Fabrication PEGDA/ANFs Biomaterial as 3D Tissue Engineering Scaffold by DLP 3D Printing Tecshnology

A. Nurulhuda, S. Izman, Nor Hasrul Akhmal Ngadiman

Abstract*: Although traditional fabrication methods (electrospinning, solvent casting, freeze drying, etc...) can be used to produce scaffold, unfortunately, each of them has many limitations such as difficulty to control distinct 3D structure and porosity. These limitations can be easily overcome by unconventional techniques such as Fused Deposition Method (FDM), Selective Laser Sintering (SLS) and Stereolithography (SLA) to produce tissue engineering scaffold. Among the three, SLA offers the lowest cost, fastest printing speed and highest resolution. Digital light processing (DLP) 3D printing process is one of the SLA techniques which has been used a lot to fabricate tissue engineering scaffold based on Poly (ethylene glycol) diacrylate (PEGDA) material. However, there is no report published on the fabrication of tissue engineering scaffold based PEGDA filled with Aramid Nanofiber (ANFs). Hence, the feasible parameter setting for fabricating this material using DLP technique is currently unknown. The aim of this work is to establish the best feasible condition to fabricate PEGDA/ANFs 3D scaffold. ANFs was synthesized first from macro size Kevlar fiber prior to crosslinking with Diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide (TPO) photoinitiator. The mixing ratio of PEGDA resin to ANFs was fixed to 9:1. The concentration of TPO was varied at 0.5, 1.0 and 1.7% wt. while the resin concentration was fixed at 30% during the mixing to produce three set of biomaterials. Calibration printing was conducted prior to actual printing with the purpose of eliminating unprintable TPO concentration. The final scaffold was printed using DLP machine (FEMTO…) at two different curing times i.e 70 and 80s to obtain a good shape and printable 3D structure. The synthesized ANFs showed that a single diameter in nano size at a range of 50 nm ~ 80 nm was able to produce. During calibration printing, it was found that 1.7%wt of TPO failed to produce a 3D profile shape. The final printing results of 0.5%wt and 1%wt of TPO were compared after being cured at 70s and 80s. It was observed that the printed 3D scaffold of 1%wt TPO at 70s curing time produces the most discernable shape of tensile specimen (ISO 37:2011) than the other three conditions. The findings from this study can be potentially used a guideline for developing a 3D structure of tissue engineering scaffold by using DLP 3D printing process.

***Index Terms*: Additive Manufacturing, 3D Printing, Tissue Engineering, Scaffold**

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I. INTRODUCTION

Bone grafting is one of the most commonly used in surgical methods to enhance bone regeneration in orthopedic procedures. Many bone grafting such as titanium alloy, ceramics and polymers, have been used as bone-substitute materials. However, each has a specific disadvantage [1]. Permanent implantations can erode due to late breakdown and causes inflammation. The acidic outcome of bone grafting and implant also negatively affected the latter-stage results of bone repair. Other than that, the available volume of bone graft from a patient is too limited, hence additional surgical procedure is required to harvest the grafting material which is associated with a significant risk of the donor [2]. Due to these limitations and constraints, researchers put their recent interest in tissue engineering technology as an alternative method for large bone defect repair.

Tissue engineering scaffold technology provides a temporary template to develop biological substitutes that restore, maintain, or improve tissue function or a whole damaged organ [3]. The tissue engineering scaffolds fabricated using ceramic and metal have been widely reported in the literature especially for bone tissue replacement. Unfortunately, their clinical applications for tissue engineering have several limitations due to their brittleness behavior and difficulties of shaping for implantation [4]. For these reasons, polymeric scaffolds become increasingly used due to their biodegradable and biocompatible properties. Group of polymeric scaffolds have unique properties such as high surface-to-volume ratio, high porosity with very small pore size, and biodegradable [5] which are suitable for tissue engineering scaffold applications. However, in actual condition the natural polymeric may produce uncontrolled impurities such as endotoxin, while the synthetic polymers are said hydrophobic and lack cell recognition, which causes limitation on the bioactivity of cells. Because of these constraints, hydrogels biopolymer materials such as poly(acrylic acid) (PAA), poly(ethylene glycol) (PEG), poly(vinyl alcohol) (PVA), polyacrylamide (PAAm), and polypeptides have been increasingly used nowadays due to high water content which is crucial as a medium to allows cell growth and encapsulation [6]. Most of

the researchers claim that Poly (ethylene glycol) diacrylate (PEGDA) hydrogel polymer has been found extensively used in biomedicine compared to other types of hydrogel biopolymer with the reasons of their excellent performance in biocompatibility and hydrophilicity [7]–[9]. Even though various PEGDA-based scaffolds have been researched, however, none of them fulfils all the requirements for tissue engineering applications [10]. Some of these materials exhibit poor mechanical properties because of the weaknesses in their physical and mechanical stability [11], [12]. On the other hands, PEGDA hydrogel also lacks in cell adhesion, which it becomes a major limitation to be used as a tissue engineering scaffold [6].

