Journal of Applied Polymer Science

REVIEW

Enhancing Expanded Poly(tetrafluoroethylene) (ePTFE) for Biomaterials Applications

A. Ian Cassady,[1] Norsyahidah Mohd Hidzir,[2,3] Lisbeth Grøndahl[2]

[1]School of Medical Science, Griffith University, Gold Coast, Queensland 4222, Australia

[2]School of Chemistry and Molecular Biosciences, University of Queensland, St Lucia, Queensland 4072, Australia

[3]School of Applied Physics, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, UKM Bangi, Selangor 43600, Malaysia

Correspondence to: L. Grøndahl (E-mail: l.grondahl@uq.edu.au)

ABSTRACT: Poly(tetrafluoroethylene) (PTFE), a fully fluorinated linear thermoplastic polymer, and in particular the porous form expanded PTFE (ePTFE) has found widespread use in biomaterials application due to its properties of high toughness, non-adhesiveness and hydrophobicity. While it performs ideally for many applications, some challenges have been identified for its use in small diameter vascular grafts and as a tissue space-filler for cosmetic reconstructions where the implant interfaces with bone. For these applications modification of the surface of ePTFE has been investigated as a means to enhance its performance. This review will focus on the applications listed above and will detail methods of evaluating the biological response, methods used to enhance the surface properties of ePTFE, and how the modified materials have performed in their intended applications. This review will focus on work published from 2004 onwards. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40533.

KEYWORDS: biocompatibility; biomaterials; grafting; polyolefins; surfaces and interfaces

Received 20 December 2013; accepted 29 January 2014

DOI: 10.1002/app.40533

INTRODUCTION TO ePTFE AND ITS USE IN BIOMATERIALS APPLICATIONS

Poly(tetrafluoroethylene) (PTFE), a fully fluorinated polymer, is polymerized from tetrafluoroethylene (CF_2=CF_2). It is a linear thermoplastic polymer, which was discovered in 1938 by Plunkett while working for Du Pont.[1] The strong C—C and C—F bonds[2,3] together with the helical structure of the polymer chain caused by the relatively larger size of fluorine atoms compared to carbon atoms[2,4] give PTFE high thermal and chemical stability. Furthermore, since the carbon atoms in the chain are enclosed within a sheath of electronegative fluorine atoms the polymer chains are very stiff and this results in a highly crystalline material.[4] PTFE exhibits good electrical insulating properties, has high toughness, is non-adhesive, has anti-frictional properties and is extremely hydrophobic.[5,6]

The expanded form of PTFE (ePTFE) was developed by Wilbert Gore with the first patent for the process granted in 1976 followed by several subsequent inventions over the following years.[7,8] The stretching process was conducted at temperatures above the lowest crystalline melting point of PTFE and resulted in an increase in amorphous content of ePTFE compared to the starting material. The ePTFE materials can be described as having a microstructure composed of nodes interconnected by fibrils. The size of the morphological features as well as the crystallinity can be tailored by the conditions used in the expansion process. This is illustrated in Figure 1 which shows the SEM images of ePTFE membranes from three different suppliers. The percent crystallinity was determined as previously reported,[9] and is indicated in the figure text.

The first reported biomaterials application of PTFE was as an artificial heart valve.[10] Shortly after, a woven textile graft of PTFE found application as a vascular graft material, however, it was found not to be ideal as it unraveled post implantation.[10] In contrast, ePTFE has proven more favorable as a biomaterial due to its antithrombotic surface and porosity which allow tissue in-growth (e.g., fibrovascular[11] and dermis[12]). Furthermore, it displays enhanced mechanical integrity.[13] PTFE is one of few (if not the only) synthetic polymer which is truly biostable and an *in vivo* study of ePTFE showed that it is stable for up to 6.5 years (length of study) after implantation.[14] Because of its overall good performance in the human body PTFE and in particular ePTFE have found numerous biomaterials applications, some of which are listed in Table I. While W. L. Gore and Associates continues to manufacture ePTFE materials and has an extensive product range in the medical implant market, other companies included in Table I likewise provide PTFE materials for biomaterials applications.

A. Ian Cassady graduated with Honors and an MSc in Biochemistry from the University of Queensland; he undertook his PhD in Molecular Biology at the University of Adelaide. He worked at the University of Queensland's Institute for Molecular Bioscience on gene regulation in macrophages and osteoclasts of bone. He is interested in bone repair and has focused on bone biomaterial development and biocompatibility. He is currently employed at Griffith University as Senior Lecturer in Clinical Biochemistry.

Norsyahidah Mohd Hidzir obtained a Bachelor degree with Honours in Nuclear Science in 2008 from the Universiti Kebangsaan Malaysia (UKM). She has worked as a tutor at UKM since 2009 and was provided with a sponsorship from UKM to pursue her PhD study in radiochemistry at The University of Queensland in 2010. She was offered a lectureship at UKM after completing her PhD. Her research interest is in radiation effects of polymer.

Lisbeth Grøndahl obtained a Cand Scient (MSc) and a PhD degree in chemistry from the University of Copenhagen. During her postgraduate studies she spent two years at the Research School of Chemistry, the Australian National University (Canberra). After completing her PhD degree in 1995 she held positions as assistant lecturer and Postdoctoral Fellow in Denmark and Australia. In 2002 she was appointed to a lectureship at the University of Queensland where she is now Associate Professor.

It has long been established that ePTFE is suitable in vascular graft applications in general, however, it cannot be used for small diameter grafts due to occlusion caused by thrombosis and intimal hyperplasia.[15] This issue has been addressed in a modified ePTFE material manufactured by W.L. Gore under the trade name Propaten® and more detail of this product will be given in the Vascular section. Another application where it has been identified that the material itself is not ideal but requires modification of its surface in order to provide an implant with optimal performance is in its use as soft tissue fillers interfacing with bone tissue. To the best of our knowledge these are the two applications where the largest research efforts have been conducted in order to improve the medical performance of ePTFE and this article therefore seeks to provide a comprehensive review of these two applications. The focus will be on research done in the period from 2004 to July 2013.

BIOCOMPATIBILITY

Definition of Biocompatibility

Studies of the interactions between biomaterials and the biological environment in which they have been implanted have

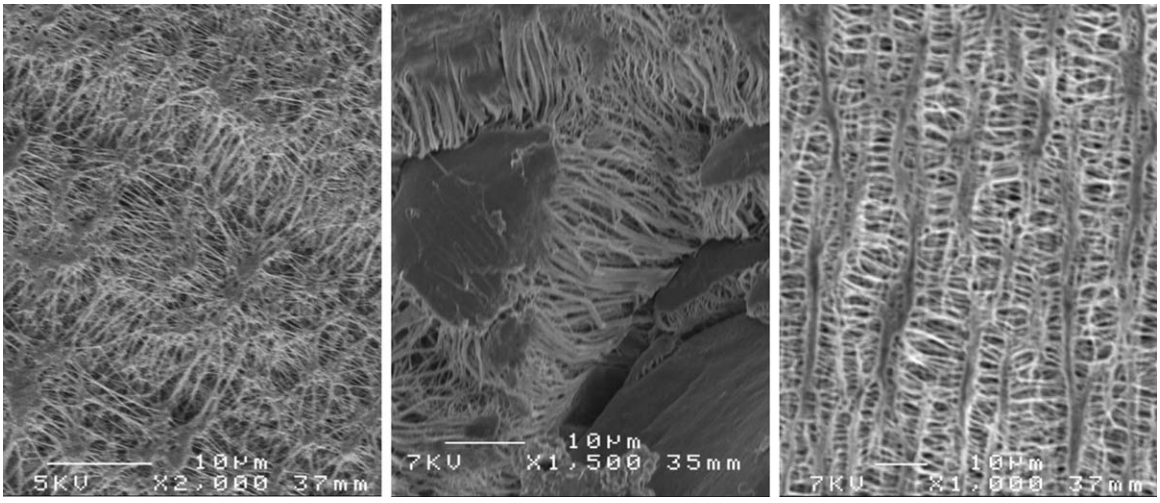


Figure 1. Examples of ePTFE structures engineered for different applications. Products from (left) Pall Corporation, (middle) W. L. Gore and Associates, and (right) Sumitomo Electric. These materials display crystallinities of 43, 14, and 24%, respectively (determined by DSC as per Ref. 9).

