PROVIDING ANTIMICROBIAL PROPERTY TO SURFACES OF ULTRA HIGH MOLECULAR WEIGHT POLYETHYLENE (UHMWPE) VIA GRAFTING BY UV INDUCED RAFT POLYMERIZATION

UV İLE BAŞLATILAN AŞILAMA YÖNTEMİ İLE RAFT POLİMERİZASYONU KULLANILARAK ULTRA MOLEKÜL AĞIRLIKLI POLİETİLEN (UMAPE) YÜZEYİNE ANTİMİKROBİYAL ÖZELLİK KAZANDIRILMASI

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ABSTRACT

PROVIDING ANTIMICROBIAL PROPERTY TO SURFACES OF ULTRA HIGH MOLECULAR WEIGHT POLYETHYLENE (UHMWPE) VIA GRAFTING BY UV INDUCED RAFT POLYMERIZATION

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Annually over 1 million patients in the US receive total joint replacements. Ultrahigh molecular weight polyethylene (UHMWPE) has been used as a load-bearing articular surface in majority of total joint arthroplasty. Periprosthetic infection (PJI) is the most threatening complication facing total joint patients. Although it occurs in 1-2% of cases, PJI is the reason of 30% of revisions and is a severe healthcare burden. Most importantly, PJI is tremendously painful and difficult for the patients. Recurrence of PJI prolongs the hospitalization with additional series of surgeries. With recurrence, treatment becomes less effective, some procedures such as arthrodesis amputation are often performed.

Approaches are needed to improve the efficacy of PJI treatment. Irrigation and debridement (I&D), liner exchange, and one-stage revision are currently used options to treat PJI. The gold standard treatment is removal of all components and the placement of antibiotic-impregnated PMMA bone cement in the joint space in a first surgery, followed by the placement of all new implant components after an

intended 6-8 weeks of antibiotic treatment. However, the bioavailability of systemic antibiotics in the bone/implant interface is very low and inefficient. On the other hand, patients are largely immobilized during treatment due to PMMA spacers not being able to bear the full weight of the patients. The 5-year success rates of I&D followed by liner exchange and two stage surgery are 38 % and 80 % of the time.

As one strategy, therapeutic agents, such as antibiotics, can be incorporated into ultra-high molecular weight polyethylene (UHMWPE) implants typically used in total joint arthroplasty for local delivery of these therapeutic agents. Because of its superior mechanical strength and markedly improved wear resistance in comparison to bone cement UHMWPE is a better candidate than PMMA bone cement as an articulating spacer and a delivery device eluting antibiotic.

Two important aspects have vital importance for an effective PJI treatment; appropriate dosage control and sustainable antibiotic treatment. They both require great attention otherwise could be devastating for patients and eventually turned into an immense public health problem. Antibiotic dosage control must be so delicate that it is not above toxicity levels and not below MIC which could lead to antibiotic resistance. Sustainable antibiotic release is essential for implants to avoid bacterial colonization which will fail patients to an additional joint replacement surgery. Therefore, it is vital to tailor a drug-releasing implant which ensures delicate dosage control and sustainable antibiotic release.

The aim of this thesis is to functionalize nonpolar UHMWPE by grafting 2-hydroxyethyl methacrylate monomer and blend resulting copolymer (UHMPWE-g-PHEMA) with commonly used antibiotic, gentamicin sulfate. RAFT polymerization was also used to synthesize UHMWPE-g-PHEMA with controlled the molecular weight and the molecular weight distribution of PHEMA to control the rate and the sustainability of gentamicin sulfate release. Alterations in the chemical properties after grafting PHEMA to UHMWPE have been investigated by using surface characterization methods, ATR-FTIR, elemental analysis, X-ray photoelectron spectroscopy (XPS), contact angle. Subsequently, antibiotic release studies from antibiotic-loaded UHMWPE and UHMWPE-g-PHEMA (prepared by conventional polymerization or RAFT polymerization) were conducted. Antimicrobial efficacy of said polymers was tested in two ways:

1. Planktonic kill in the eluent media,

2. Anticolonizing properties of polymeric surfaces.

Synthesized/prepared drug loaded polymers were tested to evaluate their mechanical strength and wear resistance by using tensile testing, IZOD impact testing and pin-on-disc wear testing.

The graft copolymer of UHMWPE-g-PHEMA showed significant increase for the GS release rate in comparison to virgin UHMWPE. The antibacterial performance of UHMWPE-g-PHEMA became more effective in parallel with release rate improvement. HEMA grafting from UHMWPE reduced its mechanical properties such as ultimate tensile strength, elongation at break and IZOD impact strength. UHMWPE-g-PHEMA synthesized via UV-initiated RAFT polymerization increased the GS release rate in a more sustainable trend compared to copolymer prepared via conventional grafting. Thus, UHMWPE-g-PHEMA exhibited better planktonic bacterial kill and non-adherent surface.

Keywords: UHMWPE, UV initiated grafting, RAFT polymerization, Periprosthetic Joint Infection (PJI), knee implant, gentamicin sulfate

ÖZET

UV ile Başlatılan Aşılama Yöntemi ile RAFT Polimerizasyonu Kullanılarak Ultra Molekül Ağırlıklı Polietilen (UMAPE) Yüzeyine Antimikrobiyal Özellik Kazandırılması

Ali Eren Atıcı

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ABD'de yılda 1 milyondan fazla hastaya total eklem değişimi gerçekleştirilmektedir. Ultra molekül ağırlıklı polietilen (UMAPE), toplam eklem artroplastisinin çoğunda yük taşıyan eklem yüzeyi olarak kullanılmaktadır. Periprostetik eklem enfeksiyonu (PPEE) total eklem hastalarının karşılaştığı en tehdit edici komplikasyondur. Vakaların %1-2'sinde görülmesine rağmen, revizyonların % 30'unun nedeni PPEE'dir ve ciddi bir sağlık yüküdür. En önemlisi, PPEE hastalar için son derece acı verici ve zordur. Tekrarlanması durumunda hastanede yatış süresini uzatmaktadır. Tekrarlayan enfeksiyon vakalarında tedavi daha az etkili olur, genellikle kurtarma için artrodez amputasyonu gibi bazı prosedürler gerçekleştirilmektedir.

PPEE tedavisinin etkinliğini arttırmak için bazı yaklaşımlara ihtiyaç vardır. Antiseptik su ile yıkama ve debridman, liner değişimi ve tek aşamalı revizyon şu anda PPEE tedavisinde kullanılan prosedürlerdir. Altın standart tedavi ise tüm protez bileşenlerinin çıkarılması ve ilk ameliyatta antibiyotik emdirilmiş PMMA

kemik çimentosunun eklem boşluğuna yerleştirilmesi ve ardından 6-8 haftalık bir antibiyotik tedavisinden sonra tüm yeni protez bileşenlerinin yerleştirilmesidir. Fakat, kemik / implant arayüzündeki sistemik antibiyotiklerin biyoelverişliliği çok düşük ve verimsizdir. Ayrıca, PMMA aralayıcının hastaların tam ağırlığını taşıyamaması nedeniyle hastalar tedavi sırasında büyük ölçüde hareketsizleşmektedir. Bununla birlikte PPEE'yi ortadan kaldırmada 5 yıllık başarı oranı sırasıyla antiseptik su ile yıkama ve debridman ve liner değişiminin için % 38, altın standart olan iki aşamalı tedavi için % 80'dir.

Başka bir strateji olarak, antibiyotikler gibi tedavi edici ajanlar, bu tedavi edici ajanların lokal salımı için tipik olarak toplam eklem artroplastisinde kullanılan ultra molekül ağırlıklı polietilen (UMAPE) implantların içerisine katılmaktadır. Üstün mekanik dayanımı ve kemik çimentosu ile karşılaştırıldığında belirgin şekilde geliştirilmiş aşınma direnci nedeniyle UMAPE, eklem aralayıcı olarak kullanılan PMMA kemik çimentosundan ve antibiyotikleri salan bir ilaç salım sisteminden daha iyi bir adaydır.

Etkili bir PPEE tedavisi için iki önemli husus hayati öneme sahiptir; uygun doz kontrolü ve sürdürülebilir antibiyotik tedavisi. Her ikisi de büyük bir dikkat gerektirir, aksi takdirde hastalar için yıkıcı etkileri olabilir ve sonunda büyük bir halk sağlığı sorununa dönüşebilir. Antibiyotik doz kontrolü, antibiyotik direncine yol açabilecek toksisite seviyelerinin üzerinde ve minimum inhibitör konsantrasyonun altında olmayacak kadar hassas olmalıdır. Hastaların ek bir eklem değişim operasyonuna daha girmesini engellemek için, implantlarda bakteri kolonizasyonundan kaçınmak için sürdürülebilir antibiyotik salınımı şarttır. Bu nedenle, hassas doz kontrolü ve sürdürülebilir antibiyotik salınımı sağlayan bir ilaç salan implantın uyarlanması hayati önem taşır.

Bu tezin amacı, polar yapıda olmayan UMAPE'ye 2-hidroksietil metakrilat monomeri aşılayarak elde edilen kopolimeri (UMAPE-g-PHEMA) yaygın olarak kullanılan bir antibiyotik olan, gentamisin sülfat ile harmanlayarak işlevselleştirmektir. RAFT polimerizasyonu, UMAPE-g-PHEMA’nın sentezinde PHEMA'nın molekül ağırlığı ve molekül ağırlığı dağılımını kontrol edebilmek ve gentamisin sülfatın salım oranını ve sürdürülebilirliğini kontrol etmek için kullanıldı. PHEMA'nın UMAPE’ye aşılanmasından sonra kimyasal özelliklerinde meydana gelen değişiklikler yüzey karakterizasyon yöntemleri, ATR-FTIR, elemental analizi,

X-ışınları fotoelektron spektroskopisi (XPS) ve temas açısı kullanılarak incelenmiştir. Daha sonra, antibiyotik yüklenen UMAPE ve UMAPE-g-PHEMA'dan (geleneksel polimerizasyon veya RAFT polimerizasyonu ile hazırlanan) antibiyotik salım çalışmaları gerçekleştirimiştir. Adı geçen polimerlerin antimikrobiyal etkinliği iki şekilde test edilmiştir:

1. Eluent ortamda planktonik öldürme,

2. Polimerik yüzeylerin antikolonize edici özellikleri.

Sentezlenmiş/hazırlanmış ilaç yüklü polimerlerin çekme dayanımı ve aşınma direncini incelemeleri mekanik testler ve aşınma testleri yapılmıştır.
UMAPE-g-PHEMA aşı kopolimeri GS salım oranını işlenmemiş UMAPE'ye kıyasla önemli miktarda arttırmıştır. UMAPE-g-PHEMA aşı kopolimerinin antibakteriyel performansı da salım oranındaki artışa parallel olarak daha etkili olmuştur. UMAPE'ye HEMA aşılanması azami çekme mukavemeti, kopmadaki uzama ve IZOD darbe dayanımı gibi mekanik özelliklerinin azalmasına sebep olmuştur. UV ile başlatılan RAFT polimerizasyonuyla sentezlenmiş UMAPE-g-PHEMA kopolimerler ise GS salımını geleneksel metodla aşılanarak hazırlanan kopolimere kıyasla daha sürdürülebilir şekilde artırmıştır. Böylelikle, UMAPE-g-PHEMA daha iyi planktonic bakteri öldürme ve yapışmaz yüzey özellikleri göstermiştir.

Anahtar Kelimeler: UMAPE, UV ile başlatılan aşılama, RAFT polimerizasyonu, Periprostetik eklem enfeksiyonu (PPEE), diz implant, gentamisin sülfat

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SYMBOLS AND ABBREVIATIONS

Symbols

μm	Micrometer
μS	Microsiemens
Å	Angstrom
Cm	Centimeter
H	Entalpy
H	Hour
kGy	Kilogray
kJ	Kilojoule
M	Molar
mm^2	Square milimeter
mm^3	Cubic milimeter
Mpa	Megapascal
Nm	Nanometer
°C	Degree celcius
Rpm	Revolution per minute
W	Watt

Abbreviations

AA	Acrylic Acid
AB	Aluminum broze
ALBC	Antibiotic-loaded bone cement
ATR-FTIR spectroscopy	Attenuated total reflectance-Fourier Transfer Infrared spectroscopy
ATRP	Atom transfer radical polymerization

CFU	Colony forming units
CPC	Calcium phosphate ceramic
CRP	C-reactive protein
DDMAT	S-dodecyl-S′-(α,α′-dimethyl-α″-asetic asid)trithiocarbonate
DSC	Diferantial taramalı kalorimetre
EAB	Elongation at break
ESR	Erythrocyte Sedimentation Rate
FDA	Food and Drug Administration
FRP	Free radical polymerization
FT-IR/ATR	Fourier-transform Infrared/Attenuated Total Reflectance
GS	Gentamicin sulfate
HDPE	High Density Polyethylene
HXLPE	Highly crosslinked polyethylene
I&G	Irrigation and debridement
ITP	Iodine transfer polymerization
LCST	Lower critical solution temperature
LDPE	Low Density Polyethylene
LLDPE	Linear low density polyethylene
MADIX	Macromolecular design via the interchange of xanthates
MALLS	Multi-angle laser light scattering detector
MIC	Minimum inhibitory concentration
MPC	2-methacryloyloxyethyl phosphorylcholine
MRSA	Methicillin-resistant *Staphylococcus Aureus*
MSIS	Musculoskeletal Infection Society
NMP	Nitroxide mediated polymerization

OEGMA	Oligoethylene glycol
OPA	o-phthaldialdehyde
PAA	Poly(acrylic acid)
PAM	Polyacryamide
PBS	Phosphate buffer saline
PDI	Polydispersity index
PDMAEMA	Poly(2-(dimethylamino)ethyl methacrylate)
PEI	Polyethylene imine
PET	Polyethylene therephthalate
PJI	Periprosthetic joint infection
PMMA	Polymethyl methacrylate
PMN	Polymorphonuclear
PNIPAAM	Poly(N-isopropylacrylamide)
POD wear test	Pin-on-disc wear test
PTFE	Poly(tetrafluoroethylene)
PVA	Polyvinyl alcohol
RAFT Polymerization	Reversible Addition Fragmentation Chain Transfer Polymerization
SEC	Size exclusion chromatography
SEM-EDX spectroscopy	Scanning electron microscopy-energy dispersive x-ray spectroscopy
SET-DTLRP	Single electron transfer-degenerative transfer living polymerization
TGA	Termogravimetrik analiz
THA	Total hip arthroplasty
TJA	Total joint arthroplasty
TSB	Tryptic soy broth

UHMWPE	Ultrahigh Molecular Weight Polyethylene
UTS	Ultimate tensile strength
UVIRP	UV-initiated radical polymerization
VAc	Vinilasetat
VH	Vancomycin hydrochloride
XPS	X-ray Photoelectron Spectroscopy

1. INTRODUCTION

Total joint replacement is a surgical procedure in which parts of a damaged joint are replaced with implant materials. Metals, polymers or ceramics are used in the design of different components of implant materials. The implant materials help to relieve the pain of the patient and to improve the loss function. Total joint arthroplasty in the hip, knee, shoulder, and other joints, produces very successful outcomes. More than 1 million new replacement and implant surgeries are performed each year in US. According to a recent study conducted by the Mayo Clinic more than 7.2 million individuals in the United States currently live with biomedical implants as a result of joint replacement surgeries (Maradit-Kremers et al. 2014). The total number of individuals with implants confirms the usefulness of these medical interventions, as these high numbers indicate increased quality of life and longevity as a result of advanced implant procedures and novel biomedical materials.

However, a joint replacement may fail for a variety of reasons within years. When this happens, the patient suffers from pain and surgical procedure must be done again to replace the joint replacement. One of the major reasons for revision is the infection of the reconstructed joint. The implants used during surgery are metallic, ceramic, or polymeric in nature and are prone to colonizing bacteria. One way to reduce the rate of infection is to improve the surfaces of the implants. For example, antibiotic coated\loaded materials can be used to inhibit bacterial adhesion and colonization. Antibiotic loaded polymethyl methacrylate (PMMA) bone cement has been in clinical use in total joint arthroplasty surgery to prophylactically reduce infections. Antibiotic loaded bone cement has been somewhat successful in reducing the infection rate. However, once infected, the implants have to be removed the joint be debrided, and in some cases temporary articulating or static spacer implants be implanted. These spacer implants are manufactured from PMMA bone cement and contain various antibiotics. Temporal release of antibiotics to the surgical site helps clear the infection (Stevens CM, Tetsworth KD, Calhoun JH, Mader JT. An articulated antibiotic spacer used for infected total knee arthroplasty: a comparative in vitro elution study of Simplex and

Palacos bone cements. J Orthop Res Off Publ Orthop Res Soc. 2005;23(1):27–33.). The spacer implants are temporary in nature and they are typically replaced within six months of implantation with permanent implants. However, PMMAs present several disadvantages such as the occurrence of chemical necrosis caused by non-polymerized monomer residues, and low toughness (Belt, H. V. D., Neut, D., Schenk, W., Horn, J. R. V., Mei, H. C. V. D., & Busscher, H. J. (2001). Infection of orthopedic implants and the use of antibiotic-loaded bone cements: a review. Acta Orthopaedica Scandinavica, 72(6), 557-571.). Patients are largely immobilized during treatment due to PMMA spacers not being able to bear the full weight of the patients.

As one strategy, therapeutic agents, such as antibiotics, can be incorporated into ultra-high molecular weight polyethylene (UHMWPE) implants typically used in total joint arthroplasty for local delivery of these therapeutic agents. UHMWPE is a better candidate than PMMA bone cement as an articulating spacer and a delivery device eluting antibiotics because of its superior mechanical strength and markedly improved wear resistance in comparison to bone cement.

Typically, acetabular liners in total hips, tibial inserts in total knees, glenoid components in total shoulders are fabricated from UHMWPE. Prophylaxis, to reduce acute and chronic infections, can also be carried out by using therapeutic agent containing UHMWPE implants not only in revision surgery but also in primary surgery.

Polymeric implants in contact with body fluids are prone to be infected by various microorganisms. In this thesis, it was aimed to compatibilize non-polar UHMWPE bulk with polar functional moieties by grafting 2-hydroxyethyl methacrylate monomer and blend resulting copolymer with commonly used antibiotic, gentamicin sulfate. The UHMWPE, the UHMPWE-g-PHEMA (prepared by conventional grafting and grafting via RAFT polymerization) powder is blended with gentamicin sulfate powder. Subsequently, antibiotic release studies from antibiotic-loaded UHMWPE and UHMWPE-g-PHEMA were conducted. Antimicrobial efficacy of said polymers was tested in two ways:

3. Planktonic kill in the eluent media,

4. Anticolonizing properties of polymeric surfaces.

Synthesized/prepared drug loaded polymers were tested to evaluate their mechanical strength and wear resistance by using tensile testing and pin-on-disc wear testing.

2. TOTAL JOINT ARTHROPLASTY

Osteoarthritis (OA) is the most prevalent subset of the arthritic conditions all across the world. Almost 27 million adults in the US and 8.5 million adults in the UK suffer from OA (Lawrence et al. 2008; National Collaborating Centre for Chronic Conditions (Great Britain) 2008). Projections predicts that symptomatic osteoarthritis will affect nearly 67 million individuals in USA by 2030 (Hootman and Helmick 2006). Prosthetic joint replacement is the golden standard for end stage treatment of osteoarthritis and is performed on more than one million patients annually for hip and knee replacement in the USA (Ayers and Franklin 2014). Polymeric materials are ubiquitously used as articular components in replacement devices. It was reported that the very first contemporary implant materials were suggested by McKee (in 1951) and Charnley (in 1958) (McKee and Watson-Farrar 1966; John Charnley 1979, 1973). Sir Charnley used Teflon as the acetabular component for an acetabulum-femur configuration of the hip implant, he then transitioned to ultra-high molecular weight polyethylene (UHMWPE) due to Teflon's high wear rate.

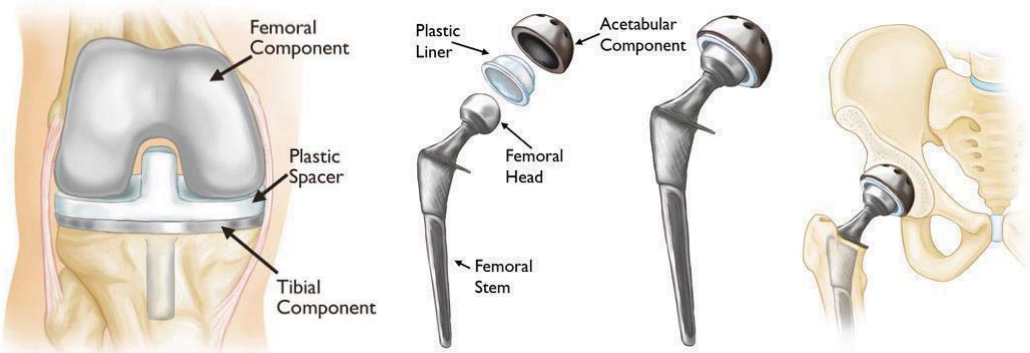


Figure 2. 1. Total Knee and Hip Implant Components (Copyright AAOS)

The femoral component of Charnley's hip implant assembly was made of stainless steel. In between 1962 and 1965 he used UHMWPE in 379 interventions and the feedbacks he received from patient surveys was excellent in terms of recovery of motion, pain relief and ability to walk (John Charnley 2005).

Existing hip implants have four main components; an acetabular component which resides in the concave cavity of the pelvis, a plastic liner that goes inside the

acetabular component, a femoral head and a femoral stem (Figure 2.1). The acetabular component and the femoral stem are made of metal whereas the articular surfaces could alternatively be made of ceramic, metal or plastic (UHMWPE). UHMWPE is most preferred liner for primary joint replacements (Muratoglu et al. 2004).

The contemporary total knee implant most commonly has three components; a femoral component, a tibial plate and a plastic tibial insert (Figure 2.1). The premises of today's knee implants emerged in 1971. It was called as the " Geometric" knee design by a group of surgeons at the Mayo Clinic (Figure 2.2) (Skollnick et al. 1976). The so-called Geometric Knee was resurrected from the Polycentric knee to adapt bicondylar knee arthroplasty. 69 % of the 10-year old Geometric knees were still usable (Rand and Coventry 1988).

Despite Geometric knee is easier to implant than Polycentric knee, fixation of the implant was challenging as in the rest of the UHMWPE based systems (Rand and Coventry 1988). Although early knee designs suffered from problems such as the leaching of the cement used for fixation in between the tibial plate and the tibial insert, the 10-year survival rate was relatively good at about 70 % (Rand and Coventry 1988). The past of the total knee arthroplasty (TKA) and total hip arthroplasty (THA) related research have witnessed UHMWPE turned an essential component of the implant assembly such as patellar component (Gunston and MacKenzie 1976), meniscus in metal tray (Collier et al. 1990), mobile bearing (Hamelynck and Stiehl 2002).

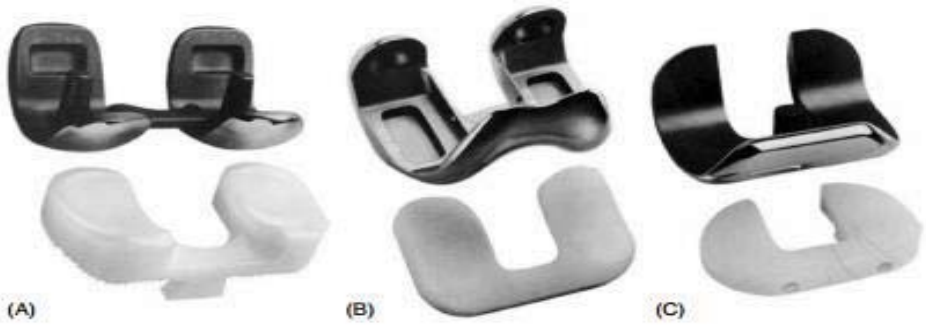


Figure 2. 2. Bicondylar knee designs, A) Geometric, B) Townley, C) Freeman-Swanson total knee prostheses with UHMWPE components (Reprinted from (Kurtz 2016)).

2.1. UHMWPE

Polyethylene is a polymer synthesized through Ziegler-Natta polymerization from its precursor ethylene gas (Cossee 1964). Polyethylenes are classified depending on the molecular weight or chain structure such as low density polyethylene (LDPE), linear low density polyethylene (LLDPE), high density polyethylene (HDPE), ultrahigh molecular weight polyethylene (UHMWPE). UHMWPE is described as having more than about $1x10^6$ Da to few millions Da. Alterations of molecular weight and chain structure brings variety on the physical properties and mechanical strength of different polyethylenes (Table 2.1).

Table 2. 1. Physical properties of HDPE and UHMWPE (Edidin and Kurtz 2000).

Property	HDPE	UHMWPE
Molecular weight (10^6g/mol)	0.05-0.25	3.5-3.7
Melting temperature (°C)	130-137	132-138
Poisson's ratio	0.40	0.46
Specific gravity	0.952-0.965	0.925-0.945
Tensile modulus of elasticity (GPA)	0.4-4.0	0.5-0.8
Tensile yield strength (MPa)	26-33	21-28
Tensile ultimate strength (MPa)	22-31	39-48
Elongation at break (%)	10-1200	350-525
Izod impact strength (J/m)	21-214	>1070 (No break)
Degree of crystallinity	60-80	39-75

Most prominent properties of UHMWPE that are distinctly superior to those of other polyethylenes are its abrasion and wear resistance. Volumetrically, HDPE

has a wear rate that is more than 4 times of the UHMWPE wear on multidirectional hip simulator (Edidin and Kurtz 2000).

Transmission electron micrographs show that long chains of UHMWPE are entangled which gets UHMWPE to have melt flow. Below melting temperature, UHMWPE chains tend to rotate along the C-C bond and fold. Crystalline lamellae of UHMWPE is thus formed. The crystalline lamellae of UHMWPE are 10-50 nm thick and 10-50 μm long on average (Figure 2.3) (Bellare, Schnablegger, and Cohen 1995).

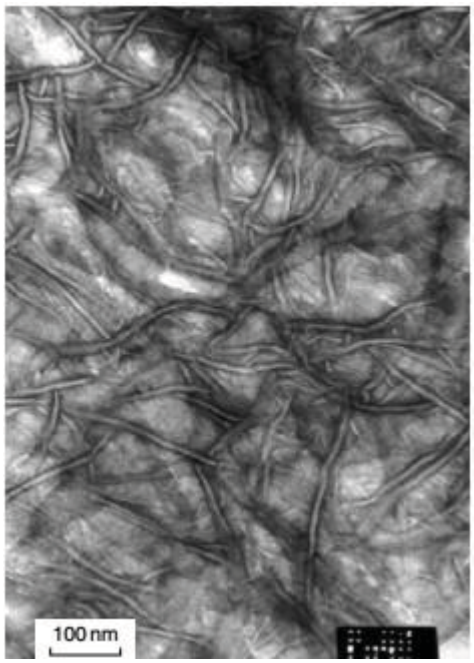


Figure 2. 3. TEM micrograph of semicrystalline UHMWPE (Bellare, Schnablegger, and Cohen 1995)

2.1.1. Synthesis of UHMWPE

The first commercialization ethylene gas polymerization was performed by a group of chemists from Max Planck Institute who had worked for a chemistry firm, Ruhrchemie AG in 1950s. The company is now known as Celanese (H.-G. Willert, Eyerer, and Buchhorn 1991).

The polymerization requires ethylene gas, hydrogen and $TiCl_4$ catalyst to take place. The polymerization is carried out in plants that could handle volatile gases and low pressure. Medical grade UHMWPE powder might have impurities as

titanium, aluminum, chlorine which comes from catalyst up to the specified concentrations specified in ASTM standard F648. Foremost medical grade resins are GUR type of resins such as GUR 1020 and GUR 1050 are produced by Celanese (Irving, TX, USA). GUR stands for “Granular”, “UHMWPE”, “Ruhrchemie” and the adduct numbers stand for properties of resin. First number is for showing bulk density where second for existence of calcium stearate, third for average molecular weight and fourth for internal code designation.

UHMWPE is often handled in fine powder form. In order to shape it to final implant, it is compression molded and machined to the final design (Figure 2.4).

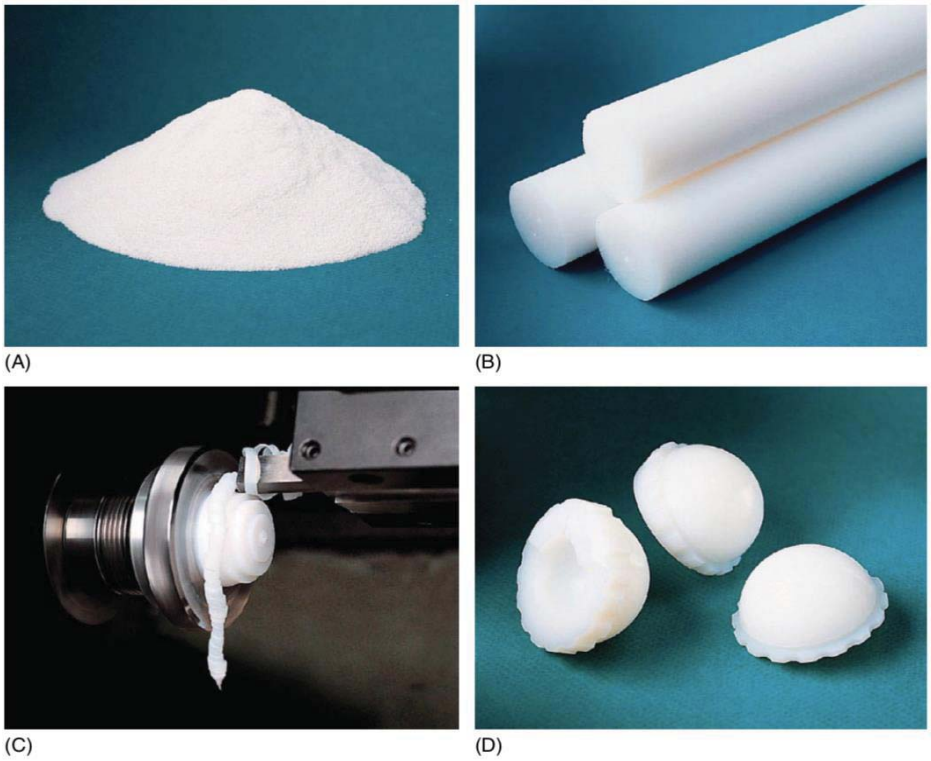


Figure 2. 4. UHMWPE processing steps UHMWPE fine powder (A), extruded UHMWPE powder (B), machining of the compression molded UHMWPE (C), molded and machined final shape of the UHMWPE acetabular heads (Biomet, Inc., Warsaw, Indiana, USA.

2.1.2. Adaption of UHMWPE to the Field of Orthopaedics

Charnley and his colleagues developed a technique to investigate the long-term wear rate of the UHMWPE as acetabular component by radiographic evaluation techniques (J. Charnley and Cupic 1973). Those who weren’t too feeble or death

and made to the follow-up examination let researchers evaluate the condition of the implants again. 106 out of 185 acetabular cups were able to be examined after 9-10 years of implantation. Certain complications were listed as infection, mechanical loosening and late dislocation (J. Charnley and Cupic 1973). The main causes for revision surgeries in TKA were aseptic loosening; infection; fracture; joint stiffness; tibio/femoral instability; patellar complications; periprosthetic fracture; and wear of UHMWPE component (Sharkey et al. 2002; Lombardi, Berend, and Adams 2014; Fehring et al. 2001; Schroer et al. 2013). Cross-linking of the UHMWPE was proposed to decrease polymeric debris caused by the wear of the inserts in TJA (Kurtz and Patel 2016). Many attempts had been made to improve UHMWPE for TJA to reduce wear and thereby increase the longevity. Cross-linking by ionizing radiation (gamma and e-beam) has largely reduced the wear of UHMWPE. The UHMWPEs are referred as “conventional” if the amount of irradiation dose was 25-40 kGy and was referred as “highly cross-linked UHMWPE (HXLPE)” if the dose was >40 kGy (Kurtz and Patel 2016). UHMWPE cross-linking affected some properties in unpremeditated manner such as reduction in ductility, fatigue and fracture resistance (H. G. Willert, Bertram, and Buchhorn 1990). In their seminal paper of 1995, the effect of nitrogen implantation of UHMWPE on the wear resistance of it articulating against titanium was investigated (Allen, Bloyce, and Bell 1995). While cross-linking improves wear resistance, in *vivo* oxidation of cross-linked UHMWPE further lowers its ductility and its ultimate strength (Kurtz et al. 2006; Medel, Rimnac, and Kurtz 2009; Wannomae et al. 2006). In *vivo* oxidation is caused by the subsequent reactions of residual free radicals after gamma irradiation and from in *vivo* lipid absorption present in the synovial fluid (Muratoglu et al. 2010; Oral et al. 2012; Regis et al. 2014). Efforts to prevent in *vivo* oxidation gave rise to the incorporation of the antioxidant vitamin E in the polymer, which is a breakthrough in the improvement of UHMWPE (Bracco and Oral 2011). Vitamin E-infused and cross-linked UHMWPE (VE-HXLPE), both oxidatively and mechanically remained stable after accelerated in vitro oxidation (Bracco and Oral 2011; Kurtz et al. 2015). In another study to prevent UHMWPE oxidation, UHMWPE was incorporated with Europium stearate which has outstanding binding affinity to oxygen (Gallardo et al. 2011). In contrast, HXLPE without VE showed a 5-fold increase in oxidation and 70-80 % decrease in mechanical strength (Kurtz et al. 2015; Bracco and Oral 2011). Vitamin E-infused

and cross-linked UHMWPE was approved by FDA in 2007 for hip and in 2008 for knee and its clinical performance is under investigation (Oral and Muratoglu 2015).

The incidence of osteolysis dropped drastically with the use of HXLPE even after ten years of implantation, which is relatively short compared to the eventual lifetime of the implants ("National Joint Replacement Registry; Hip, Knee & Shoulder Arthroplasty" 2016a). That said, the use of HXLPE did not change the incidence of periprosthetic joint infection (PJI) compared to that when conventional UHMWPE was used ("National Joint Replacement Registry; Hip, Knee & Shoulder Arthroplasty" 2016b). HXLPE has involved in revision less than UHMWPE in total knee arthroplasty ("National Joint Replacement Registry; Hip, Knee & Shoulder Arthroplasty" 2016b). As in THA, the main reasons for revision of HXLPE were aseptic loosening, pain, instability, and periprosthetic joint infection which is similar to UHMWPE ("National Joint Replacement Registry; Hip, Knee & Shoulder Arthroplasty" 2016b). PJI is a major reason for the TKA revision for the patients implanted with HXLPE ("National Joint Replacement Registry; Hip, Knee & Shoulder Arthroplasty" 2016b). Thus, it is substantial to develop better materials and techniques to battle with PJI problem.

2.2. Metals

Biomechanical properties of metals made them appropriate to use as an implant material. Besides, metallic implants can be sterilized by the common sterilization procedures that makes them easy to use. Cobalt chromium alloys, nickel alloys, gold alloys, titanium and titanium based alloys and stainless steel are used as metallic implant materials in orthopaedic applications. However, TJA components are widely made from cobalt chromium alloys. Cobalt chromium alloys have low rate of corrosion that provide long-term stability. Metal-on-metal configuration generates 13,500 times higher number of particles when compared with polyethylene particles which produced in metal-on-polyethylene configuration. However, total volumetric wear of a metal-on-metal configuration is lower than the volumetric wear of metal-on-polyethylene configuration because of the smaller size of metal particles (<50nm) compared to polyethylene particles (<0.1μm). Metal wear debris particles are found within synovial fluids and tissues and because of very small size the true extent of dissemination is not known yet.

Studies shows that orthopaedic metals induce immunological effects which cause cell mediated hypersensitivity response.

2.3. Ceramics

Ceramics are inorganic based solid materials. In orthopaedics, ceramics basically used in two forms:

1. Ceramic oxides which are used as ball heads or inserts in hip arthroplasty.
2. Calcium phosphate ceramics which are used to coat metal components for osteoconductivity.

2.3.1. Ceramic Oxides

Ceramic oxides have mainly two types, alumina and zirconia. They both exhibit corrosion resistance, hardness and stiffness. They are both chemically inert and have excellent wear resistance. Their wear debris is biocompatible (H. Liu et al. 2008).

