

Polymers for tissue engineering, medical devices, and regenerative medicine. Concise general review of recent studies

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Recently investigated applications of polymeric materials for tissue engineering, regenerative medicine, implants, stents, and medical devices are described in the present review. Papers published during the last 2 years about polymeric materials used for preparation of various polymeric scaffolds, methods of fabrication of such scaffolds and their effectiveness in providing support for cell growth and development into various tissues and enhancing or mimicking an extracellular network (ECM's) have been cited. Papers describing the use of such polymeric materials for tissue engineering of cartilage and bones were cited. The exciting developments in the field of regenerative medicine, based on application of the self-assembled biocompatible polymeric scaffolds for regeneration of tissues and organs are described in some detail. The use of the biocompatible and biodegradable collapsible polymeric stents, as well as the use of biocompatible, but not necessarily biodegradable polymeric materials for protective coatings of metallic stents and reservoirs of drugs, preventing restenosis and other post-operative complications that may occur after insertion of a stent, have been reviewed. Clinical results pointing out the advantages of such treatments, as well as results indicating their limitations, have been cited. New formulas, for coating implants, stents, and other medical devices, have been discussed. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: tissue engineering; regenerative medicine; implant; biological applications of polymers; drug delivery systems

INTRODUCTION

General reviews of polymeric materials for biomedical applications, with references to numerous review papers dealing with various specific aspects of this subject have been published by the author in 1999 and in 2003.^{1,2} Ruth Duncan published in 2003 an extensive review of the up to date achievements in the field of polymeric therapeutics, and discussed her expectations for the future. She believes that many further developments in medicine will be achieved thanks to such materials.³ A bottom-up design of biomimetic assemblies has been suggested by Tirrell and Tu.⁴ These authors pointed out that such design requires development of functional molecular building-blocks that assemble to form biologically specific components. To achieve this goal one must be able to synthesize in controlled fashion molecular fragments and to predict their ability to self-assemble so as to form structure aggregates of the required form. They suggested that creation of a toolbox of biomimetic molecules, which will self-assemble with biological precision, should be one of the goals of the research.⁴ Exciting and promising results of this kind reported by Stupp and his

collaborators and by other research groups will be discussed in a section devoted to regenerative medicine.⁵

TISSUE ENGINEERING

Peppas and Langer defined biomedical engineering as an extension of chemical engineering towards biomaterials.⁶ Tissue engineering is one of its main branches. Its concept was born when several investigators realized that when cells are placed close enough to each other they form structures identical with those formed by such cells in a living body. This, apparently, may be achieved thanks to signals that living cells are able to exchange with the neighboring ones. Various disciplines, such as materials science, cell biology, reactor engineering, as well as clinical research must contribute to tissue engineering. It requires a balanced combination of cell culture growth with biomaterials to support it and with bioactive molecules to enhance and direct it. A quite successful approach to the goals of tissue engineering involves replacement or repair of damaged or failed tissues with viable ones by creation of an environment, which promotes the native capacity of cells to integrate,

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differentiate and proliferate.^{7–9} It is more ambitious, but difficult to achieve, when the goals include generation of new tissues or organs. The creation of the required environment is usually achieved by implantation of three-dimensional (3D) scaffolds seeded with appropriate living cells secreting their own extracellular matrix (ECM). The proliferation of the cells is usually induced by addition of growth factors and other ECM components. The added bioactive molecules should also promote the intercellular communication and attachment of cells to a scaffold. Vascularization of the growing tissue is usually “a sine qua non” requirement for its successful completion. Biodegradable porous polymeric scaffolds, which support and direct generation of a tissue by the seeded cells, may be also associated with undesired effects, such as inflammatory response to the implanted materials. A novel approach known as cell sheet engineering has recently been developed to avoid such complications. It is based, either on direct transplantation of cell sheets to the host tissues, or generation of 3D structures, via assembly of individual cell sheets. Cell tissue engineering utilizes temperature responsive intelligent surfaces of various acrylamides. Yang *et al.* stated that this technique may enable one to overcome problems encountered in recreating various tissues and organs and establish a new basis for regenerative medicine.¹⁰ Among natural and synthetic polymeric materials which have been found to be suitable for tissue engineering applications, special attention has been given recently to chitosan derivatives in combination with various polysaccharides. Grafted chitosans are extensively investigated for applications in cell transplantation and for tissue regeneration.^{11–13}

Scaffolds

Advanced tissue engineering involves the use of 3D polymeric scaffolds implanted at the defective site. They provide frameworks for cells to attach, proliferate, and form ECM. Scaffolds may be endowed with a spectrum of bioactive molecules, such as ligands for adhesion of receptors to the cells, growth factors or their functional activators, hormones, enzymes and other regulators of cell behavior, which control cell attachment, growth, proliferation and differentiation. Biodegradable natural and synthetic polymers as well as some non-biodegradable polymers, which are currently used for cartilage tissue engineering, have been described by Li and Tuan.^{14a} Polymer scaffolds suitable for stem cell growth have been discussed by Godbey.^{14b} Biodegradable scaffolds should be bioabsorbed at a pre-determined rate and the space initially occupied by them should be fully replaced by the regenerated tissue.^{14c} Polylactides, polyglycolides, polycaprolactone (PCL) and their copolymers have been often used for the preparation of scaffolds. However, their hydrophobicity, the acidity of their decomposition products, and the self acceleration of their degradation, have constituted serious drawbacks. Chitosan, a non-toxic, biodegradable, biocompatible natural polymer, fully soluble at pH 5 in a very slightly acidic aqueous medium, does not have these disadvantages. It can be formed into fibers in various coagulating solutions. Chitosan fibers used for scaffold preparation seemed to combine adequate porous structure with sufficient degradability and

mechanical properties. They were produced by a wet spinning technique and treated with methanol to improve their mechanical strength. They also showed bioactive behavior important for bone repair by tissue engineering. *In vitro* studies indicated that the mesh structure of such scaffolds has been suitable for cell in-growth.¹⁵ Porous conductive chitosan scaffolds have been prepared by the phase separation technique, responsible for the dispersion of 2 wt% of polypyrrole (PPy) nanoparticles in a chitosan matrix. The conductivity of the scaffolds was 10^{-3} S/cm. They were prepared by dissolving 1.5 wt% of chitosan powder in 1% aqueous acetic acid, adding suspension of PPy, pre-evaporating it at ambient temperature and immersing the gelled membranes for 24 hr in aqueous NaOH. The porosity of a scaffold was controlled by changes in the NaOH concentrations from 0.5 to 2.5 wt%. It is expected that such conductive scaffolds will be able to support and modulate growth of various types of cells.¹⁶ It was claimed that partially de-acetylated high molecular weight chitosan was more suitable for tissue engineering applications than the unmodified natural material. The hydrophobicity, biocompatibility and maximum elongation increased, when alginate was added to chitosan.¹⁷ To evaluate the effect of release of growth factor on the formation of a cartilage, a 3D chitosan scaffold was prepared. Chitosan microspheres, loaded with the transforming growth factor, TGF-1, as well as chondrocytes, were planted into this scaffold and placed in a rabbit. It was found that the inserted TGF-1 significantly promoted regeneration of the cartilage.¹⁸ Porous scaffolds made of chitosan, poly(L-lactic acid) (PLLA), poly(D,L-lactide-co-glycolide) (PLGA), alginate, or combinations of these polymeric materials have been prepared by the freeze-gelation or freeze-extraction methods. These methods involved preparation of the diluted solution of a polymer in appropriate solvent (deionized water for alginate), freezing it between glass plates and immersing it into a gelation bath (for alginate: aqueous ethanol solution of CaCl_2 pre-cooled to -20°C). Gelation was induced by the replacement of the initial solvent by a non-solvent for a given polymer. Finally, the porous scaffolds were dried at room temperature. If the gelation takes place below the freezing point of the solvent, the method is called freeze-gelation. If it takes place above the freezing point of the solvent it is called freeze-extraction. Most pores formed were in the 60 to 150 μm range. These methods seemed to be less time and energy consuming than the generally used freeze-drying method.¹⁹ Other natural polymers, such as silk fibroin or collagen blends with PCL or with glycosaminoglycan (GAG) have been used for the preparation of scaffolds by electrospinning.^{20–22} Controlled, slow rates of the *in vivo* degradation of silks provide templating effects, which enhance bone mineral deposition during tissue formation.²⁰ The electrospun collagen-GAG scaffold had a porous nanofibrous structure. Scaffolds prepared from the uniform nanofibers, with mean diameter of 260 nm closely mimic the native ECM found in the human body and are expected to support active tissue regeneration.²² PLLA–collagen hybrid sponge seemed to be preferable to the PLLA sponge or to collagen sponge for the integration of the tissue engineered cartilage into skeletal systems. The PLLA–collagen hybrid

sponge maintained its original shape through out the implantation period of 8 weeks, while the collagen sponge collapsed. The cartilaginous tissues were more widely distributed in the PLLA-collagen scaffold than in the PLLA sponge. The former did not require pre-wetting for cell seeding. Its precise seeding efficiency has not been evaluated yet.²³ Preparation and properties of PLLA scaffolds, by thermally induced phase separation from a ternary polymer-solvent system, was also investigated. It was established for the ternary PLLA/dioxane/tetrahydrofuran (THF) system that larger pore density and higher interconnectivity of the pores has been obtained at 70% of the dioxane (good solvent), while higher void fractions, with *ca.* 90% of voids, have been obtained at 50 or 90% of dioxane.²⁴ The *in vitro* degradation, during 15-weeks storage at 37°C in a phosphate buffered saline (PBS) solution, of the 3D composite scaffold made from poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHCV) and wollastonite, was investigated. The results of these studies indicated that, in order to match the degradation rate of the polymer scaffold with the process of tissue regeneration, one should modulate the degradation rate of the composite scaffold by changing the amount of wollastonite added to the matrix.²⁵ PLGA porous tubular foams were prepared by the thermally induced phase separation process. Their application as soft-tissue scaffolds was assessed *in vivo* by subcutaneous implantation in rats for periods up to 8 weeks. They were rapidly cellularized, indicating that they may be of use in biomedical applications requiring tubular constructs.²⁶ Scaffold, hydrophilized by addition to chitosan of 10 wt% of Tween 80, has had an improved cell compatibility and could be seeded with cells without pre-wetting.²⁷ The growth of a new bone has been greatly induced by the addition of demineralized bone powder to the PLGA scaffolds.²⁸ Melt spinning from blends of PLLA with corn starch was used for the production of biodegradable fibers with adequate mechanical properties (highest modulus and strength were obtained with the 70:30 PLLA/starch blends). It was shown that this technique could be adapted for the fabrication of 3D porous meshes, with porosity and interconnectivity appropriate for tissue engineering.²⁹ In scaffolds for soft- and hard-tissue engineering, prepared using the PLLA/Bioglass (BG) composites, the effect of BG content (up to 40 wt%) was explored. An increase in cell proliferation due to the increased BG content in blends, used for fabrication of scaffolds seeded with human osteosarcoma cell line, was observed. For scaffolds seeded with human lung carcinoma cell line, a maximum in proliferation of these cells was observed at 5 wt% of BG in the blend. Results of this investigation seemed to imply that addition of the right concentration of 45S5 of BG particles to a PLLA scaffold may have a positive effect on the human endothelial lung cell adhesion and proliferation due to a favorable change of pH of the medium.³⁰ Rapid prototyping technique with selective laser sintering (SLS) was applied for the preparation of tissue engineering scaffolds in a highly reproducible fashion. Results of this study have shown that SLS-fabricated scaffolds have a good potential to be used for tissue engineering applications.³¹ Micro-porous materials were produced by gradual precipitation of PCL from its solutions in acetone. Porosity was induced by diffusion of a