Over the years, there are various traditional methods used to construct tissue engineering scaffolds include molding, solvent casting and particulate leaching, gas foaming, and electrospinning. Although a lots of traditional fabrication methods can be used to produce scaffold, unfortunately, each of these methods has limitations which they are not able to precisely control the internal topology and architecture [13], [14]. To the best of our knowledge, none of the traditional methods is satisfactory to produce scaffolds with fine control dimensions architecture, porosity, and faced the difficulty to mimic the biological function of natural tissue [13]–[16]. The technology of additive manufacturing (AM) method via DLP 3D printing is reported has the capability to fabricate high resolution and finely control in dimensions of the scaffold. DLP 3D printing technology also easy to operate, low cost and fast printing compared than traditional methods and others additive manufacturing techniques such as selective laser sintering (SLS) and fused deposition modelling (FDM) [17], [18]. However, DLP technique faced the limitation on lack of numbers biocompatible resins can be used.Therefore, the present work in this research study is aim to close the gap and by seeks into new approached Digital Light Processing technique in fabrication a novel biomaterial PEGDA filled with ANFs which are not yet been established and has not reported elsewhere. The composition of new biomaterial proposed, and the feasible printing parameter in producing 3D structure scaffold are also discussed detail.

II. METHODOLOGY

A.Materials

Chemicals used in this study were as follows: 1) Potassium hydroxide (KOH) (90% purity, Sigma Aldrich), 2) Dimethyl sulfoxide (DMSO) (QRëC), 3) Kevlar (160 mg), 4) Poly (ethylene glycol) diacrylate (PEGDA) (M_n 700, Sigma Aldrich) and 5) 2,4,6-trimethylbenzoyl-diphenylphosphine oxide (TPO) (97% purity, Sigma Aldrich).

B.Synthesis of ANFs

ANFs was synthesized based on the method reported by Guan *et. al.*, (2017) [19]. The bulk Kevlar fibers were split into aramid nanofibers by deprotonation (removal a hydrogen cation, H^+) in the solution of DMSO and KOH. Kevlar fiber (160 mg) and KOH (0.3 g) were added into 68 mL of DMSO and 12 mL de-ionized water. The solution was then magnetically stirred continuously for 1 week at room temperature until a dark red ANF/DMSO dispersion (2.0 mg/ml) was formed as shown in Fig. 1. The ANFs solution was dried under room environment in order to allow the formation of a thin sheet of ANFs. The diameter of dried single nanofiber was later observed under the Field Emission Scanning Electron microscope.

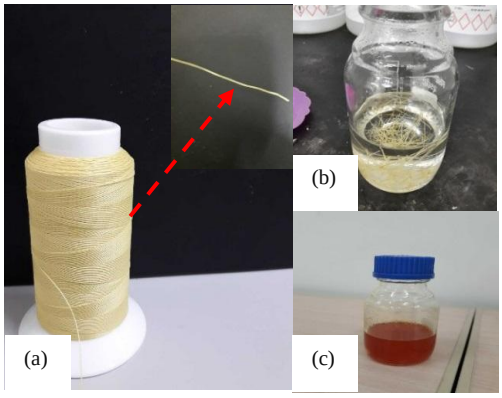


Fig. 1. Synthesizing of ANFs (a) Bulk Kevlar, (b) Kevlar dissolved in DMSO and KOH, (c) ANF dispersion in orange color after stirred one week at room temperature.

C.Resin Formulation

ANFs was first synthesized from macro size Kevlar fiber prior to the crosslinking with Diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide (TPO) photoinitiator. The mixing ratio of PEGDA resin to ANFs was fixed to 9:1. The concentration of TPO was varied at 0.5, 1.0 and 1.7% wt. while the resin concentration was fixed at 30% during the mixing to produce three set of biomaterials. PEGDA photopolymer solution was prepared by dissolving solid PEGDA (average molecular weight Mn 700) in Dimethyl sulfoxide (DMSO) to obtain concentration of 30 % wt. The photoinitiator Diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide (TPO) was also dissolved in DMSO to obtain the above concentrations.

