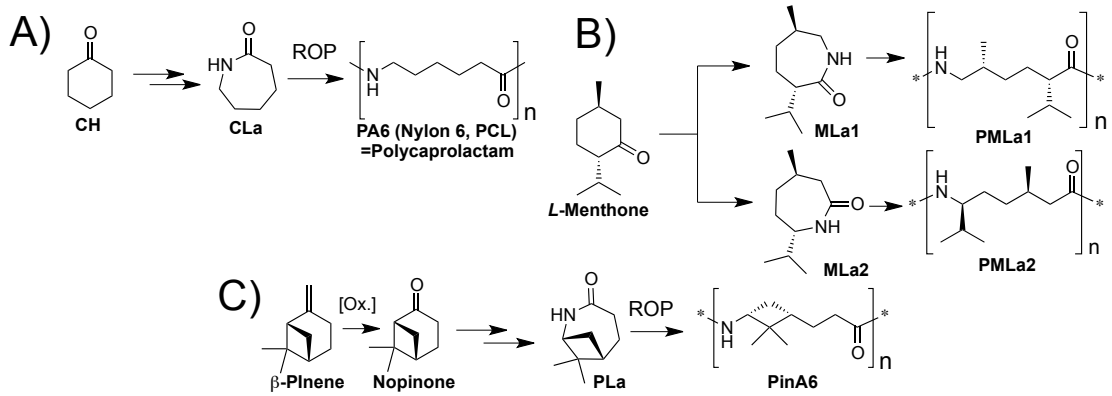
Sustainable Polyesteramides and Copolyamides Based on Substituted ε-Lactams and ε-Lactones for Possible Applications as Biomaterials

Dr. Malte Winnacker

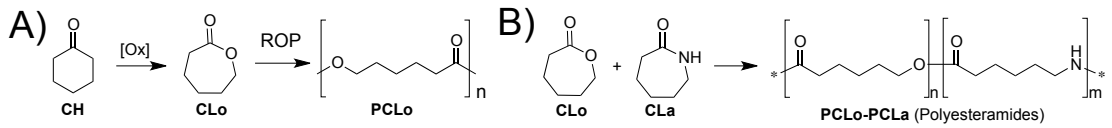
State of the art

Polyamides are very important polymers for a wide range of applications.[1] After establishing in the 1930´s with Nylon 66 and Nylon 6 (Perlon), their impact has been continuously growing. This relies on their versatility and their excellent thermal and mechanical properties, which result from e.g. amide groups and hydrogen bonding between chains. While Nylon 66 is made via polycondensation of adipic acid and hexanediamine, Nylon 6 is made via ring-opening polymerization (ROP) of ε-caprolactam (**CLa**, Scheme 1A). In the context of utilizing renewable and also structurally significant feedstock for polymer synthesis, many biobased polyamides have been developed derived from e.g. vegetable oils, carbohydrates or terpenes. An example for the latter are PAs from *L*-menthone or β-pinene, which have been investigated in our group, and which are obtained via the corresponding lactams, in analogy to the Nylon 6 synthesis (Scheme 1B,C). In addition to sustainability, interesting structural features (side groups and stereocenters) are thus introduced into the polymers, resulting in ordered microstructures and interesting properties.[2]



Scheme 1. A) Polyamide 6 (**PA6**, Nylon 6); B) and C) Terpene-based PAs.

Polyesters have also a great importance and find applications as mass plastics, but also in the biomedicine field due to their good mechanical properties, biodegradability and biocompatibility. Among them, polycaprolactone (**PCLo**), synthesized also via ROP (in this case of ε-caprolactone), is one of the most important PEs (Scheme 2A). Polyesteramides can combine the biodegradability and biocompatibility of polyesters with the excellent thermal and mechanical properties of polyamides and have thus attracted much attention. In addition to e.g. many amino-acid based PEAs, also the copolymerization of lactones and lactams (e.g. **CLo** and **CLa**) to random or block copolymers has been investigated (Scheme 2B).[3]

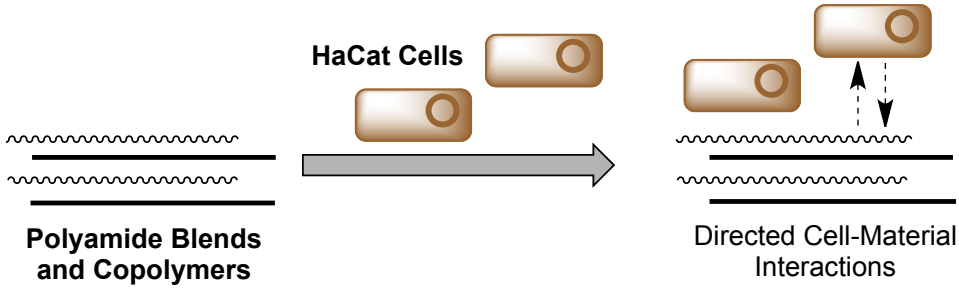


Scheme 2. A) Synthesis of polycaprolactone (**PCLo**); B) Polyesteramides via copolymerization of **CLo** and **CLa**.

