New Cumulative Damage Models for Desynchronization Rate in Cell Populations by using Stochastic Processes as Initial Damage.

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Abstract: We characterize the kinetics of two cancer cell lines: IGROV1 (ovarian carcinoma) and MOLT4 (leukemia). By means of flow cytometry, we selected two populations from exponentially growing in vitro cell lines, depending on the cell's DNA synthesis activity during a preceding labeling period.

In this paper based on generalized cumulative damage approach with a stochastic process describing initial damage for a material specimen, a broad class of statistical models for material strength is developed. The new distribution arrived can also be written as the inverse Gaussian-type distribution, which can be interpreted as the first passage of the accumulated damage past a damage threshold, resulting in material failure. For these populations we determined the time course of the cumulative damage of cells in different phases of the cycles, sampling every 9 hr for 63 hr, and connected with an interpreted real-situations.

Keywords: Accelerated testing, Branching process, Cumulative damage, Initial damage, DNA, Flow cytometric, Weibull distribution.

Subject classification: 60J85

1. Introduction

We observed that an initially semi-synchronous population (whose cells are in the same cell cycle phase) rapidly loses this feature. A variety of mathematical models have been proposed in the framework of cell population dynamics to describe this asynchronous growth [3,4,9,13] the main aim being to find under what conditions a structured population model satisfies this property [5]. Interest has also focused on cell cycle kinetics, i.e. the determination of some cellular parameters, such as DNA synthesis rates or phase transition times, related to cell cycle progression [12,14]

From the biological point of view, the speed of damage is strictly connected to some cell cycle kinetic parameters, such as the mean cell cycle duration and its variance. Many factors can influence the variability of cell cycle duration. One of them is the cell type: cells from different

tissues and with different degrees of differentiation do not have the same cycle duration; moreover, malignant cells usually have a completely deregulated cell cycle clock [11]. Cell type not only determines the internal mechanism, but also the reaction to the environment. While the proliferation of normal cells is controlled by the environment, which produces factors to stimulate growth, or inhibitory factors to stop cell proliferation, cancer cells are often able to stimulate their own growth, even when external factors are lacking. The reception of products from the outside, such as nutrients diffusing through the cell membrane from the environment, or cell cycle regulating signals from adjacent cells, is highly heterogeneous even within one cell type. This could depend on the particular spatial organization but also on the variability in distribution of intracellular substances and on inter-cell competition for growth factors [1]. Another aspect to be considered is the unequal division of mother cells, which leads to differences in cell size, protein concentration (see Darzynkiewicz et al., 1982), etc., possibly influencing the duration of the first phase of the cell cycle [15]. Some cells, comprising tumor cells in vivo, may also spend a variable period of time in a quiescent state, entailing a large effect on the coefficient of variation of cell cycle length and therefore on the speed of convergence.

From the mathematical point of view, there are many mathematical models to explain an attractive approach to the quantitative analysis of cell kinetics, the performance of an engineering and physical system. As the model is well defined, the prediction obtained from the model exactly match- with an interpreted real situations. A biological system is being continuously influenced by various biological systems and some other factors .In the absence of exact information about the contributions of such factors to the biological system, developing a model which can predict exactly is difficult.

A general class of statistical model based on cumulative damage is derived for the uses of accelerated testing situations. The approach assumes that “initial damage’’ exists in a material specimen which reduces its theoretical strength and the initial damage can be modelled by a stochastic processes that results in the distribution of the specimen’s initial strength. The power-law-weibull as an overall better fitting size-effect model than the ordinary weibull model.

2. Branching Processes and Asymptotic Properties

We follow a deterministic approach, using a simplified linear mathematical model, i.e. population model in which individuals undergo no influence what soever from each other. Other approaches could also used, such as the open proposed in [2] and based on the branching processes. Branching processes is stochastic model of individuals (cells) that live for random time and get a random number of children. “Asynchronous” reflects the property for a system to forget asymptotically its initial state. As we shall see, but non zero, amount of variability in their cell cycle length asymptotically lose their momery of the initial age configuration. For this population, we make the following assumption that:

(i) We can assume that the environmental conditions are independent of time.
(ii) The parameters of the model do not depend on the state of the system.
(iii) The population is in asynchronous exponential growth.

