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New Method for Preparation of Biodegradable Medical Materials Characterised by Highly Developed Porous Structures

Abstract
This study addresses the preparation of biodegradable and highly porous materials with the chemical purity required for medical materials. The solution method for producing porous structures with table salt was modified through the application of plasticisers in the technological process. In this paper the term medical materials includes dressing and implantable materials as well as scaffolds for tissue culture. A new method is proposed using polymers such as poly(D,L-lactide) and dibutyrylchitin to produce porous structures with enhanced absorption properties.

Key words: *biodegradable, biomaterials, porous structures, manufacturing.*

Introduction

Highly porous structures have been applied in the production of dressing and implantable materials and those associated with cell engineering. The advantage of these types of materials is their high porosity, which exceeds 95%. In porous medical materials, the distribution of pore sizes is also important because they should match the size of cells that will ultimately settle the material. Pore sizes in medical foams range from 1 to 633 μm, whereas the majority of foams are characterised by a pore distribution in the range of 50 – 300 μm [1]. Foams with oriented pores, whose arrangements facilitate the growth of cells, are particularly important [2]. Foams consist of polymers, primarily biodegradable polymers, including hyaluronic acid, alginates, poly(a-hydroxy esters), e.g., poly(L-lactic acid) and poly(D,L-lactic acid), as well as polysaccharides, e.g., chitosan and polypeptides (collagen).

Numerous foam-formation techniques have attracted considerable attention [3, 4]. For polymers with gelation capabilities, porous structure formation consists of gel matrix creation and solidification into a foam [5]. With hydrogels, cross-linking is also used in combination with cell (e.g., osteoblasts) encapsulation in a gel matrix [6]. With foams of non-gelating polymers, e.g., poly(lactic acid) (PLA), a phase separation technique is employed in which a polymer solution rapidly solidifies by thermo-coagulation [7] or solvent leaching [8]. A porous structure can also be created by incorporating table salt as a foam-forming agent and subsequent leaching after the solidification process [4, 8, 9, 10]. A gas may also be utilised as an expanding agent, e.g., CO_2, which facilitates bubble formation after incorporation into a solution. After solvent removal, the porous structure is stabilised [11]. Another process of foam formation involves creating foam directly from melt by adding surfactants [12] or expanding gases [13] to the injector. The process of polymer melt foaming is conducted with a specially developed injector [14]. Another technique used for the formation of foams or sponges is freezing out, which is described in [15].

This paper presents a new process for making biodegradable and bioactive quasi – fibrous, highly porous materials of polylactide and dibutyrylchitin by the solution method which incorporates low-molecular water-soluble expanding agents and plasticisers in a polymer solution to form a spatial structure with increased porosity compared with structures obtained by a method that employs only expanding compounds. The materials presented can be an alternative to other porous products for medical application, like 3D knitting fabrics or porous fibres.

Experimental

The bioactive and biodegradable porous structures were composed of polylactide and dibutyrylchitin, which is a well-known bioactive compound [16 - 19]. Table salt was used as the expanding agent and glycerin as the plasticiser. Tests were performed to establish the optimal composition for the polymer blend in relation to the weight content of sodium chloride and glycerin. The foams obtained were tested to determine the physical parameters describing their porous structure, the absorption of physiological saline and water absorption. Selected optimal foam variants were subjected to biological tests to determine their cytotoxicity and hemostatic ability, as well as their chemical purity.

Materials

The biodegradation rates of products composed of polylactides (PLAs) and the bioresorption rates of implants are dependent on the supermolecular structure of the material obtained. The ability of PLA to crystallise is strongly dependent on its stereochemical form and differs for the following materials: isotactic poly(L-lactide) (PLLA) or poly(D-lactide) (PDLA), syndiotactic poly (*meso-*

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lactide), atactic poly (*meso*-lactide) or poly(D,L-lactide), PLLA/PDLA stereocomplexes, and copolymers with random levels of meso-, L-, and D-lactide [18]. Consequently the physical properties of fabrics manufactured from different PLAs differ. In our study, we used PLA from NatureWorks, Blair, Nebraska, USA, with the symbol 4060D, which has a molar mass of 87 kDa and D-isomer content of 12%. It reduces the biodegradation and bioresorption capabilities of PLA considerably. This polymer was used to prepare a blend with diburyryl-chitin, with a molar mass of 139 kDa, whose chemical structure and preparation method are discussed in [20, 21].

For foam formation from PLA, ethyl acetate was used to prepare the PLA solution, whereas ethanol was selected for the dibutyrylchitin solution. PLA-dibutyrylchitin blend foams were prepared in a solution of both components in acetone. Sodium chloride with grain sizes ranging from 100 – 350 μm was employed as the expanding agent in the investigations (POCh S.A., Gliwice, Poland). An additional porous structure was obtained using glycerin (POCh S.A., Gliwice, Poland).

The porous material elaborated can be easily connected with textiles, like nonwovens or woven fabrics.

Test methods

Porous structure formation

The formation of porous, foam material from PLA 4060D and dibutyrylchitin (DBC) was conducted using a conventional method reported in literature [10, 15] which involves a salt-leaching process using sodium chloride based on a modified procedure in which various contents of glycerin are added to the solution. For foam formation from PLA, a 10% solution of polymer in ethyl acetate was prepared, and sodium chloride was added in two weight proportions: 1:1 and 1:1.5. In the modified variants, glycerin was added as a plasticiaer in quantities of 5% and 10%. Prior to solvent evaporation, the resultant polymeric mass was poured into a mold. After polymer solidification the additives were removed by rinsing the samples with distilled water.

The formation of foams from DBC was conducted in a similar manner, with ethanol used as the solvent. After preliminary tests, foams were prepared from a blend of PLA and DBC with a 50% PLA/50% DBC weight proportion, using acetone as the solvent.

