

Project # 11-Puskas Start-Up
RESEARCH PROGRAM OF THE PUSKAS GROUP

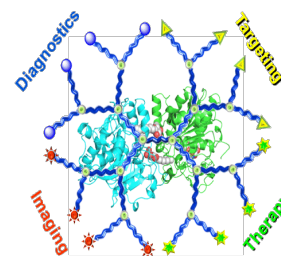
Funded by the Ohio State University



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| 1. Green Polymer Chemistry | Enzyme-catalyzed polymer functionalization
Green thiol-disulfide chemistry
Natural rubber biosynthesis
Biomimetic polymerizations |
| 2. Biorubbers | Polyisobutylene-based biomaterials
Surface modification and probing the polymer/bio Interface
Drug encapsulation and sustained release |

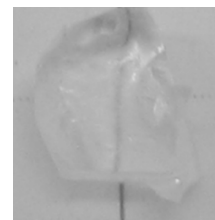
1. “Green” Polymer Chemistry. I was the first to introduce “green” polymer chemistry at the University of Akron. When I launched this platform, my students were very excited and new students applied, because young people are very interested in “green” technologies, wanting to save our environment for future generations. The program was transferred to OSU in 2019.

Enzyme-catalyzed polymer functionalization. We invented a new process for polymer functionalization using green chemistry. Specifically, we can functionalize polymers and build novel structures using enzymatic catalysis with high efficiency and region-selectivity. One example is PEG dendrimers for targeted drug delivery applications (Theranostics: diagnostic, imaging, targeting and therapeutics on the same molecule, see Figure 1a). We have been collaborating with MNIMBS and the Cleveland Clinic on folate-targeting, and recently invented a new method for exclusive gamma-conjugation of folic acid and similar drugs. The technology (US Patent 8,710,156, 2014) was licensed to my start-up company, Enzyme Catalyzed Polymers LLC (ECP). The technology was further developed in the framework of Phase I and Phase II NSF SBIRs. Three compounds were screened by the Cleveland Clinic – one showed outstanding uptake (over 80%) by triple-negative breast cancer cells. Animal studies are planned in the near future.



Dendrozyme

Green thiol-disulfide chemistry. Enzyme-catalyzed polymer functionalization was also used in my group to make biodegradable disulfide polymers and gels from dithiols (US Patents 8,552,143, 2013; 9,193,680, 2015). Polysulfidic polymers were first introduced in the patents of J.C. Patrick and N.M. Mnookin. Based on their research, Thiokol Corporation was founded and grew due to the unique adhesive and solvent resistant properties of the polymers. Today, the term ‘Thiokol’ is often used synonymously for polysulfide polymers. During the next decades, research into polysulfide polymers was dominated by the researchers at Thiokol, and the topic went seemingly unnoticed in academic investigation. Over thirty patents detailing polysulfides were awarded to Thiokol. Polysulfide chemistry experienced a rebirth recently, due to the biodegradability of the structure. The method of polysulfide polymer synthesis developed at Thiokol focuses on the reaction of α,ω -dihalogenated compounds with polysulfide salts.



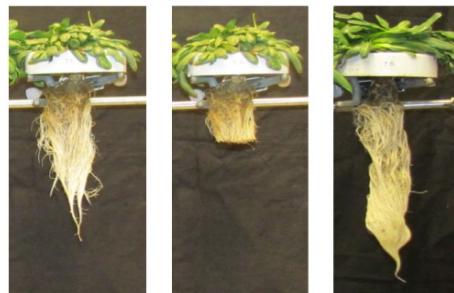
BioThioGel

Our method is “greener” (aqueous system, room temperature, recyclable catalyst) and yields high molecular weights other processes could not achieve. The polymerizations showed living character. These polymers and gels are very suitable for tissue engineering applications.

