Chapter 1

Introduction to Polymers for Biomedical Applications

1.1 Introduction

The surface of a material is the barrier between the material and the external environment. It is responsible for many properties of a substance, critical to its function i.e. adhesion, wettability, dissolution and degradation to name but a few. In the field of biomaterials the surface is of particular importance as it acts as the first point of contact in reactions with other materials. Surface analytical techniques such as time-of-flight secondary ion mass spectroscopy (ToF-SIMS), X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM) are all regularly used in biomaterial characterisation. It is vital that all biomaterials are thoroughly analysed *in vitro* to ensure their suitability for their eventual use.

New polymers are being utilised in drug delivery vectors due to the advantages their physical properties provide. This chapter will discuss the application of surface analytical techniques to polymers with biomedical applications as well as advances in controlled drug delivery systems.

1.2 Introduction to Polymers

Polymers are long chain molecules comprised of repeated monomer subunits. Each constituent of a polymer undergoes polymerisation reactions to form a multi-unit structure. Polymers can be divided into homopolymers that comprise one repeating sub unit (monomer), or copolymers which includes any polymer containing two or more different monomers[1, 2]. Copolymers can be divided into four categories; random, alternating, block and graft copolymers, a schematic of the polymer types can be found in Figure 1.1a. The different composition of homo and copolymers give rise to three main types of skeletal structures. These are linear, branched and network polymers as shown in Figure 1.1b[2].

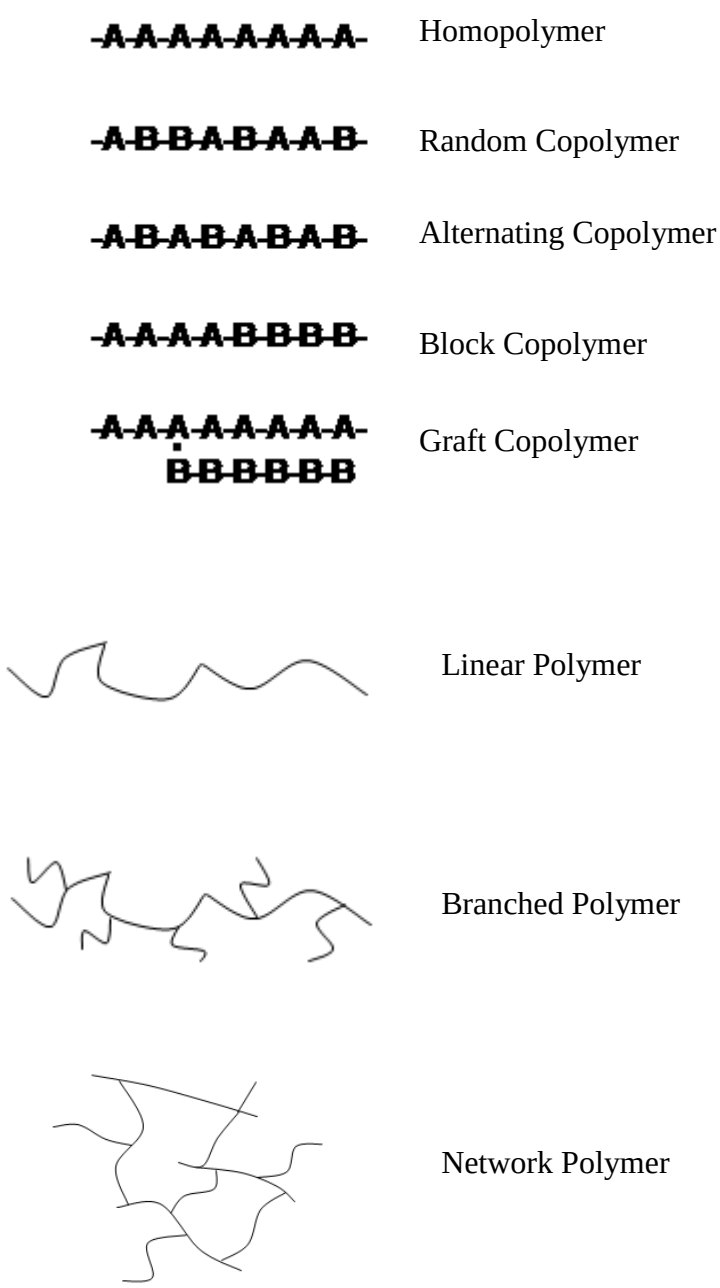


Figure 1.1 a) Schematic representation of homopolymers and copolymers (adapted from[2]). Individual monomer species are shown as 'A' and 'B'. b) Schematic of common polymer skeletal structures.

The skeletal structure is vital in determining the eventual properties of the resulting polymer. For example branched polyethylene has a lower melting point than its linear equivalent[3]. It is the understanding and manipulation of the structure of polymers which allow for advances into controlled drug release from

polymeric material. An example of such manipulation is shown in "smart" polymers whereby a therapeutic agent is encapsulated within the polymer matrix in its "closed" configuration. When encountering a specific stimulus such as a low pH environment a release of drug is observed by shifting to an "open" state[4-6]. The focus of the work in this thesis is the analysis of clinically relevant polymers, Table 1.1 shows the versatility of some polymers currently used clinically, this versatility is due to the variation in polymer architecture.

Polymer	Application
Poly(methyl methacrylate)	Rigid contact lenses, intra-ocular lens
Polymeric compounds based on methyl methacrylate	Acrylic cements for orthopedy, facial prosthesis, joint surgeries
Poly(2-hydroxyethyl methacrylate)	Flexible contact lenses, plastic surgery.
Nylon-type polyamides	Sutures
Poly(vinyl chloride)	Blood pushes, catheters
Poly(ethylene terephtalate)	Vascular prosthesis, cardiac valves
Polytetrafluoroethylene	Orthopedy, vascular clips
Polyurethanes	Catheters, cardiac pumps
Silicones	Plastic surgery, tubes, oxygenators

Table 1.1 Polymers used clinically adapted from Vert *et al.* 2007[7].

1.3 Biocompatible Polymers

Biodegradation is defined as an event which takes place through the action of enzymes and/or chemical decomposition associated with living organisms[8]. There are two classifications of biodegradable polymers which are natural, and synthetic[9]. Such polymers have use in medical and ecological applications such as reducing plastic waste from non bio-degradable polymer manufacture and

reducing costs of their disposal. A list of biodegradable polymers is shown in Table 1.2, adapted from Ikada *et al*.[9]

Polymer	Abbreviation
Poly(acid anhydride)	PAA
Poly(butylene succinate)	PBS
Poly(a-cyanoacrylate)	PCA
Poly(e-caprolactone)	PCL
Poly(DL-lactide), Poly(DL-lactic acid)	PDLLA
Poly(ester amide)	PEA
Poly(ester carbonate)	PEC
Poly(ethylene succinate)	PES
Poly(glycolide), Poly(glycolic acid)	PGA
(Poly(glycolide-co-lactide), Poly(glycolic acid-co-lactic acid)	PGALA
Poly(hydroxyalkanoate)	PHA
Poly(3-hydroxybutyrate)	PHB
Poly(L-lactide, Poly(L-lactic acid)	PLLA
Poly(orthoester)	POE

Table 1.2 List of biodegradable polymers, adapted from Ikada *et al*.[9]

The large selection of biodegradable polymers listed as well as the related copolymers have varying properties allowing for tailoring of the most suitable physical and mechanical properties for a specific application. The degradation of polymers used in treatment is often vital in their function, the three key mechanisms of degradation are surface erosion, bulk erosion and erosion front formation shown in Figure 1.2[10, 11].

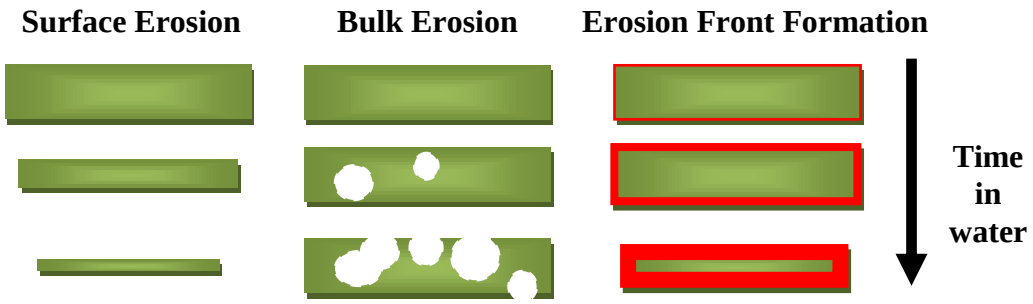


Figure 1.2 Mechanisms of polymer degradation, the green area represents polymer, red represents the erosion zone, adapted from Mathiowitz *et al*.[11]

In all degradation processes the first step is penetration of water into the polymer matrix. Surface erosion occurs when degradation of the surface occurs at a faster rate than the rate of water penetration into the bulk. This is a desirable mechanism of erosion in drug delivery due to the reproducibility of the rate of drug release as the kinetics of erosion are well understood[12]. The opposite is true for bulk erosion whereby the bulk is penetrated and degrades faster than the surface. Erosion front formation is between these two extremes and thus is more typical, whereby degradation occurs in a finite outer region termed the erosion zone[10, 11]. Both synthetic and biologically derived (natural) polymers are used commercially where they can be hydrolytically degraded (containing functional groups including esters, anhydrides, carbonates and amides) or they can be enzymatically degradable[13]. Poly(lactic acid) (PLA) is preferred for commercial use due to fewer variations in degradation between different sites and patients, therefore it is used as sutures, in drug eluting stents and in dialysis membranes[14].

As technology has allowed advances in our manipulation of such polymers, more complex polymers are being developed for human use in drug delivery[15]. As with all commercial compounds used for human treatment, extensive properties must be satisfied before being licensed for use. The key properties of polymeric biomaterials are shown below[16]:

- The material should not evoke a sustained inflammatory or toxic response upon implantation in the body.

- The material should have an acceptable shelf-life.

- The degradation time of the material should match the healing or regeneration process.

- The material should have appropriate mechanical properties for the indicated application and the variation in mechanical properties with degradation should be compatible with the healing or regeneration process.

- The degradation products should be non-toxic, and able to be metabolised and cleared from the body.

- The material should have appropriate permeability and processibility for the intended application.

Some typical examples of a biomaterial application include drug delivery systems[17-23], tissue engineering scaffolds[24-27] and temporary prosthetics[28-30]. The main advantage of a biomaterial is its improved biocompatibility and the temporary status it has in a given biomedical application. This allows for the materials hydrolysis or enzymatic degradation once its function has been accomplished.

Natural polymers are more prone to enzymatical degradation and the site of action will therefore determine the degradation rate relative to the concentration of enzyme present. Natural polymers are bioactive, however there is strong immune response associated with natural polymers derived from sources other than the patient[1]. Synthetic polymers are relatively biologically inert in comparison to natural polymers and the production of synthetic polymers forms more uniform polymer structures[31]. To counteract this hybrid polymers, which are polymers where functional groups are attached to specific locations in the polymer chain have been developed in order to alter the physical/chemical properties. An

example of this is seen in poly(ethylene-glycol) attachment (PEGylation) in an effort to achieve improved physical properties to elicit favourable biological responses[32].

1.3.1 Hybrid Polymers and PEGylation

There is a growing synergy between organic chemistry and polymer synthesis to build on the polymer architecture described in section 1.2, sophisticating molecular design to produce properties which can be tuned to satisfy a therapeutic need. Such hybrid polymers can overcome problems such as the decreased stability of biopolymers and the poor structural control of synthetic systems[33]. Poly(ethylene glycol) (PEG) polymer chains are being utilised in drug delivery by covalent attachment to another molecule as first shown by Abuchowski *et al*. in 1977[34]. PEGylation improves water solubility due to its hydrophilicity, high mobility in solution, non-toxicity above 1 kDa and it is readily cleared from the body. PEG with a molecular weight below 1 kDa has been shown to degrade into toxic metabolites but above this value, no toxicity is observed[35].

PEGylation is often carried out by the incubation of PEG with the therapeutic protein macromolecule. PEGylation acts to confuse the immune response by 'masking' the protein. This is important as proteins can elicit undesired immune responses[33]. It also increases the overall size of the molecule thereby reducing the amount of renal clearance allowing it to circulate for longer. Limitations of proteins for therapy include enzymatic degradation and limited solubility in non-aqueous solvents, which PEGylation can improve however they are also restricted by their temperature and pH stability. Other physiochemical properties of the PEGylated protein are also altered, such as the hydrophobicity, conformation and electrostatic binding, which also increase retention and binding affinity as shown in Figure 1.3.

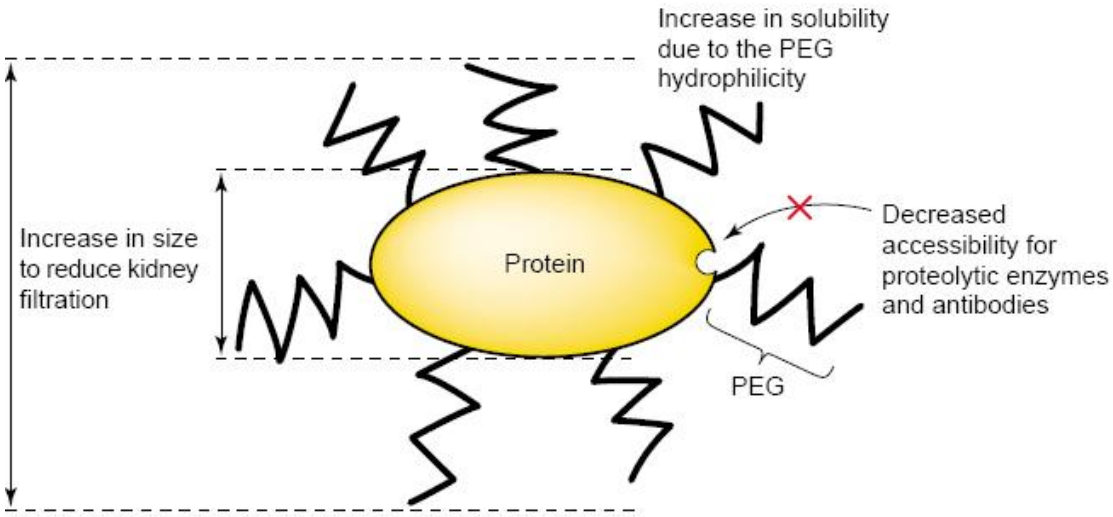


Figure 1.3 Schematic diagram illustrating the key benefits of PEGylation of protein based drug molecules[36].

Many small anti-tumour drugs have been PEGylated to avoid clearance and to increase solubility. These include campothecin, cis-platinum, doxorubicin and taxol (paclitaxel), which is used in drug loaded polymer stents to prevent restenosis[37]. In 2007 $4 billion[38] was generated from the sale of PEGylated drugs which illustrates their usefulness and suggests there is scope for further drugs to utilise PEGylation to improve treatment for patients.

1.3.2 Poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and their co-polymer poly(lactic-co-glycolic acid) (PLGA)

The first use of PLA was first patented in 1967 as a resorbable material for sutures[39, 40]. These polymers and copolymers of the two were then further developed for use in biomedical scaffolds. Now they are more commonly used as low toxicity-controlled drug delivery carriers[1]. Degradation products from PLA/PGA and PLGA are broken down into readily metabolised materials though the citric acid cycle improving their biocompatibility. As such they are FDA approved for human use. Their properties can be found in Table 1.3 below

Polymer	Crystallinity	T_g (^oC)	Degradation Rate	Typical applications
PGA	Highly crystalline (T_m = 225~230 ^oC)	35 ~ 40	2 – 3 months	Sutures, soft anaplerosis
PLA (L-form)	Semi-crystalline (T_m = 173~178 ^oC)	60 ~ 65	> 2 years	Fracture fixation, ligament augmentation
PLA (D,L-form)	Amorphous	55 ~ 60	12 – 16 months	Drug delivery systems
PLGA	Amorphous	45 ~ 55	1 – 6 months	Suture, fracture fixation, oral implant, drug delivery microspheres

Table 1.3 Properties and biomedical applications of PLA, PGA and PLGA[9, 30].

The degradation of PLA and PLGA is shown in Figure 1.4. The lactic acid produced by this degradation is easily metabolised by the body. As these polymers have high biocompatibility they are ideal candidates for sustained drug delivery[41-43].

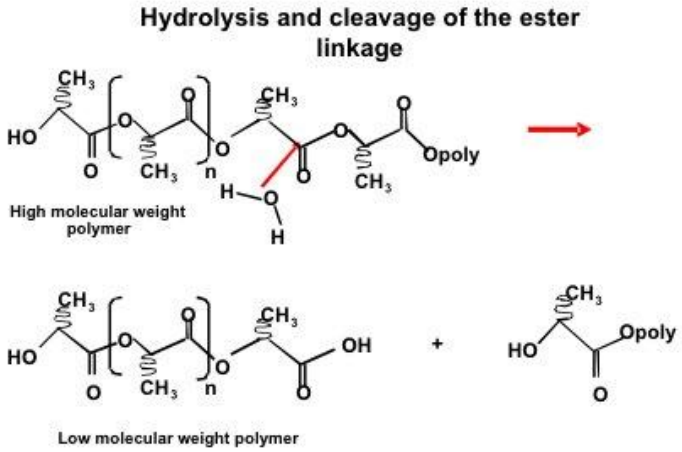


Figure 1.4 Formula of PLGA degradation yielding lactic acid**. (**Felix Simonovsky, University of Washington, Engineering).

