Evaluating the bioactivity and biocompatibility of nylon coated ultra-high molecular weight polyethylene (UHMWPE)

Nancy Hassanein
*The American University in Cairo*

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School of Sciences and Engineering

Evaluating the bioactivity and biocompatibility of nylon coated ultra-high molecular weight polyethylene (UHMWPE)

A Thesis Submitted to the
Biotechnology Master’s Program

In partial fulfillment of the requirements for the
Degree of Master of Science

By
Nancy Ahmed Hassanein
Bachelor of Pharmacy and Biotechnology

Under the supervision of
Dr. Asma Amleh
Associate Professor, Department of Biology
The American University in Cairo
November 2018
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Has been approved by

Thesis Committee Supervisor/Chair

Affiliation ________________________________

Thesis Committee Reader/Examiner

Affiliation ________________________________

Thesis Committee Reader/Examiner

Affiliation ________________________________

Thesis Committee Reader/External Examiner

Affiliation ________________________________

Dept. Chair/Director Date  Dean Date
Dedication

This work is wholeheartedly dedicated to my beloved parents, who have been my source of inspiration, who continually provided moral, spiritual, emotional and financial support. To my brother who I am truly grateful for having him in my life. To my dear husband, who have supported me during my ups and downs and who remains willing to engage with all the struggles that I face in my life. To my boy, Yassin, you are my inspiration to achieve greatness, without you I wouldn’t be where I am today.
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I would first like to thank my thesis advisor Dr. Asma Amleh, for her guidance, motivation and immense knowledge throughout my whole journey. I would like to thank Dr. Habiba Boughrera (Ryerson University/Canada) for providing the biomaterial samples for testing. I would like to thank all AUC professors who taught me a lot about biotechnology. I would like to thank Mr. Amgad Ouf for his help and support. I would like to thank Mai Omar, who taught me a lot about primary cell culture. I want to thank Ahmed Samir, who was the main reason I was able to excel in cell culture techniques. Also, I am grateful for all my fellow lab mates, who were the reason I learned how to work effectively in a team, who taught and assisted me when I needed the most and who helped me have fun in the past 3 years. Finally, I am grateful to have had the privilege of attending the American University in Cairo. This experience has granted me the opportunity to work with some of the best and brightest, who were the main reason I was able to achieve great success.
Evaluating the bioactivity and biocompatibility of nylon coated ultra-high molecular weight polyethylene (UHMWPE)

Abstract

Ultra-high molecular weight polyethylene (UHMWPE) semi-crystalline biomaterial is one of the gold standard materials that are used as a bearing surface in total joint replacement surgeries. However, wear particles generated by UHMWPE due to the relative motion between the different components of the bearing, would eventually result in osteolysis and implant failure. For this, many attempts to enhance the properties of UHMWPE were done, including coating the UHMWPE with nylon 6,6 which was reported to improve its mechanical properties and biocompatibility. In this study, the antibacterial activity, moisture and SBF absorption, pH effect, bioactivity, biocompatibility and wound healing ability of the nylon coated in contrast to the uncoated UHMWPE were assessed. The results have shown that the coated UHMWPE was more effective (P<0.001) than the uncoated UHMWPE regarding bacterial (Staphylococcus aureus and Escherichia coli) growth inhibition. Moreover, coated UHMWPE demonstrated superiority over the uncoated UHMWPE; by absorbing less moisture of both simulated body fluid and lactated Ringer's solution and by rendering the pH of simulated body fluid (SBF) less acidic. Chemical analysis of the nylon coated UHMWPE by FTIR, and morphological assessments by SEM confirmed the absence of hydroxyapatite layer and hence the inability of the nylon coated UHMWPE to be osteoconductive. The assessment of the U2-OS cell viability using MTT assay has suggested that both materials appear to not cause cell cytotoxicity and may be accelerating the cellular proliferation after 72 hours when compared to the control sample. For wound healing, nylon coated UHMWPE proved its ability to be a better orthopedic suture than the uncoated sample and control by showing a better wound closure percentage. These interdisciplinary approaches have given us the chance to investigate different features of nylon coated UHMWPE, which is a promising tool to enhance bearing surfaces in total joint arthroplasties.
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## List of abbreviations

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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>µl</td>
<td>Microliters</td>
</tr>
<tr>
<td>CF/EPOXY</td>
<td>Carbon Fiber/EPOXY</td>
</tr>
<tr>
<td>CF/PEEK</td>
<td>Carbon Fiber/ Polyetheretherketone</td>
</tr>
<tr>
<td>CF/UHMWPE</td>
<td>Carbon fiber/Ultra High Molecular Weight Polyethylene</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony forming unit</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s modified eagle medium</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>E.coli</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>EDX</td>
<td>Energy dispersive X-ray spectroscopy</td>
</tr>
<tr>
<td>FBS</td>
<td>Fetal bovine serum</td>
</tr>
<tr>
<td>FESEM</td>
<td>Field emission scanning electron microscope</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>HA/PE</td>
<td>Hydroxyapatite/Polyethylene</td>
</tr>
<tr>
<td>hrs</td>
<td>Hours</td>
</tr>
<tr>
<td>LB</td>
<td>Luria Bertani</td>
</tr>
<tr>
<td>MOP</td>
<td>Metal on Polymer</td>
</tr>
<tr>
<td>MTT</td>
<td>3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>PE</td>
<td>Polyethylene</td>
</tr>
<tr>
<td>PEEK</td>
<td>Polyetheretherketone</td>
</tr>
<tr>
<td>PET</td>
<td>Polytetrafluoroethylene</td>
</tr>
<tr>
<td>PGA</td>
<td>Polyglycolic Acid</td>
</tr>
<tr>
<td>PMMA</td>
<td>Polymethylmethacrylate</td>
</tr>
<tr>
<td>PS</td>
<td>Polysulfone</td>
</tr>
<tr>
<td>S.aureus</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>SBF</td>
<td>Simulated body fluid</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
</tr>
<tr>
<td>SR</td>
<td>Silicone Rubber</td>
</tr>
<tr>
<td>TMSCs</td>
<td>Testicular mesenchymal stromal cells</td>
</tr>
<tr>
<td>UHMWPE</td>
<td>Ultra-High Molecular Weight Polyethylene</td>
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1. Introduction and Study Objectives

1.1 Biomaterials

Biomaterials are any substance or a combination of substances that could be organic or inorganic and in continuous contact with the body fluids. It substitutes any tissue, organ or function of the body either partially or totally, that were destroyed through some pathological processes, to enhance the quality of life of an individual. These materials excludes surgical and dental instruments as well as external prosthesis such as artificial limbs or hearing aids (Bergmann & Stumpf, 2013; Agrawal, 1998).

There are numerous application areas in the field of biomaterials, such as cardiovascular medical devices, sutures, biosensors, dressings and skin substitutes, drug delivery systems, ophthalmic, orthopedic and dental applications (Williams, Cahn, & Bever, 1990). One of the most prominent application areas of biomaterials is orthopedics. This is due to the fact that weight bearing joints such as ankles, knees and hips usually lead to the feeling of severe pain when injured and usually patients are aware of the orthopedic implants that could be used to replace the diseased/injured part of the joints (Agrawal, 1998). In developed countries, bone and joint degenerative and inflammatory problems account for half of all the chronic diseases in people over 50 years of age (Ong, Yun, & White, 2015).

The biomaterials’ evolution has paved the way for scientists to develop the basis for the design and innovation of improved orthopedic clinical problems. Nowadays, there are several types of orthopedic material implants that could be used such as metals, ceramics and polymers (Williams et al., 1990); (Agrawal, 1998). There are three biomaterials generations; 1st generation (bio inert materials), 2nd generation (bioactive and biodegradable materials) and 3rd generation (materials designed to stimulate specific cellular responses at the molecular level). The first generation is still currently used in a vast number of applications. The 2nd and 3rd generations are intended to open new treatment and application possibilities, but they are not meant to replace materials from the previous generation (Navarro, Michiardi, Castaño, & Planell, 2008).
1.1.1 Metals

Metallic biomaterials are used extensively (Gilbert & Mali, 2012) in orthopedic implants because of their high strength, ductility (elasticity), tenacity, hardness, fracture toughness, corrosion resistance, formability, and biocompatibility (Sansone, Pagani, & Melato, 2013). Metal biomaterials include, stainless steel, cobalt chromium based alloys, titanium, titanium alloys and nickel (Navarro et al., 2008).

