SYNTHESIS, CHARACTERIZATION AND EVALUATION OF PH AND TEMPERATURE SENSITIVE HYDROGELS FOR BIOMEDICAL APPLICATIONS

by

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Submitted

In fulfillment of the requirements of the degree of Doctor of Philosophy

To the

INDIAN INSTITUTE OF TECHNOLOGY, DELHI

August 2007

Dedicated

to

My Parents

CERTIFICATE

This is to certify that the thesis entitled **"Synthesis, Characterization and Evaluation of pH and Temperature Sensitive Hydrogels for Biomedical Applications"** being submitted by **Ms. Dipti Singh** to the Indian Institute of Technology, Delhi, for the award of degree of **Doctor of Philosophy** is a record of bonafide research work carried out by her. **Ms. Dipti Singh** has worked under our guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to our knowledge has reached the requisite standard.

The results contained in this thesis are original and have not been submitted, in part or full, to any other University or Institute for the award of any other degree or diploma.

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ABSTRACT

Hydrogels are one of the most promising and versatile materials with enormous possibilities and potentials in the field of biomedical sciences. They have served the mankind since 1970, in the development of soft contact lenses; wound dressing material, drug release devices etc. These polymer systems might recognize a stimulus as a signal, judge the magnitude of this signal and then change their chain conformation in direct response. The stimuli sensitive hydrogels that undergo significant changes with slight changes in environment such as pH, temperature, electric field, ionic strength and combination of these, having a sensor and actuator function are also called intelligent materials.

The main aim of the work was to develop stimuli responsive hydrogels and evaluate their performance for biomedical applications. Thesis is broadly divided in to three parts. First part deals with the synthesis of interpenetrating polymer networks [biodegradable hydrogels] by gamma irradiation technique based on gelatin N-vinyl pyrrolidone: (NVP)/ or NVP- acrylic acid (AA) copolymer. These hydrogels were evaluated as drug delivery matrices using doxorubicin. Second part describes the synthesis and characterization of *N*-isopropylacrylamide (NIPAAm) homopolymer and NIPAAm-poly (ethylene glycol) methacrylate [PEGMA] copolymers by photopolymerization. Evaluation of these hydrogel films for microfluidic devices and as cell culture membranes using Hep G-2 cancer cell lines was also carried out. Third part describes the synthesis of nanoparticles based on NIPAAm and PEGMA (homopolymer and copolymers) for targeted drug delivery system. These injectable devices were evaluated in-vitro using MCF-7 cancer cell lines.

INTERPENETRATING POLYMER NETWORKS

The first part describes the synthesis and evaluation of implantable interpenetrating polymer networks (IPNs) based on NVP: gelatin (Ge) and a copolymer of NVP - acrylic acid (AA): gelatin (Ge) crosslinked using N-N' methylene bisacrylamide (BIS) (0.5, 1% w/w) and glutaraldehyde (GLU) (0.5 % v/v) as crosslinker respectively by gamma irradiation technique. GLU was incorporated after irradiation to crosslink the gelatin chains, whereas BIS was placed in the respective solutions before irradiation. Several samples were prepared by varying the composition of gelatin to NVP or by changing the ratio of NVP: AA in preparing IPNs. Swelling behavior of the hydrogels was investigated as a function of variable doses, crosslinker (BIS) concentration, copolymer composition (NVP: AA ratio) or gelatin: NVP ratio and pH of the immersion medium (3, 7.4 and 11). As expected swelling ratio increased with increasing amounts of acrylic acid and decreased with increasing BIS content. No definite trend in the swelling behavior was observed as a function of dose. The interpenetration of the polymeric chains was established by morphological and thermal characterization. The scanning electron microscopy of IPNs showed a hybrid of coral and honeycomb structures as compared to the honeycomb structure observed in the crosslinked polymers based on gelatin and NVP (G_x and PVP_x).

IPNs prepared were also evaluated for their degradation behavior, biocompatibility, drug loading and release studies. Degradation behavior of the hydrogels was investigated by immersing the samples in phosphate buffer (pH ~7.4) and in citrate borate buffer of pH 3 and 11 at a temperature of 37± 1°C. The degradation behavior was found to be dependent on composition, crosslinker concentration as well as on the pH of the immersion medium.

