Effect of Environmental Fluctuations on the Dynamic Composition of Engineered Cartilage: A Deterministic Model in Stochastic Environment

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Abstract—Dynamics of extracellular matrix (ECM) deposition and scaffold degradation in cell–polymer constructs have been studied in a random fluctuating environment created due to the applications of growth factors into the in vitro generation of cartilaginous constructs. Existing models of cell–polymer constructs for the design of engineered cartilage have been discussed and then a new deterministic scheme in random environment proposed taking into account the effects of growth factors as the environmental variability in the form of Gaussian white noise. Steady-state probability distribution of each individual component of the ECM in its homeostasis is found explicitly. The computer-simulated results of the model have been discussed and then compared with the data from a variety of scaffold systems and culture conditions.

Index Terms—Extracellular matrix (ECM), growth factor, Fokker–Planck equation, Gaussian white noise, probability density function, steady state.

I. INTRODUCTION

Three major biomolecules within the extracellular matrix (ECM) are structural proteins, specialized proteins, and proteoglycans. Structural proteins consist of collagen and elastin; specialized proteins consist of fibrillin, fibronectin, and laminin; and proteoglycans are core proteins to which is attached long chains of repeating disaccharide units termed glycosaminoglycans (GAG). The main role of the ECM is to surround and support cells. The mechanical strength of animal tissue is mainly due to collagen fibrils within the ECM and its complex three-dimensional structures. GAG molecules are good in lubrication because of their high viscosity and low compressibility properties.

This paper deals only with the deposition of different ECM molecules in cell–polymer constructs. The main objective of this paper is to develop a model that describes the accumulation of different ECM molecules in an engineered cartilage construct taking into account all environmental factors arising due to the application of growth factors.

Buschmann et al. [1] reported in the study of engineering cartilage that there exists a strong negative correlation between GAG molecule deposition and synthesis rate. A similar type of result has been obtained by Handley et al. [5] in chondrocyte culture.

Based on these experimental facts, Wilson et al. [16] proposed a mathematical model for ECM deposition

$$\frac{d(\text{ECM})}{dt} = k_1 \{(\text{ECM})_{\text{ss}} - (\text{ECM})\}$$

(1)

where $k_1$ is the rate constant, the subscript “SS” denotes the steady state, and $t$ represents time.

The solution of (1) is as follows:

$$\text{(ECM)}_t = (\text{ECM})_{\text{ss}}[1 - e^{-k_1 t}],$$

(2)

The interaction between chondrocytes and different ECM molecules has been ignored in this scheme.

It has been observed both in vivo and in vitro that some growth factors enhance the production of biomolecules and some inhibit it. For details, see [2], [7], [11], [13], and [14]. In this paper, we want to incorporate the effects of these growth factors in the form of random noise in the actual model system. Justification for this idea is mainly due to insufficient knowledge about the mechanism of the functional characteristics of the growth factors within the cell–polymer construct.

II. MATERIALS AND METHODS

A. Formulation of the Model

Let us first develop a deterministic model that describes the accumulation of ECM molecules in a cell–polymer construct based on a logistic scheme known as the Verhulst–Pearl equation [10], [15]

$$\frac{d(\text{ECM})}{dt} = \lambda(\text{ECM}) \left[ 1 - \frac{(\text{ECM})}{K} \right]$$

(3)

where $\lambda$ is the intrinsic growth rate of ECM molecules in the cell–polymer construct and $K$ is the carrying capacity within the construct.

The dimensionless form of the system (3) is as follows:

$$\frac{d\omega}{dt} = \lambda\omega(1 - \omega)$$

(4)

where

$$\text{ECM} = K^{-1}\omega$$

and

$$t = K\tau.$$
After introducing the effects of growth factors as random fluctuations in the form of Gaussian white noise into the dynamics of ECM molecule deposition, the model system (4) becomes

$$\frac{d\omega}{d\tau} = \{\lambda_0 + \rho \eta(\tau)\}\omega(1 - \omega)$$

(6)

where $\eta(\tau)$ is the Gaussian white noise. The parameters $\lambda_0$ and $\rho$ are associated with the intrinsic growth rate of matrix molecules and the amount of growth factor applied to the cell–polymer construct.

