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PREFACE

Dear Readers, Authors, Reviewers,
Members of the Scientific Committee and Section Editors,

In the last months, I once more realized that being the Editor-in-Chief of "Polimery w Medycynie – Polymers in Medicine" is not merely a function, a role, but an obligation. A commitment to people who are not with us anymore, but whose work and ideas we took up; we are developing them and adjusting them to the challenges of the 21st century – the founders of our journal.

On December 9, we celebrated the 50th anniversary of the journal – and we learned that the history of this idea is even longer, dating back to 1964. In the former times, the journal played a different role, offering access to information on the advances of polymer sci-ence in the world which would not be available from other sources. Today, knowledge is accessible instantly in Internet, but our part is no less important: we disseminate latest developments in research on the use of polymers in medicine, striving at the highest possible level of scientific and editorial professionalism.

The meeting on December 9 was a celebration among friends – we talked about our history and perspectives for the future with those who joined us in one of the lecture halls at the the Faculty of Pharmacy of Wroclaw Medical University, while many others who could not be present in person participated online. The pandemic can prevent us from meeting face to face, but it will not stop us in our efforts to provide quality scientific papers. To meet this end, we invited researchers from Poland and abroad to the Scientific Committee of the journal, and enlisted several young researchers of considerable experience and skills to help us as section editors. At the same time, our editorial staff implemented strict rules regarding due diligence in editorial and linguistic aspect of manuscript preparation. These efforts were noticed and appreciated by the Polish Ministry of Education and Science, which increased the number of points assigned in the process of journal evaluation from 20 to 70. This is just the beginning – we aim at making "Polimery w Medycynie – Polymers in Medicine" a publication platform more and more valued by researchers around the world. Such platform must be interdisciplinary – and it truly is: our successes would not be possible without cooperation with Wroclaw University of Science and Technology, represented by our Deputy Editor-in-Chief, Konrad Szustakiewicz, PhD, DSc., Eng.

Concluding, we want wish you a merry Christmas and a happy New Year – whether this part of the year is snowy, rainy or hot where you are now. We have four specific wishes: as many high-quality research published in polymer science as possible, successes and joy in your research work, inspiring and supportive people met on the way, and finally – control over the global pandemic, or at least a perspective for it.

Editor-in-Chief
Prof. Witold Musiał

Characterization and statistical optimization of γ-PGA produced by *Bacillus megaterium* UP47 isolated from *Pentaclethra macrophylla*

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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Abstract

Background. Gamma-polyglutamic acid (γ-PGA) is a microbially produced non-toxic peptide biopolymer which is gaining grounds in many biotechnological fields and has a wide range of applications.

Objectives. In this study, the characteristics of γ-PGA produced by *Bacillus megaterium* isolated from an oil seed were determined, while the nutritional requirements of the bacterium were optimized using a predictive 15 factor-16 run Plackett–Burman experimental design.

Materials and methods. The main effect of each factor, the interaction and quadratic effects of the factors on optimized production were determined from Box–Benkhen model using Dell Statistica v. 12 and 13 software. *Bacillus megaterium* UP47 produced the highest γ-PGA (16.33 g/L) out of 56 spore-forming *Bacillus* strains isolated from soil, water and fermented food samples.

Results. Hydrolysates of the produced γ-PGA had a retention factor which corresponded to the L-glutamic acid standard (retention factor (rf) 0.35), while high-definition fourier transform infrared (FT-IR) spectroscopic imaging showed characteristic peaks representative of the active bonds present in γ-PGA. The γ-PGA at a concentration as low as 50 mg/100 mL exerted antimicrobial inhibitions against test pathogens. A 2.00 w/v γ-PGA solution had 11 mm and 13 mm inhibition zones against *Staphylococcus aureus* and *Shigella dysenteriae*, respectively. A second order polynomial equation for prediction of γ-PGA was derived as:
γPGA yield = 3316.061 − 449.708A + 9.036A2 − 139.813B + 3.095B2 − 7.699C − 0.164C2 + 13.116AB − 0.087AB2 − 0.248A2B + 3.781AC − 0.076A2C − 0.394BC.
It showed an increase in γ-PGA yield with increasing L-glutamic acid and biotin, but a decrease with yeast extract.

Conclusions. *Bacillus megaterium* UP47 had a maximum γ-PGA yield of 54 g/L and 62 g/L, respectively, from the Plackett–Burman and Box–Benkhen design, thereby resulting in an appreciable increase in polymer yield after the optimization process with a 95% confidence level.

Key words: poly γ-glutamic acid, optimized production media, polymer characterization, precursor requirements, antimicrobial potentials

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Background

During the growth process of microorganisms, complex metabolic processes result in the production of natural polymers known as biopolymers. These materials include, among the others, exopolysaccharides and polyglutamic acid.[1,2] Poly-gamma-glutamic acid (γ-PGA) is a water-soluble, biodegradable, non-immunogenic, non-toxic, and unusual anionic homopolyamide and/or homopolypeptide made up of D- and L- α-amide-linked polymerized units of glutamic acid.[3–5] Figure 1A shows the structure of γ-PGA. The production of γ-PGA is influenced by different nutritional requirements of the γ-PGA-producing bacteria.[6,7] While some of these bacteria do not require glutamic acid, others grow only in its presence or require biotin.[7] The characteristics of γ-PGA biopolymer which have been exploited in its applications include its anionic, biodegradable and water-soluble attributes. What is of a great importance is the fact that γ-PGA biopolymer is edible and non-toxic to humans and the environment.[8,9] Other relevant applications of γ-PGA included their potential to thicken food, relieve bitterness in drugs, use as cryoprotective materials, in metal absorption and dye removal.[2] Reported to have a 'Generally Regarded as Safe' status (being a naturally derived food grade material),[10,11] γ-PGA is also documented to have antimicrobial characteristics.[12] Balogun-Agbaje et al. reported the antifungal activities of γ-PGA-based nanoparticles.[5] The antimicrobial potential of the γ-PGA was therefore investigated in this work to ascertain the possible suitability of the produced γ-PGA in applications such as the medical, agricultural, food, pharmaceutical, and wastewater treatment industries.

Experimental design techniques based on predictive models present more evenhanded alternative to one-variable-at-a-time (OVAT) approach for fermentation improvement in laboratory-based experimentations, such as γ-PGA production, considering the effects of time, cost and labor involved in the traditional approach.[13] In present-day biotechnology, experimental designs such as the Plackett–Burman design can be used if it is desired to screen a large number of factors (with as limited experimental run as possible) to reduce and/or identify the number of factors down to the key role-playing variables.[14–16] Furthermore, an economic factorial experimental design, the Box–Behnken model, can be used to determine the relationship between the response function and the experimental variables enlisted.[17]

Microbial γ-PGA production faces limitations due to a high production cost, low yield, diverse nature of media composition, and unique individual requirements of the producing microorganisms.[11] Since the use

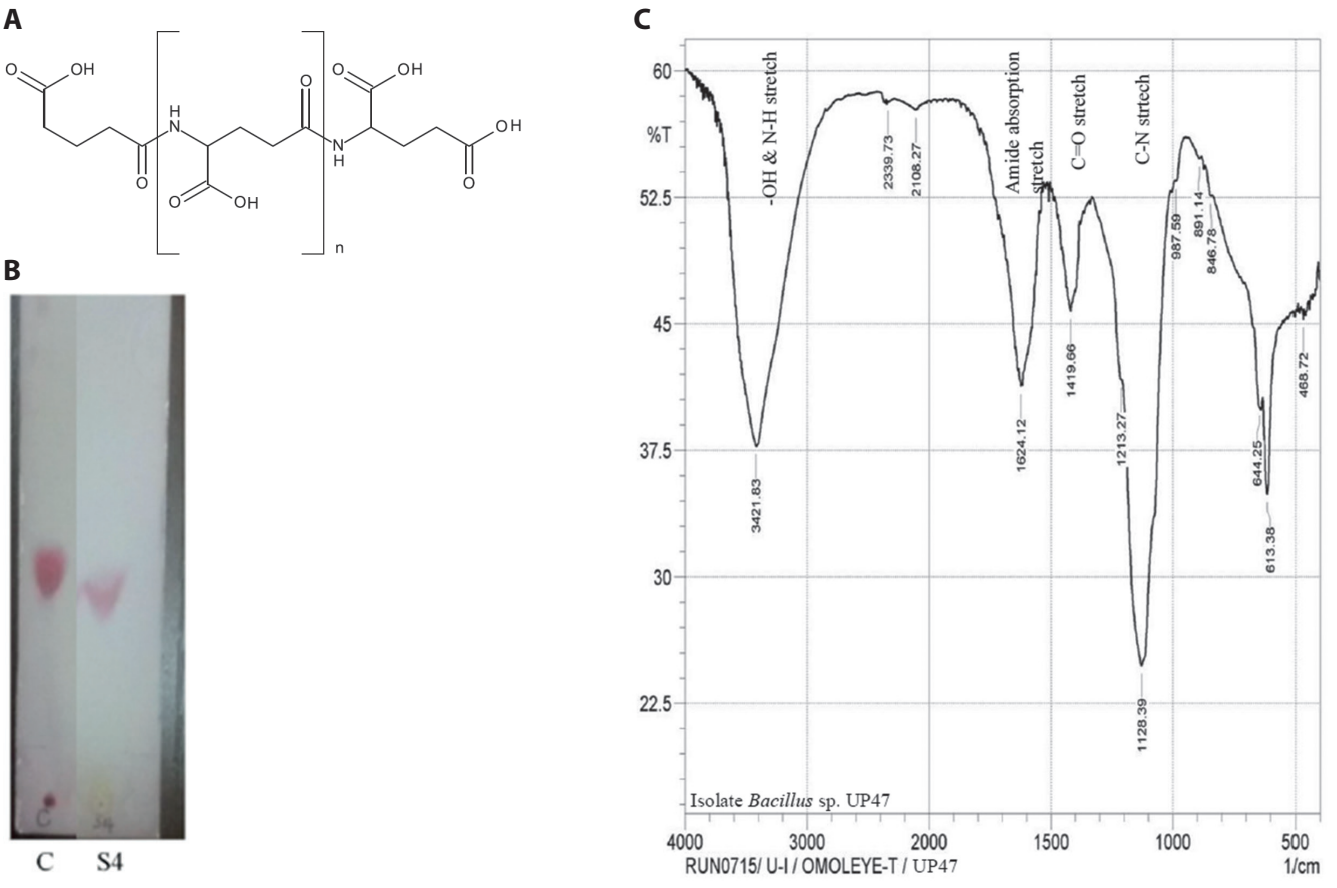

Fig. 1. Chemical structure of gamma-polyglutamic acid (γ-PGA) (A), and thin layer chromatographic (B) and spectroscopic characteristics (fourier transform infrared (FT-IR)) (C) of *Bacillus megaterium* UP47-produced γ-PGA

of predictive model provides a fast, reliable, labor-efficient, and relatively cost-effective way to obtain laboratory-based experimentation in product optimization,[18] it is expected that statistical models towards γ-PGA production could also lead to answers for cost-effective production of γ-PGA and other production problems of time consumption, unreliability and laboriousness. Hence, this work investigated the abilities of diversely sourced *Bacillus* species to produce γ-PGA, the characteristics and antimicrobial potentials of the produced polymer, and a statistical optimization of media components towards γ-PGA production.

Materials and methods

Media preparation and isolation of *Bacillus* species

All media, chemicals and reagents used were of analytical grade, and they were prepared and used according to the manufacturers' instructions. The enlisted γ-PGA production medium was the modified γ-PGA production medium (PPM) proposed by Bajaj and Singhal[18] and composed of (g/L): L-glutamic acid (20), NH_4Cl (6), citric acid (12), NaCl (25), KCl (0.66), $MgSO_4.7H_2O$ (6.8), $CaCl_2.2H_2O$ (0.2), $NaHCO_3$ (0.18), $MgCl_2.6H_2O$ (4.7), $MnSO_4.7H_2O$ (0.05), K_2HPO_4 (2), and glycerol (25 mL/L). Bacteria were isolated from samples of a laboratory fermented *Pentaclethra macrophylla* (*Ugba*) seeds, while soil and water samples were collected from 3 engine oil-contaminated sites, a maize rhizosphere, 2 domestic effluents, and 2 kettle/pot rinses. All samples (10 g or 10 mL) were heated in a boiling GFL 1083 shaker water bath (GFL Technology, Senden, Germany) for 15 min to kill off non-sporulating microbial cells and thus enhance the isolation of more *Bacillus* species, mixed with 90 mL sterile distilled water, vortexed vigorously and serially diluted. Different dilutions (1 mL) from each sample were aseptically transferred into 90 mm sterile disposable Petri dishes, plated out on nutrient agar and solidified PPM agar, and incubated at 30°C for 24–48 h.[19] Morphologically distinct bacteria from inoculated Petri dishes were subcultured onto nutrient agar plates to obtain pure cultures. The pure cultures were stored on nutrient agar slants in a refrigerator and in 25% glycerol nutrient broth at –20°C.

Screening and selection of *Bacillus* species for γ-PGA-producing potentials

The isolates obtained from the different samples were primarily screened for γ-PGA production by checking for slime-producing abilities using a sterile toothpick to touch the bacterial colony and then gently drawing upwards. The length of the stretched thread is regarded as an indication of an isolate's ability to produce more γ-PGA.[20,21]

Physiological and biochemical identification of *Bacillus* spp.

The identification of selected γ-PGA-producing strains was carried out using conventional methods of morphological and biochemical characterization, including Gram staining, spore staining, catalase reaction, oxidase test, starch hydrolysis, casein hydrolysis, citrate utilization, gelatin liquefaction, urease test, methyl red test, H_2S production, motility, growth at 6.5% and 10% NaCl, as well as carbohydrate fermentative abilities on sucrose, D-glucose, lactose, fructose, galactose, maltose, mannitol, xylose and sorbitol.[22,23]

γ-PGA production, extraction, purification, and quantification

The most promising γ-PGA producing bacterium was used in further γ-PGA production. The modified PPM medium (25 mL) was sterilized and inoculated with 10^8 CFU/mL of the selected bacterium. It was fermented at 30°C and 180 rpm for 72 h. After fermentation, the microbial cells were harvested by centrifuging the fermentation broth at 5000 rpm and 4°C for 15 min using an MSE High Speed 18 Centrifuge (MSE Ltd., Birmingham, UK). The γ-PGA was extracted from the supernatant using the ethanol precipitation method.[2] Briefly, the centrifuged cell-free broth was decanted into 3 volumes of cold ethanol, held at –4°C overnight and centrifuged again. The process was repeated 4 times to ensure increased purity of the extracted γ-PGA. The extracted γ-PGA was dried and weighed to determine the production in g/L.[24]

Characterization of the produced γ-PGA polymer

Ultraviolet spectroscopic analysis of the produced γ-PGA

The cell-free γ-PGA production broth was subjected to spectrophotometric analysis by reading the absorbance of the produced solutions between 190 nm and 900 nm, using Perkin Elmer Lambda 25 UV/Vis Spectrophotometer (Waltham, USA).[25] Isolates with the highest absorbance readings within 190 nm and 230 nm were expected to have higher γ-PGA producing abilities and were selected for further experiments.

Thin layer chromatographic analysis of the produced γ-PGA

The extracted and dried γ-PGA polymers were hydrolyzed in 7.5 M HCl and maintained in an autoclave at 121°C for 2 h 30 min. Thereafter, the acid-hydrolyzed mixtures were neutralized with 7.5 M NaOH and subjected to thin layer chromatography. The neutralized hydrolysates

were chromatographed against L-glutamic acid (Sigma-Aldrich, St. Louis, USA) as control, using ethanol-water (7:3) as the solvent system. After separation, the plates were left to dry and then, they were sprayed with 0.2% ninhydrin-acetone solution to detect the hydrolyzed amino acids bands. The retention factor (Rf) was determined using Equation 1 below.

$$Rf = \frac{\text{distance moved by spot}}{\text{distance moved by solvent front}} \quad (1)$$

The alignment of the emerging bands from the γ-PGA hydrolysates was compared with that of the L-glutamic acid standard.[21,26]

Fourier transform infrared spectroscopic analysis of the produced γ-PGA

The produced polymer was analyzed using a Shimadzu FT-IR Spectrophotometer (Shimadzu Corp., Kyoto, Japan) with 45 scans, 4 cm^{-1} resolution and Happ–Genzel apodization for its characteristic peaks and bonds. Distinctive strong and weak absorption peaks at different wavelengths characteristic of γ-PGA in the given samples were determined.[27,28]

Antimicrobial potentials of the produced γ-PGA using agar well diffusion assay

To determine the potential of the purified γ-PGA in industrial applications, the γ-PGA was used in the preparation of aqueous solutions at varying concentrations ranging from 0.5 mg/mL to 20.0 mg/mL. The appropriate concentrations of the γ-PGA biopolymer were dissolved in sterile water and their antimicrobial potential against selected laboratory stock cultures of indicator bacteria was determined. Ciprofloxacin (30 µL of a 5% concentration) was used as the control. The stock cultures of *Shigella dysenteriae*, *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli*, and *Bacillus cereus* were reactivated by subculturing from their agar slants and used as agar lawn inoculum for the agar well diffusion assay. Solidified Petri dishes containing Mueller–Hinton agar were swabbed with cell suspensions of 0.5 McFarland standard of each of the test pathogen preparations, and wells with 7 mm in diameter were bored into these swabbed plates with the aid of sterile cork borers. The different γ-PGA concentrations, as 30 µL volumes, were introduced into the agar wells and plates and were incubated overnight at 37°C. The diameter zones of inhibition exerted by the different concentrations on the test pathogens were measured and recorded in millilitres.[29] A positive inhibition was recorded as ≥2 mm inhibition zone.[30]

Optimization of γ-PGA production parameters

Media amendments and precursor requirement by selected isolate for γ-PGA production

The isolate with the best γ-PGA producing potential was cultivated in a fermentation medium supplemented with different precursors – biotin, monosodium glutamate and L-glutamic acid – using 5 media variations labelled A–E. Medium A was the γ-PGA production medium (PPM), medium B contained PPM with additional 100 µg/L biotin, medium C was PPM plus 15 g/L monosodium glutamate, while medium D was composed of PPM with biotin (100 µg/L) and monosodium glutamate (15 g/L). Medium E was composed of medium D devoid of L-glutamic acid. The fermentation setups were incubated at 30°C for 72 h at 180 rpm. The best precursors for γ-PGA production were thereafter selected.

Identification and optimization of significant factors for γ-PGA production using Plackett–Burman and Box–Behnken statistical models

An experimental design with 15 factors and 16 runs (Table 1) was set up to screen for the most important variable component that influenced γ-PGA production

Table 1. Base design for the 15 factor-16 run Plackett–Burman first order experimental design for gamma-polyglutamic acid (γ-PGA) production

Factor [units]	Code	Levels		Factor [units]	Code	Levels	
		–1 (low)	+1 (high)			–1 (low)	+1 (high)
Glycerol [mL/L]	X1	20	30	Biotin [µg/g]	X2	75	125
Glucose [g/L]	X3	25	75	L-glutamic acid [g/L]	X4	15	25
Ammonium chloride [g/L]	X5	4	8	Citric acid [g/L]	X6	9	15
NaCl [g/L]	X7	20	30	$MgSO_4.7H_2O$ [g/L]	X8	5.1	8.5
$CaCl_2.2H_2O$ [g/L]	X9	0.1	0.3	Yeast extract [g/L]	X10	2.5	7.5
Casein hydrolysate [g/L]	X11	15	25	Dummy*	X12	–	–
Corn steep liquor [mL/L]	X13	25	75	K_2HPO_4 [g/L]	X14	1	3
Inoculum size (McFarland)	X15	0.5	1.5	–	–	–	–

*X12 is an independent dummy factor; –1 indicates low value, whereas +1 indicates high value.

using Plackett–Burman experimental design[14,15] based on the first order model (Eq. 2):

$$Y = \beta_0 + \Sigma\beta_i X_i \quad (2)$$

where Y is the response (PGA yield), β_0 is the model intercept, β_i is the linear coefficient (both are constant coefficients), and X_i is the level of the independent variable.

Two factor levels (low (–) and high (+)) were considered for each independent nutritional variable in which the rows represented the trials and the columns represented the variable-independent factor. A dummy factor, whose effect on the experimental setup was not determined, was also included in the model, raising the number of trials under study from *n* to *n + 1*.

The factors that had an effect on γ-PGA yield at 95% confidence level were selected and analyzed at 3 levels (low, mid and high: –1, 0 and +1, respectively) in a Box–Behnken experimental design.[11,17] Additionally, some central points with factors fixed at their mid-levels were incorporated. The main effect of each factor, as well as the interaction and quadratic effects of the factors for the prediction of experimental yield on optimized production, were determined using Dell Statistica v. 12 and 13 (Dell Computer Corporation, Austin, USA). Based on the interaction analysis, the response surface graph of the interactions of factors against yield was plotted.[32] Some experimental trials were run with different concentrations of the factors in a scale-up experiment using increasing production volumes of 0.25 L, 0.5 L, 1.0 L and 3.0 L, to determine if the yield was commensurate with the predicted yield using the optimized medium derived for γ-PGA production. The predicted yield was calculated using the derived regression equation and compared with the laboratory experimental results. The confidence level was analyzed to determine the good fit of the equation.[17,31]

Results and discussion

From the 11 samples analyzed, 56 morphologically distinct isolates were obtained: 32.14% were isolated from the soil samples, 28.57% from the fermented food samples, 23.21% from domestic effluents, and 16.07% from the kettle/pot rinses (Table 2). Many γ-PGA producing bacteria have been recovered from diverse sources. Baxi reported the isolation of the bacteria from soils of various geographical locations, fermented flours/beans and industrial wastewater, domestic sewage, and sea water.[33]

Viscous colonies displayed mucoid consistencies which was an indication that a polymeric substance, possibly poly-γ-glutamic acid, had been produced.[34] The percentage of presumptive γ-PGA-producing isolates was low (16.1%), similarly to the reports of Baxi[33] and Ju et al.,[4] who recorded a 12.5% and 4.61%, respectively, of the isolates which possessed colonial characteristics and were similar to that of γ-PGA producers. The ultraviolet (UV) absorbance values of the cell-free γ-PGA solution ranged between 3.3 and 3.55, of which isolate UP47 had the highest absorbance, followed by isolates DS1, HE21 and EK31, respectively. The absorbance reading completely flattened out beyond 233 nm, and thus the isolate UP47 was selected for further experiments. Zeng et al. reported a UV spectrometric and high-performance liquid chromatography (HPLC) correlation of the quantification of γ-PGA at a best wavelength of 216 nm.[25]

Table 2. Colony count and screening of gamma-polyglutamic acid (γ-PGA)-producing bacteria from the analyzed samples

Sample	Sample code	CFU/mL* and number of presumptively positive γ-PGA-producing bacteria compared to selected distinct colonies		Isolate codes of selected γ-PGA producing bacteria and the corresponding absorbance for peak wavelength between 190 nm and 233 nm	
		CFU/mL	positive γ-PGA bacteria fraction	code	absorbance/γ-PGA [g/L]
Oil drainage soil site 1	DSI	1.3×10^6	1/3	DS01	3.540/11.67
Oil drainage soil site 2	DSII	1.5×10^6	1/4	DS05	3.445/7.63
Oil drainage soil site 3	DSIII	7.5×10^5	0/6	–	–
Maize farm soil	MFIV	2.4×10^7	1/5	MF10	3.435/8.67
Hostel domestic effluent	HEVI	5.4×10^6	1/3	HE21	3.502/12.00
Residential apartment domestic effluent	REIX	8.9×10^6	0/10	–	–
Pressure pot rinse water	PPVII	1.1×10^7	1/5	PP26	3.486/7.33
Electric kettle rinse water	EKVIII	7.1×10^5	1/4	EK31	3.496/12.33
Iru	IRV	2.8×10^6	2/6	IR15, IR18	3.372/8.00 3.464/10.00
Ugba fermented in plantain leaves	UPXI	1.1×10^7	1/5	UP47	3.547/16.33
Ugba fermented in aluminum foil	UAX	1.0×10^7	0/5	–	–
Bacteria ratio	–	–	9/56	–	–

* results/values are mean of triplicate readings.

Morphological and biochemical characteristics of selected isolates

Morphologically, the isolate UP47 colony was creamy, circular, mucoid, large, raised, opaque, Gram-positive rod, and a spore former. The bacterium was positive for catalase, oxidase, casein, gelatin, glucose and sucrose utilization, but negative for citrate utilization, starch hydrolysis, urease and gas production, and identified as *Bacillus megaterium* UP47. The bacterium was able to survive at high saline concentrations up to 10%. Choi et al. isolated slime-producing spore-forming bacilli from homemade Cheonggukjang for γ-PGA production,[20] and Hezayen et al. reported that most γ-PGA producing strains possessed the ability to grow in high saline conditions and thus survive hostile environments.[35]

Characterization of purified γ-PGA

The produced γ-PGA polymer was powdery and creamy-white. Thin layer chromatography of the *B. megaterium* UP47 γ-PGA hydrolysate revealed a single band with similar Rf values (0.32) as the L-glutamic acid standard (0.35), thus suggesting that the hydrolyzed polymer, reflecting a single spot, was probably composed of only glutamic acid subunits (Fig. 1B).

