Osmotic Release of Bioactive VEGF from Biodegradable Elastomer Monoliths Is the Same *In Vivo* As *In Vitro*

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Received 4 January 2011; revised 14 February 2011; accepted 16 September 2011

Published online 4 October 2011 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.22782

ABSTRACT: The feasibility of generating an extended period of linear release of therapeutic proteins from photo-cross-linked, biodegradable elastomer monolithic devices *in vitro* has been previously demonstrated. The release is driven primarily by the osmotic pressure generated upon the dissolution of the encapsulated particles within the polymer. The osmotic pressure is provided by co-incorporation into the particle of trehalose as an osmotigen. Herein, we demonstrate that the release rate of a therapeutic protein, vascular endothelial growth factor (VEGF), by this osmotic pressure mechanism is the same *in vivo* as found *in vitro*. ^{125}I-VEGF was colyophilized with trehalose and serum albumin and distributed as particles throughout a photo-cross-linked elastomer composed of trimethylene carbonate, ε-caprolactone, and D,L-lactide. The release of VEGF from the device was monitored by measuring the decrease in radioactivity within the devices *in vitro* and within explanted devices that had been implanted subcutaneously in the dorsal area of Wistar rats. The released VEGF remained bioactive *in vivo*, inducing the formation of blood vessels that contained red blood cells. Furthermore, the released trehalose was well tolerated by the surrounding tissue. © 2011 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:588–597, 2012

Keywords: vascular endothelial growth factor; *in vitro–in vivo* correlation; osmotic pressure release; degradable elastomer; angiogenesis; protein delivery; biodegradable polymer; osmosis; controlled release

INTRODUCTION

Ischemic heart disease is a leading cause of mortality. In this condition, arteries feeding the heart have become occluded, typically because of plaque deposition and/or thrombus formation.[1] Left untreated, myocardial ischemia can lead to arrhythmias, myocardial infarction, heart failure, and/or death. There are currently a number of treatment options, including drugs such as beta-blockers and nitrates, angioplasty with or without stenting, and bypass grafting surgery. Drugs only slow down the progression of the disease, and angioplasty and stenting are not always an option for a significant population of patients in whom the pathological condition is widespread or inaccessible. For patients with extensive multivessel coronary artery disease, bypass grafting is recognized as the treatment of choice, and up to 300,000 Americans have this procedure each year.[2] However, in 25% of these patients, grafting may not be completely successful because of the existence of ungraftable arteries that supply a viable, but underperfused, tissue area.[3] As a result, they have continuing symptoms of angina after surgery and a decreased survival rate. For these patients, a potentially useful strategy is to combine bypass grafting with localized growth factor delivery to generate new blood microvessels in the ischemic tissue that cannot be treated through grafting alone.[4]

Therapeutic angiogenesis can be accomplished through the controlled release of various growth factors, most notably the vascular endothelial growth factor (VEGF) family.[5] A variety of polymer device approaches have been examined for VEGF delivery. These include semisolid poly(ortho esters),[6] and poly(lactide-glycolide) (PLG) microspheres,[7] nanospheres,[8] and porous matrices.[9] A large problem with poly(ortho esters) and PLG delivery systems is maintenance of protein stability.[10] When these polymers degrade, they generate acidic oligomers and monomers. The presence of these acidic compounds

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Journal of Pharmaceutical Sciences, Vol. 101, 588–597 (2012)

decreases the local pH at the surface of the polymer and in the pores and channels of the device.[11] Such acidic degradation products have been implicated in the denaturation of VEGF[6,7,12] and may cause tissue inflammation surrounding the implant, depending on the rate of polymer degradation.[13] Hydrogels have also been explored for VEGF delivery.[14–19] Disadvantages of hydrogels include low VEGF encapsulation yields, a rapid and significant initial burst, and short release durations (typically 4–7 days).

