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RECENT ADVANCES IN THE USE OF POLYMERICS AS TOPICAL PROTEIN ANTAGONISTS

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ABSTRACT

Proteins are associated in some way with most if not all human diseases. They are either endogenous (enzymes, proteases, hormones…) or exogenous (bacteria, viruses, fungi ...) but they play a crucial role in almost all physiological functions in the body as well as in multiple pathologies. Modulating proteins therefore represents a major therapeutic approach. Due to unique physico-chemical properties, polymers have the specificity to bind with macromolecules, particularly with proteins without presenting undesirable effects, as polymers are inert, nearly nontoxic and usually non- irritant. In spite of these unique properties of polymers, still the research on polymeric drugs is in the enfant stage. This is largely related to the fact that multiple proteins are involved in the physio-pathology of a disease, which requires using multiple polymers to block only the pathological proteins, and also to the difficulties to patent and to register a combination drug. The inability of polymers to cross the gastro-intestinal barrier equally constitutes a huge obstacle. In the past 10-15 years, more and more pharmaceutical industries have been diverting their traditional research towards polymeric molecules as it is getting practically impossible to find new drugs based on chemical entities. The recent development of highly effective topical antivirals, chronic wound healing, and anti-cytokine drugs by the Vitrobio research institute in France suddenly elicited a tremendous interest in the use of polymers as the future drugs of the 21st century. This review summarizes the pros and cons of employing polymers as pure drugs and analyzes the results of new topical polymeric drugs.

KEYWORDS: polymers, protein, inhibitors, antivirals, topical.

INTRODUCTION

Human diseases are linked to many external and inner factors. Whereas foreign bodies or external invaders such as viruses and microorganisms cause various pathologies, diseases can also be induced by specific components of our body, particularly through the dysregulation of endogenous structural or functional proteins. Proteins are thus directly or indirectly involved in most diseases, and a few examples of some widespread human diseases with pathology involving endogenous or exogenous proteins are described here.

Diseases involving endogenous proteins

Enzymes: These are proteinous structures, composed of a chain of amino acids displaying catalytic activity to accelerate chemical reactions. Almost all intracellular metabolic processes need enzymes in order to occur at rates fast enough to sustain life. Like any catalyst, enzymes are not consumed in the chemical reaction, nor do they alter the equilibrium of a reaction. Enzymes differ from most other catalysts, however, by being much more specific. Other molecules can affect their activity: inhibitors (including many drugs and poisons) decrease enzyme activity whereas activators increase their activity.

Enzymes are mainly globular proteins, acting alone or in larger complexes, generally much larger than their substrates. In general, enzymes do not need additional components for their activity but some may still need non-protein molecules called cofactors to exert their activity. They may also be linked to coenzymes, which are small organic molecules, loosely or tightly bound to an enzyme. Coenzymes transport chemical groups from one enzyme to another.

Enzymes are involved in many biological processes in the body such as cellular signal transduction,[1] energy production, cellular organization, virus growth; consequently, enzymatic dysregulation can generate many different types of diseases. As enzymes act synergistically, their activity is essential for homeostasis and functioning of all body cells, and overexpression or underexpression [2] of an enzyme may lead to a pathological condition. Being very large molecules, it's nearly impossible for a standard chemical entity to block the whole enzymatic activity. Therefore, among currently available enzyme inhibiting or modulating drugs, only a few have moderate pharmacological activity and often the benefit-risk ratio of such drugs is too low.

Hormones: Hormones are derivatives of proteins, amino acids, or cholesterol, synthesized and secreted by the endocrine glands, such as the thymus, which plays an important role in

the maturation of the immune system, allows proper metabolism, use and storage of carbohydrates and fatty acids, and promotes cardiovascular properties. Consequently, hormonal dysregulations have **a** major impact on the body and can cause severe malfunctions leading to various diseases. An example is acromegaly, a condition caused by an excessive production of growth hormone by the pituitary gland, often induced by pituitary adenoma, a benign tumor, [3] and necessitating surgical treatment.

Cellular receptors: Receptors on the cell surface and their ligands constitute the basis of cellular communication. They convey various messages necessary for the functioning and survival of all human body components (organs, dermis, bone structure ...). There are three super-families of receptors: G protein coupled receptors (GPCR), enzyme-associated receptors, and ion-coupled receptors. Many diseases are linked to a modification of genes encoding the GPCRs. [4] As receptors are implicated in cellular functions and normal cellular functions are a key pre-requisite for healthy body functions, receptor dysregulation is responsible for numerous pathologies. Receptors are small and many chemical drugs are designed either to antagonize or to stimulate receptor activity.

Protein-linked topical skin and mucosa diseases: **Skin lesions**

Proteolytic enzymes such as Matrix MetalloProteinases (MMPs) play a fundamental role in the delayed healing of chronic wounds. [5] MMPs comprise a family of over 20 structurally related proteins (endopeptidases) taking a major part in tissue remodeling, cell migration, elimination of excess extra cellular matrix (ECM), and cleavage of unwanted proteins present on the surface of the wound to clean the injured surface and create a favorable ground for healing.[6,7] MMPs process enzymatically various types of proteins, including proteases, cytokines, chemokines, growth factors, adhesion molecules, but they also proteolyse matrix proteins composing the ECM support structure essential for cellular growth. Although MMP concentration is regulated through tissue inhibitors of metalloproteinases (TIMPs), in chronic wounds the balance between MMPs and TIMPs shifts in favor of MMPs, [8,9] resulting in excessive degradation of the ECM scaffolding, and stalling of the healing process. [10]

Diseases involving exogenous proteins

Many exogenous proteins, from bacteria, viruses, fungi, or from complex organisms (plants and animals), can also induce disease in our body. While pathogens are a minority in the 6 kingdoms of living beings, viruses and bacteria stand out as the main culprits, and the main exogenous protein-linked diseases are viral and bacterial infections.

Viral infections: The origin of pathogenicity and virulence of viruses resides in their surface proteins (glycoproteins on enveloped viruses and capsid proteins on naked viruses) [11-13] and proteins (nucleoproteins) enclosed within the virus. Some viruses possess enzymes (polymerase, transcriptase, integrase, and protease) that allow them to parasitize host cells. For instance, many viruses such as influenza, herpes, and HIV, present multiple mutant proteins on their capsid, which enable virus–cell attachment, virus penetration and infection.

Bacterial infections: Pathogenicity factors for bacteria are similar, and are mostly produced by the bacterium's own proteins. Thus, pili, fibria, and adhesins present on the bacterium's surface [14] will aid the reversible and fairly specific adhesion to its target cell. They allow the bacterium to either form irreversible links, as in biofilms or aggregate formations whose matrix is composed primarily of polymers and proteins, [15] or to cross the host cell membrane and develop intracellularly. Furthermore, some intracellular bacterial pathogens possess systems that directly or indirectly employ proteins to hijack the cell's machinery for their benefit, as in effector systems secretion. Another pathogenicity factor for bacteria resides in their producing and releasing toxins, most of which are of protein origin (eg, botulinum toxin, diphtheria, tetanus).

Ingested proteins: Some diseases can also be connected to proteins ingested as food or as drugs. Normally not pathogenic because most are digested in the gastrointestinal tract, in some rare cases, proteins or fractions thereof may enter systemic circulation and induce some pathological conditions.

It is thus evidenced that most human diseases are associated with proteins. Seemingly, curing a disease should therefore be as simple as inhibiting a pivotal protein implicated in the pathology or present on the surface of the infective organism to stop the pathological process.

Why it is nonetheless difficult to develop anti-protein drugs

The main reason is that, most often, multiple proteins are involved in the pathogenesis of a disease. In case of chronic wounds, for instance, over 25 MMPs are implicated in proteolytic activity. Some of these MMPs are essential for cellular modeling and cellular organization but a few others are involved in the destruction of the ECM proteins, in the absence of which the cells cannot attach and grow, and the wound cannot heal. [16] A second important factor is the location of these protein molecules. The proteins involved in a disease can be located either inside the body, circumscribed or in circulation, or on the body's surface.

Therefore, requirements for designing a topical protein antagonist cannot be the same as those for an orally or systematically administered protein antagonist. To treat topical protein-involving diseases such as viral or bacterial infections, where the protein molecules are an integral part of the pathogen, or wounds, whose surface contain many MMPs, or psoriasis, eczema or dermatitis-affected skin lesions, which contain cytokines, the chosen antagonist can be a big molecule, not needing to be metabolized: being present on the body surface, it can indeed be easily eliminated without going through the metabolic cycle. Additionally, such molecules should have no irritation or allergization potential, nor any interactions with the underlying cellular structures, to avoid any subsequent pharmacological, immunological or metabolic activity and possible side effects.

