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**Department of Mechanical Engineering and Materials Science and Engineering**

**MASTER THESIS**

**Drug release kinetics of poly(glycerol sebacate urethane) scaffolds  
for soft tissue engineering**

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**Limassol, May 2022**

CYPRUS UNIVERSITY OF TECHNOLOGY

**Department of Mechanical Engineering and Materials Science and Engineering**

Master's Thesis

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Irene Louca

Limassol, May 2022

# Approval Form

Master's Thesis

## Drug release kinetics of poly(glycerol sebacate urethane) scaffolds for soft tissue engineering

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## ACRONYM LIST

ABBREVIATION	EXPLANATION
BCA	Bicinchoninic acid
bFGF	Basic fibroblast growth factor
BSA	Bovine serum albumin
BTE	Bone tissue engineering
DL	Drug loading
DMC	Dimethyl carbonate
DMF	Dimethylformamide
DRK	Drug release kinetics
ECM	Extra cellular matrix
Hap	Hydroxyapatite
HDI	Hexamethylene diisocyanate
HFF	Human foreskin fibroblast
LDI	Lysine diisocyanate (2,6-diisocyanatohexanoate)
MDI	4,4-methylenediphenyl diisocyanate
PBA	Poly(butylene succinate)
PBS	Phosphate buffer saline
PCL	Poly(caprolactone)
PDS	Poly(p-dioxanone)
PEA	Poly(ethylene adipate)
PEG	Poly(ethylene glycol)
PGA	Poly(glycolic acid)
PGS	Poly(glycerol sebacate)
PGSU	Poly(glycerol sebacate urethane)
PLA	Poly(lactic acid)
PLGA	Poly(lactic-glycolic acid)
PU	Poly(urethanes)
ROP	Ring opening polymerization
SEM	Scanning electron microscopy
TDI	Toluene diisocyanate
TE	Tissue engineering
ATE	Adipose Tissue engineering
BAT	Brown adipose tissue
WAT	White adipose tissue
CNS	Central nervous system
FDA	Food and Drug Administration
PNS	Peripheral nervous system

## Abstract

In this study, three different poly(glycerol sebacate urethane), (PGSU) scaffolds were fabricated with an anisotropic microstructure and characterized their drug release kinetics (DRK). PGSU scaffolds with different ratios of hexamethyl diisocyanate (HDI) and polymer poly(glycerol sebacate urethane), pre-(PGS) concentration, were fabricated and investigated for their aforementioned physical properties. PGSU was synthesized with pre-PGS at concentrations 10% and 15% w/v% and HDI was added at molar concentrations (glycerol:HDI) 1:0.8 and 1:1.0. Freeze-drying with custom made moulds and ice templating, were used to form an anisotropic PGSU scaffold. The nomenclature of the samples is PGSU X-Y% where X refers to HDI ratio (0.8 or 1.0) and Y to polymer concentration (w/v%) (10% or 15%). The aim of this study was to study the DRK therefore the PGSU scaffolds were characterized for their microstructure, hydrophilicity (water contact angle and swelling rate), their drug loading efficiency, degradation rate and finally the DRK. PGSU scaffolds were characterized for their microstructure SEM and were found to exhibit an anisotropic open pore microstructure. The hydrophilicity was tested using contact angle and swelling ratio and were found to exhibit a fairly hydrophilic surface with a linear swelling rate. DRK were studied by loading BSA in scaffolds, using an in-house derived dynamic loading method which used vacuum/ventilation cycles. It resulted to a 75% drug loading efficiency, which is considered very good especially for non-hydrophilic materials. Then bovine serum albumin (BSA) was released by soaking the scaffold in phosphate buffer solution (PBS) solution on a rocker at 100 rpm for 28 days. The samples collected at different time points were analyzed using bicinchoninic acid (BCA), to determine the amount of protein released over time. A linear release rate was found and almost all samples withheld and released the protein over a period of at least 19 days. Finally, the degradation rate was studied by soaking the PGSU scaffolds in lipase enzyme and enzyme free PBS solution for 42 days in a shaker incubator, at 37°C and 100 rpm. The mass of the scaffolds was taken at multiple time points and compared with the initial mass to derive the degradation rate, the degradation rate was considered to be too slow. These results demonstrate that the glycerol:HDI molar ratio and polymer

concentration affect the properties of the scaffold, the DRK technique developed by our group was successful and the fabricated PGSU scaffolds can be used in soft TE.

