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Local release of masitinib alters *in vivo* implantable continuous glucose sensor performance

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ABSTRACT

Continuous glucose monitoring (CGM) sensors are often advocated as a clinical solution to improve long-term glycemic control in the context of diabetes. Subcutaneous sensor inflammatory response, fouling and fibrous encapsulation resulting from the host foreign body response (FBR) reduce sensor sensitivity to glucose, eventually resulting in sensor performance compromise and device failure. Several combination device strategies load CGM sensors with drug payloads that release locally to tissue sites to mitigate FBR-mediated sensor failure. In this study, the mast cell-targeting tyrosine kinase inhibitor, masitinib, was released from degradable polymer microspheres delivered from the surfaces of FDA-approved human commercial CGM needle-type implanted sensors in a rodent subcutaneous test bed. By targeting the mast cell c-Kit receptor and inhibiting mast cell activation and degranulation, local masitinib penetration around the CGM to several hundred microns sought to reduce sensor fibrosis to extend CGM functional lifetimes in subcutaneous sites. Drug-releasing and control CGM implants were compared in murine percutaneous implant sites for 21 days using direct-wire continuous glucose reporting. Drug-releasing implants exhibited no significant difference in CGM fibrosis at implant sites but showed relatively stable continuous sensor responses over the study period compared to blank microsphere control CGM implants.

1. Introduction

Nearly 350 million people (5% of the global population) suffer from diabetes (Danaei et al., 2011), including the 25.8 million Americans (8.3% of the population) who require regular glucose monitoring. Tight regulation of blood glucose has been convincingly shown to reduce diabetes morbidity and mortality (Coster et al., 2000), leading to a standard of care that demands intensive glucose monitoring. While most glucose monitoring involves painful, inconvenient finger sticks to extract blood, subcutaneous continuous glucose monitoring (CGM) sensors have been clinically available since 1999 as an alternative (Bode et al., 1999; Chase et al., 2001; Ludvigsson and Hanas, 2003; Tanenberg et al., 2004).

Currently, three commercial subcutaneous CGM systems are approved for patient self-implantation and marketed with "real-time" glucose reporting every 1–5 min, and with alarm functions for hypo- and hyperglycemia (Liao et al., 2008; Wilson and Zhang, 2010). All three FDA-approved CGMs are needle-type subcutaneous designs: Freestyle Navigator™ (Abbott Diabetes Care, Alameda, USA), Guardian Real-Time™ (Medtronic MiniMed, Northridge, USA) (Jungheim et al., 2001; Koschwanez and Reichert, 2007; Wilson and Hu, 2000), and Dexcom G4 Platinum™ (Dexcom, San Diego, USA). Most commonly measure glucose *in situ* amperometrically *via* the classic Clark glucose oxidase reaction [4–6], Abbott's Freestyle Navigator™ uses the wired enzyme principle (Feldman et al., 2003). These sensors often require multiple calibrations per day, and their signal generation often requires transport of either tissue glucose alone or both glucose and oxygen to the electrode buried within the needle sensor membrane in order to produce the essential amperometric sensing redox chemistry. Physiological or pharmacological interference with either reliable glucose or oxygen transport, or with the redox chemistry proves challenging to reliable CGM sensor calibration and glycemic reporting.

Among several clinical CGM interferents, the host foreign body

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response (FBR) to the implanted sensor remains most clinically problematic, limiting approved human CGM use to several days once implanted. The FBR involves a complex set of cellular reactions and cytokine cascades at the implant site. Initially, the acute host response is essentially a normal wound healing response to address the wound created by implant placement. This immediate inflammatory response around the CGM is believed to produce confounding influences on glucose response until the acute local tissue reaction subsides to steady state – a phenomenon called "burn in" (Gifford et al., 2006; Wilson and Zhang, 2010; Wisniewski et al., 2000). However, without implant removal or complete degradation, this acute response to the implant transitions to a chronic inflammatory response that no longer resembles wound healing; instead, the enduring tissue response has distinct features, including release of inflammatory cytokines IL-4 and IL-13 that accelerate recruitment of inflammatory and immune cells to the implant site, and their consequent activation *in situ* (Anderson et al., 2008), formation of unique foreign body giant cells, and finally, various fibroblasts that deposit excess collagen and matrix proteins (Anderson et al., 2008; Frost and Meyerhoff, 2002; Godek and Grainger, 2009; Miller et al., 1989; Murch et al., 1982; Wilson and Gifford, 2005). The endpoint of this chronic host response is a fibrous sheath that surrounds the implant, many tens to hundreds of microns thick and largely avascular (Gerritsen et al., 1999; Wilson and Gifford, 2005; Wisniewski et al., 2000). This physical collagenous barrier formation (shown in Fig. 1) frequently hinders analyte transport between host tissue and the CGM, limiting the sensing reliability and functional lifetime of this device in subcutaneous sites (Anderson et al., 2008).

