EVALUATION OF PDMS-BASED UV-CROSSLINKED HYDROGELS PROPERTIES FOR TISSUE ENGINEERING APPLICATIONS

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EVALUATION OF PDMS-BASED UV-CROSSLINKED HYDROGELS PROPERTIES FOR TISSUE ENGINEERING APPLICATIONS

by

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LIST OF ABBREVIATIONS

μm	micrometer
^{1}H-NMR	Proton nuclear magnetic resonance
2-D	Two dimensional
3-D	Three dimensional
AFM	Atomic force microscopy
AMA	Allyl methacrylate
ASTM	American Society of Testing and Materials
CROP	Cationic ring opening polymerization
DMA	Dynamic mechanical Analysis
DMAP	2, 2-dimethyl-2-phenyl-acetophenone
DSC	Differential scanning Calorimetry
ECM	Extracellular matrix
EDX	Electron-dispersive X-ray spectroscopy
ESR	Equilibrium swelling ratio
FDA	Food and drug administration
FESEM	Field-emission scanning electron microscopy
FTIR	Fourier transform infrared
GPC	Gel permeation chromatography
H_20	Water
HA	Hydroxyapatite

IPN	Interpenetrating hydrogels networks
IR	Infrared
mg	milligram
min	minutes (time)
ml	mililiter
nm	nanometer
HDPE	High density polyethylene
MA	Methacrylate
NMR	Nuclear magnetic resonance
NVP	1-vinyl-2-pyrrodinone
PAA	Poly(acrylic acid)
PCL	Poly(ε- caprolactone)
PDMS	Polydimethylsiloxane
PDMS-MA	Polydimethylsiloxane dimethacrylate
PDMS-SiH	Polydimethylsiloxane dihydrido
PEG	Polyethylene glycol
PEGDA	Polyethylene glycol diacrylate
PEO	Poly(ethylene oxide)
PGA	Poly(glycolic) acid
PHEMA	Polyhydroxyethylmethacrylate
PLA	Poly(lactic) acid

PLGA	Poly(lactic-co-glycolic) acid
PNIPAM	Poly(N-isopropylacrylamide)
PS	Polystrene
PVA	Polyvinyl alcohol
SEM	Scanning electron microscopy
TCP	tri-calcium phosphate
TE	Tissue engineering
TPA	Texture profile analysis
UV	ultra-violet
UV-vis	UV-visible spectroscopy
XRF	X-ray fluorescence
β-TCP	β-tricalcium phosphate

LIST OF SYMBOLS

%	Percentage
M_d	Weight of dried samples
M_s	Weight of swollen samples
M_n	Number-average molecular weight
M_w	Weight-average molecular weight
T_c	Crystallization temperature
T_g	Glass transition temperature
T_m	Melting temperature
°C	Degree celcius
E	Molar extinction coefficient
E'	Storage (elastic) modulus
$E^{"}$	Loss modulus
ESR	Equilibrium swelling ratio
H_z	Hertz
N	Newton
PDI	Polydispersity index
R_z	Surface roughness
SCA	Static contact angle
wt.%	Weight percentage