non-solvent (methanol) across the PCL membrane, formed *in situ* at the interface between polymer solution and a non-solvent. The non-solvent was extracted with water from the hardened polymer by immersion for 3 days in water that was replaced with fresh water every 24 hr. Hydro-apatite (HAP) could be incorporated into micro-porous membranes by dispersion in a solution of PCL in acetone. Micro-porous PCL and PCL/HAP discs were seeded with human osteoblastic cell culture. Cell/biomaterial interactions were investigated after various incubation times. Coombes *et al.* suggested that such micro-porous polymeric materials could be useful for the extended residence of cells on supports and for a slow sustained release of bioactive molecules.³² Micro-porous interconnected dextran scaffolds with controlled porosity were prepared using methacrylate coupled to dextran and radically polymerizing the former in an aqueous solution of (poly(ethylene glycol) (PEG). Porous gels were obtained as result of immiscibility of dextran-co-poly(methacrylate) (DEX-PMA) and PEG in such solutions. Both macro- and micro-porous gels could be obtained depending on the ratio between DEX-PMA and PEG and on their concentrations in an aqueous medium.³³ Biocompatible and biodegradable amphoteric poly(amido-amine) (PAA) based hydrogels have also been designed as scaffolds for tissue engineering. PAAs are amphoteric due to the tertiary amino and carboxylic groups in the main chain. They proved to be cytobiocompatible as free bases and as salts of strong and medium strong acids. The biological performance of PAAs was not affected by the nature of a counter ion. They could also be copolymerized with proteins and peptides via ϵ -lysine or their terminal amino groups. Linear PAAs, synthesized by hydrogen transfer-poly-addition of secondary amines or tertiary α,ω -diamines to bisacrylamido-aliphatic acids (BAAACs), could be converted into gels by crosslinking them with α,ω -diamines.³⁴ Two crosslinkers were used for their gelation: AG1 and AG2. The AG1 crosslinking and grafting agent was prepared by the addition of 4-aminobutylguanidine (agmatine) (3,6 meq), a bioactive molecule known to induce adhesion to cell membrane, which was followed by addition of 2-methylpiperazine (2.4 meq) and by addition of 1,10-diaminododecane (1 meq) to 2,2-bisacrylamido-acetic acid (7 meq). The AG2 crosslinker was prepared in an analogous fashion. However, PAA-NH₂ (obtained by poly-addition of an excess of ethylene diamine to BAAAC) was used for its preparation instead of the 1,10-diaminododecane. PAA hydrogels containing both *tert*-amino and carboxylic groups in repeating units can be synthesized using agmatine (a mimic of RGD peptide), as co-monomer and as a multifunctional cross-linking agent. Cell adhesion and proliferation on fibroblast cell lines was excellent for both crosslinkers and the degradation products of both hydrogels were non-toxic.³⁵ Collagen scaffolds with improved mechanical strength and stabilized microstructure could be prepared by photo-crosslinking. This technique could be used for tissue engineering of collagen scaffolds for weight-bearing tissues.³⁶ Adsorption of blood cells on porous polylactide scaffolds indicated that these scaffolds are highly thrombogenic. Lower, but still significant adsorption of blood cells was observed on the expanded polytetrafluoroethylene (ePTFE) surfaces. Therefore, they

should only be implanted *in vitro* to provide ECM support to the tissue engineered organ.³⁷ Biodegradable PLGA microspheres were used as both cell matrix and transplantation vehicle of skin cells for skin regeneration.³⁸ Injection molding/solvent evaporation technique has been used in order to form scaffolds with multi-channel geometry. Their void fraction could be modulated by changing the initial polymer concentration. Interconnectivity of voids increased with the increase in their fraction. Distinct channels in the scaffold have been seeded individually with Schwann cells, which promoted axon regeneration *in vivo*.³⁹ Advantages of supercritical fluid processing methods for preparation of scaffolds for tissue engineering involve the ability to incorporate delicate biological molecules without loss in their activity, and exclusion of organic solvents.^{40a} Supercritical carbon dioxide foaming of a blend of the SIS copolymer with tetrahydrofuryl methacrylate (THFMA) was investigated. Non-degradable scaffolds for tissue repair were obtained. THFMA-rich formulations have been found to be suitable for tissue engineering applications. A high foaming factor responsible for higher porosity and open pore structure was obtained for such formulations.^{40b} Many methods can be used for the preparation of porous scaffolds they include gas forming, salt leaching, templating, phase separation due to freezing or to solvent precipitation and electrospinning.^{41–43} (see also 19–22,33,38) A modification of the last method, involving formation of colloidal foams generated by propeller-driven high speed stirring, was used for the preparation of poly(vinyl acetate) (PVA)-amino acid (AA) scaffolds. Rotation produces waves that re-enter the PVA-AA aqueous solution, creating uniform, micrometer scale bubbles, encapsulated by a liquid shell. In order to promote cell adhesion to the scaffold, a small amount of collagen was added during the last 2 min of stirring. Following foam formation, samples were frozen at -80°C , left overnight, and lyophilized. Inherently strong, sponge like material was obtained. The mild conditions of this technique enable incorporation of various biological moieties (e.g. growth factors) and possibly incorporation of cells during scaffold formation.⁴¹ Fibroin spongy porous 3D structures were prepared by freezing and thawing fibroin aqueous solutions in the presence of a small amount of organic solvent. Sponge formation, porous structure and mechanical properties is affected by fibroin and solvent concentration, and by freezing temperature and duration. Thus prepared scaffolds can be sterilized in an autoclave and used for cell growth and handling.⁴² PDLLA, PLLA, PEG- PDLLA, and PEG-PLLA were electrospun from their solutions in 1,1,3, 3-hexafluoro-2-propanol (HFIP) to form random fused fiber topographies. Mean fiber diameters were in the 0.14–2.1 μm range. Osteoprogenitor cells were able to adhere and proliferate on them. In the presence of osteogenic factors cell density on fibers was equal to or greater than on smooth surfaces. It increased with the fiber diameter. Cells on fibers with 2.1 μm diameter extended lamella-podia along individual fibers and exhibited a higher aspect ratio, consistent with a contact guidance phenomenon. The cell morphology and proliferation is affected by the surface topology of such electrospun fibers.⁴³ A laser-based layer-by-layer photo-polymerization process was used for micro-fabrica-

tion of the poly(ethylene glycol)dimethacrylate) PEG-DMA scaffolds. PEG acrylates covalently modified with RGD peptide or with heparin sulfate (ECM component) was incorporated within the scaffolds. Cell attachment was thus facilitated and spatial sequestration of heparin-binding growth factors was allowed.⁴⁴ Mechanical strength and porosity of scaffolds prepared by room temperature compression molding of PLGA containing either cubic NaCl particles or paraffin spheres, followed by leeching was investigated. Scaffolds with spherical pores obtained in this manner showed better interconnectivity than scaffolds containing cubical pores.⁴⁵ PLGA-collagen scaffolds were prepared using pre-prepared ice particulates as porogen material.⁴⁶ Chitosan scaffolds were prepared by electrolysis of its formic or acetic acid solutions. Their pore sizes were in the 0.5–2 mm range.⁴⁷ Relatively stiff scaffolds for tissue engineering based on collagen gel containing poly(ethylene oxide) (PEO)-poly(propylene oxide) (PPO) block copolymer, modified with positively charged quaternary ammonium-MAA were prepared by radical polymerization of the aqueous solutions of the methacrylic acid (MAA) and MAA-(CH_3)₄N⁺ modified poloxamine in the presence of collagen. The cell attachment studies suggested, however, that the interaction with the cells was not sufficient to induce cell spreading. Therefore pre-adsorption of serum proteins was required.^{48a,48b} Three-dimensional porous scaffolds of complicated shape have been prepared by combining modified compression molding with conventional particulate leaching.⁴⁹ The application of such biodegradable polymers as PLA, PLGA, PGA, fibrin, copolymers of ethyl acrylate (EA) and hydroxyethyl methacrylate (HEMA), copolymers of hydroxy-butyrate-co-valerate, for the preparation of porous scaffolds and matrices and a study of their physico-chemical characteristics has also been examined by several other investigators (see for example refs.^{51–55}). Stable, non-toxic, biocompatible, mechanically strong polymeric networks have been produced by crosslinking chitosan/gelatin membranes with proanthocyanidin, a natural polyphenolic crosslinker. Changes in the chitosan and crosslinker amount may control their degradation.⁵⁶ Polylactide vascular scaffolds are highly thrombogenic. Their thrombogenicity may be reduced to some extent by hybridizing them with ePTFE. It has been recommended to insert the polylactide and surface modified ePTFE scaffolds for vascular tissue engineering after developing *in vitro* the non-vascularized tissue.³⁷ A model was developed for the diffusion of oxygen through a vascular scaffold, fed by a blood vessel at its base, with matrix elements 15–60 nm in diameter.⁵⁷ PLLA-co-ePCL (70:30) nanofiber mesh (NFM), with fiber diameter of 470 ± 170 nm and 64–67% porosity, was fabricated by electrospinning followed by plasma treatment and collagen coating. Collagen coating of the NFM enhanced spreading, viability and attachment of endothelial cells with preserved phenotype.^{58,59}

Cell seeding and delivery of ECM agents

The delivery of living cells to scaffolds and insertion of biological molecules inducing creation of an ECM, which controls all aspects of cell and tissue function providing mechanical support, is an essential part of tissue engineering.

ECM is responsible for enhancement of cell proliferation and growth, their differentiation and cell–cell interactions. The earlier described collagen-blended nanofiber mesh closely mimics, both morphologically and chemically, effects due to natural ECM. (cf. refs. 58,59). The addition of sodium ascorbate to the aortic smooth muscle cells (SMC) on a hyaluronan based vascular construct, improved cell viability, helped to maintain SMC phenotype, and enhanced secretion of the natural ECM.⁶⁰ Degradable hydrogels have been used as vehicles for delivery of growth factors and for promoting regeneration of a damaged tissue. Langer and coworkers investigated the possibility of using PEG based hydrogels, prepared by photoinitiated polymerization, for the delivery of neurotrophins, as treatment for spinal cord injury. (They found that Ciliary-neurotropic factor released from a gel above explanted retina stimulated outgrowth of a significantly higher number of neuritis than those grown without it.) They were also able to deliver simultaneously several neurotrophins with individual release rates, by using spherical particles of hydrogel/composites.⁶¹ Degradable hydrogel composites consisting of poly(PEG_(oligo)fumarate) (OPF) and gelatin microparticles (MPs) were used as vehicles for the delivery of transforming growth factor (TGF- β 1) and/or of the insulin growth factor (IGF-1) together with TGF-1. The bovine chondrocytes were embedded in composite hydrogels co-encapsulating MPs and TGF- β 1 or TGF- β 1 + IGF-1, to achieve this. Rate of release could be controlled by adding the growth factors, either to MPs or to the OPF phase of the hydrogel.^{62,63} In order to enhance activity of liver tissue cells (hepatocytes) seeded on a scaffold, one can activate them by interaction with the galactose-carrying synthetic or natural polymers, which mimic action of ECMs. Their binding through a receptor-mediated mechanism enhanced hepatocyte functions, such as albumin secretion and urea secretion and guided their adhesion. Effect due to these interactions depended strongly on the geometry as well as on the density and orientation of the attached hepatocytes to PLGA, or to poly(acrylic acid), or to alginate galactose.⁶⁴ An atomizer was developed to seed mammalian cells suspended in phosphate buffered saline solutions in the presence of pluronic F-127, or without. Cell suspension flowed in the atomizer through the inner tube and the air flowed through the outer pipe. Airborne micro-droplets were generated. Cell viability was not affected significantly by such treatment. The growth rate and appearance of the sprayed cells were comparable to pipetted samples, used as controls. The viability, growth and proliferation of cells was not affected by this method of spraying and seemed to be similar with and without addition of pluronic F-127.⁶⁵ Comparison between seeding of chondrocyte suspension on PGA or on chitosan scaffolds by the vacuum bioreactor technique and by rotation with the spinner flask was the object of another investigation. A suspension containing 5×10^6 porcine chondrocytes per scaffold was used. Yield of both seeding techniques was similar. However, the percentage of seeded cells in the center of the PGA construct was much higher than in the bioreactor, resulting in much lower uniformity of the seeding by the former technique. Formation of ECM seemed to be stimulated by seeding on chitosan in a vacuum bioreactor.⁶⁶

Ingrowth rates of tissue in PCL-co-polyurethane (PU) and in PU scaffolds was investigated by computer based histomorphometric method, involving examination of a number of computer defined concentric zones, situated a pixel from the scaffold edge. Each zone was automatically analyzed for tissue content. Penetration of a tissue into PCL-co-PU was much faster than into PU. PCL-co-PU seems to be, therefore, a promising candidate for meniscus tissue engineering.⁶⁷ Segmented PU (PCL-PU) films coated with gelatin, laminin, or collagen IV, were seeded with embryonic stem cells of cardiomyocytes. Investigation of the constructs conducted 30 days after seeding, revealed that cells seeded on laminin or collagen exhibited preferential attachment (assessed by cellular count and viability) to those seeded on gelatin. The investigators concluded that degradable elastomers, seeded with the embryonic stem cell-derived cardiomyocytes, have the potential to be used for repair of damaged heart tissue.⁶⁸ Fourier transform infrared imaging spectroscopy (FT-IRIS) was applied to the study of collagen orientation in collagen molecules in cartilage. This is an important factor determining their functionality in a connective tissue. Comparison of results, obtained by FT-IRIS technique with those obtained by polarized light microscopy (PML), revealed that zonal discrimination and orientation detected by PML in human osteoarthritic cartilage, were less obvious than those detected by FT-IRIS. Apparently, FT-IRIS detects molecular orientation changes prior to its manifestation at the microscopic level.⁶⁹