In contrast, administering a protein antagonist orally or systemically will require considerable precaution depending upon the type of disease to be treated and the exact location of the proteins to be blocked.[17] An ideal antagonist should be able to cross the intestinal barrier, withstand circulating within the body without damage, reach the destination organ and the target protein, be highly specific so as to only block the target protein without affecting other proteins' functions, be non-toxic and easily metabolized or excreted from the body, and require less frequent administrations. Finding such a drug seems a difficult task as polymers are generally large molecules, unable to cross the intestinal barrier. Moreover, their rigid structure may prevent their being metabolized after systemic administration. The best solution would therefore consist in employing either biodegradable polymers or polymers which can be excreted through the hepatobiliary route into the intestine.[18,19] Thus, current research is directed mostly towards either finding small, highly protein-specific polymers which are easily eliminated from the body in the form of protein-polymer complexes, or searching topically acting protein antagonists which do not involve systemic metabolic process. A few examples of such topically acting new protein antagonist treatments are given in the following sections.

Polymers: The best approach to antagonize proteins

It is well known that polymers, whether synthetic or natural, have strong affinity for proteins and various other macromolecules. Of particular interest is the property of polymers to form conjugates with proteins. Polymers bind to proteins with hydrogen bonds between carboxylic and carboxylate groups, through hydrophobic interactions, hydrogen interactions and sometimes sulfur bonds. [20] The specific link between a protein and a polymer is hardly

understood, but has demonstrated some selectivity, as illustrated traditionally by the tannery of leather through the binding of skin proteins by some plant tannins, or the almost specific interactions of Human IgG proteins with galactomannan polymers for instance. The capacity of some polymers to change their structure depending on their environment, reacting to pH, light, temperature, concentration and other specificities, allows them to be active at a specific place and time: those are called "smart polymers" or "stimuli responsive macromolecules", and are increasingly used in medicine, as implants (stents, prostheses), as sequestrants, as polymeric drugs (for example, synthetic plasma expanders such as poly(vinylpyrolidone) or as polymer-drug conjugates [21-23] for drug delivery, such as glucose threshold-sensitive insulin-releasing polymers). Hence, as multiple proteins are involved in various diseases, and since neutralizing specific proteins may prove useful as preventive or curative treatment for those diseases, polymers, because of their strong affinity for proteins, can be put to valuable use in a completely new therapeutic approach to treat numerous conditions.

Omnipresent polymers can be made up of virtually any molecule, come in any shape or form, and offer limitless interaction possibilities: combined with existent drugs for improved activity; designed for finely-controlled drug delivery; or customized to chase down viral proteins or bacteria and get rid of them, to name but a few examples.

Using polymers does, however, present some difficulties, mainly in terms of specificity - as target proteins may be present in different locations or on different types of micro-organisms or cells, or biocompatibility - as polymers are larger molecules than traditional drugs. Since some polymers' physical characteristics can be modified by external stimuli, such as changing states under photostimulation,[24] they could offer an interesting array of biocompatible materials for topical uses, for example: forming a protective dressing over a lesion. Polymers could thus be used to design a non-occlusive, foam, gel, or film dressing, which would allow gas exchanges for proper oxygenation of the wound, while also providing adequate hydration and fluid management to avoid either drying or maceration of tissues, and finally be capable of inhibiting pathogens such as bacteria, or regulating other particles interfering with wound healing, such as MMPs, ultimately eliminating those contaminants from the lesion's surface.

Topical possibilities of polymers seem almost endless, but polymeric structures being highly variable and big in size, they cannot cross the intestinal barrier, which limits their use as systemic drugs to a large extent. Polymers have thus been developed as biomaterials,

components in artificial organs, tissue engineering, medical devices, and dentistry. Functional polymers are also being used as delivery systems as low-toxicity, high specificity, vehicles carrying drugs to targeted sites. Some polymer-drug conjugates have already been clinically approved, notably for the controlled delivery of chemotherapeutic drugs in cancer therapy.

Notwithstanding, they have not been generally thought of as useful therapeutic agents on their own, and examples of polymers as active pharmaceutical ingredients are relatively scant. Yet, however limited their pharmacological activity may be, because of their unique physicochemical properties, they could theoretically offer some therapeutic advantages over traditional chemical molecule drugs, especially due to their lower toxicity, optimized delivery, and more defined and more specific mechanism of action. [25] Additionally, the high molecular weight characteristic of polymers offers some important benefits, including polyvalency, which allows for enhanced activity due to multiple interactions with disease targets. Among early examples, topical anticoagulant and antitumor agent Poly(ethylenesulfonate) and maleic anhydride-divinyl ether copolymer have shown some activity against various tumor cell lines,[26] through stimulation of T-cell activity and modulation of the immune system. The results, albeit modest, have set polymers forth as promising future therapeutic agents for serious diseases, including cancer. [27] But despite their promising potential, the concept of polymeric drugs remains a subject of considerable skepticism among drug discovery and development scientists and still largely remains to be explored. The systemic non-absorption of middle & high molecular weight polymers taken through oral route constitutes the biggest hurdle for the use of polymers as drugs. While awaiting new methods allowing their safe systemic circulation, they can, however, be used for molecular sequestration in the gastro-intestinal tract, or for topical application as antagonists.

Polymers for Molecular Sequestration

Many noxious substances, either coming from our food or our environment, or endogenously metabolized, circulate in the GI tract and are implicated in various conditions. Polymers that are not absorbable through the intestinal wall can thus be used to selectively sequester those substances and remove them from the GI to stop their deleterious effect on health. Customizing the physicochemical characteristics of polymers allows controlling their functions and fine-tuning their selectivity, opening a large scope of possibilities. Thus, a sodium polystyrene sulfonate polymer, Kayexalate, has now been used for decades to

sequester excess potassium ions in the GI tract to treat hyperkalemia. [28] Polymers may also bind with phosphate ions in the GI tract to treat conditions like hyperphosphatemia. Polymeric guanidinium salts bind phosphate selectively in the presence of other competing, biologically important, anions such as chloride, bicarbonate, etc. through electrostatic and possibly through hydrogen bonding interactions. After successful clinical trials, a phosphate sequestering polymer was launched in the US in 1998 under the generic name of sevelamer hydrochloride by Genzyme Corporation. The ability for improved control of serum phosphate without increasing the exposure to toxic metal ions like aluminum and eliminating the intake of additional calcium offers a number of clinically relevant benefits. Polymeric preparations have also been used to capture iron with cross-linked polymeric hydrogels containing hydroxamic acid and catechol moieties; similarly, cationic polymers cholestyramine and colestipol have also been used as bile acid sequestrants to capture low-density lipoprotein cholesterol, but showed poor efficacy. [29,30]

Topically applicable polymer drugs

Viral infection is initiated by the attachment of viruses to specific cellular receptors. This virus-cell receptor attachment process is mediated by viral attachment proteins (VAPs). This suggests that presenting polymeric ligands bearing multiple copies to bind with virus glycoproteins represents an excellent means of blocking virus entry into the cells and stopping the infection. This technique of polymer – virus glycoprotein (Gp) sequestration can only be used to treat topical viral diseases where Gps are present on the virus surface, as in influenza and herpes viruses. Influenza virus attaches to cell membranes through a process known as hemagglutination.[31] The hemagglutination process is a multivalent interaction between trimers of hemagglutinin (a carbohydrate-binding protein present on the viral surface) and multiple sialic acid groups present on the surface of the mammalian epithelial cell. These sialic acid residues are parts of cell-surface glycoproteins. Therefore, one strategy to treat influenza virus infection is to prevent the virus from binding to cells by presenting polymers bearing several sialic acid groups as competitors of cell surface ligands. As most viruses are cationic, anionic polymers should prove useful to capture them. [32] However, initial results with anionic polymers such as the poly(styrene-4-sulfonate), 2-naphthalenesulfonate-formaldehyde polymer, and acrylic acid-based polymers, were not encouraging. This may be due to the complexity and variability of Gp structures, requiring extensive testing to select the adequate polymer. Certain chemically modified natural polymers (i.e. semisynthetic) such as dextrin/dextran sulfates, cellulose sulfate, carrageenan

sulfate, and cellulose acetate phthalate, have also been investigated for this purpose. Of a number of such anionic polymers that have shown *in vitro* and *in vivo* anti-HIV activity, a couple of polymeric drug candidates have proceeded to early stage human clinical trials for safety/tolerability evaluation, but further clinical trials evaluating efficacy were not very encouraging. [33]

Polyvalent Antimicrobial Agents

The emergence of multidrug-resistant microbial pathogens, bacteria in particular, ubiquitous in both hospital and community settings, is a major public health concern worldwide. Most of these strains show multiple drug resistance factors. Using polyvalent ligands as antibacterial agents presents several potential advantages over monomeric antimicrobial agents, as polyvalence allows multiplication and potentization of weak non-covalent bonding interactions between bacterial surface receptors and the polymeric ligands. Polyvalent ligands also have the potential for aggregating and precipitating bacteria. [34,35] As with viruses, most bacterial infections are initiated by the adhesion of the microorganisms to host mucosa cell surfaces, partially mediated by bacterial protein adhesins. This suggests that appropriate polymers could competitively block microbial adhesion, and indeed some polymers have exhibited antimicrobial activity, particularly effective against *Cyptosporadium parvum* (*C. parvum*). [36,37]

Polymeric Drugs for the Treatment of Autoimmune Diseases

In simple terms, autoimmune diseases arise when the host immune system mistakenly attacks itself. Ordinarily the immune system uses a number of defense mechanisms to prevent the development of such aberrant autoimmune responses by directing defense T cells to distinguish foreign invaders. [38] Bypassing the protection against autoimmunity leads to inflammation in various parts of the human body. Inflammation may become chronic due to excessive production of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), etc. Although knowledge about the role of cytokines in these diseases has expanded over past years, no polymeric drugs have yet been developed to treat those pathologies, particularly because they would require systemic presence. Commonly used drugs are immunosuppressors and immunomodulators, with multiple side effects, such as mitoxantrone, recombinant interferon-β (e.g., Avonex®, Humira®), and, recently, glatiramer acetate. Glatiramer acetate (GA) is an amino acid-derived synthetic copolymer, developed by Weizman Institute of Science, acting as an alternative to interferon-β for the treatment of

certain forms of immunomodulation diseases, but this product works as an agonist and not as an antagonist.