PENGKAJIAN SIFAT-SIFAT HIDROGEL UV-SAMBUNG SILANG BERDASARKAN PDMS UNTUK APLIKASI KEJURUTERAAN TISU

ABSTRAK

Kajian ini menunjukkan penghasilan hidrogel berdasarkan PDMS yang mempunyai sifat-sifat yang boleh disesuaikan. Dua UV-sambung silang PDMS yang mempunyai berat molekul (M_n=1k & 6k g/mol) disintesis dahulu dan kemudian disambung-silang dengan PEGDA (M_n=0.7k g/mol) pada pelbagai nisbah berat (wt.%), dengan irgacure sebagai pemula UV. Bagi PDMS yang mempunyai M_n yang sederhana (6k), alil metakrilat (AMA) digunakan sebagai pengubah reaktif untuk meningkatkan keserasian dua polimer yang sangat tidak saling melaruti. Campuran polimer akan menjadi hidrogel selepas terdedah kepada penyinaran UV yang mempunyai rantau panjang gelombang 315-400 nm pada intensiti purata ~ 8-10 mW / cm^2 selama 30 minit. Sifat-sifat keserasian, termal, pembengkakkan, pembasahan, mekanikal, penjerapan protein dan sitotoksisiti hidrogel akan dinilaikan. Daripada kajian kalorimetri pengimbasan berbeza (DSC), walaupun dua T_g didapati bagi hidrogel yang dihasilkan dari PDMS (1k) yang mempunyai M_n rendah, hidrogel tersebut keserasian yang baik disebabkan oleh permukaan adalah homogen pada setiap PEG wt.% seperti yang ditunjukkan oleh keputusan daya atomic mikroskop (AFM). Hidrogel yang dihasilkan dari PDMS (6k) sangat tidak serasi terutamanya berlaku kepada 30 wt.% PEG dengan pemisahan fasa-makro berlaku. Permasalahan ini telah diselesaikan selepas AMA dimasukkan. Pemisahan fasa bagi hidrogel mempengaruhi sifat-sifat lain di mana permukaan hidrogel yang lebih hidrofobik telah menurunkan sifat pembengkakkan dan pembasahan disebabkan oleh jumlah domain PEG yang sedikit, lalu menjadikannya kurang hidrofilik, selepas kemasukan AMA. Penjerapan protein di permukaan hidrogel ini adalah lebih tinggi jika permukaannya dikuasai oleh permukaan PDMS, namun penjerapan masih rendah jika berbanding dengan PDMS murni. Kekakuan hidrogel bertepatan dengan rangkaian tisu lembut yang boleh diterima pada ~ 0.5-1 MPa, dan kekakuan hidrogel tersebut meningkat dengan peningkatan PEG wt.%, dan penurunan AMA wt.%. Digabungkan dengan

sitotoksisitas yang rendah, hidrogel yang dihasilkan berpotensi digunakan sebagai perancah dalam bidang kejuruteraan tisu.

EVALUATION OF PDMS-BASED UV-CROSSLINKED HYDROGELS PROPERTIES FOR TISSUE ENGINEERING APPLICATIONS

ABSTRACT

This work presents the fabrication of PDMS-based hydrogels with tunable properties via direct blending. Two UV-crosslinkable PDMS with different molecular weights (M_n=1k & 6k g/mol) were first synthesized and then UV-cured with PEGDA (M_n=0.7k g/mol) at various wt.% ratio, in the presence of Irgacure as photoinitiator. For the medium M_n PDMS (6k), allyl methacrylate (AMA) was used as reactive modifier to enhance compatibility of the two highly immiscible polymers. The liquid mixtures were converted into hydrogels after exposed to UV irradiation at a wavelength region of 315-400 nm at the average intensity of 10 mW/cm^2 for 30 minutes. Compatibility, thermal, swelling, wetting, mechanical, protein adsorption and cytotoxicity properties of these PDMS hydrogels were evaluated. From differential scanning calorimetry (DSC) study, although two T_g were observed in the hydrogels fabricated from the low M_n PDMS (1k), they were all compatible since the hydrogel surface was homogeneous at any PEG wt.% ratio, as supported by AFM result. The hydrogels fabricated from the PDMS (6k) were highly incompatible and this was especially the case for the 30 wt.% PEG with the occurrence of macrophase separation. This problem was solved with addition of AMA. The phase separation of these PDMS (6K) hydrogels affected other properties in which the more hydrophobic gel surface, after the addition of AMA, had lowered their swelling and wetting properties since there was a fewer amount of PEG domains to render the hydrophilic surface. Protein adsorption to these hydrogel was higher if the surface was dominated by the PDMS surfaces, yet the adsorption was still lower than the bare PDMS. Stiffness of the hydrogel was fall within an acceptable range of soft tissue at ~ 0.5-1 MPa, with the stiffness increased with the increased of PEG loading, and/or the decreased of AMA loading. Coupled with their non-cytotoxic property, the fabricated PDMS-based hydrogels could potentially be used as scaffolds for tissue engineering applications.