Analytical methods

Comprehensive tests were performed to determine the properties of polymeric solutions used to prepare the blends, which contained an expanding agent and plasticiser. The properties examined included the consistence coefficient k, the density of the polymeric solutions, their surface tension and the contact angle during the immersion and emergence of a porous salt plate. Coefficient k was determined by measuring the liquid rheology with a digital viscometer, series Premium from the Fungilab Company, (Barcelona, Spain). Rheological measurements were performed for both the polymeric solutions and their mixture with salt. After selecting a measurement spindle, the viscosity of liquids, η [Pa·s], was measured according to the rotational speed of the spindle, Dr [s^{-1}], performed at 20 °C. With respect to the curves, all solutions and mixtures analysed exhibited a typical shape for liquids rarefied by shearing with a flow coefficient $n < 0.6$. Therefore values of the rheological coefficients were calculated using Ostwald de Wael's equation:

$$\tau = k \cdot Dr^n$$

where:
τ – shearing stress, Pa,
k – consistence coefficient,
n – flow coefficient,
Dr – shearing rate (spindle rotational rate), s^{-1}.

The remaining parameters - the polymer solution density, surface tension and contact angle - were determined using a Thermo Scientific process tensiometer (Thermo Fisher Scientific, Waltham, USA) at 20 °C. Considering the rapid sedimentation of salt particles in the mixture with the polymer solution, tests were performed exclusively on the polymeric solutions. The contact angle and surface tension were tested by Wilhelm's plate method, in which the plate was prepared from pressed NaCl. The measurement was justified by analysing the interaction between the polymer solution and salt particles incorporated as expanding agents. To simplify the system under analysis, glycerin was not added to the solutions tested.

The quality of the foams was assessed by scanning electron microscopy (SEM). Microscopic analysis of the foams was predominantly aimed at determining their morphology, specifically, the distribution of pores and connections between the foams. Two types of specimens were prepared: one for surface observation and a second for cross-section observation. The specimens, which were dusted with gold under argon, were observed using a high-vacuum technique with a secondary electron detector and reverse dispersed electron detector (under a chamber pressure of 7×10^{-4} Pa). The observations were performed using an accelerating voltage of 25 kV and magnifications from 100 to 20 000×.

Pore distribution was determined for selected variants by mercuric porosimetry with Auto Pore IV 9500 apparatus (Micromeritics, Norcross, USA). Prior to measurement, the samples were degassed to remove moisture, air and solvent residue. Degassing was performed to obtain a vacuum of 15 μm Hg. The sample was subsequently flooded with mercury; because of the high contact angles and surface tensions, the mercury did not penetrate the porous structure of the sample. The incorporation of mercury into the sample pores was possible after increasing the pressure of the measurement system. Increasing the pressure enables pores with smaller diameters to be filled. The measurement consists of determining the relationship between the quantity of mercury incorporated (intrusion) and the pressure under which the intrusion occurs. The system allowed measurements at a maximal pressure of 413 MPa, which enabled determination of the porous structure of the foam within the range of 400 000 nm to 3.5 nm. Based on the intrusion values obtained at successive pressure values, a curve of pore distribution was plotted for the sample in which the numerical values describe the porous structure: the total pore surface and average pore size. Selection of samples for the porosity analysis was based on test results of the absorption properties.

The sorption capability of foams is a critical quality parameter for the use of the materials developed as absorption elements in dressing materials. Two methods were used to evaluate this capability: Tests were performed using the absorption method with free soaking according to Standard EN 13726-1:2005 Part 1 [22], and test liquid A - a solution with properties similar to human blood serum or wound exudate - was prepared according to the following formula: 8.298 g of

Table 1. Qualitative morphological grading of cytotoxicity [32].

Grade	Reactivity	Description of changes in cell culture
0	none	Discrete intraplasmatic granules, no cell lysis, no reduction of cell growth
1	slight	No more than 20% of the cells are round, loosely attached and without intracytoplasmatic granules, apparent changes in morphology, occasional lysed cells are present, only slight growth inhibition
2	mild	No more than 50% of the cells are round, devoid of intracytoplasmatic granules, no extensive cell lysis, no more than 50% growth inhibition
3	moderate	No more than 70% of the cell layers contain rounded or lysed cells, cell layers are not completely destroyed, more than 50% growth inhibition
4	severe	Nearly complete or complete destruction of the cell layers

NaCl and 0.368 g of calcium chloride dehydrate per 1000 ml of deionised water. The absorptions of distilled and deionised water were also tested for 24 h.

The examination of the foams' porous structure was supplemented by the assessment of the material's response to the action of compression forces. Because it is assumed that the foams will be subjected to relatively low strains during use, tests were performed using the system for sensory comfort evaluation developed by Professor Sueo Kawabata. The Kawabata Evaluation System (KES) (Kato Tech, Kyoto Japan) consists of four modules, each enabling analysis of different types of forces: bending, tensile, shearing and compressive forces, as well as the action of normal forces (measurements of friction and roughness factors). In this study the third module was employed to evaluate sensory comfort; KES FB 3 AUTO analyses the action of compressive forces. The measurement involves imposing a compressive force at a rate of 0.02 mm/s to obtain a maximal load of 50 cN/cm^2. The pressure surface area is 2 cm^2. The sample tested is unstressed. During the compression test, the hysteresis of compression is recorded, from which the percentage of elastic recovery (RC, %) is calculated as the quotient of areas under the compression and decompression curves, according to the following formula:

$$RC = \frac{\int_{Tm}^{T0} \overleftarrow{P} dT}{\int_{Tm}^{T0} \vec{P} dT} \cdot 100\%$$

where:
$\vec{P}$ – compression curve, cN
$\overleftarrow{P}$ – decompression curve, cN,
$T0$ – distance between clamps prior to measurement, mm,
Tm – distance between clamps under a load of 50 cN/cm^2, mm.

Selected variants of samples were tested for chemical purity and cytotoxic activity. Prior to the tests, the samples were sterilised with radiation at a dose of 28 kGy. The conditions of sterilisation were determined in a separate experiment.

Chemical purities of the samples selected were determined by analysing the aqueous extracts and testing the substances released from the samples. The parameters for chemical purity were determined by considering the results of a preliminary risk analysis performed according to point 2.2.1 and guidelines of Standards EN ISO 10993-18:2009 [23] and EN ISO 10993-1:2009 [24].

The contents of the eluted substances (eluted substance profile) were determined by the three-stage extraction method according to Standard EN ISO 10993-12:2009 [25] through successive use of the following liquids:

- purified water (water for injection obtained from the Baxter Company, Deerfield, USA)
- 2-propanol according to the method described in Standard PN/P-04781/06 [26]
- petroleum ether.