2.3.1.1. Alumina

Alumina is oxidized form of aluminum (Al_2O_3) (Figure 2.5). It has been in the market since 1960's. Since it has been in the market long enough, it is one of the well characterized ceramic materials (Cloyd et al. 2007). The alumina used in the orthopaedics applications is the naturally occurring alpha alumina which is known as mineral corundum. Alpha alumina is very stable against corrosion even under harsh conditions. However, its low toughness compromises it uses to 15-20 % of the ceramic implants. It is rather being used as alumina-zirconia composite.

Figure 2. 5. Biolox(R) acetabular head made of alumina

2.3.1.2. Zirconia

Zirconia was introduced in 1980's to improve toughness of alumina. Most of the effort was put to develop Magnesia and Yttria stabilized zirconia. The tetragonal-monoclinic (t-m) phase transition of zirconia helps ceramic to dissipate the fracture stress. The t-m transformation upgrades material to a tougher ceramic at macroscopic level. It was reported that zirconia was applied to UHMWPE to improve its long term stability by improving plastic deformation and wear of it (Bianchi et al. 2015).

2.3.2. Calcium Phosphate

Calcium phosphate ceramics (CPC) are known for their osteoconductive property. It was used on metallic components to increase bone-metal integration (Figure 2.6) (Peppas and Langer 1994). One important aspect which determines CPC property is calcium to phosphate ratio. The most stable CPC, Hydroxyapatite has Ca/P as 5/3 (Sims et al. 1998). The osteoconductive CPC is deposited on metallic surfaces by plasma spray technique which must take control of many crucial parameters into account such as polymorph, oxidation reactions, phase separation i.e (C. Liu, Xia, and Czernuszka 2007).

Figure 2. 6. Hydroxyapatite implant coatings (APS Materials, Daytona OH, USA).

2.3. Reasons for revision surgery

Under various circumstances knee and hip implants may fail, thus, may require revision of the materials (Figure 2.7). According to a literature review, 46 % of the

knee revisions take place in so-called early time period which is until 2-year anniversary of the implantation and the remaining 54 % occurs beyond 2 years (D. H. Le et al. 2014). Revision reasons for early group are instability (26 %), infection (24 %), stiffness (18 %), and intolerable wear (2 %). For the late group, reasons were infection (25 %), instability (18 %), stiffness (14 %), and polymeric wear (9 %) (D. H. Le et al. 2014).

Despite its relatively low incidence, implant infection remains one of the challenging problems. This PhD dissertation aimed to propose and develop a material to battle against infection of polymeric materials used in total knee arthroplasty.

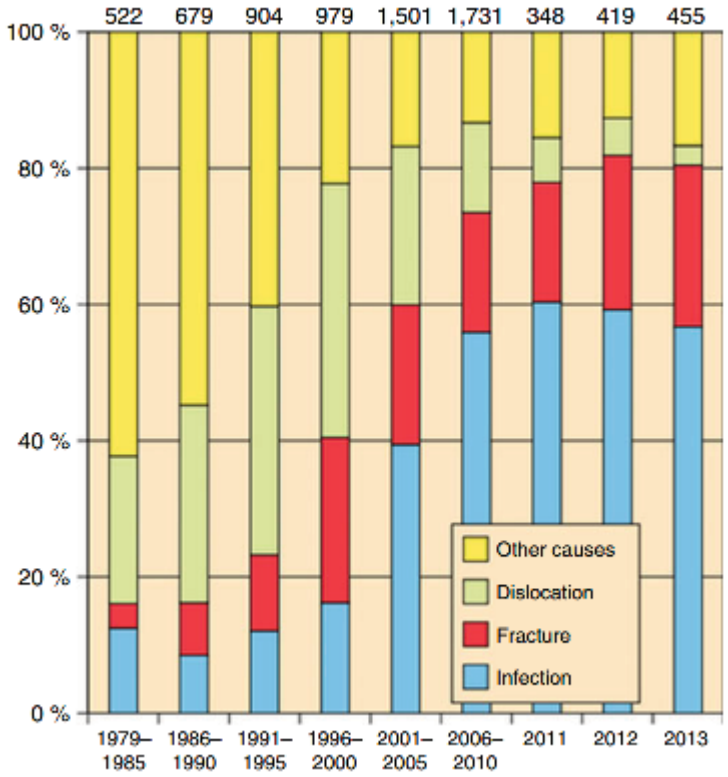


Figure 2. 7. Prevalence of various reasons for total hip arthroplasty in the Swedish Knee Arthroplasty Register (Bozic et al. 2012).

3. PERIPROSTHETIC JOINT INFECTION

Joint arthroplasty is procedure that increases the quality of life. In cases it is performed successfully, the patient takes advantages of pain relief and ease of joint motion. Despite the majority of joint arthroplasties are successful, there are cases where the prosthesis fails. The prosthesis failure usually requires another surgery. Reasons for failure could be either aseptic or infection related. The aseptic failure could arise from loss of bone-cement integration at the prosthesis-bone-cement interface, fracture of the implant, wear, malpositioning of the implant, materials fatigue. Periprosthetic joint infection (PJI) is the infection in the vicinity of implant which also involves the surrounding tissue (A. J. Tande and Patel 2014).

As defined by the Musculoskeletal Infection Society (MSIS), PJI is the condition of meeting following criteria;

(1) There is a sinus tract communicating with the prosthesis; or

(2) A pathogen is isolated by culture from at least two separate tissue or fluid samples obtained from the affected prosthetic joint; or

(3) Four of the following six criteria exist:

(a) Elevated serum erythrocyte sedimentation rate (ESR) and serum C-reactive protein (CRP) concentration,

(b) Elevated synovial leukocyte count,

(c) Elevated synovial neutrophil percentage (PMN%),

(d) Presence of purulence in the affected joint,

(e) Isolation of a microorganism in one culture of periprosthetic tissue or fluid,

(f) Greater than five neutrophils per high-power field in five high-power fields observed from histologic analysis of periprosthetic tissue at 9400 magnification" (Parvizi et al. 2011).

Although the rate of total joint arthroplasty (TJA) infection is as low as 1 %- 2%, the increase in the total number of operations and the growing aging population

are increasing prevalence of PJI. There are 7,100-15,000 patients that are affected by PJI yearly in USA and it is expected to peak 55,000-75,500 by the year 2030 (Kurtz et al. 2012). Between 2001-2009 the cost of knee and hip arthroplasties to the hospitals in United States of America rose from $320 million to $566 million annually (Berend et al. 2013). The occurrence of PJI in two-year period after implantation is greatest which is 60-70 % of all PJI cases (Pulido et al. 2008; Kurtz et al. 2010). Once the infected joint lost its functionality, it's challenging to revert back to its original state (Toulson et al. 2009). In all respects, PJI is a severe problem and a battle that none of the sides win. Hence, mitigating both the economic and the health burden caused by PJI is a worthwhile area of focus.

3.1. Pathogenesis of Prosthetic Joint Infection

The accounted causation is in three ways; bacteria seeding on the implant, hematogenous spread from rest of the body or recurrence of a previous infection (Kapadia et al. 2016). Cure for an infection related to foreign body part is compelling due to weakened immune system (W. Zimmerli, Lew, and Waldvogel 1984). The most conspicuous thing that emerges from bacterial harbouring is biofilm formation which reduces the susceptibility to antibiotics (Kapadia et al. 2016).

Biofilm is the structure where bacteria are embedded in their secreted extracellular matrix secreted by itself (Shah et al. 2015). Regardless of the type, foreign devices made of biomaterials increase susceptibility to infection (Figure 3.1). It is known from previous studies that the vulnerability of biomaterials to infection is partly because of granulocyte defect (W. Zimmerli et al. 1982; Costerton et al. 1995). The biofilm is totally a complex structure where bacteria in different layers interact with each other through quorum sensing to keep biofilm stable (Costerton, Stewart, and Greenberg 1999).

Figure 3. 1. Biofilm related failure of titanium hip acetabular implant (Lentino 2003).

The slurry which is secreted by bacteria has proteins, polysaccharides and DNA of the bacteria in it (Shah et al. 2015). The complexity of the biofilm shields bacteria from eradication, resulting in antibacterial resistance (Ceri et al. 1999; Vuong et al. 2003). Biofilm thickening stops when the nutrition and oxygen lack penetrating adequately across the biofilm (Anderl et al. 2003).

The severity of biofilm has urged the use of higher concentration of antibiotics to eradicate the biofilm by incorporating antibiotics locally (Stewart and Costerton 2001). In case of having a foreign body, it requires 10^5 times bacteria to induce infection than none foreign body existence (Puhto et al. 2014). As a matter of fact, in a mouse model, inoculation with only 100 colony forming units of *Staphylococcus aureus* was enough to cause a mild type of infection such as stitch abscess (Elek and Conen 1957). Although the susceptibility of different materials to engendering biofilm growth in vivo is not clear, it is thought that protein deposition on the implanted biomaterial *in vivo* can enhance biofilm formation through some proteins such as fibronectin acting as receptors for staphylococci (Herrmann et al. 1988; Lopes, dos Reis, and Brentani 1985).

Biofilm formation is initiated by the adherence of bacteria to implant surfaces (Gristina 1987). Thus, prevention of the microorganism adherence becomes

crucial. Adherence depends on both nonspecific physical factors (surface tension, hydrophilicity, electrostatic interactions i.e.) or specific adhesive proteins such as fibronectin (Werner Zimmerli 2014). Subsequently, other signaling molecules such as intercellular adhesion (ica) operon contributes to the adherent bacteria transforming into biofilm (Laverty, Gorman, and Gilmore 2013). Biofilms consist of microbes embedded in a secreted polymeric matrix, which is organized in complex structures similar to multicellular organisms. At the stage when the diffusion and local concentration of nutrients are limited by the growth of bacteria, quorum sensing genes are activated to stop overgrowth (Simonetti et al. 2013). This sheltered structure of biofilms makes them 1000-fold more resistant to antibiotics than planktonic bacteria (Stewart and Costerton 2001). The presence of biofilm dramatically reduces the chance from 80-90% to 30-60% for the cases that treatment is started 3-4 weeks after infection (Deirmengian et al. 2003; Laffer et al. 2006; Giulieri et al. 2004; Marculescu et al. 2006).

3.2. Bacterial Strains in PJI

68-94 % of cultures obtained from PJI patients' cultures test positive for a causative organism (Peel et al. 2012; Werner Zimmerli, Trampuz, and Ochsner 2004) and 90-95 % of those cases originate from gram-positive bacteria (Werner Zimmerli, Trampuz, and Ochsner 2004). Most of the infection episodes are caused by Staphylococci; *S. aureus* and coagulase-negative staphylococci, particularly S. epidermidis, or S. lugdunensis (Aaron J. Tande and Patel 2014). Gram-negative bacteria such as E. Coli, P. acnes and other rare bacterial strains, including Mycobacterium tuberculosis (Harwin et al. 2013), nontuberculous mycobacteria (S.-X. Wang et al. 2011), Mycoplasma (Sneller et al. 1986), Legionella (Fernández-Cruz et al. 2011) and fungal agents (Kuiper et al. 2013) can be involved in PJI. Table 3.1. outlines common microorganisms that are involved in TKA and THA.

Table 3. 1. Microorganisms in periprosthetic hip and knee infection

	Total hip arthroplasty	Total knee arthroplasty
Microorganism	n = 118[a]	n = 500[b]
Staphylococcus aureus	51/118 = 43.2%	152/500 = 30.4%
Coagulase-negative staphylococci	22/118 = 18.6%	142/500 = 28.4%
Streptococcus spp.	11/118 = 9.3%	43/500 = 8.6%
Enterococcus spp.	4/118 = 3.9%	37/500 = 7.4%
Propionibacterium spp.	1/118 = 0.8	11/500 = 2.2%
Gram-negative bacilli	7/118 = 5.9%	33/500 = 6.6%
Miscellaneous	2/118 = 1.7%	9/500 = 1.8%
Polymicrobial	9/118 = 7.6%	29/500 = 5.8%
No growth	11/118 = 9.3%	44/500 = 8.8%

[a]Giulieri *et al*. (Giulieri et al. 2004; Schinsky et al. 2008) Schinsky *et al*. (Giulieri et al. 2004; Schinsky et al. 2008).
[b]Laffer *et al*.(Laffer et al. 2006; Trampuz et al. 2004) Trampuz *et al*. (Laffer et al. 2006; Trampuz et al. 2004), Stefansdottir *et al*. (Stefánsdóttir et al. 2009).

Multiple types of bacteria often can take part at different stages of PJI. 84 % of PJI cases have *S. aureus*, coagulase-negative staphylococci and aerobic gram-negative bacili and 31 % are polymicrobial in nature (Shah et al. 2015).

Chronic PJI which reveals 1-2 years after surgery is mostly due to hematogenous infection either provoked by the *Staphyloccoci* or *Streptococci (Shah et al. 2015)* and the rest of the cases is caused by less virulent species such as coagulase-negative staphylococci and enterococci (Phillips et al. 2006). For the incidents that emerges after 2 years are usually dominated by *S. aureus (Murdoch et al. 2001;*

Parham Sendi et al. 2011).

Current state of the art prosthetic joint assembly for TKA is comprised in most cases of metal a (Co-Cr or Ti) and polymeric material (UHMWPE) articulating pair in the USA. Bacterial count on infected hip joints showed that UHMWPE has the highest amount of bacteria on it, femoral head and acetabular head has less and femoral stem has the least number of colonizing bacteria (Holinka et al. 2012). This suggests that all components of the joint implant are involved in the initiation and propagation of a bacterial infection.

3.3. Risk Factors in PJI

3.3.1. Patient-Related Risk Factors

Hematogenous seeding is obscure causation for PJI that usually generates symptoms such as dental or urinary tract infection (Gehrke and Parvizi 2014; Cruess, Bickel, and vonKessler 1975; Schmalzried et al. 1992). Patients with aforementioned symptoms of current infection should be examined and be treated immediately.

Glycaemic control is essential due diabetic patients who are in high risk group for PJI (Dowsey and Choong 2009). In a study, correlation between blood glucose level and infection occurrence was investigated and showed that blood glucose level of >200 mg/dL doubles the risk of PJI (Mraovic et al. 2011). Diabetes likely to induce PJI through impaired wound healing (McMurry 1984) and susceptibility of biofilm formation due to high glucose levels in blood (Seneviratne et al. 2013).

Meeting the conditions of dropped levels of serum albumin, lymphocyte count, serum transferrin or serum prealbumin attributes to malnourishment (Devoto et al. 2006; Cross et al. 2014). A considerable amount of literature has been published on the correlation between lack of nutrition and probability of undergoing PJI (Paul et al. 2015; Greene, Wilde, and Stulberg 1991). Collagen and proteoglycan synthesis are notably being affected by poor nutrition intake. This may result in

collapsing the wound healing process and sustained drainage may increase the risk of infection (Cross et al. 2014).

Studies have shown that high body mass index (BMI) has an effect on surgical site infection (SSI) (Dowsey and Choong 2008) and obese people have 1.5-3 times higher risk of reoperation after hip replacements (Jameson et al. 2014). In obese patients, fraction of oxygen (FiO_2) need is much higher than people in normal BMI limits (Fleischmann et al. 2005).
It has been demonstrated that alcohol consumption impacted infection in non-cardiac surgeries (Bradley et al. 2011). The infection risk inflation is conceived to be arisen from weakened immune system which is adversely affected by alcohol consumption (Tønnesen et al. 2009).

3.3.2. Prophylactic Measures for PJI

Wound healing mechanism for those who has diabetes is more impaired than those who are non-diabetics (Mraovic et al. 2011). It was found that high blood sugar gets monocytes more susceptible to apoptosis (Komura et al. 2010) and deteriorated neutrophil functioning alongside with deteriorated chemotactic, phagocytic and bactericidal capability (Turina, Fry, and Polk 2005). In order to improve neutrophil phagocytic function, a strict low glucose diet must be adapted by the patient (Rassias et al. 1999). As opposed to glucose level controlling, a massive study with 40.000 patients, showed there's no additional risk for diabetic patients (Adams et al. 2013).

As corticosteroids used to treat rheumatoid arthritis delay wound healing, they increase the incidence of wound infection (Wicke et al. 2000). A relationship between use of disease-modifying antirheumatic drugs (DMARD) and increased risk of prosthetic joint infection was shown (Moucha et al. 2011). However, the British Society for Rheumatoid (BSR) advised not to stop DMARD treatment (Luqmani et al. 2009).

Other effective steps prior the PJI operation are skin site preparation with antiseptic chlorhexidine gluconate (Johnson et al. 2013), mupirocin nasal ointment since it collapses nasal carriers of methicillin-sensitive Staphylococcus aureus

(MSSA) (Breier et al. 2011). The ultimate risk of having PJI drops 8 % in case of antibiotic prophylaxis introduction (AlBuhairan, Hind, and Hutchinson 2008). Between 57 - 87 % of organisms are being vanished when pulsatile lavage is used (Dunne et al. 2008).

3.4. Current Treatment for PJI

The present-day practice to cure PJI involves 2-stage revision, 1-stage revision and debridement, antibiotic and implant retention (DAIR) and any worse case is treated with excision arthroplasty (Drago 2017).

The standard procedure involves intravenous (IV) antibiotics route to avoid enteral resorption and ensure the highest antibiotic level in tissues. Table 3.2. summarizes bacteria-specific antibiotics (Werner Zimmerli, Trampuz, and Ochsner 2004; P. Sendi and Zimmerli 2012; Osmon et al. 2013). The duration of antibiotic therapy subject to variation from case to case depending on several parameters. Long-term treatments are planned based on the assumption of incapability of host immune to eradicate the microorganisms. Therefore, 3-month long course is suggested for debridement, antibiotic and implant retention (DAIR, explained in further sections) and one-stage, 2-3 weeks is suggested for two-stage for PJI (Werner Zimmerli, Trampuz, and Ochsner 2004; P. Sendi and Zimmerli 2012; Osmon et al. 2013; Petersen 2010). Knee PJI requires as long as 6-month long course (Werner Zimmerli, Trampuz, and Ochsner 2004; P. Sendi and Zimmerli 2012; Osmon et al. 2013; Petersen 2010),(Werner Zimmerli et al. 1998).

Table 3. 2. PJI causing bacteria and its antibiotic agent

Microorganism	Antimicrobial agent
Staphylococcus spp. Methicillin resistant	Rifampin plus, Nafcillin or oxacillin
Staphylococcus spp. Methicillin susceptible	Rifampin plus, Vancomycin or Daptomycin
Staphylococcus spp.	Rifampin plus, Levofloxacin or Ciprofloxacin or Telcoplanin or Fusidic acid or Trimethoprim-sulfamethoxazole or Minocyline or Linezolid or Clindamycin
Streptococcus spp.	Penicillin G or Cefritaxone and Amoxicillin or Clindamycin
Penicillin susceptible *Enterococcus spp.*	Pencillin G or Ampicillin or amoxicillin
Penicillin resistant *Enterococcus spp.*	Vancomycin or Daptomycin or Linezolid
Enterobacteriaceae	β-lactam and Ciprofloxacin
Enterobacter spp. And nonfermenters (e.g., *Pseudomonas aeruginosa*)	Ceftazidime or Meropenem and Ciprofloxacin
Propionibacterium spp.	Penicillin G or Clindamycin and Amoxicillin or Clindamycin
Gram-negative anaerobes (e.g., *Bacteroides spp.*)	Metronidazole
Mixed infections (without methicillin-resistant staphylococci)	Ampicillin/sulbactam or Piperacillin/tazobactam or Imipenem or Meropenem

Adapted from (Werner Zimmerli, Trampuz, and Ochsner 2004; Parham Sendi et al. 2011)

Although, the strategy to cope with PJI differs from country to country, 2-stage revision surgeries have higher success rate: 95 % (Garvin et al. 1993; Elson

1993).

DAIR treatment is most suitable in the early post-operative phase or in the case of an acute hematogenous infection with a well-functioning implant in following month after surgery. This is because complete biofilm formation can occur between 36 h to 3 weeks (Parvizi, Zmistowski, and Adeli 2010). To hinder infection recurrence antibiotics should be used for months after the treatment surgery (Osmon et al. 2013). Synergic use of antibiotic rifampin with other antibiotics such as β-lactam, glycopeptide, or fluoroquinolones was shown to leveraged efficacy of DAIR treatment against *S. aureus (Senneville et al. 2011; Vilchez et al. 2011)*. Frequently encountered DAIR treatment failure reasons are involvement of methicillin-resistant *S. aureus* (MRSA) (Soriano et al. 2006; Bradbury et al. 2009), vancomycin-resistant enterococci (VRE) and fluoroquinolone-resistant gram-negative bacilli (Jaén et al. 2012). The overall success rate of DAIR treatment for knee is 33 % and for hip is 52 % (Romanò et al. 2012; Silva, Tharani, and Schmalzried 2002).

In one-stage surgery, concurrent implication of infected implant removal and implantation of new prosthesis with antibiotic-eluting bone cement (ALBC) fixing element is performed (Shah et al. 2015). A long term antibiotic treatment is afterwards characterized, first intravenously for 4-6 weeks, then orally for 3-12 months to nullify any remaining species (Klouche et al. 2012; Ure et al. 1998; Buechel, Femino, and D'Alessio 2004). One-stage surgery is used dominantly for hip joint PJI (Klouche et al. 2012; Ure et al. 1998; Buechel, Femino, and D'Alessio 2004) and rarely for knee joint PJI (Shah et al. 2015). If there's no sinus tract infection, absence of antibiotic resistant bacteria species, soft tissue that in good shape and presence of sufficient volume of bone, the necessary conditions are provided for one-stage revision surgery (Klouche et al. 2012; Ure et al. 1998; Raut, Siney, and Wroblewski 1995, 1994).

In two-stage surgery, primarily infected implant, necrotic tissue and some native tissue are removed to ensure as much as bacteria left. Subsequently, antibiotic-eluting bone cement (ALBC) spacer is implanted to maintain joint function, aid patient's immune system to tackle with planktonic bacteria and let bone repair

completed in health conditions (Berend et al. 2013; Puhto et al. 2014). Besides ALBC, a systemic antibiotic treatment is given for 2-6 weeks under normal conditions (Mahmud et al. 2012; Bejon et al. 2010). If patient unveils any sign for detectable bacteria, debridement may be iterated and systemic antibiotic administration recommences (Mahmud et al. 2012; Bejon et al. 2010). When all detectable bacteria is cleared out of the body, a permanent prostheses is implanted by fixing it with ALBC (Mahmud et al. 2012; Bejon et al. 2010). To assure joint is bacteria-free, a local tissue biopsy is performed meanwhile, IV antibiotic is administered (Mahmud et al. 2012; Bejon et al. 2010).

3.4.1. Use of antibiotic-loaded bone cements as a PJI treatment

Poly(methyl methacrylate) (PMMA) is one of the well-recognized biomaterials (Belt et al. 2001). One of the very first fields of use for PMMA is dentistry (Munson and Heron 1941). Thereafter, it was used for hip endoprosthesis manufacture for coxarthrosis (Scales and Herschell 1945). Sir Charnley conformed PMMA to fixate hip implants and dissipate mechanical force between bone and implant material (John Charnley 1970).

The inceptive studies which conceived impregnation of antibiotics in PMMA is comprised of mixing Palacos bone cement with Gentamicin Sulfate (GS) (BUCHHOLZ and HW 1970). Bone cement is the generic name of the mixture of variational copolymer of methyl acrylate, styrene and butyl methacrylate with methyl methacrylate monomer and radiopaque (Belt et al. 2001). Often, property differences in different bone cements have its source in variability in the ratio of copolymers and methyl methacrylate monomer (Joseph, Chen, and Di Cesare 2003).

There are few acrylic bone cement brands available in the market with either premixed antibiotics or antibiotics added by surgeons in the operating room. Certain bone cement brands are Palacos (Smith & Nephew, Memphis, TN), Simplex (Howmedica, Rutherford, NJ), CMW (DePuy, Warsaw, IN), and Zimmer (Zimmer, Warsaw, IN) are the ones to be mixed with antibiotics by surgeons (Joseph, Chen, and Di Cesare 2003). Premixed bone cements are AKZ (Simplex P with colistin and erythromycin), Refobacin-Palacos R (Palacos R with GS), and Septopal (beads of Palacos R with GS) (Joseph, Chen, and Di Cesare 2003).

The way of bone cement preparation is composed of adding the liquid monomer to powder mixture (copolymer, radiopaque, antibiotic) and stirring thoroughly to proceed polymerization of methyl methacrylate (Frommelt and Kühn 2005). As polymerization continues, viscosity of the mixture increases and when it reaches to adequate viscosity to be casted, ALBC is formed into a temporary PJI device or is used for fixation of metal stem to bone (Scott, Higham, and Dumbleton 1999). Polymerization of methyl methacrylate is an exothermic reaction in its nature. During the polymerization, the temperature of the reaction milieu is elevated to 60-80 °C which urges thermostable antibiotic use (Scott, Higham, and Dumbleton 1999).

Any antibiotic candidate for ALBC must fulfill the following requirements:

1. Must have activity above both minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).
2. Must defeat breakpoint sensitivity of species to avoid bacterial resistance which usually takes 3-4 weeks.
3. The concentration of antibiotic must not be as high to cause toxicity (Belt et al. 2001).

Most of the antibiotics that are used for implant related infection treatment can be incorporated in bone cement but Rifampin. Rifampin inhibits methyl methacrylate polymerization (Edward McPherson 2011).

To treat bacterial infections even more effectively, synergistic approach of multiple antibiotic is exploited; to eradicate S*. aureus*, S*. epidermidis*, E. coli, and P. aeruginosa, GS is incorporated in ALBC together with vancomycin hydrochloride (VH) (Bertazzoni Minelli et al. 2004). Multiple antibiotic incorporation brings better anti-colonization (Gallo et al. 2005), increase in releasing rate of each antibiotic compared to single antibiotic containing ALBC (Penner, Masri, and Duncan 1996) and prolonged antibiotic release (Lewis and Janna 2006).

There are two types of PJI implants made of ALBC depending on their use; static

spacer, a load bearing implant and articulating spacer (Figure 3.2) (Shah et al. 2015). The expected functions of spacers are load bearing aid and antibiotic delivery to the surgical site. Local antibiotic delivery through ALBC gains advantage over systemic antibiotic administration in terms of effective antibiotic concentration (Masri, Duncan, and Beauchamp 1998; Sterling et al. 2003).

Figure 3. 2. Pre-made spacers made with gentamicin sulfate-loaded bone cement (InterSpace(R) Knee, Exactech, Inc., Fl USA)

The primary undesired property of ALBC is its weak mechanical strength (Dunne et al. 2008). More the antibiotics incorporated in ALBC, less mechanical strength ALBC will have (Daniëlle Neut et al. 2003). It was suggested that addition of 2 g of antibiotic in 40 g of ALBC is adequate to treat PJI (Moojen et al. 2008). When it comes to prophylactic use of bone cement, it is usually less than 2 g of antibiotic added in 40 g of bone cement (Belt et al. 2001). Although the ingredients are same, bone cement which is prepared by surgeon has weaker mechanical strength than those are sold commercially (Jiranek, Hanssen, and Greenwald 2006). A comprehensive study showed that Palamed (17 wt. % antibiotic) bone cement releases most antibiotic than Palacos (8.4 wt % antibiotic) and CMW1 (4-5.3 wt % antibiotic) (Meinardi et al. 2016). High porosity and surface roughness in accordance with surface area augment the antibiotic agent release which may entail toxicity or may shorten drug release duration (Moojen et al. 2008; Bertazzoni Minelli et al. 2004).

Another major detriment that use of ALBC arises is bacterial colonization on its surface: Upon dropping below MIC and MBC concentrations, bacteria can start attaching to the ALBC surface to form biofilm which may end up developing

antibiotic resistance (D. Neut et al. 2001). More Gentamicin resistant bacteria (88 %) was found in patients with ALBC in comparison to patients with mere bone cement spacer (16 %) (Anagnostakos et al. 2008; Hendriks et al. 2005).

Briefly, ALBC has two major functions; planktonic kill of bacteria by releasing antibiotics and inhibiting bacterial colonization on the surface.

4. ANTIMICROBIAL POLYMERIC BIOMATERIALS

Discovery of antimicrobial peptides (AMP) in the 1980s triggered the shifting use of low molecular weight antimicrobial substances paradigm. A group of researchers found out that AMPs are able to kill both Gram-negative, Gram-positive and fungi (Z. Wang and Wang 2004). The new paradigm led to a new field of antimicrobial polymers.

The chemistry of synthetic polymers is suppose to address the disruption of bacterial membrane. As reported in the literature, synthetic antimicrobial polymers must fulfill a number of requirements to be effective:

1. Adequate contact with microbes.
2. Having enough cationic charge to induce adhesion to cell envelope of the microbe.
3. Presence of hydrophobic moiety for contacting cell membrane of the microbe.
4. Being toxic for microbes only not for mammalian cells (Matsuzaki 2009).

4.1. Antimicrobial Groups in Polymers

4.1.1. N-Halamines

It could be guessed from their names that N-halamines are halo-amines which is composed of amine group attached to a halogen atom (Figure 4.1). The mechanism of action for bactericidal activity of N-halamines was explained as result of interaction between negatively charged halogen atom inhibiting the enzymatic activity of the microbial cell which drives cell death. Particularly N-halamine compounds with chloride and bromine elements showed splendid performance against a wide range of microorganisms (Sun and Sun 2004).

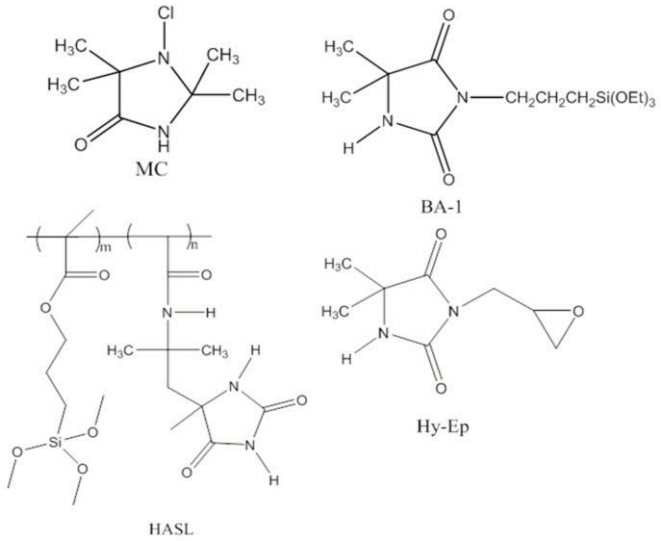


Figure 4. 1. Structure of some n-Halamines and n-Halamine precursors (Demir et al. 2017)

Chen (Chen and Sun 2006) synthesized a number of 3-alkyl-5,5-dimethylhydantoin derivatives consist bromide functionality with different alkyl chain length. Chlorination of the 3-alkyl-5,5-dimethylhydantoin derivatives yielded 1-chloro-3-alkyl-5,5- dimethylhydantoins (CADMH). Authors discovered that even low amounts of CADMH can bring remarkable antimicrobial potency.

Other halogenated structures than N-halamines such as perfluoroalkylated polymers also showed excellent antimicrobial properties. Guttard et al. (Guittard and Geribaldi 2001) synthesized micel structures composed of fluorinated bisammoniums to kill species as *S. aureus*, P. aeruginosa, C. albicans, and A. niger.

4.1.2. Peptide-like structures

Antimicrobial peptides are considered as analogous to other cationic polymers in terms of antimicrobial action. Their cationic moieties bind to anionic bacterial cell wall and disrupt it. Increased cell permeability causes apoptosis (Hancock and Sahl 2006).

Majority of the AMPs contain α-amino acids and fold to form a secondary structure when they are in contact with the cell membrane of the bacteria (Figure 4.2) (O'Neil and DeGrado 1990). Hydrophobic/cationic ratio determines the potency and selectivity of the AMP against microbes (Jiang et al. 2009). Wiradharma et al. studied antimicrobial activities of alanine, phenylalanine, leucine and charged AMPs; arginine and lysine (Wiradharma et al. 2011). They uncovered that lysine and leucine showed most selective activity.

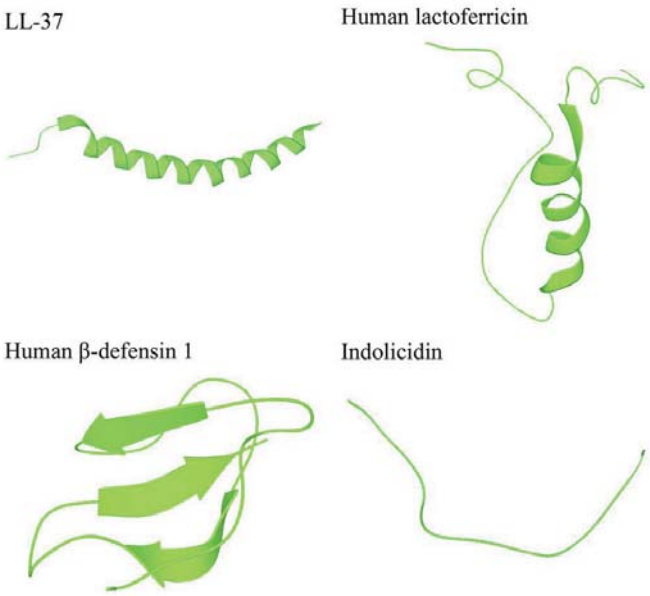


Figure 4. 2. Representative structures of three main antimicrobial peptide categories; LL-37 and human lactoferricin represent α-helical peptides, human β-defensin 1 represents β sheet peptides, indolicidin represents coiled peptides (Mahlapuu et al. 2016)

Other peptide-like polymers such as acrylamide and phenylene derivatives facilitated bacterial killing via hydrogen bonding with cell membrane (D. Liu et al. 2004) or modified amphiphilic polynorboranes by disturbing cell membranes (D. Liu et al. 2004).

4.1.3. Quaternary ammonium/phosphonium

Quaternary ammonium and phosphonium groups are another cationic chemical structure that annihilates bacterial membrane (Figure 4.3).

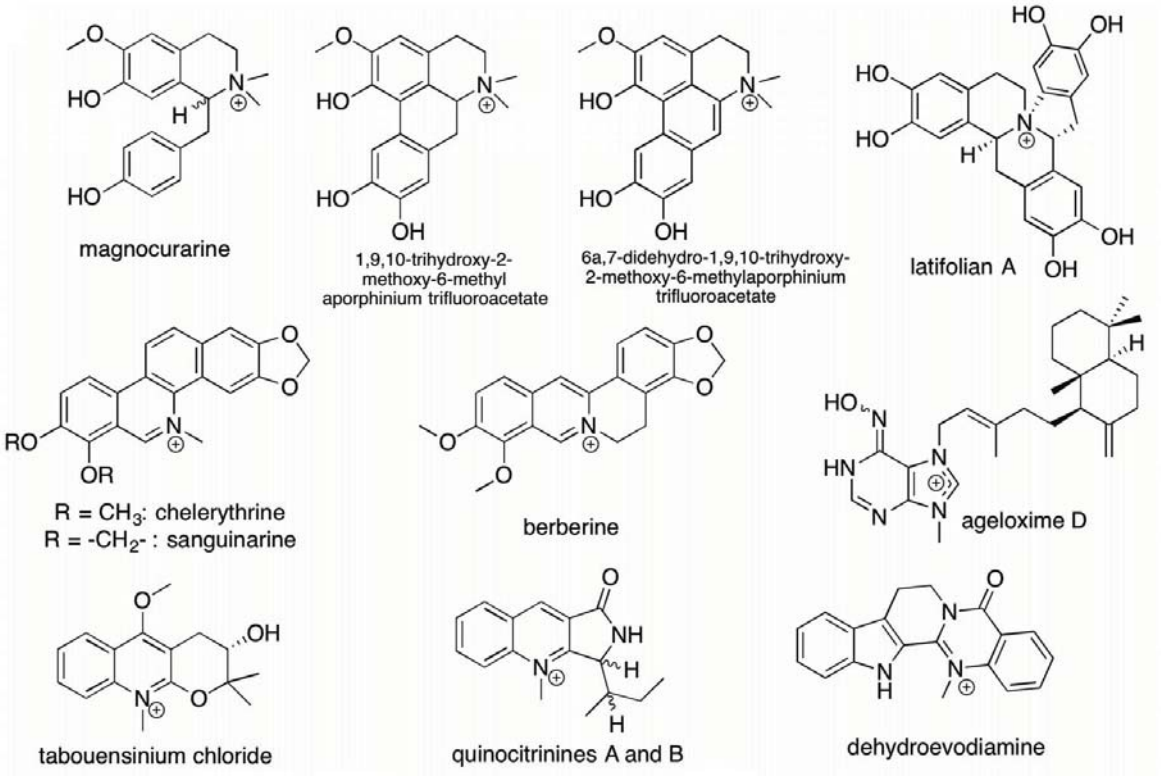


Figure 4. 3. Structures of quaternary ammonium examples (Joyce et al. 2016)

Muñoz-Bonilla and Fernández-García (Muñoz-Bonilla and Fernández-García 2012) proposed six subgroups of quaternary ammonium and phosphonium group containing structures:

1. Aromatic polymers: Quaternized poly(4-vinylpyridine) (P4VP) (Tiller et al. 2002) imidazole derivatives (E. B. Anderson and Long 2010).
2. Acrylic/methacrylic polymers: 2-(dimethylamino)ethyl methacrylate (DMAEMA) (Gottenbos et al. 2003).
3. Cationic polyelectrolytes: Poly(phenylene ethylene) (PPE) with pendant alkyl pyridinium group (Xing et al. 2009).
4. Polysiloxanes: Polysiloxanes modified with quaternary ammonium salt side group (Sauvet et al. 2003).
5. Branched polymers: Polyethylene imine (PEI) with cationic and hydrophobic functionalities (Gao, Zhang, and Zhu 2007), carbosilane dendrimers with ammonium termination (Ortega et al. 2008).
6. Polyoxazolines: Polyoxazoline with quaternary ammonium salt (Waschinski and Tiller 2005).