1.4. Biodegradable Polymers in Medicine

Polymers have increasingly seen use in medicine, the three main categories of uses are; drug delivery, implants and tissue engineering. These are discussed in this section.

1.4.1 Drug Delivery

Polymer drug delivery formulations are the most prolific use of polymers in medicine due to their versatility and improved therapeutic properties compared with traditional forms of drug delivery i.e. tablets and intravenous injections.

Such systems comprise a formulation of drug and polymer which is designed to release a drug with effective targeting and controlled release. This can either be a relatively short term delivery (hours) i.e. nicotine transdermal patches, or longer delivery (days) i.e. subdermal implants, from formulations such as those used at the surface of metallic implantable coronary stents[44]. The longer lasting formulations can provide sustained release of drug without requiring patient compliance. The main use of polymers for drug delivery however comes in the form of carriers which include hydrophilic matrix tablets[45-47], osmotic delivery systems[48, 49], membrane controlled release systems[50, 51], nano/microspheres[22, 23, 52, 53] and modified liposomes[54-56] schematic representation of such polymer drug delivery mechanisms mentioned are shown in Figure 1.5 below. A range of commercially approved drug delivery systems are summarised in Table 1.4. While PEGylation of proteins is the most common use of polymers for protein drug delivery however the versatility of polymer drug delivery extends to microsphere, thin films and implanted controlled release formulations.

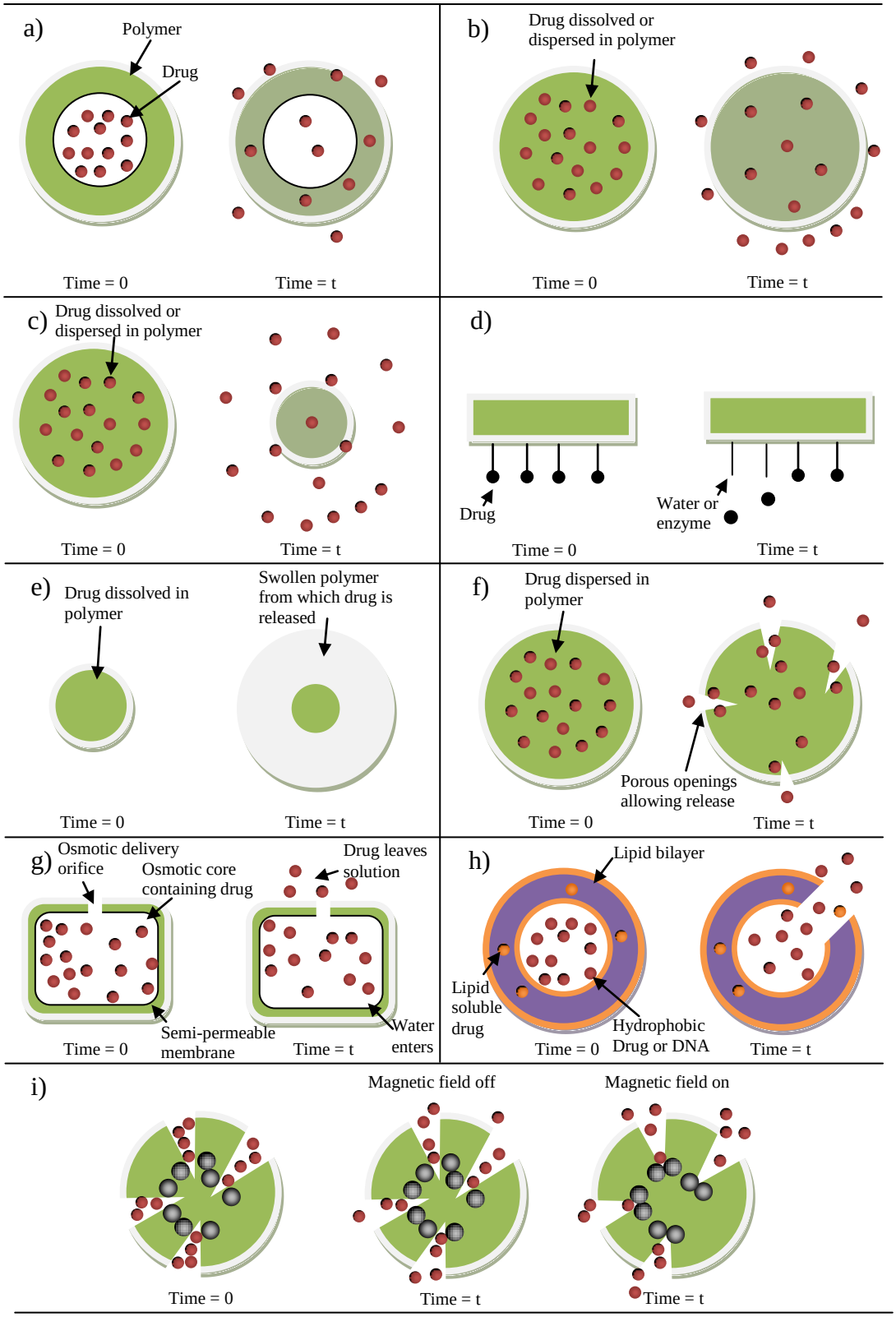


Figure 1.5 Example of drug release through a) a reservoir b) within a matrix c) polymer degradation, d) cleavage of drug from a polymer backbone, e) solvent exposure, f) osmotic pressure water permeation, g) osmotic delivery, h) liposome release and i) external stimulus mediated release, adapted from Langer, 1990[57].

Trade Name	Treatment	Polymer	Protein/Drug	Method of Admin.
Adagen	Severe combined immunodeficiency disease	PEG	Adenosine deaminase	Injectable
Oncaspar	Acute lymphatic leukemia	PEG	Asparaginase	Injectable
Duragesic	Relief of moderate to severe pain for up to 72 hrs	Copolymer of polyester/ethyl vinyl acetate	Fentanyl	Transdermal patch
Gliadel	Malignant glioma	Copolymer of polyanhydride and polifeprosan 20	Carmustine	Implanted
Zuplenz	Nausea	Hydroxypropyl methylcellulose	Ondansetron	Oral soluble thin film
Lipiodol	Hepatocellular cancer	Copolymer of Styrene and maleic acid	Neocarzinostatin	Injectable
Doxil	Cancer chemotherapy	PEGylated liposome	Doxorubicin	Injectable
Taxus	Restenosis	Styrine isoprene butadiene	Paclitaxel	Implanted
Zmax	Bacterial infections	Poloxamer 407, hydroxypropyl cellulose	Azithromycin	Microsphere, oral suspension

Table 1.4 Summary of some commercially available polymers used for drug delivery applications[58-65].

Figure 1.6 shows the release profile expected where patients take their medication at regular intervals over a period of time. There is a balance between the efficacious dose and levels of toxicity/ low drug availability that assumes regular patient compliance. Figure 1.6 also indicates the ideal situation that controlled release of drug from polymer systems could facilitate, delivering an efficacious dose over prolonged periods of time then degrading naturally.

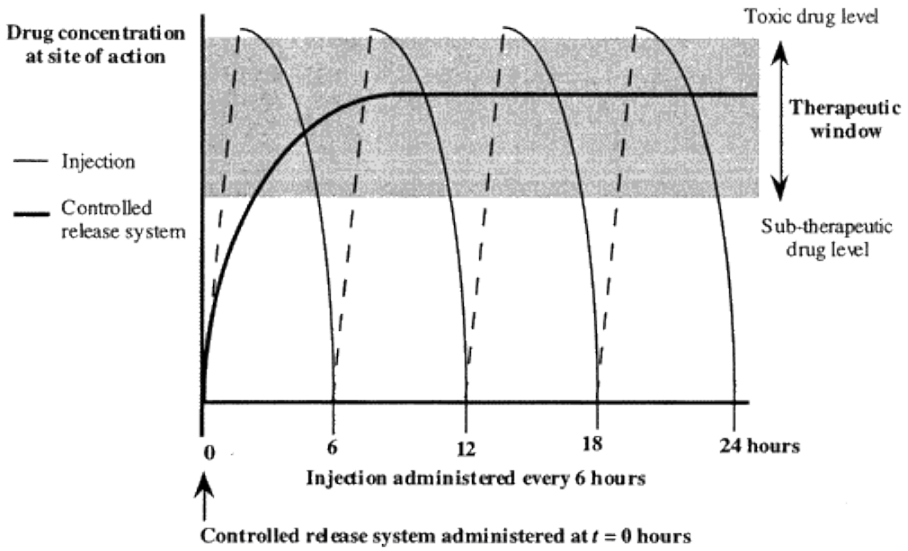


Figure 1.6 Repeat dose profile from an injection of drug X, and an ideal drug delivery profile from controlled release system[12]

There is scope for such controlled release delivery systems to play a role in providing single-administration immunisations without the need for boosters. These are often weeks apart and work by releasing low level antigens to allow antibodies to develop over time. Men *et al*. in 1995 showed using PLGA microspheres that the delivery of tetanus toxoid elicited a stronger T-cell response with fewer side effects than the currently used aluminium hydroxide adjuvant treatment[66, 67]. The work of O'Hagan *et al*. has also shown how controlled release from PLGA microspheres is capable of stimulating a 100 – 1000 fold increase in antibody and T-cell response for periods of up to two weeks in animal models[68-70]. They also showed how the DNA used for such vaccination was adsorbed to the surface of the microsphere in order to avoid the complications encountered from microencapsulation of DNA[69]. Surface analysis therefore is of interest in such systems.

PLA and PLGA have been extensively investigated for drug delivery purposes[17, 21, 22, 53, 56, 71-75]. They undergo hydrolysis on implantation, however as shown in Table 1.3, the biodegradation products are produced at a slow rate and so do not affect normal cell function. The most common method used to control the release of drug from within PLGA microspheres/films is changing the block copolymer composition and molecular weight[76]. A higher concentration of PGA in the composition and a smaller molecular weight reduces the degradation time.

Biodegradable polyesters such as PLA and PLGA microspheres and nanoparticles are currently being intensively researched in the field of targeted therapeutics utilising polymer technology[18, 60, 75, 77]. Due to their size they are ideal for adhesion to surfaces in the body such as in the gastro-intestinal tract. As different polymer structures will determine the release profile of the bound drug they can be tailored to meet the needs of the patient. Although generally they have a triphasic release profile (Figure 1.7), dosing is more difficult to control in drug delivery using microspheres[78].

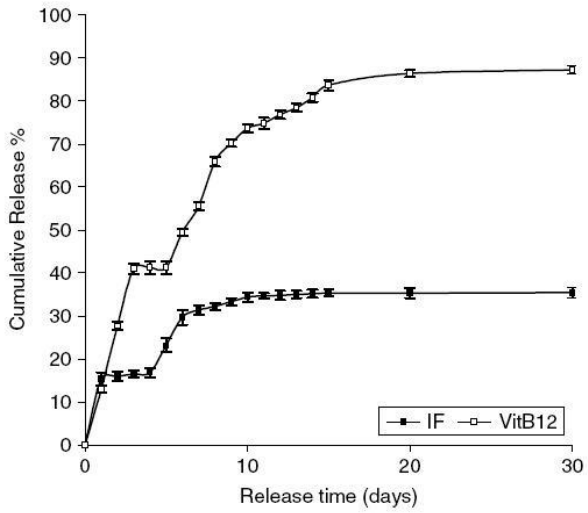


Figure 1.7 Cumulative profiles of the *in vitro* release of stomach produced protein intrinsic factor (IF) and vitamin B12 from IF-Vit B12 complex-loaded microspheres[78].

Nanoparticles are able to be internalised *via* endocytosis and are produced using a protocol which produces polymers three to four times smaller than microspheres. This property makes them useful for delivery of genes to the intracellular matrix to induce gene expression. It has been shown that DNA encapsulation into PLGA nanoparticles has been able to lead to encoding of the p53 tumour suppressor gene leading to a reduction in breast cancer cell growth[71, 79].

Certain polymers used for drug delivery exhibit changes in ionisation state upon a variation in pH, such as the hydrogel poly(N-isopropylacrylamide-co-methacrylic acid) (PNIPAA-MAA)[80]. These hydrogels often comprise weak acids or alkalis.

When exposed to different pH/ionic strength levels the polymer exhibits a conformational change causing a swelling or contraction of the structure. This depends on the attraction or repulsion of the acidic/basic groups increasing hydrophobicity in one conformation and hydrophilicity in the other[81]. This can be linked to the release of a drug molecule, providing high sensitivity to specific stimuli such as PNIPAA-MAA, where streptokinase and heparin are delivered to the site of a blood clot in response to small changes in the pH and temperature[5, 80-82].

Poly(2-hydroxyethyl methacrylate) (PHEMA) is solid in air but in water forms a hydrogel. It is commercially most commonly used for contact lenses[83-86]. However drug release characteristics from drug bound within a PHEMA matrix have also been investigated[87-89]. In these systems the drug will be released via diffusion once the hydrogel enters its swollen state, giving a good template for controlled release. The use of hydrogels with varied polymer preparation techniques and subunits provide benefits to the release of drug by changing the diffusion constant so the release profile varies.

Poly(N-isopropylacrylamide) (PNIPA) has a very narrow temperature range in which it will swell and de-swell[90, 91]. By varying the co-monomer content, PNIPA will change from a water soluble coil to a hydrophobic globule[92] at a temperature other than the 32°C, observed in a pure PNIPA polymer hydrogel[93]. It has been shown by careful control of the monomer composition of PNIPA and PEG that a block copolymer can be produced, capable of altering the lower critical solution temperature (LCST) to 37°C. Biotin ligands are attached to be released freely below the LCST or can be sequestered in the center of the collapsed hydrogel above the LCST[94]. By producing varying copolymers incorporating PNIPA, temperature sensitive controlled release formulations may be achievable[81].

It has been shown that the physical properties of a drug have a determining effect on the release profile from such hydrogels. Hydrophobic drugs were observed to decrease the swelling rate by affecting the hydrogels osmotic properties[90]. Owing to their high water content and soft consistency, hydrogels resemble natural tissue more than other classes of synthetic biomaterials. This contributes to their biocompatibility[95]. Porous hydrogel sponges have been shown to prevent capsule

formation around the implant, (part of the immune response to foreign structures described in section 1.4.2) through vascular tissue growth which improves biocompatibility. As vasculature is capable of growing throughout the network of the hydrogel sponge, drug delivery from within the hydrogel is more efficient[86].

Transdermal drug delivery formulations typically comprise a porous membrane covering a reservoir of drug. Diffusion of drug occurs into the skin through body heat triggering the melting of an adhesive such as polyacrylate[96-99]. The method of drug release is shown in Figure 1.8 below. The use of reservoirs for drug delivery however is a common technique to achieve controlled release[100-103]. A core of drug is surrounded by a polymeric membrane. The nature of the polymeric membrane therefore can determine the rate of drug elution from the system. This is exemplified by transdermal patches but also commonly in microparticulate formulations[104].

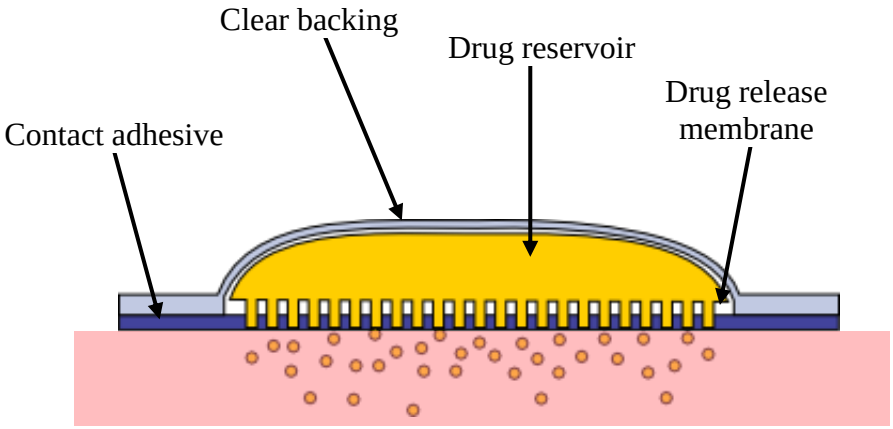


Figure 1.8 Schematic of a transdermal patch containing a drug reservoir.

Stents are tubular meshes which are inserted into occluded or constricted vessels within the body to alleviate a build up of pressure shown schematically for a bare metal stent in Figure 1.9. Stents are used clinically to treat coronary artery diseases such as atherosclerotic plaques which increase the force at which the heart has to pump to bypass a blockage. The tubular stent shown in Figure 1.9 is inserted into the occluded artery and inflated with a balloon catheter to affix the stent in place. An immune response can be mounted against the surface of the metallic stent, which can lead to smooth muscle contraction leading to re-narrowing of the coronary artery which leads to a more severe occlusion termed

restenosis[105, 106]. With drug eluting stents (DES) a polymer coating on a metallic stent can release an immunosuppressant such as the chemotherapy drug paclitaxel to the surrounding tissues. The immune response can be avoided without systemic compromise of the immune system[105, 107, 108]. More recently temporary stents made of biodegradable polymers are also being implanted which also release dispersed drug on degradation through mechanisms such as those described in Figure 1.5[106, 109, 110].

