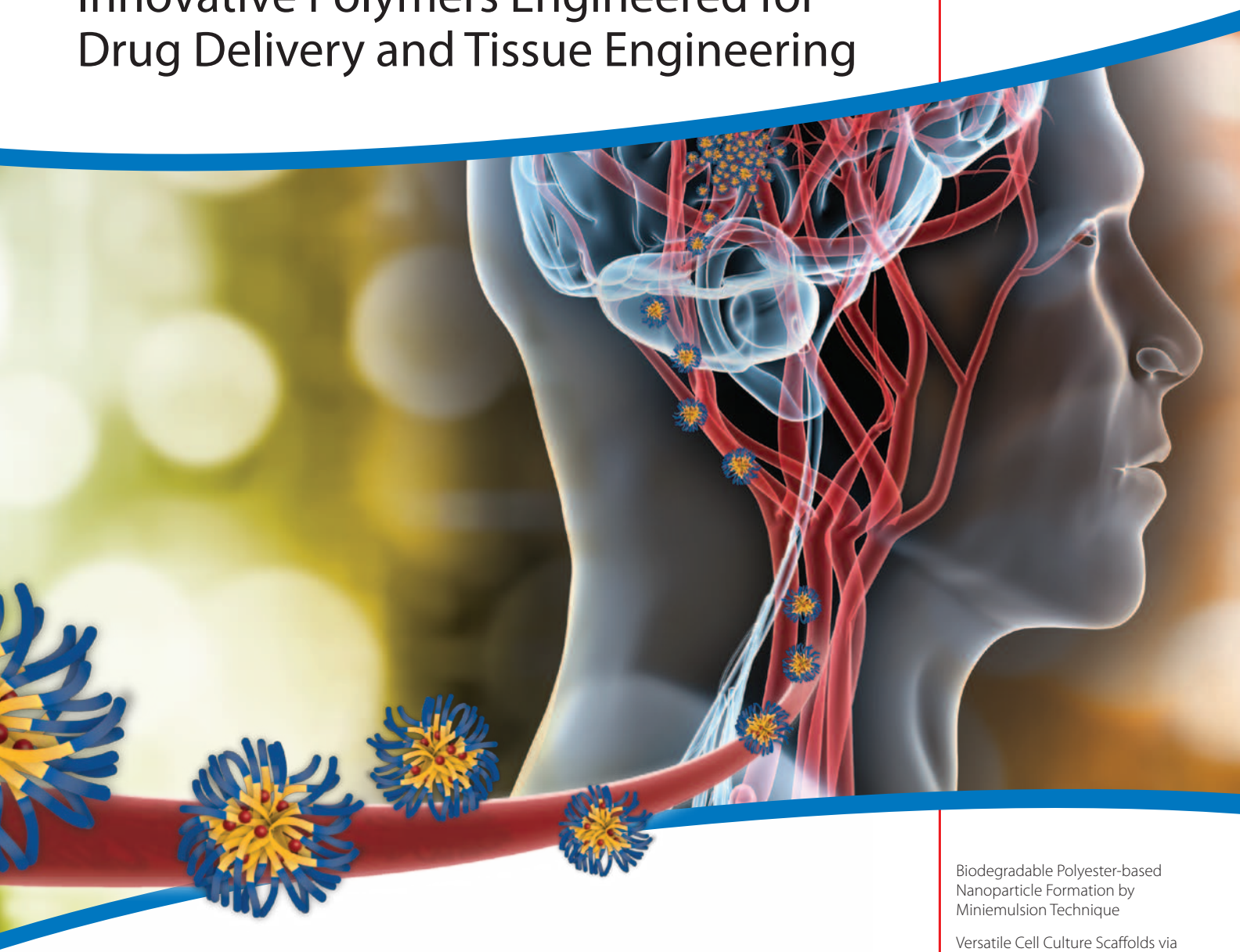
Material Matters™

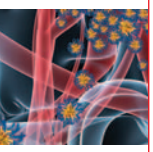
Volume 7, Number 3

ALDRICH® Materials Science

Innovative Polymers Engineered for Drug Delivery and Tissue Engineering



Targeting a better quality of life



Introduction

Sebastian Grajales, Ph.D.
Aldrich Materials Science
Sigma-Aldrich Co. LLC

Welcome to the third 2012 issue of ***Material Matters™***, focused on polymers designed for drug delivery and tissue engineering. This topic includes applications ranging from delivery of small organic molecules, biological materials, and biological signals, as well as hybrid biomaterial scaffolds. Given the vast nature of this topic and numerous significant advances in these fields, this issue aims to highlight a few key research areas focused on novel materials and techniques designed for specific targeted delivery abilities and tissue engineering needs.

In the first article, Professor Katharina Landfester (Max Planck Institute for Polymer Research, Germany) reviews biodegradable polyesters, such as polylactide, poly(lactide-*co*-glycolide), and polycaprolactone and details methods to prepare polymer nanoparticles for drug delivery. This article focuses on methods utilized to prepare polyester-based polymer nanoparticles and the degradation processes these types of particles undergo. In the case of drug delivery, both the selection of materials and nanoparticle formation technique control the incorporation of different payloads and influence the release rate.

In the second article, Professor Kristi Anseth (University of Colorado at Boulder, USA) describes a novel approach to create synthetic extracellular matrix mimics (ECM) via poly(ethylene glycol)-based scaffolds prepared through click reactions. Functionalized poly(ethylene glycol)s (PEGs) provide an opportunity to create an environment replicating the complex living tissue environment. Extracellular matrix signals found in living tissues can be mimicked by introducing capabilities using click reactions and sequential click reactions. There is extensive interest in hydrogels as cell delivery vehicles and in tissue engineering. These gels are useful models of tissue culture due to their ability to form in the presence of cells, proteins, and DNA.

In the third article, Professor Jianbiao Ma (affiliated with both Nankai University and Tianjin University of Technology, China) reviews chitosan-based biomaterials. Chitosan is derived from the second most abundant natural biopolymer, chitin, and is studied for many biomedical applications, including drug and gene delivery, tissue engineering, and hemodialysis membranes. Interest in utilizing chitosan in biomedical applications has grown rapidly due to its unique properties and ability to be functionalized forming derivatives tailored for specific utility.

In the last article, Professor Michael Malkoch from the KTH Royal Institute of Technology and Professor Andreas M. Nyström from the Karolinska Institutet, Sweden) reviews dendrimers and dendrons derived from bis-MPA (2,2-bis(methyloyl)propionic acid). Dendritic materials possess a well-defined distribution of functional groups, and of the many applications for dendrons and dendrimers this article focuses on drug delivery and surface modification. Several clickable groups are showcased to highlight the versatility and ease of application specified design.

Each article in this issue is accompanied by a list of polymers available from Aldrich Materials Science. Please contact us at ***matsci@sial.com*** if you need any material that you cannot find in our catalog, or would like a custom grade for your development work. We welcome your new product requests and suggestions as we continue to grow our polymer offering.

About Our Cover

Advances in drug delivery and tissue engineering are leading to paradigm shifts in medicine. Innovation in these fields is enabled by polymeric biomaterial research occurring at the interface between disciplines of polymer chemistry, materials science, biomedical engineering, surface science, biophysics, and biology. The cover art of this issue illustrates one of these new advances in targeted drug delivery, where a surface functionalized polymer micelle containing a therapeutic agent is being delivered to a tumor target within the body.

Material Matters™

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Sigma-Aldrich Co. LLC
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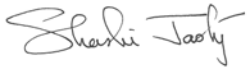

Shashi G. Jasty, Ph.D.
Director, Aldrich Materials Science

We welcome fresh product ideas from you. Do you have a material or compound you wish to see in our Aldrich® Materials Science line? If it is needed to accelerate your research, it matters. Send your suggestion to ***matsci@sial.com*** *for consideration.*

Professor William Murphy at the University of Wisconsin-Madison kindly suggested that we offer a heterobifunctional PEG containing both thiol and carboxylic acid functionality with a number average molecular weight (M_n) of 3,500 g/mole (**Aldrich Prod. No. 757837**). This functionalized PEG can be used in several applications such as bioconjugation, drug delivery, PEG hydrogel crosslinker, and general surface functionalization. These types of materials have been explored as self-assembled monolayers (SAMs) and further developed as an important tool for eliciting stem cell-material interactions.[1-2] The thiol group on one terminus of the PEG is bound to gold substrate surfaces and the pendant carboxylic acid group is later used to conjugate cell adhesion peptides to create cell adhesive environments for stem cells.[1-2] Moreover, the thiol functionality can be utilized for a wide range of applications using thiol-ene click chemistry.[3-6]

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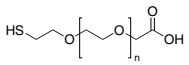
(6) Campos, Luis M.; Killops, Kato L.; Sakai, Ryosuke; Paulusse, Jos M. J.; Damiron, Denis; Drockenmuller, Eric; Messmore, Benjamin W.; Hawker, Craig J. *Macromolecules*, **2008**, *41*(19), 7063-7070.

Poly(ethylene glycol) 2-mercaptoethyl ether acetic acid

thiol-PEG-carboxylate; mercaptopoly(ethylene glycol) carboxylic acid

[165729-81-7] $HSC_2H_4O(C_2H_4O)_nCH_2CO_2H$



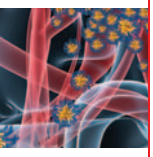
Reported applications include: bioconjugation, drug delivery, PEG hydrogel, crosslinker, and surface functionalization.

▶ **average M_n 3,500**

solid

PEG average M_n 3,400 (n~77)

757837-500MG	500 mg



Biodegradable Polyester-based Nanoparticle Formation by Miniemulsion Technique

Anna Musyanovych and Katharina Landfester
Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany
Email: landfest@mpip-mainz.mpg.de

Introduction

Aliphatic polyesters such as polylactide, poly(lactide-*co*-glycolide) and polycaprolactone, as well as their copolymers, represent a diverse family of synthetic biodegradable polymers that have been widely explored for medical uses and are commercially available.[1] These polyesters have excellent tissue compatibility and safety profile, both of which are critical for their successful utility in the human body. Moreover, these polymers have been approved by the United States Food and Drug Administration (FDA) and European Medicine Agency (EMA) for many biomedical applications such as drug delivery devices, sutures and implants. Biodegradable polymer nanoparticles have been extensively explored for delivery of both hydrophobic and hydrophilic drugs to improve bioavailability, bioactivity and control delivery. This article explores the broad range of polyester-based biodegradable polymers utilized in drug delivery and describes their physiochemical properties, methods utilized to form polymer nanoparticles, and the degradation processes these materials undergo in biological environments.

Biodegradable Polyesters

Polylactide is a thermoplastic biodegradable polyester derived from renewable resources. The properties of polylactide are dictated by its enantiomeric form. Lactic acid (or lactide) monomer is a chiral molecule with two possible enantiomers, D and L, and therefore, "polylactide" exists in the following forms: poly(L-lactide) (PLLA), poly(D-lactide) (PDLA), and the mixture of both, poly(D,L-lactide) (PDLLA). Polylactides with PLLA content >90% tend to be crystalline; whereas PDLLA is an amorphous polymer due to the random positions of its two isomeric monomers within the polymeric chain. Semicrystalline polylactide is generally preferred to the amorphous form when higher mechanical properties are required (tensile strength of 50-70 MPa). Polylactides have a glass transition temperature (T_g) and melting temperature (T_m) in the range of 50-65 °C and 175-180 °C, respectively, with higher L-lactide content contributing to increased transition temperature values.

Poly(lactide-*co*-glycolide) (PLGA) is a random copolymer with physical and mechanical properties that can be easily tuned by altering the lactide to glycolide ratio. The T_g of PLGA copolymers is reported to be above the physiological temperature (>37 °C) and hence glassy in nature. PLGAs comprised of 25-75% lactide are amorphous and hydrolytically unstable, thus they are very promising candidates for drug delivery applications. Furthermore, PLGA has been used in tissue engineering due to its excellent cell adhesion and proliferation properties. A recent, comprehensive review by Danhier et al. explores PLGA-based nanoparticles for biomedical applications.[2] Modified PLGA nanoparticles are able to cross the blood-brain barrier and have a potential use in drug delivery to the central nervous system.

Poly(ε-caprolactone) (PCL) is a semicrystalline polymer with a low tensile strength (~23 MPa), low T_g (~-60 °C) and T_m (~55-60 °C). Under physiological conditions, PCL degrades slower than polylactide and, therefore, has numerous applications in tissue engineering as an elastic material for preparation of the long-term implantable devices.[3,4] PCL-based nanoparticles are also widely explored for utility in controlled drug delivery systems.[5]

Polymer Nanoparticle Formation Methods

Nanoparticles formulated from biodegradable polymers are of great interest for drug delivery purposes. A wide variety of hydrophilic and hydrophobic drug molecules such as DNA, proteins, etc., can be encapsulated by biodegradable polymers and delivered to specific organs or cells in nanoparticle form.[6] In the past few decades, several different preparation techniques have been published describing the formulation of polyester-based particles (or capsules). The choice of a particular approach mainly depends on the physiochemical properties of the polymer (i.e., solubility and molecular weight) and the incorporated biologically active material (i.e., hydrophobicity/hydrophilicity of the drug and its sensitivity to the solvent). Currently, the most popular methods are emulsion/solvent evaporation or diffusion, double emulsion, flash nanoprecipitation and the salting out procedure, all described below.

