Keynote speaker: Ring-Opening Metathesis Polymerization-Derived Materials for Separation Science, Heterogeneous Catalysis and Tissue Engineering

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Summary: The chemistry of ring-opening metathesis polymerization- (ROMP) derived monolithic materials is summarized. Since ROMP triggered by well-defined transition metal alkylidenes is a living polymerization method, it allows for the controlled and highly reproducible synthesis of monolithic media in terms of total porosity, pore size distribution and specific surface area. In addition, the high functionality tolerance of ROMP allows for creating monolithic supports with an unprecedented diversity in terms of functional groups that may be introduced. Selected applications in the areas of separation science, heterogeneous catalysis and tissue engineering are presented and discussed within the context of ROMP.

Keywords: catalysis; monoliths; ring-opening metathesis polymerization; separation techniques; tissue engineering

Introduction

Modern, state of the art materials chemistry requires designed, high-performance polymers. This also applies to polymeric supports with regard to particle size, particle shape, porosity, pore size distribution, specific surface area, functionality and capacity, respectively. This contribution intends to summarize recent developments in metathesis polymerization-derived monolithic supports and contributions of this polymerization technique to the areas of materials chemistry, separation science and tissue engineering, respectively.

The ‘‘classic’’ approach to functional polymeric supports and scaffolds entails the use of polymer analogous reactions on crosslinked and thus insoluble polymers. Consequently, the nature of the ‘‘working‘‘ functionality of the thus prepared stationary phases is sometimes still rather based on assumption than on real analysis. In order to avoid any multi-step derivatization reactions, the polymerization of *functional monomers* therefore appeared favorable. In this context, polymerizations techniques that are both living[1,2] and highly tolerant versus functional groups were of particular interest. Generally, such polymerizations techniques allow for the full control over both the molecular weight and architecture of the resulting polymers. In due consequence, this permits the stoichiometric and thus highly reproducible design of block-copolymers including cross-linked polymers. Furthermore, this facilitates correlating the polymers’ and materials’ structure with the resulting separation properties.

Results and Discussion

We commenced our research on ring-opening-metathesis polymerization (ROMP)-derived monolithic materials some ten years ago. At that time and long after, there were only very scattered activities in the synthesis

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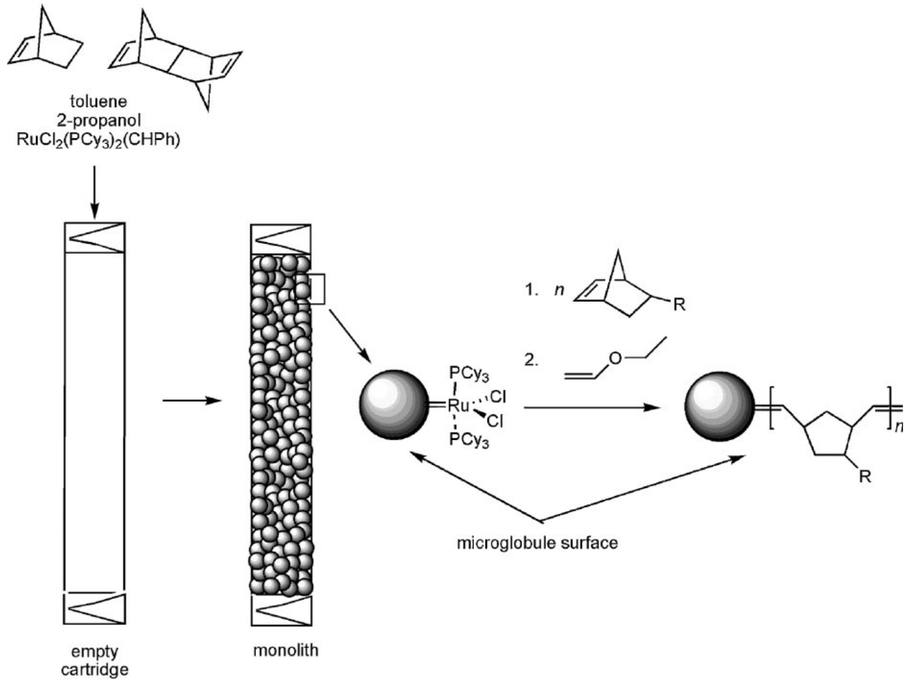
of monoliths based on living or controlled polymerization techniques.[3] In view of the impressive achievements made in ROMP at that time,[4,5] we decided to investigate the general applicability of this polymerization technique to the synthesis of monolithic supports. Using both Schrock and Grubbs-type initiators,[6] a broad range of functional monomers based on substituted norborn-2-enes and norbornadienes bearing anhydrides, esters or amides may well be polymerized in a truly living manner.