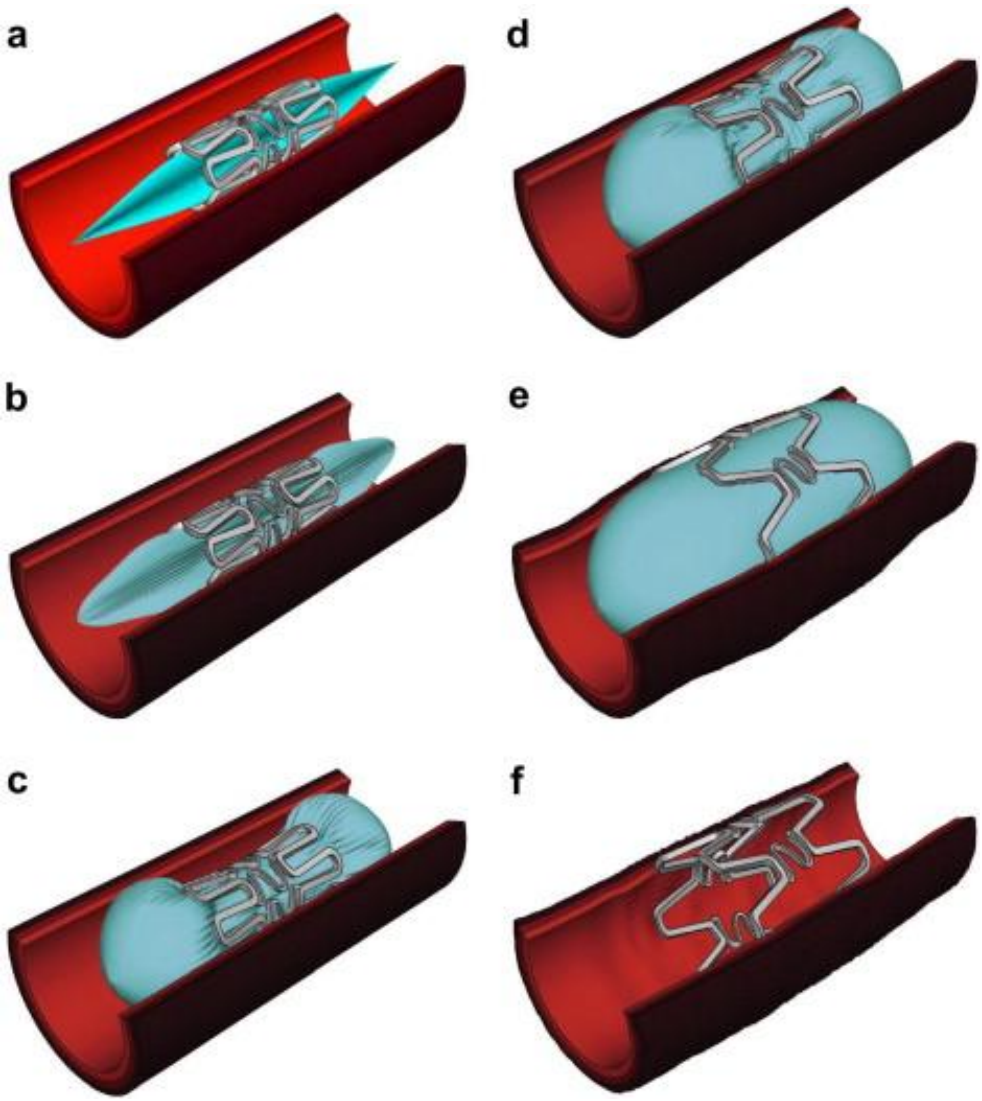


Figure 1.9 Schematic of stent implantation. A balloon catheter (blue) has a metallic drug coated stent placed over it before inflation inside the vessel[111].

Drug loaded polymer implants are ideal as drug delivery can be successfully localised when compared with drug delivery systems such as those orally administered. The use of polymers can therefore reduce systemic side effects[112].

1.4.2 Implants and Tissue Engineering

Biodegradable polymers used for implantable devices are preferable to non-biodegradable materials as there is no need for a second surgical procedure for removal. Such implants include orthopaedic devices[113-115], dental devices[116] and stents used to relieve occlusions in vessels within the body[44, 117-120]. The injectable implant Zoladex for prostate and breast cancer provides a non-pulsatile release of goserelin acetate which stimulates erratic production of testosterone and oestrogen in order to disrupt endogenous hormonal feedback systems. This leads to a reduction in the natural production of testosterone and oestrogen reducing the growth of such tumours. It is implanted by injection under the skin of the abdomen where goserelin is released over a 28 day period[121, 122]. The stability of the implant is due to due to the use of a PLGA copolymer which does not require removal as it is biodegradable adding convenience to such a treatment.

The degradation of the polymers used in orthopaedic devices provides advantages in that stainless steel implants have a tendency for re-fractures to occur after implant removal. The bone has not carried the load which the stainless steel bore whereas a biodegradable implant to aid in bone healing will gradually transfer load to the healing bone as it degrades[123]. Full hip replacements have also been undertaken, implanting an antibiotic-impregnated PMMA hip spacer in order to address infection which has a ~2% prevalence in hip replacement operations requiring treatment and additional surgery[124]. Such implants are now regarded as the gold standard compared to traditional metallic or ceramic implants which limit movement[125]. The benefits of these implants are (i) after initial implantation high levels of antibiotic concentration is reached locally. (ii) Joint mobility is maintained almost immediately after implantation and (iii) antibiotics can lead to a reduction in smooth muscle contraction (which can cause leg length discrepancies)[124]. Such polymer implants are shown in Figure 1.10a with the drug elution over a 13 day period after implantation shown in Figure 1.10b.

It is postulated that due to the physiochemical properties of the hip spacers the release of the two drugs are seen to vary in their kinetics[124]. Through the use of surface analytical techniques relevant to this thesis, such postulations can be qualified.

a) b)

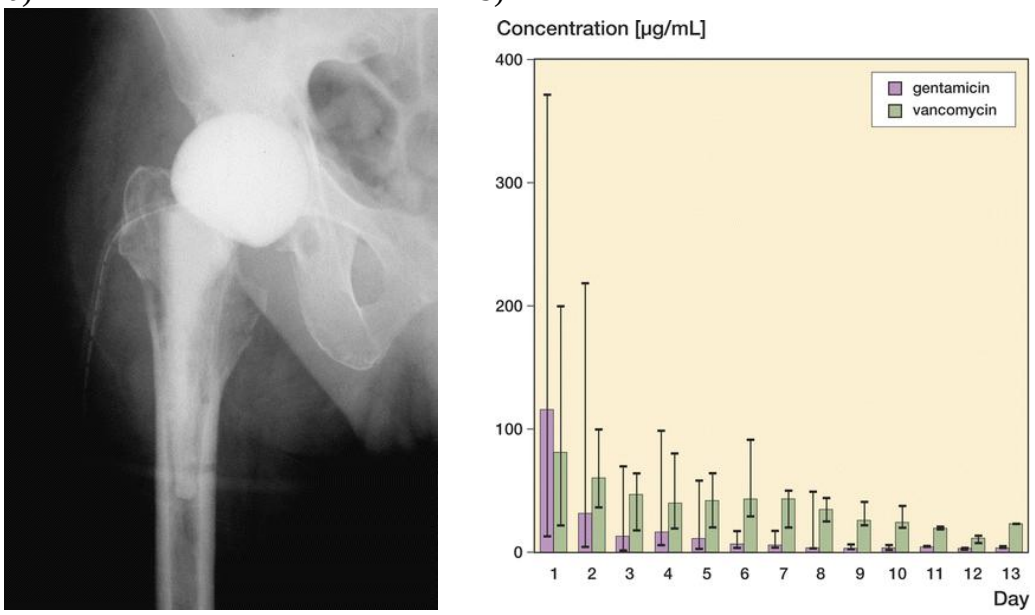


Figure 1.10 a) Antibiotic eluting PMMA hip spacer (wound drainage tube at the top of the spacer is to monitor drug release. b) Elution of antibiotics gentamicin (purple) and vancomycin (green) for 13 days postoperatively[126].

As alluded to in the previous section, implantable drug delivery systems are becoming more prevalent both in terms of drug eluting and biodegradable stents and implantable drug eluting polymer films. Such films are currently used for the controlled release of chemotherapeutic agents directly onto brain tumours[62, 127, 128] or for myocardial repair[129] allowing for a sustained release of drug over a period of time. While an invasive procedure to apply a drug delivering film is an unsustainable practice, the application of such biodegradable films postoperatively improves on therapeutic repair outcomes.

Implanted polymer formulations capable of providing localised drug delivery can also reduce systemic side effects which may be encountered with certain therapeutics while also functioning as an implantable support. Through the combination of a tissue engineering scaffold with bone morphogenic protein

(BMP) releasing microspheres, it has been shown bone formation could be stimulated[130-132]. Through the combination of a biodegradable implantable support which also releases BMP2 a great improvement to current treatment is made.

The objective of tissue engineering is to repair or replace damaged tissues by implanting tissues grown *in vitro* or assisting the growth of cells and regenerating cells *in vivo*[133, 134]. This is based on observations that isolated cells in close proximity will attempt to form tissues through cell signalling pathways first reported in 1993 by Langer and Vacanti[27]. Polymers are of great importance in this field, one that has rapidly grown in prominence over the past decade. Polymers act as a scaffold for the attachment and proliferation of cells and to assist in the formation of a synthetic extracellular matrix (ECM)[135]. This is crucial as it organises cells into a 3 dimensional (3D) architecture and to present stimuli responsible for directing the growth and formation of a desired tissue[136]. Figure 1.11 shows the growth of a fibroblast cell line (NIH3T3) on a PLA scaffold after 72 hrs.

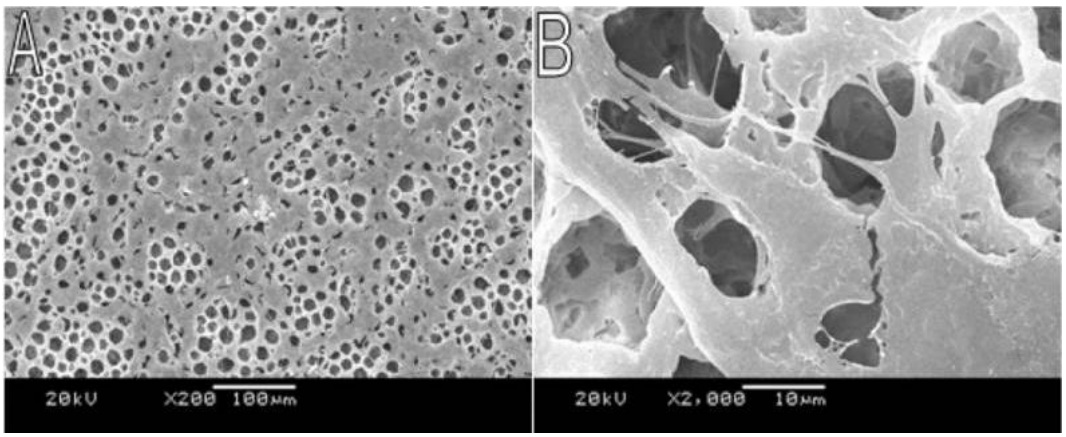


Figure 1.11 Fibroblast cell line NIH3T3 was cultured for 72 hrs shown at a) ×200 and b) ×2000 magnification. Showing good cell attachment and growth[137].

Further investigation of the adhesion and proliferation of the fibroblast cell line shown in Figure 1.11 was shown to have colonised the visible surface of the microporous PLA scaffold film shown after 4 days[137]. The surface of such polymer scaffolds is crucial as the scaffold material and its physical properties will vary depending on the type of cells being grown. As such the surface characterisation of such scaffolds is important for optimisation of the resultant

tissue[25, 138, 139]. Hydrogels have been extensively used for tissue engineering purposes due to the resemblance to natural tissues[95]. Several variables have been identified as key to adequate scaffold design/material selection. These are physical properties (degradation, mechanics and gel formation), mass transport properties (diffusion) and biological properties (cell adhesion and signalling)[140]. As synthetic biodegradable polymers typically degrade through hydrolysis and not enzyme action and the properties can be easily tailored to the properties required, they are preferred over natural polymers[27, 135, 141]. Skin[142, 143], bone[144, 145], liver[146], heart valves[147], nerves[148, 149], tendon and ligaments[150, 151] are tissues regenerated using biodegradable polymers for tissue engineering. It is hoped that in the near future permanent biostable devices used only for temporary applications will be replaced by biodegradable polymer technology.

1.5 The Importance of Surfaces

Modern surface science stems from discoveries by the Nobel laureate for chemistry in 1932, Irving Langmuir[152] who with Katherine Blodgett showed the depositing of 3001 individual monolayers and furthermore went on to describe the first use of ellipsometry to approximate the thickness of the monolayer film, (described in Chapter 2)[153, 154].

Surface phenomena play a vital role in the human body where the skin protects the body from infection. Cells lining the lungs and intestine are where nutrient transfer occurs. As such, biologically and in medicine surfaces are crucial considerations for biomedical device production. When a material comes into contact with a biological surface, a chain of interactions occur in response. In particular with implants a host-foreign body response can be mounted. This can be expressed as (i) platelet adhesion and activation leading to thrombus formation on the foreign surface and surrounding tissues which are large aggregates capable of causing a narrowing of the vessel lumen[155]. (ii) Accumulation of reactive inflammatory infiltrates, including cells capable of secreting pro-inflammatory cytokines and growth factors. Finally (iii) monocyte adhesion which can differentiate into activated macrophages to form foreign body giant cells, capable of impeding the beneficial actions of the biomaterial[156]. With detailed understanding of the biological surface/response and that of the biomedical

device, biocompatible medical devices can be produced as exemplified by drug eluting stents for localised immune response suppression[157].

For medical implants, the understanding of protein adsorption is crucial. With respect to platelet mediated thrombus formation this is most severe for arteries in the case of devices such as bare metal cardiovascular stents used to open the lumen of an already occluded artery described in section 1.4.2[158]. The attachment and colonisation of pathogenic bacteria to venous catheters via an adsorbed protein layer and the fouling of haemodialysis membranes[159] are causes of patient infection and mortality[160] to give but a few examples.

This issue is most prevalent with systems in contact with the blood. Biomaterials used in such applications must therefore be as haemocompatible as possible to avoid issues described in the previous paragraph, or otherwise be designed to mitigate such problems, i.e. the drug eluting stent for localised immune response suppression. The cascade of biological responses mediated by cell signalling agents (selectins/cytokines) activated by the pathway shown in Figure 1.12 shows the interactive nature of the biological environment posing a challenge for modern biomaterials leading to such advances as PEGylation to mitigate the biological response to non-specific interactions between protein and polymer making them non-fouling[34, 161, 162]. This process leads to the body coordinating a reaction to the original surface.

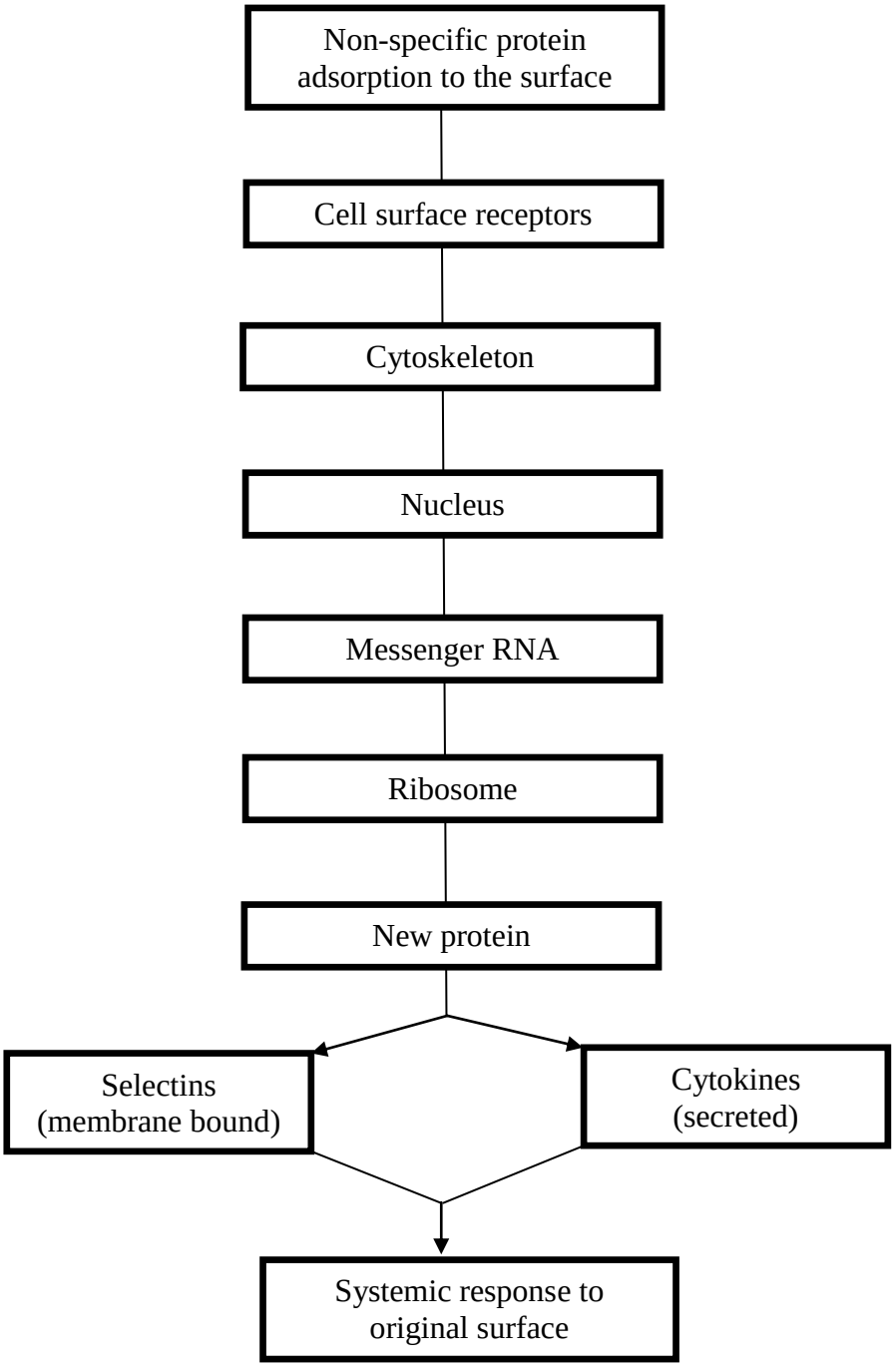


Figure 1.12 Flow diagram of signalling pathway causing a biological response, adapted from Ratner 1995[161].