Polymeric Fat Binder and Dual Acting Polymeric Lipase Inhibitor-Fat Binder

In the treatment against obesity, Orlistat and a number of other natural and synthetic inhibitors of human pancreatic and gastric lipases have been identified, some of which have progressed to clinical development [159]. They come, however, with seemingly unavoidable, mechanism-related side effects caused by the presence of non-hydrolyzed fat. However, a therapeutic intervention that could simultaneously inhibit fat hydrolysis and condense unhydrolyzed fat droplets into a less fluid form may provide a novel approach for lipase inhibitor therapy without the aforementioned side effects. Synthetic, non-absorbable, cationic functional polymers, bearing lipase-inhibiting groups and also possessing lipid-condensing properties, offer a therapeutic alternative to chemical lipase inhibitors, wherein the resulting macromolecular network may influence the state of the fat emulsion. For example, the polyelectrolyte surfactant network could provide a rigid or waxy matrix able to encapsulate or stabilize the oil droplets, preventing them from coalescing into a bulk oil phase. The polymeric fat binder could thus effectively minimize or eliminate the presence of fluid dietary fat in the lower intestine, and the resulting side effects. Although non-ionic polymers such as hydrophobically modified polyethylene glycol and polyethyleneglycol-polypropylene glycol block copolymers are known to exhibit emulsifying properties, they performed very poorly in animal models. Copolymers containing anionic and zwitterionic monomers were found to be similarly ineffective. On the other hand synthetic polycations (based on monomers containing amine and ammonium groups) exhibited considerable lipid-binding properties. Presently, several potent and non-toxic copolymer compositions are under development at Peptimmune, Inc., where they are currently undergoing human clinical trials.[39]

Currently marketed topical polymeric drugs

In spite of the known protein-binding properties of polymers, role of proteins in multiple topical and systemic diseases, and very low toxicity of polymers, the use of polymers as drugs still is a totally new field. As polymers cannot cross the intestinal barrier, their use to treat systemic diseases will need more research over the next decade, but a few new drugs to treat topical diseases (of the skin, ENT, vaginal cavity, GI tract) have already been recently launched with great success in Europe. A pharmaceutical research and development company

in France, Vitrobio, developed novel, topically acting, drugs containing polymers, which are already marketed in France, Spain, Poland, and gaining new markets in and outside Europe (Finland, Middle East, Egypt, Israel, and expanding). Those newly developed drugs are intended for the treatment of wounds and ulcers (including bedsores, diabetic ulcers, venous leg ulcers, and oral mucositis), topical viral infections (influenza-induced throat infection, rhino-sinusitis, labial and genital herpes), microbial infections such as bacterial vaginosis, or cytokine-linked diseases such as psoriasis, eczema, dermatitis, hemorrhoids, and allergic rhinitis. The strategy of Vitrobio was to identify the "proteins" involved in the origin or development of the disease, and to then employ one or more polymers to block these proteins. First, thousands of polymers were screened using appropriate *in vitro* or *in vivo* technology and were then associated with an osmotically active hypertonic filmogen solution for topical application. The hypertonic filmogen solution, derived from a glycerol base, (International patents: PCT/FR99/01340 & PCT/EP2013/061835) was used to incorporate the polymers as it is non-toxic to the underlying cellular structures, non-irritant, natural, and not liable to interact with the selected polymers. The therapeutic hypotheses, mode of action of the polymers, safety and clinical efficacy of the treatments are summarized here.

Wound healing polymeric treatments

In theory, wound healing should be extremely easy, as it simply requires the growth of deeper fibroblast and superficial epithelial cells to fill the wound cavity. In order to grow, new cells must attach onto a cushion serving as framework, called extra cellular matrix (ECM). ECM, whose composition varies according to cell type, is secreted by the specific mother cells and contains multiple proteins such as collagen, elastin, fibronectin, integrin, and laminin. [40,41] If the ECM is degraded or missing, cell attachment is prevented, and consequently cell growth and wound healing are halted. [42,43] Wounds may affect superficial skin structures only, or can expose the underlying muscles, ultimately even reaching bone tissue. Superficial skin is predominantly composed of epithelial cells, with subjacent muscular structures containing fibroblast cells. Healing deep wounds therefore requires the simultaneous growth of fibroblast and epithelial cells. [43,44] In spite of continuous research, there is currently no efficient cell growth-promoting treatment for chronic wounds as existing therapies only partially provide the conditions (hydration of surface tissues, reduction of contaminant load, antalgics) necessary for cell growth without directly promoting it. [45,46] All recent scientific works prove however that non-healing wounds critically lack ECM, and that its absence or degradation is directly linked to high concentrations of proteolytic enzymes called matrix

metalloproteinases (MMPs).[47] MMPs are proteins and comprise a family of over 20 structurally related proteins that are zinc-dependent and calcium-activated endopeptidases. Although the concentration of these MMPs is regulated through tissue inhibitors of metalloproteinases (TIMPs), in chronic wounds the balance between MMPs and TIMPs shifts in favor of MMPs,[48-51] resulting in degradation of ECM. [47,52] The excessively high concentrations of the proteolytic enzymes in chronic wounds (65-fold for MMP-1, threefold for MMP-2 proenzyme, six-fold for activated MMP-2, twofold for MMP-8, and 14-fold for MMP-9, compared to average concentrations found in biopsies of acute wounds), lead to the breakdown not only of unwanted proteins but also of ECM constituents, which ends up stalling the wound healing process totally.[52-54] Therefore, identifying and neutralizing ECM-degrading MMPs is now becoming a major research field worldwide. Except for some encouraging *in vitro* results, [55] which have led to developing collagen-based wound dressings aiming at broad spectrum MMP modulation, there isn't currently any efficacious treatment electively targeting ECM-damaging MMPs on the wound surface. [56] This is mostly related to the fact that: (1) the MMPs or the TIMPS participating in ECM destruction are not all identified yet; (2) MMPs are present on the surface of the wound where systemic treatments are not very effective; (3) some MMPs are essential to the healing process and should not be inhibited; (4) multiple MMPs may participate in ECM destruction and it is practically impossible to neutralize the totality of these MMPs with a single specific topical or systemically administered chemical drug. Vitrobio identified target MMPs and used natural plant or synthetic polymers, incorporated into the patented hypertonic filmogen solution which also contains honey to adjust viscosity, to neutralize these MMPs. *In vitro* pharmacological studies having shown that hardly 10% of natural polymers bind and neutralize over 50% of target MMPs, different polymers were therefore associated to block a group of ECM-destroying MMPs. Furthermore, it is noteworthy that the results of MMP neutralization by associations of polymers were neither strictly additive nor especially synergistic, but even slightly antagonistic in certain cases**,** indicating that a specific polymer association may be required to block one or a group of selected proteins and to cure the disease. The wound healing properties of MMP-inhibiting polymers in a solution containing glycerol & honey (AS-21) was evaluated clinically against a placebo solution containing only glycerol and honey, without polymers. 93 adult patients with one or multiple, lower extremity, deep wounds were divided randomly in two groups. 41 patients in the placebo and 52 in the active treatment groups, with respectively 49 and 69 wounds, mean surface area of 56.70 and 52.03 cm^2, and mean volume of 57.22 and 52.15 cm^3, were treated by applying the

test products topically for a period of 6 weeks. A statistically significant difference was observed between the placebo and the polymer-treated groups with respect to reduction in wound surface area (33.37% vs 97.87%) and wound volume (29.45% vs 94.17%) after 6 weeks of treatment (see fig. 1 and 2). Mean wound humidity and pain scores were also reduced, confirming that polymers are highly interesting for the treatment of chronic wounds.[57]

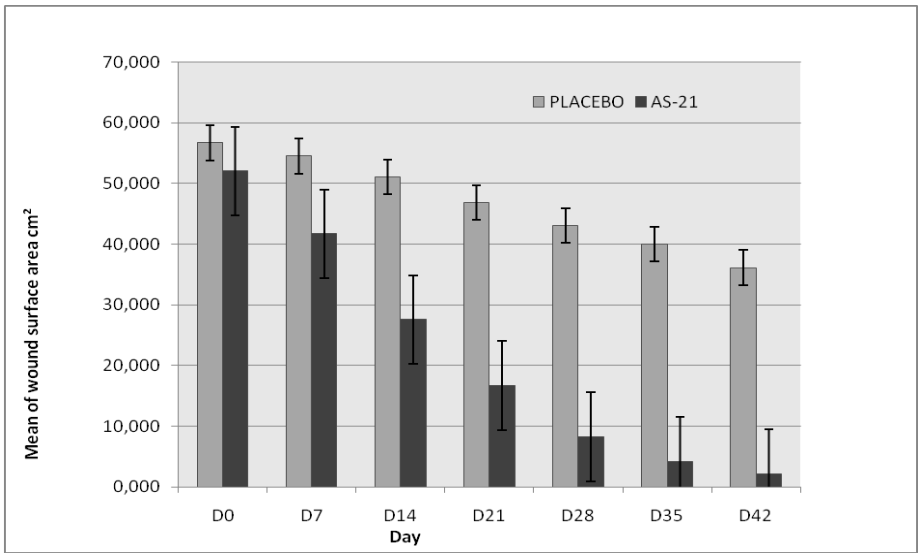

Fig. 1: Mean wound surface area (cm^2) in placebo (n=49 wounds) and in AS-21 treated (n=69 wounds) over 6-week treatment period.