3. Experiments

A leukemia cell line (MOLT4), and an ovarian carcinoma cell line (IGROV1), were analysed in order to characterize their kinetic features. Cell counts and flow cytometric (FC) data were collected every 9 hr for 63 hr, as a basis for the growth curve and the cell cycle phase cumulative damage (we recall that the cell cycle is divided into four main phases: the G1 phase, followed by S, which is the phase of DNA synthesis; the G2 phase, followed by M, characterized by the occurrence of mitosis). Asynchronicity is reached when the cell phase percentages no longer change with time, within the experimental error [8].

3.1 Growth Curve

MOLT4 cells growing in suspension and IGROV1 cells growing in adhesion in the bottom of the culture flasks were counted at each sampling time using a Coulter Counter. Cell counts were normalized to an initial number of 1000 cells. The experiment was performed during the period of exponential growth and exponential curves were fitted to the data by nonlinear least squares (Fig. 1).

From the growth curve it was possible to obtain the population cumulative damage F(s), which is an important population kinetic parameter. If the population does not have any quiescent cell, this parameter is about the same as the mean cell cycle duration, otherwise it is bigger. The cell lines we studied contained a negligible amount of quiescent cells, which disappeared after an initial lag due to the cell death or recruitment into the proliferative state.

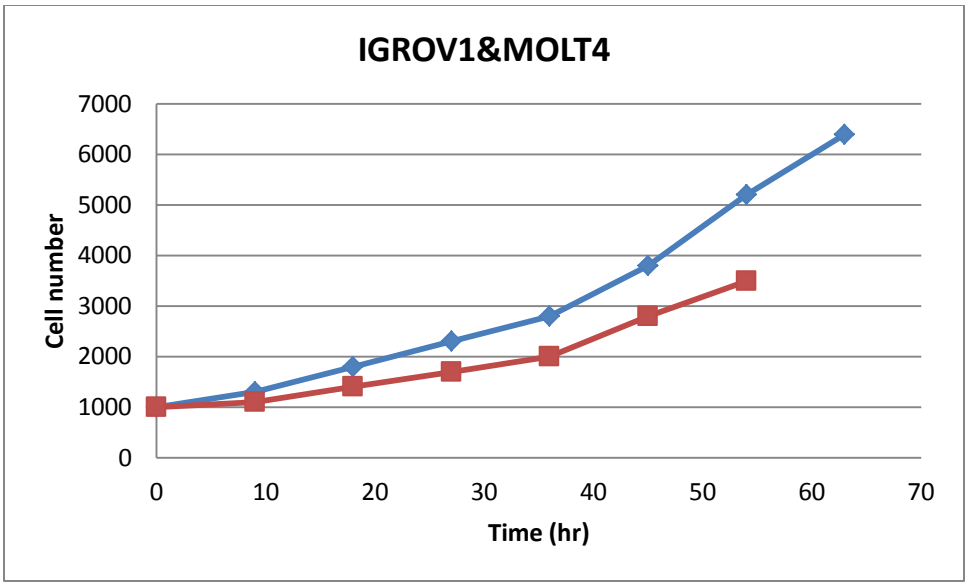

Fig.1.Growth curve of the ovarian carcinoma (IGROV1-blue), Growth curve of the leukemia (MOLT4-Red)

4. Flow Cytometric Analysis

After each count, cells were fixed in ethanol and underwent cytochemical and immunocytochemical treatments. Details have already been published (Ubezio et al., 1991). To measure DNA content, cells were stained with the fluorochrome propidium iodide (PI), which fluoresces red. To label cells in phase S at time zero, cells were incubated for 20 min with bromodeoxyuridine (BrdUrd), a thymidine analog, just before beginning data collection. As S phase cycling cells take up BrdUrd instead of thymidine in the newly synthesized DNA, the amount of BrdUrd incorporated by each cell can be related to the DNA synthesis activity of that cell. Before the flow cytometric analysis, cells were allowed to react with BrdUrd-specific monoclonal antibodies conjugated to the fluorochrome -fluorescein isothiocynate (FITC), which fluoresces green. From analysis of the first sample, related to the initial population (at time 0), cells in S phase could be distinguished, as they were positive for BrdUrd. Signals from at least 10,000 cells for each sample were collected and memorized by the FC computer. This large sample assures the representativeness of the cell population measured with respect to the whole population under study.