Surfaces are dynamic, with movement and rearrangement occurring when contacting other surfaces. This is especially prevalent in biological systems[163] which will be analysed in depth in section 1.5.1. The mechanical properties of such polymers are therefore crucial to the success of the biomaterial for its eventual use. An example of the importance of surface properties in biology is observed as linear trends for cell adhesion versus various measures of surface energy revealed a relationship between the two in the 1970s[164-166]. The improvements in surface analytical techniques since such work has allowed for advances to be made in surface science. For example, the arginine-glycine-aspartic acid (RGD) amino acid sequence in proteins has been shown to be crucial in cell adhesion. Surfaces functionalised with small peptides containing the RGD motif have been produced and analysed with XPS[167] and found to cause predictable cell adhesion to surfaces. Such knowledge has been of use for tissue engineering applications[168-171].

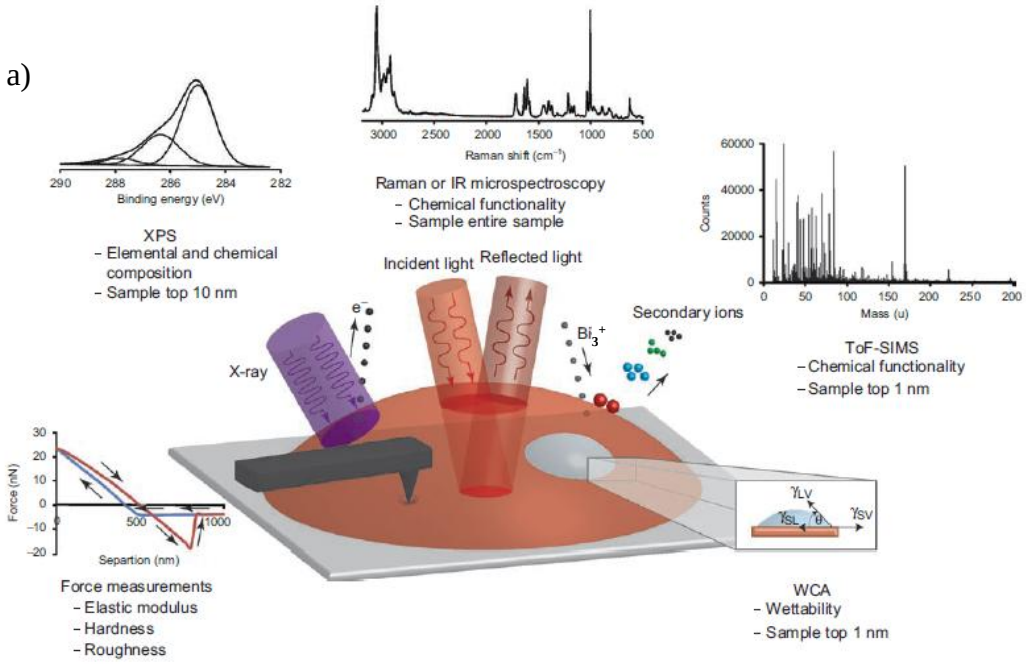
The hydration state of biomaterials is of significance. An elastomer film containing PHEMA analysed when in a frozen, hydrated state and when dehydrated exemplifies this. The XPS spectrum resembled PHEMA when hydrated but when dehydrated indicated silicone rubber[172]. The variation in the hydration state causes significant reorganisation at the surface for such polymers with polar and non-polar regions which is important to appreciate for biomaterial selection for a therapeutic application. They are exploited specifically for hydrogel drug delivery applications[141, 162, 173, 174]. Another consideration of the hydration state of synthetic biomaterials in particular is the rate of degradation through hydrolytic degradation described in section 1.3.

1.5.1 Surface Analysis of Biomaterials

This thesis exploits surface analytical techniques in order to characterise both the surface and bulk of a range of biomedically relevant formulations. The thorough characterisation of biomaterial surfaces allows for the relationship between the surface chemistry and physical properties of the biomaterial to provide understanding of biointeractions at interfaces between the two. This knowledge will further allow for optimisation of biomaterial development as implied in the

previous section. A range of surface analytical techniques are used in this thesis (explained thoroughly in Chapter 2), these are shown in Figure 1.13 below and have been used to characterise a range of new biomaterials[175-178].

a)



b)

Technique	Principle	Depth	Parameter Measured	Spatial Resolution	Sensitivity	Limitations
AFM	Deflection of a cantilever monitored	Surface	Topography, surface mechanical properties	Molecular level	Highly sensitive	Limited in the extent to which specific surface chemistries can be identified
Contact Angle	Wettability of surfaces	3-20 Å	Surface free energy	1 mm	Dependent on surface chemistry	Liquid causes sample swelling, extraction and penetration
SEM	Secondary electron eemission after electron bombardment	5 Å	Topography	40 Å	High (not quantifiable)	Sample damage, artifacts
Ellipsometry	Change in polarisation of light after reflecting off sample surface	<50 Å	Thickness, kinetics	2 Å	Highly sensitive	Affected by surface roughness and non-uniformities i.e. scratches
XPS	Secondary electron emission following X-ray bombardment	10-250 Å	Composition	10-150 µm	0.1% at.	Can be destructive to sample, complex interpretation
SIMS	Secondary ion emission following ionic bombardment	10-1000 Å	Composition	200 Å	Very high	Destructive to sample, matrix effects
Raman Spectroscopy	Vibrational spectroscopy the 'Raman Effect'	< 1 µm	Composition	1 µm	High	Limited for depths < 1 µm

Figure 1.13 a) Schematic diagram of common surface analytical techniques applied for the chemical and structural characterisation of biomedical devices[176]. b) Table comparing and contrasting the information obtained from various surface analytical techniques adapted from Kannan *et al.*[179]

Figure 1.13 illustrates some of the commonly used techniques for characterising complex material surfaces. Each technique has its own strengths and weaknesses; thus multiple techniques are often employed to complement one another.

ToF-SIMS provides highly surface sensitive mass spectra which provide qualititative chemical information. In contrast XPS is sensitive to the top ~5-10 nm of the surface and is capable of providing quantitative data regarding the chemical composition of the surface region. As such these two techniques are highly complementary in the data produced[180-182]. It is important to use a range of techniques to characterise biomedically relevant systems. As alluded to in section 1.4, a range of properties of biomaterials influence the eventual performance *in vivo*. As different surface analytical techniques provide varying levels of chemical and mechanical information a multifaceted approach is beneficial for both the surface and bulk analysis of such systems. This thesis exploits a number of techniques, however particular focus is placed on ToF-SIMS which has been used extensively for the analysis of biomaterials.

The surface characterisation of biodegradable homo-polymer systems such as polyesters[183-185], polyorthoesters[184, 186] and polyanhydrides[184, 187] have been studied with ToF-SIMS and XPS. Their fragmentation patterns, characteristic ions and the quantitative elemental composition at the surface of pure polymer have been elucidated. Such work allows for reference material for spectral identification to be undertaken and is also fundamental in understanding the fragmentation of such biomaterials to aid in the analysis of copolymers[184]. ToF-SIMS has been used to observe the hydrolysis products on both homo and co-polyesters[188, 189] as well as applied to micro and nanoparticulates for the detection of monolayers of surfactant[190] and grafted polymers[191]. The technique is now being applied more to the analysis of micro and nanoparticulate systems with standards currently being developed so recommendations for nanoparticle analysis conditions are made to the global surface analysis community. While much literature exists on the utilisation of ToF-SIMS for polymeric systems, due to the availability of the technique, its utilisation on biodegradable polymer systems has been limited. With advances in ion sources capable of the production of ever increasing resolution and less damage in organic depth profiling,[192-194] the prevalence of ToF-SIMS of biodegradable systems is ever increasing.

ToF-SIMS is a versatile technique for the analysis of polymers with biomedical applications. It has been used for the analysis of controlled-release drug delivery systems with much of the interest being directed at drug eluting stent models[17, 19,

[120, 195, 196] and controlled release from reservoir controlled-release systems[104, 197-199]. A cross section of such a solid state drug pellet is shown in Figure 1.14. The chemical localisation and high spatial resolution of drug within such solid phase drug beads allows for inferences to be drawn with regard to the release profile observed. This rationale also being applied to understand the distribution of solid phase antibiotics embedded within a block copolymer coating[200]. Through the use of surface analytical techniques it has been demonstrated drug concentration and solvent evaporation rate are key considerations in the eventual drug distribution and release profile observed. This allows for considerations to be made in the formulation stage of such drug eluting film production[200]. ToF-SIMS analysis acts as an *in situ* analysis of drug beads capable of providing both chemical and structural information simultaneously. The capability to show the spatial location of the drug, excipient and any coatings at up to ~200 nm spatial resolution[201] makes ToF-SIMS a valuable tool in the characterisation of such formulations.

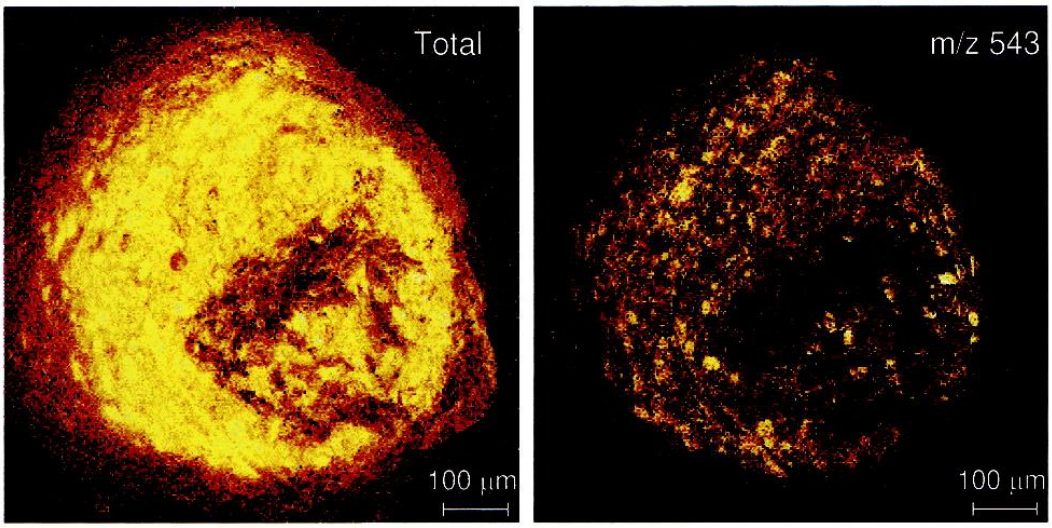


Figure 1.14 Total ion image and the ion at *m/z* 543 which is a characteristic ion for the corticosteroid prednisolone represented as an ion image of an 800 × 800 μm area [104].

A great deal of interest has been invested using ToF-SIMS to analyse protein orientation[202-207], characterise the ECM[208] for tissue engineering applications and protein release from polymer drug delivery systems[20, 207, 209, 210]. The application of multivariate statistical analysis methods to the analysis of hyperspectral data has allowed for a de-convolution of such data sets[211]. Principal component

analysis (PCA) allows for ToF-SIMS spectral interpretation by differentiating between the various statistically significant variation between the data set[212]. As all proteins specifically contain various compositions of the same 20 amino acids, techniques such as PCA are highly sensitive to rapidly highlight regions of interest from numerous spectra for identification of protein orientation and specific moieties, without the need for significant analyst expertise[202, 203, 208]. The ability to scrutinise the orientation of proteins on polymer surfaces is beneficial in order to produce surfaces with protein active sites exposed to the biological environment. Such advances have implications for biotechnology such as protein microarrays, antibody-based diagnostics, affinity chromatography and biomaterials that present protein ligands to bind cell receptors[202, 213]. Specifically it has been shown that protein orientation can influence the responses of platelets[214, 215]. The application of ToF-SIMS for the analysis of protein structure has only increased in prevalence within the last year, illustrating how advances in ToF-SIMS and its complimentary techniques have opened up new avenues of research applicable to polymers with biomedical applications.

Depth profiling is a process where a 1-2 nm thickness of the surface is analysed with ToF-SIMS followed by a layer of the surface being removed through sputtering. This process is repeated to provide a representation of polymer and drug distribution throughout the sample thickness with high chemical specificity and resolution[216]. A model stent film that has been depth profiled is shown graphically in Figure 1.15. Surface enrichment of drug is observed which is used to rationalise the ~ 54% elution of sirolimus within the first 24 hrs *in vitro*[120]. Such a representation also shows improvements in computational software have allowed for a clearer de-convolution of complex surface analytical data.

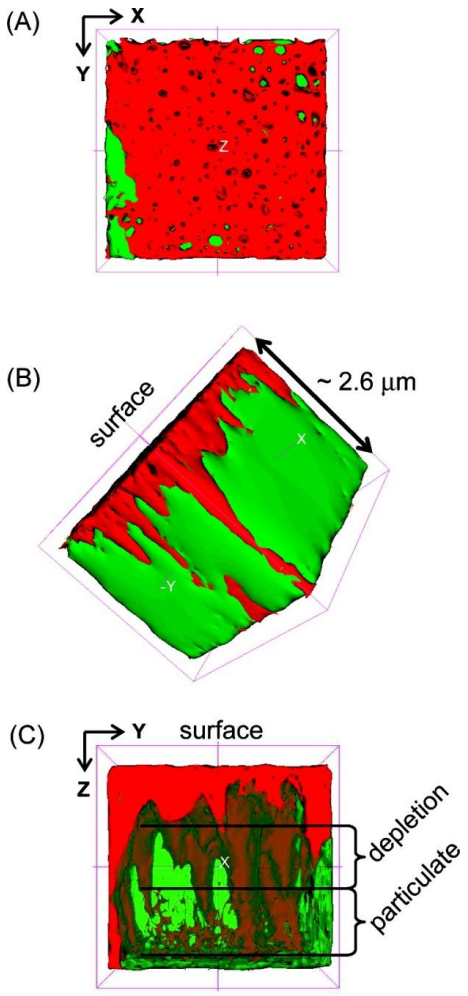


Figure 1.15 ToF-SIMS depth profile of a model DES film showing for a 200 × 200 µm area the drug sirolimus (red) and PLGA (green)from an a) xy, b) xyz and a c) xyz representation with an increase in PLGA transparency[120].

The results indicate some surface enrichment of drug. This is shown to be heterogeneously distributed in this formulation which had been spray cast onto a flat metal coupon. This analysis provides a detailed understanding of drug distribution and hence *in vivo* performance can be rationalised.

XPS is the primary technique used for the quantitative characterisation of biomedically relevant polymers with extensive reference material available for analysts[217, 218]. It measures excited electrons termed photoelectrons emitted from a sample surface after X-ray irradiation and plots a spectrum based on the binding energy of the photoelectrons detected, specific to individual atoms[216]. Controlled release formulations have been routinely characterised with XPS in order to determine the elemental composition of the surfaces. Such formulations include

nanoparticles[219-225], thin polymer films[226-228] and hydrogels[229-232]. The utility and surface sensitivity of XPS has made it a technique commonly used for representing changes in the elemental composition of various controlled release formulations as shown in Figure 1.16.

