Digital Light Processing (DLP) 3D Printing of Polyethylene Glycol (PEG) Biopolymer, Commercially available Ultra-High and Tough (UHT) Resin and Maghemite (γ-Fe_2O_3) Nanoparticles Mixture for Tissue Engineering Scaffold Application

Nor Hasrul Akhmal Ngadiman[1], Muhammad Aniq Barid Basri[1], Noordin Mohd Yusof[1,*], Ani Idris[2], Ehsan Fallahiarezoudar[3]

[1]School of Mechanical Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, Johor Bahru, Johor, 81310 Malaysia
[2]School of Chemical Engineering, Faculty of Engineering, C/o Institute of Bioproduct Development, Universiti Teknologi Malaysia, Johor Bahru, Johor, 81310 Malaysia
[3]Department of Industrial Engineering, Faculty of Engineering, East of Guilan, University of Guilan, Guilan, Roudsar, 4199613776 Iran

*Corresponding author: E-mail: noordin@mail.fkm.utm.my; Fax: (+60) 75566159

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Abstract

Digital Light Processing (DLP) 3D printing process has been used with standard, commercially available ultra-high and tough (UHT) photopolymer resin to produce for various 3D parts. Polyethylene glycol (PEG) biopolymer has been used extensively in biomedicine due to its excellent performance in biocompatibility and hydrophilicity. However, it offers low mechanical strength. The inclusion of maghemite (γ-Fe_2O_3) nanoparticles have been found to be able to increase the mechanical properties of TE scaffolds fabricated using a combination of processes. This study aims at exploring the possibility of using various mixtures which consists of different combinations UHT resin, PEG solution and γ-Fe_2O_3 nanoparticles with the DLP 3D printer system. The effects of various quantities of mixtures were investigated in terms of their mechanical and biocompatibility properties with a view of producing TE scaffolds. The results from this study proves that the simpler, DLP 3D printer system can be used with a mixture of standard photopolymer and biopolymer resins, and nanoparticles. The addition of PEG and γ-Fe_2O_3 enhanced the mechanical and biocompatibility properties of the developed structure. Copyright © VBRI Press.

Keywords: Tissue engineering scaffold, biomaterials, nanoparticle, 3D printer.

Introduction

The failure of organs or tissues due to trauma or ageing is a major concern in healthcare as they are costly and have devastating effects. 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 [**1**]. One of the more promising approaches in tissue engineering is to grow cells on biodegradable scaffold where the scaffold attempts to mimic the function of the natural extracellular matrix thus providing a temporary template for the growth of target tissues [**2**].

Tissue engineering scaffolds are unique in that they are able to establish an environment suitable for propagating cells and have specific signalling molecules that can mimic native tissue environment. The scaffolds can be natural, synthetic or a hybrid of both [**3**]. A good tissue engineering scaffold should fulfil the biological and mechanical requirements of the target tissue. The scaffold should have suitable microstructure that promote cell proliferation, contain open pore geometry with a highly porous surface that enables cell ingrowth, have suitable surface morphology and be made from a material with a predictable rate of degradation with a nontoxic degraded material [**4**]. In recent years, researchers are exploring on the use of nanofiber-based scaffolding systems which can act as scaffolds for tissue engineering application [**5**]. This is because the structures produced by nanofibers scaffolds mimic the structure of natural human tissue and thus enhance the cell growth rate. Considering these, efforts in finding the best materials and technique in the developing tissue engineering scaffolds that fulfills the aforementioned requirements are still ongoing.

Digital Light Processing (DLP) is a 3D printing process similar to stereolithography which involves the use of photopolymers. DLP 3D printing process uses the ultraviolet (UV) light as a light projector to display the entire X and Y cross sectional layer of the structure to be produced at one go onto a photopolymer resin that will turn the area exposed to UV light from liquid into solid. The solidified layer is formed on the collector which is on the Z axis. Using this process, the processing time is reduced. It is also a more versatile process with highest resolution compared to other additive manufacturing processes [**6, 7**]. It is able to create complex shapes with internal architecture, it has extremely high feature resolution and the unpolymerized resin can be easily removed. Thus, it can be used to develop the 3D structure of scaffold with any kind of shape while having good mechanical strength and can create good environment for enhancing the biocompatibility performance.