Cells which do not take up BrdUrd emit low-level green fluorescence, due to other fluorescent compounds which are naturally present in the cells and to some green fluorescence of

PI. This makes it possible to visualize BrdUrd-negative cells as well. The movement of the initial population could be followed throughout the cycle, because BrdUrd-positive cells, i.e. cells in S phase at time 0, maintain this label and pass it on to their descendants. This kind of labeling allowed us to analyse two different (and complementary) subpopulations for each cell line. The computer program previously developed for the DNA histogram analysis gave the cell phase percentages for the total population, as well as for BrdUrd-positive and BrdUrd-negative cells. Since the experiments were performed when the cell lines were in asynchronous exponential growth, the DNA histograms of the total population had more or less the same shape at each sampling time, and so did the cell phase percentages [8]. In the labeled as well as in the unlabeled groups the initial populations were to a certain extent synchronized.

Notation

$c(.)$	damage accumulation function
$m(.)$	damage model function
D	amount of damage; $D > 0$
D_n	damage to the specimen after n increments
$F_s(.)$	Cdf of S
$G_W(.)$	Cdf of W
$M_s(.)$	strength reduction model function
L	gauge length
N	number of increments until failure
n	a value of $N: n = 0,1,2$ ....
S	tensile strength; $S > 0$
s	a value of S
W	initial strength of material specimen
X_0	initial damage of material specimen
X_n	cumulative damage after n increments
Y_n	cumulative initial damage at location u
Ω_w	support of W

5. **Mathematical model**

Consider the following generalized cumulative damage model

$$X_{n+1} = (X_n) + D_n m(X_n)$$

where $c(.)$ is the nonnegative function. Because $D_n = [c(X_{n+1}) - c(X_n)] / \mathrm{m}(X_n)$, the damage incurred to the specimen after n increments of strain is

$$\sum_{i=0}^{n-1} D_i = \sum_{i=0}^{n-1} \frac{c(X_{i+1}) - c(X_i)}{m(X_i)}$$

$$\cong \int_{X_0}^{X_n} \frac{c'(x)}{m(x)} dx = M_c(X_n) - M_c(X_0) \qquad (1)$$

for large n. Here $M_c(x) = \int \frac{c'(x)}{m(x)} dx$. Then by the central limit theorem, $M_c(X_n) - M_c(X_0)$ has an approximate s-normal distribution with mean $n\mu$, and standard deviation $\sqrt{n\sigma}$: that is

$P[M_c(X_n) - M_c(X_0) \leq u] \cong \Phi\left(\frac{u - n\mu}{\sqrt{n\sigma}}\right)$ where $\Phi(.)$, denotes the standard s-normal cdf.

The following cumulative damage models are equivalent:

$$X_{n+1} = X_n + D_n X_n$$

$$logX_{n+1} = logX_n + D_n \qquad (2)$$

This shows that the additive damage model of the logarithm of cumulative damage is equivalent to the multiplicative damage model of the cumulative damage.

Let N be the number of increments of tensile strength is applied to a specimen of strength ψ until failure. Because $\left(M_c(\psi) - M_c(X_0)\right) > 0$almost surely,

We have,

N=$sup\{n: X_1 \leq \psi, \ldots\ldots\ldots X_{n-1} \leq \psi\}$

$$= sup\{n: M_c(X_1) - M_c(X_0) \leq M_c(\psi) - M_c(X_0), \ldots\ldots M_c(X_{n-1}) - M_c(X_0) \leq M_c(\psi) - M_c(X_0)\} \quad (3)$$

N=1, if the set is empty.