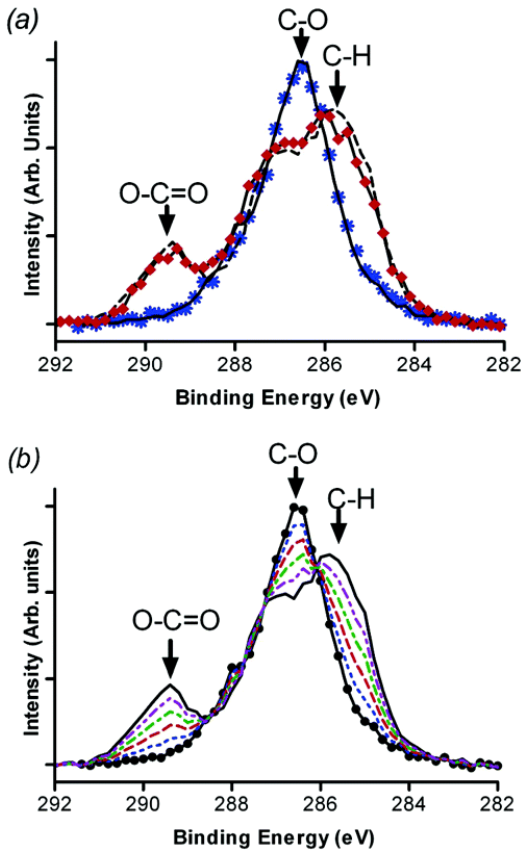


Figure 1.16 XPS C1s spectrum of a) PHEMA (---) microspheres loaded with the drug dextran (—), nondegraded dex-HEMA microspheres (*) and dex-HEMA microspheres (♦) degraded for 5.7 months (pH 7.4, 37C) plotted. b) PHEMA (black line) and dextran (●) are plotted with mixtures of PHEMA and dextran in ratios of 20/80 (blue), 40/60 (red), 60/40 (green) and 80/20 (purple) displayed[233].

For the formulation in Figure 1.16 XPS has quantitatively shown the *in vitro* degradation of dex-HEMA microspheres, suggesting surface segregation of dextran before elution experimentation. After drug elution the XPS indicates total surface dextran release represented as a close agreement after degradation between the spectrum of pure PHEMA and degraded microspheres.

XPS also has utility in the assessment of the presence of peptide sequences at the surface of polymers[234]. This is particularly relevant to tissue engineering applications, whereby a polymer substrate can be used to grow stem cells[235, 236]. Surface characterisation is capable of improving the understanding of moieties which will improve cell attachment, growth and thereby assist in the development of polymer scaffolds that promote cell-surface interactions[237-239]. Depth profiling of biomaterials using a sputter source capable of sputtering organics coupled with analysis using XPS is an application of the technique which has been gradually rising to provide quantitative bulk analysis of polymer films in recent years[120, 226, 240]. While the field of XPS depth profiling of organics is still young, it has great potential for the characterisation of thin polymer films.

AFM scans the surface of a sample, measuring minute shifts in the deflection of a cantilever in order to produce angstrom spatial resolution topographical information. AFM is limited by the extent of the chemical information it is capable of providing[216], however it is a versatile technique routinely used for characterisation of material properties such as adhesion[241], mechanical elasticity[242] and stiffness[243, 244]. Advances in the variety of cantilever tips and modes of operation have made such characterisation more routine i.e. Veeco's quantitative nanomechanical mode (QNM), capable of simultaneously recording topography, stiffness, adhesion, energy dissipation and sample deformation. This is achieved through the measurement of a force vs. distance (f-d) curve[245] which charts the attraction and repulsion of the cantilever tip from each tapping cycle (explained in Chapter 2).

This high spatial resolution (<1 nm) is complementary to the high specificity chemical characterisation found with ToF-SIMS and XPS techniques outlined previously. The molecular resolution of AFM is best described by the ability to allow monolayer identification of polymer and protein at the surface of substrates[246-248] and the ability to identify various polymorphs of drug crystals[249-251]. The surface of biodegradable polymers has previously been analysed with AFM[252, 253]. The technique has also been used in conjunction with surface plasmon resonance (SPR) to show the degradation of the semicrystalline polyanhydride poly(sebacic anhydride) (PSA)[254]. This was undertaken through monitoring the preferential loss of amorphous material over crystalline fibres. In a

similar surface erosion study, the release of the protein bovine serum albumin (BSA) from a poly(orthoester) film was observed[255], as shown in Figure 1.17. The analysis of a PVP hydrogel formulation for the release of the drug diclofenac sodium was also capable of distinguishing surface drug distribution. Similar to Figure 1.15, inferences can be made to a lesser extent as to the drug release characteristics of such films.

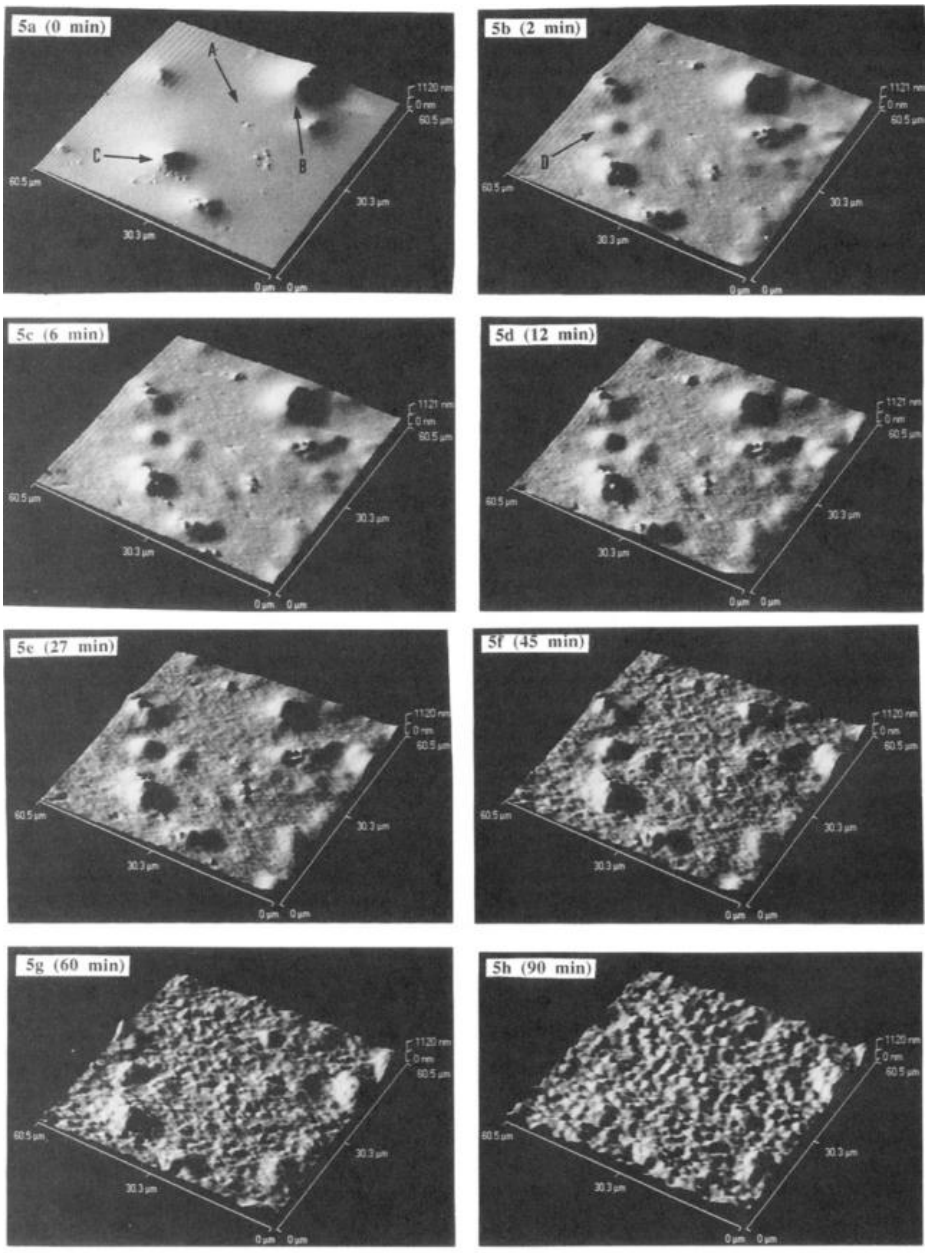


Figure 1.17 *In situ* AFM of 60.5 × 60.5 μm regions of a poly(orthoester) film containing BSA over a 90 minute period showing the degradation of the film and release of protein particles[255].

In addition to protein and drug release, AFM has been used for the analysis of both isolated proteins such as muscle fibres of actin[256] and myosin[257] as well as soluble proteins in deposited layers[255, 258, 259]. Protein adhesion using protein-modified AFM tips have also been extensively investigated using f-d curves which is of significance to the protein adhesion mediated cascade described in the flow chart of Figure 1.12[260-262]. By measuring adhesion to a range of biomaterial surfaces, candidates can be identified with naturally more repulsive surface chemistry to protein attachment. This provides benefits to the biomaterial utilised in implantable medical devices. For example phase separation in hydrated poly(urethane urea) (PUU) was correlated with molecular interactions. Both the phase separation and molecular interactions were determined with AFM for identification of novel biomaterials with application for medical implants. Previously PUU has shown good hemocompatability[263] making it a candidate for implants in contact with blood. Hard domains were shown to undergo rearrangement and enrichment at the surface when hydrated. When hydrated, PUU surfaces gradually become less adhesive to protein[264]. This phenomenon[265] has also been shown for low density polyethylene (LDPE)[266]. This study exemplifies the versatility of AFM operation for the identification of protein adhesion, its release from the surface and polymer phase separation[264]. This shows the value of AFM in biomaterial characterisation.

Complementary surface analytical techniques highlighted so far have been shown to be able to yield high throughput (HT) surface analysis for polymer microarrays as shown in Figure 1.18 for an array with autofluorescence inset for a triplicate of 576 polymer spots. Such applications of the surface analytical techniques illustrate how complementary surface analysis can rapidly be used for the identification of polymers with characteristics suitable for a range of biomedical applications.

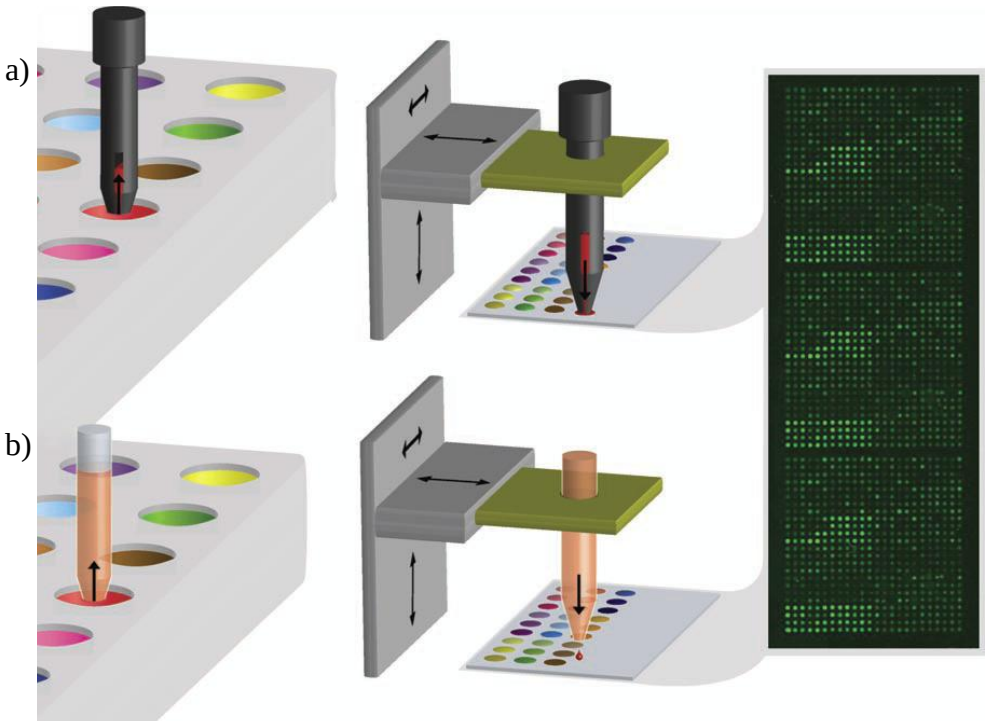


Figure 1.18 Schematic diagram of a microarray formation by a) contact (pin transfer) and b) non-contact (ink-jet) printing. The autoflourescence from a 576 polymer microarray printed in triplicate on a standard glass slide[267].

The rapid surface analysis of such a vast number of polymers using all complementary techniques illustrated in Figure 1.13 allows for correlations to be made between surface chemistry and physical performance displayed in Figure 1.19. Predictions can be drawn from such analysis based on the variation in monomer composition alone. As exemplified for a range of acrylate polymers,[268-271] the rapid analysis and identification of polymers best suited to sustain human embryonic stem cell (hES) growth is shown[272]. A range of 22 acrylate monomers were combined to produce 496 combinations (primary array) with AFM, water contact angle (WCA) measurements, ToF-SIMS and biological assays. The results of the initial array lead to the production of a secondary array of 48 polymers. Multivariate analysis of the surface analytical data obtained was used to identify chemical functionalities responsible for either enhanced or diminished stem cell colony formation. The ability to predict the performance of the surface based on this approach is shown in Figure 1.19.

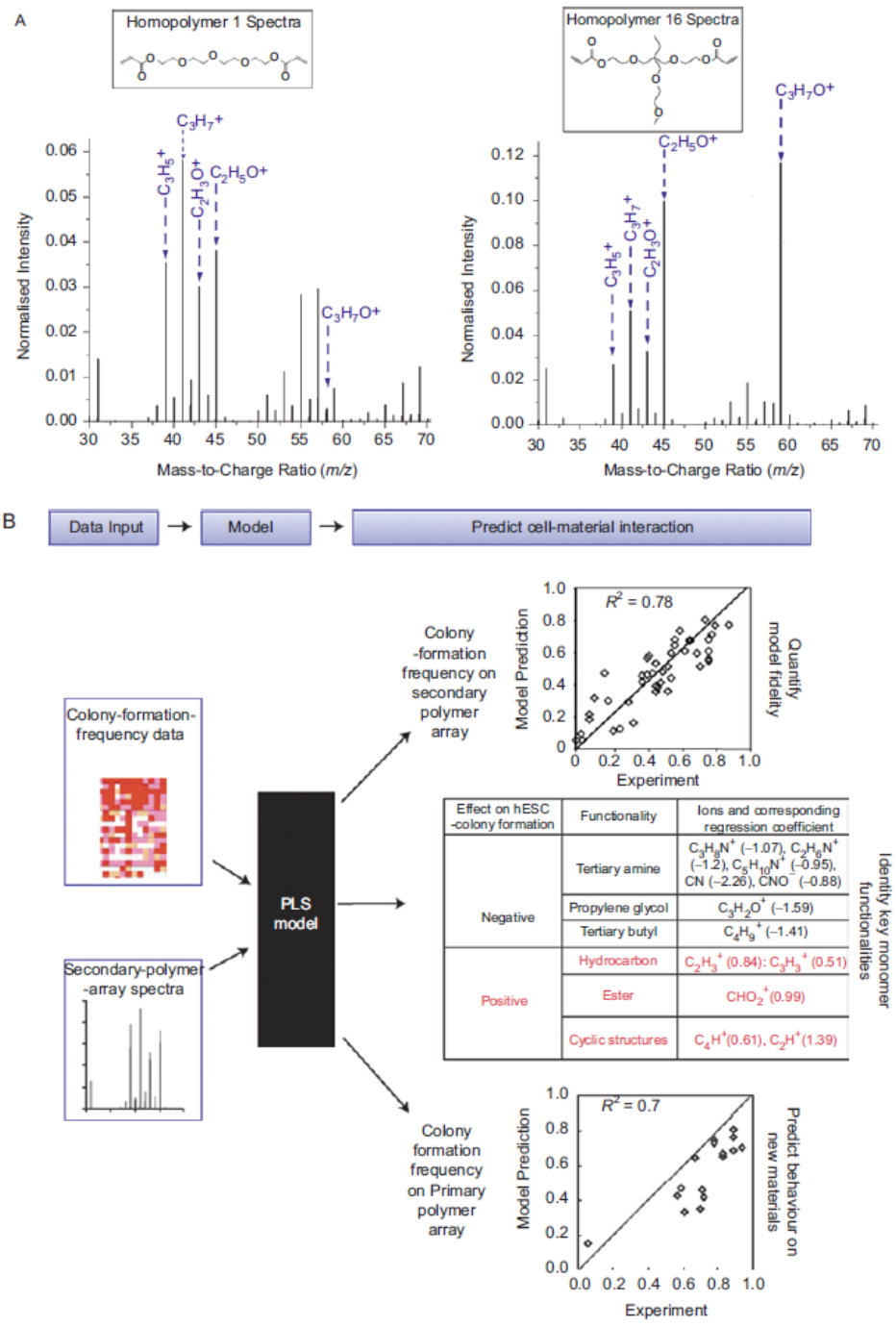


Figure 1.19 Mapping of cell behaviour to surface chemistry using arrays a) ToF-SIMS of homopolymers labelled 1 and 16. Suggesting variation in secondary ion intensity for the ions highlighted between the two polymers will cause difficulty in prediction of surface chemistry for all polymers tested. b) A multivariate partial least squares regression (PLS) model used to analyse and predict cell/material interactions by correlating ToF-SIMS spectra to their biological performance (colony formation frequency). Linear correlation of predicted versus measured colony formation frequency. Middle table: functionalities determined to promote or inhibit hES colony formation.