Recent studies showed phosphonium groups are superior to quaternary ammonium groups in terms of toxicity. They showed less toxicity to mammalian cells (Ornelas-Megiatto, Wich, and Fréchet 2012). Copolymer of styrene and divinylbenzene grafted with quaternary phosphonium group showed substantial antibacterial activity (Popa et al. 2003). Another study revealed that terpolymer which composed of polyacrylamide, polydiallyl dimethyl ammonium chloride and poly(4-penten-1-yl)triphenylphosphonium bromide and phosphonium group also exhibited antiviral activity against adenovirus (Xue et al. 2014).

4.2. Functionalization of Polymers

Conceptually grafting is one of the foremost techniques which is closely related to polymer functionalization. The goal of utilizing grafting is to impart reactive moieties to the polymeric substrate. Those reactive sites are thought to precede the reaction to generate altered monomers or polymers. Polymer functionalization techniques are inevitable to derive wider range of polymers.

4.2.1. Methods for polymer functionalization

One can define polymer functionalization as introducing reactive (mostly referred as polar groups) groups to relatively inert polymer. There are several techniques for polymer functionalization such as:

- Direct copolymerization: Two types of monomers react to form a copolymer, one with functional group such as copolymer of maleic anhydride (MA) and styrene (St) (Hurtgen et al. 2011) or polypropylene (PP) and polyethylene glycol methacrylate (PEGMA) (Kawahara et al. 2010).
- End functionalization: Chain end of the existing polymer is chemically modified, or chain growth is interrupted or first two techniques are used simultaneously.
- Grafting: There are three grafting routes; grafting from, grafting through and grafting onto. Grafting onto involves reaction of polymer with functional group on the chain end and nonfunctional polymer. Grafting through is performed by using low molecular weight polymers with polymerizable chain ends particularly vinyl groups. This technique makes it possible to construct several topologies such as brushes, adjacent grafts, centipede

and barbwire (Sheiko, Sumerlin, and Matyjaszewski 2008). Grafting from is widely being used which involves growth of monomers to polymers starting from polymer backbone (Bonilla-Cruz et al. 2008). In a major advance, 2-methacryloyloxyethyl phosphorylcholine was grafted from in order to create a lubricious UHMWPE surface (Ishihara 2019).

Some applications may require the modification of the polymer surface instead of polymer bulk. Polymer surfaces could be modified via techniques such as direct chemical modification(Chung 2002), ozone treatment, corona discharge, glow discharge, e-beam(Sakurai et al. 2004), gamma (Filho, Furtado, and Gomes 2006) and UV irradiation (W. Yang and Ranby 1996) are also used for surface modification of polymers. Surface grafting offer multilateral end products with new properties which includes biocompatibility, hydrophilicity, antifouling, lubrication, antifogging (Uyama, Kato, and Ikada 1998).

For instance, hydrophilic surface on poly(ethylene terephthalate) (PET) was created by grafting polyacrylamide (PAM) via UV-initiated grafting (Zhou et al. 2011).

Covalently attaching functional groups are capable of initiating grafting on the surface by either conventional free radical polymerization (FRP) or controlled radical polymerization.

Surfaces could also be functionalized physically. One technique is physical immobilization of targeted antimicrobial polymers on the surface, i.e., another one is layer-by-layer deposition (Guyomard et al. 2008) or dip coating (Park, Wang, and Klibanov 2006). Despite these techniques are facile to apply, they have limitations such as lack of mechanical stability against harsh conditions. Weak interfaces of layers might leach from the surface and cause rapid depletion of the biocidal structures (Gour, Ngo, and Vebert-Nardin 2014).

4.3. Chemical Modifications of Biomaterials with Antimicrobial Functionality

In order to cleanse the environment out of microorganisms, combination of multiple functionality would be more favorable such as colonization inhibition, bacterial eradication, contact-killing (Yu, Wu, and Chen 2015).

Bacteria repelling and bactericidal agent releasing materials embodies an inherent low adhesive polymeric material that demonstrate diminished bacterial adhesion (up to few orders of magnitude). Such materials are poly(vinyl alcohol)(PVA), PEG-bound copolymers, poly(acrylic acid) derivatives hydrogels. Those hydrogels are loaded with antibiotics or other bactericides to kill bacteria simultaneously while repelling them (Rodríguez-Hernández 2017). Another approach in this category is an antimicrobial coating with silver ion releasing property (Ho et al. 2004). In this approach, glass surface covalently functionalized silanoyl groups which is then tied to poly(ethylene imine) (PEI) groups to complex with silver nanoparticles. Bacterial repelling is employed by poly(ethylene glycol) (PEG) layer attached to poly(2-hydroxyethylacrylate) network (Figure 4.4).

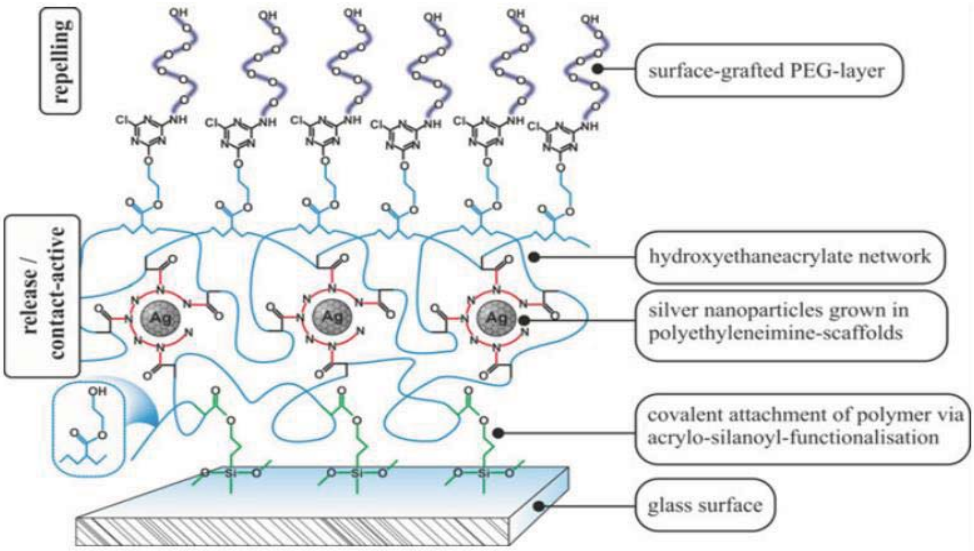


Figure 4. 4. Dual functional repelling-releasing system (Ho et al. 2004)

While PEG ends of the network repels microorganisms, PEI releases silver nanoions to kill planktonic *S.aureus.*

Another double functionality material is temperature labile polymer brushes which transition from being bactericidal to repellent (Figure 4.5) (Laloyaux et al. 2010). The structure is consisted of an antimicrobial peptide (Magainin) grafted oligo(ethylene glycol) methacrylate (OEGMA). At room temperature OEGMA chains are elongated so, Magainin groups are accessible by microbes in contact. Nevertheless, at elevated temperatures than 35 °C OEGMA chains shrink, peptide

structures become inaccessible thereby OEGMA repels bacteria away. This on/off system could be reversibly activated by heating/cooling cycles.

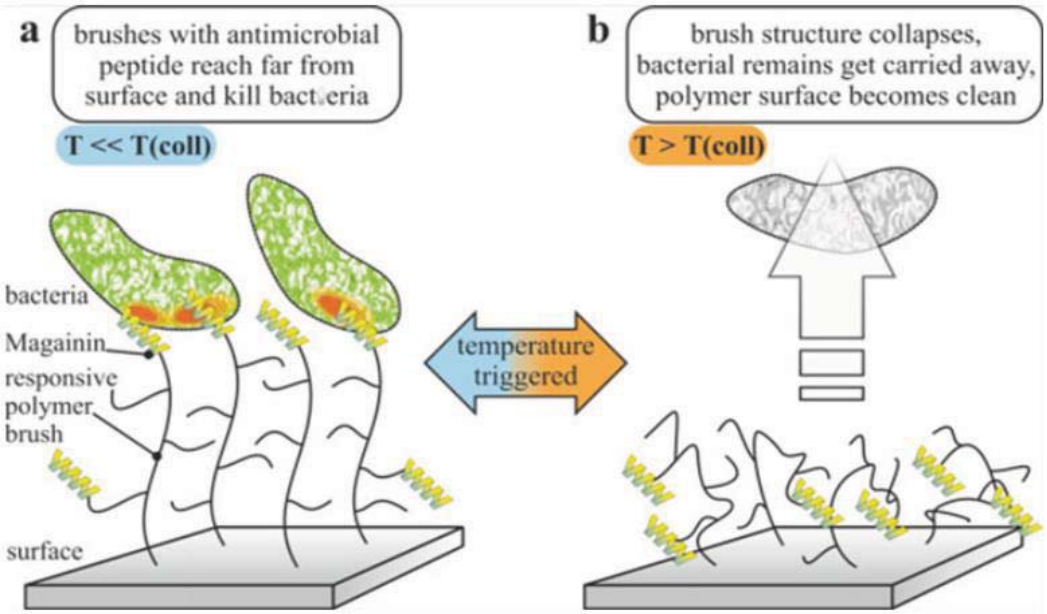


Figure 4. 5. Surface with contact-killing and repelling functions that kills bacteria below 35 °C (a) and repel above 35 °C (b) (Laloyaux et al. 2010)

Another dual function structure is bactericidal compound releasing and contact-killing type of materials. It was reported that antimicrobial N,N-dimethyl-dodecylammonium (DDA) grafted poly(2-ethyl-1,3-oxazoline ($PEtO_x$) coated by cellulose is able to kill approaching bacteria (Bieser, Thomann, and Tiller 2011). The system works as follows: When bacteria in contact with bactericidal moiety of the structure die, it unleashes an enzyme called cellulase. The cellulase enzyme degrades the cellulose layer and subsequently more DDA is released to kill the bacteria again (Figure 4.6).

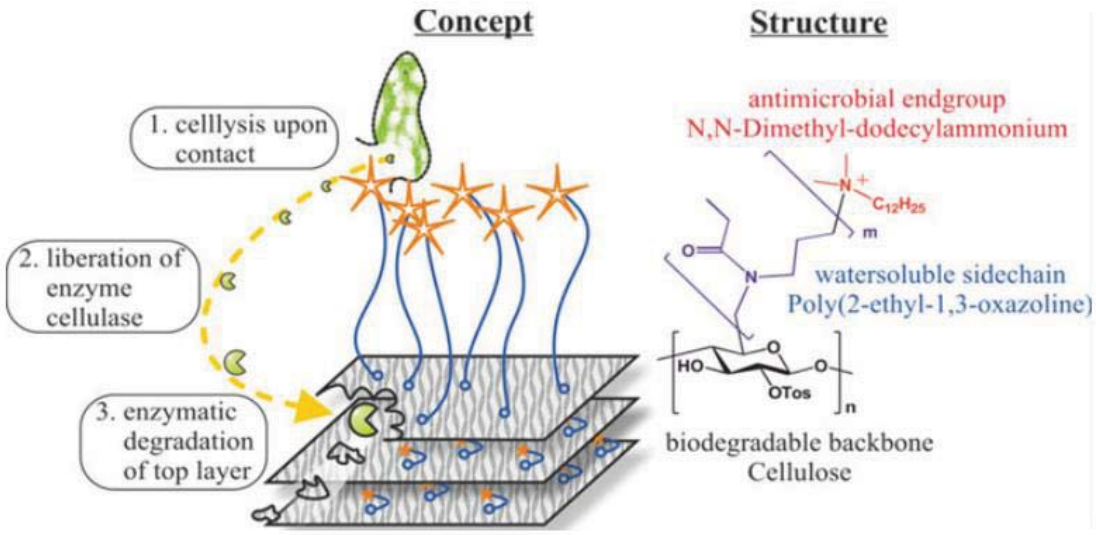


Figure 4. 6. Cellulose based coating with bactericidal DDA groups (Bieser, Thomann, and Tiller 2011)

4.3.1. Dual Functionality Antimicrobial surfaces, coatings

Having dual functionality is undoubtedly important when battling against bacteria. However, methods to fabricate such materials are even more important and of interest for many studies. Tethering polymeric brushes on the surfaces has been presented as one of the promising enabler to generate antibacterial surfaces or coatings and showed to reduce bacterial attachment on the surface (Rzhepishevska et al. 2013; W. J. Yang et al. 2012). Amongst other brush forming polymers, Poly(N-isopropylacrylamide) (PNIPAAM), a thermal stimuli responsive polymer come to fore by virtue of interchangeable structure below/above its lower critical solution temperature (LCST) (Yu et al. 2013; Cunliffe et al. 2003). In the literature, three major approaches have been extensively studied; 1) bactericidal polymer brushes, 2) polymer brushes modified with bactericidal compound or releasing bactericidal compound, 3) antifouling polymeric brushes.

Intrinsic polymer moieties which destroy bacteria is almost merely consisted of quaternary ammonium group attached polycationic structures (Figure 4.7) (Krishnamoorthy et al. 2014). One of the most important quaternary ammonium group consisting of polymers is poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) with ranging alkyl group length (Evren Özçam et al. 2012; X. Liu et al. 2012).

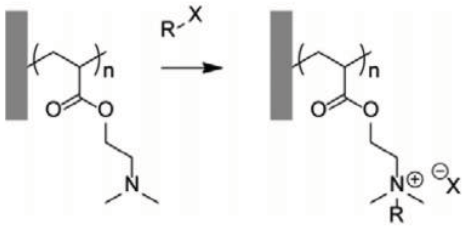


Figure 4. 7. Structure of quaternary ammonium moiety containing polymeric brush (Krishnamoorthy et al. 2014)

There is reconciliation of scientists on the mechanism of action for bacterial killing with the quaternary moieties which is the disruption of bacterial cell membrane and leakage of cell compounds such as ions and metabolites (Vooturi and Firestine 2010). For gram-negative bacteria, it was studied that polycations destabilize cell membrane through increasing the permeability of the membrane by lipopolysaccharide (LPS) crosslinking cations (Helander et al. 1997). Unalterably switchable zwitterionic poly(carboxybetaine methacrylate) (PCBMA) brushes were synthesized to kill bacteria first and then release cationic moieties upon hydrolysis to free contact-killed bacteria (Cheng et al. 2008; Zhao et al. 2013). The surfaces were able to reduce the number of bacteria 3 orders of magnitude and release 98 % of them during hydrolysis. One of the other prominent double-faced groups are acrylates such as; 2-carboxyethyl acrylate (CEA, the acrylate equivalent of 2-carboxyethyl methacrylate, CEMA) (Mi et al. 2010). At low pH values, carboxylate groups get protonated, thereby can kill bacteria and at high pH values the surface gets antifouling.

Polymeric brush functionalization with antimicrobials are two ways, either antimicrobial agents impregnated in the brush structure or covalently linked to the brush (Figure 4.8).

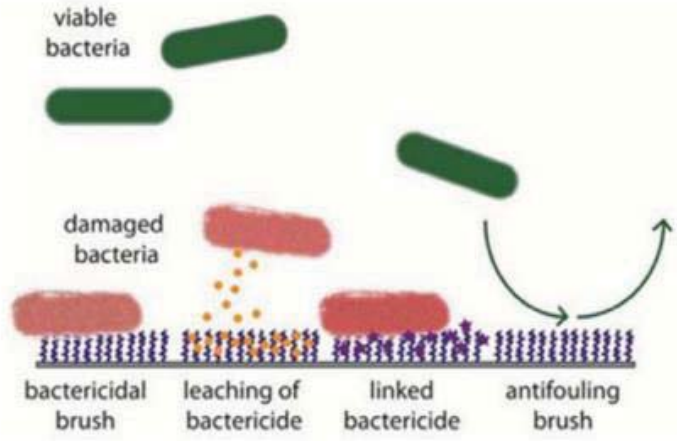


Figure 4. 8. Polymeric brushes with bactericidal functionalities (Krishnamoorthy et al. 2014)

5. COMPOSITE BIOMATERIALS

Drug release systems has been unfolded to provide temporal and spatial conveyance of drugs to the particular site of the body. Drug release systems have other roles as protecting drug from elimination, degradation and help drug to improve compliance as well as to enhance quality control in production. There are several effects on drug release mechanisms such as water diffusion, drug diffusion, drug dissolution, polymer swelling, polymer degradation.

Depending on the boundary conditions of the drug delivery device and the drug payload drug solubility ratio, four main types of drug delivery devices exist (Figure 5.1).

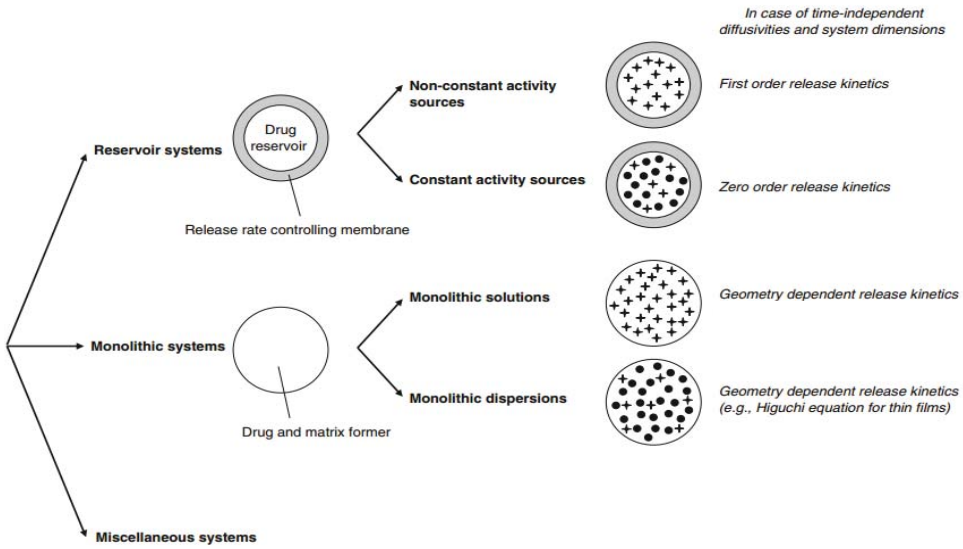


Figure 5. 1. Classification of drug delivery devices depending on drug payload and boundary conditions. (Siepmann and Siepmann 2008)

If the drug encapsulated in a shell structure and that shell act as a membrane, the device is called as reservoir system. Adversely, if the drug is somewhat dispersed in the continuous polymeric matrix, the device is then called as monolithic device. When the amount of drug in reservoir system more than its solubility, the system is considered as constant activity source since it releases drug proportionately with time. Nevertheless, for the instances reservoir system has less drug than its solubility, the device is called as non-constant activity source. Non activity sources'

release will decrease with time since the released drug in the membrane is not replaced by another drug particle immediately.

Analogously, monolithic devices also has two subtypes; when drug is more than its solubility, it is partially dissolved in the matrix, the remainder is dispersed as in solid drug form. In order for drug to diffuse out from the continuous polymeric phase, drug must principally dissolved first. For any diffusion dependent system, drug diffusion is the slowest step, therefore the rate determining step.

UHMWPE has been studied as drug carrier polymeric matrix whereas drug incorporated conceived to contribute its wear resistance, oxidation susceptibility and mechanical strength (Oral and Muratoglu 2015; Oral et al. 2017; Puértolas and Kurtz 2016). Particulates that are mixed with UHMWPE can be classified in two groups; those which are phase separated i.e. immiscible and those which aren't phase separated i.e. miscible. Immiscible additives are exemplified as sodium chloride (Maksimkin et al. 2013), graphene (Lahiri et al. 2014), carbon nanofibers (Galetz et al. 2007; Sui et al. 2009), hyaluronic acid (Fang, Leng, and Gao 2005) whilst miscible ones are peroxides (Oral et al. 2017), α-tocopherol (Oral and Muratoglu 2015; Shibata and Tomita 2005).

In the 1970's, carbon fiber brought to the fore as a filler to improve wear resistance of UHMWPE and the manufactured orthopaedic implants called Poly II (Zimmer Inc., Warsaw, Indiana, USA) even launched to the market (Wright, Rimnac, et al. 1988; Wright, Astion, et al. 1988). Although biocompatibility tests showed no significant adverse reaction after 12-15 month of implantation (Groth et al. 1978), the results were calamitous since the material had worsened ductility, crack resistance (Connelly et al. 1984), and fiber embedded surface was disturbing the metallic counterface (Peterson, Hillberry, and Heck 1988).

Notwithstanding the fact that Poly II was manufactured as commercial use, there were opposing studies which reported poor performance of injection molded carbon fiber reinforced UHMWPE due to poor integration of the components (Sclippa and Piekarski 1973). After all, investigation on the Poly II retrievals revealed that they survived both cruciate retaining (CR) and posterior stability (PS)

(Kurtz 2009).

Hyaluronan, simplest glycosaminoglycan of extracellular matrix, incorporated as micro filler to UHMWPE (Zhang et al. 2006, 2007). Hyaluronan is the lubricity source of natural cartilage. By no means, incorporation of hyaluronan into UHMWPE is not a downright task due to their water affinity difference. It was reported that when hyaluronan is complexed with quaternary ammonium cations and consequently silylated, it gains ease of diffusion into porous UHMWPE matrix (Zhang et al. 2007). Diffused modified hyaluronan is then chemically crosslinked *in situ* and hydrolyzed to hyaluronan (Zhang et al. 2007). In the last step, the microcomposite is compression molded and machined to manufacture pin for pin-on-disk (POD) test which was subsequently done and showed moderate wear reduction for multidirectional POD wear test (Zhang et al. 2007).

Along with growing interest of most studies in improvement of wear resistance and mechanical strength, biomimicking was another concern for polymeric bone substitutes (Kurtz 2009). To do that, few exploratory studies were adopted for hydroxyapatite (HA) incorporation in high density polyethylene (HDPE) (Bonfield 1988). Hydroxyapatite with its low molecular weight was used to prepare microcomposites with high density polyethylene. Given the low molecular weight components, HA/HDPE composites have multilateral practicality in terms of processability including injection molding (M. Wang et al. 2000). Studies showed HA/UHMWPE is both bioactive and biocompatible (Di Silvio, Dalby, and Bonfield 2002; S. M. Rea, Best, and Bonfield 2004; Susan M. Rea et al. 2004). One major drawback of HA/UHMWPE microcomposites is it exhibits failure under high load bearing conditions. Incorporating HA in UHMWPE was more challenging when it comes to homogenous dispersion. Referring to the literature, it was managed to disperse HA in UHMWPE matrix by various methods such as ball milling, extrusion of swollen UHMWPE with pharmaceutical grade paraffin oil (Fang, Leng, and Gao 2006). Molded UHMWPE/HA samples showed superior mechanical strength; 7 GPa of elastic modulus and 375 % of elongation at break (Fang, Leng, and Gao 2006). For UHMWPE/HA bioactive composites, to be a predicate implant device, it is required biocompatibility tests.

6. LIVING RADICAL POLYMERIZATION

Living radical polymerization (LRP) is one of the subsets of free radical polymerizations that generates radicalic terminals which don't terminate or transfer and continue polymerization as monomer is fed in the reaction medium. Through its inherent nature, radical polymerizations terminate where as LRP techniques have minimum termination step which renders the technique to have control over molecular weight and molecular weight distribution of the polymer. LRP techniques have several wished aspects such as, plausibility for wide range monomers, tolerance for various functionalities and can be carried out under mild reaction conditions. Conventional free radical polymerizations are not suitable for synthesizing polymers with complex architecture or with site specific functionality as LRP techniques are. LRPs are categorized in three groups: 1) Atom Transfer Radical Polymerization (ATRP), 2) Reversible Addition Fragmentation Chain Transfer Polymerization (RAFT), 3) Nitroxide-mediated Polymerization (NMP). Iodine transfer polymerization (ITP), single electron transfer-degenerative transfer living polymerization (SET-DTLRP), organotellurium mediated living radical polymerization (OMLRP) are other living radical polymerization techniques although to a lesser extent.

6.1. RAFT Polymerization

The RAFT polymerization has changed the era of controlled radical polymerization. It was invented simultaneously by a research team in Commonwealth Scientific and Industrial Research Organization (CSIRO) in Australia and by a group of researchers in France. The group in France called the technique as macromolecular design via the interchange of xanthates (MADIX) (Charmot et al. 1999). Despite NMP and ATRP, RAFT technique is based on a degenerative chain transfer and doesn't rely on sustained radicalic species. Drug and gene delivery, tissue engineering, regenerative medicine, membrane science, bioconjugation are the areas where RAFT technique is used (Barner-Kowollik 2008).

RAFT polymerization is governed by a versatile RAFT agent. Figure 6.1 shows the chemical structures of four generic RAFT agents. The R group is the site where monomers start to add up and grow subsequently the Z group activates thiol bond toward monomer addition and makes attached radical stable. Thereby two polymeric chains grow on both sides concurrently.

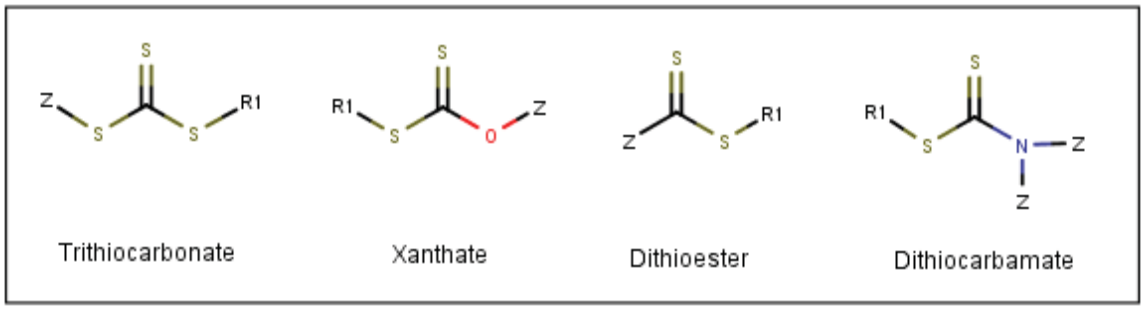


Figure 6. 1. Types of RAFT Agents

In the mechanism of RAFT polymerization, the first step is comprised of initiation through generation of a free radical (step 1) (Figure 6.2) by one of the various sources such as thermal (T. P. Le et al. 1998), UV (Lu, Yang, and Cai 2005), γ radiation (Millard et al. 2006) and pulsed laser irradiation (Junkers et al. 2005). Oligomeric radicals are formed and attack RAFT agent (step 2). The radicalic intermediate (2) has two reversible paths which it can either transform back to RAFT agent and oligomeric radical or can proceed to an oligomeric RAFT agent (3) and reinitiating R radical. Homopolymer chains grow as monomer adding up (step 3). Upon initiation, polymer chains grow on both sides of the intermediate 4 is propagation step (step 4). Termination for the RAFT polymerization occurs in two ways; 1) combination, 2) disproportionation (step 5).

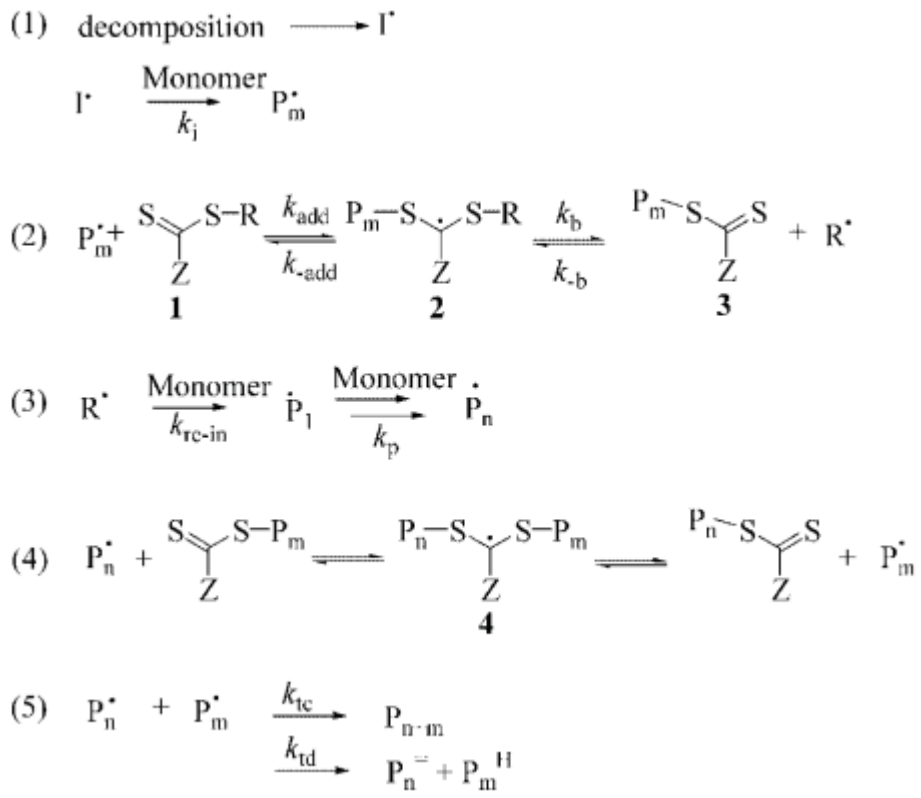


Figure 6. 2. RAFT polymerization mechanism**.**

6.2. Copolymer Synthesis by RAFT Polymerization

RAFT polymerization is a multilateral technique that enables tailoring complicated structures. It is plausible to synthesize various copolymers comprising random, alternating, block and graft copolymers.

Graft copolymers include a backbone polymer and attached branches as second polymer. Graft polymers are achieved by RAFT polymerization via two main routes; grafting to and grafting from techniques. The grafting through technique emerges as polymerization of a polymeric chain (macromonomer) through the vinyl groups on them. The grafting from technique takes place as attachment of RAFT agent to a substrate and propagating from there (Patton and Advincula 2006).

Barsbay et al. reported an efficient way of modifying polyethylene/polypropylene (PE/PP) nonwoven fabrics by RAFT mediated poly(acrylic acid) (PAA) grafting initiated by gamma rays (Barsbay and Güven 2013, 2009).

6.3. Use of RAFT Technique for Drug Delivery Applications

The foundation of precise drug delivery and nanomedicine approaches first laid in the 1980s and 1990s (Boyer et al. 2009). It is widely acclaimed that controlling hydrodynamic volume, morphology, chemical composition and structure of polymers are important aspects for precise delivery. As RAFT polymerization brings architectural variety, defined end groups or pendant functionalities, narrow polydispersity, it becomes one of the desired techniques to generate advanced polymeric systems for drug delivery (Figure 6.3) (Boyer et al. 2009).

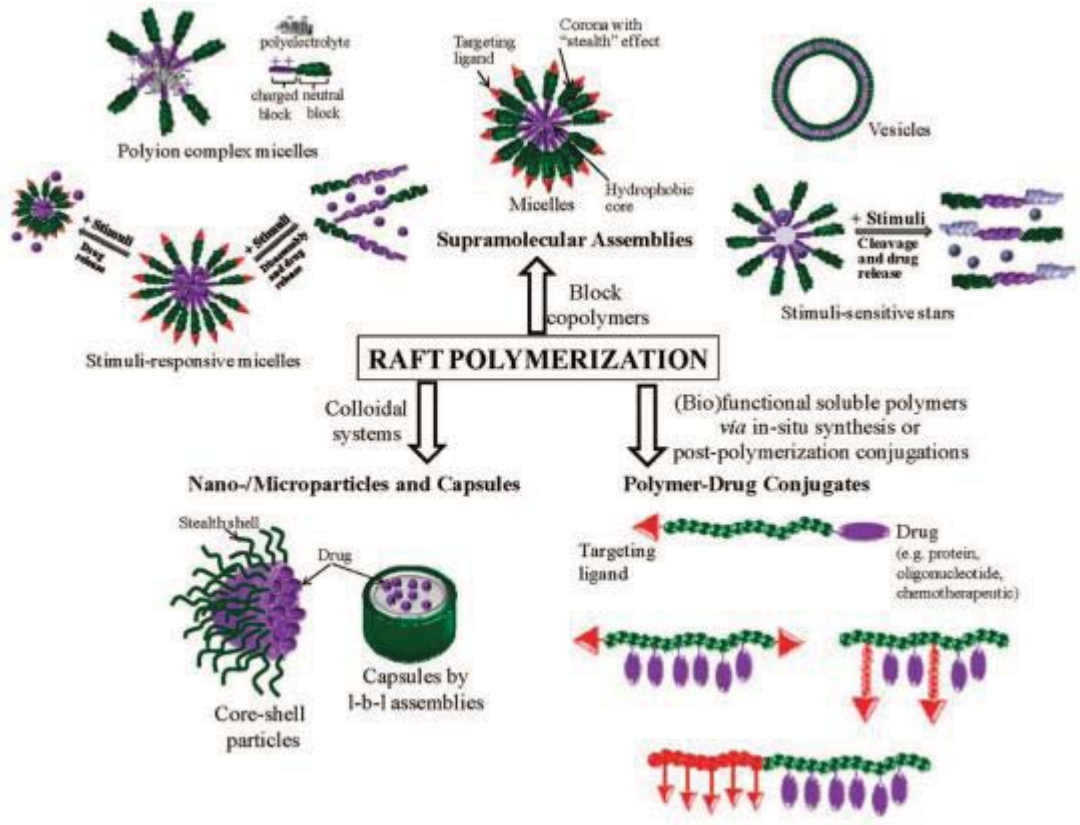


Figure 6. 3. Preparation of controlled drug delivery systems by using RAFT polymerizations (Boyer et al. 2009)

There is limited literature about RAFT agents' potential toxicity and biocompatibility (Pan et al. 2008). This problem can be eliminated by RAFT agent removal through post-polymerization treatments of polymers (Chong et al. 2007). Those post-polymerization techniques involve thermolysis, transformation to thiol, oxidation-induced end-group removal, radical-induced end-group removal and radiation-induced end-group removal.

PJI treatment could have devastating results unless two important aspects of it were taken care of; appropriate dosage control and sustainable antibiotic treatment. They both require great attention otherwise could be disastrous for patients and eventually turned into an immense public health problem. Antibiotic dosage control must be so delicate that it is not above toxicity levels and not below MIC which could lead to antibiotic resistance. Sustainable antibiotic release is essential for implants to avoid bacterial colonization which will fail patients to an additional joint replacement surgery.

Under consideration above reasons, it is vital to tailor a drug-releasing implant which ensures delicate dosage control and sustainable antibiotic release. In this thesis study, RAFT polymerization was used to synthesize UHMWPE-g-PHEMA with controlled the molecular weight and the molecular weight distribution of PHEMA to control the release rate and sustainability of gentamicin sulfate.

7. EXPERIMENTAL

7.1. Chemicals

UHMWPE with weight average molecular weight of $2x10^6$ Da. was used in the experiments (UHMWPE, GUR1020, USA) (Figure 7.1). 2-hydroxyethyl methacrylate (HEMA) monomer with 97 % purity which contains ≤250 ppm monomethyl ether hydroquinone as an inhibitor, S-dodecyl-S'-(α,α'-dimethyl-α''-acetic acid)trithiocarbonate) (DDMAT) as reversible addition fragmentation chain transfer (RAFT) agent with 98 % purity, benzophenone (BP) as Nourish Type II photosensitizer and acetone with 99.9 % purity were purchased from Sigma Aldrich, USA. Gentamicin sulfate (GS) was purchased from VWR, UK. Bone cement Palacos R-G with 61.2 g of sterile powder consisting of 50.3 g Poly(methylmethacrylate), 9g Zirconium dioxide, 0.67 g Benzoyl Peroxide, 0.75 g Gentamicin base (as sulphate) and ampoule with 30 ml of sterile liquid consisting of 27.6 g Methyl methacrylate, 0.6 g N,N-dimethyl-p-toluidine was purchased from Heraeus, USA. All the chemicals were used as received.