1.1.2 Ceramics

Ceramic biomaterials include mainly alumina, zirconia and several porous ceramics. Ceramics are used widely in orthopedics, particularly in replacing the traditional metallic femoral heads of hip prostheses by high-density and highly pure alumina ($\alpha$-Al$_2$O$_3$) (Boutin, 1972). Ceramics are also used for acetabular cups, because they show good biocompatibility, high strength, excellent wear rates and corrosion resistance (Navarro et al., 2008).

1.1.3 Polymers

Polymers are organic biomaterials that show a versatility that is not seen in metals nor ceramics (He & Benson, 2017). This versatility makes polymeric biomaterials a pioneer in the formation of permanent prosthetics devices. Polymeric materials are usually used in orthopedics as articulating surfaces of joint replacements and as inter-positional cementing material between bone and implant surfaces (Ribeiro, Monteiro, & Ferraz, 2012).

There are many types of polymers, such as, ultrahigh molecular weight polyethylene (UHMWPE), polyethylene (PE), silicone rubber (SR), acrylic resins, polyurethanes (PU), polypropylene (PP), polymethylmethacrylate (PMMA), polyetheretherketone (PEEK), poly tetrafluoroethylene (PET), polyglycolic acid (PGA), and Polysulfone (PS). Polymer composite biomaterials include, hydroxy apatite HA/PE, silica/SR, carbon fiber-ultrahigh molecular weight polyethylene (CF/UHMWPE), carbon fiber/ epoxy (CF/epoxy), and CF/PEEK (Banoriya, Purohit, & Dwivedi, 2017);(Ramakrishna, Mayer, Wintemantel, & Leong, 2001).
1.2 Ultra-High Molecular Weight Polyethylene (UHMWPE)

UHMWPE is a hydrophobic (Du et al., 2015) linear homopolymer (Kurtz, 2015) that comes under the umbrella of Polyethylenes (PE). We visualized UHMWPE sheets using scanning electron microscope (SEM) at a magnification of 27x (figure 1). UHMWPE has become one of the gold standard materials in the field of orthopedics due to its good characteristics that makes it an ideal candidate for being used in orthopedic implants. These properties include, high abrasion resistance, low friction, high impact strength, excellent toughness, low density, ease of fabrication, biocompatibility and bio stability (Shalaby & Salz, 2006). However, wearing of Polymers and specifically UHMWPE, when used in arthroplasty applications, is an inevitable consequence that must be taken into consideration (Baena, Wu, & Peng, 2015). Polyethylenes have been considered to be an indispensable part of the evolution of joint arthroplasty surgery, hence, wearing caused by them has been of much interest to scientists (Chakrabarty, Vashishtha, & Leeder, 2015), (Grieco et al., 2018).

The wearing of this biomaterial usually leads to undesirable complications. These complications are mainly linked to local and remote tissue responses. The wearing of artificial joints may lead to a continuous shedding of micron and sub-micron sized particles leading to hazardous biological reactions (Jin & Fisher, 2014) figure (2), which are usually engulfed by macrophages locally. The release of these very small particles may have a negative effect on the integrity of the cells, proteins or body fluids (Jacobs et al., 1998). In addition, the majority of the implants that are used for clinical purposes usually elicit a foreign body response (FBR), which is a specific form of non-specific inflammation (Johnston, 1988). Moreover, it has been confirmed that the wearing of PE in joint arthroplasty leads to particle induced osteolysis which leads to later failure of the implant and may require a revision surgery (Chakrabarty et al., 2015).

Figure 1. SEM micrograph of UHMWPE sheet at 27X magnification.
In 1960s, Sir John Charnley was able to develop a low friction arthroplasty using metallic femoral head and a polyethylene cup and it was a breakthrough in the field of orthopedic surgeries. UHMWPE was previously reported to have a smooth surface that allows less friction (Firouzi, Foucher, & Bougherara, 2014) and is considered to be the polymer with the lowest coefficient of friction (Panin et al., 2012). Therefore, UHMWPE is used as a liner of acetabular cups in total hip arthroplasties in tibial insert, patellar component in total knee arthroplasties, and as a spacer in intervertebral artificial disc replacement (Panin et al., 2012). UHMWPE is usually used as a part of metal on polymer (MOP), due to its high ductility, relatively low hardness and stiffness (Bhushan, 2003).

Moreover, several US patents were granted to researchers for UHMWPE to be used in orthopedic sutures (Kurtz, 2015); (US8632566B2, 2014); (EP1293218A1, 2003); (US20080021501A1, 2008). Also, it was reported earlier that UHMWPE has been used in meniscal repair techniques (Barber, Herbert, Schroeder, Aziz-Jacobo, & Sutker, 2009).

![Diagram showing major implant complication](http://smart.servier.com/)

**Figure 2.** Major implant complication involves wearing of the UHMWPE implant, leading to the release of wear debris which in return elicits immune response. Inflammation is the result of the immune response leading to implant loosening, implant failure and osteolysis. Bone image taken from: [http://smart.servier.com/](http://smart.servier.com/)
1.3 Natural and Artificial Hip Joint

The natural hip joint is the articulation of the acetabulum of the pelvis and the femoral head, together they form a ball and socket structure (figure 3). This arrangement allows for a wide variety of movements including extension, flexion, abduction and rotation. Ligaments and muscles surrounding the hip joint act as a stabilizing system to the hip, in order to support the compressive loads caused by normal walking or running. The hip bearing is characterized by the presence of a layer of smooth tissue known as cartilage on the contacting surfaces of the bones (figure 3). The cartilage allows for the distribution of the loads between opposing bones and the synovial fluid (Moreno, 2013). The synovial fluid is intended to protect the joint structure when subjected to large compressive forces.

Figure 3. The anatomy of the hip joint, image produced by Notability program.

The artificial hip joint is usually composed of 4 main parts (figure 4). The acetabular cup consists of a socket which is usually made of a metal alloy and a liner that is inserted inside the socket and is usually made from polyethylene (figure 4). The femoral ball is attached to the socket and the femoral stem is inserted into the shaft of the femur, both components are usually made from metal alloys. The point of connection between the femoral ball and the polyethylene liner is known as bearing surface (Moreno, 2013). In fact, there is a direct relationship between the longevity of the artificial hip prosthesis and the wear debris released form the implants. Recently, several researchers have been trying to enhance the joint bearing surfaces, to minimize the complications caused by these orthopedic implants.
1.4 Biocompatibility

Biocompatibility is the ability of a material to perform a specific function in the body without eliciting an immune response. The interaction of the living tissue with the man-made material at the tissue–material interface, as well as, assessing the capability of the biomaterial to co-exist with the physiological environment, could be considered as a sign of the biocompatibility of biomaterials (Szycher & Sharma, 1990). Moreover, for a material to be biocompatible, it must follow some specific criteria such as biological safety by showing no cytotoxicity, carcinogenesis, or mutagenesis. Also, it is important that a material exhibits a good bio-functionality, meaning that the material is able to perform the task that it is intended for (Morais, Papadimitrakopoulos, & Burgess, 2010).

To enhance the biocompatibility of biomaterials and for a better bio-performance between biological medium and implants, coatings are usually implemented (Ghiuţă, Cristea, Ţinţ, & Munteanu, 2015). Also, coatings are intended to enhance the lifespan, functionality (Morais et al., 2010), and help reduce the wearing of the biomaterial (Firouzi, Youssef, et al., 2014). There are several types of bioactive coatings for biomaterial implants ranging from organic to inorganic coatings. Organic coatings include alginate, chitosan, collagen, dextran and hyaluronan (del Valle, Díaz, & Puiggali, 2017). Inorganic coatings include calcium phosphate alloys (Nouri, 2015) such as hydroxyapatite and calcium phosphate cements (Mandracci, Mussano, Rivolo, & Carossa, 2016) and polyethylene oxides. Both organic and inorganic coatings could be combined. For instance the formation of a composite coating, which consists of collagen to enhance the cellular adhesion and
proliferation, and calcium phosphate minerals to promote osteoconductivity (Bosco, Van Den Beucken, Leeuwenburgh, & Jansen, 2012). Specifically speaking about UHMWPE, several coating were suggested in order to decrease the wearing of the biomaterial, including coating it with Nylon 6,6 (Firouzi, Youssef, et al., 2014).