For the testing of aqueous extracts, the following extraction method was employed: 1 g of test material per 50 cm^3 of extracting substance according to Standard EN ISO 10993-12:2009. The extraction was performed for 72 h at 37 °C. The assessment of extracts included organoleptic evaluations of transparency, colour and smell (a procedure developed by TRICOMED), and determination of pH (EN ISO 3071:2007 [27]), conductivity (TRICOMED's procedure), the permanganate value, (PN-P-04896:1984 [28]) and UV absorbance (PN-P-04990:1989 [29]). The content of heavy metals (PN-P04991:1989 [30]), chloride ions (PN-P-04895:1984) and ammonium ions (PN-P-04992:1989 [31]) in the extracts and the amount of solid residue after evaporation (TRICOMED procedure) were also determined.

These samples were subjected to biological tests, including the cytotoxicity of the materials toward fibroblast-like cells L929, conducted according to Standard PN EN ISO 10993-5 using direct material contact with the cell culture and an indirect method with polar and non-polar extracts. In the indirect tests, extracts were prepared with Eagle's culture fluid without calf serum but including 100 j/ml of penicillin, 100 mg/ml of streptomy-

*Table 2. Characteristics of PLA and DBC porous materials; * Proportions in relation to the mass of polymer solution.*

Sample symbol		Foam composition	Proportions of expanding agents added*		Absorption of liquid A after 24 h	Absorption of water after 24 h	Specific surface of pores	RC	T0
			NaCl	GI	g/g	g/g	m^2/g	%	mm
PLA 4060D	PLA 1	10% PLA + NaCl	1.0	0.00	2.890	4.388	0.117	72.92	3.000
	PLA 2		1.5		1.965	4.361	0.102	66.22	3.059
	PLA 3	10% PLA + NaCl + glycerin	1.0	0.05	9.798	10.193	0.024	53.96	2.810
	PLA 4		1.5		6.776	7.365	0.182	57.50	3.711
	PLA 5		1.0	0.10	7.615	6.580	13.745	65.07	3.740
	PLA 6		1.5		8.659	8.389	34.651	45.57	4.360
DBC	DBC 1	10% DBC + NaCl	1.0	0.00	6.610	6.110	0.201	60.14	5.334
	DBC 2		1.5		4.680	7.370	0.129	51.46	6.165
	DBC 3	10% DBC + NaCl + glycerin	1.0	0.05	10.230	10.190	0.042	61.27	5.151
	DBC 4		1.5		12.240	11.700	5.993	57.43	5.801
	DBC 5		1.0	0.10	5.560	4.030	24.282	69.69	3.840
	DBC 6		1.5		5.150	5.700	26.569	67.51	4.360

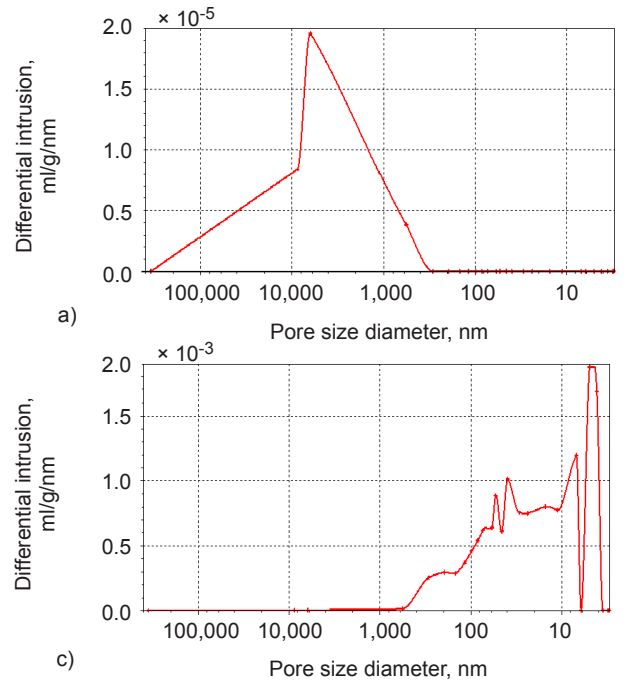

Figure 1. *Curves of pore distributions for foams composed of PLA 4060D with the addition of NaCl 1:1 in relation to the polymer solution mass with various glycerin contents: a) 0%, b) 5%, and c) 10%*

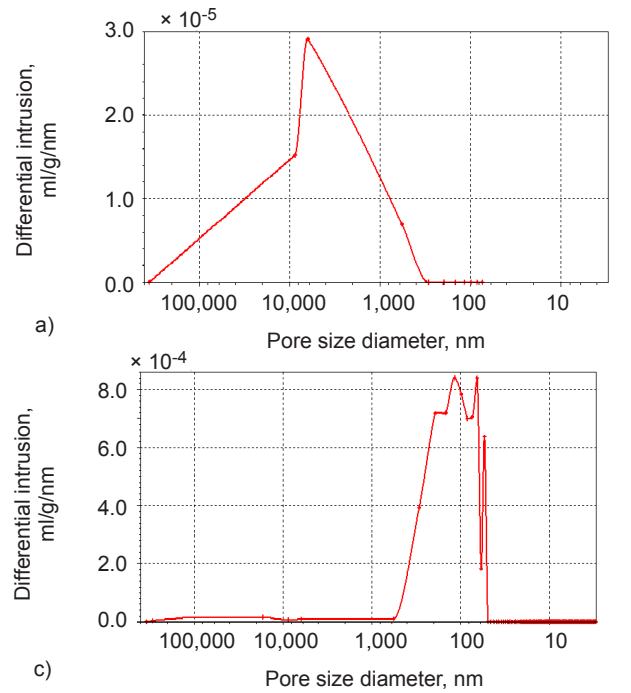

Figure 2. *Curves of pore distributions for foams composed of DBC with the addition of NaCl 1:1 in relation to the polymer solution mass with various glycerin contents: a) 0%, b) 5%, and c) 10%.*

cin and 2 mM/ml of L-lutamine. Nonpolar extracts were prepared with Eagle's fluid with the addition of 2% of calf serum, 100 j/ml of penicillin, 100 mg/ml of streptomycin and 2 mM/ml of L-lutamine. These samples were incubated for 24 h at 37 °C in 5% CO_2. Subsequently the extracts were collected in sterile test tubes for the cytotoxicity tests, which were conducted for 24, 48 and 72 h, with three repetitions for each of the methods. Cell cultures under similar conditions but without the samples or extracts were used as controls. The cultures were evaluated at a temperature of 37 °C in an atmosphere of 5% CO_2. During the tests, quantitative and morphological changes in the cell cultures were observed. The cultures were colored with trypan blue, which facilitated calculation of the number of dead cells. Depending on the changes observed, the cytotoxicity degree was determined, where 0 indicated a complete lack of toxicity, and 3 indicated acute cytotoxicity. A detailed description of the toxicity degrees is provided in ***Table 1*** [32].