However the limited number of resins that are commercially available for processing by the DLP 3D printer has often been considered the main limitation of the technique. The resin commonly used for DLP 3D printer must have the same properties as the resin used in stereolithography (SLA) 3D printer whereby the resin used must have photocurable properties that can cure under ultraviolet (UV) light. Recent works have been published on the development of 3D scaffold using DLP 3D printer from hydroxyapatite [**8, 9**] materials. In both studies, hydroxyapatite powders were added to standard commercially available photopolymer resins. However, in these studies no degradation tests results were provided eventhough good in-vitro cell culture results were reported.

Similarly in our study, standard commercially available Ultra High and Tough (UHT) photopolymer resin was used with the DLP 3D printer system. Other suitable materials will be added to enhance the properties of the 3D scaffold.

The use of polyethylene glycol (PEG) has gained much attention due to its high biocompatibility and hydrophilicity which make it suitable for biomedical applications [**10-12**]. PEG is a photocurable resin due to its double acrylate groups on the molecular chain terminals [**13**]. PEG synthetic hydrogel polymer is commonly used in tissue regeneration due to its characteristics such as non-toxic, non-immunogenic and easily cleared from the body. These hydrogels are permeable to oxygen, nutrients, and other water-soluble metabolites and have a soft consistency that makes them similar to soft tissues [**14**]. However, its drawback is that it has low mechanical strength [**15**].

Maghemite, γ-Fe_2O_3 magnetic nano particle has been used in enhancing the mechanical property of TE scaffold produced via a combination of processes involving the 3D printing process, thermal inversion phase separation (TIPS) method and electrospinning process [**16**]. The use of nano particle sized γ-Fe_2O_3 has increased the Young's Modulus value. However, as three (3) processes are involved, the TE scaffold fabrication process can be quite challenging. Further, PVA with maghemite nanoparticle was used to develop scaffold by using the electrospinning process. It was shown that the addition of maghemite nanoparticle increased the mechanical and biocompatibility properties of the scaffold developed. Also, the amount of γ-Fe_2O_3 nanoparticle must be ideal because excessive amount of nanoparticles used can also lead to the lowering of the TE scaffold mechanical properties [**17**]. The maghemite nanoparticles also will increase the Young Modulus in the hydrogel but with excessive amount of maghemite nanoparticles in the material, it will increase the stiffness of the material. The presence of nanoparticles can also increase the cell adhesion and improve the biocompatibility of the gel [**18**].

Considering all of the above, this study explores the possibility of using various mixtures which consists of different combinations of standard photopolymer (UHT) resin, polyethylene glycol (PEG) solution and maghemite (γ-Fe_2O_3) nanoparticles with the DLP 3D printer system for their intended use in hard tissues. The roles of the various process and materials are summarized in **Table 1**. The fabricated 3D structure was then tested for its mechanical and biocompatibility properties.

Table 1. Roles of the various process and materials.

No	Item	Role
1	Digital Light Processing (DLP) 3D printing	As a process for developing the 3D structure
2	Polyethylene glycol	As a based material for developing the 3D structure
3	Standard commercially available Ultra-High and Tough (UHT) resin	As a resin to bind polyethylene glycol with maghemite and react with UV light to form 3D structure
4	Maghemite (γ-Fe_2O_3)	As a filler to enhance the properties of the developed structure

Experimental

Materials / chemicals details

Chemicals used in this study were reagent grade: iron (II) chloride ($FeCl_2$) (98% purity, Sigma Aldrich), iron (III) chloride ($FeCl_3$) (45% purity, Riedel-de Haen), sulfuric acid (H_2SO_4) (QRëC), nitric acid (HNO_3) (65% purity, QRëC), ammonia solution (NH_3) (25% purity, Merck), hydrochloric acid (HCl) (37% purity, QRëC), polyethylene glycol ($C_{16}H_{34}O_9$), dichloromethane (DCM), Ultra High and Tough (UHT) resin, Dulbecco's Modified Eagle's medium (DMEM) and fetal bovine serum (FBS).