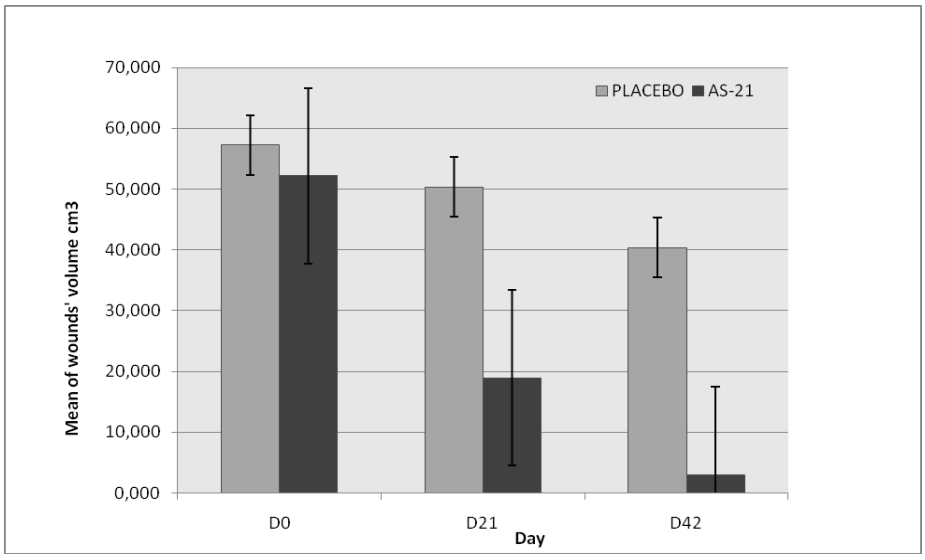

Fig. 2: Mean wound volume (cm^3) in placebo (n=49) and in AS-21 treated (n=69) groups over 6-week treatment period.

Although MMPs are implicated in ECM destruction in all sub-acute (>4-6 days) and chronic wounds, the type of MMPs and their concentration vary according to the location, etiology

and chronicity of the injury. Therefore, to verify the efficacy of polymeric drugs, the next clinical trial was conducted in patients suffering from cancer therapy-induced oral mucositis. Oral mucositis is one of the most debilitating side effects of radiation therapy and various forms of chemotherapy, particularly for head and neck cancers and hematopoietic stem cell transplants. The ulcers develop as a consequence of cytostatic effects of anticancer therapy on the fast growing oral mucosal cells. Because of the presence of some specific proteolytic MMP enzymes, known to destroy cellular matrix and inhibit cell regeneration and healing, the ulcers expand. The MMP-inhibiting plant polymers were incorporated in the filmogen glycerol solution, and the new polymeric drug's (OROSOL®) MMP neutralizing and ulcer healing properties were evaluated. The study was conducted on a group of 69 patients: 48 were treated with polymeric OROSOL® spray, 4-5 times per day for a period of 28 days, and 21 patients in the control group continued receiving other classical treatments. Overall mucositis grade, intensity of pain and burning sensation, formation of new ulcers and effect on eating impairment were evaluated before treatment, 30 minutes after first product application and on days 1, 2, 3, 4, 7, 14, 21 and 28, on a 0 to 10 scale. The results are summarized in Table 1. The overall mucositis grade started showing significantly higher amelioration in the Orosol® group compared to the group using other treatments from day 3 (26%), and this correlated with the initiation of ulcer healing which also significantly improved from day 3. However, some other individual clinical signs such as pain and burning sensation, as well as ulcer infection, began showing dramatic amelioration right after 1st application, which permitted to ease eating difficulty by day 2, and continued improving progressively throughout the study period. Orosol® group patients showed significantly higher improvement in pain, burning sensation, eating abilities, grade of infection and overall mucositis. New ulcer formation rate was not affected. Orosol® efficacy was attributed to the polymers binding the ECM-destroying MMPs, and thus restoring a favorable ground for cellular growth and ulcer healing. Although this novel approach to treating oral mucositis ulcers is extremely efficient to reduce pain, burning sensation, infection, and size of the ulcers, it has no effect on the development of new ulcers as long as the oral mucosa cells are exposed to cytostatic or anti-mitotic therapy. [58]

Table 1: Mean Mucositis Parameter Scores in Classical Treatments (CT) or Orosol® (O) Groups, % of Change Observed in each Group Compared to T0, and % Difference in Severity

Parameter	Before Tr = T0	20-30 min	Day 1	Day 2	Day 3	Day 4	Day 7	Day 14	Day 21	Day 28
Mean Mucositis Grade (0-4)										
	2.33	**2.33**	**2.19**	**2.19**	**2.33**	**2.62**	**2.52**	**2.24**	**2.14**	**2.10**
CT	*(±1.02)*	*(±1.02)*	*(±0.98)*	*(±0.98)*	*(±0.97)*	*(±0.87)*	*(±0.93)*	*(±1.00)*	*(±1.06)*	*(± 0.94)*
% Change/T0		0%	-6%	-6%	0%	+12%	+8%	-4%	-8%	-10%
Orosol®	**2.67**	**2.46**	**2.30**	**2.05**	**1.73**	**1.58**	**1.41**	**1.10**	**0.95**	**0.83**
% change/T0	*(±0.91)*	*(±0.85)*	*(±0.99)*	*(±1.00)*	*(±0.86)*	*(±0.86)*	*(±0.78)*	*(±0.73)*	*(±0.66)*	*(± 0.52)*
% Diff O/CT		-8%	-14%	-23%	-35%	-41%	-47%	-59%	-64%	-69%
	+14%	**+5%**	**+5%**	**-6%**	**-26%**	**-40%**	**-44%**	**-51%**	**-56%**	**-60%**
Pain Sensation (1-10)										
	7.14	**7.71**	**7.14**	**7.33**	**6.91**	**6.33**	***5.91***	**6.24**	**5.76**	**5.71** *(±*
CT	*(±1.98)*	*(±1.79)*	*(±1.82)*	*(±1.98)*	*(±2.00)*	*(±2.18)*	*(±1.84)*	*(±2.43)*	*(±2.39)*	*2.49)*
% Change/T0		+8%	0%	+3%	-3%	-11%	-17%	-13%	-19%	-20%
Orosol®	**7.19**	**4.71**	**4.27**	**3.67**	**3.08**	**2.65**	***2.56***	**2.41**	**2.01**	**2.05** *(±*
% Change/T0	*(±1.95)*	*(±1.86)*	*(±1.62)*	*(±1.39)*	*(±1.58)*	*(±1.44)*	*(±1.43)*	*(±1.53)*	*(±1.22)*	*1.39)*
% Diff O/CT		-34%	-41%	-49%	-57%	-63%	-64%	-66%	-72%	-71%
	+1%	**-39%**	**-40%**	**-50%**	**-55%**	**-58%**	**-57%**	**-61%**	**-65%**	**-64%**
Burning sensation (1-10)										
	6.81	**6.91**	**6.38**	**6.57**	**6.57**	**6.29**	**6.19**	**6.05**	**6.10**	**5.76**
CT	*(± 2.04)*	*(±2.10)*	*(±1.53)*	*(±1.54)*	*(±1.91)*	*(±1.52)*	*(±1.21)*	*(±1.77)*	*(±1.81)*	*(± 1.58)*
% Change/T0		+1%	-6%	-4%	-4%	-8%	-9%	-11%	-10%	-15%
Orosol®	**7.25**	**5.15**	**4.28**	**3.81**	**3.06**	**2.50**	**2.40**	**2.23**	**1.98**	**1.92**
% Change/T0	*(± 1.92)*	*(±1.99)*	*(±1.78)*	*(±1.93)*	*(±1.84)*	*(±1.74)*	*(±1.77)*	*(±1.60)*	*(±1.48)*	*(± 1.53)*
% Diff O/CT		-29%	-41%	-47%	-58%	-66%	-67%	-69%	-73%	-74%
	+6%	**-25%**	**-33%**	**-42%**	**-53%**	**-60%**	**-61%**	**-63%**	**-68%**	**-67%**
Infection (0-4)										
	2.43	**2.43**	**2.52**	**2.38**	**2.24**	**2.05**	**2.00**	**2.19**	**2.29**	**1.95**
CT	*(± 1.21)*	*(±1.21)*	*(±1.21)*	*(±1.02)*	*(±1.04)*	*(±1.07)*	*(±0.95)*	*(±0.87)*	*(±1.01)*	*(± 0.97)*
% Change/T0		0%	+4%	-2%	-8%	-16%	-18%	-10%	-6%	-20%
Orosol®	**2.10**	**1.65**	**1.44**	**1.15**	**0.83**	**0.71**	**0.60**	**0.56**	**0.48**	**0.52**
% Change/T0	*(± 1.43)*	*(±1.35)*	*(±1.18)*	*(±1.13)*	*(±1.06)*	*(±1.03)*	*(±1.03)*	*(±0.92)*	*(±0.74)*	*(± 0.74)*
% Diff O/CT		-21%	-31%	-45%	-60%	-66%	-71%	-73%	-77%	-75%
	-13%	**-32%**	**-43%**	**-52%**	**-63%**	**-65%**	**-70%**	**-74%**	**-79%**	**-73%**
New Ulcer Formation (0-4)										
	2.43		**2.48**	**2.24**	**2.38**	**2.33**	**2.57**	**2.38**	**2.52**	**2.29**
CT	*(± 0.93)*		*(±0.93)*	*(±0.77)*	*(±0.81)*	*(±0.86)*	*(±0.81)*	*(±0.87)*	*(±1.03)*	*(± 1.06)*
% Change/T0			+2%	-8%	-2%	-4%	+6%	-2%	+4%	-6%
Orosol®	**2.52**		**2.56**	**2.27**	**2.06**	**1.94**	**1.98**	**1.86**	**1.75**	**1.73**
% Change/T0	*(± 0.85)*		*(±0.90)*	*(±0.98)*	*(±1.02)*	*(±1.02)*	*(±1.02)*	*(±1.08)*	*(±1.12)*	*(± 1.14)*
% Diff O/CT			+2%	-10%	-18%	-23%	-21%	-26%	-31%	-31%
	+4%		**+4%**	**-1%**	**-13%**	**-17%**	**-23%**	**-22%**	**-31%**	**-24%**
Eating Difficulty (0-4)										

