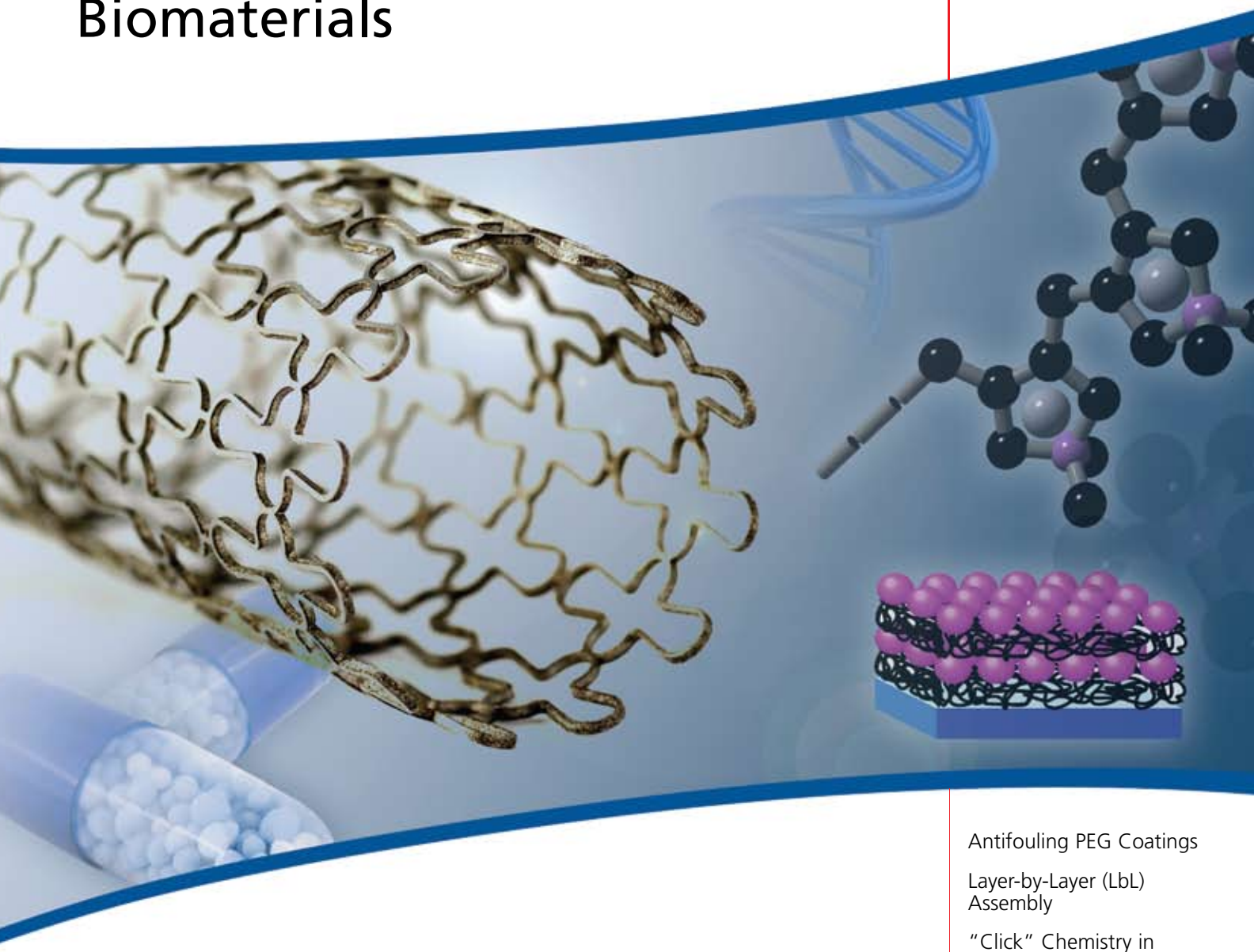
Material Matters™

Vol. 3, No. 3

ALDRICH Chemistry

Biomaterials



Better living through Chemistry

Antifouling PEG Coatings

Layer-by-Layer (LbL) Assembly

"Click" Chemistry in (Bio)Materials Science

Chemistry at Surfaces with Self-Assembled Monolayers

Bone Tissue Engineering

Ilya Koltover, Ph.D.
Aldrich® Materials Science
Sigma-Aldrich® Corporation

Introduction

Welcome to the third 2008 issue of *Material Matters*™ focusing on Biomaterials, a research area that deals with synthetic and natural materials used in contact with biological systems. The field of biomaterials is interdisciplinary and encompasses aspects of materials science as well as chemistry, biology, and medicine. It has a unique "human" aspect: few other areas of materials research can have equally direct impact on the quality of human lives by advancing medical care and diagnostics. Some results of biomaterials research are already implemented in medical practice. For example, steady research progress made over the last 50 years in structural biomaterials lead to significant improvements in quality of dental implants and artificial hip joints. Other aspects of biomaterials research are just starting to affect our lives. Novel polymeric biomaterials which encapsulate and control release rate release of drugs are used to design "high-tech" pills and to coat surfaces of cardiovascular implants. More advanced biomaterials applications are shaping on the research horizon: tissue engineering scaffolds, gene delivery devices, and biochips designed to customize medical care to an individual patient's genotype are some areas of active research.

Modern biomaterials are becoming complex, and present challenges both in terms of preparation (synthesis, fabrication, processing) of new material types, as well as analyses of finished biomaterials and their interactions with biological systems. In this issue, researchers from the Northwestern University describe new methods to prepare biomaterial surfaces resistant to fouling by biomolecules and bioorganisms. Layer-by-layer self-assembly, an innovative and versatile approach to prepare thin films of functional biomaterials, is described in the article written by Drs. Ariga and Hill from the Japan's National Institute for Materials Science. In their article on p. 57, researchers from the University of Nijmegen and Encapson B.V., Netherlands, discuss the application of "click" chemistry to make designer macromolecules that can be used as building blocks for controlled, bottom-up preparation of biomaterials. In the area of biomaterials characterization, Professor Mrksich from the University of Chicago describes SAMDI-TOF, a surface-sensitive mass spectrometry technique that can be used to measure detailed chemical composition of, for example, biochip surfaces. Finally, Professors Huster and Pretzsch from the University of Leipzig, Germany, write about application of solid state NMR to characterize engineered bone tissue — a very complex functional biomaterial central to current efforts in regenerative medicine.

Customary to *Material Matters*™, each article in this issue is accompanied by a list of Sigma-Aldrich® products helpful in the corresponding kind of biomaterials research. We at Sigma-Aldrich are pleased to combine our global expertise in high technology and life science to bring you a set of interdisciplinary tools for biomaterials research, which can be found at ***sigma-aldrich.com/biomaterials***. If you think we can improve this page or add another set of products helpful for your work, email us at ***matsci@sial.com***.

About Our Cover

Researchers are developing new methods for the preparation of biomaterials that function to improve properties of biomedical devices. Layer-by-Layer self-assembly (LbL), described in the article on page 57, is a useful method for making thin films of functional biomaterials. In the LbL technique, alternate layers of oppositely charged polymers are solution deposited to form multilayered films (see illustration on cover) on surfaces of biomedical devices (e.g. stents), or walls of controlled-release capsules (pills). Charged drugs or biological molecules (proteins, DNA) can be incorporated into the LbL films. For example, cationic poly(diallylammonium chloride) shown on the cover can be co-deposited with anionic DNA; the films can release encapsulated DNA, working as vehicles for gene delivery. See the product table on page 60 for a list of polymers for LbL applications.

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Joe Porwoll, President
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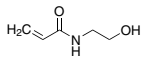
N-Hydroxyethyl acrylamide — Tool for Lab-On-A-Chip Research

Professor Annelise Barron of Stanford University kindly suggested that we offer *N*-hydroxyethyl acrylamide (HEAA), a monomer which can be used to make polymers that are useful as wall coatings and separation media for lab-on-a-chip devices. Hydrophilic poly-*N*-hydroxyacrylamide (pHEAA), synthesized by free radical polymerization of HEAA in water, forms a stable adsorbed coating on glass and fused silica microchannels, enabling excellent biomolecule separations. pHEAA coatings eliminate electroosmotic flow and greatly reduce non-specifc adsorption of biomolecules to the internal walls of microfluidic channels. DNA fragments of > 500 bases have been successfully separated and sequenced using pHEAA-assisted microcapillary electrophoresis.[1,2]

References:

(1) Albarghouthi, M.N., Buchholtz, B.A., Huiberts, P.J., Stein, T.M., Barron, A.E. *Electrophoresis* **2002**, *23*, 1429.
(2) Fredlake, C.P., Hert, D.G., Kan, C.W., Chiesl, T.N., Root, B.E., Forster, R.E., Barron, A.E. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 476.



***N*-Hydroxyethyl acrylamide (HEAA), 97%**	
697931-100ML	100 mL

Biomaterials Featured in This Issue

Fouling Resistant Biomimetic Poly(Ethylene Glycol) Based Grafted Polymer Coatings

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Accumulation of biological matter at surfaces is an inevitable event in virtually any environment in which natural and man-made materials are used. Although sometimes fouling of surfaces with biomolecules and bioorganisms has little consequence, biofouling must be minimized or controlled in order to maintain performance and safety of devices and structures. For example, in the medical environment, components of biofluids such as proteins, cells and pathogens have a propensity to strongly adhere to surfaces, altering performance with potentially hazardous outcomes. Urinary tract infections resulting from microbial colonization of catheters represents the most common hospital-acquired infection.[1] Implantable medical devices are also susceptible to microbially influenced corrosion (MIC) leading to the need for replacement surgeries with increased risk of infection.[2] Additional examples of surfaces prone to biofouling include the hulls of marine vessels which often become coated with marine organisms and their secretions, decreasing the efficiency of propulsion and contributing to higher fuel consumption.[3] This article will briefly highlight approaches for limiting biofouling of surfaces using grafted polymers, including biomimetic strategies for linking polymers onto surfaces.

A common strategy for preventing biofouling is to graft an anti-fouling polymer onto a surface as depicted in **Figure 1**.[4] Key features of such grafted polymer systems include chemical characteristics, molecular weight and architecture of the antifouling polymer, and the method by which the polymer is grafted to the surface. One of the most extensively studied anti-fouling polymers is poly(ethylene glycol) (PEG), a water soluble polymer with low toxicity and extensive history of use in medicine and drug delivery.[5] PEG is widely available to academic and industrial researchers through either direct synthesis or purchase, and with appropriate chemical derivatization can be grafted onto surfaces to reduce the nonspecific adsorption of proteins, cells and bacteria (**see Table on p. 54 of this issue**). Although the thermodynamic and molecular mechanisms for the protein and cell resistance of surface immobilized PEG are not completely understood, numerous studies have determined that steric hindrance effects, chain length, grafting density, chain conformation, and hydrophilicity of the grafted polymer play important roles in resisting protein adhesion.[6–8]

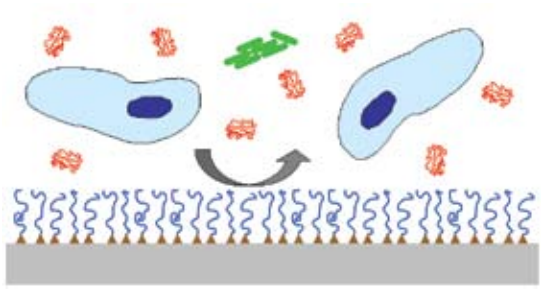

Figure 1. Grafted antifouling polymer systems consist of an antifouling polymer (blue lines) anchored by specific functional groups (triangles) onto a substrate (gray), providing a physical barrier to adsorption of proteins (red), cells (blue) and bacteria (green) onto the underlying surface.

Grafting of antifouling polymers onto surfaces can be accomplished using two basic strategies. The *graft-to* approach consists of surface adsorption of pre-synthesized polymer functionalized with a chemical anchoring group. In contrast, *graft-from* approaches rely on in-situ polymerization of polymer from a grafted initiator. Graft-to systems are typically of monolayer thickness (a few nanometers), and can be formed through physisorption or chemisorption of polymer from a solvent. Graft-from systems are generally much thicker (10–100 nanometers or more) but require pre-functionalization of the substrate with a suitable initiator. Essential to both approaches, however, is the ultimate requirement of either physisorptive or chemisorptive interactions to hold the polymer onto the surface. Physisorption relies on relatively weak Van der Waals or hydrophobic forces to tether polymers onto a surface, with an example being the adsorption of Pluronic-type block copolymers onto hydrophobic substrates.[9] Chemisorption typically provides more robust linking of polymer to substrate, and is exemplified by gold-thiolate,[10] metal oxide-silane linkages,[11] and electrostatic interactions.[12]

It has recently become apparent that strategies employed by biological organisms can provide inspiration for new approaches to grafting polymers onto surfaces. Of particular interest are unusual amino acids found in marine adhesive proteins and used to secure robust attachment to wet surfaces. Marine mussels adhere firmly to a variety of material surfaces such as rocks, wood, animals, and shells even in a wet and turbulent environment. The amino acid 3,4-dihydroxyphenylalanine (DOPA) (**D9628**) is present at concentrations of up to 27 mol% in proteins located near the interface of the mussel's adhesive pad and the substrate (**Figure 2**).[13] DOPA contributes remarkable adhesive properties as demonstrated by single molecule force spectroscopy,[14] forming strong chemical interactions with both organic and inorganic surfaces.