Preparation of PEG Solution, PEG & UHT Mixture, PEG & γ-Fe_2O_3 Solution and PEG, γ-Fe_2O_3 &UHT Mixture

As mentioned previously, polyethylene glycol (PEG) was used in this research. Aqueous PEG solution with say 40wt/v% is prepared by dissolving 40-gram PEG polymer powder in 100 ml dichloromethane (DCM) at

room temperature with constant stirring for at least 24 hours. Similarly, the other two ratios of concentration being used in this study; 20wt/v% and 30wt/v% were prepared in a similar fashion. In order to prepare the PEG and UHT mixture, 50% of the previously prepared PEG solution was added to 50% of UHT resin. In order to prepare say 1v/v% of γ-Fe_2O_3 in 40wt/v% PEG solution, 1 ml of γ-Fe_2O_3 was added to 99 ml of 40wt/v% PEG solution prepared previously. The other concentration of γ-Fe_2O_3 in the PEG solution experimented in this study i.e. 5v/v% was also prepared in a similar fashion. The preparation of the γ-Fe_2O_3 has been described elsewhere [**19**]. Finally, in order to prepare the PEG, γ-Fe_2O_3 & UHT mixture, 50% of the previously prepared PEG and γ-Fe_2O_3 solution is added to 50% of UHT resin.

Fabrication of PEG & UHT and PEG, γ-Fe_2O_3 & UHT 3D Specimen by DLP 3D Printer

Fig. 1 shows the schematic diagram of DLP 3D printing process. The PEG and UHT mixture and the PEG, γ-Fe_2O_3 and UHT mixture prepared previously will solidify when the UV light is projected onto the mixture. The specimen has been designed with a thickness of 3 mm and a length of 30 mm. Preliminary runs were performed to determine the suitable curing time for the mixture. After the printing process, the 3D structure developed undergo a post curing process in a UV light box so as to ensure that the it was fully solidified.

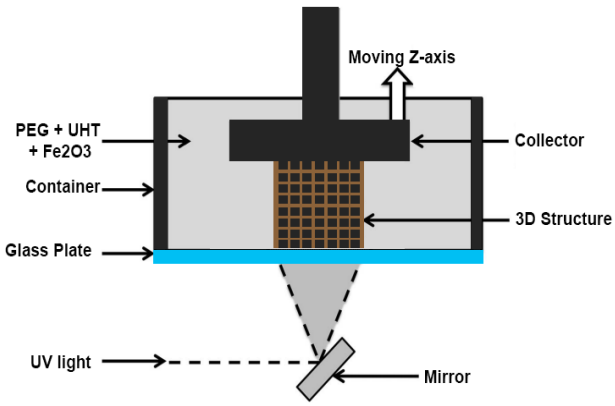

Fig. 1. A schematic diagram of DLP 3D printing process.

Mechanical property

The structure was printed with a dumb bell shape according to ISO37-2011 standard and the tensile strength test was performed using a Shimadzu Tensile Machine with a 10 mm/min crosshead speed at room temperature. At least five sample measurements were performed so as to ensure the reproducibility of the data. The result from the tensile test was used to compute the Young's Modulus using the following formula:

$$E \equiv \frac{\text{tensile stress}}{\text{tensile strain}} = \frac{\sigma}{\varepsilon} = \frac{F/A_0}{\Delta L/L_0} = \frac{FL_0}{A_0 \Delta L}$$