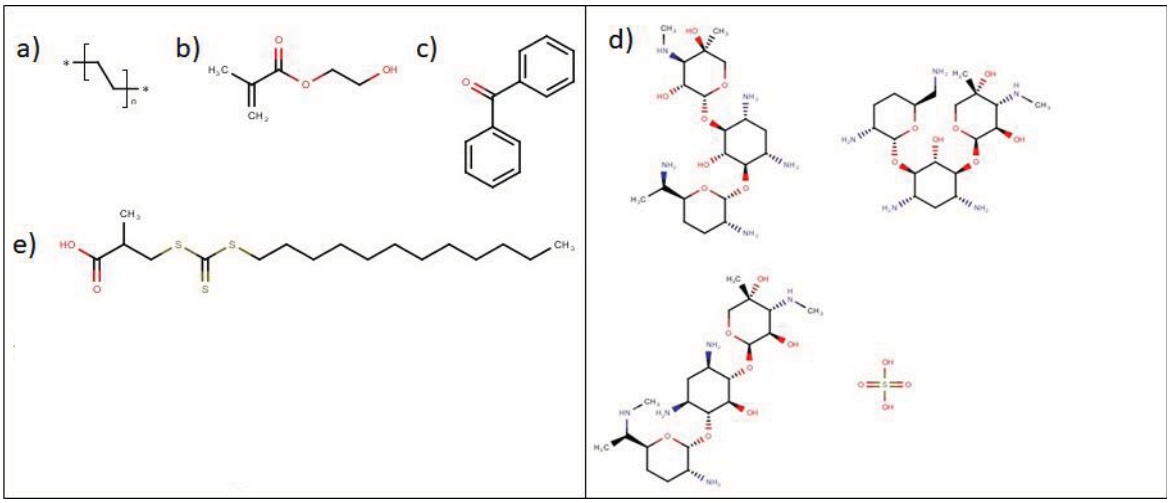


Figure 7. 1. Chemical structures of a) Polyethylene, b) 2-hydroxyethyl methacrylate, c) Benzophenone, d) Gentamicin Sulfate isomers, e) S-dodecyl-S'-(α,α'-dimethyl-α''-acetic acid)trithiocarbonate).

7.2. Grafting 2-hydroxyethyl methacrylate from UHMWPE

At the first stage of studies HEMA was grafted from UHMWPE in powder form by

UV-initiated radical polymerization (UVIRP). Grafting was optimized upon testing varying monomer concentrations in feed for polymerization, varying distances between UV lamp and reaction medium, varying durations of UV irradiation. This part of experiments was performed by using 300 W Osram Vitalux 230 V E27 UV lamp (Germany) (Figure 7.2).

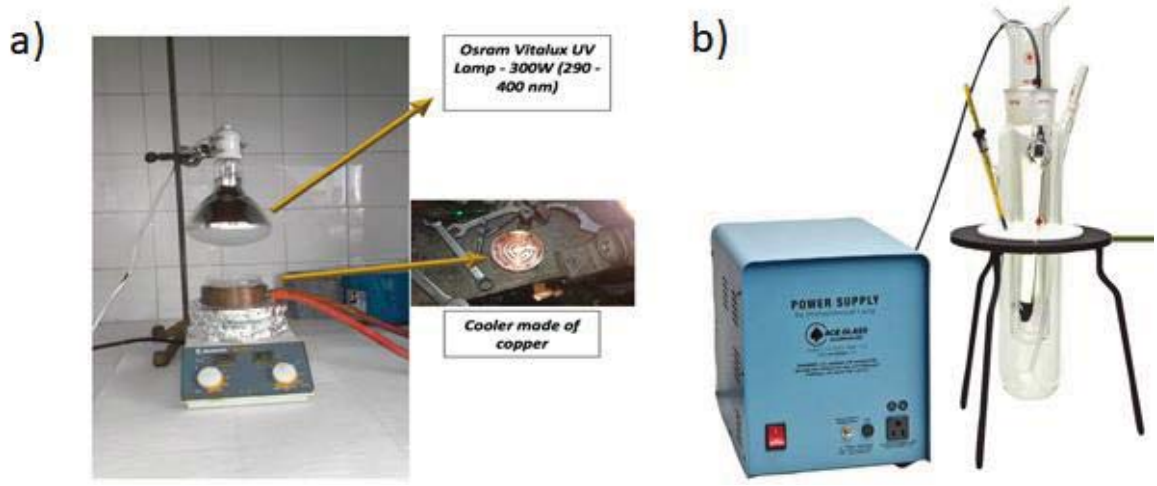


Figure 7. 2. a) Osram Vitalux UV lamp with copper cooler, b) Ace Glass jacketed UV reactor

Primarily, UHMWPE was deposited with 1% w/w BP from acetone in a fume hood at room temperature for 24 hours to condition UHMWPE for UVIRP. Then, BP deposited UHMWPE was put into a glass petri dish in water:acetone, 1:1 mixture by volume. Subsequently, HEMA was added into the petri dish. The petri dish was covered with polyethylene stretch wrap to ensure the reaction medium is sealed from ambient air. In order to keep the temperature of the reaction at 25°C (± 2°C) and minimize thermal polymerization, a cooler made of copper was placed underneath the petri dish during the reaction (Figure 7.2). Resulting copolymer of UHMWPE-g-P(HEMA) was washed for two days in ethanol. 5, 10, 15, 20, 30% were selected as HEMA percentages to control grafting yield of P(HEMA) from UHMWPE. Grafting was conducted for an irradiation time 30, 60, 90, 120, 150, and 180 min.

At the second stage of studies, grafting conditions' optimization was followed by the adoption of those conditions to larger reactor system equipped with a UV bulb, an immersion well and a reaction vessel (Figure 7.2). Both the immersion well and

reaction vessel are equipped with jacket for water cooling. Circulating water was kept at 25°C by using a circulator bath. Reaction medium was stirred thoroughly with the aid of a magnetic stirrer and a stir bar at 250 rpm. Resulting copolymer was filtered through buchner funnel and washed according to the previous protocols. In comparison to small batch experiments which was explained above, only monomer concentration and duration were optimized.

7.3. RAFT mediated grafting of PHEMA from UHMWPE via preirradiation

In this section, 2-hydroxyethyl methacrylate was grafted from UHMWPE in a jacketed UV reactor in the presence of RAFT agent DDMAT. First, BP deposited UHMWPE was added to UV reactor, then, water:acetone mixture was added. Thereafter, the reactor was purged with Argon for 30 min. The solution was irradiated for an hour to excite BP and create carbon-centered radicals on UHMWPE. The well was kept at 25 °C by circulating water. Upon completion of the irradiation step, the DDMAT in acetone:HEMA mixture was added to the medium and the reactor was kept at 60 °C by water circulation for 60 and 120 min. Grafted samples were washed in ethanol for three times for two days followed by drying in vacuum oven for three days at room temperature. For RAFT polymerization, 20 % initial monomer concentration was selected whereas three monomer/RAFT agent concentrations were chosen as ([350]/[1], [475]/[1], [800]/[1]).

7.4. Antibiotic Blending with UHMWPE and UHMWPE-g-P(HEMA)

Preparation of Gentamicin Sulfate UHMWPE/(GS) or UHMWPE-g-P(HEMA)/GS blends was comprised of:

- providing UHMWPE or UHMWPE-g-P(HEMA),
- blending UHMWPE or UHMWPE-g-P(HEMA) with GS (sieved to <75 μm) by a planetary mixer,
- dehydrating the pelletized UHMWPE/(GS) or UHMWPE-g-P(HEMA)/GS blend by vacuum treatment in a vacuum oven at 45°C or microwave treatment,
- completely consolidating the vacuum treated UHMWPE/(GS) or UHMWPE-g-P(HEMA)/GS pellet.

For UHMWPE-g-PHEMA/GS samples GS payloads were decided to be 2, 4, and 6% whereas 1, 2, 4, 6, 8, and 10% were selected for UHMWPE/(GS) samples.

7.5. Compression molding of the blends

UHMWPE and UHMWPE-g-P(HEMA) samples were consolidated by using a mold (85x55x50 cm), an insert plate and a plunger made of stainless steel. Aluminum bronze type of mold assembly was used for the samples comprising heat sensitive antibiotics (Figure 7.3). All of the consolidations were carried out in a compression molding press (Carver Auto-Series Model 3895 Press, 30 ton capacity, Carver Inc., IN, USA) at various temperatures under various loads for various durations. Synthesized copolymers and antibiotics were added to UHMWPE powder and the blend was further mixed using a mechanical mixer (Glen Mills Turbula T2F Mixer, NJ, USA) operated at 72 rpm for 30 minutes.

All of the blends were consolidated to 3 mm and 14 mm thick tibial shape blocks in a custom-made aluminum-bronze alloy mold assembly (Figure 7.3) by using an industrial press.

Figure 7. 3. Components of the aluminum-bronze alloy mold; mold (left), insert plate (middle), plunger (right).

The mold has three parts; female component with cavity with dimensions 8.5 x 5.0 x 5.0 cm, plunger and 3 mm thick insert plate. First, the insert plate was placed in the female component, then, the powder blend was poured in the mold cavity, finally, plunger was put on top. After the mold components were assembled, it was

placed between press platens which were already heated to 170 °C followed by closing platens to apply 20 MPa pressure. The molding recipe was comprised of two segments; the first segment involved heating of the mold for 8 minutes for 3 mm thick blocks and 45 minutes for 14 mm thick blocks. The second segment involved cooling down to 20 °C by water circulating in the platens for 15 minutes.

As the molding was complete consolidated block was taken out and used for further experiments.

7.6. Characterization

7.6.2. Structural characterization of PolyHEMA grafted UHMWPE

7.6.2.1. FT-IR/ATR

UHMWPE samples were structurally characterized by FT-IR/ATR (Perkin Elmer Spectrum Model 2) after grafting poly(HEMA). ATR attachment is equipped with single-reflecting diamond crystal. Spectra were collected with scan number 16 and resolution 4 cm^{-1}. To ascertain the abundance of poly(HEMA), carbonyl peaks at around 1720 cm^{-1} were compared after the C-H stretching peaks at around 2920 cm^{-1} were normalized.

7.6.2.2. CHNS/O Analysis

Elemental composition of poly(HEMA) grafted UHMWPE was analyzed by Flash 2000 NC (Thermo Scientific) elemental analyzer equipped with Al/As 3000 series autosampler. Samples were combusted in tin pans with 2,5-Bis(5-tert-butyl-2-benzoxazolyl)thiophene (BBOT) as calibration standard and vanadium pentoxide (V_2O_5) as catalyst.

7.6.2.3. X-ray Photoelectron Spectroscopy

X-ray photoelectron spectroscopy (XPS) was performed on an XPS spectrometer (Thermo Scientific K-Alpha) with monochromatic Al Kα as X-ray source. The Chamber of the equipment were kept at pressure of 2 x 10^{-9} Torr and at a temperature of 25^{O}C.

7.6.2.4. Contact Angle Measurements

Water contact angle of all pre-consolidated and powder specimens were measured from glass-contact surfaces with a goniometric contact angle measurement device (Easy Drop Gonyo-meter, Krüss, Hamburg, Germany). 10 µl deionized water was used in every measurement. Contact angles were obtained from three different spots of the sample surfaces. PHEMA grafted samples were pressed for 2 mins at 10 ton/cm^2 by a manual hydraulic press prior to contact angle measurements.

7.6.2.5. Tensile Strength Testing

Tensile specimens (type V) were stamped out of 3.2-mm-thick dog bone-shaped sections in agreement with the American Society for Testing Materials (ASTM) D638. Specimens were tested on an Insight 2 model MTS machine (Minneapolis, Minnesota, USA) at a crosshead speed of 10 mm/min. In order to determine the elongation at break (EAB), a laser extensometer was used to determine gauge displacement by reading reflective tapes mounted on the specimen. Ultimate tensile strength (UTS) and yield strength (YS) and elastic modulus (E) were calculated in accordance with ASTM D-638 standard.

7.6.2.6. IZOD Impact Strength Testing

Sample bars of 6.35 x 12.7 x 63.5 mm were double notched to a depth of 4.57 $\pm$ 0.08 mm according to the ASTM D-256 and tested according to ASTM F-648 with CEAST Instron model impact tester. All of the specimens were tested at room temperature. The impact toughness was reported as the average value of n = 3 specimens in kJ/m^2.

7.6.2.7. Pin-on-Disc (POD) Wear Testing

Cylindrical pins (9-mm diameter and 13-mm long, n=3 each) were machined using a CNC mill (ShopBot Tools Inc., NC, USA) from the materials that were supposed to be POD tested. The pins were wear tested against polished Co-Cr-Mo discs at 2 Hz in undiluted, preserved bovine serum as a lubricant that contains Penicillin-Streptomycin (Sigma, USA) and EDTA (Fisher Chemical, USA). Wear rates were calculated gravimetrically by weighing the samples every 0.15 million cycle for 1.1

MC totally. The wear rate was determined by a linear regression of the weight loss as a function of number of cycles from 0.5 to 1.1 MC.

7.6.2.8. Differential Scanning Calorimetry

The specimens for differential scanning calorimetry were prepared by cutting thin slabs (8-10 mg) for testing. The DSC specimens were weighed with a Sartorius (Sartorius Corporation, Edgewood, NY) CP 225D balance to a resolution of 0.01 mg and placed in aluminum pans. The pan was covered with an aluminum cover and placed in a Q-1000 Differential Scanning Calorimeter (TA Instruments, Newark, USA). The sample and the reference were then heated at a heating rate of 10° C/min from 20°C to 180°C, cooled to 20°C at 10°C/min, and subjected to another heating cycle from 20°C to 180°C at 10°C/ min in nitrogen gas atmosphere. Heat flow as a function of time and temperature was recorded, and the cycles were referred to as first heat, first cool, and second heat, respectively. The melting enthalpy of 100 % UHMWPE was accepted as 291 J/g and crystalline percentages of modified samples were calculated accordingly (Oral et al. 2006). The mass of PHEMA was obtained from elemental analysis and it was used to calculate the mass of UHMWPE in UHMWPE-g-PHEMA.

7.6.2.9. Scanning Electron Microscopy (SEM) - EDAX Analysis

All of the samples to be SEM-imaged were sputter coated with a layer of gold/palladium and imaged with a Zeiss ULTRA 55 (Zeiss, Oberkochen, Germany) type of microscope equipped with EDAX detector. Oxygen and nitrogen profiles were obtained.

7.6.2.10. Gravimetric Analysis of Grafting Degree

Weight gain upon polyHEMA grafting from UHMWPE indicates that the grafting took place. UHMWPE samples that were modified with polyHEMA, were weighed before and after modification. Samples were washed three times in ethanol before they were weighed. The degree of grafting was calculated by using the Equation 7.1.

$$The\ Degree\ of\ Grafting = \left(\frac{w_2 - w_1}{w_1}\right) \times 100 \qquad \text{Equation 7.1}$$

Where w_1 is the weight of pre-consolidated virgin UHMWPE and w_2 is the weight of polyHEMA-grafted UHMWPE.

7.6.2.11. Size Exclusion Chromatography-Multi-Angle Light Scattering

Molecular weight and polydispersity index of poly(HEMA) homopolymers formed simultaneously with grafting in reaction medium were determined by Size Exclusion Chromatography equipped with Multi-Angle Laser Light Scattering techniques. Instrument has both refractive index (Wyatt Optilab T-rex) and Multi-Angle Light Scattering (Wyatt Dawn Heleos II) detectors. Measurements were carried out in *N,N*-dimethylformamide (DMF) containing 0.5 mol LiBr, at a flow rate of 1.0 mL/ min (25 °C) using PL Mixed-C columns. Samples were passed through 0.1 μm PTFE filters to eliminate any contaminant. The poly(HEMA) homopolymer obtained from grafting experiments was precipitated in diethyl ether and then dried in vacuum oven at 40°C prior to molecular weight determination.

Theoretical number average molecular weights of p(HEMA) were calculated as described in Equation 7.2.

$$Mn_{theoretical} = Mw_{RAFT} + \frac{[Monomer]}{[RAFT]} \times Mw_{Monomer} \times Conversion \qquad \text{Equation 7.2}$$

7.7. Drug release studies

7.7.1. Gentamicin Release Studies

Gentamicin release over time was studied by using gentamicin containing strips (n=3, 3x5x10 mm) that were machined by CNC device (ShopBot Desktop, NC, USA). Prior to start the drug release studies, dimensions and weight of strips were individually recorded. Each sample was placed in a 3 ml sterile, disposable syringe in 1.5 ml of PBS. 1.35 ml of PBS was chosen to meet sink conditions as well as to cover the entire strip in the syringe. Syringes were placed in a 96 well plate upright and were kept in an incubator shaker at 37^{o}C with constant shaking at 100 rpm.
At pre-determined time periods, syringes were taken out, PBS solution was transferred to an HPLC vial and syringe was filled with fresh PBS to conduct drug release to next measurement time.

7.7.2. Gentamicin release quantification by fluorescence spectroscopy

Gentamicin sulfate concentration in the eluent was determined by standard calibration method. Standard solutions were prepared by dissolving gentamicin sulfate in 1X PBS with concentrations between 2 µg/mL and 40 µg/mL. Standard solutions containing gentamicin sulfate were added with fluorescent active o-phthaldialdehyde (OPA) solutions. 10 minutes after OPA solutions were added, standards in 96 well plate were measured in a fluorescence spectrometer at excitation 340 nm and emission 455 nm. Linear standard curve obtained with correlation coefficients (r^2) with 0.998 or more.

7.7.2.1. Buffer solution preparation

0.4 M Boric acid was prepared in deionized water and pH of the solution was adjusted to 10.4 by adding 8.5 M NaOH solution drop by drop.

7.7.2.2. Preparation of OPA solution

OPA solution was prepared as followed: 0.2 g of o-phthaldialdehyde (Figure 7.4) was dissolved in 1 mL of methanol and the solution mixed with 19 mL of 0.4 M boric acid followed by adding 0.4 mL of 2-mercaptoethanol and the pH was adjusted to 10.4 by adding NaOH solution. This solution was kept at 4 °C for 2-3 days for further use.

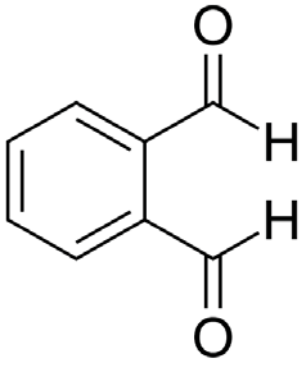


Figure 7. 4. Chemical structure of o-phthaldialdehyde

7.8. Antibacterial properties

7.8.1. Bacterial Colonization Assessment

Gentamicin sulfate incorporated UHMWPE samples were examined to ascertain their anti-adhering properties against *S. aureus*. UHMWPE and bone cement

samples (n=10) with and without gentamicin sulfate were prepared with dimensions of 3x5x10 mm and were put in PBS (1X) in 3mL sterile syringes. The syringes then were placed in a shaking incubator upright at 37^{o}C. The samples were removed from syringes at weeks 1, 2, 4, 8, 12 and placed into 1.7 mL Eppendorf tubes individually. 1 mL of 5x10^{4} CFU/mL of Xen29 in BD^{TM} Tryptic Soy Broth was added to each tube and they were incubated overnight at 37^{o}C. After incubation strips were removed and rinsed in 5 ml of PBS and placed in new Eppendorf tubes with 1 mL of PBS followed by 40-minute of sonication. Subsequently, sonicated solutions were diluted to 2-10 CFU/µL then were placed onto agar plate and spread with L-shaped bar. Upon overnight incubation at 37^{o}C, colonies formed were counted.

7.8.2. Antibacterial efficacy against planktonic bacteria

Eluted GS aliquots were sterilized under UV for 30 minutes. Antimicrobial activity of the samples against planktonic MSSA (ATCC 12600) was evaluated. Briefly, bacterial isolates were cultured in tryptic soy broth (TSB) at 35 ± 2 °C until reaching an early-stationary phase. The resulting bacterial suspension was diluted with fresh TSB to 10^{5} CFU/mL. Then, tested samples were transferred into 1.7 mL sterile tubes containing 1 mL of bacterial suspension. The analyzed samples were then statically incubated at 35 ± 2 °C. 18 hours later aliquots of the bacterial suspension were collected and analyzed by the spread-plate method in accordance with the International Standard ISO 4833-2. A total number of three replicates were performed for each measurement.

7.8.3. Determination of minimum inhibitory concentration (MIC)

The antibacterial potency of gentamicin eluted from UHMWPE was evaluated using the MIC test. Briefly, eluents were collected, and gentamicin concentration was determined using the OPA method as outlined above. Concentrations of gentamicin in the tested solutions were adjusted to the same level using PBS. MIC test was conducted according to the CLSI protocol (M07-A10). Antibacterial studies were conducted against methicillin-sensitive Staphylococcus Aureus (MSSA, ATCC 12600). Total number of 3 replicates were measured for each sample.

8. RESULTS AND DISCUSSION

Ultrahigh molecular weight polyethylene (UHMWPE) has been used as the gold standard in knee and hip replacement surgeries due to its excellent mechanical strength, fatigue resistance and ductility. Despite its excellent features, a patient's knee replacement may need to be revised because of aseptic loosening, infection, fracture, joint stiffness, tibio/femoral instability due to collateral ligament instability, patellar complications, extensor mechanism rupture and/or wear or failure of the UHMWPE components. Periprosthetic joint infection (PJI) is one of the major failures that leads to total joint arthroplasty (TJA). As PJI followed by TJA which is a reason for long-term hospitalization, significant morbidity and substantial economic burden. The total hospital cost pertaining to PJI treatment was $566 million in US in 2009. By 2020 this number has been projected to reach $1.62 billion as a consequence of 60,000 patients (Kurtz et al. 2012, 2014).

Subsequent infections after total joint arthroplasty is a perilous problem although there have been advancements in the prophylaxis and infection management in orthopaedic surgery. Most common interventions are one- and two-stage procedures. In one-stage procedure, upon removal of the infected implant, debridement and jet-lavage are performed followed by the insertion of a new prosthesis. For fixation, antibiotic-loaded bone cement (ALBC) is used and the patient is treated with systemic antibiotics for 4-6 weeks.

In the two-stage procedure, after the infected implant is removed, a preformed temporary ALBC is used for 6 months. Between infected implant removal and new implant insertion stages, the patient is treated with antibiotics topically, intravenously and with antibiotic releasing ALBC in order to eradicate the infection.

Over the past few decades, ALBC spacers have reached widespread use for the subsequent infections. The foremost advantages of ALBCs are:

- Availability of high concentrations of antibiotics in the infection site,

- Feasibility for various antibiotic load depending on the pathogen and its sensitivity,
- Maintenance of joint function,
- Protection of bone parts,
- Avoiding soft tissue contraction which causes the level discrepancy between legs,
- Convenience for permanent implant surgery.

Pre-made ALBC spacers for two-stage interventions are reported as successful devices. However, they have few side effects pertaining to their complex chemistry such as acrylate monomers derivatives and N,N-dimethyl-p-toluidine toxicity (Stea et al. 1997) and shortcomings such as low dose antibiotic elution which may induce bacterial resistance (Goltzer et al. 2015).

In this PhD thesis study, UHMWPE-g-PHEMA copolymers were prepared via UV-initiated grafting by both conventional radical polymerization and reversible addition fragmentation chain transfer (RAFT) polymerization. Copolymer synthesis was followed by gentamicin sulfate (GS) incorporation to the polymeric matrices. UHMWPE/GS and UHMWPE-g-PHEMA/GS composites were tested for GS release for the planktonic kill of the bacteria. Anticolonization properties of composites was also investigated. Both the gentamicin sulfate release performance and anticolonizing property of UHMWPE/GS, UHMWPE-g-PHEMA (conventional, RAFT) compared. Besides their drug release performance and antimicrobial activity, materials' mechanical strength and wear resistance were also investigated.

8.1. Antibiotic Selection

There are several aspects to be considered in the incorporation of antibiotics in UHMWPEs. The desired characteristics are:

- Availability of antibiotic in powder form,
- Potency against wide spectrum of strains,
- Low serum binding,
- Low adverse complications,
- Thermal stability.

Those criteria mentioned are fulfilled by glycopeptide and aminoglycoside type of antibiotics. Another important factor which may narrow down the antibiotic selection is medication use regulations depending on the country or region. For instance, the use of Vancomycin hydrochloride (VH) in Europe is mostly allowed whereas it is perceived as the last resort against Methicillin-Resistant *Staphylococcus Aureus* (MRSA).

Given that the general clinical practice in the US is to use VH, Tobramycin sulfate (TS), and Gentamicin sulfate (GS) for the treatment of orthopaedic implant-related infections. In this thesis study, GS was used due to its better thermal stability compared to VH.

8.2. Preparation of UHMWPE/GS Tibial Spacers

Gentamicin is an aminoglycoside type of antibiotic. Gentamicin has a relatively narrow therapeutic window, given the risk of toxicity in case of improper dosing, but it shows good activity against problematic pathogens such as *Pseudomonas*, *Acinetobacter*, *Streptococcus.* Tobramycin and gentamicin are the antibiotics that are prevalently being used in both primary joint arthroplasty and in periprosthetic joint infection. They both interact with the 30s subunit of the RNA and causes misreading of the genetic code (Pape, Wintermeyer, and Rodnina 2000).

Gentamicin is amorphous and relatively thermally stable antibiotic which has a hollow sphere structure that resembles raisins (Samara et al. 2017). Its spherical amorphous structure makes it look like fast-cooled liquid in microscale (Figure 8.1).

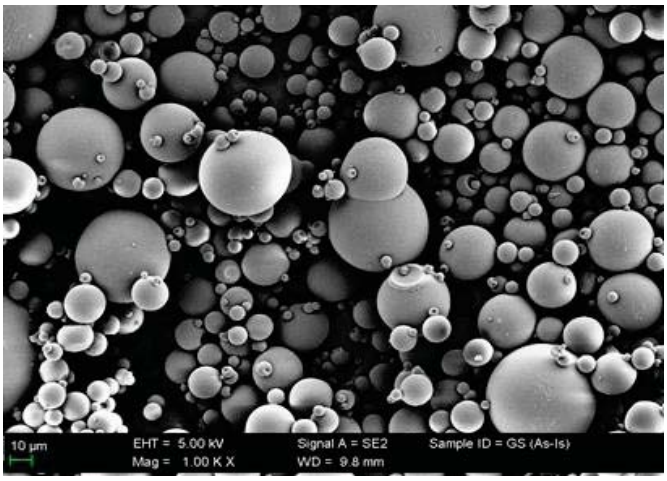


Figure 8. 1. SEM image of spherical gentamicin sulfate

Although consolidation of UHMWPE/GS blends were direct compression molded at minimum conditions required for the consolidation of UHMWPE, 3 mm-thick tibial shape blocks which were molded at 170 °C under 20 MPa pressure for only 8 minutes were discolored. Despite the fact that gentamicin sulfate is off-white/pale yellow color in its intact form, molded blends turned their color ranging from dark yellow to black depending on the molding temperature, duration of the molding cycle and amount of pressure applied (Figure 8.2).

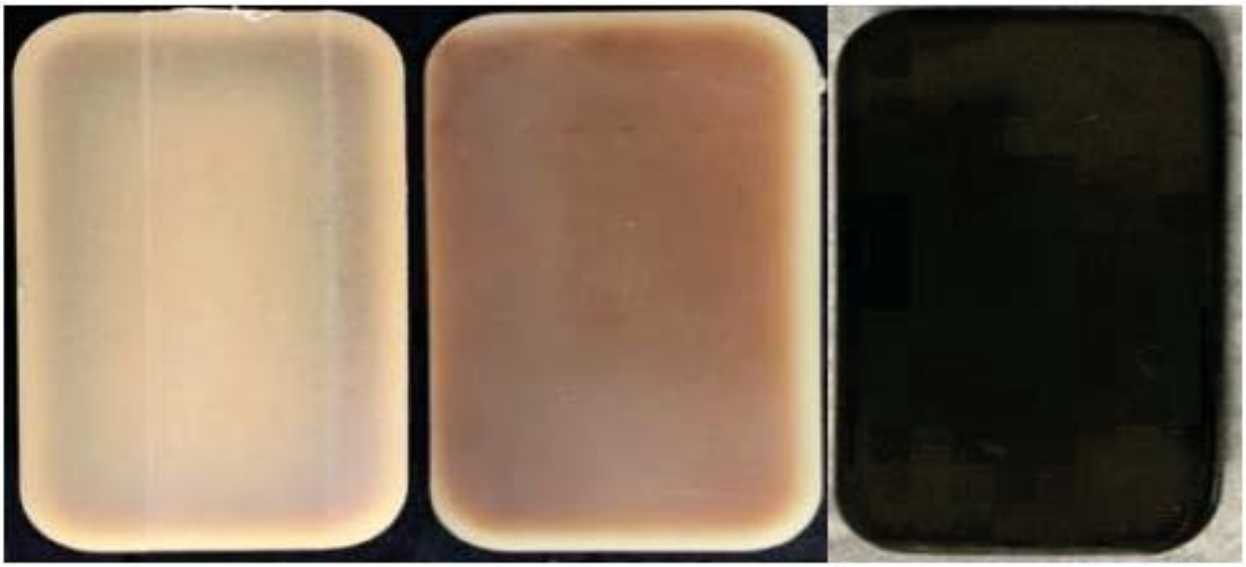


Figure 8. 2. Color change of UHMWPE loaded with 8 wt. % gentamicin sulfate molded at 170 °C for 8 min (left), 20 min (middle) and 2 hour (right)

The TGA thermogram of GS showed only 6 wt % loss which was attributed to the moisture loss (Figure 8.3). It was reported in the literature that lactose -similar chemical structure to gentamicin- undergoes thermal isomerization when processed which could be ceased by high pressure (Moreno, Villamiel, and Olano 2003). Given that, molding pressure was increased from 10 MPa to 40 MPa, and yet the area of discolored regions got widened and the hue got darker (Suppl 1). To impart thermostability by an adjuvant molecule, vancomycin hydrochloride (VH) was incorporated together with GS. However, discoloration did not become any better (Suppl 1). Vitamin E was another additive which was incorporated alongside GS to avoid discoloration in case the discoloration may be the result of oxidation. Nevertheless, the discoloration of the UHMWPE/GS block occurred.

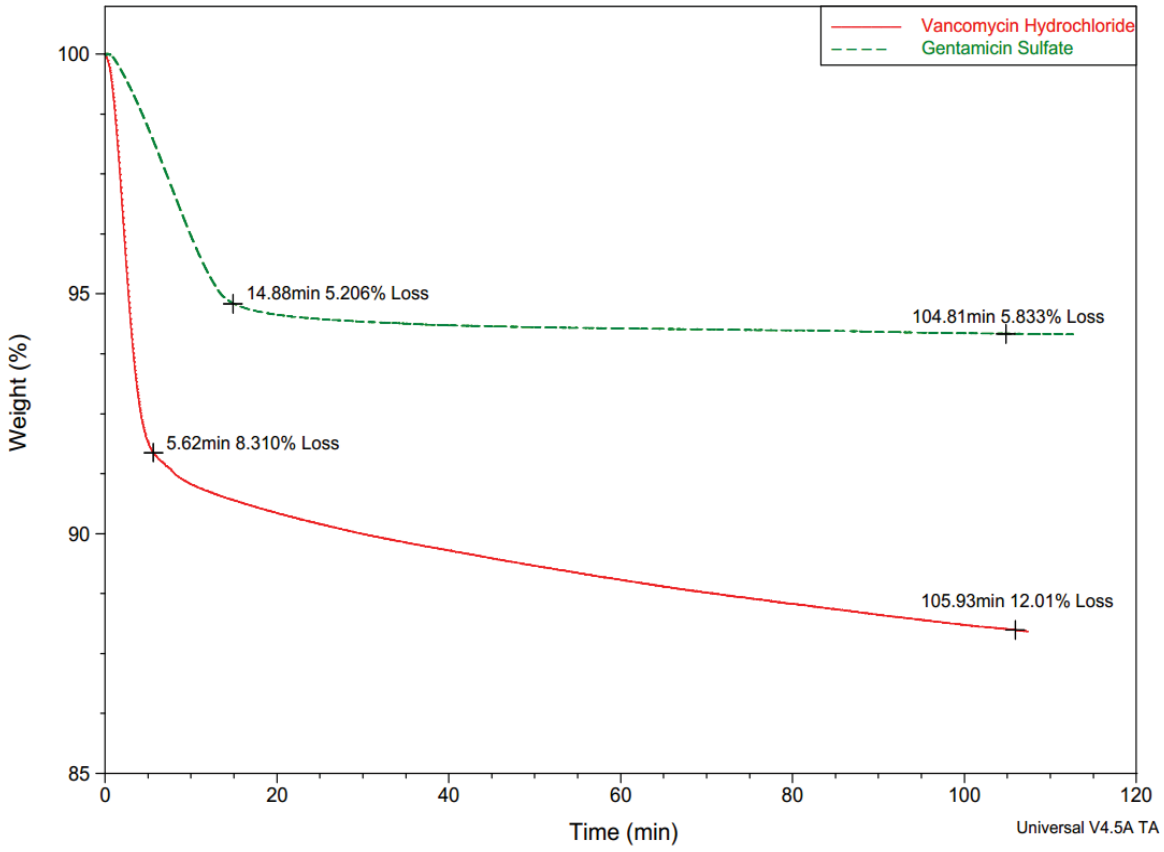


Figure 8. 3. Isothermal TGA curves of gentamicin sulfate and vancomycin hydrochloride at 170 °C for 110 min

XPS was run to characterize the chemical structure of discolored and non-discolored domains. O1s data was collected from different spots with the visibly discolored center, less discolored skin and the intersection between the skin and

the center of the powder-molded UHMWPE/GS sample. So, there are three types of oxygen in gentamicin structure, etheric oxygen in glycoside bond, oxygen in hydroxyl group and cyclic oxygen (Figure 8.4).

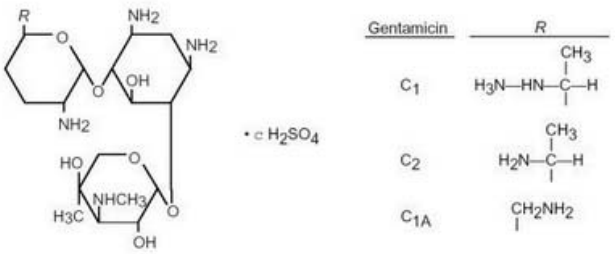
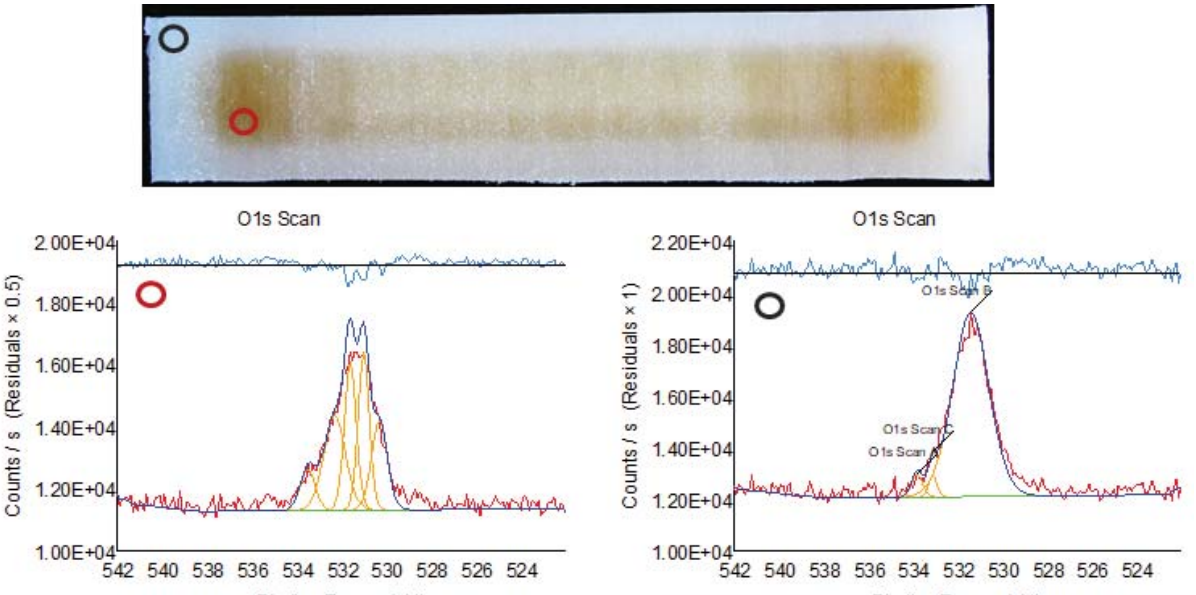


Figure 8. 4. Chemical structure of gentamicin sulfate

The peak deconvolution of O1s spectrum, however, showed five peaks. The one with binding energy 531.68 eV attributes to carbonyl oxygen (Figure 8.5).