As a result of the host-implant response and its intrinsic variations across patient populations, regulatory approvals for most of these devices in humans are several days instead of the weeks-to-months shown to characterize reliable CGM operation *in vitro*. Current USA FDA-approved sensors generally exhibit instability over the approved implantation and sensing period (3–7 days), and their pre-implant single-point calibration is thought to be good for only 12 h (Wilson and Zhang, 2010). Despite intensive research over two decades, CGM glucose sensing performance under sustained chronic implantation ($>$ 14 days) remains a major challenge primarily due to the host's acute and chronic foreign body response (FBR) to the implanted sensor. Given the current performance issues dogging CGMs, barriers to expanding their clinical utility and patient benefits are notable. Longer-term implantable CGM sensors would facilitate the development of a closed-loop glucose sensor–insulin pump system that could improve the quality of life of millions of diabetes patients as an artificial pancreas with dynamic, feedback-driven response (Cobelli et al., 2011).

Strategies to improve CGM sensor lifetimes in tissue have focused on refined signal processing (Facchinetti et al., 2010), improved surface fouling resistance by applying specific coatings to sensor surfaces to inhibit protein and cell adhesion (Wilson and Gifford, 2005; Wisniewski and Reichert, 2000), CGM device design

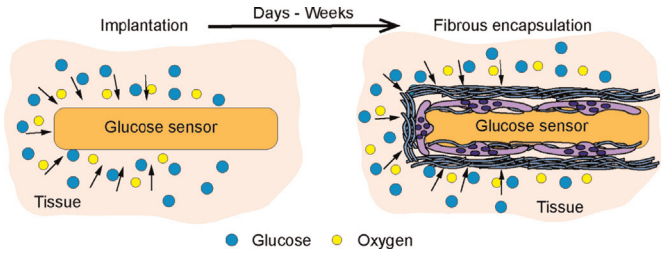

Fig. 1. Conceptual depiction of the collagenous encapsulation of implanted CGM sensors *in vivo* after subcutaneous implantation.

refinements, and modifying the CGM as a combination device that releases a drug payload locally from the implant modify local cell and tissue reactions (Avula and Grainger (In press); Hickey et al., 2002a, 2002b; Ward and Troupe, 1999; Ward et al., 2004; Wu and Grainger, 2006). To date, none of these approaches has demonstrated profound changes in the host implant site response to improve CGM functional duration.

CGM surface coatings containing bioactive nitric oxide (Hetrick et al., 2007; Nablo et al., 2005; Nichols et al., 2011), dexamethasone (Bhardwaj et al., 2007; Hickey et al. 2002a; Ju et al., 2010), and vascular endothelial growth factor (VEGF) (Golub et al., 2010; Klueh et al., 2013; Sung et al., 2009) all attempt to limit sensor fouling while exploiting a local pharmacological strategy to attenuate the intensity of the acute host inflammatory reaction. Each locally released drug and associated coating matrix approach has formulation, loading, and stability issues, different dosing requirements for given drug pharmacologies, and distinct tissue targets. Dexamethasone seeks to inhibit fibroblast production of collagen around the sensor, while VEGF prompts local angiogenesis to endow the FBR fibrotic capsule around the sensor with effective permeability, sufficiently perfused for effective transcapsular glucose and oxygen transport to the sensor. Significantly, these drug-release approaches have addressed cell targets and behaviors well downstream, as well as temporally and spatially distinct, from the early acute-phase FBR mast cell- and leukocyte-initiating reactivities around the implant.

Mast cells (MC) play a critical role in mediating acute tissue inflammatory responses. Located perivascularly throughout all tissues, MCs are mobilized during any inflammatory response (Krishnaswamy et al., 2006). MC degranulation of histamine and other pro-inflammatory mediators including heparin, cytokines (e.g., TNF-alpha), chemokines, and many proteases together with fibrinogen adsorption are recognized as powerful inducers of acute inflammatory responses to implanted biomaterials (Tang et al., 1998; Zdolsek et al., 2007). MC-released cytokines and chemotaxis along with histamine and serotonin release result in vasodilation and increased recruitment of phagocytes to the implant site. Their connection with the foreign body reaction is well recognized (Thevenot et al., 2011; Ward and Troupe, 1999; Ward et al., 2004). Specific to CGMs, Klueh et al. (2010) have recently compared MC behavior *in vivo* on CGM sensor implant performance in both wild-type and MC-deficient mice. Significantly, they confirmed using CGM signal-to-noise ratio (S/N) and analyte response time as a function of implant time that MCs play a major role in the host FBR around CGMs. Importantly, this effect was linked to subsequent fibrous capsule formation around the CGM that impedes sensor function (Klueh et al., 2010).

Elucidating how MCs orchestrate the host FBR has been elusive. One new clue is that stem cell factor (SCF), the ligand of the MC-specific c-KIT tyrosine kinase receptor, is an important growth factor regulating MC survival, proliferation, differentiation, and degranulation processes (Reber et al., 2006). The link between the MC-specific SCF/MC c-KIT pathway and the intensity the host early inflammatory response to implants appears critical to MC function and degranulation reactions (Reber et al., 2006).

Here we describe the use of a newly screened tyrosine kinase inhibitor (TKI), masitinib, shown effective in inhibiting the SCF receptor, c-KIT, on MCs. Masitinib offers potent control of MC reactivity (Dubreuil et al., 2009; Paul et al., 2010) by binding competitively to the ATP-binding c-KIT receptor, blocking its critical tyrosine kinase signaling activity. Importantly, this pharmacology stabilizes mast cells from degranulating or activating. Use of masitinib to control MC activation in the context of the FBR is unknown. We propose that its pharmacology could be exploited to benefit CGM function. We use drug formulated into a microsphere delivery system released locally from CGM sensors implanted