



THE UNIVERSITY
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**Thermoresponsive Hydrogels for
Mimicking Three-dimensional Microenvironment of
Mesenchymal Stem Cells in Cartilage Tissue Engineering**

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To my Lovely wife

Hosna

and my sweet daughter

Rasta

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Declaration for a thesis that contains publications

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Preface

This doctoral thesis is prepared in “Publication” format according to the “Specifications for Thesis (2015)” of the University of Adelaide. It includes publications that have been published, submitted for publication, or prepared in publication format:

- 1- A. Mellati and H. Zhang. Expansion of stem cells by nanotissue engineering, in Stem-cell nanoengineering (eds H. Baharvand and N. Aghdami), 2015, John Wiley & Sons Inc., Hoboken, NJ. DOI: 10.1002/9781118540640.ch14
- 2- A. Mellati, S. Dai, J. Bi, B. Jin, and H. Zhang. A biodegradable thermoresponsive hydrogel with tunable properties for mimicking three-dimensional microenvironments of stem cells. RSC Advances, 2014, 4(109): 63951-63961. DOI: 10.1039/C4RA12215A
- 3- A. Mellati, M. Valizadeh, S. Dai, J. Bi, B. Jin, and H. Zhang. Influence of polymer molecular weight on the cytotoxicity of poly (N-isopropylacrylamide). Colloid and Interface Science Communications. (Submitted and under review, Reference number: COLCOM-D-14-00131)
- 4- A. Mellati, C.M. Fan, A. Tamayol, S. Dai, J. Bi, B. Jin, C. Xian, A. Khademhosseini and H. Zhang. Microengineered 3D cell-laden thermoresponsive hydrogels as a platform for bi-zonal cartilage tissue engineering using mesenchymal stem cells. (Prepared in publication style)
- 5- A. Mellati, M. Valizadeh, S. H. Madani, S. Dai, J. Bi, B. Jin and H. Zhang. Poly (N-isopropylacrylamide)/chitosan hybrid as a three-dimensional microenvironment for stem cells in cartilage tissue engineering. (Prepared in publication style)

Some relevant components of the work have been presented in conferences:

- 1- H. Zhang, A. Mellati, S. Dai. A thermoresponsive hydrogel-based stem cell culture platform, Tissue Engineering & Regenerative Medicine International Society Asia-Pacific Annual Conference 2014 (TERMIS-AP 2014), 24-27 Sep 2014, Daegu, South Korea (Oral presentation)
- 2- A. Mellati, S. Dai, B. Jin, H. Zhang. Chitosan-g-PNIPAAm: A thermoresponsive hydrogel as a 3D microenvironment for MSCs. 2013 Royal Australian Chemical Institute SA Student Polymer & Bionanotechnology Symposium (SASPBS'13), 4 Oct 2013, Adelaide (Poster presentation)

During the PhD candidature, some relevant researches were conducted in collaboration with other researchers which led to a publication or conference presentation. The finalized works are:

- 1- S. Zheyu, A. Mellati, J. Bi, H. Zhang and S. Dai. A thermally responsive cationic nanogel-based platform for three-dimensional cell culture and recovery. 2014, *RSC Advances* 4(55): 29146-29156. DOI: 10.1039/C4RA02852J
- 2- A.W. Thomson, A. Mellati and H. Zhang. Chitosan-nanoparticle hydrogel complexes for bone tissue engineering. BioProcessing Network 2011 (BPN 2011), October 18-20, 2011, Adelaide (Poster presentation)

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- 1- Research Abroad Scholarship, The University of Adelaide, 2014 (The scholarship was awarded upon receiving an offer of pre-doctoral research fellow from Prof. Khademhosseini Lab, Harvard-MIT Division of Health Science and Technology, Cambridge, USA, May-June 2014)

2- Young Investigator Award 2011, BioProcessing Network, 2011

3- Adelaide Scholarship International, the University of Adelaide, 2011

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Abstract

Articular cartilage covers the bone heads of articulating joints to decrease the friction between bones. Unfortunately, articular cartilage has limited self-repair potential. Cartilage tissue engineering is a promising therapeutic approach, and its success strongly depends on our understanding and ability to mimic the complex three-dimensional microenvironment for cells and their surrounding native extracellular matrix (ECM) in articular cartilage. In particular, recreating the zonal organisation of articular cartilages makes the process more challenging.

My PhD thesis aims to design and develop chitosan-based thermoresponsive matrices with tailored physical, mechanical and chemical properties to fulfill microenvironmental requirements of mesenchymal stem cells (MSCs) in order to promote functional articular cartilage regeneration. The matrices are then micro-manipulated for resembling the spatially varying architecture of articular cartilage zones.

