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# Optimization and development of Maghemite ($\gamma$-$Fe_2O_3$) filled poly-L-lactic acid (PLLA)/thermoplastic polyurethane (TPU) electrospun nanofibers using Taguchi orthogonal array for tissue engineering heart valve

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A B S T R A C T

Tissue engineering (TE) is an advanced principle to develop a neotissue that can resemble the original tissue characteristics with the capacity to grow, to repair and to remodel *in vivo*. This research proposed the optimization and development of nanofiber based scaffold using the new mixture of maghemite ($\gamma$-$Fe_2O_3$) filled poly-L-lactic acid (PLLA)/thermoplastic polyurethane (TPU) for tissue engineering heart valve (TEHV). The chemical, structural, biological and mechanical properties of nanofiber based scaffold were characterized in terms of morphology, porosity, biocompatibility and mechanical behaviour. Two-level Taguchi experimental design (L8) was performed to optimize the electrospun mats in terms of elastic modulus using uniaxial tensile test where the studied parameters were flow rate, voltage, percentage of maghemite nanoparticles in the content, solution concentration and collector rotating speed. Each run was extended with an outer array to consider the noise factors. The signal-to-noise ratio analysis indicated the contribution percent as follow; Solution concentration > voltage > maghemite % > rotating speed > flow rate. The optimum elastic modulus founded to be 28.13 $\pm$ 0.37 MPa in such a way that the tensile strain was 31.72% which provided desirability for TEHV. An empirical model was extracted and verified using confirmation test. Furthermore, an ultrafine quality of electrospun nanofibers with 80.32% porosity was fabricated. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and cell attachment using human aortic smooth muscle cells exhibited desirable migration and proliferation over the electrospun mats. The interaction between blood content and the electrospun mats indicated a mutual adaption in terms of clotting time and hemolysis percent. Overall, the fabricated scaffold has the potential to provide the required properties of aortic heart valve.



## 1. Introduction

Heart is a specialized muscle in mammalian body and comprises of two separate sides that are scientifically called left and right ventricles [1,2]. The heart is working as a pulsatile pump to provide unidirectional and non-obstruct blood flow to the body organs during the life. Each ventricle has an inlet (mitral and tricuspid) and outlet (aortic and pulmonary) valve that is responsible to control the one-way direction of blood flow from the heart to the body and *vice versa* [3–5]. The function of the heart is in such a way that during the heart diastole the inlet valves open and let the blood into the ventricles and then closes. After that during the heart systole the pressure of the ventricles rises above the arteries and makes the outlet valves to be open and correspondingly eject the blood from heart chambers. The pressure drops quickly right after ejection and this makes the outlet valves closed [6,7]. This cycle is repeated roughly 75 times per minute (bpm) and is called cardiac cycling [8]. The heart valve operates under a dynamic tensile-shear-flexural loading which tolerate an elastic modulus within 10–15 MPa (in such a way that the required tensile strain is around 20–30%) [9–11]. The heart pumps around 3–5 l blood with velocity around 1.35 $\pm$ 0.35 m/s per minute [12,13]. In heart valve function elasticity and strength are the two undeniable characteristics. Any malfunction of the valves causes disorders in blood circulation that may cause serious heart disease and even death. The dysfunction of the heart valves can be due to absent/abnormality of tissue in congenital cases, rheumatic

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fever and calcification [14–16]. When one of the valves malfunction the end stage of the medical choice is to replace the defected tissue with an artificial valve.

Tissue engineering (TE) is an integrated knowledge between the life science and engineering principle that through creating three main steps a neotissue that can mimic the original tissue is developed [5, 17]. These three steps can be summarized as: (I) Fabrication of a scaffold as an initial template for the cells, (II) seeding the fabricated scaffold with appropriate source of cells, and (III) develop the seeded scaffold *in vitro* (bioreactor) prior to implementation. TE opens a new insight into biomedical engineering to overcome the limitation of artificial biomedical devices [18]. Although executable steps have been made toward developing tissue constructs that could assist as original tissue of the clinical devices, the functional properties of many of these engineered tissues fail to fully match compared to their native counterparts. Fabrication of a heart valve (HV) scaffold that can provide desirable structural, biological and mechanical properties refer to the original tissue is the main concerns. In order to fabricate the scaffold two perspectives of utilized materials and fabrication technique are debatable.

The utilized materials (polymeric/natural) to fabricate the scaffold should be biocompatible, biodegradable and compatible with dynamic function of heart valve. Synthetic biodegradable polymers such as polyglycolic acid (PGA) [19,20], polycaprolactone (PCL) [21,22], polylactic acid (PLA) [23,24], and polyglycerol sebacate (PGS) [25,26], have already been reported to be useful in tissue engineering heart valve (TEHV). All the mentioned materials are accepted by the food and drug administration (FDA) as the biocompatible polymeric materials. Sodian et al. [27] reported scaffolding of TEHV using PLA and PGA copolymers were thicker and less flexible than the native valves. Utilizing PLA solely would have resulted in a rigid scaffold and it would be in conflict with the dynamic mechanism of heart valve leaflets. Van Lieshout et al. [28] reported the application of PCL in TEHV through the electrospinning technique. The biomechanical behaviour was qualified but the low degradation rate of PCL (>2 years) fails the concept. Masoumi et al. [25] investigated the application of PGS in TEHV which demonstrates a good biodegradability, stiffness and cell adhesion compared to PGA. The PGS tensile strength tests have shown nonlinear stress-strain behaviour. The stress-strain curve associates the vulcanized rubber. The average elastic modulus of PGS was within the range of 0.025–1.2 MPa which was not sufficient for HV.

