Detailed Derivation of 1D and 2D Models

Formulation of 1D Model. We present details of bead and tissue implants simulations using the 1D model. First the derivation of the model equations is presented, and then the conversion of these equations into non-dimensional form for numerical simulations. This is followed by a discussion of the parameter values used in the simulations. We assume that Sonic hedgehog (Shh) is produced by cells in the Zone of Polarizing Activity (ZPA) and in the implant, it diffuses into the neighboring cells, binds to Patched (Ptc) receptors on the cell surface, and is also degraded. To develop the model that will describe both types of experiments, we introduce two forms of Shh, one that corresponds to the N-terminal peptide of Shh, designated N-Shh, and a form with cholesterol attached, designated N-Shh$_p$. At the outset we allow for the possibility that these diffuse at significantly different rates by virtue of the fact that the latter form may be transiently tethered to the membrane via the cholesterol. Ptc, a transmembrane protein, is the receptor for both forms of Shh. Smoothened (Smo), also a membrane-bound protein, may exist in active and inactive forms. The conversions to and from the active form are catalyzed by the Ptc-Shh complex and unbound Ptc, respectively. Michaelis Menten kinetics are used to describe the catalytic reactions, and the intermediate complexes are explicitly defined. The rate of Ptc production is assumed to be directly proportional to the active Smo concentration. The Shh-Ptc-Smo interactions are shown in Fig. 1.

Let S_c (S_n) represent N-Shh$_p$ (N-Shh), both of which bind reversibly to free Ptc (P) to form the corresponding complex $\overline{S_cP}$ ($\overline{S_nP}$). P and the Shh-Ptc complex catalyze the interconversion of active (M^a) and inactive (M^i) forms of Smo, through the formation of complexes $\overline{M^aP}$ and $\overline{M^iS_cP}$ or $\overline{M^iS_nP}$. The rate of Ptc production is assumed to be a linear function of the active Smo concentration, and k_P^* is the associated proportionality constant. k_P^0 is the basal rate of Ptc production. Uppercase letters represent the corresponding dimensional concentrations of species. k_{sub}^{sup} represent the rate constants for the reactions shown in Fig. 1, where *sub* denotes the complex formed and degraded as a result of the association (*sup* = +) or dissociation (*sup* = -) of the constituents. Absence of a superscript denotes a first-order rate constant for irreversible conversion or degradation.

Let D_c (D_n) represent the diffusivities of S_c (S_n). Let V_e and V be the extracellular and total volume, respectively, a_s the cell surface area per unit total tissue volume, and θ_f the ratio of the extracellular volume to the total volume. x_Z and x_T are used to denote the fraction of the domain occupied by the ZPA and tissue implant, respectively, f_Z and f_T represent the rate of Shh production in the ZPA and in the tissue implant, respectively, and k_P^0 is the basal rate of Ptc production. S$_j$, j = c,p is used to denote the two forms of Shh.

$$\frac{\partial S_cV_e}{\partial t} = D_c\frac{\partial^2 S_cV_e}{\partial x^2} + a_sV(-k_{s_cp}^+S_c\cdot P + k_{s_cp}^-\overline{S_cP}) - V_ek_{S_c}S_c + F_Z(1-\theta_f)Vx_Z \quad (1)$$

$$\frac{\partial S_nV_e}{\partial t} = D_n\frac{\partial^2 S_nV_e}{\partial x^2} + a_sV(-k_{s_np}^+S_n\cdot P + k_{s_np}^-\overline{S_nP}) - V_ek_{S_n}S_n + F_T(1-\theta_f)Vx_T \quad (2)$$

$$\begin{aligned}\frac{\partial Pa_sV}{\partial t} &= a_sV(-k_{s_cp}^+S_c\cdot P + k_{s_cp}^-\overline{S_cP} - k_{s_np}^+S_n\cdot P + k_{s_np}^-\overline{S_nP}\\ &\quad -k_{mp}^+M^a\cdot P + (k_{mp}^- + k_{mp})\overline{MP} + k_p^0 + k_p^*M^a - k_PP)\end{aligned} \quad (3)$$