Table I. Examples of Biomaterials Applications of PTFE and ePTFE

Application	Trade name	Supplier[a]
Vascular graft	Gore-Tex®	W. L. Gore and Associates
	AV Access Graft	BARD®
	Advanta™ Graft	Atrium Medical Corporation
	Aeos® ePTFE	ZEUS®
Bypass graft	Gore® Hybrid Vascular Graft	W. L. Gore and Associates
	Distaflo®	BARD®
Tissue space-filler in soft tissue reconstruction	Gore® DUALMESH®	W. L. Gore and Associates
	GORE-TEX® Soft Tissue Patch	W. L. Gore and Associates
	PTFE knitted mesh	SurgicalMesh™
Guided bone regeneration	Cytoplast™ micro textured high density membrane	Osteogenics Biomedical
	TefGen regenerative Membrands™	Lifecore Biomedical
Hernia membrane	Gore® DUALMESH®	W. L. Gore and Associates
	PTFE knitted mesh	SurgicalMesh™
Sutures	Gore-Tex®	W. L. Gore and Associates
Suture support	Cytoplast® monofilament	Osteogenics Biomedical
	Pledget	Santec medicalprodukte gmbp

[a] Supplier web sites: W. L. Gore and Associates: http://www.goremedical.com/; BARD® Peripheral Vascular: http://www.bardpv.com/_vascular/index.php; Atrium Medical Corporation: http://www.atriummed.com/EN/default.asp; ZEUS®: http://www.zeusinc.com/extrusionservices/materials/aeoseptfe.aspx; SurgicalMesh™: http://www.surgicalmesh.com/index.htm; Osteogenics Biomedical: https://www.osteogenics.com; LifeCore Biomedical: http://www.toothcare.gr/uploads/documents/TefGen%20Q&A.pdf; Santec medicalprodukte gmbp: http://www.santec-medical.de/e-chordae-loops-and-pledgets.htm

engendered the term, biocompatibility. Williams defined this property in its broadest terms as, "the ability of a material to perform with an appropriate host response in a specific situation."[16] Additional functional characteristics can be taken into account including, (i) that the material should do no harm, and (ii) that the tissue or cellular response elicited is consistent with the functional role of the material. In 2008, Williams proposed the following paradigm for biocompatibility: "The biocompatibility of a scaffold or matrix for a tissue-engineering product refers to its ability to perform as a substrate that will support the appropriate cellular activity, including the facilitation of molecular and mechanical signalling systems, in order to optimize tissue regeneration, without eliciting any undesirable local or systemic responses in the eventual host."[16] Both the type of biological response to a biomaterial and its extent are influenced by the intrinsic properties of the biomaterial as well as its biological context when used in an implanted device. Many variables affect the biocompatibility of a material but in this review we will restrict our examination to those variables that substantially impact upon the biocompatibility of ePTFE for vascular and bone-interfacing applications.

When a biomaterial is implanted then the material surface is the site of interaction with the biological environment. The nature of this interaction is partly governed by the surface properties. An extensively utilized approach to improve the biocompatibility of ePTFE has been to modify the surface of the material. This results in alterations of the surface chemistry, surface wettability, roughness and viscoelastic properties; all of which affect the ultimate performance of the material in a biological setting.[17] To illustrate the complexity of the interaction of a biological system with a biomaterial surface, the important consideration of material wettability will be described. An analysis of the literature by Menzies et al. found that increased biocompatibility is not necessarily associated with biomaterial surfaces that have small contact angles. They identified the requirement for a balance between surface hydrophilicity and hydrophobicity to produce optimal biocompatibility. Highly hydrophobic surfaces produce increased cell-to-cell affinity and result in reduced biocompatibility, whereas highly hydrophilic surfaces result in reduced interactions between cells.[18]

In addition to its surface properties, the three-dimensional structure of the material has a significant effect on its biocompatibility. A material can be fabricated as a solid block, as a porous scaffold or as a fibrous matrix. In fact, there is an abundance of literature reporting on the characterization of biological responses to different biomaterial conformations.[19,20] Therefore, the choice of the surface and three-dimensional structure of a biomaterial to use for a particular application rests not only on its physical properties but the biological response that it elicits at its implantation site. Assessment of the biological response of the material is a critical determinant of the value of a material for biomaterials applications.

Assessment of Biological Response

The biological response to ePTFE is dictated by the interaction with cells at the implantation site. Typically a number of stages are used to study the cellular response: (i) *in vitro* cell culture with either primary cells or cell lines, and these may also include exposure of the material to primary cells *ex vivo*; (ii) animal implantation models; (iii) provided the material has been demonstrated to be non-toxic and safe the next stage is to test it as an implant in human subjects. The assays employed

have been examined in detail in several recent reviews of the literature.[16,21–24] The basic elements of a testing scheme that is employed for any material are assessments of cytotoxicity, hemolysis, and mutagenicity. A systematic approach has been adopted to collate the physical properties, chemistry, and biological performance information in a centralized database maintained by ASM International and described by Helmus et al.[25,26] This resource is the ASM Medical Materials Database, which records an extensive set of cross-referenced articles on biomaterials that can be traced to the original publications.

The parameters that have been used for *in vitro* assays are cell adhesion; production of biologically active mediators such as NO, cytokines, thrombogenic proteins which can be detected using enzyme-linked immunosorbent assays, western blotting or protein activity assays such as through the detection of a response by secondary cells; and gene expression analyses by northern analysis, or microarray analysis. Of greatest relevance are the *in vivo* assays using animal models or human subjects that examine implant-associated wound repair, peri-implant histology, inflammation, foreign body giant cell formation, cellular infiltration, fibrosis, and scar tissue formation.

This review focuses on providing a condensed examination of the tests employed with surface-modified ePTFE. As illustrated in Figure 2, at vascular implantation sites there is a requirement for endothelial cell adherence and proliferation, a low level of platelet adherence and minimal neointimal hyperplasia. To prevent platelet adhesion many approaches design materials that are nonfouling in order to achieve a non-thrombogenic material, however, this can also reduces endothelial cell adhesion. A further requirement for vascular grafts is compliance match between the grafted vessel and the one being replaced in order to promote long-term graft patency and this relates to the bulk properties, importantly the elasticity of the material. For materials intended to interface with bone tissue upon implantation there is a requirement for osteoblast cell adhesion and proliferation. A simple evaluation of the ability for a material to from a strong interface with bone tissue *in vivo* is a noncellular so-called simulated body fluid test which investigates mineralization of the surface. In addition to the requirements specific for each of the two applications listed in Figure 2, the materials must display minimal proinflammatory response and macrophage infiltration as well as minimal immunogenic response. To provide a stable interface between the material and the biological environment it is furthermore important that the surface layer is stable to both delamination and oxidation such that long-term patency can be achieved.

MODIFICATION OF ePTFE

The surface is the site at which cells interact with the material; therefore specific modification provides the capacity to modulate cellular responses. There are many reviews concerned with surface modification of polymers in general[27–30] and specifically with surface modification of fluoropolymers.[3,31] Furthermore, a large number of reviews focus specifically on improving polymers for cardiovascular applications.[30,32–34] The reader is directed to these reviews for in-depth discussions of these topics while the current article will give an overview of surface modification techniques that have been used for ePTFE materials for applications as vascular grafts and soft tissue fillers interfacing with bone.

While PTFE has high chemical and thermal stability, its radiation stability is akin to that of hydrogen-containing polymers. The most commonly used methods for surface modification of PTFE therefore involves high energy radiation. The modification may be a surface treatment (e.g., oxidation or ablation) as the overall outcome; it may be a surface coating; or it may be a radiation-induced grafting process. In addition to these radiation processes, adsorption of e.g., hydrophobic molecules to the highly hydrophobic polymer has also been explored. In all cases the surface chemistry is altered and this may be used as the final product. Alternatively, the introduced surface chemistry may subsequently be used for the immobilization of biological molecules or fractions thereof.

Chemical Surface Treatments and Coatings

A vast range of surface treatment approaches have been used to alter the surface properties of PTFE for medical applications. Photoderivatization was explored by Williams et al. who chemically modified the protein Laminin type 1 via linker molecule with benzophenone groups which were subsequently used to bind covalently to PTFE.[35] In addition, wet chemical treatment using Na in naphthalene allows the introduction of hydroxyl

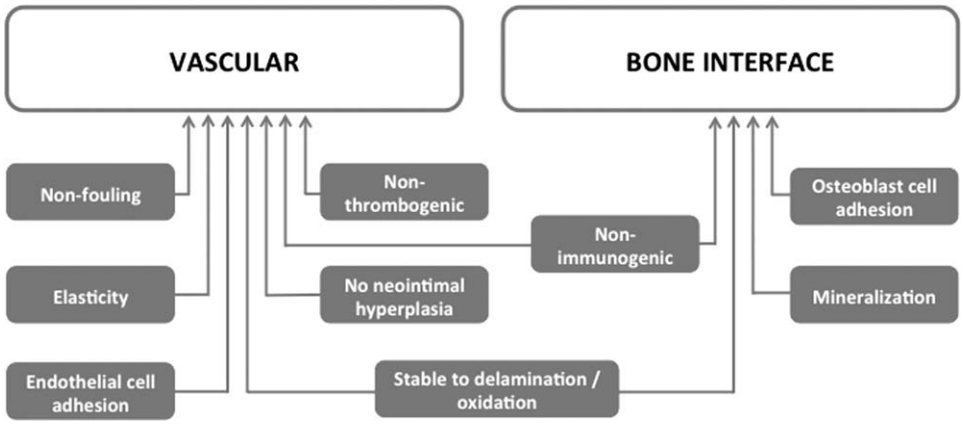


Figure 2. Schematic illustration of the desired properties of ePTFE materials for use in vascular grafts and as soft tissue fillers interfacing with bone tissue.