The active bonds of the γ-PGA polymer produced by *B. megaterium* UP47 corresponded with those found in γ-PGA (Fig. 1C). Produced γ-PGA had characteristic strong C–N group absorption peaks between 1085 cm^{-1} and 1165 cm^{-1}, weak carbonyl C=O absorption at ≈1390–1450 cm^{-1}, strong amide absorption at ≈1600–1660 cm^{-1}, characteristic aliphatic N–H stretching between 2800 cm^{-1} and 2900 cm^{-1}, and a strong hydroxyl OH absorption at ≈3400–3450 cm^{-1}. Similar findings were reported by Bhat et al.[3] and Khalil et al.[12]

The results of the bacterial inhibition exerted by the *B. megaterium* UP47 γ-PGA (Fig. 2) were an indication of its usefulness for possible applications in relevant fields. The *B. megaterium* UP47 γ-PGA showed the evidence of bacterial inhibitory activities against both Gram-positive and Gram-negative bacteria in the agar well diffusion assay. The γ-PGA concentration as low as 0.5 mg/mL was successful in exerting inhibitory activities against overnight cultures of *S. dysenteriae*, *S. aureus, P. aeruginosa* and *E. coli. Klebsiella pneumoniae* was inhibited by the γ-PGA concentration ≥1.0 mg/mL. Increasing concentrations of γ-PGA (≤20.0 mg/mL) also exerted inhibitory activity, which was most pronounced against *S. dysenteriae* (13 mm). The polymer at ≤20.0% was completely unable to exert any inhibitory effect on the *B. cereus* culture used in this study, while the effect of the control antibiotic on this bacterium was also low (3 mm). The microbial inhibitory activities reported in this work occurred with the use of very low γ-PGA concentrations. However, Lee et al. reported positive microbial inhibition by γ-PGA-adsorbed discs at 1% concentration.[36] In this study, there was a more pronounced inhibition of *S. aureus* and *S. dysenteriae* by the polymeric γ-PGA, albeit, lower than recorded using the control antibiotic. Su et al. also reported higher antibiotic activity on tested strains compared to the γ-PGA used.[37] Ajayeoba et al. reported inhibitory activities ranging between 16.6 mm and 22.5 mm on *S. aureus,* but higher concentrations of γ-PGA were used (150 mg/mL) in contrast to what was obtained with 20.0 mg/mL concentration of the *B. megaterium* UP47 γ-PGA in this work (10 mm).[38] The inhibitory activities of the γ-PGA on tested bacteria might be due to the nature and source of the specific microbes and the anionic nature of the γ-PGA, which allows it to possibly bind with materials on the bacterial cell wall and disrupt the cell content.[38,39] The bacterium could also induce stress responses to protect itself against the antimicrobial substance. Hence, increasing γ-PGA concentrations beyond 2.0% might give better antibacterial characteristics than those recorded in this research, especially against *S. dysenteriae* and *S. aureus* strains.[38]

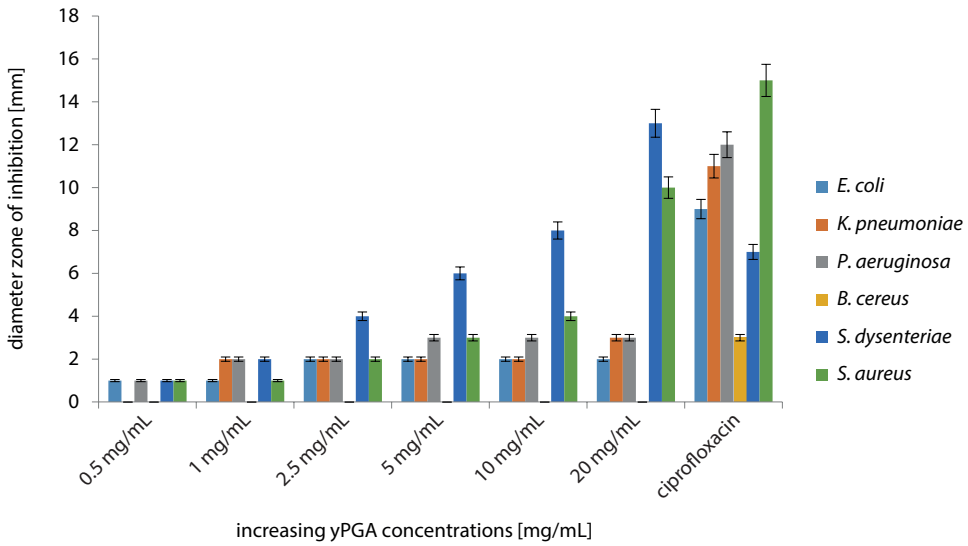

Fig. 2. Antibacterial potentials of 0.5–20 mg/mL concentrations of *Bacillus megaterium* UP47 gamma-polyglutamic acid (γ-PGA) against different test pathogens

The negligible effect of the *B. megaterium* UP47 γ-PGA observed against the tested *B. cereus* was similar to that reported by Ajayeoba et al.[38] and is suggested to be due to the biofilm-forming abilities of *B. cereus* through which it creates fibrous amyloid-like networks, which assemble structural proteins to form hydrophobic envelopes limiting the inhibitory potentials of the γ-PGA.[40]

B. megaterium UP47 precursor requirement for γ-PGA production

A precursor requirement for γ-PGA production is an important factor to be considered when screening for isolates suitable for use in a large scale production. The Randomized Complete Block Design (RCBD) showed that there was no significant difference in the production of γ-PGA by the selected *Bacillus* spp. ($p = 0.8276$), whereas in the treatment data a significant difference ($p = 5.1609$) was observed in the production of γ-PGA in 5 different media (data not shown). Analysis of variance (ANOVA) showed that the most significant difference in γ-PGA production was between media A and E and media B and E, implying that both media A and B contained components (L-glutamic acid and biotin, respectively) which greatly enhanced γ-PGA production. Thus, the *B. megaterium* UP47 was biotin- and glutamic acid-dependent. The production of γ-PGA by *Bacillus* spp. SW1-2 was enhanced by glutamic acid[13] while Goto and Kunioka reported a biotin-dependent γ-PGA producing *Bacillus subtilis* IFO 3335 which would not be produced in the absence of L-glutamic acid.[6]

Significant factors for γ-PGA production based on the Plackett–Burman analysis

The greater the coefficient, the greater the impact of a given factor on production; thus, a factor with a coefficient value close to 0 has very little impact on the production, while a factor with a high value coefficient has a large impact on the production. The optimum media composition for γ-PGA production with *B. megaterium* UP47 determined using the 15 factor-16 run Plackett–Burman analysis is shown in Table 3 and Table 4, respectively.

From the laboratory fermentation process, the highest γ-PGA yield in the experimental runs *B. megaterium* UP47 (54 g/L) was observed in run order D8 containing (g/L): glycerol (30), glucose (25), L-glutamic acid (25), ammonium chloride (4), citric acid (9), NaCl (20), $MgSO_4.7H_2O$ (8.5), $CaCl_2.2H_2O$ (0.3), yeast extract (7.5), casein hydrolysate (15), K_2HPO_4 (3), corn steep liquor (75 mL), inoculum size (0.5), and biotin (75 μg), respectively (Table 3). The lowest γ-PGA yield was observed in run order D16, which had all factors at their low levels.

The confidence level of all considered factors ranged between 80.94% and 98.00% for *B. megaterium* UP47 (Table 4). Factors X5, X6, X7, X13, X14 and X15 had negative effects on γ-PGA production by *B. megaterium* UP47; thus, a decrease in the concentration of these factors brought about an increase in production. The least yield recorded in run D16 agreed with the findings of Bajaj and Singhal.[18] Precursor factors (glucose, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$ and K_2HPO_4) included into our Plackett–Burman analysis were also considered by other authors.[13,15,18] While glucose had a positive effect on γ-PGA production by *B. megaterium* UP47, we also observed, in agreement with other authors,[13,15,18] its negative effect on γ-PGA yield. The $MgSO_4.7H_2O$ also had a positive effect on γ-PGA production by *B. megaterium* UP47, a finding corroborated by other publications.[13,15,18]

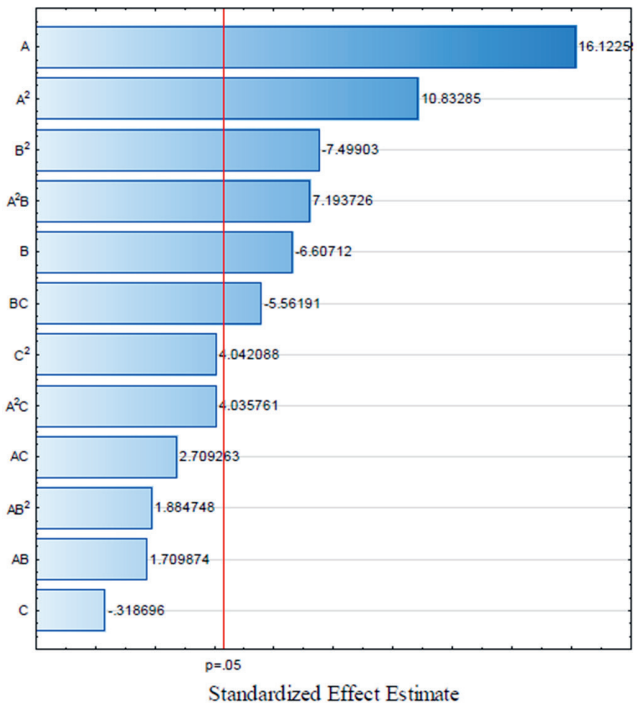

Fig. 3. Pareto chart of standardized effects of the Box–Behnken experimental analysis for *Bacillus thuringiensis* UP47-produced gamma-polyglutamic acid (γ-PGA)

Considering the effect of yeast extract on production of γ-PGA, results obtained in this work, as well as Mabrouk et al.[15] and Berekaa and Al-Otaibi,[13] showed that yeast extract had a positive effect on yield of γ-PGA. Tork et al.[34] recorded high γ-PGA yields when yeast extract and L-glutamic acid were used. For factors with a positive effect on γ-PGA production, an increase in the concentration brought about an increase in γ-PGA production. L-glutamic acid (X4), yeast extract (X10) and biotin (X2) impacted γ-PGA production by *B. megaterium* UP47 and were used in a 3 factor 1 block-15 run Box–Behnken design to determine the interaction of the factors with each other and with γ-PGA production (Table 5).

The effects of interactions of media components on γ-PGA production based on the Box–Behnken analysis showed (Fig. 3) that both the linear and quadratic relationship of L-glutamic acid and yeast extract had positive and negative effects on γ-PGA yield, respectively.

Table 3. Screening and optimization of significant variables for gamma-polyglutamic acid (γ-PGA) production by *Bacillus thuringiensis* UP47 using a 16-run Plackett–Burman design matrix

Run order	Variables															PGA* [g/L]
	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	
D1	+1	+1	+1	−1	+1	+1	+1	−1	+1	−1	−1	+1	−1	−1	−1	14.67 ±0.58
D2	−1	+1	+1	+1	−1	+1	+1	+1	−1	+1	−1	−1	+1	−1	−1	51.33 ±2.42
D3	−1	−1	+1	+1	+1	−1	+1	+1	+1	−1	+1	−1	−1	+1	−1	36.67 ±0.58
D4	−1	−1	−1	+1	+1	+1	−1	+1	+1	+1	−1	+1	−1	−1	+1	44.00 ±0.58
D5	+1	−1	−1	−1	+1	+1	+1	−1	+1	+1	+1	−1	+1	−1	−1	8.00 ±0.51
D6	−1	+1	−1	−1	−1	+1	+1	+1	−1	+1	+1	+1	−1	+1	−1	10.00 ±0.87
D7	−1	−1	+1	−1	−1	−1	+1	+1	+1	−1	+1	+1	+1	−1	+1	8.67 ±0.72
D8	+1	−1	−1	+1	−1	−1	−1	+1	+1	+1	−1	+1	+1	+1	−1	54.00 ±1.81
D9	−1	+1	−1	−1	+1	−1	−1	−1	+1	+1	+1	−1	+1	+1	+1	20.00 ±0.96
D10	+1	−1	+1	−1	−1	+1	−1	−1	−1	+1	+1	+1	−1	+1	+1	18.00 ±1.03
D11	+1	+1	−1	+1	−1	−1	+1	−1	−1	−1	+1	+1	+1	−1	+1	38.67 ±1.07
D12	+1	+1	+1	−1	+1	−1	−1	+1	−1	−1	−1	+1	+1	+1	−1	10.67 ±1.28
D13	−1	+1	+1	+1	−1	+1	−1	−1	+1	−1	−1	−1	+1	+1	+1	30.67 ±0.58
D14	+1	−1	+1	+1	+1	−1	+1	−1	−1	+1	−1	−1	−1	+1	+1	33.33 ±0.39
D15	+1	+1	−1	+1	+1	+1	−1	+1	−1	−1	+1	−1	−1	−1	+1	42.67 ±1.58
D16	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	4.00 ±0.95

* observed values are the mean value of triplicates ± standard deviation (SD) at $p < 0.05$.

Table 4. Statistical analysis of the Plackett–Burman experiment for *Bacillus megaterium* UP47

Factor	Effect	Coefficient	t	F	p-value*
Mean/Intercept	–	26.584	–	–	–
X1	6.998	3.499	7.398	54.731	0.0855
X2	14.449	7.224	10.870	118.156	0.0584
X3	11.950	5.975	8.726	76.137	0.0726
X4	32.926	16.463	31.891	1017.040	0.0199
X5	−7.226	−3.613	−6.999	48.979	0.0903
X6	−12.701	−6.351	−9.274	86.007	0.0683
X7	−13.422	−6.711	−10.098	101.963	0.0628
X8	3.049	1.525	3.223	10.389	0.1915
X9	10.384	5.192	8.797	77.389	0.0720
X10	19.850	9.925	14.285	204.057	0.0444
X11	8.879	4.439	7.025	49.355	0.0900
X13	−8.856	−4.428	−7.007	49.104	0.0902
X14	−14.315	−7.157	−10.301	106.119	0.0616
X15	−7.211	−3.605	−6.109	37.322	0.1032

* significant p-value at $p \leq 0.05$.

However, the linear relationship of biotin had a negative effect on γ-PGA and its quadratic relationship had a positive effect on the yield of γ-PGA. There were positive and negative effects of both linear and quadratic relationships of the interactions of the 3 factors with each other (Table 5) on yield, whereas there were also some redundant effects (A^2B^2, AC^2, A^2C^2, BC^2, B^2C and B^2C^2), which were not estimated. All estimated effects ranged between 21.98% and 99.61% confidence level (Table 6).

Based on these results, response surface plots of the effects of L-glutamic acid and yeast extract against yield, L-glutamic acid and biotin against yield, and yeast extract and biotin against yield were generated (Fig. 4A,B,C). The plots showed that with an increase in L-glutamic acid and a decrease in yeast extract, there was an increase in the yield of γ-PGA. An increase in L-glutamic acid and biotin brought about an increase in γ-PGA yield (Fig. 4B) while, with an increase in biotin and a decrease in yeast extract, there was

Table 5. Design and run order of the 3-factor-1 block 15-run Box–Behnken design matrix with observed gamma-polyglutamic acid (γ-PGA) production results for *Bacillus megaterium* UP47

Run order	Values			PGA yield* [g/L]
	A	B	C	
1	–1	–1	0	39.33 ±0.31
2	1	–1	0	62.00 ±1.10
3	–1	1	0	14.67 ±0.89
4	1	1	0	45.33 ±0.45
5	–1	0	–1	16.00 ±0.85
6	1	0	–1	42.00 ±1.00
7	–1	0	1	4.67 ±0.22
8	1	0	1	43.33 ±1.20
9	0	–1	–1	36.00 ±0.41
10	0	1	–1	53.33 ±0.71
11	0	–1	1	57.33 ±0.22
12	0	1	1	48.67 ±0.75
13	0	0	0	47.67 ±0.32
14	0	0	0	43.33 ±0.53
15	0	0	0	44.00 ±0.10

* observed values are the mean value of triplicates ± standard deviation (SD); A – L-glutamic acid; B – yeast extract; C – biotin.

an increase in the yield of γ-PGA (Fig. 4C). A second order polynomial equation for prediction of γ-PGA from the Box–Behnken design using the regression coefficients of the estimated effects was derived as (Eq. 3):

$$\gamma\text{PGA yield} = \begin{matrix}(3316.061 - 449.708A + 9.036A2 \\ - 139.813B + 3.095B2 - 7.699C \\ - 0.164C2 + 13.116AB - 0.087AB2 \\ - 0.248A2B + 3.781AC \\ - 0.076A2C - 0.394BC)\end{matrix} \quad (3)$$

Four trials (at 0.25 L, 0.5 L, 1.0 L, and 3.0 L) using different levels of the 3 examined factors gave laboratory yields within 95% confidence levels of the predicted yields, obtained using the second order polynomial equation derived (Table 7). The adjusted R^2 (coefficient of regression) was calculated to be 0.97905, which indicated that 97.90% of the variability in the obtained response was explained by this model. Thus, it shows that the experimental model was of good fit for γ-PGA yield prediction. The regression value (R^2) was slightly higher than the 0.95 recorded by Berekaa and Al-Otaibi.[13] There was an increase in the yield of γ-PGA polymer from 16.33 g/L to 62 g/L by *B. megaterium* UP47 when the optimized media conditions were utilized. For the factors such as L-glutamic acid (A), yeast extract (B) and biotin (C), the second order polynomial equation derived for prediction of γ-PGA yield was (Eq. 4):

$$\gamma\text{PGA yield} = \begin{matrix}(3316.061 - 449.708A + 9.036A2 \\ - 139.813B + 3.095B2 - 7.699C \\ - 0.164C2 + 13.116AB - 0.087AB2 \\ - 0.248A2B + 3.781AC \\ - 0.076A2C - 0.394BC)\end{matrix} \quad (4)$$

Conclusion

Bacillus megaterium UP47 isolated from fermented seeds of *Pentaclethra macrophylla* (*Ugba)* produced an extracellular polymeric material which was characterized as γ-PGA. At low concentrations, the polymer possessed antibacterial activities. Through the Plackett–Burman and Box–Behnken studies, the bacterium required biotin and L-glutamic acid for optimized γ-PGA production. The experiments provided us with the knowledge of specific precursor factors that the particular strain under study would require to optimally produce γ-PGA and also for scaling up γ-PGA biosynthesis. *Bacillus megaterium* UP47 γ-PGA could therefore find application in food, agriculture, pharmaceutical, medical and wastewater treatment industries, among others.

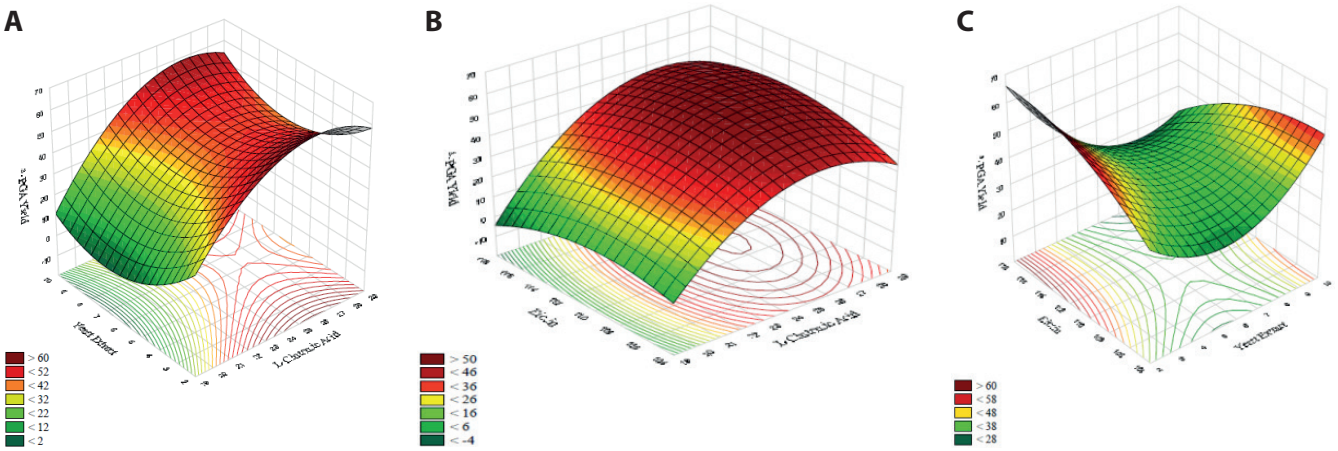

Fig. 4. A. 3D response surface plots showing the interaction of L-glutamic acid (x-axis) and yeast extract (y-axis) with their effect on the yield of gamma-polyglutamic acid (γ-PGA) (z-axis in g/L) for isolate *B. megaterium* UP47; B. 3D response surface plots showing the interaction of L-glutamic acid (x-axis) and biotin (y-axis) with their effect on the yield of γ-PGA (z-axis in g/L) for isolate *B. megaterium* UP47; C. 3D response surface plots showing the interaction of yeast extract (x-axis) and biotin (y-axis) with their effect on the yield of γ-PGA (z-axis in g/L) for isolate *B. megaterium* UP47

Table 6. Statistical analysis of the Box–Behnken experiment for gamma-polyglutamic acid (γ-PGA) production by *Bacillus megaterium* UP47

Factor	Effect	Coefficient	t	F	p-value
Mean/ Intercept	–	38.630	–	–	–
A	28.169	14.084	16.122	259.938	0.0038
A^2	13.196	6.598	10.832	117.350	0.0084
B	–11.544	–5.772	–6.607	43.654	0.0221
B^2	–9.135	–4.567	–7.499	56.235	0.0173
C	–0.555	–0.277	–0.318	0.101	0.7801
C^2	4.924	2.462	4.042	16.338	0.0561
AB	3.995	1.997	1.709	2.923	0.2294
AB^2	3.120	1.560	1.884	3.552	0.2001
A^2B	11.909	5.954	7.193	51.749	0.0187
AC	6.330	3.165	2.709	7.340	0.1135
A^2C	6.667	3.333	4.035	16.287	0.0562
BC	–12.995	–6.497	–5.561	30.934	0.0308

* significant p-value at $p \leq 0.05$; A – L-glutamic acid; B – yeast extract; C – biotin.

Table 7. Experimental and predicted gamma-polyglutamic acid (γ-PGA) yield of some production trials with the corresponding confidence levels

Trial	Laboratory experimental medium volume	Factor level*			Experimental yield**	Predicted yield	Confidence limit	
		A	B	C			–95%	+95%
1	0.25 L	20	3	105	36.00 ±0.72	33.50	19.68	47.32
2	0.50 L	28	9	116	44.00 ±0.43	35.10	22.10	48.10
3	1.0 L	28	9	105	50.67 ±0.97	46.80	32.90	60.60
4	3.0 L	20	3	116	38.67 ±0.95	35.16	22.20	48.12

* factors A, B and C indicate L-glutamic acid, yeast extract and biotin, respectively; ** the second order polynomial equation derived for prediction of γ-PGA yield is:
γPGA yield = 3316.061 – 449.708A + 9.036A2 – 139.813B + 3.095B2 – 7.699C – 0.164C2 + 13.116AB – 0.087AB2 – 0.248A2B + 3.781AC – 0.076A2C – 0.394BC.

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Antimicrobial activity of different plants extracts against *Staphylococcus aureus* and *Escherichia coli*

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Abstract

Background. Microbial pathogens, mainly bacteria, are a major cause of food spoilage resulting in several foodborne diseases. Food spoilage can be prevented by the application of chemical preservatives in the food industry but such process has harmful effects on human health and causes the introduction of chemicals in several food chains, leading to toxicity and long-term complications. Due to such adverse effects, the need to find natural preservatives that are safer to use, effective and less complicated is increasing.

Objectives. This study is based on plant extracts that play a major role in microbicidal action (the use of natural preservatives is preferred over chemical ones). Antimicrobial action of different plant extracts was assessed using *Staphylococcus aureus* and *Escherichia coli* as experimental bacterial strains.

Material and methods. Ethanolic extracts of different plants like *Punica granatum*, *Acacia catechu* and *Phyllanthus emblica* were highly effective against the both analyzed bacterial strains at a dosage of 10 mg/mL, while the extracts of *Ocimum bacilicum* and *Quercus infectoria* were effective only against *S. aureus* and *E. coli*, respectively.

Results. *Punica granatum* and *Phyllanthus emblica* extracts were found to be the most effective and exhibited bacteriostatic and bactericidal activities against the highly infectious strains of pathogenic bacteria causing food spoilage, with minimum inhibitory concentration (MIC) of 2.5 mg/mL and minimum bactericidal concentration (MBC) of 5 mg/mL.

Conclusions. The plant extracts used in the study were highly effective in reducing bacterial contamination and can be used as an alternative to chemical preservatives to avoid and control foodborne diseases and for preservation of food with no health-related hazards caused by chemicals.

Key words: *Escherichia coli*, *Staphylococcus aureus*, antimicrobial activity, plant extract

Background

There are over 1.8 million deaths worldwide per annum, mostly in young children, due to contaminated edibles, including water. According to the data of the World Health Organization (WHO), 76 million cases of foodborne diseases are recorded worldwide annually, with approx. 5000 deaths. Ingesting nutrition which has been compromised by bacteria, protozoa, toxins, and other contaminants seems to be the most prevalent source of infection and sickness. It is one of the leading causes of illness as well as mortality in poor nations. Bacteriological pollutants, primarily Gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*, are perhaps the most pronounced risk to public health.[1,2]

Certain bacterial species, such as *Bacillus cereus* and *Staphylococcus aureus*, have indeed been linked to local foodstuff spoilage.[2] Anti-targets have been continuously used in product packaging to prevent the decay of packaged foods.[2,3] Emphasis has been put on healthy, effective and natural food preservatives.[4]

Toxic substances of this kind have proven their efficiency in identifying and mitigating nosocomial infections, but their widespread use has contributed to the establishment of such hazardous substances in food chains, along with a whole slew of adverse environmental effects. Biocompatible chemicals are utilized as organic compounds against pathogenic microorganisms because they are both harmless and edible.[1,6]

Several researches have demonstrated the bioactivity of phytochemicals against disease-causing microorganisms.[7] The antibacterial efficacy of garlic, ginger and guava foliage against a diversity of social infectious agents had been studied, and it was discovered that ginger was perhaps the most efficient towards *S. aureus*, while guava and garlic proved efficacious against most of the microbes studied.[8] Antibacterial property towards *B. subtilis* and *B. cereus* was previously discovered in the foliage of *Syzygium polyanthum*.[9]

Pseudomonas aeruginosa and *Bacillus subtilis* have also been examined, and it was found that perhaps the ethanolic extracts of 4 plants (*Achyranthes aspera, Cynodon dacynodon dactylon, Lantana camara,* and *Tagetes patula*) are effective against most of the bacterial isolates, with minimum inhibitory concentration (MIC) ranging between 25 mg/mL and 125 mg/mL.[10]

The water-soluble extractions of cinnamon, aloe vera, henna, coriander, myrtle, and chamomile, as well as the hydro-alcoholic extractions of menthol, henna, ginger, chamomile, olive, myrtle, and Christ's thorn, act as their populations grow in small increments in their inhibition zones of approx. 10–28 mm.

Aquil and Ahmad discovered that ethanolic garlic extracts had almost no antibacterial activity against *E. coli* or *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, and *Shigella sonnei*.[12] In the research by Akinpelu et al., strong antimicrobial effectiveness of butanolic and pure isolates of *Persea americana* infected with *B. cereus* in foodborne illnesses was observed.[13] The minimum bactericidal concentration (MBC) of extracts varied between 3.12 mg/mL and 12.5 mg/mL, and these isolates showed antibacterial activity at doses of 25 mg/mL and 10 mg/mL. Likewise, several of these phytoconstituents have already been tested for antimicrobial effect toward microbial nutrition substances required by the organism to obtain nutrition by catabolism and anabolism.[14] Numerous therapeutic medicinal plants have been shown to counteract foodstuff-related illnesses.

