Bone Morphogenetic Protien-2 Absorption Influenced by Varying Carboxyl Surface Density via Base Hydrolysis of Poly(lactic-co-glycolic acid)

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Introduction

Bone morphogenetic protein-2 (BMP-2) is an important osteoinductive protein, is used in bone tissue engineering and regeneration applications. It has been delivered by a variety of methods, including attachment to biodegradable poly(lactic-co-glycolic acid) (PLGA) scaffolds and coatings. The effectiveness of these devices depends on their ability to uptake and release BMP-2 at suprathreshold concentrations and with retention of bioactivity.

Previous research has shown that adsorption of BMP-2 was enhanced when using PLGA that has a higher acid number (Pharm Dev Techol 4:611, 1999). An alternate approach is to directly modify the surface properties of the polymer. For example, varying the carboxyl surface density of PLGA, such as by surface hydrolysis of ester bonds, influences the physical properties that govern protein-polymer interactions. Surface hydrolysis of PLGA with subsequent changes in electrostatic properties and hydropathy may, therefore, facilitate tailoring of the polymer for protein adsorption and delivery.

The objective of this study was to investigate hydrolysis treatments that generate carboxyl groups on the surface of PLGA for modulation of can enhance BMP-2 adsorption while also preserving important PLGA bulk properties.

Materials and Methods

PLGA coatings (50:50, 11kD, aliphatic end-capped) were deposited on 12mm diameter glass coverslips via a drop-wise addition of 60µL of 0.117% (w/v) PLGA-dichloromethane solution. The coverslips were air-dried and then stored in a vacuum (-18 inches Hg) until use within two days.

Hydrolysis was accomplished by exposing the coatings to various concentrations of NaOH for increasing durations of time. Hydrolyzed coverslips were washed, dried, and stored in a vacuum until use within two days.

Carboxyl surface density was assayed using anthracenediazomomethane (ADAM) fluorophore. A stock solution contain 6.75mg ADAM per mL acetone was prepared and diluted 10x in 2-propanol. Five-hundred µL of the diluted ADAM solution was then added to coatings immersed in 1mL of 10mM SDS in 50% (w/v) isopropanol-water. The coatings were incubated 5hrs at room temperature before reading fluorescence (380nm excitation and 415nm emission).

Hydrolyzed coatings were exposed to BMP-2 fluorescently labeled with Alexa 594. BMP-2 was delivered to the PLGA coverslip coatings in 1mL of 2µg/mL PBS, pH = 7.4, for 1hr. After washing with deionized water, the amounts of bound BMP were quantified by dissolving the polymer substrate in acetone before reading fluorescence (ex = 590nm, em = 617nm).

Results and Discussion

Figure 1 shows the effect of increasing duration of hydrolysis on number of carboxyl groups per PLGA coating surface. The increase in carboxyl surface density, however, did not necessarily translate to an increase in protein adsorption. Figure 2 shows an initial decrease in BMP-2 binding to PLGA surface following short-term hydrolysis compared to non-hydrolyzed coverslips.

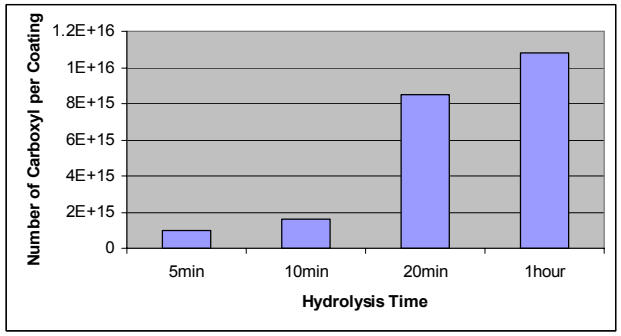

Figure 1. Carboxyl content after hydrolysis with 0.5M NaOH.

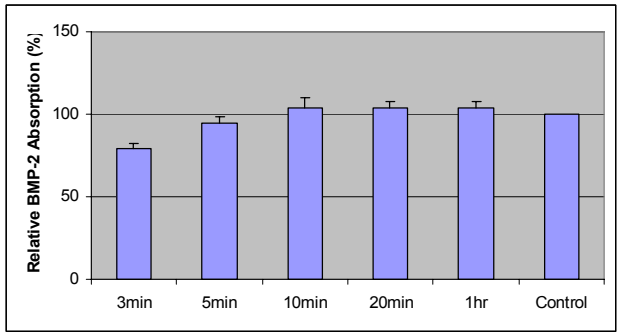

Figure 2. Binding of BMP-2 to hydrolyzed PLGA

BMP-2 may be adsorbing to PLGA via two mechanisms: both hydrophobic and hydrophilic interactions may be occurring. With the initially hydrophobic surface, protein may be denaturing, allowing nonpolar regions of the protein to interact with the non-hydrolyzed PLGA. With increasing hydrolysis, protein can adsorb via electrostatic and hydrophilic interactions with PLGA. The amount of protein bound to the surfaces is not the only factor to consider; conformation and bioactivity are essential to stimulating cell responses.

Conclusions

Hydrolysis effectively increased carboxyl surface density on PLGA, but this effect did not directly correlate with BMP adsorption. Ongoing investigations are examining bioactivity of adsorbed protein.

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