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Design of antibiotic containing hydrogel wound dressings: Biomedical properties and histological study of wound healing

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ABSTRACT

Keeping in view the antioxidant nature of the acacia gum and mucoadhesive nature of carbopol hydrogels, in the present studies, an attempt has been made to explore the potential of these materials in designing new hydrogel wound dressings meant for slow release of gentamicin, an antibiotic drug, and to enhance the wound healing potential. The hydrogel films were characterized by SEM, FTIR, XRD and swelling studies. Biomedical properties of hydrogel films like blood compatibility, antioxidant activity, mucoadhesion, antimicrobial activity, oxygen/water vapour permeability, microbial penetration and mechanical properties (tensile strength, burst strength, resilience, relaxation, and folding endurance) have been evaluated. The histological studies of wound healing were also carried out on swiss albino mice of strain Balb C and it has been observed that in case of wounds covered with hydrogel dressings shown faster wound healing, formation of well developed fibroblasts and blood capillaries as compared to open wounds. The results of biomedical properties indicated that hydrogel films are non-thrombogenic, non-haemolytic, antioxidant and mucoadhesive in nature, and are permeable to oxygen and moisture while impermeable to micro-organisms. The hydrogel wound dressings have absorbed (8.772 ± 0.184 g/g film) simulated wound fluid. Release of gentamicin drug from wound dressings occurred through Fickian diffusion mechanism in simulated wound fluid.

1. Introduction

In recent years, accumulating knowledge regarding wound healing has led to the development of numerous therapies. A plethora of novel topical preparations, dressing materials and advanced methods of debridement are now in the hands of physicians and medical experts. Wound healing is a dynamic process which normally involves systematic, coordinated and balanced activity of inflammatory, vascular, connective tissue and epithelial cells. It involves a complex series of events, lasting from the moment of injury to healing. Wounds generally produce exudate which consists of fluids, cells or other substances which slowly exuded or discharged from cells or blood vessels through small pores or breaks in cell membranes. Dry wounds tend to have higher rate of infection than moist one. Moist wound healing provides an environment that stimulates wound healing. Wound dressings are usually used to encourage the various stages of wound healing and to create better healing conditions. Wound dressings often cover the wound surface to accelerate its healing. Wound dressings have been applied to open wounds for centuries. Traditionally these were absorbents and permeable materials which could adhere to desiccated wound surface and were inducing trauma on removal. However, nowadays, new dressings have been designed to create a moist wound healing environment which allowed the wound fluids and growth factors to remain in contact with wound, thus promoting autolytic debridement and accelerating wound healing. Wound dressings are the biomaterials which promote wound healing by providing suitable micro-environment (Boateng et al., 2008). Among the wound dressings, special attentions have been given to hydrogel wound dressings due to their unique properties which can meet the essential requirements of ideal wound dressings (Higa et al., 1999).

Hydrogel dressings resemble the natural living tissue more than any other class of synthetic materials because of their high water content and soft consistency. Polysaccharide hydrogels have been observed suitable for producing flexible, mechanically strong, biocompatible, effective and economical hydrogel dressings. Hydrogel wound dressings are three-dimensional polymeric networks and are available in sheet form or as a spreadable viscous gel. Hydrogel dressings are semipermeable to gasses and water vapour. The amorphous gel formed by hydrogel dressings maintains a moist and hydrated environment (Shaheen and Yamaura, 2002; Himly et al., 1993; Saha et al., 2011). Keeping in view the antioxidant nature of the GA and mucoadhesive nature of carbopol hydrogels, in the present studies, an attempt has been made to explore

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the potential of these materials in designing new hydrogel wound dressings, for slow release of antibiotic gentamicin and to enhance the wound healing potential. Biomedical properties of hydrogel films like blood compatibility, antioxidant activity, mucoadhesion, antimicrobial activity, oxygen and water vapour permeability, microbial penetration, mechanical properties (tensile strength, burst strength, resilience, relaxation, and folding endurance), and histological studies have also been evaluated.

Gum acacia (GA) polysaccharide has been extensively used in food, pharmaceutical and cosmetic industries. It is generally recognized as safe by the United States Food and Drug Administration. It has been used for the treatment of inflammation of the intestinal mucosa and to cover inflamed surfaces (Ali et al., 2009; Wapnir et al., 2008). It possesses antibacterial (Clark et al., 1993) and antioxidant activities (Al-Yahya et al., 2009; Abd-Allah et al., 2002). Topical administration of GA can inhibit lipid peroxidation in skin (Trommer and Neubert, 2005) which stimulates wound healing and angiogenesis (Altavilla et al., 2001). It is also protective against hepatic, renal and cardiac toxicities in rats (Ali et al., 2009). On the other hand, carbopol is a hydrophilic, mucoadhesive, biocompatible crosslinked polymer of polyacrylic acid (Tang et al., 2007; Renuka et al., 2012). It has been used in biomaterials as wound dressings (Renuka et al., 2012), topical (Proniuk and Blanchard, 2002) and transdermal (Arellano et al., 1999) drug delivery systems. Gentamicin sulphate is a broad spectrum antibiotic used for the treatment of infections of the skin, bones, soft tissues and wounds. It provides highly effective topical treatment in bacterial infections of the skin. It is very effective against *Streptococcus aureus* and *Pseudomonas aeruginosa* which are most commonly recovered organisms from the wounds. Gentamicin antibiotics are potent inhibitors of protein synthesis in a wide range of bacteria.

