Porous poly-L/D-lactide copolymer scaffolds with barium titanate nanoparticles for cartilage tissue engineering application

Hanna Karjalainen[1], Ville-Pauli Karjalainen[1], Gabriela S. Lorite[1], Markus Hannula[2], Tuomo Siponkoski[1], Mikko Nelo[1], Jari Juuti[1], Ali Mobasheri[1], Minna Kellomäki[2], Jari Hyttinen[2], Simo Saarakkala[1,3], Mikko A. Finnilä[1]
[1]University of Oulu, Oulu, Finland, [2]Tampere University, Tampere, Finland, [3]Oulu University Hospital, Oulu, Finland
Corresponding author: hanna.karjalainen@oulu.fi

Disclosures: Hanna Karjalainen (N), Ville-Pauli Karjalainen (N), Gabriela S. Lorite (N), Markus Hannula (N), Tuomo Siponkoski (N), Mikko Nelo (N), Jari Juuti (N), Ali Mobasheri (9; service on the OARSI board), Minna Kellomäki (N), Jari Hyttinen (N), Simo Saarakkala (N), Mikko A. Finnilä (N)

INTRODUCTION: A suitable micro-environment is needed for chondrocytes to maintain their phenotype and capability for extracellular matrix (ECM) production in tissue engineering applications to repair cartilage defects. Pore structure, interconnectivity, as well as the ability to provide natural cellular cues, are important properties in the scaffolds. Barium titanate nanoparticles (BTNPs) can be used in composite scaffolds to tailor the scaffold properties and provide beneficial cues for cellular function in tissue engineering. With the combination of nanoparticles and the breath figure method, it is possible to create a microporous structure that enables chondrocyte migration into the pores. Additionally, piezoelectric materials, like BTNPs, can generate electrical activity in response to the applied stress thus creating an electroactive scaffold. The patterned porous structure mimics the physiological micro-environment of chondrocytes while enabling also electromechanical stimulation for the cells. This study investigated the effect of BTNP volume fraction in pore formation of poly-L/D-lactide copolymer (PLDLA) scaffolds prepared by the breath figure method for cartilage tissue engineering application.
METHODS: 96/04 PLDLA, dioleoyl phosphatidylethanolamine (DOPE) and BTNPs were used to produce porous films with the breath figure method. Four different percentages by weight of BTNP were added to PLDLA scaffolds: 10, 20, 30, and 40 wt%. Numbers 10-40 in the scaffold's names indicate the wt% of the BTNP within the scaffolds. The final nanoparticle concentration was studied with thermogravimetric thermal analysis (TGA) while scanning electron microscopy (SEM) and X-ray microtomography (µCT) were used to study the pore structure and interconnectivity of the composite scaffolds. Moreover, human articular chondrocytes (402-05a, Cell Applications Inc.) were cultured on the scaffolds for two weeks to evaluate the metabolic activity of the chondrocytes with Prestoblue assay, biocompatibility and cell morphology with LIVE/DEAD staining and finally extracellular matrix production by immunofluorescence staining of collagen II and aggrecan. Statistical testing of the Prestoblue assay results was performed with two-way ANOVA using GraphPad Prism v8.4.3.
RESULTS: The porous micropattern structure was present in all of the composite scaffolds prepared by the breath figure method. Pore diameter increased in PLDLA/BTNP scaffold approximately to 20 µm while in plain PLDLA scaffolds, it was only 2 µm. The thickness of the scaffold decreased with increasing BTNP content, while the PLDLA scaffold was the thinnest. Pore volume was the highest in PLDLA/BTNP10 scaffolds, while PLDLA/BTNP30 scaffold provided the highest interconnectivity with the second highest pore volume. All PLDLA/BTNP scaffolds were biocompatible as demonstrated by similar cell viability and metabolic activity between the scaffolds. Chondrocytes were more spherical in PLDLA/BTNP scaffolds compared to PLDLA scaffolds, but there was no difference in ECM production.
DISCUSSION: The breath figure method can be used to produce PLDLA/BTNP composite scaffolds with different percentages by weight while maintaining micropatterned porous structure. Overall, the amount of BTNP influenced the scaffold structure, however, all composite scaffolds were biocompatible and provided a suitable structure for chondrocytes.
SIGNIFICANCE/CLINICAL RELEVANCE: The increasing amount of BTNP did not have a negative effect on the chondrocytes and the porous structure enabled cell migration into the pores in the composite scaffolds. Thus, PLDLA/BTNP scaffolds are an interesting material choice for cartilage tissue engineering, which could provide novel approaches for the treatment of cartilage defects in the future.