groups (in part through oxidation of introduced vinyl moieties) which resulted in a large reduction in the advancing contact angle and these groups were subsequently used for immobilisation of a biological molecule (Attachment of Biologically Active Molecules section).[36]

A number of studies led by Laroche have used ammonia plasma treatment as the first step in multistep modifications of PTFE (see further detail Attachment of Biologically Active Molecules section).[37–40] It was observed that after immersion of the plasma-treated samples in PBS the nitrogen content halved and in addition, this method introduced a range of nitrogen functionalities with ~20% amines. The amine groups were subsequently used to attach various molecules including a range of linker molecules resulting in the introduction of carboxyl groups,[37–39] aldehyde groups,[37] maleimide derivatives,[37] thiol groups[37] as well as the cell membrane biomimic phosphorylcholine.[40] In addition, argon plasma treatment coupled with a chemical reaction with chloroacetic acid has been demonstrated to allow introduction of carboxylic acid groups which were subsequently used to link to biological molecules (Attachment of Biologically Active Molecules section).[41,42]

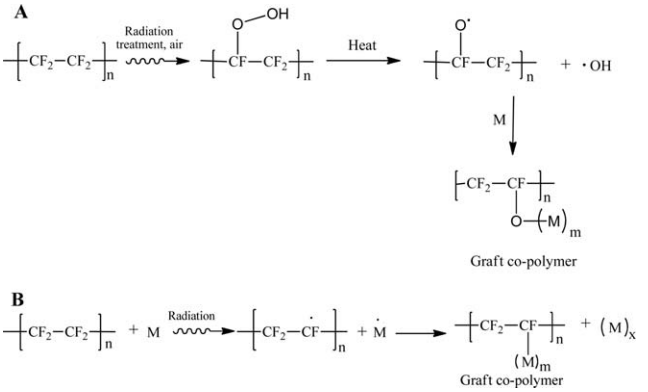
The combination of plasma activation and ion implantation, e.g., plasma immersion ion implantation (PIII), has been explored in a series of studies by Chu et al. who compared long-pulse, high frequency PIII, short-pulse, low frequency PIII and plasma exposure in all cases using oxygen.[43–45] They observed oxidation in all samples, roughening and increased hydrophobicity and these two latter effects were more pronounced for the long-pulse, high frequency PIII treatment. Moreover, chemical change cause by this treatment was found to be exclusively on the surface.[43] More recent studies investigated a variety of gases (oxygen, nitrogen, ammonia, and hydrogen) and in some cases used two consecutive PIII processes with different gases and found that both surface chemistry, roughness and wettability were greatly affected by the choice of the modification procedure. PIII has also been explored extensively by Bilek et al.[46] using a different treatment regime and thus observing somewhat different properties of a highly crosslinked subsurface and the introduction of free radicals which are useful for linker-free attachment of biomolecules (Attachment of Biologically Active Molecules section).

Coatings consisting of the bone mineral hydroxyapatite (HAP) have been applied to ePTFE in order to improve its bonding strength at the bone interface. RF magnetron sputtering has been used to deposit HAP onto PTFE substrates.[47–49] It was found that the coating composition depended on discharge power (low power needed for polymers), sputter gas composition, gas pressure, position of the sample in the chamber, the nature of the substrate and the thickness of the coat. The interfacial bond between the coating and the PTFE substrate was found to be very high (5.8 MPa) and displayed cohesive failure (i.e., within the PTFE substrate) during adhesion testing[48] and this was further improved when a Ti interlayer was deposited prior to coating deposition.[49] An electrophoretic deposition technique has been used to deposit patterns of wollastonite ($CaSiO_3$) onto a porous ePTFE substrate before biomimetic HAP growth in simulated body fluid (SBF).[50] Although these techniques are promising as methods for improving the interfacial bond of ePTFE with the underlying bone, much work still needs to be done in order to assess the biological response to the introduced coatings.

The biodegradable polymer poly(1,8-octanediol citrate) (POC) has been applied as a coating on ePTFE by initially coating a prepolymer dissolved in dioxane onto the lumen of vascular grafts followed by polymerization at elevated temperature.[51–53] The POC coating introduces carboxylic acid groups on the substrate and as a result the POC coated grafts display reduced hydrophobicity. Optimized preparation conditions allowed application of the coating without compromising the graft compliance.[51] Subsequent chemical modification of the POC coating was achieved using EDC chemistry to couple diaminohexane to the surface and this was further used to immobilize biological molecules (Attachment of Biologically Active Molecules section).[53]

Surface Grafting

Surface grafting of functional monomers can be employed to tailor the surface properties leading to new molecular functionalities incorporated onto an activated PTFE surface.[3] The peroxy/hydroxyl grafting method [Scheme 1(a)] involves exposure of PTFE to radiation in vacuum or in the presence of an inert gas followed by exposure to air. Alternatively, irradiation of PTFE in the presence of oxygen can be employed. This results in formation of hydroperoxides or diperoxides species which decompose to oxygen-centered radicals after immersion in a monomer or monomer solution at elevated temperature resulting in grafting. In the simultaneous grafting technique [Scheme 1(b)], PTFE and monomer (typically as a solution) are subjected simultaneously to radiation. The advantage of this technique is that in general higher grafting yields can be obtained as a consequent of low radical loss through decomposition reactions. However, a drawback is the concomitantly formation of homopolymer. The radiation process is most commonly carried out either using gamma irradiation (for either method) or a plasma source (for the peroxy/hydroxyl method). The exposure of PTFE to gamma irradiation leads to formation of stable radicals (mid-chain radicals, $\sim CF_2{-}\dot{C}F{-}CF_2\sim$ and end-chain radicals, $\sim CF_2{-}\dot{C}F_2$ [54]) which are believe to be located in the



Scheme 1. The mechanism of (A) the peroxy/hydroxyl grafting method, and (B) the simultaneous grafting method. M is a monomer.

crystalline-amorphous interphase region in PTFE.[3] Because the irradiation process generally leads to defluorination, oxidation and net chain scission at ambient temperature[31] alterations in bulk properties can occur which will affect the implant survival *in vivo*. Alternatively, irradiation of PTFE using a plasma source will only affect the substrate to a shallow depth, the exact penetration depending on the type and the energy of the species in the plasma chamber,[46] thereby leaving the bulk properties practically intact.

Radiation grafting has mainly been used for the purpose of enhancing the bone-bonding ability of ePTFE (further detail in Tissue Space-filler for Plastic Surgery section). Studies have used either phosphate- or carboxylate-containing monomers as these functional groups are known to induce calcification. A series of studies have used gamma irradiation induced grafting of monoacryloxyethyl phosphate (MAEP)[55–58] and 2-(methacryloyloxy) ethyl phosphate (MOEP)[57–62] in various solvent systems. It has been found that, in general, higher graft yields can be obtained using MOEP and particularly in highly nonpolar solvents.[59,60] This is related to the penetration depth of the graft-copolymer into the porous substrate[57] which was also found to be affected by the sample preparation techniques; i.e., nitrogen degassing versus vacuum. Furthermore, the grafting conditions were observed to affect the surface morphology and wettability of MOEP grafted membranes. Importantly, it was found that due to the presence of a large percentage of diene in the commercial monomers (25 %) highly branched or crosslinked graft-copolymers formed in most solvent systems,[63] however, a linear polymer topology could be achieved in a 2-phase mixed solvent system.[62] An additional complexity of using these monomers in graft polymerization reactions is the instability of the ester bonds during polymerization and detailed analysis of the graft copolymers (as well as soluble polymers produced by RAFT mediated polymerization[64]) revealed that the MOEP graft copolymer is best described as MOEP-co-HEMA while the MAEP graft copolymers are MAEP-co-AA.[57,58,60–62] More recent work has involved grafting of AA either by simultaneous gamma irradiation[9] or the peroxy/hydroxyl method using Ar plasma pretreatment.[65] These works have extensively investigated the impact of the grafting process on the mechanical properties of ePTFE and concluded that the tensile properties of AA grafted ePTFE tested under "wet" conditions were reduced to a larger extent for membranes exposed to gamma irradiation grafting compared to that obtained by plasma induced grafting and the latter is thus a preferred technique for surface modification of ePTFE used in medical applications. The plasma induced grafting technique was furthermore shown to not affect the compression modulus obtained from a half-compression test.[65] The peroxy/hydroxyl method has also been demonstrated suitable for grafting of well-defined polymer brushes using a combination of the two controlled radical polymerization methods, RAFT and ATRP[66] and this may well prove a viable method for improving the surface properties of PTFE for medical application, although, that is yet to be demonstrated.