Nevertheless, biocompatibility is not the only characteristic that a material should possess to be considered as an ideal biomaterial. An ideal biomaterial should also have other characteristics, such as; high wear resistance, low elastic modulus, excellent resistance to degradation, adequate strength, and is resistant to repeated mechanical stress. The later property, is the principal requirement for a biomaterial to be used in orthopedic surgeries. Only metals, ceramics, and polymers meet this essential requirement (Sansone et al., 2013).

1.5 Nylon 6,6 Coating on UHMWPE

Nylon 6,6 (C₁₂H₂₆N₂O₂) is considered to be a semi-crystalline polymer with a good thermal stability and mechanical strength (Vasanthan, 2012); (Navarro-Pardo et al., 2013). Nylon 6,6 is an important member of a group of polymers known as polyamides. The structural units of a polyamide are joined together by an amide, -NH-CO-, group. In fact, a coating of Nylon 6,6 on UHMWPE was originally used to enhance the penetration resistance of ultra-high molecular weight polyethylene (UHMWPE) fabric, which might be useful in manufacturing flexible body armor against spike/knife threats (Firouzi, Foucher, et al., 2014). Nevertheless, nylon coated UHMWPE might also be used to serve the main purpose which UHMWPE was intended to do; which is acting as a liner or a spacer between articulating joints.

1.6 Osteolysis

After surgeries that involve total joint replacements, biological reactions to polyethylene wear debris usually take place and may lead to osteolysis and implant loosening. Wear debris from polyethylenes usually induce progressive osteolysis by supressing the bone formation and increasing the degradation of bone (Chiu, Ma, Smith, & Goodman, 2009). Osteolysis is induced by eliciting an inflammatory responses from osteoclasts, osteoblasts, macrophages and fibroblasts (Vermes et al., 2001). Some in vitro studies have reported that the wear debris released from polyethylenes may alter the function of the proliferation of mature osteoblasts (Dean et al., 1999); (Vermes et al., 2001). It was previously reported that less osteolysis was induced by using nylon 6,6 on UHMWPE using L929 fibroblasts which might be due to decreased release of wear debris (Firouzi, Youssef, et al., 2014).
1.7 Bacterial Infection

Bacterial infection is one of the major complications in orthopedics, which may lead to implant failure (figure 5). Needless to say, the introduction of an implant, which is a foreign body, is commonly linked to a risk of bacterial infection, specifically for the joint-revision surgeries. It is a matter of fact that treating orthopedic implant infections may lead to implant replacement and, in critical cases may lead to amputation (Ribeiro et al., 2012).

One of the main causative agents in orthopedic bacterial infection is the gram-positive bacteria, *Staphylococcus aureus* (*S.aureus*). It represents two thirds of the total number of pathogens that cause infections in orthopedic implants. It uses several cell surface adhesion molecules to be able to bind to the matrix of the bone (Campoccia, Montanaro, & Arciola, 2006). Another important organism causing bacterial infection in orthopedic implants is gram negative *Escherichia Coli* (*E.coli*) (Crémet et al., 2015). *E.coli* uses adhesins which are presented at the tip of complex cell-surface structures that extend from the outer cell membrane, called pili or fimbriae (Boland, Latour, & Stutzenberger, 2000). Therefore, due to this increasing burden of infection on patients with orthopedic implants, it is essentially important to highly consider the interaction of biomaterials with bacteria when fabricating future implants.

1.8 Simulated Body Fluid (SBF) and Hydroxyapatite Formation

In 1991, (Kokubo, 1991) proposed that for an artificial material to bind to living tissues, the formation of bone-like apatite on the surface of the biomaterial has to take place inside the living body. This *in vivo* apatite formation can be mimicked *in vitro* by the use of the super-saturated simulated body fluid (SBF) or its multiples (e.g. SBF x 1.5) (Drouet, 2013), that has ion concentrations approximately equal to those of human blood plasma. This test is usually carried out for several days at 37°C. The so-called biomimetic apatite coating technique by the use of SBF has been previously used by many researchers to allow for the growth of a synthetic calcium phosphate apatite layer on the implant surface (Li, 2003); (Nagano, Kitsugi, Nakamura, Kokubo, & Tanahashi, 1996). Calcium phosphate nano-crystalline apatites (Ca$_{10-}$($\text{PO}_4$)$_6$–$\text{HPO}_4$)$x$(OH)$_{2-x}$), where $x$ depends on the condition of formation, are inorganic compounds that are recognized in several mineralized tissues.

When making bone-like apatites, the identification of the apatite phase must be carefully evaluated using the appropriate complementary characterization methods, such as scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX) and Fourier transform infrared spectroscopy (FTIR). However, it must be taken into consideration that testing the cell behavior followed by *in vitro* testing are crucial to clearly indicate the bioactivity of the sample.
This method of using SBF solution is the best option for testing the *in vitro* bioactivity of biomaterials. Moreover, this way of testing the formation of apatite layer is interesting, since it can reduce the number of animals which are usually used for *in vivo* tests.

![Figure 5. Bacterial infection caused by Staphylococcus aureus and Escherichia coli on the surface of implants. Taken from: http://smart.servier.com/](image)

### 1.9 Study objectives

The use of nylon 6,6 coating over UHMWPE was intended to enhance the characteristics of the polymer. We wanted to investigate the effect of the coating on the biomaterial, our main objective was to assess the ability of the nylon 6,6 coating on UHMWPE to improve the *biocompatibility* of the neat polymer, as well as, evaluating its *bioactivity* through determining its osteoconductive properties.
2. Materials and Methods

2.1 Biomaterial

UHMWPE and nylon 6,6 coated UHMWPE were provided by Dr. Habiba Boughrera (Ryerson University, Canada) (Firouzi, Youssef, et al., 2014), (Firouzi, Foucher, et al., 2014) in a solid form.

2.2 Samples’ Sterilization

The samples used in the experiments were in two forms, fibers and sheets (figure 6). The fibers were used in assessing the antibacterial activity, MTT cell viability assay and wound healing assay. The sheets were used in assessing the moisture and SBF absorption, the pH changes, alizarin red staining, and the morphological assessments by SEM, EDX, and FTIR. For UHMWPE and nylon coated UHMWPE fibers, the samples were cut into 2.5 cm pieces by using a sharp blade. The samples were then soaked in 97% ethanol for 5 minutes, followed by washing twice with 1X phosphate buffer saline (PBS) then left to dry for 15-20 min. For UHMWPE and nylon coated UHMWPE sheets, the samples were sterilized in the same way as the fibers, but 2 cm² of the sheets were used.

Figure 6. (a) Nylon coated UHMWPE sheet. (b) Uncoated UHMWPE sheet. (c) Nylon coated UHMWPE fiber. (d) Uncoated UHMWPE fiber.
2.3 Antibacterial Activity Assay

A glycerol stock for both gram-positive bacteria (S. aureus) and gram-negative bacteria (E. coli -Top 10) was used. For the preparation of a pre-culture, a scratch of the glycerol stock using a sterile plastic loop was added to Luria-Bertani (LB) broth (containing 10 g/l peptone 140, 5 g/l yeast extract, 5 g/l sodium chloride), which was used as a growth medium for both types of bacterial strains. The pre-culture was left overnight at 37 °C. This was followed by streaking of bacteria on Luria-Bertani (LB) agar plates (containing 10g/l peptone 140, 5g/l yeast extract, 5g/l sodium chloride and 12g/l agar). To 7 ml of LB broth, 70 µl of the pre-culture were added, and the culture was incubated at 37°C for 3 hours or till the exponential growth phase was reached. Using spectrophotometer, the optical density of the bacterial samples was measured at 600 nm to reach a final OD of approx. 0.5-0.6 (mid log phase). This was followed by incubating the fibers with the bacterial samples for 24 hours at 37°C, followed by serial dilution, then streaking 50 µl on agar plate (figure 7). The number of colonies were then counted.