	2.62	**2.62**	**2.67**	**2.62**	**2.57**	**2.43**	**2.52**	**2.48**	**2.48**	**2.38**
CT	*(±1.07)*	*(±1.07)*	*(±1.07)*	*(±1.02)*	*(±0.98)*	*(±0.93)*	*(±0.87)*	*(±0.93)*	*(±1.03)*	*(± 0.97)*
% Change/T0		0%	+2%	0%	-2%	-7%	-4%	-5%	-5%	-9%
Orosol®	**2.81**	**2.65**	**2.48**	**2.29**	**2.19**	**1.90**	**1.81**	**1.60**	**1.54**	**1.50**
% Change/T0	*(±0.98)*	*(±0.96)*	*(±0.99)*	*(±0.94)*	*(±0.92)*	*(±0.81)*	*(±0.76)*	*(±0.79)*	*(±0.80)*	*(± 0.80)*
% Diff O/CT		-6%	-12%	-18%	-22%	-32%	-36%	-43%	-45%	-47%
	+7%	**-1%**	**-7%**	**-12%**	**-15%**	**-22%**	**-28%**	**-35%**	**-38%**	**-37%**

in Orosol® Group (O) Compared to Classical Treatments Group (CT).

Tr = Treatment; T0 = pre-treatment time point; 0 to 4 and 1 to 10 scales indicate score from absent (0) or minimum (1) to severe (4 or 10) depending upon the parameter.

New anti-viral topical polymer drugs

Anti-influenza drug: A topical viral infection progresses completely differently from a systemic viral infection. During a topical external infection, as with influenza virus, a few virus particles initially come in contact with throat mucosa cells. There are practically no clinical signs at this stage. After initial infection, the virus multiplies in a few cells and millions of new virus particles are then liberated topically, infecting new cells and creating visible lesions and sore throat, [59] the condition potentially evolving towards pneumonia in severe cases. Influenza virus has no processing proteases to fuse with the host cell membrane and virus entry is determined primarily by the host cellular HA (0) processing proteases that proteolytically activate membrane fusion activity. Matrix metalloproteinases (MMPs) belong to a large family of proteases. At least seven different trypsin-type processing proteases including tryptase Clara and tryptase TL2 have been identified for HA(0) processing but probably there are many others which are not yet identified. [60-63] Intracellular virus multipication also encodes up to 11 proteins and this coding capacity demands that the virus use the host cellular machinery for many aspects of its life cycle, [64] such as help from different intracellular proteases, including specific MMPs present on the surface of the respiratory tract to enter and to infect throat cells. To restrict viral infection, our body defense mechanisms liberate anti-proteases called secretory leukoproteases in the upper respiratory tract and the pulmonary surfactants in the lower respiratory tract to reduce the amount of free proteases available for assisting viral entry. When proteases activity predominates over the activities of inhibitory compounds, virus infection cannot be stopped, [65] and it takes 5-10 days for antibodies produced by the activated body's defence mechanisms to stop virus replication. There is no topical antiviral drug on the market and current research is largely directed to searching specific proteases inhibitors as potential future therapeutic agents for the treatment of influenza.[66] Furthermore, the influenza virus surface capsid contains multiple

mutating glycoproteins such as H1 (hemagglutinin) and N1 (neuraminidase), which are directly involved in virus pathogenicity. As the virus entry-enhancing proteases and virus host cell attaching glycoproteins are proteins in nature, various *in vitro* experiments were conducted to identify specific polymers capable of binding and neutralizing both the virus glycoproteins and the proteases. The results of those *in vitro* studies showed that MMPs 1, 2, 7 and 9 are the major proteases involved in facilitating topical virus entry and that only certain specific polymers can bind with these MMPs. Among 1000s of natural plant polymers tested, hardly 6% were capable of binding either with proteases or with the virus glycoproteins, but their total antiviral activity always remained less than 50%. Therefore, individual anti-viral polymers were associated with each other to obtain 100% virus inhibition through simultaneous binding of proteases and virus glycoproteins. These results constitute a part of research work presented in patent PCT/EP2010/050236. [67] The most active plant polymer association was then incorporated in a 74% glycerol–12% honey solution (VB-Th4) for topical application by spray over the throat surface.

Clinical efficacy against influenza virus

To verify product efficacy, a single blind clinical trial was conducted at the Nexus clinical research centre in Mumbai, India, on 103 patients suffering from acute sore throat. [68] 60 patients were treated with the polymer-containing spray for maximum 14 consecutive days. 43 patients in the placebo group used other common treatments as recommended by their medical advisor. Effect on clinical signs (scoring the intensity of throat pain, irritation, and redness on a 0 to 10 severity scale), recovery time, and requirement for antibiotics, were evaluated. Patients in the VB-Th4 group were to spray the solution over their throat surface every 20-30 minutes during the 2-3 hours in the beginning of treatment and 3-4 times daily thereafter, up to complete recovery. Results showed a progressive reduction in throat pain and irritation, up to day 7 when almost all patients (55/60) had completely recovered (fig. 3 & 4).

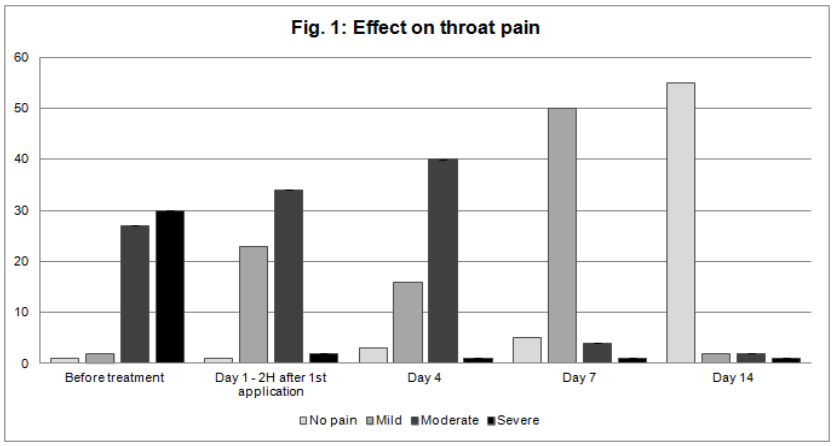

Fig. 3: Patients evaluating intensity of throat pain before treatment, 2h after 1st product application and on days 4, 7 and 14, in VB-Th4 polymer spray group (n=60).