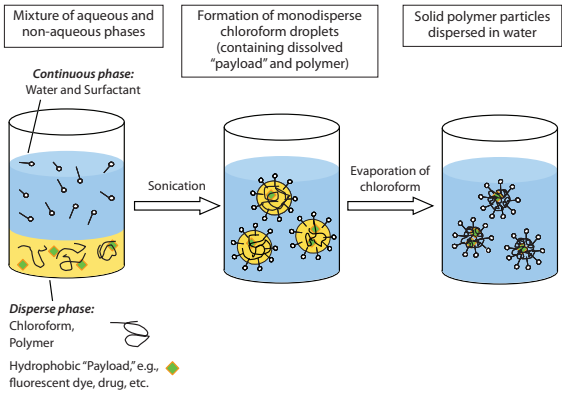
The *emulsion/solvent evaporation* method involves the preparation of an oil-in-water emulsion, where a small quantity of nonpolar organic solvent containing polymer and hydrophobic biological material (e.g., drug) (oil phase) is added to a polar solvent (water phase), containing a stabilizer.[7-9] The most common stabilizers are hydrophilic molecules such as poly(vinyl alcohol), polysorbates (TWEEN®), poly(acrylic acid), poloxamers (or Pluronic®) and sodium dodecyl sulfate. Stable nanoparticles are formed in the aqueous phase by organic solvent evaporation, under increased temperature or reduced pressure. In the *emulsion/diffusion* method, the oil phase consists of polymer dissolved in a partially water miscible solvent (e.g., ethyl acetate, propylene carbonate, etc.).[10,11] The addition of a certain volume of water to the oil-in-water emulsion induces a change in the equilibrium of the system and causes the partially water miscible solvent to diffuse from the droplets into the aqueous phase. This reduces the polymer's solubility and results in particle formation.

Another approach is the *double-emulsion,* water-in-oil-in-water, method.[12,13] The main benefit of the *double-emulsion* method is its ability to efficiently encapsulate hydrophilic drugs. In this approach, the drug, dissolved in water, is added to an organic solvent containing polymer, forming a water-in-oil emulsion. Then a small quantity of this initial emulsion is added to a second aqueous phase containing an emulsifier, such as PVA, to stabilize the particle. Polymer nanoparticles containing hydrophobic drugs are obtained by evaporation of the organic solvent. *Nanoprecipitation* (or solvent displacement) method, utilizes interfacial polymer deposition to form nanoparticles.[14] In this process, polymer and a lipophobic drug are dissolved in a water miscible solvent, semi-polar solvent such as acetone and this solution is added to an aqueous solution containing a stabilizer. Solvent diffusion gives rise to polymer precipitation on the interface between the aqueous phase and finely dispersed oil droplets, resulting in the formation of solid particles. Finally, the *salting out* method is an oil-in-water emulsion comprised of a primary aqueous phase containing stabilizer and a high concentration of salt (e.g., magnesium chloride hexahydrate) and polymer dissolved in a water miscible solvent, such as acetone or tetrahydrofuran.[15] Due to the

presence of a high concentration of salt, there is no diffusion of the solvent into the aqueous phase. Fast addition of a large amount of water to this oil-in-water emulsion reduces the ionic strength and leads to the migration of the water soluble organic solvent into the aqueous phase, inducing formation of solid particles. In this method, particles must be purified to remove residual salt and solvent prior to use. The resulting size of nanoparticles/capsules depends strongly on the preparation process, as well as on the molecular weight and hydrophilic/hydrophobic nature of the polymer used.

Combined Emulsion/Solvent Evaporation and Miniemulsion Techniques

By combining *emulsion/solvent evaporation* and *miniemulsion* techniques, it is possible to produce biodegradable nanoparticles with a controlled size and narrow size distribution.[16,17] The process consists of an emulsion comprised of water-immiscible organic phase and aqueous phase containing polymer and hydrophilic stabilizer. A *miniemulsion* is achieved using high shear rates to form monodisperse nanodroplets ~50-500 nm in diameter. Subsequently, the organic solvent is evaporated from the system causing the polymer to precipitate within the spherical nanoreactor forming size-controlled solid particles. The formation process of these well-defined polymer nanoparticles is illustrated in **Scheme 1**.



Scheme 1. Formation of biodegradable nanoparticles in the oil-in-water miniemulsion method.

A series of five different polymers were used to produce nanoparticles stabilized with the anionic sodium dodecyl sulfate (SDS) from **Scheme 1**, shown below in **Table 1**. The effect of molecular weight and polymer type used to prepare polymer nanoparticles on particle size and size distribution were studied. Particles were prepared using 72 mg of SDS and 0.3 g of polymer.

Table 1. Biodegradable polymers utilized to prepare nanoparticles with the corresponding molecular weight (M_w), abbreviation, and Aldrich product number.

Name	M_w (g/mol)	Abbrev.	Aldrich Prod. No.
Poly(L-lactide)	101,700	PLLA-1	93578
Poly(L-lactide)	67,400	PLLA-2	94829
Poly(ε-caprolactone)	115,000	PCL-1	440744
Poly(ε-caprolactone)	65,000	PCL-2	704105
Poly(D,L-lactide-*co*-glycolide) (RG 502)	15,000	PLGA	719889

The size of nanoparticles prepared using these polymers range from 76 to 165 nm in diameter. The smallest particles were obtained with PLGA and largest with PCL-2. In general, higher molecular weight results in ability to form larger particles by this technique, using SDS as a stabilizer (121 nm for PLLA-1 and 106 nm for PLLA-2). The morphology of the polymer nanoparticles was studied by scanning electron microscopy (SEM). All particles display spherical shape with narrow polydispersity. PLLA-1 particles are shown in **Figure 1**, as a representative example.

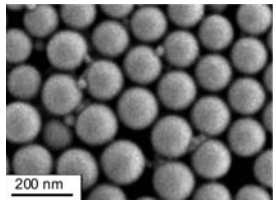


Figure 1. SEM image of PLLA-1 showing spherical shape and narrow polydispersity.

The loading efficiency of polymer particles was studied using a hydrophobic fluorescent dye marker (*N*-(2,6-diisopropylphenyl)-perylene-3,4-dicarbonacidimide) (PMI) as a model system. A series of polymer nanoparticles containing 0.5-2.98 mg of PMI per g of polymer were prepared. However, after purification of the particles the final concentration of encapsulated PMI was determined to be ~0.7 mg/g of polymer, regardless of the polymer used. Due to the presence of negative surface charges, these particles were employed as templates for the biomimetic mineralization of calcium phosphate.[18]

Nanoparticles Degradation Processes

Degradation of aliphatic polyesters follows one or more mechanisms including chemical hydrolysis, microbial, enzymatic, and thermal degradation.[19] Hydrolysis begins with water uptake, followed by hydrolytic cleavage of ester bonds. Hydrolysis in polymers can be influenced by various factors such as chemical structure (e.g., crystallinity and hydrophobicity), molecular weight and its distribution, purity, morphology, processing method, and storage conditions (e.g., temperature, pH, presence of salts, etc.).[20] Polycaprolactone degrades in at least two stages. In the first stage, non-enzymatic bulk hydrolysis of the ester bond occurs, which is autocatalyzed by carbon end groups of the polymeric chain. In this stage, PCL-devices maintain their shape and weight. The second stage starts when the PCL degrades to molecular weight fraction of ~5,000 g/mol. At this point, the rate of chain scission decreases and weight loss continues by diffusion of oligomers out of the polymer matrix. PLGA polymers are degraded into lactic and glycolic acids, which are eliminated from the body as carbon dioxide and water.

Degradation of particles PLLA-1, PLLA-2, PCL-1, PCL-2, and PLGA was estimated from the rate decrease in the weight average molecular weight (M_w). Taking into account the ultrasonication (US) step during the miniemulsion formation induces the polymer degradation, M_w of the final particles prepared without ultrasonication was also studied. Normalized values of the weight loss as a function of storage time are shown in **Figure 2**.

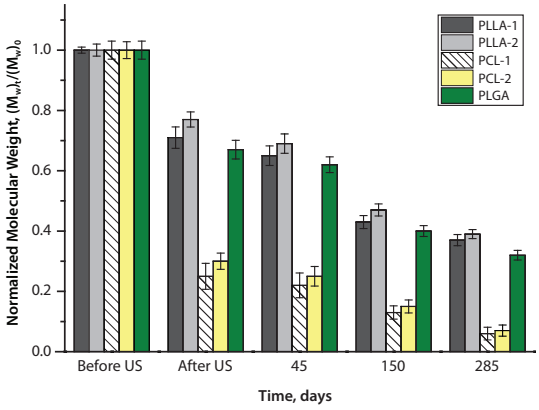
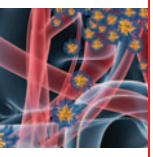


Figure 2. Degradation as a function of polymer type and molecular weight, measured before ultrasonication (US), immediately after US, then 45, 150, and 285 days after US.



All particles formed via ultrasonication showed a reduction in the polymer molecular weight, especially in the case of PCL where the molecular weight reduction was 70-75 wt. %. Polymers with high initial molecular weights (PLLA-1 and PCL-1) showed more degradation by ultrasonication than polymers with shorter chain length (PLLA-2 and PCL-2). The difference in weight loss is attributed to enhanced thermal degradation of polymers containing more chain entanglements induced by the high shear of the system during ultrasonication. During aging, molecular weight decreases rapidly as a result of random hydrolytic cleavage of ester bonds. The degradation rate of the amorphous PLGA particles was slightly faster compared to the other polymers, due to its higher permeability. Over a period of 12 months, the molecular weights of PLLA, PCL, and PLGA nanoparticles decreased by 51, 78, and 54 wt. %, respectively; whereas, particle size did not change significantly, even though molecular weight decreased.

Conclusions

Tremendous efforts have been put into research of biodegradable synthetic polyesters. Polyester-based nanoparticles remain one of the most versatile and promising class of biomaterials that can be processed to meet specific requirements in biomedical applications. Forming polymers into nanoparticles allows control over the types of drugs that can be encapsulated, such as hydrophilic drugs, hydrophobic drugs, vaccines, proteins, and nucleic acids.[2] Incorporation of different anticancer drugs, such as paclitaxel, cisplatin and hypericin in polyester-based nanoparticles (with a size range between 50 and 500 nm) strongly enhances their antitumor efficacy compared with free drugs. This enhancement is believed to be caused by the polymeric material efficiently protecting the encapsulated agent from enzymatic degradation. In addition to providing protection, nanoparticles can be used to deliver drugs at interfaces. For example, polyester-based nanoparticles can deliver growth factors to tissue engineered vascular grafts, and PLGA nanoparticle-coated stents can effectively deliver genes or drugs to vessel walls. Selection of an appropriate polymer composition and molecular weight affords the degradation kinetics and release profile of the encapsulated agent to be tuned over a broad range. Further studies on particle shape, formulation, and functionalization will lead to continuous developments in targeted delivery and theragnostics to ensure success in production of new and improved biomaterials.

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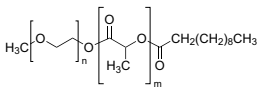
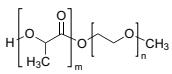
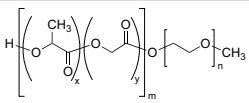
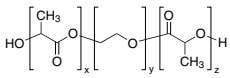
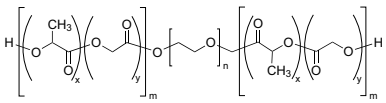
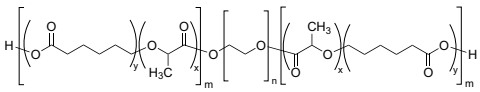
Biodegradable Copolymers

Well-defined Biodegradable Block Copolymers

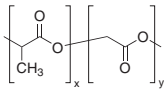
For a complete list of available biodegradable polymers, visit aldrich.com/biopoly.