Biopolymers are defined as polymers that are biobased, biogenic or biodegradable. The utilization of renewable building blocks for the synthesis of sustainable polymers has been gaining strong impact within the past decades for two main reasons: it enables independency from fossil oil, and it provides accesses to new structures that cannot be obtained so easily via fossil-based pathways.[2c]

Biomaterials are defined as materials with usage in medicine for therapeutic, diagnostic or regenerative functions, and polymeric materials find many applications in drug delivery, tissue engineering or as implants.[4,5,6] Though this definition is thus independent from the term ´Biopolymers´, there is a large overlap, and many biopolymers find applications also as biomaterials (e.g. polylactide for sutures, or nanocellulose as matrix for 3D cell culture). It is known that many material properties (nanotopography, stiffness, molecular flexibility, chemical functionality, degradability (and resulting byproducts), cell adhesivity and binding affinity) are very important for cell-material interactions and can influence cell behavior (adhesion, proliferation, clustering, …), e.g. by mimicking the biological extracellular matrix.[3,4]

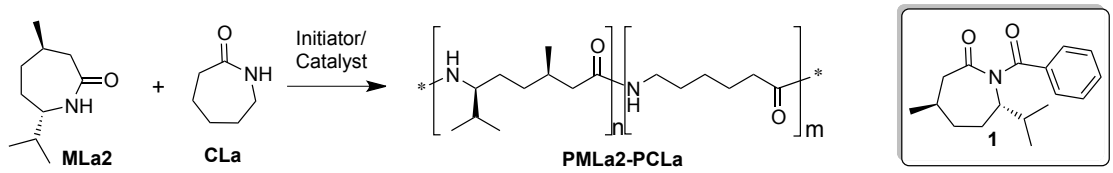
In this whole context, we investigated in this study the preparation and analysis of new terpene-based polyamide-polyester copolymers, copolyamides, polyamide-PEG, and their blends, as well as their properties. Furthermore, we explain preliminary biological results with some of these polymers - and other polymers for comparison - with regards to their interactions with cells: we prepared polymer surfaces and investigate their suitability for cell adhesion, viability and proliferation. For this, we focus on HaCaT cells (human keratinocytes) and fibroblasts with regards to possible applications in regenerative skin replacement and tissue engineering (Scheme 3).



Scheme 3. Polyamide blends and copolymers for cell-materials interactions. © 2019 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. Adapted with permission.[6]

Co-polyamides and Polyesteramides: Synthesis and Analysis

Aim of this study was the preparation of new copolymers of several lactams and/or lactones by means of different catalysts and initiators, as well as some applications. Among those, NaH was found to work out best for lactam copolymerization. The copolymerization of terpene-based lactams with ε-caprolactam (**CLa**) was performed in sealed vials and resulted in a variety of very interesting copolyamides (Scheme 4).



Scheme 4. Copolymerization of the terpene-based lactam **MLa2** with **CLa**.

As expected, the lactams show different reactivity. Different polymerization series were performed to evaluate various conditions. When using vials that are not pre-heated and without acylated co-initiator (**1**), barely polymers where formed, while the application of co-initiator yielded oligomers in small amounts (argon, NaH 60% in oil). As expected, the best results were obtained under argon (adding the compound in glovebox) with preheated vials, pure/washed NaH and with co-initiator **1**. Table 1 summarizes some of the most representative results of these copolymerizations. First, polymer yield was only determined roughly from GPC data. Later, selected samples were purified and further analyzed. Homopolymerizations were also performed for comparison (Table 1; only shown in brief here for reasons of space).

Table 1: (Co)polymerization of **MLa2** and **CLa**: selected results.