$$\frac{\partial M^aa_sV}{\partial t} = a_sV(-k_{mp}^+M^a\cdot P + k_{mp}^-\overline{M^ap} + \sum_{j=c,n}k_{s_jpm}\overline{S_jPM}) \quad (4)$$

$$\frac{\partial M^ia_sV}{\partial t} = a_sV(-\sum_{j=c,n}[k_{s_jpm}^+M^i\cdot\overline{S_jp} - k_{s_jpm}^-\overline{S_jPM}] + k_{mp}\overline{MP}) \quad (5)$$

$$\frac{\partial\overline{MP}a_sV}{\partial t} = a_sV(k_{mp}^+M^a\cdot P - (k_{mp}^- + k_{mp})\overline{MP}) \quad (6)$$

$$\frac{\partial\overline{S_jPM}a_sV}{\partial t} = a_sV(k_{s_jpm}^+M^i\cdot\overline{S_jP} - (k_{s_jpm}^- + k_{s_jpm})\overline{S_jPM}) \quad (7)$$

$$\frac{\partial\overline{S_jP}a_sV}{\partial t} = a_sV(k_{s_jp}^+S_j\cdot P - (k_{s_jp}^- + k_{s_jp})\overline{S_jP}) \quad (8)$$

The initial conditions are given by

$$\overline{MP}(x,0) = \overline{S_jP}(x,0) = \overline{S_jPM}(x,0) = S_j(x,0) = 0,\ \ M^i(x,0) = M^s,\ \ M^a(x,0) = 0,\ \ P(x,0) = 0$$

and the boundary conditions are

$$D_c \frac{\partial S_j}{\partial x}(0,t) = D_n \frac{\partial S_n}{\partial x}(0,t) = 0$$

$$D_c \frac{\partial S_j}{\partial x}(L,t) = D_n \frac{\partial S_n}{\partial x}(L,t) = 0.$$

In order to cast the equations into dimensionless form, we introduce the characteristic length and time scales L and T, a characteristic chemical concentration per unit volume U, and a characteristic chemical concentration per unit area W. We define the dimensionless concentrations as the ratio of the dimensional concentrations to the corresponding characteristic concentrations. Thus $s_c = S_c/U$, $\overline{s_c p} = \overline{S_c P}/W$, $p = P/W$, $m^a = M^a/W$, etc. The nondimensional length ξ and time τ are defined as $\xi = x/L$ and $\tau = t/T$ respectively. We also define the dimensionless groups

$$\delta_c = \frac{D_c T}{L^2} \qquad \delta_n = \frac{D_n T}{L^2} \qquad \gamma = \frac{W a_s}{U \theta_f}$$

$$\hat{k}_p^0 = \frac{T k_p^0}{W} \qquad f_T = \frac{F_T (1-\theta_f) x_T T}{\theta_f U} \qquad f_Z = \frac{F_Z (1-\theta_f) x_T T}{\theta_f U}.$$

We define nondimensional rate constants for bimolecular reactions as the dimensional constants multiplied by their corresponding reference concentrations and the reference time, and first-order nondimensional rate constants as the dimensional constants multiplied by the reference time. We use the character $\hat{}$ to distinguish between the dimensional and nondimensional forms. For example,

$$\hat{k}^+_{s_c p} = TUk^+_{s_c p} \qquad \hat{k}^-_{s_c p} = Tk^-_{s_c p} \qquad \hat{k}^+_{mp} = TWk^+_{mp}.$$