Three therapeutic phytoconstituents utilized in Nigerian traditional system of medicine were shown to have high antimicrobial activity against a broad range of foodborne infections. All had a strong antibacterial effect towards *S. aureus*, *E. coli* and *Salmonella enteritidis*, albeit to a varying extent and often with varying MICs depending on the used plant compounds and microorganisms.[15]

Mikania triangularis, often characterized as "leaf guaco", exhibits antibacterial properties against 5 fungal isolates, 3 bacterium species, as well as *Staphylococcus epidermidis*, *S. aureus, B. cereus*, and *P. aeruginosa*, among others.[16] Investigators have also studied the antibacterial efficacy of 8 natural herb isolates against *Listeria monocytogenes*, *B. cereus* and *E. coli*.[17] Methanolic extracts of *Caryophyllus aromaticus* were shown to have the highest antimicrobial effect over *S. aureus* and had been significant against those same microorganisms.[18]

Bioactive compounds from *Myrtus communis* and *Thymus daenensis* are perhaps the most effective, with MIC coefficients ranging from 0.039 mg/mL to 10 mg/mL. Many researchers have investigated and validated the antibacterial effectiveness of *Punica granatum* against germs that cause food spoilage.[19]

Verma et al. computed the antimicrobial properties of tested compounds inhospitable to nutrition pathogenic organisms, including the Citrus, Punica, as well as Allium plant extracts.[20] Thus, every crude extract tested was intrinsically efficacious against different organism like *S. aureus*, *B. cereus*, *S. typhi*, and *E. coli*; however, the *Punica granatum* isolates showed genuinely highest potency with a concentration over 500 mg/mL. Within a concentration between 30 mg/mL and 50 mg/mL, *Punica granatum* peeling ethanolic extracts have been shown to be efficacious towards *Bacillus megaterium*, *S. aureus*, *Micrococcus luteus*, and Gram-negative bacteria such as *E. coli* and *P. aeruginosa*.[21]

Because of the strong antimicrobial properties of *Punica granatum* ethanolic extracts as well as their chunk toward Gram-negative (*E. coli* and *S. typhi*) and Gram-positive bacteria (*S. aureus* and *B. cereus*) causing foodborne illnesses, those certain extracts are used as stabilizing agents in the food processing industry to protect consumers against foodborne diseases.[22]

Several spice formulations used in nutritional supplements are highly effective toward numerous food contamination microorganisms; their antimicrobial effects have been shown by many researchers.[23] For example, cinnamon isolates were shown to be an excellent antimicrobial agent against all tested bacterial strains; ginger, clove and cumin are more effective than cinnamon extracts. Cloves have been found to have antimicrobial properties against Gram-negative bacteria and other diseases.[1,4,24]

According to numerous studies, ethanolic cloves infusion may indeed be helpful against *S. aureus*, *Vibrio parahaemolyticus* and *P. aeruginosa*, but inadequate against *E. coli* and *S. enteritidis*.[25] Clove oil has been shown to be effective against all dangerous microorganisms assessed in several investigations, with perhaps the exception of *Vibrio cholerae*, *Klebsiella pneumoniae* and *S. typhi*, that also have been shown to be impervious to diluted clove extract.[26]

Furthermore, the methanolic extracts of cloves are claimed to be efficacious towards *S. aureus*, *P. aeruginosa* and *E. coli*, with MIC values ranging from 0.1 mg/mL to 2.31 mg/mL.[27] Cumin seeds (*Cuminum cyminum*) aqueous extract has been shown to have antimicrobial effect against some of the Gram-positive and Gram-negative bacteria, exhibiting varying MIC.[3,28]

Cumin extract has been shown to be efficacious towards *E. coli*, *P. aeruginosa*, *S. aureus*, and *Bacillus pumilus*, with MIC values ranging between 6.25 mg/mL and 25 mg/mL,[29] and prescribed dosages ranging from 20 mg/mL to 60 mg/mL.[30]

The effectiveness of 7 ethanolic and aqueous plant extracts was evaluated against several clinically dangerous bacteria. The ethanol extract of *Punica granatum* was shown to be efficacious against all pathogenic microorganisms assayed, with a MIC value of 0.2 mg/mL. *Thymus kotschyana* extract was shown to be efficacious towards *E. coli* and *S. aureus*; however, *Zingiber officinalis* isolate was shown to counteract *P. aeruginosa* and *K. pneumoniae*.[31]

Numerous thyme bioactive compounds have been tested for their bactericidal effectiveness against foodborne infections (*L. monocytogenes*). The prevalence of foodborne diseases have indeed been linked to dangerous microorganisms, notably Gram-negative bacteria like *E. coli*, *S. typhi* and *P. aeruginosa*, and other Gram-positive pathogens like *S. aureus* and *B. cereus*. There is a scarcity of studies in the Arabian region on the viability of *Syzygium aromaticum*, *Thymus vulgaris*, *Punica granatum*, *Zingiber officinale*, and *Cuminum cyminum* against many of the previously mentioned pathophysiological food decomposing bacteria. Therapeutic properties of many bioactive compounds against diseases caused by *S. aureus*, *B. cereus*, *E. coli*, *S. typhi*, and *P. aeruginosa* are now being evaluated in vitro.

Material and methods

Performing the Soxhlet method of alcoholic extraction

Material (galls, peels, heartwood, fruit, and whole plants) from 5 different plant species (Table 1) were obtained from the Universal Biotech Khari Baoli (Old Delhi, India). Plant parts were washed many times to remove any possible impurities. After drying, each plant material was crushed into fine powder that passed through a 100 mm sieve. Approximately 10 g of fine powder was immersed in 100 mL of ethanol and extracted for 48 h with continuous stirring, and then filtered with two-layer muslin cloth, centrifuged for 10 min at 9000 rpm, and finally filtered again using Whatman filter paper (41; Merck Millipore, Mumbai, India). The filtrates were dried using a rotary vacuum evaporator (Hahnshin Scientific, Mumbai, India) under reduced pressure at 60°C and stored in the refrigerator at 5°C. The percentage of yields was measured using the following formula[32]:

$$\text{yields of extract (\%w/w)} = R/S \times 100$$

where R – plant residues extracted weight and S – raw sample of plant weight.

Antibacterial activity of the plant extracts

Bacterial strains

Two strains of bacteria that cause food poisoning were used to test the antibacterial effectiveness of extract of each plant species. In this experiment, we used 1 strain of Gram-positive bacteria (*S. aureus*) and 1 strain of Gram-negative bacteria (*E. coli*). The strains were collected from the Biochemistry Department of Jamia Hamdard (deemed to be university), New Delhi, India.

Table 1. Ethnobotanical data of examined plant species and their extract yield percentage

Plant species	Family	Local name	Common name	Plant part used	Extract pH	Extract yield
Quercus infectoria	Fagaceae	manjakani	Aleppo oak	galls	4.8	1.2%
Punica granatum	Lythraceae	romman	pomegranate	peels	4.5	2.3%
Acacia catechu	Fagaceae	khair	black cutch	heart wood	7.2	2.1%
Ocimum basilicum	Lamiaceae	tulsi	basil	whole plant	7.8	1.98%
Phyllanthus emblica	Phyllanthaceae	amla	Indian gooseberry	fruits	3.9	2.4%

Inoculum preparation

Both strains of bacteria were left overnight for culture in Mueller–Hinton agar medium at 35°C. Using a spectrophotometer, the growth of bacteria was harvested in 5 mL of sterile saline water, and the cell count was diluted to 10^7 CFU/mL at 580 nm.

Antibacterial activity of plants extract

The antibacterial activity of each plant extract was estimated using the disc diffusion method. The residues of plant extract (50 mg) were re-dissolved in 2.5 mL of ethanol. After that, purification was performed with a Millipore filter (0.22 mm; Merck Millipore) and then loaded over a sterile disc of filter paper to reach a final concentration of 10 mg/disc. Then, 15 mL of seeded medium previously infected with bacterial suspension (100 mL of medium/1 mL of 10^7 CFU) was added to 10 mL of Mueller–Hinton agar media in Petri dishes to achieve 10^5 CFU/mL of media.

Sterile filter paper discs with extract of plant concentration (10 mg/mL) were inserted on top of the plates of Mueller–Hinton agar media in Petri dishes. Discs of filter paper containing 5 mg of gentamicin as a positive control were utilized. After this, the plates were kept for 2 h in a fridge at 5°C to allow extract of plant to diffuse before being incubated for 24 h at 35°C. The presence of inhibitory zones was measured using a Vernier caliper, recorded and interpreted as a marker of antibacterial activity.

Determination of minimum inhibitory concentrations of the effective plant extract

After 24 h of incubation, the MIC was characterized by a low concentration of antibacterial agents that prevent microbial growth. By using the method of disc diffusion, the most effective extracts of plants, having strong antimicrobial activities at 10 mg/mL, were modified to calculate their MIC and examine their efficiency in reducing bacterial strains that cause food poisoning. By dissolving 50 mg of plant extracts in 2.5 mL of ethanol, filtering it with a Millipore filter and transferring the required amount to sterile discs, effective plant extracts with different concentrations (1.25 mg/mL, 2.5 mg/mL, 5 mg/mL, 10 mg/mL, 12.5 mg/mL, and 15 mg/mL) were produced (the discs had 8 mm in diameter). Pathogenic strains of cultured bacteria were infused using Mueller–Hinton agar into sterile Petri dishes. Various amounts of extracts were obtained and loaded onto filter paper discs; Mueller–Hinton agar plates were later covered. The plates were then kept at 5°C in the fridge for 2 h and then incubated for 24 h at 35°C. By the help of Vernier caliper inhibition zones were determined and compared to the concentration of the effective extract of the plant.

Determination of minimum bactericidal concentrations of the effective plants extract

Two streaks of plates bearing MIC inhibitory zones with little concentration were collected and cultivated in tryptone soya agar (TSA) plates that displayed limited growth. Next, the plates were put for incubation at 35°C for 24 h. The growth of bacteria was observed in different plant extract concentrations. On freshly infected agar plates, MBC was defined as the extract of plant concentration that did not show any bacterial growth.

Results and discussion

Plants extraction yield

The data for the plants that have been used and their extract percentage yield are presented in Table 1. Ten grams of dried powder plant material allowed for a yield of plant extract ranging from 198 mg to 240 mg. The highest yield of plant extract was obtained from *Phyllanthus emblica* (240 mg) followed by *Punica granatum* (230 mg), while *Quercus infectoria* gave the lowest extract yield (120 mg).

Antibacterial activity of plants extract

To examine the antibacterial activity of plants against food poisoning bacteria, 5 plant species were assessed against a strain of Gram-negative bacteria (*E. coli*) and a strain of Gram-positive bacteria (*S. aureus*). Disc diffusion method was used for the assessment and to find the susceptibility of bacteria. Antibacterial action of plant extracts is presented in Table 2 and Fig. 1. It was found that the used plant extracts were very effective in counteracting the bacterial growth that leads to food poisoning.

Punica granatum was found to be the most effective extract – it has reduced the growth of pathogenic bacteria (*S. aureus* and *E. coli*) at a concentration of 10 mg/mL. *Phyllanthus emblica* was also found effective against both of the pathogenic strains. Varying antimicrobial activity

Table 2. Antimicrobial screening test of ethanolic plant extract (10 mg/mL) against some of the bacterial strains causing food poisoning

Plant species	Inhibition zones [mm]	
	Gram-positive pathogenic bacteria (*S. aureus*)	Gram-negative pathogenic bacteria (*E. coli*)
Quercus infectoria	8.6 ±0.67	0.0 ±0.0
Punica granatum	17.9 ±0.10	14.4 ±0.53
Acacia catechu	16.3 ±0.27	13.1 ±0.27
Ocimum basilicum	15.7 ±0.24	0.0 ±0.0
Phyllanthus emblica	16.5 ±0.32	14.2 ±0.21
Gentamicin (5 µg)	18.5 ±0.21	14.9 ±0.49

Data are means of 3 replicates (n = 3) ± standard error (SE).

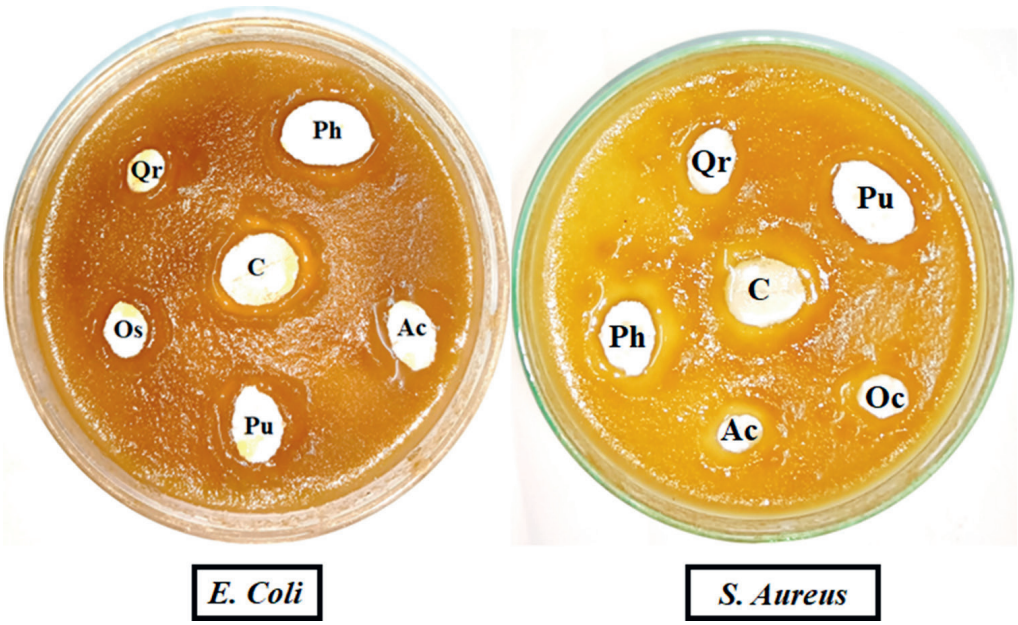

Fig. 1. Growth inhibition of bacterial strains caused by some plant extracts. *Quercus infectoria* (*Qr*), *Punica granatum* (*Pu*), *Acacia catechu* (*Ac*), *Phyllanthus emblica* (*Ph*), *Ocimum bacilicum* (*Os*), and C (positive control)

was also shown by other plants against food poisoning bacterial strains. *Acacia catechu* was proved effective against both bacterial strains, while *Ocimum bacilicum* and *Quercus infectoria* only affected *S. aureus* and had a negligible effect on *E. coli* bacteria.

Antimicrobial action of the plant extracts indicates that *E. coli* was the strain most resistant to the used plant extracts. *Punica granatum* and *Phyllanthus emblica* extracts showed highly effective antibacterial action. The experiments were conducted to find the MIC and MBC values of the food poisoning bacteria (*S. aureus* and *E. coli*).

Minimum inhibitory concentration of the effective plants extract

The MIC values of the effective plant extracts (*Punica granatum* and *Phyllanthus emblica*) were calculated using disc diffusion method to find out the bacteriostatic and bactericidal properties of the plant extracts. The concentration of the plant extracts which were found effective are presented in Table 3. The inhibiting effect of *Punica granatum* extract was detected at a concentration of 2.5 mg/mL, with the zone of inhibition at 9.5 mm and 7.5 mm for *S. aureus* and *E. coli*, respectively, while the extract of *Phyllanthus emblica* reduced the growth of bacteria at concentration of 2.5 mg/mL, forming a small inhibition zone of 8.7 mm and 5.6 mm for *S. aureus* and *E. coli*, respectively.

Minimum bactericidal concentrations of the effective plants extract

The MBC can be identified by the absence of bacterial growth streaked from the inhibition zone correlating to the tested strains' lowest MIC. *Punica granatum* was found to have an effective bactericidal activity against

Table 3. Minimum inhibitory concentrations (MICs) of the most effective plant extract against *S. aureus* and *E. coli*

Plant extract	Concentration [mg/mL]	Inhibition zones [mm]	
		Gram-positive pathogenic bacteria (*S. aureus*)	Gram-negative pathogenic bacteria (*E. coli*)
Punica granatum	1.25	0.0 ±0.0	0.0 ±0.0
	2.50	9.5 ±0.60	7.5 ±0.13
	5.0	13.7 ±0.77	10.3 ±0.21
	10.0	17.2 ±0.69	13.7 ±0.27
	12.5	20.7 ±0.32	16.9 ±0.52
	15.0	23.4 ±0.27	19.7 ±0.61
Phyllanthus emblica	1.25	0.0 ±0.0	0.0 ±0.0
	2.50	8.7 ±0.21	5.6 ±0.19
	5.0	12.3 ±0.31	9.7 ±0.21
	10.0	14.9 ±0.41	13.4 ±0.33
	12.5	17.2 ±0.56	16.3 ±0.41
	15.0	19.4 ±0.69	18.2 ±0.52

S. aureus and *E. coli*, having a MBC value of 5 mg/mL, while *Phyllanthus emblica* extract also had an MBC value of 5 mg/mL. Based on that, it can be suggested that *Punica granatum* and *Phyllanthus emblica* can be used to counteract foodborne pathogens and diseases. Bacteria used in this study play a major role in food decomposition and food poisoning. *Staphylococcus aureus* is regarded one of the most common causes of foodborne diseases, while *E. coli* is responsible for producing harmful toxins and other components that play an important role in human gastrointestinal diseases. *Punica granatum* was found effective in inhibiting the growth of all bacterial strains, while *Phyllanthus emblica* extract was found highly effective against *S. aureus* and less effective against *E. coli*.[33] Due to differences in method used for extraction, as well

as in components and bacterial strains used in the experiment, a significant variability in MIC of *Punica granatum* could be observed in comparison to other investigations, leading to variation in MIC of different plants extracts. Variations can also be caused by the properties of the chemicals like volatility and the disparities between the chemical constituents. *Phyllanthus emblica* extract was found to be effective with a 10 mg/mL concentration against *S. aureus* and *E. coli*, inhibiting their growth and forming the inhibition zones of 14.9 mm and 13.4 mm, respectively.[34] Some researchers, after studying the plant extracts and their effect on certain bacteria, suggested that plants components like terpenoids, alkaloids and phenolic compounds react with components of bacterial cell membrane and proteins present on it, causing their lysis by inducing an efflux of proton outside the cell or inhibiting important enzymes responsible for synthesis of amino acids.[35] Other study accredited the effect of plant extracts to their hydrophobic properties, which cause the reaction of protein in bacterial cell membrane with mitochondria, leading to lysis and alteration of bacterial structure, and changing its permeability.[36] This study suggests that plant extracts used in this experiment have shown effective antibacterial properties and can also be used as natural preservatives, thereby reducing the application of chemically made preservatives in food industry, which leads to several health hazards.

Conclusions

Various harmful bacteria strains can cause food spoilage. It can be prevented by the application of chemical preservatives in the food industry, but such means have harmful effects on human health and cause the introduction of chemicals in several food chains, leading to toxicity and long-term complications. Due to such adverse effects, natural preservatives that are safer to use, effective and less complicated have to be developed. Existing plant extracts which have shown their potential usefulness (*Punica granatum* and *Phyllanthus emblica*) can be employed as a natural alternative to synthetic antimicrobial agents to prevent food poisoning.

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Modifications of bacterial cellulose in wound care

Modyfikacje bakteryjnej celulozy do stosowania w opatrywaniu ran

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Abstract

Wound infection may occur in acute and chronic wounds, wounds resulting from surgery or traffic accidents, and burns. Regardless of the extent and cause of the wound, prompt treatment is essential in reducing the patient's pain and limiting the spread of contamination. Improper wound care and associated chronic diseases may hinder the therapeutic success. Bacterial cellulose (BC) is highly biocompatible and has no cytotoxic effect on cells engaged in wound healing, such as fibroblasts and keratinocytes. Its high hydration level guarantees the maintenance of a moist wound environment. High mechanical strength, flexibility and resistance to damage make BC a promising material for dressings. Unfortunately, it does not display an inhibitory effect on bacterial growth. Introducing antimicrobial agents into the structure of BC has been a subject of many studies. This paper aims to present the latest reports on the possibility of the absorption of bacteriostatic and bactericidal agents in BC, such as metal particles, essential oils, antibiotics, antiseptics, and wound irrigation solutions. Moreover, the modifications in BC culture and post-production treatments in order to improve its physical properties are discussed.

Key words: wound infection, wound, antisepsis, cellulose, biological dressings

Streszczenie

Infekcja może rozwijać się w ranach ostrych, przewlekłych, powstałych na skutek operacji, wypadków komunikacyjnych czy oparzeń. Niezależnie od rozległości ran i przyczyn ich powstania konieczne jest szybkie zastosowanie odpowiedniego leczenia, celem zmniejszenia doznań bólowych pacjenta oraz ograniczenia rozprzestrzeniania się infekcji. Choroby towarzyszące oraz niewłaściwa pielęgnacja ran mogą przyczyniać się do opóźnienia sukcesu terapeutycznego. Celuloza bakteryjna charakteryzuje się dużą biozgodnością oraz nie wykazuje działania cytotoksycznego względem komórek odpowiedzialnych za proces gojenia, takich jak fibroblasty i keratynocyty. Jej wysokie uwodnienie zapewnia utrzymanie wilgotnego środowiska rany. Wytrzymałość mechaniczna, elastyczność i odporność na uszkodzenia to cechy, dzięki którym celuloza bakteryjna rozważana jest jako potencjalny materiał do produkcji opatrunków. Niestety, bakteryjna celuloza nie wykazuje działania hamującego wzrost bakterii. Trwają badania nad możliwością wprowadzenia środków przeciwdrobnoustrojowych do struktury bakteryjnej celulozy. Celem pracy jest omówienie najnowszych doniesień dotyczących możliwości absorpcji środków bakteriostatycznych i bakteriobójczych w bakteryjnej celulozie, takich jak cząsteczki metali, olejki eteryczne, antybiotyki, antyseptyki oraz środki do przemywania ran. W niniejszej pracy omówiono również modyfikacje warunków hodowli bakteryjnej celulozy oraz zabiegi poprodukcyjne mające na celu ulepszenie właściwości fizycznych celulozy bakteryjnej.

Słowa kluczowe: zakażenia ran, rany, antyseptyka, celuloza, opatrunek biologiczny

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Introduction

The skin functions as a protective barrier against pathogens, physical injury, ultraviolet radiation, and chemicals. It also regulates the body temperature and prevents excessive water loss and desiccation. Disruption of skin integrity, caused by burns, trauma, diabetes, and venous or pressure ulcers, leads to the impairment of its essential functions and can result in hard-to-heal wounds. The risk of developing chronic wounds increases in people with diabetes and obesity, as well as in elderly patients. Severe tissue damage with an infectious process is often difficult to control. It may result in an amputation or a life-threatening injury.[1] The treatment is a long, multistep process, involving wound and periwound skin cleansing, debridement, wound edges refashioning, treatment, and dressing.[2] However, although traditional dressings protect wounds from contamination and mechanical damage, they do not promote wound healing. In addition, dressing changes are often accompanied by damage of the regenerated wound surface and are associated with patient's pain.[3] Ideally, a wound dressing should maintain moisture, absorb exudate, reduce pain, and be non-adherent, airy, sterile and antimicrobial.[3] Recently, researchers have been focused on the properties and application of bacterial cellulose (BC). This biopolymer is applied in many industries, also in the medical field, and is considered to be one of the most promising wound dressing materials.[4]

The aim of this review was to discuss research focused on the physical properties of BC and the possibilities of its application in wound infection treatment.

Properties of bacterial cellulose

Bacterial cellulose is produced by various bacterial species, such as Rhizobium, Acetobacter, Sarcina, Pseudomonas, and Escherichia.[5] Among them, *Acetobacter xylinus* spp., also named *Gluconacetobacter xylinus* spp. or *Komagataeibacter xylinus* spp., produces the most massive cellulose ribbon and is ubiquitous in BC production.[6] Presumably, cellulose provides an extra bacterial matrix, expanding the colonization and offering the advantage in the nutrient competition. Furthermore, researchers postulate that cellulose protects bacteria from unfavorable environmental conditions. For instance, BC forming on the liquid-gas interface provides a constant access to the aerobic conditions.[7]

Bacterial cellulose microfibers are formed by β-1,4-glucan chains resulting from the polymerization of uridine diphosphate glucose. Consequently, these microfibers aggregate into a fiber with a diameter from 10 nm to 100 nm and create a three-dimensional (3D) network with numerous pores due to hydrogen bonds.[8] Although the molecular formula $(C_6H_{10}O_5)_n$ is the same for bacterial and plant cellulose, their physical and chemical features differ significantly. Contrary to plant cellulose, the BC microfibrils are 100 times thicker and form a reticular structure. Plant cellulose contains lignin, pectin and hemicellulose, while BC is free of such impurities.[5] Due to its unique structure, it possesses high mechanical strength. Among its prominent features are 80–90% crystallinity, which, together with a high degree of polymerization (even up to 16,000 DPw), determines increased thermal stability and lower susceptibility to degradation.[9] Bacterial cellulose displays good flexibility, permeability, hygroscopicity, and hydrophilicity.[8] It is noteworthy that BC does not affect viability of fibroblasts and keratinocytes.[10]

There are 3 commonly applied BC production methods: static, agitation and bioreactor culture. In a static culture, BC film is produced on the gas-liquid interface in a span of 7–10 days and takes the shape of a flat gelatinous membrane. As a result of shaking, sphere-like or irregular cellulose shapes are formed. Agitation is intended to increase oxygen transport in the medium. However, according to the reports, shaking does not influence cellulose productivity.[11] Moreover, physical properties of cellulose, such as crystallinity or the degree of polymerization obtained by the agitation method differ from the properties of BC obtained in a static culture.[12] The bioreactor culture is applied in industrial bioreactors in order to reduce cultivation time and increase BC productivity. Bioreactors can be modified to provide various culture conditions such as oxygen-enriched air or rotating disks.[13] Regardless of the method, after harvesting, BC is purified in sodium hydroxide from bacterial cell debris and substances in the culture medium, and washed with water to obtain a neutral pH.[12] Interestingly, it was reported that the properties of BC produced in a commercial Hestrin–Schramm (HS) medium are comparable to those of BC cultured in different media. Therefore, obtaining BC can be inexpensive and eco-friendly.[14]

Bacterial cellulose as an antibacterial dressing

Wound healing is a physiological process whose timing depends on the area and location of the injury. Unfortunately, therapeutic success may be delayed by several factors such as wound infection or an allergic reaction to the dressing.[15,16] Wound infection may be caused by Gram-positive and Gram-negative bacteria or fungi, including multidrug-resistant strains. Therefore, the selection of an appropriate dressing is crucial to patient safety.[15] Bacterial cellulose exhibits high biocompatibility and low cytotoxic effect, and does not induce allergic reactions.[17] These properties promote the healing process and tissue regeneration.[18] However, BC does not display antimicrobial properties. In order to impart antimicrobial properties to BC, the researchers decided to take advantage of its absorbance and release potential and incorporate antimicrobial substances into the cellulose.[19]

Metals

Antibacterial activity of silver nanoparticles has been determined against Gram-positive and Gram-negative bacteria, but their mechanism of action is not well understood. It is speculated that silver ions inhibit bacterial growth by destroying the cell wall and repressing DNA transcription.[20] Jalili Tabaii and Emtiazi prepared a cellulose carrier with silver nanoparticles by soaking BC in an $AgNO_3$ solution. They reported that BC enriched with silver nanoparticles demonstrated an even 100% higher antimicrobial effect against *Staphylococcus aureus* spp. and *Escherichia coli* spp.[21] It was also reported that copper ions cause cell membrane damage and DNA impairment.[22] Copper nanoparticles incorporated into BC displayed long-term bactericidal efficacy against *S. aureus* spp. and *E. coli* spp., for up to 90 days.[23] Also, the antibacterial activity of BC chemisorbed with copper nanoparticles against *Bacillus subtilis* spp. and *Candida albicans* spp. was reported.[24] It is worth noting that BC chemisorbed with copper nanoparticles did not exhibit cytotoxicity to fibroblasts.[23] Additionally, BC chemisorbed with zinc oxide displayed antibacterial activity against *S. aureus* spp., *Pseudomonas aeruginosa* spp., and *E. coli* spp.[25] Its antimicrobial mechanism of action relies on bacterial cell disruption, cell membrane hydrophobicity change and genes downregulation. Moreover, zinc oxide generates reactive oxygen species (ROS) that induce oxidative stress, leading to damage of the cell components.[26] Additionally, zinc oxide incorporated in BC promotes wound healing and tissue regeneration.[25]

Essential oils

Recently, the antimicrobial action of essential oils (EOs) has been of interest to some researchers.[27] Dudek-Wicher et al. studied the antibiofilm efficacy of 3 essential oils: tea tree, geranium and frankincense essential oils, incorporated in BC against Gram-positive (*S. aureus* spp., *Enterococcus faecalis* spp.) and Gram-negative (*Klebsiella pneumoniae* spp., *P. aeruginosa* spp., *E. coli* spp.) strains and 1 fungal strain (*C. albicans* spp.). All tested EOs exhibited antibiofilm efficacy against the tested strains, with efficacy of nearly 80–100%, exhibited by tea tree and geranium essential oils.[27] Unlike antiseptics or antibiotics, the volatile property of EOs allows them to reach the areas neighboring the areas of application. Bacterial cellulose enriched with thymol, a component of an essential oil, was successfully applied on a third-degree burn wound closure. Bacterial cellulose chemisorbed with thymol exhibited low cytotoxicity on fibroblasts and increased cell viability.[28] Junka et al. tested 3 essential oils, namely clove, eucalyptus and thyme, against *S. aureus* spp. and *P. aeruginosa* spp.[10] All essential oils were successfully impregnated and effectively released from BC discs. Two different methods were applied to assess their antimicrobial activity. In the disk diffusion method, all tested EOs demonstrated very high efficacy. The 2nd method (Antibiofilm Dressings Activity Measurement (ADAM)), considered the penetrability index of the tested EOs. This method has demonstrated significant differences in EO activity. Minor bactericidal activity was observed for clove oil, while thyme and eucalyptus oils displayed a significantly higher activity. However, thyme and clove oils displayed a significantly higher cytotoxicity against fibroblasts than the eucalyptus oil.