ROMP-Derived Monolithic Materials

Within the context of stationary phases or catalytic supports, the term ''monolith'' applies to any single-body structure containing interconnected repeating cells or channels, which is characterized by a defined porosity and which supports interactions/reactions between this solid and the surrounding liquid phase. Such supports are either prepared from inorganic mixtures, *e.g.* silanols, or from organic compounds, *e.g.* by a crosslinking polymerization. For a profound discussion of their history, their general properties and application refer to refs. [7–12].

The suitability of ROMP for the synthesis of monolithic supports was first demonstrated by the copolymerization of norborn-2-ene (NBE) with 1,4,5,8,8a-hexahydro-1,4,5,8-*exo,endo*-dimethanonaphthalene (DMNH6) in the presence of two porogenic solvents, 2-propanol and toluene, with the 1st-generation Grubbs initiator $RuCl_2(PCy_3)_2(CHPh)$.[13] Such a setup in fact allowed for the realization of the first ROMP-derived monolithic supports (Scheme 1). Later, this synthetic approach was extended to less strained cyclic olefins such as (functional) *cis*-cyclooctenes (COEs).[14,15] There, a more active initiator, i.e. a 3rd-generation Grubbs initiator ($RuCl_2(Py)_2(IMesH_2)(CHPh)$ ($IMesH_2$ = 1,3-dimesitylimidazolin-2-ylidene)) had to be employed in order to create the monolithic structures in the desired way. COE-based monoliths, however, show a significantly reduced propensity to become oxidized over time[15]. This is



Scheme 1.
Synthesis and functionalization of ROMP-derived monolithic supports.

related to the fact that poly(NBE)-based monoliths possess tertiary allylic carbons, while poly(COE)-based ones are based on secondary allylic carbons, which are less probe to oxidation.

By variation of the polymerization mixture in terms of monomer, crosslinker, porogen and initiator content, the volume fraction of the interglobular void volume (ε_z) as well as the total porosity ε_t were successfully varied within a range of 0–50% and 50–80%, respectively. The addition of small amounts of triphenylphosphine or pyridine in the low ppm range allowed for an additional tuning the polymerization kinetics.[13,14,16] Both NBE- and COE-based monoliths prepared by ROMP displayed linear plots of pressure vs. flow rate, which confirmed that the monoliths were not compressed even at high flow velocities up to 20 mm/s. Inductively-coupled optical emission spectroscopy (ICP-OES) measurements on totally dissolved samples of various monoliths revealed that the ruthenium introduced into the monolithic matrix in form of the initiator could in fact be totally removed by the use of appropriate capping agents, resulting in Ru-concentrations $<1\ \mu g/g$, which corresponds to a metal removal >99.9%.

Monolithic Supports in the Separation of Biomolecules

Non-polar, non-functionalized polymeric surfaces are widely used as stationary phases for both reversed phase high performance liquid chromatography (RP-HPLC) and ion-pair (IP)-RP-HPLC. While the former is the method of choice for high-resolution separations of peptides and proteins, the latter is eminently suited for the separation of single- and double-stranded nucleic acids.[17] Using ROMP-derived, NBE-based monoliths, the separation of oligothymidylic acids $(dT)_{12\text{-}18}$ ranging in mass from 3638 D (dT_{12}) to 5456 D (dT_{18}) was accomplished on a microscale within 5 minutes (Figure 1).[18]

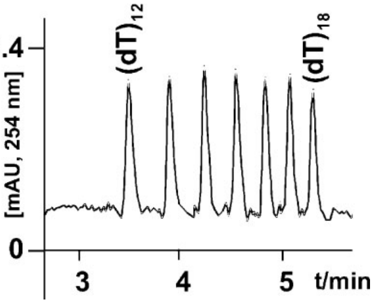


Figure 1.
Separation of oligonucleotides on a 60 x 0.20 mm i.d. NBE/DMN-H6-derived monolithic capillary column; mobile phase, (A) 0.1 M TEAA, pH 7.0, (B) 0.1 M TEAA, pH 7.0, 20% acetonitrile; linear gradient, 40–80% B in 10 min, flow rate = 9 μL/min, T = 20 °C; detection, UV (254 nm); sample: 2 ng of $(dT)_{12\text{-}18}$.