The survival probability after n increments of strain is given by

$$\mathrm{P(N} > n) = \int F_n(w)\, dG_W(w), \quad (4)$$

where $F_n(w) = P[M_c(X_n) - M_c(X_0) \leq M_c(w)]$ and $G_w(.)$ is the Cdf of

$$W = {M_c}^{-1}\big(M_c(\psi) - M_c(X_0)\big).$$

Let Y_u denote the cumulative initial damage at location u $(0 \leq u \leq L)$ along the length of the specimens. Let $H_L = max\{Y_u : (0 \leq u \leq L)\}$ denote the initial damage in terms of the severity of the inherent flaws over the specimens. That is, H_L is the random strength reduction of the specimens due to the most severe inherent flaw present before load is applied to the specimen of the gauge length L. Thus the initial strength becomes

$$W = {M_c}^{-1}\big(M_c(\psi) - M_c(H_L)\big). \quad (5)$$

The cdf of $W = {M_c}^{-1}\big(M_c(\psi) - M_c(H_L)\big)$ is given by

$$G_W(w) = P\big[{M_c}^{-1}\big(M_c(\psi) - M_c(H_L)\big) \leq w | w \epsilon \Omega_w\big]$$

$$= \frac{1 - F_{H_L}\left({M_c}^{-1}\big(M_c(\psi) - M_c(w)\big)\right)}{F_{H_L}(\psi) - F_{H_L}(0)}$$

where $\Omega_w = \{w: w = {M_c}^{-1}\big(M_c(\psi) - M_c(h)\big), 0 < h < \psi$

Finally, letting S be continuous version of N, and using the symmetry of $\Phi(.)$, as gives the failure distribution of the specimen as

$$F_S(s) = P(S \leq s) \cong \Phi\left(\frac{\sqrt{s}\mu}{\sigma} - \frac{\Lambda(\theta;L)}{\sqrt{s}\sigma}\right). \quad (6)$$

Reparameterizing the above cdf as $\mu_L = \Lambda(\theta; L)/\mu$ and $\lambda_L = (\Lambda(\theta; L)/\sigma)^2$

,we have $F_S(s) = P(S \le s) \cong \Phi\left[\sqrt{\frac{\lambda_L}{s}}\left(\frac{s}{\mu_L} - 1\right)\right]$ (7)

Note that (7) is a Birnbaum-Saunders [7] type distribution that incorporates the gauge length L. The difference between the inverse Gaussian and Birnbaum-Saunders distribution negligible when $\sqrt{\lambda_L} \gg \mu_L$ [6]. So Birnbaum -Saunders models can be approximated by the first term of an inverse Gaussian Cdf when $\sqrt{\lambda_L}/\mu_L \gg 1$. The pdf for (7) is approximated by inverse Gaussian pdf with mean parameter λ_L and scale parameter μ_L is given by,

$$f_S(s) = \sqrt{\frac{\lambda_L}{2\pi s^3}}\, exp\left[-\frac{\lambda_L(s-\mu_L)^2}{2\mu^2 L^s}\right] \quad (8)$$

Where $\mu_L = \Lambda(\theta; L)/\mu$ and $\lambda_L = (\Lambda(\theta; L)/\sigma)^2$ .Note that $\sqrt{\lambda_L}/\mu_L = {}^{\mu}/_{\sigma}$ is independent of θ & L. By selecting various form of the functions c(.) and h(.) Several new models can be obtained. We can obtain damage models with different stochastic processes describing initial damage as appropriate for the physical assumptions.

We have the following three models

$$M_A = \lambda_L\left(\theta_1 - \theta_2\sqrt{L -}\ \theta_3 L\right)^2$$

$$M_B = \lambda_L(\theta_1 - \theta_2 L - \theta_3 L^2)^2$$

$$M_c = \lambda_L\left(\theta_1 - e^{\theta_2 L - \theta_3}\right)^2$$

Where $\mu_L = \sqrt{\lambda_L/\xi}$ for all three. The model M_A includes the "Gauss-Gauss additive model'', as a special case when $\theta_3 = 0$, and the "Gauss-Gauss multiplicative model", as a special case with the constraint

$$\theta_1 = \left\{\frac{\left[log\left(\frac{\theta_2}{\theta_3}\right) - log\sqrt{8\pi}\right]{\theta_2}^2}{4\theta_3}\right\}.$$

Also note the model M_B includes the Gauss-gamma additive model as a special case when $\theta_3 = 0$.

