Tissue Failure Propagation as Mediated by Circulatory Flow

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ABSTRACT Aging is driven by subcellular processes that are relatively well understood. However, the qualitative mechanisms and quantitative dynamics of how these micro-level failures cascade to a macro-level catastrophe in a tissue or organs remain largely unexplored. Here, we experimentally and theoretically study how cell failure propagates in an engineered tissue in the presence of advective flow. We argue that cells secrete cooperative factors, thereby forming a network of interdependence governed by diffusion and flow, which fails with a propagating front parallel to advective circulation.

INTRODUCTION

The aging and death of an organism is typically attributed to subcellular mechanisms such as reactive oxygen species damage to proteins and DNA or slowing down of tissue repair due to shortening telomeres. Although this may be fundamentally true, an organism does not ultimately die because it gradually runs out of cells but rather because cellular-level failures cascade hierarchically upward, leading to malfunctions in tissues and organs and leading to a systemic catastrophe. Although microscopic mechanisms of cellular malfunction are relatively well studied (1–4), how failure propagates from the subcellular level to tissues and organs and ultimately the organism is largely unknown.

In (5), the failure of an organism was modeled as a reliability circuit in which cells within an organ are connected by OR gates (so that an organ fails when all its cells fail), and the organs are connected by AND gates (so that organism dies as soon as one organ fails). In (6), aging was described as failures taking place on a complex network of interdependent building blocks. In this model, when a node in the network malfunctions, so will those that depend on it. As a result, a few random microscopic failures can propagate to many others, ultimately leading to death. Vural et al. (6) could bridge microscopic malfunctions with their experimentally observed macroscopic manifestations, such as organismic death and population demographics, and fit experimental data such as (7) and (8) (also cf. appendix of (9)). The network approach to aging has also been insightful for understanding loss of function and frailty (10,11).

Although describing a complex organ or an entire organism as a random network of interdependencies is a useful starting point to understand how it fails (12), it is also a rather crude oversimplification. First, in real biological systems, the large-scale structure of an interdependence network is far from random. Secondly, there can be varying amounts and varying types of dependencies. In a complex organism, the exchange of signals and goods between cells occurs via either diffusion or circulation (13).

For example, interstitial flow is responsible for transporting various cooperative factors to the extracellular matrix (14),
from which cells can take in these factors \((15,16)\). The direction of flow depends on the structure and direction of capillaries and the extracellular matrix. In most cases, collagen is aligned, and the interstitial flow occurs both in the fiber direction and in the transverse direction, enabling transportation of the cooperative factors along the fibers and in and out of that particular collagen bundle \((17,18)\). Even for small flow velocities, convective flow can become more important for the transport of large molecules, which will have small diffusive range over biologically relevant timescales \((14)\). Therefore, the interdependence structure of an organism is highly dependent on its biophysical organization. As such, biophysically grounded analogs of interdependence networks are essential to understand failure propagation in living systems.

Such biophysical extensions have been investigated experimentally \((19)\) and theoretically \((20)\) to understand how failure propagates through tissues, as mediated by the loss of diffusing cooperative factors. These cooperative factors could be cytokines \((e.g.,\ \text{interleukin 15})\), growth factors \((e.g.,\ \text{epidermal growth factor})\), survival factors \((e.g.,\ \text{insulin-like growth factor 1})\), and antioxidant enzymes \((e.g.,\ \text{superoxide dismutase 3})\) \((21–29)\) diffusing across cells. However, the role of convective circulation—which is the primary mode of transport in large, complex organisms—and its role on biological failure propagation remain unknown.

The central argument of this work is that failure propagates and cascades hierarchically upward along the circulatory network of an organism, in the same direction as the advective flow (Fig. 1). To this end, we theoretically and experimentally study the mechanical nature of failure propagation through engineered tissues in the presence of advective flow and quantify how failures accumulate and propagate in relation to the flow direction. Although our experiments are conducted on mammalian tissues cultured in a microfluidic device, from here, and with the help of analytical arguments, we aim to derive more general lessons about the aging dynamics in complex organisms in vivo.

The demographic hallmark of aging is a monotonically increasing mortality rate \(\mu(t)\). In strongly aging species (like humans), the probability of death of an old individual is many times larger than a young individual. Interestingly, there are phylogenetic correlations in aging characteristics \((30)\): mammalian populations have the steepest mortality curves, whereas \(\mu(t)\) of amphibians and reptiles changes little over time, and plants tend to age even less and can exhibit mortality rates that even decrease over time. The cause behind these phylogenetic trends is not entirely clear, but one possibility may be the differences in how vital goods and signals are transported between cells, which then manifest as differences in how failures accumulate and propagate.

For example, assaulting a sizeable portion of a shrub might not kill it, because of how little the rest of the shrub depends on the assaulted portion. The interdependence structure of a shrub is highly localized. In organisms with fast and efficient circulatory systems, however, advective transport enables any cell to depend on any other, no matter how distant. A malfunction in an animal gland or organ will affect all cells that are coupled to it via bloodstream. Thus, as much as convective flow propagates goods and signals, it should also propagate failure along the same path once the goods and signals go missing.

To test these ideas experimentally, we encapsulated rat cells in a nondegradable hydrogel \((in\ which\ they\ cannot\ proliferate,\ migrate,\ or\ contact\ each\ other)\) seated in an engineered microfluidic microchannel, through which we apply unidirectional flow of media, meant to emulate blood, lymph, or interstitial flow. We then analyzed cell death rate across the channel as a function of time and flow rate. Directing cooperative factors downstream lead to a wave of failure starting near the inlet and propagating toward the outlet, which we analytically described using a mechanistic diffusion-convection-population-dynamics model.

