Synthesis and biological characterization of 3D polyhydroxybutyrate-tricalcium phosphate scaffolds

Maciej Guzik[1]*, Adam Szeligowski[1], Tomasz Witko[1], Daria Solarz[1], Szymon Skibiński[2], Ewelina Cichoń[2], Aneta Zima[2], Joanna Czechowska[2], Anna Ślósarczyk[2], Agata Gondek[3], Martyna Seta[4]

[1] *Jerzy Haber Institute of Catalysis and Surface Chemistry Polish Academy of Sciences, Poland*

[2] *Faculty of Materials Science and Ceramics, AGH University of Science and Technology, Poland*

[3] *Department of Methodology, Medical University of Warsaw, Poland*

[4] *Chair and Department of Experimental and Clinical Physiology, Laboratory of Centre for Preclinical Research, Medical University of Warsaw, Poland*

INTRODUCTION

Biopolymers are promising materials for tissue engineering. One of such examples is a group of polyesters synthesized by microorganisms, namely polyhydroxyalkanoates (PHA). These biomaterials are completely biocompatible with mammalian tissues, which makes them appropriate for tissue engineering purposes. Depending on the (*R*)-3-hydroxyacid that constituents the polymer chain, the PHAs tend to vary in their physicochemical and mechanical properties (i.e., brittle, hard to elastic), offering a wide range of potential applications. Combined with calcium-phosphate ceramics (e.g. tricalcium phosphate, TCP) 3D scaffolds these biopolymers can serve as a component that nourishes surrounding tissues. Moreover, PHAs can be applied as platforms for controlled drug release which occurs locally only in the implantation site. Here, we present a complete preparation route of 3D TCP scaffolds infiltrated by polyhydroxybutyrate (PHB) polymer along with their biological characterization to assess their applicability in bone and cartilage regeneration.

POLYHYDROXYBUTYRATE SYNTHESIS

Through microbial fermentation a PHA polymer was synthetized in 5L fermenter. *Z. denitrificans* converted glycerol to polyhydroxybutyrate (PHB). PHB was extracted form dried biomass, purified over charcoal, precipitated in methanol and resuspended in $CHCl_3$. Fermentation processes led to accumulation of PHB polymer. PHB was accumulated within 48 hours, biomass reached 96 g L^{-1} with 28% polymer content (Fig. 1). A purified PHB was used to infiltrate 3D macroporous TCP scaffolds.

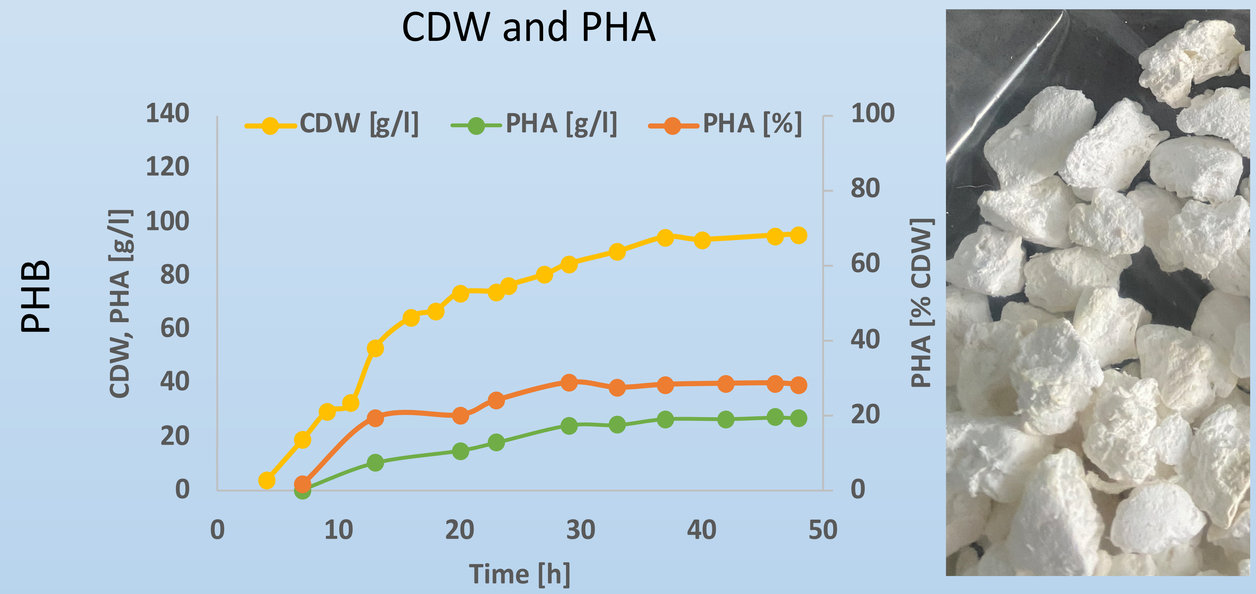

Figure 1. *Growth and PHA accumulation from glycerol by Z. denitrificans (left) and extracted and purified PHB (right)*