D.Digital Light Processing Setup

The fabrication of 3D tissue engineering scaffolds was carried out using DLP 3D printing machine (FEMTO 3D). Fig. 2 shows the set-up of this machine. SolidWorks 3D V14 software was used to model the scaffold structure design with a constant porosity value, cell size and number of unit cells based on the previous literature as summarized in Table 1. SolidWorks design file was converted into STL format for printing purpose. DLP printing process was conducted with curing time set at 70 and 80s for different TPO concentrations. At the end of printing process, the un-polymerized liquid resin was removed by post-curing within 24 hours to converts any unreacted groups of polymerization resin.

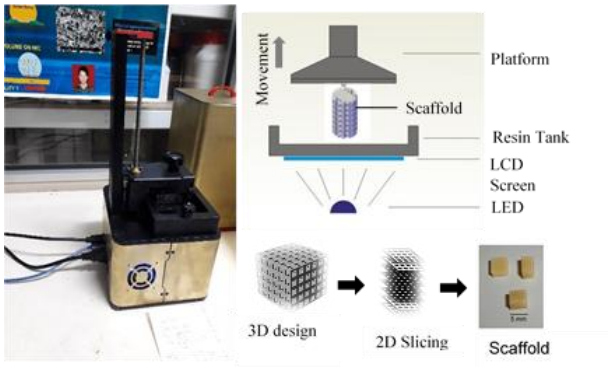


Fig. 2: The Machine Set Up For The DLP 3D Printing Process

E. Calibration of 3D Printing Process

The calibration of printing process was performed on the three set of biomaterial (0.5, 1.0, 1.7 % wt.) at varied calibration times from 20s up to 180s. The calibration printing results were visually analyzed and compared with the actual calibration 3D profile to match the dimensions and shape as per designed in the CAD software (Fig.3).

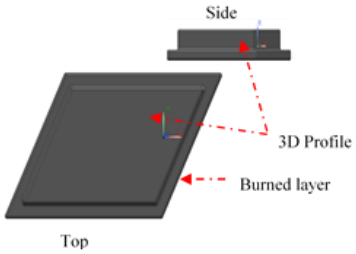


Fig. 3. A 3D profile design created in the software for calibrating the printed structure by the DLP machine.

Table 1. Unit size of scaffold design [21]

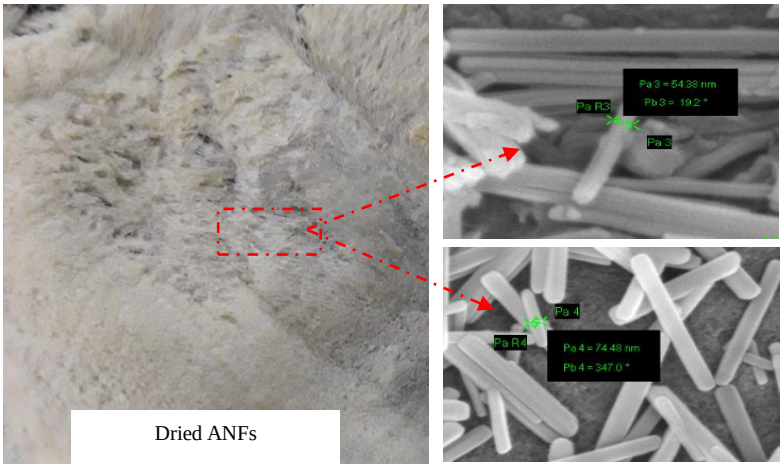
Parameters (enclosed volume = 1000mm^3)		
Lattice Name	Single lattice	Lattices with 8 of unit cells
Cell Size (l/d=1.25)		l=10, d=8
Total number of unit cell		8
Porous scaffold volume (mm^3)		1728.82
Porosity (%)		79

III. RESULTS AND DISCUSSION

A. Microstructure Analysis of Aramid Nanofibers (ANFs)

ANFs were dried at room temperature for 7 days to obtain nanofiber mats. Fig. 4a shows one of the dried ANFs mats observed under FESEM. At higher magnification (Fig. 4b), the fiber diameter size was recorded in the range of 50 nm to 80 nm. These evidences revealed that Kevlar fibers were successfully split into ANFs by deprotonation concept.