From this model, we obtained relevant length scales in terms of system parameters, determined the conditions for where a failure wave should originate, and obtained the velocity and acceleration of its propagation.

**MATERIALS AND METHODS**

**Construction of the tissue engineered model**

 fabrication of the microfluidic device

Poly(dimethylsiloxane) prepolymer and curing agent \((\text{Dow Corning})\) were mixed thoroughly at a 10:1 ratio and cast on a silicon-base mold with \(20\ \text{mm} \times 0.5\ \text{mm} \times 0.25\ \text{mm}\) (length \(\times\) width \(\times\) height) ridges and cured at \(80^\circ\text{C}\) for 30 min. The poly(dimethylsiloxane) and a glass slide were treated with air plasma for 1 min and immediately bound to each other, with the channel side facing the slide (Fig. S1 i). The device was sterilized under UV for 2 h.
Preparation of the polyethylene glycol solution

Maleimide polyethylene glycol (PEG) succinimidyl carboxymethyl ester (PEG-NHS, Mw 3400 Da; JenKem Technology) was conjugated with tyrosine-arginine-glycine-aspartic acid-serine (YRGDS; Bachem) as described previously, to obtain the PEG-RGD (19). The PEG-RGD and 4-arm PEG-acrylate (20 kDa; JenKem Technology) were mixed at a 1.5:8.5 (w/w) ratio, and a 20% (w/v) solution was prepared in culture medium (DMEM-high glucose containing 10% fetal bovine serum and 1% penicillin-streptomycin). The photoinitiator Irgacure D-2959 (Sigma-Aldrich) (final concentration: 0.1%, w/v) was added.

Cell culture and seeding

Cardiac fibroblasts were isolated from hearts of 2-day-old Sprague-Dawley rats (Charles River Laboratories) according to the IACUC guidelines with the approval of the University of Notre Dame, which has an approved assurance of compliance on file with the National Institutes of Health, Office of Laboratory Animal Welfare. Rats were sacrificed via CO2 treatment, and the hearts were immediately collected, minced, and incubated in trypsin (Life Technologies) at 4°C for 1 h with gentle agitation as described previously (19). After further digestion with collagenase type II (Worthington-Biochem) at 37°C, the tissues were strained through 40-μm filters, and the cells in filtrate were incubated at 37°C for 2 h. This brief incubation allowed exclusively fibroblasts to attach on the plate. After removal of unattached cells, fibroblasts were incubated in culture media with media change every 3 days until passage 4. When the cells were at ~80% confluency, they were detached from flasks using 0.25% trypsin-EDTA (Life Technologies), counted, and reconstituted in culture medium.

The cell suspension was mixed with the PEG/PEG-RGD solution at a 1:1 volume ratio and loaded into the microfluidic device such that the final cell density was 5 × 105 cells/mL, the PEG/PEG-RGD concentration was 10% (w/v), and the photoinitiator concentration was 0.05% (w/v) (Fig. S1 ii). The polymer was exposed to UV at 365-nm wavelength and 6.9 mW/cm² intensity for 60 s to cross-link it. We chose these parameters to minimize the possible harmful effect of UV on cells (31). The device was connected to a pump loaded with a syringe full of culture medium and perfused at a flow rate of 150 μL/h for 30 min to wash away the photoinitiator and the un-cross-linked polymer remaining in the gel. The bright field images of the gel were taken using a microscope (Hamamatsu ORCA flash 4.0; Zeiss), and the initial total cell number was determined using Fiji software (NIH).

Cell viability measurements

Cell viability was determined using the Live/Dead cell viability assay (Thermo Fisher Scientific) either daily (at days 0, 1, and 2) or in real time (every 2 h for 16 h). For the daily analysis, the microfluidic device was disconnected at each time point, perfused with PBS containing ethidium homodimer-1 (4 μM) for 30 min, and imaged under bright field (total cells) or fluorescence (dead cells, red) modes with a fluorescence microscope (Hamamatsu ORCA flash 4.0; Zeiss). The device was reconnected to the syringe pump full of culture medium and perfused at 20 μL/min flow rate until the next time points (days 1 and 2).

To monitor cell viability in real time, the device was perfused with culture medium containing ethidium homodimer-1 (4 μM) at two flow rates (20 and 120 μL/h) and imaged under bright field at time 0 (total cells) and then under fluorescence with time-lapse imaging at every 2 h for 16 h (dead cells). The cell numbers in gel areas of 0.5 mm × 1.25 mm were determined all along the gel using Fiji software (NIH). Three different measurements of the same area, the second and third being at 0.2 mm distances from the first one, were done to calculate the average cell numbers. The data from the middle portion of the gel were analyzed to eliminate the boundary effect. Cell viability was calculated as (total − dead)/total.

Statistical analysis

Statistical analyses were done using the Student’s t-test. For statistical significance, p < 0.05.

RESULTS

Experimental results

To investigate how failure propagates through a tissue under flow conditions, a microfluidic channel filled with cardiac fibroblast-laden hydrogel was perfused at various flow rates and imaged in real time every 2 h for 16 h. At a flow rate of 20 μL/min, we observed a gradual increase in the dead cell number over time in the inlet, whereas dead cell number remained relatively stable over time at the outlet (Fig. 2, a and

![Figure 2](image-url)
b). The difference between the dead cell number at $t = 2$ h and at $t = 16$ h gradually decreased toward the outlet. Thus, after 16 h of perfusion, cell viability was significantly higher at the outlet portion relative to the inlet portion (Fig. 2c).

