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Cell Biology

Leucine-Based Pseudo-Proteins (LPPs) and their Effect on Murine Macrophage/Monocytic Cell Lines: Copolymer *versus* Blend

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Abstract. This study aims to compare the physiological effects of copolymer film with those of a blend of homo-polymers 1L6 and 8L6 containing the same proportion of LPPs (70 mole % of homo-1L6 + 30 mole % of homo 8L6). To evaluate the biological properties of the two films, we used two murine monocyte/ macrophage cell lines: RAW264.7 and J774. Cell viability, migratory capacity, and the surface expression of CD115 were assessed in cultures grown on each film type for 24 hours. In parallel, the surface topography of both LPP films was analyzed using scanning electron microscopy (SEM). The results showed that, despite having similar chemical compositions (in terms of the quantity of elemental links 1L6 and 8L6), the two films revealed markedly different effects on cell physiology. The copolymer film demonstrated wound-healing-promoting properties, including enhanced cell proliferation, increased CD115 expression, and stimulated migration. In contrast, the polymer blend exhibited a significant cytotoxic effect and showed no notable influence on either cell migration or CD115 expression. SEM analysis revealed differences in porosity and pore size between the two films. Given the well-established influence of nano- and sub-micro-scale surface topography on cellular behavior, we speculate that these morphological differences may account for the observed disparity in biological responses. Of the two materials studied, the copolymer film shows the greatest potential as a wound healing material. © *2025 Bull. Georg. Natl. Acad. Sci.*

Keywords: pseudo-proteins, macrophages, wound-healing, RAW264.7, J774

Introduction

We have previously demonstrated that leucine-based pseudo-proteins (LPPs), when applied as films, exhibit excellent cell-supporting properties and also stimulate cell migration and proliferation (Ksovreli, 2023). Subsequently, we showed that LPP films promote wound healing in vitro: they not only accelerate the wound closure rate but also modulate the cytokine secretion profile in a manner beneficial for the healing process (Ksovreli, 2024).

Among the three types of LPPs used in our experiments, the most favorable results for wound

healing promotion were observed with the co-polymeric film (CoP), which was synthesized as a random copolymer of two homo-LPPs employed in our study: 1L6 (composed of carbonic acid, L-leucine, and 1,6-hexanediol) and 8L6 (composed of sebacic acid, L-leucine, and 1,6-hexanediol). The CoP consisted of 70 mol% 1L6 and 30 mol% 8L6 (or the chemical structure of the co-polymer and homo-polymers, see ref. (Ksovreli, 2023). As a next step, we aimed to evaluate whether a polymer blend made from the same LPPs in the same mole ratio (70% 1L6 and 30% 8L6) would exert comparable effects on cell cultures to those of the copolymer.

Materials and Methods

LPP synthesis was performed as previously described in Ksovreli, 2023. A 7% solution of each of the LPPs was prepared in absolute ethanol. Using 96-well plates, 40 µl of LPP solution was distributed per well. The wells were air-dried in a laminar flow hood. Using glass cover slips, each slip was covered with 100 µl of LPP solution and air-dried in a laminar flow hood.

Both types of cells (RAW264.7, J774) were cultured under standard conditions (37ºC and 5% CO_2) in DMEM supplemented with 10% fetal calf serum (FCS), 2 mM of L-glutamine, 50 U/ml of penicillin, and 50 µg/ml of streptomycin. At 80% confluence, the cells were harvested and seeded for the experiment on 96-well plates or coverslips which were pre-coated with LPPs or without LPP films. The cells were seeded at the concentration 25×10^4 per mL of cells.

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay (Mosmann, 1983) was performed according to the standard protocol described in (Kantaria, 2016). The absorbance at a wavelength of 450 nm was measured using a microplate spectrophotometer (ELx800 Absorbance reader, Biotek, USA).

For Scanning Electron Microscopy (SEM), samples were prepared as described in (Ksovreli, 2023). The registration of the images was performed using a JEOL JSM6510LV SEM. For the quantitative analysis of the polymers' micro- and nanopores via SEM, images were treated using ImageJ software with the initial scale bar set automatically by the JEOL SEM control software.