	Name	Peak BE	FWHM eV	Area (P) CPS.eV	Atomic %	Q
>	O1s Scan A	531.08	0.58	3178.87	22.97	☑
>	O1s Scan B	531.68	0.62	3192.49	23.08	☑
>	O1s Scan C	532.38	1.14	3753.52	27.15	☑
>	O1s Scan D	530.38	0.83	2571.80	18.57	☑
>	O1s Scan E	533.58	0.79	1137.15	8.23	☑

	Name	Peak BE	FWHM eV	Area (P) CPS.eV	Atomic %	Q
>	O1s Scan A	533.88	0.58	558.01	3.60	☑
>	O1s Scan B	531.48	1.89	14590.20	93.85	☑
>	O1s Scan C	533.18	0.43	396.97	2.56	☑

Figure 8. 5. Image of cross-sectional area of tibial insert made of UHMWPE/GS (top), O1s spectra of UHMWPE/GS sample from two spots

8.3. Dehydration of UHMWPE/GS

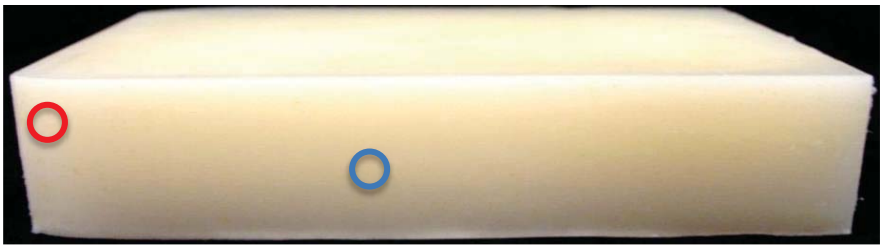
Pharmaceutical salts may innately be hygroscopic. In some circumstances they might be in moist environment. Moisture is one of the major stress factors especially when it is combined with heat. It can induce various degradations including dehydration, isomerization, oxidation and organic reactions depending on the chemical structure of the drug. To investigate the effect of any trace moisture on discoloration, UHMWPE/GS blends were dehydrated by vacuum treatment prior to consolidation. GS was sieved and blended with UHMWPE (GUR 1020) in planetary mixer. When the blend was vacuum treated in a vacuum oven at room temperature for overnight, the area of discolored domain was decreased. Whereas when the blend in a plastic jar was placed under vacuum which is 762 mmHg lower than atmospheric pressure at 45°C for overnight the block retains its pale-yellow color which is attributed to the color of gentamicin sulfate.

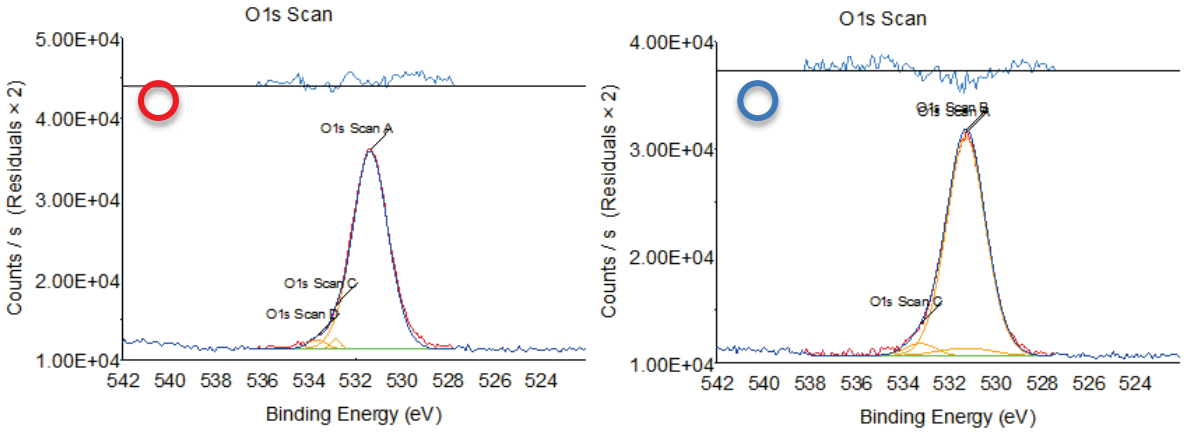
In order to ensure it is dehydration but not removal of molecular oxygen level in the vacuum chamber that vanishes the discoloration of the UHMWPE/GS block, blends were dehydrated by other methods such as microwave treatment and incorporation of a desiccant recipient alongside gentamicin sulfate. These methods also showed that the degradation of gentamicin sulfate retarded by dehydration techniques.

In conclusion, as water was driven from hygroscopic gentamicin sulfate by thermal and vacuum treatment the discoloration diminished to yield gentamicin sulfate's original color at its maximum dehydrated state (Figure 8.6.).

Figure 8. 6. Effect of dehydration on UHMWPE blocks with 8% gentamicin sulfate

In Figure 8.7, the O1s XPS spectra of UHMWPE/GS showed alterations of the oxygen character. In order to analyze if the oxygen atoms in gentamicin stay intact after dehydration, XPS spectroscopy was used as a tool. O1s spectra from different spots of cross-sectional area of UHMWPE/GS showed that all three oxygen atoms remained unchanged (Figure 8.7).





	Name	Peak BE	FWHM eV	Area (P) CPS.eV	Atomic %	Q
>	O1s Scan A	531.38	1.79	47848.61	96.44	☑
>	O1s Scan C	532.88	0.47	620.39	1.25	☑
>	O1s Scan D	533.68	0.93	1145.09	2.31	☑

	Name	Peak BE	FWHM eV	Area (P) CPS.eV	Atomic %	Q
>	O1s Scan A	531.32	1.98	43778.29	91.15	☑
>	O1s Scan B	531.28	2.87	2309.39	4.81	☑
>	O1s Scan C	533.28	1.54	1940.83	4.05	☑

Figure 8. 7. Image of dehydrated and molded UHMWPE/GS and its O1s spectra of collected from two spots specified in the cross section

8.4.1. Drug Release from UHMWPE/GS

All of the drug release studies were carried out from specimens in strip shape. Upon consolidating tibial shape blocks, they were uniformly machined/cut to strip specimens. One challenge for working with polyethylene samples for drug release is immersing the entire specimen in PBS since it floats on it. To get around this, specimens were placed in sterile 3 ml syringes filled with PBS. Tibial shape blocks were prepared with GS weight concentration 1, 2. 4, 6, 8 and 10%. All of the

samples were dehydrated prior to compression molding step. There was no discoloration appeared as a second hue observed in the molded blocks. Though the color of the samples varied between off white for 1% sample to orange for 10% sample which was the sign of increase in amount of GS (Figure 8.8).

Figure 8. 8. The display of UHMWPE/GS with various concentrations. From left to right; virgin UHMWPE, 1, 2, 4, 6, 8 and 10 % GS.

The initial release study for the samples with various GS concentration was carried out for ten days in sterile syringes filled with PBS (1X). The syringes were placed in an incubator shaker at 37 °C and 100 rpm. All of the samples showed burst release for the first day of the elution study. They then followed more steady increase for their rate of release in the latter stage of the study. Sample with 1% GS exhibited undetectable GS release after 5th day. GS release rate increased exponentially in regard to GS concentration increase. For the samples with 4% GS concentration and above, they released adequate amount of GS to match minimum inhibitory concentration for *S. aureus* which is 4 µg/ml (Swieringa et al. 2008) or more for the burst release time frame (Figure 8.9).

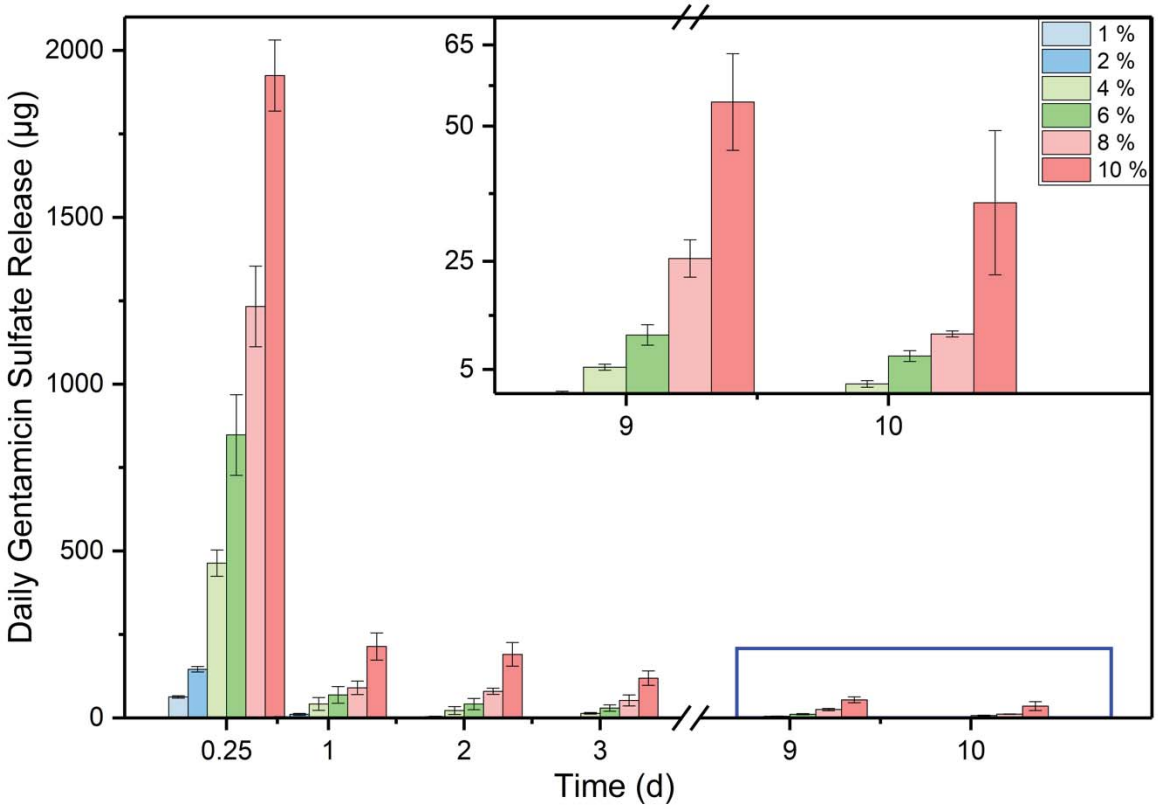


Figure 8. 9. Daily GS release from UHMWPE/GS with various GS concentrations.

The amorphous GS is embedded in continuous UHMWPE matrix. The spherical structure of the drug was retained even after consolidation took place. At relatively higher concentrations they tend to agglomerate and form random, indistinct clusters as it can be seen from SEM and EDX images (Figure 8.10).

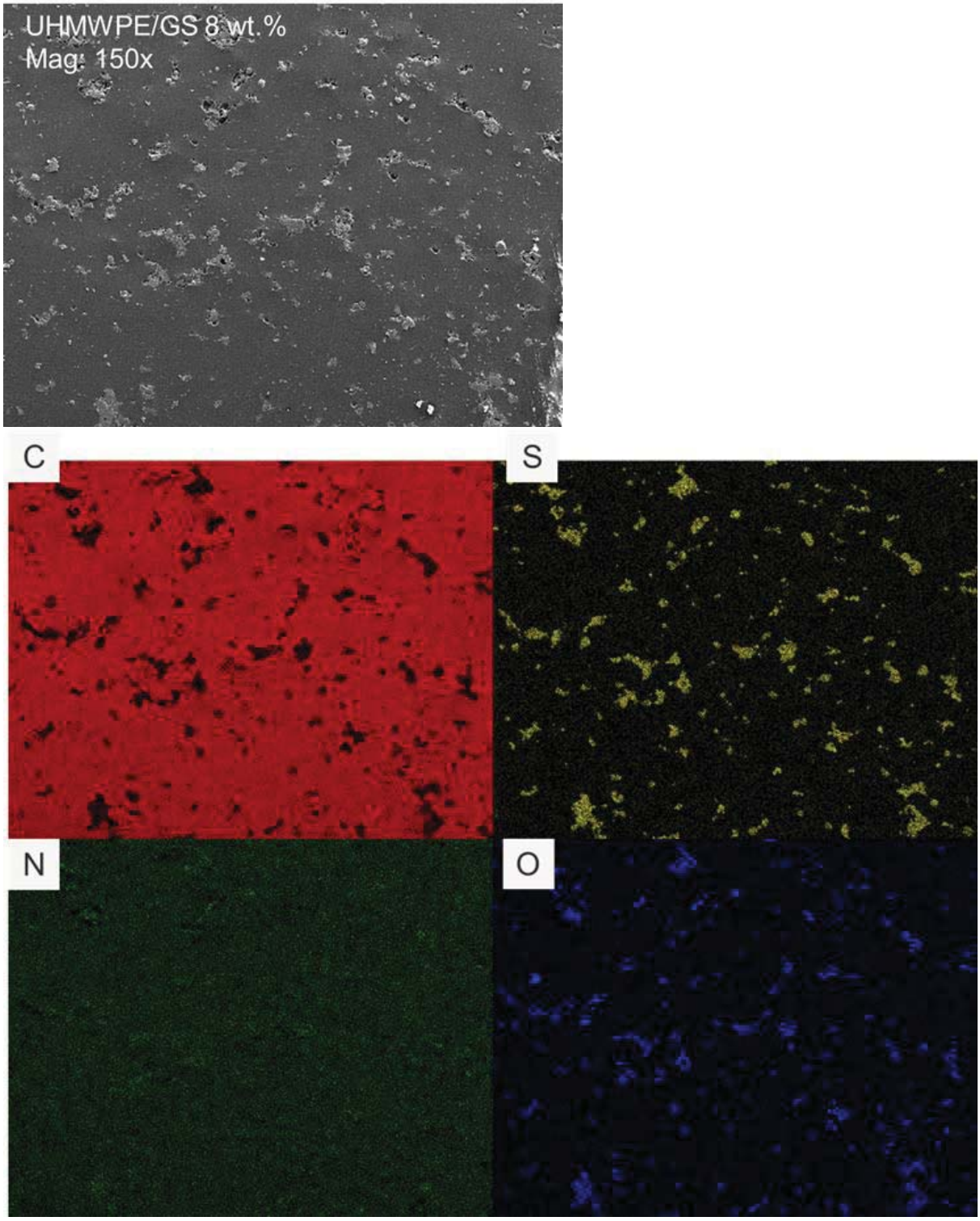


Figure 8. 10. SEM and EDX images of UHMWPE/GS (8 %) with carbon, sulfur, nitrogen and oxygen maps

The reason why the increase in release rate is not linear as the concentration of GS increases can clearly be seen in optical images that gentamicin clusters agglomerate as concentration goes up (Figure 8.11).

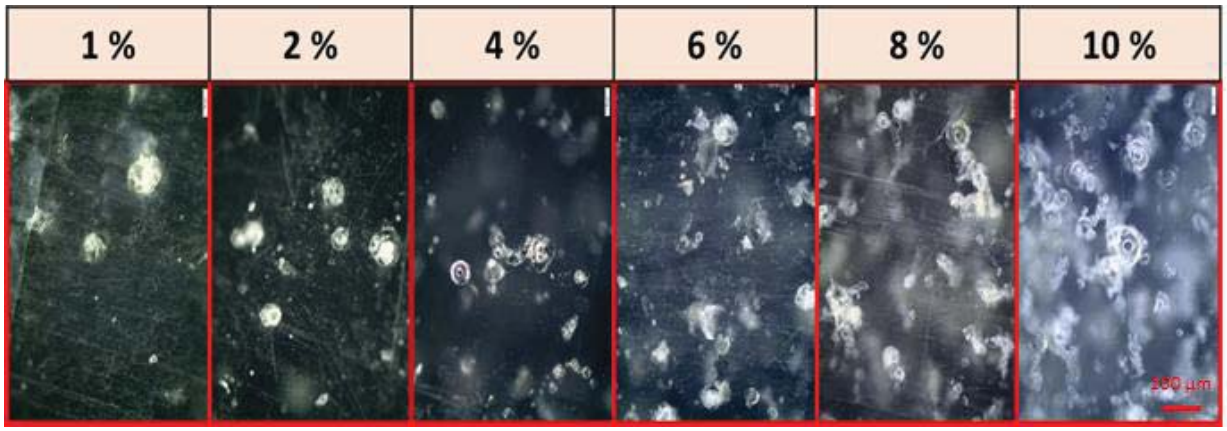


Figure 8. 11. Optical images of UHMWPE/GS with weight percentages 1, 2, 4, 6, 8, 10.

At the end of the 10th day, sample with 2% GS eluted only around 3 % of its GS content whereas sample with 10 % released around 10 % of its GS content (Figure 8.11). Cumulative GS release amount reaches their ten day-maxima by eluting 3.23 mg per implant for sample with 1 wt. % GS sample and 128 mg per implant for sample with 10 % wt. GS.

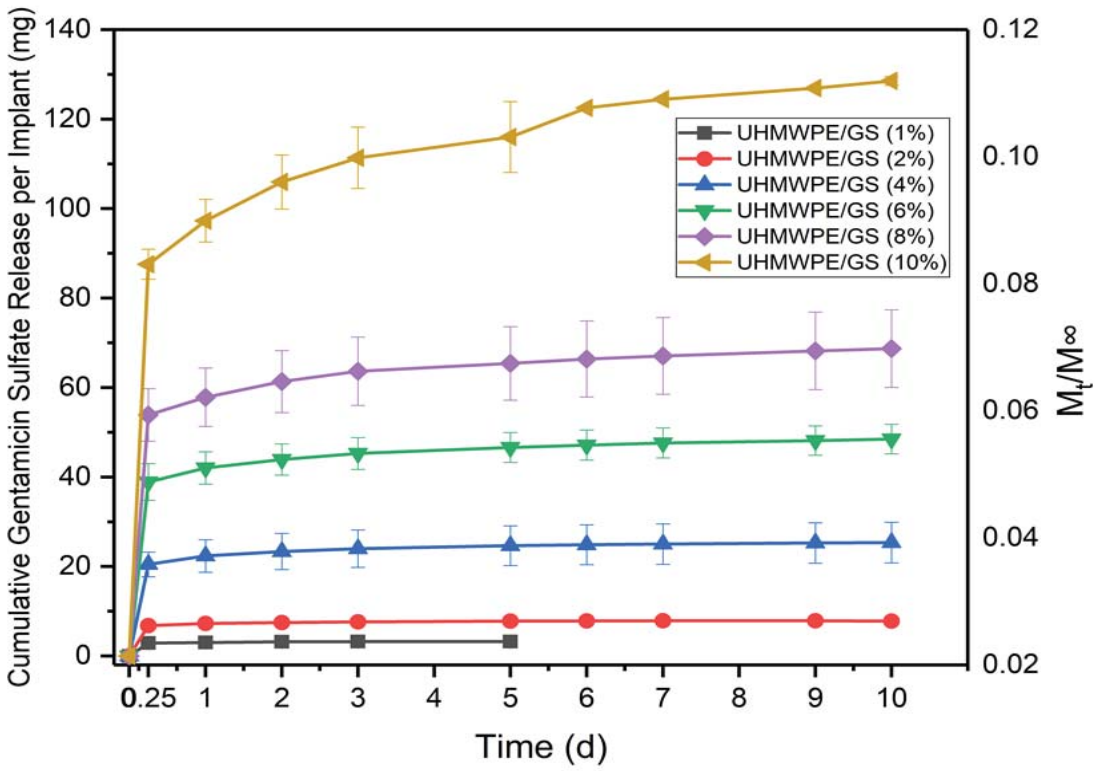


Figure 8. 12. Cumulative GS release from UHMWPE/GS samples with different GS percentages.

In order to predict clinical performance of UHMWPE as GS delivery device, GS-loaded polymethyl methacrylate (PMMA) based bone cement was taken as reference. GS-loaded PMMA was introduced to the orthopaedic field in 1970's for the treatment of infected prosthetic joints (Buchholz and Engelbrecht 1970). Since then, it has been widely used for treatment of periprosthetic joint infection as premade implants and for implant fixation purposes. To compare GS release rate, an experiment at 37 °C in PBS (pH 7.4) was conducted by using commercially available GS-loaded bone cement Palacos R-G (0.75 g gentamicin base per 75 g of net cured PMMA/GS), and UHMWPE with 4 % and 8 % GS content. This study showed that Palacos R-G releases more GS for the burst release interval. After the fourth day, UHMWPE/GS 8 % released slightly more GS than UHMWPE/GS 4 % and Palacos R-G where there's no significant difference between all three (Figure 8.13).

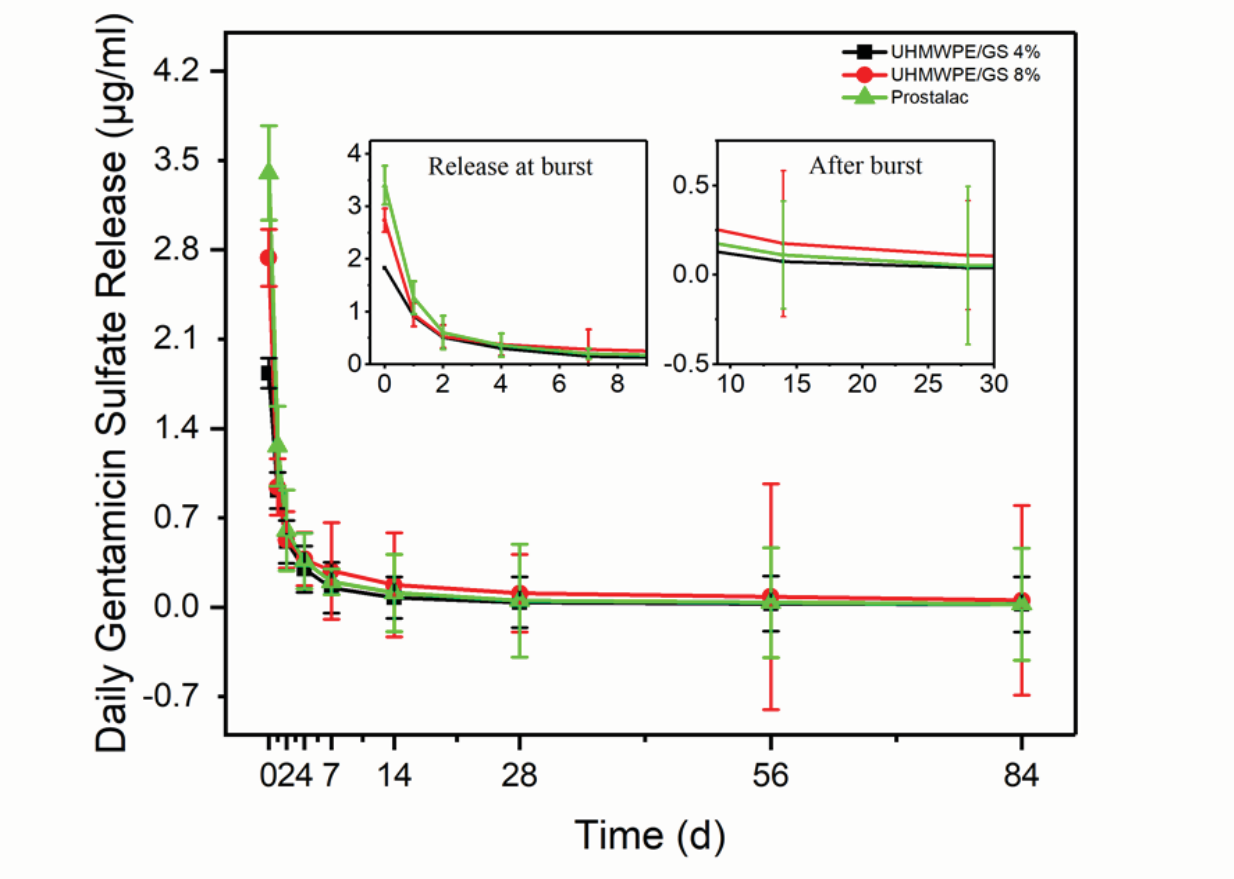


Figure 8. 13. Daily GS release from GS-loaded PMMA, UHMWPE/GS 4% and 8%.

8.4.2. Antimicrobial Performance of UHMWPE/GS

An orthopaedic implant impregnated with antibiotic must serve two major functions against infection:

- Planktonic kill of the bacteria in the close proximity of the implant
- Preventing colonization of bacterial species on implant surface.

Two separate experiments were designed to investigate those two important aspects. Planktonic bacterial kill was tested by using the elution aliquots of associated time point. Anticolonization was tested by using the elution strips which are obtained after elution halted after each time point (Muratoglu et al. 2018). Two UHMWPE/GS with GS concentration of 4 % and 8 % and Palacos R-G as bone cement reference were tested with those techniques.

For the elution aliquots, they were added S. Aureus suspension with concentration of 1E6 CFU/ml in Tryiptic Soy Broth (TSB) and incubated at 35 °C overnight. The resulting solution was plated on agar and the colonies were counted. The GS

concentration in the aliquots of all time periods points for Palacos R-G and UHMWPE/GS 8 % was adequate to be bactericidal (Figure 8.14).

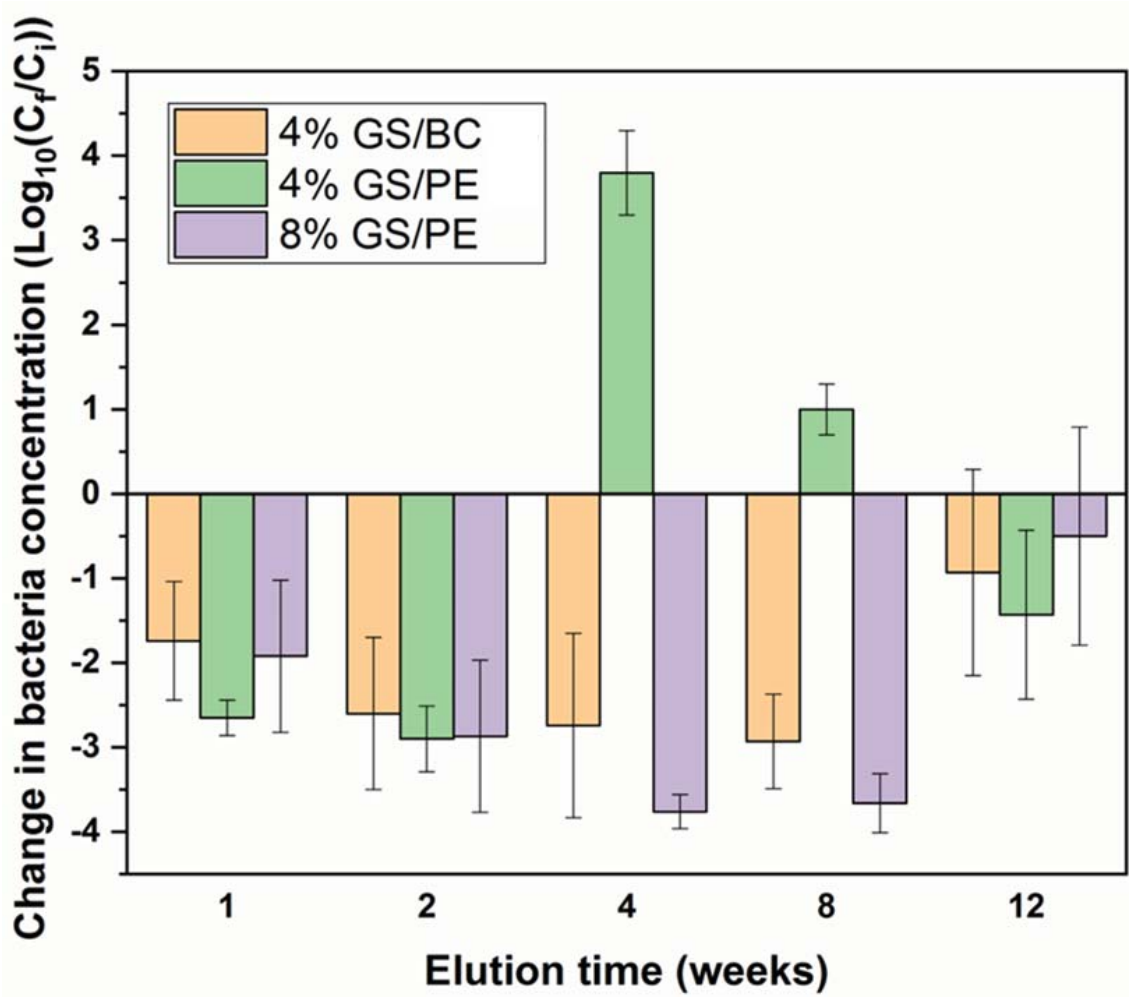


Figure 8. 14. Antibacterial performance of GS in eluents with respect to time

Seemingly there were no significant difference between UHMWPE/GS 8 % and Palacos R-G. However, 4th and 8th week aliquots of UHMWPE/GS 4 % were lacking their GS concentration to kill planktonic bacteria the poor performance of UHMWPE/GS 4 % sample recovered at 12th week and it reduced the bacterial concentration approximately one order of magnitude.

Evaluation of anticolonization property of UHMWPE/GS and Palacos R-G strips was performed by incubating them with 1E6 CFU/ml *S.Aureus* in TSB at 35 °C. As incubation was done, strips were sonicated to remove non-adherent bacteria and the adhered bacteria was counted (Figure 8.15). At 4th week UHMWPE/GS 4 % became bacteriostatic where it was bactericidal at earlier time points. Interestingly

beyond 8th week UHMWPE/GS 4 % became bactericidal again the mechanism of action of GS to kill bacteria occurs through engulfment of GS by bacteria and metabolization of it meaning the more GS is refrained from releasing, the more prone for bacterial attachment the surface gets. The upturn in bacterial killing for UHMWPE/GS 4 % might be due to unveiled fresh depots of GS releases more GS to the environment.

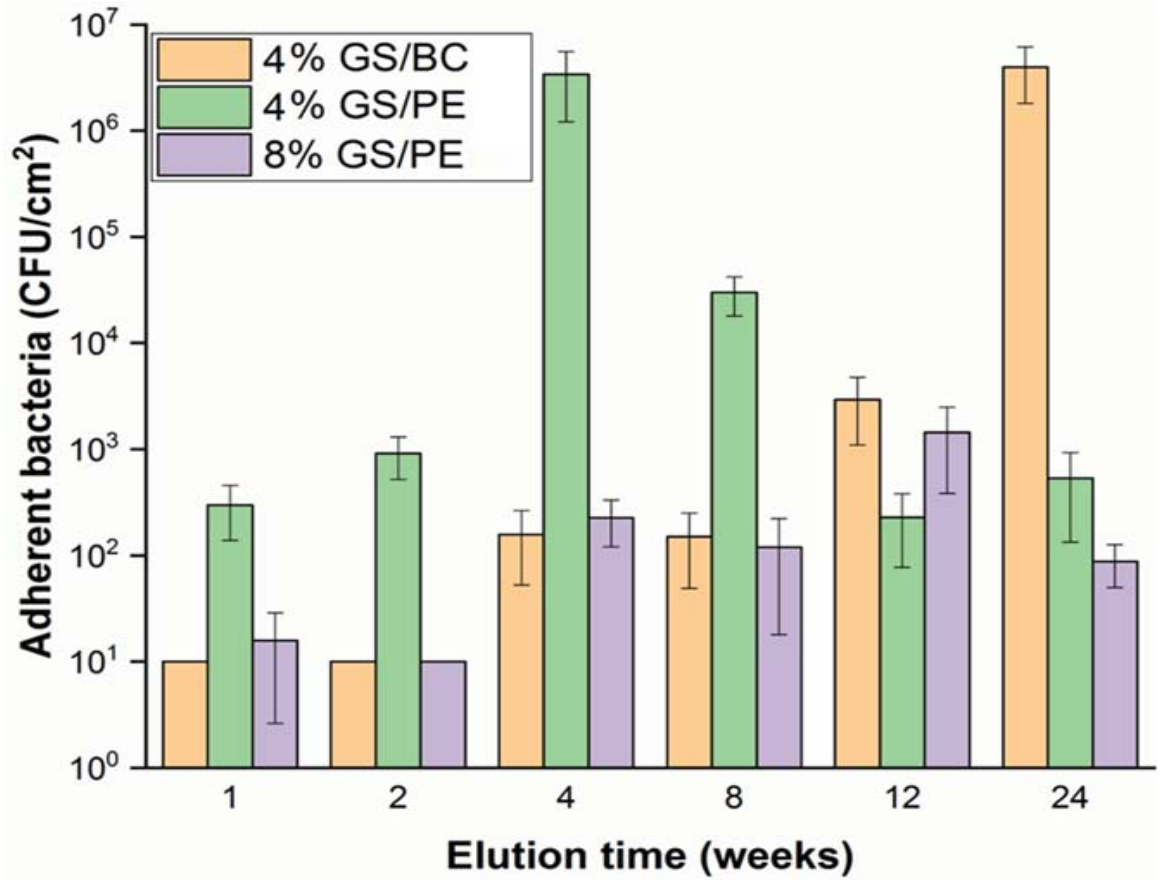


Figure 8. 15. Adherent bacterial count on drug release test strips

Palacos R-G has the greatest number of bacteria adhered on its surface at 24th week whereas UHMWPE/GS 8 % has the least number of bacteria on its surface. Although the planktonic kill is proportionately correlated with the amount of GS released, there was almost 4-log difference between UHMWPE and Palacos R-G in terms of adherent bacteria at the end of the 24th week. Bacterial attachment is overwhelmingly complicated process contributed by many factors such as chemical composition of the substrate, surface roughness, surface hydrophobicity or wettability etc. (An and Friedman 1998). So, the difference of number of

adhered bacteria might be due any of the aforementioned factors either individually or in combination.

8.4.3. Mechanical Strength of UHMWPE/GS

The longevity of implants is measured with their mechanical strength, particularly, ultimate tensile strength (UTS), elongation at break (EAB), IZOD impact strength and also its wear resistance. Mechanical strength of polymers depends on many factors such as their chemical structure, crystallinity, molecular weight, physical composition (composite i.e) etc. Since UHMWPE/GS has polymeric continuous phase of UHMWPE and discrete drug phase of GS, it must be considered under composite class. Filler particulates have vast importance on mechanical strength. Their shape, size, crystallinity, compatibility with the host polymer are factors that affect mechanical properties.

Figure 8.16 shows that the UTS was reduced with increasing GS weight percentage. Homopolymer of UHMWPE has UTS value as high as 51 MPA and UHMWPE with 10 % GS has UTS as 31 MPA. UTS highly depends on effective stress transfer in two-phase composite materials. The effective stress transfer on the other side, depends on the compatibility and the adhesion of the components in the composite material. UHMWPE is chemically inert, nonpolar polymer whereas GS has polar character. The decreasing trend in UTS could be explained with the lack of adhesion between UHMWPE and GS (Fu et al. 2008).

The EAB was also reduced with increasing weight fraction of GS in UHMWPE. The EAB decreased approximately 24 % from virgin UHMWPE to UHMWPE/GS 10 % (Figure 8.16). The continuity of the UHMWPE matrix gets intervened by the grain boundaries between GS particles and polymer phase which prevents the formation of large and uniform lamellae. It was reported that when fillers such as calcium stearate and carbon fiber present in UHMWPE matrix their boundaries with polymeric matrix act like fusion defects (Blunn et al. 1997; Sutula et al. 1995; Wright et al. 1988). Those fusion defects nucleate the crack failure of the composite and reduce the general mechanical properties of the material. In UHMWPE/GS, GS leads to the concentration of the stress at the boundary and both UTS and EAB decreased in respect to increasing GS concentration.

