Development of novel hydrogel based composites for bone tissue engineering applications

A thesis submitted for the degree of

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by

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Based on research carried out under the supervision of

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Declaration

I hereby declare that this thesis submitted to the Athlone Institute of Technology for the degree of Doctor of Philosophy, is a result of my own work and has not in the same or altered form, been presented to this institute or any other institute in support for any degree other than for which I am now a candidate.

John Killion (Date)

Dedication

I dedicate this work to my parents, Tom and Mary Killion. Their inspiration and support have made this accomplishment possible.

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Abstract

The process of tissue engineering involves replacing and assisting in the healing of damaged tissues. Specifically for bone tissue repair, a clinical demand has developed for alternative materials to replace the existing bone grafting treatments. To date, various materials have been proposed, synthesised and fabricated as potential replacements, but none have been successful. Due to the continued deficiencies of current commercially available biological bone grafts, the search for alternative substitutes has recently come to the forefront of tissue engineering.

The primary objective of this thesis involved the synthesis, photopolymerisation and characterisation of novel hydrogels and hydrogel based composite scaffolds for bone regeneration. Poly(ethylene) glycol dimethacrylate (PEGDMA) was chosen as the main macromolecular monomer for the work described herein. The first stage of the work consisted of investigating the effect of varying the concentration and molecular weight of the macromolecular monomer PEGDMA on the properties of the resultant hydrogels. Results showed the mechanical properties were tunable and predictably altered by varying the pore size and crosslink density of the hydrogel. Additionally, biocompatibility studies on selected hydrogels revealed that cell viability was greater than 86% for all extraction concentrations. Further characterisation was carried out on polymer blends of PEGDMA and polypropylene glycol dimethacrylate (PPGDMA), since homopolymers are often insufficient in terms of mechanical strength. Following these studies, there was an attempt to develop hydrogels that mimic bone in terms of water content. This resulted in the use of a hydrophobic material, i.e. polypropylene glycol. Results revealed that the incorporation of PPG into the system decreases the mechanical strength of the hydrogels, which was observed for both the compression and rheological studies. The toxicological results showed that the aforementioned set of hydrogels was not suitable for implantation unless numerous time-consuming washing steps were performed.

Following from this, the next stage of the research, synthesis of photopolymerisable maleic polyvinyl alcohol was conducted through a one step reaction between maleic anhydride and polyvinyl alcohol (PVA) in toluene sulfonic acid/formamide mixed solvent. Synthesis was confirmed by nuclear magnetic resonance (NMR) and Fourier transform infrared spectroscopy (FTIR). NMR results showed the hydroxyl groups of PVA were acylated by maleic anhydride. Subsequent photopolymerisation of the maleic PVA hydrogels resulted in a weak material that dissolved easily. As a result, PEGDMA was incorporated into the system to improve the material's strength.

In the final body of work, mechanical and bioactive properties for novel hydrogel based composites were investigated. Bioactive glass, β-tricalcium phosphate and hydroxyapatite were incorporated at varying ratios. Compression tests and rheological studies revealed that each individual bioceramic improved the compressive strength for each of the hydrogel based composites compared to the control hydrogel. The increase in compressive strength was subject to the

concentration of bioceramic and the crosslinking between individual bioceramics and PEGDMA. Biomineralisation studies revealed that the control hydrogels did not exhibit bioactive properties, as shown by the absence of an apatite layer after being submerged in simulated body fluid. An apatite layer was formed on all hydrogel based composites where a bioceramic was incorporated. Drug release studies showed that the release of the drug varied depending on the concentration of the bioceramic as well as the molecular weight of the polymer and the drug. Antibacterial studies demonstrated the ability of the hydrogel based composites to control the release of incorporated antibiotics, which could potentially reduce the risk of osteomyelitis by enabling bacterial inhibition.

Abbreviations

ACP	Amorphous calcium phosphate
ASTM	American Society for Testing and Materials
ATR-FTIR	Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy
BG	Bioactive glass
CAD	Computer-aided design
CSD	Critical size defect
CT	Computed tomography
DA	Diacrylate
DI	Distilled water
DMA	Dimethacrylate
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DMTA	Dynamic mechanical thermal analysis
DSC	Differential scanning calorimetry
ECM	Extracellular matrix
EDTA	Trypsin-ethylenediamine tetra-acetic acid
EDX	Energy-dispersive X-ray
FCS	Foetal calf serum
FDA	Food and drug administration
FDM	Fused deposition modeling
FTIR	Fourier transform infrared spectroscopy
G'	Storage modulus
G''	Loss modulus
HAP	Hydroxyapatite
HCA	Hydroxycarbonate apatite layer
HEMA	Hydroxyethylmethacrylate
IPN	Interpenetrating polymer network

Irgacure® 184	1-hydroxycyclohexylphenylketone
Irgacure 2959	4-(2-hydroxyethoxy)phenyl-(2-hydroxy-2-propyl)ketone
ISO	International standard organisation
KCl	Potassium chloride
l	Average value of the bond length between C-C and C-O bonds
LVE	Linear viscoelasticity
M_c	Molecular weight between crosslinks
M_n	Number average molecular weight
MIC	Minimum inhibitory concentration
MRI	Magnetic resonance imaging
MRSA	Methicillin-resistant Staphylococcus aureus
MTT	(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
M_W	Molecular weight
NIH 3T3	Mouse embryonic fibroblast cell line
NCCLA	National committee for clinical laboratory standards
NMR	Nuclear magnetic resonance
PBS	Phosphate buffered saline
PCL	Polycaprolactone
PDLLA	Poly-DL-lactide
PEG	Polyethylene glycol
PEGDA	Polyethylene glycol diacrylate
PEGDMA	Polyethylene glycol dimethacrylate
PLGA	Poly(lactic-co-glycolic) acid
PLLA	Polylactic acid
PMAA	Poly(methacrylic acid)
PPG	Polypropylene glycol
PPGA	Polypropylene glycol acrylate

PPGDA	Polypropylene glycol diacrylate
PPGDMA	Polypropylene glycol dimethacrylate
PVA	Polyvinyl alcohol
PVP	Polyvinylpyrrolidone
RP	Rapid prototyping
SBF	Simulated body fluid
SD	Standard deviation
Sem	Scanning electron microscope
SEM	Standard error of mean
SFF	Solid free form
SLS	Selective laser sintering
TCP	Tricalcium phosphate
TE	Tissue engineering
TGA	Thermogravimetric analysis
UV	Ultra violet
W_d	Weight of the hydrogel in the swelling state
W_s	Weight of the hydrogel in the dried state
XRD	X-ray diffraction

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