BIODEGRADABLE POLY (ETHYLENE GLYCOL) DIACRYLATE FILLED ARAMID NANOFIBER HYDROGEL THREE DIMENSIONAL PRINTED TISSUE ENGINEERING SCAFFOLD

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DEDICATION

This thesis is dedicated to my father, who taught me that the best kind of knowledge to have is that which is learned for its own sake. It is also dedicated to my mother, who taught me that even the largest task can be accomplished if it is done one step at a time.

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ABSTRACT

Digital Light Processing (DLP) process is one of the additive manufacturing techniques and has been widely used to fabricate tissue engineering scaffold based on Poly (ethylene glycol) diacrylate (PEGDA) material. However, the existing PEGDA scaffold via DLP 3D printing commonly exhibits poor mechanical and biocompatible properties. The PEGDA 3D scaffolds also have low cells viability which can cause tissue engineering failure. Therefore, this study aims to develop a novel soft tissue engineering scaffold biomaterial, using PEGDA filled with Aramid nanofibers (ANFs), with enhanced mechanical strength and biocompatible properties via DLP 3D printing technique. ANFs was first synthesized from macro size Kevlar fibre (0.2 %wt.) prior to crosslinking with Diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide (TPO) photo initiator. The mixing ratio of PEGDA resin to ANFs was fixed to 9:1. During the mixing, the concentration of TPO was varied at 0.5, 1.0 and 1.7% wt., while the resin concentration was fixed at 30% wt. to produce three sets of biomaterials. Preliminary study was conducted prior to the actual printing for the purpose of eliminating unprintable TPO concentration. The final scaffold was printed using a FEMTO3D DLP machine at two different curing times; 70s and 80s to obtain good shape and printable 3D structure. It was found that 1.7%wt of TPO failed to produce a 3D profile shape. It was observed the printed 3D scaffold of 1%wt TPO at 70s curing time produced the most discernible shape of the compression specimen (ASTM D695). Based on the printable photo initiator results, the experiments were expanded further by taking into account the PEGDA concentration, resin to ANFs ratio and DLP curing time. At this stage, both resin-PEGDA/TPO ratio and TPO concentration were fixed at 8:2 and 1.0 %wt. respectively. A two level factorial design involving three factors was used to determine the feasible printing parameter where the response is the Young's Modulus. The resin to ANFs ratio (9:1, 8:2, 7:3), PEGDA concentrations (30, 40, 50 %wt.) and curing time (70, 80, 90s) were varied during the experiments. Response surface method was used to determine the optimum setting for maximizing the Young's Modulus. The synthesized ANFs have shown a nano diameter size distributions ranging from 20 nm to 80 nm. The optimum condition was found at 7:3 resin to ANFs ratio, PEGDA concentration at 50 %wt. and at 100s curing time, which recorded the highest Young's modulus (0.55 MPa). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay showed a weak condition of the cells viability at a ratio of 10:0 (61.4 %) after 3 days of incubation. Increased ratio of ANFs enhanced the cell viability where 81.6%, 89.3 % and 96.3 % of cells viability were recorded at the ratios of 9:1, 8:2 and 7:3, respectively. Fourier Transform Infrared Spectroscopy and Diffraction Scanning Calorimetry analyses also proved that the presence of Aramid functional group in the printed PEGDA/ANFs scaffold. The optimized dried sample after freeze-drying process for 24 hours confirmed that their physical reliability with minimal volume shrinkage (30%) and 80% water content remained in the final scaffold with high interconnected internal porous structure. The mechanical strength of the optimized printed scaffold also increased at 69.1% (0.93 MPa) after the freeze dried. Overall, the mechanical and biocompatibility properties of the fabricated PEGDA filled with ANFs exhibits significant improvement as compared to PEGDA without ANFs. It has proved that the newly developed PEGDA-ANFs scaffold has a great potential to be used as an articular cartilage in soft tissue engineering applications.

