123, 124). However, regard special management, a considerable number of patients suffers from these infections, especially because the bacteria found in these wounds are eventually polymicrobial and too resistant (125, 126).

Several drugs and approaches have been reported for wound treatment through distinct mechanisms (127). Simvastatin, for instance, is a lipid lowering agent, associated to the improvement of vascular endothelial growth factor (VEGF). Being that, it stimulates angiogenesis, reduce oxidative stress and improve micro vascular and endothelial functions which rises wound healing efficiency (128).

Hydrogels can knowingly support the wound healing process, since it is able to provide moisture to the wound, preventing fluid loss (71). Hydrogel dressings can be flexible and helpful in the epidermis repair (129). In drug delivery, it offers higher flexibility and stimuli face of pH and temperature. For such properties, Poly vinyl alcohol (PVA) and Chitosan have been studied, since they have proven to have excellent mechanical properties, biocompatibility and capacity to increase the collagen synthesis (130, 131).

Treatment for more severe wounds generally requires drug administration at time intervals for long periods. To apply a release therapy extendedly would reduce dosing frequency at the same time that continuously assures drug exposure and action (132). Therefore, the combination of Simvastatin with a hydrogel, in a controlled delivery system, presents itself promising for wound healing. Such incorporation could provide prolonged release of the drug from the hydrogel while reduces dosing frequency. In that matter, therapeutic concentration in plasma would be maintained, improving the therapeutic effects of Simvastatin (114).

One treatment for burns is considered gold standard. Silver sulfadiazine (SSD) is an effective antimicrobial agent with action against Gram-positive and Gram-negative bacteria as well as fungi (117). SSD also have potent anti-inflammatory properties, which are associated to decreasing erythema and increasing healing. However, such characteristics are directly dependent on the delivery vehicle, concentration and rate of release (116).

Commercially, silver sulfadiazine is available in a cream form, which present many disadvantages. The formation of an adhesive-eschar turns difficult to differentiate it from the burn eschar and such occurrence prevents SSD to penetrate the wound (116). Increased inflammation is also observed with the use of SSD as well as toxicity towards fibroblasts and keratinocytes (117)

In vitro studies evidenced SSD's cytotoxicity, which was shown to be minimized by controlling its delivery (133). Following that direction, an interest has been put in polymeric materials, that often offer valid scope for application in drug delivery. In view of that, chitosan/Carbopol hydrogels had SSD incorporated in its matrix, in a way that enhanced burn healing while overcame the disadvantages of the current commercial formulation (117).

ORAL MUCOSA

Candida is a genre of fungi present in the normal flora of healthy individuals, being that is estimated that 45%-65% of infants and 30%-35% of adults have it. Systemic and local factors can facilitate *Candida*'s overgrowth, especially *Candida albicans*, in the oral mucosa. Such factors include the use of dentures, corticosteroids and immunomodulatory drugs, immunosuppressed states, malnutrition, leukemia, chemotherapy and radiotherapy (134).

Some other researches have applied hydrogels in the treatment of oral inflammatory diseases. Triamcinolone acetonide, a long-acting synthetic glucocorticoid, for example, was used for such management. In that matter, a formulation of poloxamer hydrogels, loaded with triamcinolone acetonide was developed, and had its release investigated. The buccal mucosa of

rats was treated with this formulation and significant histopathological changes were observed. The wound surface was covered with regenerating cells. At the same time, cytotoxicity of the drug-loaded hydrogels didn't present significant results. Therefore, this system good be an important mean for controlled oral drug release with good mucoadhesiveness, and no tissue irritation (135).

Chlorhexidine (CLX) is an antiseptic of wide spectrum, that has activity against Gram-positive and Gram-negative bacteria. It is usually used in dental applications and is presented in a variety of formulations (136). Although, the existing formulations provide short-term efficiency, which requires constant applications in order to maintain antibacterial activity. In that sense, a controlled release of CLX could reduce de amount of applications as it enhances patient compliance. Therefore, a research was conducted in order to turn it possible the study developed a drug release system combining chitosan loaded with CLX. The results showed that the system was able to control the release of CLX, in a sustained way and without modifying CLX's properties (137).

VAGINAL MUCOSA

Vaginal candidiasis is an inflammatory disorder caused by *Candida albicans* fungi. It affects about 75% of women in reproductive age and is characterized by the presence of pruritus, vaginal discharge and vulvar erythema (138). The applied treatment is usually oral or topical, being that antifungals such clotrimazole (CTZ) is used (139). CTZ is highly effective, since it causes damage to ergosterol, main component of the fugal membrane, and affects consequently the fungal functionality (140).

Treatment can be local or systemic, which depends of the occurrence's severity. There are currently available CTZ for vaginal application in the form of rings, suppositories, ointments, tables, creams and gels (141). Gels are usually more employed, since they don't irritate de mucosa, are easier to administrate. However, gels present low retention time, low dose uniformity and sensation of leaking (142).

In that sense, to develop a gel with better properties and mucoadhesitivity could improve treatment. Since mucoadhesion involves the interaction between the biological surface and a bioadhesive material, a better contact among formulation and surface of application could be achieved (143). A polymer that shows mucoadhesivity must have high molecular weight, good diffusion properties, hydrophilicity, strong adhesion with the mucus and specific adherence to the tissue (144).

Since the mucus has anionic nature and carboxylic groups, van der Waals interactions and hydrogens bond can be facilitated. For polymers to be used they must be cationic, so the interaction can happens (145). In that matter, an alternative arises, though not much studied (146)

Pullulan, is a water-soluble polysaccharide with great mucoadhsive potential. This polysaccharide is obtained from fermentation of *Aureobasidium pulllulans* starch and is non-toxic, non-mutagenic, non-immunogenic and non-carcinogenic. Pullulan have been indeed investigated for drug delivery (147, 148).

Therefore, a study was conducted in order to develop a mucoadhesive hydrogel based on Pellulan, loaded with CTZ. The studies showed that the mucoadhesivity increases with the concentration of Pullulan in the hydrogel. The hydrogel was also able to limit CTZ permeation through the vaginal mucosa, which is important in a surface infection, at the same time that released the drug in controlled manner. Such system could be an alternative treatment for vaginal candidiasis (142).

Contraceptives can be applied to the vaginal mucosa in dosage forms that include films, tables, suppositories and hydrogels. Semi-solid dosage forms are the ones more convenient for such application. They present flexible dose, good lubrification and rapid onset (149). However, semi-solid forms can't be effectively retained due to mucous secretion and turnover. Residence of conventional formulations is usually too short for bioactives to present their therapeutic role, which lowers dose and duration (150)

In attempt to overcome this, in situ hydrogels formulation have gained attention (151). Thermosensitive hydrogels show phase transition in face of temperature alterations. For vaginal administration, for instance, such formulations can be easily applied at room temperature, since they present low viscosity, which allow quickly spreading and flow into the vagina's mucosa. Besides that, hydrogels can prologue residence of the loaded drug in the vagina (152).

Thus, studies were carried out in mouse and rabbit models, for in situ release of contraception actives. It was showed that the hydrogel could form a protective layer in the mucosa of vagina's surface. This could inhibit sperm motility while it functions as lubricant for sexual activity. (153)

In that sense, poly (ethylene oxide), poly (propylene oxide), poly (ethylene oxide), in combination, (PEO-PPO-PEO), were studied for vaginal use. Therefore, tests were conducted to associate such combination with Nonoxynol-9 (N-9) (153), the most widely applied topical

spermicide (154). The results showed how the hydrogel gains hydrophilicity while suffers erosion and releases the drug in a sustained way (153).

Future perspectives and conclusions

Though hydrogels have been extensively investigated for a long time now, researches show that there is still a long wat to go. For topical use many studies are promising, but standard applications and treatments are still preferred, despite the many advantages presented by hydrogels. It is important to highlight that, in spite of that, there are hydrogels formulations available and in current use, especially for wound healing.

As new associations with hydrogels and drugs/bioactives emerge, these materials evidence their importance regularly. In a previous work, our group conducted a study that showed the functionality of PNIPAAM hydrogels to incorporate and release bromelain, a set of proteolytic enzymes with anti-inflammatory and healing properties (15). What our research and the possibilities presented in this review elucidate is that there is rich field to be yet explored.

It is evident the importance that hydrogels have been carrying out for almost 60 years. New polymers with innumerous characteristics arise frequently and for such, new techniques and developments must be performed in order to create new and alternative applications and treatments.

CAPÍTULO II

Characterization of *PNIPAAm-co-AAm* hydrogels for controlled release of bromelain

Authors

Fernanda Machado Croisfelt[1], Janaína Artem Ataíde [2], Letícia Caramori Cefali[1], Marcia de Araújo Rebelo[3], José Luis Dávila Sànchez[4], Thais Germano da Costa[5], Fernanda Camila Cruz[5], Angela Faustino Jozala[6], Marco Vinicius Chaud[3], Marcos Akira d'Ávila[4], Elias Basile Tambourgi[7], Edgar Silveira[8], Priscila Gava Mazzola[9].