To see the effect over a long time, in another experiment, the engineered tissue was perfused at a flow rate of 20 $\mu$L/h for 2 days, and the dead cells were imaged and counted at days 0, 1, and 2 (Fig. S2). Upon perfusion, more cells died at the inlet, and cell viability showed an increasing trend along the gel toward the outlet. Viability at the inlet was significantly lower than that at the outlet both for day 1 ($p < 0.0004$) and for day 2 ($p < 0.0008$).

Considering that the flow rate did not change along the microchannel, the shear stress exerted on the cells by the flow itself should be the same. Thus, we concluded that the gradual increase in cell viability toward the outlet was not because of the shear force but rather was due to accumulation of the cooperative factors.

The difference between the relative cell viability (viability at a given time relative to that at $t = 2$ h) at the inlet and outlet changed dramatically when the flow rate was altered (Fig. 3). At a flow rate of 20 $\mu$L/min, relative cell viability decreased by 2% at the outlet and 14% at the inlet over a 16-h period, with a gradual decrease at both edges (Fig. 3a). The viability difference between the inlet and outlet reached 12% at $t = 16$ h. At a flow rate of 120 $\mu$L/min, viability at the outlet decreased by 58% over a 16-h period, whereas that at the inlet decreased by 93% (Fig. 3b). Although cell viability was initially higher at the inlet (69%) than the outlet (38%), it decreased dramatically over time and became higher at the outlet (16%) than at the inlet (11%).

Therefore, at the low flow rate, cell viability was the highest, but the difference between the viability at the inlet and outlet was the smallest. With the increasing flow rate, viability decreased remarkably, especially at the inlet but also at the outlet. These observations suggest that, at lower flow rates, cooperative factors were not completely cleared from the inlet and could bind to their receptors on cell surfaces, improving cell viability outcomes. When the flow rate was increased, more cooperative factors were washed out from the inlet, giving them less time to bind to receptors on the cells and dramatically decreasing cell viability. As cells at the inlet die out, they are no longer able to produce cooperative factors to aid the survival of cells at the outlet, leading to a propagation of failure and a reduced viability of cells at the outlet.

**Analytical results**

**Model**

To characterize the dynamics of flow-mediated failure propagation in a more general setting, and to make predictions beyond the experiments described above, we developed an analytical model, the inputs of which we obtained from experiments described above. Our model assumptions, qualitatively stated, are as follows: 1) The cells do not proliferate and migrate but are under stress and die. We denote the cell density at position $x$ along the channel at time $t$, with $n(x, t)$. 2) Cells secrete some cooperative factor(s) that will diffuse, flow, and decay within the circulating fluid and help other cells survive/function. We assume that the diffusion and decay parameters for these factors are similar and denote their concentration collectively by a single quantity, $\Phi(x, t)$. These assumptions can be quantified as

\[ \dot{n} = -\alpha \frac{\phi_0 + \phi}{\phi_0 + \phi} n \]  

\[ \phi = d\nabla^2 \Phi - v \cdot \nabla \Phi - \gamma \Phi + An, \]  

where $d$ is the diffusion constant for the cooperative factors, $v$ is the flow velocity, $A$ is the rate at which cells secrete cooperative factors, $k$ describes the steepness of the response of cells to cooperative factors, and the constant $\phi_0$ quantifies the “required amount” of factors for a cell to function normally. The functional form of the right-hand side is

![Experimental data with fitted curves.](image)

*FIGURE 3* Experimental data with fitted curves. Fitted parameter values are $k = 1.87$, $\alpha = 0.25$, $\beta_{\text{inlet}} = 5.30$, and $\beta_{\text{outlet}} = 7.13$ for $v = 20$ $\mu$L/h and $\beta_{\text{inlet}} = 1.43 \times 10^{-4}$ ($\alpha$) and $\beta_{\text{outlet}} = 0.27$ for $v = 120$ $\mu$L/h ($b$). We indeed see $\beta_{\text{inlet}} < \beta_{\text{outlet}}$ for each flow rate, signifying a larger portion of cooperative factors in the vicinity of cells at the outlet. Inlet cell populations were given by averaging over measurements taken between 1.25 and 2.5 mm from the inlet for $v = 20$ $\mu$L/h and 3.75 and 5 mm from the inlet for $v = 120$ $\mu$L/h. Outlet cell populations were given by averaging over measurements taken between 5 and 6.25 mm from the inlet for $v = 20$ $\mu$L/h and 8.75 and 10 mm from the inlet for $v = 120$ $\mu$L/h. Error bars correspond to one standard deviation from the mean. To see this figure in color, go online.
hand side of Eq. 1 is the Hill function, which accurately describes a cell’s response to many different kinds of molecular agents. It is motivated by Michaelis-Menten-type reaction kinetics, fits experimental findings, and is ubiquitously used in population dynamics models. \( \alpha \) is the cell death rate when there are no cooperative factors. In a variable environment, \( \alpha \) could be time dependent; however, here we take it to be constant.