Analyses of CD115 surface marker expression via flow cytometry were conducted using monoclonal antibodies – anti-mouse CD115-APC (Santa Cruz Biotechnology, Inc., Germany) according to the manufacturer's standard protocol. Appropriately matched and fluorescently conjugated isotype control was also used. Flow cytometry was performed with a BD Accuri C6 (BD, USA).

For the examination of cell migration, the cells were seeded at a density of 3.5×10^5 on each cover slip, and after 24 h of incubation, time-lapse imaging was performed using a Nikon TMS inverted-phase contrast microscope (Nikon Corporation, Japan) with an AmScope MU300 digital camera (AmScope, USA), capturing a series of 60 images over a duration of 30 min. To analyze the data, "ImageJ" was utilized to convert the captured images into specialized formats, while an open-source software, "Cell Tracker v1.1.1" (Sacan, 2008), facilitated the manual tracking of the migration parameters.

Data are reported as means ± standard errors of the mean from at least three independent experiments performed in triplicate. Student's t-test was used to perform a statistical analysis of the differences among the experimental groups, and p-values < 0.05 were considered statistically significant.

Results and Discussion

The first physiological parameter evaluated in cells grown on LPP films was cell viability, assessed using the standard MTT assay. This assay reflects metabolic activity, which correlates to some extent with cell viability (Mosmann, 1983). While the film made from the polymer blend caused a decrease in cell viability, no such effect was observed with the copolymer film. In the case of RAW264.7 cells, the

copolymer even stimulated metabolic activity, whereas for J774 cells, no statistically significant difference from the control was detected.

An increase in the percentage of viable cells, as assessed by the MTT assay, is commonly interpreted as an indication of enhanced cell proliferation (Mosmann, 1983). In our previous work, we also observed increased proliferation of primary murine fibroblasts and RAW264.7 macrophages at two time points: 8 hours and 24 hours (Ksovreli, 2023) Therefore, the data obtained for RAW264.7 cells in this study are consistent with our earlier findings.

The lack of increased viability in the J774 macrophage cell line likely reflects a cell line-specific response. Based on these observations, we selected the RAW264.7 cell line for the subsequent experiments, as it appeared more responsive to the LPP substrate. Two additional parameters relevant to wound-healing were evaluated: migratory capacity (Fig. 2A) and the surface expression of CD115 (Fig. 2B).

Both LPP films showed a tendency to promote macrophage migration; however, a statistically significant increase in the total distance covered was observed only in RAW264.7 cells grown on the copolymer film. Since macrophage migration to the wound site is a critical step in the wound healing process (Landén, 2016), the copolymer's ability to enhance this migration is a valuable characteristic.

The data on CD115 surface expression also revealed differences between the two LPPs. In

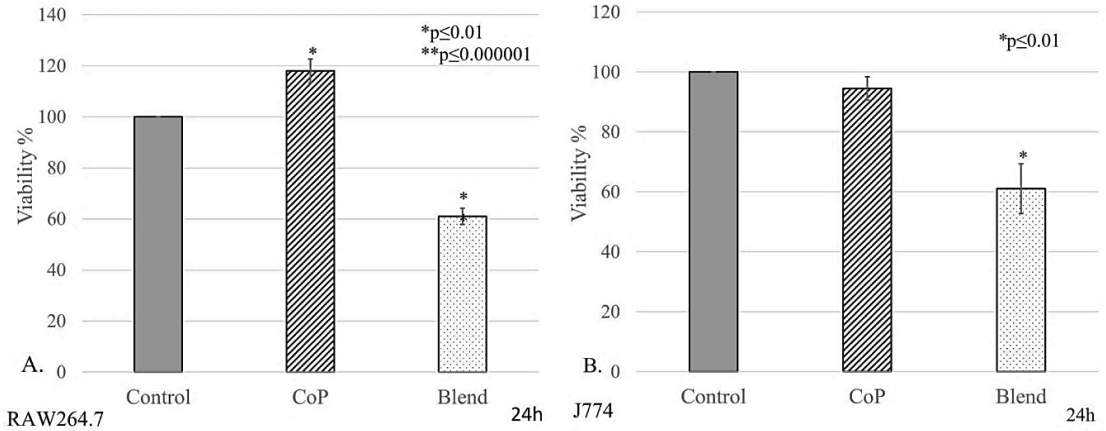

Fig. 1. Percentage of viable cells after the 24 h incubation on copolymer film (CoP) and blend film (Blend). A. RAW264.7 cells; B. J774 cells.