A good correlation was observed between the ToF-SIMS data and the colony formation frequency for each polymer in the array. Similar correlations have also been observed with WCA measurements[271] allowing for the rapid identification of new biomaterials for future biomedically relevant systems.

1.6 Scope of Thesis

In this thesis a range of surface analytical techniques are used in order to characterise polymers with biomedical relevance. Specifically ToF-SIMS is the focus of the work in this thesis. The overall aims of this thesis are to expand on the current literature to provide a greater understanding of ToF-SIMS for the analysis of polymer biomaterials and to investigate methods of improving the lateral resolution of ToF-SIMS imaging of samples with challenging topography.

With the new capability of cluster ion sources for ToF-SIMS depth profiling of organic materials, this is first applied to a simple binary blend of polymer and drug. The rationale behind this work is in order to help determine the capability of ToF-SIMS and newly developed XPS depth profiling for analysis of thin model DES films. With the use of the novel technique of XPS depth profiling a simple model system comprising PLA and a low molecular weight drug was used.

Once complete, a more advanced model film was produced of a multilayer film building on the model produced in the first segment of work. This chapter was in interest of furthering our knowledge of the capability of ToF-SIMS depth profiling of multilayer films containing drug. This was in keeping with the aims in order to build on the current literature, whilst advancing our knowledge of ToF-SIMS of drug loaded multilayer films. There is a need for such complex systems to be analysed as multilayer films are used in industry, whereby the limits of the chemical characterisation and detection capabilities are generally unknown for drug loaded multilayer films.

Finally, the expertise gained from the analysis of the first two models has been applied to the study of a real-world example of a biomedically relevant formulation of protein loaded microspheres. Microspheric samples provide considerable difficulty when analysing with techniques such as ToF-SIMS which

are highly sensitive to topographical effects. In this work AFM, ToF-SIMS, XPS and confocal Raman spectroscopy (described in Chapter 2) are applied to fully characterise such a formulation. This allows inferences to be made with respect to limitations of the production process to be determined. This chapter addressed the need to improve the lateral resolution in imaging of a topographically challenging system.

This thesis also aims to expand the readers' knowledge of surface analysis of polymers with biomedical applications. Both the results and limitations of the techniques/models used for characterisation will be discussed.

1.7 References

1. Ratner, B.D., *Biomaterials science: an introduction to materials in medicine.* 1996: Academic Press.
2. Stevens, M.P., *Polymer Chemistry: An Introduction*. 1999: Oxford University Press.
3. Young, R.J. and P.A. Lovewell, *Introduction to Polymers*. 1991: Chapman and Hall.
4. Gil, E.S. and S.M. Hudson, *Progress in Polymer Science*, 2004. **29**(12): p. 1173-1222.
5. Heath, F., P. Haria, and C. Alexander, *Aaps Journal*, 2007. **9**(2): p. E235-E240.
6. Qiu, Y. and K. Park, *Advanced Drug Delivery Reviews*, 2001. **53**(3): p. 321-339.
7. Vert, M., *Progress in Polymer Science*. **32**(8-9): p. 755-761.
8. Albertsson, A.-C. and S. Karlsson, *Chemistry and biochemistry of polymer biodegradation.*, in *Chemistry and Technology of Biodegradable Polymers*, G.J.L. Griffin, Editor. 1994, Blackie Academic and Professional: London. p. 7-17.
9. Ikada, Y. and H. Tsuji, *Macromolecular Rapid Communications*, 2000. **21**(3): p. 117-132.
10. Lee, J.W. and J.A. Gardella, *Analytical and Bioanalytical Chemistry*, 2002. **373**(7): p. 526-537.
11. Mathiowitz, E., J. Jacob, K. Pekarek, and D. Chickering, *Macromolecules*, 1993. **26**(25): p. 6756-6765.
12. Uhrich, K.E., S.M. Cannizzaro, R.S. Langer, and K.M. Shakesheff, *Chemical Reviews*, 1999. **99**(11): p. 3181-3198.
13. Li, S.M., *Journal of Biomedical Materials Research*, 1999. **48**(3): p. 342-353.
14. Hasirci, V., K. Lewandrowski, J.D. Gresser, D.L. Wise, and D.J. Trantolo, *Journal of Biotechnology*, 2001. **86**(2): p. 135-150.
15. Brown, L.R., C.L. Wei, and R. Langer, *Journal of Pharmaceutical Sciences*, 1983. **72**(10): p. 1181-1185.
16. Lloyd, A., *Medical Device Technology*, 2002. **13**: p. 18-21.
17. Belu, A., C. Mahoney, and K. Wormuth, *Journal of Controlled Release*, 2008. **126**(2): p. 111-121.
18. Brannonpeppas, L., *International Journal of Pharmaceutics*, 1995. **116**(1): p. 1-9.
19. Braun, R.M., J. Cheng, E.E. Parsonage, J. Moeller, and N. Winograd, *Analytical Chemistry*, 2006. **78**(24): p. 8347-8353.
20. Burns, S.A. and J.A. Gardella, *Applied Surface Science*, 2008. **255**(4): p. 1170-1173.
21. Chang, A.C. and R.K. Gupta, *Journal of Pharmaceutical Sciences*, 1996. **85**(2): p. 129-132.
22. Cohen, S., T. Yoshioka, M. Lucarelli, L.H. Hwang, and R. Langer, *Pharmaceutical Research*, 1991. **8**(6): p. 713-720.
23. Grenha, A., B. Seijo, C. Serra, and C. Remunan-Lopez, *Biomacromolecules*, 2007. **8**(7): p. 2072-2079.
24. Li, W.J., C.T. Laurencin, E.J. Caterson, R.S. Tuan, and F.K. Ko, *Journal of Biomedical Materials Research*, 2002. **60**(4): p. 613-621.

25. Yang, F., R. Murugan, S. Wang, and S. Ramakrishna, *Biomaterials*, 2005. **26**(15): p. 2603-2610.
26. Hutmacher, D.W., *Biomaterials*, 2000. **21**(24): p. 2529-2543.
27. Langer, R. and J.P. Vacanti, *Science*, 1993. **260**(5110): p. 920-926.
28. Bruining, M.J., A.P. Pijpers, P. Kingshott, and L.H. Koole, *Biomaterials*, 2002. **23**(4): p. 1213-1219.
29. Hinrichs, W.L.J., J. Kuit, H. Feil, C.R.H. Wildevuur, and J. Feijen, *Biomaterials*, 1992. **13**(9): p. 585-593.
30. Middleton, J.C. and A.J. Tipton, *Biomaterials*, 2000. **21**(23): p. 2335-2346.
31. Katti, D.S., S. Lakshmi, R. Langer, and C.T. Laurencin, *Advanced Drug Delivery Reviews*, 2002. **54**(7): p. 933-961.
32. Roberts, M.J., M.D. Bentley, and J.M. Harris, *Advanced Drug Delivery Reviews*, 2002. **54**(4): p. 459-476.
33. Klok, H.-A., *Journal of Polymer Science Part A: Polymer Chemistry*, 2005. **43**(1): p. 1-17.
34. Abuchowski, A., J.R. McCoy, N.C. Palczuk, T. Vanes, and F.F. Davis, *Journal of Biological Chemistry*, 1977. **252**(11): p. 3582-3586.
35. Working, P.K., M.S. Newman, J. Johnson, and J.B. Cornacoff, *Safety of Poly(ethylene glycol) and Poly(ethylene glycol) Derivatives*, in *Poly(ethylene glycol)*. 1997, American Chemical Society. p. 45-57.
36. Veronese, F.M. and G. Pasut, *Drug Discovery Today*, 2005. **10**(21-24): p. 1451-1458.
37. Pasut, G., A. Guiotto, and F. Veronese, *Expert Opinion on Therapeutic Patents*, 2004. **14**(6): p. 859-894.
38. Maggon, K., *R&D Paradigm Shift and Billion-Dollar Biologics*. Handbook of Pharmaceutical Biotechnology. 2006: John Wiley & Sons, Inc. 161-199.
39. Gilding, D.K. and A.M. Reed, *Polymer*, 1979. **20**(12): p. 1459-1464.
40. Schmitt, E.E. and R.A. Polistina, *Surgical Sutures*, U.S.P. Office, Editor. 1967: USA.
41. Mohamed, F. and C.F. van der Walle, *Journal of Pharmaceutical Sciences*, 2008. **97**: p. 71-87.
42. Mathiowitz, E., J.S. Jacob, Y.S. Jong, G.P. Carino, D.E. Chickering, P. Chaturvedi, C.A. Santos, K. Vijayaraghavan, S. Montgomery, M. Bassett, and C. Morrell, *Nature*, 1997. **386**(6623): p. 410-414.
43. Mahoney, C.M., *Analytical Chemistry*, 2005. **77**(11): p. 3570-3578.
44. Kastrati, A., J. Mehilli, N. von Beckerath, A. Dibra, J. Hausleiter, J. Pache, H. Schuhlen, C. Schmitt, J. Dirschinger, A. Schomig, and I.-D.S. Investigators, *Jama-Journal of the American Medical Association*, 2005. **293**(2): p. 165-171.
45. Baveja, S.K., K.V.R. Rao, and K.P. Devi, *International Journal of Pharmaceutics*, 1987. **39**(1-2): p. 39-45.
46. Melia, C.D., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991. **8**(4): p. 395-421.
47. Siepmann, J. and N.A. Peppas, *Advanced Drug Delivery Reviews*, 2001. **48**(2-3): p. 139-157.
48. Verma, R.K., B. Mishra, and S. Garg, *Drug Development and Industrial Pharmacy*, 2000. **26**(7): p. 695-708.
49. Verma, R.K., D.M. Krishna, and S. Garg, *Journal of Controlled Release*, 2002. **79**(1-3): p. 7-27.

50. Imanidis, G., S. Helbing-Strausak, R. Imboden, and H. Leuenberger, *Journal of Controlled Release*, 1998. **51**(1): p. 23-34.
51. Qian, F., N. Nasongkla, and J.M. Gao, *Journal of Biomedical Materials Research*, 2002. **61**(2): p. 203-211.
52. Ravi, S., K.K. Peh, Y. Darwis, B.K. Murthy, T.R.R. Singh, and C. Mallikarjun, *Indian Journal of Pharmaceutical Sciences*, 2008. **70**(3): p. 303-309.
53. Zolnik, B.S. and D.J. Burgess, *Journal of Controlled Release*, 2008. **127**(2): p. 137-145.
54. Felgner, P.L., T.R. Gadek, M. Holm, R. Roman, H.W. Chan, M. Wenz, J.P. Northrop, G.M. Ringold, and M. Danielsen, *Proceedings of the National Academy of Sciences of the United States of America*, 1987. **84**(21): p. 7413-7417.
55. Ruan, G., J.K. Ng, and S.S. Feng, *Journal of Microencapsulation*, 2004. **21**(4): p. 399-412.
56. Wang, H., P. Zhao, W. Su, S. Wang, Z. Liao, R. Niu, and J. Chang, *Biomaterials*, 2010. **31**(33): p. 8741-8.
57. Langer, R., *Science*, 1990. **249**: p. 1527-1533.
58. Drehobl, M.A., M.C. De Salvo, D.E. Lewis, and J.D. Breen, *Chest*, 2005. **128**(4): p. 2230-2237.
59. Duncan, R., *Nature Reviews Drug Discovery*, 2003. **2**(5): p. 347-360.
60. Amrol, D., *International Journal of Nanomedicine*, 2007. **2**(1): p. 9-12.
61. O'Brien, M.E.R., N. Wigler, M. Inbar, R. Rosso, E. Grischke, A. Santoro, R. Catane, D.G. Kieback, P. Tomczak, S.P. Ackland, F. Orlandi, L. Mellars, L. Alland, C. Tendler, and C.B.C.S. Grp, *Annals of Oncology*, 2004. **15**(3): p. 440-449.
62. Attenello, F.J., D. Mukherjee, G. Datoo, M.J. McGirt, E. Bohan, J.D. Weingart, A. Olivi, A. Quinones-Hinojosa, and H. Brem, *Annals of Surgical Oncology*, 2008. **15**(10): p. 2887-2893.
63. Needles, B., E. Miranda, F.M.G. Rodriguez, L.B. Diaz, J. Spector, J. Craig, G. Cohen, S. Krasnow, J. Brogden, M. Ames, and S.A.S. Grp, *Supportive Care in Cancer*, 1999. **7**(5): p. 347-353.
64. Payne, R., S.D. Mathias, D.J. Pasta, L.A. Wanke, R. Williams, and R. Mahmoud, *Journal of Clinical Oncology*, 1998. **16**(4): p. 1588-1593.
65. Kamath, K.R., J.J. Barry, and K.M. Miller, *Advanced Drug Delivery Reviews*, 2006. **58**(3): p. 412-436.
66. Men, Y., C. Thomasin, H.P. Merkle, B. Gander, and G. Corradin, *Vaccine*, 1995. **13**(7): p. 683-689.
67. Zhao, Z. and K.W. Leong, *Journal of Pharmaceutical Sciences*, 1996. **85**(12): p. 1261-1270.
68. Maloy, K.J., A.M. Donachie, D.T. Ohagan, and A.M. Mowat, *Immunology*, 1994. **81**(4): p. 661-667.
69. Singh, M., M. Briones, G. Ott, and D. O'Hagan, *Proceedings of the National Academy of Sciences of the United States of America*, 2000. **97**(2): p. 811-816.
70. O'Hagan, D., M. Singh, M. Ugozzoli, C. Wild, S. Barnett, M.C. Chen, M. Schaefer, B. Doe, G.R. Otten, and J.B. Ulmer, *Journal of Virology*, 2001. **75**(19): p. 9037-9043.
71. Prabha, S. and V. Labhasetwar, *Pharmaceutical Research*, 2004. **21**(2): p. 354-364.
72. Jain, R.A., *Biomaterials*, 2000. **21**(23): p. 2475-2490.

73. Wang, X.T., S.S. Venkatraman, F.Y.C. Boey, J.S.C. Loo, and L.P. Tan, *Biomaterials*, 2006. **27**(32): p. 5588-5595.
74. Koneracka, M., M. Muckova, V. Zavisova, N. Tomasovicova, P. Kopcansky, M. Timko, A. Jurikova, K. Csach, V. Kavecansky, and G. Lancz, *Journal of Physics-Condensed Matter*, 2008. **20**(20).
75. Yadav, A.K., A. Agarwal, G. Rai, P. Mishra, S. Jain, A.K. Mishra, H. Agrawal, and G.P. Agrawal, *Drug Delivery*, 2010. **17**(8): p. 561-572.
76. Lin, S.Y., K.S. Chen, H.H. Teng, and M.J. Li, *Journal of Microencapsulation*, 2000. **17**(5): p. 577-586.
77. Vukomanovic, M., S.D. Skapin, B. Jancar, T. Maksin, N. Ignjatovic, V. Uskokovic, and D. Uskokovic, *Colloids and Surfaces B: Biointerfaces*, 2011. **82**(2): p. 404-413.
78. Demir, S., A. Ogan, and N. Kayaman-Apohan, *Polymer International*, 2008. **57**: p. 372-377.
79. Prabhat, S. and V. Labhasetwar, *Molecular Pharmaceutics*, 2004. **1**(3): p. 211-219.
80. Brazel, C.S. and N.A. Peppas, *Journal of Controlled Release*, 1996. **39**(1): p. 57-64.
81. Peppas, N.A., P. Bures, W. Leobandung, and H. Ichikawa, *European journal of Pharmaceutics and Biopharmaceutics*, 2000. **50**(1): p. 27-46.
82. Lynn, D.M., M.M. Amiji, and R. Langer, *Angewandte Chemie-International Edition*, 2001. **40**(9): p. 1707-1710.
83. Kim, S.H., A. Opdahl, C. Marmo, and G.A. Somorjai, *Biomaterials*, 2002. **23**(7): p. 1657-1666.
84. Maldonado-Codina, C. and N. Efron, *Ophthalmic and Physiological Optics*, 2006. **26**(4): p. 408-418.
85. Opdahl, A., S.H. Kim, T.S. Koffas, C. Marmo, and G.A. Somorjai, *Journal of Biomedical Materials Research Part A*, 2003. **67A**(1): p. 350-356.
86. Dziubla, T.D., M.C. Torjman, J.I. Joseph, M. Murphy-Tatum, and A.M. Lowman, *Biomaterials*, 2001. **22**(21): p. 2893-2899.
87. Brazel, C.S. and N.A. Peppas, *European journal of Pharmaceutics and Biopharmaceutics*, 2000. **49**(1): p. 47-58.
88. Lee, P.I. and C.J. Kim, *Journal of Controlled Release*, 1991. **16**(1-2): p. 229-236.
89. Lou, X., S. Munro, and S. Wang, *Biomaterials*, 2004. **25**(20): p. 5071-5080.
90. Coughlan, D.C., F.P. Quilty, and O.I. Corrigan, *Journal of Controlled Release*, 2004. **98**(1): p. 97-114.
91. Yoshida, R., Y. Kaneko, K. Sakai, T. Okano, Y. Sakurai, Y.H. Bae, and S.W. Kim, *Journal of Controlled Release*, 1994. **32**(1): p. 97-102.
92. Shibayama, M. and T. Tanaka, *Advances in Polymer Science*, 1993. **109**: p. 1-62.
93. Heskins, M. and J.E. Guillet, 1968. **2**(8): p. 1441 - 1455.
94. You, Y.Z. and D. Oupicky, *Biomacromolecules*, 2007. **8**(1): p. 98-105.
95. Ratner, B.D. and A.S. Hoffman, *Acs Symposium Series*, 1976(31): p. 1-36.
96. Ghosh, T.K., M.J. Habib, K. Childs, and M. Alexander, *International Journal of Pharmaceutics*, 1992. **88**(1-3): p. 391-396.
97. Lufkin, E.G., H.W. Wahner, W.M. Ofallon, S.F. Hodgson, M.A. Kotowicz, A.W. Lane, H.L. Judd, R.H. Caplan, and B.L. Riggs, *Annals of Internal Medicine*, 1992. **117**(1): p. 1-9.