We study the phenomena of failure propagation by first extracting the relevant length scales. From Eq. 2, we can solve the Green’s function for a point source (Appendix B). From the Green’s function, we can then extract left \( \ell_L \) and right \( \ell_R \) length scales, given as

\[
\ell_L(v) = \left[ \frac{\lambda(v) + \frac{v}{2d}}{2d} \right]^{-1}, \quad \ell_R(v) = \left[ \frac{\lambda(v) - \frac{v}{2d}}{2d} \right]^{-1}, \quad (3)
\]

where we have defined \( \lambda(v) = \sqrt{\gamma/d + v^2/4d^2} \) and flow is taken to be from left to right \( (v \geq 0) \). These length scales correspond to the characteristic advection-diffusion-decay length of the cooperative factors given by a point source.

As the flow velocity \( v \) increases, the left length goes to zero, and the right length increases roughly linearly with respect to \( v \). This introduces a bias in the concentration of cooperative factors, and we expect to see a propagation of cell death in the direction of flow. As cells upstream die, the cooperative factors upstream also diminish, causing a propagation of death moving downstream, in the direction of flow.

**Experimental fits**

To fit our model to experimental data, we first make some simplifying assumptions to reduce our model to a solvable system. We assume the local cooperative factor concentration \( \Phi(x,t) \) will be proportional to the local cell density \( n(x,t) \). We denote this proportionality constant \( \beta \), so that \( \Phi(x,t) = n(x,t)\beta \). In the center of the microchannel, where \( n \) is roughly uniform, this constant would be given by taking the steady-state cooperative factor concentration, giving \( \Phi = An/\gamma \), so \( \beta = A/\gamma \). By the inlet, this constant will be lower in value because the area of cells contributing to the cooperative factors will be reduced.

The constant \( \beta \) will in general depend on the length scales contributing to the local cooperative factor concentration and thus will vary between the inlet and outlet regions. Specifically, we expect the value of \( \beta_{\text{inlet}} \) to scale as \( \beta_{\text{inlet}} \sim A\ell_L/(\ell_L + \ell_R) \gamma \), and \( \beta_{\text{outlet}} \sim A\ell_R/(\ell_L + \ell_R) \gamma \). Therefore, for a large flow rate, we expect the value of \( \beta_{\text{inlet}} \) to be less than \( \beta_{\text{outlet}} \) because more cells are able to contribute to the cooperative factor concentration at the outlet. With this assumption, we can write down an effective equation describing the growth of the cells over time as

\[
\dot{n} = \frac{-\alpha n}{1 + \left( \beta_{n}/n_0 \right)^k},
\]

where \( n_0 \) is the initial cell concentration. We can then solve this exactly to get

\[
n(t) = \left( n_0 / \beta \right) W\left( e^{\beta t - \beta n_0} \right)^{1/k},
\]

where \( W(z) \) is the Lambert W function, defined as the principal solution for \( w \) in \( z = we^w \), and can be computed to arbitrary numerical precision.

We then fit our model to experimental data in Fig. 3. Because we expect the growth kinetics to be the same in both experiments, with the only difference being flow, we constrain the fit parameters for \( \alpha \) and \( k \) to be the same for all fits and allow different values of \( \beta \) for inlet and outlet regions and for each flow rate \( v = 20 \mu L/h \) and \( v = 120 \mu L/h \). For Hill and decay constants, we then get \( k = 1.87 \) and \( \alpha = 0.25 \) h^{-1}. For flow rate \( v = 20 \mu L/h \), we get \( \beta_{\text{inlet}} = 5.30 \) and \( \beta_{\text{outlet}} = 7.13 \). For \( v = 120 \mu L/h \), we get \( \beta_{\text{inlet}} = 1.43 \times 10^{-14} \) and \( \beta_{\text{outlet}} = 0.27 \). We see that indeed \( \beta_{\text{inlet}} \leq \beta_{\text{outlet}} \) as expected for each flow rate. We also see the values of \( \beta \) are much smaller for the larger flow rate, with the inlet value of \( \beta \) being vanishingly small at the flow rate \( v = 120 \mu L/h \). This is because with a large flow rate, the cooperative factors are pushed much farther downstream and no longer help the cells at the inlet and may also diminish the cooperative factor concentration at the outlet.

We next study the system of Eqs. 1 and 2 and derive conditions for a propagation of failure, as well as the velocity and acceleration of failure propagation.

**Model regimes and failure propagation**

From numerical simulations and dimensional analysis, we find that the condition for failure propagation to occur is that the initial density of cells \( n_0 \) must be sufficiently large such that \( A n_0/\gamma \gg \phi_0 \). If instead \( A n_0/\gamma < \phi_0 \), we see all cells die uniformly, and there is no propagation of failure; rather, there is just a global death. This is because the local cooperative factor concentration is given by \( A n/\gamma \), and this value must be above the threshold concentration \( \phi_0 \) for cell density to not decay exponentially. If the cell concentration drops below this critical value, there will no longer be a pronounced propagation of failure. Instead, the entire population of cells will die roughly uniformly at an exponential rate of \( \alpha \).

For cases in which there is a propagation of failure, where \( A n_0/\gamma > \phi_0 \), we plot the numerical solution to our system in Fig. 4 for values of the Hill constant \( k = 1, 2, 4 \). We find that as we increase the value of the Hill constant, we get a more pronounced wave. The population dynamics is strongly determined by the initial density \( n_0 \) and the form of the response given by the Hill constant \( k \). In the case in which \( k \) is low, the bulk where \( \Phi(x) > \phi_0 \) will attenuate quicker. We find this attenuation of the bulk leads to an “acceleration” of death. We also study this attenuation and the corresponding acceleration of death propagation.

We now determine the velocity at which the death of cells will propagate. Our derivations for failure propagation
velocity, depth, and acceleration are given in detail in Appendix C. We illustrate here the procedure used in our derivations.

