Honorable Mentioned	
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Title	An Application of Nonlinear Mixed Effects Models to Cell Culturing Studies to Assess the Efficacy of Various Media
Type of Project	Capstone Research Project
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Abstract	Cell culturing studies are used to find the best media type for cell growth. However, the current method used for assessing the efficacy of the media types does not allow for formal inference on which media type is best. One complication is that cells must be passaged occasionally when cells reach confluence. This happens because when the cells grow to a certain point they will not grow any further. The scientists must take a new sample and reseed into new wells. Using a nonlinear mixed effects model, we model cell growth over time using an exponential growth model. Then, we compute the number of doublings. Using this model, we can make formal inference on whether one media type is better than the other. Also, we are able to overcome any assumption of an initial seeding density.

An Application of Nonlinear Mixed Effects Models to Cell Culturing Studies to Assess the Efficacy of Various Media

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Abstract

Cell culturing studies are used to find the best media type for cell growth. However, the current method used for assessing the efficacy of the media types does not allow for formal inference on which media type is best. One complication is that cells must be passaged occasionally when cells reach confluence. This happens because when the cells grow to a certain point they will not grow any further. The scientists must take a new sample and reseed into new wells. Using a nonlinear mixed effects model, we model cell growth over time using an exponetial growth model. Then, we compute the number of doublings. Using this model, we can make formal inference on whether one media type is better than the other. Also, we are able to overcome any assumption of an initial seeding density.

Keywords: Nonlinear Mixed Effects Model, Cell Culturing.

1 Background

Many companies are currently working on cures for diseases, such as diabetes, which involve cell therapy. In order to test these treatments, the companies must have access to a lot of cells. For example, one type of cell that companies use is called mesenchymal stem cells (MSCs) that can be taken from human bone marrow. Companies, like BD Technologies, develop methods for culturing such cell lines.

One of their key questions is: Which media type (mixture) will result in more cells in a smaller amount of time? To assess the efficacy of media types, they begin a cell culturing study. The cell culture environment begins with a base stem cell medium, a passive surface coating, and a combination of growth factors (BD Technologies, 2012). Companies that are working on these cell culturing studies are interested mainly in the number of times the cells double in the given time period.

Cell culturing studies proceed by mixing these media with cells in a large vat. The scientists will then place, by hand, what appears to be about 4000 cells/cm^2 in each of six wells per media type. Then, as the wells get to about 80% confluence, they will take the six wells and combine them in a vat. This is known as a passage. A passage represents when the wells get "too full;" so, the machine that reads the cell density cannot distinguish between the different cells any longer. They mix up the cells with the media again, then seed back into another six wells, beginning the second passage. They continue this process until there are enough passages to cover the total amount of time of interest. In our study, there were 5 passages covering about 15 days.

In this paper, we discuss the dataset used for our study. We then discuss the current methodology used to assess the efficacy of media and the flaws with this approach. Finally, we introduce a different methodology to address the question of which media allows the cells to grow the most in the time period of interest.

2 Data

A subset of data from a cell culturing study was graciously provided by BD Technologies. There were a total of 252 observations, where 126 observations came from the test media and 126 came from the positive control media. In each of the five passages, there are 6 different wells for each media type. The initial cell density in each well is supposed to be between 3000 cells/cm^2 and 5000 cells/cm^2, where they are targeting 4000 cells/cm^2 (BD Technologies, 2012). BD Technologies reseeded the cells every 3 days or at about 80% confluence. In Figure 1 below, we can see a "spaghetti plot" showing the cell growth in each passage. Points from the same well are connected by a line. Recall that different passages have different wells.

[Figure 1 about here.]

3 Current Method for Assessing Efficacy

The current way the efficacy of different media types is assessed is by comparing the number of times the cells double within a time period, D_x. This is computed by

$$D_x = \log_2(y_x/y_0), \tag{3.1}$$

where y_x is the cell density at time x and y_0 is the initial cell density. We do not observe y_x. Instead, we see y_x within a passage. Also, y_0 is not observed, but instead is targeted; therefore, it may differ across wells. Thus, D_x is not the observed response. The current method gets around this by assuming neglible growth through the first observation within each passage. In order to compute our doublings over the time period of interest, we must first compute the number of doublings D_t in each passage, where t is the total time in each passage. We then cumulate the doublings over the entire time of the study, x. That is

$$C_x = \sum_{t=0}^{x} D_t, \tag{3.2}$$

where C_x is the number of cumulative doublings at time x. The number of cumulative doublings are then plotted over time for each media type. Figure 2 shows the cumulative doublings plot using this approach.

[Figure 2 about here.]