As expected, the elution order of oligodeoxynucleotides strongly correlated with their molecular mass, suggesting that an increase in molecular mass directly translates into an increase in hydrophobic interaction of the corresponding analyte with the monolith.

Similar results were obtained with COE-derived monolithic columns. There, reproducibility of synthesis was checked by determining the relative standard deviation (RSD) in retention times (t_R), which was found to be in the range of 2.9 to 3.9% for all analytes. The long-term stability of the COE-based monolithic columns was checked for a series of proteins after more than 1000 runs at 50 °C and revealed excellent stability of the columns, thus proofing the concept of replacing NBE- by COE-based monomers and crosslinkers.[15] No significant alteration in separation performance was observed, however retention times slightly decreased after 1200 injections (approx. 1.6-7.2% for all analytes). ROMP-derived capillary monoliths were also found applicable to separation problems common in medical research.[19] Thus, insulin and various insulin analogues used in diabetic treatment were analyzed. A comparison of the separation efficiency of a monolithic capillary column with a particle-based capillary column was performed. The monolithic column showed equivalent separation efficiency compared to Vydac C4 and Zorbax

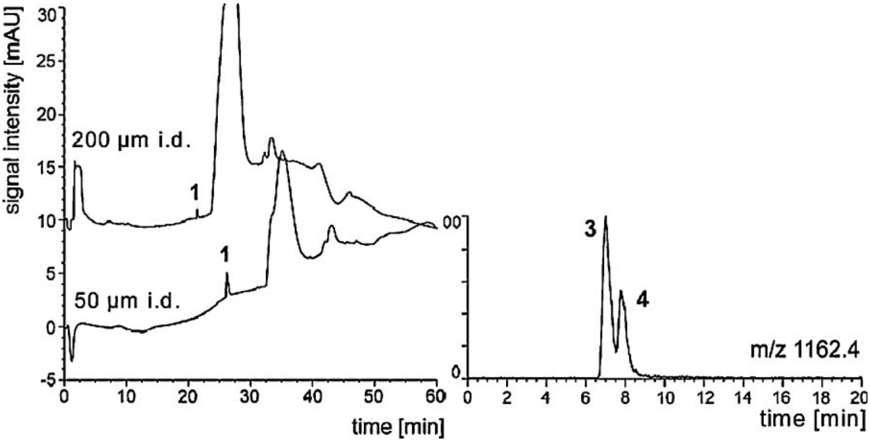


Figure 2.
Human insulin in interstitial fluid samples (100 fmol/l), injection volume 1 µl. Mobile phase: (A) 95% water, 5% acetonitrile, 0.05% TFA, (B) 20% water, 80% acetonitrile, 0.0 4% TFA; gradient: 0-30 min 0-60% B; 50-90% B within 5 min; flow (a) 1.5 µl/min, (b) 0.5 µl/min; T = 25°C; UV (190 nm).

