Tracking, Modeling and Predicting the Erosion of Fluorescently Labeled Materials Noninvasively

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Statement of Purpose: Innovation in and use of biodegradable materials are limited by the lack of reliable assays for tracking material fate. *In vitro* degradation by traditional techniques cannot always predict *in vivo* performance and often confuse erosion, absorption and degradation. Standard methods sacrifice samples or animals preventing sequential measures of the same specimen, and are artificially contaminated by swelling with fluid uptake. We harnessed fluorescence to follow material mass loss non-invasively in intact specimens, sequentially and identically *in vitro* and *in vivo*.

Methods: Our model system is an adhesive material based on polyethylene glycol (PEG)-amine and dextran aldehyde. Gels were formed following injections of the two components using a dual syringe. The gels were then submerged in 2ml PBS, shaken at 37°C and the fluorescence intensity in the media was measured to provide assessment of material loss and erosion *in vitro*. *In vivo* erosion profiles were quantified for materials implanted into the dorsal subcutaneous space of nude albino mice. Animals were imaged using Xenogen *in vivo* imaging system (IVIS) at 3 seconds exposure at various time intervals. Fluorescence intensity was determined by calculating the efficiency overlying each construct (Fig 1a). Fluorescent tagging had no effect on material properties including gelation time, adhesion strength or degradation profile.

Swelling and dissolution of polymer matrices are determined in part by construct shape and dimensions. Thus, the effect of material bulk properties on erosion was assessed by examining the material loss profile of a specific material formulation in three different shapes: disk, block, and hollow mesh cylinder.

Results: Material shape gives rise to diverse biphasic degradation profiles *in vitro*, possibly because construct dimensions can alter both swelling degree and the release of polymeric chains by diffusion from the cross-linked network.

Erosion kinetics *in vivo* illustrated identical biphasic behavior to *in vitro* kinetics for all specimens albeit at faster rates (Fig 1b-d). *In vivo* erosion was 1.77, 1.42 and 1.05 times faster compared with *in vitro* for disks, blocks and mesh cylinders respectively, with high Pearson's coefficients (Fig 1b, R^2= 0.96, 0.94 and 0.98). Erosion rates determined *in vivo* were two-fold different than rates *in vitro* but correlated linearly (R^2= 0.99).

These kinetics are fit well with a bi-exponential mathematical model that incorporates the rate of material decay and amount of starting component material. Model predictions correlate well (R^2 = 0.99) with measured material erosion profiles.

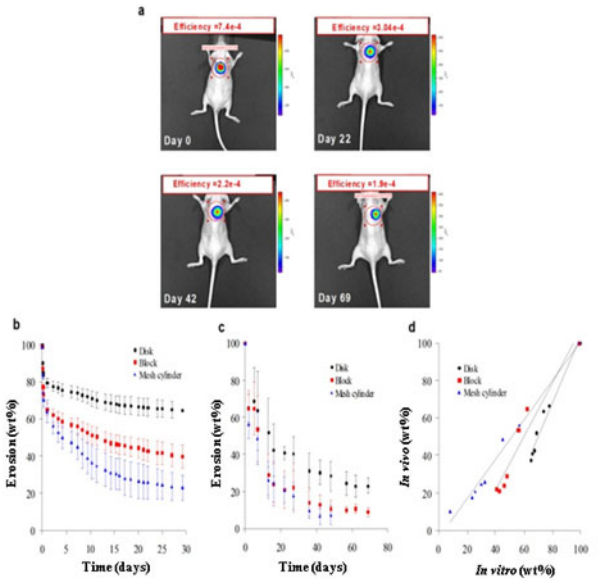

Figure 1: *In vitro* and *in vivo* erosion profiles are depicted by tracking the loss of fluorescent tag. The effect of material shape (disk, block or mesh cylinder) on degradation profile was followed *in vivo* noninvasively in mice model (a) and *in vitro* (b). The loss of fluorescent signal with time *in vivo* was converted to weight loss (c) and correlated with the *in vitro* loss (d). An excellent correlation (Pearson's coefficient >0.99) was found between *in vitro* and *in vivo* erosions.

This biphasic kinetic includes rapid sample loss that presumably arises from diffusion of non-crosslinked, unreacted PEG and dextran polymeric chains and more gradual subsequent degradation of the cross-linked co-polymer network.

Conclusions: Degradable biomaterials continue to emerge as essential components of novel biomedical devices. Yet, their erosion kinetics are poorly predicted using classic gravimetric or GPC analyses. We demonstrate that the erosion of biomaterials can be tracked *in vitro* and noninvasively *in vivo* using fluorescent labeling. *In vivo* erosion was two-fold faster than rates *in vitro* but correlated linearly. The predictability of this correlation, and its adherence to mathematical models will be of immense value in the development of new devices, their regulatory evaluation and in understanding the biological forces that guide their biological response - minimizing the time, expense and consumption of animals seen with traditional methods.

Materials with programmer erosion might now be available for broad array of applications, and for the tracking and correlation of drug release and material erosion from a polymer drug-eluting scaffold, or the fate of cells and materials within tissue-engineered formulations.