Adsorption

Adsorption is an attractive means for surface modification of PTFE as it does not involve exposure of the substrate to radiation. There is, however, a concern regarding stability of the modified layer which must be addressed before clinical applications can ensue. A number of studies have been reported using different moieties for adsorption to PTFE and introducing different functionality. Typically block copolymers, proteins and surfactants have been used with examples listed in Table II. A recent study reported the use of glutaraldehyde (GA) for crosslinking and stabilization of a LbL assembly[78] which would require careful biological evaluation when considering the well-known toxicity of this crosslinker molecule.[79,80] Stability of the adsorbed layers has been assessed in some studies using static immersion SBF for 2 weeks[69] or in water for 20 weeks[71]; shear conditions in PBS for up to 4 h,[68,70] and shear conditions in human whole blood for 1 or 96 h.[77] It should be noted that the incorporation of the biomimetic mussel inspired adhesive catechol groups (e.g., L-3,4-dihydroxy phenylanaline, DOPA) within a polymer or peptide has been explored for binding to a large variety of substrates. From the current literature it is evident that this approach of direct attachment yields adsorbed layers with high substrate affinity.[76,81]

Table II. Examples of Macromolecules Used for Adsorption onto PTFE or ePTFE

Macromolecule	Chemical moiety for adsorption	Functionality introduced	Reference
Laminin-5	Laminin-5	Laminin-5	67
Protein polymer B9 180 kDa	Elastin mimic	Elastin mimic	68
P(FB-*b*-AA)	Fluoro benzene (C_6F_5)	Carboxylate groups (PAA)	69
PVAm(Dex:FC)	Perfluroundecanoyl ($CF_3(CF_2)_9C(O)$-)	Dextran	70
PVAm(Pep:FC)	Perfluroundecanoyl ($CF_3(CF_2)_9C(O)$-)	Peptides	71–73
Proteins	L-3,4-dihydroxy phenylanaline (DOPA)	Pvfp-1 mimic	74,75
P(MPC-co-NPEM) modified by dopamine	Catechol	PC	76
Two layer construct	Poly(monoaminomethyl-para-xylene) (parylene)	DNA-oligonucleotide	77
LbL assembly of PEI, $(HEP:COL)_5$	PEI	Anti-CD34 antibody (using GA)	78

FB: fluoro benzene; PVAm: polyvinyl amine; Dex: dextran: FC: perfluroundecanoyl; MPC: methacyloyloxyethylphosphorylcholine; NPEM: nitrophenyleny-loxycarbonlypoly(thhylene glycol) methacrylate; PC: phosphorylcholine; PEI: polyethylene imine, HEP: heparin; COL: collagen; GA: glutaraldehyde.

Attachment of Biologically Active Molecules

One consequence of the chemical modification of the surface of ePTFE is an alteration in its capacity to bind biologically active molecules. The binding capacity is altered through changes in the surface charge and surface chemistry of the material and the provision of functional groups that permit the specific covalent attachment of biological molecules. Some studies, as outlined in Adsorption section, have used adsorption of biological molecules for the modification of PTFE, however the use of covalent linkages between the surface and the biological molecule has the advantage of high stability resulting in a surface layer that remains upon implantation. Biological molecules applied include those that modulate specifically the inflammatory response, cell adhesion and tissue repair.

A commonly used method for attaching biological molecules to surfaces involves the use of carbodiimide-mediated formation of an amide bond (between amine and carboxyl groups). The most commonly used reagent is EDC which is sometimes in combination with NHS. A recent review by Coad et al.[82] pointed out a number of issues with this method including formation of side-products, limited life-time of the intermediate(s) formed and the concomitant crosslinking occurring within the biological molecule when the reaction is done *in situ* (i.e., the presence of biological molecule and EDC reagent in same solution). They also stipulated that it is plausible that crosslinked protein multimers precipitate onto surfaces leading to the erroneous conclusions of covalent coupling. It was thus advised that alternative coupling chemistry be used to attach biological molecules to surfaces. Amongst the studies involving attachment of biological molecules to surface modified PTFE, a number of studies do indeed use the *in situ* approach for peptides[41,42] and HEP (also containing both carboxyl and amine groups).[53] In addition, the alternative approach of preactivation of the surface with EDC followed by washing prior to attachment of a biological molecule has been reported in a series of studies for the attachment of BSA and fibronectin.[37–39] It was shown in these studies that this approach gave a greater activity of fibronectin on the surface than when it was coupled directly to a maleimide derivatized surface.[37,39] The use of alternative coupling chemistry was demonstrated by Gabriel et al. who used hexamethylenediisocyanate to react to the introduced hydroxyl groups followed by attachment of an RGD peptide.[36]

An attractive means for the covalent immobilization of biomolecules in the absence of a chemical linker has been studied in depth by Bilek et al.[46] They have shown that PIII modification of PTFE creates free radicals which allow covalent attachment of various proteins (e.g., collagen I[83] and tropoelastin[84]). The process avoids denaturation of the proteins and high biological activity results.

ENHANCEMENT IN PERFORMANCE

Vascular

Disorders of the cardiovascular system are classified into those primarily affecting the blood, heart, or blood vessels. Atherosclerosis is a pathological condition that is central to cardiovascular disorders and is characterized by a build-up of plaque on the interior surface of the coronary arteries. The decrease in luminal diameter that is caused by atherosclerosis results in reduced blood flow and poor cardiac performance. Ultimately atherosclerosis can lead to cardiac ischemia and myocardial infarction. The significance of coronary artery disease is that it is the most common cause of premature mortality in the Western world with total deaths of ~750,000 from cardiovascular disease in the US in 2009.[85]

The repair and regeneration of coronary blood vessels as well as restoration of blood flow are a primary focus of biomaterial science and the devices used in these approaches include vascular grafts, stents, and rotary blood pumps. Synthetic vascular grafts are used to provide a bypass of occluded arteries in coronary artery bypass graft surgery as well as the replacement of dilated aortic aneurysms. Commercial ePTFE-based prosthetic vascular grafts with an internal diameter >6 mm have proved to be successful in bypass grafts and are in routine clinical use (see Table I). The current clinical challenge is the supply of blood vessels with an internal diameter <6 mm for use in grafts. The challenge with narrow vessels occurs because these vessels can be occluded more readily and the increased likelihood of blood coagulation (thrombosis). The coagulation cascade of molecular events is initiated in grafts by protein adsorption as well as surface-induced conformational changes. Blood platelets respond to graft-induced stimuli including thrombin activation that in turn causes platelet activation with the formation of a stable thrombus that can occlude the blood vessel.[34]

Several approaches have been examined to optimize the performance of ePTFE graft materials and these are listed in Table III. In addition to these studies, commercial products exist on the market and these will be described separately at the end of this section. One approach that has been extensively studied for vascular grafts in general has also been applied to ePTFE and involves the modification of the ePTFE with the biomimetic phosphorylcholine (PC) moiety or PC-copolymers. These have been anchored to the ePTFE surface either covalently[40] or adsorbed using the strongly adhesive catechol groups.[76] Both studies resulted in reduced protein adsorption and platelet adhesion in an *in vitro* protein and cell-based assay. In addition, the study using the covalently attached PC moieties showed increased fibroblast adhesion.