Antibiotics

The antibiotics most commonly applied in wound treatment are ceftriaxone, gentamycin, vancomycin, ciprofloxacin and tetracycline. Topical application of antibiotics in compresses previously immersed in an antibiotic solution is not recommended for wound infection treatment.[29] The reasons are the difficulties in determining the drug concentration and the uncontrolled rate of drug release to the infected skin, which may increase the percentage of resistant strains. Antibiotic incorporation in BC makes it possible to determine the amount of the drug bound and to control the drug release rate from the membrane.[30]

Lazarini et al. compared the release time of ceftriaxone from BC produced in media with different sugar compositions. Bacterial cellulose produced in the medium supplemented with sugarcane molasses displayed a higher fiber density.[31] Hence, the release time of ceftriaxone from chemisorbed BC was prolonged by 5 h, as compared to BC cultured in a standard HS medium.[31] Junka et al. successfully impregnated BC with gentamycin. The antibiotic concentration was 2 g/L, corresponding to the commercially available collagen gentamycin sponge.[32] The release rate of gentamycin from BC was lower than from the collagen sponge. Even after 48 h of incubation, a small amount of gentamycin was released from BC. In contrast, 90% of gentamycin was released up to 8 h during the experiment from the collagen sponge.[32] Bacterial cellulose dressings, chemisorbed with gentamicin in 2 concentrations of 2 g/L and 0.006 g/L, were administered on rat bones. Despite their uneven and porous surface, BC chemisorbed with gentamycin effectively inhibited the growth of *S. aureus* spp., restraining the bacterial biofilm development.[32] Furthermore, vancomycin and ciprofloxacin are widely applied in wound infection treatment. It was reported that BC dressings impregnated with these antibiotics effectively impede infection development.[33] A high bactericidal effect of vancomycin and ciprofloxacin was determined against *S. aureus* spp. and *K. pneumoniae* spp. strains. Nevertheless, a significant amount of the antibiotics was released from BC in the 1st hour of the experiment. The above may lead to low usefulness of vancomycin and ciprofloxacin in BC in long-term wound treatment.[33] However, Cacicedo et al. slowed the release rate of ciprofloxacin from BC by incorporating chitosan (Chi) into the cellulose structure.

Bacterial cellulose/Chi modification prolonged the release time by more than 6 h and exhibited antimicrobial activity against *S. aureus* spp. and *P. aeruginosa* spp. Adding ciprofloxacin to the hybrid significantly enhanced the bactericidal effect.[34] Shao et al. incorporated different tetracycline hydrochloride concentrations into BC.[30] The lowest concentration of tetracycline hydrochloride in BC (0.41 mg/dm^2) was sufficient to restrain the growth of *S. aureus* spp., *E. coli* spp. and *B. subtilis* spp. However, the inhibition zone of *C. albicans* spp. was detectable only in the highest tested concentration (10.17 mg/dm^2). The fast release stage of tetracycline was up to 1 h after the beginning of the experiment. Interestingly, the percentage of the antibiotics released from the cellulose was increased in the BC/tetracycline hydrochloride with higher concentrations of the incorporated antibiotic. Additionally, free tetracycline was released slower than when incorporated in BC, which suggests the BC usefulness for controlled drug dosage.[30]

Antiseptics and wound irrigation solutions

In recent years, antiseptics have attracted considerable attention in wound care. The most commonly used wound healing agents include polyhexamethylene biguanide hydrochloride (PHMB), octenidine dihydrochloride (OCT), iodine povidone (PVP-I), and chlorhexidine gluconate (CHX).[35,36] Their broad spectrum of action includes both Gram-positive and Gram-negative bacteria in vegetative and spore forms, as well as fungi, viruses and multidrug resistant strains. Most antiseptics are characterized by a high ability to eradicate the biofilm. Additionally, resistance mechanisms to these antimicrobials are not recorded.[35]

Dydak et al. presented the results of antimicrobial activity for all of the abovementioned antiseptics in the biocellulose disk diffusion method against Gram-positive (*S. aureus* spp., *Staphylococcus epidermidis* spp., *Enterococcus faecium* spp.), Gram-negative (*K. pneumoniae* spp., *E. coli* spp., *Acinetobacter baumannii* spp., *P. aeruginosa* spp., *Enterobacter cloacae* spp.) and fungal strains (*C. albicans* spp.). The most significant inhibition zones were found for PVP-I and CHX, followed by PHMB and OCT, against all tested bacteria. Also, the largest zone of inhibition of fungal strain growth was determined for PVP-I. Moreover, the high antifungal efficiency was shown for CHX and PHMB, whose inhibition zones were comparable.[37] Wiegand et al. reported that PHMB incorporated in BC exhibited a higher bactericidal activity against *S. aureus* spp. than PVP-I in BC. However, better biocompatibility in human keratinocytes was obtained for BC/PVP-I than for BC/PHMB.[38] The BC/OCT displays a similar antimicrobial activity against *S. aureus* spp. and *P. aeruginosa* spp. Its high growth inhibitory efficacy was assessed using the disk diffusion method, in which the inhibition zones were 22–23 mm and 20–22 mm, respectively.[39] Also, Inoue et al. reported high inhibitory activity of BC/CHX against *S. aureus* spp., *E. coli* spp. and *C. albicans* spp., using the disk diffusion method.[40] Superoxidized hypochlorite solutions are considered wound irrigation solutions. However, there are reports of their very low efficacy in growth inhibition and biofilm eradication.[41,42] The lack of antimicrobial efficacy of hydrochloride solutions has been demonstrated in microtiter plate studies and chemisorbed BC.[37,41–43] A wound irrigation product containing 0.004% of sodium hypochlorite and 0.004% of hypochlorite acid was evaluated in the research on the antimicrobial efficacy of antiseptics.[37] No growth inhibition was detected with the disk diffusion method for most of the strains of the tested species of *S. aureus* spp., *S. epidermidis* spp., *Enterococcus faecium* spp., *K. pneumoniae* spp., *E. coli* spp., *P. aeruginosa* spp., *E. cloacae* spp., *A. baumannii* spp., and *C. albicans* spp.

The antimicrobial efficacy of antiseptics increases with prolonged real-contact time.[44] The incorporation of antiseptics in BC makes it possible to extend the contact time of microorganisms exposed to antimicrobial agents. The insertion and release time depend on the molecular mass of the compound.[38] As the molecular mass of the compound increases, the time of its release from the cellulose also increases. The PVP-I release from BC was slightly delayed compared to PHMB, due to higher molecular mass of PVP-I. For both compounds, rapid release rate lasted up to 8 h. Furthermore, release with a slower rate continued up to 24 h for PHMB and 48 h for PVP-I.[38] The time of rapid release of octenidine was equal in the case for PHMB and PVP-I, the release with a slower rate continued even up to 96 h.[45] Moreover, 2 release stages of chlorhexidine from BC were observed by Inoue et al. The stage of rapid release took 15 min from the beginning of the experiment. After this time, the release rate slowed down and remained constant for 48 h.[40] The rapid release stage of 15 min was observed for PHMB and PVP-I by Krasowski et al.[19] Octenidine incorporated in BC maintains its activity for an extended period. The BC/OCT stored for over 6 months presented similar features (low cytotoxicity and high antimicrobial activity) as a freshly prepared dressing.[45] Alkhatib et al. supplemented BC with poloxamer to prolong OCT release time from BC. Poloxamer is a nonionic triblock copolymer which, when incorporated in BC, does not affect the bactericidal efficacy of BC/OCT. Moreover, BC/poloxamer is highly biocompatible. The incorporation of poloxamer into BC enables extending the release time of OCT up to 8 h which can be employed for long-term treatment of chronic wounds.[39]

Additionally, incorporating antimicrobial substances into BC may influence the material structure, thus improving its physical properties. Adding PVP-I caused structural changes of BC, which led to an increased comprehensive strength when compared to native BC. In contrast, adding PHMB and OCT did not cause any changes in the cellulose structure.[38,45]

Table 1. Effect of natural and synthetic polymers on selected physical properties of bacterial cellulose

Parameter	Modification	Value without modification	Value with modification	Reference
Temperature of thermal degradation	bacterial cellulose/chitosan	263°C	366°C	51
	bacterial cellulose/collagen	262°C	352°C	53
	bacterial cellulose/polyacrylonitrile	296°C	560°C	58
	bacterial cellulose/poly(ethylene glycol)	263°C	293°C	59
Young's modulus	bacterial cellulose/chitosan	6.0 GPa	1.8 GPa	51
	bacterial cellulose/collagen	4.5 GPa	9.5 GPa	53
	bacterial cellulose/poly(ethylene glycol)	6.35 GPa	4.12 GPa	59

Modifications of physical properties of bacterial cellulose in order to improve dressing design

Bacterial cellulose exhibits excellent absorption properties. It consists of 99.9% water, which fills the pores formed by the nanofibrils structure. The pore size varies, depending on the cellulose culture time. As the culture time extends, the pore volume in the cellulose decreases, and thus, the surface area of the membrane is reduced.[46] Pore-forming agents added to the culture, such as agarose microparticles, can improve water holding capacity of BC.[47] Increased carrying capacity of BC and constant release of antibiotics from BC can also be obtained by changes in the culture medium carbon source.[31] Also, laser piercing can be applied to enhance the amount of water trapping sites.[48] The absorption ability of BC may involve exudate assimilation from the wound environment.

Bacterial cellulose can be functionalized with different natural and synthetic polymers to improve some of its mechanical properties. Chitosan is a biopolymer that adversely affects bacterial viability.[49] Its mechanism of action relies on bonding to teichoic acids in the Gram-positive bacteria cell wall or disrupting the nutrients intake in Gram-negative strains.[50] Cai et al. reported that BC supplemented with chitosan (BC/Chi) showed enhanced thermal stability (temperature of thermal degradation increased from 263°C to 366°C) and lower tensile strength (Young's modulus decreased from 6.0 GPa to 1.8 GPa).[51] One of the best-known natural polymers is collagen, whose drawbacks are high cost, poor mechanical properties and no antimicrobial effect. However, collagen is highly biodegradable and its surface offers excellent cell-binding properties. Therefore, collagen is studied as a scaffold for BC.[52] Zhijiang and Guang reported that combining BC with collagen (BC/Col) increased thermal stability (temperature of thermal degradation changed from 262°C to 352°C) and tensile strength (Young's modulus increased from 4.5 GPa to 9.5 GPa), and decreased crystallinity (from 87% to 75%). The BC/Col scaffolds enabled fibroblasts to adhere and proliferate, contrary to native BC, on which fibroblasts did not show enhanced growth after being adhered to the surface.[53] Pasaribu et al. proposed a BC wound dressing functionalized with collagen and Chi in different configurations.[54] The BC/Col/Chi and BC/Chi/Col demonstrated similar moisture and antibacterial activity. However, BC/Chi/Col had smaller pores and displayed lower thermal stability than BC/Col/Chi (the temperatures of maximum mass-loss were 329°C and 338°C, respectively). According to the report, a higher chitosan-to-collagen ratio in the dressing provides a better potential for wound dressing. Gelatine, a collagen denaturation product, is an alternative to collagen as a BC matrix. The BC/gelatine shows good adhesiveness and biocompatibility, promotes cell adhesion and is inexpensive.

Poly(acrylic acid), which inhibits bacterial growth, is among the synthetic polymers considered for incorporation into BC for wound dressing.[55,56] Mohamad et al., who investigated burn wounds, reported significantly higher wound reduction in the group where BC with acrylic acid were enriched with fibroblasts and keratinocytes than in the control group including BC with acrylic acid only. Additionally, more significant collagen deposition was noted for BC loaded with cells, as compared with other treatments.[57] Another example of synthetic polymer incorporated in BC is polyacrylonitrile. Xiao et al. reported that BC/polyacrylonitrile in 25:75 ratio displays higher thermal stability (temperature at weight losses of 50% increased from 296°C to 560°C). Also, comparing dynamic contact angles, the BC/polyacrylonitrile composite has shown a high hygroscopicity property, playing a decisive role in exudate absorption.[58] The BC/poly(ethylene glycol) composite was characterized by better adhesion and proliferation of fibroblast than pure BC. In addition, this polymer improved the thermal stability of BC (from 263°C to 293°C), while tensile strength tended to decrease (Young's modulus decreased from 6.35 GPa to 4.12 GPa).[59]

The effects of natural and synthetic polymers on selected physical properties of bacterial cellulose are presented in Table 1.

Other biomedical applications

The research on the use of BC does not finish with the development of skin wound dressings. The usefulness of BC has been studied by scientists from various fields of medicine. Its high flexibility, persistence and good biocompatibility allow BC to conform to uneven surfaces and treat infections in hard-to-reach areas. There are reports of potential use of BC in dental therapies, treatment of inflammatory lesions, or after dental procedures, like extraction, root canal treatment or mucosal transplantation.[19,60,61] Depending on the application, BC can be made stable or degradable, allowing it to be placed in the body permanently or temporarily.[62] Some studies were carried out on the application of BC in bone engineering and cartilage implants.[63,64] Due to the excellent biocompatibility and slow degradation, BC is considered a composite bone repair material.[63] Moreover, BC coated with hydroxyapatite displays enhanced mechanical properties.[63] It is excellently moldable, so that it can be applied in soft tissue reconstructions. It has frequently been reported that BC has potential to become a material for artificial blood vessels, for instance in replacement of atherosclerotic coronaries.[64] Innovatively, Binnetoglu et al. applied BC tubes in facial nerve repairment, which allowed for robust myelinated fibers regeneration.[65] An intriguing adaptation of BC was applied in eye therapeutics. Bacterial cellulose with convex shape can be used as contact lenses. Additionally, BC loaded with drugs may be applied in eyeball infections.[66]

Conclusions

Bacterial cellulose displays high absorption capacity, which makes it suitable for the incorporation of many antimicrobial substances, both hydrophilic and hydrophobic. Small molecules, such as metal ions, as well as high-molecular compounds with bactericidal activity, are being tested. Numerous studies have shown that antibiotics and antiseptics applied in wound infection treatment display high antimicrobial effect within release time from BC. Moreover, BC seems to be a proper carrier for essential oils that consist of antibacterial compounds and which may be used in wound care. Additionally, its physical properties such as crystallinity, thermal stability and tensile strength can be easily modified by specific changes implemented in a culture method or incorporation of natural or synthetic polymers. The aforementioned advantages of BC make it a promising material for wound dressing development.

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Ultrasonic-treated fucoidan as a promising therapeutic agent

Fukoidan poddany obróbce ultradźwiękowej jako obiecujący środek terapeutyczny

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Abstract

Fucoidans represent the sulfated heteropolysaccharides that possess a wide range of important pharmacological properties. The properties of a fucoidan depend on several factors, including the molecular weight and the way of extraction. However, the selection of an optimal depolymerization method is necessary to enhance its therapeutic applications. Reducing the molecular weight of fucoidans will make it possible to use them in creating nanoparticles and nanocarriers for, among others, the targeted drug delivery. The molecular mass of the polymer can be changed by means of various methods of depolymerization. In this work, the possibility of application of ultrasonic destruction for decrease in the size of fucoidan molecules for the purpose of expansion of opportunities and spheres of their therapeutic application is considered. This is one of the simple and effective methods of depolymerization of fucoidan, which leads to a decrease in molecular weight without significant structural changes in macromolecules. In addition, methods and potential applications of the ultrasonic extraction of fucoidan from seaweed and the possibilities of their combination are discussed, as well as other physical or chemical methods of extraction.

Key words: antioxidant activity, fucoidan, depolymerization, ultrasonic treatment, ultrasonic extraction

Streszczenie

Fukoidany to siarczanowane heteropolisacharydy o szerokim zakresie farmakologicznie ważnych właściwości. Właściwości fukoidanu zależą od wielu czynników, w tym masy cząsteczkowej i metody ekstrakcji. Jednak w celu poszerzenia możliwości zastosowania terapeutycznego konieczny jest dobór optymalnej metody depolimeryzacji. Zmniejszenie masy cząsteczkowej fukoidanów pozwoli na ich wykorzystanie do tworzenia nanocząstek i nanonośników, w tym do ukierunkowanego dostarczania leków. Masę cząsteczkową polimeru można zmienić przy użyciu różnych metod depolimeryzacji. W niniejszej pracy rozważono możliwość wykorzystania destrukcji ultradźwiękowej do zmniejszenia wielkości cząsteczek fukoidanu. Metoda ta jest jedną z prostych i skutecznych metod depolimeryzacji fukoidanu, która prowadzi do spadku masy cząsteczkowej bez istotnych zmian strukturalnych w makrocząsteczkach. Omówiono również metody i możliwości ekstrakcji ultradźwiękowej fukoidanów z alg, a także możliwość łączenia ich z innymi fizycznymi lub chemicznymi metodami ekstrakcji.

Słowa kluczowe: aktywność przeciwutleniająca, ekstrakcja ultradźwiękowa, fucoidan, depolimeryzacja, obróbka ultradźwiękowa

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Introduction

Fucoidans are of a great interest among biopolymers of marine origin. Fucoidan is a branched sulfated heteropolysaccharide isolated from brown algae and some marine invertebrates.[1] The main monomeric unit of fucoidan is L-fucose. However, the presence in the structure of some amounts of residues of glucose, mannose, xylose[2], galactose[3,4] and glucuronic acid was also established. The structure of fucoidans itself is not uniform and the 2 most common types of backbone can be distinguished. The 1st type is the 1→3-related residues of α-L-fukopyranose, the 2nd type is alternating 1→3- and 1→4-related residues of α-L-fukopyranose.[6] In addition to sulfate groups, which are usually located at the C-2, C-3 and/or C-4 carbon atom of the fucose ring,[7,8] there are acetate groups at the positions C-4 (at the 1→3-related fucose residues) and C-3 (at 1→4-bonds).[8] The properties of the polysaccharide depend on the structural characteristics of the polysaccharide determined by a group of factors (the place of growth, the raw material, the time of its collection, the method of extraction, etc.) and require the selection of an optimal method for obtaining its extraction. The molecular weight of such polysaccharides can vary widely. Low-molecular-weight (3–8 kDa), medium- (from 30 kDa) and high-molecular-weight fucoidans are isolated (may exceed 2000 kDa). Fucoidan has a wide range of biological activity, including immunomodeling,[9] antimicrobial,[10] anti-inflammatory,[11] anticancer,[12] and antiviral activity.[13] It enhances the activity of natural killers, macrophages, dendritic cells and T-cells,[14] and stimulates hemopoies.[15] There is an increasing interest in the possibility of its use as an adjuvant. An adjuvant effect is observed when the vaccine is administered orally.[16] Immunomodulation is observed after absorption of the vaccine in the small intestine. The presence of sulfate ester groups confers a negative charge on the skeleton[17] and in general, the mechanism of action of this polysaccharide in biological interactions with various targets is based on the charge density and chemical properties of the biopolymer itself.[17,18]

The industrial production of fucoidan and functional products with its contents is expanding.[19] For these purposes, fractions of low-molecular-weight fucoidan are used more often, since some high-molecular-weight fucoidans have a strong branching of the molecule, which leads to an increase in viscosity[20] and a decrease in the absorption of the polysaccharide due to "limited transport" through the cell membrane".[21]

Furthermore, low-molecular-weight fucoidans exhibit higher biological activity.[22] In such a way, for the use of fucoidan in the pharmaceutical and food industries, it is necessary to develop a quick and easy way to produce low-molecular-weight fucoidan with specified physicochemical properties. Ultrasonic exposure can lead to faster reactions and processes. For polysaccharides, ultrasound is used to extract them from raw materials or through depolymerization process,[23] since the generated acoustic energy is sufficient both to destroy the cell wall of the raw materials[24] and to break bonds in polymer structures.[25]

Ultrasound as a depolymerizing factor

Low-frequency ultrasonic exposure is applicable to the depolymerization of polymeric materials, including naturally occurring ones, and has been used to depolymerize various biopolymers, including polysaccharides, DNA, etc., without altering their chemical structure.[26]

Ultrasonic degradation is characterized by a high decomposition rate of large molecules with a narrow molecular weight distribution,[27] which allows to obtain an aqueous polymer solution without introducing additional purification steps. To reduce the molecular weight of polymers, ultrasonic waves with a frequency of 16 kHz are used.[23] Ultrasonic processing is based on the phenomenon of cavitation. As a result of cavitation, shock waves, intense local heating (about 5000°C) and high pressure (about 1000 atm) are created (Fig. 1).[28] The energy is released to break bonds in any polymeric material,[25] including glycosidic bonds in polysaccharides.[29]

The primary, secondary, and physical sonochemical effects are isolated. The primary effect is associated with all processes occurring in the gas phase inside the bubble, the secondary effect in the solution phase, and the physical effect is caused by the shockwave.[30] It is assumed that polymer chain rupture as a result of sonolysis is not random, but is carried out in the middle of the molecule, with a greater effect observed when exposed to low-frequency ultrasound.[31] Polysaccharides have also been found to depolymerize more rapidly in dilute solutions and with the increased ultrasound time.[32]

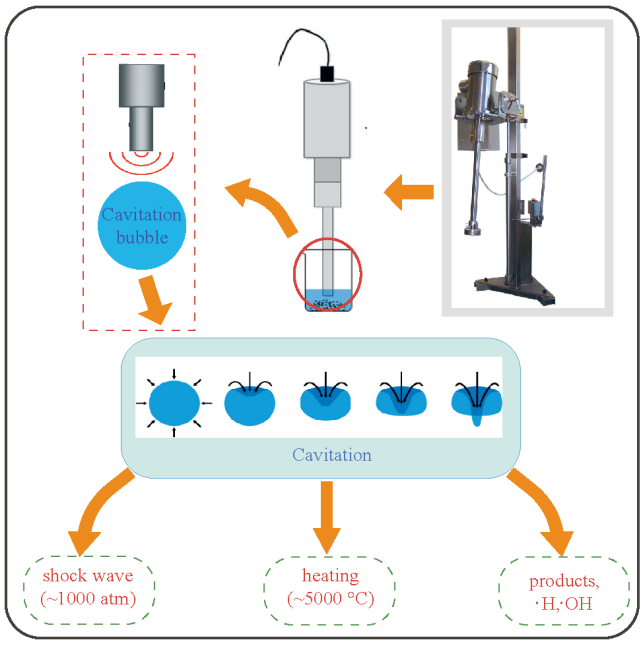

Fig. 1. Types of ultrasonic emission

In addition, it is known that with prolonged and intense exposure to an ultrasonic field, the energy transfer is dampened and fragments of polysaccharides larger than 20 kDa are formed.[33] Therefore, to increase the effectiveness of ultrasonic exposure, a combination of other physical methods is possible, such as radical depolymerization,[34] or introducing additional chemicals into the treatment medium.[35] The ultrasonic treatment itself is possible in a neutral, acidic and alkaline medium. Upon ultrasonic exposure at 300 W to a chitosan solution in acetate buffer with pH 4.4 and a concentration of 0.2%, 0.8%, 1.4% and 2.0%, the polydispersity decreased from 10.10 to 2.11, 3.11, 4.04, and 5.09, respectively.[36] The use of an alkaline medium is possible with low solubility of the depolymerized component or complex, where cleavage occurs from the surface of the swollen particles.[37]

Hydroxyl radicals generated using ultraviolet cavitation also make a great contribution to bond breaking, including glycosidic ones. Therefore, in polysaccharide depolymerization, additional introduction of hydrogen peroxide (H_2O_2) is possible, which catalyzes radical hydrolysis under the action of ultrasonic waves.[38] This ultrasonic treatment leads to the formation of products with a low polydispersity index (PI = 1.38 ±0.001).[38] The efficiency of the depolymerization process itself is improved. High molecular weight (MW) exopolysaccharide produced by a deep-sea hydrothermal bacterium *Alteromonas macleodii* subsp. *fijiensis* biovar deepsane is halved compared to ultrasonic treatment without hydrogen peroxide (MW = 204.5 – 112.7 g/mol).[34] Metal ions can be used as catalysts to increase the amount of hydroxyl radicals in the system. An example of such ions are Fenton systems where Fe^{2+} ions act as a catalyst for the production of such radicals.[33] Ultrasound synergistically increases the effectiveness of the Fenton reaction in decomposing pectin from 448 kDa to 5.5 kDa in just 35 min,[33] and heparin from 14,8 kDa to 4.87 kDa within 20 min.[39] In the process of such depolymerization, no significant chemical changes in the backbone occur, including fucoisylated chondroitin sulfate and loss of sulfate groups.[40] Moreover, when Fenton depolymerization based on the H_2O_2/ascorbic system acid is used, the increase in efficiency of decomposition of fucosylated chondroitin sulfate without loss of fucoidan branches is observed.[41]

Ultrasonic depolymerization of fucoidans

The main property determining the functional value of the polymers is the MW distribution.[42] Unlike acid hydrolysis, ultrasonic depolymerization leads to the production of fucoidan oligomers without changing the monomer composition and quantitative content of sulfo groups.[19] However, the decrease in MW must be controlled because a decrease below the optimal value for a given activity can lead to the loss of this activity.[21] Therefore, it is known that the inhibition of α-amylase activity is possible by fractions of fucoidan with a molecular weight of 637 kDa and 2351 kDa. Fractions with a molecular weight below 43 kDa no longer possess this ability.[21,43]

Ultrasonic degradation has been found to not lead to significant structural changes in fucoidan macromolecules.[44] When sonochemically treated in an aqueous medium, fucoidan isolated from sea cucumber at an intensity of 508 W/cm^2 and a frequency of 21–25 kHz retained repeated linear tetrasaccharide blocks only with partial destruction of unsulfated fucose units.[45] After 220 min of such treatment, the average molecular weight of fucoidan decreased from 338 to 91 kDa. Depolymerization of fucoidan from *Sargassum fulvellum* by high-intensity low-frequency treatment (25 kHz, 200 W) in the presence of H_2O_2 leads to the acceleration of the decomposition of fucoidan. The resulting product retained the structural features of the original biopolymer without altering the functional groups, such as sulfate and monosaccharide units.[46] The infrared (IR) spectra of the treated fucoidan were identical to the spectrum of the native polysaccharide, with the exception of the peak of the bond stretch absorption band C=O (1730 cm^{-1}) and the peak of asymmetric bond stretch COO^- (1630 cm^{-1}).