ABSTRAK

Pemprosesan cahaya digital (DLP) adalah salah satu teknik pembuatan bahan tambahan yang semakin banyak digunakan untuk membuat perancah kejuruteraan tisu berdasarkan bahan *Poly (ethylene glycol) diacrylate* (PEGDA). Walau bagaimanapun, perancah 3D PEGDA yang di hasilkan melalui percetakan DLP biasanya menunjukkan sifat mekanik dan biokerasian yang lemah. Perancah 3D PEGDA juga mempunyai daya tahan sel yang rendah yang boleh menyebabkan kegagalan tisu kejuruteraan. Oleh itu, kajian ini di jalankan bertujuan untuk meningkatkan keupayaan bahan bio dalam pembuatan perancah kejuruteraan tisu lembut baru, menggunakan PEGDA yang diisi dengan gentian nano *Aramid* (ANFs) dengan kekuatan mekanikal dan sifat biokerasian yang dipertingkat melalui teknik percetakan 3D DLP. ANFs disintesis terlebih dahulu dari serat *Kevlar* bersaiz mikro (0.2 *%wt*.) sebelum dicampurkan dengan fotopemula *Diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide* (TPO). Nisbah campuran damar PEGDA ke ANFs ditetapkan pada 9:1. Pembolehubah kepekatan TPO ditetapkan pada kepekatan berat 0.5, 1.0 dan 1.7%, sementara kepekatan damar ditetapkan pada 30% untuk menghasilkan tiga set bahan bio. Kajian awal dilakukan sebelum percetakan sebenar bagi mengenal pasti kepekatan TPO yang dapat dicetak. Perancah terakhir dicetak menggunakan mesin percetakan FEMTO3D DLP pada dua masa percetakan yang berbeza iaitu pada 70 dan 80 saat untuk memperoleh bentuk dan struktur 3D yang baik. Didapati bahawa kepekatan TPO pada 1.7% gagal menghasilkan susuk 3D. Diperhatikan bahawa perancah 3D yang dicetak dengan kepekatan berat TPO pada 1% pada masa 70 saat telah menghasilkan bentuk susuk 3D mengikut saiz piawai ujian mampatan (ASTM D695). Berdasarkan hasil fotopemula yang dapat dicetak, ujikaji diperluas dengan mempertimbangkan kepekatan PEGDA, nisbah damar ke ANFs dan tempoh masa percetakan DLP. Pada tahap ini, kedua-dua nisbah damar PEGDA/TPO dan kepekatan TPO masing-masing ditetapkan pada 1.0% dan 8:2. Satu reka bentuk ujikaji faktoran penuh dua tahap yang melibatkan tiga faktor telah digunakan untuk menentukan parameter percetakan yang boleh dimana tindak balasnya adalah Modulus Young. Nisbah damar kepada ANFs (9:1, 8:2, 7:3), kepekatan PEGDA (30, 40, 50%) dan masa cetakan (70, 80, 90 saat) telah dipelbagaikan semasa ujikaji. Kaedah permukaan tindakbalas telah digunakan bagi menentukan tetapan optimum bagi menghasilkan Modulus Young yang maksimum. ANFs yang disintesis menunjukkan taburan ukuran garis pusat bersaiz nano pada ukuran 20 nm hingga 80 nm. Keadaan optimum diperoleh dengan catatan Modulus Young tertinggi (0.55 MPa) adalah pada nisbah 7:3 damar ke ANFs, kepekatan PEGDA pada 50% dan percetakan pada 100 saat. Ujian ke atas *3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide* menunjukkan kebolehjayaan sel pada nisbah 10:0 adalah sebanyak 61.4% pada 3 hari pengeraman. Peningkatan nisbah ANFs telah meningkatkan perkembangan sel yang mana 81.6%, 89.3% dan 96.3% perkembangan sel masing-masing dicatatkan pada nisbah 9:1, 8:2 dan 7:3. Spektroskopi Infra-merah Jelmaan Fourier dan Analisis Permeteran Kalori Pengimbasan Kebezaan juga membuktikan kewujudan kumpulan berangkap *Aramid* dalam perancah PEGDA/ANFs yang telah dicetak. Sampel optimum yang telah di sejuk-beku setelah proses pengeringan selama 24 jam telah menunjukkan kebolehpercayaan fizikal dengan pengecutan isipadu minimum (30%) dan kandungan air 80% kekal dalam perancah serta mempunyai struktur berliang yang saling terhubung. Kekuatan mekanikal optimum perancah juga meningkat pada 69.1% (0.93 MPa) selepas process pengeringan beku. Secara keseluruhan, sifat mekanikal dan biokerasian PEGDA yang diisi dengan gentian ANFs menunjukkan peningkatan yang ketara berbanding dengan PEGDA tanpa ANFs. Dibuktikan bahawa, perancah PEGDA-ANF yang baru berpotensi besar untuk digunakan sebagai tulang rawan bersendi dalam aplikasi kejuruteraan tisu lembut.