An alternative approach investigated to optimize ePTFE for vascular applications has been to modify the polymer surface by attaching specific peptides to modulate the responses of cells interacting with the surface. The P15 peptide represents the collagen type I cell adhesion sequence and attachment to ePTFE was found to promote endothelial cell attachment and proliferation.[41,42] While this aim is desirable the promotion of thrombogenic activity by ePTFE was a major obstacle to its widespread use in vascular repair. Similarly, the attachment of peptides containing the RGD recognition sequence for integrin cell adhesion proteins has been utilized to optimize cell attachment and surface colonization,[36,71–73] however, again raises concerns of producing a nonspecific biological response. Other biological molecules that have been attached to ePTFE for vascular graft applications include the biologically specific VEGF[38] and elastin mimetic protein[68] as well as less biologically specific moieties

Table III. Recent Approaches to Assessing the Biocompatibility of Surface-Enhancements of ePTFE for Vascular Grafts

Year	Surface modification	Moiety attached or applied	Biological context	Biological assays	Properties	Reference
2004	Ammonia plasma and chemical modification	Covalent binding of fibronectin to modified surface	*In vitro*: human cell line	HT1080 fibrosarcoma cell culture and cell count	↑ Cell adhesion	37
2004	Plasma modification, grafting, covalent attachment of peptides	Branched and linear cell-binding P15, MAP4-I, MAP4-II peptides	*In vitro*: human primary ECs	*In vitro*: HUVEC and HUASMC cultures; cell adhesion, proliferation and morphology	↑ EC adhesion and proliferation with MAP4 peptides; small ↑ in SMC cells	41
2005	As above	P15 cell-binding peptide	*In vitro*: human primary ECs; *in vivo*, sheep vascular graft	*In vitro*: as above; *In vivo*: extent of NH in arteriovenous vascular grafts at 14 and 28 days	*In vitro*: ↑ EC and ↓ SMC adhesion and proliferation; *in vivo*: ↑ endothelialization	42
2005	Extra cellular matrix coating	HaCaT cell conditioned medium	*In vivo*: mouse subcutaneous implant	*In vivo/in vitro*: Adherent cell RNA isolation, cDNA probe labelling, microarray analysis of gene expression; histological staining	↑ Diversity in expression of gene clusters	67
2005	Ammonia plasma and chemical modification	Phosphorylcholine	*In vitro*: human primary platelets and neutrophils	*In vitro*: hemocompatibility, platelet adhesion and aggregation; thrombelastography; neutrophil adhesion; SEM; fibroblast culture and adhesion	↓ Thrombogenicity index; ↓ platelet aggregation; ↓ neutrophil adhesion	40
2005	Flow-mediated protein adsorption	Laminin-5-enriched conditioned medium	*In vitro*: human squamous ECs; *in vivo*, mouse subcutaneous implant	*In vitro*: HMVEC adhesion; SEM; *in vivo*: histology and IHC for EC and macrophage; inflammation; vascular density	↑Neovascularization; ↑ EC attachment	86
2005	Ammonia plasma and chemical modification	Electrostatically bound VEGF to modified surface	*In vitro*: human primary ECs	*In vitro*: HUVEC culture; Boyden chamber cell migration assay	No effect on EC adhesion; ↑ EC migration	38
2006	Adsorption	Peptide fluorosurfactant polymer; integrin-binding RGD peptides	*In vitro*: human primary pulmonary artery ECs	*In vitro*: EC culture, adhesion, and proliferation; cytoskeleton CC; stain; lipoprotein uptake assay; phenotypic IHC; prostacyclin assay	↓ Receding contact angle; ↓ EC attachment; ↑ EC growth; EC phenotype maintained	72
2007	Adsorption	Peptide fluorosurfactant polymer biomimetic with EC-selective peptide ligands	*In vitro*: human pulmonary artery EC	*In vitro*: anti-integrin cell binding specificity assay; EC culture, adhesion and proliferation; shear stability assay; EC phenotypic ELISA; platelet adhesion assay	↓ Platelet adhesion; ↑ EC attachment; EC phenotype maintained	71

Table III. *Continued*

Year	Surface modification	Moiety attached or applied	Biological context	Biological assays	Properties	Reference
2007	Adsorption	Elastin mimic protein polymer	*Ex vivo*: baboon femoral arteriovenous shunt	*Ex vivo*: ^{111}In-labeled platelet adhesion assay; ^{125}I-labeled fibrinogen assay for fibrin deposition	↓ Platelet adhesion; ↓ fibrin deposition	68
2007	Ammonia plasma and chemical modification	Covalent binding of fibronectin to modified surface	*In vitro*: bovine primary aortic EC	*In vitro*: BAEC culture; cell adhesion and viability	↑ EC adhesion	39
2009	Adsorption	Fluorosurfactant polymer biomimetic with dextran	*In vitro*: human whole blood	*In vitro*: platelet adhesion under static and dynamic laminar flow; fluorescence microscopy	↓ Platelet adhesion and ↓ platelet activation under dynamic laminar flow	70
2009	Adsorption	Peptide fluorosurfactant polymer; integrin-binding RGD peptides and M-7 polysaccharide	*In vitro*: human pulmonary EC; human primary platelets	*In vitro:* static and dynamic shear stress cell adhesion assay; fluorocytochemical staining and microscopy	Peptides: ↓ Platelet adhesion and ↑ EC attachment; M-7: ↓ Platelet adhesion and ↓ EC attachment	73
2010	Coating by spin-shearing method	POC attached onto luminal nodes and fibrils	*In vivo*: porcine common carotid artery graft anastomosis	*In vivo*: MRA and duplex ultrasonography patency assay; contrast angiograpy; histology; H&E stain; vWF IHC; CD45RA IHC for leukocytes; MAC387 IHC for macrophages; SEM	Neointimal hyperplasia; leukocyte and macrophage infiltration; ↑ EC growth	52
2011	Wet chemical treatment and covalent attachment of RGD peptide	Integrin-binding RGD peptides	*In vitro*: human primary ECs	*In vitro*: HUVEC culture and adhesion; calcein CC staining	↑ EC adhesion and proliferation with RGD peptides	36
2011	Covalent coupling to surface via photoderivatization	Covalently attached chemically modified laminin type 1	*In vivo*: rat interpositional aortic graft anastomosis	*In vivo*: histology; H&E stain; Gs1 lectin staining for EC, monocytes, macrophages; SEM	Antithrombogenic cells on lumenal flow surface; ↑ neovascularization of interstices of laminin-modified grafts	35
2012	Adsorption	PMNC polymer with phosphoryl-choline and catechol	*In vitro*: human primary platelets	*In vitro*: platelet adhesion under static conditions; SEM	↓ platelet adhesion	76
2012	Adsorption to parylene adsorbed layer	DNA-oligonucleotide	*In vitro*: murine T17B embryonic EPC line; human primary ECs	*In vitro*: dynamic cell adhesion assay; vinculin and integrin α_5 cell adhesion marker IHC; F-actin and DNA CC staining; cell viability at 1,2,5,8,12,14 days; thrombin-antithrombin complex, PMN-elastase, β-thromoglobulin assays	↑ Murine EC adhesion, ↑ HUVEC adhesion, ↑ human saphenous vein EC adhesion; ↓ thrombogenicity and good hemo-compatibility	77

Table III. *Continued*

Year	Surface modification	Moiety attached or applied	Biological context	Biological assays	Properties	Reference
2013	Cell impregnation in bioreactor	SVF cell sodding	*In vitro*: 3D-constructed human blood vessel mimic	*In vitro*: H&E staining; vWF IHC for EC, smooth muscle α-actin IHC for SMC, vimentin IHC for mesenchymal cells; SEM; nuclear CC stain	Complete lining of cells on inner surfaces of grafts	87
2013	LbL assembly of PEI, (HEP:-COL)$_5$ cross-linked to anti-CD34 antibody	Anti-CD34 antibody-functionalized HEP/COL multilayer	*In vivo*: porcine common carotid artery implant anastomosis	*In vivo*: fluid dynamic analysis; ultrasonography; histology; H&E staining, vWF IHC for EC	Thromobosis unchanged; EC adherence and proliferation unchanged	78
2013	Coating by spin-shearing method	POC attached onto luminal nodes and fibrils; HEP covalently bound	*In vitro*: human BOEC, PB-MNC, whole blood, platelet-enriched plasma; HUVEC; HASMC	*In vitro*: platelet adhesion assay; cell morphology; SEM; cell viability; vWF and CD144 IHC for EC; α-actin IHC for SMC; NO production assay	↓ Thrombo-genicity and platelet adhesion; ↑ EC adhesion and proliferation; normal NO production; ↑ SMC phenotypic marker and ↓ SMC proliferation	53

BAEC = bovine aortic endothelial cell; BOEC = blood outgrowth endothelial cell; CC = cytochemical; COL = collagen; EC = endothelial cell; ELISA = enzyme linked immunosorbent assay; HASMC = human aortic smooth muscle cell; HEP = heparin; HSVEC = human saphenous vein endothelial cells; HUVEC = human umbilical vein endothelial cell; HUASMC = human umbilical artery smooth muscle cell; IHC = immunohistochemistry; MRA = Magnetic resonance angiography; NH = neointimal hyperplasia; NO = nitric oxide; PB-MNC = peripheral blood mononuclear cell; PEO = poly(ethylene oxide); PMN-elastase = polymorphonuclear-elastase or neutrophil elastase; PMNC = poly[2-(methacryloyloxy)ethylphosphorylcholine]; POC = poly(1,8-octanediol citrate); SEM = scanning electron microscopy; SMC = smooth muscle cell; SVF = stromal vascular fraction; vWF = Von Willebrand factor.

including fibronectin,[37,39] laminin,[35] dextran,[70] and a DNA olionucleotide.[77]