Degradation test

For the degradation test, the 3D structure was modelled to a cylindrical shape (Ø10mm and height 30mm) using Solidworks. The specimens printed were then weighed and immersed in a water bath at a temperature of 37^0C. The temperature must be exactly 37^0C which corresponds to the human body temperature. The specimens were observed until it dispersed. Prior to that, at different time intervals (after 1-day, 3-days and 7-days), a specimen were taken for evaluation. The degraded sample was then rinsed with distilled water and dried at room temperature before being weighed. The dried sample weight before and after degradation were recorded. The equation for weight loss is as follows:

$$Weight\ loss = \frac{weight\ before - weight\ after}{weight\ before} \times 100$$

MTT assay

MTT (3 - (4, 5 – dimethylthiazol – 2 – yl) – 2, 5 diphenyltetrazolium bromide) was used to assess cell viability. MTT assay involves metabolically active cells that react with tetrazolium salt in the MTT agent. Human skin fibroblast cells were used in order to produce formazon dye solution that can be absorbed at a wavelength of 490 nm [**20**]. All the experiments were performed in triplicate.

Results and discussion

Curing time

Before printing the 3D specimen, curing time of the mixture prepared was determined in order to ensure that the sample was perfectly cured. The curing time was determined using the DLP 3D printer. In this study, the curing time investigated was in the range of 20 to 80 seconds with increments of 10 seconds. After making optical observation and evaluation, 30 seconds of curing time was needed to cure the PEG with UHT mixture as it produces the most perfect and precise rectangular shape (**Fig. 2**). The same curing time was also applicable for the PEG, γ-Fe_2O_3 & UHT mixture.

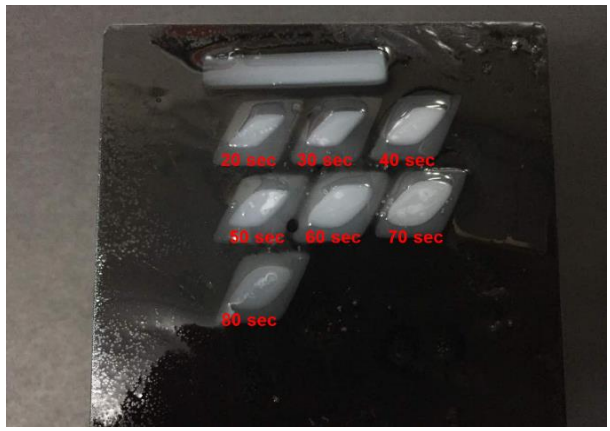

Fig. 2. Curing time evaluation.

Tensile test

In this study, the mechanical property investigated was in terms of the Young's Modulus of the structure which was 3D printed initially using PEG and UHT mixture and subsequently using the PEG, γ-Fe_2O_3 and UHT mixture. The initial experimental results help determine the PEG concentration which results in a high Young's Modulus value. The PEG concentration determined was used for the subsequent experiments involving varying quantities of γ-Fe_2O_3.

Table 2 and **Fig. 3** present numerically and graphically the Young's Modulus results for the structures developed. It can be seen clearly that as the concentration of PEG increases, the strength of the structure developed also increases. The average Young's Modulus value of pure UHT is 15.19 MPa. By adding 20wt/v% of PEG it increases to 19.09 MPa. The value of Young's Modulus increases to 22.88 MPa and to 26.97 MPa when the concentration of PEG increased from 30wt/v% to 40wt/v%. The higher concentration of polymer will increase the mechanical properties due to the entanglement between chain. So in order to rupture, more polymer bonds need to be broken and the polymer will absorb more energy before failing [**17**]. The concentration of PEG used in the subsequent section was 40wt/v% of PEG as it results in the highest Young's Modulus value. Maghemite nanoparticles were then added to the solution in stages starting with 1v/v% and then 5v/v%.

Table 2. Young's Modulus result of PEG + UHT.

