

## Antimicrobial peptides-loaded smart chitosan hydrogel: release behavior and antibacterial potential against antibiotic resistant clinical isolates

Nourollah Rezaei<sup>1,2</sup>, Hatef Ghasemi Hamidabadi<sup>1,2</sup>, Sadjad Khosravimelal<sup>3,4,5</sup>, Maria Zahiri<sup>6</sup>, Zahra Aliakbar Ahovan<sup>7</sup>, Maryam Nazm Bojnordi<sup>1,2</sup>, Behnaz Sadat Eftekhari<sup>8,9</sup>, Ali Hashemi<sup>7</sup>, Fatemeh Ganji<sup>3,4</sup>, Shahram Darabi<sup>10</sup>, Mazaher Gholipourmalekabadi<sup>3,4\*</sup>

<sup>1</sup>Immunogenetic Research Center, Department of Anatomy & Cell Biology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

<sup>2</sup>Department of Anatomy & Cell Biology, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

<sup>3</sup>Cellular and Molecular Research Centre, Iran University of Medical Sciences, Tehran, Iran

<sup>4</sup>Department of Tissue Engineering & Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran

<sup>5</sup>Department of Medical Biotechnology, Faculty of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran

<sup>6</sup>The Persian Gulf Marine Biotechnology Research Center, The Persian Gulf Biomedical Sciences Research Institute, Bushehr University of Medical Sciences, Bushehr, Iran.

<sup>7</sup>Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

<sup>8</sup>Department of Biomedical Engineering, Amirkabir University of Technology, Tehran, Iran.

<sup>9</sup>Department of Physiology and Institute for Medicine and Engineering, University of Pennsylvania, USA.

<sup>10</sup>Cellular and Molecular Research Center, Qazvin University of Medical Science, Qazvin, Iran

\*Dr. Mazaher Gholipourmalekabadi (ORCID: 0000-0001-6287-6831)

Dr. Mazaher Gholipourmalekabadi

Department of Tissue Engineering & Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran 1449614535, Iran

Tel: (+98 21) 8862 2755; Fax: (+98 21) 8862 2533

E-mail: mazaher.gholipour@iums.ac.ir; mazaher.gholipour@gmail.com

### Abstract

In this study, we synthesized thermo-responsive chitosan (TCTS) hydrogels, and loaded with different concentrations of antimicrobial peptide (AMP) (0, 4, 8 and 16  $\mu\text{g}\cdot\text{ml}^{-1}$ ) to fabricate an antibacterial wound dressing against resistant clinical isolates. Physico-chemical properties, release behavior, cytobiocompatibility and antibacterial activity of the AMP-TCTS hydrogels against standard strain and resistant *Acinetobacter baumannii* were fully determined *in vitro*. The TCTS-40%  $\beta$ -glycerolphosphate hydrogels showed a gelation time of 15 min at 37 °C. 80% weight loss at day 35 with no changes in pH value was observed. AMP-TCTS hydrogels showed a burst release of AMP (around 40%) at day 1, and a controlled release up to day 7. A dramatic water uptake was observed at first 4 h, and then continued for 10 h in a steady manner. All the AMP-TCTS hydrogels showed excellent cytobiocompatibility for human fibroblasts. The TCTS showed no antibacterial activity against both standard strain and clinical isolates. All the AMP-TCTS hydrogels had strong antibacterial activity against standard strains, but only 16  $\mu\text{g}\cdot\text{ml}^{-1}$  showed antibacterial behavior against resistant *A. baumannii*. Our results strongly suggest the 16  $\mu\text{g}\cdot\text{ml}^{-1}$  AMP-TCTS hydrogel as an excellent antibacterial wound dressing against resistant *A. baumannii*, and now promises to proceed with pre-clinical investigations.

**Keywords:** chitosan; hydrogel; thermos-responsive; resistant bacteria, post-wound infection; wound dressing;