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STUDY OF MACROPHAGE SURVIVAL DURING CO-CULTIVATION WITH BACTERIA IN A NANOFIBER SCAFFOLD 3D CELL CULTURE SYSTEM

Michaela Burvalova[1*], Monika Zouharova[2], Nathália Oderich Muniz[3], Ales Pavlik[1], Petr Slama[1]

[1]*Affiliation: Department of Animal Morphology, Physiology and Genetics, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic*

[2]*Affiliation: Department of Infectious Diseases and Preventive Medicine, Veterinary Research Institute, Hudcova 70, 621 00 Brno, Czech Republic.*

[3]*Affiliation: Biomécanique et Bioingénierie (BMBI) - UMR 7338, University of Technology of Compiègne (UTC), 60280 Compiègne, France.*

[*]*xburvalo@mendelu.cz*

Abstract: This study investigates macrophage survival and apoptosis during co-cultivation with *Staphylococcus aureus* and *Streptococcus uberis* on nanofiber scaffolds made from polycaprolactone (PCL) and PCL combined with silk fibroin (PCL/SF), compared to traditional 2D culture. Macrophages, differentiated from CD14+ peripheral blood monocytes using GM-CSF, were analyzed using flow cytometry. Results showed that macrophages on PCL/SF scaffolds had significantly higher survival rates and lower necrosis percentages than those on PCL and 2D cultures, especially under bacterial stress at 1, 3, and 24-hour intervals. The PCL/SF scaffolds provided a superior environment for macrophage growth and resilience, suggesting promising applications for future research and cell culture techniques.

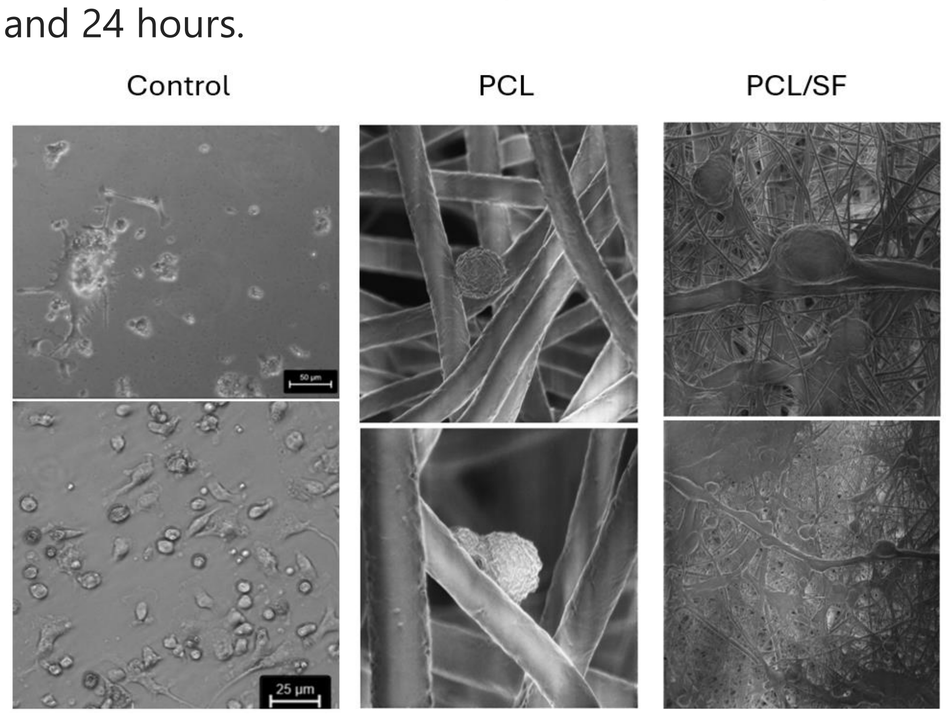
• Introduction

Macrophages are essential for engulfing and destroying pathogens, and their resilience under bacterial stress is vital for immune defense. Traditional 2D cultures often lack the complexity of *in vivo* environments. Bovine mastitis, caused by bacteria like *S. aureus* and *Str. uberis*, highlights the need for accurate models. Using nanofiber scaffolds to create a 3D culture system can better mimic natural conditions, enhancing cell viability and resilience. This approach is crucial for advancing immune cell research and improving treatments for bovine mastitis.

• Material and method

CD14+ cells were isolated from bovine blood using gradient centrifugation and magnetic separation. These cells were cultured in RPMI 1640 medium with fetal bovine serum, GM-CSF, and antibiotics at 37°C and 5% CO2. Cultivation was performed on different types of nanofiber membranes (PCL, PCL/SF) and in control 2D cultures.

After 7 days of cell differenciation macrophages analyzed using electron microscopy and flow cytometry. Co-cultivation with bacterial suspensions was analyzed at 1, 3, and 24 hours.

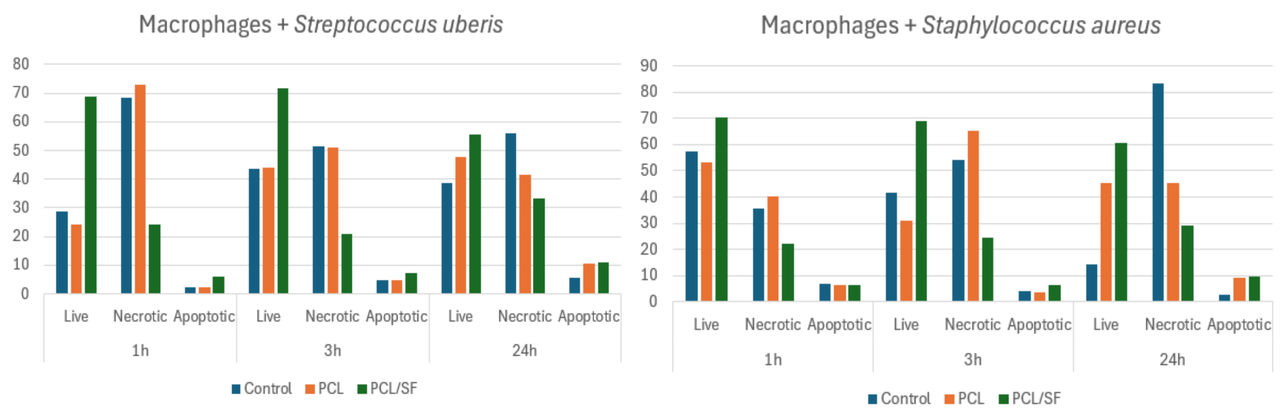


• Results and discussions

Isolated CD14+ cells were successfully differentiated into macrophages. 3D cultivation systems using nanofiber membranes (PCL, PCL/SF) were tested. PCL had poor cell adhesion. Combining PCL with fibroin improved cell adhesion and viability of macrophages.

These macrophages were co-cultivated with *S. aureus* and *Str. uberis*. Results showed that macrophages on nanofiber scaffolds were more resilient to bacterial pressure, especially on PCL/SF.

The three-dimensional environment provided by nanofiber scaffolds enhances the physiological relevance of *in vitro* models, making them more relevant to *in vivo* conditions. PCL/SF material improves cell adhesion and viability for macrophages. These findings suggest potential applications in studying immune responses and developing more accurate *in vitro* models for disease research.



• Conclusions

Our findings confirmed that providing a three-dimensional environment with addition of non-synthetic polymer (silk fibroin) for cultured cells increases the physiological relevance of the *in vitro* model. This relevance can be further enhanced by adding additional factors present in the *in vivo* environment of the organism.

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