**PHARMACY UPDATES**

**CHITOSAN/HYALURONIC ACID POLYELECTROLYTE COMPLEX HYDROGELS IN THE MANAGEMENT OF BURN WOUNDS**

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CHITOSAN/HYALURONIC ACID POLYELECTROLYTE COMPLEX HYDROGELS IN THE MANAGEMENT OF BURN WOUNDS (Abstract): **Aim:** This paper is a short review on the state-of-the-art of the use of polyelectrolyte complexes containing polysaccharides for wound and burn healing and treatment using the chitosan/hyaluronic acid polyelectrolyte complex (PEC) hydrogel. **Results and conclusions:** PEC is suitable for wound healing because a wet treatment can be realized and both components of PEC contribute by their properties to the enhanced antimicrobial activity, promote wound healing and prevent wound damage during treatment. **Keywords:** WOUND HEALING, DRESSING, CHITOSAN, HYALURONIC ACID.

Contamination and infection remain serious complications of wound burns and sepsis is a leading cause of mortality and morbidity in patients with major burns. Several factors contribute to the colonization of wounds, including the presence of: serous exudates that contain several proteins providing nutrients for bacteria, necrosed tissue that enhances the proliferation of micro-organisms and their invasion of the underlying viable tissues, and a compromised immune response that prevents the full expression of natural defenses (1). Initially, a burn is sterile, but rapidly becomes colonized, predominantly by Gram positive bacteria in the first instance (2). Once bacterial density reached up to $10^5$/g of tissue, wound infection and sepsis are likely to occur. Early application of topical antimicrobial agents is the most effective way of preventing invasive burn infection when combined with early eschar excision (3).

Wound healing is an intricate process in which the skin (or another organ-tissue) repairs itself after injury. It is a particular biological process related to general phenomenon of growth and tissue regeneration. Wound healing progresses by a series of independent overlapping steps such as hemostasis, inflammation, migration/granulation/proliferation and maturation phases of tissue formation, matrix remodeling/reshaping/ and reepithelialization, in which a variety of cellular and matrix components act together to re-establish affected tissue integrity and to replace the
lost tissue (4).

Dressings are applied on open wounds for centuries to protect them from injuries and bacterial invasion. Recently, dressings made from novel polymers are used for the delivery of drugs to acute, chronic, and other types of wounds. Many types of dressings, of occlusive or semi-occlusive nature have been developed. These include collagen/glicosaminoglycan sponges, alginate gels, hydrocolloids, hydrogels, polyurethane, collagen, chitosan (CS), pectin and hyaluronic acid-based biomaterials. CS has a beneficial influence in all stages of wound healing (5). Wet dressings have a superior quality because the controlled wet medium at the interface wound-bandage favors wound healing (6).

**CHITOSANS** (CSs) are polycationic copolymers of β-(1-4)-2-acetamido-2-deoxy-D-glucopyranose (deacetylated units) and 2-amino-2-deoxy-D-glucopyranose N-acetyl-D-glucosa-mine (acetylated units) as chitin derivatives with different deacetylation degrees (from 70 to 95%) and average molecular weight ranges from 10 to 1.000 kDa (7, 8) (fig. 1).

Chitosan exhibits a good biocompatibility and biodegradability (9) which allows its wide medical applications as topical formulation in ophthalmology, as implants (10) or injection (11), and facilitates the penetration of hydrophilic drugs across mucosal epithelia. Because of the positive charges at physiological pH, CS is a good bioadhesive, increasing the retention to the application sites, efficient for wound healing (12) also because of its bacteriostatic effect (13).

Many studies have reported on the use of chitin, CS, and their derivatives as wound healing accelerators (>75 %) (14), acting in each stage of the healing process by enhancing the functions of the inflammatory cells polymorphonuclear leukocytes, macrophages, fibroblasts or osteoblasts, and by increasing the resistance to wound extension (15).

To further improve the CS properties, chemical modification and copolymerization are applied. These techniques could lead to the formation of entities with unknown toxicological profiles, therefore they should be avoided and the strategies based on non-covalent bonding preferred, this being achieved by electrostatic forces, hydrophobic or hydrogen bonding. Amino groups C₂ position in glucopyranosic units of CS can electrostatically interact with anionic groups (e.g. carboxyl) of polyanions of natural origin (such as pectine, alginate, carrageenan, xanthan gum, carboxymethyl cellulose, chondroitin sulphate, dextran sulphate, hyaluronic acid) or syn-
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thetic origin (e.g. polyacrylic acid, polyphosphoric acid, poly(L-lactide) forming polyelectrolyte complexes (PEC) (16).