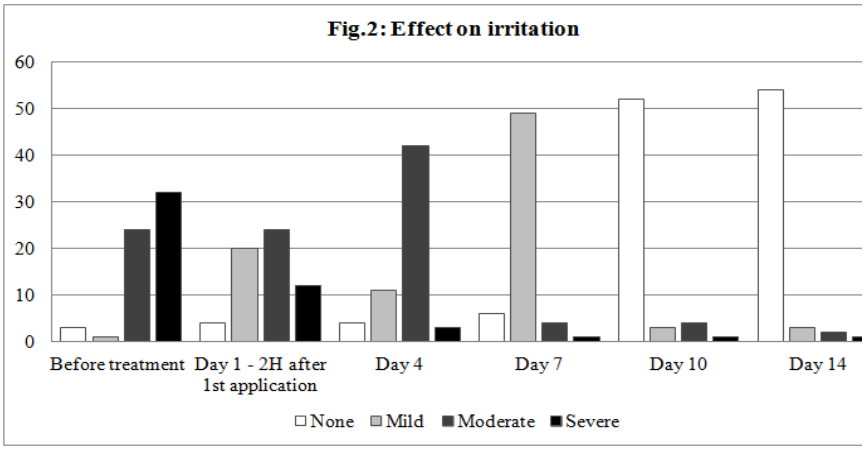

Fig. 4: Patients scoring intensity of throat irritation before treatment, 2h after first product application and on days 4, 7, 10 and 14 in VB-Th4 polymer spray group (n=60).

31% of patients in the VB-Th4 polymer solution group (n=60) stopped all treatment after 2 days as they had completely recovered, compared to only 11% in the placebo group (n=43) treated with antiseptic sprays (28/43), salt water gargles (13/43) or expectorants (2/43). On day 7, 61% VB-Th4 participants had recovered compared to 25% in the placebo group. On day 10, almost all VB-Th4 patients (95.0%) had no further need for treatment (57/60) compared to 28/43 patients (65.1%) in the placebo group. These results clearly show the virus and protease neutralization effects of antiviral polymers. As a result of this rapid recovery, during the 14-day study period, only 4/60 VB-Th4 patients (6.66%) necessitated antibiotherapy, for an average duration of 7.1 days, compared to 14/43 patients (32.56%) in the placebo group for an average period of 9.8 days. No side effects or any undesirable reaction were observed in any of the patients, and haematological, blood biochemical, or renal parameters were not affected in the VB-Th4 group.

Table 2: Number & percentage of patients recommended antibiotherapy by their medical doctor from the date of entry into the study, respectively in each group.[a]

Group	Before treatment	Day 1	Day 2	Day 7	Day 10	Day 14
VB-Th4 % population	60	0	2 3.33	3 5.0	4 6.66%	1 1.66
Placebo group % population	43	2 4.65%	13 30.23	14 32.56	8 18.60	7 16.28

a: Patients not having recovered completely after the day 14 were not followed.

Clinical efficacy against Rhinovirus

Rhinoviruses belong to the picornavirus family, responsible for common cold infections in humans. [69] These non-enveloped viruses consist of one single-stranded, positive-sense RNA (~7200 bases) and an icosahedral capsid. The rhinovirus capsid contains 60 protomers, each composed of four proteins. Three larger proteins (VP1–3, ~30 kDa each) are located on the external surface of the virus and one smaller protein (VP4, ~7 kDa) lines the inner surface, interfacing with VP1–3 and RNA. Polymers having strong affinity for the proteins can bind with these and neutralize the virus. The antiviral properties of polymers were evaluated *in vitro* as described above and a suspension of those polymers was prepared in water and glycerol (Nesospray) for topical application as spray in the nasal cavity. A clinical trial was conducted to evaluate the anti-rhinovirus potential of the product compared to standard treatments in adult volunteers suffering from symptoms of nasal sinus infection. After initial screening, 109 selected patients suffering from acute and chronic sinusitis were given either Nesospray (active treatment group) or a saline-containing spray (placebo group), and 103 final results were obtained: 58 in NESOSPRAY group and 45 in placebo group. The active treatment or the placebo product (both in 20ml containers) was applied as nasal spray 3-4 times per day for a maximum period of 21 consecutive days or up to complete recovery. Parameters evaluated included: 1. Effect on nasal congestion, cough, and runny nose, 2. Effect on pain upon pressure around the nasal sinus surface, 3. Overall condition of the patient with respect to sinus infection, 4. Antibiotics administration (upon medical recommendation): number of patients and duration of antibiotherapy treatment were recorded so as to assess whether Nesospray treatment influences requirements for antibiotics. Study parameters were recorded before treatment, 30 minutes after 1st application, and on days 1, 2, 3, 4, 7, 14 and 21, using a 1 to 4 rating system: 1 indicating excellent condition, 2 good condition, 3 poor and 4 very poor condition.

In both groups, most patients had strong nasal congestion and cough, and comparable rhinorrhea (mean score 3.37/4 in placebo group and 3.22/4 in Nesospray group). In both

groups, nasal congestion was noticeably reduced (by nearly 30%) as soon as treatment was started, but then remained nearly unchanged in the placebo group between days 1 and 5, before diminishing progressively through day 21. In the Nesospray group, however, symptom intensity decreased markedly from day 1 with nearly 50% more reduction in nasal congestion compared to placebo. Sinus pain and overall rhinosinusitis condition diminished concomitantly.

Table 3: Mean scores for nasal congestion, runny nose, sinus pain and overall rhinosinusitis grade, in placebo saline solution (PSS, n=45) and NS-2 Nesospray (n=58) groups. % change indicates mean difference within same group compared to day 0 (pre-treatment) values or between the NS-2 and the PSS group at same time-point.

		Day 0	30min	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 14	Day 21
Nasal congestion	PSS	3,378	2,689	2,756	2,778	2,733	2,711	2,400	2,244	2,356	2,289	1,867
	± *SD*	*0,535*	*0,557*	*0,802*	*0,517*	*0,751*	*0,626*	*0,657*	*0,83*	*0,484*	*0,626*	*0,786*
	NS-2	3,224	2,224	1,914	1,707	1,379	1,293	1,172	1,086	0,845	0,569	0,241
	± *SD*	0,893	0,773	0,904	0,918	0,952	0,817	0,861	0,884	0,834	0,652	0,432
	% change NS-2 vs day 0	-	**-31,02%**	**-40,63%**	**-47,05%**	**-57,23%**	**-59,89%**	**-63,65%**	**-66,32%**	**-73,79%**	**-82,35%**	**-92,52%**
	% change PSS vs day 0	-	**-20,40%**	**-18,41%**	**-17,76%**	**-19,09%**	**-19,75%**	**-28,95%**	**-33,57%**	**-30,25%**	**-32,24%**	**-44,73%**
	% change NS-2 vs PSS	**-4,56%**	**-17,29%**	**-30,55%**	**-38,55%**	**-49,54%**	**-52,31%**	**-51,17%**	**-51,60%**	**-64,13%**	**-75,14%**	**-87,09%**
	p-value	**0.1198**	**0.0004**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**
Runny nose	PSS	1,400	1,844	1,667	1,222	1,289	1,400	1,644	1,289	1,644	1,111	0,867
	± *SD*	*0,72*	*0,737*	*1,168*	*0,85*	*0,661*	*0,751*	*0,883*	*0,727*	*0,957*	*0,714*	*0,726*
	NS-2	1,224	3,241	2,638	1,845	1,621	1,466	1,397	1,241	0,569	0,569	0,414
	± *SD*	0,974	0,683	0,873	0,854	0,97	0,754	0,954	0,924	0,624	0,652	0,563
	% change NS-2 vs day 0	-	**164,79%**	**115,52%**	**50,74%**	**32,43%**	**19,77%**	**14,13%**	**1,39%**	**-53,51%**	**-53,51%**	**-66,18%**
	% change PSS vs day 0	-	**31,71%**	**19,07%**	**-12,71%**	**-7,93%**	**0,00%**	**17,43%**	**-7,93%**	**17,43%**	**-20,64%**	**-38,07%**
	% change NS-2 vs PSS	**-12,57%**	**75,76%**	**58,25%**	**50,98%**	**25,76%**	**4,71%**	**-15,02%**	**-3,72%**	**-65,39%**	**-48,78%**	**-52,25%**
	p-value	**0.0364**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**0.0005**	**0.3397**	**0.0010**	**0.4644**	**P<0.0001**	**P<0.0001**	**P<0.0001**
Sinus pain	PSS	2,667	2,622	2,600	2,600	2,444	2,222	1,800	1,444	1,244	0,778	0,311
	± *SD*	*0,674*	*0,65*	*0,889*	*0,863*	*0,624*	*1,106*	*0,842*	*0,967*	*0,857*	*0,902*	*0,468*
	NS-2	2,793	2,6	1,155	0,966	0,586	0,379	0,259	0,345	0,414	0,397	0,138
	± *SD*	0,614	0,598	1,04	0,878	0,531	0,489	0,442	0,479	0,563	0,591	0,348
	% change NS-2 vs day 0	-	**-6,91%**	**-58,65%**	**-65,41%**	**-79,02%**	**-86,43%**	**-90,73%**	**-87,65%**	**-85,18%**	**-85,79%**	**-95,06%**
	% change PSS vs day 0	-	**-1,69%**	**-2,51%**	**-2,51%**	**-8,36%**	**-16,69%**	**-32,51%**	**-45,86%**	**-53,36%**	**-70,83%**	**-88,34%**
	% change NS-2 vs PSS	**4,72%**	**-0,84%**	**-55,58%**	**-62,85%**	**-76,02%**	**-82,94%**	**-85,61%**	**-76,11%**	**-66,72%**	**-48,97%**	**-55,63%**
	p-value	**0.5276**	**0.7538**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**
Overall rhinosinusitis	PSS	3,156	2,444	3,200	3,378	3,267	3,067	2,956	2,800	2,600	1,556	0,822
	± *SD*	*0,638*	*0,624*	*0,588*	*0,49*	*0,688*	*0,688*	*0,796*	*0,815*	*0,751*	*0,867*	*0,777*
	NS-2	2,914	2,793	1,19	1,138	0,879	0,655	0,759	0,741	0,586	0,466	0,138
	± *SD*	0,904	0,744	0,712	0,868	0,818	0,608	0,802	0,664	0,726	0,503	0,348
	% change NS-2 vs day 0	-	**-4,15%**	**-59,16%**	**-60,95%**	**-69,84%**	**-77,52%**	**-73,95%**	**-74,57%**	**-79,89%**	**-84,01%**	**-95,26%**
	% change PSS vs day 0	-	**-22,56%**	**1,39%**	**7,03%**	**3,52%**	**-2,82%**	**-6,34%**	**-11,28%**	**-17,62%**	**-50,70%**	**-73,95%**
	% change NS-2 vs PSS	**-7,67%**	**14,28%**	**-62,81%**	**-66,31%**	**-73,09%**	**-78,64%**	**-74,32%**	**-73,54%**	**-77,46%**	**-70,05%**	**-83,21%**
	p-value	**0.0365**	**0.0205**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**