The degradation pattern showed bulk as well as surface erosion supporting typical behavior of IPNs. Among various IPNs studied, GV(0.07)* and GV(0.14)* [i.e. IPNs prepared at dose of 0.07 and 0.14 Mrad using gelatin : NVP ratio as 1:1 (w/w) with 0.5 % (w/w) BIS] degraded within 11 days in phosphate buffer of pH 7.4. MALDI analysis showed that the degradation products had mass in the range of 200- 1600 g/ mole depicting easy phagocytosis or excretion of degradable materials in-vivo.

Drug loading (carried out by immersing the hydrogels in doxorubicin solution [prepared in water: alcohol (70:30)] and release studies in-vitro were carried out in phosphate buffer (pH 7.4 and 6.4).

Furthermore histo and cytotoxicity was evaluated using Wistar rats and MCF-7 cancer cell lines respectively. Biocompatibility studies showed that in sample GV(0.07)*, inflammation was much less and local compared to IPNs prepared at dose of 0.14 Mrad [GV(0.14)]. Additionally, no fibrosis, capsule formation was found. The sample degraded with a total mass loss within two months. Cytotoxicity studies on MCF-7 cancer cell lines elicited ~93% cell viability with GV(0.07) sample.

TEMPERATURE SENSITIVE HYDROGELS BY PHOTOPOLYMERIZATION

The second part describes the synthesis, characterization and evaluation of temperature sensitive NIPAAm homopolymers and its copolymers with PEGMA. Photopolymerization of NIPAAm monomer and NIPAAm-PEGMA using water (homogeneous conditions at ~7°C and heterogeneous conditions at ~40°C) or a mixture of water/ethanol (50/50, heterogeneous at ~7°C) as solvent was carried out using 1-[4-(2-hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one (hydrophilic) or 2-hydroxy-2-methyl propiophenone (hydrophobic) photoinitiator. In order to investigate the

effect of temperature and cross-link density, polymerization was carried out at ~7°C [below Lower Critical Solution Temperature (LCST)] and ~40°C [above LCST] using varying amounts of *N,N'*-methylene bisacrylamide (BIS) ranging from 1 – 4 % (w/w). Hydrogels prepared at ~7°C using hydrophobic photoinitiator and water/ethanol (50/50) as solvent, showed much higher degree of swelling at all levels of cross-link density as compared to hydrogels prepared at ~7°C using hydrophilic photoinitiator and water as solvent. LCST determined by optical microscopy and DSC was found to be dependent on solvent system (homogeneous/heterogeneous), temperature of polymerization and cross-link density. Homopolymers showed large effect of reaction parameters on morphology. NIPAAm homopolymers were also used for patterning which may find applications in microfluidic devices.

Copolymerization of NIPAAm with varying amount of PEGMA (ranging from 1-20% w/w) was also carried out using similar conditions as mentioned for homopolymers. NIPAAm-PEGMA copolymer showed increased swelling ratio with increasing PEGMA content. LCST increased from 35 to 39 °C as PEGMA content in copolymers increased from 1- 20 % (w/w). Morphology of copolymers was found to be independent of the reaction conditions. Copolymer films having an optimum combination of swelling and performance properties were evaluated as switchable cell culture membranes. Hepatic cancer cell lines (Hep G-2) was used to study the cell growth and detachment. Cell growth and detachment were found to be dependent on the copolymer composition. Cell viability was found comparable to trypsin which also supports application of these films as cell culture membranes.

PREPARATION OF NANOHYDROGELS

The third part describes the synthesis and characterization of temperature sensitive nanoparticles for cancer targeting. Nanoparticles having core-shell morphology were prepared by copolymerizing N-isopropylacrylamide with varying amounts of poly(ethylene glycol) methacrylate (PEGMA) of molecular weight 526 g/mol. The nanoparticles were synthesized by dispersion polymerization using ammonium persulfate as an initiator at 80 ^{0} C (much above the LCST). The effect of reaction conditions i.e. temperature, concentration of initiator, monomer feed composition and time of polymerization was investigated on the particle size, particle size distribution and morphology. Particle size and size distribution was determined using dynamic light scattering method. Stability of emulsions was also established. Particle size decreased with increasing amounts of PEGMA in the feed and increased with increasing initiator amount.

The polymeric particles having an optimum particle size and LCST close to body temperature were further modified by preparing folic acid conjugates and loaded with doxorubicin. The in-vitro studies on drug release and cytotoxicity was performed on MCF-7 cancer line. Microscopic studies showed more uptake of particles after conjugation with folic acid by MCF-7 cancer cell lines. The proposed approach provides a better and more effective site-specific method of active targeting to improve the efficacy of highly cytotoxic anticancer drugs.

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