98. Wang, C., R.S. Swerdloff, A. Iranmanesh, A. Dobs, P.J. Snyder, G. Cunningham, A.M. Matsumoto, T. Weber, N. Berman, and G. Testosterone Gel Study, *Journal of Clinical Endocrinology & Metabolism*, 2000. **85**(8): p. 2839-2853.
99. Kusamori, K., H. Katsumi, M. Abe, A. Ueda, R. Sakai, R. Hayashi, Y. Hirai, Y.S. Quan, F. Kamiyama, T. Sakane, and A. Yamamoto, *Journal of Bone and Mineral Research*, 2010. **25**(12): p. 2306-2315.
100. Al-Saidan, S.M., Y.S.R. Krishnaiah, D.V. Chandrasekhar, J.K. Lalla, B. Rama, B. Jayaram, and P. Bhaskar, *Skin Pharmacology & Physiology*, 2004. **17**(6): p. 310-320.
101. Chen, S.X. and R.T. Lostritto, *Journal of Pharmaceutical Sciences*, 1997. **86**(6): p. 739-746.
102. Matsumoto, A., Y. Matsukawa, T. Suzuki, and H. Yoshino, *Journal of Controlled Release*, 2005. **106**(1-2): p. 172-180.
103. Shimono, N., T. Takatori, M. Ueda, M. Mori, and Y. Nakamura, *Chemical & Pharmaceutical Bulletin*, 2003. **51**(6): p. 620-624.
104. Belu, A.M., M.C. Davies, J.M. Newton, and N. Patel, *Analytical Chemistry*, 2000. **72**(22): p. 5625-5638.
105. Park, S.J., W.H. Shim, D.S. Ho, A.E. Raizner, S.W. Park, M.K. Hong, C.W. Lee, D.H. Choi, Y.S. Jang, R. Lam, N.J. Weissman, and G.S. Mintz, *New England Journal of Medicine*, 2003. **348**(16): p. 1537-1545.
106. Westedt, U., M. Wittmar, M. Hellwig, P. Hanefeld, A. Greiner, A.K. Schaper, and T. Kissel, *Journal of Controlled Release*, 2006. **111**(1-2): p. 235-246.
107. Braun, R.M., J. Cheng, E.E. Parsonage, J. Moeller, and N. Winograd, *Applied Surface Science*, 2006. **252**(19): p. 6615-6618.
108. Eunah Kang, H.W.I.K.K.Y.-H.S.K.K.K.M.M.J.B.J.-X.C.K.P., *Journal of Biomedical Materials Research Part A*, 2008. **87A**(4): p. 913-920.
109. Alexis, F., S.S. Venkatraman, S.K. Rath, and F. Boey, *Journal of Controlled Release*, 2004. **98**(1): p. 67-74.
110. Xue, L.A., S.Y. Dai, and Z. Li, *Biomaterials*, 2010. **31**(32): p. 8132-8140.
111. Zunino, P., C. D'Angelo, L. Petrini, C. Vergara, C. Capelli, and F. Migliavacca, *Computer Methods in Applied Mechanics and Engineering*, 2009. **198**(45-46): p. 3633-3644.
112. Jain, J.P., S. Modi, A.J. Domb, and N. Kumar, *Journal of Controlled Release*, 2005. **103**(3): p. 541-563.
113. Salgado, A.J., O.P. Coutinho, and R.L. Reis, *Macromolecular Bioscience*, 2004. **4**(8): p. 743-765.
114. Rokkanen, P.U., O. Bostman, E. Hirvensalo, E.A. Makela, E.K. Partio, H. Patiala, S. Vainionpaa, K. Vihtonen, and P. Tormala, *Biomaterials*, 2000. **21**(24): p. 2607-2613.
115. Thomson, R.C., M.J. Yaszemski, J.M. Powers, and A.G. Mikos, *Biomaterials*, 1998. **19**(21): p. 1935-1943.
116. Deb, S., *Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine*, 1998. **212**(6): p. 453-464.
117. Stone, G.W., S.G. Ellis, D.A. Cox, J. Hermiller, C. O'Shaughnessy, J.T. Mann, M. Turco, R. Caputo, P. Bergin, J. Greenberg, J.J. Popma, M.E. Russell, and T.-I. Investigators, *New England Journal of Medicine*, 2004. **350**(3): p. 221-231.
118. Mahoney, C.M. and A.J. Fahey, *Analytical Chemistry*, 2008. **80**(3): p. 624-632.

119. Stettler, C., S. Wandel, S. Allemann, A. Kastrati, M.C. Morice, A. Schomig, M.E. Pfisterer, G.W. Stone, M.B. Leon, J.S. de Lezo, J.J. Goy, S.J. Park, M. Sabate, M.J. Suttorp, H. Kelbaek, C. Spaulding, M. Menichelli, P. Vermeersch, M.T. Dirksen, P. Cervinka, A.S. Petronio, A.J. Nordmann, P. Diem, B. Meier, M. Zwahlen, S. Reichenbach, S. Trelle, S. Windecker, and P. Juni, *Lancet*, 2007. **370**(9591): p. 937-948.
120. Fisher, G.L., A.M. Belu, C.M. Mahoney, K. Wormuth, and N. Sanada, *Anal Chem*, 2009. **81**(24): p. 9930-40.
121. Bolla, M., D. Gonzalez, P. Warde, J.B. Dubois, R.O. Mirimanoff, G. Storme, J. Bernier, A. Kuten, C. Sternberg, T. Gil, L. Collette, and M. Pierart, *New England Journal of Medicine*, 1997. **337**(5): p. 295-300.
122. Kotake, T., M. Usami, H. Akaza, K. Koiso, Y. Homtna, K. Kawabe, Y. Aso, S. Orikasa, J. Shimazaki, S. Isaka, O. Yoshida, Y. Hirao, E. Okajima, S. Naito, J. Kumazawa, H. Kanetake, Y. Saito, Y. Ohi, Y. Ohashi, and Z.S. Group, *Japanese Journal of Clinical Oncology*, 1999. **29**(11): p. 562-570.
123. Athanasiou, K.A., C.M. Agrawal, F.A. Barber, and S.S. Burkhart, *Arthroscopy: The Journal of Arthroscopic & Related Surgery*, 1998. **14**(7): p. 726-737.
124. Anagnostakos, K., O. Furst, and J. Kelm, *Acta Orthopaedica*, 2006. **77**(4): p. 628-637.
125. Akgun, R.C., B. Atilla, A.M. Tokgozoglu, and A.M. Alpaslan, *J Bone Joint Surg Br*, 2006. **88-B**(SUPP_I): p. 83-d-84.
126. Anagnostakos, K., P. Wilmes, E. Schmitt, and J. Kelm, *Acta Orthopaedica*, 2009. **80**(2): p. 193-197.
127. Brem, H. and P. Gabikian, *Journal of Controlled Release*, 2001. **74**(1-3): p. 63-67.
128. Westphal, M., D.C. Hilt, E. Bortey, P. Delavault, R. Olivares, P.C. Warnke, I.R. Whittle, J. Jaaskelainen, and Z. Ram, *Neuro-Oncology*, 2003. **5**(2): p. 79-88.
129. Miyagi, Y., L.L.Y. Chiu, M. Cimini, R.D. Weisel, M. Radisic, and R.-K. Li, *Biomaterials*, 2011. **32**(5): p. 1280-1290.
130. Wang, E.A., V. Rosen, J.S. D'Alessandro, M. Bauduy, P. Cordes, T. Harada, D.I. Israel, R.M. Hewick, K.M. Kerns, and P. LaPan, *Proceedings of the National Academy of Sciences of the United States of America*, 1990. **87**(6): p. 2220-2224.
131. Illi, O.E. and C.P. Feldmann, *European Journal of Pediatric Surgery*, 1998. **8**(4): p. 251-255.
132. Wei, G.B., Q.M. Jin, W.V. Giannobile, and P.X. Ma, *Biomaterials*, 2007. **28**(12): p. 2087-2096.
133. Quirk, R.A., R.M. France, K.M. Shakesheff, and S.M. howdle, *Current opinion in solid state and materials science*, 2004. **8**: p. 313-321.
134. Langer, R., *Pharmaceutical Research*, 1997. **14**(7): p. 840-841.
135. Jagur-Grodzinski, J., *Polymers for advanced technologies*, 2006. **17**: p. 395-418.
136. Yang, S., K.-F. Leong, Z. Du, and C.-K. Chua, *Tissue Engineering*, 2001. **7**(6): p. 679-689.
137. Shi, S.A., X.H. Wang, G. Guo, M. Fan, M.J. Huang, and Z.Y. Qian, *International Journal of Nanomedicine*. **5**: p. 1049-1055.
138. Santiago, L.Y., R.W. Nowak, J.P. Rubin, and K.G. Marra, *Biomaterials*, 2006. **27**(15): p. 2962-2969.

139. Riboldi, S.A., M. Sampaolesi, P. Neuenschwander, G. Cossu, and S. Mantero, *Biomaterials*, 2005. **26**(22): p. 4606-4615.
140. Drury, J.L. and D.J. Mooney, *Biomaterials*, 2003. **24**(24): p. 4337-4351.
141. Peppas, N.A., J.Z. Hilt, A. Khademhosseini, and R. Langer, *Advanced Materials*, 2006. **18**(11): p. 1345-1360.
142. Adekogbe, I. and A. Ghanem, *Biomaterials*, 2005. **26**(35): p. 7241-7250.
143. Kumbar, S.G., S.P. Nukavarapu, R. James, L.S. Nair, and C.T. Laurencin, *Biomaterials*, 2008. **29**(30): p. 4100-4107.
144. Hutmacher, D.W., J.T. Schantz, C.X.F. Lam, K.C. Tan, and T.C. Lim, *Journal of Tissue Engineering and Regenerative Medicine*, 2007. **1**(4): p. 245-260.
145. Porter, J.R., T.T. Ruckh, and K.C. Popat, *Biotechnology Progress*, 2009. **25**(6): p. 1539-1560.
146. Ohashi, K., T. Yokoyama, M. Yamato, H. Kuge, H. Kanehiro, M. Tsutsumi, T. Amanuma, H. Iwata, J. Yang, T. Okano, and Y. Nakajima, *Nature Medicine*, 2007. **13**(7): p. 880-885.
147. Mendelson, K. and F.J. Schoen, *Annals of Biomedical Engineering*, 2006. **34**(12): p. 1799-1819.
148. Ghasemi-Mobarakeh, L., M.P. Prabhakaran, M. Morshed, M.H. Nasr-Esfahani, and S. Ramakrishna, *Materials Science & Engineering C-Materials for Biological Applications*, 2010. **30**(8): p. 1129-1136.
149. Yang, F., R. Murugan, S. Ramakrishna, X. Wang, Y.-Z. Ma, and S. Wang, *Biomaterials*, 2004. **25**: p. 1891-1900.
150. Kimura, Y., A. Hokugo, T. Takamoto, Y. Tabata, and H. Kurosawa, *Tissue Engineering Part C-Methods*, 2008. **14**(1): p. 47-57.
151. Stoll, C., T. John, M. Endres, C. Rosen, C. Kaps, B. Kohl, M. Sittinger, W. Ertel, and G. Schulze-Tanzil, *Journal of Orthopaedic Research*, 2010. **28**(9): p. 1170-1177.
152. Langmuir, I., 1932.
153. Blodgett, K.B. and I. Langmuir, *Physical Review*, 1937. **51**(11): p. 0964-0982.
154. Langmuir, I. and V.J. Schaefer, *Journal of the American Chemical Society*, 1937. **59**: p. 1406-1406.
155. Gorbet, M.B. and M.V.M.V. Sefton, *Biomaterials*, 2004. **25**(26): p. 5681-5703.
156. Kavanagh, C.A., Y.A. Rochev, W.M. Gallagher, K.A. Dawson, and A.K. Keenan, *Pharmacology & Therapeutics*, 2004. **102**(1): p. 1-15.
157. Liu, X.S., Y.M. Huang, C. Hanet, M. Vandormael, V. Legrand, J. Dens, J.L. Vandenbossche, L. Missault, C. Vrints, and I. De Scheerder, *Catheterization and Cardiovascular Interventions*, 2003. **60**(2): p. 172-178.
158. McLean, D.R. and N.L. Eiger, *Rev Cardiovasc Med*, 2002. **3 Suppl 5**: p. S16-22.
159. Lin, W.-C., T.-Y. Liu, and M.-C. Yang, *Biomaterials*, 2004. **25**(10): p. 1947-1957.
160. Costerton, J.W., P.S. Stewart, and E.P. Greenberg, *Science*, 1999. **284**(5418): p. 1318-1322.
161. Ratner, B.D., *Surface and Interface Analysis*, 1995. **23**(7-8): p. 521-528.
162. Castner, D.G. and B.D. Ratner, *Surface Science*, 2002. **500**: p. 28-60.
163. Ratner, B.D., *Surface and interface analysis*, 1995. **53**: p. 521-528.

164. Neumann, A.W., M.A. Moscarello, W. Zingg, O.S. Hum, and S.K. Chang, *Journal of Polymer Science Part C-Polymer Symposium*, 1979(66): p. 391-398.
165. Yasuda, H., B.S. Yamanashi, and D.P. Devito, *Journal of Biomedical Materials Research*, 1978. **12**(5): p. 701-706.
166. Mohandas, N., R.M. Hochmuth, and E.E. Spaeth, *Journal of Biomedical Materials Research*, 1974. **8**(2): p. 119-136.
167. Porte-Durrieu, M.C., C. Labrugere, F. Villars, F. Lefebvre, S. Dutoya, A. Guette, L. Bordenave, and C. Baquey, *Journal of Biomedical Materials Research*, 1999. **46**(3): p. 368-375.
168. El-Ghannam, A.R., P. Ducheyne, M. Risbud, C.S. Adams, I.M. Shapiro, D. Castner, S. Golledge, and R.J. Composto, *Journal of Biomedical Materials Research Part A*, 2004. **68A**(4): p. 615-627.
169. Ito, A., K. Ino, T. Kobayashi, and H. Honda, *Biomaterials*, 2005. **26**(31): p. 6185-6193.
170. Sawyer, A.A., K.M. Hennessy, and S.L. Bellis, *Biomaterials*, 2005. **26**(13): p. 1467-1475.
171. Tiwari, A., H.J. Salacinski, G. Punshon, G. Hamilton, and A.M. Seifalian, *Faseb Journal*, 2002. **16**(8).
172. Ratner, B.D., *Biosensors and Bioelectronics*, 1995. **10**(9-10): p. 797-804.
173. Lin, C.C. and A.T. Metters, *Advanced Drug Delivery Reviews*, 2006. **58**(12-13): p. 1379-1408.
174. Geever, L.M., C.C. Cooney, J.G. Lyons, J.E. Kennedy, M.J.D. Nugent, S. Devery, and C.L. Higginbotham, *European journal of Pharmaceutics and Biopharmaceutics*, 2008. **69**(3): p. 1147-1159.
175. Djordjevic, I., N.R. Choudhury, N.K. Dutta, and S. Kumar, *Polymer*, 2009. **50**(7): p. 1682-1691.
176. Hook, A.L., D.G. Anderson, R. Langer, P. Williams, M.C. Davies, and M.R. Alexander, *Biomaterials*, 2010. **31**(2): p. 187-198.
177. Ma, Z., Z. Mao, and C. Gao, *Colloids and Surfaces B: Biointerfaces*, 2007. **60**(2): p. 137-157.
178. Chandler-Temple, A.F., E. Wentrup-Byrne, H.J. Griesser, M. Jasieniak, A.K. Whittaker, and L. Grøndahl, *Langmuir*, 2010. **26**(19): p. 15409-15417.
179. Kannan, R.Y., H.J. Salacinski, D.S. Vara, M. Odlyha, and A.M. Seifalian, *Journal of Biomaterials Applications*, 2006. **21**(1): p. 5-32.
180. Briggs, D., *Surface analysis of polymers by XPS and static SIMS*. 1998, Cambridge: Cambrige University Press. 198.
181. Mahoney, C.M., J.X. Yu, A. Fahey, and J.A. Gardella, *Applied Surface Science*, 2006. **252**(19): p. 6609-6614.
182. Norrman, K., K.B. Haugshoj, and N.B. Larsen, *Journal of Physical Chemistry B*, 2002. **106**(51): p. 13114-13121.
183. Davies, M.C., R.D. Short, M.A. Khan, J.F. Watts, A. Brown, A.J. Eccles, P. Humphrey, J.C. Vickerman, and M. Vert, *Surface and interface analysis*, 1989. **14**: p. 115-120.
184. Briggs, D., A. Brown, and J.C. Vickerman, *Handbook of Static Secondary Ion Mass Spectrometry*. 1989, Chichester: John Wiley & Sons.
185. Davies, M.C., R.D. Short, M.A. Khan, J.F. Watts, A. Brown, A.J. Eccles, P. Humphrey, J.C. Vickerman, and M. Vert, *Surface and Interface Analysis*, 1989. **14**(3): p. 115-120.
186. Davies, M.C., R.A.P. Lynn, J.F. Watts, A.J. Paul, J.C. Vickerman, and J. Heller, *Macromolecules*, 1991. **24**: p. 5508-5514.

