Short thesis for the degree of doctor of philosophy (Ph.D.)

Production possibilities of biodegradable polyurethane-based scaffolds

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1. Introduction

Nowadays, plastics have appeared in all areas of life. They can be widely used as building materials, as auxiliary materials in the food industry, or even the pharmaceutical industry likes to use them. In medicine, they are widespread as prostheses, sutures and various tissue supports (scaffolds). The requirement for this use is that the given substance is biocompatible. It can also be important that the aid is absorbed after the task is completed. This is called biodegradation.

During my research work, my goal was to produce polymers that could be suitable for the production of tissue support materials that support the growth of tissue at the site of bone tissue cysts and that completely decompose after completing the task. Polyurethanes seem to be the most suitable for this task, including those made by using aliphatic isocyanate. The polyol, which gives the main mass of the polymer, is a polycaprolactone (PCL), polylactic acid (PLA) or polyethylene glycol (PEG) derivative. By using polyols separately or together, we can endow the frame material with "programmable" properties. Examples include the decomposition time and water absorption affecting the release of the active ingredient. During my research, I compared these versions, examined their physical properties, and studied how the dental bone stem cells in the body react to these materials.

2. Experimental methods

The structural analysis of the produced polyol raw materials and the finished polyurethane samples was carried out by size exclusion chromatography (SEC), infrared spectroscopy (FTIR-ATR), nuclear magnetic resonance spectroscopy (1H-NMR) and matrix-assisted laser desorption/ionization mass spectrometry (MALDI- TOF). The mechanical properties were explored using tensile tests. Dynamic mechanical analysis (DMA) and differential scanning calorimetry (DSC) investigated the thermomechanical and shape memory properties. The cross-linking density of the polyurethane samples with different compositions was also determined using swelling experiments and a DMA device. The surface of the samples, as well as the internal morphology of the scaffolds, were identified using scanning electron microscopy (SEM). We mapped the effects of the samples on the body by culturing them with dental bone stem cells.

3. New scientific results

Linear and cross-linked polyurethanes containing a new type of poly-ω-pentadecalactone were produced. I established a close correlation between the composition of the produced polyurethanes and their mechanical and thermal properties.

For the production of polyurethanes (PUs) containing ω-pentadecalactone, We first synthesized poly-ω-pentadecalactone (PPDL) by ring-opening polymerization. The synthesis is illustrated in Figure 1. The polymers produced in this way were then synthesized into linear and cross-linked polyurethanes using 1,6-hexamethylene diisocyanate (HDI) and PCL. I created the cross-linked structure with the help of an allophanate bond. Tables 1 and 2 and Figure 2 show the composition and production of polyurethane.

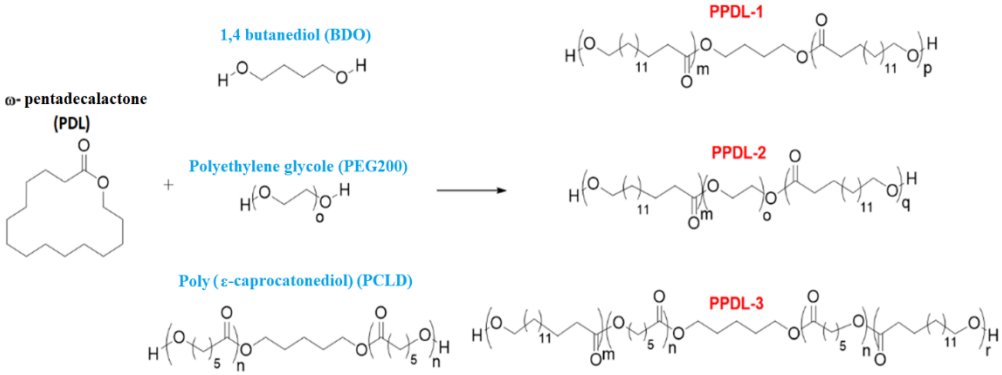

Figure 1. Synthesis of PPDL 1-3 samples

Table 1. The composition of the completed linear PU samples containing PPDL

Sample	Initial composition with molar ratios
PU- 1	PCL (50) – HDI – (PPDL- 1) / 1 : 3 : 1
PU- 2	PCL (50) – HDI – (PPDL- 2) / 1 : 3 : 1
PU- 3	PCL (50) – HDI– (PPDL- 3) / 1 : 3 : 1