Cartilaginous tissue engineering

Cartilage is a connecting soft elastic tissue found in various parts of the body. It is generated by chondrocytes. Its network constituents are primarily Type II collagen, proteoglycans, water and mobile ions. It is able to lubricate joints and withstand static and dynamic loads remarkably well. In order to describe lubrication and load bearing characteristics of cartilage, Bassar and Horkay proposed to treat cartilage ECM as a composite medium, with a proteoglycans phase exerting swelling pressure and collagen phase resisting it. The two constituents have distinct biological roles.⁷⁰ Chondroitin sulfate proteoglycan—aggrecan forms complexes with hyaluronan, stabilized via its N-terminal G-domain link-protein. They provide cartilage with its load bearing properties. Similar aggregates of chondroitin sulfate proteoglycans (containing other members of the link-protein family, such as versican, may contribute to the structural integrity of other tissues, such as skin and brain.⁷¹ Ear-shaped constructs, mimicking human-ear shape, were prepared by mixing chondrocytes with fibrin polymer and gelling it. The constructs were implanted into athymic mice and extensively stented for 6 weeks. Implants histology and mechanical properties were checked after 12 weeks. These studies demonstrated that it is possible to engineer a cartilage construct similar to the human ear not only in shape, but also in size and flexibility.⁷² Effect of hydrogels of alginate, of type I collagen, of methylcellulose (MC), and of pluronic F-127 on the production of tissue engineered cartilage on poly(glycolic acid) (PGA) scaffolds was evaluated and compared with controls prepared without hydrogels. Cartilage matrix deposition in the presence of type I collagen,

methyl cellulose (MC), or pluronic F-127 was significantly improved as compared to alginate. Quantitative ECM analysis did not show, however, that combined seeding of chondrocytes and hydrogels leads to significantly better performance than seeding without hydrogels. Nevertheless, it was concluded on the basis of the effect of chondrocytes proliferation on cartilage regeneration that type I collagen or MC were the best candidates for this purpose. Pluronic F-127 was the next best because it also did not affect negatively cartilage matrix secretion and did not prevent chondrocytes from maintaining their condition in balance with cellular morphology.⁷³ The effect of fabrication variables on the potency of poly(ethylene imine)(DNA-PEI)-loaded collagen films was tested. Conditions leading to various levels of transgenic expression were identified.⁷⁴ Self-diffusion of PEG in bovine nasal cartilage was determined by pulsed-field gradient NMR. At low PEG concentrations (<10 wt%) the effect of restricted polymer diffusion was negligible. Its values have primary been determined by water content and molecular-weight (MW) of PEG.⁷⁵ The investigation of structure of the PEG-PPO-PEO (F-127) in the mammalian cell media (MEM) revealed that the gel-sol transition temperature and concentration, corresponding to the formation of close-packed cubic assembly of spherical micelles, is shifted down in comparison to pure water. At 25°C the critical micelle concentration (CMC) in MEM decreased significantly. However, its basic structure remained the same in MEM and in water.⁷⁶ A novel 3D fiber deposition technique was designed to form porous interconnecting scaffolds containing, either homogeneously spaced pores (fiber spacing: 1 mm, pore size ~680 µm diameter), or pore size gradients (fiber spacing: 0.5–2 mm, pore size ~200–1650 µm in diameter), but with similar overall porosity and volume fraction available for cell attachment and ECM formation. Such pore distribution closely resembled cell distribution observed in native cells.⁷⁷

Cartilage can be tissue engineered in rabbits using poly(glycolic acid) (PGA) mesh embedded with autologous chondrocytes, encapsulated in alginate. This study demonstrated that the reconstruction of tracheal defects by cartilage engineering is feasible.⁷⁸ The application of PLGA MPs as biodegradable injectable scaffold for cartilage tissue engineering was also investigated. Chondrocytes were injected to subcutaneous space in mice in the presence and in the absence of PLGA MPs. Progressive formation of a cartilage, increase in the amount of GAG and uniformity of type II collagen deposition was observed in samples containing MPs. Larger mass of cartilage formed and higher content of proteoglycans was obtained when MW of PLGA was higher or/and contained methyl ester end groups. However, in a latter case the mass distribution was not homogeneous. Mercier *et al.* believed that their data show promise for utilization of the injectable PLGA-chondrocytes system in tissue engineering applications.⁷⁹

A mathematical model was developed in order to examine strong interactions between profiles of diffusing oxygen and cell distribution within cartilaginous constructs. Predictions of this model were in agreement with experimental results. Both the experimental and the predicted results showed that cell-scaffold constructs that rely solely on diffusion for the

supply of nutrients will produce proliferation dominated regions near the outer edge of the scaffold, when oxygen level or/and cell density exceeded critical level. Conventional methods of enhancing oxygen supply may help to reduce the non-uniform cell distribution.⁸⁰ The electro-spun meshes from PU, were coated with films of different relative surface charge: hydrofluoropropylene (HFP) (neutral), *N,N*-dimethylaminoethyl methacrylate (AMA) (positive charge), and methacrylic acid (MMA) (negative charge). An uncoated mesh had also a slightly negative surface charge. Samples were implanted in rats for 5 weeks. Fibrous capsule presence around the implants, cell nuclei density, and the number of vessels, were assessed. There was no significant difference between the four groups in cell nuclei density and coating. However the number of generated vessels was higher for the uncoated and for the MA-coated samples, than for the HFP- and AMA-coated ones. This suggests that an ingrowth of vessels was favored in the negatively charged samples.⁸¹ Cartilaginous tissue formed *in vitro* is deficient in collagen and has only a fraction of the compressive strength of natural cartilage. Waldman *et al.* proposed to improve its quality by long-term intermittent stimulation. They harvested chondrocytes from bovine metacarpal-carpal reticular cartilage and prepared an *in vitro*-formed tissue. In order to determine the optimal stimulation parameters, they submitted this tissue to various stimulation protocols. They found that 5% compressive amplitude, applied every second day during a 4 week period, at a frequency of 1 Hz for 400 cycles, resulted in *ca.* 40% increase in collagen synthesis, 30% increase in proteoglycans, and a two to three-fold increase in compressive mechanical properties. Accumulation of the ECM was accelerated. These studies showed that short intermittent stimulations improve significantly the compression properties of cartilaginous tissue. Prolongation of such intermittent treatment, by application of 2000 cycles instead of 400 cycles, did not affect the result significantly, though changes in compression amplitude did occur.⁸²

Bone tissue engineering

Applications of polymers for bone tissue repair and reinforcement, for regeneration of cartilage associated with bones, for helping in partial replacement of bones by metallic parts, and as carriers of antibiotics to the infected bone tissues, have been extensively investigated. Relevant investigations, conducted during the last 2 years, will be reviewed in this section. Re-absorbable or degradable biomaterials containing the high MW hyaluronan (HA)-based polymers have been extensively explored for tissue engineering applications. HA-based biomaterials with excellent histocompatibility and biodegradability were prepared for the synthetic bio-skin and biosynthetic osteo-cartilage used for the reconstruction of tissues. The addition of growth factors and cytokines promoted cell migration, proliferation and formation of a new tissue.⁸³ The synthetic polyester poly(cyclohexyl sebacate) (PCS) and two natural polyesters D400G and D600G, containing 8 and 12% of hydroxyvalerate, respectively, were tested for repair of the musculoskeletal tissues. *In vivo* experiments indicated that PCS caused a more extensive inflammatory reaction and yielded lower vascular

densities than D400G and D600G. Giavaresi *et al.* concluded, on the basis of *in vitro* experiments with fibroblast cultures and *in vivo* subcutaneously implanted tissues in rats, that D400G may perhaps be the most suitable polymeric material for this application.⁸⁴ Repair of skeletal defects with vascularized bone grafts was much more successful than with non-vascularized free grafts. Osteoblastic cells were seeded into a non-woven multifilament of PGA, which provided 3D support during *in vitro* generation of a culture. After 2 weeks these constructs were implanted around femoral vessels of athymic nude rats. New bone formation was evident in 10 out of 12 seeded implants. After 6 weeks the tissue was primarily composed of cartilage enveloping small islands of osteoids. It became later an organized bone with vascular pedicle.⁸⁵ The natural repair of osteochondral defects can be enhanced by using biocompatible materials that support repair process and are biocompatible. HA-based scaffolds seem to allow faster cell infiltration leading to faster tissue formation than the synthetic polylactides and their copolymers. This may be due to the polysaccharides provided by the natural polymers.⁸⁶ A water soluble formulation based on sulfated poly(*N*-acetyl glucosamine) has been shown to enhance healing of osteochondral defects in animal models.⁸⁷ Magnetic iron oxide nanoparticles, colloiddally stabilized by addition of oleic acid as a long chain surfactant, were coated with transferrin. Cell proliferation, cytoskeletal organization and formation of an ECM was enhanced when super-paramagnetic, transferrin coated nanoparticles, were added to the human dermal fibroblast. Moreover, many genes in the cells were up-regulated, particularly in the area of cytoskeleton and cell signaling. Though the transferrin coated particles were attached to cell membranes they were not internalized by them and did not instigate receptor mediated endocytosis.⁸⁸ The effect on cell morphology and expression of surface markers of osteogenic cells, due to cell-polymer interactions with bioresorbable polylactides and with their copolymers with glycolic, was investigated. Differential effects on the bone-progenitor cells, due to expression of factors involved in cell-cell and cell-matrix interactions, have been demonstrated.⁸⁹ Development of bone analogues on the biomimetic PCL/ceramic polymer (CaP) was explored. Human marrow stem cells (MSCs) were seeded together with fibrin glue on PCL/CaP scaffolds and cultured *in vitro* for periods of up to 8 weeks. MSCs were able to adhere, migrate, and differentiate along the osteogenic lineage of these biodegradable scaffolds, which degraded 27 times faster than PCL alone.⁹⁰ Osteochondral graft based on a microsphere scaffold, prepared from a hybrid of a polymeric hydrogel with a bioactive glass composite (PLGA-BG), improves healing of osteochondral defects and promotes integration with the host tissue. This graft consists of three regions: gel only, gel/composite interface, composite only. Effects of composition of a scaffold on chondrocytes response were also investigated.⁹¹ Scaffolds consisting of two layers: bone forming layer on the top and cartilage forming layer at the bottom, were used. These scaffolds were based on OPF and various amounts of TGF- β 1, added to the cell culture. They support healthy tissue growth in rabbit osteochondral defects, and undergo biocompatible degradation. Tissue quality improved over time with hyaline cartilage filling the

chondral region and mixture of a compact trabecular bone, filling the sub-chondral region. New bone tissue was completely integrated with the surrounding bone after 14 weeks and no bone up-growth into the chondral region was observed.⁹² A study of the influence of TGF- β 1 on the bone-like tissue formation in 3D cultures of rat MSCs seeded on the PLGA fiber meshes, showed that addition of TGF-1 has a positive effect on the formation of procollagen type I, collagen type I, and collagen type V. This was confirmed in a long-term study of formation of an ECM. Matrix mineralization was also improved by the TGF- β 1 treatment. However, study of the differentiation of the marrow stromal cells into osteoblastic phenotype, in a dynamic cell culture, revealed that while bone sialoprotein increased with the increase in the dose of TGF- β 1, the osteonectin was not affected by it. Moreover, both alkaline phosphate activity and osteocalcin formation were suppressed by high doses of TGF- β 1. In view of these findings and considering the beneficiary effect of TGF- β 1 on matrix formation and mineralization, and on cell differentiation, it was decided that addition of only 1 ng of TGF- β 1 a week for the first 2 weeks may be optimal for the formation *in vitro* of bone-like tissue.⁹³ The chitosan-alginate scaffolds were prepared from solutions at physiological pH, which provide favorable environment for incorporating proteins. Bone forming osteoblastic cells attached readily to chitosan-alginate scaffolds, proliferated well, and deposited calcified matrix. *In vivo* studies showed that a hybrid scaffold has a high degree of tissue compatibility. Calcium deposition appeared 4 weeks after insertion. These investigators believed that the biological and mechanical properties of these scaffolds warrant clinical trials.⁹⁴ Scaffolds suitable for bone tissue engineering were prepared by combining ceramic materials with biocompatible, water soluble polymers based on *N*-vinylpyrrolidone and acrolein diethyl acetal. The aldehyde groups on the copolymer may conjugate with proteins and peptides controlling cell adhesion and growth. The introduction of positively charged lissyl ligands further increased the adsorption capacity of the composite scaffolds. The compatibility with bone tissue was enhanced by the mineral components. These scaffolds were found to be non-cytotoxic.⁹⁵ Several other research groups also investigated, during the last 2 years, bone tissue formation on scaffolds prepared from composites, such as the calcium metaphosphate, hydroxyapatite, carbonated fluoroapatite, or phosphate glass, combined with such biodegradable polymers, as polylactides and their copolymers, PCL etc., which were seeded with osteoblastic cells.⁹⁶⁻¹⁰¹ Articular cartilage is a dense white tissue that covers surfaces of bone joints. It shows limited reparative capacity after injury. An attempt was made to repair it by seeding the appropriate chondrocyte cells in biocompatible composite scaffolds, culture them *in vitro*, and then implant the cell material complex in the damaged tissue. Several investigations dealing with this problem have recently been published.¹⁰²⁻¹⁰⁷