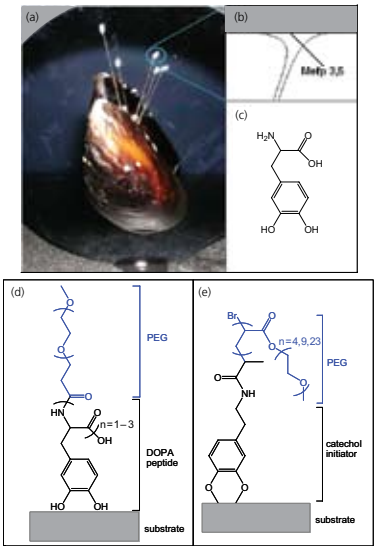

Figure 2. Biologically inspired strategies for grafting antifouling polymers onto surfaces. (a) Image of a mussel adhered to a substrate, (b) Schematic of the adhesive pad and interfacial location of mussel adhesive proteins (Mefp3, Mefp5) with the highest content of the amino acid 3,4-dihydroxyphenylalanine (DOPA), (c) Chemical structure of DOPA, (d) Example graft-to biomimetic polymer showing an adhesive peptide anchor and antifouling PEG polymer, (e) Example graft-from biomimetic polymer showing a surface bound biomimetic initiator and polymerized PEG polymer.

Chemical versatility and robustness of DOPA have recently been exploited in a number of ways to link antifouling polymers onto surfaces.[15] Polymers such as monomethoxy-terminated PEG conjugated with DOPA containing peptides combine the attractive antifouling features of PEG with an adhesive anchor allowing for convenient attachment to surfaces via the *graft-to* approach.[16] Linear PEG polymers derivatized with 1–3 DOPA residues (Figure 2) were found to adsorb to titanium oxide (TiO_2) and gold substrates,[17] and conferred high resistance to serum components as measured by optical waveguide spectroscopy (OWLS) and spectroscopic ellipsometry (ELM). In-situ adsorption of serum revealed that the TiO_2 control surface accumulated 250 ng/cm^2 of serum protein while the surfaces modified with mPEG-$DOPA_3$ only accumulated less than 1 ng/cm^2 confirming the excellent nonfouling characteristics of PEG conjugated with DOPA.[17] More sophisticated peptide anchors have also been developed; for example, a decapeptide analog of *Mytilus edulis* foot protein-1 was conjugated to PEG and used to confer resistance to cell attachment on gold surfaces (**Figure 3**).[16] Peptide conjugated polymers are synthesized by solid phase methods in cases where an exact amino acid sequence is desired, or through polymerization of *N*-carboxyanhydride monomers via initiation from a monoamine functionalized polymer to create an oligomeric peptide anchor with a statistical distribution of amino acids. Recently, related catechol based adhesive anchors inspired by the biological iron chelator anachelin have been used in a similar way,[18] further expanding the chemical diversity of anchoring groups available for attaching PEG onto surfaces.

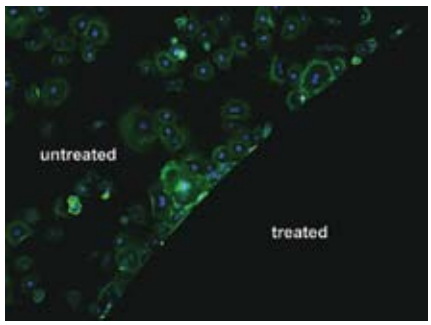

Figure 3. Fibroblast cell attachment to untreated gold (upper left) and gold grafted with PEG derivatized with a mussel adhesive protein analog decapaptide Ala-Lys-Pro-Ser-Tyr-Hyp-Hyp-Thr-DOPA-Lys (lower right).

The graft-from approach has also been employed to create antifouling coatings through polymerization of PEG macromonomers from surface-bound initiators. For example, atom transfer radical polymerization (ATRP) has been employed to polymerize acrylate-functionalized PEG from a gold surface modified with an initiator functionalized self-assembled monolayer (SAM).[19] The resulting grafted polymer coating, like most other *graft-from* coatings, was considerably thicker than *graft-to* coatings and exhibited excellent protein and cell fouling resistance. With respect to biomimetic anchors, a dopamine-based ATRP initiator was synthesized for preparation of antifouling coatings on metal oxides.[20] In this case, the catechol functional group found in the side chain of DOPA was exploited for adsorption onto titanium oxide and stainless steel, from which polymerization of PEG macromonomers via ATRP yielded thick fouling resistant coatings.

A hybrid *graft-from/graft-to* approach with remarkable versatility in its ability to functionalize different materials with antifouling polymers was recently reported.[21] The method involves two steps, the first relies on polymerization of dopamine hydrochloride (H8502) at alkaline pH to form a thin (50 nm or less) adherent polydopamine coating on the surface of virtually any material. The coating process utilizes a readily available reagent and can be applied to complex shaped objects via simple dip-coating. Formation of the polydopamine coating exploits reactions reminiscent to those occurring during solidification of mussel adhesive proteins and during formation of the biological pigment melanin. The resulting coating harbors latent reactivity toward nucleophiles, facilitating a second coating step involving covalent grafting of amine or thiol functionalized PEG onto the polydopamine coated surface (**Figure 4**). This new approach to modification of materials promises a convenient, cost-effective and versatile strategy to confer antifouling properties onto surfaces, does not require complicated surface preparation steps, and combines elements of both *graft-from* (Step 1) and *graft-to* (Step 2) approaches.

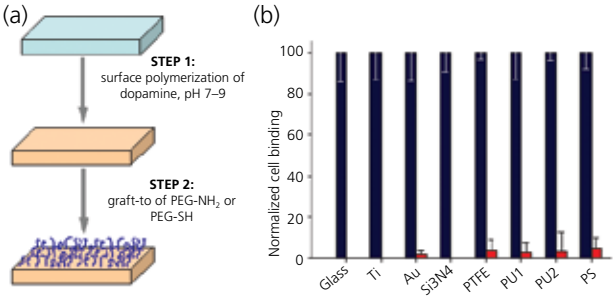

Figure 4. Facile grafting of antifouling polymers to material surfaces using a two-step approach. (a) Schematic of the surface grafting method, consisting of surface mediated polymerization of dopamine followed by graft-to of an amine or thiol functionalized PEG. Using this approach, antifouling grafted PEG coatings can be applied to many different surfaces, including inorganic and organic materials. (b) Normalized cell attachment before (black bars) and after (red bars) modification of glass, titanium, gold, silicon nitride, Teflon (PTFE), polyurethanes (PU1, PU2) and polystyrene (PS) with polydopamine and PEG-NH_2.

Grafted PEG coatings have also been investigated for inhibiting attachment of marine organisms to surfaces.[22–24] Several grafted polymer designs have been employed, including hyperbranched fluoropolymer-PEG composites,[22] hydrophobic polymers with PEG side chains,[23] and linear PEG.[24] These studies have to date emphasized fouling of diatom (*Navicula perminuta*) and green algae (*Ulva linza*) species and show enhancement of antifouling performance with increasing PEG content and favorable performance compared to standard silicone fouling-release coatings. Silicone fouling-release coatings are currently used by the marine industry to facilitate hydrodynamic removal of biofouling agents but these agents are not completely effective against all marine fouling and do not work well on slow-moving vessels. With further development, PEG-based coatings may provide effective fouling performance in the marine environment.

In summary, grafted PEG coatings can provide effective resistance to fouling of surfaces by proteins, cells, bacteria and other biological organisms. Biologically inspired anchoring moieties offer convenient, versatile and robust approaches to linking PEG onto surfaces. PEG coatings can be used to control fouling of implantable medical device surfaces, contact lenses, surgical tools, biosensors, electrophoresis capillaries for bioseparations, and fouling-prone surfaces of water treatment facilities and ship hulls.

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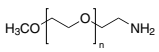
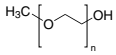
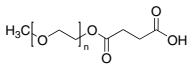
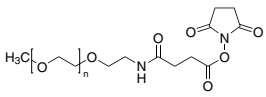
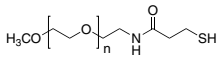
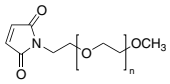
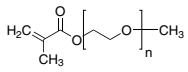
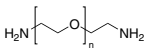
Functionalized poly(ethylene glycols)

Poly(ethylene glycol) (PEG) polymers are available with a wide range of molecular weights and end-functions. The table below lists a selection of functional linear PEG molecules commonly used in biomaterials research. For a complete list of available PEGs, including additional molecular weights, end-groups, as well as un-functionalized and branched (star-shaped) PEG polymers, please visit ***sigma-aldrich.com/biopeg***.

End-function (R)	Structure	Molecular Weight (Avg. M_n)	Prod. No.
Monofunctional PEGs			
$-NH_2$		2,000	06676-1G
		5,000	06679-1G
			06679-5G
		10,000	07965-1G
–OH		1,000	17738-250G
			17738-1KG
		2,000	202509-5G
			202509-250G
			202509-500G
		5,000	81323-250G
			81323-1KG

End-function (R)	Structure	Molecular Weight (Avg. M_n)	Prod. No.
–COOH		2,000	17928-5G
		5,000	17929-1G
			17929-5G
N-succinimidyl		2,000	41214-1G
–SH		5,000	11124-250MG
			11124-1G
		20,000	63753-250MG
Maleimide		5,000	63187-1G
			63187-5G
Methacrylate		1,100	447951-100ML
			447951-500ML
		2,080, 50 wt.% in H_2O	457876-250ML
			457876-1L
Homobifunctional PEGs			
–NH_2		2,000	14501-250MG
			14501-1G
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			14502-1G
		6,000	14504-250MG
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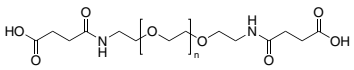
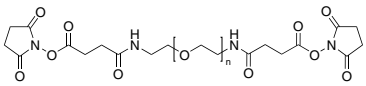
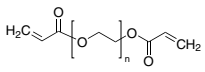
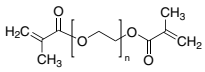
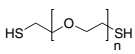
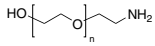
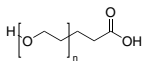
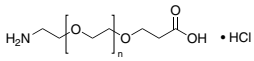
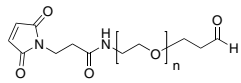
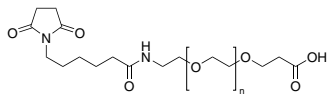
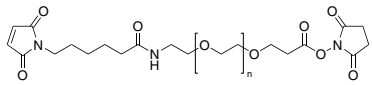
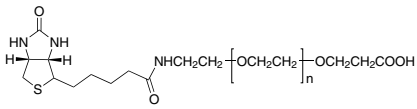
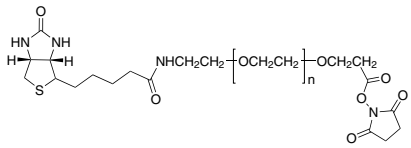
		10,000	14508-250MG
			14508-1G
–COOH		2,000	14565-1G
		3,000	14567-250MG
		6,000	14569-1G
		10,000	14571-1G
N-succinimidyl		3,000	15961-1G
Acrylate		2,000	701971-1G
		6,000	701963-1G
Methacrylate		2,000	687529-1G
		6,000	687537-1G
–SH		1,500–1,800	704369-1G
		3,400–3,600	704539-1G
		10,000–10,300	705004-1G

Heterobifunctional PEGs

R1	R2	Structure	Molecular Weight (Avg. M_n)	Prod. No.
–OH	–NH_2		3,000	07969-250MG
				07969-1G
			5,000	672130-100MG
				672130-500MG
			10,000	671924-100MG
				671924-500MG
–OH	–COOH		3,000	670812-100MG
				670812-500MG
			5,000	670936-100MG
				670936-500MG
			10,000	671037-100MG
				671037-500MG
–NH_2	–COOH		3,000	671487-100MG
				671487-500MG
			5,000	671592-100MG
				671592-500MG
–COH	Maleimide		3,000	579319-250MG
–COOH	Maleimide		3,000	670162-250MG
N-succinimidyl	Maleimide		3,000	670278-100MG
–COOH	Biotin		3,000	669946-250MG
N-succinimidyl	Biotin		3,000	670049-100MG

Layer-by-Layer (LbL) Assembly, A "Gentle Yet Flexible" Method Toward Functional Biomaterials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Introduction

Scientists spend considerable time and effort on the development of functional materials and systems. On the other hand, Nature has taken many millenia to evolve highly functional materials, which can be referred to as biomaterials. Perhaps as a reflection of the huge difference in development times, artificial materials are often inferior in their efficiencies and specificities when compared with biomaterials. Therefore, from the point of view of a rational materials design, the integration of biomaterial functions into desired systems should be advantageous. Unfortunately, biomaterials are prone to either attenuation of properties or decomposition under harsh chemical and physical conditions. Of course, biomaterials have not evolved in Nature for use as artificial device components. A mild, yet adaptable approach is necessary for successful immobilization of biomaterials within artificial structures. One possible method involves accommodating the biomaterials in biomembrane-like thin films such as Langmuir-Blodgett (LB) films or self-assembled monolayers (SAMs). Although these membranes serve as reasonable media for biomaterials to function, the films are neither simple to construct nor versatile. Recently, layer-by-layer (LbL) assembly has emerged as a versatile, gentle and, simple method for immobilization of functional molecules in an easily controllable thin film morphology.[1,2] In this short review, we introduce recent advances in functional systems fabricated by using the mild, yet adaptable LbL technique.