Table 2. The composition of the cross-linked PU samples containing PPDL

Sample	Initial composition with molar ratios
PU- 4	PCL (50) – HDI – (PPDL- 1) – HDI / 1 : 3 : 1 : 1
PU- 5	PCL (50) – HDI – (PPDL- 2) – HDI / 1 : 3 : 1 : 1
PU- 6	PCL (50) – HDI – (PPDL- 3) – HDI / 1 : 3 : 1 : 1

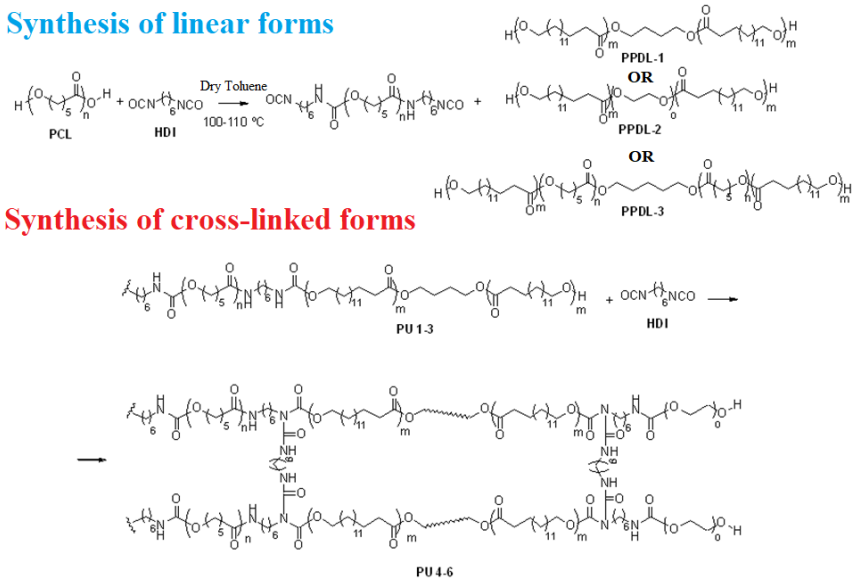

Figure 2. The synthesis route of PPDL-containing polyurethanes

We found that the high molecular weight of the used PCL segments (Mn=50 kg/mol) increased flexibility. The partial opalization visible during drawing supports our idea that the sample has several stages of hardening. In addition, the PPDL units function as hard segments, but the initiators forming the basis of the chain show a softening effect as their chain length increases. However, in the case of cross-linked samples (PU 4-6), this tendency can no longer be detected. In the case of PU 4-6 samples, the crystallization of the PPDL segments is inhibited by the formed cross-linked structure, so their flexibility remains, but due to their fixed nature, their elongation at break value decreases.

In the case of the cross-linked samples, stress relaxation was also investigated, and based on this, we set up the SLS (Standard Linear Solid) model of the samples, which is important from the point of view of molecular engineering.

The DSC and DMA tests of the samples revealed that the crystalline parts of the PCL and PPDL segments softened in well-separated regions, so we also assumed their shape-memory properties, which is confirmed in Figure 3. The shape retention rate of the tested PU 4 sample was 99% and the shape recovery rate was 81%.