At an ultrasonic depolymerization of a fucoidan from *Sargassum muticum*, the shift of peaks of molecular weight from 80 kDa and 40 kDa up to 65 kDa and 25 kDa, accompanied with the increase in antioxidant properties of polysaccharide, with the maximum value of indicators of samples processed at 80 kHz within 120 min is observed. These samples have also shown the inhibiting action on growth of cells of carcinoma of a neck of the womb (HeLa 229).[47]

The further use of depolymerized fucoidan is possible in various fields, including the creation of nanoparticles and nanocarriers. At the same time, the use of high-molecular-weight fucoidan leads to the production of only large particles. For example, when high-molecular-weight fucoidan reacts with chitosan, large aggregates are formed.[48] With a decrease in the size of fucoidan, the size of particles formed with chitosan also decreases. Thus, with a decrease in molecular weight from 340 kDa to 123 kDa in a ratio of fucoidan to chitosan 1:1, there is a drop in the size of the formed particles by 50–70 nm. Furthermore, an increase in the quantitative content of fucoidan in the system at some stages led to an increase in the difference of nanoparticles size.[48]

Ultrasonic extraction of fucoidan

The main method for extracting fucoidans from raw materials is the use of acid solutions at a temperature of 70–100°C with the separation of alginates using Ca^{2+}.[49] The H_2O_2 can be used to remove polyphenolic compounds. However, it is now increasingly popular to use physical

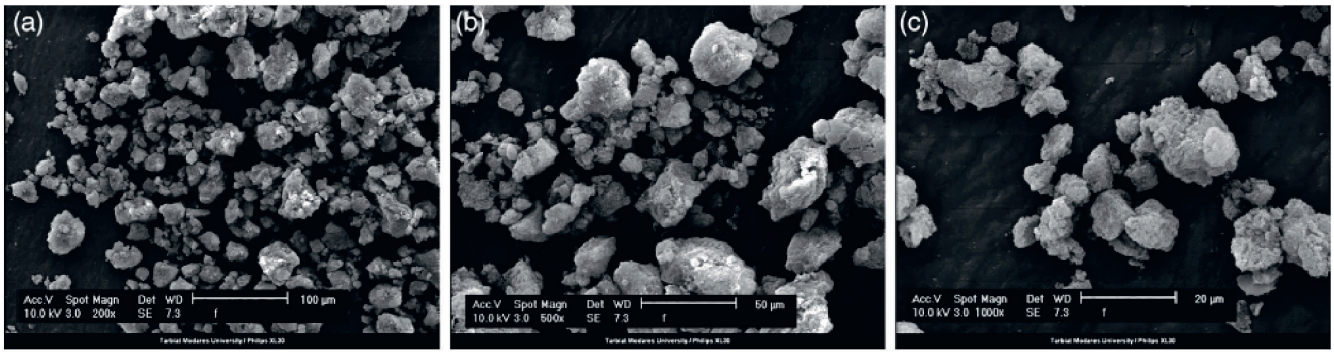

Fig. 2. Scanning electron microscope (SEM) picture (×200, ×500 and ×1000) of fucoidan extracted with ultrasonics[54]

extraction methods to reduce or eliminate the complete use of toxic solvents with an increased extraction efficiency.[21] However, it is necessary to control and optimize the ultrasonic processing time of the raw material to avoid damage to the target compound.[50]

The ultrasonic extraction of fucoidan from the raw material is carried out under the influence of low-frequency ultrasound with a short duration in time, possibly in an acidified medium. In addition, both aqueous and alcoholic systems may be used as the treatment medium. Treatment of *Sargassum muticum* in water with ultrasound at a frequency of 40 kHz, with a power of 150 W for 5–30 min at 25°C led to the production of fucoidan with a high yield (147.6 ±8.0 g/kg of raw materials).[51] The combination of such extraction with ion exchange chromatography allowed the total fucoidan fraction from the *Fucus evanescens* brown algae to be divided into 2 fractions at a ratio of 1:0.2.[52] The fractions differ in structural characteristics, namely the presence and location of the acetate group, the content of galactose and xylose residues.[52] With respect to the structural features, fucoidan isolated by ultrasound has a lower fucose content.[53] The fucoidan obtained by ultrasonic extraction from *Nizamuddinia zanardinii* spp. at a ratio of water to raw materials of 80:1 (power 196 W, extraction temperature 70°C for 58 min) showed a noticeable inhibition of the growth of cancer cells HeLa (62.36%) and HepG2 (56.83%) (Fig. 2).[54]

In the work of Okolie et al., the extraction was carried out by ultrasound at a frequency of 20 kHz for 35 min in an aqueous medium containing 0.01 M HCl, followed by the treatment of the extract with 2% (w/v) $CaCl_2$ and 4 volumes of 95% ethanol.[55] The fucoidan obtained from *Ascophyllum nodosum* spp. showed a high prebiotic activity similar to that of the standard prebiotic inulin. The addition of fucoidan extracts to MRS (de Man, Rogosa and Sharpe) broth, with a final concentration of 0.1 and 0.5%, significantly ($p < 0.05$), but not dose-dependently, improved the growth of *Lactobacillus delbrueckii* subsp. *bulgaricus* strain.[55] When alcohol was used as a solvent in ultrasonic extraction, the fucoidan yield increased by 16.8%, compared to the extraction with hot water. In this way, fucoidan was obtained from *Sargassum mcclurei* at a solvent to algae ratio of 24:1, the extraction time of 49 min at 54°C, with the ultrasound power being 360 W.[56]

Ultrasonic exposure is also known to lead to an increase in enzyme activity when used together. The enzymatic ultrasonic extraction method allows for obtaining a lower molecular weight polysaccharide with a higher fucoidan yield compared to the ultrasonic method (from 3.6% to 7.87%).[57] The average molecular weight of fucoidan isolated by the ultrasonic method was 1020.85 kDa and enzymatic ultrasonic was 443.70 kDa.[57]

In addition to chemical agents, ultrasonic exposure during extraction can be combined with other mechanical effects. For example, the combination of ultrasonic exposure (200 W, 20 kHz, 55°C) and microwave exposure (700 W, 90°C) resulted in an increase in the fucoidan sulfate content of 27.16%.[58]

Conclusions

Due to the biological activity of fucoidans, interest in them remains high. However, the selection of an optimal depolymerization method is necessary to enhance their therapeutic applications. Reducing the molecular weight of fucoidans will make it possible to use them in creating nanoparticles and nanocarriers for, among others, the targeted drug delivery. Ultrasound destruction is applicable for these purposes. This is one of the simple and effective methods of fucoidan depolymerization, which leads to the decrease in molecular weight without significant structural changes in macromolecules. This approach is simple and can be used on an industrial scale. The ultrasonic extraction method is useful for extracting fucoidans from algae. Yet, the use of the ultrasonic method in combination with chemical (for example, acidic, enzymatic) and physical (for example, microwave radiation) methods allows to increase the yield of the desired product.

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Is additive manufacturing a magic bullet to resupply lacking PPE? Producing respirators and face shields during COVID-19 pandemic: A systematic review

Czy druk 3D to skuteczny sposób na dostarczenie brakujących środków ochrony osobistej? Produkcja maseczek ochronnych i przyłbic podczas pandemii COVID-19 – przegląd systematyczny

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Abstract

Coronavirus Disease 2019 (COVID-19) pandemic caused an increase in the demand for personal protective equipment (PPE) and disruptions in production chains, resulting in an acute shortage of PPE. A possible solution to this problem was additive manufacturing (AM) technology – allowing for a quick start of the production of PPE and potentially able to meet the demand until the production is restored. In addition, AM allows for the production of PPE prototypes with potentially greater comfort of use or degree of protection. In order to assess the production of PPE in AM during the COVID-19 pandemic, previously published articles in this field were analyzed. After analyzing abstracts and full texts, 30 original works were selected from the initially collected 487 articles.

Based on the analyzed literature, it was found that there are not enough studies comparing traditional and AM PPE as well as not enough comparisons of the different types of AM PPE with each other. In many cases, researchers focused only on the subjective assessment of the comfort of using PPE, without assessing their effectiveness in preventing infections. Despite that, AM has a great potential to quickly produce lacking PPE. Respirators and shields made by AM were rated by the vast majority of users as comfortable to wear. Some of the respirators could be adapted to a specific user, by designing on the basis of a face scan or after warming up the finished print and modeling the shape.

Key words: COVID-19, personal protective equipment, three-dimensional printing

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Streszczenie

Pandemia COVID-19 doprowadziła do jednoczesnego wzrostu zapotrzebowania na środki ochrony indywidualnej (ŚOI) oraz przerwania łańcuchów produkcji, co poskutkowało dotkliwym niedoborem ŚOI. Możliwym rozwiązaniem tego problemu okazała się technologia druku 3D, pozwalająca na szybkie rozpoczęcie wytwarzania ŚOI i potencjalnie mogąca zaspokoić popyt do czasu przywrócenia produkcji dotychczasowymi metodami. Ponadto technologia druku 3D pozwala na wykonanie prototypów ŚOI o potencjalnie większym komforcie użytkowania lub stopniu ochrony.

W celu oceny produkcji ŚOI w technologii druku 3D w trakcie pandemii COVID-19, przeanalizowano dotychczas opublikowane artykuły w tej dziedzinie. Po analizie abstraktów oraz pełnych tekstów, z początkowo zebranych 487 artykułów wyłoniono 30 oryginalnych prac.

Na podstawie przeanalizowanego piśmiennictwa stwierdzono, że brakuje badań porównujących tradycyjne oraz wydrukowane ŚOI oraz porównań wykonanych już ŚOI między sobą. Ponadto w wielu przypadkach badacze skupili się jedynie na subiektywnej ocenie komfortu użytkowania ŚOI, bez oceny ich skuteczności w ochronie przed zakażeniem. Pomimo tych zastrzeżeń druk 3D ma duży potencjał szybkiego wyprodukowania brakujących ŚOI. Wykonane w tej technologii maseczki oraz przyłbice ochronne były oceniane przez zdecydowaną większość użytkowników jako komfortowe w noszeniu. Część maseczek ochronnych dawała możliwość dostosowania do konkretnego użytkownika poprzez zaprojektowanie na podstawie skanu twarzy lub po rozgrzaniu gotowego wydruku i wymodelowaniu kształtu.

Słowa kluczowe: COVID-19, środki ochrony osobistej, druk trójwymiarowy

Introduction

In December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first isolated in Wuhan, China. Since then, the virus has spread all over the world causing a global outbreak. In March 2020, the World Health Organization (WHO) announced the current situation as pandemic with overall 118,000 cases worldwide.[1] The increasingly high numbers of Coronavirus Disease 2019 (COVID-19) cases made it difficult to ensure protection not only for patients but also for healthcare workers. Wang et al. proved that 29% of in-hospital infections had health professionals involved.[2]

The virus spreads by respiratory droplets, e.g., coughing and sneezing, and is present in the upper respiratory tract for approx. 2–10 days before any symptoms appear.[3] Face shields combined with additional mouth and nose masks have been recommended to reduce the risks of inhalational exposure, specifically when performing activities with aerosol formations. Considering that no causative treatment is available, prevention has become the main objective. These aspects made it a necessity to cover the face and disinfect all used surfaces.[4,5] The global situation made it necessary for everyone to gain access to personal protective equipment (PPE). The pandemic created a great shortage in PPE and additive manufacturing (AM) was an alternative solution to this problem. Face shields are PPE devices used in many professions for protection of the face area from splashes and sprays of body fluids. Nonetheless, to be effective during the COVID-19 pandemic, they should be used with other protective equipment.[6,7]

The traditional manufacturing industries almost shut down because of lockdown measures, so AM stepped in to supply medical professionals. The steps for AM production of the PPE consist of creating or obtaining the project for the parts, and producing and assembling them with the additional required supplies. Additive manufacturing is a process which involves adding the material layer by layer in line with a computer-aided design model.[8] Models can be created using numerous 3D design software. Eventually, the designed model is conveyed to a slicer software to set production parameters, such as the height of the layers and the thickness of the shell of the models. Finally, the AM machine uses the resulting file to manufacture the model. The last step is post-processing: for example, removal of supports and sanding.[9] There are many methods of AM such as fused deposition modeling (FDM), stereolithography (SLA) and digital light processing. However, most of the analyzed papers described the use of FDM technology.

Materials and methods

A systematic literature review was done using the PubMed and Scopus databases. The search strategy was extensive to ensure that no significant articles were missed. The search algorithm consisted of 3 conditions, all of which had to be met: 1) connected with AM ("additive manufacturing", "3D printing" and names of individual AM techniques); 2) connected with COVID-19 pandemic ("covid 19", "sars cov 2", "pandemic", "coronavirus", "severe acute respiratory syndrome coronavirus 2"); 3) date of publication between November 1, 2019 and July 31, 2021, to exclude publications from before the outbreak of the COVID-19 pandemic. Also, in the case of the Scopus database, due to its multidisciplinary characteristics, the search criteria have been narrowed down to the Medicine category.

Only original papers were selected. Publications written in languages other than English or without a full article available were excluded from the analysis. Papers about virtual 3D rendering or modeling without manufactured AM parts were not included. Only articles where AM technology was used to produce or assist with testing and designing face respirators or face shields during the COVID-19 pandemic were selected.

The research team was divided into 2 workgroups. At each step, where biased selection had to be taken into consideration (2 stages: abstract screening and full-text screening), each group separately performed a manual selection. After that, the differences in the assessment were summarized and a discussion was held between both groups to establish a consensus. If no consensus was possible to achieve at the abstract screening step, the final decision was postponed until the full text was analyzed.

The articles that passed the full-text review were analyzed in detail using the table of evidence to present the relevant features and results of the study. Based on the results commonly reported in literature, the following variables were included: AM-made PPE types, analyzed PPE parameters, number of participants, test duration, test results, AM technology, and machinery and materials used. Detailed procedure of the article screening is presented in Fig. 1.

Face mask design

Many mask designs have come to light during the pandemic. Most of them consist of 3 reusable parts: the mask base, the filter grill and the filter insert (Fig. 2A). The mask straps are assembled using phlebotomy straps, Velcro/elastic bands or simple strings.[10–13] The disposable filter is placed between the filter insert and the filter grill. The filters inserted were: nano-sized Cumminis, IsoGuard filters, FFP2/FFP3 and HEPA. The FDM mask offered the possibility of reshaping, using both microwave and hot water since it is thermoplastic – this ensures a better fit on a user's face.[14] Face masks can be also custom-made by using face scanning programs. The obtained data are then used to design a 3D model.[11]

The limitation of available commercial standard masks is the poor variety of face shape. To ensure better protection, the design can be personalized. Fit testing of respirators is mandatory in some workplaces.[15] In particular, it is essential while performing procedures posing high risk of virus exposition, such as nasolaryngoscopy.[16]

Many researchers took advantage of computer-aided design software to implement some improvements in their projects. Helman et al. used the open-source design and modified it, enclosing more of the midface and adding 2 ports (they used software such as Blender (www.blender.org) and Fusion 360 (https://www.autodesk.com/products/fusion-360/overview)).[17] Piombino et al. used Meshmixer (www.meshmixer.com), which is available for free – that was important during the episodes of lack of PPE during the COVID-19 pandemic.[18] Shaheen et al. and Swennen

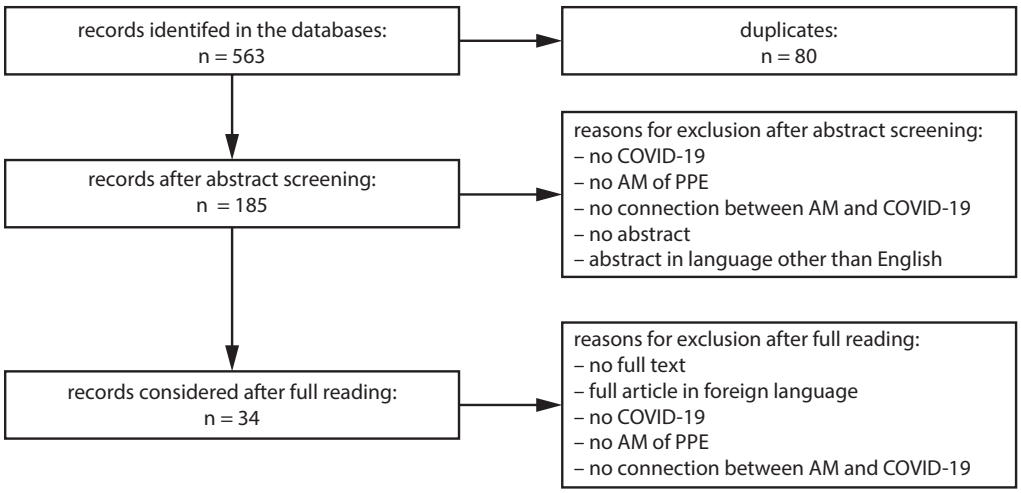

Fig. 1. Algorithm of articles selection

COVID-19 – Coronavirus Disease 2019; AM – additive manufacturing; PPE – personal protective equipment.

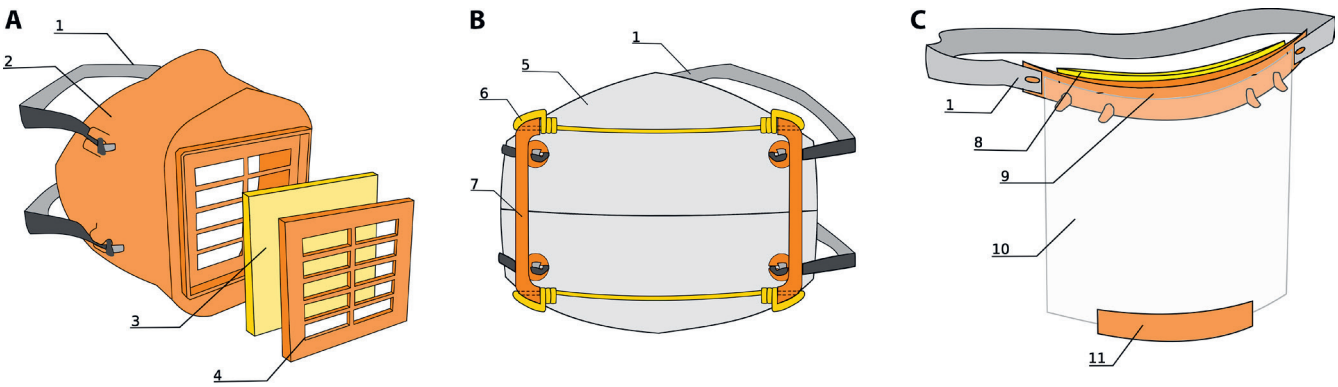

Fig. 2. A. An example of additive manufacturing (AM)-made face mask: 1 – straps, 2 – mask base, 3 – filter insert, 4 – filter grill; B. An example of AM-made face frame: 1 – straps, 5 – commercially available respirator, 6 – wire, 7 – frame; C. An example of AM-made face shield: 1 – straps, 8 – adhesive foam, 9 – headband, 10 – transparent layer, 11 – chin support

et al. used VECTRA Face Sculptor® to automatically put key landmarks on soft tissues on the face.[5,11] Davies et al. added port to existing face mask model (Copper 3D Nanohack; Copper 3D, Santiago, Chile) with Materialise Mimics software (www.materialise.com).[16]

There are various methods of minimizing the risk of virus transmission. Some designs (e.g., Helman et al.) were filled with vacuum seal between the face and the mask.[17] As tightness of the face mask increases, the protection becomes more effective. Another way to optimize the fitting of PPE is 3D face scanning. Swennen et al. used new generation smartphone with 2 cameras supported with Bellus3D app to generate individual 3D face scans.[11] Shaheen et al. used 3D camera VECTRA® H1 (Canfield Scientific Inc., Parsippany, USA),[5] while Piombino et al. used smartphone and Bellus3D application (Bellus3D, Campbell, USA).[18] These customized face masks can be complemented with a disposable filter membrane support, also designed using computer-aided design – those masks become consistent PPE after quick assembly. Both elements are connected with each other using screw fixation, which also improves tightness.[11]

Another interesting design concept is a project whose originators are Ng et al. Their design was adopted from a simple silicon respirator and was modified and produced with silicone injection molding. This one provides tightness and good filtration; it is also equipped with a port for a pleated-membrane respiratory filter.[19]

Anwari et al. designed a reusable mask, called a "simple silicone mask" (SSM). The SSM was invented for the appropriate fitting to a user's face shape. This was achieved by designing a special harness added to the basic mask construction and using silicone that provided air-tight seal. The design was supplied with a heat-moisture exchange filter. The mask was cast using original FDM molds.[20]

Bezek et al. found that the application of an epoxy sealant to the Stopgap respirator (made of polylactic acid (PLA)) increased the filtration efficiency from ~55% to a peak of ~75%.[21]

Face mask accessories

Not only masks but also accessories improving their quality and effectiveness, such as mask adapters, can be produced using AM technology. The design created by Imbrie-Moore et al. consisted of 3M N95 face mask (3M, Saint Paul, Minnesota) and SLA rigid cartridge with sealing flange.[22] Another PPE which was complemented with AM-made accessory, the adapter, was a full-face diving mask.[23]

Davies et al. designed face masks and adapters that may be used by patients undergoing medical procedures, such as nasal endoscopy. When examining a patient equipped with AM-made PPE, healthcare workers are less exposed to viruses.[16]

McAvoy et al. described the development of the design of the FDM face frames (Fig. 2B) that prolongs the lifespan of masks and allows them to be reused. The simple design enables it to be easily molded for customization.[24]

Face shield design

Most of the face shields consist of 3 main parts: the head band, the transparent layer attached to the band, and string/straps and additional parts that include optional chin support and adhesive foam (Fig. 2C). In addition to the manufactured parts, straps and transparent film are needed to assemble the shield. During the usage of face shields one should have in mind that they are inadequate as an individual protection, and will not be sufficient without a face mask.[10,25]

In the designing process, it is crucial to adjust further properties of the face shields to special medical allocation of this PPE. Hence, the design must be adapted to the needs of medical staff during the COVID-19 pandemic, the shortage of materials and lack of time for PPE preparation. Moreover, AM can make PPE more customized. Thanks to individual design modifications, AM-made face shields have features not present in commercially available ones.[26,27] For example, Critical Cover Coverall Face Shield by AlphaProTech (Markham, Canada) does not provide adequate liquid protection at the top and on the sides of the visor.[26]

Most of the studies focus on such parts of the design as: efficient protection (e.g., from aerosols and liquids), comfort of wearing (especially when worn for a long time), the possibility of fast assemblage of a PPE, and making it easy to manufacture. There are different methods that help to achieve these goals, for example, adjusting the length of the face shield to clinicians' needs, easy attachment, limitation of holes in the PPE, and adding the lip above the visor.[26,27] The most popular face shield design in analyzed studies was PRUSA RC2.[6,26,28]

A much more complicated design was presented in the study conducted by Huang et al. – a design consisting of 4 elements: goggles, lenses, exact face shield, and elastic bands. The goggles are the most comprehensive part of this design, because, as any other part of the shield they must fit the user properly. They were designed in 3 sizes (large, medium and small).[27]

Lemarteleur et al. designed a face shield that required 3 h to manufacture. The project was inspired by the open-source PRUSA RC2 and PRUSA RC3 models (Prusa, Prague, Czech Republic). The headband consisted of 2 arches: one to support the forehead, the other one to deflect the shield from the face. This construction prevented from fogging and was obtained with FDM using polylactic acid (PLA).[29]

It is crucial to mention that not only the significant features of face shields, but also the method and efficiency of production and a potential for large-scale manufacturing are the key elements in evaluation of a particular project.[6,25,26] Stacking – producing multiple parts on top of each other (they require post-processing for separation) – may be a factor providing effectiveness and reducing the costs of production.[25,26] The lack of this feature may be an exclusion criterion for the design due to its impracticability. A detailed comparison of face shield designs is presented in Table 1.

Table 1. Different face shields design comparison[6,26,30–32]

Parameters	Prusa RC1	Prusa RC2	3D Face Shield V3 (Budmen)	Easy 3D Face Shield	Modified Prusa (PanFab)
Tools for assembling	DIN A4 perforator	DIN A4 perforator	DIN A4 perforator	none	additional laser cutting or rotary die cutting of the transparent shield
Weight [g]*	39	51	42	30	no exact data, but it was mentioned that the design was based on RC2
Wearing comfort according to analyzed articles	worse because of a clamp for head frame (might be too tight)	increased (when compared to RC1) due to no clamp for head frame	less comfortable for medical staff than RC2 (e.g., due to rigidity)	similarly comfortable to RC2 (e.g., due to lower weight)	perceived as more comfortable for medical staff (e.g., due to reduction of tightness) than RC2
Anchor point	placed lateral to the headband	similar to RC1	similar to RC1	visor is put into a small continuous slot with clamping retention	placed in line with headbands – reduction of tightness
Dimensions and print volume requirements [mm^2]	240 × 240	144 × 191	requirements as in RC2	requirements as in RC2	240 × 305
Attachment (shield to strap)	four-point attachment; necessity of perforation	similar to RC1	similar to RC1	visor is put into a small continuous slot with clamping retention	six-point attachment; necessity of perforation
Protection from liquids (during special medical procedures) on the top and sides of visor	restricted in the area on the top of the visor	similar to RC1	no data	no data	fin on the top of headband and additional plastic lip
Additional equipment	lower space between face and visors (when compared to RC2)	increased space between face and visors (when compared to RC1) – easier to put eye or mouth-nose personal protective equipment (PPE) (goggles, masks)	space between face and visors comparable to RC2	space between face and visors comparable to RC2	no direct data, but space between face and visors regarded as comparable to RC2
Possibility of stack printing	no	yes	no	no	no exact data

* fused deposition modeling (FDM) technology was used; the weight can differ depending on the type of used materials or print parameters (e.g., the number of walls, infill density).

Face masks – materials

The transmission of SARS-CoV-2 occurs primarily by droplets. Most of the studies for testing filtration efficiency use particles with the size of 300 nm, whereas viruses are slightly smaller.[33]

Swennen et al. designed a reusable face mask design, which consisted of 4 elements, 2 of which were produced using selective laser sintering (SLS) and were reusable. The most important component was the mask itself, which was made of polyamide composite (PA11-SX 1450). This material has ISO/USP Class VI medical certification, which proves that there are no negative, long-term effects on the organism resulting from its use. The replaceable elements were the head fixation band and the polypropylene particle filter membrane (Moldex 8080).[11]

A filter project published by He et al. assumed the use of nanofiber mat made of 10% PLA (polylactic acid) solution dissolved in chloroform and n,n-dimethylformamide. The main body of the filter, on which the nanofiber mat was embedded in order to strengthen and avoid damage to its fibers, was made of the same solution, previously dried for 12 h at 80°C. The optimal printing temperature was 210°C. Higher temperatures led to a loss of transparency and filtration efficiency. The effectiveness of the surgical mask (filtration efficiency at least 55% for 700 nm mass median aerodynamic diameter) was exceeded with the use of 1 layer. The use of 2 layers allowed to achieve over 80% (FFP1 criteria), and the use of 4 layers – over 94% (FFP2), in some cases even above 95% of filtration efficiency (KN95/N95).[33]

In case of face masks, in order to prevent the virus from getting into the respiratory tract, a suitable, close fit to the face is necessary. Rendeki et al. describe the Face Mask v. 2.0 model, in which a layer of silicone has been added to reduce air leakage at the point of contact between the mask and the face. Disinfection does not adversely affect the mechanical properties of this material, and moreover, it is long-lasting and durable.[34]

The design of individual face masks on the basis of a 3D face scan was proposed by Shaheen et al. VeroClear

photopolymer, which is characterized by hardness and transparency, was used to print 6 components. The last element, a soft rim, was made of TangoPlus photopolymer, a soft transparent rubber-like material.[5]

Imbrie-Moore et al. shared an idea to transform one N95 face mask into 4 new masks with the same properties. They used SLA technology. The main materials of the mask adapter were multi-purpose polyurethane and biocompatible silicone. The filter was made of an N95 mask and it was attached using a nontoxic thermoplastic adhesive.[22]

Proper disinfection is a challenge that must be faced when using PPE made with AM technology. Vaňková et al. published an article comparing the disinfection efficiency of PLA using 96% ethanol, 70% isopropanol or 0.85% sodium hypochlorite. A suspension of bacteria and viruses (SARS-CoV-2) was applied to the reference object made of PLA. All 3 agents were effective in terms of complete decontamination against SARS-CoV-2. It is worth noting that in the case of ethanol, there was also a slight melting of filaments made of PLA. It led to a decrease in the distance between individual filaments, an increase in density and, presumably, the improvement of the filtration properties.[35] Welch et al. tested several materials, including PA12, acrylonitrile butadiene styrene (ABS) and PLA. A single application by wipe of quaternary ammonium (Sani-Cloth germicidal disposable wipe), 3% H_2O_2 and 10% bleach resulted in a complete inactivation of tested viruses, including SARS-CoV-2. However, a single wipe of 70% isopropyl alcohol led only to >95% inactivation, as compared to >99% effectivity of other compounds. For complete virus inactivation, stronger application might be required.[36]

Table 2 provides a summary of the properties, advantages and disadvantages of the filaments used in the production of face shields and masks using FDM technique.