Outline of LbL: why and how gentle for biomaterials?

A general process of LbL assembly is illustrated in **Figure 1a** using assembly between cationic polyelectrolytes and anionic proteins as an example. Deposition of the cationic polyelectrolyte at the negatively charged surface of a solid support usually causes over-adsorption, resulting in surface charge reversal. Subsequent adsorption of anionic proteins again reverses the surface charge so that alteration of the surface charge permits continuous fabrication of the layered structure. Because this mechanism can be applied to various charged substances, there is a vast choice of available biomaterials including proteins, nucleic acids, saccharides, and virus particles. The assembly process, resulting in films of nanometer-scale thickness, can be conducted in an aqueous solution under mild ambient conditions, using only beakers and tweezers. The driving force for the LbL assembly is not necessarily limited to electrostatic interaction. Other interactions such as hydrogen bonding and metal coordination can be used for the assembly.[3] Biospecific interactions, for example recognition between lectins and sugars, could provide more specific film construction.[4]

A milestone in LbL assembly innovation was the introduction of template synthesis to this process.[5] **Figure 1b** illustrates LbL assembly preparation involving colloidal particles and subsequent hollow capsule formation. In this strategy, the LbL films are assembled sequentially, similarly to the conventional LbL assembly, but on a colloidal core. Destruction of the central particle core results in hollow capsules. Polyelectrolyte wrapping by LbL assembly on enzyme crystals followed by dissolution of the enzyme crystals, leads to extremely high enzyme loading in a nano-sized capsule.[6] If LbL assembly of biomaterials is conducted at the interior of a porous alumina template, subsequent dissolution of the template results in formation of microtubes composed of biomaterials.[7]

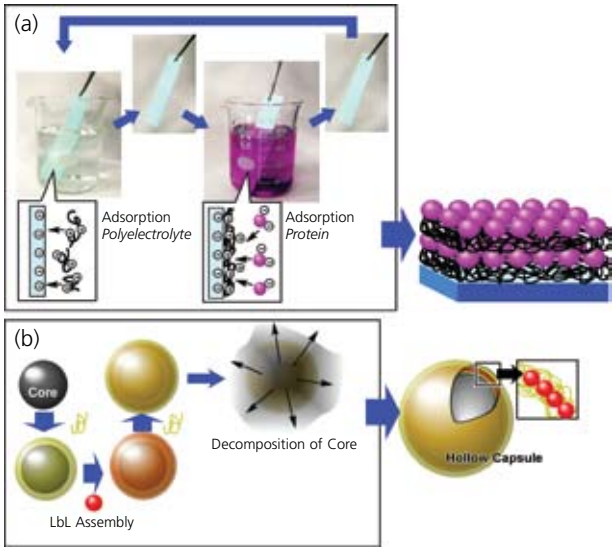

Figure 1. Process of LbL assembly (a) on a solid substrate; (b) on a colloidal core. Reprinted with permission from Ariga, K. et al. *Phys. Chem. Chem. Phys.* **2007**, *9*, 2319. © 2007, Royal Society of Chemistry.

Bio-related usage: reactors and sensors

The wide freedom available in LbL film construction can be advantageous for the preparation of thin-film enzyme reactors designed for specific reaction sequences. **Figure 2** shows one successful example of a dual-enzyme reactor containing glucose oxidase and glucoamylase prepared on an ultrafilter.[8] When a substrate starch solution was passed through the reactor, hydrolysis of the starch glucose bond by glucoamylase produced glucose, that was subsequently converted to gluconolactone by glucose oxidase with H_2O_2 as a byproduct. Reactor efficiency can be optimized by adjusting the number of layers, layering sequence, and layer separation.

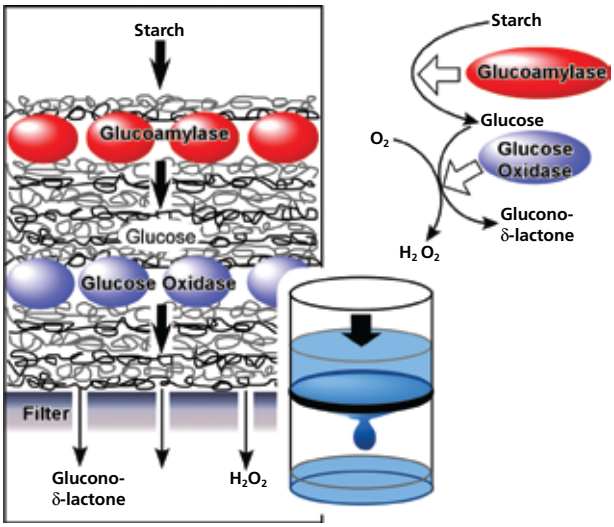

Figure 2. LbL assembled multienzyme reactor. Reprinted with permission from Ariga, K. et al. *Phys. Chem. Chem. Phys.* **2007**, *9*, 2319. © 2007, Royal Society of Chemistry.

Performance of single-enzyme reactors with glucose oxidase, prepared by LbL assembly, when compared against LB films containing the same enzyme, showed significant advantages.[9] Increases in thickness in the former did not alter efficiency of the enzyme reaction, while the enzyme activity in thicker LB films was drastically reduced. Polyelectrolyte layers used in LbL assembly seem to be more permeable to the reaction precursor than the condensed lipid phase in LB films. Durability, thermostability, and stability against pH changes were also examined for the single component LbL films. The LbL reactor with glucose oxidase showed increased stability in all these aspects. Gentle fixing of the enzyme molecules within a soft polyelectrolyte cushion could be acting to prevent conformational changes even upon external perturbations of the reactor.

Sensor applications are some of the most important potential uses for LbL biomaterial assemblies. The LbL method can be easily used to fabricate thin films of active enzyme structures on solid surfaces of sensor device components such as electrodes or transistors. For example, Rusling and coworkers performed pioneering research in this field, which they described in a recent account.[10] They developed a detection system for DNA damage using DNA and enzyme (myoglobin or cytochrome P450).[11] The enzyme in the film generates the metabolite styrene oxide from styrene upon activation by hydrogen peroxide, and the resulting styrene oxide reacts with double stranded DNA in the same film. This process can be regarded as mimicking metabolism and DNA damage in human liver. Square wave voltammetry based on electrochemistry of a Ru-complex and a Co-complex was used to detect DNA damage. The methodology could find wide application for in vitro screening of organic precursor and metabolite toxicities.

Advanced medical applications

Because the LbL technique is very simple and versatile, many practical applications will be developed in the near future. Some biomedical applications in drug delivery and cell technology have already been realized.

Many approaches were developed to make capsule structures using the LbL method. The capsules can be used as carriers for controlled delivery and release of drugs.[12] For example, Lvov and coworkers devised a unique approach for DNA encapsulation at the interior of a biocompatible polyelectrolye microshell, with retention of the natural double-helix structure of the DNA (**Figure 3**).[13] DNA degradation is a serious problem in gene delivery. This makes it critical to encapsulate DNA in an appropriate carrier, prepared from environmentally friendly materials. In their method, $MnCO_3$ particles, used as the template core materials, were suspended in DNA solution. Addition of spermidine solution into the stirred $MnCO_3$/DNA mixture caused precipitation of a water-insoluble DNA/spermidine complex onto the $MnCO_3$ particles. The mixed component $MnCO_3$/DNA/spermidine cores were then covered by an LbL assembly comprised of biocompatible polyarginine and chondroitin sulfate. Subsequent core dissolution was carried out in two steps. First, $MnCO_3$ template particles were dissolved in deuterated 0.01 M HCl, resulting in biocompatible capsules containing DNA/spermidine complex, with the second step involving further treatment with 0.1 M HCl resulting in decomposition of the DNA/spermidine complex. After the second process, low molecular weight spermidine was released into the capsule interior to leave a DNA-entrapped biocompatible capsule. As the permeability of entrapped materials through the polyelectrolyte membranes can be regulated by perturbing factors, such as pH change, additional solvent, and temperature jump, controlled release of the trapped DNA is possible.

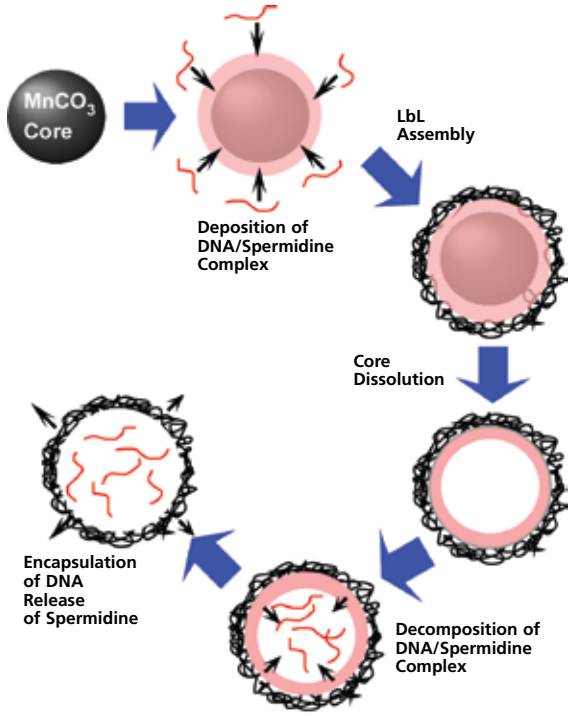

Figure 3. DNA entrapped in an LbL assembled capsule. Reprinted with permission from Shchukin, D. G.. et al. *J. Am. Chem. Soc.* **2004**, *126*, 3374. © 2004, American Chemical Society.

Use of planar LbL films in drug delivery applications was also proposed. For example, Lynn and coworkers prepared LbL films up to 100 nm thick containing plasmid DNA, encoded for enhanced green fluorescent protein, with a synthetic degradable cationic polymer on the surfaces of planar silicon and quartz substrates.[14] Upon degradation of the latter polymer, the plasmid DNA from the LbL films was released and

became transcriptionally active to promote the expression of high levels of enhanced green fluorescent protein in the cell. Recently, Yu, Ariga, and coworkers reported hollow-capsule-containing LbL films for controlled materials release.[15] The prepared films referred to as "mesoporous nano-compartment films" were composed of silica particles and hollow silica capsules (**Figure 4**). The resulting mesoporous nano-compartment films possess special molecular encapsulation and release capabilities so that stimuli-free, auto-modulated, stepwise release of water or drug molecules was achieved through the mesopore channels of robust silica capsule containers embedded in the film. Reproducible stepwise release of entrapped molecules was observed that originates in non-equilibrated rates between evaporation of water from the mesopore channels to the exterior and the capillary penetration of water from container interior to the mesopore channels. These nano-compartment films are promising materials for drug delivery since they allow gradual release of therapeutic agents with likely related improvements in their efficacy.

