

# HUMAN COLLAGEN-BASED HYDROGEL WITH ZINC OXIDE NANOPARTICLES FOR ARTICULAR CARTILAGE ENGINEERING

Cobzac Vitalie<sup>1,2</sup>, Cojocari Stefan<sup>2</sup>, Jian Mariana<sup>1</sup>, Stoian Alina<sup>1</sup>, Nacu Ana-Maria<sup>1</sup>, Motelica Ludmila<sup>3</sup>, Trusca Roxana<sup>3</sup>, Fikai Anton<sup>3</sup>, Nacu Viorel<sup>1,2</sup>

<sup>1</sup> Nicolae Testemitanu State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

<sup>2</sup> Clinical Hospital of Traumatology and Orthopaedics, Chisinau, Republic of Moldova

<sup>3</sup> National University of Science and Technology Politehnica Bucharest, Bucharest, Romania

**Introduction.** Articular cartilage tissue engineering has a long history of using three-dimensional matrices seeded with cells of chondrogenic potential. Recent studies show that chondrogenesis is influenced not only by growth factors, but also by the biochemical microenvironment, including ions such as Zn<sup>2+</sup> and Cu<sup>2+</sup>.

**Materials and methods:** With approval from the Ethics Committee of Nicolae Testemitanu State University of Medicine and Pharmacy and under a collaboration agreement with the Human Tissue Bank of the Clinical Hospital of Traumatology and Orthopaedics, articular cartilage was collected from a donated femoral head, along with processed umbilical-placental complex. Collagen hydrogels (3 mg/ml) were prepared in 24-well plates containing ZnO NPs (<50 nm; Sigma-Aldrich, USA) at 0 (control), 1, 10, 20, 40, 60, 80, and 100 µg/ml (n=4). Their elastic modulus (EM) was measured using a TX-BLM probe and a TX-700 texture analyzer (Lamy Rheology, France). For viability testing, human chondrocytes (1×10<sup>5</sup> cells/ml) were cultured on hydrogels containing 0 (control), 10, 50, or 100 µg/ml ZnO NPs. Resazurin (10 µg/ml; Acros TFS, Belgium) was used as the viability reagent, and blanks without cells were prepared for each concentration and control group (n=3). Measurements were performed at 24, 48, and 72 hours after 6 h incubation at 37°C and 5% CO<sub>2</sub>. Absorbance was recorded at 570 and 600 nm using a Synergy H3 spectrophotometer (BioTek, USA).

**Results and Conclusions.** ZnO NPs concentration markedly affected the mechanical properties of the hydrogels. Significant differences were found between the control group (470.73 ±38.00 N/m<sup>2</sup>) and most ZnO-loaded groups. EM was significantly reduced at 60, 80, and 100 µg/ml ZnO (p <0.05), whereas at 1, 10, and 20 µg/ml it was significantly higher than in the control group (p <0.05). No significant difference was observed at 40 µg/ml (p >0.05). The resazurin assay showed a dose-dependent effect of ZnO NPs on chondrocyte viability. The highest viability was recorded at 10 µg/ml ZnO, remaining above 70% over three consecutive days, while higher concentrations caused a marked decrease in viability (p < 0.05). Therefore, 10 µg/ml ZnO appears to be the most promising concentration, offering a favorable balance between mechanical strength and cell viability.

**Keywords:** cartilage engineering, collagen hydrogel, ZnO nanoparticles, human chondrocytes

**Acknowledgements:** research funded by young researchers project #25.80012.8007.05TC