2. Materials and methods

2.1. Materials used

GA and carbopol 940 (Loba Chemie Pvt. Ltd., Mumbai, India), N,N′-methylenebisacrylamide (NN-MBA) (Acros organics, New Jersey, USA), ammonium persulphate (APS) (Qualigens Fine Chemicals, Mumbai, India), gallic acid (Himedia Laboratories Pvt. Ltd., Mumbai India), nitroblue tetrazolium chloride, riboflavin, methionine, glycerol (S.D. Fine Chemical Ltd., Mumbai, India), Folin–Ciocalteu (F–C) reagent (Merck Specialities Pvt. Ltd., Mumbai, India), bovine serum albumin (Bio Basic Inc., Canada), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma–Aldrich, Munich, Germany), and gentamicin sulphate (Ranbaxy Lab. Ltd., New Delhi, India) were used as received.

2.2. Synthesis of hydrogel films

The solution of definite concentration of GA (5%, w/v) and carbopol (2%, w/v) was prepared and kept for 12 h for hydration. Then this solution was stirred at constant speed (100 rpm) for definite time period (45 h). Then definite concentration of NN-MBA (1.62×10^{-3} mol/L), APS (5.48×10^{-3} mol/L) and glycerol (0.14 mol/L) was added to the reaction mixture and contents were stirred for 3 h. The polymer films were prepared by solution casting method and were named as acacia-*cl*-carbopol hydrogel films. These films were washed with distilled water and ethanol to remove the soluble fraction left therein. The optimum reaction conditions were evaluated by varying the reaction parameters. The carbopol was varied from 0.5 to 2.5% (w/v), NN-MBA was varied from 1.62×10^{-3} to 11.34×10^{-3} mol/L and glycerol was varied from 0.070 to 0.47 mol/L during the synthesis of hydrogels. The optimum [carbopol], [NN-MBA] and [glycerol] were obtained 2% (w/v), 8.10×10^{-3} mol/L, and 0.34 mol/L respectively.

2.3. Characterization

SEMs were taken on FEI QUANTA 250 (Switzerland). FTIR spectra were recorded in KBr pellets on Nicolet 5700 FTIR THERMO (USA). XRD measurements were made using PAN-analytical X'Pert Pro powder diffraction system (The Netherlands). Swelling studies of hydrogels were carried out by gravimetric method (Singh and Sharma, 2009).

2.4. Drug release studies

The release profile of drug from the drug loaded polymer films was determined. The loading of a drug into the polymer matrix was carried out by swelling equilibrium method. The hydrogels were allowed to swell in solution of known concentration (1000 μg/mL) for 24 h at 37 °C and then were dried to obtain the drug loaded hydrogels. In vitro release studies of the drug were carried out by keeping the dried and drug loaded samples in definite volume of releasing medium at 37 °C temperature. The amount of drug released was measured spectrophotometrically in distilled water, PBS and simulated wound fluid after every 30 min up to 300 min in each case and then after 24 h. The absorbance of the solution of drug was measured on the UV visible spectrophotometer (Cary 100 Bio, Varian). The amount of gentamicin drug release was determined from the calibration curves prepared at λ_{max} 255 nm using UV visible spectrophotometer (Singh and Sharma, 2009). All the studies were carried out in triplicate. Based on the relative rate of diffusion of water into polymer matrix and rate of polymer chain relaxation, swelling of the polymers and the drug release profile from the drug loaded polymers have been classified into three types of diffusion mechanisms. Ritger and Peppas (1987) showed that the power law expression (Eq. (1)) could be used for the evaluation of drug release from swellable systems.

$$\frac{M_t}{M_\infty} = kt^n \quad (1)$$

where M_t/M_∞ is the fractional release of drug in time t, 'k' is the constant characteristic of the drug–polymer system and 'n' is the diffusion exponent characteristic of the release mechanism. M_t and M_∞ are the amount of drug released at time 't' and at equilibrium respectively.

2.5. Biomedical properties of hydrogel wound dressings

2.5.1. Blood compatibility

The haemocompatibility was evaluated by studying the two types of blood–polymer interactions i.e. thrombogenicity and haemolytic potential. The evaluation of thrombus formation on polymeric surfaces was carried out using a gravimetric method (Imai and Nose, 1972). The haemoglobin release by haemolysis was measured by the optical density (OD) of the supernatant at 540 nm using a UV visible spectrophotometer (dos Santos et al., 2006). All studies were carried out in triplicate.

2.5.2. Antioxidant activity

Oxidative stress and excess free radical production at the wound surface impair wound healing. Different mechanisms (like free radical scavenging and metal chelation) act at different levels, independently or in combination, to bring about the wound healing effects (Akkol et al., 2011). Consequently, in the present studies, antioxidant activity of acacia-*cl*-carbopol hydrogel films was determined by F–C reagent assay, superoxide radical ($O_2^{\bullet -}$) scavenging