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Targeting homeostasis in drug delivery using bioresponsive hydrogel microforms

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Succinyl-alanyl-alanyl-prolyl-phenylalanine-4-nitroanilide (PubChem CID: 5496888)
4-Nitroaniline (PubChem CID: 7475)
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (PubChem CID: 2723939)
Hydroxy-2,5-dioxopyrrolidine-3-sulfonicacid sodium salt (PubChem CID: 23697313)

ABSTRACT

A drug delivery platform comprising a biocompatible, bioresponsive hydrogel and possessing a covalently tethered peptide–drug conjugate was engineered to achieve stasis, *via* a closed control loop, of the external biochemical activity of the actuating protease. The delivery platform contains a peptide–drug conjugate covalently tethered to the hydrogel matrix, which in the presence of the appropriate protease, was cleaved and the drug released into the bathing environment. This platform was developed and investigated *in silico* using a finite element modeling (FEM) approach. Firstly, the primary governing phenomena guiding drug release profiles were investigated, and it was confirmed that under transport-limited conditions, the diffusion of the enzyme within the hydrogel and the coupled enzyme kinetics accurately model the system and are in agreement with published results. Secondly, the FEM model was used to investigate the release of a competitive protease inhibitor, MAG283, *via* cleavage of Acetyl-Pro-Leu-Gly|Leu-MAG-283 by MMP9 in order to achieve targeted homeostasis of MMP-9 activity, such as in the pathophysiology of chronic wounds, *via* closed-loop feedback control. The key engineering parameters for the delivery device are the radii of the hydrogel microspheres and the concentration of the peptide–inhibitor conjugate. Homeostatic drug delivery, where the focus turns away from the drug release rate and turns toward achieving targeted control of biochemical activity within a biochemical pathway, is an emerging approach in drug delivery methodologies for which the potential has not yet been fully realized.

1. Introduction

Hydrogels are polymers synthesized from highly hydrophilic monomers and/or pre-polymers into networks that imbibe large amounts of water, are passively biocompatible due to their favorable interaction with extracellular matrix (ECM) proteins, and are highly versatile in their range of possible biotechnical applications that exploit their swelling and de-swelling dynamics (Ottenbrite et al., 2010). Because of these characteristics, hydrogels have been extensively studied for a wide range of biomedical applications that include controlled drug delivery (Qiu and Park, 2012; Servant et al., 2012; Siepmann and Peppas, 2012); tissue engineering and regenerative medicine (Hoffman, 2012; Nicodemus and Bryant, 2008); and diagnostic biomedical biosensors (Kotanen et al., 2012a,b; Pistol et al., 2011; Viter et al., 2011). Hydrogels have also evolved over the years through several generations wherein increased conferred functionality, control of polymer architecture and improved processing has enabled new and innovative applications resulting in the moniker of "smart materials". Among

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these smart applications is one wherein the ability to sense and react with a biological entity is integrated with the physicochemical responses of the hydrogel (Bhat et al., 2013; Chung et al., 2008). Biologically responsive, or bioresponsive, hydrogels operate by first sensing the chemical activity of a biological specie in its environment, through catalysis or binding, and then couples the biomolecular recognition to transduction of that information with a response that seeks to restore equilibrium or a pseudo-steady state within the hydrogel (Wilson and Guiseppi-Elie, 2012). In this sense, they are Generation 2 drug release platforms that supersede Generation 1 drug release platforms, which rely on loading and passive release of a drug from an engineered microform. Of particular interest is the use of micro- and nano-hydrogels to sense and respond to spatiotemporal perturbations of biological activity as this enables the development of therapeutics and diagnostics at molecular and cellular length scales. Such systems are well described and are actively pursued in the literature. The development of drug delivery systems that are based on self-regulation and that are made responsive to an externally derived or environmentally based biological activity that dictates the release rate of the drug has long been a goal of drug delivery designers (Sanchez and Peppas, 2010; Wilson et al., 2012). This is particularly relevant in glucose-responsive insulin delivery, wherein the rate of release of insulin may be governed by the bathing concentration of glucose, analogous to an all synthetic artificial pancreas (Guiseppi-Elie et al., 2002). Recently, an *in vivo* demonstration of this objective that uses the glucose(sense)–insulin(deliver) system was reported; however, engineering control of key delivery parameters of such systems are unexplored (Miyata et al., 2009).

Generation 3 drug delivery systems go beyond environmental control of the release rate to focus attention on achieving physiological stasis of the biological specie that is the target of drug action. Self-regulated delivery systems imply a nascent ability to change release profile pursuant to the amount of or extent of release. Generation 3 systems use the released drug to modulate the activity of the actuating biological factor. In this sense, it is distinguishable from self-regulating systems whereupon the release kinetics are controlled by the environmental conditions. The Generation 3 drug delivery system actually controls the environmental conditions and seeks to achieve homeostasis, that is, it targets achievement of a particular activity of the actuating biological specie within the environment that had elicited the release response in the first place. This requires the implementation of feedback control.