Samples	UHT (MPa)	20wt/v % PEG + UHT (MPa)	30 wt/v % PEG + UHT (MPa)	40 wt/v % PEG + UHT (MPa)
1	15.32	18.84	22.97	27.18
2	14.96	19.33	22.73	26.97
3	15.21	19.26	22.86	26.78
4	15.29	19.03	22.9	27.07
5	15.17	18.97	22.96	26.86
AVG	**15.19**	**19.09**	**22.88**	**26.97**

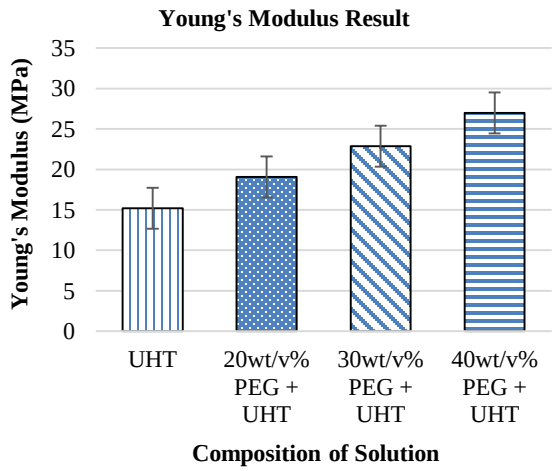

Fig. 3. Bar graph of Young's Modulus result of PEG + UHT.

From **Table 3** and **Fig. 4**, the addition of maghemite, γ-Fe_2O_3 nanoparticles increase the Young's Modulus of the structure developed. By adding 1v/v% of maghemite, the value of Young's Modulus increase from 26.97 MPa to 32.94 MPa and up to 34.89 MPa as the amount of γ-Fe_2O_3 nanoparticle increase to 5v/v%. This is due to the characteristic of maghemite which enhanced the mechanical properties of the polymer when added as a filler in the solution [**21**].

Table 3. Young's Modulus result of PEG + UHT + γ-Fe_2O_3.

Samples	40 wt/v % PEG + UHT + 0% Fe_2O_3 (MPa)	40 wt/v % PEG + UHT + 1% Fe_2O_3 (MPa)	40 wt/v % PEG + UHT + 5% Fe_2O_3 (MPa)
1	27.18	33.14	34.55
2	26.97	32.69	35.17
3	26.78	33.08	34.83
4	27.07	32.94	34.9
5	26.86	32.87	35.02
AVG	**26.97**	**32.94**	**34.89**

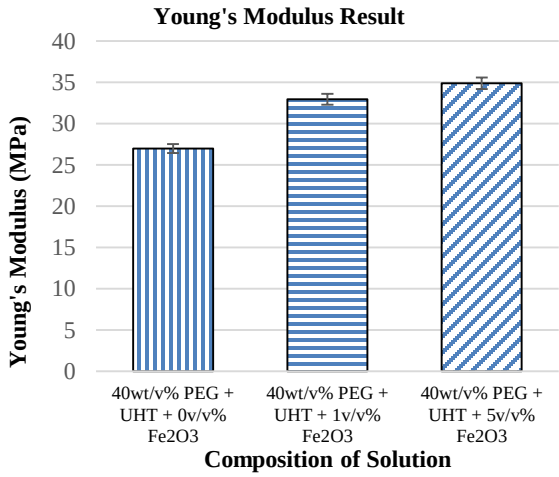

Fig. 4. Young's Modulus result of PEG + UHT + γ-Fe_2O_3.

Degradation test

The degradation of samples was carried out in ionize water. One sample, 40wt/v% concentration of PEG and another sample with the same concentration of PEG sample added with 1v/v% maghemite were placed in a 20ml of plastic container containing 5ml of ionize water. The samples were stored at room temperature for one month. At a week interval, the weight of the remaining sample was dried and weighed. Then, the samples were placed again in a new ionize water. **Fig. 5** shows the comparison of the two samples in a graph.