Affiliation

[1]Biology Institute, State University of Campinas (UNICAMP), Campinas, SP, Brazil

[2]Faculty of Medical Sciences, State University of Campinas (UNICAMP), Campinas, SP, Brazil

[3]Laboratory of Biomaterials & Nanotechnology, University of Sorocaba (UNISO), Sorocaba, SP, Brazil

[4]Faculty of Mechanical Engineering, State University of Campinas, Campinas, SP, Brazil

[5]Faculty of Bioprocess Engineering and Biotechnology, University of Sorocaba (UNISO), Sorocaba, SP, Brazil

[6]Department of Technological and Environmental Processes, University of Sorocaba (UNISO), Sorocaba, SP, Brazil

[7]School of Chemical Engineering, State University of Campinas (UNICAMP), Campinas, SP, Brazil

[8]Genetics and Biochemistry Institute, Federal University of Uberlândia (UFU), Uberlândia, MG, Brazil

[9]Faculty of Pharmaceutical Sciences, State University of Campinas (UNICAMP), Campinas, SP, Brazil

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Abstract: Hydrogels are materials known for decades, with applications in wound healing, contact lenses, biosensor and tissue engineer. Thermosensitive materials, such as PNIPAAm are considered for the development of hydrogels for controlled release, especially for topical use. Bromelain is a set of proteases found in plants of the *Bromeliceae* family, being that pineapples represent the main source of these enzymes. In burns, bromelain acts by hydrolyzing the devitalized tissue, both *in vivo* and i*n vitro*, which increases the healing capacity. Therefore, the combination of such hydrogels and bromelain could point to a topical application of interest. For this work, NIPAAm and AAm were co-polymerized and the hydrogel synthesized taken to swelling tests for 24 hours. The best formulation, was loaded with bromelain solutions. Pineapple residues were processed and precipitated with ethanol for posterior freeze-drying and use. After loading which was nearly 30%, the hydrogel discs were dryed and immersed in phosphate buffer pH 7.0 for release. The amount of protein and enzymatic activity were measured from the buffer after 60, 90 and 180 minutes and after 48 and 96 hours. Fourier transform infrared (FTIR) was performed to determine the specific chemical groups of the purified bromelain and the hydrogels, pure and containing bromelain. Micrographs of the hydrogels both pure and loaded with bromelain showed the appearance of the polymeric chains and how the loading clearly appears. The hydrogels were also submitted to mucoadhesion tests in order to assure the material's retention, spread and bioavailability. Rheology and resistance experiments were applied to verify the samples' physical behavior in face of stress. Cell viability assay showed percentage of death at 50% after 48h. In that sense, the characterization performed in these tests points to the possibility of association of thermosensitive hydrogels and a bioactive product, such as bromelain,

1. Introduction

Hydrogels are polymeric networks able to absorb large amounts of water (23). Their tridimensional configuration allows the polymer to be present in aqueous medium without dissolving in it. Hydrophilic groups make such behavior possible, at the same time that crosslinks hold the polymeric chain together (16).

Covalent and hydrogen bonds, physical tangles or *van de Waals* and hydrophobic interactions form the crosslinks, that structurally allow substances to be entrapped in the polymeric matrix, which is viable for the developing of controlled release systems (155). Hence, hydrogels are good candidates to be vehicles for pharmaceuticals, bioactive products, proteins and cells (156) by facilitating their delivery in stimuli to environmental conditions such as temperature, pH, ionic strength and light (157).

Hydrogels that respond to temperature alterations are called thermosensitive (157), being that, temperature changes may increase or decrease the rate of swelling of a hydrogel. When the level of swelling rises with the temperature, the hydrogel presents higher solubility in water due to the predominance of hydrophilic groups in its structure. Hydrogels that the level of swelling lowers with the increase of temperature tend to shrink, since hydrophobic interactions increases (20). The temperature that determines such behavior in hydrogel is denominated lower critical solution temperature (LCST) (158, 159).

The most studied hydrogels are the thermosensitive (19) being that the ones made of poly (N-isopropylacrylamide) (PNIPAAm) have been scientific target for years (160). Such hydrogels have the ability to swell and shrink in face of alterations of the external temperature (161) which is due to their LCST (162-164). PNIPAAm hydrogels have LCST around 34ºC, that is very close to the normal temperature of the human body. Under this temperature the polymer absorbs aqueous solution and becomes swollen. Once a higher rate is reached the hydrogel collapses and expels the content trapped in its matrix (163).

Therefore, PNIPAAm hydrogels are an alternative to be considered for controlled release, especially for topical use. Our group has already proposed a delivery system by associating these hydrogels with bromelain, a set of proteolytic enzymes found in plants of the *Bromeliaceae* family, mainly pineapples (15).

Bromelain preparations are used since Seligman showed its action as an anti-inflammatory agent (1). Bromelain have been pointed in the treatment of rheumatoid arthritis, bruises, oral inflammations, diabetic ulcers, rectal and perirectal inflammations, athletic injuries. In all cases, bromelain causes significant reduction of pain and swelling, and decreases

healing time by half the time compared to conventional treatment (7). Bromelain is also still highlighted in the treatment of breast and ovarian cancer in which is associated with a decrease in metastasis and increase in nocitoxicity and neutrophilic activity (3). In burns, bromelain acts by hydrolyzing the devitalized tissue, both *in vivo* and i*n vitro*, which increases the healing capacity (7, 165).

Thus, the study of such hydrogels, in association with bromelain, becomes interesting for use in topical occurrences. In that matter, it is important to determine physical and chemical-physical characteristics of this material in order to ensure its efficacy and applicability.

2. Materials and Methods

2.1. Materials

N-isopropylacrylamide (NIPAAm) (97%), N, N, N', N', tetramethylethylenediamine (TEMED), Bradford reagent, and Azocasein were purchased from Sigma-Aldrich (São Paulo, Brazil). Acrylamide (AAm), N, N'-methylene-bis-acrylamide (BIS), ammonium persulfate (APS), tris (hydroxymethyl) aminomethane (TRIS), polyethylene glycol (PEG) 400 and glucose were purchased from LABSynth (São Paulo, Brazil). **Trichloroacetic aci**d (TCA) was purchased from Dinâmica Química Contemporânea (São Paulo, Brazil). The pineapple residues were gathered from local markets. NIPPAAm was prepared at 10% and Bis-Acrylamide at 30% of acrylamide and 0.8% of N, N′-methylene-bis-acrylamide for crosslinking reaction. Tris reagent, used to buffer the formulation, was titrated with HCl to pH intended**.**

2.2. Methods

2.2.1. Hydrogel

a. Formulation

Hydrogels were prepared by redox polymerization, according to Schild (166). NIPAAm and AAm were co-polymerized in pre-determined concentrations and pH. TEMED was used as the accelerator and APS worked to initialize the reaction. The solution was gently mixed and polymerization occurred in 12 well-plate (diameter = 21,4 mm) and 60mm plates for 20 minutes at room temperature. Once synthesized, the gels were immersed in distilled water for 24h, to wash unreacted compounds. Following that, samples were dried for 8h in oven at 60 °C.

b. Swelling capacity

Discs of dried hydrogels were hydrated in distilled water for 24h to reach swelling equilibrium at room temperature, based on methodology described by Wang et al, 2009 (163).

Swelling ratios were determined by the ratio of the weights of the swollen gel (Ws) to that of the corresponding dried gel (Wd) (167). The formulation with the best swelling ratio was selected for the experiments with bromelain.

2.2.2. Bromelain

a. Preparation

Pineapple residues (bark and stalk) were processed in a kitchen juicer (Philips, WALITA Koninklijke Philips N.V., 2004 - 2018) and then centrifuged (Centrifuge by Nova Instruments, Piracicaba, Brazil) at 4000 rpm for 10 min at 4 °C to remove insoluble particles.
The procedure
was repeated twice. The resulting supernatant (crude enzyme extract) was taken to precipitation process.

b. Precipitation

Bromelain precipitation followed as described by Englard, 1990 (168), with some alterations. Initially, ethanol was cooled at 1 °C and added in a dropwise manner to the crude enzyme extract until desired concentration (25% to 65% v/v). The solution was centrifuged at 4000 rpm for 15 min at 4 °C. The supernatant was discarded and the precipitated was washed in distilled water to be resuspended and lyophilized. The lyophilized was kept in freezer at –4ºC to posterior use.

c. Loading

Bromelain was incorporated to the hydrogels using the methodology of diffusion by turgidity. Dried discs of hydrogel were immersed in 10mL solution containing 0,5% of bromelain, 10% of PEG and 5% of glucose at 25ºC for 24h. Protein quantification[1] and enzyme activity[2] assays were performed prior and after the incorporation.

