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Quantitative ToF-SIMS Studies of Protein Drug Release from Biodegradable Polymer Drug Delivery Membranes

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Abstract

Biodegradable polymers are of interest in developing strategies to control protein drug delivery. The protein that was used in this study is Keratinocyte Growth Factor (KGF) which is a protein involved in the re-epithelialization process. The protein is stabilized in the biodegradable polymer matrix during formulation and over the course of polymer degradation with the use of an ionic surfactant Aerosol-OT (AOT) which will encapsulate the protein in an aqueous environment. The release kinetics of the protein from the surface of these materials requires precise timing which is a crucial factor in the efficacy of this drug delivery system.

Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS) was used in the same capacity to identify the molecular ion peak of the surfactant and polymer and use this to determine surface concentration. In the polymer matrix, the surfactant molecular ion peak was observed in the positive and negative mode at m/z 467 and 421, respectively. These peaks were determined to be $[AOT + Na^+]$ and $[AOT - Na^+]$. These methods are used to identify the surfactant and protein from the polymer matrix and are used to measure the rate of surface accumulation. The second step was to compare this accumulation rate with the release rate of the protein into an aqueous solution during the degradation of the biodegradable film. This rate is compared to that from fluorescence spectroscopy measurements using the protein autofluorescence from that released into aqueous solution.

1. Introduction

Biodegradable polymers are widely used to provide materials for cell and tissue scaffolding in tissue engineering and for controlled release of drugs.¹ Our laboratory has developed methodology to interpret the degradation kinetics of biodegradable polymers, used for tissue engineering and drug delivery.^{2–7} The goal of our present research program is the development of a bioengineered membrane that will be used as a temporary interface between the connective tissue and the epithelium during the human trachea/bronchia healing process after surgery or traumatic injury.⁸ This membrane contains protein growth factors that would be delivered in a tuned sequence, degrade at a controlled rate, and provide a barrier for reepithelialization to occur. There are multiple growth factors involved with this process but the primary used for this study is Keratinocyte Growth Factor (KGF-1). It has been shown in previous studies that KGF-1 is an integral protein the wound healing process due to its significant effect on

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[1]Mahoney, C. M.; Fahey, A.; Gardella, J. A. Jr. , SIMS depth profiling of polymer blends with protein based drugs” *Applied Surface Science* 2006, 252, 6609-6614.

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reepithelialization.⁹ This paper summarizes approaches to characterization of degradation mechanisms in these complex membrane constructs using ToF-SIMS. Recent work from our laboratory has utilized ToF-SIMS to simultaneously determine the degradation kinetics of polymers and, in collaboration with scientists from NIST, to depth profile complex polymer membrane composites to determine the in-depth distribution of the components.^{4, 7}

Docusate Sodium Salt (AOT, Aerosol-OT) is a biocompatible anionic surfactant which was incorporated into the biodegradable polyester drug delivery implant system in order to improve the stability of the protein. This study used blends of Poly (l-lactic acid) (PLLA) and Poly (lactic-co-glycolic acid) with AOT in order to stabilize the hydrophilic drug during the formulation and degradation process.¹⁰ Numerous studies have been done into the stabilization effect of surfactants on proteins within an organic solvent by reverse micelle.^{6, 11, 12} The strategy used for this study was to formulate a hydrophilic drug within a biodegradable polymer matrix using the surfactant for a stabilization effect. Hydrophilic drugs are not typically formulated into a biodegradable polymer due to their hydrophobic nature.

The present study uses fluorescence spectroscopy to determine the release rate of a protein from a biodegradable polymer into solution. These measurements were coupled with time-of-flight secondary ion mass spectrometry (ToF-SIMS) which was used to determine the accumulation rate of the surfactant at the surface of the biodegradable membrane. The surfactant acts as an encapsulation for the protein and therefore the rate at which accumulation occurs at the surface will match with the release rate of protein into solution.