REGENERATIVE MEDICINE

The aim of regenerative medicine is to regenerate the soft and hard tissues, organs, and nerves responsible for main human

disabilities. Though it initially looked as an extension of tissue engineering, it is now considered to be one of the major interdisciplinary scientific challenges. It requires integration of emerging knowledge in the physical and life sciences with bioengineering and with clinical medicine so as to learn how to trigger the failed human tissues and organs.¹⁰⁸ Several investigators recently came to the conclusion that in order to achieve a breakthrough in this field one must mimic the natural biological processes.^{4,5} Self-assembly, based on supramolecular interactions, plays a prominent role in biology. Fulfillment of this requirement is, of course, not an easy task, but spectacular results achieved recently by Stupp and his coworkers, briefly discussed later, give hope that this goal may, indeed, be achieved in the future. They reported on the preparation and properties of self-assembling nanostructures composed of peptide amphiphiles in which cells encapsulated *in vitro* within a bundle of amphiphilic nanofibers survived organization and growth of the nanofibers around them forming regular 3D structures. The encapsulated cells proliferated and differentiated rapidly, when peptides acting as cell stimulating epitopes, that promote receptor based interactions with cells, were used for the synthesis of the amphiphile molecules.¹⁰⁹ Constructs self-assembled from such molecules mimicked natural ECMs. However, possible problems related to formation of such constructs in living organisms have not been investigated in depth, if at all. The immunogenicity of the constructs or of their parts may constitute a hurdle difficult to overcome. Perhaps, generation *in vitro* of the entire organ by self-assembly of a construct seeded with appropriate cell cultures and its subsequent partial degradation prior to the *in vivo* implantation of an organ may provide solutions to such problems. Stupp prepared a number of self-organized nanostructures that are highly regular in size and shape. He used amphiphilic triblock copolymers. Repulsive forces among some segments of these triblock molecules have been responsible for the regularity and finite size of the supramolecular units.¹¹⁰ Formation of nanoribbons¹¹¹ that may be twisted to generate nano-helices has been reported by these investigators.¹¹² Nanofibers can form double layer patterns.¹¹³ The self-assembling peptide amphiphiles contain a hydrophobic peptide covalently coupled to lipid tails. As such, they are analogous to phospholipids and other membrane-forming amphiphiles. The self-assembled aggregates were precursors of various supramolecular structures: from individual fiber-like micelles to larger aggregates and membrane mimics. Cylindrical structures, of the N-acylated oligopeptides joined with palmitic acid, were formed by self-assembly in aqueous media. The ionizable amino acids in the peptide segment drive the self-assembly through hydrophobic collapse into high aspect ratio cylindrical nanostructures, with dimensions of the molecular components in the 6–8 nm range. The repeated residues of such amino acids, as alanine, leucine, or valine, promoted formation of β -sheet like structures, which stabilized the aggregates and imparted them with a high degree of order. These cylindrical structures can display signaling to cell epitopes on their surface.^{113,114} Chemical formulae of the self-assembling peptide amphiphiles used in such experiments are shown in Figs. 1 and 2. Study of the

properties of these constructs revealed significant sequestration by them of hydrophobic molecules, as well as sufficient salvation allowing diffusion and release of sequestered species into the local environment. These results demonstrated the potential of such constructs, as carriers of drugs and proteins.¹¹⁵

Scaffolds that have been made of PLLA had satisfactory mechanical properties, but their wettability and cell adhesion were poor. Attempts made in the past to correct this situation by depositing on them amphiphilic block copolymers, by using Langmuir–Blodgett technique, were not very successful. Stupp *et al.* approached this problem by coating PLLA with the self-assembled triblock oligomer consisting of the rigid cholesteryl segments followed by the L-(lactic acid) oligomers and the second generation L-lysine dendrons. The self assembly of amphiphilic peptides into nanofibers is depicted in Figs. 3 and 4. The adhesion of cells and their proliferation on the modified PLLA scaffolds was significantly enhanced.

Apparently, the deposition of the bioactive multilayers on the surfaces may trigger cell response, which may be useful in tissue engineering, and for generation and transplantation of cell cultures.^{116,117} Suspension of the neural progenitor cells in physiological media was mixed with 1% aqueous solution of amphiphilic peptides consisting of a pentapeptide isoleucine-lysine-valine-alanine-valine (ILnVAV) combined with four alanine and three glycine residues, followed by an alkyl tail of 16 carbons. The ILnVAV segment (also found in laminin) is known to promote neurite sprouting and direct its growth. Mixing of neural progenitor cell suspension with the solution of the amphiphilic peptide promoted self-organization of the molecules into a solid construct. The cells survived the growth of nanofibers around them. Scaffolds formed from these nanofibers induced rapid differentiation of the cells into neurons, while formation of astrocytes was discouraged. Area of the cell body and the rate of growth of neuritis on the bioactive scaffolds were significantly larger and faster than for cell cultures grown on PDL or laminin-coated substrates.¹⁰⁹ It has also been shown by Li and Stupp that peptide based nanofibers can serve as scaffolds for the one-dimensional assembly in organic solvents of lipophilic inorganic nanoparticles.¹¹⁸ Stupp stated during the FBPS 05 meeting in Granada, Spain, that his group is involved in the *in vivo* testing of performance of such systems for bone regeneration, angiogenesis, regeneration of heart tissue, and transplantation of insulin productive tissues. He claimed that the initial results were very encouraging.¹¹⁹

Self-assembly of other amphiphilic peptides and of the amphiphilic peptide nucleic acids has also been investigated during the last 2 years by other research groups.^{120–123} Several investigators were particularly interested in using these techniques for the preparation and/or investigation of bioactive constructs and related materials as artificial ECMs. Self-assembled nanofibrous scaffolds have a high surface-to-volume ratio that enhances cell adhesion and proliferation. They are expected to provide more efficiently acting ECMs than those in the lowest range of the natural ECMs. On the other hand, cross-sections of nanofibers generated from collagen by the phase separation and by electrospinning methods are in the middle and in the upper range,

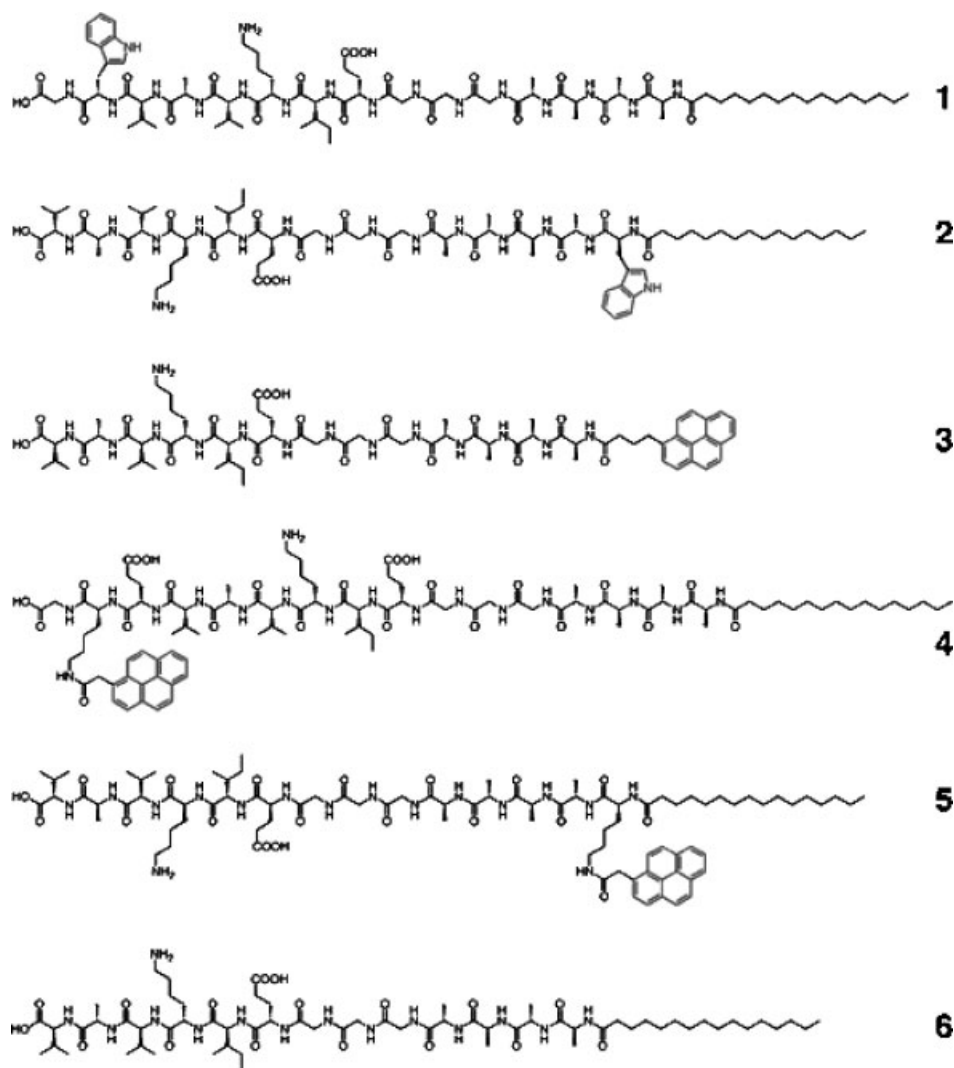


Figure 1. Chemical structures of the self-assembling amphiphilic peptide. Reproduced by permission from chart 1 from J.D. Tovar, *et al. J. Am. Chem. Soc.* 2005; **127**(20): 7337–7345. Copyright © 2005 American Chemical Society.

respectively, of natural collagen ECMs.¹²⁴ Zhang and his coworkers found that also peptides consisting of periodic repeats of alternating domains of the hydrophobic and of the ionic hydrophilic AAs self-assemble when exposed to physiological salt containing solutions.¹²⁵ Peptides that self-assembled with pores of a size suitable for accommodation of cells, were used by Zhang and his coworkers for the preparation of 3D scaffolds acting as ECMs for the attached cells. Thus formed 3D matrices supported cell attachment, induced differentiation of a variety of mammalian primary tissues and/or tissue cultured cells, and enhanced their survival.^{126–129} Tissue engineered human blood vessels reconstructed using the self-assembling approach were tested for the presence of endothelin vasopressor ET_A receptors, and for functionality in such reconstructed blood vessels. Reverse transcriptase polymerase chain reaction studies demonstrated that mRNA of the ET_A receptor was indeed present and functional in vessels developed in self-assembled constructs, as was also the endothelin converting enzyme, responsible for the formation of the biologically active endothelin peptides. However, mRNA of the ET_B receptor was missing.¹³⁰ One of the major present tasks of

regenerative medicine is replacement of damaged human nerve tissue. Two papers were recently published within the framework of the nerve regeneration attempts. Outcome of nerve regeneration studies conducted on peripheral nerve injury of rat models demonstrated that nerve guidance channels prepared, by using coil reinforced PHEMA-MMA hydrogels, show equivalence to nerve autografts. However, second surgery for the removal of such non-biodegradable tubes was required after nerve regeneration was completed. Authors of this report declared that they are investigating the possibility of using biodegradable polymeric tubes for the repair of the peripheral nerve of the spinal cord.¹³¹ Extension of neonatal rat dorsal root ganglion neurons, cultured on grooved substrates of polydimethylsiloxane (PDMS) coated with laminin and poly-L-lysine, across micropatterned grooves, tens of microns in size, has been investigated. The size of a groove corresponded to the size of pores in a scaffold and depended on the design of the biomaterial. Such studies have been of interest for understanding the cytoskeletal dynamics and designing biomaterials for axon guidance.¹³² Population of neurons showed a unique growth pattern with neuritis bridging across grooves between adjacent

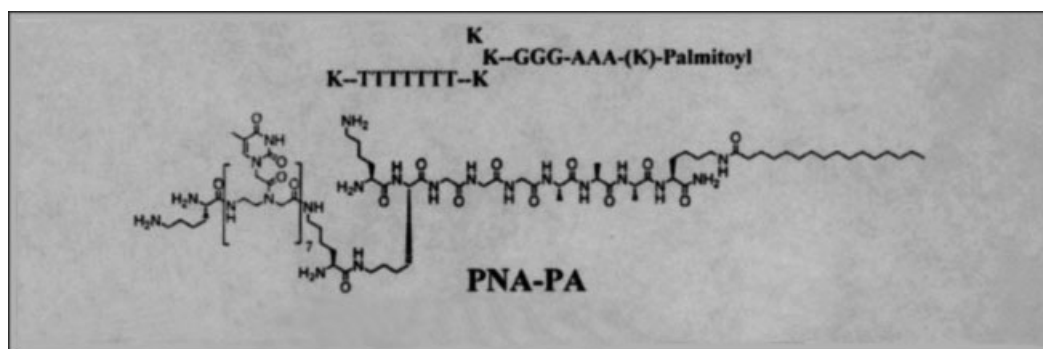


Figure 2. Structure of peptide nucleic acid/peptide amphiphile conjugate (PNA-PA). The thymine nucleic acid base was attached through an acetyl linker to an *N*-(2-aminoethyl) glycine PNA derivative, shown in gray. Reproduced by permission from Guler *et al. Bioconjugate Chem.* **16**(3): Fig. 1 pp. 501–503. Copyright © 2005 American Chemical Society.