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LIST OF ABBREVIATIONS

AM	-	Additive Manufacturing
ANFs	-	Aramid Nanofibers
BAPO	-	Bis(2,4,6-trimethylbenzoyl) phenyl-phosphine oxide
BMA	-	Butyl methacrylate
CCD	-	Central Composite Design
CNT	-	Carbon Nanotube
CQ	-	Camphorquinone
DEF	-	Diethyl Fumarate
DLP	-	Digital Light Processing
DSC	-	Differential Scanning Calorimetry
ECM	-	Extracellular Matrix
FAME	-	Fumaric acid mono ethyl ester
FDM	-	Fused Deposition Modelling
LAP	-	Phenyl-2,4,6-trimethylbenzoylphosphinate
NVP	-	N-vinyl-2-pyrroli
OLMA	-	Methacrylated oligolactones
PCL	-	Poly (e-caprolactone)
PEGDA	-	Poly(ethylene glycol) diacrylate
PLA	-	Polylactide
PPF	-	Poly (propylene fumarate)
RSM	-	Response Surface Methodology
SLA	-	Stereolithography
SLS	-	Selective Laser Sintering
TGA	-	Thermogravimetric Analysis
TPO	-	Diphenyl (2,4,6-trimethylbenzoyl) phosphine oxide
UDMA	-	Urethandimethacrylate
UV	-	Ultraviolet

LIST OF SYMBOLS

α	-	Alpha
Δ	-	Delta
D	-	Diameter
H	-	Height
H	-	Hour
λ	-	Lambda
mm	-	Millimetre
%	-	Percentage
s	-	Second

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CHAPTER 1

INTRODUCTION

1.1 Introduction

This chapter discusses the background of study, research problems, objectives, scopes, and significance of research. Its ends with outline of thesis.

1.2 Background of Problem

The failure of organs or tissues due to trauma or ageing is a primary concern in healthcare as they are costly and result in devastating problems. This has led to the development of tissue engineering (TE), which aims to create biological substitutes to repair or replace the failing organs and tissues (Hassanajili et al., 2019). One of the best approaches in tissue engineering is by growing biodegradable scaffold cells, which attempts to imitate the natural extracellular matrix function and provide a temporary tissue growth template (Mondschein et al., 2017).

Tissue engineering scaffolds are unique in a way; as they are able to establish three-dimensional environments for propagated cells and provides specific recognition molecules which capable of mimicking the environment of natural tissues. The scaffolds can be either natural, synthetic or hybrid (Annabi et al., 2016). Tissue engineering scaffold should fulfil the biological and mechanical target tissue requirements. The scaffolds should have an appropriate microstructure to support cell proliferation, contained with open-porous geometry with a highly porous surface enabling cell growth, appropriate surface morphology and a predictable degradation rate of non-toxic material (Miri et al., 2019).

Tissue regeneration in scaffolds through cell implantation relies primarily on the scaffold structure and the nature of the biomaterials (Heljak et al., 2020). The interaction between biomaterials and surrounding tissues is a crucial concern when choosing the proper material for scaffolds. Although the specific material requirements depend on the nature of the application, all biomaterials must be evaluated for their biocompatibility (Bil et al., 2010). The biomaterial scaffolds allow cells containment and signalization of molecules as a transplant, to enable functional tissue regeneration in the host as an alternative to common organ transplantation and tissue reconstruction practices (Annabi et al., 2016). Essentially, an optimal biomaterial scaffold could mimic the natural structure and process tissue regeneration (Lim et al., 2017).