CHAPTER ONE

INTRODUCTION

1.1 Research background

Hydrogels are polymeric materials that have three-dimensional network structure with the ability of absorbing abundant water while maintain their integrity due to chemical and/or physical crosslinking (Xie et. al., 2017). Hydrogels have been widely investigated in the past few decades, due to their exceptional potential in wide range of applications, ranging from food industry (Xie et. al., 2017), agricultural (Vundavalli et. al., 2015), pharmaceutical (Peppas et. al., 1999) and tissue engineering (Munoz-Pinto et. al., 2012; Hou et. al, 2010). Among these applications, hydrogels for tissue engineering applications have become a major area of interest with several commercial products already developed, mostly in skin reconstruction (Chu et. al., 2002; Falanga & Sabolinski, 1999; Fitton et. al., 2001). Some unique properties that make hydrogels applicable in the field of tissue engineering include their excellent water-absorbing capabilities, a degree of softness that highly resemble to the natural tissues, biocompatibility and other attractive characteristic. During the last two decades, natural hydrogels were gradually replaced by synthetic hydrogels which has long service life, high capacity of water absorption, and high gel strength (Ahmed, 2015). In addition, synthetic hydrogels usually have well-defined structure that can be modified to yield tailorable functionality and degradability. Many synthetic hydrophilic polymers such as PEG, PVA, PAA, PNIPAAM and other synthetic polymers, well fits the definition of hydrogels. Among them, PEG which possess many unique properties likes hydrophilicity, flexibility, non-toxicity, non-immunogenicity and low non-specific proteins adsorption, has been widely employed as hydrogels in the field of tissue engineering (Varghese et. al., 2009).

Compared with the single-component hydrogels, researchers nowadays prefer to use multi-component hydrogels, since single-component hydrogels cannot fulfil all the criteria required for an ideal scaffold, such as they are mechanically fragile and non-degradable.

Micro- or nano-composite hydrogels, copolymeric hydrogels, multipolymer interpenetrating polymeric hydrogels (IPN), semi-IPN hydrogels and polymer blends are some examples of promising multi-component hydrogels that are widely investigated due to their sustainability in the field of tissue engineering. In these hydrogels, new properties that are different from the intrinsic properties of the original materials can be easily endowed by combining two or more components together. For instance, organic-inorganic PEG/PDMS hydrogels has been fabricated by Hou et. al. (2010) and they found that these hydrogel scaffolds demonstrated the ability to guide mesenchymal stem cells (MSCs) towards osteogenic differentiation with increased levels of PDMS microparticles. Besides, a nanocomposite made up of PEG and clay has been developed by Varghese et. al. (2009) and they elucidated that the overall mechanical properties of PEG hydrogels were improved by adding up to 10 wt.% of clay. It is worth to mention that one of the similarity between both studies is that the hydrogels were prepared by photocrosslinking technique in their respective projects. As compared to thermal or redox initiated crosslink mechanisms, photo-induced free radical hydrogen crosslinking produces less heat while allowing for improved spatial and temporal control (Hou et. al, 2010), due to short-term UV-exposure, i.e. within a minutes. Hence, photopolymerization is generally considered as a safe method to encapsulate cells. Another advantage of in situ polymerization is that specific shapes can be tailored made to fit exactly the tissue defects need to be repaired.

UV-curable PDMS-based hydrogels is another class of hydrogels, which are widely used as contact lenses (Lin et. al., 2014). Compared to the hydrogels which made up of only hydrophilic chains, these hydrogels is mainly consisted of hydrophobic PDMS. They also possess the unique properties of PDMS, such as biocompatible, high gas permeability, low T_g and unique viscoelastic properties when lightly crosslinked. PDMS have been used in various biomedical applications, such as transdermal (Mikolaszek et. al., 2016), antifouling coating (Gu et. al., 2016), ultrafiltration, drug delivery system (Racles, 2013) and tissue engineering applications (Munoz-Pinto et. al., 2012; Sung et. al., 1999; Pedraza et. al., 2013;

Si et. al., 2016). Although the potential uses of PDMS as scaffolds have been widely studied, their hydrophobicity always hinders their applicability in biomedical applications, due to non-specific protein adsorption following implantation (Wong & Ho, 2009; Zhang & Chiao, 2015). This phenomenon is highly unfavourable since protein adsorption to the hydrophobic surface is often irreversible and proteins will denature once they absorb to the surface. Surface modification of PDMS is a facile method to endow the surface hydrophilic, but it is often involved a complex process which consumes time and the uses of solvents which is toxic. Compared to surface modification, blending of PDMS with PEG is a simple and time saving method to impart hydrophilicity not only in the surface, but also within the bulk. Regard to this, this project focuses on the fabrication of two-component hydrogels that are comprised of two different acrylate-functionalized polymers, which is PDMSMA as the major phase and PEGDA as the minor phase, by means of photocrosslinking reaction. By the end, it should be able to translate the PDMS-PEG products into hydrogels by varying the number-average molecular weight (M_n) of PDMS and the composition of PDMS and PEG.