Name	Structure	Molecular Weight	PDI	Degradation Time	Prod. No.
Poly(ethylene glycol) methyl ether-*block*-poly(D,L Lactide)-*block*-decane (PEG-PDLLA-decane)		PEG average M_n2,000 PLA average M_n2,000 average M_n 4,000 (total)	< 1.2	2-5 weeks	764736-1G
Poly(ethylene glycol) methyl ether-*block*-poly(D,L lactide) (PEG-PDLLA)		PEG average M_n2,000 PLA average M_n2,000 average M_n 4,000 (total)	≤ 1.4	2-4 weeks	764779-1G
Poly(ethylene glycol) methyl ether-*block*-poly(lactide-*co*-glycolide) (PEG-PLGA)		PEG average M_n5,000 PLGA average M_n10,000 average M_n 15,000 (total)	< 1.6	1-4 weeks	765139-1G
		PEG average M_n5,000 PLGA average M_n55,000 average M_n 60,000 (total)	< 1.2	1-4 weeks	764752-1G
		PEG average M_n2,000 PLGA average M_n15,000 average M_n 17,000 (total)	≤ 1.6	1-4 weeks	764760-1G
		PEG average M_n2,000 PLGA average M_n4,000 average M_n 6,000 (total)	< 1.4	1-4 weeks	764825-1G

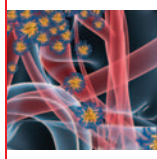
Name	Structure	Molecular Weight	PDI	Degradation Time	Prod. No.
Polylactide-*block*-poly(ethylene glycol)-*block*-polylactide (PLA-PEG-PLA)		PEG average M_n900 PLA average M_n1,500 average M_n 3,900 (total)	< 1.2	<12 months	659630-1G
		PEG average M_n10,000 PLA average M_n1,000 average M_n 12,000 (total)	< 1.2	<12 months	659649-1G
Poly(lactide-*co*–glycolide)-*block*-poly(ethylene glycol)-*block*-poly(lactide-*co*–glycolide) (PLGA-PEG-PLGA)		PEG average M_n1,000 PLGA average M_n1,500 average M_n 4,000 (total)	≤ 1.2	1-2 weeks	764787-1G
		PEG average M_n1,000 PLGA average M_n1,500 average M_n4,000 (total)	≤ 1.2	2-3 weeks	764817-1G
Poly(lactide-*co*–caprolactone)-*block*-poly(ethylene glycol)-*block*-poly(lactide-*co*–caprolactone) (PLCL-PEG-PLCL)		PEG average M_n5,000 PLCL average M_n6,000 average M_n 17,000 (total)	< 1.2	1-2 months	764833-1G

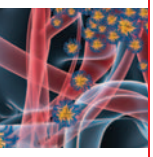
Poly(Lactide-*co*-Glycolide) Copolymers

For a complete list of available biodegradable polymers, visit aldrich.com/biopoly.



Synonym(s)	End Group	Lactide:Glycolide (x:y)	Molecular Weight	Degradation Time	Prod. No.
RESOMER® RG 502 H	acid terminated	50 : 50	M_w 7,000-17,000	<3 months	719897-1G 719897-5G
RESOMER® RG 503 H	acid terminated	50 : 50	M_w 24,000-38,000	<3 months	719870-1G 719870-5G
RESOMER® RG 504 H	acid terminated	50 : 50	M_w 38,000-54,000	<3 months	719900-1G 719900-5G
RESOMER® RG 653 H	acid terminated	65 : 35	M_w 24,000-38,000	<5 months	719862-1G 719862-5G
RESOMER® RG 752 H	acid terminated	75 : 25	M_w 4,000-15,000	<6 months	719919-1G 719919-5G
RESOMER® RG 502	ester terminated	50 : 50	M_w 7,000-17,000	<3 months	719889-1G 719889-5G
RESOMER® RG 503	ester terminated	50 : 50	M_w 24,000-38,000	<3 months	739952-1G 739952-5G
RESOMER® RG 504	ester terminated	50 : 50	M_w 38,000-54,000	<3 months	739944-1G 739944-5G
RESOMER® RG 505	ester terminated	50 : 50	M_w 54,000-69,000	<3 months	739960-1G 739960-5G
RESOMER® RG 756 S	ester terminated	75 : 25	M_w 76,000-115,000	<6 months	719927-1G 719927-5G
PLGA	ester terminated	85 : 15	M_w 50,000-75,000	<6 months	430471-1G 430471-5G
RESOMER® RG 858 S	ester terminated	85 : 15	M_w 190,000-240,000	<9 months	739979-1G 739979-5G
PLGA	acrylate terminated	50 : 50	M_n 25,000	<3 months	764841-1G
PLGA 5 arm star with a glucose core	hydroxyl terminated	50 : 50	M_n 30,000	2-5 weeks	764868-1G
PLGA 5 arm start with a glucose core	hydroxyl terminated	50 : 50	M_n 50,000	3-6 weeks	764876-1G





Biodegradable Homopolymers

Well-defined Biodegradable Polylactides

For a complete list of available biodegradable polymers, visit aldrich.com/biopoly.

Name	Molecular Weight (M_n)	PDI	Degradation Time	Prod. No.
Poly(L-lactide)	5,000	< 1.2	>3 years	764590-5G
	10,000	< 1.2	>3 years	765112-5G
	20,000	< 1.2	>3 years	764698-5G
Poly(D,L-lactide)	5,000	< 1.2	<6 months	764612-5G
	10,000	< 1.2	<6 months	764620-5G
	20,000	< 1.2	<6 months	767344-5G

Poly(L-Lactide)s

For a complete list of available biodegradable polymers, visit aldrich.com/biopoly.

Name	End Group	Molecular Weight	Degradation Time (years)	Prod. No.
RESOMER® L 206 S	ester terminated	-	>3	719854-5G 719854-25G
Poly(L-Lactic acid)	ester terminated	M_n 50,000 M_w 67,000	>3	94829-1G-F 94829-5G-F
	ester terminated	M_n 59,000 M_w 101,000	>3	93578-5G-F
	ester terminated	M_n 99,000 M_w 152,000	>3	81273-10G
	ester terminated	M_n 103,000 M_w 259,000	>3	95468-1G-F 95468-5G-F

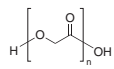
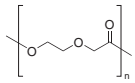
Poly(D,L-Lactide)s

For a complete list of available biodegradable polymers, visit aldrich.com/biopoly.

Name	End Group	Molecular Weight	Degradation Time (months)	Prod. No.
RESOMER® R 202 H	acid terminated	M_w 10,000-18,000	<6	719978-1G 719978-5G
RESOMER® R 203 H	acid terminated	M_w 18,000-24,000	<6	719943-1G 719943-5G
RESOMER® R 202 S	ester terminated	M_w 10,000-18,000	<6	719951-1G 719951-5G
RESOMER® R 203 S	ester terminated	M_w 18,000-28,000	<6	719935-1G 719935-5G

Other Biodegradable Homopolymers

For a complete list of available biodegradable polymers, visit aldrich.com/biopoly.

Name	Structure	Inherent Viscosity (dL/g)	Degradation Time (months)	Prod. No.
Polyglycolide, PGA		1.1-1.7 (0.1 % (w/v) in hexafluoroisopropanol at 25 °C)	6-12	457620-1G 457620-5G 457620-10G
Poly(dioxanone), RESOMER® X 206 S		1.5-2.2 (0.1 % (w/v) in hexafluoroisopropanol at 30 °C)	<6	719846-1G 719846-5G

Versatile Cell Culture Scaffolds via Bio-orthogonal Click Reactions



[1,2]Malar A. Azagarsamy, [1]Navakanth R. Gandavarapu, [1,2]Kristi S. Anseth*
[1]Department of Chemical and Biological Engineering and [2]the Howard Hughes Medical Institute, University of Colorado at Boulder, Boulder, CO 80303
Email: Kristi.Anseth@colorado.edu

Introduction

Devising biomaterial scaffolds that are capable of recapitulating critical aspects of the complex extracellular nature of living tissues in a three-dimensional (3D) fashion is a challenging requirement in the field of tissue engineering and regenerative medicine. Hydrogels are one class of materials that have been widely studied and developed as such synthetic extracellular matrix mimics (ECM). Hydrogels, due to their high water content, elasticity, and transport properties, are suitable for culturing cells in 2D and 3D environments, and simulate some of the critical mechanical aspects of soft tissue environments.[1-5]

From a basic perspective, hydrogels can be fabricated from a wide range of natural and synthetic precursors that crosslink via physical, ionic, or covalent interactions.[2] When cells are cultured in such systems, hydrogels formed from natural polymers provide a plethora of biological signals (e.g., cell adhesion proteins, depot of growth factors, proteolytically degradable domains), but often lack flexibility in controlling physicochemical properties of the final material in a reproducible manner. Alternatively, hydrogels formed from synthetic macromolecules allow precise engineering of critical properties, such as mechanics, swelling, and degradation, but typically use chemistries that are unrecognizable to cells. Thus, there is a growing interest to develop strategies to synthesize gels as cell delivery vehicles and culture systems that integrate the benefits of both synthetic and naturally derived materials. The perspective provided herein will discuss some of these concepts in the context of poly(ethylene glycol) (PEG)-based hydrogels inherently. PEG hydrogels have been widely used for 2D and 3D cell culture, and various strategies have been employed to control PEG gel properties and integrate cellular cues.[3-5] Of further significance is that PEG materials minimize non-specific protein interactions, which enable researchers to better understand how cells receive specific signals from their extracellular environment.

Chemistry and Molecular Design Principles for PEG Hydrogel Synthesis

PEG hydrogels have been formed using linear or branched/star polymeric chains that are either covalently or non-covalently crosslinked to each other, typically under aqueous conditions. Physically crosslinked block copolymers of PEG and poly(propylene oxide) (e.g., Pluronics) have enjoyed widespread use as *in situ* forming hydrogels with lower critical solution temperature (LCST) tuned around 37 °C;[6] however, these non-covalently assembled hydrogels have a limited range of mechanical properties. As an alternative, covalent crosslinking methods have become an indispensable tool for construction of highly stable PEG hydrogels.[2] To build covalently crosslinked networks for cell culture, one must also be mindful of the cytocompatibility of the crosslinking method. In this work, two polymerization mechanisms have been widely used: (i) chain growth and (ii) step-growth.[4,5] Chain polymerization of end-functionalized PEGs is robust and simple,[4] but results in a complex network structure that is structurally heterogeneous with a broad distribution of kinetic chain lengths and degradation that is limited to the network crosslink points. More recently, step-growth polymerization of PEGs has emerged as a facile method to achieve controlled and more uniformly crosslinked polymeric networks, while simultaneously providing a simple route to introduce biological signals. Typically for a PEG based step-growth hydrogel, a multi-functionalized molecular system is crosslinked using bi-functionalized molecules in a stoichiometric manner; in general, the average functionality must be greater than two to form a gel. Depending upon the required mesh size or crosslinking density, any one of these molecules or both can be derived from a PEG-based polymer (**Figure 1**). For hydrogels aimed at 3D cell culture and tissue regeneration, physiological conditions, and highly efficient, non-toxic chemical crosslinking methods are the critical requisites since gels often have to be formed in the presence of cells and proteins. These complexities have prompted bioengineers to explore a variety of chemical reactions for step-growth hydrogel formation that are mild and specific, but still proceed at an appropriate rate under physiological pH and temperature.

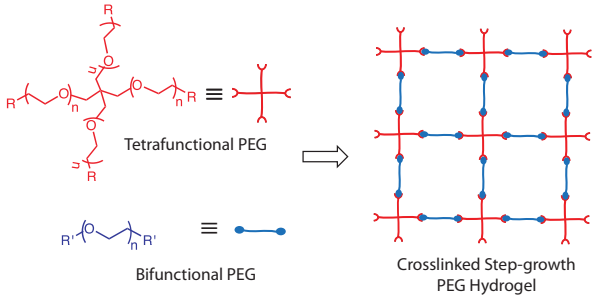
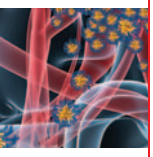


Figure 1. Structures of 4-arm and linear PEG precursors and a schematic illustration of the resulting step-growth hydrogel formed using these PEG macromolecules.

Click Reactions in the Development of Complex, Yet Well-defined Scaffolds

One of the recent chemical strategies that has brought significant advances to the field of biomaterials is 'click chemistry', introduced by Sharpless and co-workers to describe reactions that are rapid, highly selective, and high yielding in connecting two molecular components together under aqueous conditions.[7] While copper catalyzed azide-alkyne cycloaddition (CuAAC) (**Figure 2A**) was the first to be recognized as a click reaction, a variety of chemical reactions, including Michael additions (**Figure 2B**), photo-initiated thiol-ene reactions (**Figure 2C**), Staudinger ligation, and strain promoted azide-alkyne cycloaddition (SPAAC), have been later identified to fulfill the concept of click chemistry.[7-9] Since this group of reactions is not only orthogonal to each other, but also orthogonal to functional groups in biological systems, they have emerged as superior chemical tools for biomaterial scientists to construct complex material scaffolds that are well-controlled and highly defined.



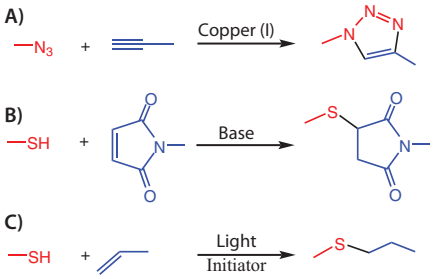


Figure 2. Popularly known click reactions: **A)** Copper catalyzed azide-alkyne cyclo-addition; **B)** Base catalyzed Michael addition reaction between thiol and maleimide; **C)** Photoinitiated thiol-ene coupling.