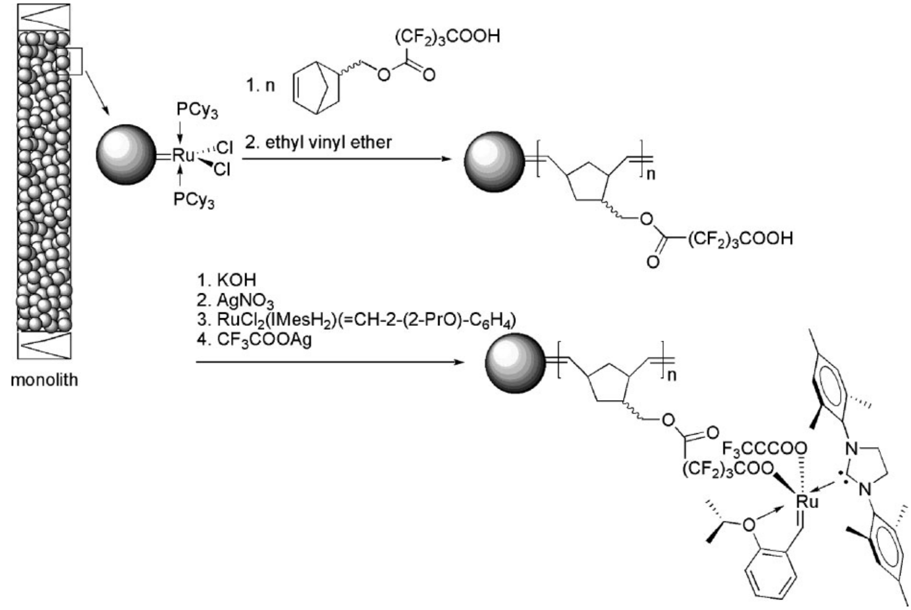
C3-based stationary phases. Moreover, the high permeability of monoliths enabled chromatographic separations at higher flow rates, thus shortening analysis times to about one third without any loss in separation efficiency. For the analysis of insulin in human biofluid samples, enhanced sensitivity was achieved by using a 50 µm i.d. ROMP-derived monolith (Figure 2). Coupling to mass spectrometry allowed for analyzing 0.1 atomol amounts of insulin. For purposes of completeness, it should be mentioned that ROMP-derived, NBE-based columns have also been successfully applied in voltage-assisted capillary LC.[20]

Functionalized ROMP-Derived Polymeric Monoliths

ROMP triggered with Schrock or Grubbs-type initiators is a living polymerization process. Using the active initiator covalently bound to the surface of the structure-forming microglobules after completed rod-formation, various functional monomers have been grafted onto the monolith surface by simply passing solutions thereof through the mold (Scheme 1). This way, linear polymer chains were attached to the inner surface of the monolith. This approach generally offers some advantages. First, the structure of the ''parent'' monolith is not affected by the functional monomer and can be optimized regardless of the functional monomer used later. Second, solvents other than the porogens, e.g. methanol, CH_2Cl_2, dimethylformamide, can be used for this ''*in situ*'' derivatization.[21] The versatility of this concept was demonstrated by grafting various functional NBE- or 7-oxanorbornene-based monomers, e.g. ester-, amine-, phenol-, ß-cyclodextrin- (ß-CD), imidazolium salt-, carboxylic acid-containing ones onto the monoliths' surface. Using a ß-CD-derivatized monolith, the chiral separation of proglumide was accomplished.[13]

Monolith-Immobilized Catalysts[22]

In order to overcome the problems commonly related to catalysts immobilized to beaded catalysts, we aimed on the synthesis of non-porous supports suitable for continuous flow experiments, e.g. monolithic supports. Using a ROMP-based protocol, we were able to synthesize functionalized monoliths.[13,16,23] These were designed in a way that only interparticle porosity was generated, whereas the structure-forming microclobules were virtually non-porous. Taking advantage of the ''living'' character