Calculation of the colony forming unit (CFU) was done using the following equation:

\[
\text{CFU/mL} = \frac{\text{(no. of colonies x dilution factor)}}{\text{volume of culture plate}}
\]

To convert the results to log values:

\[
\text{Log (CFU/ml)}
\]

2.4 Moisture Absorption

2.4.1 Lactated Ringer’s solution and simulated body fluid (SBF)

The test samples were used as sheets with a size of 2 cm x 2 cm. The surface area of the polymer was the same for all the samples. All test samples were weighed to an accuracy of ±0.01 gm prior to immersion in either of the solutions. Lactated Ringer’s solution (Otsuka, Egypt) was used as an isotonic solution.

Simulated body fluid was used as it contains ion concentrations that is approximately equal to the human blood plasma. Kokubo method was used for the preparation of the SBF solution (Kokubo, 1991). The SBF solution was prepared using the following reagents, sodium chloride (NaCl), sodium bicarbonate (NaHCO₃), potassium chloride (KCl), potassium phosphate dibasic trihydrate (K₂HPO₄.3H₂O), magnesium chloride hexahydrate (MgCl₂.6H₂O), calcium chloride dehydrate (CaCl₂.2H₂O), and sodium sulphate (Na₂SO₄) into distilled water and buffered with tris (hydroxyl-methylamino-methane, NH₂C(CH₂OH)₃) and hydrochloric acid (HCl) to pH 7.4 at 37 °C. The concentrations of the ions used in comparison to the human blood plasma are shown in Table (1) and the amounts of reagents used for the preparation of SBF are shown in Table (2).
Three samples from both the nylon coated and uncoated UHMWPE were placed in 5 ml of both solutions and incubated for 60 days at 37 °C. The weight of the samples was measured after the removal of the solution from the surface by patting the samples on a filter paper. An illustration of the protocol used is shown in (figure 8). The percentage change in weight % at time (t) resulting from the absorption of lactated Ringer’s solution and SBF was determined by the following equation:

\[
\text{% weight change} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100
\]

Figure 7. **Antibacterial activity assay illustration.** The figure illustrates the protocol used in this experiment. Falcon tubes and Eppendorfs were drawn using Inkscape program.
Table 1. Different ion concentration in SBF fluid and the human blood plasma.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Simulated body fluid</th>
<th>Blood plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$</td>
<td>142.0</td>
<td>142.0</td>
</tr>
<tr>
<td>K$^+$</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>148.8</td>
<td>103.0</td>
</tr>
<tr>
<td>HCO$_3^-$</td>
<td>4.2</td>
<td>27.0</td>
</tr>
<tr>
<td>HPO$_4^{2-}$</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table 2. Reagents for preparing SBF solution (pH 7.40, 1L)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>7.996 g</td>
</tr>
<tr>
<td>NaHCO$_3$</td>
<td>0.350 g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.224 g</td>
</tr>
<tr>
<td>K$_2$HPO$_4$·3H$_2$O</td>
<td>0.228 g</td>
</tr>
<tr>
<td>MgCl$_2$·6H$_2$O</td>
<td>0.305 g</td>
</tr>
<tr>
<td>1M-HCl</td>
<td>40 mL</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>0.278 g</td>
</tr>
<tr>
<td>Na$_2$SO$_4$</td>
<td>0.071 g</td>
</tr>
<tr>
<td>(CH$_2$OH)$_3$CNH$_2$</td>
<td>6.57 g</td>
</tr>
</tbody>
</table>
2.5 pH Measurements

UHMWPE and nylon 6,6 UHMWPE sheets that were used for the SBF absorption and lactated Ringer’s solution experiment, were tested for pH changes using pH meter (Thermo scientific) over a period of 60 days at 37°C. The starting pH for lactated Ringer’s solution was 6.5 and that for the SBF was 7.4.

Figure 8. Illustration of the protocol used for assessing moisture and SBF absorption, pH changes and visualization of SEM. The test tubes are drawn using Inkscape program. The pH meter is taken from creative commons (open source)
2.6 SEM – EDX

The morphology of the surface of the sheets immersed in SBF solution for 60 days were assessed using Zeiss high resolution field emission scanning electron microscope (FESEM) (LEO SUPRA). Before the visualization process, the samples were washed thoroughly with 1X PBS and were allowed to dry for 24 hours. To avoid poor images, the samples were coated with gold (Gold sputtering) using a current of 15 mA for 200 sec to prevent charge build-up by the electron absorption. The study was conducted to assess the distribution of Hydroxy-apatite on the surface of the samples which might have been induced due to the SBF solution, Also, to visualize any fractures, cracks, or pores formed on the surface. The FESEM is equipped with EDX detector for elemental analysis. EDX was used to investigate the presence of certain elements and to identify the composition of any layer formed on the surface of the biomaterial. The generated data from EDX represents a spectra that corresponds to all the different elements found in the sample. Each element shows a characteristic peak of unique energy. EDX analysis can be used as a quantitative analysis, to assess the percentage of concentration of each element with respect to other elements, or as qualitative analysis to assess the type of elements.

2.7 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR provides a detailed information of characteristically vibrational energies of various groups in a molecule. The FTIR (Nicolet 380, Thermo-scientific) was used to detect the functional groups that are present in the nylon coated and uncoated UHMWPE sheets before and after immersion in SBF solution. The analysis was recorded in the mid-infrared spectrum region (400-4000 cm\(^{-1}\)). The samples were added to the IR cell and the spectrum was recorded. The IR is a very useful tool for the identification of the chemical nature of the polymer under testing and to determine its composition. In order to assess the spectrum produced from the samples, it is important to address that the structure of nylon 6,6 which is Poly[imino (1,6-dioxohexamethylene) iminohexamethylene].

2.8 Cell Culture

The cell line of choice was U2-OS human bone osteosarcoma cell line (kindly provided by Dr. Andreas Kakarougkas, department of biology, AUC). The cell line is derived from a 15 years old, Caucasian female. The cells were cultured in media composed of DMEM (Dulbecco’s Modified Eagle Medium) (Invitrogen, USA), supplemented with 10% fetal bovine serum (FBS) (Invitrogen), 100 units/ml penicillin and 100 mg/ml streptomycin (Invitrogen). Cells were incubated in a humidified
CO₂ incubator (Hera cell CO₂ incubator, Thermo-fisher) at 37°C and 5% CO₂. Routinely, the cell line was maintained by regularly splitting the cells after reaching 80% confluency. The cells were washed with phosphate buffer saline (PBS), then detached using 0.05% Trypsin-EDTA solution (Invitrogen, USA). Cells were visualized using inverted microscope (Olympus IX70, USA).

2.9 Alizarin Red Staining

Nylon coated UHMWPE and uncoated UHMWPE sheets (2 cm²) were sterilized and inserted in 35 mm² plates. The cells in DMEM were added to the plate with a cell density of 1x10⁵ cells. The cells were left with the material for 21 days, and the media was changed every 2 days. To test for osteogenesis, alizarin red staining was used. The sheets were aseptically removed and the plates were washed three times with 1X PBS and the wells were fixed in 4% paraformaldehyde for 15 min, then stained with 1% alizarin red solution (Sigma-Aldrich) for 45 minutes. This was followed by washing the wells with distilled water until it didn’t appear orange. The plates were visualized using inverted microscope (Olympus IX70, USA).

2.10 Conditioned Media Preparation

Sterilization of the fibers was done as previously described. After they are completely dry, 2.5 cm per 3 ml of DMEM media were used. During the conditioning process the media didn’t contain any FBS nor Penicillin/Streptomycin. The samples were kept with the media at 37°C whilst shaking at 20 rpm for 72 hours. After the specified incubation time, the conditioned media was filtered using 0.22 µm nylon syringe filters (Corning). FBS and Penicillin/Streptomycin were then added to the conditioned media just before incubation with cells.