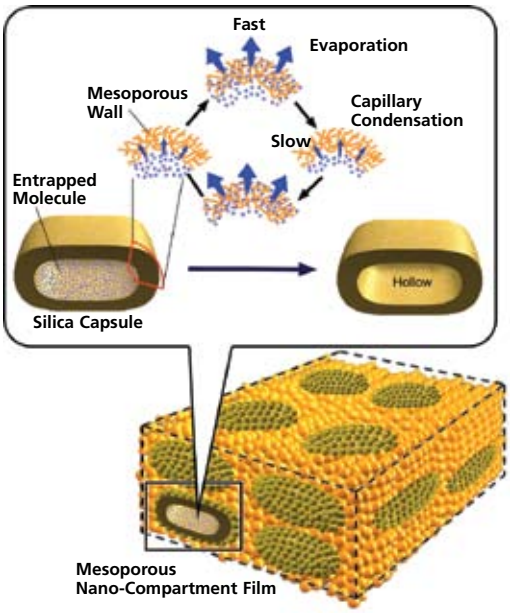

Figure 4. Mesoporous nanocompartment film. Reprinted with permission from Ji, Q. et al. *J. Am. Chem. Soc.* **2008**, *130*, 2376. © 2008, American Chemical Society.

Applications of the LbL method in cell technology have very demanding prerequisites. Several pioneering research works related to this have been summarized in a recent review by Kotov and coworkers.[16] For example, Jan and Kotov demonstrated the potential use of LbL films in stem cell technology, where the differentiation of environment-sensitive neural stem cells, both as neurospheres and single cells, on LbL films of carbon nanotubes and polyelectrolytes was examined.[17] Benkirane-Jessel and coworkers demonstrated control of cellular apoptosis by bone morphogenetic protein and its antagonist, Noggin, which were embedded in LbL films of poly-L-glutamate (P4761)and poly-L-lysine (P9404).[18] This is a good demonstration illustrating the possibility of in situ control of apoptosis during tooth differentiation, mediated by reagents embedded in a polyelectrolyte multilayer film. De Smedt and coworkers conducted in vivo studies on cellular uptake, degradation, and biocompatibility of LbL polyelectrolyte microcapsules that had been fabricated from dextran sulphate (**D6924**) and poly-L-arginine (**P7762**) layers on a template of calcium carbonate microparticles.[19] Most of the microcapsules were internalized by the cells and started to degrade within sixteen days after subcutaneous injection, indicating that LbL microcapsules made from degradable polyelectrolytes would be suitable for drug delivery.

Future perspectives

In this short review, we have briefly introduced several aspects of the LbL method involving biomaterials. Its mildness is one of its most pronounced characteristics and makes the method applicable to delicate biomaterials. Its other features of simplicity and versatility are important for the performance of gentle fabrication procedures. The LbL method can be combined with existing top-down microfabrication and nanofabrication strategies.[20] The simplicity of the LbL assembly method makes it compatible with microfabrication techniques such as photolithography, ink-jet lithography, and other patterning techniques for adaptation to a wide range of purposes. Fusion between the LbL method and top-down fabrication methods will lead to the integration of biomaterials into microfabricated structures and result in the next generation of bio-nanodevices and ultrafine biodevices, for example biosensor microarrays and micro-tip reactors.

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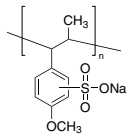
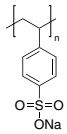
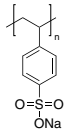
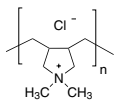
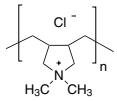
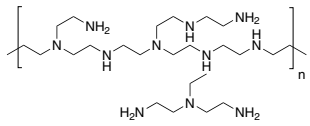
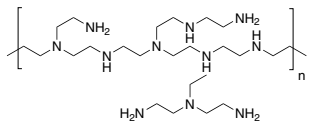
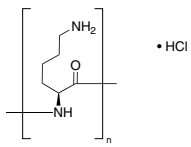
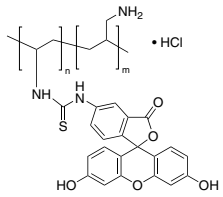
Polyelectrolytes

Anionic (negatively charged) and cationic (positively charged) polymers, commonly referred to as polyelectrolytes, are the key enabling materials for layer-by-layer (LbL) self-assembly. The following table gives a selection of materials commonly used in LbL research. For a complete list of polyelectrolytes and the latest products, visit ***sigma-aldrich.com/poly***.

Name	Structure	Property	Prod. No.
Anionic Polyelectrolytes			
Poly(anetholesulfonic acid, sodium salt)		Avg. M_v 9,000–11,000	444464-5G 444464-25G
Poly(sodium 4-styrenesulfonate) (PSS)		Avg. M_w ~ 70,000	243051-5G 243051-100G 243051-500G
		Avg. M_w ~ 1,000,000	434574-5G 434574-100G 434574-500G
Poly(sodium 4-styrenesulfonate) solution (PSS)		Avg. M_w ~ 70,000, 30 wt.% in H_2O	527483-100ML 527483-1L
		Avg. M_w ~ 200,000, 30 wt.% in H_2O	561967-500G 561967-4KG
		Avg. M_w ~ 1,000,000, 25 wt.% in H_2O	527491-100ML 527491-1L
Poly(vinyl sulfate), potassium salt		Avg. M_w ~ 170,000	271969-1G 271969-5G
Poly(vinylphosphonic acid, sodium salt) solution		25 wt.% in H_2O, technical grade	278424-5ML 278424-250ML 278424-1L
Poly(acrylic acid, sodium salt) (PAA)		Avg. M_w ~ 2,100	420344-100G 420344-500G
		Avg. M_w ~ 5,100	447013-100G 447013-500G
Poly(acrylic acid, sodium salt), solution (PAA)		Avg. M_w ~ 1,200, 45 wt.% in H_2O	416010-100ML 416010-500ML
		Avg. M_w ~ 8.000, 45 wt.% in H_2O	416029-100ML 416029-500ML
		Avg. M_w ~ 15,000, 35 wt.% in H_2O	416037-100ML 416037-500ML
Cationic Polyelectrolytes			
Poly(allylamine hydrochloride) (PAH)		Avg. M_w ~ 15,000 (vs. PEG std.)	283215-5G 283215-25G
		Avg. M_w ~ 70,000 (vs. PEG std.)	283223-1G 283223-5G 283223-25G
Poly(diallyldimethylammonium chloride) solution (PDDA)		Avg. M_w < 100,000 very low molecular weight, 35 wt.% in H_2O	522376-25ML 522376-1L

Name	Structure	Property	Prod. No.
		Avg. M_w 100,000–200,000 low molecular weight, 20 wt.% in H_2O	409014-25ML 409014-1L 409014-4L
		Avg. M_w 200,000–350,000 medium molecular weight, 20 wt.% in H_2O	409022-25ML 409022-1L 409022-4L
		Avg. M_w 400,000–500,000 high molecular weight, 20 wt.% in H_2O	409030-25ML 409030-1L 409030-4L
Polyethylenimine solution (PEI)		Avg. M_w ~ 1,300 (by LS), 50 wt.% in H_2O	482595-100ML 482595-250ML
		Avg. M_w ~ 2,000 (by LS), 50 wt.% in H_2O	408700-5ML 408700-250ML 408700-1L
		Avg. M_w ~ 750,000 (by LS), 50 wt.% in H_2O	181978-5G 181978-18KG 181978-100G 181978-250G
Polyethylenimine, branched (PEI)		Avg. M_w ~ 25,000 (by LS)	408727-100ML 408727-250ML 408727-1L
Poly-L-Lysine hydrochloride		Avg. M_w ~ 15,000–30,000	P2658-25MG P2658-100MG P2658-500MG P2658-1G
		Avg. M_w > 30,000	P9404-25MG P9404-100MG P9404-500MG P9404-1MG
Fluorescently Labeled Polyelectrolytes			
Poly(fluorescein isothiocyanate allylamine hydrochloride)		Avg. M_w ~ 15,000, PAH:fluorescein 50:1	630217-250MG
		Avg. M_w ~ 70,000, PAH:fluorescein 50:1	630209-250MG

"Click" Chemistry in (Bio)Materials Science



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Introduction

Combining biomolecules and synthetic polymers into a new class of versatile biohybrid materials with many (future) fields of application, has gained much interest in recent years. One of the reasons is the current availability of a synthetic toolbox to conjugate biomolecules and synthetic polymers in a controlled fashion. A particularly useful candidate from this toolbox is the copper(I)-catalyzed azide-alkyne cycloaddition, or "click" chemistry methodology, due to the efficiency and specificity of this reaction, along with the possibility of introducing the required functionalities in polymers of both biological and synthetic origin. In this article, an overview is given of the application of "click" chemistry in macromolecular engineering and the synthesis of polymer biohybrids.

Polymer bioconjugate synthesis

Natural polymers like DNA and proteins possess a level of structural control that is undisputedly superior to current synthetic materials. The well-defined three-dimensional organization stems from a highly controlled arrangement of nucleotides or amino acids, and gives biopolymers their specific functionality. This three-dimensional structure is often responsible for the function associated with a biopolymer, and any conformational changes that can readily occur will result in a loss of function. Synthetic polymers, on the other hand, are lacking this absolute level of control, yet they are easier to prepare in a wide range of distinct topologies and compositions adapted to a specific application environment.

A logical strategy that has surfaced recently is to combine the structural control of biopolymers, leading to properties like programmed assembly, recognition and bioactivity, with the versatility of synthetic polymers. Most of the currently applied biohybrid polymers are synthesized using traditional chemistry, which relies on reactivity towards amine or thiol groups present in lysine and cysteine residues of proteins, respectively. Although impressive results already have been obtained with the biohybrids, these residues, especially lysine, are commonly present in proteins, resulting in multiple additions of the synthetic polymer building blocks. Hence, in order to prepare well-defined biohybrid polymers, very specific coupling chemistry is demanded, which is inert towards other functionalities present in biomolecules.

In this respect, "click" chemistry is highly suitable for the conjugation of biomolecules and synthetic polymers. "Click" chemistry is a term coined by Sharpless for a set of chemical reactions that are *modular, wide in scope, give very high yields*, and generate only *inoffensive byproducts* that can be removed by nonchromatographic methods.[1] The most well known example of a "click" reaction is the copper(I)-catalyzed azide-alkyne cylcoaddition (CuAAC), which yields a 1,4-disubstituted five-membered 1,2,3-triazole ring[2], as depicted in **Figure 1**. This reaction between azides and alkynes offers high yields, and other functionalities do not interfere with the reaction pathway. Both azide and alkyne functionality can be introduced at specific locations in biomolecules and synthetic polymers using currently available techniques. This has allowed "click" chemistry to revolutionize the fields of polymer chemistry and bioconjugation. Some of these advances will be highlighted in the following sections.

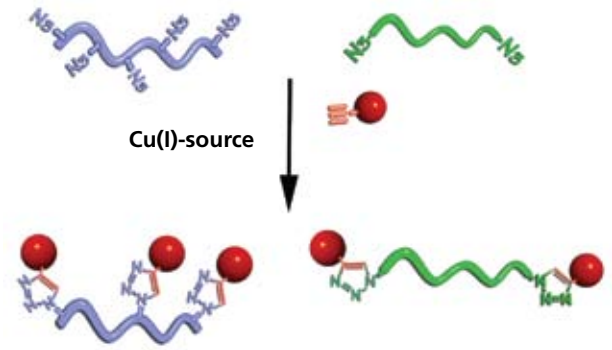

Figure 1. Schematic illustration of the functionalization of side and end groups of polymers using "click" chemistry.

The emerging role of "click" chemistry in macromolecular engineering

Living/controlled polymerization techniques that are currently available, such as ring-opening metathesis polymerization (ROMP), nitroxide mediated radical polymerization (NMP), reversible addition-fragmentation chain transfer polymerization (RAFT) and atom transfer radical polymerization (ATRP) allow precise control over the polymerization process; the degree of polymerization can be predetermined and the polydispersity is low. Furthermore, tailoring of macromolecular architectures with respect to functionality in the side chains and end groups, composition (*e.g.* block, graft and gradient copolymers) and topology (*e.g.* comb, star and dendritic structures) is possible.[3] Because of the fact that azide and alkyne moieties can be readily introduced at different locations in polymer chains by adopting controlled polymerization methodologies, the highly efficient CuAAC reaction has become a powerful tool in macromolecular engineering.[4–8]

The CuAAC reaction has been applied to modularly construct a myriad of well-defined polymer topologies from azide and alkyne functionalized building blocks, in high yields. In **Figure 2**, a selection of synthesized architectures is given, ranging from block, graft and star copolymers to polymer networks like hydrogels. For example, by application of

ATRP it was demonstrated that azide and alkyne groups can be introduced at polymer chain ends.[9] This was achieved by using initiators containing the alkyne functionality, and by post-polymerization substitution with an azide of the halogen, which is always present at the terminus of a polymer prepared via ATRP (see application note on p. 66). This resulted in functionalized polymer building blocks which could be coupled in a quantitative fashion using a copper(I)-catalyst.