## Περίληψη

Σ' αυτή τη μελέτη έγινε σύνθεση τριών ικριωμάτων poly(glycerol sebacate urethane) PGSU με ανισοτροπική πορώδη μικροδομή και χαρακτηρίστηκαν για τον ρυθμό της απορρόφησης της πρωτεΐνης, της κινητικής απελευθέρωσης της πρωτεΐνης, για την υδροφιλία τους και τον ρυθμό βιοδιάσπασης του ικριώματος. Τα ικριώματα PGSU που παρασκευάστηκαν είχαν διαφορετικές αναλογίες της συγκεντρώσεως του γλυκερόλη:HDI με pre-PGS. Το pre-PGS είχε χρησιμοποιηθεί σε συγκεντρώσεις 10%w/v, 15%w/v και η γλυκερόλη:HDI σε αναλογία 1:0.8 και 1:1.0. Η μέθοδος σύνθεσης ήταν η λυοφιλίωση, σε ειδικά δοχεία σε συνδυασμό με τη μέθοδο παγώματος, δημιουργώντας πάγωμα του ικριώματος από κάτω προς τα πάνω, δημιουργώντας την ανισοτροπική δομή του ικριώματος. Τα ικριώματα χαρακτηρίστηκαν για την μικροδομή τους με το ηλεκτρονικό μικροσκόπιο σάρωσης, το οποίο έδειξε να έχουν πορώδη ανισοτροπική δομή. Η υδροφιλία τους χαρακτηρίστηκε με μέτρηση της γωνίας επαφής και του λόγου διόγκωσης, τα αποτελέσματα έδειξαν ότι τα ικριώματα έχουν σχετικά υδρόφιλη επιφάνεια, με ευθύγραμμη αύξηση του ρυθμού διόγκωσης ως προς το χρόνο. Ο ρυθμός κινητικής απελευθέρωσης (DRK) της πρωτεΐνης αλβουμίνης (BSA), μελετήθηκε με μία δυναμική μέθοδο η οποία σχεδιάστηκε από την ομάδα μας, στην οποία χρησιμοποιήσαμε επαναλαμβανόμενους κύκλους, αφαιρώντας αέρα και προσθέτοντας αέρα (vacuum/ventilation cycles) στο ικριώμα με σκοπό να απορροφηθεί η πρωτεΐνη. Η μέθοδος αυτή είχε 75% απορρόφηση πρωτεΐνης, το αποτέλεσμα θεωρήθηκε ικανοποιητικό ειδικά για υλικά που έχουν πιο υδρόφοβες επιφάνειες. Στη συνέχεια η πρωτεΐνη αλβουμίνη (BSA), απελευθερώθηκε βυθίζοντας τα ικριώματα σε ρυθμιστικό διάλυμα φωσφορικών (PBS) για 28 μέρες. Η πρωτεΐνη που απελευθερώθηκε μελετήθηκε με τη μέθοδο του δικινχονικού οξέος (BCA). Ο ρυθμός απελευθέρωσης της πρωτεΐνης ήταν ευθύγραμμος και όλα τα δείγματα απελευθέρωσαν πρωτεΐνη σε διάστημα 19 μερών. Στο τέλος μελετήθηκε ο ρυθμός βιοδιάσπασης του υλικού, βυθίζοντας τα ικριώματα σε διάλυμα ενζύμου λιπάσης και σε ρυθμιστικό διάλυμα φωσφορικών για 42 μέρες στους

37°C και με συνεχή ανάδευση στις 100 rpm. Μετρήθηκε η μάζα των ικριωμάτων σε διαφορετικές χρονικές στιγμές και συγκρίνοντας την με την αρχική μάζα του ικριώματος υπολογίστηκε ο ρυθμός βιοδιάσπασης. Τα αποτελέσματα έδειξαν ότι ο ρυθμός βιοδιάσπασης του υλικού είναι πολύ αργός. Από τα αποτελέσματα συμπεραίνουμε ότι η αναλογία της γλυκερόλη:HDI με την συγκέντρωση του pre-PGS έχει πολύ σημαντικό ρόλο στις ιδιότητες του ικριώματος, στη μικροδομή, υδροφιλία, στον ρυθμό απορρόφησης και απελευθέρωσης της πρωτεΐνης και στο ρυθμό βιοδιάσπασης του υλικού. Επίσης η τεχνική για σύνθεση των ικριωμάτων PGSU και η τεχνική για απορρόφηση και απελευθέρωση της πρωτεΐνης η οποία σχεδιάστηκε από την ομάδα μας είχαν ικανοποιητικά αποτελέσματα, αποδεικνύοντας ότι το ικρίωμα PGSU είναι κατάλληλο για επούλωση μαλακών ιστών.