University of Technology, Sydney

The Characterisation of Adipose Derived Stem Cells on Coralline Scaffolds for Bone Tissue Engineering.

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy: Science

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Abbreviations

2-Dimenstional-Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis	2D-SDS-PAGE
Adipose Derived Stem Cells	ADSCs
3,3´-diaminobenzidine	DAB
Bovine Serum Albumin	BSA
Beta-tricalcium phosphate	β-TCP
Calcium Carbonate	CC
Coralline hydroxyapatite	cHA
Dulbecco's Modified Eagle's Medium	DMEM
Embryonic Stem Cells	ESCs
Extracellular Matrix	ECM
Fetal Bovine Serum	FBS
Hour	h
Hexamethyldisilazane	
Hydroxyapatite	HA
Inductively coupled plasma-mass spectroscopy	ICP-MS
Kilodalton	kDa
Liquid Chromatography – Tandem Mass Spectrometry	LC-MS/MS
Matrix-Assisted Laser Desorption/Ionization	MALDI
Phosphate Buffered Saline	PBS
Polyvinylidene Fluoride	PVDF
Rat Adipose Derived Stem Cells	rADSCs
Room Temperature	RT
Scanning Electron Microscopy	SEM

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis	SDS-PAGE
Stromal Vascular Fraction	SVF
Time of Flight	TOF

Abstract

Skeletal injuries affect millions of people worldwide, making it one of the most common causes of severe chronic pain and physical disability while also being a heavy burden on Australian healthcare, costing approximately $700 million a year. Over the past decades, biodegradable coralline biomaterials have been considered as an alternative implant material for bone regenerative therapy. This is because coralline materials have been found as being clinically advantageous due to their biocompatibility, osteoconductivity and scaffold resorbability. Additionally, coating coralline material with autologous stem cells is desirable for tissue ingrowth to occur rapidly as possible to provide the implant with structural integrity and eventual complete scaffold resorption. Adipose Derived stem cells (ADSCs) are considered promising biological tools for regenerative medicine as they are an accessible and abundant source of stem cells that have shown to be able to differentiate into bone tissue.

Recent *in vivo* and *in vitro* studies of coralline materials seeded with mesenchymal stem cells have produced conflicting results that range from demonstrating complete fracture repair to ineffective tissue regeneration [1-3]. This is because the underlying biological mechanism behind the clinically advantageous properties of coralline material is not well understood.

This PhD project has therefore been developed in order to address the problems outlined above. This work has investigated the effect of seeding rat adipose derived stem cells (rADSCs) and human adipose derived stem cells (hADSCs) onto biomimetic coralline scaffolds. The data presented here demonstrates that ADSCs can be successfully cultured onto coralline scaffolds, which provide a suitable microenvironment for ADSCs to proliferate. Additionally, the research I have undertaken shows that ADSCs seeded on coralline scaffolds undergo a proteomic change that resembles osteogenic cells, without the addition of any external osteoinductive factors.

This project also investigated the effects of different coralline scaffolds such as coralline carbonate, converted coralline hydroxyapatite (cHA), nanoporous cHA, macroporous cHA and high-density cHA on hADSCs where I showed that seeded cHA induced a stronger osteogenic response than seeded coralline calcium carbonate. Furthermore, I identified a unique immunomodulatory response from each seeded coralline scaffold that suggested a microenvironment rich in pro-inflammatory and pro- angiogenic factors which is a physiological feature commonly noted during *in vivo* fracture repair.

Overall this PhD project has contributed significantly to a wealth of biological knowledge about the effects of coralline scaffolds on ADSCs. Future work can utilise what is described here to either fabricate a coralline implant to harness the biological responses we have recorded or apply the data towards a safe and effective animal model for future therapeutic applications.

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