We have explored the potential of osmotic pressure driven release from biodegradable elastomer monoliths prepared through photo-cross-linking for VEGF delivery. The osmotic pressure release mechanism is governed by the osmotic activity of the embedded particles and the mechanical properties of the elastomer (Fig. 1).[20] This release mechanism can provide a linear and sustained release of bioactive growth factors for a period of longer than 2 weeks with a low initial burst.[22] Photo-cross-linked elastomers have been prepared from terminally acrylated *star*-poly(ε-caprolactone-D,L-lactide), *star*-poly(trimethylene carbonate-ε-caprolactone), and *star*-poly(trimethylene carbonate-D,L-lactide) and found to degrade effectively *in vivo* without inducing an extensive inflammatory response.[23–25] Photo-cross-linked elastomers made of a terpolymer prepolymer of terminally acrylated *star*-poly(trimethylene carbonate-ε-caprolactone-D,L-lactide) with a molar composition of (50:25:25) and number average molecular weight of 4000 Da were able to release highly bioactive VEGF for over 2 weeks *in vitro*.[26] However,

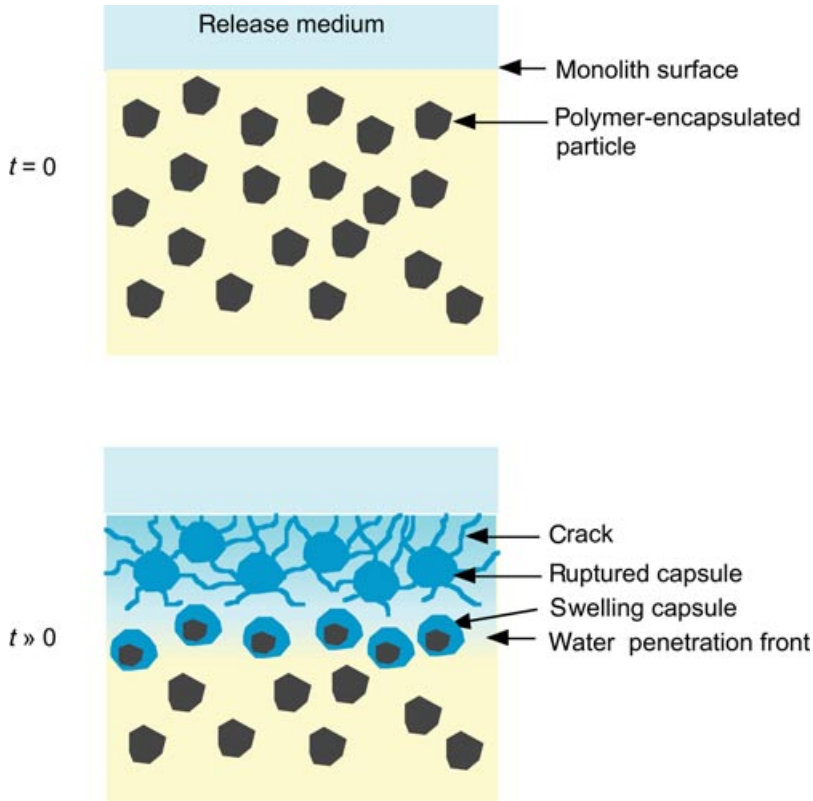

Figure 1. Schematic of the osmotic pressure driven release mechanism. The particles are initially distributed as discrete entities throughout the polymer matrix at a volumetric loading level of less than 30%. Upon implantation of the device, water absorbs into and diffuses through the polymer until it reaches a polymer-encapsulated particle. The water dissolves the particle, generating a saturated solution. Water is then drawn into the capsule formed under the reduced water activity gradient. A pressure is generated in the capsule, equal to the osmotic pressure of the solution. This pressure pushes against the surrounding polymer, which resists until it eventually cracks. The cracks formed propagate throughout the matrix, forming an interconnected network of channels that ultimately reach the device surface. The polymer contracts under the reduction in pressure, forcing the dissolved drug solution through the channel network. The process repeats itself in a particle layer-by-layer fashion, moving toward the center of the device. Once the center of the device has been reached and all those particles whose encapsulating polymer thicknesses are thin enough to allow crack formation have been connected to the network, the remaining dissolved solute in the crack network is released by diffusion.[20,21]