We then may write the governing equations in dimensionless form as follows

$$\frac{\partial s_c}{\partial \tau} = \delta_c \frac{\partial^2 s_c}{\partial \xi^2} + \gamma(-\hat{k}^+_{s_c p} s_c \cdot p + \hat{k}^-_{s_c p} \overline{s_c p}) - \hat{k}_{s_c} s_c + f_Z \tag{9}$$

$$\frac{\partial s_n}{\partial \tau} = \delta_n \frac{\partial^2 s_n}{\partial \xi^2} + \gamma(-\hat{k}^+_{s_n p} s_n \cdot p + \hat{k}^-_{s_n p} \overline{s_n p}) - \hat{k}_{s_n} s_n + f_T \tag{10}$$

$$\begin{aligned} \frac{\partial p}{\partial \tau} = & -\hat{k}^+_{s_c p} s_c \cdot p + \hat{k}^-_{s_c p} \overline{s_c p} - \hat{k}^+_{s_n p} s_n \cdot p + \hat{k}^-_{s_n p} \overline{s_n p} \\ & -\hat{k}^+_{mp} m^a \cdot p + (\hat{k}^-_{mp} + \hat{k}_{mp}) \overline{mp} + \hat{k}^0_p + \hat{k}^*_p m^a - \hat{k}_p p) \end{aligned} \tag{11}$$

$$\frac{\partial m^a}{\partial \tau} = -\hat{k}^+_{mp} m^a \cdot p + \hat{k}^-_{mp} \overline{m^a p} + \sum_{j=c,n} \hat{k}_{s_j pm} \overline{s_j pm} \tag{12}$$

$$\frac{\partial m^i}{\partial \tau} = -\sum_{j=c,n} [\hat{k}^+_{s_j pm} m^i \cdot \overline{s_j p} - \hat{k}^-_{s_j pm} \overline{s_j pm}] + \hat{k}_{mp} \overline{mp} \tag{13}$$

$$\frac{\partial \overline{mp}}{\partial \tau} = \hat{k}^+_{mp} m^a \cdot p - (\hat{k}^-_{mp} + \hat{k}_{mp}) \overline{mp} \tag{14}$$

$$\frac{\partial \overline{s_j pm}}{\partial \tau} = \hat{k}^+_{s_j pm} m^i \cdot \overline{s_j p} - (\hat{k}^-_{s_j pm} + \hat{k}_{s_j pm}) \overline{s_j pm}) \tag{15}$$

$$\frac{\partial \overline{s_j p}}{\partial \tau} = \hat{k}^+_{s_j p} s_j \cdot p - (\hat{k}^-_{s_j p} + \hat{k}_{s_j p}) \overline{s_j p}) \tag{16}$$

The initial conditions are given by

$$\overline{mp}(\xi,0) = \overline{s_j p}(\xi,0) = \overline{s_j pm}(\xi,0) = s_j(\xi,0) = 0, \;\; m^i(\xi,0) = 1, \;\; m^a(\xi,0) = 0, \;\; p(\xi,0) = 0$$

and the boundary conditions are

$$\frac{\partial s_j}{\partial \xi}(0,\tau) = \frac{\partial s_n}{\partial \xi}(0,\tau) = 0$$

$$\frac{\partial s_j}{\partial \xi}(1,\tau) = \frac{\partial s_n}{\partial \xi}(1,\tau) = 0.$$

Table 1 lists the parameters (dimensional and dimensionless forms) used for the simulations.

Discussion of Parameter Values. We let $L = 0.1$ cm, the approximate width of the limb bud, and $T = 24$ h, the time scale of the experiments. The reference extracellular concentration U is taken to be 1 nM, and the reference membrane concentration of proteins (W) is taken to be 5×10^{-3} nM-cm, which corresponds to a concentration of 1×10^4 molecules per 5μm radius cell.

It can be shown that

$$\frac{a_s}{1-\theta_f} = \frac{a_{cell}}{V_{cell}} = \frac{3}{r}.$$

We choose a_s to be 5357 cm^{-1}, which is $\approx 90\%$ of the surface-area-to-volume ratio of an isolated sphere with a radius of 5μm. We choose a void fraction of 0.11 that satisfies the above relation for a radius of 5μm, allowing for tightly packed cells with 11% volume taken up by the extracellular matrix.

F_Z and F_T: We assume a production rate for the specialized ZPA cells of 800 Shh molecules per cell per minute. This is a modest rate compared to the rate of production of proteins such as ovalbumin, which has been reported to be $\approx 10^6$ molecules per cell per minute (1). As the cells used for the tissue implants are derived from ZPA cells, we assume that they have properties identical to the native ZPA.