1.2 Problem statement

Controlled synthesis of PDMS with a well defined M_n is a prerequisite for the success of this project since physical and chemical properties of a crosslinked polymer is mainly governed by the M_n of matrix. Cationic ring opening polymerization (CROP) is a facile method to obtain the predesigned molecular weight and the molecular architecture, as described elsewhere (Toskas et. al., 2006). D_4 monomers is widely used to afford the linear homopolymer PDMS chains and the SiH containing endcappers is used to terminate the growing chains at the end of reaction (Bi et. al., 2007). Many groups have used this chemical scheme to synthesis a myriad of functionalized PDMS. For example, Hou et. al. (2010) had further functionalized the SiH-terminated PDMS chains with allyl methacrylate (AMA) via hydrosilylation reaction to endow them the photocrosslinking moieties.

The fabrication of PDMS-based hydrogels via solution polymerization technique is highly unfavourable since aqueous solvent is not miscible with PDMS (Lee et. al., 2003). On

the other hand, organic solvent is not often used due to its cytotoxicity. Therefore, the crosslinking reaction of PDMS is usually done in bulk polymerization technique. Conversely, one issue dealing with the bulk technique is the effects of molecular weight and/or viscosity on the curing efficiency of a polymer, particularly referred to the high M_n homologs. Regard to this matter, reactive diluents has been widely used to induce dilution effect during the crosslinking reaction of a polymer, mostly in epoxy coating (Li et. al., 2014). However, only few research papers have been reported on the use of reactive diluents in PDMS, due to the fact that PDMS has limited miscibility with most reactive diluents, such as acrylate- and methacrylate based reactive diluents. Therefore, microphase separation was identified by the observation of two T_g due to the separated reactive diluent phases (Yu et. al., 1985; Pouget et. al., 2009). In this project, AMA is proposed to be incorporated into the PDMS curing formulation to facilitate the crosslinking reaction since it fulfils the basic definition of reactive diluent (Ash, 2007). AMA should be incorporated in the right amount to avoid the phase separation problem, even though the functionalized methacrylate (MA) moieties in the terminal ends of PDMS chains made it more miscible with the reactive diluent.

PDMS is inherently hydrophobic, making it difficult for water to penetrate into its crosslinked structure. Therefore, PDMS should be blended with PEG in order to transform them into hydrogels. One of the issues dealing with this technique is the degree of miscibility or phase separation when blending the two polymers together. Macro-phase separation will occur if the PDMS-PEG blends suffer a change of composition, which force them into non-stable region. To avoid the phase separation problem, the M_n of PDMS and the amount of PEG loading should be carefully designed to get a desirable compromise between swelling properties and material's compatibility. Besides, PEG was used to switch the PDMS surface from hydrophobic to hydrophilic one. Nevertheless, micro-phase segregation of PEG to the surface remains a challenge since PEG tends to buried within the PDMS matrix for their low surface energy (Gu et. al., 2016; Wang et. al., 2011). Therefore, surface properties of these

hydrogels were carefully controlled by varying the PDMS-PEG composition, until PEG is micro-phase segregated to render the surface hydrophilic.

1.3 Objectives of the study

The objectives of this study are simplified as follows.

1. To synthesize PDMSMA precursors with a well-defined molecular weight (M_n)
2. To study the effects of different M_n of PDMS and different reactive diluent loading on the curing characteristics of the PDMSMA precursors
3. To investigate the effects of different ratio of PDMS to PEG on the compatibility, swelling, surface, mechanical, protein adsorption and toxicity properties of the PDMS-PEG hydrogels

1.4 Research scope

The acid-catalyzed CROP process was used to synthesize PDMS precursors with low PDI value. The equilibrium chain redistribution during the reaction was likely to impart variations on the PDI value of the PDMS precursors, especially for the high M_n homologs (12000 g/mol). Therefore, the PDI value of PDMS precursor was first determined by GPC and ^{1}H-NMR end group analysis, respectively. UV-crosslinking of the PDMS precursor was only further proceeded if the PDI value obtained was less than or equal to 1.5.

Stickiness of the pristine PDMS gel can cause handling problem during sample characterization, especially referred to the PDMS with M_n of 6000 g/mol and 12000 g/mol. Besides, this issue might cause cytotoxicity in *in-vitro* testing as the stickiness of the gels also reflected to a considerable amount of leftover unreacted oligomer. The issue should be first addressed before it is further blended with PEG. Therefore, curing profile was first developed to determine the optimized curing time (t_c) as the function of different M_n of PDMS and different reactive diluent loading, in order to fabricate the PDMS gels with the least unreacted oligomers in the shortest possible time. However, PDMS precursors with M_n