Tracking and Unraveling the Mechanism of Nanostructured Porous Si Carriers Erosion in Neoplastic State

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Statement of Purpose: The benefits from localized delivery of therapeutic agents, serve as a driving force for the design and synthesis of biomaterials for medical applications. The design of erodible biomaterials relies on the ability to program *in vivo* material retention time, which necessitates real-time monitoring of erosion. Loss of material integrity, structure and eventually mass, progress dependently over time but are dominated by different environmental forces *in vitro* and *in vivo*. Thus, *in vivo* residence times and *in vitro* durability of degradable materials may differ dramatically, affecting biomaterial performance. Specifically, the performance of porous Si (PSi)-based drug delivery systems is critically dependent on the degradation behavior of the Si scaffold[1,2]. Most studies have investigated the behavior of these carriers *in vitro*; however, clinical applications of these nanomaterials would require establishing clinically relevant *in vitro* conditions under which one can attain similar behavior in the two domains. We have recently shown that the degradation profile of biomaterials *in vitro* and *in vivo* varies[3]. In the present work the effect of tissue microenvironment on PSi degradation is studied in healthy and neoplastic states. We show that correlation between *in vitro* and *in vivo* erosion persists only under specific conditions that enable prediction of *in vivo* erosion from *in vitro* data. These conditions take into consideration local oxidative stress manifested by the tumor microenvironment that enhances silicon degradation compared to healthy state.

Methods: PSi carriers were prepared by electrochemical etching and further ultrasonication into microparticles. The resulting porous particles were chemically-modified by surface alkylation with undecylenic acid, followed by fluorophore tagging through covalent attachment of Texas-Red Hydrazide (TRH) dye molecules *via* EDC/sulfo-NHS chemistry. The modified particles degradation was followed *in-vitro* by plate reader for fluorometric analysis and ICP-AES-the gold standard method for measuring Si mass changes. Breast cancer tumor was induced by injecting MDA-MB-231 luciferase expressing cells in to the mammary fat pad tissue of SCID female mice. The modified particles were injected intratumorally and followed using In Vivo Imaging System (IVIS).

Results: Recently, it has been demonstrated that the fluorescent intensity of dye molecules attached to the pore walls of PSi depends on its oxidation level[4]. Dye molecules attached to fresh carriers present low fluorescence, due to the physical proximity between the dye and the Si matrix, which acts as an energy acceptor. As the PSi oxidizes, the oxide layer increases and so does the distance, consequently, increasing the fluorescent intensity. We have found that the observed differences in the fluorescence of TRH-PSi particles injected into healthy and cancerous tissues can be ascribed to the profound differences between the physiological microenvironments in terms of their oxidative stress. To study the effect of oxidative stress *in vitro*, we have used 3-morpholinosydnonimine N-ethylcarbamide (SIN-1) to generate physiologically relevant levels of peroxynitrite ($OONO^-$), a highly reactive oxygen species (ROS) involved in human carcinogenesis. Interestingly, only under specific conditions that mimic physiological oxidative stress, one can recapitulate the *in vivo* conditions and attain a linear correlation between the fluorescent signal *in vitro* and *in vivo* (Figure 1a,b).

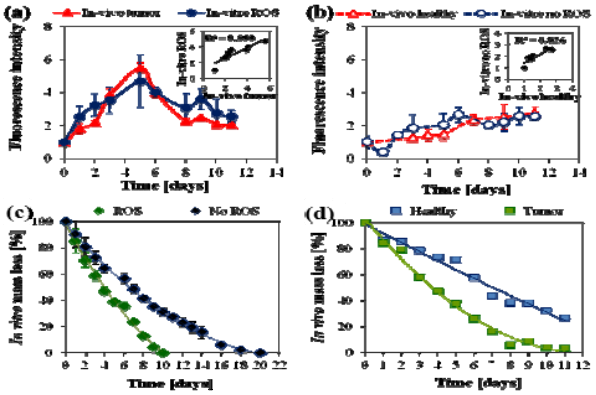

Fig. 1: (a) The fluorescent intensity of TRH-labeled PSi particles injected intratumorally follows the intensity measured *in vitro* in the presence of ROS (R^2 = 0.800) and (b) The fluorescent intensity of particles injected into healthy mammary fat pad follows the intensity measured *in vitro* without ROS (R^2 = 0.826) (c) *In vitro* Si mass loss profiles measured by ICP-AES in PBS buffers with and without ROS and (d) *In vivo* Si mass loss profiles calculated for healthy and tumor environments.

Si erosion is enhanced in the presence of ROS, resulting in a complete degradation within 10 days. In comparison, the erosion profile in PBS depicts a gradual degradation, lasting for ~20 days (figure 1c). The *in vivo* Si mass loss of the labeled particles is calculated based on the relationship between mass loss (by ICP) and fluorescence (Figure 1d). These correlations allow the assessment of *in vivo* Si mass loss without the need to sacrifice animals at each time point of the experiments, presenting a generic methodology to infer device performance directly from the observed *in vivo* fluorescence.

Conclusions: We present a noninvasive method that enables tracking silicon erosion *in vivo*. Comparison and correlation between *in vivo* and *in vitro* material mass loss unravels the impact of physiological microenvironment in determining device fate. This in turn allows one to engineer material properties to control and predict material in vivo performance.

References:

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