Since it is also possible to introduce an azide as well as an acetylene end group in the same polymer building block, the "click" coupling concept was extended to allow consecutive functionalization of both end groups of a single polymer.[10] In order to selectively perform the CuAAC reaction on one end of a polymer, the acetylene moiety at the other terminus was protected with a triisopropylsilyl (TIPS) group that could be easily removed afterwards making it accessible for the second "click" coupling. Using this modular approach a poly(methyl acrylate)-*block*-polystyrene-*block*-poly(*tert*-butyl acrylate) ABC-type triblock copolymer (see reaction I in Figure 2) was prepared.[12] An extension of this modular approach is having blocks prepared by different polymerization mechanisms which are also linked relatively easily.

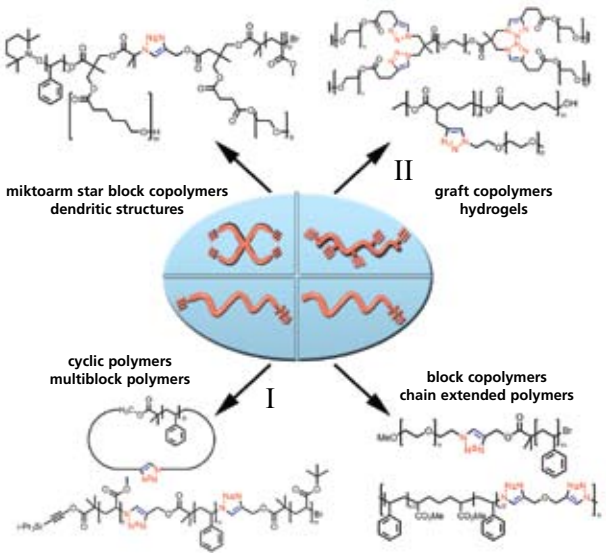

Figure 2. Examples of macromolecular architectures prepared by "click" chemistry: block copolmyers[9,10], chain extended polymers[11], cyclic polymers[12], graft copolymers[13], hydrogels[14] and miktoarm star block copolymers[15].

Apart from generating a variety of polymer architectures, the CuAAC reaction can also be exploited to introduce functionality along polymer chains and in networks.[4–6] Bulky pendant groups can be grafted onto polymer chains by employing azide or alkyne functionalized monomers, (see reaction II in Figure 2), something that can be problematic when polymerizing pre-functionalized monomers. In general, controlled polymerization techniques and "click" chemistry form a powerful couple with almost unlimited possibilities for the introduction of different functionalities in polymer side chains and end groups.

Biohybrid polymer synthesis via "click" chemistry

In addition to the fact that the CuAAC reaction is very efficient, making it possible to couple large molecules such as polymers, in high yields, the "click" process is also highly specific. This implies that the used azide and alkyne groups are inert towards other functionalities and merely react with each other in the presence of a copper(I)-catalyst. This specificity combined with the fact that the reaction can be performed in an aqueous environment at ambient temperatures, makes "click" chemistry an ideal tool for the conjugation of biomolecules, like peptides, proteins, carbohydrates and DNA, to synthetic polymers.[16,17] One of the first examples of this approach was the conjugation of an azide terminated polystyrene and the alkyne-functionalized protein bovine serum albumin (BSA),[18] as depicted in **Figure 3**. For this purpose, the azide end functionality was quantitatively introduced by nucleophilic substitution of the halogen terminus of polystyrene, which was prepared by ATRP. The required alkyne function was introduced by Michael addition of an alkyne bearing maleimide to the thiol functionality of a cysteine residue (Cys-34) located on the exterior of BSA, yielding a singly alkynated protein. Subsequently, a "click" reaction was performed between the synthetic polymer and the protein by addition of cupric sulfate and ascorbic acid which serves as a reductor to generate the catalytic Cu(I)-species *in situ*. Interestingly, as a consequence of the amphiphilic character of the isolated biohybrid polymer, micelles were formed in an aqueous environment as established with transmission electron microscopy (Figure 3).

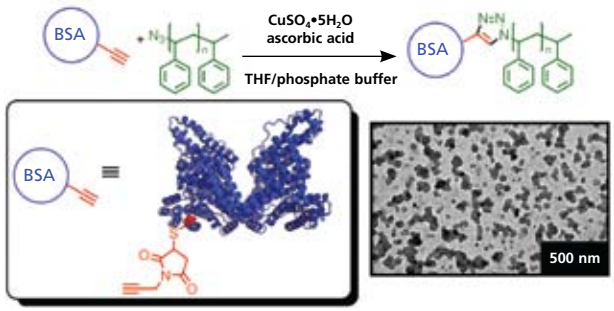

Figure 3. Illustration of the "click" reaction between polystyrene and the protein bovine serum albumin (BSA).[18] The resulting amphiphilic biohybrid displayed micelle formation in an aqueous environment as visualized in the transmission electron microscopy image. Reprinted with permission from Ref. (18). Copyright 2005 The Royal Society of Chemistry.

In addition to the bioconjugation of proteins, the CuAAC reaction has also been used to couple carbohydrates, such as mannose and galactose, to linear and dendritic polymers.[19,20] These biohybrids, containing multivalent binding sites, can be adopted to interfere in very specific pathways in cell-cell recognition and cell-protein interaction processes. Moreover, carbohydrates serve as targeting ligands for hormones, antibodies and toxins, making these materials suitable candidates for use in medicine or as biosensors. Additionally, "click" reactions have been performed onto more complex biological entities such as viruses, bacteria and cells, clearly underlining the power of this chemistry.

To maintain the function of biomolecules as well as the reproducibility of the biohybrid synthesis, it is of the utmost importance that the necessary alkyne and azide groups be incorporated at desired locations in biomolecules via protein engineering techniques. One approach is the so-called multi-site replacement, in which auxotrophic bacterial strains that lack the ability to produce one of the proteinogenic amino acids, are used. If a non-canonical amino acid, *e.g.* azidohomoalanine, is added to the growth medium, it can be incorporated in place of the natural substrate.[21] This methodology has been applied to introduce azide functionality in the enzyme *Candida antarctica* Lipase B (CalB) which was subsequently coupled by means of a CuAAC reaction to poly(ethylene glycol) containing an alkyne terminus.[22]

Functionalizing molecular assemblies

The application of click chemistry in bioconjugation reactions not only works well on molecularly dissolved species, but can also be used to functionalize molecular assemblies. For example, amphiphilic block copolymers are capable of self-assembling into vesicular structures in solution. These polymer vesicles, also referred to as polymersomes, are spherical shell structures that exhibit remarkable stability and can be used to encapsulate a wide variety of compounds. For these nanocontainers to serve as drug delivery vehicles or nanoreactors, they would need to be equipped with targeting ligands or enzymes. To illustrate this approach, polystyrene-*block*-poly(acrylic acid) (PS-*b*-PAA) was prepared and the bromide terminus substituted for an azide moiety.[23] Upon slow addition of water to a solution of the block copolymer in dioxane, this amphiphilic block copolymer self-assembled into polymersomes with the azide functionalities exposed on the outside of the vesicle. After extensive dialysis against water to remove the organic solvent, several alkyne-functionalized substrates, including enhanced green fluorescent protein (eGFP), were coupled to the exterior of the vesicles (**Figure 4**). The fluorescent behavior of the eGFP-tethered polymersomes was visualized by confocal laser-scanning microscopy, as illustrated in Figure 4. In a control experiment in which the addition of a copper-catalyst was omitted, no fluorescence was observed leading to the conclusion that eGFP was covalently bound to the vesicles.

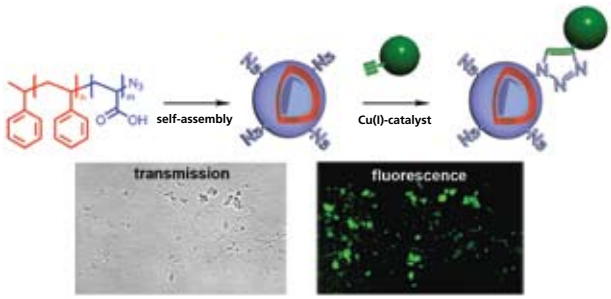

Figure 4. Schematic representation of the polymersome formation from azide functionalized polystyrene-*block*-poly(acrylic acid) and the subsequent functionalization of its periphery with enhanced green fluorescent protein using "click" chemistry, as visualized by confocal laser-scanning microscopy.[23] Reprinted with permission from Ref. (23). Copyright 2007 The Royal Society of Chemistry.

In subsequent research, semiporous polymersomes composed of polystyrene-*block*-poly[*L*-isocyanoalanine(2-thiophen-3-yl-ethyl)amide] (PS-*b*-PIAT) were functionalized at the periphery with the enzyme CalB by employing "click" chemistry.[24] Since in this case the polar PIAT block could not be equipped with a desired alkyne moiety, an alkyne terminated polystyrene-*block*-poly(ethylene glycol) block copolymer was coaggregated within the vesicles, thereby providing a handle for further functionalization. The enzyme retained its activity after attachment to the polymersome.

Opportunities in biomaterial science

Biohybrid polymers have been recognized for many years as versatile materials for application in drug delivery, nanotechnology and bioengineering. Since pure biomolecules are susceptible to conformational changes which, in general, involve loss of function, bioconjugate research will be an important theme in the coming years, leading to new and improved material applications. In this respect, "click" chemistry has a promising future as it has been proven to be a very efficient, specific and biologically compatible method for coupling two compounds. Together with parallel advances in polymer chemistry and protein engineering scientists now have a comprehensive toolbox with which to prepare biohybrid macromolecules with well-defined architecture and properties.

References

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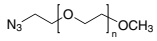
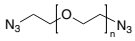
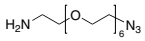
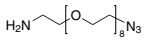
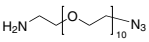
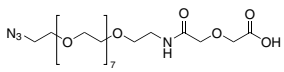
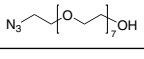
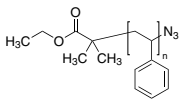
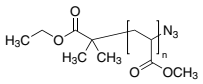
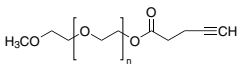
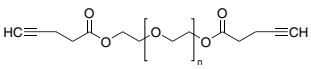
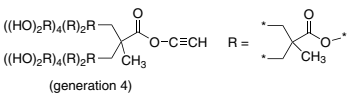
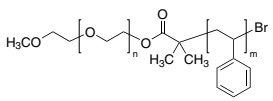
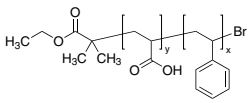
Click-Compatible Biomaterials

For a complete selection of "click" chemistry reagents, please visit ***sigma-aldrich.com/click***.

Name	Structure	Molecular Weight (Avg. M_n)	Prod. No.
Azide-Functionalized			
Methoxypolyethylene glycol azide		2000, M_w/M_n < 1.2	689807-250MG
			689807-1G
		5,000, M_w/M_n < 1.2	689475-250MG
			689475-1G
Polyoxyethylene bis(azide)		2000, M_w/M_n < 1.2	689696-250MG
			689696-1G
		5,000, M_w/M_n < 1.2	689580-250MG
			689580-1G
O-(2-Aminoethyl)-*O'*-(2-azidoethyl) pentaethylene glycol		350	76172-500MG
O-(2-Aminoethyl)-*O'*-(2-azidoethyl) heptaethylene glycol		440	76318-500MG
O-(2-Aminoethyl)-*O'*-(2-azidoethyl) nonaethylene glycol		530	77787-500MG
O-(2-Azidoethyl)-*O*-[2-(diglycolyl-amino) ethyl]heptaethylene glycol		550	71613-500MG
O-(2-Azidoethyl)-heptaethylene glycol			689440-250MG
Polystyrene, azide terminated		2,000, M_w/M_n < 1.3	699772-500MG
Poly(methyl acrylate), azide terminated		2,000, M_w/M_n < 1.3	699764-500MG
Acetylene-Functionalized			
Poly(ehtylene glycol) methyl ether, acetylene terminated		2,000	699802-500MG
Poly(ethylene glycol), bis-acetylene terminated		2,000	699810-500MG
Polyester-8-hydroxyl-1-acetylene bis-MPA dendron	 (generation 4)	869, generation 3	686646-250MG
		1,798, generation 4	686638-250MG
		3,656, generation 5	686611-250MG
Polymersome-Forming Polymers			
Poly(styrene)-*block*-(poly(ehtylene glycol)		800–1,200 (PEG) 20,700–25,300 (PS)	686476-500MG
Poly(styrene)-*block*-poly(acrylic acid)		1,890–2,310 (PAA) 5,580–6,820 (PS)	686794-500MG

Introduction of Terminal Azide and Alkyne Functionalities in Polymers

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"Click" chemistry, and the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) in particular, is a powerful new synthetic tool in polymer chemistry and material science. Success of the CuAAC in the engineering of (bio)polymer architectures stems, in part, from the possibility of introducing the required azide and alkyne functionalities at predetermined locations in macromolecular building blocks, is a result of advances in controlled polymerization techniques.