plateaus. In absence of underlying solid support, neurons were pulled 50 μm above the initial adhesion point in order to suspend themselves between groves. In order to do this in nature neurons must navigate a complex 3D matrix of molecules and cells. Attempts to promote cell regeneration must take this into account. One must also remember that neurons and glial cells have the capacity to grow counter to the intended direction of growth. Authors of this report stated that their results will influence the future design of scaffolds supporting nerve cell regeneration.¹³³ It has been indicated in the introduction to this review that a novel method—cell sheet engineering—has recently been devised for generation or regeneration of tissues from cell cultures. This method is based on either direct transplantation of cell sheets from Petri dishes to the host tissues, or generation on host tissues of 3D structures, via assembly of individual cell sheets. It eliminates complications due to the application of scaffolds or carrier substrates. In this way host inflammatory response to the implanted polymeric materials can be avoided. Cell sheets have been generated by placing cells on Petri dishes coated with temperature responsive polymers. Cells will adhere to the coated surface and form a cell sheet at initial temperature. But upon change in temperature the entire sheet will be released. At present, the prospective applications of this method include regeneration of periodontal, heart, corneal, liver tissues, as well as reconstruction of the bladder. It is believed that this approach will overcome

problems that in the past have limited the traditional methods. This will establish a new basis for some aspects of regenerative medicine.¹³⁴ A novel multi-functional linear, terblock copolymer, poly(*N*-isopropylacrylamide-co-acrylic acid)-*b*-(lactic acid) (NAL) was synthesized for cell sheet engineering applications. Spin-cast NAL films were thermo-responsive. At 37°C their surface was more hydrophobic and rougher than at 22°C. At the lower temperature it was hydrophilic and smooth. Murine osteoblastic MC3T-E1 cells displayed comparable adhesion but slower proliferation on NAL films than on PLLA or on end-silylated polystyrene. A well-established MC3T-E1 cell sheet was successfully detached from NAL films by lowering the temperature from 37°C to room temperature. NAL copolymer may be applicable for non-invasive two- or three-dimensional cell sheet harvesting. Apparently, it may also have potential for controlled release of therapeutic agents, while supporting growth of the cells.¹³⁵

IMPLANTS, STENTS AND MEDICAL DEVICES

Implants

Implants for bone repair and reconstruction

Bone implants are the most common body implants. They are implanted in order to replace surgically removed parts or minimize or, if possible, eliminate damages or defects due to

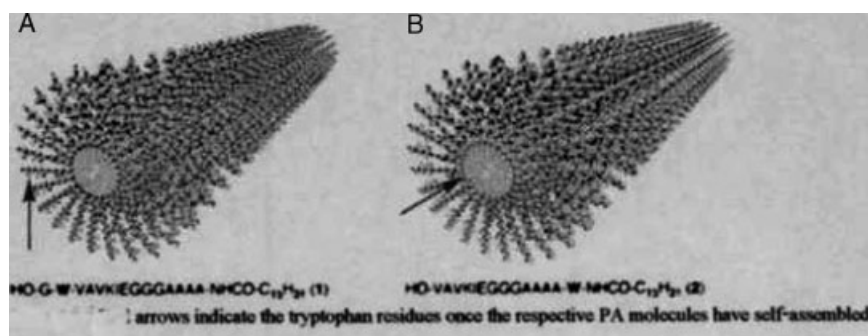


Figure 3. Self-assembled amphiphilic peptides. Black arrows indicate the tryptophan residues in the self-assembled molecules. Reproduced by permission from chart 2 in a paper by J.D. Tovar, *et al. J. Am. Chem. Soc.* 2005; **127**(20): 7337–7345. Copyright © 2005 American Chemical Society.

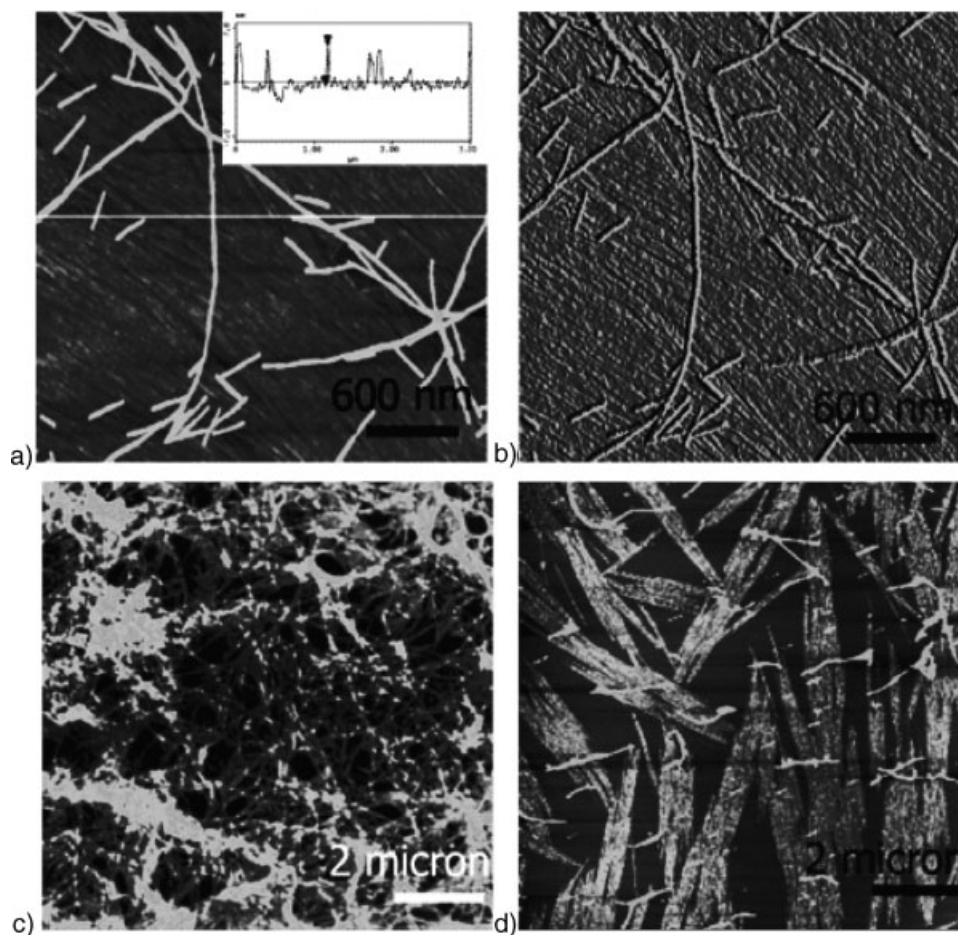


Figure 4. Atomic force microscopy tapping mode images of PA nanofibers. (a) Topographical an. (b) phase images of isolated PA nanofibers lying on a top of a close-packed layer of nanofibers. (c) Topographical image of random network of nanofibers. (d) Topographical image of "patches" of close-packed layer of monolayers, with isolated single fibers on top. Reproduced by permission from Fig. 1 in a paper by H.J. Jiang, *et al. Langmuir* 2005; **21**: 5242–5246. Copyright © 2005 American Chemical Society.

diseases, accidents, birth defects, crimes or wars. Polymeric materials have often been used for such purposes. Chitosan has been crosslinked by reaction of its amino groups with PEG₆₀₀ terminated at its two ends with carboxyls. Thus covalently crosslinked networks are obtained forming pH-sensitive hydrophilic hydrogels. The *in vivo* studies revealed that such hydrogels facilitated bone regeneration. When the degradation of the implant was completed, bone defects filled with such hydrogels were completely regenerated.^{136a} Tests conducted *in vivo* on sheep, with microfracture defects in the knees, showed that implants of chitosan-glycerol phosphate plus full autologous blood can improve the structural and compositional properties of repaired cartilage.^{136b} The application of the hydroxy-apatite/collagen-alginate (APC) composite as bone filler and carrier of recombinant human bone morphogenetic protein (rh-BMP2) was investigated by Shinomiya and coworkers.¹³⁷ Five weeks after filling bone defects by implantation with APC plus rh-BMP2, it was found that bone was formed through the implant without any obvious deformation. The compression strength of APC sponges was high enough to prevent squeezing out of the rh-BMP2 protein during bone formation. However, its brittleness in a dry state imposed limitations on its use. Shinomiya and coworkers concluded

that APC may be useful as a drug carrier.¹³⁷ Acrylic bone cement is generally used as a delivery system for depot administration of antibiotics to the infected bones. However, it is not biodegradable and requires secondary procedure for its removal. Though many biodegradable polymeric and natural materials have been investigated, none has yet been approved for clinical use in the US.¹³⁸ An implant made of PLA with the carbonated calcium phosphate/CaCO₃, was prepared by combination of hot pressing and gas forming. It contained the macroporous, fast degradable poly(D,L-lactic acid) + CaCO₃, on the inside, which promoted the ingrowth of bone cells. To ensure mechanical stability and protection of the implant, it contained the slowly degradable compact PLLA + the carbonated calcium phosphate on the outside. Calcium carbonate neutralized lactic acid formed as a result of degradation of PLA. Good proliferation of the human osteoblastic cells was observed *in vitro*, testifying to the biocompatibility of this implant for cranial reconstruction.¹³⁹ Satisfactory results were obtained, when PLLA implants were used for healing 23 human patients, with scaphoid failure of broken bones.^{140a} The application of implants, prepared from 80:20 poly((L/DL)-lactide) instead of the commercially available 30:70 poly((L/DL)-lactide), was investigated for fixation of fractures of bones in regions of low

load. The higher chemical strength and loading capacity, due to higher crystallinity, promised advantages for long-term implantation. They were tested *in vivo* to promote bone regeneration of circular cranial defects in white rabbits. Short-term studies demonstrated that there was almost complete regeneration of cranial defects after an 8 week period. However, long-term studies must be conducted to confirm the expected advantages of this formulation.^{140b} The glycerol-L-lactide polymer, obtained by ring-opening polymerization (ROP) of L-lactide in the presence of glycerol, could not be used for the fibroblast factor (BFBF)-coated hydroxyapatite discs, because it did not have pores enabling ingrowth of a tissue into the implant.¹⁴¹ HAPEX and AWPEX, composites of high-density polyethylene with hydroxyapatite or with glass-ceramic apatite-wollastonite, showed potential as orthopedic implant materials. HAPEX had clinical successes. In order to explore their biological responses, samples of these implants were immersed at 36.5°C for up to 14 days in the simulated body fluid (SBF), with ion concentration similar to that of human blood plasma. Osteoblast-like cells proliferated on both composite materials. However, preferential attachment to ceramic particles was evident on AWPEX. There was no development of apatite within 14 days of immersion of HAPEX in SBF, while immersion of AWPEX in SBF produced a layer of apatite after 7 days. Its thickness and crystallinity increased continuously during the subsequent 7 days. This study showed that AWPEX, apparently, had a higher level of bioactivity than HAPEX. Both promoted growth of osteoblast-like cells contact guided by the topography of their surfaces, with preferential attachment to ceramic particles.¹⁴² Deformities of the facial skeleton in the maxillofacial region have been reconstructed using high-density porous polyethylene. Its fixation was easy and did show neither tissue ingrowth nor capsule formation. However, placement under the skin without a facial envelope posed the risk of an early or late exposure. Authors of this report stated that autogenic implants instead of allogenic material may be preferable.¹⁴³ Modulus mismatch between bone and the implanted material alters load distribution transferred through the bone, and may cause reduction of stress in some regions of the remaining bone. This may lead to a loss of bone. The unnatural stresses at the bone implant interface may cause bone hypertrophy. Polymeric cups have been developed to eliminate such problems. Brooks *et al.* evaluated body responses to the use for such cups of carbon-fiber reinforced poly(butylene terephthalate). They did not observe any adverse effects and found good bone binding to the hyaluronan coated prostheses.^{144a} The new acetabular cup consists of three zones: surface of the implant is 100% ultra high molecular weight polyethylene (UHMWPE), zone 2 consists of a blend of UHMWPE with PDMS, zone 3, which is the shock absorbing layer of the cup, consists of PDMS only.^{144b} Deformation of the acetabular cup can be decreased and its strength increased by exposing UHMWPE to a 60–66 KGy dose of β -radiation.^{144c} Metal implants are in general not compatible with the human body. In particular, blood exhibits haemostatic response to them. The adverse effects may be minimized by coating prostheses with HA. Pitt *et al.*