[1]Protein quantification was performed by method described by Bradford (169)

[2]Enzymatic activity was determined using the azocasein method, according to Coelho et al, (170). In this method, bromelain was set to cleave the substrate, azocasein, at 37°C for 10min. TCA was added to stop the reaction as precipitate non-hydrolyzed azocasein. Tyrosine residues, released by the cleavage of bromelain, were detected at 440 nm by spectroscopy (spectrophotometerc Genesis 10s UV-Vis, by Thermo Fisher Scientific, Waltham, USA) in order to calculate de amount of bromelain needed to process azocasein per min at 37ºC. Enzymatic activity was calculated in activity units (U/mL).

d. Release

After loading, the discs were softly dried in filter paper and immersed in phosphate buffer pH 7.0. The discs were dried from time to time as the buffer was replaced. The amount of protein (169) and enzymatic activity (170) were measured from the buffer. Buffer changes occurred after 60, 90 and 180 minutes and after 48 and 96 hours, in adaptation of Zhang et al, 2004 (171). Tests were performed at 25ºC and 37ºC.

2.2.3. Physicochemical characterization

a. Scanning electron microscopy (SEM)

Discs of hydrogel, pure and loaded with bromelain, were frozen in liquid nitrogen at –196ºC and then lyophilized. The samples were fractured and placed in stubs, fixed with carbon double-sided tape. The stubs went through Sputtering at 30ºC in Sputter Coater EMIECH, model K450 (Kent, United Kingdom). Afterwards, micrographs were taken in Scanning Electron Microscope with X-ray Dispersive Energy Detector, LEO Electron Microscopy (model Leo 440i, Cambridge, England).

b. Infrared spectroscopy (FTIR)

The characterization of the specific chemical groups of the purified bromelain and the hydrogels, pure and containing bromelain, was performed by Fourier transform infrared (FTIR) spectroscopy (Shimadzu, FTIR IRAffinity-1S, Kyoto, Japan) (172). Briefly, bromelain was characterized using KBr tablets and the hydrogels by attenuated total reflection (ATR), both in transmittance mode. The spectra were obtained in the wavelength range 4000 to 600 cm^{-1} after 128 scan and resolution of 4 $cm^{-1.}$ The spectra were normalized and the vibration bands were associated to the main chemical groups.

c. Rheological behavior

The rheology was assessed using a modular compact rheometer (ANTON PAAR, MCR 102, Sao Paulo, Brazil) and sensor cone/plate (C35/2º Ti). The data were analyzed with the RheoCompass^{TM} Software. The rheological behavior was determined using amplitude and frequency scanning assays to analyze the dynamic viscosity (η) and stocking module (G) of the hydrogels, pure and loaded with bromelain. For the tension-scanning test, a sheering tension range of 39.4 to 22,200 Pa was used (173). The frequency-scanning test was performed using a frequency range of 0.1 to 240 rad/s at a tension of 6,300 to 9,100 Pa. Thus, the elastic module

(G') and viscous module (G") were determined (173-175) All assays were performed in triplicate at 25 ± 0.5 ºC and at 37ºC ± 0.5 ºC.

d. Mucoadhesion, drilling and resilience

The parameters used for the tests of mucoadhesion, drilling and resilience are presented in **Table 1**.

Table 1. Parameters used to evaluate the mechanical properties of pure hydrogel and loaded with bromelain

Parameters	Mucoadhesion	Drilling	Resilience
Aparate	Film Support Rigg Part Code HDP/FSR Batch n 1305	Film Support Rigg Part Code HDP/FSR Batch n 1306	Film Support Rigg Part Code HDP/FSR Batch n 1307
Test mode	Compression	Compression	Compression
Pre-test speed	0,5 mm/s	1 mm/s	1 mm/s
Test speed	0,5 mm/s	2 mm/s	3 mm/s
Post-test speed	10 mm/s	10 mm/s	11 mm/s
Target mode	Distance	Distance	Distance
Distance	-	15 mm	5 mm
Trigger type	Auto	Auto	Auto
Trigger force	0,049 N	0,049 N	0,049 N

The mucoadhesive capacity of the hydrogel, pure and loaded with bromelain, was evaluated by the force necessary to detach the mucin disc from the hydrogel. Mucin disks with 8mm diameter were prepared by compression (Lemaq, rotary compressor machine, Mini Express LM-D8, Diadema, BR). The hydrogel samples were fixed on appropriate platform (A / MUC) and kept in water bath set at 37ºC. The mucin disk was previously hydrated and attached to the lower end of the analytical probe (P/10). The mucin disc was compressed in the apical → basal direction on the surface of the samples, with a force of 0.049 N. The time of contact of the disc with the surface of the samples was 200 s. The displacement of the analytical probe in the basal - apical direction was programmed to 0.5 mm/s. The force required to detach the

mucin disc from the surface of the hydrogel was determined by the ratio time (in seconds) x force (N).

In order to evaluate the mechanical strength of the hydrogels, the samples were fixed between two perforated plates (HDP/90) previously fixed to the platform of the equipment. The probe (P/5S) was compressed towards the apical → basal direction on the surface of the samples, with compressive force of 0.049 N. To measure the drilling resilience, the distance covered by the probe was, respectively, 10 mm and 5 mm.

2.2.4. Cell viability - Mitochondrial activity assay (MTS)

For performing the analyzes for the application of a V79 lineage (normal Hamster lung cells), as cells were plated approximately 5.4 x 10 4 cells / well in 24 well plates, as were incubated at 37ºC - 5% CO_2 after 24h and total adherence of the cells were exposed to the material which was washed in PBS prior to the above. One exposed in 24 hours, 48 and 72 hours in incubation 37 ° C - 5% CO_2.

After treatment, the culture medium and membrane were removed and 500 µl culture medium and 100 µl MTS (3- (4,5-dimethylthiazol-2-yl) -5- (3-carboxymethoxyphenyl) -2- (4-sulfophenyl) -2H-tetrazolium bromide) and incubated for 2 h at 37ºC - 5% CO_2. After this solution period for withdrawal and placement in 96-well plates for reading. The reading was performed using the microplate reading equipment at 570nm.

3. Results and discussion

3.1 Hydrogel

The redox polymerization of NIPAAm and AAm happened in the presence of APS as the initiator and TEMED as the accelerator. BIS is usually the choice in crosslinking, mainly due to its structural similarity to NIPAAm. For this formulation, BIS was due to its well established use in polyacrylamide gels for electrophoresis (176).

The inherent viscosity of PNIPAAm is independent of pH below 6.8, but increases at higher values (166). For the experiments pH 5.0, 7.0 and 9.0 were tested. Such behavior showed that in the tested concentrations for the polymers a more neutral pH favors the formation of the polymeric chain.

At the same time, the association of the polymers concentration and the pH has already been discussed by our group in a previous publication, by the use of the surface response methodology (RSM) (15). Such methodology elucidated the best results regarding the

association of NIPAAM and AAm, while buffered with TRIS-HCl. By the use of the RSM, we could determine that the best concentrations for these polymers lies over 8%.

For this work, we tested concentrations of 11% and 12% for NIPPAm and AAm, respectively. Once the hydrogels were successfully polymerized, the parameter used to determine the best formulation was the swelling ratios.

The weight variation was used to estimate swelling ratios according to **Eq. 1**.

$$\mathbf{P = \frac{Wd}{Ws}\ x\ 100} \qquad \mathbf{(1)}$$

The hydrogel was able to reach swelling ratio of 340%. The interaction between NIPAAm and AAm showed to be crucial in order to ensure higher rates of swelling. The formulation that showed the best swelling behavior presented pH 7.0, which is also relevant for the incorporation of bromelain, since it presents better activity in pH between 6.5 and 7.5 (177).

3.2. Bromelain load and release

To evaluated the incorporation of bromelain by the hydrogels, the solution (initial solution) was prepared and had protein amount and enzymatic activity measured (as shown in item 2.2.2.) After 24h the volume not absorbed by the hydrogel (final solution) went through the protein and enzymatic assays once again. To determine the values of protein and enzymatic activity in the hydrogel, **Eq 2** was applied.

$$\mathbf{Hydrogel = initial\ solution - final\ solution} \qquad \mathbf{(2)}$$

The registered results are shown in **table 2**.

Table 2. Protein amount and enzymatic activity of bromelain after 24h incorporation at room temperature (n=20).