To simplify our analysis, we used a boxcar approximation to the Green’s function for the cooperative factors, where left and right lengths are given by Eq. 3, and assume chemicals quickly reach steady state. The total area of the Green’s function corresponds to the secretion rate per cell density at steady state. We therefore have \( G(x) = (A/\gamma)\Theta(x + \ell_L)\Theta(\ell_R - x), \) where \( \Theta(x) \) is the Heaviside step function.

We then convolve this boxcar Green’s function with a semi-infinite initial cell concentration, \( n(x, 0) = n_0\Theta(x) \). This gives a cooperative factor concentration profile that increases linearly up until \( x = \ell_R \), after which \( \Phi(x) \) saturates to a constant given by \( \Phi(x > \ell_R) = A_0/\gamma \). Assuming the initial cell density is sufficiently large such that \( A_0/\gamma > \phi_0 \), we can find the position \( \Delta \) such that \( \Phi(\Delta) = \phi_0 \). To the left of this value, cells are expected to decay exponentially at a rate \( \alpha \). The cooperative factor concentration will then update to this new concentration of cells, and we can reiterate this to get the next decay of cells. The death of cells will therefore continue to propagate by an amount \( \Delta \) at a rate \( \alpha \), giving a first approximation to the failure propagation velocity as \( v_d = \alpha \Delta \). We find this gives good agreement for large \( \Delta \), where the cell death rate behaves closer to a step function, and for short times.

We then further improve these calculations by performing a second iteration with an updated approximate cell concentration, taking into account the region where \( \phi_0 < \Phi(x) < A_0/\gamma \), as well as taking into account the attenuation of the bulk \( n_b(t) = n(x > \ell_R, t) \). Details of this procedure are given in the Appendix C. Our final result for the velocity of failure propagation is

\[
v_d = \frac{\alpha v}{2\gamma} + \frac{\alpha \sqrt{v^2 + 4d\gamma}}{2\gamma(1 + 2\gamma)} \left[ 1 - 2^k + 2^{1+k} \left( \frac{u}{1 - \alpha k t} \right)^{1/k} \right], \tag{4}
\]

where \( u = [\gamma \phi_0/(A_0)]^{1/\gamma} \). The initial cell density will then see a failure propagation at the inlet end at this velocity, as well as an attenuation of the bulk density \( n_b(t) \). For the evolution of the bulk density, we note that \( \Phi(x) \) in this region is given as \( A_0/(\gamma) \). We then expand the Hill function in Eq. 1 about infinity (for \( A_0/(\gamma) \gg \phi_0 \)) and solve for \( n_b(t) \). We then can get for the bulk density

\[
n_b(t) = n_0(1 - \alpha k t)^{-1/k}. \tag{5}
\]

We compare our results with numerical simulations for the failure penetration depth and bulk attenuation over time for values of \( k = 1 - 4 \) in Fig. 5, a and b and see good agreement with analytical formulas.

Note that the velocity diverges when the term in the innermost square bracket vanishes in Eq. 4. This happens at a critical time:

\[
t_c = 1/(\alpha k u). \tag{6}
\]

This corresponds to the time at which the bulk goes below the initial critical cell density, that is, when \( n_b(t) \) from Eq. 5 vanishes. After this, there is no more pronounced wave. From the expression for the death propagation velocity, we can also determine a death depth. Depending on the tissue length \( L \), this death depth may occur before or after the time the bulk collapses.

The attenuation of the bulk also leads to an “acceleration” of failure propagation. This effect is largest for low \( k \) because the bulk decays faster as the Hill function saturates slower. We can get this acceleration \( a_d \) by taking a time derivative of \( v_d \):

\[
a_d = \frac{\alpha^2 \sqrt{v^2 + 4d\gamma}}{\gamma(2 + \gamma)} \left( \frac{u}{1 - \alpha k t} \right)^{(1+k)/k}. \tag{7}
\]

Because \( A_0/\gamma \gg \phi_0 \), we see as \( k \to \infty \), the first term in the square brackets grows much faster than the linear term \( \alpha k t \). Because the exponent is overall negative, this corresponds to a large positive term in the denominator as \( k \to \infty \), and the death acceleration goes to zero.

We compare our analytical results for the velocity and acceleration of death with numerical simulations and get good agreement (Fig. 5, c and d).
DISCUSSION

Here, we studied how failure propagates in a system in which interdependence is mediated by flow. These results emphasize the importance of intercellular processes on aging.

We performed experiments with engineered tissues filled with cardiac fibroblast-laden PEG hydrogels in a microchannel and observed that flow can help increase the lifespan of cells downstream of the flow (Figs. 1 and 2). We explained this observation with cooperative factors, which were carried by the flow toward the outlet. Cooperative factors are known to promote cell survival (19–26), cardioprotection (21,22), and angiogenesis (22,23), and cells failing to receive the necessary factors from the neighboring cells go through apoptosis (32). Motivated by these results, we developed an analytical model to describe the death of cells that communicate via diffusive cooperative factors in a flowing environment. We assumed in our model that cells do not proliferate or migrate but are under stress and die. The probability of death per unit time was assumed to have a Michaelis-Menten-Hill form dependent on a cooperative factor secreted by cells. We assumed a single cooperative factor for a minimal model. The cooperative factor was assumed to diffuse, advect, and decay along with the fluid medium.