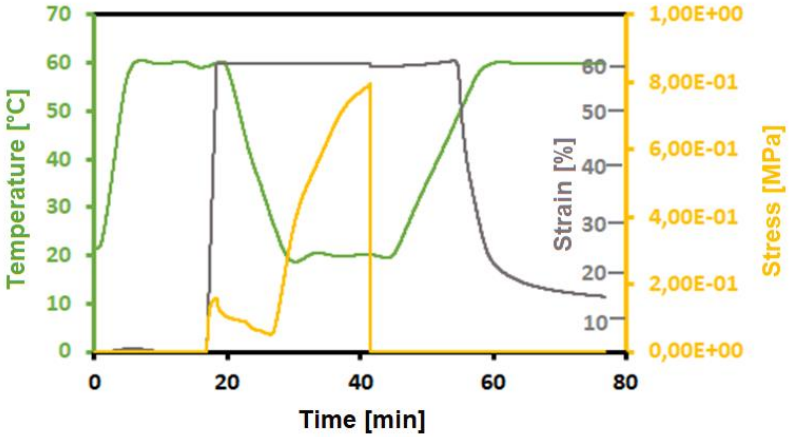

Figure 3. Examination of the shape memory effect of the PU 4 sample using a DMA

3.2. Linear and cross-linked polyurethanes containing potentially biodegradable, biocompatible sucrose-HDI co-oligomers were produced. We established a close relationship between the composition of the produced polyurethanes and their mechanical and thermal properties.

For their production, we first determined the optimal reaction conditions of the sucrose-HDI co-oligomer. Polyols containing isocyanate end groups of different molecular weights were added to the reaction mixture containing the produced sucrose-HDI co-oligomer. The reaction scheme and the compositions of each sample are shown in Figure 4 and Table 3.