Entry	Monomers CLa:MLa2	Initiators (eq.)	Conditions	M_n/M_w [a)]	Yield % (polymer)[b)]	Remarks
1	Only **MLa2**	NaH (0.1)	4h / 250 °C	Mainly monomers	2-3	c)
2	1:1	NaH (0.1), **1** (0.1)	4h / 250 °C	2262/3248	~ 6	c)
3	1:1	NaH (0.1), **1** (0.1)	4h / 200 °C	2730/8570	>75	d)
4	1:1	NaH (0.1), **1** (0.1)	4h /150 °C	Mainly monomers	1-2	c)
5	1:1	NaH (0.1), **1** (0.1)	4h / 250 °C	1200/4420	>75	d)
6	1:1	NaH (0.1), **1** (0.1)	4h / 225 °C	2800/9110	>50	d)
7	1:1	NaH (0.05), **1** (0.05)	4h / 225 °C	4470/14050	>55	d)
8	1:2	NaH (0.05), **1** (0.05)	1h / 225 °C	1270/3120	>55	d)
9	1:2	NaH (0.05), **1** (0.05)	4h / 225 °C	1630/3380	>65	d)
10	1:2	NaH (0.05), **1** (0.05)	14h /225 °C	1670/4360	>45	d)
11	1:2	NaH (0.05), **1** (0.05)	24h /225 °C	1690/4550	>65	d)
12	Only **MLa2**	NaH (0.1), 1 (0.1)	4h / 250 °C	1630/2020	>25	d)

a) Determined by GPC/SEC, first exact, then up-rounded values; b) estimated from raw-GPC; c) Vials not pre-heated, but argon atmosphere during reaction; d) pre-heated vials, weighting in glovebox.

For these copolyamides, analysis was performed e.g. by means of gel permeation chromatography (GPC) and nuclear magnetic resonance (NMR, for selected purified samples) as exemplarily shown in Figure 1.

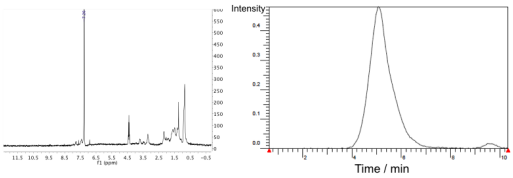
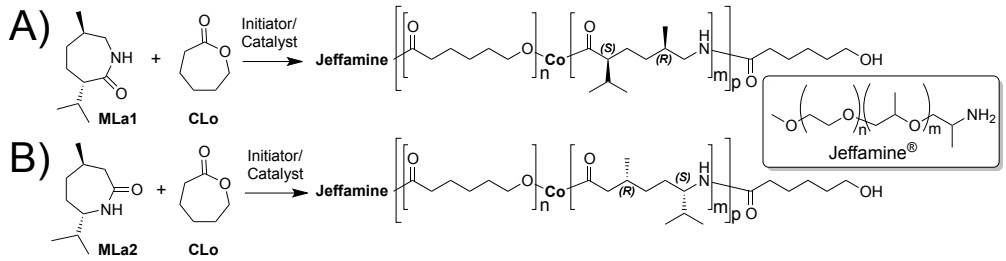


Figure 1. ^{1}H-NMR spectrum (left) and GPC elugram (right) of a copolymer **PMLa2-PCLa**.

For the synthesis of polyesteramides, Jeffamine® was used as initiator to start the ring-opening of the cyclic monomers. Reactions were performed with menthone-based regioisomers **MLa1** and **MLa2** (easier available) and **CLo** (Scheme 5).



Scheme 5. Copolymerizations of terpene-based lactams **MLa1** and **MLa2** with **CLo**.

Many conditions were tested, and copolymers with good MW were obtained, purified and analyzed. Many reactions were performed with the easier available **MLa2** (Table 2). Copolymerizations of **CLa** and **CLo** (Scheme 2B) and homopolymerizations were also performed for comparison (not shown here for space reasons).

Table 2. Copolymerization (random) of **MLa2** and **CLo**: selected results. a) Determined from NMR.

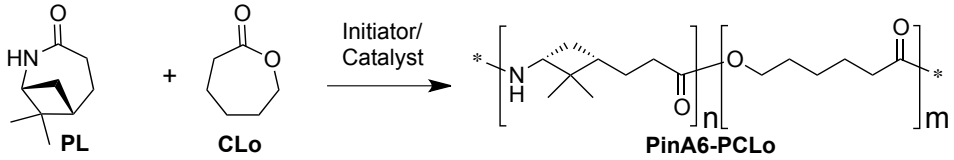
	Initiator	Conditions	M_n/M_w x10^3	PDI	Yield (%)	PE/PA[a)]
1	$SnOct_2$	4h, 250 °C	4.5/9.3	2.1	15	3:2
2	$SnOct_2$	4h, 150 °C	6.3/9.6	1.5	89	n.d.
3	$SnOct_2$	4h, 200 °C	7.8/9.6 (+x)	1.2	99	n.d.
4	H_3PO_2	4h, 250 °C	2.3/5.4	2.3	52	15:4
5	H_3PO_2	7h, 250 °C	6.8/8.8	1.3	75	7:2

For **MLa1**, H_3PO_2 was found to be the most effective catalyst (Table 3).