Due to fast symptomatic improvement, Nesospray patients (21%) needed nearly 50% less antibiotherapy than placebo patients (40%). Average antibiotherapy duration in the placebo group was 10.5 days versus 7.41 days in the Nesospray group. Except for slight initial local irritation, no other side effects were noticed in any patient. These results show that the use of a specific anti-rhinovirus polymer-containing solution represents a new, efficient, safe and cost-effective approach to treat rhinosinusitis. [70]

Clinical efficacy against Genital Herpes & vaginal infection

Herpes simplex virus 1 and 2 (HSV-1 and HSV-2) are enveloped DNA viruses belonging to the family Herpesviridae that infect humans. In most cases, over 50% men and women,[71] the virus remains dormant in nerve cells. Under stressful conditions, the latent virus migrates towards the skin or mucous membranes, causing labial or genital herpes. These viruses contain an external envelop with several proteins (glycoproteins) on their surface. The HSV envelope is a highly complex structure containing many types of surface gps (gps) such as gB, gD, gH, gL, gC,gI, gE as main proteins. [72] To infect the cells, it is postulated that the C and/or B gps (gC and gB respectively) and probably the gH glycoprotein, bind to the heparan sulfate receptors on the cell surface. [73] This allows virus fusion with the host cell membrane and to create an opening or *pore*, through which the virus enters the host cell. [74] The host cell lyse liberates a large amount of virus particles over the skin or mucosal surface which infect new cells and lead to the development of herpes lesions.[75] Therefore, cells that are devoid of heparan sulfate receptors are not susceptible to HSV.[76] One possible treatment for topical HSV infection would be to block cellular heparan receptors, but this may interfere with cellular functions and induce side effects. Another possibility would therefore be to neutralize virus glycoproteins to prevent virus–host cell membrane interaction. Recent scientific evidence shows that in addition to viral glycoproteins, some MMPs also play an important role in facilitating topical viral entry into cells. The use of proteases by viruses during intracellular multiplication was already known but it was recently established that HSV also takes the help of certain proteases for cellular penetration.[77] Proteases, or proteolytic enzymes, are particularly found in the vicinity of damaged tissues, and play a vital role in protein catabolism to clean the infected lesions from damaged protein molecules which interfere with healing. Hundreds of proteases, found topically or intracellularly, can be divided into four major groups according to the character of their catalytic active site and conditions of action: metalloproteinases (MMPs), serine proteinases, cysteine (thiol) proteinases, and aspartic proteinases. Many proteases may be found topically on a virus-

infected skin or mucous membrane, including MMPs, pepsin, trypsin, chymotrypsin, subtilisin, cystin proteinase (cathepsin B,H,K,L,S), aspartic proteinase (cathepsin D), and clotting factors, but MMPs are particularly abundant in the open herpes lesions or on genital herpes-infected mucous membranes.[78,79] This is the reason why MMPs such as gelatinase A (MMP-2), gelatinase B (MMP-9), collagenase (MMP-1), collagenase 3 (MMP-13), stromelysin-1 (MMP-3), stromelysin-3 (MMP-11), MT-1 MMP (MMP-14), macrophase metalloelastase (MMP-12) and surely many others which are not yet discovered are considered to be involved in topical viral infections. It has been shown that in the open HSV lesions, levels of MMP-2 and MMP-9 increase during early infection [80] and that certain protease inhibitors reduce the activity of HSV.[81] As all currently available treatments are intracellular virus growth inhibitors,[82] one means of stopping HSV progression may entail neutralizing the MMPs which assist HSV entry into cells. Taking into consideration the amount of free virus particles on an HSV-infected surface, different types and sub-types of virus surface Gps and the large variety of proteases present in the lesion, it is practically impossible to find a specific drug which can simultaneously block all the factors involved in virus entry and in subsequent progression of HSV infection. Viral glycoproteins and proteases being proteinous in nature, Vitrobio's research objective was to find non-specific protein-binding agents which may neutralize not only proteases but also multiple viral Gps in order to stop HSV infection. [83]

Clinical confirmation: A multicentric, open label, single arm, prospective pilot study was conducted by the Nexus Clinical Research Pvt Ltd in Mumbai, India, in 2010. 60 women with visible lesions of genital herpes were treated with the polymeric solution Hp-Gy (10ml per day) for 14 consecutive days. Product was administered daily into the vaginal cavity and GHSV symptoms were evaluated before treatment, 2 h after 1st application, and on days 4, 7, and 14. GHSV lesions smears were collected to quantify virus-loaded multinucleated giant cells using Tzanck test.

RESULTS

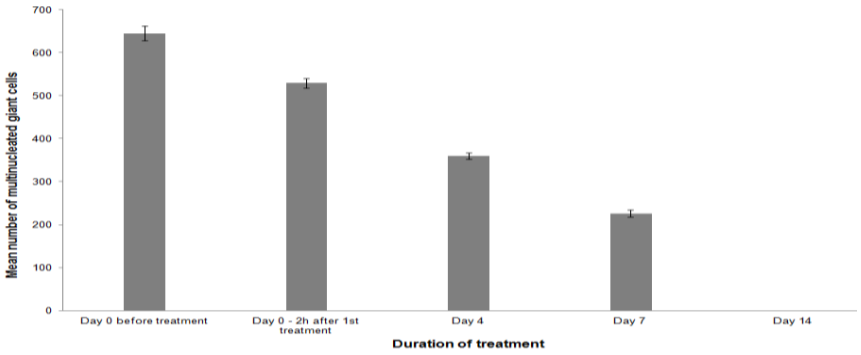

Fig. 5: Number of virus-loaded multinucleated giant cells per field in virus-positive patients after Tzanck staining of the HSV lesion smears.

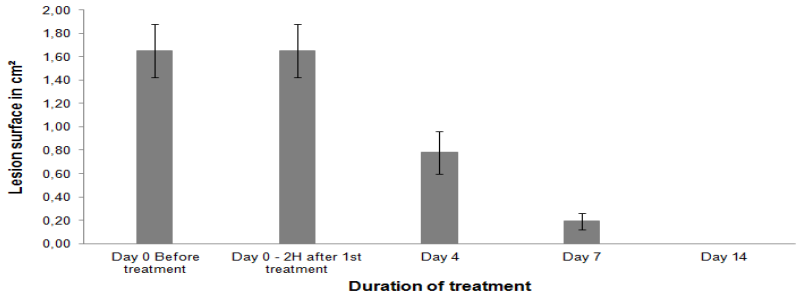

Fig. 6: Mean surface area of 60 lesions. Lesions were photographed before treatment and on days 4 and 7. All selected lesions were completely healed by day 14.

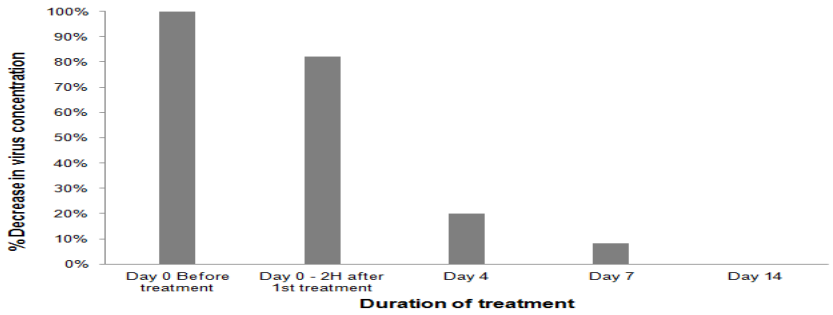

Fig. 7: Virus concentration proportionally to lesion surface: Mean % decrease in number of virus-loaded cells per field compared to lesion surface, 2h after 1st treatment, and on days 4 and 7.