Controlled polymerizations result in polymers with well-defined end-groups, which can be subsequently converted into terminal azide and alkyne functionalities. Examples demonstrating conversion of the hydroxyl terminus of poly(ethylene glycol) (PEG) (**295906**) into an azide or an alkyne terminal polymer are depicted in **Scheme 1**. The azide functionality can be introduced by treating the alcohol with pyridine (**676772**) and tosyl chloride (TsCl) (**240877**) to afford the easily substitutable tosyl-activated alcohol, which is subsequently reacted with sodium azide (NaN_3) (**438456**) to provide the terminal azide polymer. The alkyne is introduced on PEG via esterification with pentynoic acid (**232211**) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (**E7750**) and 4-dimethylaminopyridine (DMAP) (**522805**) (**Scheme 1**).[1]

The atom transfer radical polymerization (ATRP) process generally yields halide-terminated polymers and therefore this method can be exploited for further functionalization due to the inherent susceptibility of halides to undergo nucleophilic substitution reactions.[2] For example (**Scheme 2**), after ATRP, the terminal bromide of polystyrene (PS) can be quantitatively exchanged for an azide moiety by treatment with azidotrimethylsilane (Me_3SiN_3) (**155071**) and tetrabutylammonium fluoride (TBAF) (**216143**).[1,3]

Success of post-polymerization end-group modification procedures depends on suppression of termination reactions during polymerization. Additionally, the end-group manipulation must be quantitative to prevent incomplete introduction of the desired functionality. Functional initiators can be useful in implementing a quantitative introduction of the functionality, with the condition that no side reactions occur during the polymerization process.

An example of using a functional initiator to incorporate an alkyne functionality is shown in **Scheme 3**. The alkyne bearing α-bromoester initiator is protected with a TIPS protecting group to prevent complexation with the copper-catalyst during ATRP. The azide can be used for a "click" reaction and the TIPS group can be removed following polymerization, allowing the alkyne to be used for a second "click" reaction.[4]

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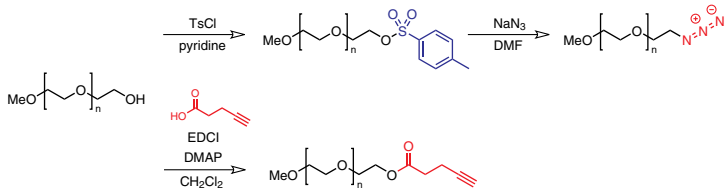
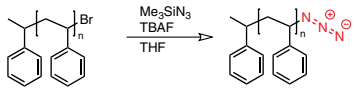
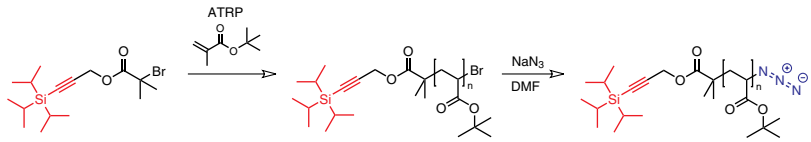


Scheme 1. Transformation of the hydroxyl terminus of poly(ethylene glycol) into azide and alkyne functionalities.[1]



Scheme 2. Polystyrene prepared by ATRP contains a bromide end group which can be readily substituted for an azide.[1,3]



Scheme 3. Synthesis of heterotelechelic poly(tert-butyl acrylate) comprising both a protected alkyne and an azide terminus.[4]

Chemistry at Surfaces with Self-Assembled Monolayers and SAMDI-ToF Mass Spectrometry

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Modern synthetic chemistry permits the construction of elaborate molecular structures and has been vital to the development of pharmaceuticals, catalysts, and functional polymers. Chemists no longer ask whether, but rather how efficiently, a target structure can be prepared. A vastly different expectation holds when these same reactions are applied to the elaboration of surfaces. While surfaces having well-defined structures and that present a broad range of functional groups are easily prepared, the difficulty in characterizing the products of interfacial reactions makes it difficult to carry out even the simplest transformations. Ironically, interfacial reactions are substantially easier to perform than corresponding homogeneous phase reactions—because the reaction workup requires only that the surface be rinsed of the reaction mixture; however, the characterization of products, yields and rates is enormously more difficult. For reactions performed in solution, the products are easily isolated, purified and characterized using NMR, IR and other spectroscopic methods. The small amount of product present when these same reactions are performed at a two-dimensional interface makes these analytical tools useless and instead requires the use of several methods that are sensitive but limited in the structural information they provide.[1]

This article describes the combination of **s**elf-**a**ssembled monolayers with **m**atrix-assisted laser **d**esorption-**i**onization **m**ass **s**pectrometry—a technique termed SAMDI MS—that permits a rapid characterization of products resulting from interfacial reactions.[2] The self-assembled monolayers are an attractive platform for surface chemistry because they are easily prepared by immersing a gold-coated substrate in a solution of terminally-substituted alkanethiols under normal laboratory environments and because they are synthetically flexible. This latter property stems from the compatibility of the assembly process with alkanethiols carrying a wide range of terminal functional groups—since the chemisorption of thiol for gold is quite specific—and because the resulting monolayers are thermally stable and compatible with a wide range of solvents and reagents.[3] The growing availability of alkanethiol reagents from commercial suppliers (**see Table on page 70 of this issue**) and the lack of a need for significant instrumentation and facilities make these substrates accessible to synthetic chemists. The development of the SAMDI MS technique finally provides a rapid and routine method of characterizing reactions of molecules attached to self-assembled monolayers and could prompt a significant growth of basic and applied research in molecular surface chemistry.

The first examples of characterizing self-assembled monolayers with laser desorption mass spectrometry came from the laboratories of Hemminger, Fritsch, Wilkins and Hanley.[4–6] Fritsch and Wilkins, for example, used a 308 nm laser to desorb monolayers and were able to observe alkanesulfonates that result from oxidation of alkanethiolates. At the time of that work, the limited availability of commercial instruments for laser desorption mass spectrometry and the still limited number of examples of interfacial reactions on monolayers prevented a wider adoption of the method. Some years later, when challenged by the difficulties in characterizing interfacial reactions, we found that commercial instruments for matrix-assisted laser desorption-ionization mass spectrometry could be applied to self-assembled monolayers that were functionalized with a broad range of molecular groups.[7] In a typical experiment, a solution of the common matrix molecules used in SAMDI MS is applied to a monolayer and allowed to dry. Irradiation of the monolayer with a nitrogen laser results in efficient release of the alkanethiols—or the analogous disulfides—from the gold substrate and reveals the masses of these molecules (**Figure 1**). In a first example, we used this to characterize the cycloaddition of pentamethylcyclopentadiene to a monolayer-tethered maleimide group, the formation of an amide, and the deprotection of a *tert*-butyl ester. In each case, the SAMDI spectrum showed peaks that correspond to the substituted alkanethiols, or their disulfides, before and after the reaction. SAMDI complements other spectroscopic techniques in that it provides information on the total mass of the alkanethiols, rather than on the identities of functional groups present in the molecules. Further, the rapid acquisition of a SAMDI spectrum—the time to load and analyze a sample is less than ten minutes—makes this method useful for assessing reaction yields and confirming the presence of the anticipated product.

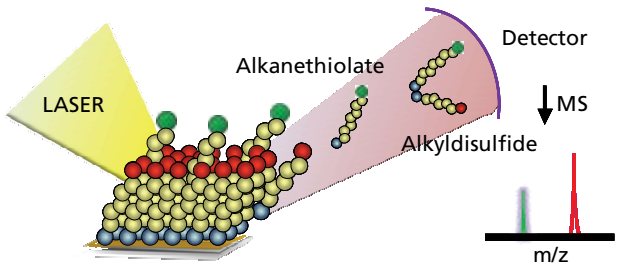

Figure 1. Self-assembled monolayers can be characterized using matrix-assisted laser desorption-ionization mass spectrometry in a technique termed SAMDI MS. A nitrogen laser is used to desorb the monolayer to give alkanethiolate and the corresponding disulfide molecules. The mass spectrometer reports the mass-to-charge ratio for these molecules and can provide information on the products, yields and rates of interfacial reactions.

Since this early report, SAMDI has been used in a broad range of applications, including the characterization of products resulting from electrochemical reactions of the monolayer,[8] the combinatorial discovery of reactions,[9] and many examples of enzyme-mediated transformations.[10–13] Three examples, described below, demonstrate the unique capability that SAMDI offers in characterizing the products that result from interfacial reactions at self-assembled monolayers, and in turn enabling a broad range of studies that use functionalized surfaces.

The first example addresses the reactions of monolayers that present a terminal alkyne functional group. The base-promoted exchange of the terminal hydrogen atom with a deuterium atom represents a straightforward reaction yet one that is very challenging to characterize when performed on a monolayer. The SAMDI spectrum of a monolayer prepared from a mixture of an alkyne-terminated and a methyl-terminated monolayer revealed peaks corresponding to the mixed and the symmetrical disulfides. Treatment of the monolayer with sodium hydride and then deuterium oxide afforded exchange of the terminal hydrogen atom, as verified by the increase in mass of the disulfides by one and two Dalton, respectively (**Figure 2**). This example reveals both the straightforward application of mass spectrometry to characterizing an interfacial reaction and also the good mass resolution inherent to the method. This method was also used to demonstrate the hydration of the alkyne to give a methylketone and a Sonagashira coupling to give the phenylacetylene.

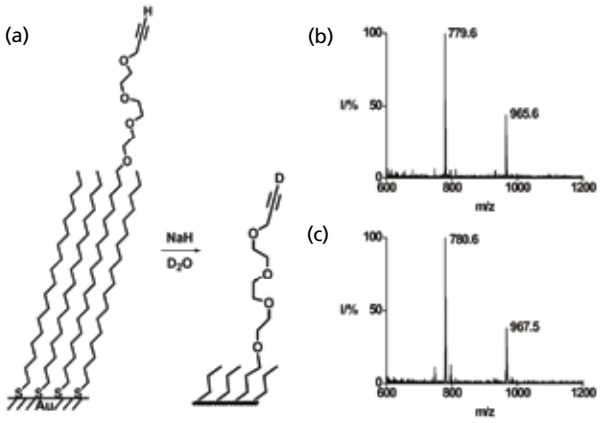

Figure 2. SAMDI MS is used to characterize the exchange of hydrogen for deuterium in a terminal alkyne. The mass spectrum prior to reaction shows a peak at m/z of 779.6 that shifts to 780.6 after the reaction. The corresponding disulfide shows the expected mass increase of two Daltons.

The second example addresses the development of multi-step synthetic sequences for elaborating the structures of self-assembled monolayers. Monolayers are frequently used in 'biochip' applications, where surfaces that present an array of peptides, carbohydrates or small molecules are used to identify substrates for enzymes or ligands for proteins from a large set of possible interactions.[14] In these applications, the molecules that are tethered to the monolayer are often first synthesized using established methods and then immobilized to a monolayer using appropriate conjugation chemistries. For certain classes of molecules—for example, oligosaccharides—the time and expense required to prepare the molecules limit the sizes of arrays that can be prepared.

Early work with peptide and oligonucleotide arrays took advantage of methods to directly synthesize the molecules on the substrate and thereby rapidly and efficiently assemble arrays of hundreds to thousands of biomolecules on the surface.[15] These examples required a substantial effort to develop and optimize the sequences of interfacial reactions used in array synthesis, with the most significant challenges being in characterizing the products and yields of the interfacial reactions. We recently used SAMDI to develop routes to preparing arrays of oligosaccharides by performing multi-step syntheses directly on the monolayer.[16]

The strategy used to synthesize an array of disaccharides is shown in **Figure 3** and begins with a monolayer that presents a phenol group at a density of 5% against a background of tri(ethylene glycol)-terminated alkanethiolates. The former serves as the nucleophile for attaching the carbohydrate building blocks and the latter are effective at preventing the non-specific adsorption of protein and therefore are important for subsequent biochemical assays of the immobilized saccharides.[17] A monosaccharide that was triacetylated and had the fourth hydroxyl group protected as the levulinate ester—to permit a selective deprotection in a following step—was prepared as its trichloroacetimidate and activated with trimethylsilyl trifluoromethanesulfonate for coupling to the phenol group. The monolayer was then treated with hydrazine to remove the levulinate group and then treated with a second monosaccharide reagent to give the fully protected disaccharide. Treatment with sodium methoxide led to removal of the acetyl groups and gave the immobilized disaccharide. SAMDI spectra showed that each step proceeded in high yield and gave the expected products (Figure 3). This sequence was applied to the preparation of an array of twenty four distinct disaccharides—which could be accomplished in less than six hours—and then used to profile the substrate specificities of a glycosyltransferase enzyme. Again, the use of mass spectrometry allowed the products of the enzyme reaction to be identified directly.