Fat, skin, tendon, muscle, articular cartilage, nerves, fascia, intervertebral disc, synovium, joint capsule, and blood vessels are all included in the category of soft tissues. These tissues generally surround, support, or connect the body's structure and organs. Currently, autologous implantation has been the major way of treating the defects or illnesses of soft tissue. However, autologous tissue is easily absorbed and rapidly lost in volume, leaving just 40–60 percent of soft tissue cells alive. Other than that, autologous transplantation is also limited by donor site allogeneic response and morbidity. Therefore, tissue engineering has been used to produce novel biological replacements for healing and regenerating injured soft tissues, overcoming the limitations of current clinical treatments (Pei et al., 2017).

Nanoscale fibrous structures have gained much interest in tissue engineering field application such as musculoskeletal tissues (including bone, cartilage, ligament, and skeletal muscle), skin tissue engineering, neural tissue engineering, vascular tissue engineering, and controlled delivery drug, protein, and DNA (Stocco et al., 2018). Nanofiber has emerged as promising biomimetic candidates of scaffold due to their small size (1–100 nm), which is comparable with peptides and small proteins. The high surface-to-volume ratio of nanofibers for tissue engineering applications is extremely desired due to quickly spread through membranes (Xue et al., 2019). This nano-environment allows for cells grow and it has potential to promote cell adherence, proliferation, migration and cell differentiation, which similar to natural extracellular matrices (ECM) of tissues and organs (Rasouli et al., 2019). Nanoparticles are also

extremely mobile when in a free state, which results in incredibly slow sedimentation rates (Hasan et al., 2018). Therefore, the use of the right type of nanofibers in tissue engineering can significantly improve the biological and mechanical properties of scaffolds, depending on the application.

There are two main methods of fabricating scaffold, viz. through the use of conventional and additive manufacturing processes. Conventional fabrication methods include electrospinning, phase separation, freeze drying, self-assembly, solvent casting, textile technologies, and material injections. Additive manufacturing, commonly referred to as 3D printing, includes stereolithography (photopolymerization), inkjet printing, bioprinting, fused deposition modeling (FDM), extrusion, laser beam melting, selective laser sintering (SLS), digital light processing (DLP), electron beam melting, and polyjet (Roseti et al., 2017). Even though these method are commonly used in manufacturing industries, however, they become a new technique in biomedical industries due to their several advantages including the ability to create complex geometries, multiple materials, and a wide range of biomaterials can be used compare to conventional technique (Jammalamadaka and Tappa, 2018).

1.3 Problem Statement

Current research is tremendously focused on the development of light-curable and highly biocompatible resin under Digital Light Processing (DLP) 3D printing. The resin used in DLP printing process is usually composed of photoinitiator, polymerizable oligomers, and additives (Ronca and Ambrosio, 2017). To date, common biocompatible photopolymers, such as poly(ethylene glycol) diacrylate (PEGDA) (Eshel et al., 2016), poly(ethylene glycol) dimethacrylate (Burke et al., 2019), gelatin methacrylate (Na et al., 2018), and poly(propylene fumarate) (Mishra et al., 2016) have been successfully used in DLP printing.

Among them, poly(ethylene glycol) diacrylate (PEGDA) hydrogel polymer has been extensively used as tissue engineering scaffold in comparison to other biopolymers due to its excellent performance in biocompatibility and hydrophilicity

properties (Gigli et al., 2016; Palaganas et al., 2017; Scaffaro et al., 2016). Even though various 3D printed PEGDA scaffolds were reported, none of them fulfill all the requirements for tissue engineering applications (Kotturi et al., 2017). The existing 3D printed PEGDA scaffolds show low cell's viability and exhibit poor mechanical properties due to the weakness in their physical and mechanical stability, which becomes the main shortcoming for it to be used as tissue engineering scaffolds (Naahidi et al., 2017; Park et al., 2017; Sivashankari and Prabaharan, 2016; Turnbull et al., 2018).