Maghemite ($\gamma$-$Fe_2O_3$) nanoparticle, which is a novel biocompatible material, has recently been used in biomedical applications such as magnetic cell seeding, cell expansion and drug delivery and the results are quite promising. The outstanding bio-behaviours (mechanical and biological) of maghemite have been reported in previous researches [17,29,30]. Maghemite nanoparticles filled nanofibers such as polyvinyl alcohol (PVA) were used previously for composite reinforcement purpose [31,32]. Furthermore, maghemite filled polyvinyl alcohol was reported as a potential materials for bone tissue engineering purpose which resulted in higher tensile strength and better cell proliferation [33].

On the other hand, the scaffold proper structure and morphology is related to fabrication technique. The scaffold structure should be hierarchical with interconnected pores that facilitate the access to nutrients and oxygen for the cells [5,34,35]. Electrospinning is a well-known method to fabricate a nanofiber based scaffold through easy and cost-effective process. As a common setup for electrospinning, the desired rate of conductive polymer solution is supplied using a syringe pump into the spinneret nozzle. High electrical field is then applied to transform the emerging solution into the fibers. Application of electrospinning yields in a porous (interconnected pores) scaffold with desirable structure as the initial template for cell growth. The microstructure such as fibers diameter and subsequently the macro properties of the electrospun mats can be modified by varying the involved parameters in the process (electrospinning) and system (solution) [36,37].

**Table 1**
List of utilized materials in this research.

| No. | | Type of materials | Purity | Brand |
|---|---|---|---|---|
| 1 | Polymers | Poly-L-lactic acid (PLLA) (70 kDa Mw) | 99 + % | Ingeo™ Biopolymer 4032D |
| 2 | | Thermoplastic polyurethane (TPU) (90 kDa Mw) | 99 + % | Wenzhou City Sanho Co., Ltd |
| 3 | Maghemite Synthesis | Acetone | 99 + % | Sigma Aldrich |
| 4 | | Iron (II) chloride ($FeCl_2$) | 98% | Sigma Aldrich |
| 5 | | Iron (III) chloride ($FeCl_3$) | 45% | Sigma Aldrich |
| 6 | | Ammonium hydroxide ($NH_4OH$) | 25% | Sigma Aldrich |
| 7 | | Nitric acid ($HNO_3$) | 65% | Sigma Aldrich |
| 8 | | Ferric nitrate ($Fe(NO_3)_3.9H_2O$) | 70% | Sigma Aldrich |
| 9 | | Hydrochloric acid (HCl) | 37% | Sigma Aldrich |
| 10 | Solvents | Dichloromethane (DCM) | ≥99.5% | Merck, Co. |
| 11 | | Dimethylformamide (DMF) | ≥99.5% | Merck, Co. |
| 12 | | Dimethyl sulfoxide (DMSO) | ≥99.5% | Merck, Co. |
| 13 | Biocompatibility test | Dulbecco's modified Eagle's medium (DMEM) | – | Gibco USA |
| 14 | | Phosphate buffered saline (PBS) | – | Gibco USA |
| 15 | | Fetal bovine serum (FBS) | – | Gibco USA |
| 16 | | Penicillin-Streptomycin | – | Gibco USA |
| 17 | | Trypsin-EDTA | – | Sigma Aldrich |
| 18 | | Trypsin inhibitor | – | Sigma Aldrich |
| 19 | | MTT powder | – | Sigma Aldrich |
| 20 | Cell & blood | Human aortic smooth muscle cells (HAOSMCs) | – | Sigma Aldrich |
| 21 | | Human blood (donor 28 male) | | – |

Here ($\gamma$-$Fe_2O_3$) nanoparticles filled poly-L-lactic acid (PLLA)/thermoplastic polyurethane (TPU) electrospun nanofibers was developed using electrospinning technique. The involved parameters on the process (such as flow rate, voltage and collector rotating speed) and on the system (such as solution concentration and percentage of maghemite in the content) were investigated using Taguchi orthogonal array to optimize the elastic modulus of electrospun mast. In our very recent work, Fallahiarezoudar et al. [37] five different ratios of PLLA/TPU electrospun mats containing 1% (*w*/*v*) maghemite were characterized in terms of structural, biological and mechanical properties to tune the ratio of PLLA and TPU that proposes the best performance for TEHV. The initial analysis exhibited an overall satisfaction on 50:50% (*v*/v) PLLA/TPU electrospun mats. The founded elastic modulus for that scaffold measured to be 4.759 ± 0.52 MPa (tensile stress, 2.57 ± 0.11 MPa and tensile strain, 54 ± 7%). Thus, this research was carried out to optimize the fabrication process to obtain the required elastic modulus for TEHV. The previous reports revealed a reverse relationship between elastic modulus/porosity and fibers diameter [31,38]. On the other hand, a direct relationship was reported between porosity and cell attachment/growth [17]. Therefore, optimization of elastic modulus has resulted in fewer diameter distributions of the fibers and correspondingly higher porosity and better cell proliferation. This combination of materials has never been reported in combination with electrospinning process.

**Table 2**
Factors and levels for Taguchi experimental design.

| | No. | Factors/levels | Level (1) | Level (2) |
|---|---|---|---|---|
| Controllable Factors | 1 | **A**-Flow rate (ml/h) | 2 | 3 |
| | 2 | **B**-Voltage (kV) | 20 | 30 |
| | 3 | **C**-Maghemite (%) | 1 | 3 |
| | 4 | **D**-Concentration (wt%) | 10 | 15 |
| | 5 | **E**-Rotating speed (rpm) | 1000 | 2000 |
| Noise Factors | 6 | Temperature | Heater ON | Heater OFF |
| | 7 | Humidity | Aircon. ON | Aircon. OFF |
| | 8 | Nozzle type | Aluminium | Steel |