Table 3. Copolymerizations of **MLa1** and **CLo**: selected results. a) Calculated from ^{1}H-NMR spectra.

	Initiator	Conditions	M_n/M_w x10^3	PDI	Yield (%)	PE/PA[a)]
1	$SnOct_2$	4h, 250 °C	6.2/8.0	1.3	23	n.d.
2	$SnOct_2$	4h, 150 °C	5.5/9.6	1.8	99	n.d.
3	H_3PO_2	4h, 250 °C	7.7/10.0	1.3	35	5:2
4	H_3PO_2	1h, 250 °C	7.5/9.8	1.3	34	15:2

As it turned out in previous homo-polymerization studies that the pinene-based lactam **PL** polymerizes easier than **MLa**, we focused more detailed on this lactam for the copolymerization with **CLo** (Scheme 6).



Scheme 6. Copolymerization of **PL** and **CLo** (Jeffamine® structure not shown here).

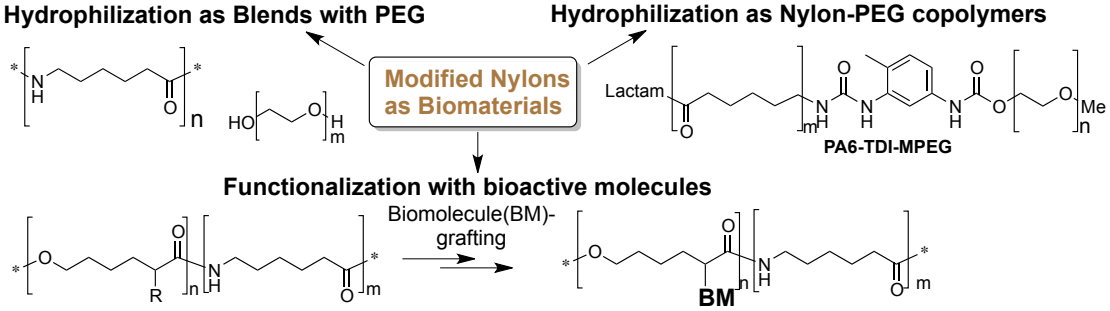
We focused on $SnOct_2$ as the catalyst, which afforded the best results. Some experiments were also performed with n-$Bu_2SnLaurate_2$ and with H_3PO_2, and different rations and conditions were applied and compared (Table 4; only selected results are shown for space reasons). Homopolymers and block-copolymers were also synthesized. Some of these (co)polymers were also shown to have defined glass transition temperatures (T_g; between -71.5 and +79.1 °C).

Table 4. Copolymerization (random) of **PL** and **CLo** by means of the catalyst/initiator system $SnOct_2$/Jeffamine (J) or H_3PO_2: selected and preliminary results; a) also other times, temperatures etc. were tested; b) as calculated from ^{1}H-NMR-spectra; preliminary results; c) exact, later rounded values.

	Catalysts (mol%)	PL:CLo (%)	[M]/[J]	Conditions[a)]	M_w/M_n	Yield (%)	PA/PE (PL/CLo)[b)]
1	$SnOct_2$ (0.25)	70:30	25	24h, 250 °C	3949/1833[c)]	38	57/43
2	$SnOct_2$ (0.25)	70-30	50	24h, 250 °C	3180/1130	54	65/35
3	$SnOct_2$ (0.5)	70-30	25	24h, 250 °C	4250/1870	42	56/44
4	$SnOct_2$ (0.5)	70-30	50	24h, 250 °C	3610/1620	57	59/41
5	$SnOct_2$ (0.25)	50-50	25	24h, 250 °C	3500/1700	44	t.b.d.
6	$SnOct_2$ (0.5)	50-50	25	24h, 250 °C	5020/1670	44	19/81
7	$SnOct_2$ (0.25)	30-70	25	24h, 250 °C	4600/1920	34	35/65
8	$SnOct_2$ (0.5)	30-70	25	24h, 250 °C	5820/2180	65	14/86
9	$SnOct_2$ (0.5)	only **CLo**	25	24h, 250°C	7480/2550	95	homopolymer
10	$nBu_2SnLaurate_2$	50-50	0.5	24h, 250 °C	5030/1900	51	19/81
11	H_3PO_2 (0.25)	50-50	10	1h, 250 °C	10030/7840	53	13:2
12	H_3PO_2 (0.25)	50-50	10	2h, 250 °C	15520/10500	64	24:5
13	H_3PO_2 (0.25)	50-50	10	8h, 250 °C	16500/11200	99	12:5
14	H_3PO_2 (0.25)	50-50	10	8h, 200 °C	9340/7200	99	6:1