In order to enhance the performance of PEGDA scaffold, some researchers added PEGDA hydrogels polymers using different types of nanofiller such as carbon nanotube (CNT), nano-silica, laponite nanoparticle, and others (Mishra et al., 2015; Palaganas et al., 2017; Vashist et al., 2018). It has been revealed that nanofibers are capable of enhancing cell adhesion, increase cell proliferation, and improve mechanical properties of tissue engineering scaffold (Barhoum et al., 2019). Incorporation of certain types of nanofibers into scaffolds can significantly increase scaffold's surface area and surface wettability. These hydrophilicity behavior provide favorable conditions for cell adhesion and the same time enhanced the cellular behavior which result in enrichment of proliferation rate and cells formation (Udomluck et al., 2020; Zhu et al., 2020). The mechanical strength and decreasing degradation rate of scaffold can be enhanced by crosslinking the nanofibers with the biomaterial (Nemati et al., 2019). Thus, the use of nanofibers can produce new tissue engineering scaffolds that possess optimal mechanical and biological features.

Unfortunately, the current types of nanofiller used have several limitations. For example, bioceramic and bioglass nanofillers appear to have brittle properties, fragile and low fracture strength (Munir et al., 2019). These materials are also challenging to fabricate due to poor flexibility properties. Low fatigue strength behavior also makes them incompatible with being used in the formulation of tissue engineering hydrogels (Mondschein et al., 2017). Other than that, the use of carbon nanotube (CNTs) as a filler also faced limitation due to lack in their biodegradability properties. CNTs are nonbiodegradable and may remain in an organism as reported by researchers (Gao et al., 2017; Raphey et al., 2019). High concentration of CNT used will also contribute

to negative effect for cell proliferation (Peng et al., 2020). On the other hand, one of obvious limitation of CNTs application in tissue engineering is their high stiffness properties, which never be able to mimic the mechanical properties of tissues and considered to be critical for the proliferation of cell (Newman, 2016).

Though a lot of works on application of PEGDA as tissue engineering scaffold has been published, but there is still no literature reported on the development of PEGDA hydrogels biomaterials filled with Aramid nanofibers (ANFs) as a tissue engineering scaffold. Previous studies only discussed issues related to kinetic crystallization and morphology of PEGDA hydrogel with Kevlar fibers in a macro scale. Aramid nanofibers (ANFs) offer an excellence biodegradable properties, good mechanical strength and relatively favorable hydrophilicity (Wang et al., 2018; Yang et al., 2019). ANFs are also light, strong, resistance to fatigue, and stress rupture which make it suitable for tissue engineering scaffold applications (Rho et al., 1998; Yang et al., 2019). However, the application of PEGDA filled with ANFs as a tissue engineering scaffold also has not been reported elsewhere, and therefore their mechanical and biodegradable properties remain unknown. In addition, the capability to print PEGDA/ANFs scaffold via DLP 3D printing process also has not been reported in any literature to date. Thus, the present work is aimed to close the gap.

In practice, conventional methods such as molding, solvent casting, and particulate leaching, gas foaming, and electrospinning are used to construct tissue engineering scaffolds. Although many traditional fabrication methods can be used to produce scaffold, unfortunately, each method has their own limitations precisely the internal topology and architecture. Based on the reviewed literature, none of the traditional methods are able to produce scaffolds satisfactory with fine control architecture dimensions, porosity, and face the difficulty to mimic the biological function of natural tissue (Mondschein et al., 2017; Osama and Darwish, 2011; Wei et al., 2016).

As an alternative to conventional scaffold fabrication methods, additive manufacturing techniques have been developed in tissue engineering such as rapid prototyping by which a 3D scaffold is fabricated by laying down multiple, precisely formed layers in series. Subia et al. (2010) has claimed that the rapid prototyping technique (RP) has drawn tremendous attention with its potential to overcome most of the limitations faced by conventional technique for the fabrication 3D scaffolds (Subia et al., 2010). Even though there are numerous additive manufacturing processes, DLP 3D printing (photopolymerization) technology has become the easiest method with lowest cost and fast printing speed compared to other additive manufacturing techniques such as Selective Laser Sintering (SLS) and Fused Deposition Modelling (FDM) (Geng and Shan, 2015; Stansbury and Idacavage, 2016).

It is noteworthy that photopolymerizable system in DLP 3D printing is usually composed of photopolymer, photoinitiator and additives (Yang et al., 2020). The addition of ANFs in photopolymerization system would affect the curing time printing parameter and the properties of printed scaffold. Therefore, further studies on their printability are necessary. The properties such as printing fidelity, internal structures, mechanical and biocompatibility properties of the produced scaffold also need to investigate.