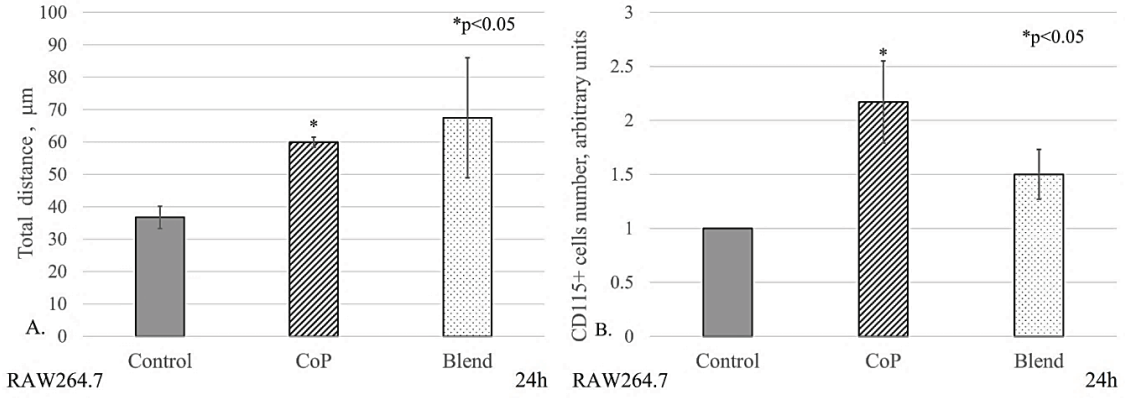

Fig. 2. Additional physiological parameters evaluated for RAW264.7: migratory capacity (A) and a surface expression of CSF-1 receptor–CD115 (B): A. Total distance (in µm) covered during 30 min after 24h growth on LPP films; B. CD115+ cells number after 24h growth on LPP films.