2. Experimental

2.1 Materials

Poly (l-lactic acid) (MW = 100000) and Poly (lactic-co-glycolic acid) (80:20) were purchased from Sigma (St. Louis, MO). Recombinant Human Keratinocyte Growth Factor (KGF-1, FGF-7) was purchased from Recombinant Protein Technologies (Israel) and used as received. Aluminum weigh dishes (Sigma, St. Louis, MO) were cut into 1 cm × 1 cm pieces and cleaned with n-hexane, methanol, and triply distilled water prior to use as substrates for ToF-SIMS analysis. HPLC grade chloroform was used for the preparation of PLGA/AOT and PLLA/AOT blends in a series of ~3% w/v samples. The concentration of AOT present was kept constant at 1.5×10^{-2} M. KGF concentration in these samples was kept constant at 2.0×10^{-4} M (500ng/mL). AOT was water loaded prior to the addition of KGF protein using the equation ($R = [\text{water}]/[\text{AOT}]$) where $R=11$. The KGF was dissolved in triply distilled water prior to introduction to the Polymer/AOT blend. These samples were emulsified in a water/oil emulsion to encapsulate the protein within the AOT. These solutions were spin cast at 3000 rpm for 30 s onto the aluminum substrate. The spin-cast procedure was carried out under the guidelines of Frank et al. and Xie et al.^{13, 14} The films were then dried further in a vacuum oven (National Appliance Company Model 5830 vacuum oven, Portland, OR at room temperature under reduced pressure (107 Pa) for 72 hrs. Samples used for depth profiling were prepared as described except cast onto a silicon substrate. The samples were then removed from the vacuum oven and placed in 5mL of Phosphate Buffered Saline (PBS) Solution (Sigma, St. Louis, MO) at pH 7 for a period of 1,2,3,4, or 5 days. The pH of the PBS was determined using an Oakton Acorn Series pH/mV/°C meter (Vernon Hills, IL). The samples were degraded in a recirculating water bath (Fisher Circulator Model 73) at 37°C. The film samples for ToF-SIMS analysis were removed from the water bath and washed in an ultrasonic cleaner (RAI Research Model 450, Long Island City, New York) of triply distilled water for 60s prior to being vacuum dried for 120 hours for ToF-SIMS analysis. The PBS solution containing KGF were stored at 5°C until analysis. These samples were analyzed when the degradation experiment was complete. L-Tryptophan residue (99%) used to determine the calibration curves for fluorescence experiments were purchased from AlfaAesar (Ward Hill, MA).

2.2 Fluorescence Analysis

The PBS solution containing KGF was placed in a standard 1-cm² fused silica cuvette (35°C). A spectrofluorometer (SLM Instruments model 48000 MHF) was configured to excite the tryptophan residues within the KGF (270 nm) and collect its emission (320 nm) in the solution. The film itself was not illuminated during these experiments; only fluorescence from the buffer. The total KGF concentration over time was determined from the observed fluorescence intensity and a series of standard tryptophan calibration curves.

2.3 ToF-SIMS analysis

ToF-SIMS static and depth profiling experiments were performed on an Ion-ToF V (Münster, Germany) time of flight secondary ion mass spectrometer equipped with Bi, Cs, and C₆₀ primary ion beam sources. The Bi¹ ion beam was utilized for the static study since both spectra and image analysis could be performed with this source and was operated at 25kV. The target current was ~ 1 pA with a raster size of 500μm × 500μm. Target current was measured by a Faraday cup. Each spectrum was averaged over 50 scans with a pulse width of 5.1 ns and a cycle time of 155μs. Both positive and negative secondary ions were extracted from the sample into a reflection-type time of flight mass spectrometer. The secondary ions were then detected by a microchannel plate detector with a post-acceleration energy of 10 kV. The Bi³⁺⁺ ion beam was utilized for depth profiling analysis while sputtering with the C₆₀₊₊₊ source. The target current was ~0.3 pA for the Bi³⁺⁺ ion gun. The analysis area was 100×100 μm with a sputter area of 400μm. The analysis was done in negative mode with a cycle time of 300 μs.