From the degradation profile, in the first three weeks the degradation rate is consistent then after 4 weeks, the degradation rate is increased. This is because, initial attack on the PEG and PEG/ γ-Fe_2O_3 chains was consistent with a random endocleavage and at the same time the occurrence of oxidative reactions of the tertiary carbon atoms of PEG and PEG/γ-Fe_2O_3 chain was established [**22, 23**]. When the polymer chain starts to break, the degradation rate increased. From the initial

data of degradation rate, it shows that combination of PEG/ γ-Fe_2O_3 have a good profile of degradation rate and seems to be compatible with the tissue engineering scaffold degradation rate profile [**24**].

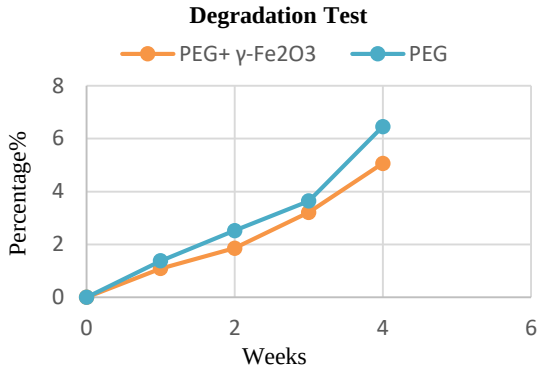

Fig. 5. Degradation test results.

MTT assay

Fig. 6 shows the results of relative cell viability towards different composition of structure. The proliferation rate of the cell on PEG / γ-Fe_2O_3 structure (96.38%) was higher than the PEG structure without γ-Fe_2O_3. The presence of magnetic nanoparticles develops a great number of tiny magnetic fields, which would subsequently express osteoinductive effect of static magnetic fields. It can be said that each magnetic nanoparticle acts as a single magnetic nanofield. Therefore, when it was integrated with the matrix, it created the micro-environment in the pores or on the surface of the blend which in turn produced the great number of tiny magnetic fields which promote the cell proliferation rate [**16**].

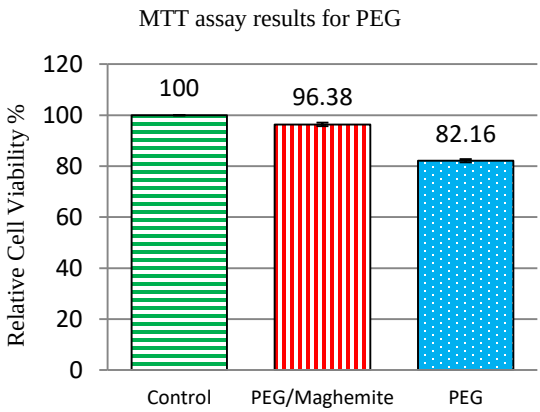

Fig. 6. MTT assay results.

Conclusion

PEG with concentration of 40% offered higher mechanical properties due to the higher crosslinked structure. The γ-Fe_2O_3 reinforcement has increased the mechanical properties and resulted in a slower degradation rate of the specimen, which is desirable. In this study, the mechanical properties of UHT can be increased by addition of PEG and γ-Fe_2O_3 from 15.19 MPa to 34.89 MPa. Moreover, the cell proliferation rate under MTT assay was greatly enhanced. The results revealed that the addition of PEG and γ-Fe_2O_3 enhanced the mechanical properties and biocompatibility properties of the structure developed. The successful use of PEG biopolymer, UHT resin and γ-Fe_2O_3 nanoparticles in producing strong and high proliferated specimen via DLP 3D printing provided evidence of their great potential for use in producing TE scaffolds for various biomedical industry applications.

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Author's contributions

Conceived the Plan: Prof Noordin & Dr Hasrul; Performed the experiments: Dr Hasrul, Dr Ehsan & Mr Aniq; Data analysis: Prof Noordin, Prof Ani & Dr Hasrul; Wrote the paper Prof Noordin, Prof Ani & Dr Hasrul. Authors have no competing financial interests.

Conflicts of interest

There are no conflicts of interest to declare.

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