Fitting this model to experiment, we saw indeed that the proportion of cooperative factors downstream of the flow were larger than those upstream. This then leads to a faster death of cells by the inlet and longer lifespan for cells by the outlet.

Next, we investigated further the consequences of our analytical model. We found analytical conditions for a

“wave” of failure propagation to occur in the direction of flow. As cells die out upstream, this failure will propagate and increase the mortality rate of cells downstream. The conditions for this propagation to occur were found to be a sufficient density of cells and a nonzero flow rate. Once cell density decreases below a critical threshold, the cells will die uniformly at roughly the same exponential rate. Failure propagation has already been studied previously when cells are coupled through diffusion (19,20). Here, we analyzed the failure propagation under flow and developed an analytical model that describes the depth, velocity, and acceleration of failure propagation. This is physiologically more relevant because cooperative factors are carried

FIGURE 5 Analytical theory and numerical simulations for failure propagation and bulk death versus time. (a) Bulk cell concentration normalized by initial cell concentration $n_0/n_0$ versus time. For lower values of the Hill constant $k$, the cell concentration in the bulk decays quicker. At a critical time $t_c$, the bulk density falls below the critical value $n_0 < \gamma / \phi_0 / \alpha$ and dies out exponentially. (b) Propagation of failure versus time for Hill constants $k = 1, 2, 3, 4$ is shown. For lower Hill constants, the failure depth rapidly increases up to a critical time $t_c$, where the bulk collapses before the propagating wave reaches the end of the domain. For larger Hill constants, the velocity remains roughly constant and reaches the end of the bulk before time $t_c$. (c) Failure propagation velocity versus flow velocity is shown. The failure propagation velocity $v_0$ increases roughly linearly with the flow velocity $v$. (d) Failure propagation acceleration versus Hill constant. As the Hill constant $k$ increases, the failure propagation becomes more pronounced and moves at a more constant speed. The acceleration then decreases roughly exponentially as $k$ increases. Solid lines are obtained from numerical simulations throughout, and dotted lines are obtained from analytical theory. To see this figure in color, go online.
via flow in the body. For many large molecules, convective transport is more important than diffusion over biologically relevant timescales (14). For example, paracrine factors are transported via interstitial flow (18,33).

Because in our experiments the flow rate was kept constant, we concluded the difference in cell viability observed was not due to shear force. However, shear stress is known to affect cell viability (34). Thus, shear force might therefore also include the mechanical interplay of the extracellular matrix and influence death patterns (35). Additionally, the cells themselves also play important roles in maintaining flow. For example, cells help maintain the integrity and tension of the extracellular matrix and therefore help to regulate the interstitial fluid pressure (14). A more sophisticated model might therefore also include the mechanical interplay of flow and cell viability.

Fluid flow is known to mediate communications within microbial communities and influence death patterns (35). Thus, although our model was motivated by experiments on mammalian tissues, we might expect similar results to also hold for eukaryotic colonies and bacterial biofilms in which diffusive and advective forces are responsible for the communication of interdependent members of the population (13).

**APPENDIX A: ADDITIONAL EXPERIMENTAL RESULTS**

To see the effect over a long time, the engineered tissue was perfused at a flow rate of 20 μL/h for 2 days, and the dead cells were imaged and counted at days 0, 1, and 2 (Fig. S2 ai). At day 0, bright field images were also taken to account for the initial total cell number. Cell viability at day 0 was similar throughout the gel, except the viability at the inlet (point 1.25 mm) was significantly higher than the other points (p < 0.0006) when compared with the outlet (point 7.5 mm) (Fig. S2 aii, aiiii). Upon perfusion, more cells died at the inlet, and cell viability showed an increasing trend along the gel toward the outlet. Viability at the inlet was significantly lower than that at the outlet both for day 1 (p < 0.0004) and for day 2 (p < 0.0008).

In another experiment, we kept all the parameters the same but reversed the flow direction after day 1 (Fig. S2 bii). At day 0, cell viability was the same all along the gel. Upon perfusion for 1 day, more cells died at the inlet (left side of the gel) than the outlet (right side), and cell viability followed an increasing trend toward the outlet (Fig. S2 biii). After day 1, flow direction was changed; perfusion was applied from the right side of the gel. After perfusion for another day (but in the reverse direction), we again observed an increasing cell viability toward the outlet (left side of the gel). Although at day 1, cell viability was significantly higher at the right side of the gel (outlet) than the left (p < 0.0006), it was higher at the left side than the right at day 2 (p < 0.0009) (Fig. S2 aiiii).

**APPENDIX B: CHEMICAL GREEN’S FUNCTION**

We assume the simplified case of a constant flow profile, \( v = v_0 \). Assuming a constant flow profile, we can use a shift of coordinates to simplify the chemical dynamics. If we let \( x_0 \) be the coordinate in the direction of flow and let \( z = x_0 - vt \), the chemical equation reduces to

\[
\frac{\partial \phi}{\partial t} = D \nabla^2 \phi - \gamma \phi + A_n,
\]

where now \( \nabla = \sum_{i=1}^{d-1} \partial_{x_i} + \partial_{z} \).