A widely adopted technique uses the anticoagulant polysaccharide heparin to inhibit the thrombogenic response for an extended period of time. The efficiency of this approach relies upon several factors including the extent of heparin coating of the material, whether the attached heparin coating has retained its biological activity and the stability of the surface coating. Early techniques used for the attachment of heparin to ePTFE included silyl-heparinization, that produced a coating with greater thromboresistance than unmodified ePTFE but had a rapid and major loss of its heparin coating.[88] More recently, a POC-heparin coated vascular graft was evaluated through an array of cellular *in vitro* assays and found to be thromboresistant as well as promoting adhesion, viability, and proliferation of endothelial cells.[53]

Vascular prostheses containing ePTFE coated with heparin have been commercially available since 2002. Initial studies using proprietary Carmeda® BioActive Surface technology achieved good heparin coating of the intimal surface that improved luminal patency, absence of thrombus formation, and heparin stability over a 12-week period using an *in vivo* canine vascular interposition model.[89] Likewise, an early study of the Carmeda® BioActive Surface coating of ePTFE in a baboon model found greatly reduction of platelet deposition and no side effects in a 4-week study.[90] Individual studies and clinical surveys have been undertaken to compare the performance of surface modified vascular ePTFE grafts with autologous and unmodified ePTFE grafts. In a study of the thromobogenicity of heparin-coated ePTFE using a human *ex vivo* perfusion model, a reduction was observed in the production of fibrinopeptide A by thrombin with time for the heparin coating in comparison to unmodified ePTFE graft and neither platelets nor fibrin were associated with the heparin coated graft.[91] The gold standard comparison for a synthetic vascular implant is the responses *in vivo* to an autologous vein graft. In a multicenter comparison of vein grafts in diabetic patients, heparin-coated ePTFE had poorer primary patency than autologous vein grafts but heparin-coated ePTFE performed satisfactorily in secondary patency.[92] The thickening of the intimal lining of vessels is a characteristic response to vessel injury and is a major contributor to vascular graft failure through restenosis. The use of the heparin coating on ePTFE has reduced neo-intimal hyperplasia (NH) and in combination with anti-platelet drug therapy completely prevented NH on vein walls in a recent preclinical study using porcine arterovenous grafts.[93] Similarly, of patients with heparin-bonded PTFE Propaten® grafts an overall reduction of the risk of graft failure of 37 % was found in a 569 patient study.[94]

Tissue Space-Filler for Plastic Surgery

Plastic surgery constitutes a vast and sometimes criticized industry but also includes the important challenge of trauma repair. Of the patients who encounter serious trauma, 5–33% are affected by facial injury.[95] Maxillofacial traumas and facial soft tissue injuries are rarely life threatening, however, they can bring tremendous impact especially to the patient's confidence and self-esteem including impact on social function which may eventually lead to chronic stress and depression.[96] The repair and regeneration process of facial injury are many and complex and remain a challenge for the public healthcare service due to high financial cost for a treatment, the high incidence rate and the requirement for a multidisciplinary team of surgeons. One problem encountered in facial repair is the short- to medium-term lifetimes of many soft tissue replacement materials which leads to volume changes that are highly visible in the face and this has led to the widespread use of alloplastic materials.[97] In some applications, when these materials are used as a soft-tissue substitute they are required to interface with the underlying bone; an illustration of how such a soft-tissue substitute is placed during surgery is shown in Figure 3.

The surface of ePTFE can be functionally modified by the attachment of chemical moieties that alter the interaction of ePTFE with both the solvent, ions in biological fluids, biological molecules, and cells. A series of studies in our laboratory involving grafting of phosphate-containing monomers to modify the surface chemistry of ePTFE (described in Surface Grafting section) have to a large extend been evaluated using the so-called SBF test to study *in vitro* mineralization as an indication of the surface's ability to integrate with bone tissue. An early study of a MAEP-grafted ePTFE substrate revealed that not HAP but rather the more acidic calcium phosphate phase brushite or monetite formed predominantly.[56] Subsequent studies of *in vitro* mineralization of MOEP-grafted ePTFE revealed that the solvent used during the grafting process directly influenced the mineralizaiton outcome; a surface where the grafting process was done in methanol induced nucleation and growth of HAP while mixed or unknown calcium phosphate phases formed on other substrates where grafting were done in less polar solvents.[59] The mineralizaiton outcome was further improved and a thick coat of HAP formed on a MOEP-grafted ePTFE substrate that was formed in the aqueous phase of a two-phase solvent system.[62] Considering the different topology of the graft copolymers which form in the different solvents (Surface Grafting section) it was concluded that linear brushes favored HAP mineralization while highly crosslinked copolymers resulted in mixed phases and/or sparse mineralization. In addition to these *in vitro* mineralization studies, we have shown that the grafted ePTFE membranes produced increased protein adsorption and osteoblast attachment which are both important for improving bone tissue integration.[59] Finally, in our recent work we have demonstrated that *in vitro* macrophage response is affected by the types of proteins that adsorb from serum and can be minimized by careful selection of monomer and solvent combinations during the grafting process.[58]

In the work by Chu et al. the use of a long pulse, high frequency oxygen PIII treatment of PTFE has been found to produces surfaces which supported cell growth.[43–45] Despite the treatment causing the substrate to have increased hydrophobicity compared to pristine PTFE, additional effects of surface roughening and oxidation appeared to overall benefit cell attachment and proliferation both in MC3T3-E1 murine derived osteoblastic cells,[43] in rat calvaria osteoblasts[44] and in

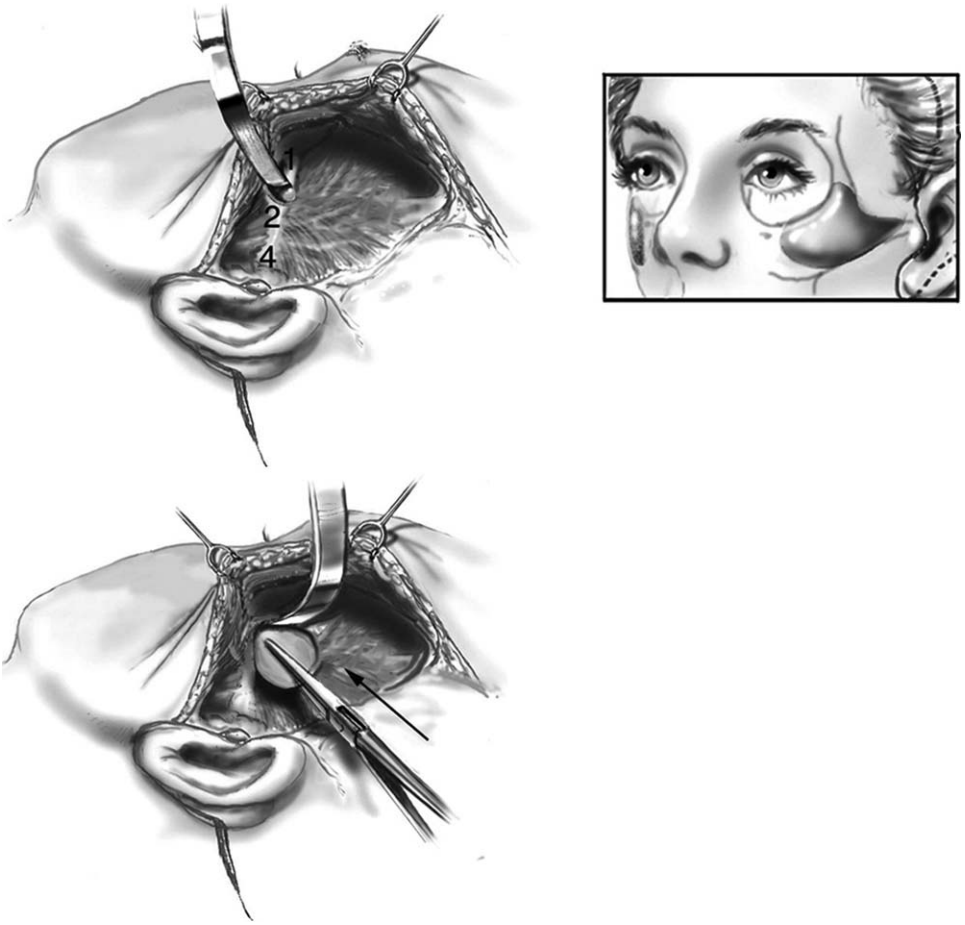


Figure 3. Example of placement of an implant (e.g., ePTFE). A small incision is made through the soft tissues across the side approach of malar bone which intersects with the zygomatic arch. (Reproduced from Ref. 97, with permission from Taylor & Francis Group LLC).

mesenchymal stem cells (MSCs).[45] When the surface treatment involved a second ammonia PIII treatment, resulting in introduction of diverse chemistry including amine functionalities, the MSC displayed the best proliferation and osteogenic differentiation. Zhang et al. have taken the approach of linking peptides to the PTFE surface.[74,75] Stable attachment was achieved by incorporation of DOPA in the peptide and the group identified peptides which improved osteoblastic (MC3T3-E1) cell adhesion and spreading and were nontoxic. It should be noted, that both the PIII and peptide adsorption studies were done on PTFE rather than ePTFE.

