The Effect of Polymer End-Group Capping on Protein Release and Degradation of Poly(D,L-lactic-co-glycolic) Based Particles

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ABSTRACT SUMMARY
PLGA particles of different diameters (around 0.3, 1 and 20 µm) and loaded with bovine serum albumin as a model protein were prepared using a solvent evaporation technique. PLGA 50:50 was either acid terminated (PLGA-COOH) or capped with lauryl alcohol (PLGA-capped). In vitro degradation and release studies showed a substantial slower degradation and also incomplete release of BSA from particles based on PLGA-capped compared to those based on PLGA-COOH, demonstrating a remarkable influence of the capping group on protein release and degradation behaviour.

INTRODUCTION
A frequently studied strategy to obtain a prolonged release of therapeutic proteins and peptides after injection is their entrapment in polymeric nano- and microparticles. Particularly, poly(D,L-lactide-co-glycolide) (PLGA) has gained tremendous attention over the last thirty years for the design of protein formulations because of its biocompatibility and biodegradability. Many studies have shown that the release profile of proteins from PLGA nano- and microcarriers and the degradation of the polymeric matrices are dependent on the molecular weight of the polymer and the copolymer composition 1-4. However, less attention has been given on the effect of capping group of the terminal carboxylic acid unit on degradation and release properties of PLGA matrices 5. The aim of this study was to investigate the protein release behaviour and the degradation kinetics of PLGA nano- and microparticles prepared from capped (with lauryl alcohol) and uncapped PLGA.

EXPERIMENTAL METHODS
PLGA capped and uncapped (lactide:glycolide, molar ratio=50:50, IV=0.4 dl/g) were obtained from Sigma-Aldrich. PLGA nano- and microparticles loaded with and without BSA-loading were prepared by a double emulsion solvent evaporation technique. The BSA loading efficiency of the particles was measured by BCA Protein Assay. In vitro release and degradation studies were carried out by incubation of placebo and BSA-loaded particles in 150 mM PBS buffer at 37 °C. At appropriate time intervals the amount of BSA released was measured by Ultra Performance Liquid Chromatography (UPLC), whereas the particles residues were analyzed in order to monitor changes of dry mass, copolymer composition, and molecular weight.

RESULTS AND DISCUSSION
Table 1: Size, loading efficiency and loading capacity of the PLGA particles used in this study.

<table>
<thead>
<tr>
<th>Polymer used in particle preparation</th>
<th>Size (µm)</th>
<th>LE (%)</th>
<th>LC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGA-capped</td>
<td>20±5</td>
<td>67±4</td>
<td>3.3±0.2</td>
</tr>
<tr>
<td>PLGA-COOH</td>
<td>15±3</td>
<td>83±2</td>
<td>4.1±0.1</td>
</tr>
<tr>
<td>PLGA-capped</td>
<td>1.0±0.2</td>
<td>61±2</td>
<td>3.0±0.1</td>
</tr>
<tr>
<td>PLGA-COOH</td>
<td>1.0±0.2</td>
<td>70±3</td>
<td>3.35±0.15</td>
</tr>
<tr>
<td>PLGA-capped</td>
<td>0.32±0.18</td>
<td>48±16</td>
<td>4.8±1.6</td>
</tr>
<tr>
<td>PLGA-COOH</td>
<td>0.30±0.16</td>
<td>67±3</td>
<td>6.7±0.3</td>
</tr>
</tbody>
</table>
Table 1 shows that the BSA loading efficiency increased with particle size from 67 to 83% and from 48 to 67% for PLGA-COOH and PLGA-capped, respectively. The higher loading for particles based on PLGA-COOH is likely attributed to ionic interactions between BSA and free carboxylic groups of the polymer, which prevents the premature diffusion of the protein from the organic phase into the external water phase.

GPC analysis showed that the molecular weight of both PLGA-COOH and PLGA-capped slowly decreased in time due to hydrolysis of ester bonds. It was further shown by NMR analysis of the degrading particles that the lactic content, and for PLGA-capped also the lauryl alcohol content, increased in time. It was further shown that BSA-loaded and placebo particles had the same degradation profile (dry mass loss). Figure 1 shows that particles of 20 µm degraded much faster than those with a size of 0.3 µm. Likely, acid degradation products were more slowly extracted from the bigger particles resulting in acidification of the particles, which in turn causes faster polymer degradation.

Importantly, particles based on PLGA-capped degraded slower than those based on PLGA-COOH, likely because of the more hydrophobic nature of the capped PLGA.

Particles based on PLGA-COOH showed, after a small burst, a sustained and complete release of BSA during 60-70 days (figure 2). On the other hand the particles based on PLGA-capped showed a much slower and incomplete release accompanied by the presence of an insoluble residue remaining even after 200 days.

Conclusio?

FTIR analysis of the residue confirmed the presence of the protein and considering the polymer enrichment in lauryl alcohol, the incomplete release observed for PLGA-capped particles is likely attributed to interactions between the protein and the lauryl end group.

**CONCLUSION**

Particles based on PLGA-COOH showed, in contrast to those of the corresponding lauryl-capped polymer, complete degradation as well as quantitative release of an entrapped protein. Uncapped PLGA is therefore preferred for the design of protein formulations.

**REFERENCES**