

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Lactosylated Polymers for Tissue Engineering

A thesis presented in partial fulfillment of the requirements for the degree of

Master of Science in Chemistry

At Massey University

Shi Zheng

February 2006

Abstract

This thesis describes the synthesis of new polymers suitable for the support of in vitro hepatocyte growth. The key design feature of the monomers investigated was the incorporation of a galactose residue, expected to promote the adhesion and proliferation of hepatocytes in vitro, through the regioselective derivation of lactose.

A graft polymerisation strategy was investigated as an alternative to copolymerization, since it proved difficult to isolate the carbohydrate-derived monomers in sufficient purity. This led to the formation of a glutaraldehyde-crosslinked lactosylated polyamine hydrogel, which was found to perform well in initial cell trials.

Acknowledgements

Firstly, I would like to thank my supervisors, Roger Lins and David Harding, for the help and guidance I have received over the last two years. I can honestly say that this thesis would not have been completed with the help and support of Roger and David.

I also wish to extend my thanks to others in the group Darren Englebretsen, Veronika Kristova and Libei Guo Bateman. They were wonderful people to work with.

Ms L Green and Associate Professor K Pedley, Institute of Food Nutrition and Human Health (see Appendix: p. 77-81) are thanked for their efforts on the cell studies associated with my research.

Thanks to Pat Edwards and David Lun, for helping me with NMR and MS.

Last but definitely not least, I would like to thank my parents for everything they have done for me and for making me what I am today.

Table of Contents

Abstract.....	ii
Acknowledgements.....	iii
Table of Contents	iv
List of Figures.....	vi
List of Schemes.....	vii
List of Tables.....	viii
List of Abbreviations.....	ix
Chapter 1: Introduction.....	1
1.1 Tissue Engineering.....	1
1.2 Hydrogels and Hydrogels in Tissue Engineering.....	3
1.3 Design Parameters for Hydrogels in Tissue Engineering.....	6
1.4 Hydrogels for Cell Immobilization	7
1.5 Problems of Hepatocytes Outside the Organ and Specific Requirements for Hydrogels.....	10
1.6 Choice of Lactose to Build a Sugar-based Polymer Scaffold.....	12
1.7 Hydrogels from Synthetic Polymers – Copolymer of Lactose and N-isopropylacrylamide.....	13
1.8 Objectives for this Study	14
Chapter 2: Preparation of monomers and synthetic polymers	16
2.1 Oxidation of lactose.....	16
2.2 Preparation of Monomer one (51).....	27
2.3 Preparation of Monomer two (57).....	31
2.4 Preparation of Monomer three (58).....	37
2.5 Preparation of Synthetic Polymers.....	41
Chapter 3: Conclusion and Future studies	46
3.1 Conclusion.....	46
3.2 Future studies.....	48
Chapter 4: Materials, Methods and Experimental.....	49

4.1 Reagents.....	49
4.2 Methods.....	49
4.3 Experimental.....	52
4.3.1 Synthesis of lactone (46).....	52
4.3.2 Synthesis of Monomer one (51).....	54
4.3.3 Synthesis of Monomer two (57).....	57
4.3.4 Synthesis of Monomer three (58).....	62
4.3.5 Synthetic Polymer.....	63
4.3.6 Preparation of Films and Sponges.....	67
References:.....	69
Appendix: Initial Cell Trial Results.....	77
A.1 HeLa cell trials (bright field).....	77
6.2 HeLa cell trials (fluorescence).....	79
6.3 HepG2 cells trials.....	80

List of Figures

Figure 1.1.	General strategy for tissue engineering	2
Figure 1.2.	Kinetics and sugar specificity of rat hepatocyte binding to polyacrylamide gels.....	11
Figure 1.3.	The structure of lactose.....	13
Figure 1.4.	Ideal scaffold for binding hepatocytes.....	14
Figure 1.5.	Objective hydrogel for ECM.....	15
Figure 2.1.	Starting point - fresh lactobionic acid (45) in D ₂ O.....	22
Figure 2.2.	Different percentage of lactone (46) in different solvents.....	23
Figure 2.3.	Starting point - fresh lactone (46) in MeOH in D ₂ O.....	24
Figure 2.4.	Starting point - fresh lactone (46) in <i>t</i> -butanol in D ₂ O.....	24
Figure 3.1.	Monomer one (51).....	46
Figure 3.2.	Monomer two (57).....	47
Figure 3.3.	Monomer three (58).....	47
Figure A.1.	HeLa cell trial on polyamine film.....	77
Figure A.2.	HeLa cell trial on lactosylated polymer film.....	78
Figure A.3.	HeLa cell BCECF trial on polyamine film.....	78
Figure A.4.	HeLa cell BCECF trial on lactosylated polymer film.....	79
Figure A.5.	HepG2 cell trial on lactosylated polymer film 1.....	80
Figure A.6.	HepG2 cell trial on lactosylated polymer film 2	81