CONCLUDING REMARKS

A vast amount of research has gone into enhancing ePTFE for vascular graft applications and heparinized commercial products exist on the market. However, a number of studies appear to make poor choices either in the chemistry used to introduce biological molecules or in the overall approach used being nonspecific. In the area of tissue fillers for plastic surgery the approaches used are to a large extend well-designed, however, here work is still needed to prove the benefit of the surface modification approach through more detailed *in vitro* as well as *in vivo* studies.

REFERENCES

1. Kauffman, G. B. *Educ. Chem.* **1988**, November, 173.
2. Sperati, C. A.; Starkweather, J. R. *Adv. Polym. Sci.* **1961**, *2*, 465.
3. Dargaville, T. R.; George, G. A.; Hill, D. J. T.; Whittaker, A. K. *Prog. Polym. Sci.* **2003**, *28*, 1355.
4. Scheirs, J, Ed. Modern Fluoropolymers: High Performance Polymers for Diverse Applications; Wiley: Chichester, **1997**.
5. Sperati, C. A. In High Performance Polymers: Their Origin and Development; Seymour, R. B., Kirshenbaum, G. S., Eds.; Elsevier Science Publishing: New York, Amsterdam, London, **1986**; p 267.
6. Gore, R. W. US Patent 3,953,566, **1976**.
7. Chandler-Temple, A.; Wentrup-Byrne, E.; Grøndahl, L. *Chem. Aust.* **2008**, *75*, 3.
8. Gore, R. W. US Patent 3,962,153, **1976**.
9. Hidzir, N. M.; Hill, D. J. T.; Martin, D.; Grøndahl, L. *Polymer* **2012**, *53*, 6063.
10. Kannan, R. Y.; Salacinski, H. J.; Butler, P. E.; Hamilton, G.; Seifalian, A. M. *J. Biomed. Mater. Res. B Appl. Biomater.* **2005**, 74B, 570.
11. Constantinides, M.; Galli, S. K. D.; Miller, P. J. *Laryngoscope* **2001**, *111*, 2114.

12. Bota, P. C. S.; Collie, A. M. B.; Puolakkainen, P.; Vernon, R. B.; Sage, E. H.; Ratner, B. D.; Stayton, P. S. *J. Biomed. Mater. Res. A* **2010**, *95A*, 649.
13. Catanese, J., III; Cooke, D.; Maas, C.; Pruitt, L. *Biomed. Mater. Res.* **1999**, *48*, 187.
14. Guidoin, R.; Maurel, S.; Chakfe, N.; How, T.; Zhang, Z.; Therrien, M.; Formichi, M.; Gosselin, C. *Biomaterials* **1993**, *14*, 694.
15. Bezuidenhout, D.; Zilla, P. In Encyclopedia of Biomaterials and Biomedical Engineering; Wnek, G. E., Bowlin, G. L., Eds.; Marcel Dekker, Inc.: New York, **2004**; p 1715.
16. Williams, D. F. *Biomaterials* **2008**, *29*, 2941.
17. Dubiel, E. A.; Martin, Y.; Vermette, P. *Chem. Rev.* **2011**, *111*, 2900.
18. Menzies, K. L.; Jones, L. *Optometry Vis. Sci.* **2010**, *87*, 387.
19. Voskerician, G.; Gingras, P. H.; Anderson, J. M. *J. Biomed. Mater. Res. A* **2006**, *76*, 234.
20. Ainslie, K. M.; Bachelder, E. M.; Borkar, S.; Zahr, A. S.; Sen, A.; Badding, J. V.; Pishko, M. V. *Langmuir* **2007**, *23*, 747.
21. Gentile, P.; Chiono, V.; Tonda-Turo, C.; Ferreira, A. M.; Ciardelli, G. *Biotech. J.* **2011**, *6*, 1187.
22. Zeller, T. *Vasc. Med.* **2007**, *12*, 223.
23. Sigler, M.; Paul, T.; Grabitz, R. G. *Zeitschrift fur Kardiol.* **2005**, *94*, 383.
24. de Mel, A.; Jell, G.; Stevens, M. M.; Seifalian, A. M. *Biomacromolecules* **2008**, *9*, 2969.
25. Helmus, M. N.; Gibbons, D. F.; Cebon, D. *Toxicol. Pathol.* **2008**, *36*, 70.
26. ASM-International (2006) from http://products.asminternational.org/meddev/index.aspx.
27. Poncin-Epaillard, F.; Legaeay, G. *J. Biomater. Sci. Polym. Edn.* **2003**, *14*, 1005.
28. Nasef, M. M.; Hegazy, A.-S. A. *Prog. Polym. Sci.* **2004**, *29*, 499.
29. Solouk A.; Cousins B. G.; Mirzadeh H.; Seifalian A. M. *Biotech. Appl. Biochem.* **2011**, *59*, 311.
30. Wise, S. G.; Waterhuse, A.; Kondyurin, A.; Bilek. M. M.; Weiss, A. S. *Nanomedicine* **2012**, *7*, 1907.
31. Kang, T.; Zhang, Y. *Adv. Mater.* **2000**, *12*, 1481.
32. Kidane, A. G.; Salacinski, H.; Tiwari, A.; Bruckdorfor, K. R.; Seifalian, A. M. *Biomacromolecules* **2004**, *5*, 798.
33. Sololuk, A.; Cousins, B. G.; Mirzadeh, H.; Seifalian, A. M. *Biotech. Appl. Biochem.* **2011**, *58*, 311.
34. Qi, P.; Maitz, M. A.; Huang, N. *Surf. Coat. Technol.* **2013**, *233*, 80.
35. Williams, S. K.; Kleinert, L. B.; Patula-Steinbrenner, V. *J. Biomed. Mater Res. A* **2011**, *99*, 67.
36. Gabriel, M.; Dahm, M.; Vahl, C. F. *Biomed. Mater.* **2011**, *6*, 035007.
37. Gauvreau, V.; Chevallier, P.; Vallieres, K.; Petitclerc, E.; Gaudreault, R. C.; Laroche, G. *Bioconjug. Chem.* **2004**, *15*, 1146.
38. Crombez, M.; Chevallier, P.; Gaudreault, R. C.; Petitclerc, E.; Mantovani, D.; Laroche, G. *Biomaterials* **2005**, *26*, 7402.
39. Vallieres, K.; Petitclerc, E.; Laroche, G. *Macromol. Biosci.* **2007**, *7*, 738.
40. Chevallier, P.; Janvier, R.; Mantovani, D.; Laroche, G. *Macromol. Biosci.* **2005**, *5*, 829.
41. Li, C.; Zheng, Y.; Imran, M. *J. Biomed. Mater. Res. A* **2004**, *71*, 134.
42. Li, C.; Hill, A.; Imran, M. *J. Biomater. Sci. Polym. Ed.* **2005**, *16*, 875.
43. Tong, L.; Kwok, D. T. K.; Wang, H.; Wu, L.; Chu, P. K. *Adv. Eng. Mater. B* **2010**, *12*, 163.
44. Wang, H.; Kwok, D. T. K.; Wang, W.; Wu, Z.; Tong, L.; Zhang, Y.; Chu, P. K. *Biomaterials* **2010**, *31*, 413.
45. Wang, H.; Kwok, D. T. K.; Xu, M.; Shi, H.; Wu, Z.; Zhang, W.; Chu, P. K. *Adv. Mater.* **2012**, *24*, 3315.
46. Bilek, M. M.; McKenzie, D. R. *Biophys. Rev.* **2010**, *2*, 55.
47. Feddes, B.; Vredenberg, A. M.; Wolke, J. G. C.; Jansen, J. A. *Surf. Coat. Techn.* **2004**, *185*, 346.
48. Feddes, B.; Wolke, J. G. C.; Vredenberg, A. M.; Jansen, J. A. *Surf. Coat. Techn.* **2004**, *184*, 247.
49. Feddes, B.; Wolke, J. G. C.; Weinhold, W. P.; Vredenberg, A. M.; Jansen, J. A. *J. Adhes. Sci. Techn.* **2004**, *18*, 655.
50. Yamaguchi, S.; Yabutsuke, T.; Hibino, M.; Yao, T. *J. Mater. Sci. Mater. Med.* **2008**, *19*, 1419.
51. Yang, J.; Motlagh, D.; Allen, J. B.; Webb, A. R.; Kibbe, M. R.; Aalami, O.; Kapadia, M.; Carroll, T. J.; Ameer, G. A. *Adv. Mater.* **2006**, *18*, 1493.
52. Kibbe, M. R.; Martinez, J.; Popowich, D. A.; Kapadia, M. R.; Ahanchi, S. S.; Aalami, O. O.; Jiang, Q.; Webb, A. R.; Yang, J.; Carroll, T.; Ameer, G. A. *J. Biomed. Mater. Res. A* **2009**, *93*, 314.
53. Hoshi, R. A.; Van Lith, R.; Jen, M. C.; Allen, J. B.; Lapidos, K. A.; Ameer, G. *Biomaterials* **2013**, *34*, 30.
54. Forsythe, J. S.; Hill, D. J. T. *Prog. Polym. Sci.* **2000**, *25*, 101.
55. Grøndahl, L.; Cardona, F.; Chiem, K.; Wentrup-Byrne, E. *J. Appl. Polym. Sci.* **2002**, *86*, 2550.
56. Grøndahl, L.; Bostrom, T.; Cardona, F.; Chiem, K.; Wentrup-Byrne, E. *J. Mater. Sci. Mater. Med.* **2003**, *14*, 503.
57. Chandler-Temple, A.; Wentrup-Byrne, E.; Whittaker, A.; Griesser, H.; Jasieniak, M.; Grøndahl, L. *Langmuir* **2010**, *26*, 15409.
58. Chandler-Temple, A.; Kingshott, P.; Wentrup-Byrne, E.; Cassady, A. I.; Grøndahl, L. *J. Biomed. Mater. Res. A* **2013**, *101*, 1047.
59. Suzuki, S.; Grøndahl, L.; Leavesley, D.; Wentrup-Byrne, E. *Biomaterials* **2005**, *26*, 5303.
60. Wentrup-Byrne, E.; Grøndahl, L.; Suzuki, S. *Polym. Int.* **2005**, *54*, 1581.
61. Chandler-Temple, A.; Wentrup-Byrne, E.; Whittaker, A.; Grøndahl, L. *J. Appl. Polym. Sci.* **2010**, *117*, 3331.
62. Wentrup-Byrne, E.; Suzuki, S.; Suwanasilp, J. J.; Grøndahl, L. *Biomed. Mater.* **2010**, *5*, 045010.
63. Grøndahl, L.; Suzuki, S.; Wentrup-Byrne, E. *Chem. Commun.* **2008**, 3314.
64. Suzuki, S.; Whittaker, M. R.; Grøndahl, L.; Wentrup-Byrne, E.; Monteiro M. J. *Biomacromolecules* **2006**, *7*, 3178.