Now we make the replacement which follows directly from the interpretation of the integral of $\eta(\tau)$ [4], [9], i.e.

$$dW(\tau) = W(\tau + d\tau) - W(\tau) = \eta(\tau)d\tau$$

(7)

where

$$dW(\tau) \sim N(0, d\tau).$$

(8)

For simplicity, we will only consider the linear term of the fluctuation part of (6). As such, (6) can be rewritten as

$$d\omega = \lambda_0 \omega(1 - \omega)d\tau + \rho(1 - \omega)dW.$$  

(9)

The corresponding Fokker–Planck (FP) equation [4], [9], [12] is

$$\frac{\partial P(\omega, \tau)}{\partial \tau} = -\frac{\partial}{\partial \omega}\left[\lambda_0 \omega(1 - \omega)P(\omega, \tau)\right] + \frac{1}{2} \frac{\partial^2}{\partial \omega^2}\left[(\rho(1 - \omega))^2 P(\omega, \tau)\right]$$

(10)

where $P(\omega, \tau)$ is the probability density function of ECM deposition at time $\tau$. Then in the steady state, the probability density function of ECM deposition $P_\infty(\omega)$ proportional to

$$(1 - \omega)^{-2(\rho^2 + \lambda_0^2)} \exp\left\{-\frac{2\lambda_0}{\rho^2} \omega\right\}.$$  

(11)

The constant of proportionality $C$ can be obtained from

$$C = \frac{1}{\int_0^1 (1 - \omega)^{-2(\rho^2 + \lambda_0^2)} \exp\left\{-\frac{2\lambda_0}{\rho^2} \omega\right\} d\omega}.$$  

(12)

Therefore, the steady-state density function for GAG molecules within the ECM is

$$P_\infty(\text{GAG}) = C_1(1 - \text{GAG})^{-2(\rho^2 + \lambda_0^2)} \times \exp\left\{-\frac{2\lambda_0}{\rho^2} \text{GAG}\right\}$$

(13)

and that of collagen is

$$P_\infty(\text{Collagen}) = C_2(1 - \text{Collagen})^{-2(\rho^2 + \lambda_2^2)} \times \exp\left\{-\frac{2\lambda_2}{\rho^2} \text{Collagen}\right\}$$

(14)

where $C_1$ and $C_2$ are two constants of proportionality.

$\lambda_1$ and $\lambda_2$ are intrinsic growth rates of GAG and collagen, $\rho$, the effect of growth factor, is assumed to be same for both the matrix molecules.

For simplicity, we assume that the growth factor does not affect the scaffold dynamics. As such, we will use the same first-order decay kinetics for scaffold degradation as proposed by Wilson et al. [16]

$$\frac{d(\text{Scaffold})}{dt} = -\gamma(\text{Scaffold})$$

(15)

where $\gamma$ is the scaffold’s decay rate.

We further assume that cell mass is constant (although this is not the case in reality [7]); then, the mean total engineered cartilage construct mass in the steady state can be obtained from

$$\bar{M}_{\text{ss}} = (\text{Cell mass}) + (\text{GAG}_{\text{ss}} + (\text{Collagen})_{\text{ss}}$$

$$+ \lim_{t \to \infty}(\text{Scaffold})_{\text{initial}} e^{-\gamma t}. ($$

(16)

B. Parameter Choice

There is no such explicit data available to calculate natural growth and decay rates of matrix molecules in engineered constructs. Obradovic et al. [8] have estimated the intrinsic synthesis rate $k$ (difference of natural growth rate–natural death rate) based on GAG synthesis in engineered constructs [3].

The sixth-order polynomial (see the trend line in Fig. 1) is given by

$$y = -0.0001 x^6 + 0.0025 x^5 - 0.0191 x^4 + 0.0253 x^3$$

$$+ 0.2567 x^2 - 0.9537 x + 1.3023.$$  

(17)

In (17), $x$ stands for GAG and $y$ stands for its synthesis rate. The fifth-order polynomial (see the trend line in Fig. 2) is given by

$$v = 0.0374 u^5 - 0.7857 u^4 + 0.1561 u^3$$

$$- 22.056 u^2 + 34.878 u - 17.026.$$  

(18)

where $u$ stands for collagen and $v$ represents synthesis rate.

From a mathematical point of view, both polynomials strongly suggest that there exists a functional relationship of the type

$$\text{Synthesis rate} = \frac{1}{\alpha + \delta(\text{ECM})^\theta}$$

(19)

where $\alpha$, $\delta$, and $\theta$ are chosen arbitrarily in order to show the type of relationship exhibited by the trend lines (17) and (18). Based on these trend lines, one can estimate the intrinsic growth rates of GAG and collagen molecules.