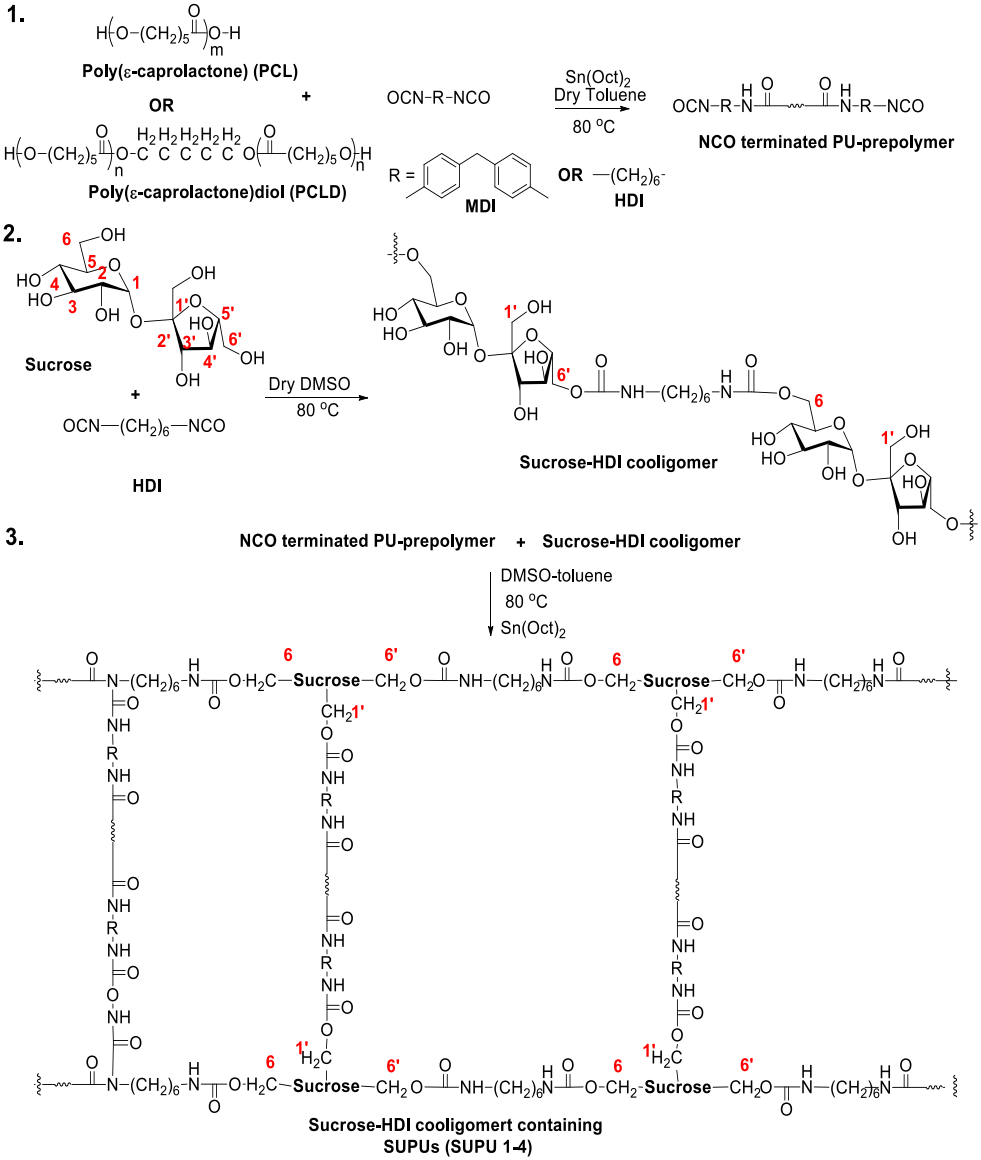

Figure 4. Synthetic route for the preparation of cross-linked polyurethanes containing sucrose-HDI co-oligomers

Table 3. Composition of the cross-linked polyurethanes containing sucrose-HDI co-oligomers

Sample	Sucrose-HDI co-oligomer		PU-prepolymer			
	Sucrose g/(mmol)	HDI (mL)	PCL(10) g/(mmol)	PCLD(2) g/(mmol)	HDI (mL)	MDI g/(mmol)
SUPU 1	0,5/(1,5)	0,24	3,65/(0,4)	–	–	0,18/(0,8)
SUPU 2	0,5/(1,5)	0,24	3,65/(0,4)	–	0,12	–
SUPU 3	1,5/(4,4)	0,70	–	2,2/(1,1)	–	0,55/(2,4)
SUPU 4	1,5/(4,4)	0,70	–	2,2/(1,1)	0,36	–

In the case of SUPU 1-3 samples containing MDI, we observed a higher modulus of elasticity and tensile strength, indicating the aromatic isocyanate's effect as a chain stiffener. We found that in the case of cross-linked samples, the elastic modulus of the SUPU 1-2 samples containing high molecular weight PCL units was higher than the versions containing low molecular weight PCLD segments. This can be explained by the higher degree of crystallinity of the PCL parts. An extended SLS model was set up for the tensile curves of the tested samples, which could also describe the hardening that occurred under stress.

By DSC examination of the samples, we established that in the case of the samples containing PCL units, the formed cross-linked structure reduced the crystalline nature of the formed polyurethane, as it inhibited segment movement. This idea is also supported by the fact that the degree of crystallinity of the SUPU 4 sample - in which no crosslinks could be detected - does not differ significantly from the crystallinity of pure PCLD.

3.3. PCLD-based cross-linked polyurethanes modified with PEG-600 and PLAD-1000, which can be used for dental purposes, were synthesized and their mechanical and thermomechanical properties were determined in detail.

PCL-based medical aids are widely used, either as implants or as drug carriers. In the case of this type of material, it is important that they break down in the body, i.e. they are biodegradable. Our goal was to produce copolymers that can be programmed to decompose or release medicinal substances. During the

synthesis, we, therefore, used PLAD, which increased biodegradation, and PEG, which increased biocompatibility, in addition to poly ε-caprolactone. Aliphatic isocyanate was used for the synthesis, so that the decomposition products of the scaffold were not toxic. The synthesis path of the products is illustrated in Figure 5, and the completed compositions are listed in Table 4.