ACKNOWLEDGEMENTS: This project was funded from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 814558 "RESTORE". Imaging was performed at the Biocenter Oulu Light Microscopy Core Facility and Centre for Material Analysis, University of Oulu, Oulu Finland.

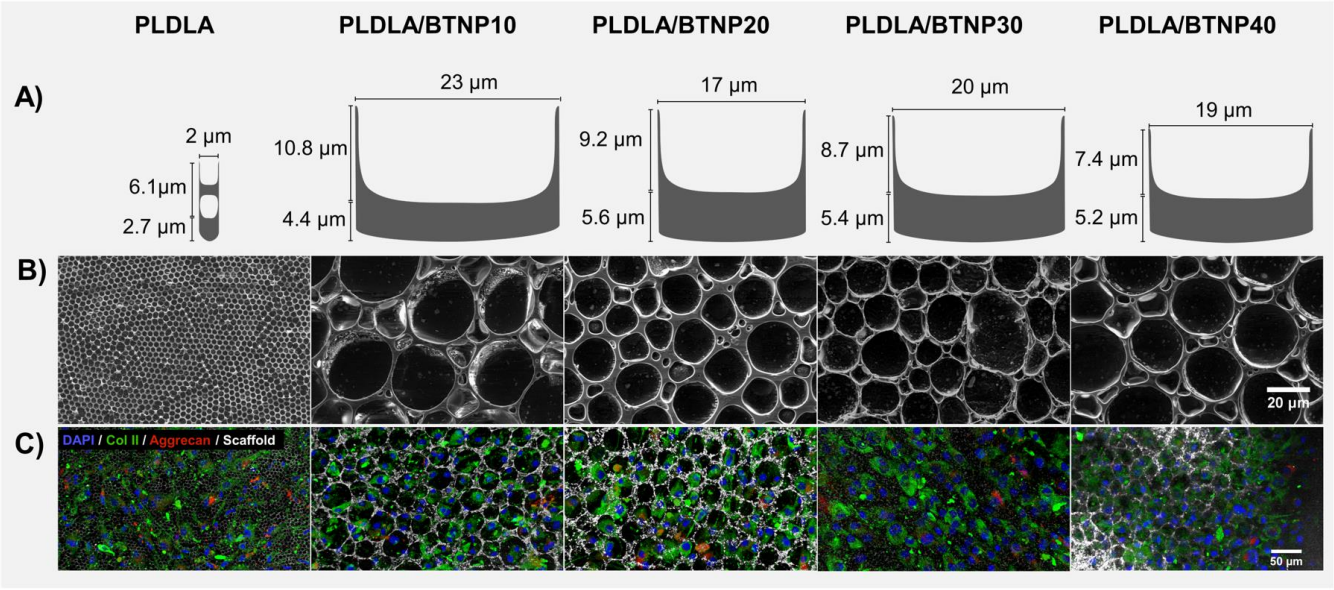

Figure 1. A) Illustrated pore sizes of the scaffolds based on SEM and µCT data. **B)** SEM images of the scaffolds show increased pore size in the PLDLA/BTNP scaffolds compared to PLDLA scaffold. Scale bar 20 µm. **C)** Immunofluorescence staining of chondrocytes cultured on the scaffolds for two weeks revealed no difference in collagen II and aggrecan production between the samples. Scale bar 50 µm. Numbers 10-40 indicate the wt% of the BTNP within the composite scaffolds.