Results and discussion

Formation of porous material from PLA and DBC solutions

Characteristics of the morphological, mechanical and sorption properties of the foams according to the formation parameters are listed in ***Table 2***. ***Figures 1*** and ***2*** illustrate typical curves of the pore dis-

tributions for the PLA and DBC foams. The foam structures are illustrated in ***Figures 3*** and ***4***.

Based on the data listed in ***Table 2***, the addition of sodium chloride in weight ratios of 1:1.5 and 1:1 to the mass of the 10% solution of PLA 4060D causes the development of a porous foam structure, with specific surface areas of the pores of 0.102 and 0.117 m^2/g, respectively. Using the method of foam preparation in the literature, we obtained a material with water absorption in the range of 4.36 – 4.39 g/g after 24 h, where the absorption of test liquid A ranged from 1.96 – 2.89 g/g. The addition of NaCl in a 1:1 proportion and the addition of glycerin in a 1:0.05 proportion causes a reduction in the pore specific surface area to 0.024 m^2/g, which may be associated with an increase in the pore radius and the absorption of the liquids tested in the range of 10.19 – 9.79 g/g. ***Figure 1.b*** shows that this material contains pores with a wide range of diameters, from 0.1 mm to 100 nm. In the case of foams formed from PLA 4060D, this method of formation is optimal with respect to the liquid absorption capability of the foam. An increase in the quantity of glycerin added to the polymeric solution in a weight proportion of 1:0.1 caused a rapid increase in the development of the pore specific surface area to 13.74 m^2/g with a NaCl content of 100% by wt. of the polymer solution mass and to 34.65 m^2/g with a NaCl content of 150% in relation to the polymer solution mass. A review of the pore distribution plot, which is shown in ***Figure 1.c***, indicates that the material is characterised by a high content of pores with dimensions ranging from 577 nm to 7.3 nanometers. Such a structure results in decreased water absorption values of 6.58 g/g for variant PLA5 and 8.39 g/g for variant PLA6. A similar trend was observed in the case of foam formation from DBC. The highest absorption is observed for foams formed with a NaCl content at levels from 100% in weight relation to the polymer solution mass and 5% glycerin in relation to the polymer solution mass, in which case the absorption exceeds 10 g/g. As observed for PLA, the addition of 10% glycerin in relation to the polymer solution mass to the mixture of the DBC solution and NaCl causes a significant increase in the specific surface areas of pores in the range of 24–26 m^2/g, which results in a shift in the pore diameter distribution curve towards pores smaller than 500 nm, resulting in a decrease in the absorption of the liquids tested to 4.03 to 5.70 g/g. For foams used for filling bone defects, the elastic recovery of the foam after compression is an important parameter. For PLA foams, the greatest reduction is exhibited by that containing no glycerin. For this type of foam, 27 – 34% of the energy is dissipated during compression. A reverse phenomenon was observed during the compression of DBC foam. The addition of approximately 10% glycerin in relation to the polymer solution mass causes a slight increase in the energy recovered during compression from 67 – 69%, i.e., the energy loss ranges from 31 – 33%.

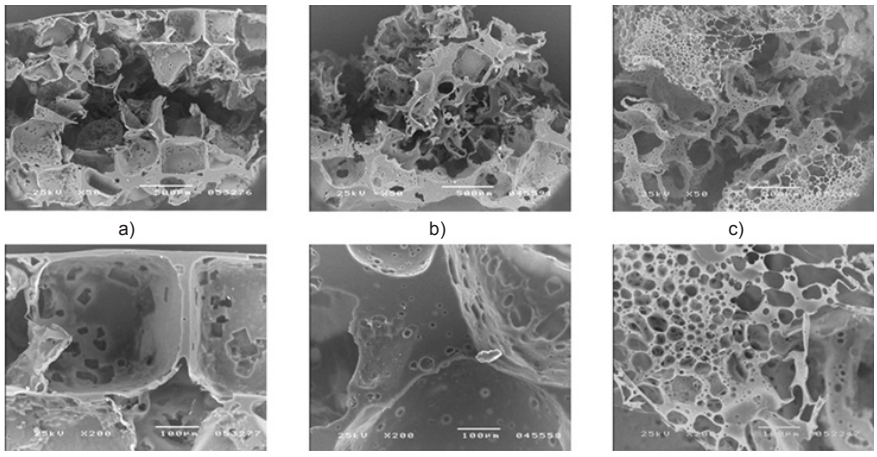

Figure 3. *SEM images of PLA foams containing NaCl 1:1 in relation to the polymer solution mass with various glycerin contents: a) 0%, b) 5%, and c) 10%.*

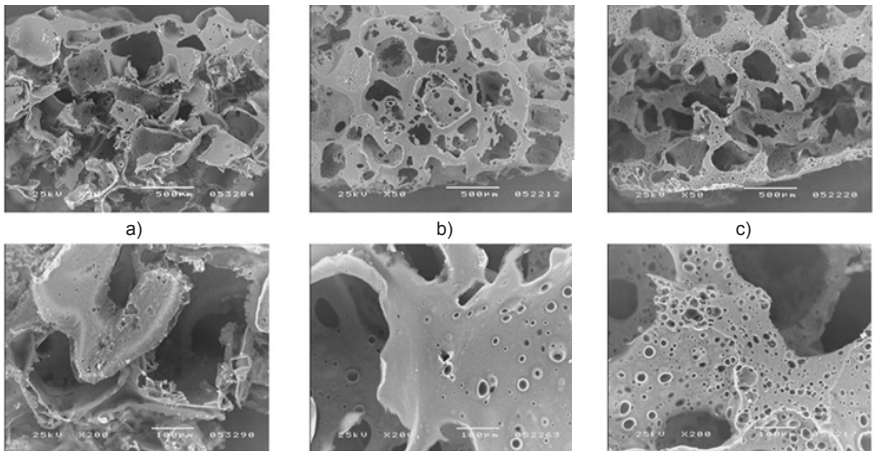

Figure 4. *SEM images of PLA foams containing NaCl 1:1.5 in relation to the polymer solution mass with various glycerin contents: a) 0%, b) 5%, and c) 10%.*