The real development of nanoscale reinforcement can be more challenging due to the difficulties arise from exfoliation and dispersion of existing materials. The nature of dissimilar chemical between nanofillers and polymeric resin material was said as the main reason for the imperfect filler dispersion, which in directly contributes to the low mechanical performance of composites. A homogeneous dispersion of filler is also critical in polymer nanocomposites. Agglomeration may occur and results in poorly dispersed nanofiller creating micron-sized aggregates. Previous study revealed that moderate interaction between filler and polymer creates optimum dispersion of filler. At low interaction, filler tends to attract each other causing agglomeration, while strong interaction between polymer and filler creating flocculation due to strong polymer adsorption to its neighbouring filler [22]. However, the result obtained from this research shows that ANFs dissolve completely in resin solution (PEGDA/TPO) without incurring any formation of precipitation and coagulation (Fig. 5). The formulation ratio used in this study demonstrate excellence results whereby the proposed new biomaterial resin able to be produced without any imperfect filler dispersion condition.



(a) (b)

Fig. 4: Dried ANFs completely dissolved in resin (PEGDA/TPO) without any coagulation formation.

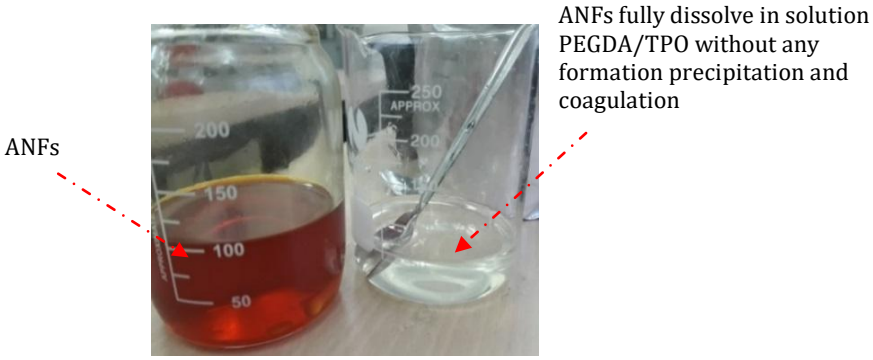


Fig. 5: Anfs Completely Dissolved In Resin (PEGDA/TPO) Without Any Coagulation Formation

B. Effects of Photoinitiator Concentration on the DLP 3D Printed Profile

Fig. 6(a-c) illustrates the 3D profile results of calibration printing using three different TPO concentrations, i.e. 0.5%wt, 1.0%wt and 1.7%wt respectively. In general, TPO concentration has a significant effect on the 3D profile formation. It is clearly seen that at high concentration of TPO, 1.7%wt, a thin layer of

printed profile is able to form. It is believed that a localization of high free radical initiation closer to the surface causes the penetration depth of the photons laser decreased and produces a tightly cross-linked and thin cured profile [23][24]. However, the printed 3D profile produced by 0.5 % wt TPO forms a non-uniform and inconsistent shape which merged each other. At 1.0 % wt concentration of TPO, it exhibits a lot better 3D profile than 0.5 %wt of TPO.

C.Printable Results of PEGDA/ANFs 3D Tissue Engineering Scaffold

Table 2 shows the printed results of 3D scaffold structure using DLP machine based on 0.5%wt. and 1.0%wt. of TPO at 70s and 80s curing time. It is observed that at the condition of 0.5 % wt. TPO for 70s and 80s curing time, the produced 3D profiles were not according to the designed 3D structure. It is completely out of tensile specimen shape and dimension. The printed profile formed are still in semi-solid phase and not fully cured, perhaps due to lower initiator concentration [25]. The most acceptable and accurate dimension of printed 3D tensile sample is shown under 1.0 %wt TPO at 70s. Under the same concentration with 80s curing time, the 3D structure produced seems to be over cured and incomplete. Based on this result, it was decided to print the final 3D scaffold structure of PEGDA/ANFs with pores by using the formulation composition at 1.0 %wt TPO-30% PEGDA cured for 70s due to its ability to form the most acceptable 3D profile and dimensions.

The results of 3D printed scaffold with pores structure can be seen in Fig. 7. The novel biomaterial resin of PEGDA/ANFs is successfully printed via DLP technique with precise pore size and dimensional accuracy. After post-curing within 10h, the sample of scaffold structure becomes more stiffer due to complete photopolymerization during post-curing process.

