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Responsive hydrogels produced via organic sol–gel chemistry for cell culture applications

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ABSTRACT

In this study, we report the synthesis of novel environmentally responsive polyurea hydrogel networks prepared via organic sol–gel chemistry and demonstrate that the networks can stabilize pH while releasing glucose both in simple aqueous media and in mammalian cell culture settings. Hydrogel formulations have been developed based on the combination of an aliphatic triisocyanate with pH-insensitive amine functional polyether and pH-sensitive poly(ethyleneimine) segments in a minimally toxic solvent suitable for the sol–gel reaction. The polyether component of the polyurea network is sufficiently hydrophilic to give rise to some level of swelling independent of environmental pH, while the poly(ethyleneimine) component contains tertiary amine groups providing pH sensitivity to the network in the form of enhanced swelling and release under acidic conditions. The reaction of these materials to form a network is rapid and requires no catalyst. The resultant material exhibits the desired pH-responsive swelling behavior and demonstrates its ability to simultaneously neutralize lactic acid and release glucose in both cell-free culture media and mammalian cell culture, with no detectable evidence of cytotoxicity or changes in cell behavior, in the case of either SA-13 human hybridomas or mouse embryonic stem cells. Furthermore, pH is observed to have a clear effect on the rate at which glucose is released from the hydrogel network. Such characteristics promise to maintain a favorable cell culture environment in the absence of human intervention.

1. Introduction

The in vitro culturing of primary tissues or established cell lines is a well-characterized technique widely used in biomedical laboratories and pharmaceutical industries worldwide. Precise control of the cell culture environment allows for the regulation of survival and growth of different cell populations, and for directing specific differentiation programs. Cell culture studies have greatly increased the understanding of cellular functions and complex signaling pathways, have enabled the production of therapeutic proteins, vaccines and biopolymers, and have been routinely used for toxicity screening of new compounds. All cell culture studies hinge on the capacity to maintain a suitable cell culture environment. Cell culture media are therefore formulated to provide optimal nutrition and physiological parameters to support the growth of specific types of cells [1]. Such media provide for cell growth only so long as optimal pH and nutrient levels are maintained and the build-up of toxic concentrations of cellular metabolites is prevented. Indeed, all cells consume nutrients from the media (e.g. glucose) as they proliferate and simultaneously release metabolic waste (e.g. lactic acid) [2]. Unchecked, acidic waste rapidly decreases pH, and although most media contain buffers to minimize changes in pH, weak buffers such as bicarbonate are only effective over short periods (e.g. 24–48 h) and stronger buffers such as N-(2-Hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid (HEPES) can be toxic to certain cultured cells [3]. Nutrients such as glucose and glutamine may rapidly be exhausted over similar time periods, loss of which can cause multiple cell types to undergo apoptosis [4–6]. The most common solution is to replenish the environment by serial passage into fresh culture medium. While this is easily managed in small cultures, it becomes a severe problem in large-scale culturing operations, such as those used in bioreactors for the production of bioreactive compounds [7]. Likewise, even short-term culturing of cell lines requiring serum-free media (i.e. for the production of human therapeutics, human embryonic

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stem cell research, etc.) can be problematic [8], and adds additional costs due to the expensive components required to replace those contained in animal serum. Small fluctuations in environmental conditions can profoundly affect cellular functions, including growth, viability, productivity and differentiation (particularly in the case of stem cells) [6,9]. Regular medium changes also increase the likelihood of contamination, compromising results, increasing costs and risking loss of cell lines. Laboratory automation technologies such as bench-top bioreactors can help, but media conditions still fluctuate, and their cost may be prohibitive for small-scale cultures [10]. Continuous-flow bioreactors have also been designed to provide continuous supplies of nutrients and limit pH changes during long-term cell expansion, but such systems require complicated monitoring and injection systems too costly for most academic laboratories. Genetic engineering has been used to sustain higher cell densities in the absence of cell death, but such manipulations are time consuming, labor intensive and impractical for the production of biotherapeutics [11–13]. As a result, nutrient supplementation remains the preferred means of maintaining cell viability, productivity and differentiation characteristics in most cell culture applications.

To address this issue, we describe the preparation of hydrogels designed to prolong optimal culturing conditions in the absence of intervention. Hydrogels consist of a polymer network swollen in an aqueous solution without loss of its three-dimensional structure, thanks to the presence of chemical or physical crosslinks. As their name implies, hydrogels possess a high water content [14], with control of their composition and structure allowing for tunable swelling kinetics and the ability to respond to external stimuli such as temperature, pH and ionic strength [15]. Relevant to cell culture, the ability of well-designed hydrogels to controllably entrap and release small molecules (e.g. nutrients or metabolic waste) in a responsive fashion makes them an attractive solution to the problem of sustaining cell viability and productivity for periods longer than 24–48 h.

