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Using Taguchi method for the optimization of processing variables to prepare porous scaffolds by combined melt mixing/particulate leaching

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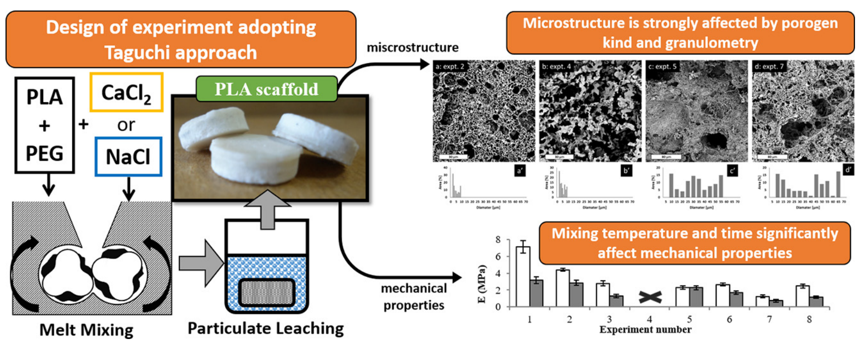
HIGHLIGHTS

- Polylactic acid porous scaffolds were successfully fabricated by combining melt mixing and particulate leaching.
- Mixing temperature and time are the most significant variable affecting mechanical properties of the scaffolds.
- Pores morphology was found to be affected by the kind of porogen salt (i.e. NaCl or $CaCl_2$) and their dimension.

GRAPHICAL ABSTRACT



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ABSTRACT

Synthetic biopolymers have made significant inroads into the development of devices for tissue regeneration. In this context, a challenge is the achievement of appropriate properties mimicking the natural extracellular matrix by fabricating scaffolds presenting mechanical properties, specific surface, porosity and pore interconnection adequate for the final application. This study involved a systematic procedure based on Taguchi method for parameters optimization of melt mixing/particulate leaching combined processes aiming to enhance the performance of the scaffolds. In particular, it was evaluated the effect of time and temperature of melt mixing of the poly(lactic acid) matrix with two water-soluble inorganic porogen agents (i.e. NaCl or $CaCl_2$) with two different pore size and poly(ethylene glycol). Thereafter, the blends were compression molded and water-leached for different time and at different pH. By adopting L_8 Taguchi orthogonal array, seven control factors, each at two levels, were tested, and ANOVA was applied to find the statistically significant factors and the combination of their optimal levels. The results revealed that the mixing temperature had the highest effect on mechanical properties. Moreover, the internal architecture of the scaffolds was studied by morphological analysis, finding that it is affected by the kind of porogen salt and by mixing time.

1. Introduction

Over the past decades, one of the primary aims of tissue engineering was to develop scaffolds using biodegradable and biocompatible materials for repairing bone or tissue defects by a regenerative process [1]. Due to the complexity of the human body, mimicking the structural

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and biological function of native extracellular matrix (ECM) requires materials with manifold characteristics [2–5]. Biomaterials used for this aim are ceramics, bioglass, polymers, composites either of natural or synthetic origin and, rarely, metals [6,7]. The current challenge is to find an ideal device with the following characteristics: (i) mechanical behaviour matching that of replacement tissue; (ii) suitable surface that actively supports cell attachment, proliferation and differentiation to build up the new tissue; (iii) appropriate pore dimensions, porosity and interconnection between pores in order to allow the transport of cellular nutrients and metabolites [8–10]; (iv) biodegradation rate comparable with tissue regeneration rate and innocuous degradation products; (v) sterilizability to avoid toxic contamination [6]. Actually, the most promising materials are biopolymers, widely studied because they are nontoxic, biocompatible and possess desirable mechanical and physical properties [4,9–11]. Among biodegradable polymers of synthetic origin, poly(lactic acid) (PLA) has been extensively studied for several decades, thanks to its chemical-physical properties and easy processability [5,12–14]. One of the most efficient plasticizers for PLA is poly(ethylene glycol) (PEG) hydrophilic and biocompatible polymer [13,15–18].

Since the above mentioned characteristics required for the scaffolds depend both on the polymer used and on the fabrication method [19], a wide variety of techniques have been developed to prepare scaffolds. Generally, those techniques can be divided into two groups: conventional and advanced [20]. Conventional techniques include combined melt mixing/particulate leaching [5,13,17], thermally induced phase separation [21], combined solvent casting/particulate leaching (SC/PL) [22], combined high-pressure moulding/salt leaching [23], gas antisolvent [8]. Advanced techniques include, among the others, electrospinning [9,24] and 3D printing [25]. Each of these techniques has strengths and weaknesses regarding resolution control and manufacturability; e.g., the widely used SC/PL method, on the one hand can effectively control the porosity by varying the amount and size of the pore former on the other hand it often uses toxic solvent [13].