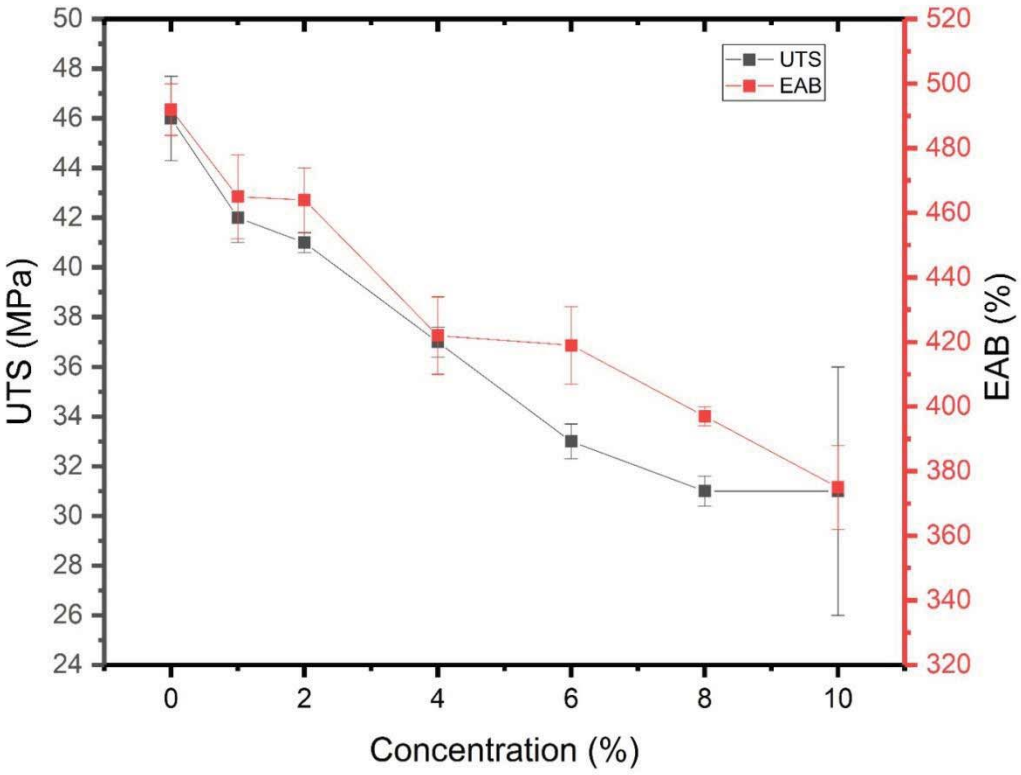


Figure 8. 16. The trend in ultimate tensile strength and elongation at break of UHMWPE/GS with increasing GS percentage

The double-notched IZOD impact testing was used to analyze impact strength of UHMWPE/GS with various weight concentration. The virgin UHMWPE showed impact strength of 166 kJ/m^2 (Figure 8.17). Subsequent addition of 10 % GS in the UHMWPE decreased its strength to 92 kJ/m^2. This reduction could be explained as the increasing concentration of GS leads to more stress concentration around GS which initiates and accelerate the fracture formation and propagation. Thus, the impact strength was reduced. Notched impact strength relies on the contributions which affect the propagation of fracture initiated around notch tip where stress concentration is high.

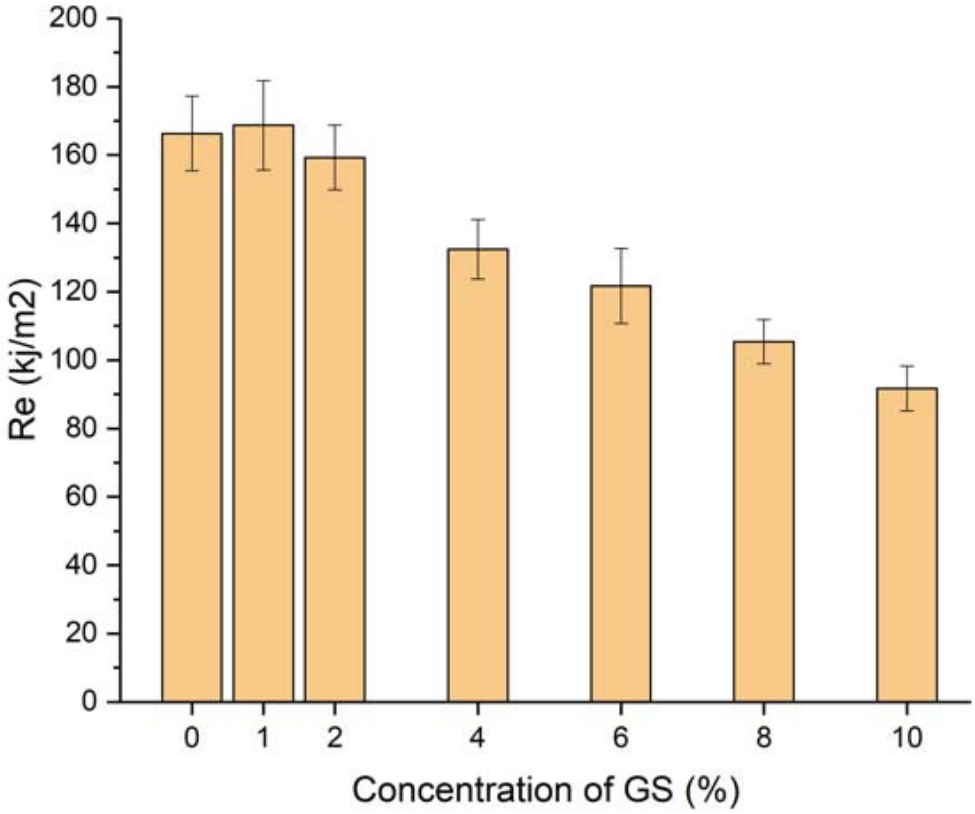


Figure 8. 17. IZOD impact strength of UHMWPE/GS with increasing GS weight percentage.

8.4.4. Bidirectional Wear Test of UHMWPE/GS Samples

The wear resistance of samples with varying GS content was determined by testing pins using bidirectional pin-on-disc wear tester. The wear tester was operated at a frequency of 2 Hz using a Paul-type curve with maximum stress of 4.8 MPa per pin. Wear rate is determined gravimetrically by weighing pins every 0.157 million cycle (MC) until it becomes 1.2 MC. The wear rates of pins were calculated as the linear regression of weight change against the number of cycles in between 0.5-1.2 MC. All of the UHMWPE samples with GS showed wear rate around 10 mg/MC (Figure 8.18).

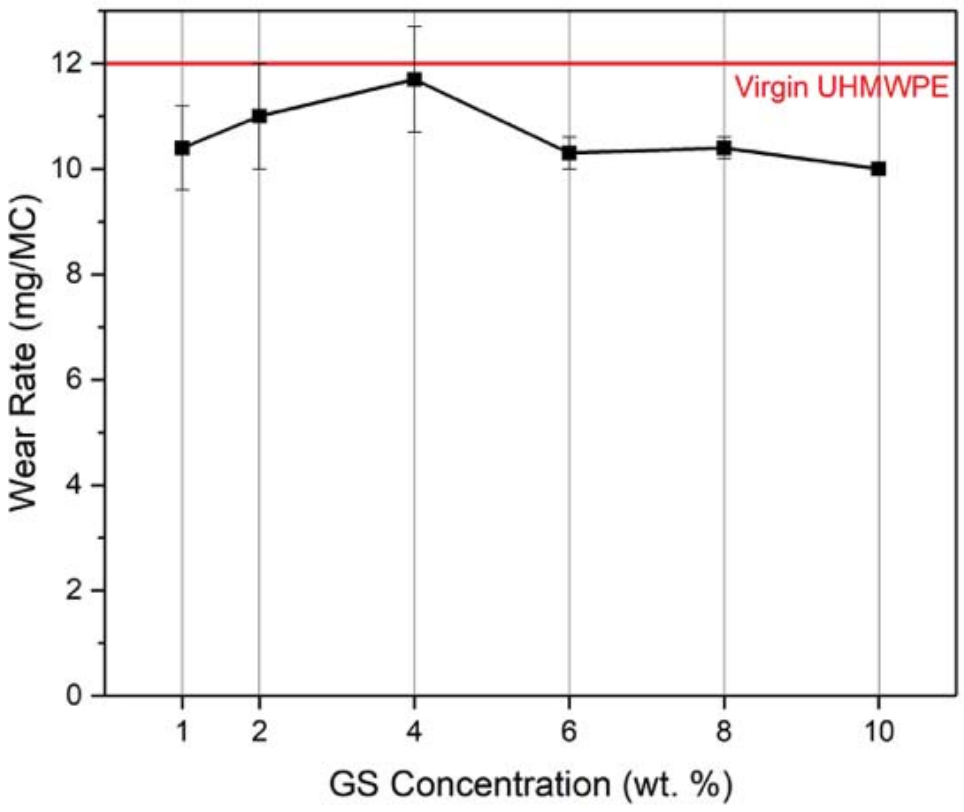


Figure 8. 18. Pin-on-disc wear test of UHMWPE/GS with increasing GS weight percentage

Since, the virgin UHMWPE pins exhibited wear rate around 12 mg/MC, it is possible to confer that incorporation of GS in UHMWPE reduced the wear rate. This reduction is thought to be the GS-loaded UHMWPE has thicker layer of lubricants between articulating surfaces of Co-Cr disks and UHMWPE/GS pins. One should note that the wear test medium got thicker during one-week long test due to GS release from all six pins. Thicker lubricant is also thought to be one of the reasons which reduced the wear rate. Recent evidence suggests that another hydrophilic drug, bupivacaine hydrochloride, reduced the wear rate of UHMWPE under cyclic load (Suhardi 2017, 166–167). The fluorescence imaging of bupivacaine hydrochloride-loaded UHMWPE under compression showed that there was a thicker layer of lubricant film on the material's surface compared to virgin UHMWPE which reduced the wear rate of the drug loaded UHMWPE. The thicker layer of lubricant was stated due the surface extrusion of lubricants during

load bearing. The wear rate of UHMWPE/GS needs to be considered total weight change after the release of wear debris of UHMWPE and GS to the bovine serum. In conclusion, GS slightly reduced the wear rate despite its release.

8.5. 2-hydroxyethyl Methacrylate Grafting from UHMWPE

The grafting of monomer to the surface of a polymeric substrate is a versatile way to change the surface properties and to functionalize the surface of the substrate. UV initiated surface photografting is one of the widely used methods to graft polymers to the substrates. Photoinitiators need to be used to initiate the reactions. In this study benzophenone which is a TYPE II photoinitiator is used. Each photoinitiator has specific and different absorption characteristics and can react most efficiently when exposed to a certain UV wavelength or wavelength range. For photoinitiation to proceed efficiently, the absorption bands of the photoinitiator must match with the emission spectrum of the light source. In this PhD study, the photo-induced graft polymerization was carried out with a UV lamp that works in the range 290-400 nm. Benzophenone has a π–π* transition at 250 nm and a much weaker n–π* transition at 350 nm. Benzophenone is excited to triplet state when exposed to UV light. In triplet state, BP abstracts H atoms from the UHMWPE surface to generate surface radicals. Monomers attack to the radicals on UHMWPE surface and initiate grafting process (Figure 8.19).

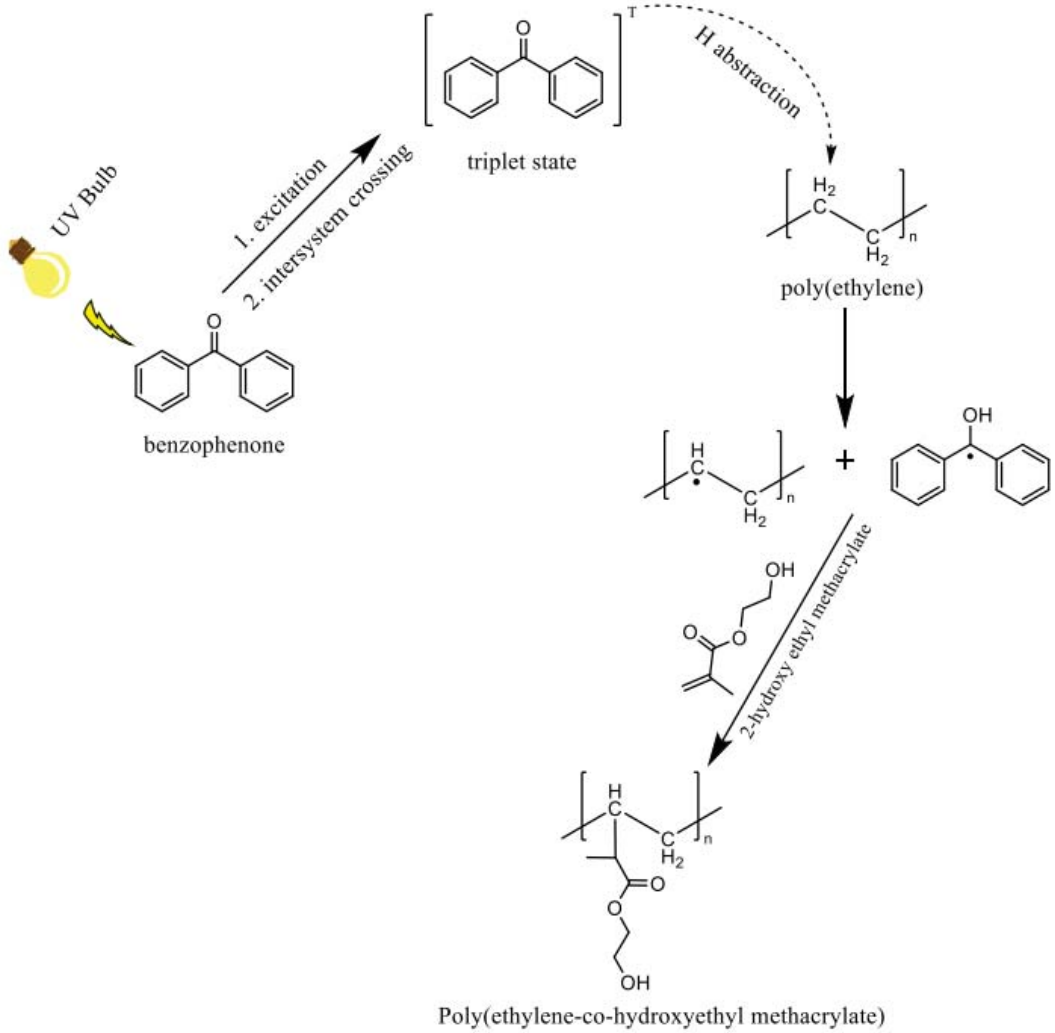


Figure 8. 19. Scheme of 2-hydroxyethyl methacrylate grafting from UHMWPE by UV-initiated radical polymerization

There are two decisive parameters investigated for grafting HEMA from UHMWPE which were initial monomer concentration and UV irradiation duration. To determine appropriate monomer concentration for grafting studies, three different initial monomer concentration was tried; 10, 20, and 30 % (v/v). The extent of grafted HEMA was monitored by analyzing the peak area of carbonyl group in FT-IR spectra of copolymer prepared with said initial monomer concentrations. C-H stretching peak around 3100 cm^{-1} was normalized in order to compare carbonyl peak areas at 1724 cm^{-1}. As the initial monomer concentration increased, carbonyl peak area increased proportionally (Figure 8.20). It was also possible to track the increase in grafted HEMA moieties by -OH peak strengthening at around 3300 cm^{-1}.

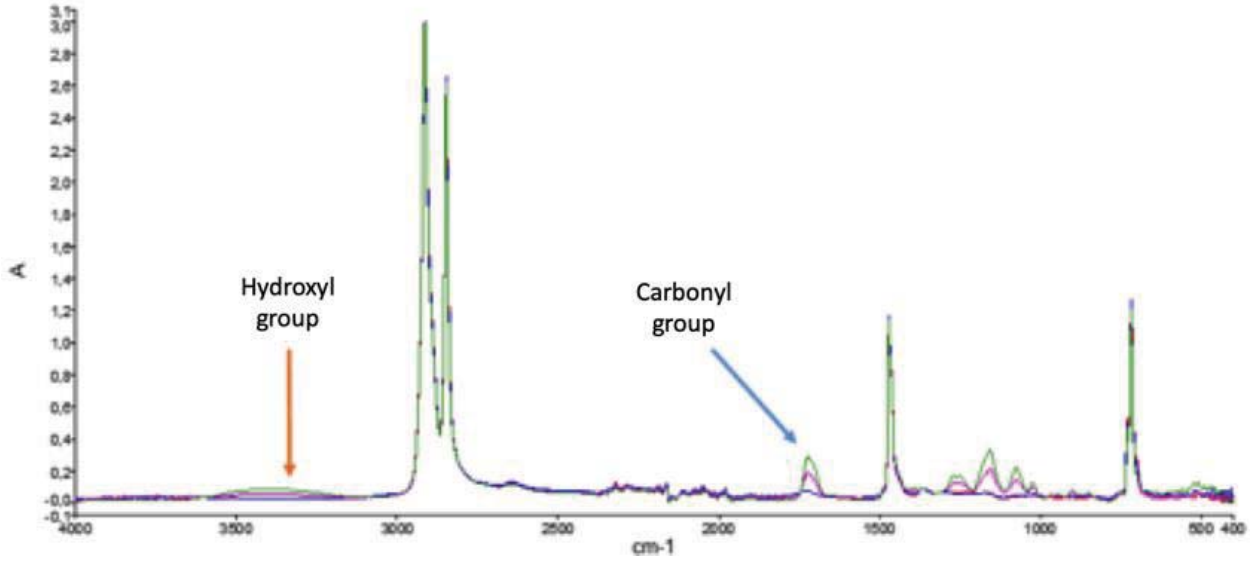


Figure 8. 20. Change in carbonyl and hydroxyl peak intensities of UHMWPE-g-PHEMA with various initial monomer feed irradiated for 60 min. From bottom to top 10, 20, and 30 % HEMA concentration

Other important parameter to control the extent of HEMA grafting is the irradiation duration. The polymerization medium prepared with 20 % (v/v) initial monomer concentration was irradiated for 60 min, 90 min and 120 min. The area under the carbonyl peak was compared. The carbonyl peak area increased with increasing irradiation duration. Both the carbonyl and hydroxyl peaks were boosted upon 120 min of irradiation (Figure 8.21).

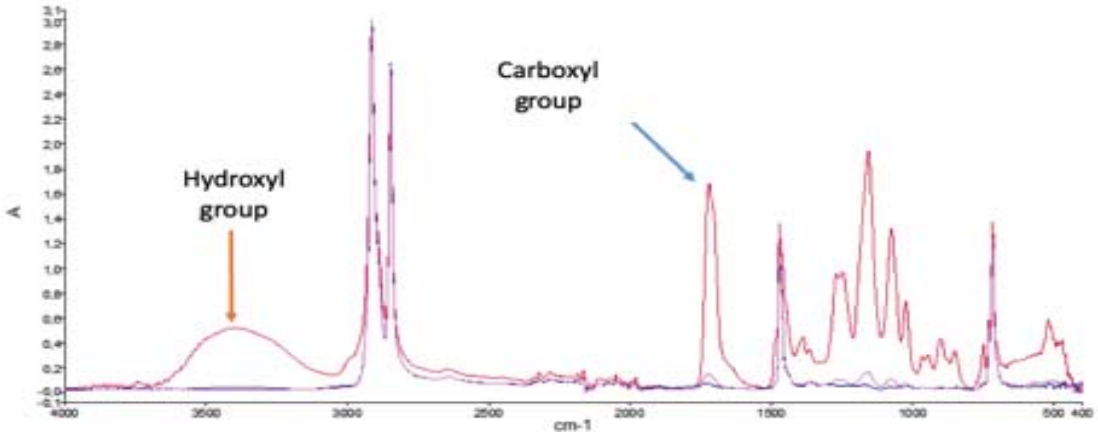
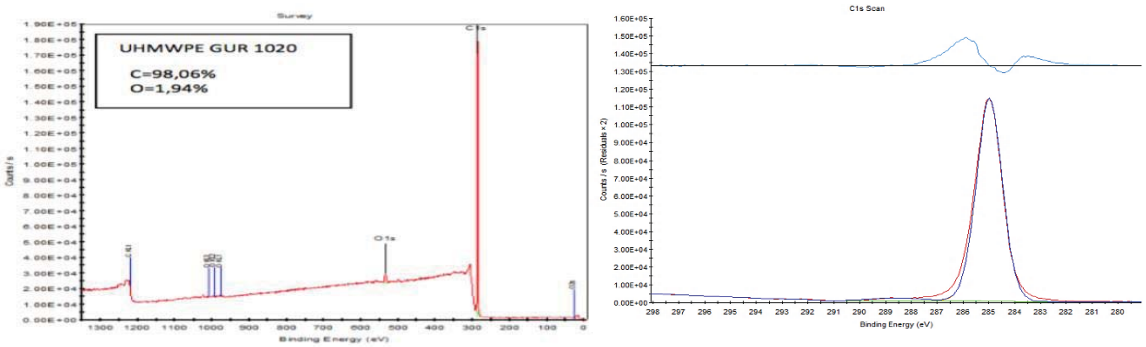


Figure 8. 21. Change in carbonyl and hydroxyl peak intensities in regard to irradiation time from bottom to top; 60 min, 90 min, 120 min (with 20 % (v/v) initial monomer concentration)

The presence of grafted PHEMA groups on UHMWPE was proven supportively with X-ray photoelectron spectroscopy (XPS). The XPS survey showed that there are two major elements, oxygen and carbon on the UHMWPE-g-PHEMA surface. C1s spectrum of the UHMWPE-g-PHEMA has yielded four peaks upon deconvolution. Two of them belong to C-C bond with different chemical character. C-O and C=O bonds also showed up besides C-C bond. The C-O peak at 296.3 eV and C=O peak at 298 eV showed the grafting was successfully done (Figure 8.22). The oxygen peak which was detected in virgin UHMWPE GUR 1020 was attributed to carbonyls, hydroperoxide and catalyst residues in commercial UHMWPE.

a) b)



c) d)

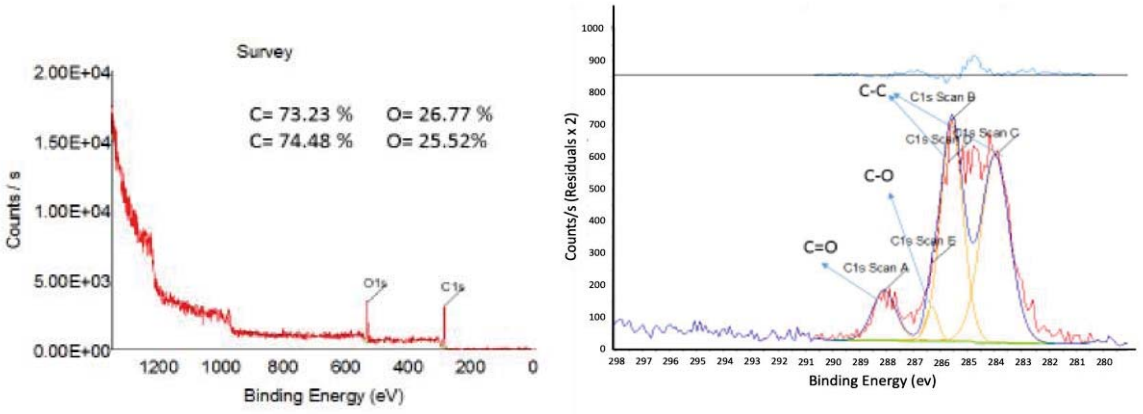


Figure 8. 22. XPS surveys and C1s spectra of a, b) Virgin UHMWPE, c, d) UHMWPE-g-PHEMA (with 20 % (v/v) initial monomer concentration, irradiated for 120 min)

To determine absolute chemical composition of the copolymers C, H, N, S analysis (elemental analysis) was used. The amount of oxygen for PHEMA grafted UHMWPE sample is calculated by subtracting the sum of other elements (C and H) that were directly measured in the analysis from 100%. The grafting percentage of copolymer was calculated using the following equations (1) and (2). Table 8.1 shows that the virgin UHMWPE has 0.3% oxygen due to carbonyls, hydroperoxide and catalyst residues that come from the manufacturing process. The oxygen content of copolymer increased with increased initial monomer feed and irradiation time. The grafting percentages calculated by the Equation 2 were increased with increased initial monomer concentration.

$$\frac{m_{oxygen}x3xb}{m_{UHMWPE\ unit}\times a+m_{PHEMA\ unit}xb}\times 100 = \%Oxygen \qquad \text{Equation 1}$$

$$\frac{m_{PHEMA\ unit}}{m_{UHMWPE\ unit}\times a+m_{PHEMA\ unit}xb}\times 100 = \%Grafting \qquad \text{Equation 2}$$

where ‘a‘ is the repeating unit of UHMWPE and ‘b’ is the repeating unit of PHEMA in a copolymer.

Table 8. 1. Grafting percentage of UHMWPE-g-PHEMA synthesized by conventional radical polymerization by using 10 % and 20 % monomer feed for 60, 90, 120 min

% Initial mon. con. (v/v), irr. time (min)	Carbon (%)	Hydrogen (%)	Oxygen (%)	Grafting (%)
Non-irr.	85	14.7	0.3	
10%, 60	86.4	10.8	2.8	7.7
10%, 90	84.6	10.6	4.1	11.2
10%, 120	83.7	10.2	7.7	20.8
20%, 60	84.9	10.6	4.5	12.1
20%, 90	83.4	10.4	6.2	16.7
20%, 120	79	9.9	11.1	30.1

Prolonging the irradiation duration at both 10 % and 20 % monomer concentration increased both oxygen content and degree of grafting. Beyond 20 % initial monomer feed and two hours of irradiation resulted excessive homopolymer formation which is featured with hydrogel formation which embeds UHMWPE particulates and impairs the course of grafting. Hence, grafting was limited to 20 % monomer concentration and 2 hours of irradiation time.

It was theoretically expected that the higher the grafting degree for PHEMA the more hydrophilic the copolymer gets. To verify this, contact angle of water was measured for copolymer prepared with various initial monomer feed. Prior to contact angle measurements, copolymer in powder form was pressed to thin pellets by a hot press under 10 MPa of pressure. Hydrophobic surfaces exhibit greater water contact angles than those hydrophilic surfaces.

Nonetheless, duration of grafting as long as two hours with grafting degree 30 % decreased contact angle from 100.6 to 62 degrees (Figure 8.23).

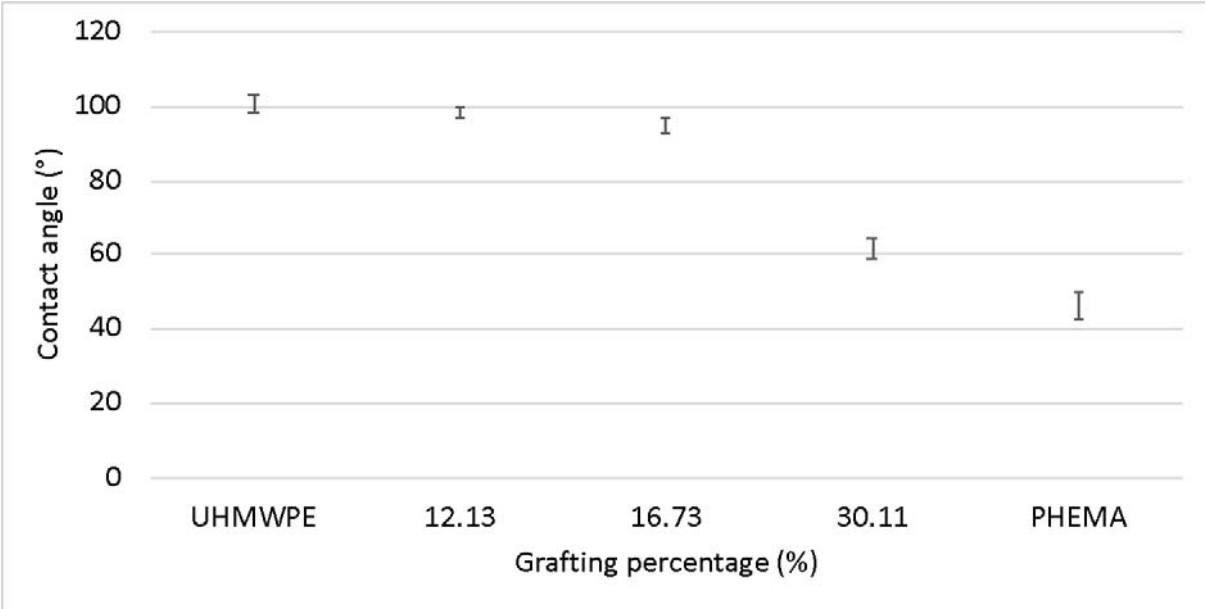


Figure 8. 23. Contact angle of virgin UHMWPE, UHMWPE-g-PHEMA with grafting degree 12.13, 16.73, 30.11 and PHEMA

Mechanical strength is one of the major properties that needs to be assessed during the characterization of an implant material. Crystallinity is one of the key factors in this regard. To evaluate whether the crystallinity changes in UHMWPE-g-PHEMA compared to virgin UHMWPE, differential scanning calorimetry analysis was conducted (Figure 8.24). UHMWPE is a semi-crystalline polymer with 47.4 % crystallinity and melting temperature of 137 °C. Upon incorporation of PHEMA chains to the UHMWPE both the crystallinity and the melting temperature decreased. Among the copolymers prepared with various initial monomer concentration, copolymer with 30 % grafting percentage showed the lowest melting temperature as 132 °C and lowest crystallinity was calculated as 42 % by using Equation 3 (Table 8.2).

$$X_c = \frac{\Delta H_{UHMWPE-g-PHEMA} \times \frac{(100 - Grafting\ \%_{UHMWPE-g-PHEMA})}{100}}{\Delta H_{UHMWPE}} \qquad \text{Equation 3}$$

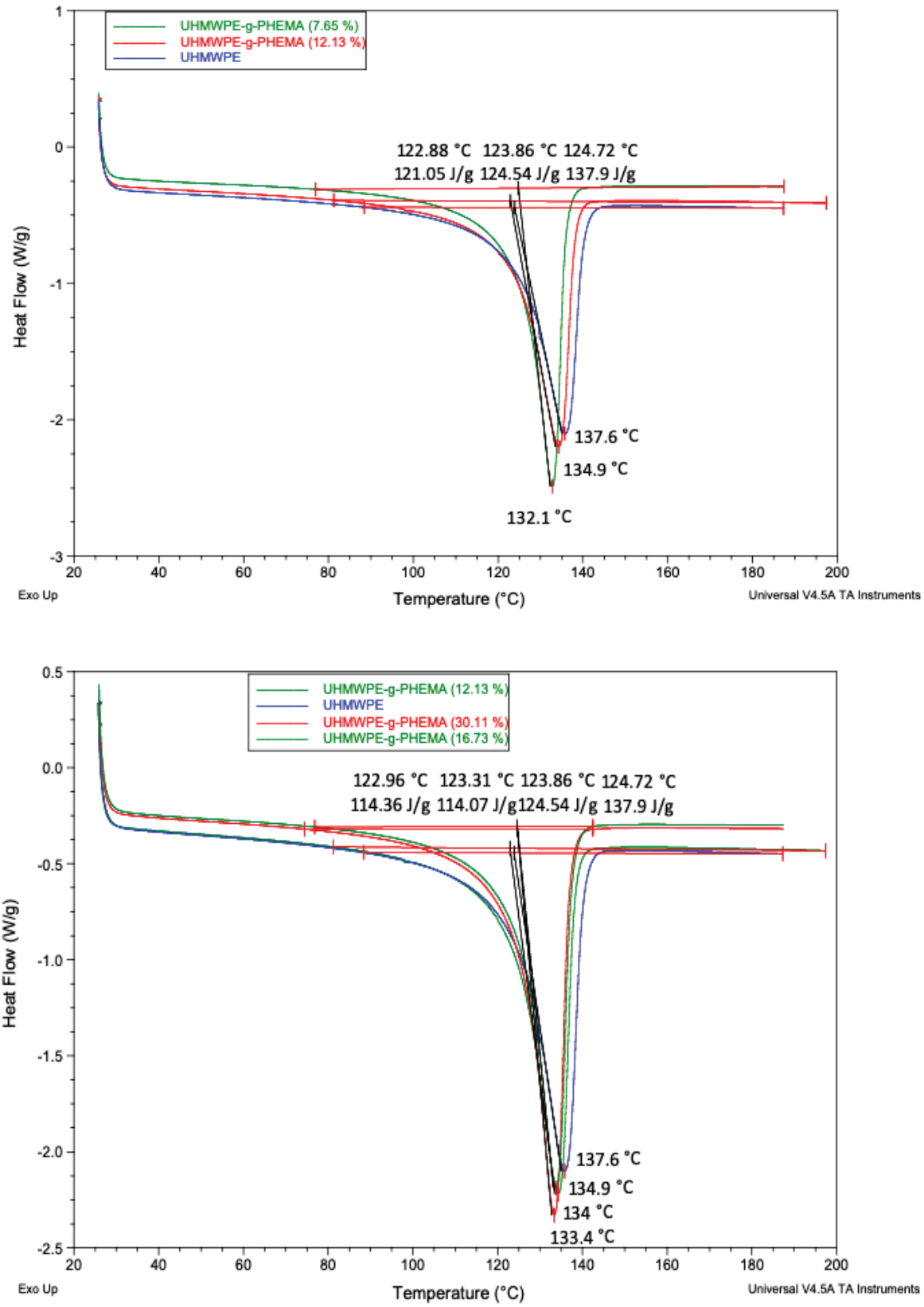


Figure 8. 24. DSC curves of virgin UHMWPE and UHMWPE-g-PHEMA with various grafting percentage

Table 8. 2. Melting temperatures and crystallinity percentages of virgin UHMWPE and UHMWPE-g-PHEMA with increasing degree of grafting

Initial Monomer Concentration (%),	Irradiation Duration (min)	Grafting (%)	T_m(℃)	% X_c
0	**0**	**0**	137.6	47.4
10	**60**	**7.65**	132.1	41.6
20	**60**	**12.13**	134.9	42.8
20	**90**	**16.73**	134	39.2
20	**120**	**30.11**	133.4	39.3

Duration of grafting also decreased melting temperature to 133 °C and crystallinity to 39 % for the copolymer prepared with grafting degree 30 % (Table 8.2).

Polymer chains are packed closely in crystal domains which promotes secondary interactions. Mechanical strength of polymers increases with increasing crystallinity since the secondary interactions are increased. It is seen in Table 8.2 that crystallinity decreased with increased grafting degree which decreased the mechanical strength of the UHMWPE-g-PHEMA.

To ascertain the mechanical strength of the UHMWPE-g-PHEMA/GS copolymers, tensile testing and IZOD impact testing were performed. Dog bone specimens made of UHMWPE-g-PHEMA/GS with degree of grafting 21 % and 30 % were tested. Elongation at break and ultimate tensile strength were compared to UHMWPE/GS counterpart. Both copolymers were loaded with three GS concentration; 2 %, 4 % and 6 % by weight. As the tensile strength of the

UHMWPE/GS samples showed decreasing trend with increasing GS content, the tensile strength of the UHMWPE-g-PHEMA (GD 21 %) samples also showed decreasing trend with increased GS content (Figure 8.25).