coupled HA with the stainless steel surfaces via a silane coupling layer. To achieve this they dipped clean metal surfaces in the aqueous emulsion of crosslinked silane. After drying they dipped it again in an epoxidized silane, and oxidized epoxy to aldehyde by exposing the surfaces to sodium periodate. Subsequently, a layer of the low molecular weight HA, modified by reaction with adipic dihydrazide (ADH), was attached. Aldehydic groups reacted directly with the hydrazide linker. The response of platelets to the HA coated surfaces was passive and non-adhesive.¹⁴⁵ Adhesion of the *Staphylococcus aureus* bacteria to the implanted surfaces has been the main cause of post-implant infections. Coating the TiO₂ layer on the surface of the implanted titanium with PEG grafted on poly(L-lysine) (PLL-g-PEG), reduced adhesion of bacteria by 89%. However, such coating also caused a decrease in adhesion of osteoblastic and fibroblastic cells. They could be restored by functionalizing PLL-g-PEG layer with RGD type peptide (Arg-Asp-Gly). Coating obtained by interacting PLL-g-PEG with PEG-RGD did not affect adhesion of the osteoblastic and fibroblastic cells, while reducing by 69% adhesion of *S. aureus*. Such coatings may be used in the orthopedic and dental implantology and for other medical devices.¹⁴⁶ Another variant of this approach involves embedding an antibacterial peptide between multilayered PLL-g-PEG coats. Positive results were achieved when the positively charged lysine was the outermost layer of the film.^{147a} Fully absorbable osteogenic material was generated by mixing porous β -Ca₃PO₄ with the recombinant human bone morphogenic protein (rh-BMP2), block-copolymerized with D,L-lactic acid (PLA), which contained randomly inserted *p*-dioxane (DX)-PEG segments. These studies indicated that rh-BMP2(PLA-DX-PEG) β -Ca₃PO₄ composite seems to be an efficient enough agent for repairing large bone defects.^{147b} The α -MSH, a melanocortin derivative, attached to the negatively charged PGA, inserted between two positively charged layers of PLL conferred the anti-inflammatory properties on the coating.¹⁴⁸ When protein body fluids are in contact with an implant, and protein adsorption occurs, it is followed by cell adhesion, which may cause undesirable biological responses, such as inflammation, thrombus formation, and encapsulation. In order to minimize cell adhesion, poly(ethylene terephthalate) (PET) surfaces were grafted with a linear-PEI or with poly(allyl amine), and reacted with PEG, which had chain ends functionalized with epoxy or aldehyde groups. Absorption of proteins was eliminated, when PET surface was completely covered by the PEG chains.¹⁴⁹ The properties of bone implants are a crucial factor for their successful osseointegration, though they must also satisfy many other requirements that cannot be satisfied by a single material. Composite materials may provide the desired solutions. Relatively high values of push-out force and ultimate shear strength have been observed for composite implants of the poly(methyl methacrylate) (PMMA)/hydroxyapatite reinforced by E-glass fibers coated by PHEMA.¹⁵⁰ Successful fixation between bone and implant material in knee arthroplasty was achieved by filling titanium fibers, surrounding a titanium implant, with hydroxyapatite slurry prepared by soaking its powder in an ethanol solution of hydroxy-propyl-cellulose. As result of

such treatment significantly higher bonding strength was obtained.¹⁵¹ Durability of the composite of polysulfone (PSu) with carbon fibers (which contained 15% of short and 45% of long fibers) in body fluids environment was investigated and compared with that of PSu. It was found that PSu and its composites can work safely at a level of 30–40% of initial strength, under long-term load, for a period required for bone fixation. Degradation processes were accelerated in body fluids by the presence of carbon fibers.¹⁵² PCL, that has been successfully applied for the delivery of anti-inflammatory agents and protein for bone regeneration, seemed to be a leading candidate for application in case of musculoskeletal pathologies. Giaveresi *et al.* decided to compare with PCL the biocompatibility, biodegradation rate, and vascular density induced by four polyester copolymers and by the substituted poly(organo) phosphazene (POP). All investigated polyesters were in some respects superior and in others inferior to PCL, with the exception of poly(cyclohexyl-sebacate) that was definitely inferior to it. However, POP substituted by phenylalanine and imidazole in 80:20 ratio, showed increase in vascular density in comparison to PCL, while a mild tissue reaction caused by it was similar to that due to contact with PCL.¹⁵³ The investigation of adhesion of the *Escherichia coli* bacteria to surfaces of PMMA, low-density polyethylene (LDPE), and poly(vinyl chloride) (PVC) showed that adhesion to PVC surface was the highest one, apparently due to its acidity. The adhesion of *E. coli* to the surface of PMMA was much lower and was strongly affected by surface purity. After cleaning the PMMA surface with tetrachloroethane it was even lower, possibly due to the effect of trace amounts of the residual solvent.¹⁵⁴

Other implants

Polyisobutylene-based thermoplastic elastomers are considered to be prospective implant materials for replacement and reconstruction of soft tissues. Presently, silicones, PET, PTFE, PE, and PUs, which are relatively inert and biocompatible, are being used for such purposes.¹⁵⁵ The formation of fibrous capsules around implants, which require communication/interaction with the surrounding tissue or with the circulatory system, render them ineffective. It is essential for such implants not only to be surrounded by a highly vascularized tissue, but also to be penetrated by it. Microporous PHEMA hydrogels grafted with PEG have an interconnected pore structure and high porosity for a given fraction of active solvent. PHEMA sponges, prepared with at least 70% of a solvent, are capable of supporting *in vitro* tubule formation, essential for vascularization. Though attachment of the endothelial cells to PEG grafted networks is reduced, it is not crucial for vascularization of a hydrogel. PEG containing networks were better suited for tubules formation than those without PEG. (Apparently, due to the increased interconnectivity of the pores and greater porosity.¹⁵⁶) Intracortical neural implants, with embedded microfluidic channels, were fabricated on the basis of poly(benzocyclobutene) (BCB). It did not exert *in vitro* any toxic effects on the cultured cells, and was not impedimental to their growth and adhesion. Stiff BCB electrodes with silicon backbone penetrated pia of a rat without buckling.¹⁵⁷ Novel chronically implantable cortical electrodes, based on poly-

imide biopolymer, were devised. The load distribution provided flexibility for micro-motion compliance between brain tissue and skull at brain/implant interface, but were stiff enough for surgical handling. A 5–10 nm thick silicone backbone layer was attached to the tip of an electrode to enhance its structural stiffness.^{158,159}

Sutures made of poly(hydroxy butyrate) (PHB) or of its blend with poly(hydroxy valerate) (PHV) were compared with sutures made of silk and catgut. High purity PHB or PHB/PHV (85:15) sutures remained sufficiently strong through the period of healing. Post-traumatic inflammation, lasting up to 4-weeks, was caused by all four investigated implants, Fibrous capsules (less than 200 nm thick) encircled them as result of tissue reaction to the implants. Thickness of capsules formed around the silk and catgut implants did not decrease with time. However, PHB and PHB/PHV capsules later become 40–60 nm thin. Adverse effects, such as suppurating, inflammation, necrosis, calcification or malignant tumor formation, have not been observed even when the intramuscular PHB and PHB/PHV sutures remained in the body for an extended period of time.¹⁶⁰ On the basis of experiments involving implantations of a single polypropylene microfiber into subcutaneous tissue of rats, Sanders and Rochefort noted that vertical separation of collagen fibers was induced by distortion of ECM for fibers with vertical dimensions higher than 5.9 nm. Distortions adjacent to the fibers created dead space regions that attracted cells and initiated encapsulation of an implant.¹⁶¹ Gellan-based scleral implants of indomethacin were evaluated *in vitro* and *in vivo*. Ethylene and propylene glycol were chosen as plasticizers of these implants. The pharmacodynamic studies showed a considerable improvement in the eyes of rabbits with the induced uveitis, as the result of injection of the indomethacin loaded implants into their sclera. Such implants survived in sclera up to 3 weeks.¹⁶² The thermosensitive polydepsipeptide poly(Glc-Asn-NIPPAAm) can be degraded *in vitro* at room temperature by cleavage of the ester bonds in the main chain. It is non-toxic, and so are products of its degradation. Its degradability, lack of toxicity, and cloud point at 29°C (between room and body temperature) make it attractive for implants and other biomedical applications.¹⁶³ Biodegradable implant polymeric materials with shape-memory have been developed for application in biomedicine. These polymers have the capability to change their shape when stimulated by changes in environmental factors such as temperature. The shape-memory effect may be due to minute changes in polymer structure and morphology. Examples of thermoplastic materials or of the covalently crosslinked elastomers have been given.¹⁶⁴ An implantable glucose micro-biosensor for the determination of level of glucose in blood has been developed. It consists of an electrodeposited redox polymer/glucose sensing membrane and of an overcoat, analyte flow, regulating membrane made of the highly hydrophilic poly(4-vinyl pyridine-co-acrylic acid). The response time in amperometric measurements was less than 10 sec. Good correlation was obtained between glucose level in the blood and the readings of a subcutaneously injected sensor.¹⁶⁵ PHEMA hydrogels were modified to improve their *in vitro* potential and their *in vivo* biocompatibility¹⁶⁶ by blending them with methacryloyloxyethyl

phosphorylcholine (MPC) and with PEGylated methyl methacrylate (PEGMA), and crosslinking the modified PHEMA with 3 mol% of tetraethylene glycol diacrylate (TEGDA). PHEMA with 5–10 mol% of TEGDA and 0.5 mol% of PEGMA displayed proton adsorption reduced by 64% in comparison with the unmodified PHEMA, when incubated with such ECM proteins as collagen, fibronectin, or laminin. Optimal viability (>80%) [but low proliferation (<40%)] and lack of cytotoxicity characterized these hydrogels.¹⁶⁷ Higher values of push-out force and ultimate shear strength were observed for implants of the PMMA/hydroxyl-apatite composites reinforced by E-glass fibers coated by PHEM.¹⁶⁸ The biocompatibility of implanted chemical sensors was enhanced by coating them with nitric oxide releasing/generating polymers.¹⁶⁹

Injectable liquids in situ forming semi-solid implants

The hydroxy acids ricinoleic (RA) and lactic (LA) have been copolymerized by three methods: (a) random polycondensation, (b) trans-esterification followed by their repolymerization, (c) ROP of the respective cyclic anhydrides. The composition of the copolymers prepared by different methods and their thermal properties differed. ROP yielded a predominantly lactic acid homopolymer, and contained a small fraction only of the LARA segments. It was highly crystalline and melting points ranged from 162°C for a homopolymer prepared with pure LA in the feed to 137°C for a copolymer prepared with 50:50 LA/RA in the feed and contained 6% of LA-RA segments. Melting points of copolymers prepared by the trans-esterification-repolymerization ranged from 147°C (for a copolymer with 90, 8, and 2% of LA-LA, LA-RA, and RA-LA segments, respectively) to a liquid at room temperature prepared with the 50:50 feed. Copolymers prepared by direct polycondensation were liquid at room temperature when prepared with feed that contained only 20% of the RA monomer. These investigators stated that the biocompatible liquid copolymers may be used as injectable implants for local drug delivery to diseased tissues.^{170,171} Domb and coworkers reported that the injection of a mixture of the liquid biocompatible copolymer with an anti-cancer drug into a body, solidifies on contact with body fluids to form a depot implant. They claimed that such injectable therapeutic implants may offer a more effective and safer alternative to standard chemotherapy. They prepared biocompatible and biodegradable liquid polyester-anhydrides by trans-esterification of RA and sebacic acid. Its mixture with paclitaxel or with *cis*-platinum injected into animal models, solidified *in vivo* and formed a solid implant, which released the drug in a controlled manner. It provided high local concentration of the anti-cancer drug, which was able to destroy malignant cells, with minimal systemic drug distribution.¹⁷² Various physicochemical principles can be used to prepare injectable biocompatible liquids (liquid embolics) that solidify *in situ* to form semi-solid implants. Solubility changes, responsible for precipitation of water insoluble polymers, may be used for certain therapeutic applications. Other methods include ionic or thermal crosslinking or/and polymerization. The use of such implants for the minimally invasive, image-guided treatment of vascular lesions was recently described

by Jordan *et al.* When liquid embolics reach the vascular space, they block it by hardening or by inducing a thrombotic effect. These authors discussed polymers that can be applied for such implants, mechanism and reasons for solidification, and criteria for selecting effective injectable implants, such as cyanoacrylates polymerize with blood clots, partially hydrolyzed PVA dissolved in 50% alcohol, poly(HEMA-co-MMA) in 75% alcohol precipitated in contact with blood, alginate gels crosslink ionically, and so on. Criteria for choosing a particular material for a liquid implant include its biocompatibility, non-toxicity, biodegradability, visibility under a fluoroscopy, viscosity before setting, setting time, and its eventual bioactivity.^{173,174} The preparation and properties of the reversibly thermo-responsive polymers for the *in situ* generation of implants was investigated by Cohn and his collaborators. They pursued two strategies to reach this goal:

- (A) The PEO-PPO-PEO triblocks (known as F-127) were end-capped with tri-ethoxysilane. Their mechanical properties could be further improved by crosslinking with dimethacrylate. The mechanical properties of the hydrated gels prepared by this procedure increased with time as the result of an increase in the degree of crosslinking following their hydrolysis. The initial value of ~1.3 MPa for compression modulus of 30% solution of such a gel increased after a week to 4 MPa.
- (B) The PEO₆₀₀₀ segments were coupled with PPO₃₀₀₀ segments by phosgene (ClCOCl) as a coupling agent. Viscosity of a 20% aqueous solution of thus prepared polyether carbonate was very low below 30°C and jumped above it to very high values (its value approached 100 000 Pa at 37°C).¹⁷⁵ Obviously, such a solution, which may contain a water soluble drug, converts in the body into a semi-solid implant after it is percutaneously injected at room temperature.