Face shields – materials

Several tools and components are required to make the face shield. In most cases, only the main body of the face shield is made using AM techniques, to which the remaining accessories are then attached.[25]

The face shield project designed and published by Amin et al. contained a main body made of PLA filament. The protective barrier was a transparency film made of plastic. The face shield was attached to the head with 2 Velcro strips. In order to increase the comfort of use, a sponge was glued to the contact point of the AM-made headband with the forehead. The authors allow the use of Super Sani-Cloth® Germicidal Disposable Wipes (PDI, Woodcliff Lake, USA) for disinfecting and cleaning the face shields.[25]

A model designed by Armijo et al. consisted of 2 elements produced with FDM technology: a headband and a chin piece, which were manufactured using PLA. A transparent polyvinyl chloride (PVC) sheet and a head strap were installed to the headband. It is also possible to use plexiglass or laminating foil as a protective layer. The biggest disadvantage of PVC was the gradual loss of transparency due to the used cleaning solution. Optionally, in order to increase the comfort of use, the authors recommend gluing the foam to the inside of the headband. However, this will make it impossible to reuse the face shield after sterilization, which is done by disassembling the model and then dipping individual elements into the dilute bleach solution, followed by drying.[10]

Wesemann et al. published an article comparing 4 face shield designs. The main body of each of them was made of the biodegradable material Extrudr Green-TEC PRO (Extrudr GmbH, Lauterbach, Germany; carbon filament based on lignin). The protective layer was a transparent foil made of polyethylene terephthalate. In order to keep the face shield on the head, an elastic polyester strap was used.[6]

In the article published by Rendeki et al., scientists tested the disinfection of face shields manufactured with PLA, and the transparent protective layer made of poly(methyl methacrylate) (PMMA). As a disinfectant, they used a solution consisting of sodium perborate and tetraacetylethylenediamine. One disinfection cycle was run at 24°C and

Table 2. Properties, advantages and disadvantages of various filaments used for the production of face shields and masks using fused deposition modeling (FDM) technique[6,10,33,37,38]

Material property	PLA polylactic acid	ABS acrylonitrile butadiene styrene	PET polyethylene terephthalate	Green-TEC PRO based on lignin
Printing temperature	180–220°C	230–255°C	220–235°C	160–190°C
Durability	brittle	durable	durable	stable
Warp deformation*	little	prone	moderate	little
Autoclave sterilization – temperature stability	volume change	not recommended – low heat resistance	volume change	dimensionally stable
Other	high-speed low-cost material; ease of use; completely biodegradable	possibility of generating toxic gas fumes during printing; higher risk of shrinkage during cooling; not biodegradable	absorbs water – needs to be stored in specific conditions; not biodegradable	biodegradable

*warp deformation – bending towards the energy source caused by inner stresses resulting from the contraction of layer, lack of pre-heating the base plate, non-uniform distribution of temperature inside the build chamber, or improper control of process parameters.[39]

lasted for 1 h. Scientists compared the face shields after 0, 5 and 10 cycles, in terms of light transmission (using spectrophotometry – measurability of light transmission) and mechanical parameters: flexibility (tensile strength) and brittleness (three-point bending test). In terms of mechanical parameters and visibility, no significant changes were observed after 5 and 10 disinfection cycles.[34]

Perez et al. in their review article brought up the topic of sterilization of items made using AM technology. There are 4 main sterilization techniques commonly used in medicine: autoclave, gamma radiation, hydrogen peroxide gas plasma, and ethylene oxide gas. The following different materials were used for the tests: PC-ABS, ABS-ESD7, ABS-M30i, ABS-M30, and ABSi. From each of them, 30 specimens of the ASTM D638 Type I design were made and sterilized using the above methods. Then, each of the samples (including test samples, not sterilized) was placed in an airtight glass container in 60 mL of tryptic soy broth and incubated for 14 days. Efficacy evaluation was performed through the observation of tryptic soy broth, which became cloudy after the contamination with fungal or microbial agent. The authors report that individual samples gave a positive test result, but note that the contamination could have occurred after sterilization, during the transfer of the material to the incubation site. Of all the methods, it is worth noting the disadvantages of the autoclave as a method of sterilization of samples made of acrylonitrile butadiene styrene (ABS) derivatives. Humidity and high temperature had a negative effect on this material, leading to indentations, bending and color change.[37]

Sterilization of products made with the FDM technique using ultraviolet light is ineffective because these objects are not watertight or airtight.[10]

Noguera et al. tested possible damage of PPE due to 0.1% sodium hypochlorite, 70% ethanol and H_2O_2-quaternary ammonium salt mixture. The FDM face shields headbands were made using different materials (including PLA, ABS and polyethylene terephthalate glycol (PETG)) and layer thickness. Visors were made of 0.5 mm PETG, 0.5 mm poly(ethylene terephthalate) (PET), 0.75 mm polycarbonate, or 0.5 mm/0.75 mm polyethylene glycol (PEG). Disinfection was done using gas soaked in chemical solution, 30 times to headbands and 40 times to visors, followed by spontaneous drying. Researchers observed no physical changes in visual integrity to tested models.[40]

A comparison of commonly used materials for AM is presented in the previous chapter (Table 2).

Discussion

Most of the analyzed articles were peer-reviewed (excluding the article by McAvoy et al.[24]). There are concerns about the technical aspects of AM in this field, in particular FDM, as it was the dominant technology in the analyzed articles. For example, Gomes et al. defines the process speed as 100%,[28] which is a relative parameter, and therefore it is difficult to estimate the absolute value of the speed. It should be reported in millimeters per second with acceleration and jerk values. Moreover, it should be taken under consideration that any comparison of production times is also relative as there are many parameters that can be modified to accelerate production, often with (acceptable) decrease of quality.

Neijhoft et al. assessed the quality of the manufactured parts by assigning each FDM machine a different filament color.[41] Unfortunately, there is no information about the selected production temperatures, but it should be noted that the correct production temperature of PLA varies depending on the color of the filament.[42] It adds additional complication to the production – instead of quality assurance, each machine should be using individually modified file, for example with a simple marking (number of the machine) on the surface of the model.

Bezek et al.[21] describe a lot of flaws in their AM-made mask respirators, without any concern about possible errors during the manufacture process. Afinia H800 3D printer (Afinia, Chanhassen, USA) was used in their research. It is a fully enclosed FDM machine and as the picture of their product made of PLA suggest, a problem with the part cooling had occurred. The air cooling the model (during the printing) is warmer due to enclosure (air is taken from the inside); enclosure is used to provide slower cooling of the model (and usually is used without active cooling). There is a possibility that this problem affected PLA mask respirators manufactured by this team.

Parameters that were taken into account during face mask analyses included qualitative fit testing,[24] quantitative fit testing,[19,22,43] filtration efficiency,[13,21] and overall comfort or discomfort (Table 3).[18,19,43]

It is worth emphasizing that the use of 3D face scanning techniques allows for producing a mask with the highest degree of adhesion, and thus, tightness.[5,11,18] In order to increase the comfort wearing of a face mask obtained using AM techniques, the use of soft rim, most often made of silicone, has become common.[5,22,34] Designs of a modified full-face diving mask with AM-made additives and an additional filter were also described.[13,23] To increase the fit, microwave and hot water can be used, which allows for reshaping of an FDM face mask made of PLA.[14]

The methodology of the papers analyzing face shields varied (Table 4). Only 2 of them compared different types of AM-made face shields,[6,44] other assessed only 1 type,[26,27,45–47] and 1 compared N95 mask, goggles and face shield to modified full-face snorkel mask (with AM-made elements).[48] Number of participants (face shields testers) was between 9 and 300. The observation time was from 30 to 60 min in most cases, but multiple articles lack this information. The parameters included in the analyzes were COVID-19 infection,[27] participants' physiological parameters such as blood pressure,[48] fogging and splash protection,[26] but most of all, the individual evaluation

Table 3. Review of validations of additive manufacturing (AM)-made face respirators studies (including AM-made respirators accessories). Analyzed parameters included: qualitative fit testing (QLFT), quantitative fit testing (QNFT), filtration efficiency, and subjective users' opinion on the respirator

Study	Compared face respirators types	Parameters	Number of participants	Duration of test	Results	AM technology, machine, material	Comments
McAvoy et al.[24]	AM-made mask frames combined with masks: – 1860 N95 – 8210 N95 – KN95 – Kimberly-Clark duckbill	– QLFT – QNFT	45	unknown	passing rates with the frame in the absence of the original straps (in case of proper fit with original straps) were: – 1860 N95: 24/30 – KN95: 11/12 – Kimberly-Clark duckbill 12/15 – 8210: N95 9/9	FDM, no data, PLA	not every mask type was tested on each participant
Liu et al.[49]	AM-made adapter for the 3M 7501 and 3M 6200 elastomeric respirators to interface with anesthesia circuit filters	– end-tidal carbon dioxide – respiratory rate – self-reported of discomfort	8	60 min	mean end-tidal carbon dioxide and mean respiratory rate were not statistically different ($p > 0.05$); 4/8 (50.0%) subjects self-reported discomfort	FDM, Ultimaker S5, PLA (Premium PLA, Formfutura BV)	all participants passed qualitative positive and negative pressure leak testing, quantitative and qualitative fit testing
Bezek et al.[21]	AM-made masks: – Montana – Factoria – Stopgap; masks were manufactured once with each method	– filtration efficiency – masks were compared before and after post-processing	not applicable	not applicable	Factoria respirator provided the highest observed performance, with a filtration efficiency 90–95%; post-processing modifications to the produced respirators generally improved performance	FDM, Afinia H800, ABS and PLA; SLS; DTM Sinterstation 2500 Plus, nylon-12; FDM, Fortus 400 mc, ULTEM 9085	–
Gierthmuehlen et al.[13]	– AM-made COVID-19 MASK v. 2.0 – modified scuba-diving mask (Easybreath®) – mask sewn from a vacuum cleaner bag	– filtration efficiency	not applicable	not applicable	filter efficacy: – sewn mask: 69.76% – AM-made COVID-19 MASK v. 2.0: 39.27% – scuba-diving mask: 85.07%	FDM, Ender 3 pro Printer, PLA (Primacreator Primavalue); FDM, custom core XY machine, Tefabloc TPE (Verbatim), PLA (Verbatim) and PLA (Filamentworld)	–
Piombino et al.[18]	AM-made person-tailored Mask 3D	– skin comfort – respiratory comfort – quality of work shift whilst wearing the mask (five-point Likert scale) in 3 localizations: surgery room, medical clinic and maxillofacial surgery ward	6	7 days	overall rating: – in the surgery room: 3/6 very good, 3/6 good; – in the medical clinic: 2/6 very good, 4/6 good; – in the maxillofacial surgery ward: 2/6 very good, 4/6 good	FDM, Ultimaker 2 Extended+, TPU (Rubber TPU D27 (Bioflex, Bioalfa, Soria Vecchia) and PLA (Eco PLA, 3DJake Italia, Niceshops GmbH)	for such small test group results should be presented individually for each test subject
Davies et al.[16]	AM-made modified Copper 3D Nanohack (added a central port to permit attachment of bronchoscope adapter)	– spread of phosphor fluorescent dye during simulated bronchoscopy	1	not applicable	AM-made mask reduced to zero spread of phosphor fluorescent dye	SLA, Form 2, biocompatible photopolymer resin (Dental SG, Formlabs Inc.)	–

Table 3. Review of validations of additive manufacturing (AM)-made face respirators studies (including AM-made respirators accessories). Analyzed parameters included: qualitative fit testing (QLFT), quantitative fit testing (QNFT), filtration efficiency, subjective users' opinion on the respirator – cont

Study	Compared face respirators types	Parameters	Number of participants	Duration of test	Results	AM technology, machine, material	Comments
Imbrie-Moore et al.[22]	AM-made cartridge with an inner ridge and soft silicone base used to seal 1/4 of a 3M 1860 N95 mask	– QNFT	6	not applicable/no data	overall fit factor was 148 ±29	SLA, Carbon M2, biocompatible Silicone (SIL 30, Carbon) and Multipurpose Polyurethane (MPU 100, Carbon)	–
Ballard et al.[43]	AM-made 5 rigid and 5 flexible mask prototypes of own design	– QNFT – comfort level	4	7 min	2 designs produced with flexible polymers passed QNFT with a mean fit factor of 138; comfort level was similar to N95 respirators	SLA, Form 2; elastic and flexible (V2) resins (FormLabs); PolyJet; Stratasys J750; Agilus30, Biocompatible Clear MED610, Tango and Vero; FDM Makerbot 5th Gen; PLA	–
George et al.[50]	SNAP – AM-made, single-use, valved endoscopic port, retrofitted to any surgical mask	– spread of fluorescein – adverse effects	9	no data	no spread of fluorescein; no adverse effects	FDM, Flashforge Creator Pro 3D, no data	–
Ng et al.[19]	AM-made reusable silicone-molded face mask (SSM), N95 3M face mask	– QNFT – comfort – breathability	40	not applicable/no data	SSM scored 3.5/5 and 4/5 for comfort and breathability; overall passing rates in disposable and SSM respirators on QNFT were 65% and 100%	Mold: FDM; PRUSA I3 MK3S, no data; Harness FDM, PRUSA I3 MK3S, PLA and PETG	–
Felinska et al.[23]	modified Easybreath full-face diving mask (addition of filter), standard surgical mask	– time until proficiency – number of attempts until proficiency (laparoscopic suturing) – Objective Structured Assessment of Technical Skills scores (laparoscopic cholecystectomy) – comfort	40	not applicable	no statistically significant difference	FDM, no data, PLA	participants were laparoscopically naive medical students
Helman et al.[17]	AM-made endoscopic mask	– surgical freedom – test of aerosolization	not applicable	not applicable/no data	mask reduced particle spillage: – by 86% for anteriol surgery – by 71% for posterior surgery – the trocar system reduced spillage by 97%; mask allowed for an appropriate surgical range of motion	FDM, Ultimaker 2 and Pulse XE, TPU and Polyamide 12 (NylonX, MatterHackers)	tested on 2 cadavers

FDM – fused deposition modeling; PLA – polylactic acid; SSM – simple silicone mask; TPU – thermoplastic polyurethane.

Table 4. Review of validations of additive manufacturing (AM)-made face shields studies. Analyzed parameters included: fogging testing, splash protection, users' body parameters like respiratory rate, and subjective users' opinion on the respirator

Study	Compared AM-made face shields types	Parameters	Number of participants	Duration of test	Results	AM technology, machine, materials
Wesemann et al.[6]	RC1, RC2, Budmen V3, Easy 3D	– fit – comfort – wearing – protection – overall evaluation (Visual Analogue Scale (VAS))	10	60 min	overall Easy 3D (87 ±4) and RC2 (81 ±5) received the highest scores, which differed significantly from those for RC1 (63 ±6) and Budmen V3 (56 ±4) ($p = 0.001$)	FDM, Prusa I3 MK3S, PLA (GreenTEC PRO, Extrudr)
Sapoval et al.[45]	3D4Care face shield (modified RC2)	– ability to perform the assigned intervention as usual – quality of visual comfort – musculo-skeletal tolerance (1–5 Likert scale)	38	mean time 59 min	ability to perform the assigned intervention as usual was 1.7 ±0.8 (SD); mean visual tolerance rating was 1.6 ±0.7 (SD); the mean tolerability rating was 1.4 ±0.7 (SD)	FDM, no data, PLA and ABS
Celik et al.[46]	own unnamed design	– functionality – design – quality – satisfaction of use – first impression – ergonomics – originality of design – material quality (insufficient/poor/average/good/excellent)	15	no data	in most questions about 80% good or excellent answers	FDM, no data, PLA
Chaturvedi et al.[47]	own unnamed design	i.a.: – ease of use – visibility during the procedures – comfort during the procedures – ease of assembly – ease of disassembly – ease of cleaning – confidence to reuse (0–10 scale)	37	no data	overall mean score was 7.92 ±2.13 (SD)	FDM, no data, PLA
Kusano et al.[48]	set (N95 mask, goggle and face shield), modified full-face snorkel mask (with AM-made elements)	– blood pressure – pulse – oxygen saturation – respiratory rate (assessed twice: before and after the procedure of endoscopy)	9	30 min	statistically significant: set decreased oxygen saturation by 0.9 percent point; modified snorkel mask increased respiratory rate by 1.5 breaths/min	no data
Mostaghimi et al.[26]	PanFab face shield (modified RC2)	– fogging testing (min. 30 min) – testing splash protection (subjective) – durability – ease of use – comfort (five-point Likert scale)	92	30–60 min	average scores were: – splash protection – 4.7 – durability – 4.6 – ease of use – 4.3 – comfort – 4.4	FDM, Ender 3 Pro, PLA (Hatchbox)
Huang et al.[27]	own unnamed design	COVID-19 infection	over 300	no data	none of participants were reported to be infected with COVID-19	SLA, no data
Desselle et al.[44]	Prusa RC3, MSD headband	– presence of visible contamination on the face and forehead	5 participants, 10 reviewers	no data	overall pass rates: MSD headband 75%, Prusa RC3 100%	no data

SD – standard deviation; FDM – fused deposition modeling; PLA – polylactic acid; ABS – acrylonitrile butadiene styrene.

of the face shield model by participants.[6,26,45–47] Most of the researches tested only if AM-made face shields are comfortable to wear, not if they protect against infection (especially COVID-19). Huang et al. mentioned that none of the users of their face shield were reported to be infected with COVID-19, but they did not report the methodology for this finding. There is no information if the participants of that study were tested for SARS-CoV-2 on a regular

basis. There is lack of control group of non-AM-made shields users to determine the true usefulness of this PPE.[27]

Wesemann et al. found out that Easy 3D (https://www.thingiverse.com/thing:4233193) and Prusa RC2 achieved better overall score than Prusa RC1 and Budmen V3 (IC3D Printers, Columbus, USA).[6] Desselle et al. proved that the Prusa RC3 is better than the MSD headband (University of Melbourne, Australia).[44] In the remaining papers, researchers tested 3D4Care face shield (modified RC2; 3D4Care, Paris, France), PanFab face shield (also modified RC2; Greater Boston Pandemic Fabrication Team, USA) and other, unnamed designs. All of them received a subjective positive rating.

Proper disinfection, which allows AM-made PPE to be reused, is a major challenge. The polymers used for FDM production are prone to high temperature and humidity (autoclave). Therefore, the most common decontamination method, especially against SARS-CoV-2, was the use of 96% ethanol, 70% ethanol, 70% isopropanol, 0.85% or 0.1% sodium hypochlorite, 3% H_2O_2, 10% bleach and quaternary ammonium, or H_2O_2-quaternary ammonium salt mixture.[36,40]

The quality control of the finished part should be considered. It is difficult to compare between the analyzed papers regarding which models are the fastest and cheapest to produce, due to the differences between used machines, materials, process parameters and models included in the comparison, and is beyond the scope of this article.

The authors of the analyzed articles did not provide any information whether the used materials and processes are certified (or verified) for skin contact, apart from Swennen et al. (PA11-SX 1450 which meets USP Class VI requirements).[11] The additional research of materials used in the reviewed articles did not reveal any other skin-safe materials.

Conclusions

In the design phase, it is crucial to focus on effective protection, comfortable wearing and the possibility of easy production of the PPE. It is also commendable to make the project publicly available for free, with open source data. In addition to helping to produce PPE, it encourages global collaboration to improve the design. The current situation requires efficient cooperation of the scientific community to overcome the challenges posed by the COVID-19 pandemic.

The face masks are the most important element of PPE, as the transmission of SARS-CoV-2 occurs primarily by droplets. They can be produced with AM to replace shortages, but also can be personalized for potential better comfort or protection and a better fit than commercially available ones (e.g., SSM respirator). Presumably, personalized masks would be more expensive, and in a crisis such like the COVID-19 pandemic, there would be no time or resources for it.

Unfortunately, most researchers have only tested 1 type of AM-made respirators and did not compare them with other respirators (AM-made or commercially available). In comparison, the Factoria respirator provided the highest performance observed, with a filtration efficiency of 90–95%.

Furthermore, the face shield is an important part of the PPE utilized during the COVID-19 pandemic. It can be concluded that, with certain limitations, AM-made face shields can be designed and manufactured. There is no research comparing commercially available face shields and AM-made ones. Basing on insufficient data (mainly questionnaires), RC2 (and its newer version – RC3 or their modifications) is the best choice for the FDM face shield model. It has good fitting and wearing comfort, and offers space for additional PPE and stacking possibility.

Additive manufacturing is not adequate for high-volume production of PPE, but it is still useful. It has the potential to temporarily fix the broken supply chains and it is useful in designing new PPE products. It allows for the production of personalized PPE or accessories to improve existing PPE (e.g., frames for better fit of face masks). A significant limitation of AM-made PPE in the analyzed papers is the lack of data on the safety of skin contact of the produced PPE.

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Historical article

An exceptionally long line: 50 years of "Polymers in Medicine"

Wyjątkowo długa linia: 50 lat "Polimerów w Medycynie"

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Streszczenie

Historia „Polimerów w Medycynie" odzwierciedla nie tylko rozwój zastosowania tego typu materiałów w medycynie i farmacji, ale także przemiany w polskich czasopismach naukowych – rozpowszechnianie wyników badań naukowych i życie naukowe zawsze odbywają się w określonym kontekście zarówno językowym, jak i społeczno-politycznym. Artykuł prezentuje historię czasopisma od powstania biuletynu informacyjnego „Tworzywa Sztuczne w Medycynie", poprzez I Międzynarodową Konferencję Krajów Członkowskich RWPG „Zastosowanie tworzyw sztucznych w medycynie", która odbyła się w październiku 1969 roku w Warszawie oraz założenie „Polimerów w Medycynie" w latach 1970–1971, aż po współczesność. Przedstawiono zmiany na stanowisku redaktora naczelnego, przemiany szaty graficznej, a przede wszystkim zmieniającą się tematykę czasopisma, które początkowo poświęcone było przede wszystkim materiałom polimerowym w ogólności, protetyce ortopedycznej i produkcji sprzętu medycznego. Zmienny rytm wydawania czasopisma został omówiony na tle przemian gospodarczych czasów schyłkowej PRL i początków III RP. Jako ważny symbol przemian w globalizującym się świecie nauki wskazano języki, w których publikowano artykuły i materiały dodatkowe w „Polimerach w Medycynie" – początkowo cztery (polski, angielski, rosyjski, niemiecki), od 1986 trzy (bez niemieckiego), od 1997 roku dwa (zniknął rosyjski), a od 2021 roku jeden (angielski).

Słowa kluczowe: polimery, czasopismo naukowe, tworzywa sztuczne, historia nauki, języki

Abstract

The history of "Polymers in Medicine" reflects not only the development of utilizing such materials in medicine and pharmaceutics, but also changes in Polish scientific journals – dissemination of results of scientific research and broader scientific activity always takes place in a specific linguistic and sociopolitical context. The paper presents a brief historical sketch of the journal, starting from the establishment of the information bulletin "Plastics in Medicine", through the 1st International Conference of the COMECON "Utilization of plastics in medicine", which took place in Warsaw in October 1969, and the founding of "Polymers in Medicine" in 1970–1971, until the present day. Subsequent editors-in-chief are introduced, along with transformations of the layout, and above all, the evolution of issues described in the published papers, which initially concerned chiefly polymer materials in general, orthotics and plastic medical equipment. The changing rhythm of publication of the journal is discussed on the background of economic transformations during the decline of Polish People's Republic and the early days of modern Poland. Languages in which articles and additional materials were published in "Polymers in Medicine" can be regarded as a symbol of changes in the globalizing world of science: between 1964 and 1986 four languages (Polish, English, Russian, and German), then three (without German) until 1997, then two (Russian also disappeared) and – since 2021 – one (English).

Key words: polymers, scientific journal, plastics, history of science, languages

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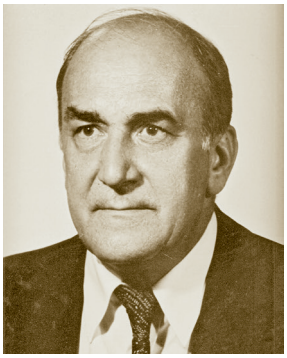
Początek przed początkiem

Obchodzimy pięćdziesięciolecie „Polimerów w Medycynie", ponieważ pod takim tytułem czasopismo to ukazuje się od 1971 roku. Dopiero eksploracja zasobów Biblioteki Uniwersytetu Medycznego we Wrocławiu wykazała, że nasze czasopismo stanowi kontynuację wychodzącego w latach 1964–1969 kwartalnika „Tworzywa Sztuczne w Medycynie", wydawanego w tej samej instytucji i w dużej części przez ten sam zespół redakcyjny. Inicjatorem był prof. (wówczas doc. dr hab.) Henryk Kuś (ryc. 1), jeden z najwybitniejszych wówczas w Polsce specjalistów w dziedzinie chirurgii ręki i chirurgii urazowej w ogóle, a czasopismo wydawano w Zakładzie Chirurgii Eksperymentalnej i Badania Biomateriałów, funkcjonującego w ramach Katedry Chirurgii Urazowej Akademii Medycznej we Wrocławiu w szpitalu przy ul. Poniatowskiego 2. Zakład był wydawcą do 2003 roku – od tego momentu rolę tę przejęła uczelnia jako całość, czyli do 2012 roku Akademia Medyczna we Wrocławiu, a po zmianie nazwy Uniwersytet Medyczny im. Piastów Śląskich. W „Tworzywach…" i wczesnych numerach „Polimerów…" podanych jest kilku wydawców: Centralne Laboratorium Naukowo-Doświadczalne Przemysłu Ortopedycznego, Zjednoczenie Przemysłu Ortopedycznego, a także Resortowy Ośrodek Informacji Centralnego Ośrodka Techniki Medycznej. Czasem dopiero sięgnięcie



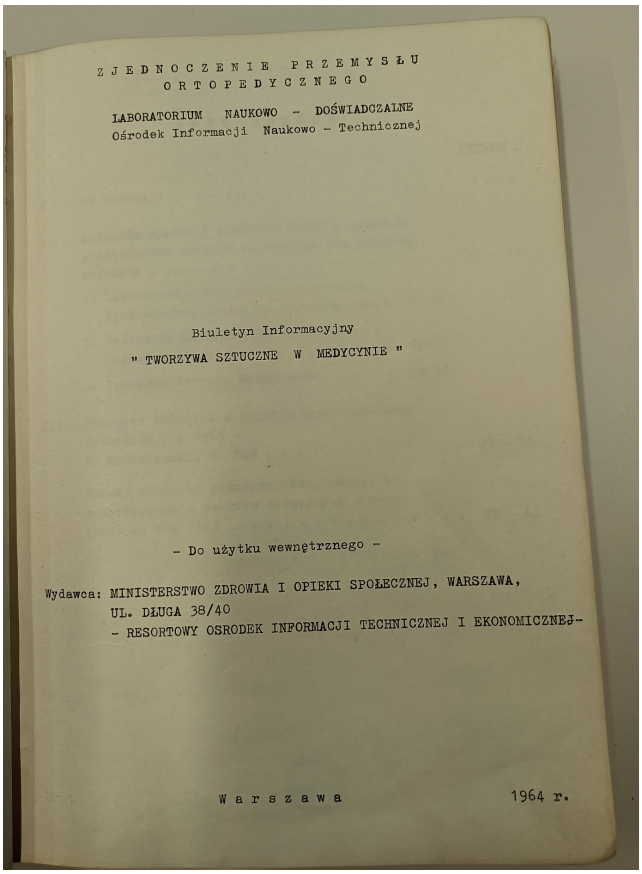
Ryc. 1. Prof. dr hab. Henryk Kuś, redaktor naczelny „Tworzyw Sztucznych w Medycynie" i pierwszy redaktor naczelny „Polimerów w Medycynie"

Fig. 1. Prof. Henryk Kuś, PhD, DSc., editor-in-chief of „Plastics in Medicine" and first editor-in-chief of „Polymers in Medicine"

do innych źródeł informacji pozwala stwierdzić, czy w tym gąszczu nazw charakterystycznych dla scentralizowanej gospodarki uspołecznionej mamy do czynienia z różnymi podmiotami, czy z różnymi komórkami tej samej organizacji.