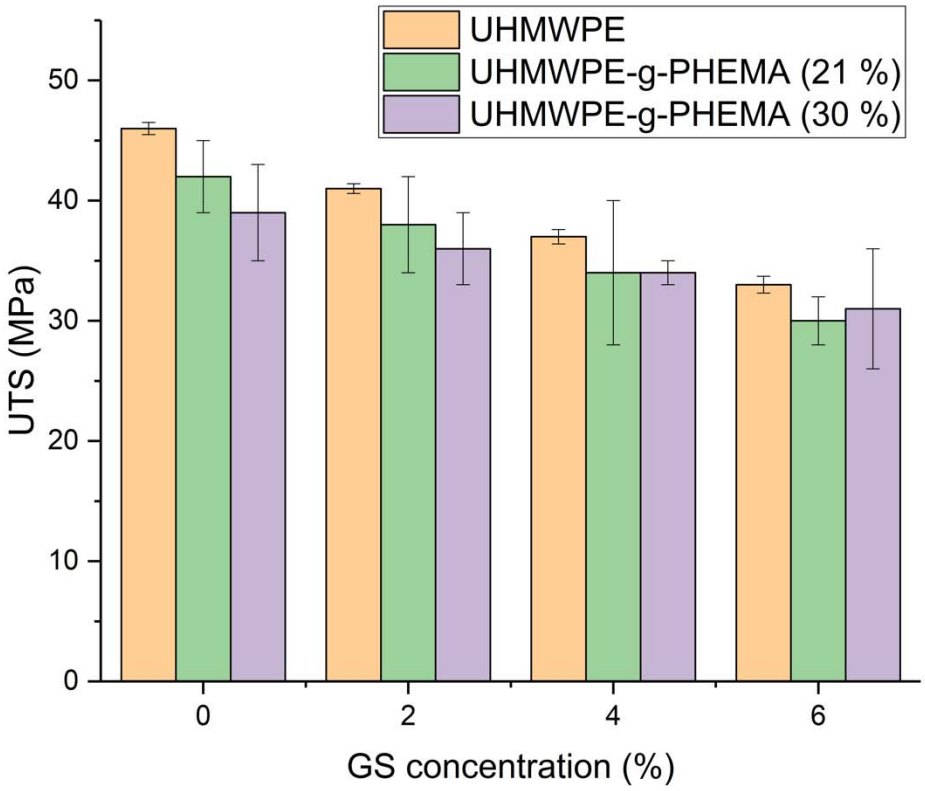


Figure 8. 25. Ultimate tensile strength of virgin UHMWPE and UHMWPE-g-PHEMA (21 % and 30 %) with/without GS

Both ultimate tensile strength and elongation at break decreased as GS concentration in UHMWPE increased. UHMWPE-g-PHEMA (grafting degree 21 %) has ultimate tensile strength of 41 MPa which drops to 30 MPa after incorporation of 6 % GS. Elongation is the other property that declined from 456 % for UHMWPE-g-PHEMA (grafting degree 21 %) to 387 % for UHMWPE-g-PHEMA/GS (6%). GS-loaded UHMWPE-g-PHEMA (grafting degree 30 %) showed similar strength to its counterpart with grafting degree 21 % (Figure 8.26). UTS of GS-loaded UHMWPE-g-PHEMA (grafting degree 30 %) drops from 36 MPa for 2 % GS-loaded copolymer to 31 MPa for 6 % GS-loaded copolymer and EAB drops from high 418 % for 2 % GS-loaded copolymer to low 391 % for 6 % GS-loaded

copolymer (Figure 8.26). IZOD impact strength results of showed similar trend with UTS and EAB results that grafted HEMA slightly decreased the impact strength whereas GS incorporation caused more sharper decrease. The virgin UHMWPE has impact strength of 180 kj/m^2, UHMWPE-g-PHEMA (30 %) has around 170 kj/m^2 whilst their 6 % GS-loaded versions have impact strength around 130 and 140 kj/m^2 respectively (Figure 8.27).

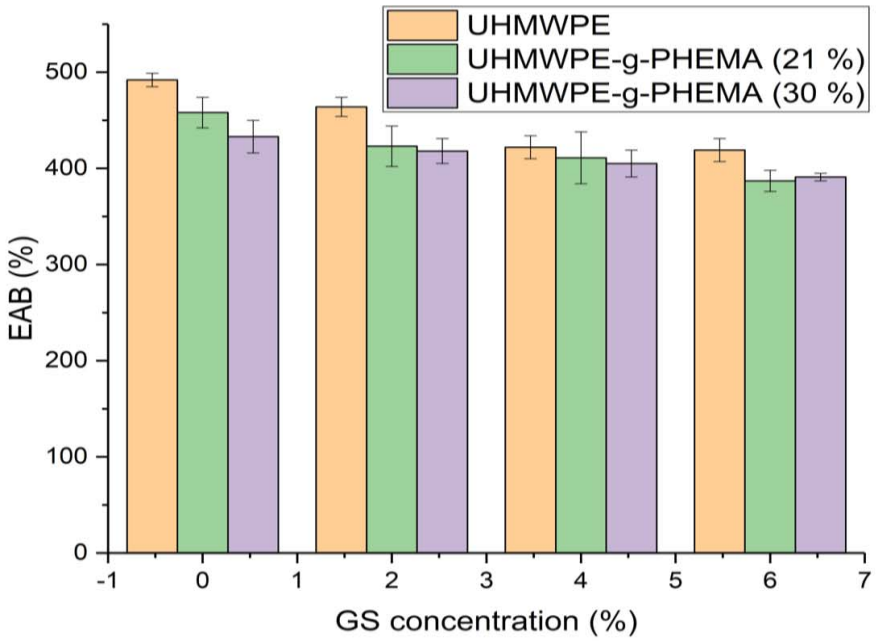


Figure 8. 26. Elongation at break of virgin UHMWPE and UHMWPE-g-PHEMA (21 % and 30 %) with/without GS

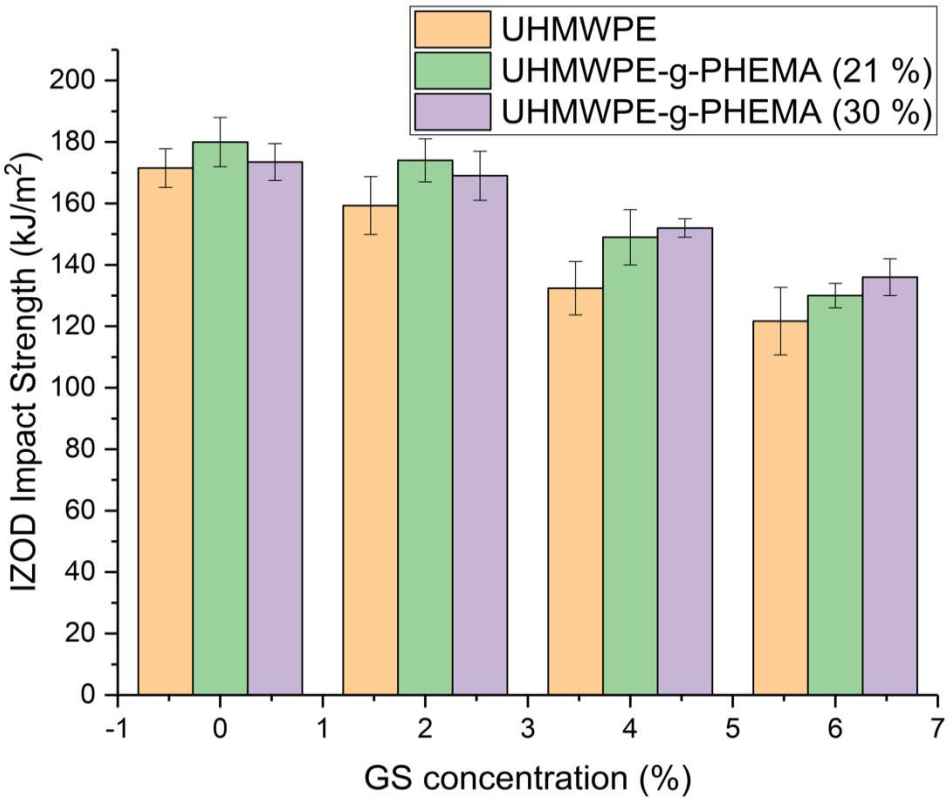


Figure 8. 27. IZOD impact strength of virgin UHMWPE and UHMWPE-g-PHEMA (21 % and 30 %) with/without GS

Functionalization of UHMWPE with PHEMA showed slight weakening in ultimate tensile strength and elongation at break.

In order to study drug release, GS was incorporated in both UHMWPE-g-PHEMA copolymers with grafting degree of 21 % and 30 %. Three different GS concentration was chosen as 2, 4 and 6 % by weight. Both copolymers with three different GS concentration had a burst release in first day regardless of their grafting degree followed by drastic decrease (Figure 8.28 and 8.29). The lowest released GS per day is 12 µg released by UHMWPE-g-PHEMA/GS (2 %) with grafting percentage 21 % after the 4th day of the elution.

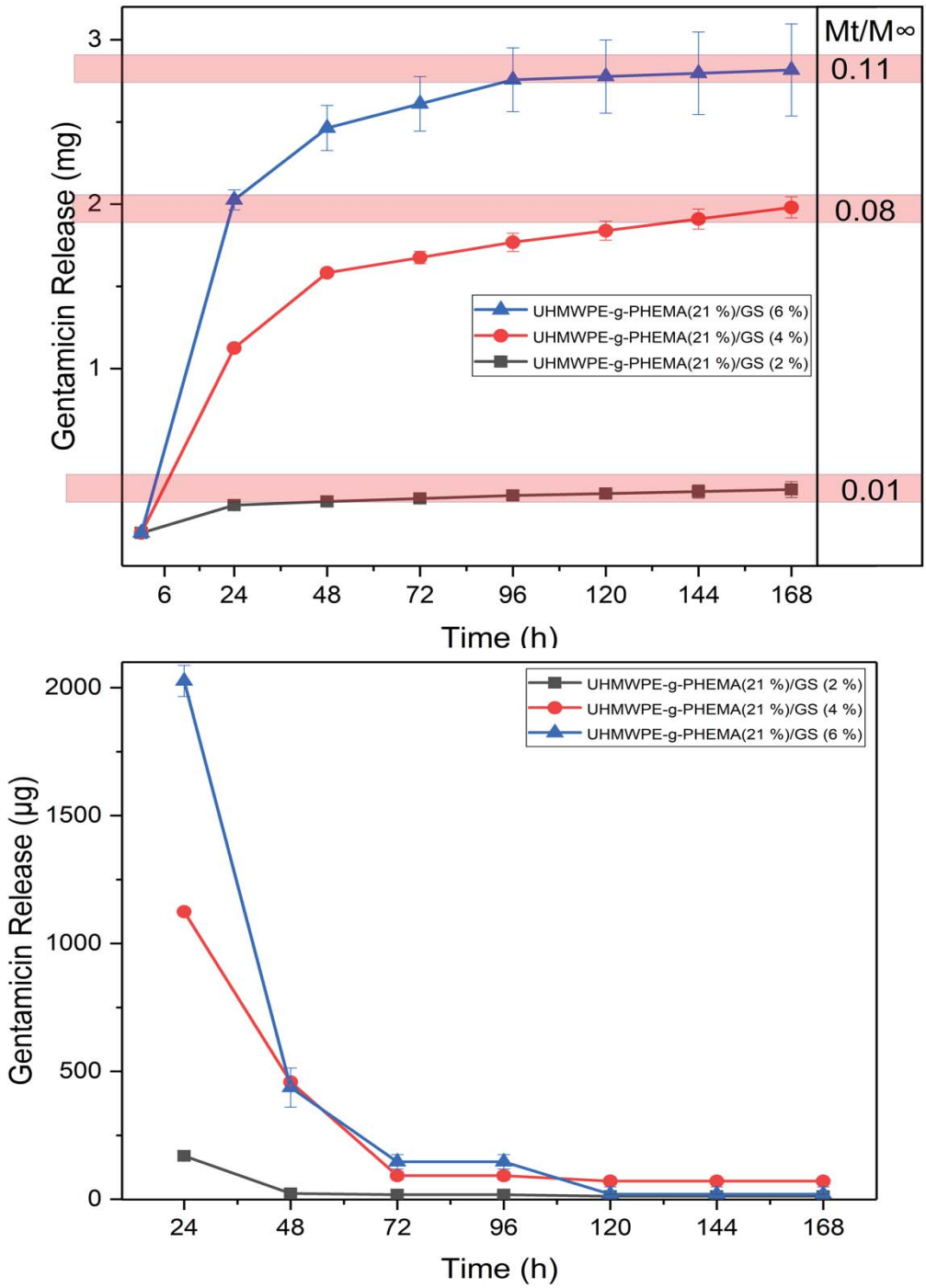


Figure 8. 28. Cumulative (a) and daily (b) GS release from UHMWPE-g-PHEMA (21 %) loaded with 2, 4, 6 % GS

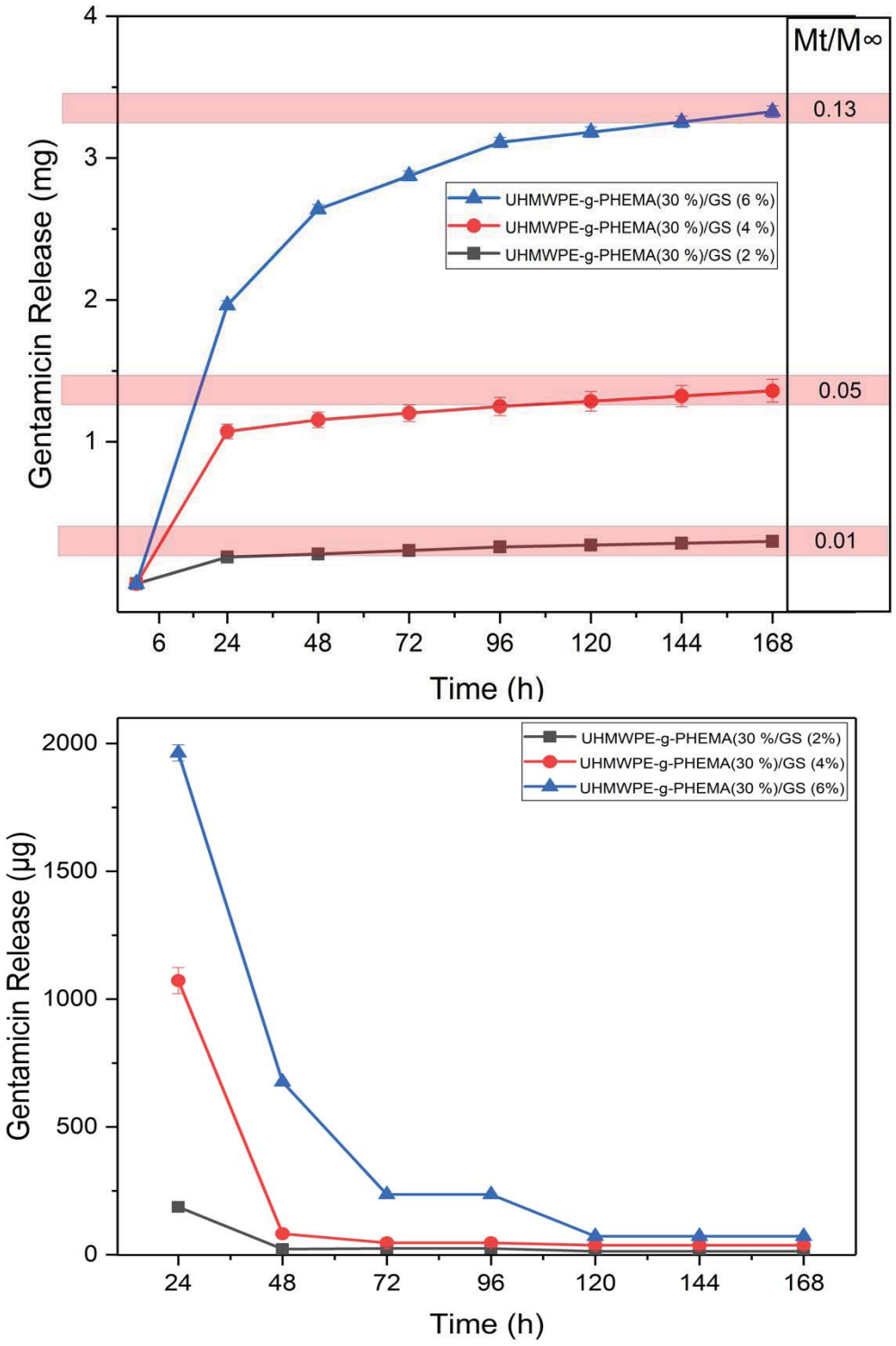


Figure 8. 29. Cumulative (a) and daily (b) GS release from UHMWPE-g-PHEMA with grafting degree of 30 % and loaded with 2, 4, 6 % GS

Granted the drug release specimens are approximately one in two hundredths of actual tibial implant surface area, which released sufficient GS to match MIC (4 µg/ml) unless the volume of joint fluid doesn't exceed 600 ml. Both of the

copolymers with grafting percentages 21 % and 30 % released more GS than virgin UHMWPE**.** However, drug release profiles haven't changed. The increase in released GS amounts was thought to be due to the orientation and distribution of the GS clusters in copolymer matrices. The optical images of GS-loaded copolymer displayed structures that resembles cobblestone (Figure 8.30). The increase in cluster connectivity of gentamicin in UHMWPE-g-PHEMA matrix was thought to be the result of copolymer behaves as an amphiphilic structure and gentamicin resides closely with HEMA moiety of copolymer.

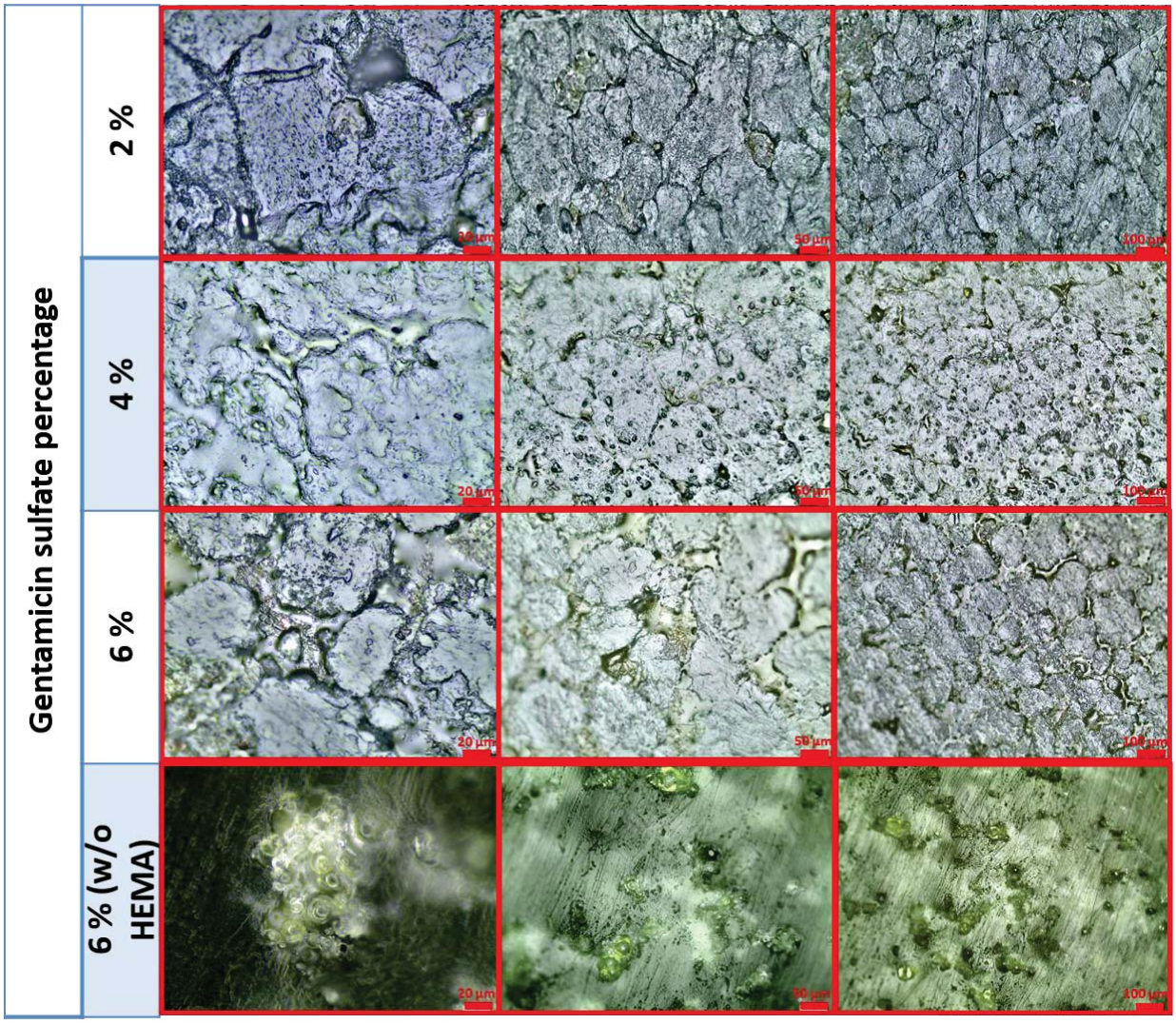


Figure 8. 30. Optical images of UHMWPE-g-PHEMA/GS (30 %) and UHMWPE/GS

8.6. 2-hydroxyethyl Methacrylate Grafting from UHMWPE via RAFT Polymerization

Introduction and advancement of living radical polymerization (LRP) techniques recently represented a major avenue in polymer chemistry. These techniques brought countless opportunities to synthesize tailor-made structures, thereby it is likely to have significant progress in future applications of polymeric materials. Adaption of CRP techniques to industry depends on their feasibility, versatility and their extra cost for the target material. Among those CRP techniques, RAFT polymerization has particular importance since it combines the advantages of conventional polymerization and CRP. The RAFT polymerization could be applied with many monomers. The RAFT polymerization is also very effective on controlling average molecular weight and molecular weight distribution. It is also useful to control chain-end functionality and macromolecular architecture. In this study, RAFT polymerization was particularly used to control molecular weight and its distribution so the interaction between gentamicin sulfate and the polymeric matrix can be improved which would enable the release of gentamicin sulfate in a controlled manner. More specifically, 2-hydroxyethyl methacrylate is grafted from UHMWPE and copolymer was incorporated with gentamicin sulfate. Given the functional groups of P(HEMA) of UHMWPE-g-P(HEMA) (UHMWPE-g-PHEMA prepared via RAFT polymerization is denoted as R-UHMWPE-g-PHEMA beyond this part) and gentamicin sulfate will have secondary interactions, release mechanism was investigated.

Some of the RAFT agents have chromophore moieties that absorb UV light (Lu et al. 2006). It was reported that DDMAT has two absorption peaks in UV region (Figure 8.31) (Bakar Atıcı 2018).

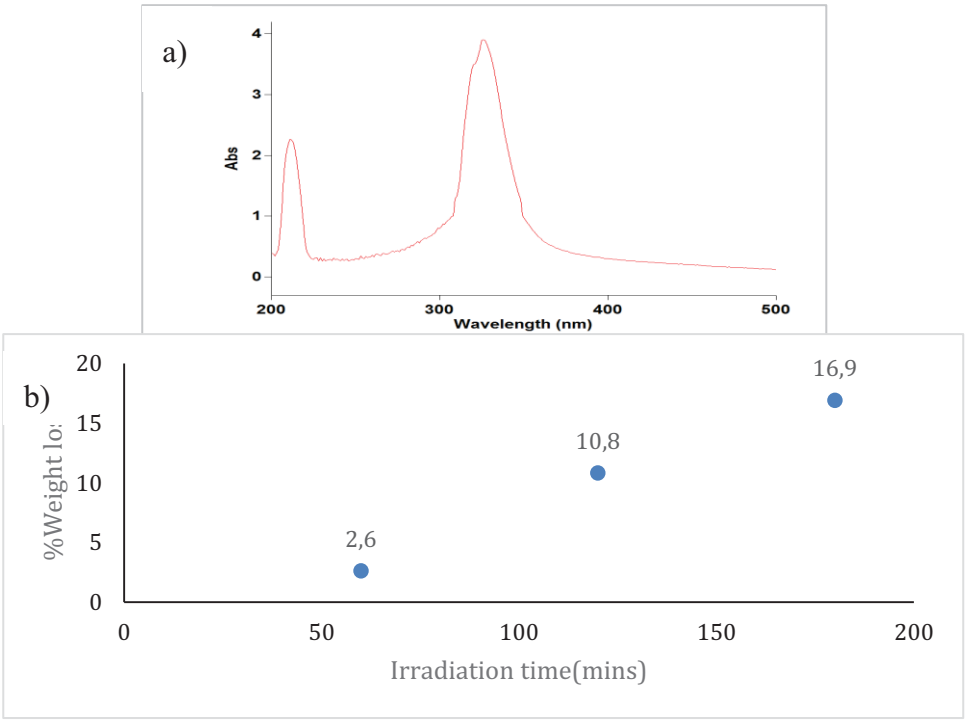


Figure 8. 31. a) UV-Vis spectra of DDMAT and b)weight loss of DDMAT after various irradiation time (Bakar Atıcı 2018).

Once the RAFT agent is photolyzed, it is important to know the amount of the intact RAFT agent in order to calculate the actual quantities of the intact agent. In comparison to dithioester type of RAFT agents, trithiocarbonate type of RAFT agents are more stable against photolysis (Lu et al. 2006; Bakar Atıcı 2018). Yet, in this dissertation study, it was chosen to utilize pre-irradiation technique for RAFT agent's stability. Due to its trithiocarbonate character and compatibility with 2-hydroxyethyl methacrylate monomer S-dodecyl-S′-(α,α′-dimethyl-α″-acetic acid) trithiocarbonate (DDMAT) was chosen as chain transfer agent for the RAFT polymerization.

All of the grafting experiments were performed in water:acetone (1:1) solvent system which dissolves DDMAT but not benzophenone. As benzophenone stays deposited on the UHMWPE surface it became possible to impart UHMWPE surface with macroinitiator character.

First and foremost feature of RAFT polymerization is the possibility of setting the molecular weight of the polymer by adjusting the [monomer]/[RAFT agent] ratio. As this ratio increases, molecular weight of the polymer increases proportionally.

$$M_{n,theoretical} = M_{w_{RAFT}} + \frac{[HEMA]}{[DDMAT]} \times M_{w_{HEMA}} \times Conversion \qquad \text{Equation 4}$$

The longer the polymer chains and the more grafting degree, the higher the molecular weight of the grafted polymer (Barsbay and Güven 2013). It is a well-known fact that RAFT polymerization often yields shorter chains with lower molecular weight compared to its grafting via conventional radical polymerization counterpart, so it is important to determine monomer concentration. Subsequently, other important aspect to be determined is the degree of conversion as seen from above given Equation 4.

Since the grafting degree with 20 % is higher than 10 %, experiments for grafting via RAFT polymerization were conducted with 20 % HEMA concentration. Samples were irradiated for one hour in pre-irradiation step and then thermally treated at 60 ℃ for 60 min and 120 min. Three different [HEMA]/[DDMAT] ratio was chosen as 350, 475 and 800.

The grafting degree was calculated gravimetrically by weighing the synthesized, washed and dried UHMWPE-g-PHEMA samples. Table 8.3 shows that grafting degree varies between 2 % for [HEMA]/[DDMAT] ratio 350 for 60 min and 13.6 % for [HEMA]/[DDMAT] ratio 800 for 120 min. Thus [HEMA]/[DDMAT] ratio was chosen as 800 and samples were irradiated for 120 min since these conditions yielded optimum grafting degree.

Table 8. 3. Grafting percentage of UHMWPE-g-PHEMA (20 %) synthesized by RAFT polymerization with various [HEMA]/[DDMAT] for 60 and 120 min

	Grafting Degree (%)		
[HEMA]/[DDMAT]	350	475	800
Time (min)			
60	2	3.2	5.7
120	6.3	9.1	13.6

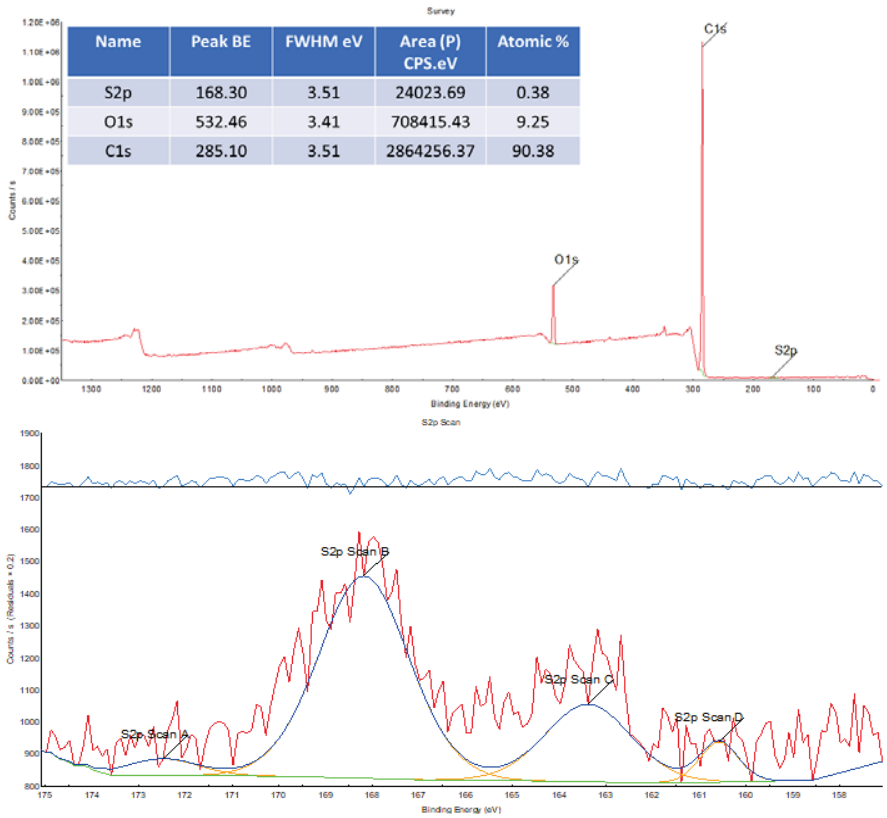
The degree of grafting was also calculated by C, H, N, O analysis. Table 8.4 shows the relation of grafting degree with [monomer]/[RAFT agent] ratio for 120 min irradiated samples. The degree of grafting, as expected, increased with increased [monomer]/[RAFT agent] ratio. The rate of RAFT polymerization and its kinetics are different than its corresponding conventional radical polymerization. RAFT polymerization often requires longer polymerization time to match the grafting degree of its conventional polymerization analogue (Barner-Kowollik et al. 2005). The grafting degrees (%) calculated from elemental analysis were higher compared to grafting degrees obtained with gravimetric methods. Although filtration of UHMWPE-g-PHEMA was carried out carefully, loss of material that might have occurred during the filtration can be the reason of this result. It should also be noticed that, RAFT agents expected to be at chain ends when RAFT agents are used in polymerization However, despite the RAFT agent contains sulfur atom, it wasn't detected in elemental analysis. The amount of sulfur atoms stayed under the detection limit (1%) of this method.

Table 8. 4. C, H, N, S analysis of R-UHMWPE-g-PHEMA samples polymerized in 120 min with various [HEMA]/[DDMAT]

	C, H, N, S Analysis				Gravimetric
[HEMA]/[DDMAT]	**% Carbon**	**% Hydrogen**	**% Oxygen**	**% Grafting**	**% Grafting**
350	86.6	10.8	2.6	7.1	6.3
475	84.6	10.6	4.8	12.9	9.1
800	83.7	10.5	5.8	15.6	13.6

Surface characterization of samples prepared with three different [HEMA]/[DDMAT] ratio for 120 min was done by XPS (Figure 8.32).

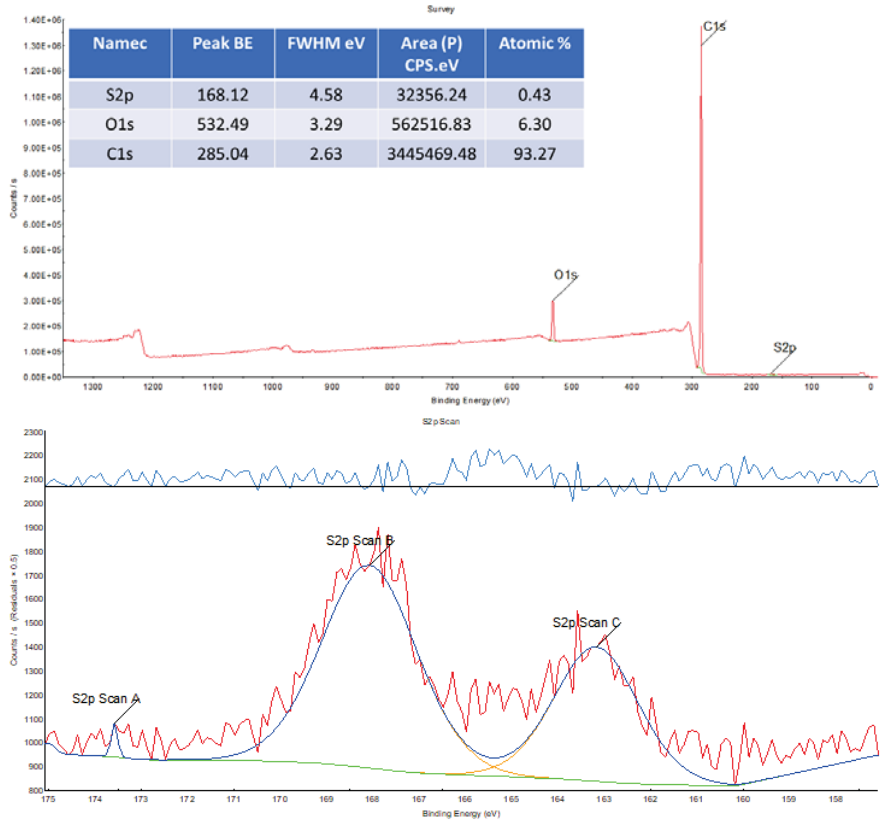
a) [HEMA]/[DDMAT], [350]\[1]



Name	Peak BE	FWHM eV	Area (P) CPS.eV	Atomic %
S2p	168.30	3.51	24023.69	0.38
O1s	532.46	3.41	708415.43	9.25
C1s	285.10	3.51	2864256.37	90.38

b) [HEMA]/[DDMAT], [475]\[1]

Namec	Peak BE	FWHM eV	Area (P) CPS.eV	Atomic %
S2p	168.12	4.58	32356.24	0.43
O1s	532.49	3.29	562516.83	6.30
C1s	285.04	2.63	3445469.48	93.27



c) [HEMA]/[DDMAT], [800]\[1]

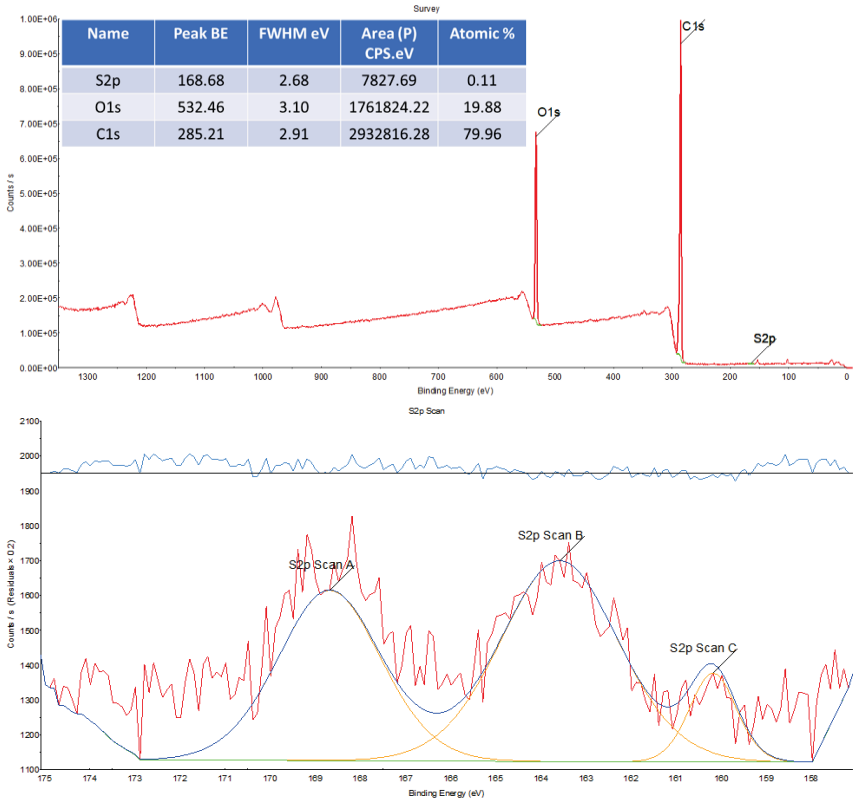


Figure 8. 32. The XPS survey and sulfur spectra of UHMWPE-g-PHEMA (20 %) prepared via RAFT polymerization with [HEMA]/[DDMAT] (350:1 (a), 475:1 (b), 800:1 (c))

The XPS survey showed O 1s at 532 eV, C 1s at 285 eV and S 2p at 168 eV. Although very small, S 2p attributes to the existence of DDMAT at the chain ends which demonstrates that RAFT polymerization took place. The O 1s peak was contributed by both HEMA monomer and DDMAT RAFT agent. The grafting degree results calculated by elemental analysis (Table 8.4).

Both the C, H, N, S analysis and XPS revealed that the degree of grafting increases as [HEMA]/[DDMAT] ratio increases. It was reported in the literature that the homopolymer that is formed during grafting via controlled radical polymerization has approximately the same average molecular weight to those grafted (Barsbay et al. 2007). For this reason, the average molecular weight and polydispersity of grafted PHEMA chains were measured by analyzing the homopolymer of PHEMA by Size Exclusion Chromatography (SEC) equipped with Multi-angle Laser Light Scattering detector (MALLS). Figure 8.33 shows the

chromatograms of PHEMA homopolymers prepared with [HEMA]/[DDMAT] ratios 350, 475 and 800 with conversion 45.1, 51.7, 53 % respectively.