This research aimed to develop a novel biomaterial 3D printed PEGDA/ANFs scaffold via DLP 3D printing process. The experiment were designed thoroughly to evaluated the curing time printing parameter, mechanical and biocompatibility properties, stability and internal pores structures of 3D printed PEGDA/ANFs scaffold.

1.4 Research Objectives

The objectives of the research were:

(a) To develop a new biomaterial PEGDA/ANFs with enhanced mechanical and biocompatibility properties for soft tissue engineering scaffold.

(b) To determine feasible curing time of DLP 3D printing for providing high Young's modulus value of 3D printed PEGDA/ANFs scaffold.

(c) To modify 3D printed PEGDA/ANFs scaffold with internal pores structure via freeze drying technique and investigate their shrinkage behavior and mechanical strength.

1.5 Scopes of Research

The scopes of research were as follow:

i. Digital Light Processing (DLP) 3D printing process was used to develop Poly(ethylene glycol) diacrylate (PEGDA) filled with Aramid nanofibers (ANFs) for 3D soft tissue engineering scaffold

ii. Preliminary study was carried out to identify the feasible printable curing setting. The testing were limited to 10:0, 9:1, 8:2, and 7:3 for resin to ANFs ratio while PEGDA concentration was limited to 30, 40 and 50% wt.

iii. Investigation on the mechanical properties was limited to Young Modulus by compression technique.

iv. Biocompatibility studies only involved biodegradation, swelling ratio and MTT assay tests.

v. Freeze drying technique was carried in freeze drier machine at -58 °C within 24 hours in order to enhance the internal porous structure of printed scaffold.

1.6 Significance of Research

A novel combination of biomaterial PEGDA containing ANFs is useful in the fabrication of biodegradable tissue engineering scaffold. The PEGDA/ANFs scaffold developed has both the required mechanical strength and biocompatibility to function as a tissue engineering cartilage. In addition, the novel PEGDA/ANFs 3D tissue engineering scaffold also has the potential to minimize the frequency a patient has to undergo implant surgery and can minimize complications after surgery. Other than that, the information on the DLP 3D printing process parameters to produce optimum Young's modulus value and biodegradation properties of 3D PEGDA/ANFs scaffold with porous structure has been disclosed. These findings can also improve time and cost of fabrication.

1.7 Thesis Outline

General information on research, objectives and scope is presented in the first chapter of this thesis. Chapter 2 summarizes the literature review on tissue engineering, including the previous analysis of the microstructure, function and mechanical properties of human tissue. Chapter 3 provides a research framework and a detailed description of each process to explain the methodology of the experiments performed. Chapter 4 presents the outcomes of this study. This chapter is divided into three main sections: preliminary discussion, DLP 3D Printing process optimization using Design of experiment (DOE) and Response surface measurement (RSM) analysis study, and the last part in Chapter 4 is material characterization and fabrication of 3D scaffold with internal porous structure. In Chapter 5, the conclusion was made according to the results obtained

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LIST OF PUBLICATIONS

Indexed Journal

1. **Nurulhuda, A**., Izman, S., Ngadiman, N.H.A. (2020) 'Fabrication a novel 3D tissue engineering scaffold of Poly (ethylene glycol) diacrylate filled with Aramid Nanofibers via Digital Light Processing (DLP) technique ', *Journal of Mechanical Engineering*, 9, pp. 1-12. (I**ndexed by Scopus**)
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3. **Nurulhuda, A**., Izman, S., Ngadiman, N.H.A. (2019) 'Fabrication PEGDA/ANFs biomaterial as 3D tissue engineering scaffold by DLP 3D printing technology', *International Journal of Engineering and Advanced Technology (IJEAT)*, 6(8), pp. 751-758. (I**ndexed by Scopus**)

Research and Innovation

1. **Nurulhuda, A.,** Izman, S., Ngadiman, N.H.A (2019) 'A Novel 3D Scaffold of PEGDA/ANFs for Tissue Engineering by DLP 3D Printing Process', *International Research Conference and Innovation and Exhibition 2019* (*IRCIE 2019*). (**Gold Award**)