Table 4. The composition of PCLD-based cross-linked polyurethanes containing PEG and PLAD

Sample	**Prepolymer**	**PCLD-PEG / Sucrose molar ratio**
	PCLD / PEG / HDI molar ratio	
SUPUR 1	0,9/0,1/2	10/1
SUPUR 2	0,8/0,2/2	10/1
SUPUR 3	0,7/0,3/2	10/1
SUPUR 4	0,6/0,4/2	10/1
SUPUR 5	0,5/0,5/2	10/1
	PCLD / PLAD / HDI molar ratio	**PCLD-PLAD / Sucrose molar ratio**
SUPUR 6	0,9/0,1/2	10/1
SUPUR 7	0,8/0,2/2	10/1
SUPUR 8	0,7/0,3/2	10/1
SUPUR 9	0,6/0,4/2	10/1
SUPUR 10	0,5/0,5/2	10/1

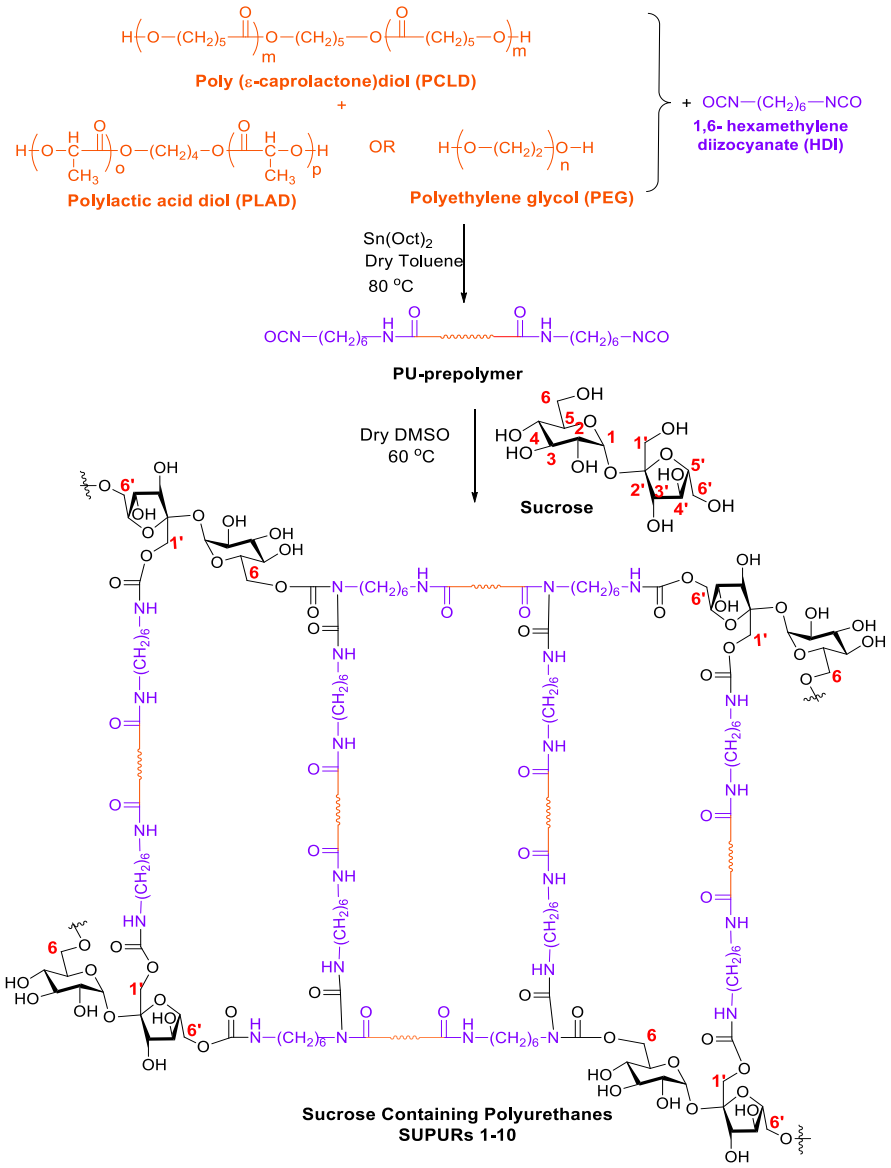

Figure 5. Synthetic route for the preparation of cross-linked PCLD-based polyurethanes containing PEG or PLAD

Based on our observations, with the increase in the molar ratio of PEG, initially, the tensile strength did not change significantly, and the modulus of elasticity showed a decreasing trend, however, at 40 n/n % a significant decrease in tensile strength was already visible, and in the case of 50 n/n %, the physical parameters changed drastically. Based on the bleaching of the sample and the results of the subsequent DSC measurement, significant crystallization started in the samples. Thus, PEG acted as a plasticizer to a small extent, but as a hard segment to a larger amount. This is due to the crosslinking points being too close to each other. In order to design the materials, I also applied the previously introduced extended SLS model to the tensile curves.