With the above introduction to PEG hydrogels and click chemistry, the remaining sections of this article will focus on: (i) the utilization of various click reactions in constructing PEG hydrogels for cell culture applications and (ii) how the orthogonality of click reactions has been exploited to precisely functionalize these materials with selective biological epitopes to direct cellular functions.

Michael Additions

Michael-type additions are attractive click reactions to form hydrogel materials in the presence of cells, owing to their mild reaction conditions and favorable reaction rates. This type of reaction proceeds due to the nucleophilic attack of thiolate anion (Michael donors) on electron deficient double bond of α, β-unsaturated ketones (Michael acceptors) as shown (**Figure 2B**). Vinyl sulfones, maleimides, and acrylates are widely used Michael acceptors to form hydrogels. Hubbell and co-workers[10,11] were one of the first to develop step-growth hydrogels using 4-arm PEG tetra vinyl sulfone and cysteine flanked peptides (**Figure 3**) for cell culturing applications. Since de-protonation of the thiol to the thiolate anion is critical for these reactions and requires addition of base, Hubbell and co-workers circumvented this issue by simply adjusting the pH of the buffer. This synthetic route allowed them to incorporate cysteine-containing peptides without requiring additional modification. By this route they demonstrated numerous peptide epitopes to control cell adhesion (e.g., Arg-Gly-Asp-Ser (RGDS), Ile-Lys-Val-Ala-Val (IKVAV)), as well as a plasmin sensitive peptide crosslinker that allowed degradation by cell-secreted proteases. Recently, Garcia and co-workers[12] reported maleimide functionalized multi-arm PEGs with faster reaction kinetics using very low concentrations of base to crosslink the di-thiol peptide crosslinkers, which provides additional benefits when encapsulating more sensitive cell types.

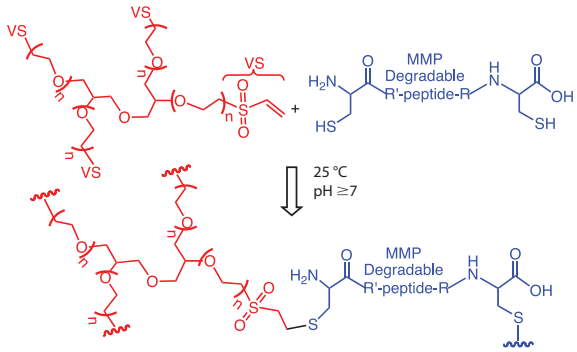


Figure 3. Step-growth hydrogel formation between 4-arm PEG tetra vinyl sulfone and cysteine end functionalized MPP cleavable peptide via Michael addition.

Thiol-ene Chemistry

Unlike Michael addition reactions (i.e., anionic), thiol-ene reactions proceed via a radical mechanism in which a thiyl radical can add over to either an electron deficient or electron rich carbon-carbon double bond (termed -ene).[8] A typical thiol-ene mechanism involves (**Figure 4A**): (i) abstraction of a hydrogen atom from a thiol by an initiating radical, (ii) the resultant thiyl radical adding over to an alkene functionality creating a carbon radical, and (iii) chain transfer of the carbon radical to another thiol, regenerating a thiyl radical which then propagates to a new alkene. While the thiyl radicals can be generated from thermal, photo, and redox initiators, photoinitiators are especially attractive for cell relevant applications, as cytocompatible photoinitiation conditions have been widely reported[8] and also allow the user to control the reaction spatially and temporally.

Our group first reported the synthesis of peptide-functionalized PEG hydrogels using photoinitiated thiol-ene chemistry.[13] In our approach (**Figure 4**), norbornene-functionalized multi-arm PEGs and cysteine containing chymotrypsin cleavable peptides were crosslinked to create PEG hydrogels, using lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) as a water-soluble photoinitiator and 365-420 nm light in PBS buffer under physiological pH conditions. Both gel forming conditions and the resultant hydrogel system supported high levels of cell viability (>95%). More importantly, because this chemistry is based on light, photopatterning of cell signals, such as the cell-adhesive RGDS, at specific locations of the gel was achieved (**Figure 4**), which ultimately dictated local cell interactions and morphology. This facile incorporation of any thiolated cell signaling moieties (e.g., thiolated proteins) on hydrogel scaffolds post cell encapsulation enables experimenters the possibility of understanding and directing cellular functions (e.g., differentiation) in a spatio-temporal fashion. In a similar line of inquiry, thiol-acrylate polymerizations have also been employed to incorporate thiolated affinity peptides that retain certain cell-survival proteins in the scaffold[14], as well as proteins that mimic cell-cell interactions,[15] thereby, integrating additional functional properties of the ECM.

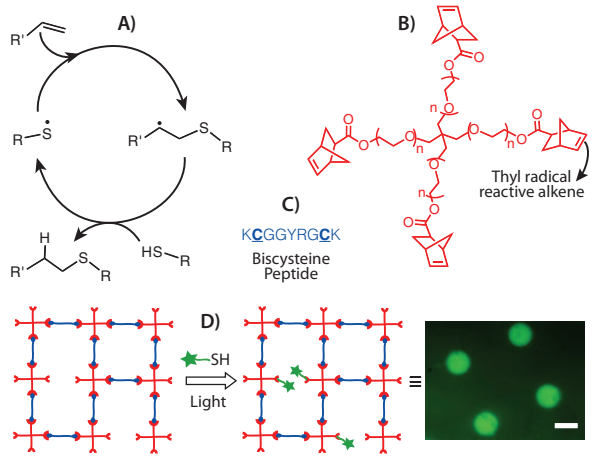


Figure 4. Thiol-ene photochemistry: **A)** General mechanism of the thiol-ene reaction; **B)** Structure of a commonly used ene, 4-arm PEG tetra norbornene; **C)** A cysteine containing chymotrypsin cleavable peptide crosslinker; **D)** Schematic of photopatterning networks formed via thiol-ene polymerization by reacting a network off-stoichiometry and subsequently conjugating a thiolated cell adhesive ligand at specific locations within the hydrogel scaffold; Image obtained from photopatterning of Alexafluor488 labeled RGD, scale bar: 500 µm.

Strain-promoted Azide-Alkyne Cycloaddition Reactions

Among various click reactions, copper (I) catalyzed azide-alkyne cycloaddition (CuAAC) to form 1,2,3-triazole (**Figure 2A**) has emerged as a widely utilized reaction for applications ranging from material to biomedical sciences, because azides, alkynes, and triazoles are largely inert to various reaction conditions and to most biological systems. While this click reaction gained popularity only after the introduction of a copper (I) catalyst into classical Huisgen cycloaddition, the toxic nature of this transition metal considerably restricted its utility in biological systems. Particularly for cell culture applications, CuAAC produced hydrogels simply remained as a prefabricated cell culture substrate rather than a cell encapsulation scaffold,[16,17] while being the icebreaker for the emergence of sequential click reactions (see *Sequential Click Reactions* section) techniques in hydrogel scaffolds.[18]

To overcome this limitation of CuAAC, Bertozzi and co-workers pioneered the development of strain-promoted azide-alkyne cyclo-addition (SPAAC) between cyclooctynes and azides (**Figure 5**) which proceeds without the need for copper catalyst, making it as an ideal bio-orthogonal reaction suitable for cell encapsulation.[9] Building from the concept of copper-free click chemistry, PEG hydrogels have been synthesized using 4-arm PEG tetraazide (**Figure 6A**) and di-cyclooctyne-terminated matrix metalloproteinase (MMP) degradable peptides.[19,20] Here, the gel is crosslinked using a degradable peptide to enable network degradation and remodeling, as dictated by cell-secreted MMPs. As one example, a gem-difluorocyclooctyne (DIFO)[9,19] was employed for its faster kinetics due to the presence of electron withdrawing fluorines, along with the characteristic ring strain (**Figure 5**). Gelation occurs in less than 5 minutes under physiological conditions in the presence of cells.

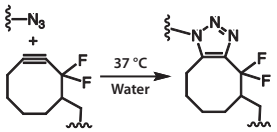


Figure 5. SPAAC between an azide and a difluorocyclooctyne eliminates the need for a copper catalyst and allows material fabrication under physiological conditions.

Sequential Click Reactions

In numerous biological applications, it is often desired to introduce functional cues at selected time points and/or in spatially defined regions. These signals may include survival cues (e.g., integrin-binding molecules), growth factors (e.g., affinity ligands to sequester cytokines), or cell-specific degradation sites (e.g., MMP-cleavable linkers to release signals or allow cell motility). This has led to the emergence of sequential click reactions: forming gels using one click chemistry and subsequently modifying it in a spatiotemporally controlled fashion using an additional click chemistry. Since thiol-ene chemistry is driven by light, it has become a powerful and versatile approach to achieve such well-defined and highly sophisticated incorporation of cues in PEG hydrogel cell niches.

Exploiting the orthogonality between SPAAC and thiol-ene click reactions: our research group has demonstrated the formation of PEG hydrogels via SPAAC (**Figure 6A**) followed by the creation of spatio and temporal photopatterns using the thiol-ene click reaction, in which case the peptide crosslinker carried a pendant allyl group (**Figure 6A**) to allow post-gelation photopatterning.[19] Cells encapsulated within this hydrogel matrix not only expressed >90% cell viability, but also responded to the patterned biochemical cues. For example, spatial control over cell morphology and adhesions were established upon patterning RGD peptide at specific locations of the gel (**Figure 6A**). Similarly, when a self-quenching diflourescein collagenase cleavable peptide, which shows enhanced fluorescence upon cleavage, was photopatterned, much greater fluorescence was observed at regions of high collagenase activity i.e., around the cell and allowed to visualize the local activity of cells in real time.

More recently, we achieved sequential patterning and depatterning of biologically relevant cues using orthogonal photocoupling and photo-cleavage reactions (**Figure 6B** and **6C**).[21] Spatio and temporal control over coupling and cleavage were executed using light of different wavelengths using a thiolated RGD peptide that contained a nitrobenzyl ether linker[21-23] to enable the photocleavage (**Figure 6D**) and subsequently allowed us to externally direct cell adhesion and detachment of human mesenchymal stem cell (hMSC) populations at specific locations within the hydrogel (**Figure 6B** and **6C**). Such dynamic control over biochemical cues on hydrogel scaffolds enables one to not only spatially control release of specific signals and cell types for cell delivery applications in regenerative medicine, but also provide opportunities to tune stem cell differentiations by controlling the presentation of specific and multiple cues in a spatio-temporal manner.

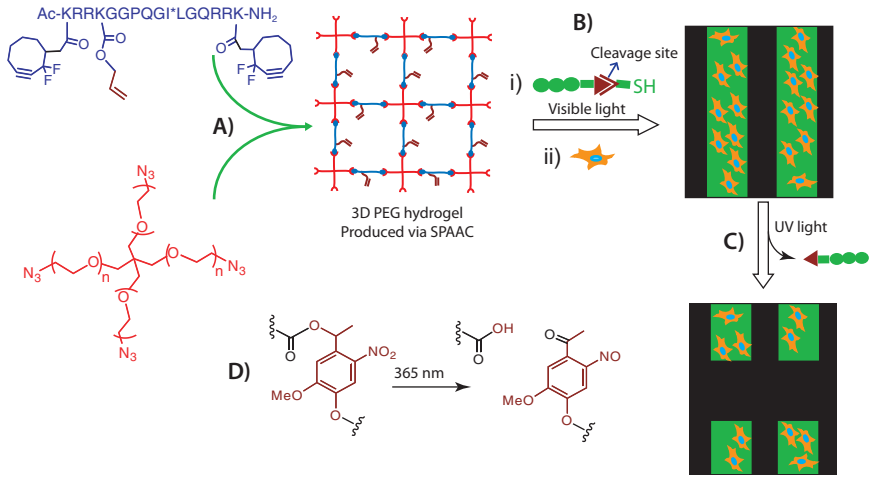
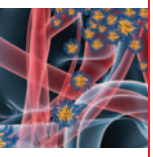


Figure 6. SPAAC Hydrogel Chemistry: **A)** 3D step-growth hydrogel formation via SPAAC click chemistry using 4-arm PEG tetra azide and dicyclooctyne MMP degradable peptide (* shows the cleavage site); **B)** Thiol-ene photopatterning on SPAAC produced hydrogel and subsequent cell adhesion; **C)** Spatial removal of patterned cues and resultant cell detachment; **D)** Nitrobenzyl ether photocleavage reaction.



Conclusion

Over the past decade, click chemistry has revolutionized the field of biomaterial scaffolds for cell culture and delivery because of its extreme convenience, versatility, and bio-orthogonal nature in building complex material scaffolds for regenerative medicine applications.[24] This repertoire of reactions not only enables the creation of a highly functional class of PEG hydrogels, but also opens up innovative pathways, such as sequential click reactions to incorporate biochemical signals at specific locations of the hydrogel scaffold in a temporally controlled manner. However, many innovations in materials development are yet to emerge from this chemistry rich field, especially in the area of biomaterial scaffold design. For example, while these elegant chemical approaches have been greatly utilized to fundamentally understand and control cellular properties, including morphology and adhesion in a 3D environment, their utilization to better understand spatio-temporal complex cues in a stem cell niche and direct more efficient differentiation of stem cells in a controlled manner *in vitro* remains a great opportunity and challenge for biological engineers.