3D MACROPOROUS TCP SCAFFOLDS

TCP powder was synthesized by a wet chemical method. Next, a ceramic slurry was prepared and 3 types of polyurethane sponges (with small medium and large pores) were thoroughly covered with it. Then ceramic specimens were dried and sintered at 1150 °C. The 3D TCP scaffolds were infiltrated with 5% PHB solution in CHCl3 and air-dried TCP scaffolds (Fig.2). Ceramic sinters were uniformly covered with the biopolymer which were evidenced by SEM observations. Preliminary studies revealed no cytotoxicity of TCP as well as TCP/PHB scaffolds.

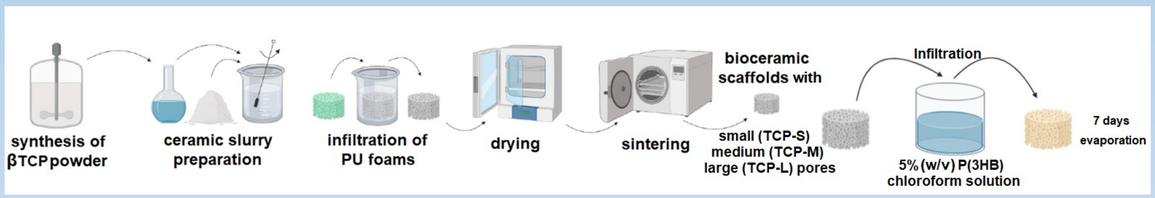

Figure 2. *Procedure for synthesis of the 3D scaffolds*

ACKNOWLEDGMENTS

Research funded by and the National Centre for Research and Development, Poland, grants TECHMATSTRATEG2/407507/1/ NCBR/2019 and LIDER/27/0090/L-7/15/NCBR/2016.