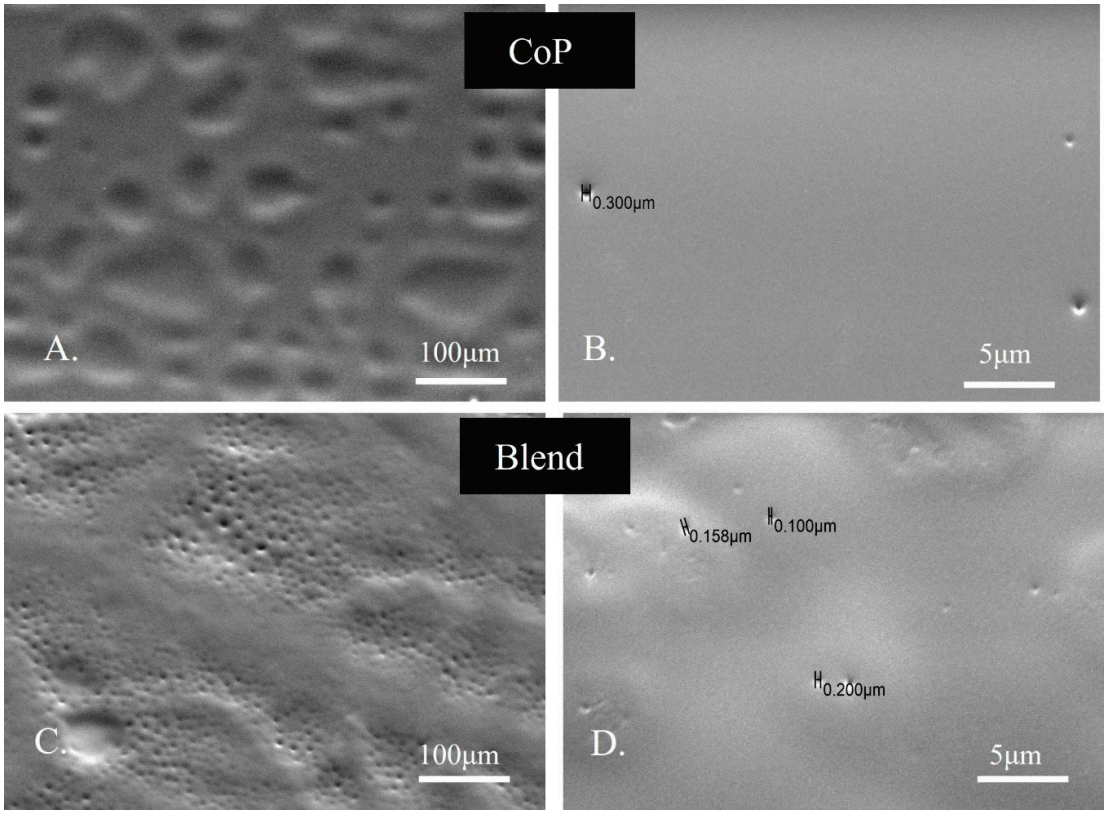

Fig. 3. LPP surface characterizations via SEM. Representative SEM images of the LPPs' surfaces: (A,C) ×200; (B,D) ×4000; (A,B) copolymer; (C,D) blend.

RAW264.7 cells, CD115 expression was significantly increased only in response to the copolymer film. CD115 is a receptor for colony-stimulating factor 1 (CSF-1), which is produced by fibroblasts. The CD115–CSF-1 signalling axis mediates macrophage–fibroblast interactions at the wound site. Enhanced CD115 surface expression facilitates more effective communication between these cell types, contributing to the wound healing process. Thus, the observed increase in CD115 expression further supports the wound healing potential of the copolymer LPP.

To explain the differences in cellular responses to the two LPP films, we analysed their surface topologies using SEM (Fig. 3A–D), evaluating both pore size and pore density (number of pores per µm²). The quantitative data are summarized in the Table.

As shown, despite having similar chemical compositions (in terms of the quantity of elemental links 1L6 and 8L6), the two LPP films differ markedly in their sub-micrometre surface topologies. It is well established that surface characteristics at the nano- and sub-micro levels can influence various aspects of cell physiology by modulating adhesion complexes. These, in turn, affect cytoskeletal dynamics, nuclear lamina structure, and ultimately gene expression – impacting cell proliferation, migration, activation state, and cytokine production (Rausch, 2021). We therefore speculate that differences in surface morphology account for the disparity in biological responses observed between the two LPP films.

Table. The LPP films' surface porosity values, measured at a sub-micro level. SEM images were analyzed using ImageJ software

	Number of pores per 1²µm	Pore size
Copolymer	0.2±0.05	0.181±0.021
Blend	1.88±0.5	0.171±0.031

Conclusion

In summary, of the two materials studied, the copolymer film demonstrates superior potential as a wound-healing material due to its ability to enhance cell proliferation, migration, and the communication between macrophages and fibroblasts.

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უჯრედული ბიოლოგია

ფსევდო-პროტეინები ლეიცინის (ფპლ) საფუძველზე და მათი ეფექტი მაკროფაგ/მონოციტურ უჯრედულ ხაზებზე: თანაპოლიმერი *versus* პოლიმერული ნარევი