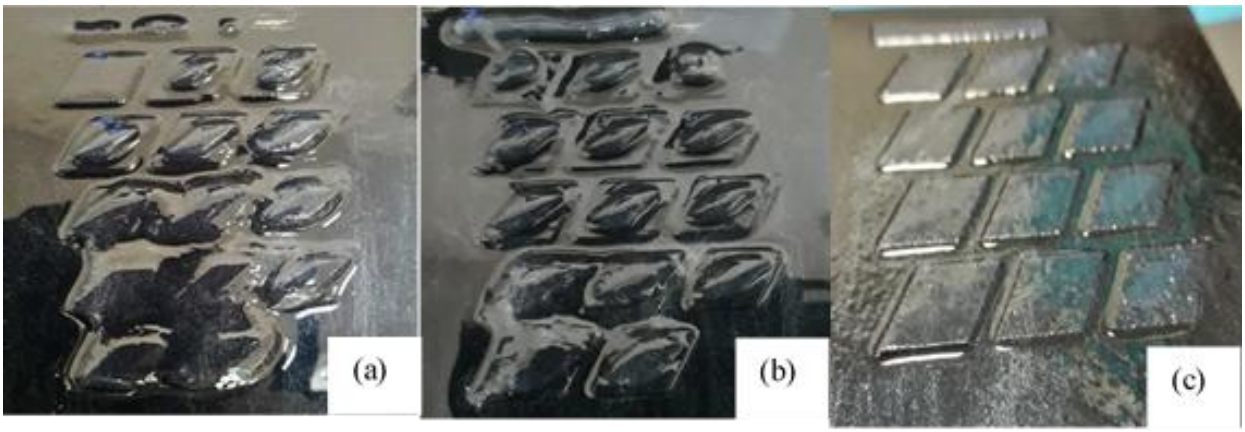
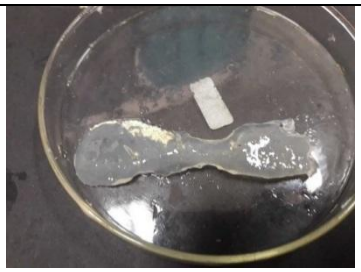


Fig. 6. Calibration of printed profile resin: ANFs at constant 30% wt PEGDA with varied concentration of TPO; (a) 0.5 %wt,(b) 1.0 %wt and (c) 1.7 %wt.

Table 2. Results Of 3D Sample Print By DLP 3D Printing With Different Concentrations Of %Wt. TPO

Composition	Concentration	Curing Time(s)	Printing Result
PEGDA	TPO		
30% wt	0.5 % wt	70	
30% wt	0.5 % wt	80	
30% wt	1 % wt	70	
30% wt	1 % wt	80	

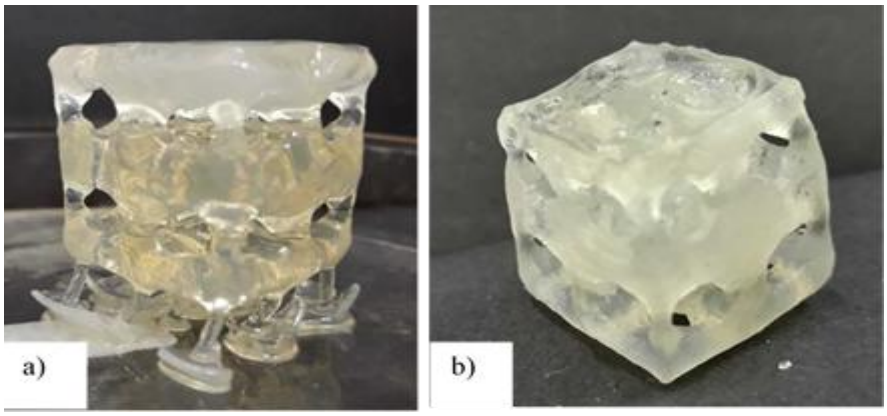


Fig. 7. 3D tissue engineering scaffold PEGDA/ANFs with porous structure; (a) before post curing (b) after post curing.

III. CONCLUSION

The present work evaluates the feasible TPO concentration and curing time for printing a novel 3D structure made of PEGDA/ANFs bio-material via DLP 3D printing process. A nanofiber diameter of ANFs in the range of 50 – 80nm is successfully synthesized from the Kevlar fiber. Three TPO concentrations are tested and it is found that 1%wt TPO-30%wt PEGDA in resin to ANFs ratio of 9:1 with 70s curing time produces the most discernable 3D scaffold structure compared to the rest of other conditions. This condition is proven able to construct a porous 3D structure after post curing.

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