187. Davies, M.C., M.A. Khan, A. Domb, R. Langer, J.F. Watts, and A.J. Paul, *Journal of Applied polymer Science*, 1991. **42**: p. 1597-1605.
188. Lee, J.-W. and J.A. Gardella.Jr, *Macromolecules*, 2001. **34**: p. 3928-3937.
189. Lee, J.-W. and J.A. Gardella.Jr, *Journal of American society for Mass Spectrometry*, 2002. **13**: p. 1108-1119.
190. Scholes, P.D., A.G.A. Coombes, L. Illum, S.S. Davis, J.F. Watts, C. Ustariz, M. Vert, and M.C. Davies, *Journal of Controlled Release*, 1999. **59**(3): p. 261-278.
191. Lynn, R.A.P., M.C. Davies, S.S. Davis, P. Humphrey, and J. Hearn, *Journal of Pharmacy and Pharmacology*, 1988. **40**(SUPPL): p. 130P.
192. Nagy, G. and A.V. Walker, *International Journal of Mass Spectrometry*, 2007. **262**: p. 144-153.
193. Weibel, D., S. Wong, N. Lockyer, P. Blenkinsopp, R. Hill, and J.C. Vickerman, *Analytical Chemistry*, 2003. **75**(7): p. 1754-1764.
194. Winograd, N., *Analytical Chemistry*, 2005. **77**(7): p. 142A-149A.
195. Mahoney, C.M., S. Roberson, and G. Gillen, *Applied Surface Science*, 2004. **231-2**: p. 174-178.
196. Mahoney, C.M., J. Yu, A. Fahey, and J.A. GardellaJr, *Applied Surface Science*, 2006. **252**: p. 6609-6614.
197. Prestidge, C.A., T.J. Barnes, and W. Skinner, *Journal of Pharmacy and Pharmacology*, 2007. **59**(2): p. 251-259.
198. Edge, S., A.M. Belu, U.J. Potter, D.F. Steele, P.M. Young, R. Price, and J.N. Staniforth, *International Journal of Pharmaceutics*, 2002. **240**(1-2): p. 67-78.
199. Davies, M.C. and A. Brown, *Archiv Der Pharmazie*, 1987. **320**(9): p. 914-914.
200. McDermott, M.K., D.M. Saylor, R. Casas, B.J. Dair, J. Guo, C.S. Kim, C.M. Mahoney, K. Ng, S.K. Pollack, D.V. Patwardhan, D.A. Sweigart, T. Thomas, J. Toy, C.M. Williams, and C.N. Witkowski, *Journal of Pharmaceutical Sciences*, 2010. **99**(6): p. 2777-2785.
201. Sodhi, R.N.S., *Analyst*, 2004. **129**(6): p. 483-7.
202. Baugh, L., T. Weidner, J.E. Baio, P.C.T. Nguyen, L.J. Gamble, P.S. Slayton, and D.G. Castner, *Langmuir*, 2010. **26**(21): p. 16434-16441.
203. Weidner, T., J.S. Apte, L.J. Gamble, and D.G. Castner, *Langmuir*, 2009. **26**(5): p. 3433-3440.
204. Kempson, I.M., A.L. Martin, J.A. Denman, P.W. French, C.A. Prestidge, and T.J. Barnes, *Langmuir*, 2010. **26**(14): p. 12075-12080.
205. Wagner, M.S., T.A. Horbett, and D.G. Castner, *Langmuir*, 2003. **19**(5): p. 1708-1715.
206. Wang, H., D.G. Castner, B.D. Ratner, and S. Jiang, *Langmuir*, 2004. **20**(5): p. 1877-1887.
207. Xia, N., C.J. May, S.L. McArthur, and D.G. Castner, *Langmuir*, 2002. **18**(10): p. 4090-4097.
208. Barnes, C.A., J. Brison, R. Michel, B.N. Brown, D.G. Castner, S.F. Badylak, and B.D. Ratner, *Biomaterials*, 2011. **32**(1): p. 137-143.
209. Rafati, A., A. Boussahel, A.G. Shard, K.M. Shakesheff, P.T. Whiteside, S. Rigby-Singleton, C.J. Roberts, X. Chen, D.J. Scurr, M.R. Alexander, and M.C. Davies, *Journal of Pharmacy and Pharmacology*, 2010. **62**(10): p. 1348-1349.
210. Mantus, D.S., B.D. Ratner, B.A. Carlson, and J.F. Moulder, *Analytical Chemistry*, 1993. **65**(10): p. 1431-1438.
211. Wagner, M.S. and D.G. Castner, *Langmuir*, 2001. **17**(15): p. 4649-4660.

212. Lee, J.L.S. and I.S. Gilmore, *The application of multivariate data analysis techniques in surface analysis*, in *Surface Analysis The Principal Techniques*, J.C. Vickerman and I.S. Gilmore, Editors. 2009, John Wiley & Sons: London.
213. Horbett, T.A., *Colloids and Surfaces B: Biointerfaces*, 1994. **2**(1-3): p. 225-240.
214. Kiaei, D., A.S. Hoffman, T.A. Horbett, and K.R. Lew, *Journal of Biomedical Materials Research*, 1995. **29**(6): p. 729-739.
215. Tsai, W.-B., J.M. Grunkemeier, and T.A. Horbett, *Journal of Biomedical Materials Research*, 1999. **44**(2): p. 130-139.
216. Vickerman, J.C. and I.S. Gilmore, *Surface Analysis: The Principal Techniques*. Vol. 2. 2009: John Wiley & Sons Ltd.
217. Beamson, G.B., D, *The XPS of Polymers Database in High Resolution*. 2000, SurfaceSpectra Ltd.
218. Beamson, G. and D. Briggs, *High Resolution XPS of Organic Polymers - The Scienta ESCA300 Database*. 1992, Chichester: Wiley.
219. Calvo, P., C. RemunanLopez, J.L. VilaJato, and M.J. Alonso, *Pharmaceutical Research*, 1997. **14**(10): p. 1431-1436.
220. Cheng, F.Y., C.H. Su, Y.S. Yang, C.S. Yeh, C.Y. Tsai, C.L. Wu, M.T. Wu, and D.B. Shieh, *Biomaterials*, 2005. **26**(7): p. 729-738.
221. Dong, Y.C. and S.S. Feng, *Biomaterials*, 2004. **25**(14): p. 2843-2849.
222. Feng, S.S. and G.F. Huang, *Journal of Controlled Release*, 2001. **71**(1): p. 53-69.
223. Mu, L. and S.S. Feng, *Journal of Controlled Release*, 2003. **86**(1): p. 33-48.
224. Shakesheff, K.M., C. Evora, I. Soriano, and R. Langer, *Journal of Colloid and Interface Science*, 1997. **185**(2): p. 538-547.
225. Gref, R., Y. Minamitake, M.T. Peracchia, V. Trubetskoy, V. Torchilin, and R. Langer, *Science*, 1994. **263**(5153): p. 1600-1603.
226. Rafati, A., M.C. Davies, A.G. Shard, S. Hutton, G. Mishra, and M.R. Alexander, *Journal of Controlled Release*, 2009. **138**(1): p. 40-44.
227. Kim, K., S. Park, Y. Byun, and H.T. Moon, *Macromolecular Research*, 2010. **18**(5): p. 519-525.
228. Porter, S.C. and L.A. Felton, *Drug Development and Industrial Pharmacy*, 2010. **36**(2): p. 128-142.
229. Yan, S.F., J.B. Yin, L. Tang, and X.S. Chen, *Journal of Applied polymer Science*, 2011. **119**(4): p. 2350-2358.
230. Castro, G.R., B. Panilaitis, and D.L. Kaplan, *Bioresource Technology*, 2008. **99**(11): p. 4566-4571.
231. Huang, L.Y. and M.C. Yang, *Journal of Magnetism and Magnetic Materials*, 2007. **310**(2): p. 2874-2876.
232. Van Tomme, S.R., A. Mens, C.F. van Nostrurn, and W.E. Hennink, *Biomacromolecules*, 2008. **9**(1): p. 158-165.
233. Vlugt-Wensink, K.D.F., X. Jiang, G. Schotman, G. Kruijtzer, A. Vredenberg, J.T. Chung, Z. Zhang, C. Versluis, D. Ramos, R. Verrijk, W. Jiskoot, D.J.A. Crommelin, and W.E. Hennink, *Biomacromolecules*, 2006. **7**(11): p. 2983-2990.
234. Santiago, L.Y., R.W. Nowak, J. Peter Rubin, and K.G. Marra, *Biomaterials*, 2006. **27**(15): p. 2962-2969.
235. Belu, A.M., D.J. Graham, and D.G. Castner, *Biomaterials*, 2003. **24**(21): p. 3635-3653.

236. Poulsson, A.H.C., S.A. Mitchell, M.R. Davidson, A.J. Johnstone, N. Emmison, and R.H. Bradley, *Langmuir*, 2009. **25**(6): p. 3718-3727.
237. Brown, B.N., C.A. Barnes, R.T. Kasick, R. Michel, T.W. Gilbert, D. Beer-Stolz, D.G. Castner, B.D. Ratner, and S.F. Badylak, *Biomaterials*, 2010. **31**(3): p. 428-437.
238. Yin, M., Y. Yuan, C. Liu, and J. Wang, *Biomaterials*, 2009. **30**(14): p. 2764-2773.
239. Smith, B.S., S. Yoriya, L. Grissom, C.A. Grimes, and K.C. Popat, *Journal of Biomedical Materials Research Part A*, 2010. **95A**(2): p. 350-360.
240. Tanaka, K., N. Sanada, M. Hikita, T. Nakamura, T. Kajiyama, and A. Takahara, *Applied Surface Science*, 2008. **254**(17): p. 5435-5438.
241. Louey, M.D., P. Mulvaney, and P.J. Stewart, *Journal of Pharmaceutical and Biomedical Analysis*, 2001. **25**(3-4): p. 559-567.
242. Dimitriadis, E.K., F. Horkay, J. Maresca, B. Kachar, and R.S. Chadwick, *Biophysical Journal*, 2002. **82**(5): p. 2798-2810.
243. Schön, P., K. Bagdi, K. Molnár, P. Markus, B. Pukánszky, and G. Julius Vancso, *European Polymer Journal*, 2011. **In Press, Corrected Proof**.
244. Davies, M., A. Brindley, X.Y. Chen, M. Marlow, S.W. Doughty, I. Shrubb, and C.J. Roberts, *Pharmaceutical Research*, 2005. **22**(7): p. 1158-1166.
245. Butt, H.J., B. Cappella, and M. Kappl, *Surface Science Reports*, 2005. **59**(1-6): p. 1-152.
246. Kyun Kim, H., K. Kim, and Y. Byun, *Biomaterials*, 2005. **26**(17): p. 3435-3444.
247. Yamamoto, S., Y. Tsujii, and T. Fukuda, *Polymer*, 2001. **42**(5): p. 2007-2013.
248. Green, R.J., J. Davies, M.C. Davies, C.J. Roberts, and S.J.B. Tendler, *Biomaterials*, 1997. **18**(5): p. 405-413.
249. Cao, X., C. Sun, and T.J. Thamann, *Journal of Pharmaceutical Sciences*, 2005. **94**(9): p. 1881-1892.
250. Danesh, A., X.Y. Chen, M.C. Davies, C.J. Roberts, G.H.W. Sanders, S.J.B. Tendler, P.M. Williams, and M.J. Wilkins, *Pharmaceutical Research*, 2000. **17**(7): p. 887-890.
251. Danesh, A., X. Chen, M.C. Davies, C.J. Roberts, G.H.W. Sanders, S.J.B. Tendler, P.M. Williams, and M.J. Wilkins, *Langmuir*, 2000. **16**(2): p. 866-870.
252. Leadley, S.R., K.M. Shakesheff, M.C. Davies, J. Heller, N.M. Franson, A.J. Paul, A.M. Brown, and J.F. Watts, *Biomaterials*, 1998. **19**(15): p. 1353-1360.
253. Shard, A.G., K.M. Shakesheff, C.J. Roberts, S.J.B. Tendler, and M.C. Davies, *Surface characterisation of bioerodible polymers using XPS, SIMS and AFM*, in *Handbook of Biodegradable Polymers*, A.J. Domb, J. Kost, and D.M. Wiseman, Editors. 1997, Harewood Academic Publishers: Amsterdam. p. 417-450.
254. Chen, X., K.M. Shakesheff, M.C. Davies, J. Heller, C.J. Roberts, S.J.B. Tendler, and P.M. Williams, *Journal of Physical Chemistry*, 1995. **99**(29): p. 11537-11542.
255. Shakesheff, K.M., M.C. Davies, J. Heller, C.J. Roberts, S.J.B. Tendler, and P.M. Williams, *Langmuir*, 1995. **11**: p. 2547-2553.
256. Weisenhorn, A.L., B. Drake, C.B. Prater, S.A. Gould, P.K. Hansma, F. Ohnesorge, M. Egger, S.P. Heyn, and H.E. Gaub, *Biophysical Journal*, 1990. **58**(5): p. 1251-1258.

257. Hallett, P., G. Offer, and M.J. Miles, *Biophysical Journal*, 1995. **68**(4): p. 1604-1606.
258. Ohnishi, S., M. Hara, T. Furuno, and H. Sasabe, *Biophysical Journal*, 1992. **63**(5): p. 1425-1431.
259. Valle, F., J.A. Derose, G. Dietler, M. Kawe, A. Plückthun, and G. Semenza, *Journal of Microscopy*, 2001. **203**(2): p. 195-198.
260. Moy, V., E. Florin, and H. Gaub, *Science*, 1994. **266**(5183): p. 257-259.
261. Allen, S., X.Y. Chen, J. Davies, M.C. Davies, A.C. Dawkes, J.C. Edwards, C.J. Roberts, J. Sefton, S.J.B. Tendler, and P.M. Williams, *Biochemistry*, 1997. **36**(24): p. 7457-7463.
262. Allen, S., J. Davies, A.C. Dawkes, M.C. Davies, J.C. Edwards, M.C. Parker, C.J. Roberts, J. Sefton, S.J.B. Tendler, and P.M. Williams, *Febs Letters*, 1996. **390**(2): p. 161-164.
263. Xue, L. and H.P. Greisler, *Journal of Vascular Surgery*, 2003. **37**(2): p. 472-480.
264. Xu, L.C. and C.A. Siedlecki, *Journal of Biomedical Materials Research Part A*, 2010. **92A**(1): p. 126-136.
265. Sethuraman, A., M. Han, R.S. Kane, and G. Belfort, *Langmuir*, 2004. **20**(18): p. 7779-7788.
266. Xu, L.C. and C.A. Siedlecki, *Biomaterials*, 2007. **28**(22): p. 3273-3283.
267. Davies, M.C., M.R. Alexander, A.L. Hook, J. Yang, Y. Mei, M. Taylor, A.J. Urquhart, R. Langer, and D.G. Anderson, *Journal of Drug Targeting*, 2010. **18**(10): p. 741-751.
268. Yang, J., Y. Mei, A.L. Hook, M. Taylor, A.J. Urquhart, S.R. Bogatyrev, R. Langer, D.G. Anderson, M.C. Davies, and M.R. Alexander, *Biomaterials*, 2010. **31**(34): p. 8827-8838.
269. Mei, Y., K. Saha, S.R. Bogatyrev, J. Yang, A.L. Hook, Z.I. Kalcioglu, S.W. Cho, M. Mitalipova, N. Pyzocha, F. Rojas, K.J. Van Vliet, M.C. Davies, M.R. Alexander, R. Langer, R. Jaenisch, and D.G. Anderson, *Nature Materials*, 2010. **9**(9): p. 768-778.
270. Urquhart, A.J., D.G. Anderson, M. Taylor, M.R. Alexander, R. Langer, and M.C. Davies, *Advanced Materials*, 2007. **19**(18): p. 2486-2491.
271. Urquhart, A.J., M. Taylor, D.G. Anderson, R. Langer, M.C. Davies, and M.R. Alexander, *Analytical Chemistry*, 2007. **80**(1): p. 135-142.
272. Anderson, D.G., S. Levenberg, and R. Langer, *Nat Biotech*, 2004. **22**(7): p. 863-866.