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დადგენილია, რომ ლეიცინის საფუძველზე შექმნილი ფსევდო-პროტეინული (LPP) ფირები წარმოადგენს პერსპექტიულ მასალებს ჭრილობების მოვლის გაუმჯობესებისთვის. კვლევის შედეგად მიღებული მონაცემების თანახმად, სამ სხვადასხვა LPP ფირს შორის თანაპოლიმერის ფირი (რომელიც შედგება 70 მოლ% 1L6-ისა და 30 მოლ% 8L6-გან) არა მხოლოდ აჩქარებდა ჭრილობების დახურვას, არამედ ხელს უწყობდა რეგულირებულ შეხორცების პროცესს. კვლევის მიმდინარე ეტაპზე გადავწყვიტეთ შევადაროთ თანაპოლიმერის ფირის ეფექტი უჯრედების ფიზიოლოგიაზე მსგავსი ქიმიური შემადგენლობის მქონე პოლიმერული ნარევისგან მიღებული ფირის ეფექტს: 70 მოლ. % 1L6 + 30 მოლ. % 8L6. LPP ფირების თვისებების შესაფასებლად გამოყენებულ იქნა თაგვის მონოციტ-მაკროფაგული უჯრედების ორი ხაზი: RAW264.7 და J774. დავაკვირდით უჯრედების სიცოცხლისუნარიანობას, მიგრაციულ უნარებს და CD115-ის ზედაპირულ ექსპრესიას, როგორც თანაპოლიმერის, ისე ნარევის ფირებზე გაზრდილ უჯრედებში. ორივე ტიპის ფირის ზედაპირის ტოპოგრაფია შეფასდა მასკანირებელი ელექტრონული მიკროსკოპიით (SEM). მიღებული შედეგების მიხედვით, მიუხედავად ქიმიური შემადგენლობის მსგავსებისა (ელემენტური რგოლების 1L6 და 8L6 რაოდენობის თვალსაზრისით), ორივე ფირი უჯრედების ფიზიოლოგიაზე არსებითად განსხვავებულ გავლენას ახდენს. თანაპოლიმერის ფირი იწვევს დადებით რეაქციას – სტიმულირდება

უჯრედების პროლიფერაცია და CD115-ის ექსპრესია, აგრეთვე იზრდება მიგრაციის უნარი. ამასთან, ნარევის ფირი გამოხატავს მნიშვნელოვან ციტოტოქსიკურ ეფექტს, არ შეინიშნება უჯრედების მიგრაციის ან CD115-ის ექსპრესიის მნიშვნელოვანი ცვლილება. SEM-ანალიზმა ფირების ზედაპირზე გამოავლინა ფორების რაოდენობისა და ზომის განსხვავება. ცნობილია, რომ ზედაპირის ტოპოლოგია ნანო- და სუბ-მიკრო დონეზე მნიშვნელოვნად მოქმედებს უჯრედების ფიზიოლოგიაზე, შესაბამისად, ვვარაუდობთ, რომ უჯრედებზე ფირების ზემოქმედების განსხვავება შეიძლება აიხსნას სწორედ ამ ტოპოგრაფიული მახასიათებლების სხვაობით. შედეგად, შეგვიძლია დავასკვნათ, რომ ორი შედარებული ფირიდან თანაპოლიმერის ფირი წარმოადგენს უფრო პერსპექტიულ მასალას ჭრილობების შეხორცების ხელშესაწყობად.

REFERENCES

Buechler, MB., Fu, W., & Turley, SJ. (2021). Fibroblast-macrophage reciprocal interactions in health, fibrosis, and cancer. *Immunity, 54*(5), 903-915.

Kantaria, T., Kantaria, T., Kobauri, S., et al. (2016). Biodegradable nanoparticles made of amino-acid-based ester polymers: Preparation, characterization, and in vitro biocompatibility study. *Appl. Sci., 6*, 444.

Ksovreli, M., Kachlishvili, T., Mtiulishvili, T., et al. (2023). Leucine-based pseudo-proteins (LPPs) as promising biomaterials: A study of cell-supporting properties. *Polymers,* 15, 3328.

Ksovreli, M., Kachlishvili, T., Skhvitaridze, M., et al. (2024). Wound closure promotion by leucine-based pseudo-proteins: An in vitro study. *Int. J. Mol. Sci., 25*, 9641.

Landén, N.X., Li, D., Ståhle, M. (2016). Transition from inflammation to proliferation: A critical step during wound healing. *Cell. Mol. Life Sci., 73*, 3861-3885.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods, 65*, 55-63.

Rausch, M.A., Shokoohi-Tabrizi, H., et al. (2021). Impact of implant surface material and microscale roughness on the initial attachment and proliferation of primary human gingival fibroblasts. *Biology, 10*, 356.

Sacan, A., Ferhatosmanoglu, H., Coskun, H. (2008). Celltrack: An open-source software for cell tracking and motility analysis. *Bioinformatics, 24*, 1647-1649.

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