3. Results and Discussion

3.1 ToF-SIMS Results

Identification of the AOT molecular peak within the sample spectrum was done in order to determine the surface concentration. The molecular peak for AOT in the positive spectrum, [AOT + Na⁺] was identified at an m/z of 467 amu (Figure 1). This was done by analyzing a sample of pure AOT and was used as a reference peak in this study. AOT was also analyzed using the negative mode to determine if a molecular peak could be identified. A peak was observed at an m/z of 421 which corresponds to the peak [AOT – Na⁺][–]. These reference spectra were used to identify AOT within the blended samples of PLLA/AOT/KGF or PLGA/AOT/KGF. The molecular ion peak was observed in these samples and the area under the peak was used to determine the surface concentration of the AOT. Figure 2 shows a representative spectrum from this study where the AOT molecular peak from the reference spectrum can be observed in the blend of both PLGA and PLLA.

The degraded samples were analyzed in a method developed by Lee *et al* by which two peak intensities representative of the AOT and PLA monomer were integrated to normalize the AOT peak.⁴ The PLA monomer fragment of [C₃H₄O]⁺ at m/z = 56 was chosen since it was observed at high counts as a peak in both the PLLA and PLGA blended samples. This occurred in the PLGA as well as the PLLA since PLA is the major component in this copolymer blend. The results from this normalization are graphed in Figure 3. The positive peak was used for this study since higher counts were observed. Negative peaks were observed and the accumulation of AOT matched that of the positive spectra.

The accumulation kinetics of AOT to the surface is extremely varied based on the type of polymer being blended. The PLGA shows the highest surface concentration of AOT at 72 hours degradation time followed by a sharp decrease to no surface concentration. This type of surface concentration is not observed in the PLLA until 96 hours degradation in the PLGA. The decrease to no surface concentration of AOT in the PLGA was confirmed by the fluorescence data which showed a leveling out in the concentration of KGF present. This is evidence that

in the PLGA film, the KGF is associated with the AOT. The data for the PLLA showed extreme variation in the amount of KGF present in solution versus the accumulation of AOT. AOT is consistently present at the surface of the PLLA with an increase at the 96 hour degradation time. The fluorescence data shows a consistent release rate where there is no increase as observed for the AOT. The structural differences between these polymers could account for this difference in AOT accumulation and how the surfactant is encapsulating the KGF. PLLA is a semicrystalline polymer while PLGA is amorphous therefore allowing more water intake into the polymer in a shorter time period. This would cause the AOT surfactant to be conducted to the surface of the polymer.

Depth profiling of the blended films was performed to determine the association of KGF and AOT. This analysis was done in the negative mode since the $[\text{CN}]^-$ and $[\text{CNO}]^-$ peaks could be used to identify the protein in the polymer blend. The PLA, AOT, and KGF peaks were summed in the depth profile of each polymer blend at 0 hours degradation (Figure 5). In both profiles, an increased amount of AOT is observed at the surface of the polymer. The PLGA sample shows the AOT increase at the surface and then a leveling out through the bulk of the sample. This matches the KGF concentration which remains consistent the bulk of the sample. The PLLA shows consistency of the KGF through the bulk of the sample but this is not matched with the AOT. The level of AOT at the surface and bulk of the sample does not correlate with the KGF concentration. This AOT level is shown at 0 hours and 24 hours degradation time. At 24 hours degradation, the AOT concentration is still not level or consistent with the KGF. This results in the difference in AOT accumulation at the surface versus KGF release into solution observed in the fluorescence data.

3.2 Fluorescence Results: Comparison to ToF-SIMS

Figure 4 represents the concentration of KGF that was measured in the PBS solution after degradation. The release data of KGF from the PLGA film shows a dramatic increase in concentration at 72 hours degradation time. This data correlates to the ToF-SIMS data since highest AOT concentration is observed at 72 hours. The KGF concentration in solution after release from the PLLA does not show a correlation of AOT accumulation and KGF release into solution. The release rate of KGF for the PLGA can be compared to the accumulation of AOT at the surface.

Conclusions

A comparison between the accumulation of AOT at the surface of a biodegradable polymer membrane and the release rate of KGF protein in solution was performed. The AOT molecular fragment was used to successfully follow the accumulation pattern in the ToF-SIMS spectra in the PLLA and PLGA samples. The KGF protein was monitored using the autofluorescence of the tryptophan residue and a release rate was determined. The comparison between the data was stronger for the PLGA/AOT/KGF blend than the PLLA/AOT/KGF blend.