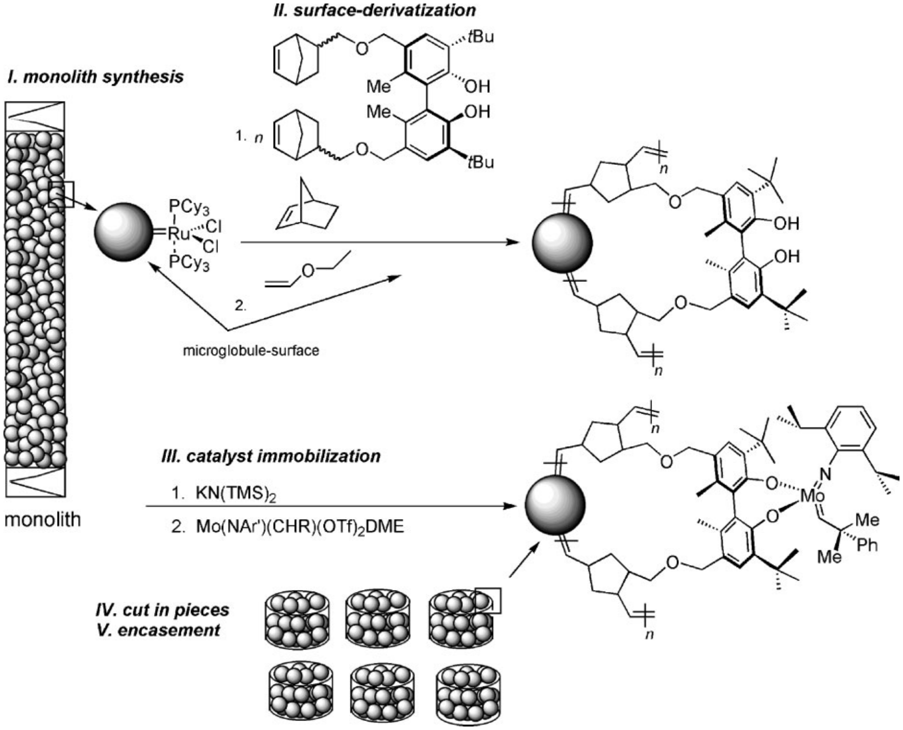


Scheme 2.
Synthesis of a monolith-bound 2^nd^-generation Grubbs-Hoveyda-type catalyst based on fluorocarboxylates.

of ruthenium-catalyzed ROMP used for their synthesis and the high tolerance of the catalytic system towards different functional monomers we were able to graft a norborn-2-ene-based perfluorinated acid onto the surface. Tentacle-like polymer chains attached to the surface were formed. Then the free acid was deprotonated and converted in the corresponding silver salt. Reaction of the thus modified support with $RuCl_2(IMesH_2)(=CH\text{-}2\text{-}(2\text{-}PrO)\text{-}C_6H_4)$ resulted in the formation of a supported metathesis catalyst.

Finally, any unreacted Cl-groups were reacted with CF_3COOAg to yield the corresponding bis-carboxylate substituted catalyst (Scheme 2).[24] This monolith-supported version of $Ru(CF_3COO)_2(IMesH_2)(=CH\text{-}2\text{-}(2\text{-}PrO)\text{-}C_6H_4)$[25] was used in continuous flow experiments.[26] A TOF_0 of $0.13\,s^{-1}$ was observed in the RCM of diethyl diallyl malonate, turn-over numbers (TONs) were >500. It is worth mentioning that both the turn-over frequencies (TOFs) and TONs obtained with this supported version are very comparable to the ones obtained with the homogeneous analogues. In order to immobilize a chiral metathesis catalyst on a monolithic support, a similar protocol as described above was applied. After rod formation was complete and unreacted monomer had been removed from the support, (R)-5,5′-bis-(norborn-5-ene-2-ylmethyleneoxymethyl)-3,3′-di-*t*butyl-6,6′-dimethyl-biphen-2,2′-diol[27] was grafted to the surface (Scheme 3).

In contrast to molybdenum-initiated polymerizations, no protection of the free phenolic groups was necessary.[28] The grafted phenolic moieties were then deprotonated by adding $KN(Si(CH_3)_3)_2$ and subsequently reacted with the catalyst precursor $Mo(N\text{-}2,6\text{-}i Pr_2\text{-}C_6H_3)(CHCMe_2Ph)(OTf)_2 \cdot DME$ ($OTf = triflate = CF_3SO_3^-$). Following this protocol, a loading of 0.7 wt.-% of the chiral Schrock catalyst on the monolithic support was achieved. The catalyst-loaded monolith was then cut in pieces 1 cm in height and encased in a way that polymer-encased membranes, which may directly be used as reaction vessels/filtration units in commercially



Scheme 3.
Immobilization of a chiral ligand on a monolithic support and formation of a chiral Schrock catalyst for use in high-throughput screening.

available machines for parallel synthesis and high-throughput screening (HTS), respectively, were obtained.

