Supplementary Materials

The following five equations describe the interactions of albumin and S1P in a hydrogel with volume V_{gel} in contact with a solution with volume V_{wash}. Immobilized albumin, alb_i, is assumed to interact with S1P with the same affinity as dissolved albumin, alb. Dissolved albumin is assumed to reside only in the solution phase. After substituting equations 3-5 into equations 1 & 2, the two resulting equations were solved iteratively for the concentration of S1P bound to immobilized albumin $[S1P - alb_i]$ and the concentration of S1P bound to dissolved albumin $[S1P - alb]$. These calculations illustrate that, in a well-mixed system, gel and solution concentrations of S1P would not approach equilibrium during any wash with 0.4% or 4% FAF-BSA (Suppl. Table 1). It is highly likely that wash solutions without dissolved FAF-BSA did become saturated with S1P, but these release measurements may have been influenced by the release of small amounts of unreacted albumin from the gels. The results for 0.01% FAF-BSA are related to postloading of S1P into the hydrogels. Thus, postloading of 59% of S1P at 24 h indicates that either the process was not at equilibrium, or the K_D for the interaction is above 10 μM.

$$K_D^i = \frac{[S1P][alb_i]}{[S1P - alb_i]} \quad [1]$$

$$K_D = \frac{[S1P][alb]}{[S1P - alb]} \quad [2]$$

$$S1P^{TOT} = [S1P]*(V_{gel} + V_{wash}) + [S1P - alb_i]*V_{gel} + [S1P - alb]*(V_{gel} + V_{wash}) \quad [3]$$

$$alb^{TOT} = [alb]*(V_{gel} + V_{wash}) + [S1P - alb]*(V_{gel} + V_{wash}) \quad [4]$$

$$alb_i^{TOT} = [alb_i]*V_{gel} + [S1P - alb_i]*V_{gel} \quad [5]$$

Supplementary Table 1: Predicted equilibrium release from preloaded 50 μL PEG-OVS/albumin hydrogels into 1.5 mL 0.4% or 4% fatty acid free-bovine serum albumin (FAF-BSA) in PBS, or equilibrium loading of S1P into gels from 0.01% FAF-BSA.

Equilibrium dissociation constant (K_D)	*Percent release from gel at equilibrium (0.4% FAF-BSA in soln)*	*Percent release from gel at equilibrium (4% FAF-BSA in soln.)*	*Percent S1P postloaded in gel at equilibrium (0.01% FAF-BSA in soln.)*
1 nM	54.4	92.3	97.1
10 nM	54.4	92.3	97.1
100 nM	54.4	92.3	96.9
1 μM	54.8	92.3	95.1
10 μM	58.4	92.4	80.3

The presence of a large excess of albumin in the gel, and in solutions with 0.4% and 4% FAF-BSA, allows the assumption of constant [alb_i] and [alb], resulting in equations 6 and 7.

$$\frac{K_D^i}{[alb_i]} = \frac{[S1P]}{[S1P - alb_i]} = \frac{1}{R_i} \quad [6]$$

$$\frac{K_D}{[alb]} = \frac{[S1P]}{[S1P - alb]} = \frac{1}{R} \quad [7]$$

where R_i and R are partition coefficients defined by S = RC, where S is the concentration of albumin-bound S1P and C is the concentration of S1P free in solution, following the nomenclature of Crank.[1] With this simplification, the ratios of solution S1P concentrations to gel S1P concentrations were calculated using a K_D of 1 μM, to determine the validity of the perfect sink condition used in the calculation of effective diffusion coefficients. In the release experiments, none of the release solutions contained more than 0.5 nmol S1P. As shown in Suppl. Table 2, the assumption of perfect sink conditions is not expected to substantially impact the calculation of the effective diffusion coefficient for 4% FAF-BSA release solutions. However, the concentration gradient of

free S1P between the gel and 0.4% FAF-BSA may deviate from the perfect sink condition by up to ~10% if any particular wash contained 0.5 nmol S1P, even in a well-mixed system.