	Bromelain loading					
	Initial solution		**Final solution**		**Hydrogel**	
Protein amount (mg)	21,92	±9,2	10,9	±1,73	11,01	±10,85
Enzymatic activity (U)	392	±90,1	295,6	±14,07	96,39	±87,56
Incorporation (%)					42,5	±27,82

The period of 24 hours is the time necessary by PNIPPAm hydrogels to reach their equilibrium state (164). In such period the hydrogel gets to its most swollen state, where it absorbs water until the maximum point handled by the polymeric chain. Passing that point the hydrogel stops absorption. With the bromelain solutions, the hydrogel behaved at the same manner. The initial solution was prepared to be 10 mL of volume. After absorption, the hydrogel incorporated 2,8 mL of bromelain solution. For all samples tested, this was the highest value of volume integrated to the hydrogel.

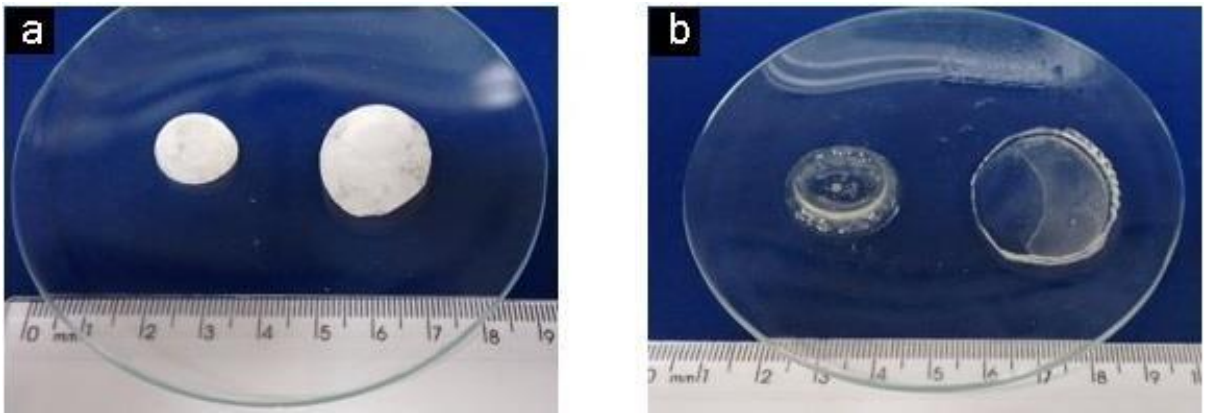

Fig 1. Discs of dried hydrogel **(a)** and loaded with bromelain **(b)**.

By analyzing that and the tests of protein quantification and enzymatic activity, the hydrogel was able to incorporate about half the protein that was present in the initial solution. At the same time bromelain didn't lose considerable activity after the incorporation (final solution) when compared to the initial solution. Such occurrence may be in result of the addition of PEG (400) and glucose. Both can be pointed as stabilizers, when in phosphate buffer pH 7.0, of bromelain's activity, as highlighted by Soares, 2012 (178). Such stability could also be observed in the process of release from the hydrogels, as shown in **Fig 2 a** and **b.**

The graphics show that both temperature and time did not affect the amount of protein released neither the enzymatic activity of bromelain. We could observe a small drop of activity right after 90 minutes of release. This may be associated to first change of buffer. However, bromelain appeared to be stable in pH 7.0, which according to the literature represents a favorable condition for its activity (177).

The best temperature for release from PNIPAAM hydrogels is around 34ºC (179). This temperature represents the range of PNIPPAm's LSCT. We tested 37ºC mainly because this value is found in such range at the same time that is the human body's temperature, which could make it suitable for topical application. Since the hydrogel showed practically the same behavior both at 25ºC and 37ºC, we can assume that for bromelain, at least, in combination with PNIPAAm, release can me modulated for temperatures different of the LSCT.

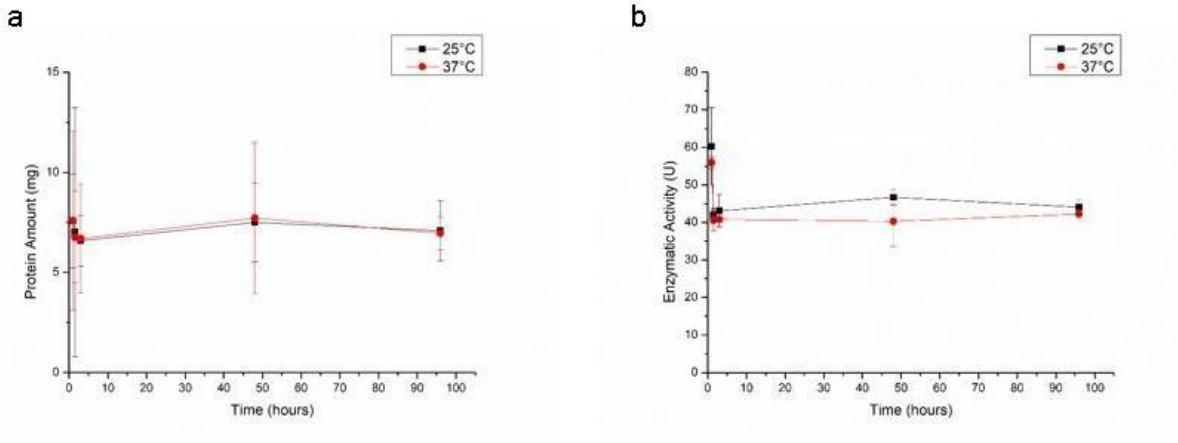

Fig 2. Analysis of bromelain releasing at 25ºC and 37ºC by quantification of protein amount **(a)** and measurement of enzymatic activity **(b)**. .

3.3. Scanning electron microscopy (SEM)

Figure 3 shows micrographs of hydrogels samples, pure, before loading with bromelain. **Fig 3a** displays the hydrogel's surface, where the matrix's pores are highlighted. Thought the pores present different sizes, they can be considered macrospores, since their diameter is greater than 50 nm (180). In **3b** it is possible to observe a cross section of the hydrogel disc. In the image the polymeric network is out in evidence, which makes feasible to see the spaces where the hydrogel holds the water content.

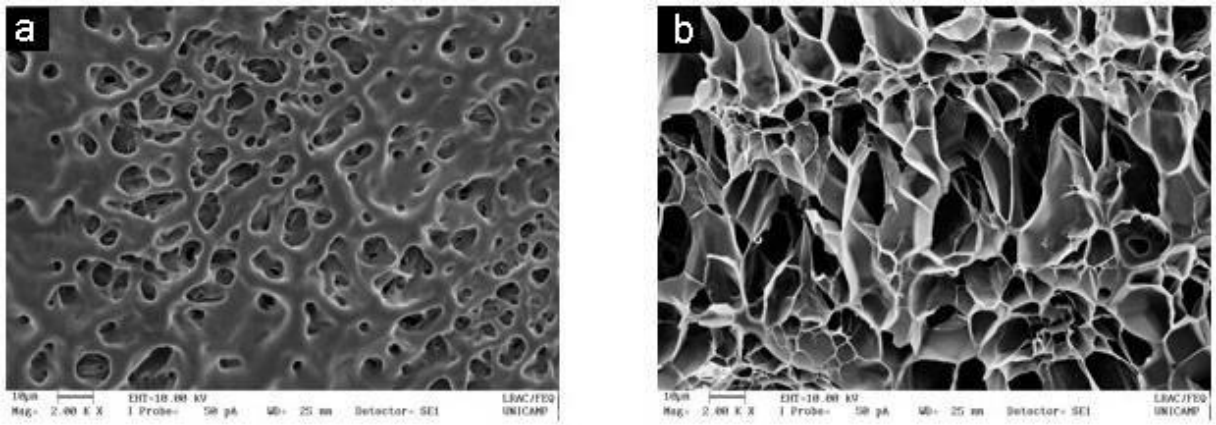

Figure 3. Micrographs of hydrogel without bromelain, after freezing at – 196ºC and lyophilization. **(a)** Surface cut 2000x. **(b)** Transversal cut 2000x.

Figure 4 shows micrographs of hydrogels samples loaded with bromelain. In **4a** the hydrogel's surface in once displayed, as most of the pores are filled with bromelain solution. It is possible to see the difference by comparing it with **Fig 3a**. In **Fig 4b** lighter parts of the image

highlight the presence of bromelain among the hydrogel's matrix. The difference is visible when compared to **Fig 3b**.