Then these systems were used for enantioselective ring-closing metathesis (RCM) using various prochiral substrates such as 3-allyloxy-2,4-dimethylpenta-1,4-diene, 2,4-dimethyl-3-(dimethylvinylsilyloxy)-penta-1,4-diene, 2,4-dimethyl-3-(dimethylvinylsilyloxy)-penta-1,4-diene, 3-allyloxy-3-phenylisobut-1-ene. Moreover, desymmetrization reactions with N-allyl-N-(2-methyl-4-phenylbut-1-en-4-yl)aniline and N-(isobut-1-en-3-yl)-N-(4-phenylbut-1-en-4-yl)aniline were carried out. Using 9-13 mol-% of supported catalyst, excellent yields up to 100% and high enantiomeric excess ($ee \leq 88\%$) were observed. Noteworthy, metal (Mo) leaching into the products was low ($\leq 2\%$ with respect to the amount of catalyst).

Monolithic Materials for Tissue Engineering[29,30]

In search of alternative scaffolds suitable for tissue engineering, we again focused on monolithic supports. The molding processes that are feasible thereby significantly reduce restrictions in shape.

By using a 20:20 wt.-% mixture of NBE and pentaglycerol bis(7-oxanorborn-5-ene-2-ylcarboxylate) acrylate (PGBA) in a microporogen (toluene) and a macroporogen (2-propanol, 5:10 wt.-% ratio), monolithic structures were realized with the aid of $RuCl_2(pyridine)_2(IMesH_2)(CHPh)$ (0.03 wt-%) and low amounts of additional pyridine as regulator.[29] Scheme 4 illustrates the basic polyreaction. In order to enhance the mechanical stability of the supports, nano-scaled inorganic particles, i.e. $CaCO_3$ and hydroxylapatite, were added. The Grubbs-type initiators used

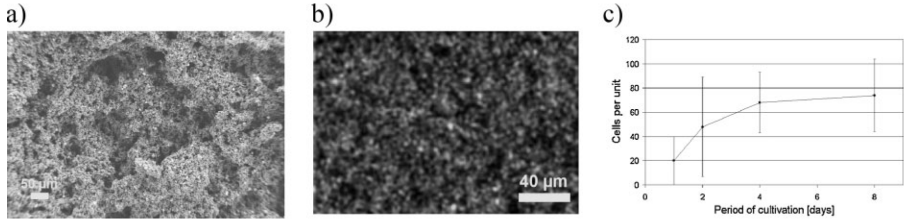
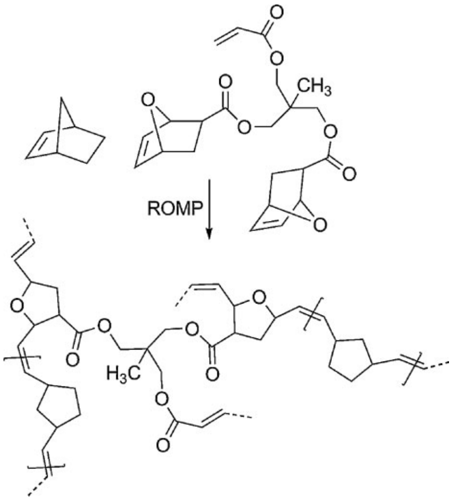


Figure 3.
a) Porous, ROMP-derived monolithic scaffolds, b) EDX mapping of Ca verifying the homogeneous distribution of the nanoparticles within the matrix in course of the rod-formation process, c) cell growth study.



Scheme 4.
Synthesis of 7-oxanorborn-2-ene-based, ROMP-derived monoliths.

did not only tolerate the presence of the inorganic nanoparticles, equally important particle loadings up to 12 wt.-% did not interfere with the phase separation process, allowing for the realization of monolithic nanocomposites. Figure 3a shows a typical structure of a nanoparticle-filled monolith, Figure 3b illustrates the homogeneous distribution of the inorganic filler. Preliminary studies of the cell growth are shown in Figure 3c.[31]

Conclusion

Originally designed for high-speed separations, monolithic supports have successfully entered other fields of chemistry and material science. In parallel, polymer chemistry itself helped to further broaden the spectrum of monolithic devices in terms of the chemistry of the supports, porosities as well as of functionalization. In this context, ring-opening metathesis polymerization-derived monolithic supports have gained strong positions. Particularly the living character and thus the high control over polymerization as well as the high tolerance versus functional groups and the ease of functionalizing the final monolithic supports make this polymerization technique highly attractive. The ongoing initiator developments will most certainly allow for extending the area of monoliths to other interesting applications.

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