2.11 Trypan Blue Exclusion Method

To assess the number of viable cells, trypan blue method was used. For which, 10 µl of the cell suspension was mixed with 10µl of 0.4% w/v trypan blue (Serva, Germany), the 10 µl of the mixture was loaded to each chamber of the hemocytometer (Hauser Scientific, USA). The cells seen in the four outer squares were counted and the number of viable cells per milliliters (ml) was determined according to the following equation (Strober, 2001):

\[
\text{Viable cells/ml} = (\text{Total number of viable cells/total number of squares}) \times \text{dilution factor} \times 10,000
\]

After the counting procedure, the cells were seeded in 96-well plate (Greiner Bio-one, Germany). U2-OS cells were seeded at a density of 5x10³/well (in two different plates). The cells were incubated for 24 hours to allow for attachment to the plate. Then the media was removed and replaced.
with the conditioned media for both the nylon coated and uncoated samples. One plate was incubated for 48 hours and another for 72 hours prior to cell viability measurements. In each plate the control has represented untreated cells that were only exposed to regular DMEM media and not the conditioned one, and the resulting values of absorbance for the control condition, were considered as 100 % viability.

2.12 MTT Cell Viability Assay

The viability of U2-OS cells incubated with nylon coated and uncoated conditioned media (conditioned media was prepared using fibers) was determined using MTT viability assay. MTT (Serva, Germany), 3-(4, 5-dimethylthiazoly1-2)-2, 5-diphenyltetrazolium bromide, is a yellow tetrazolium compound that interacts with the mitochondrial dehydrogenase enzymes of the viable cells to produce a purple color (Riss et al., 2004). A final concentration of 5 mg/ml of MTT was prepared by dissolving the powder in DMEM media, and then sterile filtering the produced solution. After the incubation of the cells with the conditioned media either for 48 or 72 hours, the media was removed and a mixture of 20µl of MTT solution (5mg/ml) and 100 µl DMEM media were added to the cells and incubated for 3 hours. The media was discarded and 100 µl of Dimethyl sulfoxide (DMSO) (Sigma Aldrich, USA) were added to each well to solubilize the formed purple formazan crystals. The absorbance was measured at 595 nm using SPECTRO star nano microplate reader (BMG LABTECH). The percentage cell viability was calculated by using the absorbance of the test wells as a percentage of the control wells.

2.13 Wound Healing

The wound healing assay was done to test the cell migration ability of U2-OS cells when incubated with conditioned media from nylon coated and uncoated UHMWPE sheets. The experiment was performed in 24 well plate, a cell density of 7x10^4 was added to each well and incubated for 24 hours to allow for the attachment of the cells to the plate. At a confluency of approx. 90%, two perpendicular scratches were made in the monolayer using a sterile 20 µl pipette tip. Cells were then washed twice with 1X PBS to get rid of any cell debris or dead cells, then fresh DMEM media was added. Using the inverted microscope (Olympus IX70, USA), pictures were taken at time zero and at 24 hours after the scratch formation. The percentage wound closure was measured using image J software.
The following equation (Boleman et al., 2012) was used to calculate percentage wound closure:

\[
\text{Percentage wound closure} \% = \left(\frac{\text{wound area at zero time} - \text{wound area at 24 hrs}}{\text{wound area at zero time}}\right) \times 100
\]

2.14 Data Analysis

The statistical analysis, of the antibacterial activity assay, moisture and SBF absorption, pH measurements, MTT cell viability assay and wound healing assay, was done using Graph pad prism 8.0. Data were presented as the mean value ± Standard deviation (SD) from three independent experiments. The P-values for statistical significance were computed using Tukey’s multiple comparisons test, Bonferroni's multiple comparisons test, or Sidak’s multiple comparison tests (depending on the experiment). A p-value<0.05 was considered significant (* for P<0.05, ** for P<0.005, *** for P<0.001)
3. Results

3.1 Antibacterial Activity Assay

The antibacterial effect of nylon coated UHMWPE and uncoated UHMWPE were investigated by a comparison of the viable *S. aureus* or *E. coli* after being in contact with the biomaterial. Figure (9 a, b) show the colony forming unit (CFU)/ml. After 24 hours of incubating the biomaterial with the bacterial cells, the antibacterial activity of the coated UHMWPE was higher than the uncoated UHMWPE and the control with respect to *E. coli* (Gram negative). There was a significant difference (P-value <0.001) between both Coated UHMWPE with a value of 4.3x10^7 CFU/ml and control with a value of 8.2x10^7 CFU/ml Also, there was a significant difference between the coated UHMWPE and uncoated UHMWPE with a value of 9.6 x10^7 CFU/ml. For *S. aureus*, it was observed that the number of viable *S. aureus* (Gram positive) bacterial cells were seen to be three orders of magnitude less than the control with a value of 2.7 x10^6 CFU/ml after being in contact with both the coated with a value of 5.5x10^7 CFU/ml and the uncoated UHMWPE with a log value of 6.4x10^7 CFU/ml, showing a significant difference (P-value<0.001). To confirm the reproducibility of the results, the antibacterial activity assay was repeated in triplicates.

![Antibacterial Activity Assay](image)

Figure 9. Antibacterial Activity Assay. (a) Number of viable adherent *E. coli* & (b) *S. aureus* cells incubated with coated UHMWPE and uncoated UHMWPE fibers for 24 hours in colony forming units per milliliters. Presented data represent mean ±SD of three independent experiments (n=3). The P-values for statistical significance were computed relative to the control sample using Tukey’s multiple comparisons test, *** for P<0.001.
Moisture Absorption

3.2.1 Lactated Ringer’s Solution

The moisture absorption of nylon coated, and uncoated UHMWPE were evaluated using lactated Ringer’s solution. Figure (10a) shows the change in weight for coated and uncoated UHMWPE stored at 37°C plotted against square root of time. This plot (line graph) was specifically used to follow Fick’s law of diffusion which postulates a linear relationship between the moisture uptake against the square root of time (Cussler, 1997). Both samples show an increase in the percentage weight change during the first 6 hours with the highest slope. However, the percentage weight increase of the coated sample was only 18.7% and that of the uncoated sample was 44% which resembles more than double the increase in weight with a statistical significance (P-value ≤0.001). After 6 hours (2.44 in square root of hours), there was a slower rate of water absorption. The coated sample started to plateau for the next 14 days (18.3 in square root of hours), whereas the uncoated sample plateaued only till 24 hours (4.89 in square root of hours) then started to decrease then fluctuate. This was followed by a very sharp decrease in the percentage weight change at 14 days for the uncoated sample. After 14 days, the coated sample showed a slight decrease in the percentage weight change till 45 days followed by a slight increase. For the uncoated sample, there was a plateau seen between 30 days (26.8 in square root of hours) and 45 days (32.8 in square root of hours) followed by sharp increase. It was seen that the pattern after 14 days for both the coated and the uncoated samples were almost the same, however the uncoated sample was showing larger changes in percentage weight change when compared to the coated sample.

3.2.2 Simulated Body Fluid (SBF)

SBF solution mimics the ion concentration of the human plasma (Macuvele et al., 2017); (Kokubo & Takadama, 2006), and helps in characterizing the bioactivity of biomaterials by confirming the formation of hydroxyapatite (HA) layer on the surface of the samples. Figure (10b) shows the effect of the immersion of coated UHMWPE and uncoated UHMWPE in SBF solution for a period of 2 months (60 days) at 37°C. The results show a faster rate of SBF uptake (42.7% increase) for uncoated UHMWPE till 6 hours (2.4 in square root of time) followed by a fluctuation in the percentage weight change. The coated UHMWPE showed a slower rate (only 22.8% increase) of SBF absorption till 6 hours. The variation in percentage weight change for the coated sample was approximately half that of the uncoated samples. For the coated sample, the SBF uptake process involved a rapid Fickian process that is linear to \( t^{1/2} \) followed by a slower rate of uptake (almost plateau) till 72 hours (22.9 in
square root of hours), then followed by a slight gradual increase during the period from 72 hours till 60 days (37.9 in square root of hours).