To achieve these aims, chitosan-g-poly (N-isopropylacrylamide) (CS-g-PNIPAAm) hydrogel with a random chain length of grafts was synthesized through free radical polymerization. The influence of various polymerization conditions on physical and mechanical properties was systematically investigated. Its suitability for mimicking microenvironment for MSC culture was studied using cell viability assays. The best CS-g-PNIPAAm in terms of its cell compatibility and cell culture performance was used in fabrication of microengineered constructs for regenerating the superficial zone and the middle zone of articular cartilage. Chondrogenic differentiation of embedded MSCs was evaluated through ECM components (glycosaminoglycan (GAG), total collagen and collagen type II) analysis. Cellular organisation and morphology within microchannels were determined using cell alignment and elongation quantification methods. To further control the chain length of PNIPAAm, well-defined and narrow-dispersed molecular weights of PNIPAAm were synthesized through atom transfer radical polymerization (ATRP). Influence of the polymer molecular weight on cytotoxicity and the cell death mechanisms were investigated through standard assays. Finally, chitosan/well-defined PNIPAAm (CSNI) hybrids were prepared using low/no toxic PNIPAAms. MSCs mixed with PNIPAAm solution and were seeded in the voids of the chitosan scaffolds. The phase separation of PNIPAAm at 37 °C led to a hybrid matrix for MSCs. The structural characteristics of the hybrids were studied and chondrogenic

differentiation of incorporated MSCs was evaluated through measuring GAG and total collagen deposition.

Various copolymerization conditions of CS-g-PNIPAAm have been optimised to obtain the best hydrogel for MSC culture with desired physical and mechanical properties. After MSC proliferation, MSCs can be recovered by separating cells from the polymer solution at room temperature using the sol-gel thermo-reversible property of the CS-g-PNIPAAm copolymer. It has been demonstrated that the CS-g-PNIPAAm copolymer hydrogel can provide an appropriate microenvironment for 3D cultivation of MSCs.

Biochemical analysis demonstrates that the CS-g-PNIPAAm hydrogel can support the embedded MSCs differentiation into chondrocytes in 3D. Histological and immunohistochemical stainings also confirm the increasing accumulation of GAG and collagen type II. The CS-g-PNIPAAm hydrogel with manipulated properties can be micropatterned for regenerating the superficial zone and the middle zone of articular cartilage. The cell-laden hydrogel micropatterned in 50-100 μm constructs can organize cells along the microchannel horizontal axis. The cell shape and alignment in the constructs is very similar to the superficial zone of chondrocytes of the native cartilage. Meanwhile, cells in the microchannel with the gap above 150 μm are randomly distributed which can be used to mimic the middle zone of the cartilage tissue.

The cytotoxicity of PNIPAAm is molecular weight dependent, and varies with the PNIPAAm chain length. Low molecular weight PNIPAAm (degree of polymerization (DP) = 35) is inherently toxic to cells, and necrosis is the dominant cell death mechanism. Moderate-sized PNIPAAms with their DP between 100 and 200 are non-cytotoxic. For the PNIPAAm with a higher molecular weight (DP = 400, P-400), cell viability is dependent on the assay method. The P-400 hydrogel is detrimental to stem cells when the cells are covered with a thick layer of gel, and this layer may become a barrier for nutrient or oxygen delivery to cells.

The CSNI hybrid matrices composed of chitosan scaffolds and well-defined PNIPAAm with a degree of polymerization of 400 (CSNI400) can provide a supporting platform for 3D stem cell culture and cartilage tissue engineering. Matrix characterization shows improved structural properties of CSNI400 in comparison with CSNI100 and the chitosan-alone scaffold.

In conclusion, we are able to create and refine 3D microenvironment for stem cells through manipulation of matrices in order to enhance cell proliferation and chondrogenic differentiation. Our results reveal that graft copolymer of chitosan and PNIPAAm with tailored properties and microengineered architecture is appealing for zonal cartilage tissue engineering. The hybrid matrices from chitosan scaffolds with well-defined PNIPAAm hydrogels promote chondrogenesis, better than the graft copolymer.

Graft copolymerization of chitosan and well-defined PNIPAAms (CS-g-W-PNIPAAm), microengineering of CSNI hybrids, and CS-g-W-PNIPAAm, stacking the microengineered constructs to form a macroscale 3D cartilage tissue, and *in vivo* implantation of engineered tissues should be addressed in future projects.

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