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(3) Tibbitt, M. W.; Anseth, K. S. *Biotech. Bioeng.* **2009**, *103*, 655-663.
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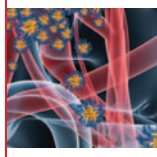
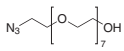
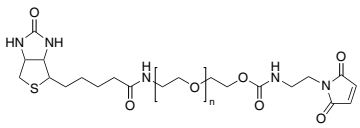
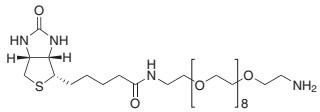
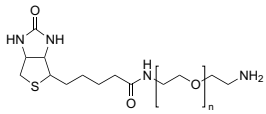
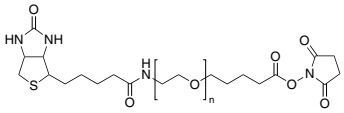
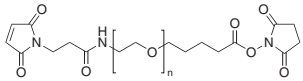
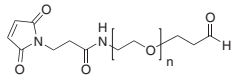
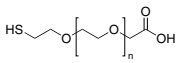
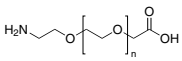
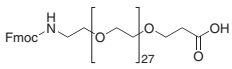
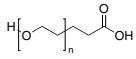
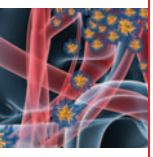


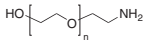
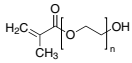
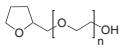
Poly(Ethylene Glycol)s

For your convenience, we list the polymers using the α and ω to refer to the terminal end groups of the polymer. For a complete list of available PEG materials, visit aldrich.com/PEG.

Heterobifunctional PEGs

α-end	ω-end	Structure	Molecular Weight	Prod. No.
Azide	OH		average M_n 400	689440-250MG
Biotin	Maleimide		PEG average M_n3,400 (n~77) average M_n 3,800	757713-100MG
			PEG average M_n5,000 (n~110) average M_n 5,400	757748-100MG
Biotin	NH_2		average M_n 700	689882-100MG
			PEG average M_n2,000 (n~45) average M_n 2,300	757756-100MG
			average M_n 3,700	757764-100MG
			PEG average M_n5,000 (n~110) average M_n 5,300	757772-100MG
			PEG average M_n3,400 (n ~ 77) average M_n 3,800	757799-100MG
			PEG average M_n5,000 (n~110) average M_n 5,400	757802-100MG
Maleimide	NHS ester		average M_n 4,000	757853-100MG
Maleimide	Formyl		average M_n 3,000	579319-250MG
COOH	SH		PEG average M_n1,000 (n~22) average M_n 1,100	757810-500MG
			PEG average M_n2,000 (n~45) average M_n 2,100	757829-500MG
			PEG average M_n3,400 (n~77) average M_n 3,500	757837-500MG
			average M_n 5,100	757845-500MG
COOH	NH_2		PEG average M_n1,000 (n~22) average M_n 1,100	757861-100MG
			PEG average M_n2,000 (n~45) average M_n 2,100	757888-100MG
			PEG average M_n3,400 (n~77) average M_n 3,500	757896-100MG
			average M_n 5,100	757918-100MG
			PEG average M_n10,000 (n ~230) average M_n 10,100	757705-100MG
COOH	Fmoc		average M_n 1,600	689653-100MG
COOH	OH		M_p 10,000	671037-100MG 671037-500MG
			M_p 3,000	670812-100MG 670812-500MG

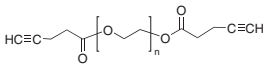
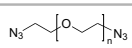
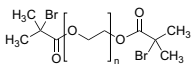
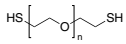
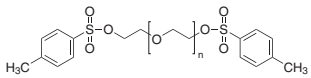
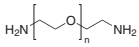
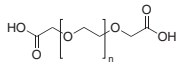
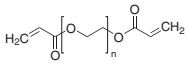
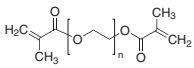
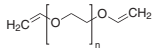
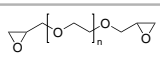


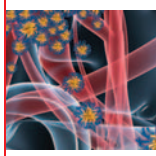
α-end	ω-end	Structure	Molecular Weight	Prod. No.
NH_2	OH		M_p 5,000	672130-100MG 672130-500MG
			M_p 10,000	671924-100MG 671924-500MG
Methacrylate	OH		average M_n 360	409537-5ML 409537-100ML 409537-500ML
			average M_n 500	409529-100ML 409529-500ML
Tetrahydrofurfuryl ether	OH		average M_n 200	309524-25G

PEG-core bis-MPA Dendrons

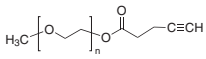
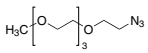
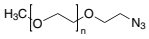
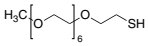
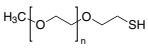
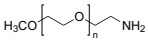
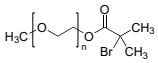
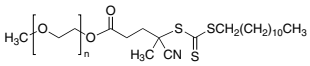
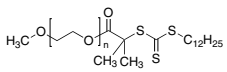
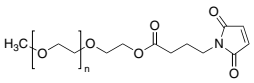
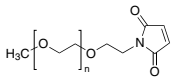
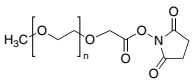
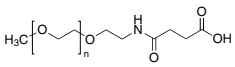
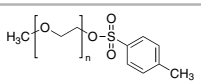
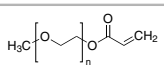
Please refer to the dendron table on page *24* for selected dendrons with a PEG core.

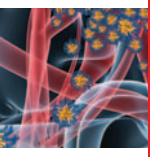
Homobifunctional PEGs

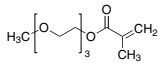
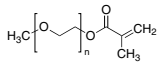
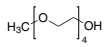
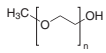
α- and ω-ends	Structure	Molecular Weight	Prod. No.
Acetylene		average M_n 2,000	699810-500MG
Azide		average M_n 10,000	767530-1G
2-Bromoisobutyrate		average M_n 700	767573-1G
SH		average M_n 1,000	717142-1G
		average M_n 3,400	704539-1G
		average M_n 8,000	705004-1G
Tosylate		average M_n 1,300	719080-5G
		average M_n 3,500	701750-5G
		average M_n 10,000	705047-5G
NH_2		M_w 2,000	14501-250MG 14501-1G
		M_w 3,000	14502-250MG 14502-1G
		M_w 20,000	14509-1G-F
COOH		average M_n 250	406996-100G
		average M_n 600	407038-250ML 407038-1L
Acrylate		average M_n 250	475629-100ML 475629-500ML
		average M_n 1,000	729086-1G
		average M_n 6,000	701963-1G
		average M_n 10,000	729094-1G
		average M_n 20,000	767549-1G
Methacrylate		average M_n 550	409510-250ML 409510-1L
		average M_n 6,000	687537-1G
		average M_n 10,000	725684-1G
		average M_n 20,000	725692-1G
Vinyl		average M_n 250	410195-5ML 410195-25ML
Glycidyl		average M_n 500	475696-100ML 475696-500ML



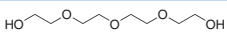
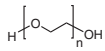
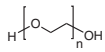
Monofunctional PEGs

ω-end	Structure	Molecular Weight	Prod. No.
Acetylene		average M_n 2,000	699802-500MG
Azide		average M_n 200	712590-100MG
		average M_n 400	767557-1ML
		PEG average M_n1,000	733407-1G
		PEG average M_n2,000	689807-250MG 689807-1G
		PEG average M_n5,000	689475-250MG 689475-1G
		PEG average M_n10,000	726168-250MG
		PEG average M_n20,000	726176-250MG
SH		PEG average M_n350	672572-250MG
		average M_n 1,000	729108-1G 729108-5G
		average M_n 5,000	729159-1G 729159-5G
NH_2		average M_n 400	767565-1ML
2-Bromoisobutyrate		average M_n 600	767581-1ML
4-Cyano-4-pentanoate dodecyl trithiocarbonate		average M_n 1,400	752487-1G 752487-5G
		average M_n 2,400	751634-1G 751634-5G
		average M_n 5,400	751626-1G 751626-5G
DDMAT		average M_n 1,100	740705-1G
Maleimide		average M_n 2,000	731765-1G 731765-5G
		PEG average M_n750	712558-250MG
		PEG average M_n5,000	63187-1G 63187-5G
		PEG average M_n10.000	712469-250MG
NHS ester		PEG average M_n5,000	85973-1G
Succinylamine		PEG average M_n20,000	21954-250MG 21954-1G
Tosylate		average M_n 1,000	729116-5G
		average M_n 5,000	729132-5G
Acrylate		average M_n 2,000	730270-1G
		average M_n 5,000	730289-1G



ω-end	Structure	Molecular Weight	Prod. No.
Methacrylate		average M_n 200	729841-25G
		average M_n 300	447935-100ML 447935-500ML
		average M_n 500	447943-100ML 447943-500ML
		average M_n 950	447951-100ML 447951-500ML
		average M_n 2,000	457876-250ML 457876-1L
		average M_n 4,000	730327-1G
OH		average M_n 200	705276-25G
		average M_n 550	202487-5G 202487-250G 202487-500G
		average M_n 750	202495-250G 202495-500G
		average M_w 2,000	81321-250G 81321-1KG
		average M_n 5,000	81323-250G 81323-1KG
		average M_n 10,000	732621-5G 732621-25G
		average M_n 20,000	732613-5G 732613-25G

Glycols, Other PEGs, and PEOs

Name	Structure	Molecular Weight	Prod. No.
Tetraethylene glycol		average M_n 200	110175-100G 110175-1KG 110175-3KG 110175-20KG
Pentaethylene glycol	$HOCH_2CH_2(OCH_2CH_2)_4OH$	average M_n 250	335754-5G 335754-25G
Hexaethylene glycol		average M_n 300	259268-5G 259268-25G
Poly(ethylene glycol)		average M_n 1,500	81210-1KG 81210-5KG
		average M_n 2,050	295906-5G 295906-250G 295906-500G
		average M_n 3,350	202444-250G 202444-500G
		average M_n 10,000	309028-250G 309028-500G
		average M_n 20,000	81300-1KG 81300-5KG
Poly(ethylene oxide)		average M_v 100,000	181986-5G 181986-250G 181986-500G
		average M_v 200,000	181994-5G 181994-250G 181994-500G
		average M_v 600,000	182028-5G 182028-250G 182028-500G

Chitosan-based Biomaterials



Guolin Wu[1], Hui Gao[2], and Jianbiao Ma[1,2]*
[1]Key Laboratory of Functional Polymer Materials of MOE, Institute of Polymer Chemistry, Nankai University, Tianjin 300071 (PR China)
[2]School of Chemistry and Chemical Engineering, Tianjin University of Technology, Tianjin 300191, P. R. China
*Email: jbma@nankai.edu.cn

Introduction

Chitin, a natural polysaccharide, is the second most abundant natural biopolymer in the world, after cellulose. It is the primary, high modulus fibrous component of the exoskeleton of arthropods including crab, shrimp and lobsters, as well as some fungi. Structurally, chitin is an insoluble, linear, highly crystalline polymer, composed of isotactic poly-*N*-acetyl-D-glucosamine (**Figure 1A**). Chitosan, the only naturally occurring cationic amino polysaccharide (**Figure 1B**), is derived from the *N*-deacetylation of chitin, resulting in a copolymer of *N*-acetylglucosamine and glucosamine. Chitosan is characterized by the degree of deacetylation (DDA), which allows it to be soluble in dilute acetic acid when the DDA is >40%. Chitosan has been widely studied for a variety of biomedical applications such as wound healing, hemodialysis membranes, drug and gene delivery systems, implant coatings and tissue engineering/regeneration due to its good biocompatibility, biodegradability with no acidic degradation products and non-toxic nature. Specific functionality designed for biomedical applications is achieved on chitosan by grafting pendant functional groups through primary amine or hydroxyl groups, without modification of the polymer backbone in order to preserve original properties.

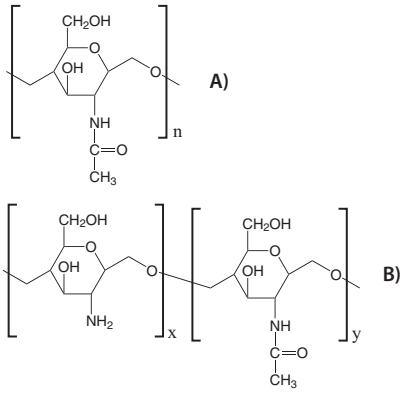


Figure 1. Chemical structure of **A)** chitin and **B)** chitosan.