DSC and DMA measurements were performed for all samples, and based on the results, no significant degree of crystallinity could be detected in the samples other than the previously mentioned. Based on the DMA measurements, we also determined the crosslink density values, which fell within an order of magnitude, varying between $3.9 *10^{-3}$ and $1.9 *10^{-3}$ mol/cm3.

3.4. We made scaffolds from PCLD-based cross-linked polyurethanes modified with PEG-600 and PLAD-1000 and proved their biological compatibility.

Tissue supports were prepared from the individual compositions using the salt dissolution technique. The electron micrographs of the finished support materials are illustrated in Figure 6. The average pore size of the SUPUR samples was determined using software analysis of the recordings. After sterilization, the samples were subjected to dental bone stem cell culture. The results are shown in Figure 7. After culturing, the samples were labelled with cell staining to visualize live and dead cells. The results of the test are shown in Figure 8. Based on the results, it can be concluded that the number of cells on the surface of the frame material does not differ from the data on the control surface and their viability also shows a good agreement, on this basis it can be said that the frame material is not toxic.

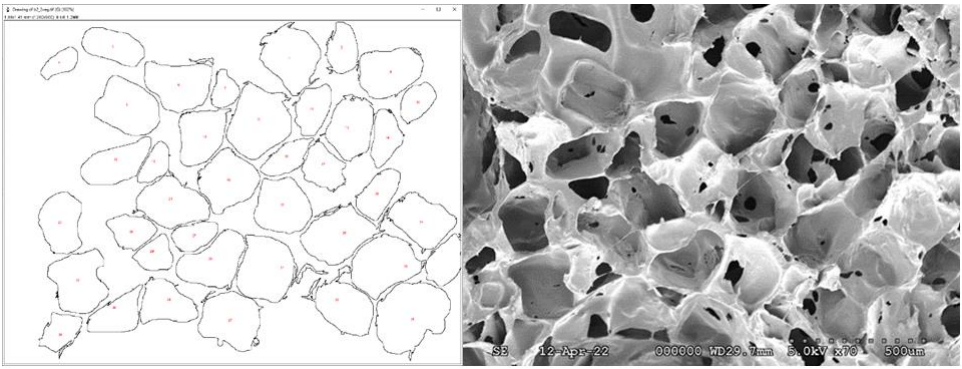

Figure 6. SEM image of the SUPUR 2-based frame material (right) and the outline of the voids recognized by the software (left)