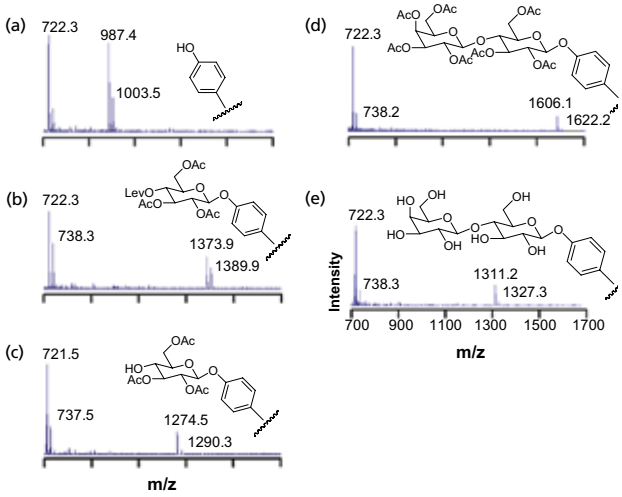

Figure 3. SAMDI MS was used to characterize the surface after each step of a disaccharide synthesis on a monolayer substrate: (a) monolayer presenting the phenol group; (b) coupling of the first carbohydrate; (c) selective removal of the levulinate protecting group; (d) coupling of the second carbohydrate; (e) final deprotection.

A third example addresses reactions of DNA oligonucleotides. DNA arrays that contain tens of thousands of distinct sequences are now commonly used to profile patterns of gene expression in cell cultures, and more recently have been used to identify protein-DNA binding interactions.[18] The arrays use fluorescent labels to detect binding interactions and for this reason have not been applicable to studies of chemical reactivity including, for example, the covalent adducts formed on treatment of DNA with reactive small molecules. Our approach to use SAMDI to characterize the reactions of immobilized DNA begins with a monolayer that present a maleimide group at 5% density against a background of tri(ethylene glycol) groups.[19] To attach the DNA, we first immobilized a biotin-terminated alkanethiol and then bound a streptavidin protein to give a surface that was used to capture a biotin-labeled oligonucleotide duplex. We treated an array of duplexes with the anti-cancer drug cis-platin and used SAMDI to identify the mono- and di-adducts formed in the reaction (**Figure 4**). The flexibility of the SAMDI method, together with the mature methods for preparing and applying DNA arrays may enable a significant extension of the applications that can be pursued with the arrays.

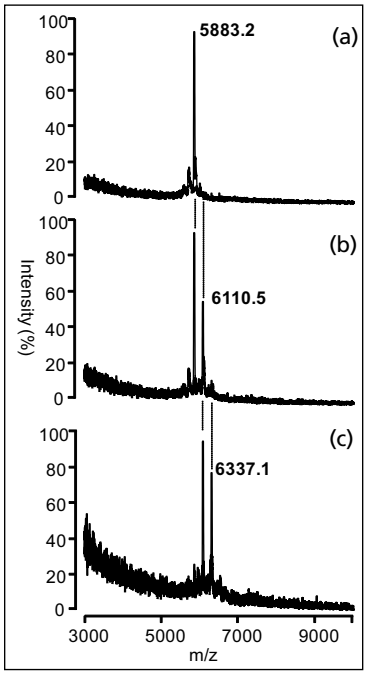

Figure 4. SAMDI MS was used to characterize the products resulting from reaction of a biotinylated duplex DNA (5′-biotin-TTT TAT ATA CGT ATA TCG) with *cis*-[$Pt(NH_2)_2Cl_2$]. Spectra are shown for reaction times of 0, 4 and 21 hr (A, B and C, respectively).

The examples summarized in this review illustrate the value of SAMDI for rapidly assessing the products and yields of interfacial reactions at self-assembled monolayers. No methods, or combinations thereof, yet exist that can provide comprehensive information on the structures of molecules attached to surfaces, as do the techniques of NMR and x-ray diffraction for molecules prepared in larger quantities. Currently, studies in surface chemistry instead use several methods to assemble an understanding (often times incomplete) of interfacial structure, including infrared spectroscopy to identify functional groups, x-ray photoelectron spectroscopy to determine the elemental composition and ellipsometry to measure the thickness of a monolayer. By providing the molecular weight of the alkanethiols, SAMDI complements these methods and provides molecular information that is not available with other methods. Most significantly, this method is straightforward to use and provides the synthetic chemist with information that can be used to develop and implement a wide range of chemistries on monolayers. These benefits should enable the development of surfaces having complex structures for basic and applied studies in a broad range of disciplines.

References

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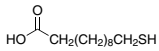
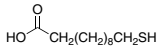
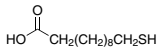
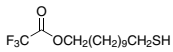
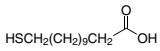
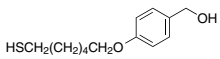
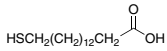
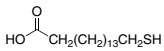
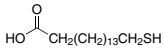
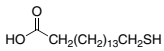
Materials for Molecular Self-Assembly on Gold Surfaces

The following thiols can be used to create functional self-assembled mono-layers (SAMS) on gold surfaces. For a complete selection of self-assembly materials, visit ***sigma-aldrich.com/selfassembly***.

Chain Length	Name	Purity	Structure	Prod. No.
10	1-Decanethiol	96%	$CH_3(CH_2)_8CH_2SH$	D1602-50ML D1602-250ML
	11-Mercaptoundecanoic acid	99%		674427-500MG
	11-Mercaptoundecanoic acid	95%		450561-5G 450561-25G
	NanoThinks™ ACID11	Ethanol solution		662925-100ML
	1H,1H,2H,2H-Perfluorodecanethiol	97%	$CF_3(CF_2)_7CH_2CH_2SH$	660493-5G 660493-25G
11	11-Amino-1-undecanethiol hydrochloride	99%	$HSCH_2(CH_2)_9CH_2NH_2 \cdot HCl$	674397-50MG
	11-Mercapto-1-undecanol	99%	$HSCH_2(CH_2)_9CH_2OH$	674249-250MG
	11-Mercapto-1-undecanol	97%	$HSCH_2(CH_2)_9CH_2OH$	447528-1G 447528-5G
	11-Mercaptoundecyl trifluoroacetate	99%		674230-50MG
	NanoThinks™ ALCO11	Ethanol solution	$HSCH_2(CH_2)_9CH_2OH$	662224-100ML
	1-Undecanethiol	98%	$CH_3(CH_2)_9CH_2SH$	510467-5G
12	1-Dodecanethiol	≥98%	$CH_3(CH_2)_{10}CH_2SH$	471364-100ML 471364-500ML 471364-2L 471364-18L
	tert-Dodecylmercaptan	98.50%		471585-100ML 471585-500ML 471585-2L 471585-18L
	12-Mercaptododecanoic acid	96%		675067-1G
	4-(6-Mercaptohexyloxy)benzyl alcohol	97%		673560-50MG
14	15-Mercaptopentadecanoic acid	97%		675091-5G
	1-Tetradecanethiol purum	≥98.0%	$CH_3(CH_2)_{12}CH_2SH$	87193-5ML 87193-25ML
15	16-Mercaptohexadecanoic acid	99%		674435-250MG
	16-Mercaptohexadecanoic acid	90%		448303-1G 448303-5G
	NanoThinks™ ACID16	Ethanol solution		662216-100ML
	1-Pentadecanethiol	98%	$CH_3(CH_2)_{13}CH_2SH$	516295-1G
16	1-Hexadecanethiol	99%	$HS{-}CH_2(CH_2)_{14}CH_3$	674516-500MG

Chain Length	Name	Purity	Structure	Prod. No.
18	NanoThinks™ 18	Ethanol solution	$CH_3(CH_2)_{16}CH_2SH$	662194-100ML
	1-Octadecanethiol	98%	$CH_3(CH_2)_{16}CH_2SH$	01858-25ML 01858-100ML
20	Triethylene glycol mono-11-mercaptoundecyl ether	95%	$HSCH_2(CH_2)_9CH_2O$–(O–CH$_2$–CH$_2$)$_3$–OH	673110-250MG
23	[1-(Methylcarbonylthio)undec-11-yl] tetra(ethylene glycol)	95%	H_3C–C(=O)–S–$(CH_2)_{11}$–(O–CH$_2$–CH$_2$)$_4$–OH	674176-250MG
24	(1-Mercaptoundec-11-yl) tetra(ethylene glycol)	95%	$HSCH_2(CH_2)_9CH_2O$–(O–CH$_2$–CH$_2$)$_4$–OH	674508-250MG
29	(1-Mercaptoundec-11-yl) hexa(ethylene glycol)	96%	$OCH_2(CH_2)_9CH_2SH$–(O–CH$_2$–CH$_2$)$_6$–OH	675105-250MG

Bone Tissue Engineering Based on Calcium Phosphate Ceramics

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Bone is a composite material with a remarkable combination of elasticity and stability. The tissue is composed of extracellular inorganic (≈50–60 wt. %) and organic (≈30–40 wt. %) matter, water (≈10 wt. %), and several cell types. This extracellular matrix (ECM) is produced by cells called osteoblasts, and mostly consists of bioapatite and collagen. The bone mineral can be characterized as a carbonated apatite consisting of hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) with about 4–8% CO_3^{2-} and other trace elements. Collagen Type I is the largest component of the bone tissue. The platelet-like bioapatite crystals are inserted in a parallel fashion into the collagen fibrils, replacing the water found in other tissue collagens (**Figure 1**).

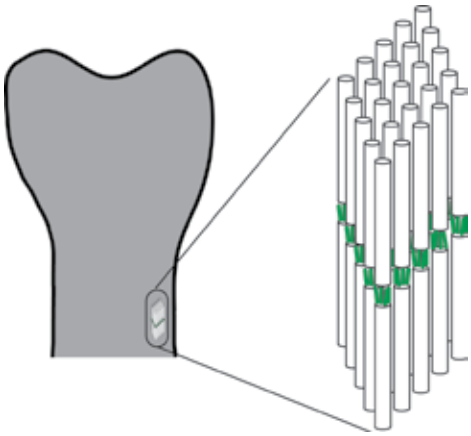

Figure 1. Schematic drawing of a mineralized collagen fiber. Crystalline bone mineral (green) is incorporated where collagen triple helices (white cylinders) meet.

In principle, bone has a good self-healing capacity. However, for defects larger than a certain size ("critical size defect") spontaneous healing of bone injury is not possible. Such defects can occur in many diseases such as osteoarthritis, bone cysts and tumors, or as a result of surgical procedures, for instance osteolyses associated with loosened endoprostheses or osteotomies. The gold standard for the treatment of bone defects is an autologous bone transplantation; however, disadvantages of the method include follow-up operations that are necessary in addition to the bone harvesting from the iliac crest, which are connected with significant comorbidity.[1] Availability of natural bone is restricted and often not sufficient to heal large bone defects, while maintenance of an extensive bone bank is expensive and complicated by long-term tissue preservation issues. To overcome these difficulties, a number of synthetic and partial synthetic bone substitute materials have been developed. In clinical practice, the most important class of materials are HA ceramics, because the inorganic component of the bone matrix consists largely of HA. Materials such as HA obtained typically from marine coral,[2] glass-reinforced HA,[3] brushite,[4] tricalcium phosphate,[5] and mixtures of these materials (composites)[6] are in use. Of central importance is the structure of the ceramics because an interconnecting pore system is required for reasonable osseous integration.[7] A resorption of pure HA ceramics and substitution with bone (*restitutio ad integrum*) does not take place. However, bioceramics made of HA and tricalcium phosphate (TCP) provide better scaffold resorption.[8]

While there are a number of available bone substitution materials with sufficient bioactivity to treat small defects, it remains difficult to stimulate formation of bone ECM, necessary to treat larger injuries. This ECM re-growth, or osteoinductive effect, can be achieved by seeding implants with mesenchymal stem cells (MSCs).[10] MSCs are easily harvested from the iliac crest and are suitable for regenerative therapies due to their high duplication capacity (up to forty times), ability to withstand preservation by freezing and capacity to build new tissue in a defect.[11] In numerous animal[12,13] and even human[14] in-vivo studies, improved healing of critical size defect was observed using HA implants seeded with MSCs. However, reliable regenerative bone therapy remains a challenge with additional experiments needed to address the slow HA implant degredation[15], choice of optimal MSCs and implant materials, as well as surgical procedures an patient follow-up.