Vascular stents

Stenosis and/or formation of thrombus on arteries bringing blood to the heart may be a precursor of cardiac infarct. Medical treatment may involve insertion of a stent into the obstructed blood vessel, which is expected to restore normal blood flow. The use of bare metallic stents for such treatments may, however, cause severe problems. Most of such problems, caused by restenosis, may develop several months after insertion of an implant, however, some may develop within a few days as a result of slight accidental injury to the walls of a blood vessel during insertion of a metallic stent. Inflammation, which may develop on the injured spot, may be followed by formation of a thrombus that can severely obstruct blood flow into the heart. More common problems, apart from the not very frequent accidents, are due to the response of the immune system to the stent. Obstruction of a blood vessel by deposition of platelets on the inserted metallic surface may eventually lead to stent restenosis. A close loop system, perfused with platelets-reach but low-grade thrombogenic plasma, at flow shear rates much below the value at which it induces thrombocytes activation, was recently used to compare hemocompatibility of polymer coated stents with that of

the bare steel stents. Such comparison revealed that the number of activated circulating thrombocytes and their platelet reactivity were much higher for bare steel stents than for polymer coated ones. Moreover the number of platelets adhering to the steel surface was also much higher for the bare steel stents.¹⁷⁶ As a matter of fact, the incidence rate of 30 to 50% of restenosis after implantation of bare metallic stents represents a serious limitation to the success of coronary angioplasty involving application of such stents.¹⁷⁷ Coating of metallic stents with a thin layer of biocompatible polymeric material was believed to be an answer. The application of several polymeric materials, such as hemocompatible copolymers of phosphorylcholine and methacrylates, copolymers of derivatives of the poly(paracyclophane), coated using the chemical vapors deposition (CVD) technique, have been tested (cf. p. 24 in ref. 2). Such polymeric coatings reduced the occurrence of restenosis only to some extent. It has been proposed, therefore, to combine coating of a stent with release of a drug. Use of the coated polymeric layer as a reservoir of drugs seemed to provide an obvious solution, though quantities of drug which can be stored in the coated layer are rather limited. Polymeric coating of a stent and the drugs eluted from it must satisfy many physical, biological, and regulatory criteria. The stability of a coating, its biocompatibility, good adhesion to the metal surface, and slow controlled release of a drug dissolved or occluded by the polymeric matrix are among the key requirements. *In vivo* studies on animal models must be conducted before clinical trials are permitted. Many relevant investigations have been conducted during the last 2 years. The promising *in vitro* and *in vivo* experiments conducted on small animals, were eventually continued on porcine models.^{178–182} In view of the positive results of some of these investigations, they were followed by clinical trials of various drug-eluting stents (DESs). Investigated were such polymeric materials as: blends of segmented polyurethanes (SPUs) combined with copolymers of the methacryloylphosphorylcholine-co-lauryl-co-hydroxypropyl-g-tetramethylsilyl methacrylates; derivatives of poly(paracyclophane) deposited by chemical vapor vacuum deposition technique;² polylactic acid and its copolymers;¹⁸³ polyalkylmethacrylates and polyacrylate copolymers;¹⁸⁴ glycidylxypropyl-3-methoxy-silane combined with polyether- and polycarbonate-PU;¹⁸⁵ ethylene- or butyl methacrylate-co-vinyl acetate and poly(vinyl pyrrolidone)(PVP);¹⁸⁶ ePTFE;¹⁸⁷ triblock copolymer with isobutylene block in the center and cyclohexyl vinyl ether-vinyl alcohol blocks on the outsides;¹⁸⁸ triblock copolymer with isobutylene block in the center and hydroxystyrene, or styrene blocks on the outside.^{189,190}

Poly(bis(trifluoroethoxy)phosphazene) (PTFEP) as a prospective late in-stent stenosis reducing and non-thrombogenic coating was tested *in vivo* on rabbits.¹⁹¹ Effects due elution of such anti-proliferative and anti-inflammatory substances and therapeutics, such as Sirolimus (rapamycin), Tacrolimus, QP2, Everolimus, Biolimus, Paclitaxel, ABT-578, Methotrexat, Dexamethasone, 17-beta-Estradiol, Batimastat, Angiopeptin, Actinomycin-D, Tyrosinkinase inhibitors, were tested. Both non-clinical and clinical parameters were evaluated in such trials. In 2004 only three polymer coated

DES models satisfied all requirements of such trials. Namely: Cypher stent (Cordis, J&J) releasing Sirolimus from the copolymer of poly(*n*-butyl methacrylate) and poly(ethylene vinyl acetate) (PEVA); Taxus (Boston Sci. Corp.) releasing Paclitaxel from the poly(hydroxystyrene-*b*-polyisobutylene-*b*-hydroxystyrene) (HSIBHS) or poly(styrene-*b*-isobutylene-styrene) (SIBS) triblock copolymer^{189,191} and, apparently, Dexamet stent (Abbott) releasing Dexamethasone from the PU coating. Since the goal of these tests is to improve patient outcome, the choice of DES for routine treatment must be directed by results of the randomized clinical studies, based on clinical primary end-points such as MACE (major adverse cardiac event) and TVF (target vessel failure), obtained after an appropriately long-time interval. These criteria have been met last year only by CYPHER¹⁹² and TAXUS.^{179,191,193} In March 2005 results were published of a large clinical trial of the commercial DES systems (sponsored by Johnson & Johnson, producer of the Sirolimus eluting CYPHER stent) showing that efficacy and safety of CYPHER is nearly equal to that of TAXUS Express2. Incidence of stent thrombosis due to CYPHER was lower than to TAXUS Express2. Moreover, clinical trials conducted by Windecker *et al.* and published in August 2005, favored Sirolimus over Paclitaxel. Namely, occurrence of MACE during 9 months period after stent insertion was 6.2% in the first group and 10.8% in the second.¹⁹² Controlled release of Paclitaxel by the TAXUS Express2 stent coated with triblock elastomer SIBS, which have had microphase-separated morphology, was investigated by Ranade *et al.*¹⁹² References to other papers, reporting on the reduction in adverse effects caused by a percutaneous coronary angioplasty, due to elution of Paclitaxel or Sirolimus from polymer coated stents, are cited in refs. 194–199. Papers, in which short-term benefits due to prevention of stent restenosis by these DESs have in principle been confirmed, but doubts have been expressed, either on their beneficiary effect to diabetic patients, or about their possible long-term disadvantages, are cited in refs. 177 and 200–209. For example, Virmani *et al.* cautioned against total disregard of various complications that may be related with the implantation of these stents. Such as increased possibility of the development of a DES induced thrombosis, or an in-stent restenosis, due to hypersensitivity of certain individuals to the polymeric coating or to the eluted drug. They pointed out 290 cases of sub-acute thrombosis (60 fatal), which followed Cypher deployment and were confirmed by “Cordis” and posted in an FDA (Federal Drug Administration) alert, and to reported cases of hypersensitive reactions. They also pointed out that not all results obtained by the *in vivo* testing on animals were unqualifiedly positive. They observed that it is very unlikely that the polymers and drugs used are fully benign. They concluded their review of this subject with the following statement: “Drug-eluting stents are not a panacea as suggested by many interventional cardiologists”,²⁰² Virmani *et al.* cited in another report the case of a 58 year old man who died of late state thrombosis 18 months after receiving two Cypher stents for unstable angina. They pointed out that the aneurismal dilation of the stented arterial segment discovered during his autopsy and the presence in this area of giant T lymphocytes and eosinophiles suggested that reaction of his body to the

polymer caused the late stent thrombosis. They recommended careful long-term follow-up after Cypher stent placement.^{202b} Radke *et al.* pointed out that conventional intracoronary radiation (ICR) treatment of the long-term in-stent restenosis caused by bare and by Paclitaxel-eluting stents led to comparable results.²⁰⁶ Steffel *et al.* recently reported that their studies revealed that rapamycin (Sirolimus) enhanced endothelial cell expression, induced by thrombin and tumor necrosis factor. These effects may favor the development of thrombus formation as the result of deployment of Sirolimus-eluting stents. Particularly after withdrawal of anti-thrombotic drugs.²⁰⁷ Serruys *et al.* pointed out that the marked reduction of in-stent restenosis on the implanted TAXUS II stents is not associated with a decrease in edge stenosis. Actually, the reduction of the lumen loss at the distal edge was significantly smaller after implantation of TAXUS II than after implantation of a bare stent.²⁰⁸ Wessely *et al.* pointed out that since current commercially available DESs use non-biodegradable polymers, some investigators suspect the possibility of development of late stent thrombosis in patients who received them. Moreover, the development of late in-stent restenosis is still an unresolved issue. They reported two cases in which each patient received one bare stent and one Sirolimus-eluting stent. During the first 7 months the minimal lumen diameter remained constant in the blood vessel stented with the Sirolimus-eluting stent and significantly decreased in the vessel with a bare stent. However, during the following 11–14 months it slightly increased in the bare stent stented vessel, while it drastically decreased in the Sirolimus-eluting one. As a result recurrent angina at exertion occurred, which had to be treated by percutaneous intervention. They recommended further studies of clinical relevance of this phenomenon and the determination of the length of follow-up for patients receiving DESs.²⁰⁹ Stents with a composite coating, consisting of Everolimus or Tacrolimus (Sirolimus-like agents) mixed with an equal quantity of a biodegradable poly(hydroxy acid), were recently introduced by Biosensors International Inc. Preclinical trials of these stents, in which non-clinical criteria were used in evaluation, were encouraging. However, results of some clinical trials were disappointing. Nevertheless, the initial clinical experience of Grube and Buellesfeld with Everolimus-eluting stents, coated with bioabsorbable polymer, indicated that this may be a safe and efficacious method for reducing in-stent neointimal hyperplasia and restenosis.²¹⁰ Actinomycin D-eluting stents investigated by Serruys *et al.* have shown increase instead of decrease of restenosis after percutaneous coronary angioplasty. However, the rate of survival after coronary incident increased. Perhaps a small percentage of this drug could be added to drugs that effectively reduce restenosis.²¹¹ Jeong and coworkers studied the use of Abciximab, an inhibitor that blocks the final pathway of platelet aggregation and which, even when applied systemically, may decrease to some extent the short- and long-term event rates after percutaneous coronary intervention. It was attached to the surface of a stent functionalized by plasma polymerization of diamino-cyclohexene. Amidation of the carboxylic group of Abciximab produced a link to the layer of the polycyclohexene on the stent surface. Clinical trials with

the Abciximab-coated stents demonstrated that they were effective in prevention of the intimal hyperplasia and of stent thrombosis, even in patients with myocardial infarctions and unstable angina. Jeong and coworkers suggested that vasculo-protective agents such as Abciximab may provide an alternative to the antiproliferative agents.²¹² Kenan and coworkers proposed to consider the use of the environment-responsive (TRP) hydrogels, based on NIPAAm, as stent coatings. They pointed out that these hydrogels show an ability to incorporate and release drugs, modify activity of platelets, enhance proliferation of smooth muscle cells, and promote attachment of vascular endothelial cells to the walls of an artery. He admitted, however, that some important issues must be clarified before such use of the TRP hydrogels may be attempted.²¹³ Phospholipid-like copolymers based on 2-methacryloyloxyethyl phosphorylcholine (MPC) copolymerized with other methacrylic segments (see also ref. 2 pp. 24–25) were modified by incorporation of 5 to 20% of the positively charged choline methacrylate (CMA). This modification imparted it with the ability to absorb/adsorb many drugs, proteins, nucleotides, and to release them in a controlled fashion. Coronary stents coated with the CMA containing polymer were loaded with such compounds as the cell migration inhibitor-Batimastat, the anti-proliferative agent-angiopepsin, the IIIb/IIIa receptor inhibitor-Abciximab, anticoagulant-heparin, plasmid DNA for gene therapy and several other oligonucleotides. *In vivo* studies have indicated that these compounds can be delivered to the stented section of the vessel, while very small quantities were delivered outside the target area. Moreover, coatings remained stable after the elution of a drug and after 6 months implantation *in vivo*. After successful *in vitro* and *in vivo* experiments, these stents were sent for clinical evaluation to several hospitals and pharmaceutical companies.^{214,215} A stent produced by BiodivYsio, coated with MPC based polymer, was loaded with phVEGF 2-plasmid, which contained DNA encoded for vascular endothelial growth factor (VEGF)-2. It was tested *in vivo* by implantation in rabbits. The reendothelialization was nearly completed after 10 days in the VEGF stent group and was significantly greater than in the control group. Moreover, after 3 months the lumen cross-sectional area was significantly greater and a narrowing significantly lower for VEGF stents implanted in hypercholesterolemic rabbits than for the control. Losordo and coworkers concluded on the basis of these results that reendothelialization via VEGF-2 gene-eluting stents provides an alternative treatment for prevention of restenosis. It may be applied alone or as combination therapy.²¹⁶ Radioactive devices have been obtained by coating plasma functionalized surfaces of stents or catheters, made of TiNi alloy or of Teflon, with a HA-diethylenetriamine pentaacetic acid (HA-DTPA) conjugate complexed with yttrium or indium. The edges of the stent can be made more radioactive to eliminate edge effects, related to the re-narrowing of an artery at stent edges. These investigators suggested that combination of the hemocompatibility of a HA-coated stent with the anti-proliferative effects of radiotherapy will help to alleviate restenosis induced by existing devices.²¹⁷ Caprolactone was grafted *in situ* onto the activated surface of a metallic stent. Good adhesion of the biodegradable aliphatic polyester