Images of the foams indicate that a significantly low NaCl content can cause the formation of an external layer with decreased porosity, which is a film on the surface, as shown in ***Figure 5.b***. This occurrence may result from the lack of liquid access to the internal foam layers of higher porosity. ***Table 2*** demonstrates that the addition of 150% sodium chloride to the DBC solution mass with a glycerin content of 5% increases the liquid absorption from 10 g/g to 12 g/g, which may be attributed to improved conditions for the formation of a porous external layer of foam, which is denoted by DBC 4 (***Figure 6.b***).

Formation of porous material from solutions of the PLA/DBC mixture

The next experiment used a mixture of both polymers. The proportion of PLA/BDC mixture components was 1:1. The objective of these tests was to determine the effect of polymeric solution concentration on the properties of the foam structures formed. To assess the effect of glycerin on the microporous structure, an additional experiment was conducted without glycerin, which is denoted by PLA/DBC 1a. To prepare the foams, solutions of PLA and DBC in acetone (an adequate solvent for both components) were prepared at concentrations of 5, 10 and 15%. The solutions were mixed in a 1:1 ratio, and a quantity

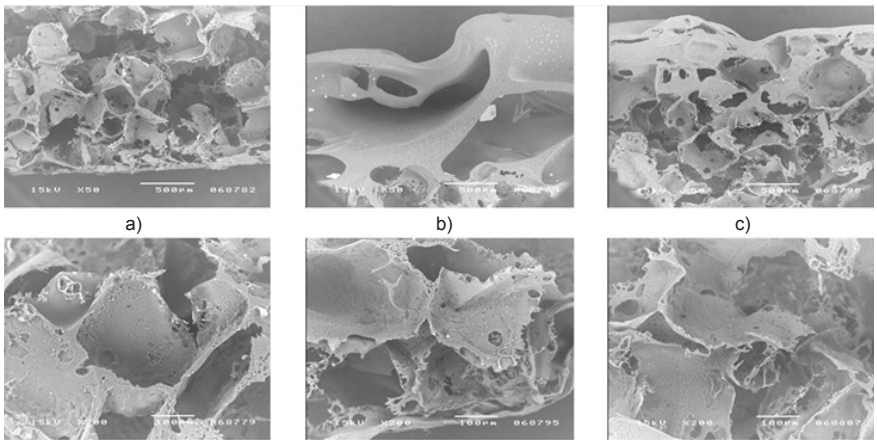

Figure 5. *SEM images of DBC foams containing NaCl 1:1 in relation to the polymer solution mass with various glycerin contents: a) 0%, b) 5%, and c) 10%*

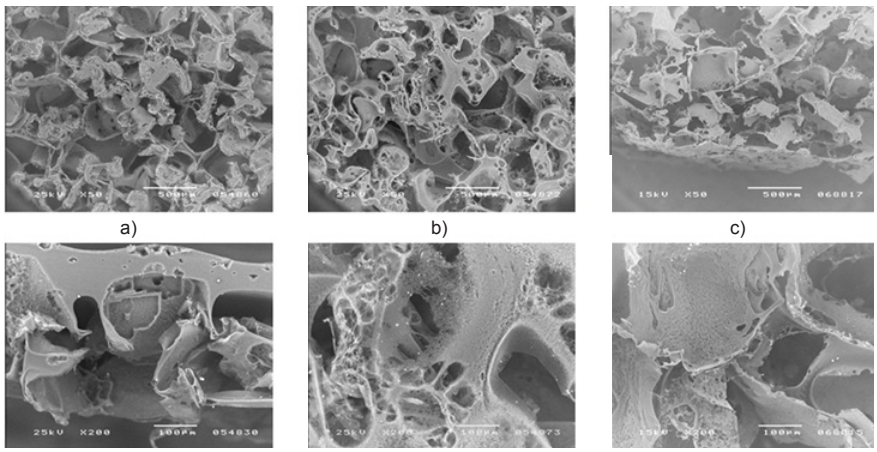

Figure 6. *SEM images of DBC foams containing NaCl 1:1.5 in relation to the polymer solution mass with various glycerin contents: a) 0%, b) 5%, and c) 10%.*

of 150% NaCl in relation to the polymer solution mass was added; 5% of glycerin was also added in three trials. Solidification of the foam occurred by solvent evaporation, and the material was rinsed with distilled water to remove the expanding agents. The resulting structures of the foams and their absorption properties are listed in ***Table 3.*** Note that the incorporation of a new solvent considerably affected the density of the polymer solution. Consequently the systems, with a 1:1 ratio of salt content to the polymer solution mass, underwent phase separation. The foam formed contained a clearly distinct foil layer (***Figure 7***), which caused the stiffening of the system, and its strain increased the material brittleness and decreased the liquid absorption.