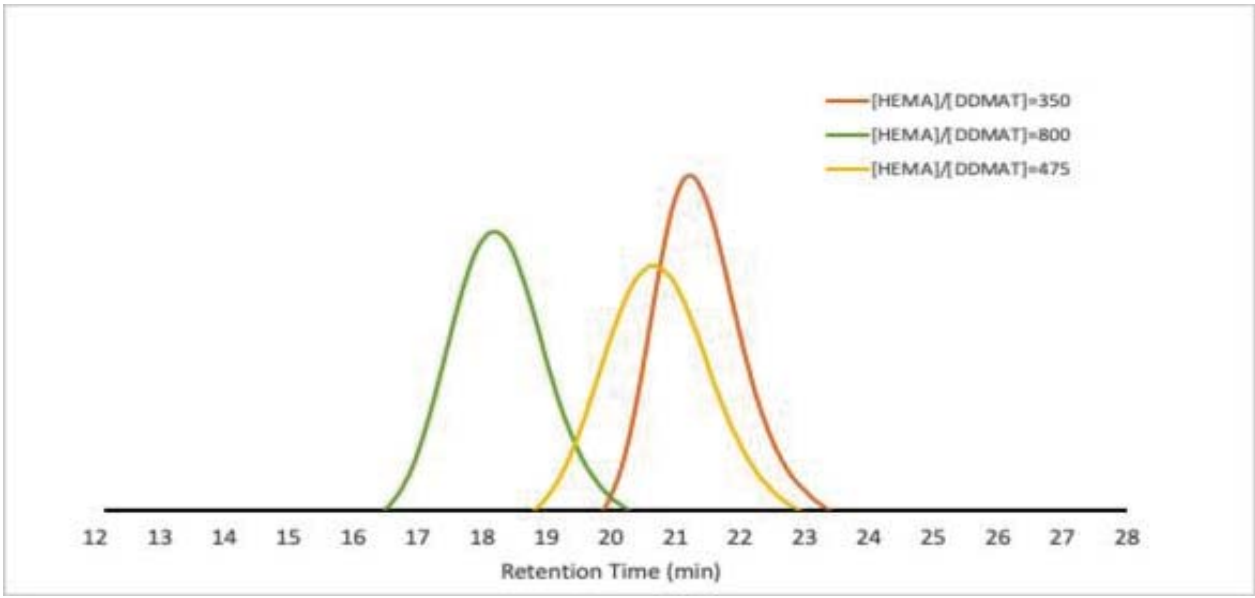


Figure 8. 33. GPC chromatograms of PHEMA homopolymer formed in the grafting medium

Table 8.5 shows the $M_{n,\ theoretical}$,$M_{n,\ experimental}$ and polydispersity (D) of homopolymer of PHEMA. As seen from the chromatograms, the greater the [HEMA]/[DDMAT] ratio is the less retention time the copolymer exhibits. It is due to the increase in average molecular weight. All of the PHEMA homopolymers synthesized via RAFT polymerization had low polydispersity.

Low PDI indicated that RAFT polymerization took place to control the molecular weight distribution. Two factors play vital role to reduce the PDI; [monomer]/[RAFT agent] and degree of conversion. Higher the denominator in the ratio, more the amount of chain transfer agent which ensures relatively low PDI. Conversion on the other hand is another key element to control PDI. The initial broad polydispersity in RAFT polymerization is high due to low transfer constant of RAFT agent. So, the PDI of the polymer decreases as the conversion of the monomer takes place. Since, the PDI is inversely proportional with the DDMAT concentration and the degree of conversion increased with increased RAFT agent

concentration, the homopolymer synthesized with [HEMA]/[DDMAT] : 800 has the highest PDI. The scarcity of DDMAT would result higher PDI since it is the chain transfer agent in the system (Sütekin and Güven 2018).

The RAFT polymerization make relative control of molecular weight of the polymers plausible by varying the [monomer]/[RAFT agent] and conversion. Table 8.5 shows that the $M_{n(theoretical)}$ and $M_{n(experimental)}$ are in good agreement. This shows that grafting proceeded via RAFT polymerization.

Table 8. 5. Monomer to polymer conversion, theoretical and experimental M_ns and polydispersity of PHEMA homopolymer formed in the grafting medium

[HEMA]/[DDMAT]	% Conversion	M_n (theoretical)	M_n (experimental)	PDI
350	45.1	20907	22764	1.24
475	51.7	32324	34894	1.29
800	53	55544	56543	1.31

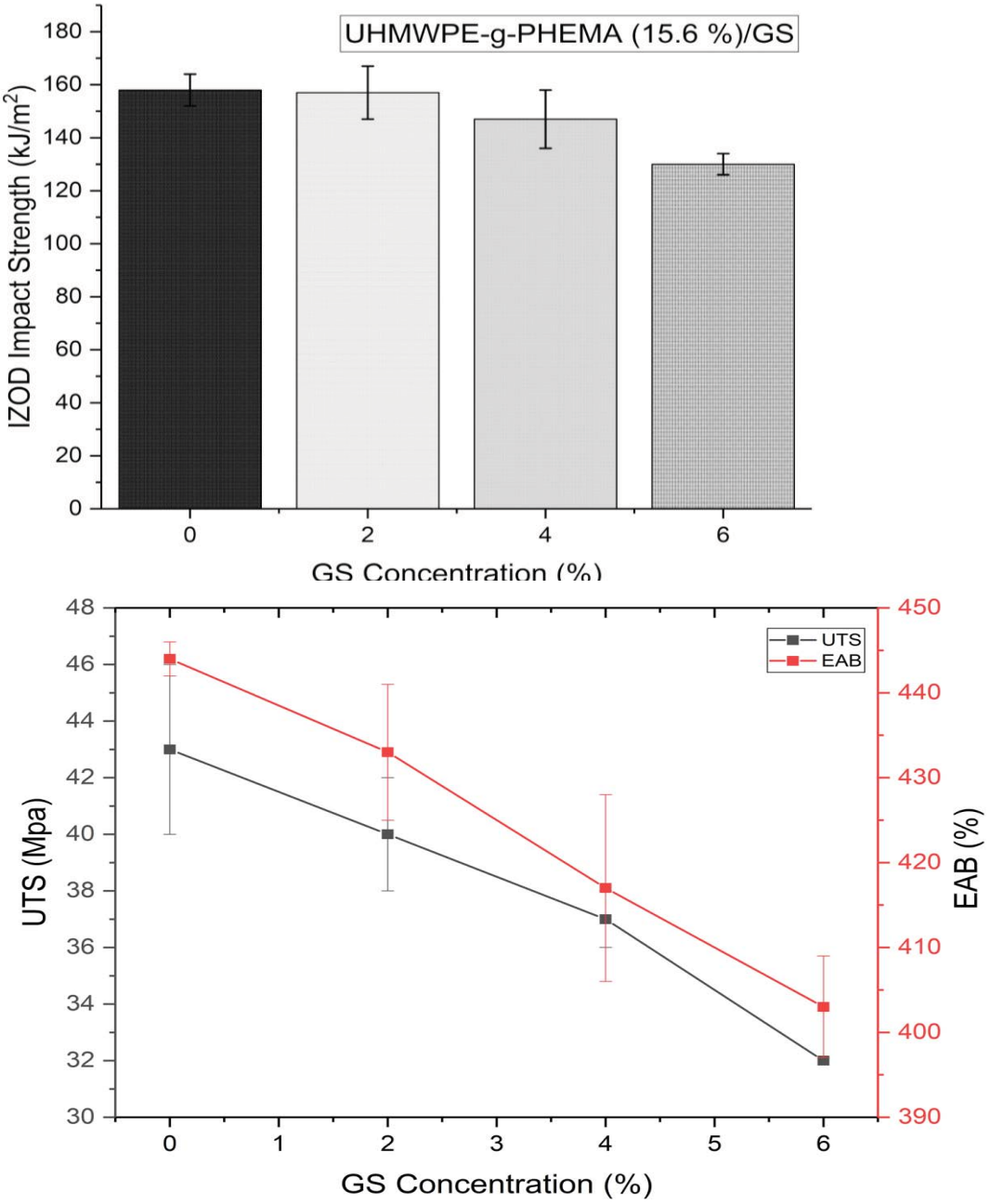


Figure 8. 34. Ultimate tensile strength, elongation at break and IZOD impact strength of UHMWPE-g-PHEMA (15.6 %) prepared via RAFT polymerization loaded with 2, 4, 6 % GS

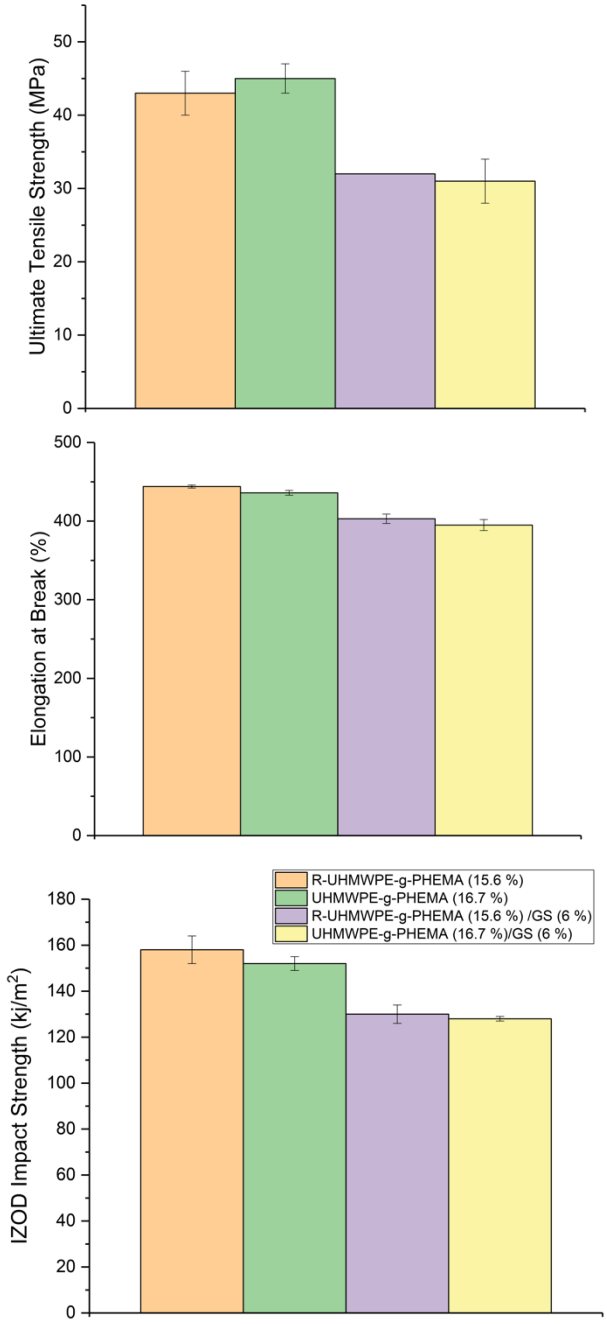


Figure 8. 35. UTS, EAB and IZOD impact strength comparison of UHMWPE-g-PHEMA with grafting degrees 15.6 % (RAFT) and 16.7 % (conventional)

Figure 8.35 shows the comparison of UTS, EAB and IZOD impact strength of UHMWPE-g-PHEMA prepared via RAFT and conventional techniques. The results showed that samples with similar grafting degree (15.6 % vs 16.7 %) exhibited similarly in mechanical testing and incorporation of GS decreased their overall strength. Both samples have UTS around 45 MPa which dropped to nearly 30 MPa upon GS loading. Similarly, both samples elongated around 450 % and

dropped to nearly 400 % after addition of GS. Their IZOD impact strength lost almost 30 kj/m^2 after GS incorporation.

Hydrogels and hydrogel coated surfaces have gained vast attention for bio-applications (Kopeček and Yang 2007). PHEMA particularly is one of the earliest hydrogels used in bio-applications. Its versatile nature such as biocompatibility, permeability to small molecules and more importantly decent lubricant properties makes it a good candidate for articulating implant surfaces. Pin-on-disc wear test of UHMWPE-g-PHEMA samples with and without gentamicin sulfate in them were performed. The POD test showed that having PHEMA in conjunction with UHMWPE as articulating surface improved wear resistance of the material (Figure 8.36).

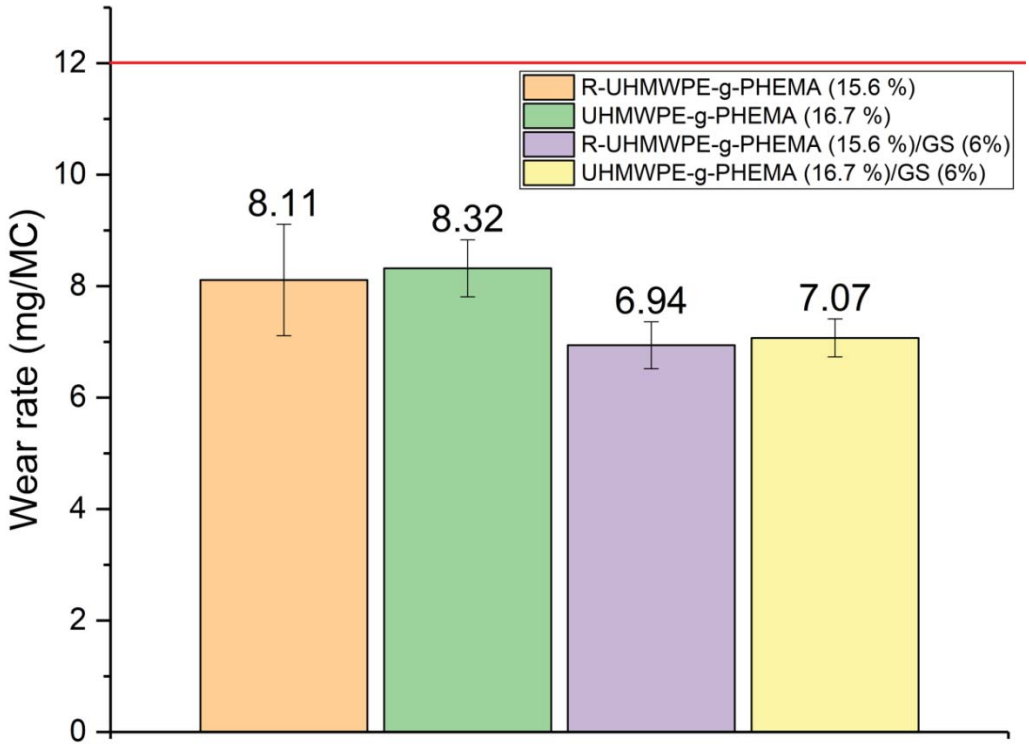


Figure 8. 36. Wear rates of UHMWPE-g-PHEMA with similar grafting degree with and without GS prepared via conventional radical polymerization vs RAFT polymerization (the solid red line denotes the wear rate of UHMWPE)

UHMWPE-g-PHEMA with similar grafting degree showed similar wear rate around 8 mg/MC (Figure 8.36). Incorporation of GS decreased the wear rate even beyond the copolymers itself to 7 mg/MC as it was observed in GS loaded virgin UHMWPE. Overall the reduction of wear rate was thought to be because of PHEMA has ability to reduce the contact stress and promote fluid film lubrication (Bavaresco et al. 2008).

UHMWPE-g-PHEMA samples with similar grafting degrees prepared with RAFT and conventional techniques were chosen in order to compare drug release and antibacterial properties. The UHMWPE-g-PHEMA (grafting degree 15.6 %) prepared with [HEMA]/[DDMAT] : 800 ratio with UV induced preirradiation and RAFT mediated grafting was chosen for drug release and antibacterial efficacy studies. This sample was compared with UHMWPE-g-PHEMA (grafting degree 16.7 %) prepared with conventional technique. Both copolymers were loaded with three GS concentration; 2, 4, 6 %. The amount of GS released for a week were 8 %, 4 %, 1 % for the copolymer with GS mass percentage 6, 4, 2 % respectively (Figure 8.37).

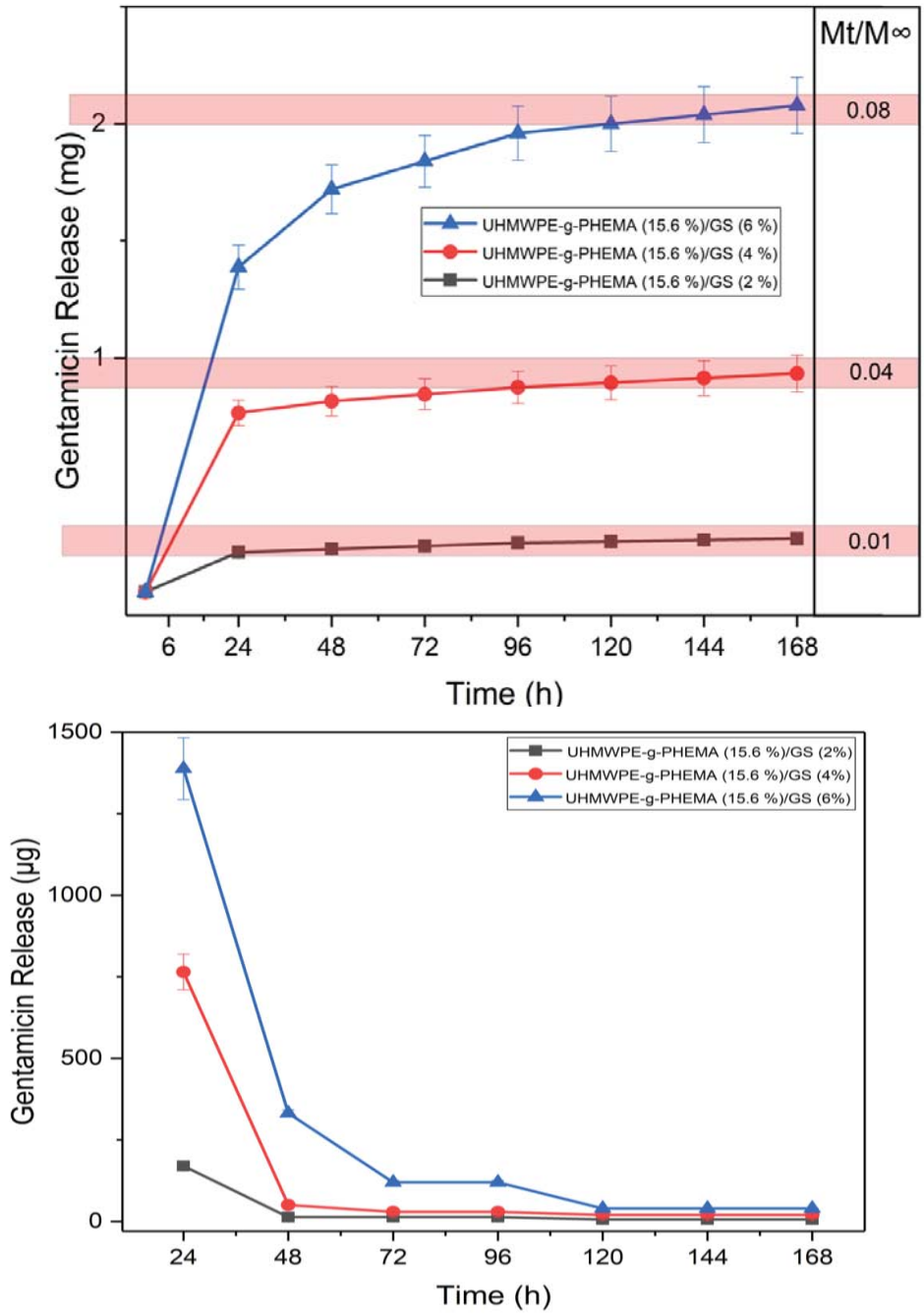


Figure 8. 37. Cumulative (a) and daily (b) GS release from GS-loaded UHMWPE-g-PHEMA (degree of grafting 15.6 %) prepared via RAFT polymerization

The copolymer with GS concentration 2 % released two-fold of the MIC of GS whereas it was five-fold for 4 % and ten-fold for 6 % GS-loaded samples at 7th day (Figure 8.37). In general copolymer synthesized via RAFT polymerization released GS less than copolymer prepared with conventional radical polymerization but more than virgin UHMWPE loaded with GS.

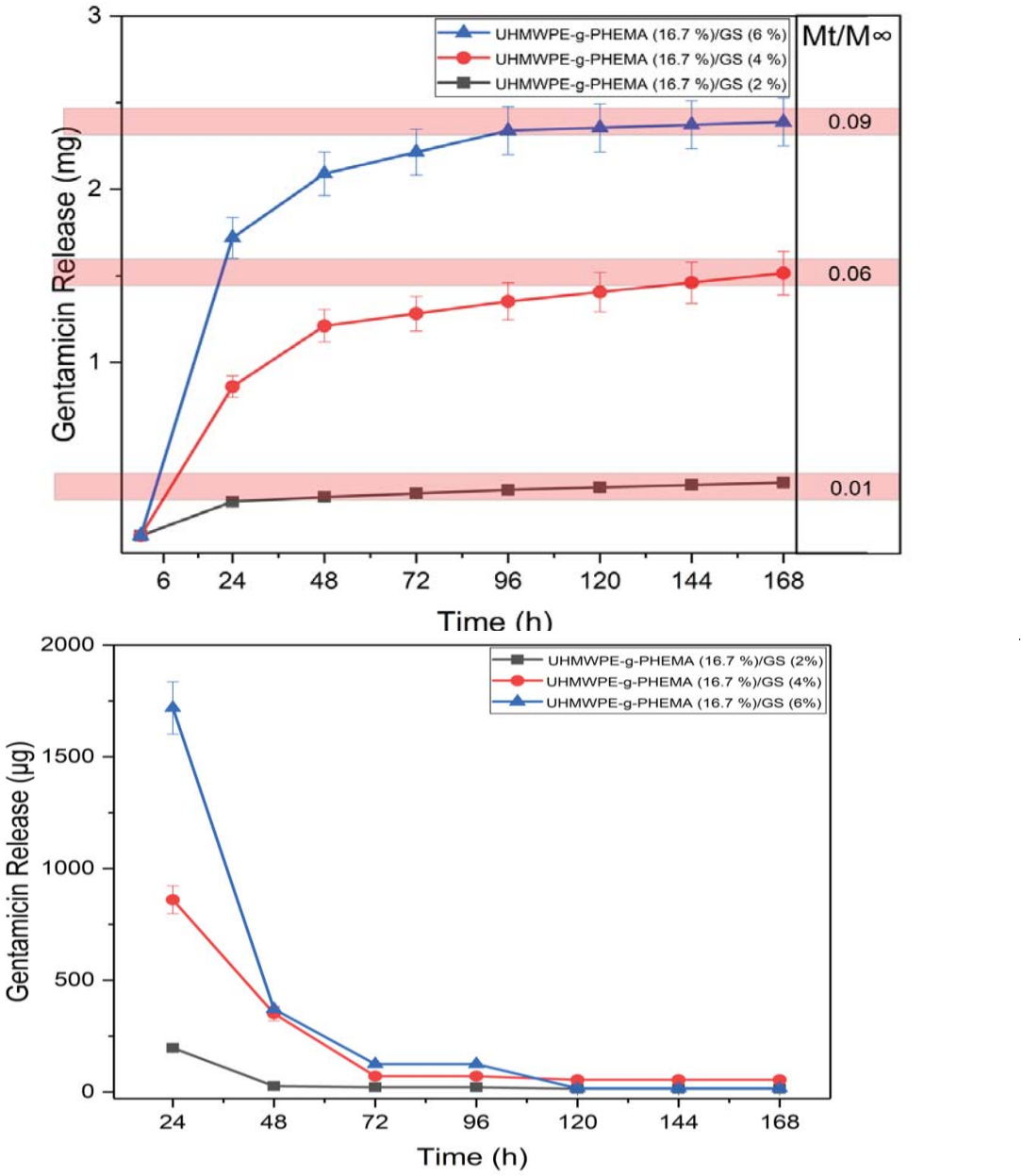


Figure 8. 38. Cumulative (a) and daily (b) GS release from GS-loaded UHMWPE-g-PHEMA (degree of grafting 16.7 %) prepared via RAFT polymerization

Figure 8.38 shows cumulative and daily GS release from UHMWPE-g-PHEMA with similar grafting degree prepared with RAFT and conventional technique. It was understood that both copolymers has burst release until 3rd day followed by more steady state GS release. Sample with grafting degree 16.7 % and loaded with 6 % GS released 20 % more GS as 2.21 mg per test strip in the first three days. At the end of 7th day sample prepared with conventional technique (grafting degree of 16.7 %) released more GS for all three concentrations.

The UHMWPE-g-PHEMA/GS is not a contact killing material, yet, its antimicrobial property comes from the released GS and it is correlated to the amount of it. So, the more GS is released from the UHMWPE-g-PHEMA/GS the more antimicrobial efficiency it will have. The ideal device would have a steady, sustainable release rate just above the MIC of the relevant bacterial strains. Grafting HEMA from UHMWPE has not changed the drug release profile but the release rate. Higher release rates augmented UHMWPE-g-PHEMA/GS to have its surface with less attached bacteria compared to UHMWPE/GS (Figure 8.39).

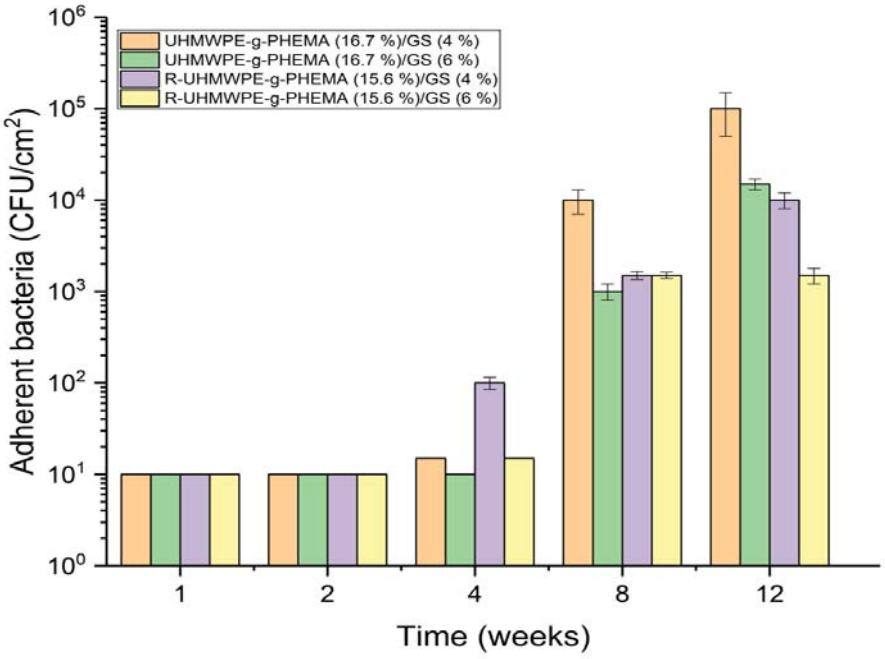


Figure 8. 39. Adherent bacteria count for 12 weeks for the UHMWPE-g-PHEMA/GS (grafting degrees 15.6 and 16.7 %) prepared via conventional radical polymerization vs RAFT polymerization

Over the course of first two weeks there weren't any difference between samples in terms of bacterial adhesion. At 8^{th} and 12^{th} weeks, surprisingly samples with 4 % GS showed better results compared to samples with 6 % GS. Copolymers prepared with RAFT technique exhibited results superior to samples prepared with conventional technique. UHMWPE-g-PHEMA (15.6 %, RAFT) loaded with 6 % GS had around 10^2 CFU adhered bacteria whereas UHMWPE-g-PHEMA (16.7 %,

conventional) loaded with 6 % GS had two orders of magnitude more bacteria (Figure 8.39).

In overall antimicrobial performance comparison of UHMWPE-g-PHEMA/GS to UHMWPE/GS, it was understood that grafting PHEMA from UHMWPE surfaces improved the GS release rate and hence bacterial colonization was inhibited more in those copolymers. Another factor that aided the antimicrobial efficacy of copolymer could be PHEMA is inherently able to inhibit bacterial colonization (Lee et al. 2018).

CONCLUSIONS

The main goal of this dissertation was to create an articulating surface for tibial implants which avoids bacterial colonization for revision surgeries. Virgin UHMWPE has been succesfully used in the total joint arthroplasty for decades now. However, it is known that synthetic materials are vulnerable to infection. Gentamicin sulfate is one of the foremost antibiotics that is used in approaches to treat PJI. In this study, we developed gentamicin sulfate-releasing UHMWPE and UHMWPE-g-PHEMA as a temporary tibial spacer for knee arthroplasty. We investigated the gentamicin sulfate release and antibacterial performances of gentamicin sulfate-loaded virgin UHMWPE and UHMWPE-g-PHEMA prepared via conventional and RAFT grafting techniques.

The UHMWPE/GS and UHMWPE-g-PHEMA/GS blends were dehydrated prior to compression molding for reduce the water stress on gentamicin sulfate. Since, high temperature (170°C) was used to fabricate gentamicin sulfate-loaded UHMWPE and gentamicin sulfate-loaded UHMWPE-g-PHEMA, we showed that the chemical structure of gentamicin sulfate was not comprimised by XPS.

Gentamicin sulfate and UHMWPE were observed as phase seperated based on the optical microscope images. The gentamicin sulfate clusters showed tendency to agglomerate in the polymeric matrix as the concentration increased. UHMWPE/GS with 1, 2, 4, 6, 8, 10 wt. % GS were prepared and used for drug release, antibacterial and mechanical testing studies. All of the GS-loaded virgin UHMWPE showed a burst release in the first six hours followed by more steady state release. UHMWPE/GS (1 wt. %) released 3.23 mg GS and UHMWPE/GS (10 wt. %) released 128 mg GS from full size tibial implant in five days which is approximately 800 and 32000-fold of the MIC respectively for *S. aureus.*

The UHMWPE/GS formulation with 8 wt. % GS exhibited similar drug release profile to clinically used gentamicin sulfate-loaded bone cement (Palacos R-G). Both UHMWPE/GS (8 wt. %) and Palacos R-G have remained as bactericidal against 10^5 CFU/ml for 12 weeks.

The UHMWPE/GS had fewer adherent bacteria on its surface than gentamicin sulfate-loaded bone cement over the course of the 24^{th} week of the test.

The UHMWPE-g-PHEMA was synthesized via UV-initiated grafting with two

approaches: 1) Conventional grafting, 2) Grafting via RAFT polymerization.

UHMWPE-g-PHEMA with grafting percentages 7.65, 12.13, 16.73, 30.11 were obtained by controlling the monomer concentration and irradiation duration of conventional grafting.

The RAFT agent, DDMAT, absorbs the UV light at around 330 nm and decomposes. Hence, it was chosen to preirradiate samples before conducting grafting via RAFT polymerization. The grafting via RAFT polymerization technique was carried out at 60°C. Grafting HEMA via RAFT technique reduced PDI of the grafted HEMA down to as low as 1.24 with [HEMA]/[DDMAT], 350/1 with 45.1 % conversion. GS release from UHMWPE-g-PHEMA/GS with similar grafting degree (15.6 % for RAFT grafting vs 16.7 % for conventional grafting) prepared via conventional and RAFT grafting techniques was compared. Both copolymers have shown decrease in their wear rate (around 8 mg/MC) compared to virgin UHMWPE (around 12 mg/MC). Both copolymers obtained via conventional grafting and grafting via RAFT polymerization were loaded with 2, 4, 6 wt. % GS to analyze their drug release and antibacterial performances.

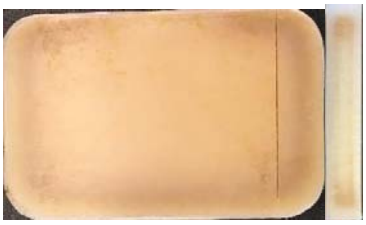
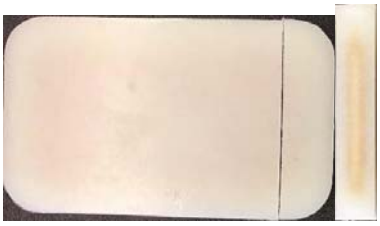
UHMWPE-g-PHEMA/GS prepared via conventional and RAFT technique showed similar GS release of all three GS concentrations for the first week. GS-loaded UHMWPE-g-PHEMA copolymers were compared for their anticolonization properties against *S. aureus*. They all killed 10^5 CFU/mL bacteria regardless from the type of grafting technique and GS concentration for the first two weeks. At 12^{th} week, R-UHMWPE-g-PHEMA had almost two orders of magnitude less bacteria adhered than UHMWPE-g-PHEMA.

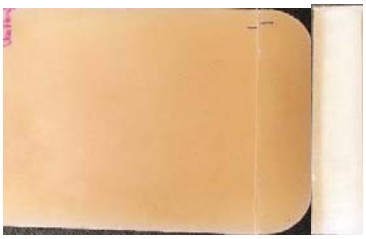
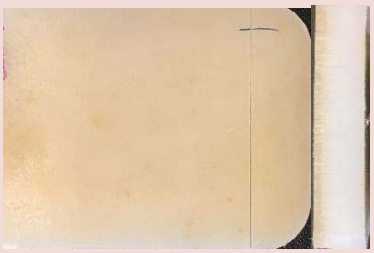
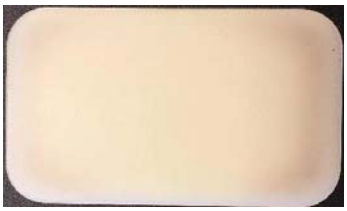
In this study, we succesfully showed that we could change the GS release amounts and thereby the antibacterial performance by modifying UHMWPE spacers. Copolymer prepared via RAFT polymerization had relatively less drug release for the first week and substantially less adhered bacteria at 12^{th} week.

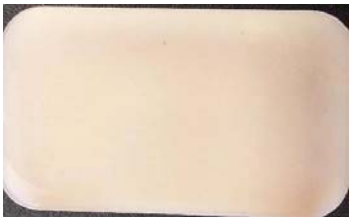
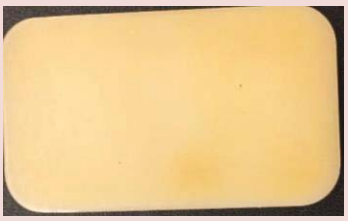
This study could be improved to make higher antibacterial efficacy against a broad spectrum of bacterial species possible by possibly incorporating multiple antibiotics in both UHMWPE and UHMWPE-g-PHEMA. To convert a temporary spacer to a permanent one, the wear rate of the UHMWPE and UHMWPE-g-PHEMA needs to be improved.

SUPPLEMENTARY

Supplementary 1. Images (top and cross-section) and molding conditions of consolidated, tibial shape UHMWPE blocks loaded with GS/GS+VH

Composition	Duration	Temperature	Pressure	Thickness	Mold Type	Image
4.22 % UHMWPE/GS	6 min.	170 °C	20 Mpa	8.98	AB	
2.11%-2.11% UHMWPE/GS/VH	6 min.	170 °C	20 Mpa	8.48	AB	
4.22 % UHMWPE/GS	6 min.	170 °C	40 Mpa	5.1	AB	
4.22 % UHMWPE/GS	6 min.	170 °C	20 Mpa	8.94	AB	

4.22 % UHMWPE/GS	6 min.	170 °C	10 Mpa	9.55	AB	
Layered, 4.22 % UHMWPE/GS	Ramp to 155 °C and kept for 4 min.	Top Platen: RT, Bottom Platen: 160 °C	20 Mpa	10.52	AB	
Layered, 4.22 % UHMWPE/GS	Ramp to 160 °C and kept for 4 min.	Top Platen: 170 °C, Bottom Platen: 160 °C	20 Mpa	10.34	AB	
4.22 % UHMWPE/GS 0.2 % Vit E	6 min.	170 °C	20 MPa	8.18	AB	
4.22 % UHMWPE/GS, 0.2 % Vit E	6 min.	170 °C	20 MPa	5.32	AB	

4.22 % UHMWPE/GS, 0.2 % Vit E	6 min.	170 °C	20 MPa	8.2	AB	
4.22 % UHMWPE/GS	6 min.	170 °C	20 MPa	7.25	AB	
4.22 % UHMWPE/GS	6 min.	170 °C	20 MPa	8	AB	
4.22 % UHMWPE/VH	6 min.	170 °C	20 MPa	9.03	AB	

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