The power-law-Weibull model as an overall better fitting size-effect model than the ordinary Weibull model [10]. The power-law Weibull Cdf is given by

$$F_S(s) = [1 - \exp[-L^{\theta}\left(\frac{s}{\beta}\right)^{\alpha}],\ s>0\ . \qquad (9)$$

6. Application

We can use the characteristic cell kinetic population gives an approximation of cumulative damage. Using the experimental data, we can obtain the cumulative distribution function. Show that the cell population of IGORV1 and MOLT4 cells grows exponentially, followed by flow cytometric analysis and additional cell count, providing an independent way to estimate the population of cell damage. Either BrdUrd- labeled or unlabeled cells exactly match with this distribution function associated with one of the cell cycle phases. We will focus here on the damage is of S phase cells within the population of BrdUrd labeled cells. Different BrdUrd concentrations times may be used depending on the cell type and aim of the experiment (pulse, pulse followed cumulative BrdUrd labeling over long periods of time). Some cell types or lines may be overly sensitive to BrdUrd, and their cell cycle may be perturbed at higher concentrations and longer exposure times. DNA susceptibility to denaturation varies depending on cell type, pilot studies should be done to find optimal conditions for a particular cell type by testing different temperatures of DNA denaturation (80–100°C). The denaturation results in cell damage and may lead to a significant cell loss. A glance that Fig.2 shows a tendency that breaking strengths decrease as gauge length increase by an accelerated test.

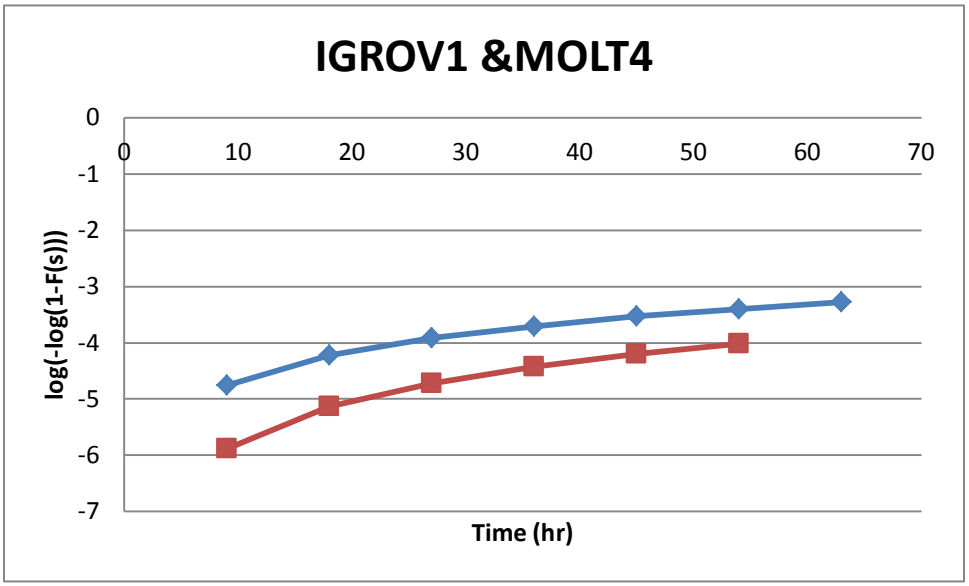

Fig.2 : $log[-log(1-F_S(s))]$ changes due to BrdUrd-DNA by using power law weibull probability plots IGROV1 &MOLT4(Blue & Red colours)

7. Conclusions

An analytical approach was described for estimating some cell kinetic parameters in the case where inter-cell variability in cell cycle duration. We aimed at assessing the damage of desynchronization of a cell population and reached this goal by using a mathematical model and by considering the damage cell cycle duration of the main significant parameters. Consider the cell kinetics of the two lines. The leukemia cell line has a longer cell cycle, but less inter-cell variability than the ovarian carcinoma cell line, even though the ovarian carcinoma cell cycle is about 9 hr shorter. Even if the cells are all of the same kind and grow in homogeneous conditions, each cell is an individual, with its own particular features; the cell cycle, for example, does not last exactly the same for every cell. A cumulative damage model represents the power-law-weibull cumulative distribution function is characterized by the failure $F_S(s)$. By using $F_S(s)$ along with the time intervals the plotted data scattered and the curve defined a failure as initial damage by a power-law- weibull distribution. The model is developed to find the levels of an experimental data and the results have been obtained.

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