Quantitative analytical tools need to be developed to monitor the formation of bone ECM in the various implant materials under appropriate conditions. We have used solid-state NMR spectroscopy (at a magnetic field of 17.6 T) to study the formation of ECM in bone implants. To this end, β-TCP ($Ca_3(PO_4)_2$) ceramics were loaded with osteogenically differentiated MSCs to heal a critical size defect in the femoral condyle (knee joint) of a rabbit model.[16] The MSC's were isolated from aspirated bone marrow, cultured, and osteogenically differentiated. Porous β-TCP cylinders (6 mm × 10 mm) were seeded with the MSCs for up to 7 days. The cylinders were transplanted into a 6 mm hole that was drilled into the rabbit bone (metaphyse of the distal femoral condyle). The animals were sacrificed after three months and the bone implants were removed for analysis.

Typically, the NMR spectra of solid materials are characterized by broad, anisotropic line shapes. However, the application of magic angle spinning (MAS) averages the anisotropic contribution to the chemical shift and collects the spectral intensity into a narrow centerband and a number of spinning sidebands. In the centerband of the NMR spectrum, the characteristic signals are separated according to their isotropic chemical shifts. Depending on the nucleus that is observed in these spectra, the organic and inorganic components of the newly formed bone ECM could be detected. **Figure 2** shows ^{31}P NMR spectra of (a) rabbit bone, (b) pure β-TCP, and (c) a rabbit implant removed after three months. The NMR spectra of rabbit bone and TCP consist of a single or two lines, respectively. The ^{31}P NMR signal of the rabbit implant can be described by a

superposition of the line shapes of rabbit bone bioapatite and the β-TCP matrix material. This spectrum indicates that (i) the characteristic carbonated bioapatite mineral was synthesized in the implant and (ii) the β-TCP matrix material was not fully resorbed after three months of implantation.

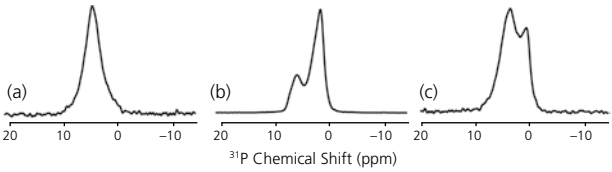

Figure 2 161.9 MHz solid-state ^{31}P MAS NMR spectra of (a) rabbit bone, (b) pure β-TCP, and (c) a rabbit implant removed after 3 months. Spectrum (c) can be simulated from a superposition of spectra (a) and (b) at a 49:51 ratio. The NMR experiments were carried out in a 4-mm MAS rotor using a Hahn echo pulse sequence at 37 °C and a MAS frequency of 8 kHz. Spectra were acquired with a 1.9 µs 90° pulse and a relaxation delay of 400 s.

The solid-state NMR results can be quantified to measure the composition of the implant material, provided a few prerequisites are fulfilled. First, all ^{31}P nuclei need to be polarized identically, as in a single pulse excitation experiment. Second, the chemical shift anisotropies of the signals must be similar; otherwise all sideband intensities have to be included into the analysis. Third, the spin-lattice relaxation times of the molecular species must be at least five times shorter than the repetition time of the experiment. Under these conditions, the line shape of the implant spectrum can be decomposed into a superposition of rabbit bone and the β-TCP spectrum at a 49:51 ratio.

From the ^{31}P NMR spectrum of the bone implant, the contribution from the apatite can be filtered out. Since bone apatite contains hydroxyl groups, the ^{31}P NMR spectrum can be obtained by the cross-polarization (CP) technique, in which, first, the ^{1}H nuclei are polarized and subsequently, the polarization is transferred to ^{31}P. While no ^{31}P CP MAS spectrum of β-TCP can be acquired due to the absence of ^{1}H nuclei, the ^{31}P CP MAS of a rabbit implant consists only of a single line, while the directly polarized ^{31}P NMR spectrum of the same implant yields the superposition of the β-TCP and the rabbit bone NMR spectrum (**Figure 3**). Thus, it could be demonstrated that the newly formed inorganic bone ECM in the implant is indeed bioapatite containing hydroxyl groups.

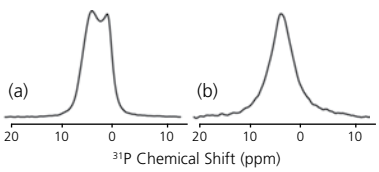

Figure 3. 161.9 MHz ^{31}P MAS NMR spectra of a β-TCP implant removed from a rabbit's femoral condyle after three months. Spectrum (a) was directly polarized using a single 90° pulse, while spectrum (b) was cross-polarized using a contact time of 1408 µs. All spectra were measured at 37 °C.

In addition to the inorganic contribution of bone ECM, solid-state NMR can also detect the organic component in the implants using ^{13}C NMR. Because of the lack of ^{13}C in β-TCP, no ^{13}C NMR spectrum can be acquired (not shown). However, the rabbit bone implants recovered after three months showed a ^{13}C CP MAS NMR spectrum with characteristic signals from protein amino acids (**Figure 4**). For comparison, the NMR spectra of rabbit bone and collagen type I are given. Clearly, a good correspondence is visible between the three NMR spectra indicating that the ^{13}C NMR spectrum of the implant can be explained solely by the organic collagen component. According to the isotropic chemical shifts of the signals in the ^{13}C NMR spectra, the most abundant amino acids of collagen type I (glycine, alanine, proline, hydroxyproline, and glutamate) can be identified. The crucial peak confirming that the NMR spectrum of the bone implant indeed refers to collagen is the HyPro Cγ peak at 71.1 ppm. There is no other aliphatic ^{13}C NMR signal of a regular amino acid with this chemical shift indicating that collagen type I was synthesized by the osteoblasts in the implant. Furthermore, the isotropic chemical shifts of the peaks in the spectra of the implant, native rabbit bone, and isolated collagen type I are identical indicating that no alterations of the collagen structure in the β-TCP implants occurred as isotropic chemical shifts are a very sensitive marker of protein secondary structure.

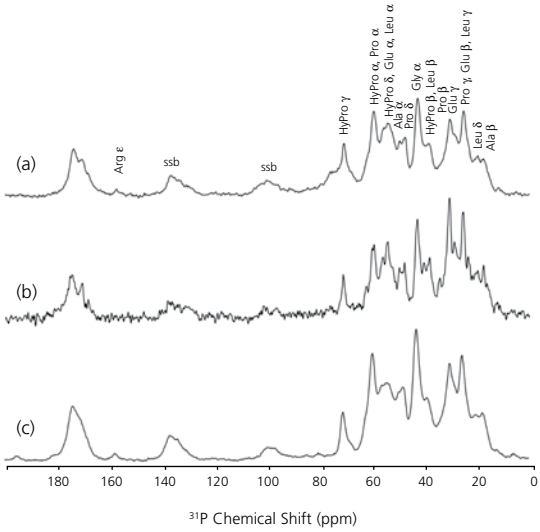

Figure 4. ^{1}H decoupled 188.5 MHz ^{13}C CP MAS NMR spectra of (a) native rabbit bone, (b) a β-TCP implant removed from a rabbit's femoral condyle after three months, and (c) isolated collagen type I. Spectra were recorded at 37 °C and a MAS frequency of 7 kHz. The peak assignment was taken from the literature.[17,18]

In summary, it was shown that hydroxylated apatite and collagen was produced in implants of inorganic ceramics that were loaded with MSCs. The ^{31}P NMR analysis of the inorganic ECM formation in the implants allows quantitative detection of bone mineral formation. The ^{13}C NMR analysis of collagen represents a unique fingerprint of the molecule and its dynamic properties. These results suggest that solid-state NMR is a useful analytical tool to monitor the formation of bone ECM quantitatively. The method can be used to overcome challenges faced in bone engineering including the choice of the appropriate scaffold material, possible surface modifications of these scaffolds, and the right growth conditions. Since tissue engineering of bone faces increasing demand, current procedures may benefit from an atomistic and quantitative understanding of bone synthesis in implant materials by solid-state NMR spectroscopy.

Acknowledgement

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Biocompatible Ceramics

The following table gives a short selection of ceramic particulate materials commonly used in biomaterials and biomedical research, including bone engineering and dental materials.

For a complete list of available ceramics, visit ***sigma-aldrich.com/ceramics***.

For a complete list of available metal nanoparticles, visit ***sigma-aldrich.com/nano***.

Name	Physical Form	Particle Size	"Powder Purity/ Dispersion Concentration"	Prod. No.
Aluminum oxide				
(alumina, Al_2O_3)	Powder	–100 mesh	99.9%	319767-25G
				319767-100G
		10 µm (average)	99.7%	265497-25G
				265497-500G
				265497-2.5KG
	Nanopowder	< 50 nm (BET)		544833-10G
				544833-50G
	Dispersion	< 50 nm (BET)	10 wt.% in H_2O, pH 5–7	642991-100ML
			10 wt.% in isopropanol	702129-100G
				702129-500G
Zirconium (IV) oxide				
(zirconia, ZrO_2)	Powder	5 mm	99%	230693-100G
				230693-500G
				230693-2KG
	Nanopowder	< 100 nm (BET)		544760-5G
				544760-25G
	Dispersion	< 100 nm (BET)	10 wt.% in H_2O	643025-100ML
				643025-500ML
Calcium Phosphate Ceramics				
Hydroxyapatite ($Ca_5(OH)(PO_4)_3$), synthetic	Powder		99.999%	574791-5G
				574791-15G
	Suspension		~ 25 wt.% in 1mM phosphate buffer, pH 6.8	H0252-10G
				H0252-25G
				H0252-65G
				H0252-250G
	Nanopowder	< 200 nm (BET)	≥97%	677418-5G
				677418-10G
	Nanopowder, 5% silica doped	< 200 nm (BET)		693863-5G
	Dispersion	< 200 nm (BET)	10 wt.% in H_2O	702153-25ML
Calcium phosphate ($Ca_2O_7P_2$), amorphous	Nanopowder	< 100 nm (BET)		693871-5G
β-Tricalcium phosphate ($Ca_3O_8P_2$)	Powder (unsintered)		≥98%	13204-10G
				13204-100G
	Powder (sintered)		≥98%	49963-10G
				49963-100G
Tricalcium phosphate hydrate ($C\alpha,(PO_4)_2 \bullet \times H_2O$)	Nanopowder	< 200 nm (BET)		693898-5G

Biocompatible Metals: Titanium

Titanium (Ti) is the most commonly used metal in biomaterials research. It is used is many orthopedic implants and, therefore, titanium foils, meshes and wires are often introduced as cell culture supports in bone engineering experiments. The following table gives a selection of available titanium materials. For a complete list, visit ***sigma-aldrich.com/metals***.

Physical Form	Dimensions	Purity	Quantity Equivalency	Prod. No.
Crystalline				
	5–10 mm (chunks)	99.99+ %	–	305812-25G
			–	305812-100G
Foil				
	thickness 2.0 mm	99.7%	22.5 g = 50 x 50 mm	369489-22.5G
			90 g = 100 x 100 mm	369489-90G
			200 g = 150 x 150 mm	369489-200G
	thickness 0.5 mm	99.99%	1.4 g = 25 x 25 mm	34805-1.4G
			5.6 g = 50 x 50 mm	34805-5.6G
	thickness 0.25 mm	99.99%	700 mg = 25 x 25 mm	267481-700MG
	thickness 0.25 mm	99.7%	25.2 g = 150 x 150 mm	267503-25.2G
	thickness 0.127 mm	99.99+%	1.5 g = 50 x 50 mm	460397-1.5G
			6.2 g = 100 x 100 mm	460397-6.2G
	thickness 0.127 mm	99.7%	13 g = 150 x 150 mm	348791-13G
	thickness 0.1 mm	99.99%	280 mg = 25 x 25 mm	348813-280MG
			1.1 g = 50 x 50 mm	348813-1.1G
	thickness 0.25 mm	99.98%	280 mg = 50 x 50 mm	348848-280MG
			1.1 g = 100 x 100 mm	348848-1.1G
Wire				
	diameter 2.0 mm	99.99%	1.4 g = 10 cm	348856-1.4G
			7 g = 50 cm	348856-7G
	diameter 1.0 mm	99.99%	350 mg = 10 cm	266035-350MG
			3.5 g = 100 cm	266035-3.5G
	diameter 0.81 mm	99.7%	23 g = 10 m	267902-23G
			115 g = 50 m	267902-115G
	diameter 0.5 mm	99.99%	450 mg = 50 cm	348864-450MG
			2.7 g = 300 cm	348864-2.7G
Rod				
	diameter 6.35 mm	99.99%	7.2 g = 50 mm	347132-7.2G
			36 g = 250 mm	347132-36G
	diameter 6.35 mm	99.7%	25 g = 16.7 cm	266051-25G
			100 g = 66.8 cm	266051-100G
	diameter 3.2 mm	99.97%	–	266043-1G
Sponge				
	2–12 mm	99.5%	–	268526-250G
				268526-1KG

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