References

1. Martin P. Wound Healing-Aiming for Perfect Skin Regeneration. *Science* 1997;276(5309):75–81. [PubMed: 9082989]
2. Lee JW, Gardella JAJ. In Vitro Hydrolytic Surface Degradation of Poly(glycolic acid): Role of the surface segregated amorphous region in the induction period of bulk erosion. *Macromolecules* 2001;34(12):3928–3937.
3. Chen J, Gardella JAJ. Time-of-Flight secondary ion mass spectrometry studies of in Vitro hydrolytic degradation of biodegradable polymers. *Macromolecules* 1999;32(22):7380–7388.

4. Lee JW, Gardella JA. Simultaneous Time-of-Flight Secondary Ion MS Quantitative Analysis of Drug Surface Concentration and Polymer Degradation Kinetics in Biodegradable Poly (l-lactic acid) Blends. *Analytical Chemistry* 2003;75:2950–2958. [PubMed: 12964738]
5. Lee WK, Gardella JAJ. Hydrolytic kinetics of biodegradable polyester monolayers. *Langmuir* 2000;2000(16):7.
6. Melo EP, Aires-Barros MR, Cabral JM. Reverse micelles and protein biotechnology. *Biotechnology Annual Review* 2001(7):87–129.
7. Mahoney CM, Yu J, Fahey A, Gardella JAJ. SIMS depth profiling of polymer blends with protein based drugs. *Applied Surface Science* 2006;252:6609–6614.
8. Cho EJ, Tao Z, Tang Y, Tehan EC, Bright FV, Hicks WL, Gardella JA, Hard R. Tailored delivery of active keratinocyte growth factor from biodegradable polymer formulations. *Journal of Biomedical Materials Research* 2003;66A(2):417–424. [PubMed: 12889013]
9. Werner S, Peters KG, Longaker MT, et al. Large Induction of Keratinocyte Growth Factor Expression in the Dermis During Wound Healing. *Proceedings of the National Academy of Sciences of the United States of America* 1992;89(15):6869–6900.
10. Ikada Y, Tsuji H. Biodegradable polyesters for medical and ecological applications. *Macromolecular Rapid Communications* 2000;21:117–132.
11. Ono T, Goto M. Application of reversed micelles in bioengineering. *Current Opinion in Colloid Interface Science* 1997;2:397–401.
12. Luisi PL, Giomini M, Pileni MP, Robinson BH. Reverse micelles as hosts for proteins and small molecules. *Biochimica y Biophysica Acta* 1988;947:209–246.
13. Frank CW, Rao V, Despotopoulou MM, Pease RFW, Hinsberg WD, Miller RD, Rabolt JF. Structure in thin and ultrathin spin-cast polymer films. *Science* 1996;273:912–915. [PubMed: 8688068]
14. Xie FC, Zhang HF, Lee FK, Du BY, Tsui OKC, Yokoe Y, Tanaka K, Takahara A, Kajiyama T, He TB. Effect of low surface energy chain ends on the glass transition temperature of polymer thin films. *Macromolecules* 2002;35:1491–1492.

Figure 1.

(a) 3.4×10^{-3} M AOT cast onto a silver substrate in positive mode. The peak at 467 represents [AOT + Na⁺] which are the major peak in the spectrum. (b) 3.4×10^{-3} M AOT cast onto a silver substrate in negative mode. The peak at m/z 422 represents [AOT - Na⁺]⁻ which is the major peak in the spectrum.

Figure 2.

(a) Positive spectrum of PLGA/AOT/KGF blend. (b) Positive spectrum of PLLA/AOT/KGF blend.

Figure 3.
Accumulation kinetics of AOT to the surface of the PLLA or PLGA film.

Figure 4.
Release Kinetics of KGF into PBS buffer as measured by Fluorescence Spectroscopy.

Figure 5.

(a) Negative spectrum depth profile of PLGA/AOT/KGF blend at 0 hour degradation. (b) Negative spectrum depth profile of PLLA/AOT/KGF blend at 0 hour degradation. (c) Negative spectrum depth profile of PLLA/AOT/KGF blend at 24 hours degradation.