Do użytku wewnętrznego

Gdy przyjrzeć się biografii naukowej prof. Kusia, można wysunąć przypuszczenie, że do założenia czasopisma skłoniły go dwa wydarzenia: stypendium w Bordeaux w 1963 roku i – po powrocie – objęcie stanowiska kierownika Zakładu Badań Tworzyw Sztucznych AM we Wrocławiu. Decyzję o wydawaniu kwartalnika – z inicjatywy


ZJEDNOCZENIE PRZEMYSŁU
ORTOPEDYCZNEGO

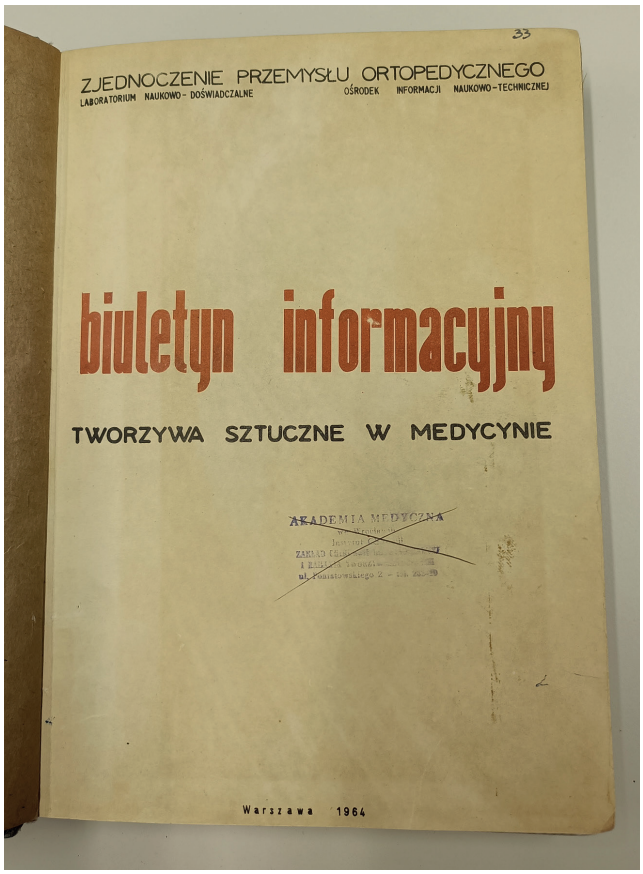
LABORATORIUM NAUKOWO - DOŚWIADCZALNE
Ośrodek Informacji Naukowo - Technicznej

Biuletyn Informacyjny
" TWORZYWA SZTUCZNE W MEDYCYNIE "

- Do użytku wewnętrznego -

Wydawca: MINISTERSTWO ZDROWIA I OPIEKI SPOŁECZNEJ, WARSZAWA,
UL. DŁUGA 38/40
- RESORTOWY OSRODEK INFORMACJI TECHNICZNEJ I EKONOMICZNEJ-

Warszawa 1964 r.



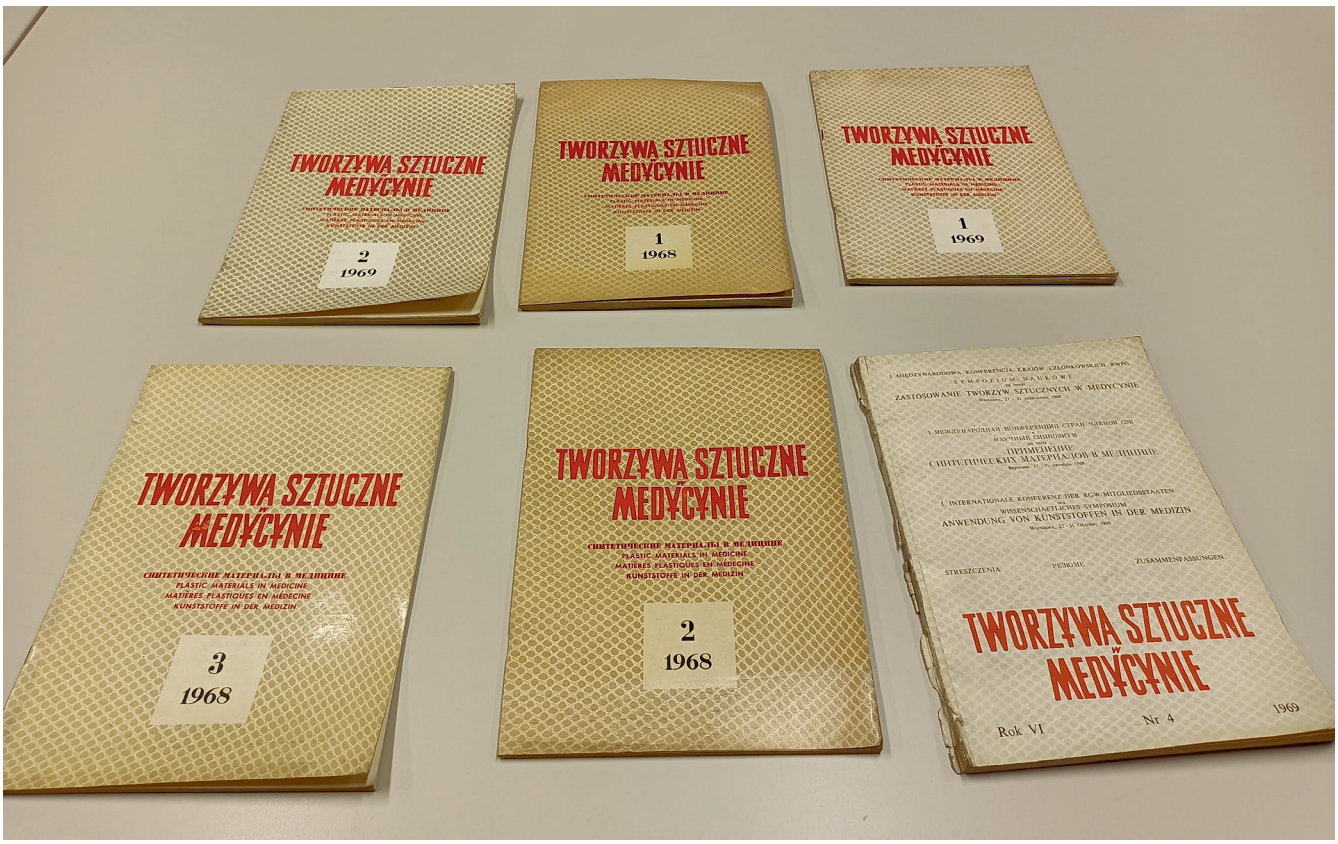
Ryc. 2. Karty tytułowe pierwszego i drugiego numeru „Tworzyw Sztucznych w Medycynie" (numery 1/1964 i 2/1964)

Fig. 2. Title pages of the 1st and 2nd issue of „Plastics in Medicine" (issues 1/1964 and 2/1964)

dr. Kusia – podjęła Rada Naukowa Zjednoczenia Przemysłu Ortopedycznego. Zawartość pierwszych numerów „Tworzyw Sztucznych w Medycynie" z 1964 roku – omówienia zachodnich publikacji oraz polskich i zagranicznych patentów, a także polskie zestawienia bibliograficzne oraz doniesienia z konferencji i kongresów – wskazuje, że początkowo czasopismo to było pomyślane jako próba zintegrowania środowiska badaczy w kraju i przybliżenia im zagranicznego piśmiennictwa, do którego nie mieli dostępu, nie zaś jako typowe czasopismo naukowe. Nie bez powodu nosiło zresztą podtytuł „Biuletyn Informacyjny", a adnotacja na stronie tytułowej mówiła, że przeznaczone jest wyłącznie do użytku wewnętrznego. Umiędzynarodowienie nauki z punktu widzenia wielu polskich badaczy było wówczas słabsze niż dzisiaj, a w przypadku krajów Bloku Wschodniego często ograniczało się do innych krajów RWPG. Jednak w ciągu roku czasopismo wyraźnie się sprofesjonalizowało. Pierwsze dwa numery (ryc. 2) przypominają publikacje wydawane poza cenzurą – to maszynopisy odbite nawet nie w drukarni, a w powielarni. Natomiast już w numerze 1/1965 layout, czyli postać wizualna czasopisma, nie odbiega od ówczesnych standardów edytorskich (ryc. 3–5). Zawartość czasopisma stanowią już głównie prace oryginalne, nie brakuje wysokiej jakości ilustracji czarno-białych (drukowanych w technice rastra), a od numeru 4/1966 sporadycznie pojawiają się ilustracje barwne – głównie przedruki z książek lub innych czasopism – na wklejkach (kolorowe ryciny na stałe zagościły w „Polimerach w Medycynie" dopiero od 2010 roku). Niektóre wykresy, schematy i rysunki intrygują nie tylko treścią, ale także rzadko dziś spotykaną elegancją, wręcz stylem. W jednym z numerów z 1965 roku pojawia się też interesujący passus w instrukcji dla autorów – o wynagradzaniu za teksty według „przyjętych stawek". Stanowiłoby to przeniesienie zwyczajów z prasy ogólnej (gdzie autor otrzymuje honorarium) do prasy naukowej (gdzie – jeśli w ogóle jest mowa o pieniądzach – to autor płaci). Niestety dla autorów, kolejne instrukcje aż do początku lat 90. wyraźnie mówiły, że publikowanie na łamach „Polimerów w Medycynie" nie wiąże się z wynagrodzeniem.

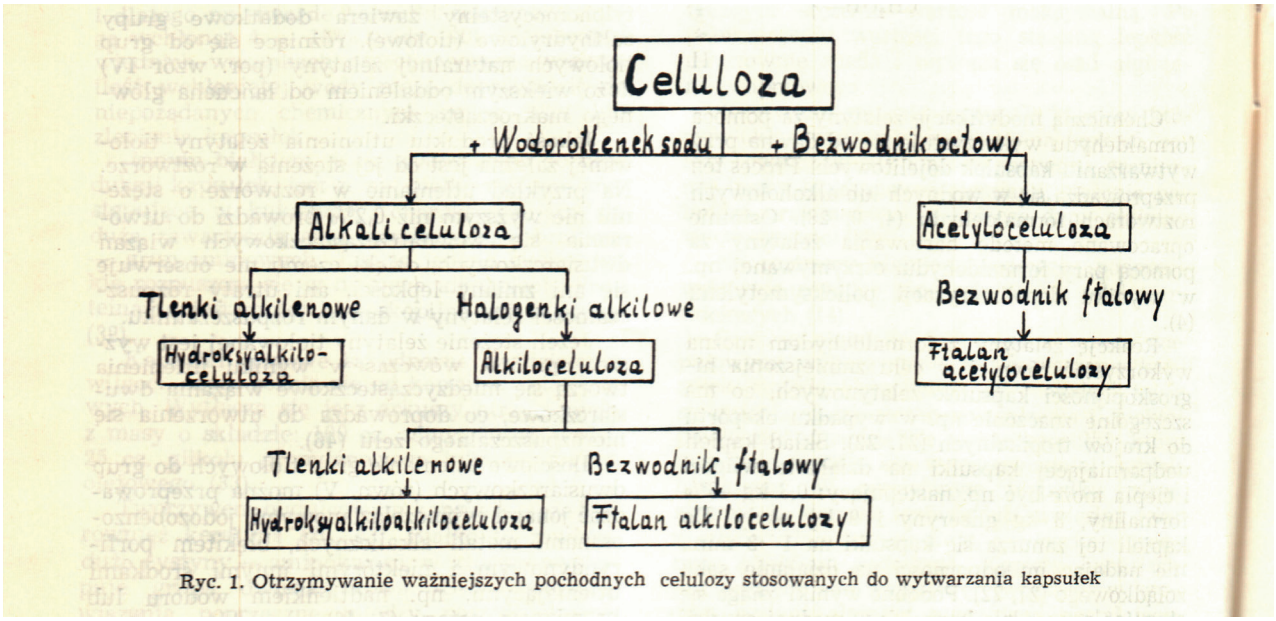
Cenzura zezwala

Ostatni numer „Tworzyw Sztucznych w Medycynie" trafił do rąk czytelników na początku 1970 roku – kilkumiesięczne opóźnienia w druku były normą. W tym samym roku pod redakcją prof. Kusia ukazał się liczący ponad 800 stron tom, również zatytułowany „Tworzywa sztuczne w medycynie", będący zbiorem artykułów, częściowo powstałych na zamówienie, a częściowo stanowiących plon kilku konferencji, oraz tekstów nadesłanych do czasopisma, zakwalifikowanych do druku, a nieopublikowanych już z braku miejsca. Jednocześnie 22 lutego 1970 roku Akademia Medyczna we Wrocławiu złożyła w Głównym



Ryc. 3. „Tworzywa Sztuczne w Medycynie" w formacie A4 w latach 1968–1969

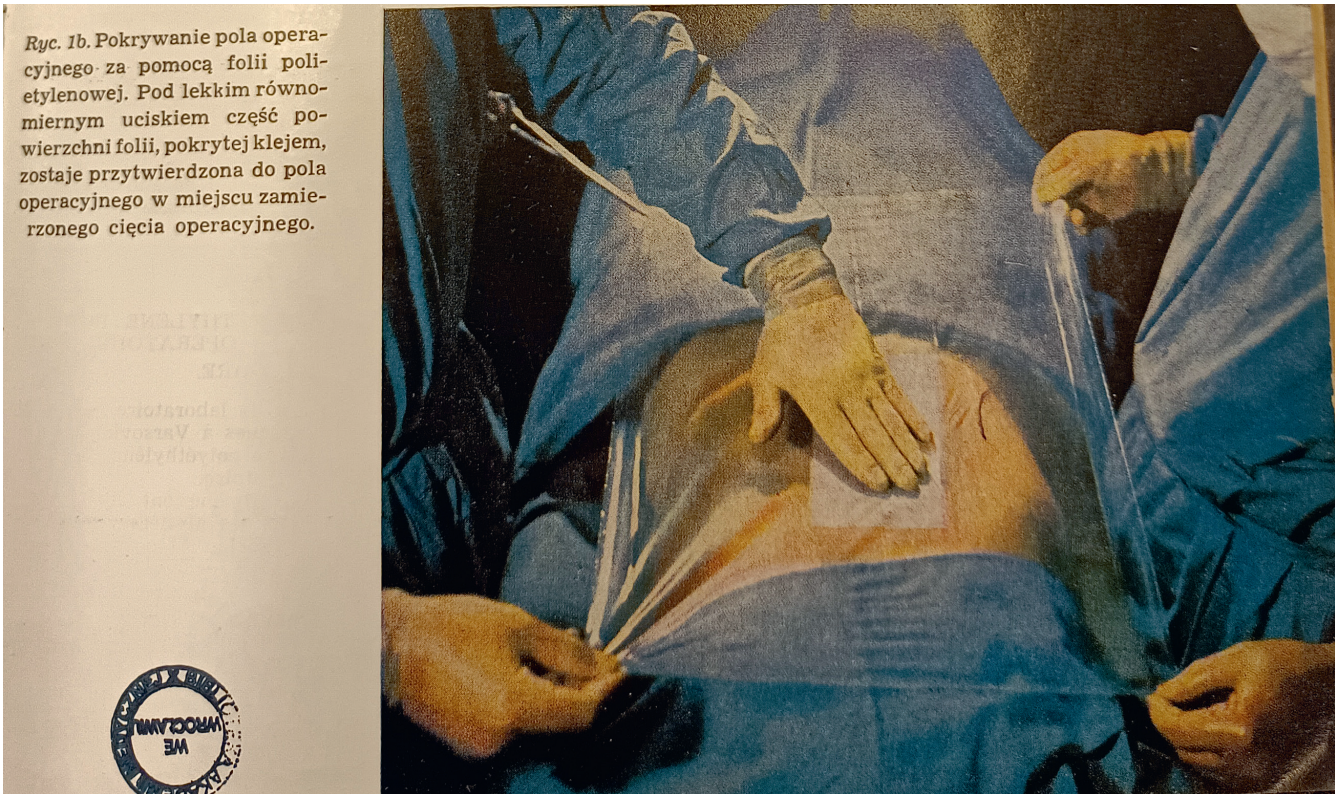
Fig. 3. „Plastics in Medicine" in A4 format (1968–1969)



Ryc. 1. Otrzymywanie ważniejszych pochodnych celulozy stosowanych do wytwarzania kapsułek

Ryc. 4. Ręcznie rysowany schemat zamieszczony w numerze 1/1968 „Tworzyw Sztucznych w Medycynie". Zwraca uwagę staranne, wręcz kaligraficzne pismo

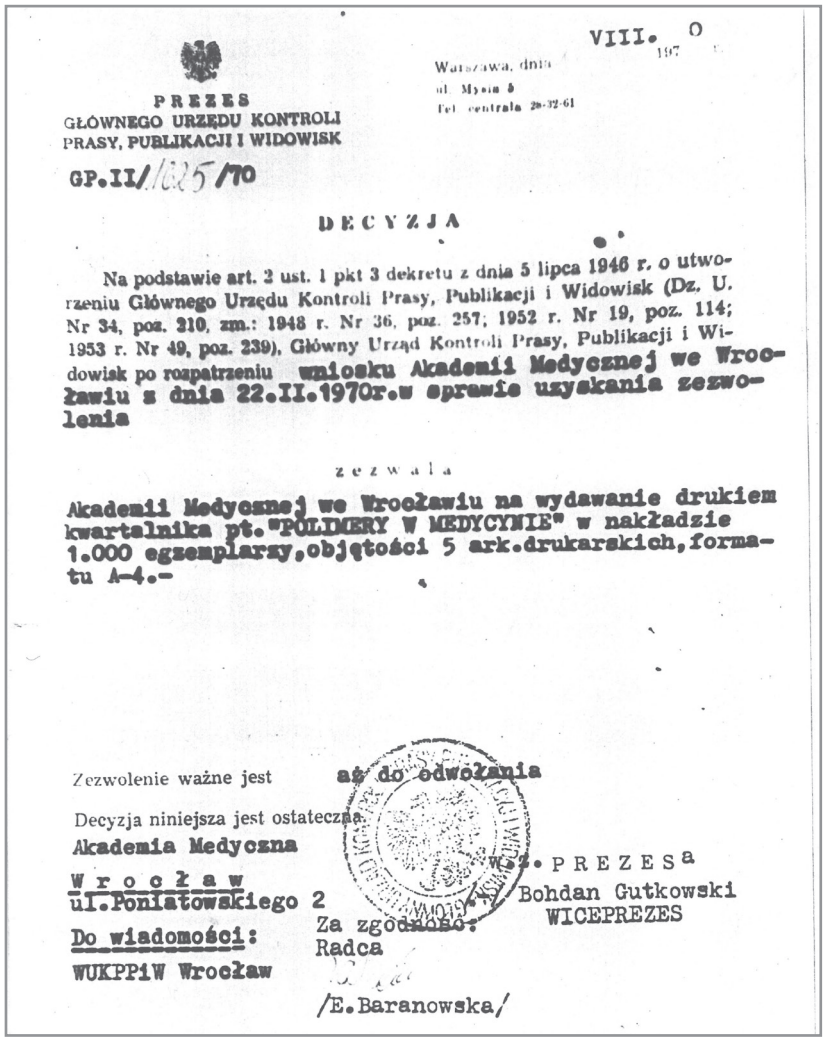
Fig. 4. Hand-drawn chart published in „Plastics in Medicine" 1/1968. It should be noted that the handwriting is very meticulous, almost calligraphic


Ryc. 1b. Pokrywanie pola operacyjnego za pomocą folii polietylenowej. Pod lekkim równomiernym uciskiem część powierzchni folii, pokrytej klejem, zostaje przytwierdzona do pola operacyjnego w miejscu zamierzonego cięcia operacyjnego.

Ryc. 5. Pierwsze kolorowe ilustracje – „Tworzywa Sztuczne w Medycynie" nr 1/1966

Fig. 5. One of the first color illustrations in „Plastics in Medicine" – issue 1/1966

Urzędzie Kontroli Prasy, Publikacji i Widowisk (tj. cenzurze) wniosek o zgodę na wydawanie czasopisma „Polimery w Medycynie" – do 1990 roku oprócz rejestracji tytułu w sądzie wymagana była taka zgoda. Decyzja urzędu cenzorskiego (ryc. 6) nie precyzowała częstotliwości ukazywania się czasopisma, wspominała natomiast o jego nakładzie (1000 egz.), objętości (5 arkuszy drukarskich) i formacie (A4). Spośród tych parametrów dotrzymywany był tylko nakład – format czasopisma zmniejszył się do B5 (ryc. 7), a objętość była zmienna z numeru na numer.



PREZES
GŁÓWNEGO URZĘDU KONTROLI
PRASY, PUBLIKACJI I WIDOWISK

Warszawa, dnia VIII. 1970
ul. Mysia 5
Tel. centrala 28-32-61

GP.II/1625/70

DECYZJA

Na podstawie art. 2 ust. 1 pkt 3 dekretu z dnia 5 lipca 1946 r. o utworzeniu Głównego Urzędu Kontroli Prasy, Publikacji i Widowisk (Dz. U. Nr 34, poz. 210, zm.: 1948 r. Nr 36, poz. 257; 1952 r. Nr 19, poz. 114; 1953 r. Nr 49, poz. 239), Główny Urząd Kontroli Prasy, Publikacji i Widowisk po rozpatrzeniu wniosku Akademii Medycznej we Wrocławiu z dnia 22.II.1970r.w sprawie uzyskania zezwolenia

zezwala

Akademii Medycznej we Wrocławiu na wydawanie drukiem kwartalnika pt."POLIMERY W MEDYCYNIE" w nakładzie 1.000 egzemplarzy,objętości 5 ark.drukarskich,formatu A-4.-

Zezwolenie ważne jest aż do odwołania

Decyzja niniejsza jest ostateczna.

Akademia Medyczna
W r o c ł a w
ul.Poniatowskiego 2

Do wiadomości:
WUKPPiW Wrocław

z-ca PREZESa
Bohdan Gutkowski
WICEPREZES

Za zgodność:
Radca
/E.Baranowska/

Ryc. 6. Zezwolenie Głównego Urzędu Kontroli Prasy, Publikacji i Widowisk (tj. cenzury) na wydawanie „Polimerów w Medycynie" z roku 1970

Fig. 6. A permit from the Central Control Office of the Press, Publications and Public Performances (state censorship in communist Poland) from 1970, allowing the publication of „Polymers in Medicine"

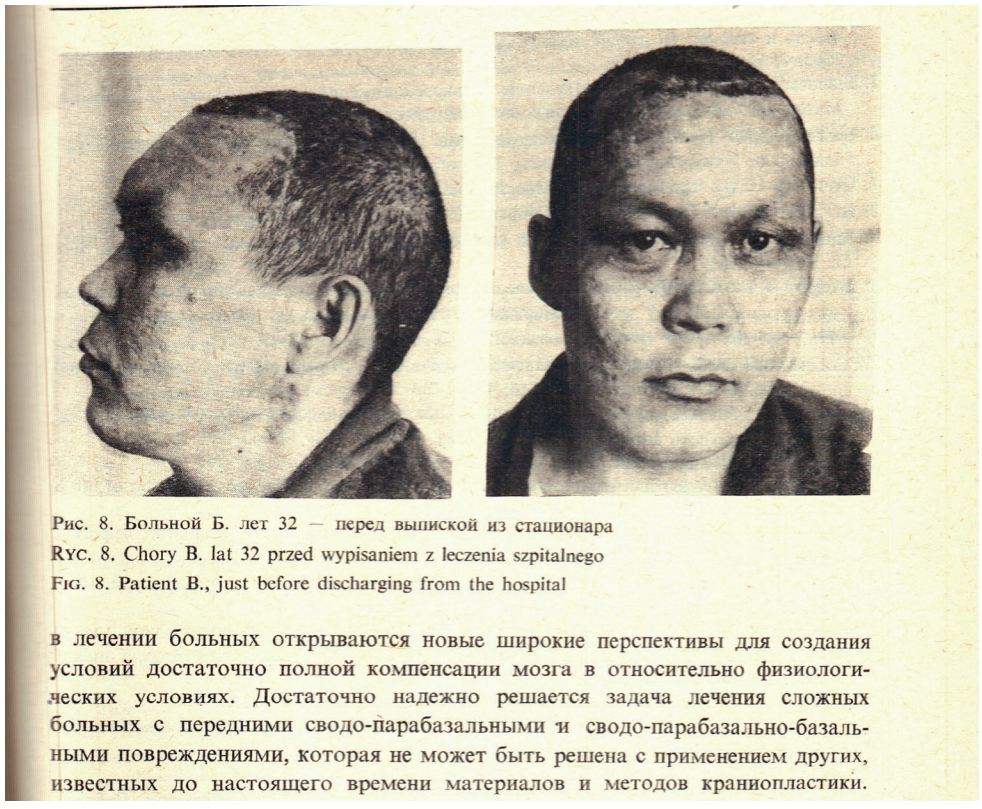


Рис. 8. Больной Б. лет 32 — перед выпиской из стационара
Ryc. 8. Chory B. lat 32 przed wypisaniem z leczenia szpitalnego
Fig. 8. Patient B., just before discharging from the hospital

в лечении больных открываются новые широкие перспективы для создания условий достаточно полной компенсации мозга в относительно физиологических условиях. Достаточно надежно решается задача лечения сложных больных с передними сводо-парабазальными и сводо-парабазально-базальными повреждениями, которая не может быть решена с применением других, известных до настоящего времени материалов и методов краниопластики.