Supplementary Table 2: Solution concentrations of free (unbound) S1P as a function of the total amount of S1P released from the gel, assuming a well-mixed release solution that is never replaced. Asterisks indicate release that would exceed the equilibrium condition (equilibrium release would be found when the concentration of S1P free in the gel equaled the concentration of S1P free in solution). A K_D of 1 μM was assumed, but the ratio of solution to gel S1P concentrations is relatively insensitive to K_D between 100 nM and 10 μM.

Total S1P remaining in gel (nmol)	5	4.5	4	3	2	1
Total [S1P] + [S1P-alb$_i$] in gel (nM)	100,000	90,000	80,000	60,000	40,000	20,000
[S1P] free in gel (nM)	66.1	59.6	52.9	39.7	26.4	13.2
[S1P] free in 0.4% FAF-BSA (nM)	0	5.45	10.9	21.8	*	*
[S1P] free in 4% FAF-BSA (nM)	0	0.553	1.11	2.21	3.31	4.42

The previous analyses assumed a well-mixed system. Release of S1P from the hydrogels into an unstirred system was also considered. For this analysis, it was assumed that, within the gel, equilibration between free S1P and S1P-bound to albumin was instantaneous. Transport of S1P in the gel was described by:

$$\frac{\partial[S1P]}{\partial t} = D_{AB}\frac{\partial^2[S1P]}{\partial x^2} - \frac{\partial[S1P - alb_i]}{\partial t}, \qquad [8]$$

If [S1P-alb$_i$] = R$_i$[S1P] (as in equation 6) then,

$$\frac{\partial[S1P]}{\partial t} = \frac{D_{AB}}{R_i + 1}\frac{\partial^2[S1P]}{\partial x^2}.^{1} \quad [9]$$

The effective diffusion coefficient here is precisely the same as the one calculated from the S1P release data:

$$D_{AB}^{eff} = \frac{D_{AB}}{R_i + 1} \quad [10]$$

Using the Wilke-Chang correlation, the diffusion coefficient of S1P in water at 37 °C was calculated to be 4.16 x 10^{-6} cm^2/sec. For the Wilke-Chang correlation, the molar volume of S1P was estimated by the Le Bas method to be 525.4 cm^3/mol (substituting the molar volume given for sulfur as the molar volume of phosphorous; a molar volume for phosphorous is not specified for the LeBas method).[2] R_i was then estimated from equation 10 using the diffusion coefficient calculated from the Wilke-Chang correlation as D_{AB}, and the effective diffusion coefficient, D_{AB}^{eff}, calculated for release of S1P into 4% FAF-BSA. The K_D for the interaction between S1P and albumin was calculated from R_i using equation 6. Using this method, we found R_i = 5907 and K_D = 256 nM. This approach assumed that the diffusion coefficient in the gel was identical to that found in water, and that 4% FAF-BSA maintained a perfect sink condition. These assumptions were then tested using FEMLAB 2.3 (Comsol, Inc.) to solve the diffusion equations in the gel and in solution, accounting for the exchange of wash solutions at the times listed in Fig. 6a. A one dimensional model was used with a gel height of 0.025 cm and a wash solution height of 0.75 cm. Diffusion in the gel was described by equation 9. The initial concentration of free S1P was $[S1P]_{tot}/(R_i + 1)$. The concentrations of free S1P and S1P-alb_i in the solution were initially zero. At each time step, the flux of S1P from the gel was calculated from the concentration gradient in the gel near the interface from:

$$N_{S1P}\Big|_{gel-wash} = -(R_i + 1)D_{AB}^{eff}\left.\frac{\partial[S1P]}{\partial x}\right|_{gel-wash} \quad [11]$$