The basal rate of Ptc production, k_p^0 is set to be 10 molecules per cell per h. Reaction rate constants correspond to typical values for receptor-ligand binding and degradation constants (2) have been used. It has been shown that the two forms of Shh have similar binding rate constants, but the Shh response caused by S_c is 30-fold that caused by S_n (3). This is the reason for assuming that the specific degradation rate $k_{s_c pm}$ of the intermediate complex formed during the catalytic activation of Smo by $\overline{S_c P}$ is 10-fold faster than the specific degradation rate $k_{s_n pm}$ for degradation of the intermediate complex formed during the catalytic activation of Smo by $\overline{S_n P}$. Notice that all the rate constants are almost identical. This is because most constants correspond to the initial guess value used to simulate the Ptc profiles and morphology corresponding to the control experiments.

Formulation of 2D model. A detailed formulation of the model used in the 2D simulations is presented in ref. (4). For completeness, we state the equations used in the model and the values of the parameters used for the simulations presented in this work. The reader is referred to ref. (4) for details.

Continuity Equation:

$$\frac{\partial u_x}{\partial x} + \frac{\partial u_y}{\partial y} = S([Fgf], x, y) \tag{17}$$

$$S([Fgf], x, y) = \frac{s_m [Fgf](x,y)}{K_m + [Fgf](x,y)} \tag{18}$$

Navier-Stokes Equations:

$$\rho\Big(\frac{\partial u_x}{\partial t} + u_x\frac{\partial u_x}{\partial x} + u_y\frac{\partial u_x}{\partial y}\Big) = -\frac{\partial p}{\partial x} + \mu\Big(\frac{\partial^2 u_x}{\partial x^2} + \frac{\partial^2 u_x}{\partial y^2} + \frac{1}{3}\frac{\partial S}{\partial x}\Big) + F_x \tag{19}$$

$$\rho\Big(\frac{\partial u_y}{\partial t} + u_x\frac{\partial u_y}{\partial x} + u_y\frac{\partial u_y}{\partial y}\Big) = -\frac{\partial p}{\partial y} + \mu\Big(\frac{\partial^2 u_y}{\partial x^2} + \frac{\partial^2 u_y}{\partial y^2} + \frac{1}{3}\frac{\partial S}{\partial y}\Big) + F_y \tag{20}$$

F_x and F_y are the x- and y-components of the body force density.

Body Force Density:

$$\mathbf{F}(\mathbf{x},t) = \int_\Gamma \mathbf{f}(s,t)\delta(\mathbf{x}-\mathbf{X}(s,t))ds \tag{21}$$

The force density at each boundary element is given by

$$\mathbf{f}_k = \sum_{j=1}^{3} \mathbf{f}_k^j \tag{22}$$

$$\mathbf{f}_k^1 = \sum_{p=k-1,p\neq k}^{k+1} S_1(\|\mathbf{X}_k-\mathbf{X}_p\|-L_0)(\mathbf{X}_k-\mathbf{X}_p)/\|\mathbf{X}_k-\mathbf{X}_p\| \tag{23}$$

$$\mathbf{f}_k^2 = S_2(\mathbf{X}_k-\mathbf{X}_k^0) \tag{24}$$

$$\mathbf{f}_k^3 = S_3(\|\mathbf{X}_k-\mathbf{X}_p\|-D_w)(\mathbf{X}_k-\mathbf{X}_p)/\|\mathbf{X}_k-\mathbf{X}_p\| \tag{25}$$

Eq. 24 is used only for the tethered points along the proximal boundary of the limb bud. Instead of the simple spring forces used for elements across the width of the limb (Eq. 25), we use a better representation comprised of a spring and dashpot combination. The Dirac delta function is approximated as

$$\delta(\mathbf{x}-\mathbf{X}) \approx d(x-X)d(y-Y) \tag{26}$$

$$d(r) = \begin{cases} \frac{1}{4h}\left(1+\cos\frac{\pi r}{2h}\right) & |\ r\ | < 2h \\ 0 & |\ r\ | \geq 2h \end{cases} \tag{27}$$

h is the mesh size used in the numerical simulations.