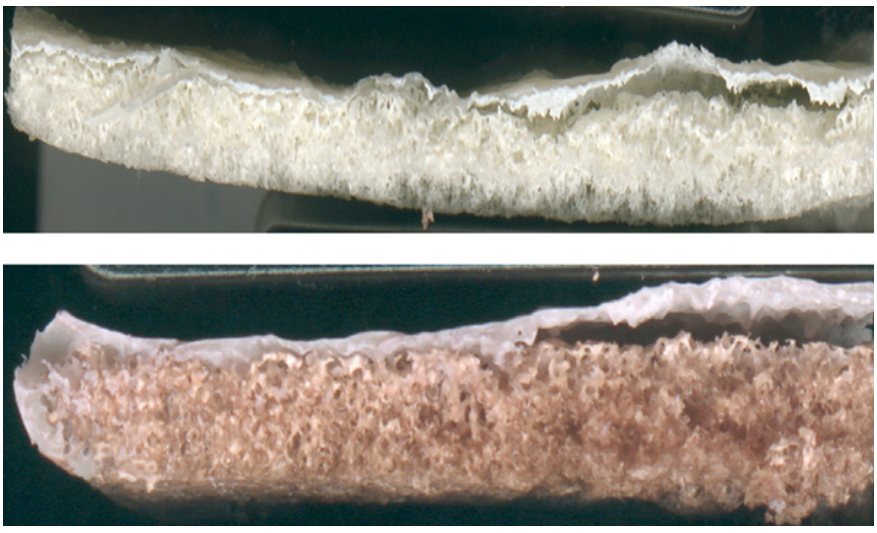

Figure 7. *Microscopic image of PLA/DBC foam containing 1:1 NaCl in relation to the polymer solution mass and 5% glycerin.*

Using a simple test with the use of a selective solvent, the separating foil layer was determined to exclusively consist of PLA. This test consisted of depositing ethanol on the surface of the foil formed. Ethanol dissolves DBC but not PLA**.**

The morphological, mechanical and sorption characteristics of the foams, which are dependent on the formation process parameters, are listed in ***Table 3*** (see page 126). ***Figure 8*** displays typical curves for pore distribution in PLA and DBC foams. The foam structure is illustrated in ***Figure 9***.

Based on the images and pore distribution curves obtained, foams from the solutions with higher concentrations are more compact and contain a higher number of closed pores, which are difficult to detect by the mercury porosity technique. Considering the results of the initial foam formation trials, investigations were performed using foams that contained NaCl in a quantity of 150% in relation to the polymer solution mass. The results indicate that the pore size decreases and the specific surface area of pores increases with increasing concentrations of the polymer solution. This change in foam structure results in the decreased liquid absorption and increased elastic recovery of the foams after compression. Comparing the absorption of test liquids for PLA/DBC 1 and PLA/DBC 1a foams, it can be concluded that with the 5% polymer solution, which contains 150% sodium chloride, the addition of 5% glycerin caused a decrease in the specific surface areas of the pores from 0.088 m^2/g to 0.017 m^2/g; pores with a greater diameter and accessibility to liquids were produced. Therefore the addition of glycerin contributed to increased water absorption after 24 h, which changed from 5.88 g/g to 10.75 g/g for liquid A and from 5.13 g/g to 12.63 g/g for water. As in the case of previous DBC foams, the incorporation of glycerin increased the elastic recovery of the foams from 38% to 45% after compression.

To explain the differences in salt behavior in the solution of the DBC/PLA mixture,

Table 3. *Results of the liquid absorption and porous structure for foams composed of the PLA-DBC mixture; *proportions in relation to the polymer solution mass.*

Sample symbol	Foam composition	Proportions of expanding agents added*		Liquid A absorption after 24 h	Water absorption after 24 h	Specific surface of pores	RC	T0
		NaCl	Gl	g/g	g/g	m^2/g	%	mm
PLA/DBC 1	5% PLA+DBC+NaCl+glycerin	1.5	0.05	12.630	10.750	0.017	45.27	3.370
PLA/DBC 1a	5% PLA+DBC+NaCl		0.00	5.130	5.880	0.088	38.05	4.290
PLA/DBC 2	10% PLA+DBC+NaCl+glycerin		0.05	9.098	8.440	2.697	66.05	5.530
PLA/DBC 3	15% PLA+DBC+NaCl+glycerin		0.05	2.994	4.640	32.584	72.26	5.190