Chitosan for Drug and Gene Delivery Systems

Chitosan's unique physiochemical and biological properties make it ideal for utility in drug delivery systems. It has been reported to enhance drug permeation across the intestinal, nasal, and buccal mucosa[1]. Chitosan nanoparticles encapsulated with insulin were first prepared in 1997 by Alonso et al. for nasal delivery in rabbits[2]. Studies show that chitosan enhances the absorption of peptides such as insulin and calcitonin across the nasal epithelium[3]. Chitosan nanoparticles have also been developed for applications in gene delivery[4]. It is the most significant natural polymer-based gene delivery vector due to its biodegradability, biocompatibility and unique ability to form a complex with DNA through electrostatic interactions when protonated in acidic solution[5,6]. Mumper et al. first described chitosan as a delivery system for plasmids[7]. Chitosan/plasmid DNA nanoparticles were readily formed by complexation between the positively charged amine groups on chitosan and negatively charged phosphate groups on DNA. Molecular weight and the degree of deacetylation (DDA) have significant impact on chitosan's performance in gene delivery. High molecular weight chitosan leads to more chain entanglements with DNA, creating stronger complexes and gives rise to improved gene delivery efficiency[8]. The DDA, typically between 40-100%, affects the physiochemical properties of the chitosan. Moderate DDA increases the transfection efficiency of chitosan in gene delivery system, but for pure chitosan its transfection efficiency is still relatively low compared to viral vectors. Numerous chemical modifications such as quaternization[9], deoxycholic acid modification[10], galactosylation[11], PEI grafting[12] and thiolation[13] have been carried out on chitosan in an attempt to improve its gene transfection efficiency. Modified chitosan and its nucleic acid loaded nano-complexes have created a new method for fabricating gene therapy for management of various diseases.

Chitosan for Tissue Engineering

Studies suggest that chitosan and its derivatives are promising candidates as supporting material for tissue engineering applications. Chitosan-based tissue scaffolds include skin[14] (**Figure 2**), bone[15,16] (**Figure 3**), cartilage[17], liver[18,19], nerve[20] and blood vessel[21,22]. The versatility of chitosan is due to an intrinsic antibacterial nature, minimal foreign body reaction, wound-healing activity, as well as the ability to form porous structures and gels. Additionally, chitosan has a high affinity to *in vivo* macromolecules, suitable for cell ingrowth and osteoconduction. Importantly, chitosan has been found to have an acceleratory effect on the tissue engineering processes due to the polycationic nature which enhances cellular attraction to the polymer.

One example of chitosan in skin tissue engineering and use as a dermis substitute is the scaffold formed by the bilayer structure of chitosan film and sponge. The scaffold was processed successively via the formation of a dense chitosan film by solution casting, followed by lyophilization to form a porous chitosan sponge. Porogens such as sodium chloride, glucose and sucrose were used to create large pores in the chitosan sponge layer (**Figure 2**). When human neofetal dermal fibroblasts were seeded within the chitosan sponge layer, cells grew and proliferated in an extended shape on the bottom of large pores. After the culture fibroblasts could bind tightly with the sponge layer via newly formed extracellular matrices to give a living cell-matrix-chitosan composite. During cell culture, the bilayer chitosan material remained stable; however, contraction is typically observed in collagen sponge material used as a tissue engineering scaffold. The results suggest that chitosan

utilized as scaffolds in skin tissue engineering would be good alternatives to some collagen materials[14]. It has been found that the degree of cell attachment also depends on the percent of deacetylation of the chitosan[22].

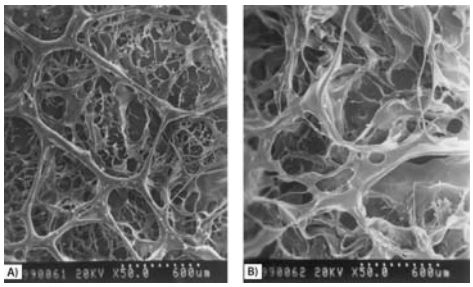


Figure 2. The SEM images of bilayer chitosan sponges designed as an artificial skin prepared by the sucrose leaching method: **A)** 4.5% sucrose in chitosan solution as a porogen; **B)** 6% sucrose in chitosan solution as a porogen.

Chitosan has also been explored for utility in tissue engineering for bone repair. Phosphorylated chitosan (P-chitosan) can be mixed into a calcium phosphate cement and implanted into a bone defect. For example, P-chitosan cement implanted in rabbit bone defects shows that all wounds healed gradually and the rabbits were active with no post-surgery complications. After implantation for 1, 4, 12, and 22 weeks, X-ray radiographs (**Figure 3**) show that the P-chitosan containing samples permitted stabilization of the defected bones and maintenance of correct position. New bone formation and implant biodegradation in the bone defects could be clearly reflected by the gradually weakened umbral on the radiographs. During the entire process, bone repair in controls progressed less perfectly than those with phosphorylated chitosan reinforced implants.

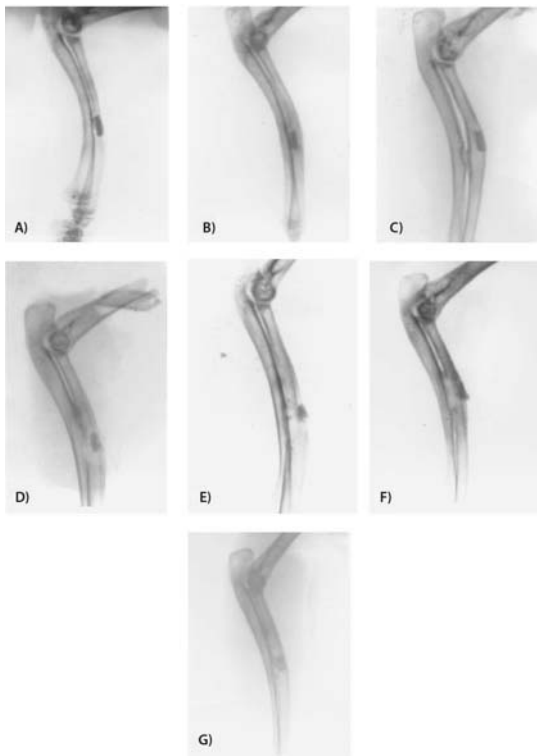


Figure 3. Radiographs of the prehardened phosphorylated chitosan (P-chitosan) reinforced calcium phosphate cements after implantation: **A)** 1 week (P-chitosan: 0.12 g/mL); **B)** 4 weeks (P-chitosan: 0.12 g/mL); **C)** 12 weeks (P-chitosan: 0.12 g/mL); **D)** 12 weeks (P-chitosan: 0.07 g/mL); **E)** 12 weeks (P-chitosan: 0.02 g/mL); **F)** 12 weeks (P-chitosan: 0 g/mL); **G)** 22 weeks (P-chitosan: 0.12 g/mL).

Recently, injectable polymeric hydrogels have become very attractive in tissue engineering. Injectable hydrogels have particular advantages:

1. Implantation surgery can be replaced by a simple minimally invasive injection procedure
2. Cells and bioactive moieties like growth factors can be readily incorporated *in situ*
3. Gels can be formed in any desired shape with good alignment to the surrounding tissue
4. Gels can enable sustained or stimuli responsive drug release
5. Gels reduce the frequency of administration and thus lighten the burden for the patients

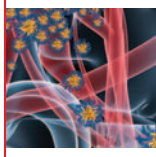
In the past years, many injectable chitosan-based hydrogels have been developed for tissue engineering. These hydrogels are prepared by either physical[24] or chemical crosslinking methods, such as redox-initiated[25], photo-initiated[26] and enzymatic[27] crosslinking. Moreover, crosslinked hydrogels parameters such as gelation time, gel modulus and hydrogel degradability can be adjusted by the molecular weight of polymers and the crosslinking densities.

Chitosan for Hemodialysis Membranes

Hemodialysis is an extracorporeal blood purification procedure for the treatment of patients with late-stage renal disease, which utilizes a polymeric semipermeable membrane to remove desired amounts of toxic biological metabolites from blood. Chitosan has been identified as a material to serve as an artificial kidney membrane because of its suitable permeability to urea and creatinine, in addition to its high tensile strength. However, pure chitosan membranes are impermeable to serum proteins and possess inadequate compatibility. Various modifications improve the compatibility, selectivity and dialysis rates for medium- and large-size molecules for chitosan hemodialysis membranes. For example, chitosan with pendant 2-hydroxyethylmethacrylate (HEMA) grafts utilized in dialysis membranes showed enhanced permeation to glucose, urea, and albumin relative to the unmodified chitosan films[28]. Heparin and dextran sulfate were also used to modify chitosan membranes to improve their blood compatibility in hemodialysis[29]. The chitosan-*graft*-poly(vinyl acetate) (chitosan-*g*-PVAc) membranes were found to exhibit higher permeability to creatinine, urea, and glucose compared with the commercial cellulose membrane and are impermeable to the essential nutrient albumin[30]. Due to all these excellent properties, potential applications like artificial organs, such as kidneys and pancreas, are envisaged from these chitosan-based membranes.

Conclusions

Chitosan and chitosan derivatives have received considerable attention as a functional biopolymer for pharmaceutical and biomedical applications. They can be formulated as nano-carriers for drug and gene delivery, hydrogels for tissue engineering, and membranes for hemodialysis. There are still many potential applications which need to be developed for chitosan and its derivatives, indicating that they have yet to be utilized to reach their full potential.



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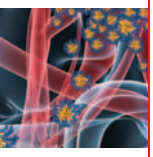
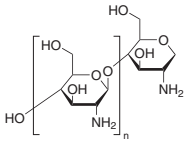


Chitosans

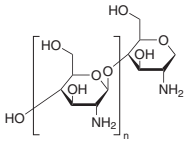
For a complete list of available natural polymers, visit aldrich.com/natural.

High Purity Chitosans (White Mushroom Origin)



Name	Molecular Weight (M_V)	Degree Of Acetylation	Prod. No.
KiOmedine-CsU® D	140,000-220,000	≤40 mol. %	740179-1G 740179-5G
KiOmedine-CsU® C	110,000-150,000	≤40 mol. %	740500-1G 740500-5G
KiOmedine-CsU® B	60,000-120,000	≤40 mol. %	740063-1G 740063-5G

Chitosans (Animal Origin)



Name	Inherent Viscosity (cP)*	Degree Of Deacetylation	Prod. No.
Chitosan	800-2000	>75%	419419-50G 419419-250G
Chitosan	200-800	75-85%	448877-50G 448877-250G
Chitosan	20-300	75-85%	448869-50G 448869-250G
Chitosan	>200	≥ 75%	417963-25G 417963-100G
Chitosan oligosaccharide lactate	-	> 90%	523682-1G 523682-10G

* 1 wt. % chitosan solution in 1 vol. % acetic acid, 25 °C, Brookfield

Biocompatible Dendritic Building Blocks for Advanced Biomedical Research

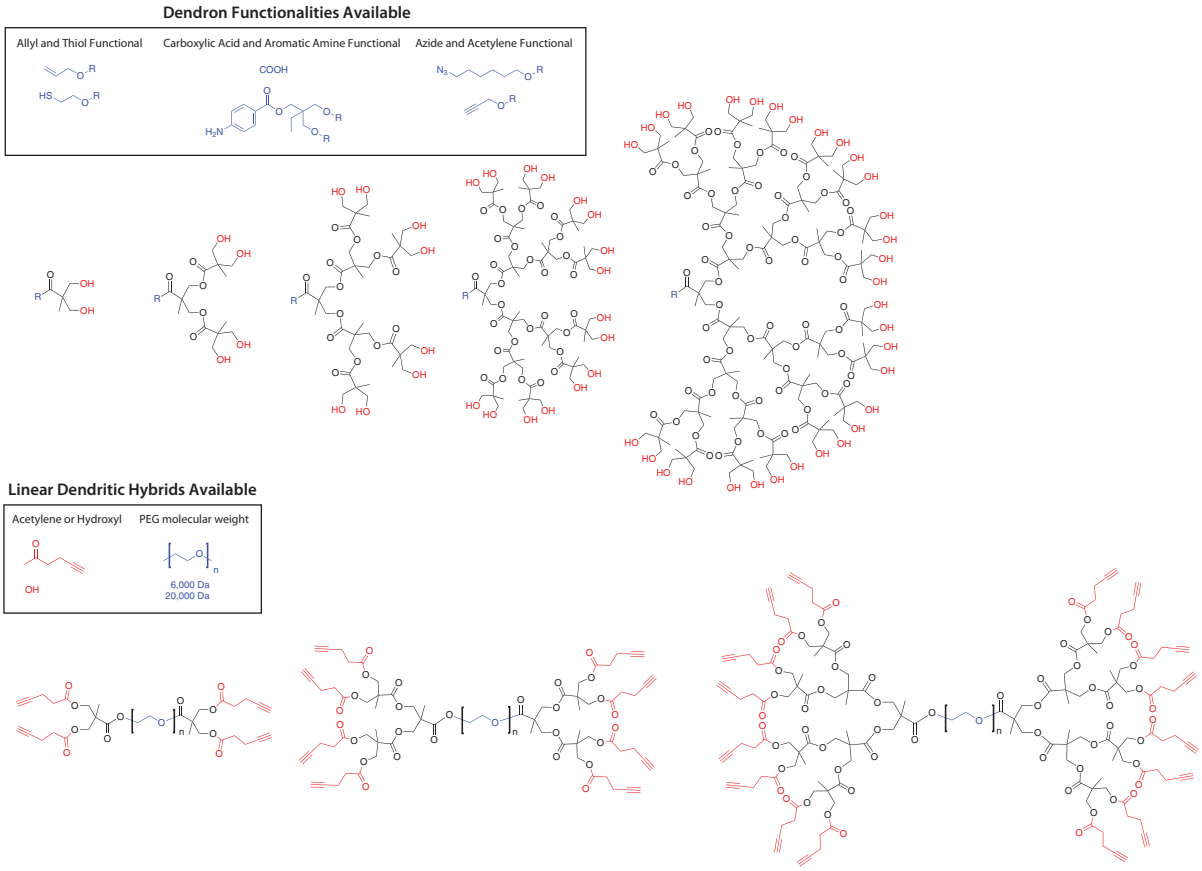

Dr. Michael Malkoch, Associate Professor, Division of Coating Technology, Fibre and Polymer Technology, KTH Royal Institute of Technology, and Chief Technology Officer Polymer Factory Sweden AB
*Dr. Andreas M. Nyström, Associate Professor of Nanomedicine, Swedish Medical Nanoscience Center, Karolinska Institutet, Chief Executive Officer Polymer Factory Sweden AB
*Email: andreas.nystrom@polymerfactory.com