Figure 10. Moisture and SBF absorption of nylon 6,6 UHMWPE and uncoated UHMWPE. (a) Changes in weight of nylon coated and uncoated UHMWPE sheets due to moisture absorption at 37°C over time. Both samples were aged in lactated Ringer's solution for 60 days. Both samples experienced an increase in weight. The uncoated UHMWPE experienced a greater increase in weight when compared with the coated sample. (b) Changes in weight of nylon coated and uncoated UHMWPE sheets due to SBF absorption at 37°C over time. Both samples were immersed in SBF solution for 60 days. Both samples experienced an increase in weight. The uncoated UHMWPE experienced a greater increase in weight when compared with the coated sample. Presented data represent mean ±SD of three independent experiments (n=3). The P-values for statistical significance were computed relative to the uncoated sample using Bonferroni's multiple comparisons test, * for P<0.05, ** for P<0.005, *** for P<0.001.
3.3 **pH Measurements**

The pH of nylon coated and uncoated UHMWPE in SBF and Ringer’s solution, over a time of 60 days, was assessed. Figure (11) shows the pH values of the Ringer’s solution as a function of the immersion time of the nylon coated and uncoated UHMWPE. The variations in pH of the Ringer’s solution with the nylon coated UHMWPE were milder than the uncoated samples. On the first day of immersion, the pH has increased from 6.5 to 7.2 this was followed by minor changes till reaching a pH of 7.4 at 60 days. As for the uncoated UHMWPE, the pH was seen more fluctuating than the nylon coated UHMWPE, however no significant differences were seen between both samples.

Figure (11), shows the pH values of the SBF solution as a function of the immersion time of the nylon coated and uncoated UHMWPE. The variations in pH between both samples were similar up until 72 hours, showing approximately no change in pH. After 3 days of immersion till 14 days, a sharp decrease in the pH from 7.4 to 5.7 was observed for the uncoated UHMWPE sample with a significant difference between the coated UHMWPE at 7 and 14 days (P-value < 0.001). After 14 days of immersion there was a sharp increase till 30 days with a significant difference between the coated UHMWPE and uncoated UHMWPE at 30 days (P-value <0.001), followed by a decrease till reaching PH 6 at 60 days. The observed fluctuations with the uncoated samples was not noted with the nylon coated samples. The recorded data indicates that the decrease in pH from 72 hours till 30 days has occurred gradually from pH 7.4 to pH 6.2. This was followed by a decrease then an increase to pH 7.2 and then a decrease to pH 6.3.

3.4 **Structural Analysis of nylon 6,6 coated and uncoated UHMWPE**

3.4.1 **Scanning Electron Microscope (SEM)**

SEM was used to assess the bone bonding ability of the coated and uncoated UHMWPE, which is usually evaluated by testing the formation of the apatite layer on the surface of the material after immersion in SBF fluid for a prolonged period of time .The nylon coated and uncoated UHMWPE samples that were immersed in SBF and the samples that were not immersed in SBF were visualized under the microscope. Figure (12), shows the smooth surface of the nylon coated UHMWPE that was visualized at 1000X and at 3000X, in addition to, the nylon coated UHMWPE surface immersed in SBF solution for 60 days that was visualized at 1000X and 3000X. The surface of the nylon coated immersed in SBF appears to be rougher when compared to the control sample. At a magnification of 3000X some pores were seen in the control sample with a size less than 10 μm. after immersion in SBF solution, these pores have increased in size. The uncoated UHMWPE samples without SBF and that immersed in SBF for 60 days were visualized at 3000X and at 5000X. Figure (13) shows that the
surface of both samples was seen to be smooth at both magnifications. However, the sample immersed in SBF showed several nano-cracks propagating parallel to each other.

![Average PH changes in SBF and lactated Ringer’s solutions](image)

Figure 11. **Changes in pH of SBF and lactated Ringer’s solution after incubation with nylon coated UHMWPE and uncoated UHMWPE sheets** over time (60 days). The pH of lactated Ringer’s solution incubated with the uncoated UHMWPE showed more fluctuations when compared to the nylon coated UHMWPE. Presented data represent mean ±SD of three independent experiments (n=3). No significant difference was seen between both samples. The pH of SBF solution incubated with the uncoated UHMWPE showed more sharp fluctuations when compared to the nylon coated UHMWPE. Presented data represent mean ±SD of three independent experiments (n=3). The P-values for statistical significance were computed relative to the uncoated sample using Sidak’s multiple comparison tests, * for P<0.05, ** for P< 0.005, & *** for P<0.001.
Figure 12. Scanning electron microscope micrograph (SEM) (a) Surface of nylon coated UHMWPE sheets (1000X). (b) Surface of nylon coated UHMWPE sheets after being immersed in SBF solution for 60 days (1000X). (c) Surface of nylon coated UHMWPE sheets (3000X). (d) Surface of nylon coated UHMWPE sheets after being immersed in SBF solution for 60 days (3000X). The orange circles indicate the size of the pores which increased in diameter after immersing the sample in SBF solution.
Figure 13. Scanning electron microscope micrographs. (a) Uncoated UHMWPE (3000X) (b) Uncoated UHMWPE immersed in SBF solution for 60 days (3000X). (c) Uncoated UHMWPE (5000x). (d) Uncoated UHMWPE immersed in SBF solution for 60 days (5000X). Both samples show a smooth surface. The surface appeared to be smooth with several micro-cracks that propagate parallel to each other (orange arrows).
3.4.2 Energy Dispersive X-ray Spectroscopy (EDX)

The ability of solid surfaces to favor the nucleation and growth of “bone-like” apatite upon immersion in supersaturated fluids such as SBF is commonly used as one evaluation index of the “bioactivity” of such surfaces. The results for the coated UHMWPE revealed a sharp calcium peak and a sharp chloride peak with no evidence of a phosphorus peak (figure 14 a). Whereas, the coated UHMWPE immersed in SBF solution showed a short peak of calcium and chlorine (figure 14 b). The percentage weight of elements detected with the coated UHMWPE shows that calcium was 39.32%, chlorine was 48.92 % and oxygen was 11.76 %. The percentage weight of elements detected with the coated UHMWPE immersed in SBF shows that calcium was 9.03%, chlorine 36.36%, and oxygen 54.61%.

Similarly, the uncoated sample showed a peak of calcium (Figure 14 c) whereas the uncoated sample immersed in SBF solution showed no calcium peak (Figure 14 d), while both are also showing a chloride peak figure (14 a, b, c, d). Moreover, a sharp peak of calcium was seen in (figure 14 a, b, c, d) at 0 KeV. The percentage weight of elements detected with the uncoated UHMWPE show that calcium was 31.27 %, chlorine was 33.83 % and oxygen was 34.90%. The percentage weight of elements detected with the uncoated UHMWPE immersed in SBF shows that chlorine was 50.53%, oxygen was 49.40% and calcium was 0.00%.

3.4.3 Fourier-Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of nylon coated UHMWPE sheets before and after immersion in SBF solution is shown in figure (15 a,b). The frequency region of 1500-4000 cm\(^{-1}\) which resembles the functional group region and the frequency regions of 450-1500 cm\(^{-1}\) which resembles the finger print regions of nylon 6,6 UHMWPE were assessed. All the results were compared to a reference of nylon 6,6 FTIR (Pramanik, Alam, Kh, & al, 1970a)

For the finger print region figure(15a), the peak assigned for C=O stretching band of amide in nylon 6,6 (amide I) was seen only with the unimmersed sample at 1696 cm\(^{-1}\).However, the sample immersed in SBF has shown one shoulder peaks at 1650 cm\(^{-1}\) of C=O stretching and at 1899 cm\(^{-1}\) which represents the carboxylic carbonyl group. The peak for in plane N-H deformation band (amide II) was observed at \sim1530 \text{ cm}^{-1} for both nylon coated UHMWPE immersed in SBF and un-immersed in SBF solution. The nylon coated UHMWPE immersed in SBF has shown a peak at 1143 cm\(^{-1}\) that was not detected in the un-immersed sample. Moreover, two important crystalline bands are 935 cm\(^{-1}\) (CO–NH in-plane vibration) and 1200 cm\(^{-1}\)(CH\textsubscript{2} twist-wag vibration) which are attributed to the crystalline phase of nylon 6,6, were detected in both immersed and un immersed nylon coated
UHMWPE samples. No peaks were detected at 1045 cm\(^{-1}\) which resemble the P-O stretching band of hydroxyapatite for both samples.