Hyaluronic acid (HA) was discovered by Meyer and Palmer in 1934 in the vitreous humor of bovine eyes. Hyaluronic acid (also called hyaluronan or hyaluronate) is the only nonsulphated glycosaminoglycan found in the extracellular matrix throughout connective, epithelial and neural tissues. Hyaluronic acid is a naturally occurring physiological constituent of the connective tissue, especially in the gingival mucosa (17). It is a glycosaminoglycan, a linear anionic polysaccharide, composed of repeating disaccharides, $\alpha$-(1,4)-D-glucuronic acid, and $\beta$-(1,3)-N-acetyl-D-glucosamine linked by $\beta$-(1 - 3) bonds (fig. 2).

![Fig. 2. The hyaluronic acid structure](image)

HA is produced by bacterial fermentation of a Streptococcus sp. or is extracted for commercial purpose from rooster combs, umbilical cords, synovial fluids, or vitreous humour. It has been used in ophthalmic surgery, arthritis treatment, in tissue engineering, component of scaffolds for wound healing and implants devices (18). The implants covered with HA and its derivatives reduce adsorption, adhesion and cellular proliferation of Staphlococcus aureus at least 100 times (19).

HYDROGELS are macromolecular networks swollen in water or biological fluids. Hydrogels are often divided into three classes depending on the nature of their network, namely entangled networks, covalently cross linked networks, and networks formed by physical interactions, but a clear differentiation between them does not exist. Chemical hydrogels are formed by irreversible covalent bonds, while physical hydrogels are formed by various reversible links. These can be ionic interactions, as in ionically cross linked hydrogels and polyelectrolyte complexes (PEC), or secondary interactions, as in CS/poly (vinyl alcohol) (PVA) complexed hydrogels, grafted CS hydrogels and entangled hydrogels. The latter are formed by solubilization of CS in an acidic aqueous medium and their use is limited by their lack of mechanical strength and tendency to dissolve (20).

Due to the potential toxicity of free unreacted covalent cross linkers, which require an additional purification step during hydrogel manufacturing, the development of alternative types of hydrogels was necessary and desirable. Hydrogels formed by direct interaction between polymeric chains without the addition of cross linkers were prepared by complexation with another polymer or by aggregation after grafting.
POLYELECTROLYTE COMPLEX HYDROGELS (PECs)

Polyelectrolyte complex hydrogels are formed by ionic crosslinking (of ions or ionic molecules with high molecular weight, and in the case of polyelectrolytes with a certain distribution of molecular weight) and are generally biocompatible networks exhibiting interesting swelling characteristic, being an alternative to covalently crosslinked hydrogels. Electrostatic attraction between amino cationic groups of CS and anionic carboxyl group of HA is the main and the strongest interaction in this PEC (fig. 3).

BIO-SMART HYDROGELS rapidly respond to the action of external stimuli (or local environmental variations) by changes in the internal structure, size, volume, refraction index, and mechanical properties of the network. They are considered superabsorbent materials because they can absorb a high amount of water (solvent), hundred times their weight. Compared to covalently crosslinked hydrogels, PEC has a higher swelling sensitivity, especially to pH changes, which led to a wide variety of applications. Bio-smart polysaccharidic hydrogels show good biocompatibility and bacteriostatic properties necessary for wound healing (20).

PEC PREPARATION

CS or its positively charged derivatives, such as glycol CS (21) or N-dodecylated CS (22), and HA are used. The reaction occurs in aqueous solution, this being the main advantage over covalent crosslinking, because biocompatibility is assured and purification before delivery avoided. Both polymers should be in ionized state and bear opposite charges, therefore the reaction can only occur at a pH value close to the pK interval of both polymers; pKₐ of CS is ~ 6.5 (23) while that of HA is about 3.0 (24). During complexation, polyelectrolyte may coacervate or form a less compact hydrogel; if interactions are very strong precipitation occurs. Precipitation is very frequent and hinders hydrogel formation. It can be avoided by weakening the electrostatic interaction, adding salts such as NaCl. The presence of salts reduces the interaction between electrolytes by the contribution of the ions in the reaction environment. Phase separation usually does not occur, and a viscous, macroscopically homogeneous mixture, which may gel
when temperature is lowered, is obtained (25). Other factors affecting PEC properties are: pH, temperature, ionic strength, order of mixing, as well as polymer flexibility, molecular weight, degree of deacetylation of CS, and substitution degree of both polymers when derivatives are used. Injectable hydrogels for in vitro delivery of an adipogenic factor of insulin and adipose tissue engineering have been prepared by Schiff-base reaction between N-succinyl-CS and aldehyde of hyaluronic acid (26) and HA/CS polyelectrolyte complexes were obtained also by electro spinning (27).