We can see from this plot that the doublings do not show a smooth growth over time. Currently, this plot is used to graphically assess media types and leads us to conclude that the test media type is better than the positive control. However, without an estimate of the error, we cannot provide formal inference; therefore, we cannot say one media type is statistically better than another. This approach also does not consider known cellular growth models that science may provide. Finally, the correlation structure (multiple observations per well) in the data is ignored.

4 A Nonlinear Mixed Effects Approach to Assessing Efficacy

We can address some of the limitations from the current method by considering a nonlinear mixed effects model for cellular growth. Nonlinear mixed effects models allow us to consider data that are not independent. We can use this method to model each trajectory of the cell growth in each well, which is the individual model. We can then use a population model to allow for the trajectories to vary across media type and passages. The population level model allows the parameters of the individual level

model to vary. In addition it accounts for any random effects (Davidian and Giltinan, 1995). We consider using an individual level model that follows a two-parameter exponential growth curve. Specifically, our individual level model is

$$y_i = \beta_{0,i} \exp\left\{\beta_{1,i} t\right\}, \tag{4.1}$$

where t is the time from the start of each passage and y_i is the cell density in the ith well.

Our individual level model captures the growth of each well in each passage within each media type. In other words, we are considering an exponential growth model for each trajectory in each passage. We then want to model the differences in each trajectory using our population level model, which is

$$\beta_{0,i} = \beta_{0,0}(1 - m_i) + \beta_{0,1} m_i + b_{0,i} \tag{4.2}$$

$$\beta_{1,i} = \beta_{1,0}(1 - m_i) + \beta_{1,1} m_i + \beta_{1,2} p_i (1 - m_i) + \beta_{1,3} m_i p_i, \tag{4.3}$$

where

$$b_{0,i} \sim \mathcal{N}(0, \sigma_b^2).$$

Here, we let m_i equal 1 for the test media type and 0 for the positive control, and p_i represents the passage number. We fit our model via maximum likelihood under the assumption of normality. The likelihood was approximated by linearization (Lindstrom and Bates, 1990).

Our population level model allows for the initial cell density to differ across wells, which is captured by the random effect b_0 in the model for β_0. Our model for β_1 allows for differences in the trajectory for each media type, as well as, across the passages. In other words, as we move to each passage, we see a decline in the number of doublings that occur, and our model takes that into account.

We then use our estimates from the nonlinear mixed effects model to compute doublings using (1). Specifically, our equation for the number of doublings, D_x at time x is

$$D_x = \sum_{p=1}^{5} (\beta_{1,0}(1-m) + \beta_{1,1} m + \beta_{1,2} p(1-m) + \beta_{1,3} pm) \frac{(\min(x, t_p) - t_{p-1})\, \mathbb{I}(x > t_{p-1})}{\log(2)}, \tag{4.4}$$

where t_p is the maximum time point observed in passage p and $t_0 = 0$. This converts our model from "passage time" to overall time in the study. Again, we can plot these doublings over the entire study, where total time is measured using x. Figure 3 shows the estimated doublings using our nonlinear mixed effects model.

[Figure 3 about here.]

Comparing Figure 2 and Figure 3, we can see that Figure 3 shows a smoother growth curve. In addition, the final D_x is larger. We no longer had to assume negligible growth within the first period, allowing for the doublings to grow continuously over time. Finally, we could compute confidence intervals for the curves shown in Figure 3, if desired.

[Figure 4 about here.]

In Figure 4, we see the difference in the number of doublings between the positive control media and the test media (and 95% confidence band). A 95% confidence interval for the difference in the doublings at 15 days is (-1.53, 0.02), which corresponds to a p-value of 0.056. This confidence interval does contain 0, but all previous points show that the two media are statistically different from one another.

5 Discussion

Our results using nonlinear mixed effects modeling are different from the results achieved using the current approach. We find that the two media types are not statistically different from one another. Although they are not statistically different from one another, we can see that the test media trends toward a larger number of doublings. We can also see from Figure 4 that although the two media types are not different from one another at the last end point, they are statistically different before the end time point of 15 days. This implies that the test media is better than the positive control media, but at the end of the study, the positive control media "catches up" with the test media.

Generalizations of our strategy include fitting the model using a two-stage approach instead of the likelihood approach we considered. At the individual level, we specified a two-parameter exponential model. Other specifications for the individual model are possible. The model for the shape parameter $\beta_{1,i}$ could also include random effect, which we did not consider. We also specified the population model as being linear in the passage effect, which may not hold. We also assume normality of the response for estimation purposes and assume constant variance, both of which could be relaxed.