For the functional group region figure (15b), no absorbance was recorded between 3700-4000 cm\(^{-1}\). A peak was seen in the nylon 6,6 UHMWPE unimmersed in SBF at 3329.4 cm\(^{-1}\) which represents the N-H stretching of the amide group of nylon 6,6, which was absent in the sample immersed in SBF. Peaks that represent CH\(_2\) symmetric stretching and CH\(_2\) asymmetric stretching (aliphatic chain of nylon 6,6) were observed only with the nylon 6,6 UHMWPE unimmersed in SBF at 2870 cm\(^{-1}\) and 2984.8 cm\(^{-1}\), respectively.

![Figure 14: Percentage weight of elements detected in EDX elemental analysis](image.png)

Figure 14. **Percentage weight of elements detected in EDX elemental analysis** (a) EDX observations for coated UHMWPE sheets unimmersed in SBF solution, with percentage weight of each element. The figure is showing a high calcium peak. (b) EDX observations for coated UHMWPE sheets immersed in SBF solution for 60 days with percentage weight of each element. The figure is showing a shorter calcium peak than that of the coated sample. (c) EDX observations for uncoated UHMWPE sheets unimmured in SBF solution with percentage weight of each element. The figure is showing a short calcium peaks in the uncoated sample. (d) EDX observations for uncoated UHMWPE immersed in SBF solution for 60 days with percentage weight of each element. The uncoated sample immersed in SBF is showing no calcium peaks.
Table 3. The Characteristic band assignment for nylon 6,6 UHMWPE immersed and unimmersed in SBF

<table>
<thead>
<tr>
<th>Band Frequency/cm$^{-1}$</th>
<th>Band Assignment</th>
<th>Nylon6,6 UHMWPE unimmersed in SBF</th>
<th>Nylon6,6 UHMWPE immersed in SBF</th>
</tr>
</thead>
<tbody>
<tr>
<td>~3330</td>
<td>N-H stretching</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>~3087</td>
<td>N-H bending/angular deformation in plane</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>~2870</td>
<td>CH$_2$ symmetric stretching</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>~2985</td>
<td>CH$_2$ asymmetric stretching</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>~1696</td>
<td>C=O stretching of amide (Amide I)</td>
<td>Present</td>
<td>+“substituted by a shoulder band at 1650 cm$^{-1}$ and 1899 cm$^{-1}$ of carboxylic carbonyl</td>
</tr>
<tr>
<td>~1530</td>
<td>In plane N-H deformation (Amide II)</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>~1143</td>
<td>Chain defects</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>~935</td>
<td>CO–NH in-plane vibration</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>~1200</td>
<td>CH$_2$ twist-wag vibration</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>~1045</td>
<td>P-O stretching (corresponds to hydroxyapatite)</td>
<td>Absent</td>
<td>Absent</td>
</tr>
</tbody>
</table>
Figure 15. FTIR analysis for nylon 6,6 coated UHMWPE immersed and unimmersed in SBF solution. (a) Merged FTIR (finger print region) for coated UHMWPE (green) and coated UHMWPE immersed in SBF solution for 60 days. (b) Merged FTIR (functional region) for coated UHMWPE and coated UHMWPE immersed in SBF solution (Purple) for 60 days. The chemical nature of the deposited mineralized matrix was assessed using FTIR for coated UHMWPE immersed in SBF solution, the figure is showing no peaks at 1045 cm⁻¹ that corresponds to P-O stretching band and this confirms no apatite layer formation.
3.5  Assessment of Bone Mineralization Alizarin Red S Staining

U2-OS osteosarcoma cell line was cultured with UHMWPE sheets for a period of 7, 14 and 21 days to assess whether wear debris from the nylon coating would enhance the process of osteogenesis or not. By visualization under the microscope, it was seen that the cells were adhering to the surface of the plate. Also, the sheets and the cells were seen to be in different planes. Alizarin red stain was used to determine the calcium phosphate mineralized deposits of the cells, after the removal of the sheets from the plate wells, by colorimetry (Chiu & Goodman, 2012). The results indicate no calcification after 21 days with both the coated and uncoated UHMWPE figure (16a, b). A positive control of Alizarin staining of Testicular mesenchymal stromal cells TMSCs (as a reference from previous lab data) figure (16c).

Figure 16.  Alizarin red staining. (a) U2-OS cells cultured with nylon coated UHMWPE sheets for 21 days. (b) U2-OS cells cultured with uncoated UHMWPE sheets for 21 days. (c) Positive control using Testicular mesenchymal stem cells.
3.6 MTT Cell Viability Assay

U2-OS osteosarcoma cell line was incubated with the conditioned media prepared from nylon coated and uncoated UHMWPE fibers. The results after 48 hours indicated similar viability between the coated and the uncoated UHMWPE, 95% and 93% respectively Figure (17). The results after 72 hours, showed a comparable viability of the coated samples 115% and the uncoated samples 112% with both showing a significant difference when compared to the control (P-value <0.001).

![MTT assay graph](image)

Figure 17. MTT cell viability assay. (a) Percentage viability of the cells cultured in conditioned media for 48 hours. (b) Percentage viability of the cells cultured in conditioned media for 72 hours. Presented data represent mean ±SD of three independent experiments (n=3). The P-values for statistical significance were computed relative to the uncoated sample using Bonferroni’s multiple comparisons test, *** for P<0.001.

3.7 Wound Healing

The wound healing experiment was done to assess the cell migration ability of U2-OS osteosarcoma cells when incubated with conditioned media of coated and uncoated UHMWPE figure (18 a, b, c, d, e, f). Images of the wound was taken at zero time and at 24 hours. The results show that there is an increase in the percentage wound closure in case of the coated with a percentage of 80% when compared to the uncoated and control. Both the uncoated and control samples showed a similar percentage of wound closure 60% figure (18g).
Figure 18. **Wound healing assay.** (a) Migration of U2-OS cells cultured in DMEM media at zero time. (b) Migration of U2-OS cells cultured with conditioned media of the uncoated sample at zero. (c) Migration of U2-OS cells cultured with conditioned media of the coated sample at zero time. (d) Migration of U2-OS cells cultured in DMEM media after 24 hours. (e) Migration of U2-OS cells cultured with conditioned media of the uncoated sample after 24 hours. (f) Migration of U2-OS cells cultured with conditioned media of the coated sample after 24 hours. (g) Percentage wound closure of U2-OS cells when incubated with conditioned media of coated and uncoated UHMWPE fibers after 24 hours. Coated UHMWPE is shown to increase the percentage wound closure when compared to uncoated UHMWPE and control.
4 Discussion

4.1 Antibacterial Activity Assay

The antibacterial activity of both the coated UHMWPE and the uncoated UHMWPE were assessed by comparing the number of viable *E.coli* and *S.aureus* cells after being in contact with the biomaterial. Both bacterial strains were deemed appropriate for testing the antibacterial activity of biomaterials as they are considered to be one of the most common bacteria associated with biomaterials bacterial infections (Juan, Zhimin, Anchun, Lei, & Jingchao, 2010).

Materials that possess an antibacterial activity have beneficial advantages to implant applications. The antibacterial activity of nylon coated UHMWPE and uncoated UHMWPE sheets against *E.coli* (Gram negative) and *S. aureus* (Gram Positive) bacteria were evaluated quantitatively by counting the number of viable cells on agar plates. It was observed that the nylon coating on UHMWPE possess an antibacterial activity against both *E.coli* and *S.aureus* when compared to the control. Also, it was shown that nylon coated UHMWPE was behaving better than the uncoated UHMWPE with respect to *E.coli* bacteria where it has shown a significant antibacterial activity. Whereas the uncoated UHWME possess an antibacterial activity against *S.aureus* only when compared to the control.

Several strategies have been raised to lower peri-implant infection rates by the inhibition of bacterial growth, which include the use of coating to enhance the antibacterial activity. In fact, it was previously reported that Polyethylenes show a minimal decrease in the bacterial growth when tested with both *E.coli* and *S.aureus* (Seyfriedsberger, Rametsteiner, & Kern, 2006). So, we wanted to test the ability of Nylon 6,6 to enhance the antibacterial activity of UHMWPE.

