

THE DEVELOPMENT OF AN *IN SITU* SOLIDIFYING DEGRADABLE SCAFFOLD THROUGH THE UTILISATION OF THE BIOTIN-AVIDIN BINDING MECHANISM.

A.K. Salem, J.R. Mitchell¹, M.C. Davies, S.J.B. Tendler, C.J. Roberts, P.M. Williams and K.M. Shakesheff

School of Pharmaceutical Sciences, University of Nottingham, Nottingham, NG7 2RD, UK

¹National Centre for Macromolecular Hydrodynamics, University of Nottingham, Sutton Bonnington LE12 5RD, UK.

e-mail: paxaks@gmail.nottingham.ac.uk

ABSTRACT SUMMARY

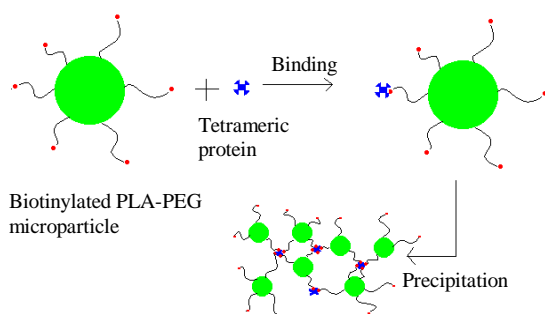
The cross linking of poly (lactic acid)-poly (ethylene glycol)-biotin (PLA-PEG-biotin) microparticles and biotin-PEG-biotin solutions with avidin has been evaluated for the purpose of developing an injectable scaffold that solidifies within the body.

Keywords: biodegradable, biomaterial, injectable, biotin, avidin, PLA-PEG, drug delivery, tissue engineering

INTRODUCTION

PLA-PEG copolymers and their use as an effective biomaterial in drug delivery and tissue engineering have been well characterised.^{1,2} These materials have proven to be useful as thermo sensitive injectable gels.³ A cross linking PLA-PEG-biotin and avidin scaffold has potential as an attractive alternative to current injectable systems because of their potential ability to form biodegradable scaffolds *in situ* with good mechanical properties, a porous structure to allow cell infiltration, desirable surface chemistries through the utilisation of biotinylated peptides and the ability to prevent non-specific interactions through the use of polyethylene glycol.⁴ (figure 1.)

Figure 1. A schematic describing the aggregation of biotinylated microparticles through avidin immobilisation.



EXPERIMENTAL METHODS:

PREPARATION OF PLA-PEG-BIOTIN:

α -hydroxy- ω -amine PEG (1g) was dissolved into acetonitrile (2ml), methylene chloride (1ml) and Et₃N (80 μ l). After addition of NHS-Biotin (0.250g), the reactants were stirred overnight under argon. The reaction was worked-up by the slow addition of diethyl ether (40ml) to precipitate the polymer, which was then filtered through a Buchner funnel and washed with diethyl ether. The isolated material was then dissolved in hot isopropanol (70°C) to give an opaque-cloudy solution.

The polymer was re-precipitated on cooling; this product was then analysed for biotin attachment by ¹H-NMR spectroscopy. The polymer (350 mg) was dried azeotropically and left under vacuum. Lactide (2g) was transferred into the round-bottom flask with Biotin-PEG-OH (0.35g), diluted with 10ml toluene, Sn(Oct)₂/toluene (0.1g in 1ml) was then added (after being purged with nitrogen) and the reaction was then brought to reflux at 110°C for 4 hours under argon. The reaction mixture was precipitated from a dichloromethane solution into a cold diethyl ether. The final product was isolated by vacuum filtration and lyophilised overnight. Final product was assessed by gel permeation chromatography and ¹H-NMR spectroscopy.

PREPARATION OF BIOTIN-PEG-BIOTIN

α - ω -bis(amine) PEG (1g) was dissolved into acetonitrile (2ml), methylene chloride (1ml), Et₃N (80 μ l) and NHS-Biotin (0.50g). The reactants were stirred overnight under argon and then worked-up by diethyl ether (40ml) to precipitate the polymer. The isolated material was then dissolved in hot isopropanol (70°C) to give an opaque-cloudy solution. On cooling the product was filtered and lyophilised.

PREPARATION OF NANO/MICROPARTICLES:

500mg of PLA-PEG-biotin was dissolved in 20ml of dichloromethane (DCM) to produce a 25mg/ml solution. PVA (250000 Mw) [88% hydrolysed] was dissolved into ELGA water (0.25g into 250 ml) to make a 0.1% w/v solution. The PLA-PEG-biotin solution was then added to a homogenised PVA solution using a 5ml Gilson pipette. The mixture was homogenised for a further 10 minutes at 5000 rpm and then left stirring overnight for DCM to evaporate and microparticles to form. Nanoparticles were prepared as above with a 7% PVA solution and homogenisation at 7000 rpm and a 5mg/ml solution of PLA-PEG-biotin

PARTICLE SIZE ANALYSIS:

Nanoparticle size was determined using photon correlation spectroscopy (PCS) (Malvern S4700 PCS system, Malvern Instruments Ltd, Malvern, UK). The analysis was performed at a scattering angle of 90°C and a temperature of 25°C using samples diluted with filtered water (0.2 μ m filter, Minisart®, Germany). For each sample, the mean diameter \pm standard deviation of six determinations were calculated applying multimodal analysis. Values shown are the mean diameter for two replicate samples

MICROSCOPY

Phase contrast images were taken on an optical microscope (Leica DMIRB) attached to a Leica Q500 IW using a colour video camera (JVC TK-C1380).