Hydrogels are one of the most extensively researched materials for delivery and release systems [16]. Most applications take advantage of specific hydrogel characteristics such as transport properties, thermal sensitivity and biodegradability. In the context of this work, we report the synthesis and swelling behavior of a novel polyurea hydrogel composed of a combination of pH-insensitive polyether segments and pH-sensitive polyimine segments. The presence of these segments in the hydrogel network gives rise to pH-dependent swelling and release behavior, as well as the ability to simultaneously take up the contents of any solution it swells in. The hydrogel networks described here are engineered to contain alkaline (amine) groups that preferentially absorb acidic molecules, thereby helping to maintain pH within an optimal range (typically 7.0–7.7), although some cell lines have been reported to tolerate levels as low as 5.5 [17,18]. By loading the hydrogels with a nutrient such as glucose, the hydrogels can be designed to help maintain nutrient levels as they are being consumed by the cells. The desired behavior of these hydrogels is shown schematically in Fig. 1.

While there are many ways to prepare polymer networks and hydrogels, we use as a basis for the efforts described here an organic sol–gel method our group has previously developed and applied in a range of contexts [19–26]. Here we focus on polyurea network formation because of the robust and versatile nature of these polymers, coupled with the fact that the kinetics of the exceptionally fast isocyanate–amine reaction are moderated in solution, allowing for rapid but controlled formation of homogeneous networks at room temperature and in the absence of other additives. Likewise, the isocyanate groups are readily deactivated by water to form amines plus carbon dioxide. These two characteristics minimize the possibility of toxic extractables and/or unwanted residual functional groups. In addition, the sol–gel technique enables the use of solid prepolymers without heating, and is able to substantially suppress phase-separation of the multiple network components that might otherwise occur in the neat state, thus ensuring the formation of a homogeneous material. This report describes the generation of a prototype responsive polyurea network via organic sol–gel chemistry and its potential to increase the time that optimal cell culture conditions can be maintained without human intervention, and creates a basis for the design of a range of networks tailored to the specific needs of various cell culture applications.

2. Experimental section

2.1. Materials preparation

Hydrogel networks were prepared based on amine-terminated poly(propylene oxide (PO)-*block*-ethylene oxide (EO)-*block*-propylene oxide) (Jeffamine ED-2003, 2000 g mol^{-1}, 39:6 EO:PO molar ratio, moisture content $\leqslant$ 0.35% as received, donated by Huntsman Corporation, The Woodlands, TX) and poly(ethyleneimine) (M_n = 10,000 g mol^{-1}, M_w = 25,000 g mol^{-1}, primary/secondary/tertiary amine ratio = 25/50/25, moisture content $\leqslant$ 1% as received, Polysciences, Warrington, PA), referred to generically as PEO and PEI segments, respectively, and shown in Fig. 2A and B. The primary and secondary amine groups in this PEO/PEI mixture were reacted with the isocyanurate of hexamethylene diisocyanate (Desmodur N3300A, donated by Bayer Materials Science, Pittsburgh, PA; shown in Fig. 2C) in acetone (extra dry, Acros Organics, Morris Plains, NJ). All materials were used as received, with special care taken to minimize moisture uptake by the network forming components. In particular, when not in use, the PEO and PEI components were stored in a Labconco fiberglass vacuum desiccator cabinet with attached vacuum gauge connected to a Welch DryFast Ultra Chemical Duty dry vacuum pump (model 2031B-01, 32 l min^{-1}, 2 torr ultimate vacuum). The cabinet was kept under vacuum when closed, with the relative humidity minimized using a mixture of indicating and non-indicating 10–20 mesh Drierite (W. A. Hammond) and additionally confirmed via a 10–60% relative humidity card (W. A. Hammond) affixed to the inside of the door. Finally, the Desmodur N3300A was purged with ultrahigh-purity nitrogen (Airgas) and the cap sealed with laboratory sealing film following each use.

In a typical reaction, the amounts of prepolymer (PEO and PEI), crosslinker, and solvent used were calculated to ensure a total solution volume of 30 ml, a total solids content of 10% (w/v), equal masses of PEO and PEI, and a 1:1 M ratio of isocyanate to (primary and secondary) amine groups. Given the rapidity of the network formation reaction, separate solutions of both prepolymers (PEO and PEI) and of the isocyanate crosslinker were prepared and, following complete dissolution of all components, combined and mixed rapidly in a 60 ml straight-sided polypropylene jar, which was then sealed. Gelation occurred in a matter of minutes, but the reaction vessel was allowed to stand for approximately 24 h at room temperature. Subsequently, the vial was then opened and the solvogel was allowed to dry in a fume hood. Following the loss of the majority of the solvent (as confirmed through monitoring of the sample mass over time), the material was further dried in a Fisher Isotemp 281A vacuum oven at 50 °C under vacuum using a Welch DryFast Ultra Chemical Duty dry vacuum pump (model 2031B-01, 32 l min^{-1}, 2 torr ultimate vacuum) for 12 h to yield a fully dense, solvent-free xerogel, consisting of a polyurea based PEO/PEI network (formation reaction shown in Fig. 2D). Alternatively, to obtain consistent sample size and constant surface to volume ratio, the same procedures were followed but the