Wharton's Jelly-Derived Extracellular Vesicles Promote Chondrocyte Proliferation and Chondrogenesis, and Mitigate Oxidative Stress

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INTRODUCTION: Articular cartilage tissue engineering (ACTE) using chondrocytes has long been explored as a strategy to repair articular cartilage defects. To obtain a sufficient number of cells for clinical use, in vitro cell expansion of chondrocytes is commonly performed. However, the in vitro expansion of chondrocytes often leads to a loss of their native chondrogenic phenotype, primarily due to oxidative stress. This phenotypic drift is widely recognized as a key contributor to fibrocartilage formation following implantation clinically. To overcome the limitations of chondrocyte-based ACTE, Wharton's jelly-derived mesenchymal stem cells (WJMSCs) have emerged as a promising alternative. These cells offer several advantages, including robust proliferative capacity, potential for chondrogenic differentiation, noninvasive harvesting procedures, and minimal ethical concerns. Nonetheless, their clinical application remains constrained by challenges such as stringent storage and transport requirements, potential tumorigenicity, risk of disease transmission, and possible immune rejection. Recent studies suggest that the therapeutic benefits of MSCs may be mediated not only by direct differentiation, but also through their secreted extracellular vesicles (EVs). Some studies indicate that EVs are capable of mimicking the numerous therapeutic functions of MSCs, offering a promising acellular approach for ACTE. In recent years, studies have highlighted the beneficial effects of WJMSC-derived EVs (WJMSC-EVs) on articular cartilage, demonstrating their anti-inflammatory properties, ability to reduce cartilage degradation, and capacity to attenuate chondrocyte senescence. However, there remains a lack of focused cellular-level investigations, especially concerning the direct interactions between WJMSC-EVs and chondrocyte function. Based on these findings, we hypothesize that WJMSC-EVs may mitigate oxidative stress in chondrocytes and help preserve their chondrogenic phenotype. Therefore, WJMSC-EVs could potentially be applied in chondrocyte-based ACTE. In this study, we investigated the effects of WJMSC-EVs on chondrocyte proliferation, survival, chondrogenesis and oxidative stress response.

METHODS: WJMSCs-EVs used in this study were generously provided by Kao-Ho Hospital in Kaohsiung. To confirm their identity and quality, WJMSCs-EVs were characterized based on particle count, size distribution, morphology and the presence of canonical surface markers CD9, CD63, and CD81. Human chondrocytes derived from the human articular cartilage were purchased from Lonza Bioscience (cat. no. CC-2550; NHAC-kn Articular Chon CGM, cryo amp; Walkersville, MD, USA). Chondrocytes were maintained at 37 °C in a humidified incubator with 5% CO2, and the culture medium was replenished every two days until reaching confluency for passaging. For all of the experiments, chondrocytes were used after six passages. Chondrocytes were allocated into two experimental groups: 1. Control group–received no WJMSC-EVs treatment. 2. WJMSC-EVs group–treated with WJMSC-EVs at the indicated concentrations. Subsequent analyses focused on WJMSC-EVs uptake by chondrocytes and their impact on cellular behavior, including survival, proliferation, chondrogenesis, and oxidative stress. Chondrogenesis was assessed by measuring mRNA levels of SOX-9, collagen type II (Col-II), Aggrecan, Col-I. Oxidative stress was assessed by measuring mitochondrial superoxide levels and the expression of the antioxidant enzyme superoxide dismutase 2 (SOD2).

RESULTS SECTION: The mean particle sizes of the WJMSC-EVs were 79.8 ± 19.05 nm (Figure. 1). Transmission electron microscopy (TEM) revealed that WJMSC-EVs exhibited a spherical morphology (Figure. 1). The presence of CD9, CD63, and CD81 confirmed the identity of WJMSC-EVs, with α-tubulin undetected (Figure. 1). UCMSC-EVs were intake by chondrocytes after treatment (Figure. 2). UCMSC-EVs maintained chondrocyte survival, and increased chondrocyte proliferation after intake by chondrocytes (Figure. 3). WJMSC-EVs upregulated mRNA levels of SOX-9, Col-II, and Aggrecan, while decreasing Col-I levels (Figure. 4). WJMSC-EVs reduced the oxidative stress of chondrocytes by reducing mitochondrial superoxide production and increasing protein levels of SOD-2 and Sirt-3 in chondrocytes (Figure. 5).

DISCUSSION: Chondrocyte-based ACTE has been extensively studied for its potential to regenerate articular cartilage, but it is limited by fibrocartilage formation. For example, the clinical outcomes of patients treated with autologous chondrocyte im-plantation (ACI) are not optimal because many of them form fibrocartilage. It is suggested that fibrocartilage formation is due to chondrocyte loss and their function caused by oxidative stress. Therefore, developing new methods to enhance chondrocyte function is required. Overall, we found that WJMSC-EVs enhance chondrocyte function, and WJMSC-EVs-treated chondrocytes can be used as a potential strategy for chondrocyte-based ACTE.

SIGNIFICANCE/CLINICAL RELEVANCE:

WJMSC-EVs promote chondrocyte proliferation and chondrogenesis, and mitigate oxidative stress.

Figure 1

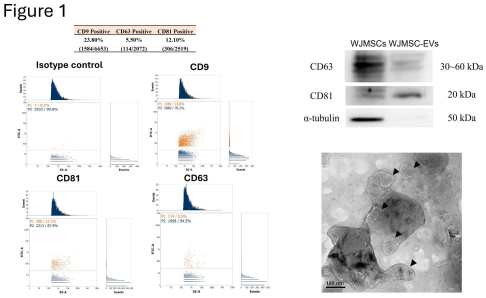

Figure 2

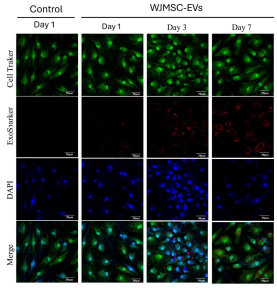

Figure 3

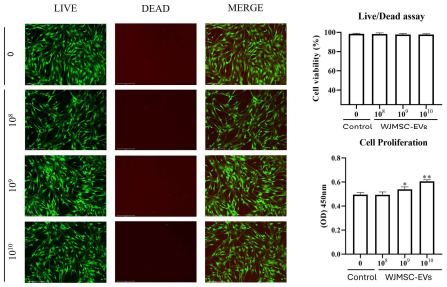

Figure 4

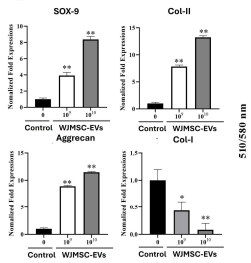

Figure 5