The calculated flux was used as a boundary condition for the concentration of unbound S1P in solution. The binding of S1P to albumin in the wash solution was described assuming k_{off} = 1 sec^{-1},[3] while k_{on} was determined using the K_D calculated above (k_{on} = k_{off}/ K_D). The diffusion coefficient for dissolved albumin (and thus S1P bound to dissolved albumin) was calculated to be 6.40 x 10^{-7} at 37°C.[4] The albumin concentration in solution was assumed to be constant, due to its large excess. The boundary condition for the concentration of S1P in the gel at the gel-solution interface was set equal to the calculated concentration of S1P in solution. The use of this boundary condition at the gel-solution interface was necessitated by the discontinuity in calculated S1P flux at the gel solution interface that is described by equation 11. The equations were evaluated using the time-dependent solver. The fraction of S1P remaining in the gel after one hour was estimated by integration of [S1P] over the gel sub-domain. Subsequently, to consider the effects of changing the wash solutions, the concentration profile of S1P within the gel at the end of a simulation was used as the initial condition for the gel in the subsequent simulation. At the start of each new simulation, the solution concentrations of S1P and S1P-alb were set to zero, consistent with addition of fresh wash solution. Release was simulated over the time spans that were used in the release experiments described in Fig. 6a. In Suppl. Fig. 1a, the results for the calculated release of S1P are compared to the measured release of S1P.

To examine another extreme in the diffusion coefficient for S1P within the gel, the calculated effective diffusion coefficient for sulforhodamine was used as the diffusion

coefficient for S1P in the gel (leading to R_i = 89.2 and K_D = 16.9 μM; see Suppl. Fig. 1c). To examine an intermediate value for the diffusion coefficient, a K_D of 1 μM was assumed, leading to R_i equal to 1510 and D_{AB} in the gel of 1.06 x 10^{-6} cm^2/sec (Suppl. Fig. 1b).

The computational results illustrate that the assumption of perfect sink conditions for 4% FAF-BSA is not correct, but the deviation is relatively small. The benefit of using this assumption was that it allowed us to avoid fitting both K_D and the S1P diffusion coefficient in the gel from our limited amount of data.

It was also found that the effective diffusion coefficient calculated for sulforhodamine probably does not reflect S1P diffusion coefficient within the gel. In this case, the predicted release curves for 0.4% and 4% FAF-BSA were found to overlap. A system with a K_D of 16.9 μM thus would not lead to the differences observed between release of S1P into 0.4% and 4% FAF-BSA, according to the proposed release mechanism that does not depend upon diffusion of albumin from solution into the gel. Even with K_D = 1 μM, the observed difference in release of S1P between 0.4% and 4% FAF-BSA is not fully realized. However, for K_D = 256 nM, the differences observed with 0.4% and 4% FAF-BSA are explained by the absence of convection and stirring in solution during release.

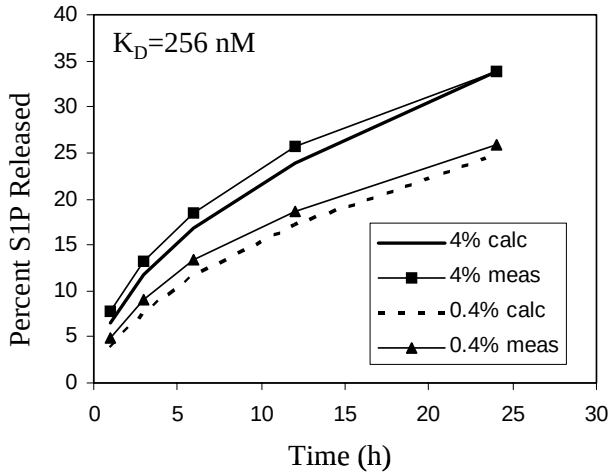
An alternative explanation for the observed differences in release of S1P between 0.4% and 4% FAF-BSA is that the release of S1P is dependent upon diffusion of dissolved albumin into the gel from solution. The dissolved albumin would likely not be washed out during solution changes and would serve to shuttle S1P throughout the gel. Indeed, albumin has been observed to aid extraction of lipids from plasma membranes in