Introduction: Click-ready Dendritic Building Blocks

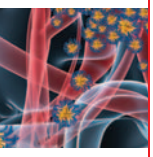
Dendrimers and dendrons are symmetrically branched polymer structures that possess a well-defined spatial distribution of functional groups. These dendritic materials have attracted considerable interest due to their monodisperse nature and other unique properties such as enhanced solubility and reduced viscosity, relative to their linear analogues. A range of advanced applications have been proposed for dendrimers and dendrons in diverse areas such as drug delivery, diagnostic imaging, hydrogels, catalysis, and as optical materials.[1]

Despite the breadth of applications available, most of the current research activities of dendrimers and dendrons has, up to recent years, been 1) restricted to exclusive academic research groups with in-depth knowledge in dendrimer chemistry and 2) limited to a small number of commercially accessible dendritic building blocks, mainly PAMAM dendrimers. Recently, a range of polyester dendrons and poly(ethylene glycol) (PEG) dendrimers based on the bis-MPA (2,2-bis(methylol) propionic acid) building block were made available to researchers globally by Aldrich® Materials Science. Polymer Factory, offering hyperbranched polymers, dendrimers, and dendrons to the market since 2006, is known for high quality dendritic structures and materials. This article will showcase some recent advances and applications of these materials, utilizing different types of click chemistries including thiol-ene and azide-acetylene conjugations.[2]

Click chemistry was introduced in 2001 by Prof. K. B. Sharpless[3] and today covers a set of highly reliable and synthetically simple chemical reactions known to proceed in a wide range of solvents, including organic and aqueous conditions, with high conversions in short reaction times. The introduction of popular "clickable groups" such as primary acetylenes (-C≡CH), organic azides (-N_3), thiols (-SH), or unsaturated vinylic (-CH-CH_2) groups to dendritic structures enables their exploration by non-chemists as well. Using these functional groups, dendrimers and dendrons based on bis-MPA have been successfully conjugated to bioactive moieties, such as carbohydrate, disaccharides, fluorescent dyes, etc. and then evaluated in solution as well as on surfaces.



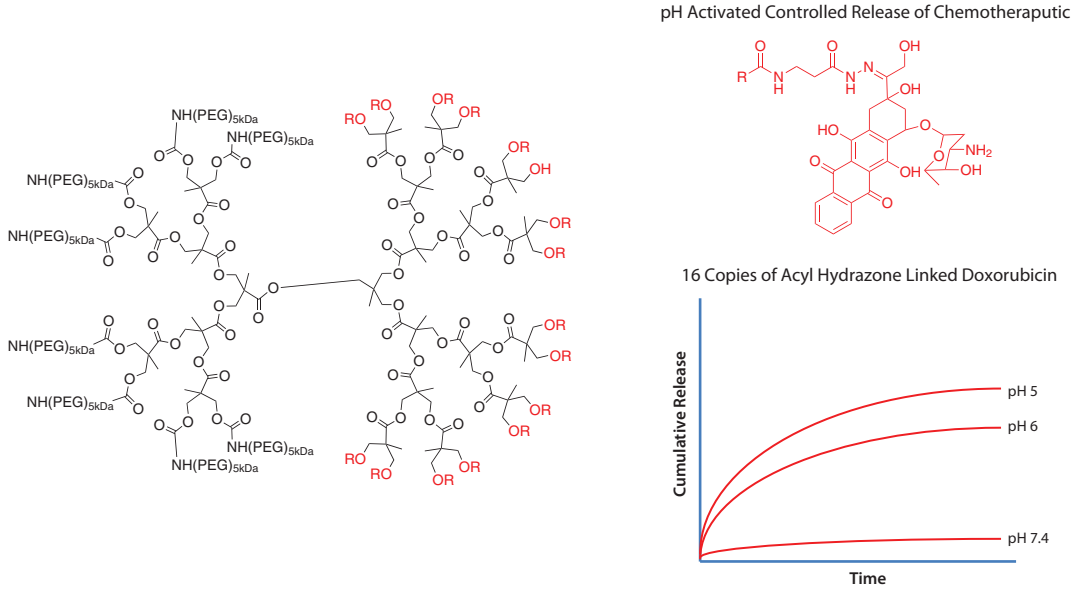
Scheme 1. Click-ready dendritic building blocks.



Biological Applications

Polyester dendrimers and dendrons based on the bis-MPA building block have been extensively explored for biological applications involving *in vitro*[4] toxicological studies and *in vivo*[5-10] via radionuclide labeling and optical imaging. As recently shown in a study utilizing human primary macrophages as a model for immunoactivation and toxicity,[4] bis-MPA based dendritic materials and building blocks have low or no toxicity, are degradable under physiological conditions, and do not show immunogenic properties, which is of utmost importance in biomedical applications. In the *in vivo* setting, biodistribution (BioD) studies of high generation dendrons (up to 7th generation) show these materials do not demonstrate any specific organ accumulation in healthy rats via radiolabeling of the focal point and imaging via single-photon emission computed tomography (SPECT).[9] Fréchet and his research team[11] spent considerable efforts to modulate blood circulation time of chemotherapeutic conjugated bis-MPA dendrimers by coupling PEG chains to the surface of the dendrimer.[7] The dendrimers were tuned with respect to circulation time allowing for substantial increases in tumor specific uptake and low bystander tissue uptake (**Scheme 2**). A dendrimer based drug delivery system with one of the best efficacy and survivability rates reported in the literature was developed by combining dendrimers with a potent and often clinically employed chemo-therapeutic, such as doxorubicin.[7]

The monodisperse nature of dendrons allows researchers to create their own dendritic-drug conjugate with considerably lower variation in molecular weight compared to linear polymers, while allowing the drug candidate to be modified in a modular fashion using various forms of chemistry tailored to the diverse set of functionalities offered on the dendrons, -SH/-allyl, -N_3/-acetylene, -COOH/-NH_2.



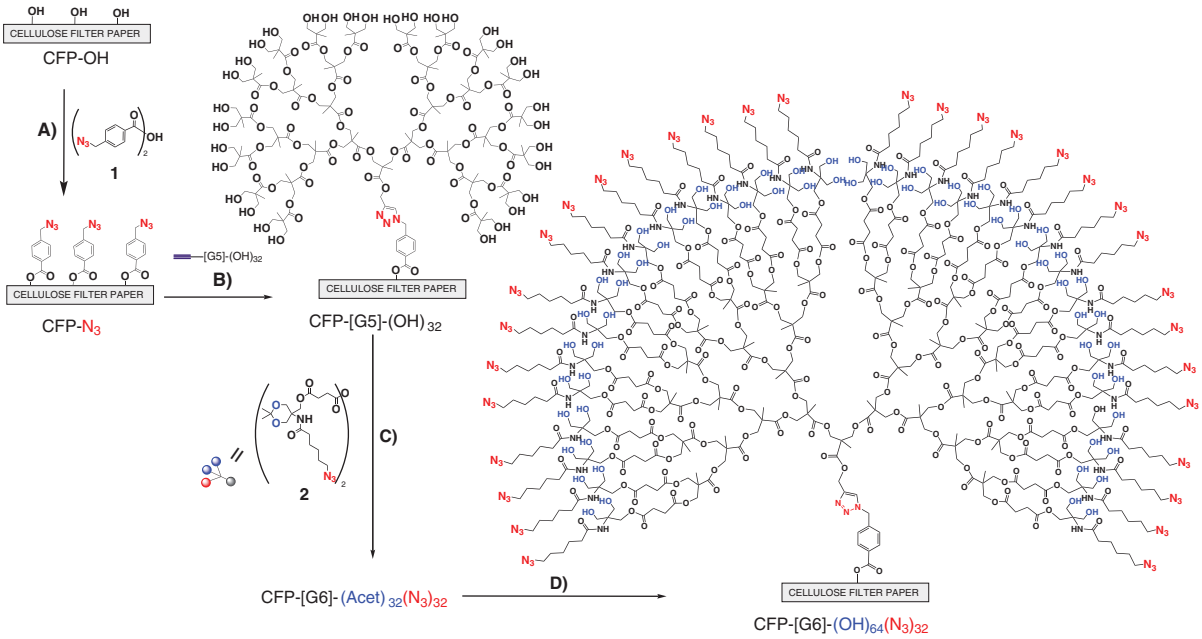
Scheme 2. Drug delivery applications of bis-MPA dendrimers, (left) dendritic bow-tie structure with poly(ethylene glycol) chains on one side for tailored pharma-co-kinetics and chemotherapeutics (Doxorubicin) on the other side. (Right, top) structure of Doxorubicin, (right, bottom) typical release behavior of a hydrozone linked pH triggered pharmaceutical.[7]

Engineering Applications and the Construction of Multivalent Surfaces and Gels

Dendron building blocks are highly interesting as scaffolds for the creation of multivalent binding systems in biosensor applications, as well as the construction of surfaces where the high density of functional groups can be advantageous. A recent paper by Malkoch et al. attached acetylene functional bis-MPA dendrons to filter paper via copper catalyzed click chemistry.[2] The surface groups of the dendrons were post-functionalized with mannose, creating a lectin binding sensor with increased sensitivity compared to mono-mannosylated surfaces (**Scheme 3**).[12] Similarly, asymmetric dendrimers can be coupled to a Janus-type structure using complementary azide/acetylene dendrons bridging different functionalities.[13] Thiol-containing dendrons can be utilized in copper-free thiol-ene click chemistry for the construction of advanced functional nanomaterials, including complementary dendrons bearing thiol and ene focal points. Thiols also allow for functionalization of gold surfaces in a robust manner, where high functional group density allows for post-functionalization of gold surfaces.[14]

Other advanced applications include dip-pen nanolithography based click chemistry with bis-MPA dendrons bearing focal point azides and acetylene functional silicon wafers as substrates.[15] This general strategy can be further developed from its initial laboratory proof of principle to dip-pen lithography of surface functional dendrons that offer both precise tailoring of the spatial resolution and high density functionality. These functional dendrons can also be used for the creation of tailored linear-dendritic polymers with tunable hydrophilicity to create novel block copolymers that form ordered isoporous membranes.[16] Gilles et al. have recently shown several beautiful examples of effective post-functionalization of nanoscale vesicles, polymer micelles,[17] and iron oxide nanoparticles[18] bearing click-ready groups on the surface. They tuned the nanostructures using functional bis-MPA dendrons bearing fluorescent moieties. Such applications open a new area for nanoparticle functionalization, utilizing the dendrons to provide multivalent inter-actions for tissue specific targeting in nanomedicine applications.

In addition to functional dendrons, dendritic PEGs are another type of click ready material now commercially available. Materials comprised of a PEG core flanked by functional dendrons have been used to construct advanced hydrogels with superior mechanical properties such as high elongation at break and flexibility in terms of additives that can be incorporated into the gels prior to crosslinking.[19,20] Aida et al. have explored such structures for the construction of aerogels in which guanidinium groups on PEG bis-MPA dendrimers exfoliate nanoclay and form free-standing moldable and self-healing gels with only 2-3 weight % of solid content.[21]



Scheme 3. Click chemistry applications of bis-MPA dendrons of surface functionalization.[12]

Outlook

A complete set of dendrons and linear-dendritic PEG hybrids made commercially available with varying size and functionalities opens a horizon of new possibilities for researchers to explore and develop novel dendritic materials. Dendritic polymers, due to their unique properties, have been successfully utilized in a range of advanced applications from hydrogels for engineering and biomedical applications, to nanoscale surface modification, and the development of novel biosensors and molecular imaging agents. Furthermore, bis-MPA based materials have shown superior properties in terms of their lack of *in vitro* toxicity, *in vivo* compatibility, and high efficacy towards cancer in drug delivery systems.

