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**LOW-INTENSITY PULSED ULTRASOUND DIRECT CHONDROGENIC DIFFERENTIATION OF ADIPOSE-STROMAL CELLS IN 3D PIEZOELECTRIC HYDROGELS**

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**Introduction:** A major challenge in cartilage tissue engineering (TE) is to develop scaffolds capable of providing an instructive biomimetic environment to effectively drive mesenchymal stromal cells (MSCs) differentiation [1]. Hydrogels have emerged as promising biomaterials for this purpose, due to their biocompatibility and ability to mimic the tissue extracellular matrix [2]. Recently, graphene oxide (GO) emerged as a promising nanomaterial for cartilage TE due to chondroinductive properties when embedded into polymeric formulations [3]. It has been also shown that piezoelectric nanomaterials, like barium titanate (BaTiO<sub>3</sub>) nanoparticles, can be exploited as nanoscale transducers capable of inducing cell growth/differentiation [4]. Ultrasound waves are an interesting tool to facilitate chondrogenesis. In particular, it has been demonstrated that low-intensity pulsed ultrasound (LIPUS) regulates the differentiation of adipose mesenchymal stromal cells (ASCs) [5].

The aim of this study was to investigate whether dose-controlled LIPUS is able to direct chondrogenic differentiation of ASCs embedded in a 3D piezoelectric hydrogel.

**Methodology:** Human adipose mesenchymal stromal cells at  $2 \times 10^6$  cells/mL were embedded in 3D VitroGel RGD® hydrogel with or without nanoparticles (GO, 25 µg/ml, BaTiO<sub>3</sub>, 50 µg/ml) and exposed to LIPUS stimulation (frequency: 1 MHz, intensity: 250 mW/cm<sup>2</sup>, duty cycle: 20%, pulse repetition frequency: 1 kHz, stimulation time: 5 min) every 2 days, until day 10 of culture. Hydrogels were cultured and chondrogenic differentiated for 2, 10 and 28 days. At each time point cell viability (Live&Dead), cytotoxicity (LDH), gene expression of collagen type 2 (COL2), aggrecan (ACAN), SOX9, and collagen type 1 (COL1), electron microscopy, histology and immunohistochemistry (COL2, aggrecan, SOX9, and COL1) were evaluated.

**Results:** In both 3D hydrogels we evidenced that LIPUS treatment did not affect negatively the viability of the embedded cells. LIPUS boosted the chondrogenic differentiation of ASCs laden in 3D piezoelectric hydrogel: the chondrogenic genes and proteins markers (COL2, aggrecan and SOX9) were increased while the fibrotic marker COL1 was decreased compared to control samples (non piezoelectric hydrogels and piezoelectric hydrogels not stimulated with LIPUS).

Conclusions: These results suggest that the combination of LIPUS and piezoelectric hydrogels push the differentiation of ASCs encapsulated in a 3D hydrogel and represent a promising tool in the field of cartilage TE.

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keywords: Low-intensity pulsed ultrasound (LIPUS), Adipose mesenchymal stromal cells (ASCs), Hydrogels Chondrogenic differentiation