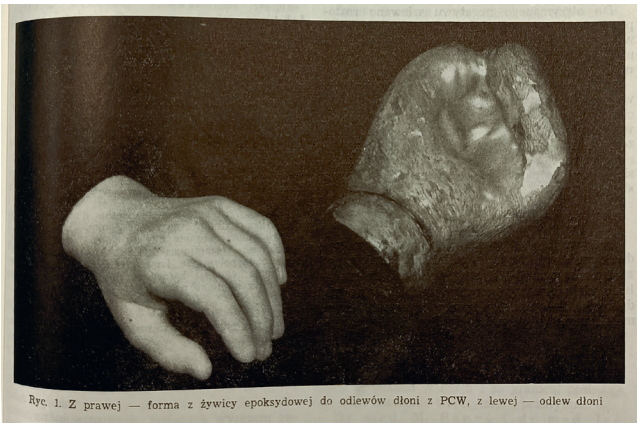
Ryc. 7. Przykład artykułu w języku rosyjskim w numerze 2/1975 „Polimerów w Medycynie". Zwraca uwagę layout podobny raczej do książki niż czasopisma (format B5)

Fig. 7. An example of a paper in Russian published in the 2/1975 issue of „Polymers in Medicine". Of note, the layout resembles more a book than a journal (B5 format)

Impulsem do przekształcenia „Tworzyw Sztucznych w Medycynie" w szerzej zakrojone „Polimery w Medycynie" była I Międzynarodowa Konferencja Krajów Członkowskich RWPG pod tytułem „Zastosowanie tworzyw sztucznych w medycynie", która odbyła się w październiku 1969 roku w Warszawie – prof. Kuś i inni członkowie zespołu redakcyjnego „Tworzyw…" byli jej współorganizatorami. Dwa specjalne zeszyty „Tworzyw…" z 1969 roku zawierały program konferencji w trzech językach (po polsku, rosyjsku i niemiecku) wraz z mapką oraz zdjęciem miejsca obrad (Pałacu Staszica) na czwartej stronie okładki. Natomiast pierwszych sześć numerów „Polimerów…" (1–4/1971 i 1–2/1972) to po prostu komplet materiałów z tej konferencji (z podziałem na poszczególne posiedzenia) oraz relacje z wydarzeń towarzyszących (wystaw, dyskusji panelowych itp.).

Proszę nie odbiegać od tematu

Gdy przyjrzeć się tematyce „Tworzyw…" i „Polimerów...", zwraca uwagę przede wszystkim dominacja trzech obszarów. Pierwszym była protetyka ortopedyczna – sporo artykułów prezentuje innowacyjne protezy nóg i rąk, dopasowane do indywidualnych potrzeb konkretnych pacjentów dzięki zaawansowanym technikom obróbki polimerowych tworzyw sztucznych (ryc. 8). Drugi obszar to szeroko rozumiany sprzęt medyczny wykonywany z materiałów polimerowych – od strzykawek, poprzez cewniki, urządzenia do pobierania i magazynowania próbek tkanek, nici chirurgiczne, na narzędziach chirurgicznych skończywszy. Szczególnie widoczna jest obecność produktów zaspokajających potrzeby służby krwi i transfuzjologii – od pojemników na krew konserwowaną do eksperymentalnych preparatów krwiozastępczych – oraz chirurgii (alloplastyka, kranioplastyka). Wreszcie trzecia dziedzina to różnego rodzaju materiały opatrunkowe, nierzadko bardzo specjalistyczne – np. przeciwodleżynowe, przeznaczone do opatrywania oparzeń i odmrożeń albo stosowane po zabiegach chirurgicznych (zwłaszcza z zakresu chirurgii urazowej) w celu przyspieszenia gojenia się ran. Znaczącą grupę stanowią artykuły o wytwarzaniu i przetwarzaniu polimerów jako takich – zdecydowanie z obszaru chemii, a nie zastosowania polimerów w medycynie. Pojawiały się też szeroko zakrojone, przeglądowe studia o zastosowaniach tych samych tworzyw polimerowych w różnych gałęziach medycyny. Ważnym zagadnieniem była ewentualna toksyczność wyrobów polimerowych – zarówno w procesie produkcji, jak i przy ich stosowaniu. Tematyka farmaceutyczna, dziś wyraźnie obecna w czasopiśmie, zaznacza się mocniej dopiero po roku 2000, a protetyka stomatologiczna – w drugiej połowie lat 90. Znacznie częstsze niż obecnie były studia przypadków – np. osób, które doznały różnorodnych urazów. Współcześnie dla badaczy publikujących w „Polimerach…" naturalnym środowiskiem jest laboratorium, nie zaś sala chorych czy dom pacjenta. Specjalizacja w nauce jest tu widoczna na wyrazistym przykładzie.


Ryc. 1. Z prawej — forma z żywicy epoksydowej do odlewów dłoni z PCW, z lewej — odlew dłoni

Ryc. 8. Forma z żywicy epoksydowej do odlewania protez dłoni z PVC oraz proteza odlana za jej pomocą – typowa tematyka w „Tworzywach Sztucznych w Medycynie" i wczesnych rocznikach „Polimerów w Medycynie"

Fig. 8. An epoxy mold for casting PVC hand prostheses and a prosthesis made using this mold – typical topic in „Plastics in Medicine" and early issues of „Polymers in Medicine"

Oprócz prac oryginalnych i poglądowych w czasopiśmie znaleźć można było materiały innego typu:
- relacje z konferencji, sympozjów itp. oraz zapowiedzi takich wydarzeń (w tym na Zachodzie – nawet w USA),
- informacje o pracach polskich i zagranicznych ośrodków badawczych,
- testy nadesłanego do redakcji sprzętu medycznego,
- informacje o asortymencie producentów takiego sprzętu,
- recenzje i streszczenia książek (szczególnie zagranicznych, do których czytelnicy mieli utrudniony dostęp),
- wykazy wniosków patentowych,
- opisy wniosków racjonalizatorskich,
- a także listy do redakcji, nierzadko polemizujące z tezami opublikowanych wcześniej prac oryginalnych.

W epoce przedcyfrowej uzyskanie wielu z powyższych informacji było skomplikowane – wymagało czasu i zachodu, a nierzadko znajomości języków obcych czy wręcz kontaktów za granicą. Wspólny wysiłek społeczności badaczy pozwalał zgromadzić tę wiedzę w jednym źródle.

Reklama i plan

Najbardziej zaskakującym elementem wydają się jednak reklamy – zarówno krajowych producentów sprzętu medycznego, jak i zagranicznych koncernów (amerykańskich – np. 3M, brytyjskich, zachodnioniemieckich); w instrukcjach dla autorów nazywano je informacjami handlowymi (ryc. 9). Materiały promocyjne polskich zakładów publikowano w kilku językach, co świadczy o tym, że międzynarodowy obieg czasopisma był faktem. Paradoksalnie, reklamy zniknęły z czasopisma w połowie lat 90., czyli wraz z wolnorynkowymi przemianami. Wyraźniej też zaznaczała się obecność samego zespołu redakcyjnego – ukazywały się relacje z jego posiedzeń, a w 1976 roku

Ryc. 9. Jedna z reklam (zwanych wówczas informacjami handlowymi) zamieszczonych w numerze 1/1973 „Polimerów w Medycynie" – anglojęzyczna reklama nieistniejącej już amerykańskiej firmy Davis & Geck

Fig. 9. One of the advertisments (then called „trade information") in „Polymers in Medicine" 1/1973 – an English-language advertisment of the now-defunct company Davis & Geck from the USA

nawet skromny fotoreportaż. Można też dostrzec, że prace redakcji były precyzyjnie planowane – wczesne numery z lat 70. zawierają informacje o nadesłanych artykułach, które dopiero przechodzą recenzję, a na ostatniej stronie okładki prezentowano zapowiedź kolejnego numeru. W tym okresie zdarzały się też numery tematyczne w obrębie zwykłej numeracji zeszytów, a nie tylko jako suplementy. Redaktorzy zatem nie tylko zbierali i oceniali teksty, ale też planowali na co najmniej pół roku do przodu.

Suplementy i gospodarka niedoboru

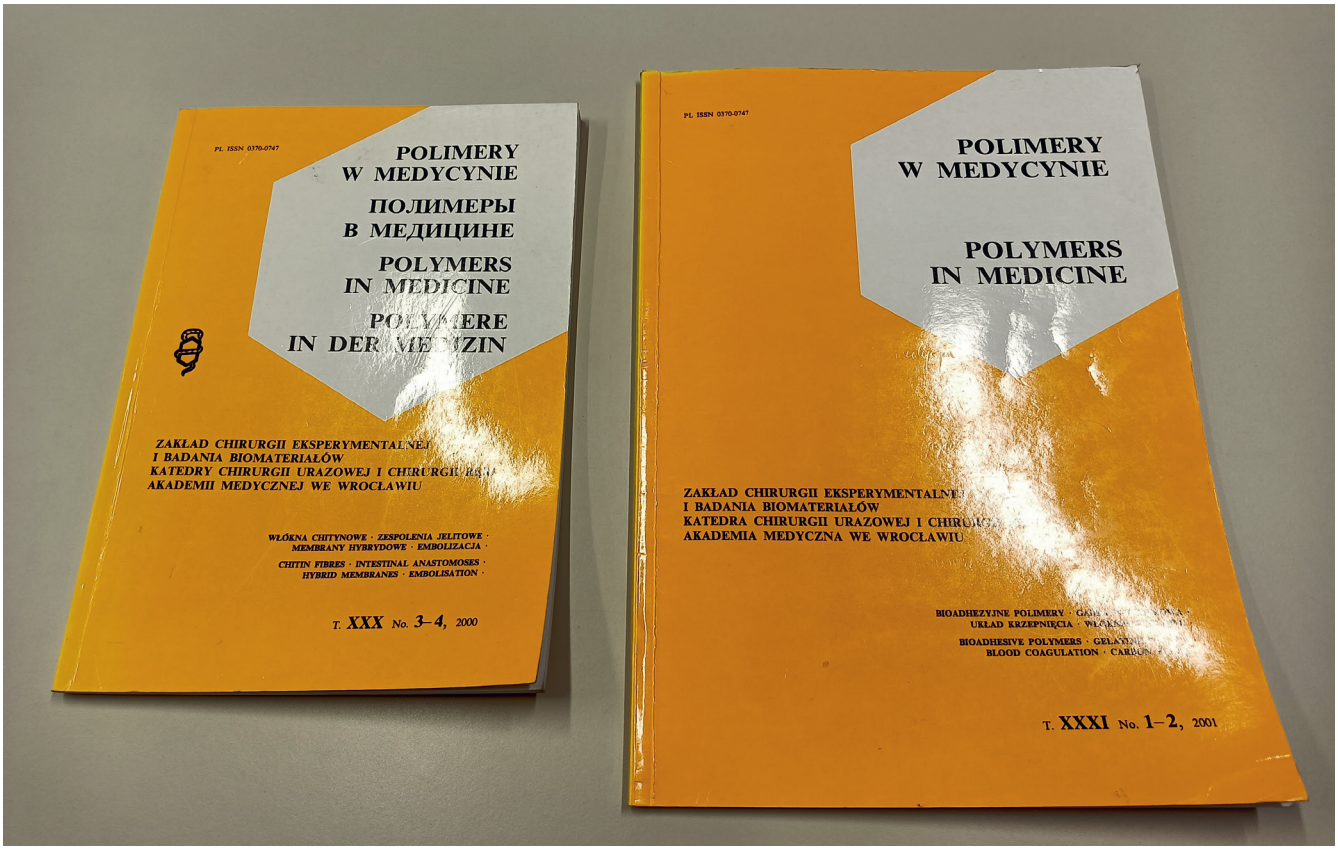
W latach 70. „Polimery w Medycynie" współpracowały z polskimi zakładami produkującymi różnego typu materiały medyczne – m.in. kleje i opatrunki. W latach 1976–1977 roku wydano trzy zeszyty-suplementy przygotowane we współpracy z zakładami „Polfa" – pierwszy zawierał artykuły o nowo wówczas wprowadzonym na rynek polskim kleju tkankowym „Chirurcoll", drugi był plonem zorganizowanej przez „Polfę" konferencji o klejach tkankowych, trzeci zaś – konferencji o zastosowaniu PVC w medycynie. Kryzys gospodarczy, który ogarnął PRL w drugiej połowie lat 70., uwidocznił się w spadającej częstotliwości wydawania czasopisma – wynikało to, co warto podkreślić, z ograniczeń przydziału papieru i wielorakich problemów w branży drukarskiej, nie zaś z braku materiałów do publikacji. W latach 1981–1983 i 1985–1989 wydawano dwa łączone numery (1–2 i 3–4), a w 1984 roku wyszedł tylko jeden numer (1–4), który w dodatku trafił do odbiorców dopiero wiosną 1985 roku. Po upadku komunizmu problemem stało się raczej finansowanie – w 1990 roku także wyszedł tylko jeden łączony zeszyt, a aż do 2003 roku publikowano dwa łączone numery co pół roku. Rosnąca liczba czasopism naukowych sprawiła zaś, że po 1990 roku liczba nadsyłanych manuskryptów zaczęła spadać – czasopismo wyraźnie „schudło" w drugiej połowie lat 90., a od 2015 roku jest wydawane jako półrocznik. Jednocześnie wymusza to jednak surowszą selekcję nadsyłanych manuskryptów.

Kto tu dowodzi?

Profesor Kuś stał na czele zmieniającego się zespołu redakcyjnego przez 25 lat (a wliczając poprzednie czasopismo – 32 lata), aż do śmierci – zmarł nagle 16 lipca 1996 roku w wieku 71 lat. Zastąpił go ówczesny zastępca redaktora naczelnego – prof. Roman Rutowski, specjalizujący się w mikrochirurgii i chirurgii ogólnej, który w 1995 roku przejął od prof. Kusia kierownictwo Katedry i Kliniki Chirurgii Urazowej AM. On także sprawował tę funkcję do śmierci (zmarł 12 czerwca 2013 roku w wieku 66 lat). W 2013 roku na stanowisko to została powołana prof. Magdalena Krajewska, która jednak w 2018 roku musiała zrezygnować z uwagi na objęcie funkcji kierownika Katedry i Kliniki Nefrologii i Medycyny Transplantacyjnej. Profesor Krajewska w piśmie do rektora sama zasugerowała prof. Mariusza Kusztala, który dokończył za nią kadencję (prof. Krajewska była powołana do 2020 roku). Wreszcie, od początku 2021 roku, „Polimerami w Medycynie" kierują prof. Witold Musiał z Uniwersytetu Medycznego we Wrocławiu oraz dr hab. Konrad Szustakiewicz z Politechniki Wrocławskiej.

Forma i zmiana

Zmieniała się też postać czasopisma. „Tworzywa Sztuczne w Medycynie" były w latach 1964–1969 wydawane w formacie A4, natomiast „Polimery w Medycynie" miały od początku bardziej kieszonkowy format B5. Wpływało to też na trwałość, gdyż mniejsze składki po 16 stron można było łączyć oprawą szytą nicią z miękką okładką tekturową (tzw. oprawa broszurowa) – zszywanie metalowymi zszywkami, dawniej częste w tygodnikach i miesięcznikach, jest mniej trwałe, a format A4 nie mieści się na wielu regałach. Zwraca uwagę sam layout czasopisma – wyglądało ono zdecydowanie bardziej jak książka niż czasopismo (współcześnie taką postać periodyku spotkać można w przypadku niektórych czasopism humanistycznych). Do formatu A4



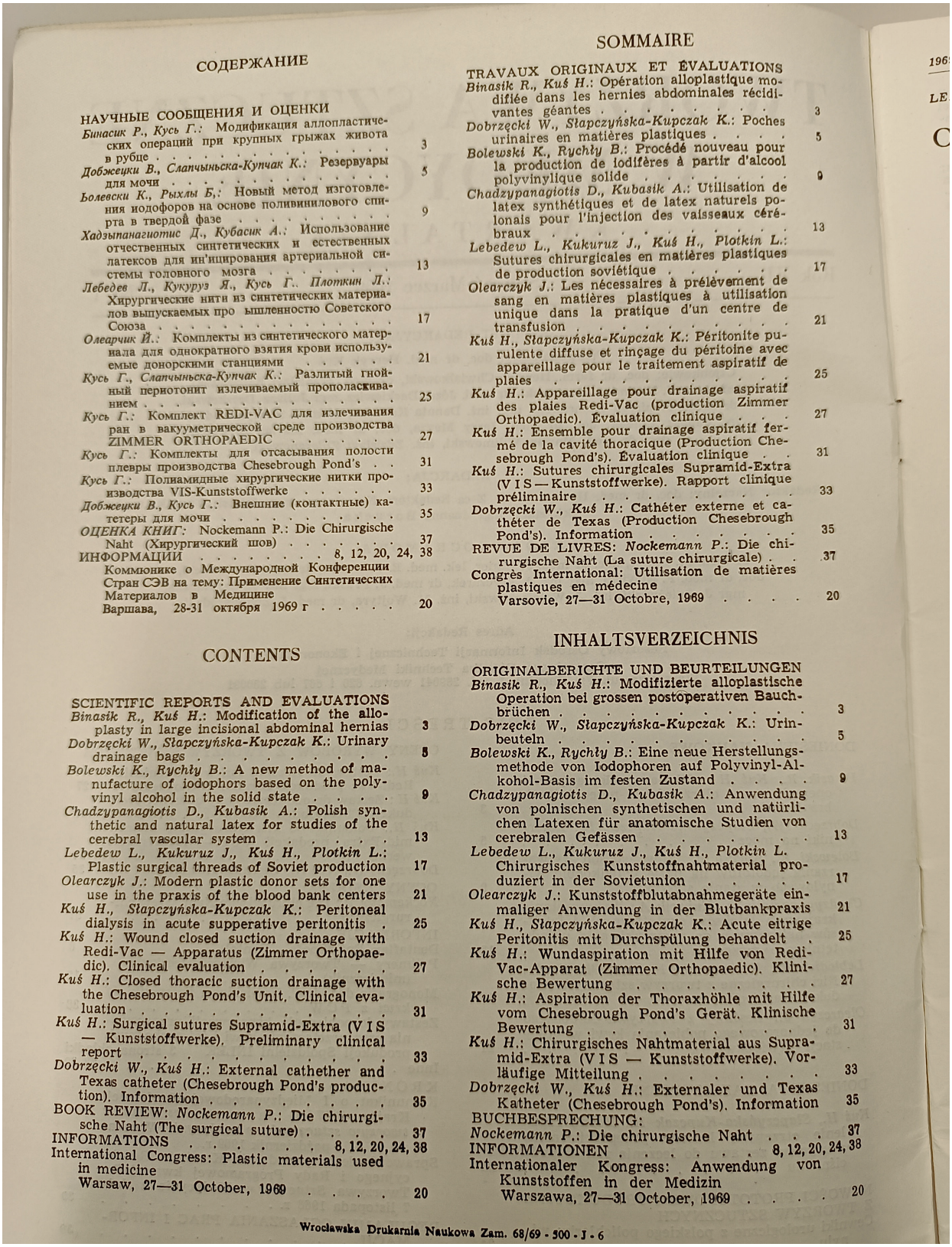
Ryc. 10. Zmiana formatu „Polimerów w Medycynie" z B5 do A4 i zastąpienie czterojęzycznego tytułu przez tytuł w dwóch językach z początkiem roku 2001

Fig. 10. A change of format of „Polymers in Medicine" from B5 to A4, and reducing the number of languages in which the title is given from four to two, beginning from the 1–2/2001 issue

powrócono w 2001 roku (ryc. 10) z uwagi na standaryzację w drukarniach – druk czasopism, zwłaszcza w niewielkich nakładach, w formacie innym niż A4 stał się po prostu droższy (w tym okresie wiele czasopism różnego typu znanych z nietypowych formatów po prostu zniknęło z rynku). Mniej więcej w tym samym czasie „Polimery w Medycynie" zaczęły też – początkowo nieśmiało – wkraczać w cyberprzestrzeń. W numerze 1–2/2001 po raz pierwszy podano redakcyjny e-mail, a w instrukcjach dla autorów pojawił się wymóg nadsyłania artykułu w pliku Word na dyskietce. Nie udało się ustalić, od kiedy istnieje podstrona „Polimerów w Medycynie" na stronie internetowej uczelni, natomiast jasne jest, że elektroniczne wydanie czasopisma – od początku w trybie *open access* – jest na niej umieszczane od początku 2009 roku. Od 1 stycznia 2017 roku wersją pierwotną wszystkich opublikowanych artykułów jest wersja elektroniczna, a wersja drukowana jest przeznaczona głównie dla bibliotek, które wymagają egzemplarza obowiązkowego.

W językach mówimy stu

Ważnym wskaźnikiem przemian w polskim piśmiennictwie naukowym w ciągu ostatnich 50 lat są języki, w których publikowano na łamach „Polimerów w Medycynie". Obecnie tytuł czasopisma jest dwujęzyczny – tak właśnie jest ono zarejestrowane w sądzie. Gdy jednak spojrzymy na okładkę w zeszytach z lat 1971–2000, zobaczymy tytuł w czterech językach – polskim, angielskim, niemieckim i rosyjskim. Do lat 70. znajomość angielskiego wśród naukowców nie tylko z krajów Bloku Wschodniego, ale także zachodnioniemieckich czy francuskich nie była wcale oczywista; z drugiej strony liczni badacze brytyjscy i amerykańscy znali tylko ojczysty angielski, a radzieccy – rosyjski (który był ich językiem ojczystym lub nie; zdarzało się też, że byli w stanie czytać po polsku, gdyż w naszym kraju cenzura była nieco łagodniejsza). W krajach RWPG nie byli rzadkością specjaliści znający w stopniu umożliwiającym uczestnictwo w nauce francuski, niemiecki lub rosyjski – ale nie angielski. Ten ostatni stał się *lingua franca* dopiero w następstwie II wojny światowej i w wielu krajach wśród osób wykształconych wciąż dominowała znajomość francuskiego. Profesor Kuś i jego współpracownicy zdecydowali się na radykalne z dzisiejszego punktu widzenia, a wówczas często praktykowane rozwiązanie i stworzyli czasopismo wielojęzyczne. Instrukcja dla autorów z pierwszego numeru „Polimerów w Medycynie" z 1971 roku stanowi nawet, że artykuły można nadsyłać „w dowolnym języku", ale teksty w bardziej egzotycznych językach albo nigdy nie nadeszły, albo nie zdecydowano się na ich publikację. W „Tworzywach Sztucznych w Medycynie" ukazywały się teksty po



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Ryc. 11. Obcojęzyczny (w czterech językach) spis treści „Tworzyw Sztucznych w Medycynie" w numerze 4/1969

Fig. 11. Table of contents in four langauges in „Plastics in Medicine" 4/1969

polsku i rosyjsku, a towarzyszyły im streszczenia po rosyjsku lub po polsku (w przypadku artykułów po rosyjsku), angielsku i francusku (ryc. 11). „Polimery w Medycynie" były zaś przez pierwszych 15 lat istnienia konsekwentnie czterojęzyczne – artykuły po polsku, angielsku, rosyjsku i niemiecku, i streszczenia w pozostałych językach. Partie

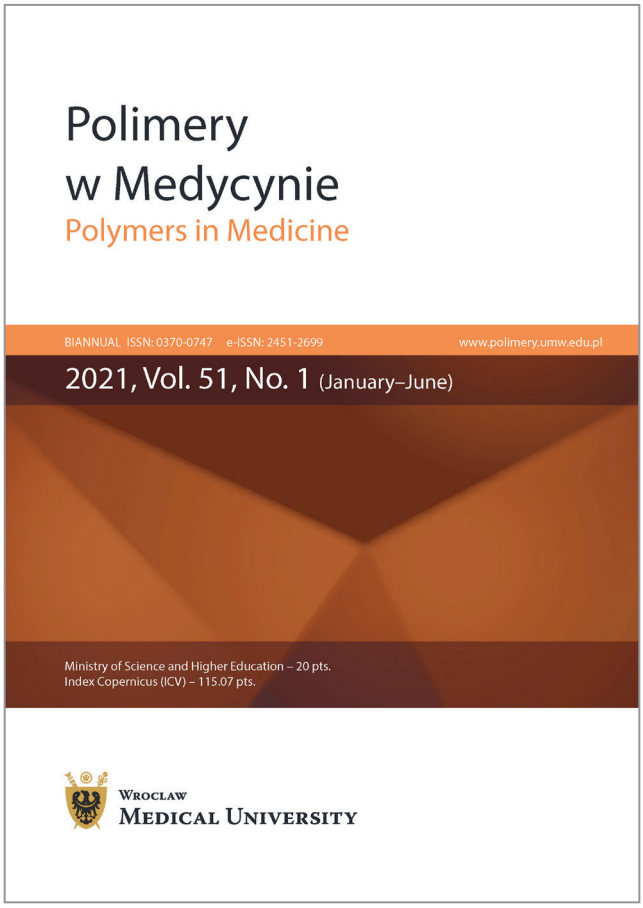
po rosyjsku wyglądały szczególnie stylowo, gdyż w alfabecie cyrylickim obowiązują nieco odmienne reguły składu – jest on na ogół gęstszy, z mniejszym kerningiem (odstępami między literami). Stosowano też inny, bardziej pasujący krój pisma, a nie cyrylicką wersję kroju, którym złożono teksty w alfabecie łacińskim. Z czasem jednak coraz większą rolę zaczął odgrywać angielski i tylko uczeni z ZSRR długo stawiali temu opór. Język niemiecki znika z kart „Polimerów w Medycynie" począwszy od numeru 1–2/1986, choć czterojęzyczny tytuł na okładce zachowano do końca 2000 roku. Ostatnie prace po rosyjsku pojawiają się pod koniec lat 80., ale spis treści i abstrakty w tym języku znikają dopiero w numerze 3–4/1997. Od tego czasu jesteśmy czasopismem dwujęzycznym – początkowo po polsku i po angielsku, teraz już praktycznie wyłącznie po angielsku – jedynie pracom napisanym przez polskich autorów mogą towarzyszyć polskojęzyczne abstrakty. Z jednej strony jest to naturalny skutek globalizacji nauki, z drugiej strony jako osobie znającej także niemiecki i trochę rosyjski pozostaje mi westchnąć nad utratą takiego bogactwa.

Długie trwanie

Od dawna nie żyją już wszystkie osoby zaangażowane w stworzenie „Polimerów w Medycynie", a najmłodsi autorzy artykułów z wczesnych roczników czasopisma przekroczyli siedemdziesiątkę. „Polimery w Medycynie" funkcjonują już praktycznie wyłącznie w Internecie – w każdej chwili i za darmo mają do nich dostęp badacze z Europy, USA, Chin, Indii czy Afryki Subsaharyjskiej. Jesteśmy jednak wciąż tym samym czasopismem – profesjonalnym i przybliżającym badaczom ważne osiągnięcia innych badaczy. Pięćdziesiąt lat temu sekretarz redakcji, mgr Joanna Borucka, potrafiła dzwonić do zalegających z korektą autorów na domowy telefon – a zmotywowani przez nią spóźnialscy przywozili „szczotki" z naniesionymi poprawkami syrenami, wartburgami czy dużymi fiatami. Pięćdziesiąt lat później piszący te słowa wysyła autorowi z Kioto sprawdzony artykuł. U mnie jest 15.00, u autora 22.00. Po godzinie przychodzi odpowiedź:

– Everything is OK. Thank you so much, Mr. Editor. One problem less. I can go to bed now. A long day closes.
– How is it now in Kyoto?
– Peaceful. The street is empty, I can hear the cicadas and it's a little foggy.

Gdzieś w Kioto zmęczony japoński lekarz zamyka laptopa i kładzie się spać. Gdzieś we Wrocławiu zmęczony polski redaktor zamyka laptopa i idzie na spacer z córką.



Ryc. 12. Współczesna okładka „Polimerów w Medycynie"

Fig. 12. Modern cover of „Polymers in Medicine"

Artykuł powstał na podstawie analizy archiwalnych egzemplarzy „Tworzyw Sztucznych w Medycynie" i „Polimerów w Medycynie" dostępnych w Bibliotece Uniwersytetu Medycznego im. Piastów Śląskich we Wrocławiu. Dziękuję pracownikom biblioteki – szczególnie Czytelni Czasopism – za pomoc i życzliwość. Konsultacją przy zapoznawaniu się z materiałami w języku rosyjskim służyła dr Ija Sudopłatowa.

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