Lipocalin 2 Loaded Polyanhydride Microspheres Accelerate Cell Migration

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Statement of Purpose: Degradable biomaterials composed of polyanhydrides based on 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) and 1,6-bis(*p*-carboxyphenoxy)hexane (CPH) are a class of surface and bulk eroding macromolecules that have been studied extensively for applications in drug delivery[1]. These materials are particularly appealing because they provide a protein-friendly environment and tunable release kinetics. Microspheres based on this polymer system have been widely investigated for use in sustained drug delivery[2]. The goal of this research was to study the ability of CPTEG:CPH microspheres to stabilize and release a multi-functional acute phase protein, Lipocalin 2 (Lcn2). Lcn2 is thought to play an important role in wound healing by promoting epithelial cell migration[4].

Materials/Methods: CPTEG:CPH microspheres encapsulating Lcn2 were fabricated by a previously described method[3]. The release of recombinant mouse Lcn2 was studied for 20 days and quantified using SDS PAGE and flamingo fluorescent gel stain.

The cell migration assay used the human colonic epithelial cell line, HCT 116. The cells were grown to confluency in calf serum-containing medium and then incubated in serum free medium for 24 h. Following the incubation period, a wound line was created in the monolayer of cells by scraping with a pipette tip. This created a void space into which the cells could migrate. Following wounding, the cells were washed three times to remove non-adherent cells and 3 mL of serum-free medium was added. Along the wound line uniquely independent marks were made incrementally with a sterilized scalpel on the same plane as the cells for imaging at differential time points for the duration of the assay. At this time, the treatments (Table 1) were added to the cells. Serum free medium, SFM, was the negative control while serum containing medium, SCM, was the positive control. The cells were imaged at time=0 h and time=24 h and the cell migration distance assessed using Adobe Photoshop.

Table 1. Treatments, including the amount of Lcn2 and microspheres, used to stimulate HCT116 cells post wounding.

Treatments	Lcn2 (µg)	Microspheres (mg)
Serum free medium, SFM (negative control)	-	-
Serum containing medium, SCM (positive control)	-	-
Recombinant mouse Lcn2	45, 30, 15, & 9	-
50:50 CPTEG:CPH blank microspheres	-	1.5
Lcn2-loaded 50:50 CPTEG:GPH microspheres	15	1.5
50:50 CPTEG:CPH blank microspheres + free Lcn2	30	1.5

Results: The release profile of the encapsulated Lcn2 from 50:50 CPTEG:CPH microspheres followed a zero order release after an initial burst, which is in good agreement with previous work[3]. Several treatments, listed in Table 1, were tested for their influence on migration of HCT116 cells. In correlation with previous findings [4], the cells were found to respond to Lcn2 in a dose dependent manner, with the response plateauing at doses greater than 10µg/mL. The blank 50:50 CPTEG:CPH microspheres had the same effect on cell migration as serum-free medium alone. However, the 50:50 CPTEG:CPH microspheres with encapsulated Lcn2 significantly enhanced cell migration over the 10 µg/mL Lcn2 dose administered in the absence of microspheres (Figure 1).

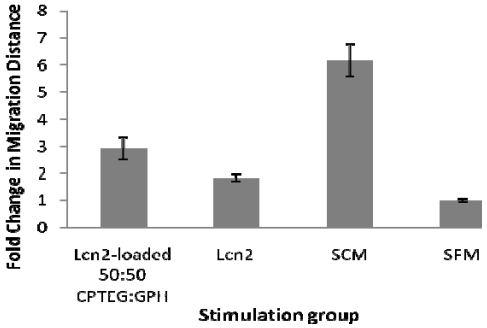

Figure 1: Histogram showing the average fold change in distance over the negative control (SFM) that wounded HCT116 cells migrated in 24 h after being exposed to the Lcn2-loaded 50:50 CPTEG:GPH microspheres (Table 1).

An additional treatment, blank 50:50 CPTEG:CPH microspheres plus 10 µg/mL of unincorporated Lcn2 was tested to evaluate possible synergism between the microspheres and the Lcn2. This treatment stimulated cell migration no more than the equivalent dose of Lcn2 alone, negating the possibility of a synergistic effect between the groups. This emphasized the importance of the controlled release of stable Lcn2 from the 50:50 CPTEG:CPH microspheres to help significantly enhance cell migration.

Conclusions: We have demonstrated the ability of 50:50 CPTEG:CPH microspheres to successfully release Lcn2 in a sustained manner while maintaining its functionality. The Lcn2 released from the microspheres enhanced cell migration over Lcn2 added in the absence of microspheres, suggesting the importance of a controlled release system for healing wounds. These findings add to the large body of evidence supporting the use of polyanhydrides as drug delivery devices.

References: [1] (Shen, EE. Biomat. 2001;22:202-210.) [2] (Putney, DS. Curr Opin Chem Biol. 1998;2:548-552) [3] (Torres, MP. Biomat. 2007;28:108-116 [4] (Playford RJ. Gast. 2006;131:809-817.)