Using our nonlinear mixed effects model allowed us to consider a known growth model — the two-parameter exponential model. It also allowed us to model the correlation structure of the data using our individual level and population level models. We were able to model the cell growth without assuming negligible growth in the first time period — allowing the different wells to have different intial cell densities. Using this approach, we were able to provide formal inference and say that the two media are not statistically different from one another at the last time point of 15 days.

References

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Lindstrom, M. J. and Bates, D. M. (1990). Nonlinear mixed effects models for repeated measures data. *Biometrics*, 46:673–687.

List of Figures

Figure 1: Plot of the Observed Cell Growth in Each Passage

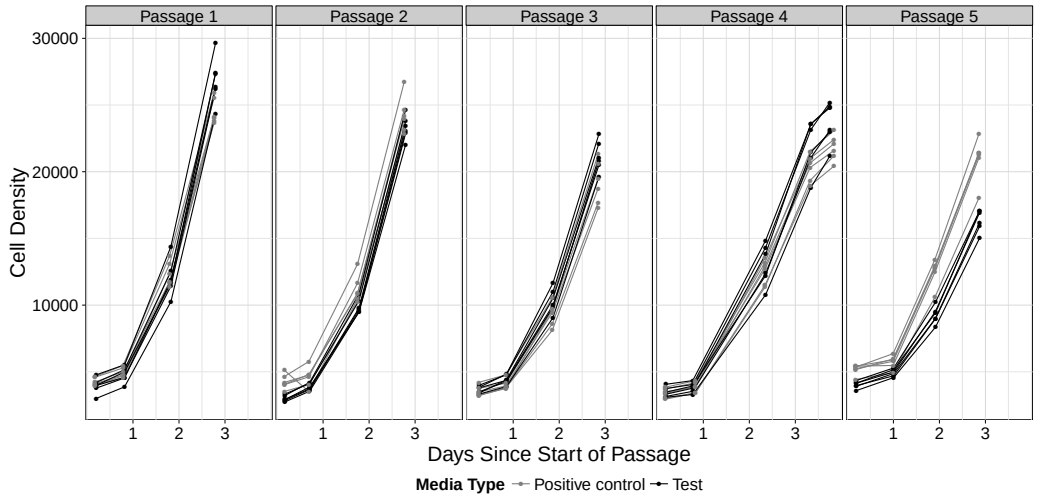

Figure 2: Cumulative Doublings Using Current Methods, Assuming No Growth Over Initial Observation of Each Passage

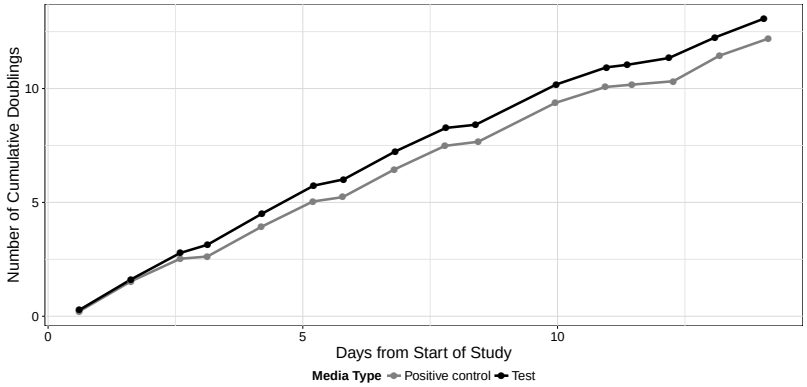

Figure 3: Cumulative Doublings After Modeling Cell Growth

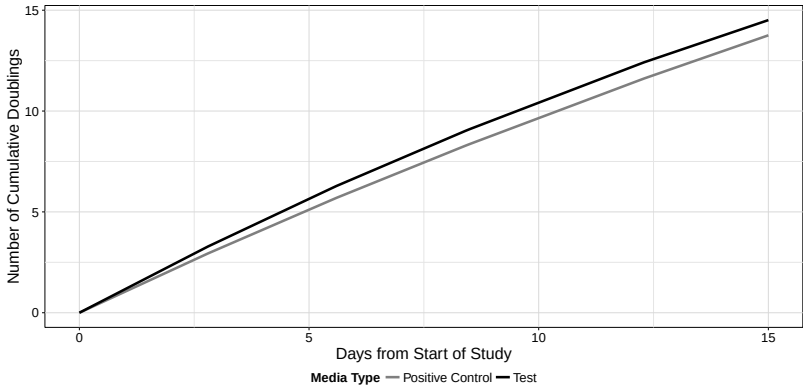

Figure 4: Difference in Doublings with 95% Confidence Interval