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Applicability of the Taguchi Method to Mechanobiology-Based Experiments

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Abstract

Design of experiments (DOE) methods are very commonly used for process validation. In this study, we employed the Taguchi approach in order to develop an optimal process for the maximization of oscillatory shear stress environments (OSS). OSS has been associated with increase in cellular activity and has been linked to healthy gene expression and tissue formation. Therefore, OSS may be a key factor for many tissue-engineering applications. The results obtained through the Taguchi method identified the best combination of factors and levels associated with higher OSS. These conclusions were found to be in agreement with computational fluid dynamics (CFD) results that we previously found concerning optimal experimental set-up for OSS maximization.

Introduction

Process validation plays a very important role in the development of methodologies that can be deemed effective. This is commonly accomplished through techniques grounded in the design of experiments (DOE), such as full factorial and fractional designs. The full factorial design approach tests the considered variable against all other combination of variables in the experiment (1). However, this can be expensive and time-consuming. More cost and time effective approaches are fractional design concepts, such as the Taguchi method (1-3). This technique has been widely used in order to determine which experimental set-up will succeed regardless of any interactions that may exist between the monitored parameters (2-3). It is an effective and efficient method for multi-parameter optimization. It also permits acquisition of more data from only a few experimental trials (3).

In general, the Taguchi method can be described by the following sequence of processes:

1. State the goal of the experiment. Identify the parameters that need to be maximized or minimized.
2. Develop a plan to measure the monitored parameters, also called quality characteristics. It is important to have a suitable unit system, instrumentation, and personnel.
3. Identify the major contributing factors that are believed to play a role in changing the quality characteristics.
4. Identify the factor levels. These are usually one low and one high-level for each factor.
5. Choose an appropriate orthogonal array. Arrays can be determined using $A = l^f$, where A is the array number, l is the number of levels, and f is the number of factors. For an experiment with three factors and two levels, an L8 array is needed. A typical L8 is shown in Table I.

Table I: An example of a Typical L8 Orthogonal Array. The top row contains the factor or interactions between them. These are represented by letters (second row). For example, C signifies factor 3. The 1 and 2 values that fill up the 8x8 matrix represent the level of the factor.

	Factor 1	Factor 2	Interaction Between Factor 1 and 2	Factor 3	Interaction Between Factor 1 and 3	Interaction Between Factor 2 and 3	Interaction Between Factor 1, 2, and 3
Trial #	A	B	C	D	E	F	G
1	1	1	1	1	1	1	1
2	1	1	1	2	2	2	2
3	1	2	2	1	1	2	2
4	1	2	2	2	2	1	1
5	2	1	2	1	2	1	2
6	2	1	2	2	1	2	1
7	2	2	1	1	2	2	1
8	2	2	1	2	1	1	2

6. The next step is to perform the experiment in the eight different trials. Typically five sample tests are performed at each trial for a total of forty data samples. This is important because it includes some of the variability that happens within trials (3).
7. Arrange the values inside the correct L8 array cells. A common mistake is to include the values for the Factor 3 in the C column (interaction between Factor 1 and 2).
8. Analyze the data. This is usually done by arranging the rows in a descending order with respect to the monitored parameter.
9. Identify the set-up with the highest parameter value (top row). This is the best combination of factors, the best-case scenario in order to maximize the desired parameter. Likewise, if minimization is preferred, then identify the set-up for the bottom row. Additionally, other analysis tools include interaction and cube plots.

The Taguchi method has been widely used in a variety of biomedical related fields, such as genetics, orthopedics, and pharmaceutics (4-8). In this study, we wished to show that the Taguchi method can be used as a validation tool for methods designed for oscillatory shear stress (OSS) maximization. OSS has been shown to influence bone marrow derived mesenchymal stem cell (BMSCs) proliferation and up-regulate transcription factors responsible for phenotypic regulation (9-13). Li et al. (14) reported that OSS caused triggering of intracellular calcium mobilization in BMSCs and regulation of osteogenic gene expression. This evidence suggests that OSS may play a critical role in the development of tissue-engineering constructs. In a recent study, Salinas et al. (15) performed a computational fluid dynamics simulation in a U-shaped bioreactor housing rectangular scaffolds (16). The goal was to determine what conditions resulted in maximum and most uniform OSS distributions on inner and outer walls of the specimens (see Figure 1). It was determined that samples kept in a straight configuration and subjected to pulsatile flow showed higher OSS environments compared to configurations with a bent-shape (For more details on this simulation, please refer to Salinas et al. [15]). The goal of this study was to apply the Taguchi method to arrive at a prediction of the ideal OSS experimental set-up and compare it to the Salinas et al. (15) CFD results.