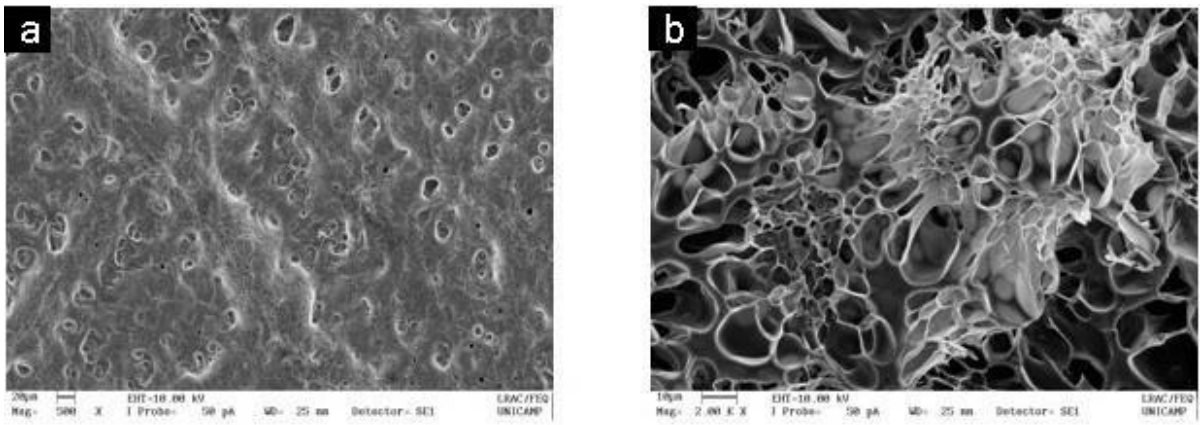

Figure 4. Micrographs of hydrogel loaded with bromelain, after freezing at – 196ºC and lyophilization. **(a)** Surface cut 500x. **(b)** Transversal cut 2000x.

3.4. Infrared spectroscopy (FTIR)

The purified bromelain is characterized by the C-N groupings (1250 cm^{-1} e 1517 cm^{-1}), which refers to the non-conjugated amines, C=O (1635 cm^{-1}) which refers to the amide I, N-H (1541 cm^{-1}) which refers to the amide II and the enzymatic peptide bonds (3423 cm^{-1}). For the hydrogel, pure (b), the stretch in 1641cm^{-1} refers to the bond C=O of the polymer and the stretch in 3390 cm^{-1} refers to the grouping -OH. The hydrogel loaded with bromelain presented the same stretches of the groups C-N (1250 cm^{-1} e 1510 cm^{-1}), C=O (1537 cm^{-1}), N-H (1635 cm^{-1}) and peptide bonds (3408 cm^{-1}) (c). In the regions of 1537 cm^{-1} e 3408 cm^{-1} there is overlap of the polymer's groupings, with stretches referred to amide I and enzymatic peptide bonds (172, 181, 182).

The analysis indicated that the incorporation of bromelain by the hydrogel happened by the formation of hydrogen bonds while it did not interfere with NIPAAm or AAm intermolecular polymeric chains. Such result, shows that the bromelain entrapment into hydrogel point to a strategy for skin applications.

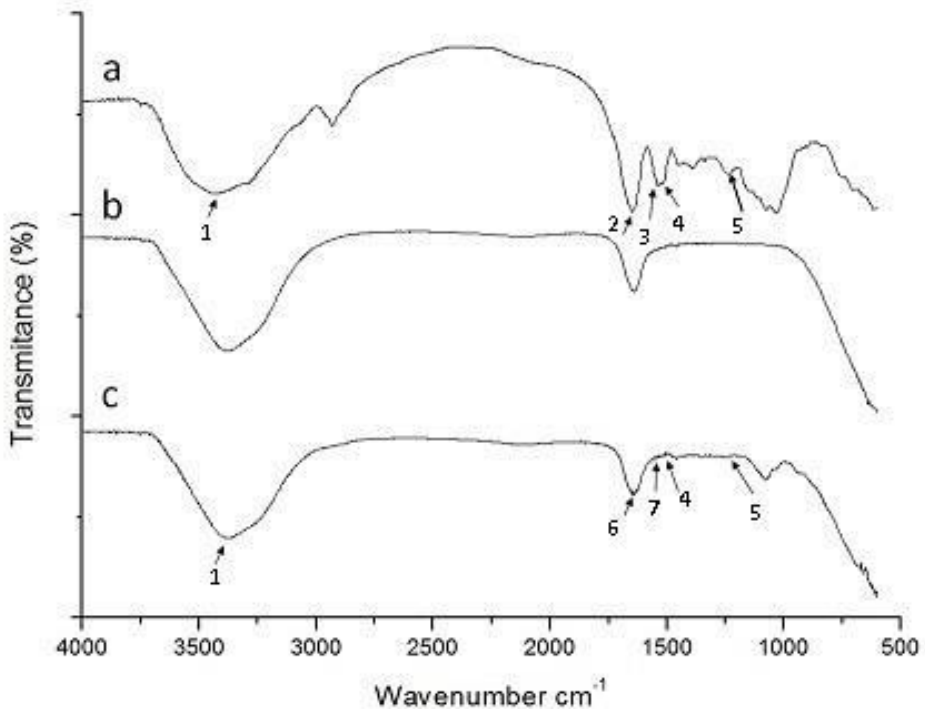

Fig 5. FTIR spectra of free bromelain **(a)**, pure hydrogel **(b)** and hydrogel loaded with bromelain **(c).** The number 1 correspond to stretch at 3390 cm^{-1}, number 2 at 1541 cm^{-1}, number 3 at 1635 cm^{-1}, numbers 4 and 5 correspond to stretches at 1517 cm^{-1} and 1250 cm^{-1}, respectively. The number 6 correspond to stretch at 1537 cm^{-1} and number 7 correspond to stretch at 1633 cm^{-1}.

3.6. Rheological behavior

The rheological analysis evaluates the Newtonian or non-Newtonian behavior of materials. This analysis verifies whether the fluid is plastic, pseudoplastic, or dilatant, or if it exhibits thixotropy and presents viscoelastic behavior (183).

According to Ribeiro, 2004 (184), the elastic and viscous properties of a material determines if the same has the ability to recover its elasticity after the shearing tension ceases. In this work, we could observe such behavior by the hydrogels, as they were submitted to amplitude and frequency scanning tests.

The amplitude scanning test was performed in order to determine the shearing tension values. In that sense, at 25ºC or 37ºC, the hydrogel samples did not suffer deformation within a range of linearity. Such test is important to select the linear viscoelastic region, a range of shear stress in which the material structure is not disrupted during the creep and recovery

analysis. Therefore, information regarding intermolecular and interparticle forces of the material tested can be obtained.(185)

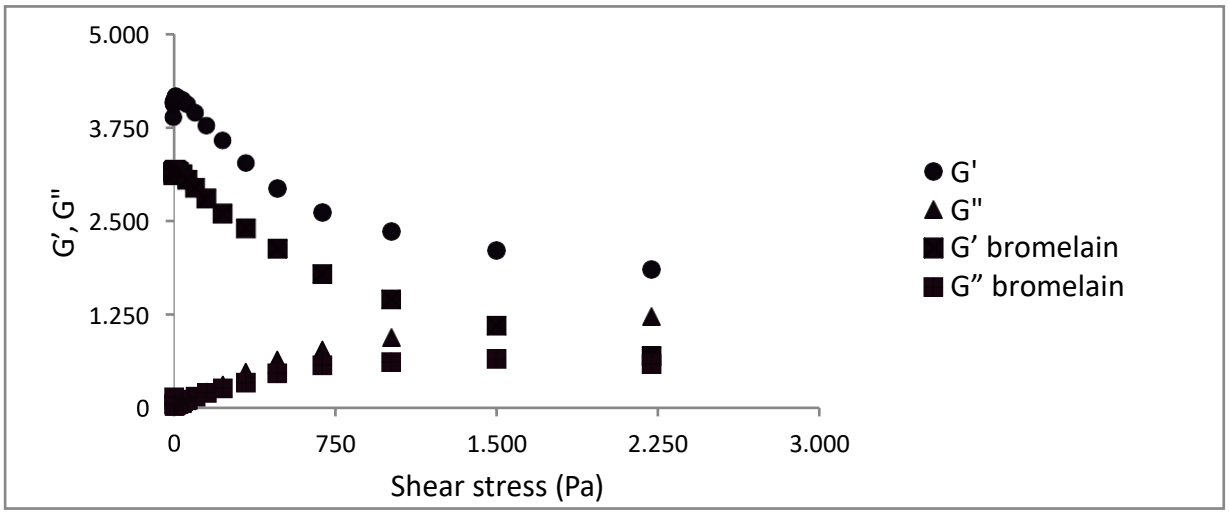

Fig 6. Rheological behavior (amplitude scanning) of hydrogels pure and loaded with bromelain at 25ºC.