Since each method present peculiar processing variables, considerable effort has been directed in searching the relationships between these parameters and the final performance of the scaffolds [19]. Therefore, determining the influence of the process conditions by appropriate optimization methods, such as statistical methods, represents a useful strategy to improve specific target properties. Among the statistical methods, Taguchi's method is a useful tool applied to investigate the effect of multiple variables on the observed properties. Recently, Taguchi's approach has been successfully applied in combination with the analysis of variance (ANOVA) for the optimization of process parameters of several processes, e.g. investigation of the optimal design of ternary activators activated slag [26], production of metal-composite overlap joints [27], fabrication of hybrid plastic-metal joints for medical device applications [28], synthesis of minimal-size ZnO nanoparticles [29], tribological analysis of composite fibers [30], electrochemical machining [31], plastic materials processing [32,33], steel manufacture [34,35], engineering ceramics [36,37]. Eddie et al. [38] used Taguchi method and ANOVA in the parametric study of the biopotential equation for breast tumour identification and, finally, Lin et al. [39] and Dar et al. [40] applied such method in conjunction with others statistical approach. Up to date, Vigneswari et al. have been the only ones who applied Taguchi method and ANOVA in the design of scaffolds for tissue engineering to investigate the effect the significant process parameters on the hydrophilicity of the scaffolds and to determine their optimal values [41].

For the first time, our study applied Taguchi's experimental design and ANOVA to maximize the mechanical properties of porous scaffolds fabricated by combining melt mixing and particulate leaching; this combined manufacturing method offers several advantages in terms of low cost production, industrial scalability and absence of dangerous/toxic solvents. The process was optimized by using Taguchi's orthogonal arrays (OAs), a simple and easy tool to investigate a large number of variables by performing a workable number of experiments. More in detail, the effect of seven process variables, i.e. porogen salt, salt granulometry, mixing temperature and mixing time, leaching temperature, leaching time and leaching pH, was investigated. This study analyses the effect of these parameters at two levels on the compressive Young's modulus in air and in phosphate buffered saline (PBS). The final goal is the determination of optimal values of the statistically significant processing variables, in order to maximize the compressive elastic modulus and to give immediate information and a precious tool to researchers for future studies. The results obtained have been validated by confirmation experiment.

2. Theoretical background: statistical methods, Taguchi approach and ANOVA

In 1920, for the first time the English statistician R. A. Fisher studied the effect of multiple variables simultaneously and developed a powerful statistical technique, the Design of Experiments (DOE) [42]. A design in which every setting of every factor (variable) appears with every setting of every other factor, identifying all possible combinations for the given set of factors, is a full factorial design [43,44]. The discrete values of the factors are named levels. If there are k factors, each at 2 levels, a full factorial design has $N = 2^k$ experiments [45]. However, since most industrial experiments usually involve a relevant number of factors, a full factorial design would often result in a too large number of experiments. The fractional factorial design reduces the number of experiments by selecting only a small set from all the possible experimental combinations. In this issue, to reduce the number of experiments to a practical level, Taguchi's approach inserts in the science of DOE by selecting only a small set of orthogonal arrays (OAs), from all possible experimental combinations, which includes all the independent combinations of the factors chosen [46].

Before beginning the experimental runs, Taguchi's approach decides the entire process, i.e. the factors, the levels, how run the tests, how analyse the results. These steps are in contrasts with the traditional techniques in which some initial ideas are tested, followed by other ideas and tests and so on. Taguchi's approach has consistency and reproducibility rarely found in any other statistical methods and it can be employed to obtain the best values of process variables, called optimal values in Taguchi's terminology, with the minimum number of investigations.

Analysis of variance (ANOVA) is the most common statistical method applied to results, in a factorial design of experiments, to determine which factors examined have most influence on the property of interest. The effect of one factor represents the variation of the response of interest of the process varying the levels of the same factor. ANOVA computes quantity called sum of squares, degree of freedom, variance and variance ratio and organizes in standard tabular format [47]. ANOVA uses a F-test to determine the statistically significant factors within the confidence interval examined. A more detailed explanation about ANOVA application is provided in the Supporting information (SI).

3. Experimental

3.1. Design of experiments using the Taguchi approach

The experiments were carried out with seven variables (factors) and two discrete values (levels) for each variable: type of porogen salt, salt granulometry, temperature and time of melt mixing, temperature, time and pH of leaching. Table 1 shows the factors selected and their levels.

It may be expected that the porogen salt factor may change the pore shape and therefore the microstructure of the scaffold, in addition to the leachability, while salt granulometry may dominates the pore size. It is reasonable to suppose that mixing temperature and time will vary the viscosity and the molecular weight of thermo-mechanically biodegradable PLA, which influence the mechanical properties. Finally, the factor