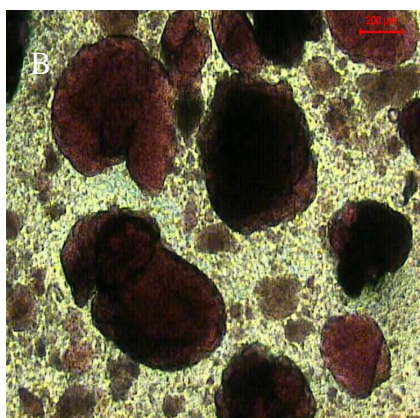
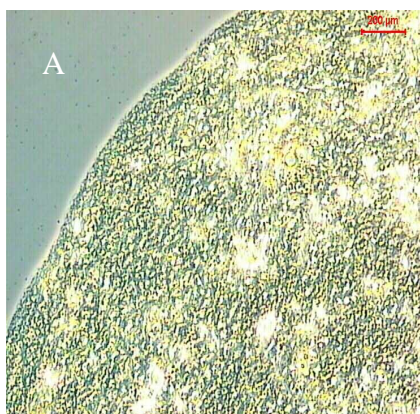
SPECTROPHOTOMETER TURBIDITY STUDIES

10 mg/ml PLA-PEG-biotin microparticles (≈ 10 micron) were prepared with varying concentrations of avidin (0 – 10 mg). Kinetic studies over a period of 60 minutes were carried out on the Beckman DU640 spectrophotometer with an analytical wavelength of 700 nm at 37°C with a read average time of 5 seconds. Gradients were calculated using linear lines of best fit.

RESULTS AND DISCUSSION

Preliminary microscopy results using rhodamine-conjugated avidin with PLA-PEG-biotin microparticles have shown an aggregation process with 10 micron particles aggregating to sizes up to and in excess of 1mm (figure 2).

Figure 2. Microscope images of PLA-PEG-biotin microparticles before [A] and after addition of avidin [B]

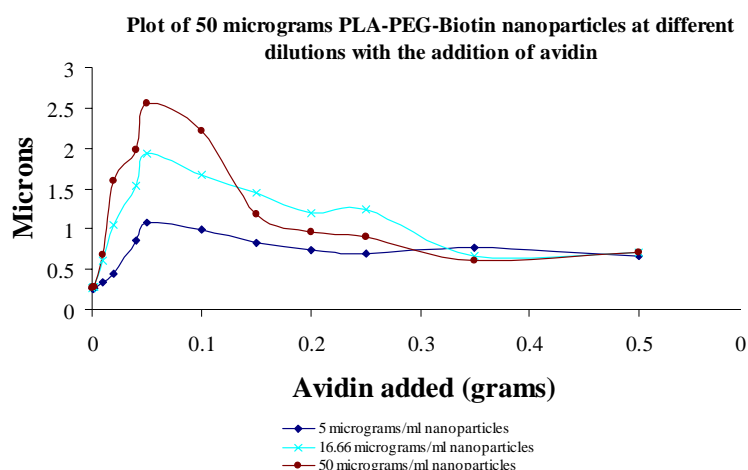


Kinetic turbidity studies on the sedimentation rate of PLA-PEG-biotin microparticles with varying concentrations of avidin have been used to elucidate critical concentrations of avidin for optimum aggregation.

A gradient of -0.0293 was achieved for 10 mg of PLA-PEG-biotin microparticles (10 microns) with 2.5 mg avidin in 1ml ELGA purified water.

The manufacture and study of PLA-PEG-biotin nanoparticles provide supporting data. Analysis of these nanoparticles by PCS shows an aggregating action due to avidin.

PCS results indicate that the aggregation process is also dependent on the concentration of the nanoparticles with an increased concentration leading to larger aggregates and a critical concentration region of avidin before particles are saturated by avidin (figure 5).



Viscometry work by rotational rheometry and capillary viscometry with biotin-PEG-biotin has shown an increase in viscosity upon the addition of avidin when compared to non-biotinylated PEG suggesting a cross linking of the PEG-biotin chains.

CONCLUSIONS

This study has illustrated the possibility of developing a novel in-situ solidifying scaffold through the interaction of biotin and avidin. Optimum ratios of avidin to PLA-PEG-biotin nano/microparticles have been elucidated by PCS and spectrophotometry with the ratio dependent on concentration and size of particles. Further work will now emphasise on refining the process by combining the biotin-PEG-biotin and PLA-PEG-biotin microparticles with avidin and testing cell viability on such scaffolds.

REFERENCES

- 1) Li, Y. X.; Kissel, T. *Journal of Controlled Release* **1993**, *27*, 247-257.
- 2) Agrawal, C. M.; Athanasiou, K. A.; Heckman, J. D. *Materials Science Forum* **1997**, *250*, 115-128.
- 3) Jeong, B.; Bae, Y.H.; Lee D.S.; Kim, S.W, *Nature* **1997**, *388*, 860-862
- 4) Cannizzaro, S. M.; Padera, R. F.; Langer, R.; Rogers, R. A.; Black, F. E.; Davies, M. C.; Tendler, S. J. B.; Shakesheff, K. M. *Biotechnology and Bioengineering* **1998**, *58*, 529-535.

ACKNOWLEDGEMENTS

We thank EPSRC for funding