Now for an arbitrary source function \( S(x,t) = An(x,t) \), the chemical profile will be given by

\[
\Phi(x,t) = G(x,t) * S(x,t),
\]

where \( * \) is the convolution operator. The Green’s function \( G(x,t) \) is the solution to

\[
\left( \partial_t - D \nabla^2 + \gamma \right) G(x,t) = \delta(x) \delta(t).
\]

Taking a Fourier transform with respect to space \( x \), we get

\[
\left( \partial_t + k^2 D \nabla^2 + \gamma \right) \tilde{G}(k,t) = \delta(t).
\]

The solution to this is given by

\[
\tilde{G}(k,t) = \Theta(t) \exp\left[ - (k^2 D + \gamma) t \right].
\]

We recognize this as the Green’s function for the diffusion operator times a decay factor of \( \exp[-\gamma t] \). We then get, for the Green’s function in \( d \) dimensions,

\[
G(x,t,v) = \Theta(t) \left( \frac{1}{4 \pi Dt} \right)^{d/2} e^{-r^2/4Dt} e^{-\gamma t},
\]

where \( r^2 = \sum_{i=1}^{d-1} x_i^2 + (x_d - vt)^2 \).

If we now have a stationary point source, we can get the steady-state Green’s function by convolving with a source, \( S(x,t) = \delta(x) \) constant in time.

In the one-dimensional case, we get

\[
\Phi(x,v) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} G(x' - x, t') \delta(x') dx' dt'
\]

\[
= \exp \left[ x \left( \frac{v}{\sqrt{2d}} - \text{sign}(x) \sqrt{\gamma/d + v^2/4d^2} \right) \right] / (2d\lambda),
\]

where we have defined \( \lambda(v) = \sqrt{\gamma/d + v^2/4d^2} \). From here, we can get diffusion-advection-decay lengths given by

\[
\ell_L(v) = \left( \frac{\lambda(v)}{2d} \right)^{-1} \quad \ell_R(v) = \left( \frac{\lambda(v) - v}{2d} \right)^{-1},
\]

assuming the flow is from left to right \( v \geq 0 \).
APPENDIX C: FAILURE PROPOGATION VELOCITY DERIVATION

We begin by assuming that the cells respond to the chemical concentration “sharply.” This is the case as \( k \to \infty \), where the response function becomes a step function. We then later relax this assumption.

To simplify our analysis, we use a boxcar approximation to the Green’s function for the cooperative factors, where left and right lengths are given by Eq. B.3, and assume chemicals quickly reach steady state. The total area of the Green’s function corresponds to the secretion rate per cell density at steady state. We therefore have

\[
G(x) = (A / \gamma) \Theta(x + \ell_L) \Theta(\ell_R - x), \tag{C.4}
\]

where \( \Theta(x) \) is the Heaviside step function.

To get a first approximation to the cooperative factor concentration, we convolve the boxcar Green’s function (Eq. C.4) with an initial semi-infinite concentration of cells, \( n(x,0) = n_0 \Theta(x) \). This gives us a linearly increasing chemical profile, with slope \( H/W \), that saturates to a maximal value of \( H \) after a length of \( \ell_L \) into the microchannel, where \( H = A n_0 / \gamma \) and \( W = \ell_L + \ell_R \). Specifically, we get

\[
\phi(x) = \begin{cases} 
0, & x < - \ell_L \\
\frac{H}{W} (x + \ell_L), & -\ell_L < x < \ell_L \\
H, & x > \ell_L 
\end{cases}, \tag{C.5}
\]

Now, if the response to the chemicals is a step function (as is the case when \( k \to \infty \)), and if \( H > \phi_0 \), cells for which \( \phi(x) \) is below \( \phi_0 \) will die at a rate \( \alpha \), and cells for which \( \phi(x) \) is above \( \phi_0 \) will survive. The cell density will then shift to the right by an amount \( \Delta \) given by setting \( \phi(x) - \phi_0 = \phi_0 \) or

\[
\Delta = \frac{1}{2A n_0} \left[ A n_0^v + (2 \gamma \phi_0 - A n_0) \sqrt{4d \gamma + v^2} \right]. \tag{C.6}
\]

The cooperative factor concentration will then update with this new concentration of cells, and we can then reiterate this to get the next death of cells. The death of cells will then continue to propagate by an amount \( \Delta \) at a rate \( \alpha \). The death propagation velocity is therefore given as \( v_d = \alpha \Delta \). We find this gives good agreement for large \( k \) and short times but fails to capture the strong time dependence for small \( k \).

We then improve on our analytical results by considering the death of the bulk (in the region \( x > \ell_L \)). We replace the initial cell concentration \( n_0 \) in Eq. C.6 by a time varying function \( n_0(t) \) for the bulk cell concentration. To get \( n_0(t) \), we approximate the chemical concentration in the bulk as the steady state, nonspatial concentration, \( \Phi = A n_0(t) / \gamma \). We then expand the Hill form about infinity (high concentration limit) to get

\[
\frac{1}{(\Phi / \phi_0)^k + 1} \approx \left( \frac{\phi_0}{\Phi} \right)^k - \left( \frac{\phi_0}{\Phi} \right)^{2k} + \left( \frac{\phi_0}{\Phi} \right)^{3k} - \ldots
\]

Taking the first order term and plugging in \( \Phi' \), we get for the attenuation of the bulk cell density

\[
n_b(t) = \left[ n_b^0 - \alpha \left( \frac{\gamma \phi_0^k}{A} \right) \right]^{1/k}.
\]

Plugging this into the above expression for \( v_d = \alpha \Delta \), we get

\[
v_d = \frac{\alpha v}{2\gamma} + \frac{\alpha \sqrt{4d \gamma + v^2}}{\gamma} \left\{ \left( \frac{A n_0}{\gamma \phi_0^k} \right) - \alpha k t \right\}^{\frac{1}{2}} - \frac{1}{2}.
\]

This expression for the velocity better captures the acceleration of velocity over time but grows in error over time for small \( k \). This is because we fail to consider the region where \( \Phi > \phi_0 \) but not yet fully saturated. This gives another region where \( n(x,t) \) does not die out exponentially but dies out faster than \( n_0(t) \) and will lead to an increase in the velocity \( v_0 \).