Reaction-Diffusion-Convection: Equations for S_c, S_n and fibroblast growth factor (FGF) concentration take the form

$$\frac{\partial c}{\partial t} + cS + \left(u_x\frac{\partial c}{\partial x} + u_y\frac{\partial c}{\partial y}\right) = D_c\left(\frac{\partial^2 c}{\partial x^2}+\frac{\partial^2 c}{\partial y^2}\right) + \mathbf{R_c} \tag{28}$$

where c is the concentration of the particular protein, D_c is the corresponding diffusion coefficient and $\mathbf{R}_c$ is the reaction rate term associated with the protein. In the case of Shh, the reaction rate term includes exactly the same degradation rates and binding rates that were used in the 1D simulation. The production rate, which was treated as a constant (F_Z or F_T) in the 1D simulation, is now modeled as dependent on the local FGF concentration. As in the 1D simulation, the production rate is zero in the region outside the ZPA or tissue implant response. Similarly, for FGF, the production rate is nonnegative only in the apical ectodermal ridge (AER) region. The FGF production rate in the AER (F_A) depends on the local Shh concentration. Both forms of Shh are assumed to affect FGF production to the same extent. The production rates are given by

$$F_Z = F_T = V_Z\frac{[FGF]}{K_Z+[FGF]} \tag{29}$$

$$F_A = V_A\frac{[S_c]+[S_n]}{K_A+[S_c]+[S_n]} \tag{30}$$

Equations for the concentration for all the other species (Ptc, Smo, etc.) are the same as Eq. 28 with the diffusion coefficient set to zero. The reaction terms are exactly the same as used for the 1D simulation. In the equation for Ptc production, a time lag of 30 min is used to approximate the time required for the signal transduction mechanism (phosphorylation, DNA binding, transcription and translation of *ptc* transcripts). Changing this lag time to zero does not change the qualitative nature of the results.

Discussion of Parameter Values. The values of the parameters used in the 2D simulations are given in Table 2. The tissue density is assumed to be equal to that of water. The viscosity of chick limb tissue has been measured (5) experimentally and used to set the range for the value used in this study. Details of the numerical simulation are given in ref. (4). Briefly, simulations were started with the given initial conditions for a period of 6 h in the absence of the implant. The implant was activated after 6 h, and simulations were carried out for an additional 20 h. As in the 1D simulations, except the implant Shh concentration and diffusivity, all the other parameters are unchanged for the wild-type (no implant), bead-implant and tissue-implant simulations.

Computations on a fixed 2D domain reveal that the bead implant results are qualitatively obtained even in this system. The response seen for the tissue implant depends on interactions of the implant with the AER. In a simulation using a nongrowing domain where the AER is always at a fixed distance from the implant site, the Ptc expression does not show any decline as the FGFs from the AER can quickly reestablish Shh production in the implant. This shows that the particular values of the fluid property parameters and growth parameters used do not significantly affect the qualitative nature of the bead-implant results which are influenced only by interactions along the A-P axis. However, the cyclic *ptc* expression profile depends on the location of the tissue implant and the growth of the tissue.

1.Palmiter, R. D. (1975) *Cell* **4**, 189–197.

2.Lauffenburger, D. A. & Linderman, J. J. (1993) *Receptors: Models for Binding, Trafficking and Signaling*. (Oxford Univ. Press, New York).

3.Pepinsky, R. B., Zeng, C., Wen, D., Rayhorn, P., Baker, D. P., Williams, K. P., Bixler, S. A., Ambrose, C. M., Garber, E. A., Miatkowski, K., Taylor, F. R., Wang, E. A., et al. (1998) *J. Biol. Chem.* **273**, 14037–14045.

4.Dillon, R. & Othmer, H. G. (1999) *J. Theor. Biol.* **197**, 295–330.

5.Forgacs, G., Foty, R. A., Shafrir, Y., & Steinberg, M. S. (1999) *Biophys. J.* **74**, 2227–2234