PLA, PGA, or PLGA deposited on the PCL layer grafted onto a metal surface was achieved. Coated stents loaded with a therapeutic active compound, such as Rapamycin (Sirolimus) or CVT313-aCDK2 inhibitor, could control their slow release for a period of several months. The biocompatibility of these coated stents was investigated *in vitro* in cell cultures and *in vivo* in pigs. However, it is not clear that these investigators were aware of the fact the TAXUS stent (produced by Boston Sci.) originally used the copolymer of ϵ -caprolactone and LA, which was later replaced by currently used non-biodegradable hydrocarbon copolymers [probably because of some not disclosed negative results!! (cf. p. 316 in ref. 202)]. The relationship between properties of the coated stent and system performance was analyzed.²¹⁸ Release of Paclitaxel and Rapamycin loaded into the collapsible stents coated with PDLLA and PDLLGA was investigated *in vitro* by Venkatraman and coworkers. They showed that the initial burst of the drug can be eliminated by judicious selection of the polymer/drug combination. Drugs were released in a sustained fashion for a month from PDLLGA and for several months from PDLLA.²¹⁹ Bioresorbable expandable polymeric stents were prepared from the high MW PLLA fibers. They were coated with poly(DL-lactide-co-glycolide) (PDLGA) loaded with albumin. Stents were immersed for 3 sec in 70:30 CH₂Cl₂/ethanol solution to slightly dissolve their surface layer and then dipped in microsphere powders containing albumin. The initial radial compression strength of these stents was 200 KPa and it started to decrease only after immersion for 8–12 weeks, at 37°C in buffered saline solution. A reservoir of albumin in microspheres enabled a sustained slow release over periods of several months.²²⁰ Self-expanding biodegradable stents made from PLA+P(D,L)LA or of PLA + PCL loaded with Dexamethasone or with Simvastatin were tested *in vivo* on porcine model. They appear to be biocompatible and reliable [(?)see exclamation signs in the discussion of ref. 219]. They caused only a minimal neointimal hyperplasia. However, these investigators stated that these stents should be further investigated, to prove their safety and efficacy.²²¹ Inhibitory effect of heparin on formation of aggregates of leukocytes with platelets and platelets-monocyte conjugates, in acute myocardial infarction patients, implanted with heparin coated stents was demonstrated. This seems to indicate that implantation of the heparin coated stents is less thrombogenic than balloon angioplasty alone.²²² Collagens weak antigenic character makes it an attractive candidate for stent coating or vascular grafts. However its thrombogenic nature limits this application. Heparinization, which makes it less thrombogenic, performed by crosslinking with heparin, extensively oxidized by periodate, was performed to solve this problem. Results of this study indicated that immobilization of heparin on collagen prevents thrombus formation.²²³ Paclitaxel derivative 7-hexanoyltaxol was loaded into novel polymer-coated stent QuaDDS supplied by Quantum Medical Corp./Boston Scientific Corp. Clinical tests on patients, after percutaneous coronary angioplasty, led to the conclusion that the safety profile of this stent was unacceptable, because of the increased rates of stent thrombosis, myocardial infarctions, and cardiac death.²²⁴ Stents coated with poly(hydroxy-methyl-*p*-xylene-co-propy-

lene) were tested *in vitro* and *in vivo* for their biocompatibility. Results of this study indicated that this coating is unacceptable for medical applications.²²⁵

Catheters and other stents

A review of the current status of biomaterials available for the urinary tract, their biocompatibility, and clinical use, were discussed by Denstedt *et al.* They pointed out that the ongoing research in this field is involved in optimizing their biocompatibility and the biomaterials related complications such as infections and encrustation within the urinary tract.^{226a} Another paper of Denstedt and coworkers provides a concise review of the use of stents in urology and of the progress made in development of a bioactive infection and thrombosis resistant catheter.^{226b} The range of materials currently used for manufacturing Foley catheters from both latex and silicone was described. Problems associated with their hospital-acquired infection, encrustation and blockage were outlined. The use of slow release polymers introducing disinfectants and antibacterial agents is proposed. Attempts to use hydrogel, PTFE, or silicon coatings based on silver were described. It was pointed out, however, that problems still remain with the design and materials currently used to manufacture catheters.²²⁷ A stent coated with pu-urea (PUU), which was crosslinked *in situ*, was loaded with glucocorticoid prednisolone by equilibrium immersion in its solution. It was submitted to ion implantation, in order to improve kinetics of release of the drug, by destroying it in a surface layer. The toxicity caused by its initial fast release was minimized. Moreover, the PUU matrix was modified by ion implantation, which introduced oxidized groups and double carbon bonds.²²⁸ Teflon coated guide wires for catheterization were tested for the bending force of the tip and the shaft, tip puncture force, and force required to pull it out. The measurements indicated safety of the tip.²²⁹ A new tubular mesh configuration of self-reinforced biodegradable polylactic urethral stents was developed. Their biocompatibility and degradation time was tested in rabbit urethra. Changes caused by operative trauma gradually abated in biodegradable stent groups, while chronic inflammatory changes and fibrosis was evident even after 6 months in metallic stents. These biodegradable, self-expanding braided stents have been found to be suitable for clinical studies.²³⁰ Catheter-related infection may be minimized by coating catheters with a silicon or pu layer loaded with silver nanoparticles. *In vitro* tests of the Pu matrix impregnated with silver nanoparticles demonstrated its hydrophilic character and its antibacterial activity. The adherence of bacteria to the surface of such catheters was minimal, proliferation of bacteria adhered to it was prevented, as was formation of a biofilm.²³¹ The alterations of chemical, biological, immunological and physical properties of polymeric materials used for central venous catheters have been extensively investigated to prevent their bacterial colonization. The current state-of-the-art strategies to minimize risk of catheter-related bloodstream infections (CRBSIs) are analyzed. Progress made in developing infection- and thrombosis-resistant dialysis catheters have been described.²³² A novel biodegradable fluoroquinolone polymer has been developed. It releases malidixic acid (an antibiotic) slowly and continuously and

prevents catheter-induced infection during drainage of cerebrospinal fluid. It was prepared from diisopropylcarbodiimide, PCL, and malidixic acid. The released antibiotic and its derivatives are active for 3 months against *E. coli*, *S. aureus* and *Salmonella typhi*. Application of this polymer will enable manufacture of drainage catheters that will resist catheter-induced infection by delivering antibiotics for more than 1 month.²³³

A polymer-based bioabsorbable biocompatible urethral stent was developed. The efficacy of this stent loaded with methotrexate was tested *in vitro* before and during stent degradation.²³⁴ Tenenbaum-type Teflon coated stainless steel stents for distal malignant biliary obstruction were clinically tested as an alternative to the presently used polyethylene stents. However, this study did not indicate that its patency was better than that of the presently used one, and its resin will be attempted.²³⁵

Polymeric and polymer coated medical devices

Implantable medical devices are still a major source of hospital acquired infections. A new approach based on impregnation of silicon made devices with silver nanoparticles. To achieve this, silicon was impregnated with silver nanoparticles in supercritical carbon dioxide.²³⁶ *In vitro* degradation of self-reinforced PLGA 80L:20G material was studied in artificial urine and in phosphate buffer solutions. The measured changes in the bending, shear, and compression strength after immersion in these solutions for 6, 8, 12, and 15 weeks coincided with clinical tests. These results seemed to indicate that, for urological purposes, it was possible to make sufficiently accurate models of the degradation rate of bioabsorbable polymers, on the basis of the *in vitro* studies.²³⁷ A polymeric microreservoir device for controlled pulsatile drug delivery is based on the rate of degradation of a thin PLGA membrane that seals each reservoir. *In vitro* measurements indicated correlation between the time at which maximum swelling of the membranes was observed and the release time of various molecules. Changes in MW values and in the hydrophilicity or hydrophobicity of drugs loaded into such a device does not appear to affect neither time of their release or membrane swelling. Burst of a membrane occurred when the MW of PLGA went down to the 5000–15000 Da region.²³⁸ Programmed polymeric devices for pulsed drug delivery rely either on the membrane degradation, or on their osmotic burst, or on both. In systems using spontaneous hydrolysis of membranes, rate of release is controlled by bulk erosion of PLGA or by the surface erosion of dextran-HEMA or of PLA. Systems, which depended on an enzymatic degradation, have also been designed. Systems relying on osmotic bursting have a reservoir of NaCl or other salts attached. Dextran systems rely on biodegradation plus osmotic bursting. Osmotic pumping involves permeation of water through a semi-permeable membrane into a solid salt reservoir. The increase in osmotic pressure, due to dissolution of a salt, stretches a membrane until it opens an orifice of an elastic cap through which a drug is released as a pulse. Osmotic pressure goes down, cap closes and a new cycle starts again.²³⁹ Models may be constructed to predict absorption rates of bioabsorbable devices as a function of

the initial degradation properties. The degradation rate of PLLA/HA screw was predicted on the basis of a decrease in the MW value and flexural strength during 55 weeks of degradation *in vitro*.²⁴⁰ Self-lubricating silicone biomaterial has been prepared by crosslinking silicone elastomers with tetra-olexyloxy-silane. Malcolm *et al.* found that this crosslinking agent not only imparted an inherently lubricious character to the condensation-cure silicone elastomers but also enhanced the release of the antibacterial drug metronidazole loaded into it. This effect was due to the increased solubility of the drug in oleic alcohol as compared to its solubility in silicon.²⁴¹ SPU has been found to be the best synthetic material for artificial heart valves. However, in long-term implantations serious problems may develop due to calcification of its surface. Apparently, calcification can be prevented by grafting PDMS onto a SPU surface. The grafted PDMS showed no cytotoxicity and was compatible with fibroblast cells. Adhesion of platelets to the grafted surface and to SPU seemed to be unchanged.²⁴² A new dual acting trilayer polymeric coating that combines NO release with effect due to the surface-immobilized heparin was prepared. It contained a dense bottom polymer layer, a middle layer (polymer matrix doped with the NO-donor), and upper polymeric layer (aminated PVC or aminated medical grades PU) to which heparin was bound. It was confirmed that the surface bound heparin did not significantly affect the level/release duration of NO complexed with the NO-donor in the middle layer. This coating is supposed to mimic the action of the endothelial cell layer that lines the inner wall of blood vessels. Greater thromboresistivity is expected from polymers utilizing either NO-release or immobilized heparin alone.²⁴³ State of the art of the novel, NO-releasing or generating polymers, in respect to their preparation, properties, and application as coatings of medical devices to render them highly biocompatible, have been reviewed.²⁴⁴ The long-term safety of the coated stainless steel or Nitinol stents, which contain chromium or nickel as additives, is controversial, since their eventual corrosion may lead to the release of potentially toxic and carcinogenic compounds. Plasma deposition on the metallic surface of an ultra-thin layer of a fluorocarbon has been proposed to prevent such harmful developments. A thin strongly adherent layer of an inert polymeric material will provide complete isolation of the device from the body fluids.²⁴⁵

CONCLUDING REMARKS

Numerous papers published during the last 2 years on the application of polymers in the fields of tissue engineering, regenerative medicine, and medical devices, attest to the great and still growing interest of the scientific community in the biomedical applications of macromolecules. Very considerable progress has, indeed, been made recently in these fields. Very optimistic assessments of benefits that such discoveries, for example self-assembly of certain biomolecules, will make in the near future to the well-being of mankind seem to be, however, a bit exaggerated. Solution of problems involved in these very complicated subjects is difficult and many obstacles are probably still ahead. A very enthusiastic response to the introduction of the DESs seems also to be a bit premature.

Many very favorable reports on the results of their implantation have been cited in the present concise review. However, a few papers calling for caution and pointing out the adverse effects to certain individuals and to the possibility of enhanced late thrombogenicity, have also been included.

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