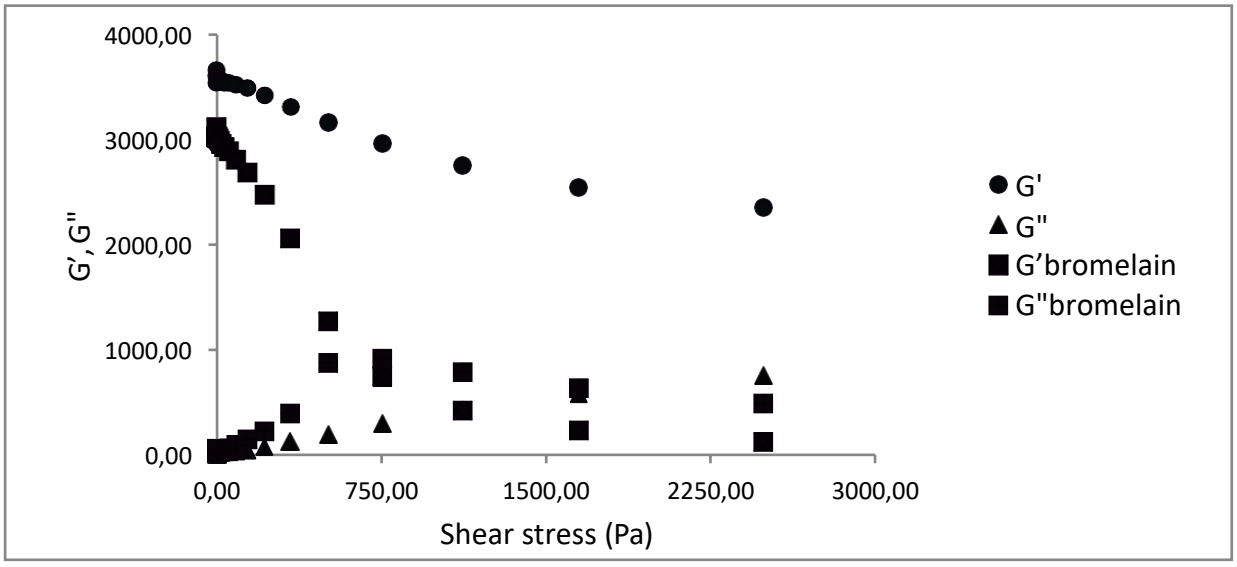

Fig 7. Rheological behavior (amplitude scanning) of hydrogels pure and loaded with bromelain at 37ºC.

According to figures 6 and 7, as observed by by Seddiki and Aliouche, 2013 (186) in the range from 39.4 to 22,200 Pa, the sample didn't go under deformation, as the G' and G" values remained linear in the shearing tension range. This shows the stable behavior of hydrogels when submitted to tension.

The difference observed from the tests ate 25ºC, was that the crossover (change in the rheological behavior of the elastic to viscous material, that is, when the material became more fluid) of the sample with bromelain. This occurred in a percentage of amplitude lower at 37ºC (25.3% of amplitude), as for 25°C it occurred in 100%. For the sample without bromelain, no change was observed and the crossover occurred above 100% of amplitude in the two temperatures. Then, bromelain presence interfered in the rheological behavior of hydrogels developed above 25ºC, becoming it more fluid.

In the frequency scanning test, it was possible to determine the G' (storage module) and G" (loss module) of the hydrogels. Usually, a material has higher G' than G'' values, which shows its elastic behavior (175). According to what is shown in figures 8 and 9, the hydrogels presented such property, at 25ºC and 37ºC, which is favorable for topical products. For the hydrogel, especially, this behavior will promote bromelain's action on the skin.

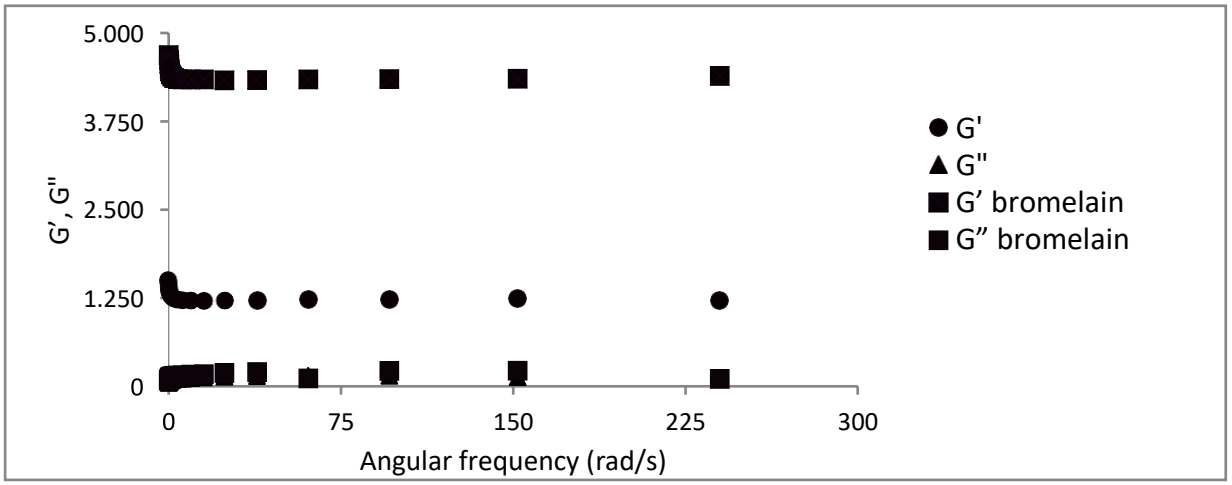

Fig 8. Frequency scanning test of hydrogels loaded with bromelain at 25ºC.

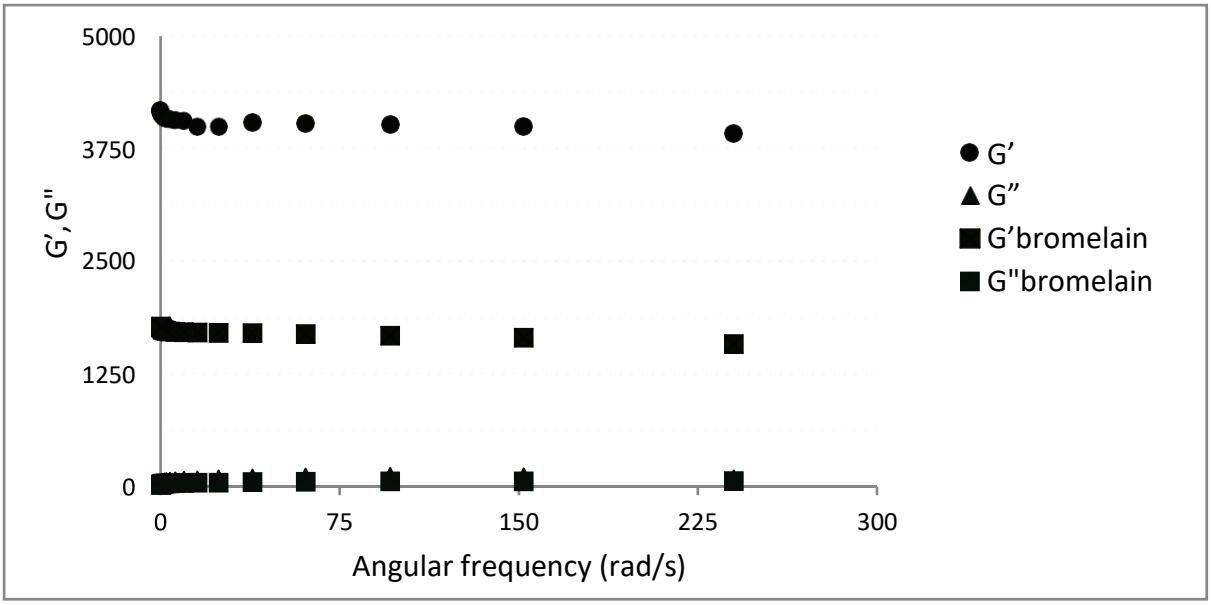

Fig 9. Frequency scanning test of hydrogels loaded with bromelain at 37ºC.

In both assays, it was possible to observe that the bromelain didn't interfered to G' and G" values of hydrogel, being that, the material elastic behavior was maintained when submitted do stress e/or frequency range. For materials such hydrogels, the determination of mechanical properties is extremely important, since these formulations, for topical use, undergo physiological conditions.

In that matter, bromelain can alter the hydrogel's behavior turning it more fluid at 37ºC. However, in the amplitude test, the temperature didn't change the behavior of the samples with and without bromelain. There was no crossover in any of the situations.

3.7. Mucoadhesion

Mucoadhesion is a property related to the ability of a material, biological or synthetic, to adhere a biologic tissue during a period of time. Such aspect is considered important for gels specially, in order to guarantee its retention, spread and bioavailability (187).

In our experiments, we tested the mucoadhesive properties of the developed hydrogels, both pure and loaded with bromelain, at 25ºC and 37ºC. It was possible to observe that the ability of the hydrogel to adhere the mucin discs decreases when the temperature applied in the samples from 25ºC to 37ºC. This is mainly due to the fact the higher temperatures cause the raise of hydrophobic bonds. According to Karolewicz, 2016 in mucoadhesion tests, hydrogels interact to the tissue applied by hydrogen bonds, which are facilitated by the presence of hydrophilic groups as majority in the polymeric chains. (188)

As for the hydrogels loaded with bromelain, though the values of mucoadhesion registrated were much lower, from 25ºC to 37ºC there was an increase of this property. Since bromelain solution was mainly aqueous, that contributed to the increase the presence of hydrophilic bonds in the hydrogel. In that sense, hydrogen the formation of hydrogen bonds was facilitated. For drug delivery systems this occurrence is important since guarantee the permanence of the hydrogel in the skin for the determined times and according to necessity (189).