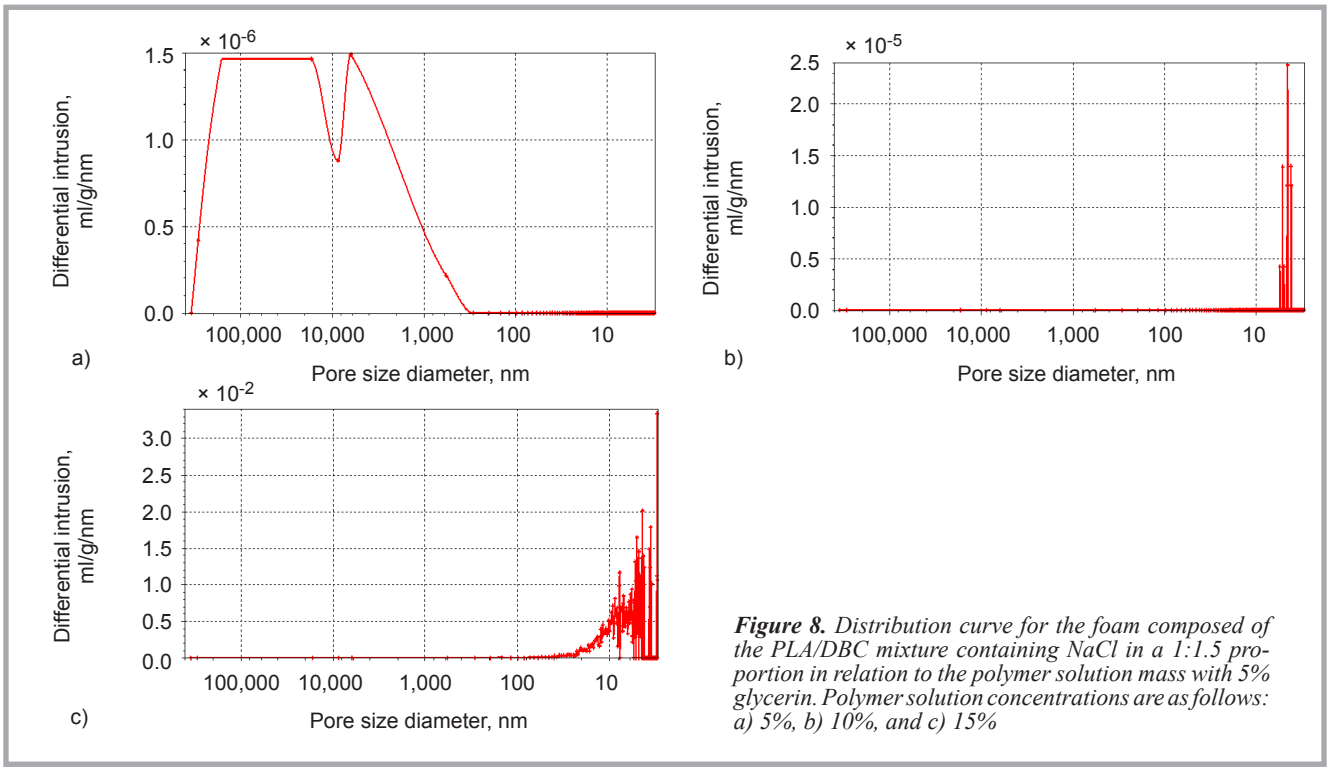

Figure 8. *Distribution curve for the foam composed of the PLA/DBC mixture containing NaCl in a 1:1.5 proportion in relation to the polymer solution mass with 5% glycerin. Polymer solution concentrations are as follows: a) 5%, b) 10%, and c) 15%*

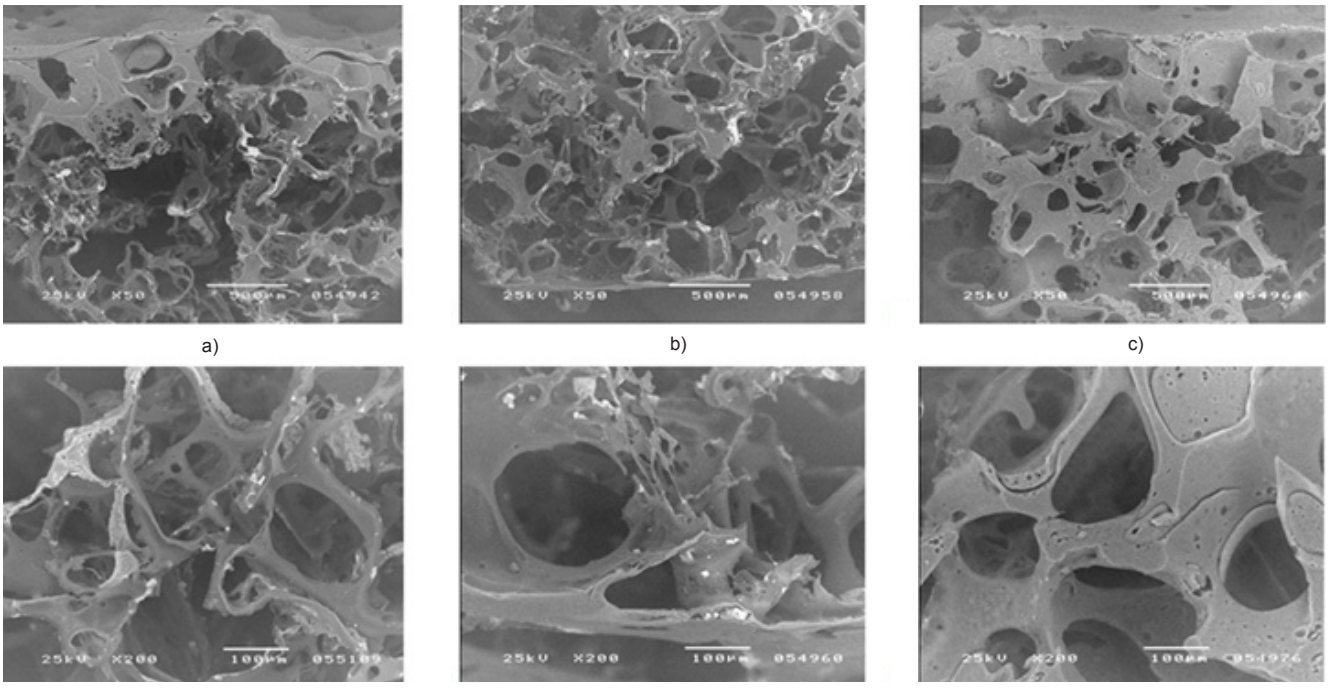

Figure 9. *SEM images of PLA/DBC foams containing NaCl 1:1.5 in relation to the polymer solution mass with 5% glycerin. Polymer solution concentrations are as follows: a) 5%, b) 10%, and c) 15%.*

the properties of these solutions were determined. Test results for rheological properties of the solutions and mixtures are listed in ***Table 4***, and results of the density, surface tension and contact angle of the polymeric solutions used to make the foams are listed in ***Table 5***.

The results of rheological measurements of the polymeric solutions used for foam preparation reveal considerable differences in consistence coefficient k values of the PLA solution in ethyl acetate and the DBC solution in ethanol. The use of a common solvent and the reduction of the solution concentration to 5% enabled the creation of a homogeneous mixture in which the polymers were properly mixed.

From the analysis of the contact angle values, the greatest difference between the contact angles during the immersion and emergence of the salt plate was observed in the case of DBC. Such a result suggests that strong adhesion forces act between the surface of the salt and polymer solution, which causes the deposition of solution on the salt plate surface and changes the contact angle values. In the case of the PLA and PLA/DBC solutions, the difference between the contact angles during immersion and emergence was considerably lower. DBC, whose molecules exhibit a greater adhesion to salt molecules, migrates in the mixture and pulls the salt particles, which results in the formation of a non-porous PLA film layer on the foam surface (confirmed by the insolubility test in ethanol). To prevent the formation of a non-porous layer, the salt content in the mixture should be sufficiently high to fill the entire volume of the polymer solution. In the case of the PLA solution in ethyl acetate, which also exhibits a minor difference between contact angles during immersion and emergence, there is a lack of force for phase separation. Therefore the process of salt precipitation from the PLA/DBC mixture is slower than that of salt precipitation in the case of the PLA/DBC/NaCl mixture in acetone, whereas the time for solvent evaporation is similar to that for foam solidification.