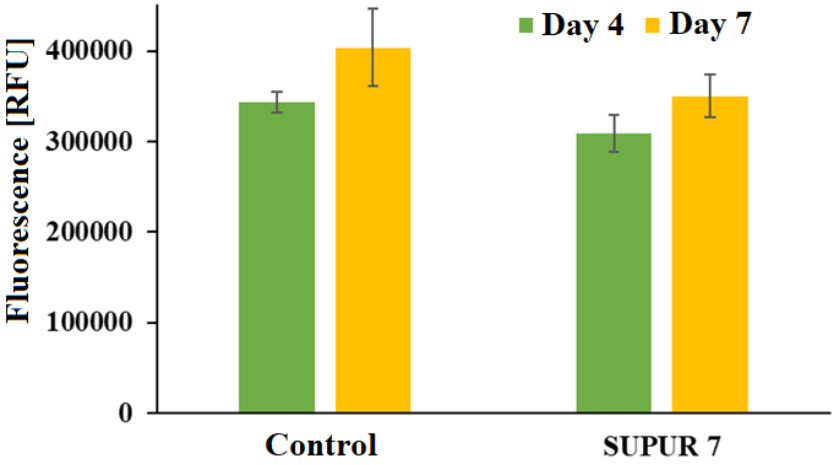

Figure 7. Results of the SUPUR 7 sample viability test

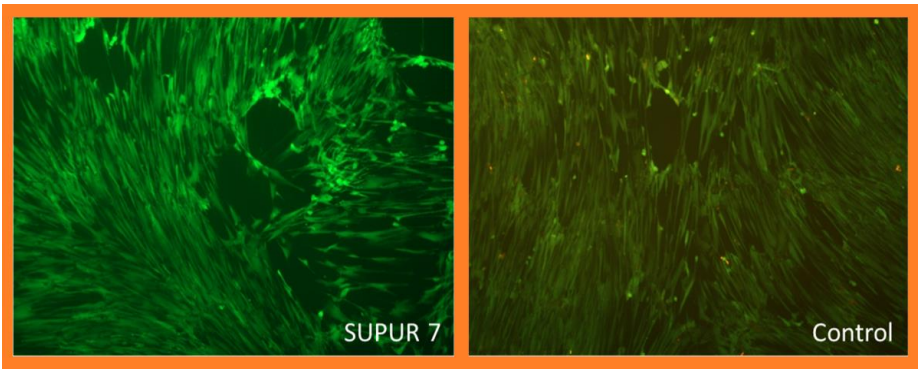

Figure 8. Live/dead assay of glass surface control and SUPUR 7 by optical microscopy

4. Applications of results

The linear and cross-linked polyurethane containing a new type of poly-ω-pentadecalactone produced by us has good mechanical properties and, thanks to its complex macromolecular structure, it also has shape memory properties. Based on these, the material we produce can be used in itself or as a sandwich structure, as a composite, for the production of medical devices after successful experiments in the appropriate biological environment.

In the field of biomedicine, the PLAD and PEG-modified polyurethane synthesized by us is another significant step forward, because by using these modifying polyols, it becomes possible to change the release time of the drug and control the degradation time. With the use of the mentioned raw materials, it was also possible to create tissue support materials, the biological compatibility of which was confirmed with the help of dental bone stem cells.

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List of publications related to the dissertation

Foreign language scientific articles in international journals (3)

1. **Kordován, M. Á.**, Hegedűs, C., Czifrák, K., Lakatos, C., Kálmán-Szabó, I., Daróczi, L., Zsuga, M., Kéki, S.: Novel Polyurethane Scaffolds Containing Sucrose Crosslinker for Dental Application.
   Int. J. Mol. Sci. 23 (14), 1-18, 2022. ISSN: 1661-6596.
   DOI: http://dx.doi.org/10.3390/ijms23147904
   IF: 5.6

2. Lakatos, C., **Kordován, M. Á.**, Czifrák, K., Nagy, L., Vadkerti, B., Daróczi, L., Zsuga, M., Kéki, S.: Synthesis of Sucrose-HDI Cooligomers: new Polyols for Novel Polyurethane Networks.
   Int. J. Mol. Sci. 23 (3), 1-13, 2022. ISSN: 1661-6596.
   DOI: http://dx.doi.org/10.3390/ijms23031444
   IF: 5.6

3. Czifrák, K., Lakatos, C., **Kordován, M. Á.**, Nagy, L., Daróczi, L., Zsuga, M., Kéki, S.: Block Copolymers of Poly(omega-Pentadecalactone) in Segmented Polyurethanes: Novel Biodegradable Shape Memory Polyurethanes.
   Polymers. 12 (9), 1-17, 2020. EISSN: 2073-4360.
   DOI: http://dx.doi.org/10.3390/polym12091928
   IF: 4.329

Hungarian abstracts (3)

4. **Kordován, M. Á.**, Hegedűs, C., Kálmán-Szabó, I., Czifrák, K., Lakatos, C., Zsuga, M., Kéki, S.: Biokompatibilis építőelemeket tartalmazó poliuretánok előállítása és biológiai vizsgálata = Production and biological testing of polyurethanes containing biocompatible building blocks.
   In: XXVII. Nemzetközi Vegyészkonferencia = 27th International Conference on Chemistry.
   Ed.: Majdik Kornélia, Erdélyi Magyar Műszaki Tudományos Társaság (EMT), online [Kolozsvár], 1, 2021, (ISSN 2734-7109)