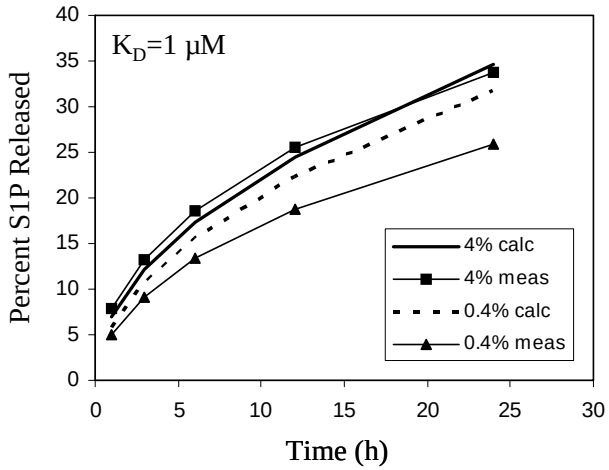
cells and may be necessary to extract S1P from immobilized albumin.[3] The combination of diffusional release of S1P as analyzed above with the diffusion of soluble albumin into the gel could result in non-Fickian release behavior, similar to what might be observed with a deswelling gel. However, if the diffusion of albumin into the gel was the major limiting factor for S1P release, Fickian release behavior would also be expected. Further study of albumin diffusion into the gels could address this hypothesis.

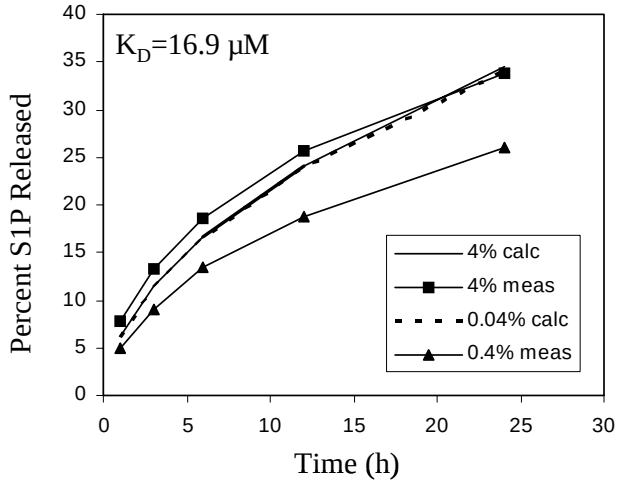
The release of S1P into solutions containing no dissolved FAF-BSA was also modeled using FEMLAB, using the same conditions as above, except the concentration of albumin in solution was set to zero. Due to the potential for release of unreacted albumin from the gel, determinations of K_D using this data may be unreliable. However, if the computational model predicts release greater than the rate observed experimentally for a particular value of K_D, then that value and greater values are likely implausible. A K_D of 16 μM led to a rate of release into buffer without dissolved albumin that greatly exceeded that which was observed (22.3% release of S1P in 24 h), while a K_D = 256 nM or K_D = 1 μM led to predicted rates of release less than what was observed experimentally (data not shown).

Supplemental Figure 1: Using FEMLAB, rates of S1P release were predicted for 50 μL PEG-OVS/albumin hydrogels in 1.5 mL of buffer containing 0.4% or 4% FAF-BSA. The amount of S1P in the gel was initially 5 nmol (100 μM). It was initially assumed that the 4% release solution represented perfect sink conditions. Using the effective diffusion coefficient calculated for release into 4% FAF-BSA (listed in Table 1), we could calculate R_i and K_D. (A) Assuming that the diffusion coefficient in the gel was identical to that in water led to R_i = 5907 and K_D = 256 nM. Release of S1P calculated for 0.4% (dotted line) or 4% (bold solid line) FAF-BSA was similar to experimental data. While it is expected that the calculated and the experimental curve should be quite close for the case of 4% FAF-BSA (due to the method of parameter estimation), note that the calculated curve for 0.4% FAF-BSA was not fit to the 0.4% FAF-BSA data. (B) Assuming that the 4% release data represented perfect sink conditions and a K_D of 1 μM, we calculated R_i = 1510 and D_{AB} in the gel = 1.06×10^{-6} cm^2/sec. (C) Using the effective

diffusion coefficient calculated for sulforhodamine as the gel diffusion coefficient for S1P led to $R_i = 89.2$ and $K_D = 16.9$ μM. Note that the curves for the predicted release into 0.4% and 4% FAF-BSA overlap in this case.







Supplemental Figure 1

References

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(4) M. E. Young, P. A. C., R. L. Bell,, Estimation of diffusion coefficients of proteins. *Biotechnology and Bioengineering* **1980,** 22, (5), 947-955.