65. Hidzir N. M.; Hill, D. J. T.; Taran E.; Martin D.; Grøndahl L. *Polymer* **2013**, *54*, 6536.

66. Yu, W. H.; Kang, E. T.; Neoh, K. G. *Langmuir* **2005**, *21*, 450.

67. Schwartz, M. A.; Stone, A. L.; Greer, K. A.; Hoying, J. B.; Williams, S. K. *J. Biomed. Mater. Res. A* **2005**, *73*, 30.

68. Jordan, S. W.; Haller, C. A.; Sallach; R. E.; Apkarian, R. P.; Hanson, S. R.; Chaikof, E. L. *Biomaterials* **2007**, *28*, 1191.

69. Suzuki, S.; Whittaker, M. R.; Wentrup-Byrne, E.; Monteiro, M. J.; Grøndahl, L. *Langmuir* **2008**, *24*, 13075.

70. Wang, S.; Gupta, A. S.; Sagnella, S.; Barendt, P. M.; Kottke-Marchant, K.; Marchant, R. E. *J. Biomater. Sci. Polym. Ed.* **2009**, *20*, 619.

71. Larsen, C. C.; Kligman, F.; Tang, C.; Kottke-Marchant, K.; Marchant, R. E. *Biomaterials* **2007**, *28*, 3537.

72. Larsen, C. C.; Kligman, F.; Kottke-Marchant, K.; Marchant, R. E. *Biomaterials* **2006**, *27*, 4846.

73. Tang, C.; Kligman, F.; Larsen, C. C.; Kottke-Marchant, K.; Marchant, R. E. *J. Biomed. Mater. Res. A* **2009**, *88*, 348.

74. Jiang, Z.; Du, L.; Ding, X.; Xu, H.; Yu, Y.; Sun, Y.; Zhang, Q. *Mater. Sci. Eng. C* **2012**, *32*, 1280.

75. Jiang, Z.; Yu, Y.; Du, L.; Ding, X.; Xu, H.; Sun, Y.; Zhang, Q. *Colloids Surf. B Biointerfaces* **2012**, *90*, 227.

76. Gong, Y.-K.; Lui, L.-P.; Messersmith, P. B. *Macromol. Biosci.* **2012**, *12*, 979.

77. Schleicher, M.; Hansmann, J.; Elkin, B.; Kluger, P. J.; Liebscher, S.; Huber, A. J.; Fritze, O.; Schille, C.; Müller, M.; Schenke-Layland, K.; Seifert, M.; Walles, H.; Wendel, H.-P.; Stock, U. A. *Int. J. Biomater.* **2012**, 2012, 397813.

78. Lu, S.; Sun, X.; Zhang, P.; Yang, L.; Gong, F.; Wang, C. *Perfusion* **2013**, *28*, 440.

79. Takigawa, T.; Endo, Y. *J. Occup. Health* **2006**, *48*, 25.

80. Zeiger, E.; Gollapudi, B.; Spencer, P. *Mutat. Res.* **2005**, *589*, 136.

81. Faure, E.; Falentin-Daudre, C.; Jerome, C.; Lyskawa, J.; Fornier, D.; Woisel, P.; Detrembleur, C. *Prog. Polym. Sci.* **2013**, *38*, 236.

82. Coad, B. R.; Jasieniak, M.; Griesser, S. S.; Griesser, H. J. *Surf. Coat. Technol.* **2013**, *233*, 169.

83. Bax, D. V.; McKenzie, D. R.; Weiss, A. S.; Bilek, M. M. *Biomaterials* **2010**, *31*, 2526.

84. Bax D. V.; Wang, Y.; Li, Z.; Maitz, P. K. M; McKenzie, D. R.; Bilek, M. M. *Biomaterials* **2011**, *32*, 5100.

85. Go, A. S.; Mozaffarian, D.; Roger, V. L.; Benjamin, E. J.; Berry, J. D.; Borden, W. B., et al. *Circulation* **2013**, *127*, e6.

86. Kidd, K. R.; Dal, P. D.; Stone, A. L.; Hoying, J. B.; Nagle, R. B.; Williams, S. K. *Tissue Eng.* **2005**, *11*, 1379.

87. Touroo, J. S.; Dale, J. R.; Williams, S. K. *Tissue Eng. C Methods* **2013**, *19*, 307.

88. Laredo, J.; Xue, L.; Husak, V. A.; Ellinger, J.; Greisler, H. P. *Am. J. Surg.* **2003**, *186*, 556.

89. Begovac, P. C.; Thomson, R. C.; Fisher, J. L.; Hughson, A.; Gallhagen, A. *Eur. J. Vasc. Endovasc. Surg.* **2003**, *25*, 432.

90. Lin, P. H.; Chen, C.; Bush, R. L.; Yao, Q.; Lumsden, A. B.; Hanson, S. R. *J. Vasc. Surg.* **2004**, *39*, 1322.

91. Heyligers, J. M.; Verhagen, H. J.; Rotmans, J. I.; Weeterings, C.; de Groot, P. G.; Moll, F. L.; Lisman, T. *J. Vasc. Surg.* **2006**, *43*, 587.

92. Dorigo, W.; Pulli, R.; Piffaretti, G.; Castelli, P.; Griselli, F.; Dorrucci, V.; Ferilli F.; Ottavi P.; De Blasis G.; Scalisi L.; Monaca V.; Battaglia G.; Vecchiati E.; Casali G.; Pratesi C. *J. Cardiovasc. Surg.* **2012**, *53*, 187.

93. Gessaroli, M.; Bombardi, C.; Giunti, M.; Bacci, M. L. *J. Vasc. Surg.* **2012**, *55*, 192.

94. Lindholt, J. S.; Gottscalksen, B.; Johannesen, N.; Dueholm, D.; Ravn, H.; Christensen, E. D.; Viddal, B.; Flørenes, T.; Pedersen, G.; Rasmussen, M.; Carstensen, M.; Grøndal, N.; Fasting, H. *Eur. J. Vasc. Endovasc. Surg.* **2011**, *41*, 668.

95. Goodisson, D.; MacFarlane, M.; Snape, L.; Darwish, B. N. *Zealand Med. J.* **2004**, *117*, 1.

96. Landau, T. Identity in About Faces; Doubleday: New York, **1989**; p 36.

97. Terino, E. O., Ed. Three Dimensional Facial Sculpting, 1st ed.; Informa Healthcare: New York, **2007**; p 43.