List of Schemes

Scheme 2.1.	General plan for making lactosylated polymer.....	16
Scheme 2.2.	Procedure for amide bond formation.....	17
Scheme 2.3.	Oxidation equations of D-galactose and D-ribose.....	18
Scheme 2.4.	Oxidation equations of D-mannose and maltose.....	18
Scheme 2.5.	Oxidation equation of potassium hypoiodite-methanol procedure.....	18
Scheme 2.6.	EDC coupling reaction.....	20
Scheme 2.7.	Coupling reaction from potassium lactobionate to lactone.....	21
Scheme 2.8.	Reaction of allylamine with lactone (46).....	25
Scheme 2.9.	Polymerization of allyl double bond.....	25
Scheme 2.10.	Behavior of ethylene diamine at different pH conditions.....	26
Scheme 2.11.	Mechanism for the elimination reaction in acetylation of lactobionic acid.....	38
Scheme 2.12.	ABX coupling system.....	39

List of Tables

Table 1.1.	Some examples of polymers that are used in the preparation of hydrogels.....	5
Table 1.2.	Hydrogels that have been used for cell immobilization.....	9

List of Abbreviations

ALG	Ca-alginate gels
Azo88	1,1'-azobis (cyclohexanecarbonitrile)
Boc	<i>tert</i> -butoxycarbonyl
CD	cyclodextrin
¹³ C DEPT135	carbon distortionless enhancement by polarization transfer
¹³ C NMR	carbon nuclear magnetic resonance
2D COSY	two-dimensional correlation spectroscopy
DCM	dichloromethane
DIEA	<i>N,N</i> -di-isopropylethylamine
DMF	<i>N,N</i> -dimethylformamide
ECMs	extracellular matrices
EDC	1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide
EG	ethylene glycol
ES MS	electron spray mass spectroscopy
FPLC	fast protein liquid chromatography used here for gel filtration chromatography
GC	galactosylated chitosan
HA	hyaluronic acid
HMQC	heteronuclear multiple quantum coherence
¹ H NMR	proton nuclear magnetic resonance
HPLC	high performance liquid chromatography
IR	infra-red
LCST	lower critical solution temperature
MeOH	methanol
Mw	molecular weight
NVP	<i>N</i> -vinyl-2-pyrrolidone
P (.....)	poly (.....)
PAAc	poly (acrylic acid)
PAAm	poly (acrylamide)

PAN	poly (acrylonitrile)
PBO	poly (butylene oxide)
PCL	poly (caprolactone)
PE	propylene fumarate
PEG	poly (ethylene glycol)
PEMA	poly (ethyl methacrylate)
PEO	poly (ethylene oxide)
PGEMA	poly (glucosylethyl methacrylate)
PHB	poly (hydroxy butyrate)
PHEMA	poly (hydroxyethyl methacrylate)
PHPMA	poly (hydroxypropyl methacrylamide)
PLA	poly (lactic acid)
PLGA	poly (lactic-co-glycolic acid)
PMMA	poly (methyl methacrylate)
PNIPAAm	poly (<i>N</i> -isopropyl acrylamide)
PNVP	poly (<i>N</i> -vinyl pyrrolidone)
PPO	poly (propyleneoxide)
PS	poly (styrene)
PVA	poly (vinyl alcohol)
PVAc	poly (vinyl acetate)
PVLA	poly (<i>N-p</i> -vinylbenzyl-4- <i>o</i> - β -D-galactopyranosyl-D-gluconamide)
RGD	arginine-glycine-aspartic acid sequence
RT	room temperature
Sty	styrene
TE	tissue engineering
TEMED	<i>N,N,N',N'</i> -tetramethyl-ethylenediamine
TFA	trifluoroacetic acid
UPD	under potential deposition
UV-Vis	ultraviolet-visible
VAc	vinyl acetate
Mc	Molecular wight of the crosslinked gel

TBTU

O-(Benzotriazol-1-yl)-*N,N,N',N'*-
tetramethyluronium tetrafluoroborate