Methodology and Results

The monitored parameter was an oscillatory shear index (OSI) proposed by He and Ku (17). OSI is a unitless number ranging from zero to 0.5 wherein zero means unidirectional shear stress and 0.5 indicates full shear stress reversal. (For a complete review of the bioreactor set-up, refer to

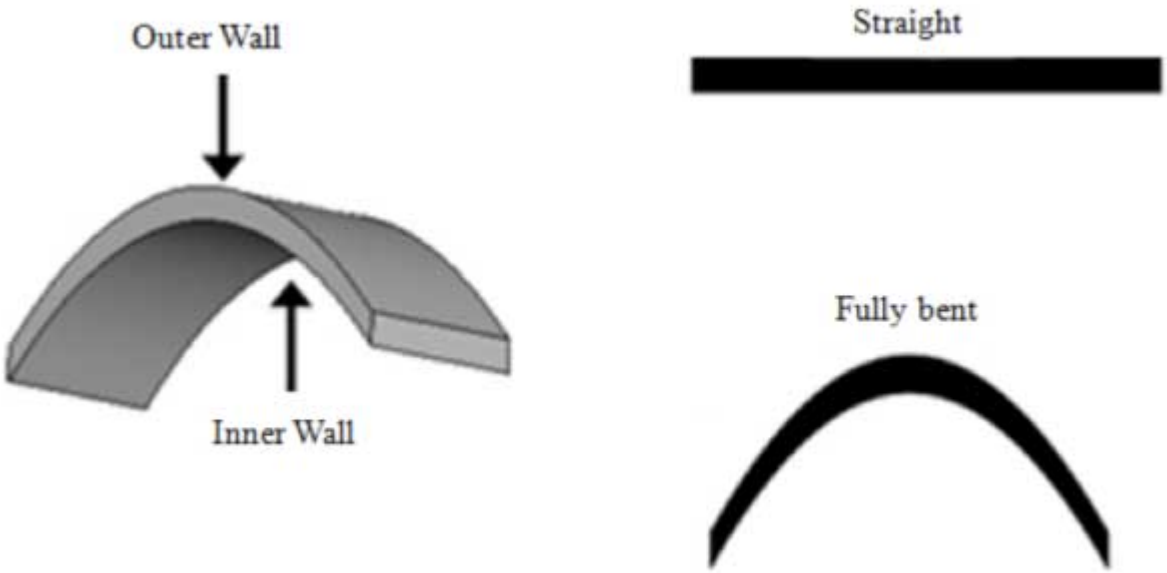
Salinas et al. [15])

OSI can be obtained by using the following equation:

$$OSI = (\frac{1}{2})(1 - \frac{\int_0^T \tau dt}{\int_0^T abs(\tau) dt})$$

The three accounted factors were: 1) type of flow, 2) geometry position, and 3) sample wall.

Figure: Geometry of a Straight and Fully-Bent Sample Situated in the Bioreactor.



The levels were either pulsatile or steady, straight or fully bent, and inner or outer wall as shown in Table II.

Table II: The Three Factors and Levels Considered in the Taguchi Method in Prediction of the Experimental Set-up that Could Maximize OSS.

Control Orthogonal Array						
Type of Flow	Geometry Position	Interaction Between Factor 1 and 2	Wall Side	Interaction Between Factor 1 and 3	Interaction Between Factor 2 and 3	Interaction Be Factor 1, 2,
Trial #	A	B	C	D	E	F
1	1	1	1	1	1	1
2	1	1	1	2	2	2
3	1	2	2	1	1	2
4	1	2	2	2	2	1
5	2	1	2	1	2	1
6	2	1	2	2	1	2
7	2	2	1	1	2	2

8	2	2	1	2	1	1

As shown in Table III, the top row shows higher OSI. This configuration belongs to the pulsatile flow, straight sample, and inner wall.

Discussion

OSS environments have been shown to play an important role in cell activity. One study (18) reported an increase in cell activity expressed by Ca2+ secretion. Cells exposed to OSS showed significantly higher Ca2+ secretion. Li et al. (140 reported osteoblastic differentiation of human BMSCs. Vermot et al. (19) reported marked increase in klf2a expression by valve endothelial cells that OSS was found to directly regulate. The gene klf2a is associated with healthy valve formation. Therefore, there is a growing amount of evidence to suggest that inherent OSS that act on various native tissues (e.g., heart valves) can translate to a conditioning parameter in directing stem cell differentiation and, potentially, growth of robust engineered tissues for application in replacement of diseased and or damaged tissues.

In an effort to maximize OSS environments for the development of protocols for tissue-engineering applications, Salinas et al. (15) developed a CFD model of a U-shaped bioreactor and simulated different set-ups for the maximization of OSS. In this study, we wanted to demonstrate the utility of the Taguchi method in being able to predict the experimental set-up to maximize physiologically relevant OSS. Our results indicated complete agreement between the Taguchi method prediction and our previously found CFD results described in Salinas et al. (15) for maximizing OSS. Indeed, the Taguchi method clearly showed that OSS could be maximized by inducing pulsatile flow on the inner wall of straight samples that essentially was validated by a similar finding determined by our previous CFD analysis (i.e., that samples kept in a straight configuration and subjected to pulsatile flow showed higher OSS environments compared to configurations with a bent shape) (15). This finding is not intuitive since one would generally have expected greater OSS to occur when there is greater variation in the sample geometry (i.e., a bent configuration to permit greater flow oscillations to occur). This, of course, was found to not be the case in neither the Taguchi method prediction reported here nor the CFD findings previously described by Salinas et al. (15).

In closing, owing to its simplicity and straightforward implementation, the Taguchi method is a powerful tool that can be used in predicting the optimal experimental design conditions for native or engineered tissue mechanobiology-based investigations. Its utility will be best served as an “early indicator” of maximizing a specific stress environment, which can be achieved during the early planning stages and as a prerequisite to time consuming CFD simulations or relatively expensive mechanobiology experiments. We thus conclude that the Taguchi method will serve as a useful tool that can help in the guidance of the experimental set-up to maximize mechanical conditioning parameters in mechanobiological investigations.

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