Control of Alginate Microbead Physical Characteristics for Sustained Delivery of Angiogenic Proteins

Monica L Moya[1], Emmanuel Opara[1], Howard P Greisler [2,3], Eric M Brey [1,3]
1. Illinois Institute of Technology, Chicago, IL; 2. Loyola University Medical Center, Chicago, IL;3. Hines Veteran's Hospital, Chicago, IL

Statement of Purpose

Despite the many advances in engineering of tissues, a recurring challenge in their application and success is the need to provide adequate supply of nutrients to these tissues. Stimulating neovascularization using growth factors is a promising solution, however attempts to stimulate therapeutic angiogenesis have met with limited success in part to an inability to control vascular response both spatially and temporally. While bolus delivery of proteins stimulates a cellular response, sustained local delivery of low levels of proteins is required for a persistent response.

In order to control delivery of angiongenic factors, our research focuses on developing methods for the formulation of alginate encapsulated proteins for release at a desirable rate for the optimal stimulation of angiogenesis. Alginate is a natural, biocompatible acidic polysaccharide extracted from algae composed of β-D-mannuronic acid and α-L-guluronic acid. Using alginate as a biomaterial for the delivery proteins will allow for a tunable system as the functional and physical properties of alginate beads can be controlled by alginate chemistry and synthesis conditions. We have previously shown that our calcium-alginate microbeads can encapsulate and deliver proteins that remain fully active following encapsulation. By varying alginate composition as well as the micro-encapsulator set up, the goal of this research is to study the effects of synthesis parameters on the physical properties of alginate microbeads with the final objective of obtaining an optimal formulation for sustained low level release of angiogenic proteins.

Materials and Methods

To predict the effects of varying alginate synthesis parameters on protein release, a semi-empirical mass transport model based on Fick's 2nd law of diffusion was constructed to describe the microbeads. We are specifically interested in the release of HBGAM-FGF-1 an angiogenic chimera of FGF-1 with increased specificity for endothelial cells over wild type FGF-1[1]. To describe the movement of HBGAM-FGF-1 (stokes radius, r_s=2.06 nm) an obstruction model[2] that takes into account the presence of impenetrable polymer chains as hindrances to protein diffusion was used to calculate the diffusion coefficient.

Fick's 2nd Law

$$\frac{\partial C}{\partial t} = \frac{D}{r^2}\left(\frac{\partial}{\partial r}\left(\frac{\partial C}{\partial r}r^2\right)\right)$$

Amsden's Model[2]

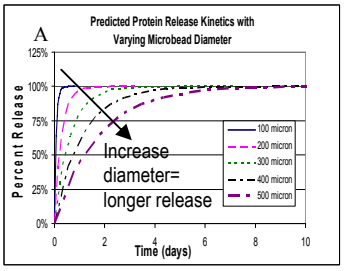
$$\frac{D_g}{D_o} = \exp\left(-\frac{\pi}{4}\left(\frac{r_s + r_f}{\bar{r} + r_f}\right)^2\right) \qquad 2\bar{r} = k_s \varphi^{-1/2}$$

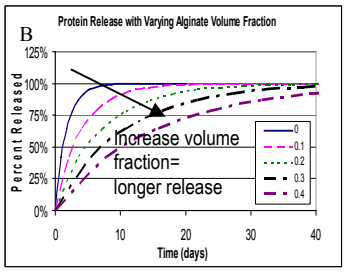
k_s polymer solvent constant
r_f radius of the fiber
r average radius of the openings between the polymer chains
φ volume fraction
r_s radius of the solute

Two types of alginate were tested to experimentally determine the effect of alginate composition on physical characteristics: high guluronic acid content (LVG) and high mannuronic acid content (LVM) acquired from Novamatrix (Norway). Alginate mixed with or without protein was loaded into a 2-channel air droplet micro-encapsulator where droplets expelled through syringe needle (varying gauge size 20-27) cross-linked with 1.1% $CaCl_2$ solution forming solid spherical alginate beads. Empty microbeads of varying concentrations (1-3%) of LVM and LVG were used to vary volume fraction as well as the diameters of the microbeads. To calculate volume fractions, microbeads were measured initially and after being dried out in a dry incubator for at least 24 hours.

Results and Discussion

Using a transport model of diffusion in alginate, protein release kinetics can be controlled by varying microbead diameter (A) and varying alginate volume fraction (B)





These physical properties can be controlled by alginate chemistry and synthesis conditions. Microbeads' volume fraction was increased by increasing alginate concentration. In addition, microbeads formulated using alginate of higher G content yielded microbeads with smaller volume fraction then high M content. **(Table 1)**

Table 1: Volume fractions of LVM and LVG alginate

% Alginate	LVM	LVG	p value
3	0.049 ± .004	0.032 ± .004	2.44E-.05
2	0.033 ± .011	0.023 ± .003	.052
1	0.028 ± .003	0.015 ± .003	.002

Diameters of microbeads were varied using different gauge needles and alginate composition. LVM beads were found to form statistically significant smaller beads than LVG. As gauge of needle was increased, smaller beads were formed. (**Figure2**)

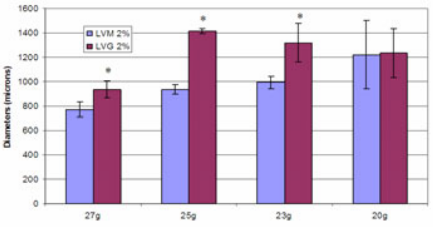


Figure 2: Varying Diameters of Beads with Alginate Type and Needle Gauge (*) p <. 05

Conclusion

A mass transport model of protein release from alginate microbeads suggests release can be controlled by modifying volume fraction and microbead diatemeter. Volume fraction of beads was found to vary with different G content as well as alginate concentration. Diameters of beads was varied using different gauge needles. At same concentration of alginate, beads formed using LVM alginate had smaller diameters than those made with LVG. Current ongoing work investigates the effects of varying alginate parameters on protein release *in vitro*.

References

1. Xue L, et al.. *Journal of Vascular Surgery*. 33, 554-560 (2001)
2. Amsden, B. *Macromolecules* Vol 31. No 23. (1998)