DEVELOPMENT OF BIOCOMPOSITE SCAFFOLDS AND INJECTABLE BIOCEMENT FOR BONE REGENERATION

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Abstract

To repair massive bone defects caused by disease and trauma, a bone grafting procedure is required. The limitations associated with the use of autografts (tissue grafts from one point to another of the same individual's body) and allografts (tissue grafts between genetically nonidentical individuals) have boosted the research and development of bone graft substitutes.

Calcium phosphate cement (CPC) is a promising bone graft substitute because of its bioactivity, osteoconductivity and bone replacement capability. However, difficulties with injectability and slow resorption rate have limited the wider applications of CPC. To overcome these limitations, premixed and injectable calcium deficient apatite biocement (cd-AB) were prepared in the initial phase of this study. Using a non-aqueous solution as the liquid phase, the resulting premixed cd-AB had the advantage of remaining stable in the syringe and only hardened following delivery to the defect site. As well, when injected into an aqueous environment, this premixed cd-AB exhibited improved washout resistance when compared to the conventional cd-AB using water as the liquid phase. However, the premixed cd-AB required a longer setting time and developed a reduced compressive strength compared to the conventional cd-AB. The hydration products of premixed cd-AB were a mixture of calcium deficient hydroxyapatite (cd-HA) and PLA. *In vitro* Tris-HCl immersion tests demonstrated that the premixed cd-AB was degradable. The results revealed

that the premixed cd-AB was cytocompatible and no adverse effects were observed after attachment and proliferation of MG-63 osteoblast-like cells *in vitro*. The most distinct advantages of premixed and injectable PLA-modified cd-AB were its excellent washout resistance and *in vitro* degradability, suggesting that it may be a promising candidate for future bone reconstruction.

In recent years, bone tissue engineering has emerged as a promising approach for the repair of bone damage and defects. In this approach, a scaffold is normally used alone or in combination with growth factors and/or cells to guide bone regeneration. Among the synthetic polymers used as scaffold materials, poly(ε-caprolactone) (PCL) has been widely used given its excellent biocompatibility and ease of processing. However, the use of PCL scaffolds is limited as a consequence of potential drawbacks including a slow degradation rate and their hydrophobic surface. These disadvantages may be overcome by incorporating additional natural polymer or inorganic fillers into the PCL matrix.

In the second section of this study, porous scaffolds of zein/PCL biocomposite were fabricated and characterized. These scaffolds were prepared using the particulate leaching method with sodium chloride particles as porogen. Porous biocomposite scaffolds with porosity around 70% and well-interconnected network were obtained. The incorporation of zein into PCL led to an improvement of the surface hydrophilicity as confirmed by the results of water contact angle measurement.

Following immersion in a phosphate buffered saline solution (PBS) *in vitro* for 28 days, it was observed that the zein/PCL scaffolds degraded more rapidly than the PCL scaffolds and the degradation rate could be controlled by adjusting the amount of zein in the composite. These results demonstrated the potential of the zein/PCL biocomposite scaffolds to be exploited in tissue engineering strategies for the repair of bone defects.

In the final section of this study, porous scaffolds using a magnesium phosphate (MP)/PCL biocomposite were developed for bone tissue engineering applications. The composite scaffolds were fabricated by the particulate leaching method again using sodium chloride particles as porogen. The resulting scaffolds had interconnected macroporous structure with porosity around 73%. The surface hydrophilicity of the scaffolds was enhanced by the incorporation of MP component and confirmed by water contact angle measurement. The results from subsequent *in vitro* degradation experiments showed that the MP/PCL composite scaffolds degraded faster than PCL scaffolds in a PBS solution. An additional benefit was that the degradation rate of the scaffolds could be tuned by adjusting the content of MP component in the composite. These results indicated that the MP/PCL composite scaffolds have potential application in bone tissue engineering.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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List of Publications

Journal papers

F. Wu, Y. Ngothai, J. Wei, C. Liu, B. O'Neill, Y. Wu. Premixed, injectable PLA–modified calcium deficient apatite biocement (cd-AB) with washout resistance. Colloids and Surfaces B: Biointerfaces, 2012, 92, 113–120.

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F. Wu, C. Liu, B. O'Neill, J. Wei, Y. Ngothai. Fabrication and properties of porous scaffold of magnesium phosphate/polycaprolactone biocomposite for bone tissue engineering. Applied Surface Science, 2012, 258, 7589–7595.

Conference paper

F. Wu, Y. Ngothai, C. Liu, J. Wei, B. O'Neill, R. Musgrove. Preparation and characterization of macroporous magnesium phosphate scaffold for bone regeneration. CHEMECA 2011, Sydney, Australia, September 2011.

Conference abstract

F. Wu, Y. Ngothai, C. Liu, J. Wei, B. O'Neill, R. Musgrove. PLA–modified magnesium phosphate bone cement with anti-washout property and degradability. CHEMECA 2011, Sydney, Australia, September 2011.

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ANOVA	analysis of variance
cd-AB	calcium deficient apatite biocement
CPC	calcium phosphate cement
DMSO	dimethyl sulfoxide
ECM	extracellular matrix
FBS	fetal bovine serum
FDA	Food and Drug Administration
FTIR	Fourier transform infrared
HA	hydroxyapatite
HCA	hydroxycarbonate apatite
HPMC	hydroxypropyl methylcellulose
hMSCs	human mesenchymal stem cells
LPR	liquid-to-powder ratio
micro-CT	micro-computed tomography
MgO	magnesium oxide
MP	magnesium phosphate
MPC	magnesium phosphate cement
MTT	methyl thiazolyl tetrazolium
NMP	N-methyl-2-pyrrolidone
OD	optical density
PDLA	poly(D-lactide)
PDLLA	poly(DL-lactide)
PLLA	poly(L-lactide)
P/L	powder-to-liquid
PBS	phosphate buffer saline
PEG	polyethylene glycol

PHA	precipitated hydroxyapatite
PLA	polylactide
PCL	polycaprolactone
PTFE	polytetrafluoroethylene
RH	relative humidity
rpm	revolutions per minute
SBF	simulated body fluid
SD	standard deviation
SEM	scanning electron microscopy
TCP	tissue culture polystyrene
TCP	tricalcium phosphate
Tris	tris(hydroxymethyl)aminomethane
WCA	water contact angle
XRD	X-ray diffraction