Table 3. Mucoadhesive properties of NIPAAm-co-AAm hydrogels pure and loaded with bromelain (n=3).

Hydrogel	**Temperature**	**M-Fmax (N)**
Pure	25ºC	0,533 ± 0,125
Loaded with bromelain	25ºC	0,50 ± 0,191
Pure	37ºC	0,335 ± 0,256
Loaded with bromelain	37ºC	0,87 ± 0,13

M – Mucoadhesion

3.8 Drilling and resilience

Mechanical resistance to drilling corresponds to the force required to break the interlocking of the polymer chains from the hydrogel. Resilience is the elastic property of the hydrogel and is related to the structural mobility of the polymer network.

Table 4 contains the results of the drilling and resilience tests performed.

Table 4. Mechanical properties of NIPPAm-co-AAm hydrogels pure and loaded with bromelain (n=3).

Hydrogel	**D-Fmax (N)**	**R-Fmax (N)**
Pure	-	0,815 ± 0,07
Loaded with bromelain	1,304 ± 0,03	0,851 ± 0,05

D - Drilling, R- Resilience

In the absence of bromelain, it was not possible to measure the mechanical resistance of the hydrogel to the perforation because the probe instead of drilling the hydrogel caused rupture of the membrane. While in the presence of bromelain, the mechanical resistance to drilling was equivalent to 1,304 ± 0,03. This result suggests that bromelain increases crosslinking of the polymers, but does not significantly interfere in the elastic property of the hydrogel. The behavior presented by the hydrogels loaded with are characteristic of materials with lower structural mobility.

4. Cell viability (Mitochondrial activity assay - MTS)

The results presented by the NPA- (hydrogel without bromelain) and NPA+ (hydrogel loaded with bromelain) membrane exposure accused significant death of cells after 24 hours. The percentage of death was higher after 48h, reaching 50% (**fig 10**).

However, a previous study has shown that the cell viability of PNIPAAm could reach 88%. The combination with arginine was responsible for enhancing the cell viability of the hydrogels synthetized. Such combination was able to promote biocompatibility, make it more suitable for wound dressing, for example (190).

As for our object of study, it is possible that AAm might had being the responsible for turning PNIPAAm more toxic. However, in research performed by Cooperstein and Canavan, 2013 (191) PNIPAAm showed viability of < 70% after 24h and 48h. After 96h the cell viability was registered. at 90%. It appeared that the cells had not fully attached to the PNIPAAm coating before that time. The uneven coverage on the surfaces, and the possibility of the presence of traces of other materials on the surface (such as ethanol used in the polymerization process) seemed to no promote cell adhesion. After 96h cells had enough time to attach the surface and divided, which resulted in higher values of viability.

In that sense, our results point to need to perform more tests in order to confirm the possibilities for the developed hydrogel.

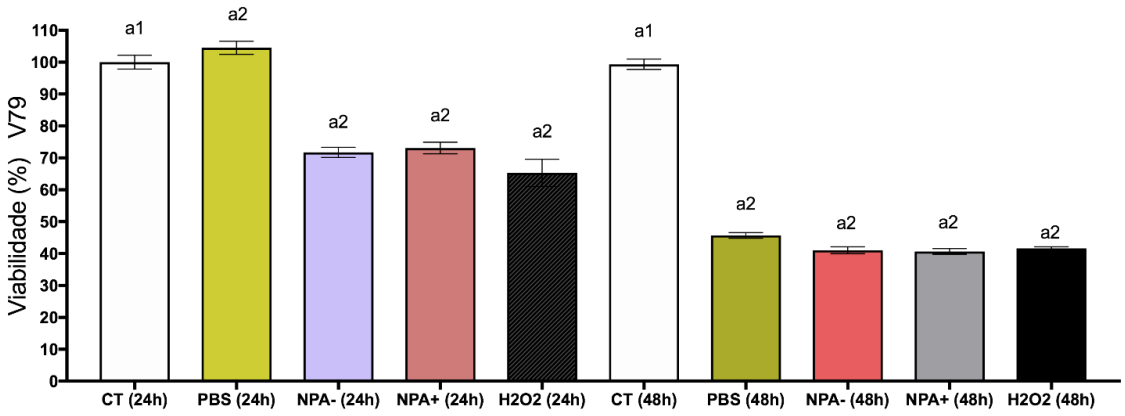

Fig 10. MTS assay results after 24h and 48h (NPA- and NPA+)

5. Conclusions

Hydrogels are important in pharmaceutical and biomedical applications. Many approaches have been done and as result of it, new products may be released soon, in order to improve diseases outcomes and give patients better alternatives. In that sense, the characterization performed in these tests highlight the possibility to create a new system evolving hydrogels and bromelain for wound healing.

6. Acknowledgements

The authors would like to acknowledge FAPESP and CAPES for their support.

7. Conflict of Interest

The authors declare no commercial or financial conflicts of interest.

DISCUSSÃO GERAL

A polimerização do NIPAAm com a AAm na presença de persulfato de amônio e TEMED são escolhas estabelecidas para a formulação deste tipo de hidrogel (192). BIS geralmente é utilizada na reticulação do polímero por sua semelhança diante da acrilamida e comportamento já conhecido em géis de poliacrilamida para eletroforese (192). Neste sentido, o hidrogel desenvolvido apresentou características esperadas ´para tal formulação, de acordo com o que consta na literatura e fora também destacado no artigo de revisão que constitui o capítulo inicial desta dissertação.

Para a seleção do melhor pH de atuação do hidrogel desenvolvido, a viscosidade do NIPPAm fora determinante (166), assim como o comportamento da bromelina, que exibe melhor atividade em pHs entre 6,5 e 7,5 (193). Para melhor associação do hidrogel e da bromelina o pH 7,0 foi efetivo para o favorecimento da formação da cadeia polimérica assim como para a ação da bromelina.

Neste sentido, foi importante medir a concentração de proteínas e atividade enzimática das soluções de bromelina utilizadas na formação do sistema de liberação. Com a possibilidade de uma aplicação tópica do hidrogel estudado a manutenção das características da bromelina são cruciais. Sendo assim, mantê-la estável e atuante se faz imprescindível.

Desde modo, a adição de PEG (400) e glicose (194) à solução de bromelina contribuiu consideravelmente para manutenção das propriedades da bromelina diante da incorporação e liberação. Fator comprovado pela pequena perda de concentração proteica e atividade enzimática após o teste de liberação, sob temperaturas possivelmente desnaturantes.

As micrografias mostraram o comportamento esperado para o hidrogel, visto que o mesmo foi capaz de reter a bromelina em sua rede polimérica, como mostras as imagens. A organização física do hidrogel observada nas micrografias evidencia como o polímero é estruturado, ao se observar espaços onde o hidrogel retém a água, e neste caso, a bromelina.

De acordo com Ribeiro, 2004 (205), as propriedades elásticas e viscosas de um material determinam se o mesmo tem a capacidade de recuperar sua elasticidade após aplicação de uma tensão e quando a mesma nessa. No teste de tensão foi possível observar que o hidrogel não sofreu deformação dentro de uma faixa de linearidade. Este teste é importante para obter informações sobre forças intermoleculares e interpartículas do material testado (206).

Neste sentido, os hidrogéis testados apresentaram comportamento elástico (175), o que é favorável para aplicação tópica. Para o hidrogel especificamente, esse comportamento

promovera a ação da bromelina na pele. Visto que a bromelina não interferiu no comportamento do hidrogel quando submetido a estresse sua associação a este tipo de material considera a possibilidade da manutenção das características do hidrogel.

Para materiais como os hidrogéis, a determinação das propriedades mecânicas é extremamente importante, uma vez que essas formulações, para uso tópico, sofrem condições fisiológicas. Nesse sentido, o hidrogel passou por análise de reologia também a 37ºC para avaliar seu possível comportamento em condições fisiológicas.

O hidrogel continuou a exibir comportamento elástico maior do que viscoso, condição era esperada para este material, uma vez que os hidrogéis possuem propriedades viscoelásticas, mas mais elásticas do que viscosas. A bromelina, no entanto, pode alterar o comportamento do hidrogel tornando-o mais fluido a 37ºC, o que não altera a possibilidade de possível aplicação tópica.

