Degradable Thermo-responsive Polymeric Hydrogel Biomaterials as Non-Invasive Cell & Drug Delivery Vehicles for Retinal Degenerative Diseases

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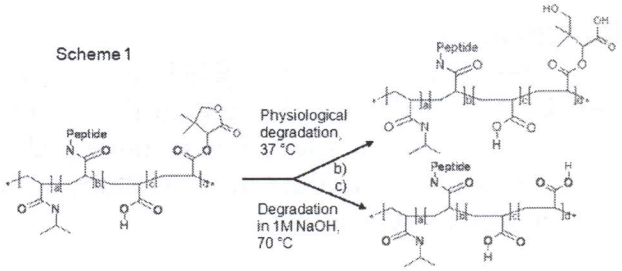
Introduction

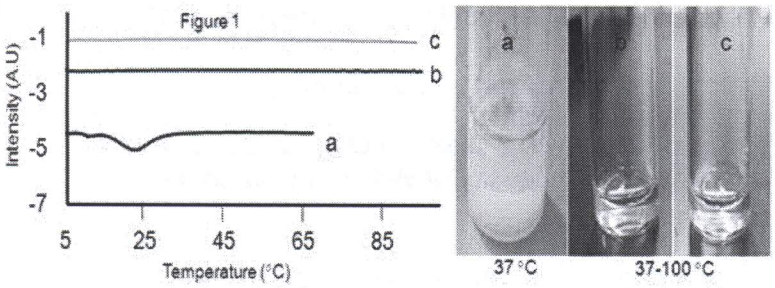
Recent advances in cell-based therapies have demonstrated tremendous potential in combating retinal degenerative diseases. However, delivery of therapeutic cells and/or drugs into the delicate subretinal space is a particularly challenging task. Hydrogels, since their development by Wichterle and Lim[1], have generated significant interest as drug/ cell delivery scaffolds. Currently they represent one of the most suitable methods of delivering drug/cells to tissues due to their good compatibility with blood, body fluids and tissues[2]. Hydrogels are also attractive due to their potential for changes in shape and volume under such environmental stimuli as pH, temperature, the presence of enzymes and electrical field[3]. Of these, significant research has been conducted on temperature sensitive hydrogels particularly on poly(N-isopropylacrylamide) gels (pNIPAAm)[4] as they are capable of gelation by a simple alteration of temperature. There is no need for chemical /photo-crosslinking that may raise a cytotoxcity issue. In addition, thermo-responsive hydrogels may be easily delivered *in vivo* using procedures such as minimally invasive surgery[5]. However, the major disadvantage of pNIPAAm hydrogels is their non-degradability. Ideally a delivery scaffold should degrade over the time and be cleared from the body. In this work, several biodegradable thermo-responsive, cell adhesive amine reactive pNIPAAm based hydrogel scaffolds have been synthesized, which should clear from the body after degradation upon changing the confirmation of co-monomer without loss of components. Their solution properties and potential application in the non-invasive delivery of retinal pigment epithelial (RPE) cells and model protein/drugs into the subretinal space will be discussed.

Materials and Methods

Copolymers of N-isopropylacrylamide (NIPAAm), N-acryloxysuccinimide (NAS), acrylic acid (AA) and acryloyloxy dimethyl butyrolactone (DBA), denoted as pNNAD, were synthesized via free radical polymerization in 1, 4- dioxane using benzoyl peroxide (BPO) as an initiator. The resultant pNNAD were modified with RGDS to examine potential application of these polymers as temporary cell scaffolds for retinal therapy.



The structural and chemical compositions of the resultant copolymers were determined by FT-IR and ^{1}H NMR respectively. Gel permeation chromatography (GPC) was used to determine the molecular weight (MW) of hydrolysed copolymers derived from individual polymeric materials. Accelerated partial and complete hydrolysis of the copolymers was performed following ISO 10993[6] (Scheme 1). Time dependent degradation rates of the copolymers were examined by observing change in mass as a function of time when samples were incubated in PBS (pH 7.4) at 37 °C.



Phase transition properties (LCST) of different intact and degraded copolymers were characterized by DSC, turbidity testing and by assessing gelling kinetics (Figure 1). Water content of these hydrogels was measured gravimetrically.

Drug release kinetics were observed by loading copolymers with bovine serum albumin (BSA) as a model protein and dextramethasone as a model drug while incubating in PBS at 37 °C. The structural feature of various hydrogels as a function of degradation time was visualized using scanning electron microscopy (SEM). RPE cell compatibility was tested with these polymeric scaffolds.

Results

The composition of all copolymers closely mirrored the co-monomer feed ratios, confirmed by ^{1}H NMR (Figure 2). It showed that LCST of all copolymers strongly influenced by DBA content, and were found to be below physiological temperature, thus allowing the delivery of a liquid suspension of cells that gels *in situ* to form a scaffold. Turbidity testing confirmed DSC findings and gave insight into phase transition behaviour. The modified thermo-responsive PNIPAAm scaffolds demonstrated rapid gelling kinetics, which is essential for localization of treatment. Upon optimum partial and complete degradation, there was no observable phase transition between 0-100 °C. RPE cells demonstrated excellent viability when cultured within the bulk matrices.

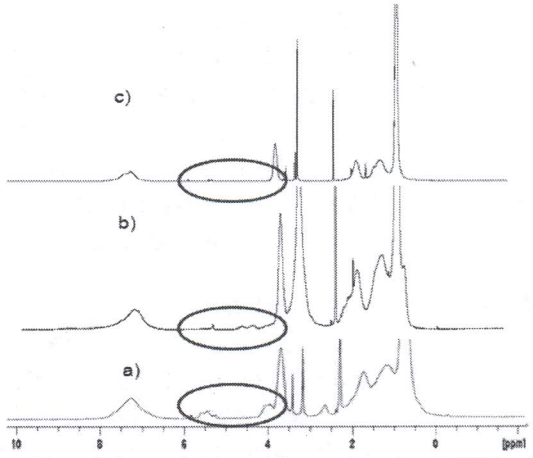


Figure 2: Sequential degradation confirmed by 1H NMR

Conclusions

Degradable thermo-responsive pNIPAAm based cell and drug carrying biomaterials offer a relatively non-invasive means to deliver therapeutic drug and cells into the subretinal space and provide adhesion-dependant RPE cells with an artificial extracellular matrix for temporary support. These degradable polymeric scaffolds can be used as solid drug reservoirs delivered into the vitreous using minimally invasive techniques and slow degradation kinetics should allow long-term delivery, reducing the frequency of treatment.

References:

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