Natural rubber biosynthesis. NR, arguably the most important polymer produced by plants, is a strategically important raw material used in many thousands of products, including hundreds of medical devices. Based on the above comprehensive review and analysis of the chemical, polymer chemical and biochemical literature pertaining to the biosynthesis of NR, we developed a new mechanistic view of this process. A combination of two critical parameters, livingness and carbocationic intermediates, leads us to propose that the biosynthesis of NR proceeds by a natural living carbocationic polymerization mechanism. This new mechanism is a combination of chain- and step-growth, as explained in detail in my textbook (Introduction to Polymer Chemistry: a bio-based Approach). Understanding the mechanism led us to a new discovery: in recent field experiments in Brazil we have also shown that tapping the tree under nitrogen with a catalytic amount of IP increased the rubber yield by 20-50%!

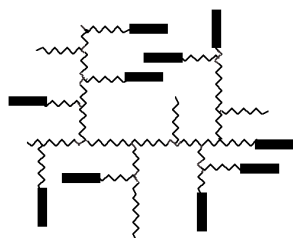
A potential new domestic crop is being developed by my friend Katrina Cornish (<https://cornishlab.cfaes.ohio-state.edu/>) the Buckeye Gold! Most promising is hydroponic cultivation – imagine that you cut the roots that contain the rubber, and it grows back to even larger root mass!

Biomimetic polymerizations. Based on natural rubber biosynthesis we studied a series of biomimetic polymerizations in the framework of an NSF-IUPAC international project – more details are given in relevant publications.

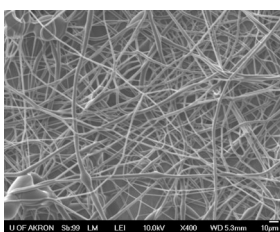


2. Biorubbers are rubbery materials that are well-tolerated by the human body. The most well-known biorubber is silicon rubber. I have been developing polyisobutylene-based biomaterials.

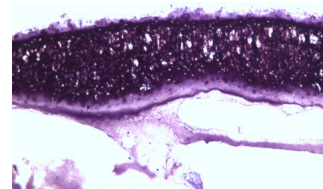
Polyisobutylene-based biomaterials. The first generation, linear triblock poly(styrene-b-isobutylene-b-styrene), SIBS for short, was developed at the University of Akron and first disclosed in 1990 (US patent 4946,899; J.P.Kennedy, G. Kaszas, J. E. Puskas, W. G. Hager). Boston Scientific Co. uses this polymer, under license from The University of Akron, to coat drug-eluting coronary stents. These stents revolutionized interventional cardiology; since their introduction more than one million have been implanted. We then developed the third generation with a branched polyisobutylene core capped with polystyrene or its derivatives/copolymers (J.E.Puskas *et al.*, US patent 6,747,098, 2004). The fourth generation, poly(alloocimene-b-isobutylene-b- alloocimene) (Allomatrix© for short)



Arbomatrix©



electrospun fiber mat



scaffold/chondrocytes after 7 weeks



SIBS and Allomatrix

received patent protection in 2017 (US Patent 9,790,301). Diblock and multiblock copolymers were also made. Alloocimene is a renewable monomer and its polymer blocks are biodegradable. These PIB-based block copolymers self-assemble at the nanoscale, so they are transparent, resembling silicone

rubber. In many applications they potentially can replace silicone rubber because they are biocompatible and biostable. In addition, they can be processed as plastics and can be electrospun to make fiber mats for tissue culture and other applications. We demonstrated excellent chondrocyte development on electrospun scaffolds.

Surface modification and probing the polymer/bio Interface. Using novel technologies of “modular” surface modification we were able to vary surface chemistry and topology independently, including “pegylation of the surface and the creation of superhydrophobic surface (water contact angle ~ 150) via electrospinning. We use homo- and block copolymers functionalized via enzymatic catalysis, invented in our lab. We have shown that fibrinogen adsorption can be reduced to 14 ng/cm², creating essentially non-fouling surfaces with this simple method. Together with the Cleveland Clinic we are probing the tissue reaction (polymer/bio interface) of our biomaterials *in vitro* and *in vivo*.

Drug encapsulation and sustained release Using electrospinning and electrospraying drugs were encapsulated into our biomaterials. The release profiles were controlled by polymer architecture and composition. Anti-inflammatory effects were demonstrated *in vitro*. *In vivo* experiments just started in a rat model.

The polyisobutylene-based biomaterials platform opens new avenues in medical device and tissue engineering development.