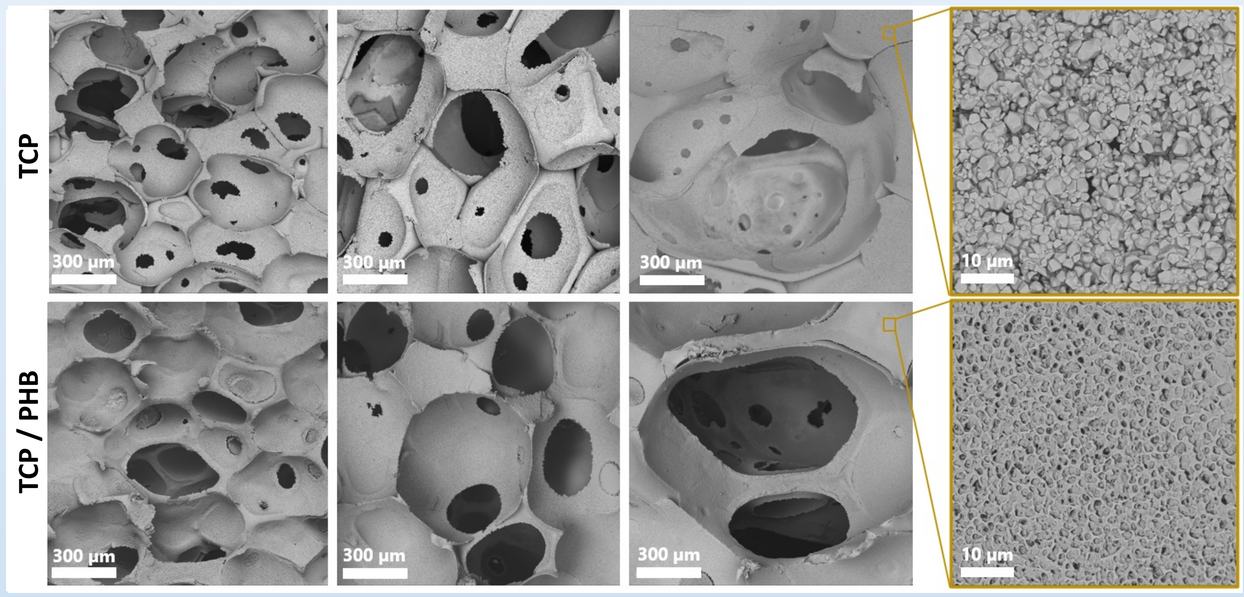

Figure 3. *Microstructure evidenced by SEM imagining of TCP scaffolds infiltrated with PHB*

BIOLOGICAL ASSESSMENT OF 3D SCAFFOLDS

Scaffolds were tested using human Mesenchymal Stem Cells (hMSC). hMSC adhesion, growth and differentiation were assessed. hMSCs adhere, grow and proliferate on both materials (viability over 85% at 7 and 21 DIV). Both types of scaffolds led to an increase in the number of cells in 21 DIV if compared to 7 DIV. It was found that for TCP scaffolds the average number of cells in the field of view increased from 61 to 165, from 70 to 80 and from 54 to 105 for material with small, medium and large pores, respectively. In case of TCP/PHB scaffolds with small pores a decrease in the number of cells from 46 to 13 was observed. While for materials with medium and large pores an increase in the number of cells from 29 to 38 (medium) and from 28 to 38 (large) was noticed. What is more, in the case of scaffolds with large pores (both TCP and TCP/PHB), the cells penetrate easily the materials (even 650-700 µm into the scaffold). The results prove appropriate material architecture.

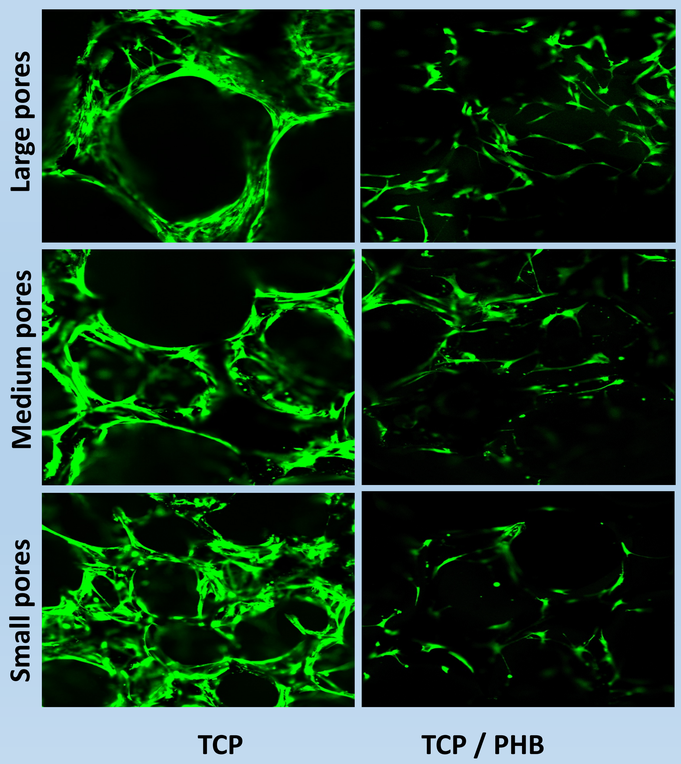

Figure 4. *hMSC proliferation on 3D materials*

CONCLUSIONS

Fermentation process allows to obtain a wide range of PHA polymers, here a PHB polymer. This material can serve as a component of ceramic-polymer composites which can be obtained by the infiltration of ceramic TCP scaffolds with PHB. These composites act as substates for cell attachment and growth, as evidenced by preliminary studies employing hMSC. In future, the developed materials can serve as substitutes for bone regeneration purposes.

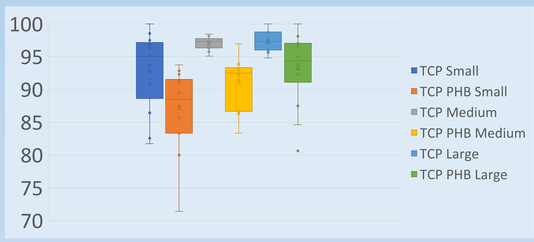

Figure 5. *Cell viability in % (Day 21)*