Scaffold degradation rate in cell–polymer constructs may be dependent upon the chondrocyte population and ECM molecule deposition, but at this stage there is not much information...
available about the functional relationship between the scaffold, chondrocytes, and the ECM molecules; as such, it is hard to develop a concrete mathematical formulation for the scaffold degradation term. At this point, one could estimate it by taking the average of gradients at different times over the total culture period of the scaffold concentration curve.

The rate at which growth factors act on the system dynamics is a totally externally controlled parameter. Depending on application, one can estimate the parameter \( \rho \). Steady-state cell mass can be obtained by cell number density per square units.

### III. COMPUTER SIMULATIONS AND COMPARISON WITH EXPERIMENTAL DATA

The probability density function of collagen molecule accumulation, as mentioned in (14), within ECM deposition is shown in Fig. 3.

The mean value of the steady-state collagen accumulation can be calculated from the following formula:

\[
(Collagen)_{ss} = \int_{0}^{1} (Collagen) P_{ss}(Collagen) d(Collagen),
\]

For a fixed set of values of \( \lambda_2 \) and \( K_2 \), we have the following relationship:

\[
\text{Mean}_{SS}^{\text{Collagen}} = F(\rho).
\] (20)

From (20), one can say that the stochastic fluctuations may alter the steady-state accumulation of collagen molecules. Both from mathematical and computational results, it is evident that depending on the level of stochastic fluctuations, i.e., for \( 0.2 < \rho < 0.7 \) collagen accumulation is less than its theoretical deterministic steady-state value \( K_2 \). Fig. 4 clearly demonstrates this feature.

Now we compare our mathematical outcomes with experimental findings.

Pie et al. [11] reported from their experimental work on \textit{in vitro} generation of cartilaginous constructs that the combination of growth factors TGF-\( \beta_1 \) and FGF-2, applied sequentially from day 3 to day 10, inhibits the collagen accumulation in ECM deposition remarkably. At the same time, it enhances the chondrocytes' proliferation. For more evidence, see [6].

The working principles of growth factors within cell–polymer constructs are largely unknown. Researchers in these areas do understand that the growth factors have a huge influence on the dynamics of matrix molecules, but the actual deterministic physical laws behind this phenomenon are yet to be identified.

From a mathematical point of view, we consider the effects of growth factors in the system dynamics as a noise (Gaussian white noise) in the environment.

Now, by comparing our mathematical outcomes with the experimental findings, one can come to the conclusion that both the results have the same qualitative behavior.

Next, we discuss GAG molecules. A similar type of probability density function can be obtained for GAG molecule accumulation in ECM deposition. For simplicity, we used the same set of parameters for GAG molecule accumulation dynamics as we did for collagen. As such, we obtained the same set of curves.
for mean and standard deviation of GAG molecule accumulation for different dose levels of growth factor. Therefore, it is evident that, like collagen accumulation, GAG molecule accumulation is also less than its theoretical steady-state value $K_I$, depending upon the dose levels of the growth factor.

It was observed in the experimental work on in vitro generation of cartilaginous constructs [11] that the combination of the growth factors TGF-$\beta_3$ and FGF-2, applied sequentially from day 3 to day 10, inhibits the GAG molecule accumulation in ECM deposition.

Now the question is, does the growth factor alter the overall construct mass in the steady state? The answer is probably no. The reason is that those growth factors which act as an inhibitor of the ECM molecules promote or enhance the chondrocytes’ proliferation. As such, overall construct mass may not be altered.

On the other hand, if we assume cell mass is constant, as the other researchers [16] did, then we can see some changes in the construct mass from our theoretical outcomes (see Fig. 5), provided that no interactions exist between the matrix molecules.

IV. CONCLUSION

It is well known now from the experimental findings that the growth factors have some effect on the dynamics of the cell–polymer construct. Unfortunately, until now there is very little knowledge about its working principles. Researchers are trying to investigate the mechanisms by doing different types of trial-and-error methods, but no one has come up with a definitive conclusion. To overcome this complex issue, we are trying to incorporate the effects of growth factor as a stochastic fluctuation into the system’s environment and then study the effects of this fluctuating environment on the system dynamics.

Computer simulations show that due to the application of the growth factor, both GAG and collagen accumulations within the ECM deposition are less than the normal steady-state value (without growth factor). In this situation, growth factor inhibits the steady-state production of GAG and collagen molecules. Similar types of results have been reported by Pie et al. [11] and Horton et al. [6].

Only steady-state analysis has been performed in this paper. Transient-state analysis of the previously described system is under construction.

REFERENCES


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