Therefore, for a better approximation to the velocity, we also take into account the region where the cooperative factor concentration is larger than the threshold \( \phi_0 \) but is not yet saturated. The cell concentration in this region (denoted \( n_1(x,t) \)) will decay faster than the cell concentration in the saturated region, \( n_0(t) \).

We then approximate the cell concentration \( n(x,t) \) as \( n_1(t) \) in the region where \( \phi_0 < \Phi(x) < A n_0 / \gamma \) and \( n_0(t) \) where \( \Phi(x) > A n_0 / \gamma \), as shown in Fig. 3.

Taking the convolution of this updated cell concentration with our boxcar Green’s function, we get another piecewise function for \( \Phi(x) \) with five regions. Our second iteration of \( \Phi(x) \) then gives

\[
\Phi = \begin{cases} 
0, & x < - \ell_L \\
\frac{H_1}{W} (x + \ell_L), & -\ell_L < x < -\ell_L + \delta \\
\frac{H_2}{W} (x + \ell_L - \delta) - \frac{\delta(H_2 - H_1)}{W}, & -\ell_L + \delta < x < \ell_L \\
\frac{H_3 + \delta}{W} (x + \ell_L - \delta), & \ell_L < x < \ell_L + \delta \\
H, & x > \ell_L + \delta 
\end{cases}
\]

where \( H_1 = A n_0 / \gamma \) and \( H_2 = A n_0 / \gamma \). We now solve again for \( \Phi(\Delta) = \phi_0 \) to get a new expression for \( \Delta \). We assume \( \delta \) is small and use the center condition to solve for the threshold crossing. This assumption works for large velocities as \( \Delta \to \ell_L \) and \( \delta = \ell_L - \Delta \to 0 \). Solving for the threshold crossing then gives, \( \Delta = \left( W \phi_0 - H_2 (\ell_L - \ell_R) - H_2 (\ell_0) / (2H_2 - H_1) \right) / (2H_2 - H_1) \), which in terms of original parameters gives

\[
\Delta = \frac{An^v(2n_0 - n_3) + \sqrt{v^2 + 4d \gamma (2 \gamma \phi_0 - A n_0)}}{2A \gamma (2n_0 - n_3)}.
\]

Note that this reduces to our original formula if we set \( n_3 = n_0 \) as expected.

Now, to get the velocity propagation, we need an expression for \( n_0 \). We could in principle obtain this from solving for \( n(x,t) \) with a linear approximation for \( \Phi(x) \). We instead use a simplified expression from the following arguments. As the Hill constant \( k \to \infty \), the cell concentration will remain constant for any value of \( \Phi \) above the threshold \( \phi_0 \), and the two regions should remain the same, \( n_0(t) = n_0(\ell_L) \). For \( k = 1 \), we approximate \( n_0 \approx \frac{n_0^0}{A} \). We therefore approximate \( n_3(x,t) \) as

\[
n_3(t) = \left[ 1 - \left( \frac{1}{2} \right)^t \right] n_0(t).
\]

Now, taking the velocity to be \( v = \alpha \Delta \) and substituting Eqs. 7 and 9 for \( n_0 \) and \( n_3 \), we get
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\[ v_d = \frac{\alpha v}{2\gamma} \]

\[ + \frac{\alpha \sqrt{v^2 + 4d^2}}{2(1 + 2^k)} \left( 1 - 2^k \frac{\gamma \phi_0}{A} n_0^k - \alpha k t \left( \frac{\gamma \phi_0}{A} \right)^k \right)^\frac{1}{k} \]  

(C.10)

We find this gives good agreement with numerical simulations of the model.

We can also integrate this to get the failure penetration depth over time, \( x_d(t) = \int_0^t v_d(t) dt \), giving

\[ x_d = \frac{\sqrt{v^2 + 4d^2}}{2(1 + 2^k)A \gamma} \left( \alpha t + 4 \log \left( 1 - \alpha t \left( \frac{\gamma \phi_0}{A} \right)^k \right) \right) \]

for \( k \neq 1 \) and

\[ x_d = \frac{\alpha v t - \sqrt{v^2 + 4d^2}}{6}\alpha \gamma \left( \alpha t + 4 \log \left( 1 - \alpha t \left( \frac{\gamma \phi_0}{A} \right)^k \right) \right) \]

for \( k = 1 \). Also, taking the derivative of Eq. C.10 gives the acceleration

\[ a_d = \frac{2^k \alpha v \phi_0 \sqrt{v^2 + 4d^2} \left( \frac{\gamma \phi_0}{A} \right)^k \left( n_0^k - \alpha k t \left( \frac{\gamma \phi_0}{A} \right)^k \right)^{\frac{k}{k+1}}}{(1 + 2^k)A} \]

### SUPPORTING MATERIAL

Supporting Material can be found online at [https://doi.org/10.1016/j.bpj.2020.11.004](https://doi.org/10.1016/j.bpj.2020.11.004).

### AUTHOR CONTRIBUTIONS

G.U., G.B., P.Z., and D.C.V. formulated the problem, G.B. and P.Z. designed and carried out the experiments, G.U. and D.C.V. designed and carried out the theory, G.U., G.B., and D.C.V. wrote the article.

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