Table 4. *Consistence coefficients of the solutions and mixtures used to make the foams.*

Polymer solution	NaCl content	Consistence coefficient k
10% DBC/ethanol	-	3833
	NaCl 1:1	8236
	NaCl 1:1.5	32208
10% PLA/ethyl acetate	-	332
	NaCl 1:1	1583
	NaCl 1:1.5	12955
5% DBC/acetone	-	294
	NaCl 1:1	5475
	NaCl 1:1,5	65578
5% PLA/acetone	-	56.9
	NaCl 1:1	3899
	NaCl 1:1.5	14224
5% DBC/acetone + PLA/acetone	-	166
	NaCl 1:1	3109
	NaCl 1:1.5	14135

Table 5. *Values of surface tension, density and contact angle of the solutions used to make the foams.*

Polymer solution	Consistence coefficient	Surface tension, mN/m	Density of polymer solution, g/cm^3	Contact angle, degree	
				advanced	reverse
5% DBC/acetone	294.15	24.31	0.7218	89.25	70.16
5% PLA/acetone	56.88	23.09	0.7018	79.83	79.25
5% DBC/acetone+ +PLA/acetone	165.85	23.22	0.7107	78.78	77.61
10% DBC/ethanol	3833.75	26.10	0.8451	87.82	75.08
10% PLA/ethyl acetate	332.25	24.29	0.9343	79.26	73.63

Chemical purity and cytotoxicity tests

One foam was chosen from each material group for chemical purity and cytotoxicity tests. The absorption and elastic recovery values of the foams were the criteria for selection. The following foams were selected: PLA 3, DBC 4 and PLA/DBC 1. The test results are listed in ***Table 6***.

Based on the results listed in ***Table 6***, foams containing DBC in their structure contain higher specific moisture contents than the PLA foams. The contents of soluble substances in ether, water and 2-propanol are within admissible ranges; foams that contain DBC show increased contents of soluble substances in 2–propanol. This effect is due to the limited solubility of DBC in 2-propanol. Skin and properly healed wounds have a slightly acidic pH of 5 – 7. Both acidification and alkalisation of the skin surface can cause adverse effects, such as decreased skin resistance to the action of micro-organisms or increased risk of irritation. The pH level of the foam that contains DBC is within the standard limits, whereas those of the foams that contain PLA exceed 7, which may result from the solvent residues (ethyl acetate) that remain in the foam structure, suggesting the necessity of thorough rinsing of the foam to remove them from the PLA. The lower pH levels of the foams that con-

Table 6. *Results of chemical purity and cytotoxicity tests of selected foams;* ***Abbreviations****: Z_{et} – Content of soluble substances in petroleum ether, Z_{prop} – Content of soluble substances in 2-propanol, Z_{H_2O} – Content of soluble substances in water, pH – pH of aqueous extract, O_2 – Permanganate value, A_{max} – maximum absorbance in UV, A(245) – Absorbance at a wavelength of 245 nm, Cl$^-$ – Content of chloride ions, NH_4^+ – Content of ammonia ions, Pb_2^+ – Content of heavy metals, S – Conductivity, Ms – Solid residue, Ct – Degree of cytotoxicity.*

Foam symbol	Specific moisture content	Z_{et}	Z_{prop}	Z_{H2O}	pH	O_2	Amax	A(245)	Cl-	NH4+	Pb2+	S	Ms	Ct
		%	%	%	pH	mgO_2/g	-	-	mg Cl-/g sample	mg NH4+/g sample	mg Pb2+/g sample	µS/cm	mg/g dry sample	-
PLA 3	3.51	0.107	1.838	0.243	7.70	0.100	0.0811	0.0509	> 0.04	< 0.02	<0.02	>100	1.75	0
DBC 4	4.578	0.226	12.020	0.215	5.57	0.824	0.1800	0.1165	< 0.04	> 0,02	<0.02	45.5	1.153	-
PLA/DBC 1	4.246	0.231	12.221	0.114	6.36	2.696	0.1965	0.,1571	> 0.04	> 0.02	<0.02	151.7	2.69	0

tain DBC indicates that these materials will create a more favorable medium for wound healing. The permanganate value, which indicates the presence of oxidizable compounds, is the highest for foams composed of the DBC/PLA mixture and lowest for PLA foams. The content of potentially toxic heavy metals in the materials tested is significantly low. The increased content of chloride ions may result from inadequate rinsing of NaCl, which is not reflected in an increase in the cytotoxic effect.

None of the foams created exhibit a cytotoxic effect. In all the cultures, cells exhibit proper morphology and proliferation; however, the content of dead cells was extremely low, which was similar to the level in the control culture.

Conclusions

Based on the experiments performed, the following conclusions can be made:

1. A new process for porous, quasi – fibrous material preparation was developed that employs a solution of polylactide, dibutyrylchitin or a mixture of both polymers in addition to the use of two expanding agents: sodium chloride and glycerin. The materials created under optimal conditions for the given variant are characterised by the absorption of water and physiological saline at a level above 10 g/g after 24 h.

2. The incorporation of glycerin in the material-forming polymeric mass causes reconstruction of the porous structure of the product. Increasing the glycerin content to 5% in the mixture of PLA and sodium chloride initially causes a decrease in the specific pore surface area compared to the materials formed without glycerin, which can result from the increased pore diameter. The result of this phenomenon is an increase in the absorption of both water and physiological saline for all foam variants investigated.

3. A maximum increase of 10% in the glycerin content in the mixture of polymer and sodium chloride causes an abrupt increase in the specific surface areas of the pores, which is caused by a decrease in their size to several hundred nanometers. The result of this phenomenon is a decrease in the absorption of test liquids for all variants of the foams investigated.

4. The contents of NaCl crystals, which are responsible for the formation of porous structures, should be higher than 100% of the mixture of PLA 4060D and DBC solutions. Otherwise the mixture is delaminated, which results in a heterogeneous foam structure across its entire thickness.

5. An increase in the concentration of the polymeric solutions used for preparation of the PLA/DBC foam-forming mixture with sodium chloride and glycerin causes the reconstruction of the porous structure toward small pores with dimensions of ten nanometers and an increase in the elastic recovery of the materials after compression.

6. The materials developed do not exhibit any cytotoxic action *in vitro* compared with the fibroblast-like cells of series L929.

7. The chemical purity test, which is one of the criteria for optimal material selection with respect to the composition and structure of the dressing material, must be confirmed by biocompatibility *in vitro* and *in vivo*.

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