The information flow from sensing to response in Generation 2 bioresponsive hydrogels often involves dynamical concomitant perturbations such as chemical reactions (Guiseppi-Elie et al., 2002) or binding events (Miyata et al., 2009); diffusive, convective and/or migratory transport of species (Kang et al., 2008; Li et al., 2005); mechanical deformation (Yoo and Mitragotri, 2010); optical density changes (Zhao et al., 2010); and redox state changes (Wilson et al., 2010). Due to the high interdependency of such changes within bioresponsive hydrogels, explicit solutions derived from constitutive mathematical relations are invariably difficult and more often unresolved. Thus, the approach to engineering and optimization of bioresponsive hydrogels is commonly *in vitro* or *in vivo* experimentation coupled with mathematical simplifications for analysis. This approach, while effective, can be time intensive and may result in suboptimal systems. Fortunately, the exponential increase in computational power over past decades is enabling numerical resolutions to constitutive relations, which were previously inaccessible (Voller and Porte-Agel, 2002). The coupling of finite element modeling (FEM) with bioresponsive hydrogels to investigate previously difficult or inaccessible relations has and will continue to be a robust and fruitful area of research (Galdi and Lamberti, 2012; Plontke et al., 2007; Villalobos et al., 2006). The complexity of the models has scaled in parallel to increasing transistor density and has offered insights into these systems. Initially, due to limited computational power, only uni-physical models were explored, such anisotropic drug transport in complex geometries (Wu and Zhou, 1998; Zhou and Wu, 1997). However, complete multiphysics models are currently being developed *in silico* to inform the engineering of bioresponsive hydrogels (Vernerey et al., 2012).

To this point, an *in silico* model of enzymatically mediated release from a bioresponsive hydrogel was developed and is presented. The three-step process of release occurs by (1) diffusion of an enzyme to and into a hydrogel matrix, (2) enzymatic cleavage of a tethered peptide–prodrug conjugate that is a substrate specific to the transported enzyme, and (3) subsequent diffusive release of the active drug into the local environment. *In vitro* variations of enzyme mediated release include the use of pendent or polymer-backbone integrated substrates (Chau et al., 2004; Kopeček, 2010), degradation or gelation of the hydrogel matrix (Fu and Kao, 2010; Wang et al., 2010), and dynamic swelling or collapsing of the hydrogel matrix (Liu et al., 2012; Zhang and Wu, 2002). This work explores a non-degradable bioresponsive hydrogel with pendant peptide substrate–prodrug which when cleaved, activates and releases an active drug. A non-degradable formulation was selected as a first premise to allow investigation of the interrelated transport with reaction and feedback control without the additional complexity of bio-erosion. The model was validated by direct comparison of its results with previously published *in vitro* results achieved for the release of a model chromogen, para-nitroaniline, by the enzyme chymotrypsin for bioactive peptide hydrogels (Wilson et al., 2012).

Furthermore, the utility of the model was demonstrated by the design and optimization of a drug delivery platform that focuses on achieving stasis in the biological activity of the external enzyme that is inhibited by the released active drug. This platform requires the implementation of, in addition to the above three steps, closed-loop feedback control that couples the release of the drug with the activity of the sense enzyme. The foregoing is a requirement for Generation 3 classification. The particular implementation was parameterized for the release of a competitive protease inhibitor, MAG283, *via* cleavage of Acetyl-Pro-Leu-Gly|Leu-MAG-283 by MMP9 in order to achieve targeted homeostasis of MMP-9 activity *via* closed-loop feedback control. This is relevant for the pathophysiology of chronic wounds where normal metabolic activity has been elevated to levels incompatible with acute wound healing. It is estimated that 1–2% of the population will suffer from a chronic wound in their lifetime (Gottrup, 2004). In 2009 it was estimated that chronic wounds affect 6.5 million patients and the annual treatment cost is estimated to be $25 billion. Compounding the impact on society are disabilities, such as limb amputation, and loss of wages, estimated at $39 billion, that result from the inability to quickly and appropriately treat the disease to mitigate these problems (Sen et al., 2009). The developed model identified key engineering parameters for the delivery platform, which could address a chronic wound environment. Homeostatic drug delivery, where the focus is no longer just using drug release rate to achieve targeted control of biochemical activity within a biochemical pathway, represents a new paradigm in controlled drug delivery and may be called Generation 3 Drug Delivery Devices (Wilson and Guiseppi-Elie, 2012).

2. Methods

2.1. Modeling approach and setup

The *in silico* model was designed to match the physical, chemical, and reactive properties of a previously synthesized *in vitro* bioresponsive hydrogel (Wilson et al., 2010, 2012). Briefly, the