

Selective Laser Sintered Polyethylene Scaffolds Enhance Chondrogenesis in Pediatric Patient-Derived Auricular Chondrocytes: A Promising Tissue Engineering Approach for Microtia

Alhussain A Ojaym, PhD^{1,2}, Hope C Ball, PhD³, Trinity A Kronk, B.S.^{1,2}, Gabrielle T Robinson B.S.^{1,2}, Fayez F. Safadi, PhD^{1,2,3,4,5}, Ananth S. Murthy, MD³
¹Department of Biomedical Sciences, Northeast Ohio Medical University, Rootstown, OH, USA, ²Musculoskeletal Research Group, NEOMED, Rootstown, OH, USA, ³Akron Children's Hospital, Akron, OH, USA, ⁴Rebecca D. Considine Research Institute, Akron Children's Hospital, Akron, OH, USA, ⁵University Hospitals, Cleveland, OH, USA

Introduction: Microtia is a pediatric congenital deformity characterized by the absence or underdevelopment of the external ear, often accompanied by atresia of the external auditory canal. This condition leads to hearing impairment and significant psychosocial challenges. Current reconstructive options are limited by the availability of donor tissue and aesthetic variability, thereby driving interest in scaffold-based regenerative approaches. This study introduces a novel, plasma-enhanced 3D-printed scaffold strategy that promotes chondrogenesis while addressing critical clinical and aesthetic needs in microtia reconstruction.

Method: In this study, we evaluated multiple 3D-printed scaffold formulations for their capacity to support chondrogenesis using auricular chondrocytes isolated from five patients with microtia, with approval from the Institutional Review Board (IRB) at Akron Children's Hospital (ACH) and Northeast Ohio Medical University (NEOMED). Cells were seeded onto selective laser sintered (SLS) polyethylene (PE) scaffolds, either with or without plasma surface treatment (see Table 1). Scaffolds were then evaluated for biocompatibility (MTT assays at 24, 48, and 72 hours), chondrogenic gene expression (qPCR for COL2A1, SOX9, and Aggrecan), and extracellular matrix deposition (Alcian Blue staining) over 7 and 14 days.

Result: The biocompatibility and chondrogenic potential of the PE scaffolds were assessed using the MTT assay for cell viability at 7 and 14 days across three time points (24, 48, and 72 hours) (Figure 1- 48 hours Only). Quantitative PCR (qPCR) was performed to assess the expression of chondrogenic genes, and histochemical analysis was conducted using Alcian Blue staining. Gene expression analysis demonstrated the upregulation of chondrogenic markers, including SOX9 and Aggrecan (Figure 2), along with the downregulation of catabolic and inflammatory markers such as MMP13, ADAMTS4, and IL-1 β (data not shown), indicating a favorable microenvironment for cartilage matrix formation. Histochemical evaluation using Alcian Blue staining revealed substantial extracellular matrix deposition, particularly in the plasma-treated groups, reflecting enhanced glycosaminoglycan (GAG) synthesis.

Discussion: Plasma-treated SLS PE + RxG and SLS PE + RxC scaffolds exhibited superior chondrocyte viability and matrix deposition compared with untreated scaffolds, indicating the critical role of plasma modification in enhancing scaffold bioactivity. Enhanced gene expression and histochemical outcomes further suggest that plasma treatment improves cell-scaffold interactions and promotes chondrogenesis. These results support the translational potential of plasma-modified scaffolds, particularly SLS PE + RxG + Plasma and SLS PE + RxC + Plasma, as promising platforms for auricular cartilage tissue engineering in microtia reconstruction.

Significance/Clinical Relevance: Microtia reconstruction remains one of the most challenging areas in pediatric plastic and reconstructive surgery due to limitations in autologous rib cartilage harvest, complications with synthetic implants, and aesthetic inconsistencies. By demonstrating improved chondrocyte viability, gene expression, and extracellular matrix deposition, plasma-modified 3D-printed scaffolds offer a clinically relevant strategy that could reduce the need for invasive donor-site procedures, improve cosmetic outcomes, and expand therapeutic options for children with microtia. This work highlights the translational value of combining patient-derived cells with advanced scaffold technologies to achieve reliable, functional, and aesthetically acceptable auricular reconstruction.

Table 1: List of the 3D-printed selective laser sintered (SLS) polyethylene (PE) scaffolds

Scaffold (No plasma)	Scaffold (+ plasma)
(1) SLS PE	(1) SLS PE
(2) SLS PE + RxG	(2) SLS PE + RxG
(3) SLS PE + RxC	(3) SLS PE + RxC

Figure 1:

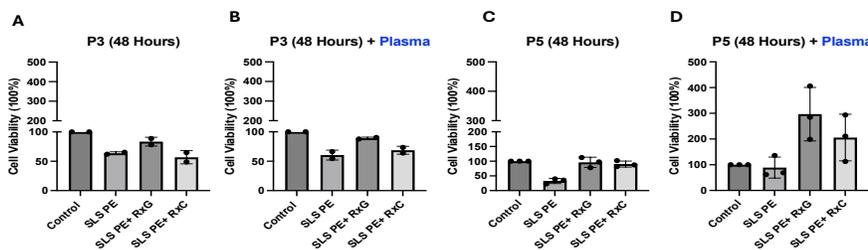


Figure 1: MTT assay evaluating the viability of chondrocyte cells isolated from patients #3 and #5. (A, B) Patient #3 (P3) cells were seeded on scaffolds with or without plasma treatment. (C, D) Patient #5 (P5) cells were seeded on scaffolds with or without plasma treatment. The results indicate enhanced cell attachment and viability in plasma-treated scaffolds compared to scaffolds without plasma or untreated controls. Data presented are from two independent experiments (two patients) for the scaffold without plasma and three replicates per condition.

Figure 2:

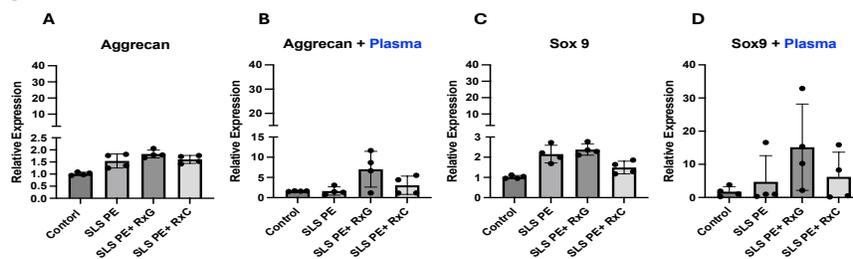


Figure 2: Quantitative PCR (qPCR) analysis of chondrogenic gene expression in auricular chondrocytes cultured on 3D-printed scaffolds with or without plasma surface treatment. Auricular chondrocytes isolated from two patients with microtia were evaluated for the expression of chondrogenic markers at Day 14. Relative mRNA expression levels of SOX9 (A, B) and Aggrecan (C, D) were quantified. The data presented are the mean \pm SEM from three independent experiments (three patients) with three replicates per experiment.