5. Lakatos, C., **Kordován, M. Á.**, Czifrák, K., Nagy, L., Vadkerti, B., Zsuga, M., Kéki, S.: Új típusú szacharóz-hexametilén-diizocianát (HDI) oligomer tartalmú poliuretánok előállítása és vizsgálata = Synthesis and characterization of new types of polyurethanes containing sucrose-hexamethylene diisocyanate (HDI) oligomer.
In: XXVII. Nemzetközi Vegyészkonferencia = 27th International Conference on Chemistry. Ed.: Majdik Kornélia, Erdélyi Magyar Műszaki Tudományos Társaság (EMT), Kolozsvár, 1, 2021, (ISSN 2734-7109)

6. **Kordován, M. Á.**, Nagy, L., Nagy, M., Vadkerti, B., Daróczi, L., Deák, G., Zsuga, M., Kéki, S.: Új típusú poliuretánok előállítása szacharóz, 1,6-hexametilén diizocianát (HDI) és (epszilon)-kaprolakton felhasználásával = Preparation of New Type Polyurethanes Containing Sucrose,1,6-hexamethylene Diisocyanate (HMDI) and epsilon-Caprolacton.
In: 25th International Conference on Chemistry = XXV. Nemzetközi Vegyészkonferencia. Szerk.: Majdik Kornélia, Erdélyi Magyar Műszaki Tudományos Társaság (EMT), Kolozsvár, 55, 2019, (ISSN 1843-6293)

Foreign language abstracts (1)

7. Lakatos, C., **Kordován, M. Á.**, Czifrák, K., Nagy, L., Vadkerti, B., Daróczi, L., Zsuga, M., Kéki, S.: Monitoring and characterization of sucrose-HDI cooligomer formation by MALDI-TOF mass spectrometry.
In: International Mass Spectrometry Conference 2022 : Poster Abstract book, [S. n.], Maastricht, 49-50, 2022.

List of other publications

Hungarian abstracts (2)

8. Kuki, Á., Nagy, T., Nagy, L., Deák, G., **Kordován, M. Á.**, Róth, G., Zsuga, M., Kéki, S.: Új irányok a környezetbarát fém-levegő akkumulátorok fejlesztésében = Development of novel environmentally friendly metal-air batteries.
In: XXVIII. Nemzetközi Vegyészkonferencia = 28th International Conference on Chemistry. Szerk.: Majdik Kornélia, Erdélyi Magyar Műszaki Tudományos Társaság (EMT), Kolozsvár, 30, 2022, (ISSN 2734-7109)

9. **Kordován, M. Á.**, Nagy, L., Vadkerti, B., Batta, G., Fehér, P. P., Zsuga, M., Kéki, S.: A biokompatibilis poliuretán alapú polimerek térhálósítójaként használt szacharóz reaktivitásának vizsgálata fenil-izocianát jelenlétében.
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Foreign language abstracts (2)

10. Czifrák, K., Lakatos, C., **Kordován, M. Á.**, Nagy, L., Daróczi, L., Zsuga, M., Kéki, S.: Application of MALDI-TOF MS for the structural identification of new polyol building blocks for polyurethanes.
In: IMSC 2022 : Poster Abstract book

11. **Kordován, M. Á.**, Nagy, T., Nagy, L., Kuki, Á., Erdélyi, Z., Baradács, E., Deák, G., Zsuga, M., Kéki, S.: Környezetbarát akkumulátorok fejlesztése 3D nyomtatással támogatva = Development of environmentally friendly secondary battery supported by 3D printing technology.
In: XXVIII. Nemzetközi Vegyészkonferencia = 28th International Conference on Chemistry. Szerk.: Majdik Kornélia, Erdélyi Magyar Műszaki Tudományos Társaság (EMT), Kolozsvár, 29, 2022.

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