Polyamide/PEG Blends, PA-PEG Copolymers and further polymers

The further modification of suchlike polymers by the introduction of hydrophilic PEG (PEGylation) or additional functionalities is important for (bio)medical applications and was investigated (Scheme 7). Many blends and copolymers with PEG were prepared with **PinA6** or Nylon 6. The copolymerization of **CLa** with chloro-substituted lactones was also performed. Due to the excellent suitability of the other (above mentioned) copolymers as biomaterials for the desired purposes and the difficulty to copolymerize substituted lactones at high temperatures, this copolymerization was so far only investigated in some initial experiments (status September 2019).



Scheme 7. Modification strategies for polyamides. R = Cl, N_3, …; **BM** = Biomolecule.

Polymers were purified by washing or precipitation and analysed by NMR and GPC.

Cell Adhesion and Viability Tests on the polymers and blend surfaces

Selected polymer blends in different ratios, as well as copolymers, were solvent-casted into well plates. Then, cells were seeded onto them, incubated for a certain time and then investigated via e.g. light microscopy and viability assays (e.g. MTT, a special dye that indicates cell viability). For instance, hydrophilized **PA/PEG** surfaces show better adhesion than on **PA6** alone, with regards to elongated cell morphologies and cell clustering (Figure 2).

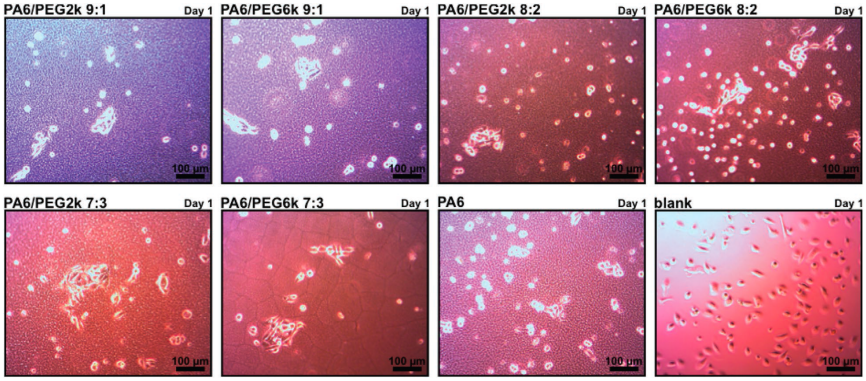


Figure 2. Attachment of HaCat cells (keratinocytes) on different PA/PEG blends.

Cell attachment on different **PinA6/PEG** blends is also very good and can be regulated via surface composition and roughness (Figure 3). The relation between cell movement and clustering and surface properties has also been investigated.

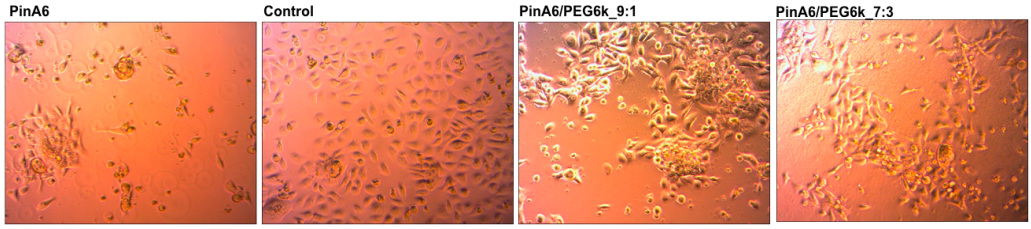


Figure 3. Cell attachment of HaCat cells on different **PinA6/PEG** surfaces (blends).

Conclusion and outlook

In this study, we have successfully prepared a series of novel biopolymers based on terpenes. Due to several prosperous properties, many of them can be applied also as biomaterials for directed cell-material interactions. For this, we have also performed several cell tests and showed the suitability of these biomaterials for the convenient regulation of cell adhesion, clustering and growth. Further suchlike studies are ongoing. Some of these results have already been published in reference 6, others are planned to be published soon.

Literature

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