A mucoadesão foi testada para avaliar a capacidade do hidrogel em aderir um tecido biológico durante um período de tempo. Este ensaio se prova necessário visto que se objetiva uma aplicação tópica ao final do desenvolvimento completo da formulação. No caso deste hidrogel, quanto carregado com bromelina, tem sua capacidade de mucoadesão aumentada a 37ºC.

Isso pode ser explicado pela característica termossensível do polímero, pois, enquanto colapsa e expulsa a bromelina da sua cadeia polimérica, o hidrogel fica mais em contato com o tecido. Na ausência de bromelina, não foi possível medir a resistência mecânica do hidrogel à perfuração, porque o mesmo rompeu-se frequentemente nos testes. Enquanto na presença de bromelina, os resultados sugerem a mesma aumenta a reticulação dos polímeros, mas não interfere significativamente na propriedade elástica do hidrogel.

Os resultados demonstraram então, que o hidrogel desenvolvido possui composição e propriedades adequadas para polimerização satisfatória. A termossensibilidade e a grande absorção de água esperadas pelo PNIPAAm foram observadas, evidenciando a aplicabilidade desse polímero diante do objetivo proposto. Além disso, o hidrogel mostrou-se capaz de reter a solução de bromelina e libera-la quase em sua totalidade, quando necessário, e diante de temperatura próxima à do corpo humano. Sendo assim, um sistema de liberação controlada entre o hidrogel de PNIPPAm e bromelina pode indicar uma nova aplicação terapêutica para a mesma.

CONCLUSÃO

Dado o exposto neste trabalho, é relevante apontar para a possibilidade da formação de um sistema de liberação controlada de bromelina. As condições estudadas afunilam para apenas este conjunto de enzimas, mas abrem vértices para a utilização de outros ativos de ação biológica. Neste sentido, a utilização de hidrogéis é promissora, como a literatura exibe há décadas, e para esta aplicação pelo menos podemos apontar para uma possível aplicabilidade tópica, como na cicatrização de feridas, por exemplo.

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APÊNDICE

Bromelina

Bromelina é o nome coletivo de enzimas proteolíticas ou proteases encontradas nos tecidos de plantas da família *Bromeliaceae* e cuja fonte melhor conhecida é o abacaxi (*Ananas comosus*). Essa complexa mistura de enzimas é constituída de diferentes tiol-endopeptidases e outros compostos ainda não caracterizados, como fosfatases, glicosidases, peroxidases, celulases, glicoproteínas, carboidratos e cálcio.

A bromelina foi descoberta em 1957, e desde então é muito estudada, mas muitos autores se referem à uma só bromelina, quando de fato, existem preparações derivadas do talo do abacaxi e também da fruta. A bromelina do talo é a mais pesquisada e é uma cisteína protease, enquanto que a da fruta é uma glicoprotease. A bromelina do talo e da fruta possuem diferentes atividades proteolíticas e características distintas, como a massa molecular, que é de 38kD e 20kD, respectivamente. O ponto isoelétrico também difere, sendo 10 para a bromelina do talo e 4,6 para a da fruta A temperatura ideal de ação da bromelina está na faixa de 37ºC e o pH entre 6,5 e 7,5 A bromelina possuir caráter hidrofílico e sua atividade proteolítica pode ser determinada com diferentes substratos, como caseína, hemoglobina e gelatina

Proteases são uma classe de enzimas hidrolíticas capazes de clivar ligações peptídicas de cadeias proteicas e são essenciais à processos fisiológicos Nas plantas, as proteases estão envolvidas no desenvolvimento, degradação de proteínas danificadas e resposta à estresses bióticos e abióticos, mas sua aplicabilidade em diferentes atividades já foi demonstrada

Na indústria, a bromelina é utilizada em alimentos, principalmente no amaciamento de carne sendo também aplicada como agente purificante em cosméticos na indústria têxtil e na formulação de detergentes. Em aplicações médicas é utilizada no tratamento do câncer de mama e de ovário, em que está envolvida na diminuição de massas cancerosas, decréscimo da metástase aumento da atividade neutrofílica e aumento da nocitoxicidade Em feridas causadas por queimaduras, a bromelina é capaz de hidrolisar o tecido desvitalizado, *in vivo* e *in vitro*, sem prejuízos ao tecido normal, aumentando a capacidade de cicatrização. A cicatrização de tecidos envolve ainda processos inflamatórios e de oxigenação. A melhora da oxigenação do tecido em feridas de queimadura, é uma chave para total cicatrização, e como aprimorar essa oxigenação a partir do uso da bromelina ainda deve ser melhor elucidado A bromelina possui

ação comprovada em processos antiinflamatórios, na inibição de agregação plaquetária, na atividade fibrinolítica, na modulação de citocinas e na assistência à digestão.

ANEXOS

Anais em congressos

WCCE10
ECCE11
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Barcelona, Spain
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POSTER PRESENTATION CERTIFICATE

This is to certify that

Priscila Gava Mazzola

has presented the poster:

NIPAAm HYDROGEL AS CARRIER OF BROMELAIN FOR PHARMACEUTICAL USE

Fernanda Croisfelt (State University of Campinas, Brazil) / Elias Basile Tambourgi (State University of Campinas, Brazil) / Edgar Silveira (Federal University of Uberlândia, Brazil) / Priscila Gava Mazzola (State University of Campinas, Brazil)

during the 10th World Congress of Chemical Engineering held in Barcelona (Spain) from 1th to 5th October, 2017.

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Scientific Steering Committee Secretary

Technical Secretariat GRUPO PACIFICO. Maria Cubi, 4 - Pral. - 08006 Barcelona (SPAIN)
Phone: +34 932.388.777 / wcce10@wcce10.org

ABSTRACTS BOOK

Pharmceutical sciences in an emerging economy: Challenges for a sustainable world

[1551] MORPHOLOGICAL CHARACTERIZATION OF HYDROGEL FOR CONTROLLED RELEASE OF BIOACTIVE

Croisfelt, F. [1] , Tambourgi, EB. [2] , Silveira, E. [3] , PG, Mazzola. [4]

[1] Biology Institute, State University of Campinas, Campinas, SP, Brazil , [2] Faculty of Chemical Engineering, State University of Campinas, Campinas, SP, Brazil , [3] Institute of Genetics and Biochemistry, Federal University of Uberlandia, Uberlandia, MG, Brazil , [4] Faculty of Pharmaceutical Sciences, State University of Campinas, Campinas, SP, Brazil

ABSTRACT

INTRODUCTION: Hydrogels are polymers that present the ability to absorb large amounts of water, without dissolving in it. Due to this capacity, hydrogels have similarities to living tissues. They have been extensively described in medical and pharmaceutical applications. As carriers of bioactive products, hydrogels are good candidates since they can release it under controlled environment conditions, such as temperature and pH. Bromelain denominates the set of proteolytic enzymes found in tissues of plants from the Bromeliaceae family, especially in pineapples. Bromelain's therapeutic applications have been studied mainly due its anti-inflammatory and healing properties. OBJECTIVE: Morphologically evaluate bromelain-containing hydrogel by scanning electron microscopy (SEM). METHODOLOGY: Hydrogels were prepared by the co-polymerization between NIPAAm and Bis-acrylamide. Finished the polymerization, the hydrogel samples were hydrated for 24h and then dried for 8h. After that, one sample was immersed in water and another was immersed in bromelain solution. The samples stayed immersed for 24h and then were frozen at -180°C and lyophilized. Following that, samples were attached to stubs and metallized with a gold-palladium alloy. Interferents were removed by argon, followed by vacuum. The samples were observed and digitally photographed by SEM. RESULTS: Photographs of both samples, with and without bromelain showed good visibility and clarity. It was possible to see how the network was connected. Such result exhibited the main characteristic of hydrogels, which is its ability to form polymeric chains that are able to shelter substances, such as bromelain. It was very clear that the bromelain was trapped in the hydrogel, and may have had interacted with the polymer. Therefore, hydrogels were able to hold bromelain, keeping it prompt to release. CONCLUSION: Towards what as shown, and the reason why the combination of hydrogel and bromelain was made, such system can be applied for controlled release, since they behaved as expected.

KEYWORDS

Hydrogel, bromelain, controlled release

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Fernanda M. Croisfelt

Assinatura do Aluno:
Fernanda Machado Croisfelt

Profa. Dra. Priscila Gava Mazzola
Matrícula 293994
Faculdade de Ciências Farmacêuticas
UNICAMP

Assinatura do Orientador:
Professora Doutora Priscila Gava Mazzola

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Assinatura: Fernanda M. Croisfelt

Nome do(a) autor(a): **Fernanda Machado Croisfelt**

RG n°. 485253884 SSP/SP

Profa. Dra. Priscila G. Mazzola
CRF 33311 - Matr. 293594
Faculdade de Ciências Farmacêuticas
UNICAMP

Assinatura:

Nome do(a) autor(a): **Priscila Gava Mazzola**

RG n.° 306978775 SSP/SP