Statistically significant reduction in vaginal itching, redness, pain, dryness, discharge, presence of blisters, were observed, as well as normalization of vaginal pH. Marked improvement was observed within 2h of 1st application. This study's results clearly show that topical application of specific virus GP-neutralizing plant polymers in an osmotically active solution **(Hp-Gy)** represents a highly promising new scientific approach to treat GHSV. [84]

Clinical efficacy against Labial Herpes

Another multicentric, prospective pilot study was conducted by the Nexus Clinical Research Pvt Ltd in Mumbai, India, between 06-2009 and 12-2010, to evaluate a plant polymer–glycerol solution activity against labial herpes. 60 patients were to apply 3-4 drops of the test product directly onto open labial herpes lesions, three times per day, up to complete healing or for a maximum period of 14 consecutive days. Patients rated sensations of itching, burning and pain on a scale of 0 to 3 (0 = no symptom, 1= mild, 2 = moderate, 3 = severe) at the start of treatment, 2h after 1st product application and on days 4, 7, and 14. Mean lesion surface and virus concentration per lesion were also measured and compared at each time-point to before treatment values. Results and absence of side effects led to the conclusion that neutralizing and eliminating free virus particles from the open lesion is one of the best strategies to treat labial herpes. [85]

Table 4: Mean virus concentration per lesion and % of change compared to before treatment values (± SD); mean lesion surface (cm^2); % change in lesion surface compared to pre-treatment values and relative concentration of free virus particles adjusted according to mean lesion surface (n= 60) before treatment, 2h after 1st treatment and on days 4, 7, and 14 (mean ± SD)

Mean Values	Day 1 Before treatment	Day 1 2h after first treatment	Day 4	Day 7	Day 14
Mean number of virus-infected cells per field	>750 *(± 17.72)*	465 *(±10.82)*	359 *(± 6.35)*	226 *(± 10.22)*	0
% change	*0*	*- 38%*	*- 52.13%*	*- 69.87%*	*-100%*
Surface area CM^2	1.528 *(± 0.72)*	1.528 *(± 0.73)*	0.683 *(± 0.36)*	0.193 *(± 0.11)*	0
% change	*0%*	*0%*	*-55.3%*	*-87.37*	*-100%*
% change in virus concentration compared to the surface area of the lesion	0%	-38.0	-76.69	-91.18%	-100%

Table 5: Effect on the sensation of itching, burning and pain

	Number of replies (n=60)				
Parameter : Itching sensation	**None**	**Mild**	**Moderate**	**Severe**	***p-value***
Day 1 before treatment	07	19	20	14	*0.0698*
+ 2h	07	39	19	13	*0.0460*
Day 4	10	27	14	09	*0.0033*
Day 7	22	20	18	0	*0.8187*
Day 14	56	04	0	0	*<.0001*
Parameter : Burning sensation					

Day 1 before treatment	05	09	20	26	*0.0003*
+ 2h	06	10	21	23	*0.0033*
Day 4	12	15	16	17	*0.8174*
Day 7	27	19	14	0	*0.1165*
Day 14	60	0	0	0	.
Parameter : Pain sensation					
Day 1 before treatment	12	26	14	08	*0.0074*
+ 2h	15	28	12	05	*0.0003*
Day 4	28	21	09	02	*<.0001*
Day 7	45	07	08	0	*<.0001*
Day 14	60	0	0	0	.

Clinical efficacy against cytokine-induced dermatological diseases

Finally, a 6-week, single blind clinical study was undertaken to verify the hypothesis of protein inhibition by polymers applied to dermatological diseases: [86] polymeric solution VB-DERM was applied three times a day over open skin lesions of patients suffering from psoriasis, eczema or dermatitis (PED), and effects were compared against Commonly Prescribed Drugs (CPD) consisting of corticosteroids, antibiotics, or anti-inflammatory drugs. 56 patients in the VB-DERM group and 51 in the CPD group rated their symptoms' severity on a 0 to 4 scale at T0, 1, 2, 4, and 6 weeks. Topical application of the test product induced an exudation of liquid from the injury during the first 5-10 minutes following application, and resulted in strong wound healing properties. Compared to the control treatments, VB-DERM demonstrated high efficacy in treating all symptoms associated with PED skin diseases as it significantly reduced signs of erythema, pruritus, oedema, oozing, dryness, and itching, without any undesirable effects or allergic reactions, resulting in significant relief and amelioration of quality of life of the patients, right from the second week after the start of treatment. VB-DERM can therefore be considered a very safe topical wound cleaning medical device for the treatment of PED lesions. [87]

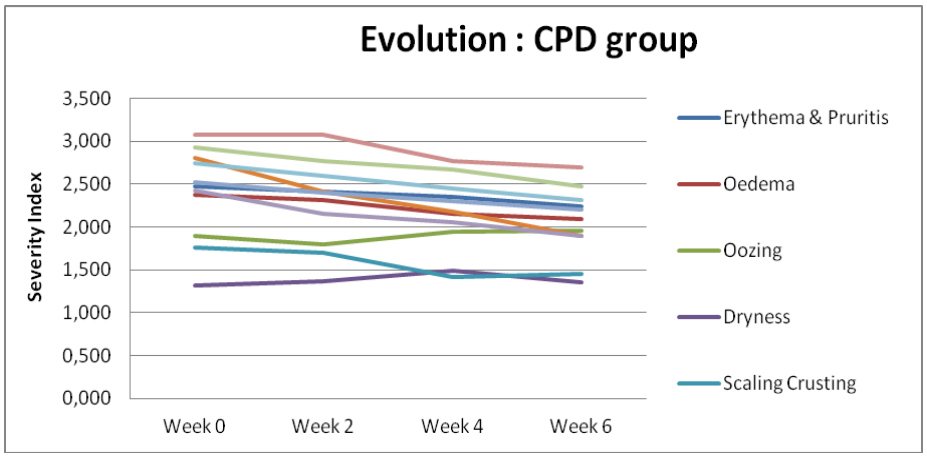

Fig. 8: Evolution of symptoms in standard treatments group over the 6-week period

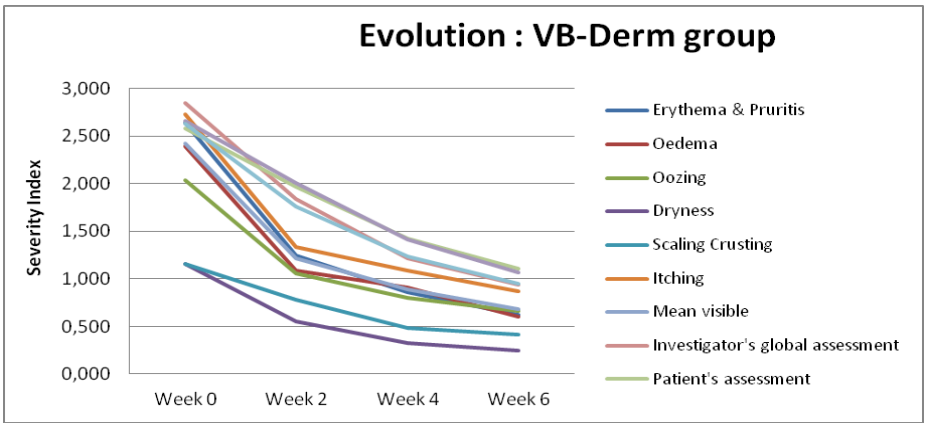

Fig. 9: Evolution of symptoms in VB-DERM group over the 6-week study period

DISCUSSION

Numerous diseases involve proteins either as primary causative agents or in supporting roles, at various stages of the physiopathology. Therefore it makes sense that those proteins should be considered as a viable target for pharmaceutical design aiming to inhibit or modulate their functions. For example, inhibiting or modulating proteins such as proteolytic enzymes, which break down protein substrates and have a major influence on many physiological processes, represents a possible therapeutic doorway to treat certain diseases.

Hence, in the absence of any efficient chemical or biological curative treatment, the use of protein inhibitors, meant to attach and neutralize the pathological protein, may play a key role in the treatment of various diseases.

As novel as this approach is, and despite the suspicion with which the introduction of the first biotechnology and polymer-based products has been greeted before the turn of the century, just as modern chemotherapy once had, it is notable that present-day industrial pharmaceutical research and development focuses on low-molecular-weight drugs (both natural product extracts and synthetic drugs) and prodrugs, particularly orally administered, patentable and patient-friendly drugs. [88]

While the lack of oral bioavailability and chemical complexity of macromolecular drugs, including polymers, thwarted their large-scale industrial development till two decades ago, news that some polymeric drugs had been successfully launched recently, suddenly diverted the attention of pharmaceutical research towards polymeric therapeutics as new future drugs.[88]

For clinical use, it is essential to identify biocompatible synthetic polymers that will not be harmful in relation to their route, dose and frequency of administration. The general

cytotoxicity, haematotoxicity and immunogenicity (cellular and humoral) of water-soluble polymers has been widely studied over many years, and they were found to be much less toxic, or even nontoxic, compared to chemical drugs. New clinical trials, conducted with multiple topical polymers by Vitrobio Pharma, France, clearly show that polymer therapy can have selective effects on the proteins affecting a diverse range of biochemical processes. These topical polymeric treatments have now demonstrated that polymers can satisfy the stringent requirements of safety, efficacy and regulatory approval.

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