PEC PROPERTIES

Stoichiometric PECs contain equal amounts of oppositely charged polyions, so the resulted PEC will have zero overall net charge, are usually insoluble and precipitate from solution (28). CS properties are maintained in PEC and hydrogels, therefore these are very well tolerated by living organisms and can be used in various applications: controlled release systems, cell cultures, enzymes immobilization, tissue regeneration and wound healing. CS-HA PEC protects HA against enzymatic hydrolysis, but only at pH values different of the optimum for enzymatic activity. Cells proliferation and activity in wound healing is reduced comparatively with that of CS (29). Both swelling and release profiles are pH-responsive. In acidic medium polyacid is neutralized, and because of free amino groups of CS positive charges will be present within gels. Mutual repulsion and water penetration together with contraions to neutralize these charges will determine the swelling. In case of a prolonged immersion in water a shrinkage is observed, because of segmental mobility of polyelectrolyte chains in swollen state, which allow the completion of interpolyelectrolyte reaction (30). In basic medium the mechanism is the same, but swelling is induced by negative charges of the polyacid (31). This is the logical reason for sensibility of the swelling to the ions presence and swelling rate is controlled by diffusion of mobile ions and changes in ionization degree. Osmotic pressure and electrostatic repulsion are responsible for swelling, which control the network forces depending on elasticity. If swelling becomes too important, dissolution happens at certain pHs. To prevent dissolution, covalent crosslinking is applied, but this will create biocompatibility problems.

TISSUE REGENERATION AND WOUND HEALING USING CS/HA CONTAINING FORMULATIONS

CS, with a structure similar with that of glycosaminoglycans (GAG) is indicated for preparing PEC hydrogels with GAG polyanions, such as chondroitin sulphate or HA, useful in cartilage reconstruction and wound healing, which mimic the GAG-rich extracellular matrices of articular chondrocytes (32).

In vitro tests showed that these PECs could be used as carriers for chondrocyte transplant and/or scaffold for cartilage-like tissue engineering. PEC CS/HA as sponges and films allow the culture of various specific cells, such as keratocytes, which produces skin matrix accelerating wound healing after skin ablation without inflammatory reactions and toxicity for animals (29).

When PEC is obtained from chitosan and other biocompatible polymers, it can be applied as bandages or powders, which protect the wound, accelerate healing and prevent bacterial contamination (33).

Composite CS/HA films with high transparency can be prepared using glass or
polymethylmethacrylate substrates, but not with PTFE. With increasing HA amount, the films become rougher, increasing the water contact angle and water uptake and decreasing water vapor permeability, BSA adsorption and fibroblasts adhesion, all these being desirable characteristics for wound bandages. Comparatively with Vaseline, CS/HA films are more efficient in wound healing because they do not cause wound damage when the bandage is removed. (34). Rossi et al. (35) prepared blends of CS hydrochloride, 5-methyl-pyrrolidinone CS and HA by freeze-drying. Chlorhexidinediacetate was used as antimicrobial agent. HA decreased the hydration capacity but modulated the antimicrobial agent release. It was suggested that the mechanism of the antimicrobial activity of CS consists in the interaction of positively charged CS molecules with negatively charged microbial cell membranes, which leads to disruption of bacterial membrane and loss of protein and intracellular constituents (36).

CONCLUSIONS
PECs of chitosan with hyaluronic acid are suitable for wound healing because a wet treatment can be realized and both components of PEC contribute through their properties to the enhanced antimicrobial activity, promote wound healing and prevent wound damage during treatment. Polyelectrolyte complexation occurs under mild reaction conditions and biocompatible PEC systems are obtained. PEC hydrogels exhibit a highly pH-sensitive swelling, therefore they can be used for pH-controlled drug delivery in different conditions.

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