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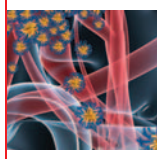
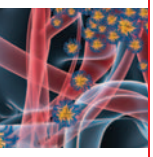
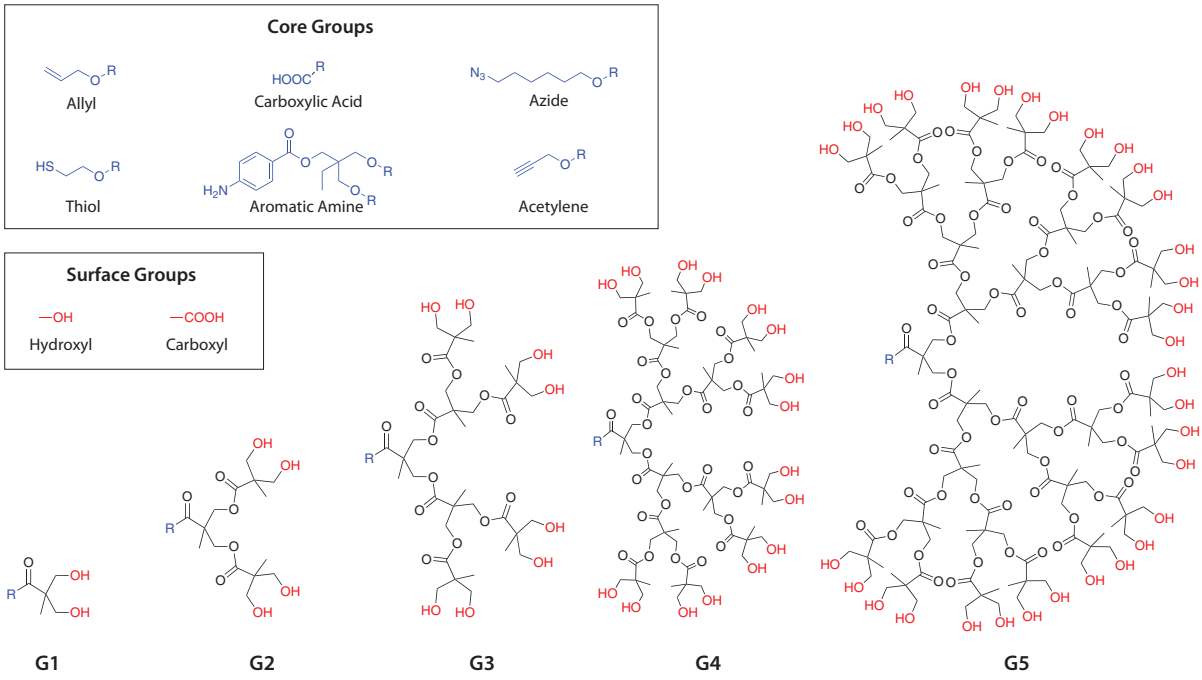




Dendrons

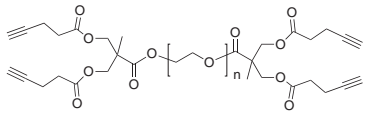
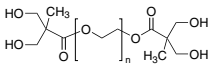
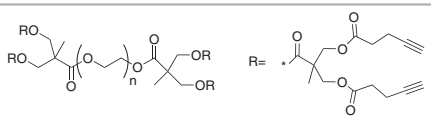
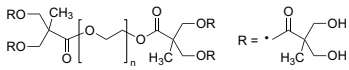
For your convenience, we list the dendritic material based on bis-MPA. For a complete list of available dendrons and dendrimers, visit aldrich.com/dendron.

bis-MPA Dendrons



Focal Point	Generation	Surface Groups	No. of Surface Groups	Prod. No.
acetylene	1	hydroxyl	2	767328-250MG
	2	hydroxyl	4	767301-250MG
	3	hydroxyl	8	686646-250MG
	4	hydroxyl	16	686638-250MG
	5	hydroxyl	32	686611-250MG
azide	1	hydroxyl	2	767220-250MG
	2	hydroxyl	4	767212-250MG
	3	hydroxyl	8	767204-250MG
	4	hydroxyl	16	767190-250MG
	5	hydroxyl	32	767182-250MG
allyl	3	hydroxyl	8	767298-250MG
	4	hydroxyl	16	767271-250MG
	5	hydroxyl	32	767255-250MG
thiol	3	hydroxyl	8	767131-250MG
	4	hydroxyl	16	767123-250MG
	5	hydroxyl	32	767115-250MG
amine	3	hydroxyl	8	767263-250MG
	4	hydroxyl	16	767247-250MG
	5	hydroxyl	32	767239-250MG
	3	carboxyl	8	767174-250MG
	4	carboxyl	16	767166-250MG
	5	carboxyl	32	767158-250MG
carboxylic acid	3	hydroxyl	8	686670-250MG
	4	hydroxyl	16	686662-250MG
	5	hydroxyl	32	686654-250MG

PEG-core bis-MPA Dendrons

Structure	Generation	Surface Groups	No. of Surface Groups	Molecular Weight	Prod. No.
	1	acetylene	4	PEG average M_n6,000 average M_n 6,300	760900-500MG
	1	acetylene	4	PEG average M_n20,000 average M_n 20,000	760935-500MG
	1	hydroxyl	4	PEG average M_n6,000 average M_n 6,300	752401-500MG
	1	hydroxyl	4	PEG average M_n20,000 average M_n 20,300	752398-500MG
	2	acetylene	8	PEG average M_n6,000 average M_n 6,900	760919-500MG
	2	acetylene	8	PEG average M_n20,000 average M_n 21,000	760943-500MG
	2	hydroxyl	8	PEG average M_n6,000 average M_n 6,700	760862-500MG
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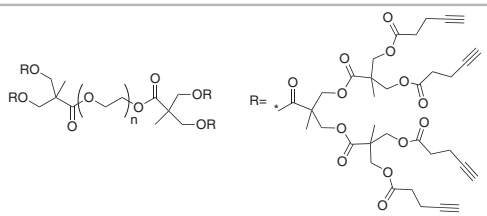	3	acetylene	16	PEG average M_n6,000 average M_n 8,000	760927-500MG
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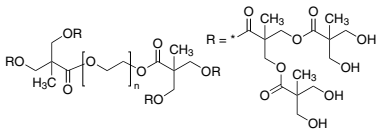	3	hydroxyl	16	PEG average M_n6,000 average M_n 8,000	752371-500MG
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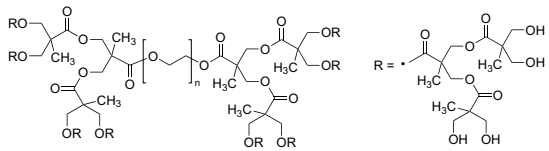	4	hydroxyl	32	PEG average M_n6,000 average M_n 9,500	760870-500MG
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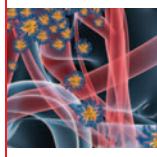

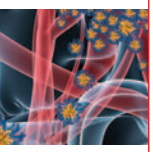

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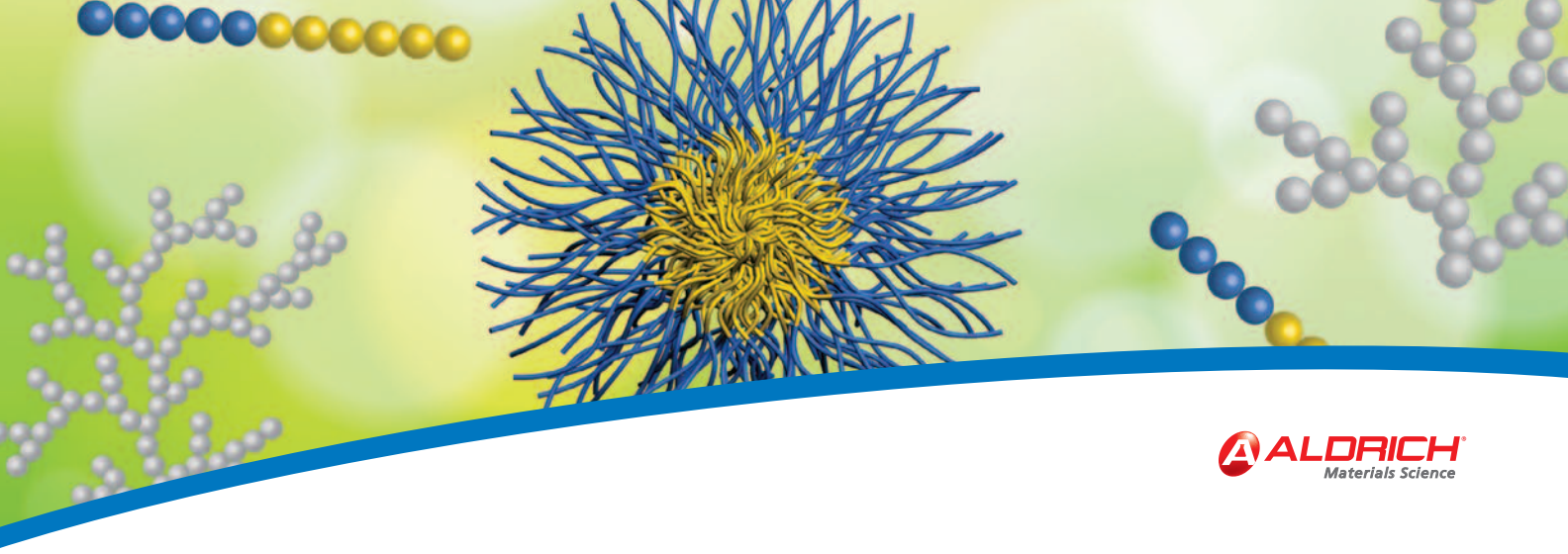

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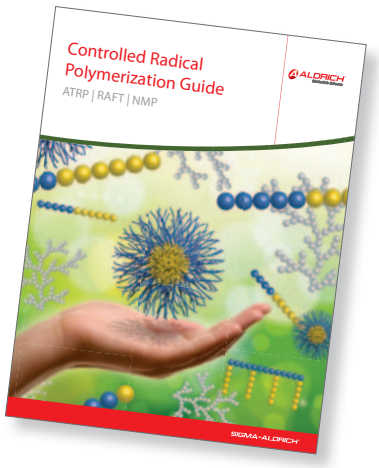
  - Reversible Addition/Fragmentation Chain Transfer (RAFT) agents
  - Atom Transfer Radical Polymerization (ATRP) ligands and monomers



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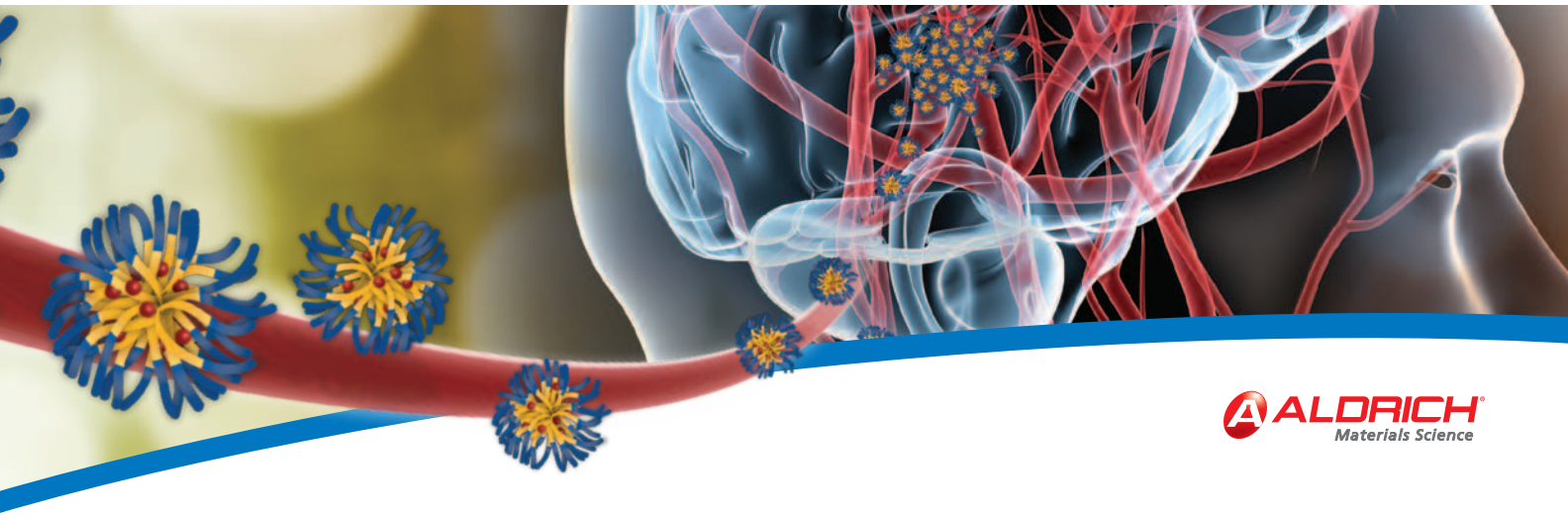

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