In fact, Nylon is generally used in fabrics and textiles. The antibacterial activity of Nylon is crucial for this industry. It was previously reported that nylon 6,6 treated textiles does not promote the growth of *S.aureus* bacteria, when put in contact with the bacteria (Saihi, El-Achari, Caze, & Ghenaim, 2006) which agrees with our results.

Moreover, It was previously reported that reinforcing UHMWPE with nano-ZnO has significantly improved the antibacterial activity against *E.coli* and *S. aureus* (Chang, Akil, Nasir, Bandara, & Rajapakse, 2014).
4.2 Moisture Absorption

Lactated Ringer’s solution that was used in this experiment mimics the interstitial fluids of the body and has approximately the same concentration of chloride ions (Cl⁻) found in the body (Deb, Braden, & Bonfield, 1995). The change in moisture uptake/aging was evaluated due to its great importance in polymers that are intended to be used for clinical purposes. Any changes in the properties of the material will negatively affect the performance of the material and hence this may lead to implant failure. Very few numbers of articles have discussed the aging of polymers using lactated Ringer’s solution. In vitro conditions were employed in this experiment with similar temperature, moisture conditions and solution concentration, to mimic the in vivo conditions as much as possible.

Despite the fact that UHMWPE is a hydrophobic polymer (Guo, Wang, Yin, Li, & Li, 2017), the sharp increase in weight during the first 6 hours was due to the diffusion of the lactated Ringer’s solution into the polymer sheets. It was previously reported that Polymethyl methacrylate polymer (PMMA) that is also hydrophobic is capable of swelling when submerged in water which agrees with the case of both the coated and uncoated UHMWPE (Ayre, Denyer, & Evans, 2014). Because of the presence of nylon coating on the surface of UHMWPE, the swelling of the material was less than that of the uncoated one (uncoated UHMWPE was seen fluctuating for a considerable amount of time during the experiment). After 6 hours there was a slower rate of diffusion. Also, the data suggested that the sharp decrease in the uncoated sample between 14 and 30 days was due to full saturation of the fibers with the lactated Ringer’s solution, followed by diffusion of lactated Ringer’s solution from an area of high concentration (fibers) to an area of low concentration (solution), resulting in a sharp decrease in the weight of the sample. The similarity in trend between both samples between 14 and 30 days confirms that the nylon coating has improved the behavior of UHMWPE regarding moisture absorption.

4.3 Simulated Body Fluid (SBF)

A method used to assess the in vitro bioactivity of biomaterials, which involves the immersion of the biomaterial in SBF solution for a certain period of time. SBF solution mimics the ion concentration of the human plasma (Macuvele et al., 2017); (Kokubo & Takadama, 2006), and confirms the formation of hydroxyapatite (HA) layer on the surface of the samples by inducing carbonate apatite formation on the surface of the biomaterial (Macuvele et al., 2017). The volume of SBF solution absorbed by the fibers of the material is controlled by diffusion following Fick’s law of diffusion followed by a slower rate of uptake that is indicated as matrix saturation. These results agree with the results of Poly (ε-caprolactone)/Hydroxyapatite PCL fibers when immersed in SBF solution.
and showed an increase in the weight percentage followed by a plateau (Hassan, Sultana, & Hamdan, 2014). On the contrary, the uncoated sample showed fluctuations during the whole time of the experiment. This clearly indicates that the nylon coating is of great advantage to the polymer as it prevents the increased absorption of the SBF and provides a steady rate of absorption and hence may decrease the risk of implant degradation and hence failure.

4.4 pH Measurements

It was previously reported that a relatively high local pH (weak alkaline environment) is essential for the bone formation process (i.e. osteoblast formation) (Ruan et al., 2017). However, this is not the case when using polymeric biomaterial, as the released wear debris from the polymer will lead to lowering of the pH of the surrounding solution and hence making it acidic and enhancing osteoclast generation (Liu et al., 2016). We recorded the local pH for both the lactated Ringer’s solution and SBF solution over a period on 2 months. These experiments were mainly carried out to investigate the effect of the wear particles that are released from both nylon-coated and uncoated UHMWPE polymer on pH of different solutions.

Despite its slightly acidic pH of 6.5, lactated ringer’s solution is considered to be an alkalizing agent (Smith, 2014). During the first 24 hours, the pH of lactated Ringer’s solution has increased from 6.5 to 7.0 with both coated and uncoated UHMWPE. This is suggested to be due to the sodium lactate (\(\text{CH}_3\text{CH(OH)CO}_2\text{Na}\)) which neutralizes the acidity caused by the wear debris of both samples. The pH started to increase gradually with both samples till reaching the optimum pH of the body 7.4, with a steadier pace in case of the nylon coated UHMWPE. This suggests that the lactated Ringer’s solution acts as a buffer and that the nylon coating has further supported the effect of the solution by showing approximately no fluctuations in the pH.

As for the pH of the SBF solution, it was seen dropping after 72 hours of the immersion of the nylon coated and uncoated UHMWPE. This drop in the pH is suggested to be due to the acidity caused by the wear debris released from both biomaterials. However, in case of the uncoated UHMWPE, the drop in the pH was seen to be with a sharp slope from 72 hours to 14 days. Whereas the drop of pH in case of the nylon coated UHMWPE was considerably regulated and took a longer time to decrease (from 72 hours to 30 days). This further supports the fact that the nylon coating over UHMWPE produces less wear debris than the uncoated UHMWPE. Also, this is a support that nylon coated UHMWPE avoids fluctuations in pH and allow for steady changes in pH.
4.5 Scanning Electron Microscope (SEM)

The influence of the immersion of nylon coated and uncoated UHMWPE was successfully evaluated using SEM. The micrographs obtained from SEM were intended to assess the biological properties and specifically the ability of the polymer to induce mineralization through the growth of the Hydroxy-apatite layer. The samples were immersed in SBF for 60 days, which is of importance to allow for the formation of the apatite layer. It was previously reported by (Ahmed, Punshon, Darbyshire, & Seifalian, 2013) that the immersion time of the biomaterial in SBF is of importance for the growth of the apatite layer.

The micrographs of the nylon coated UHMWPE immersed in SBF showed a rough layer of covering the surface when compared to the un-immersed nylon coated UHMWPE sample. It was previously reported that uncontrolled parameters such as pH or substrate reactivity might be the cause for changes in the surface of the polymer (Drouet, 2013). In fact, the pH changes and specifically a decreased pH was reported to cause a decreased mineralization and hence decreases bone formation (Brandao-Burch, Utting, Orriss, & Arnett, 2005), which agrees with our previous results of the pH measurements of SBF solution (see section 4.4).

Moreover, it is important to mention that the pore size, is a crucial morphological property of biomaterial for bone regeneration (Karageorgiou & Kaplan, 2005). An increase in the size of the pores (> 5µm) on the surface of the nylon coated UHMWPE sheets after the immersion in SBF was noticed. The presence of an increased sized pores in sample with no apatite layer formation agrees with the results of Juraski et al., 2017, which states that if the pore diameter is greater than 5µm, this will provide an inappropriate surface for osteoblastic adhesion and proliferation. This supports the fact that UHMWPE is used as a bearing surface that doesn’t require being osteoconductive.

The uncoated UHMWPE sample showed a smooth surface even after immersion in SBF fluid. These results are in agreement with previously reported data by (Aparecida, Fook, & Guastaldi, 2009), which stated the it was not possible to induce the formation of apatite layer of the surface of UHMWPE using the standard SBF solution.

Another important topological feature that was detected on the surface of the uncoated UHMWPE is micro cracks. The detection of these cracks is of crucial importance to ensure the performance reliability of the polymer, as their presence is a catastrophic biomaterial failure. One of the already reliable ways of detecting cracks in polymers is the non-destructive tests (NDT) such as visual testing using microscopes such as SEM (Awaja, Zhang, Tripathi, Nikiforov, & Pugno, 2016). The SEM micrograph showed micro cracks propagating parallel to each other. These cracks are suggested to be due to the precipitation and swelling phenomena that has occurred due to the stress.
caused by the immersion in SBF (Drummond, 2008). The cracks were not seen in the nylon coated samples that were immersed in SBF; because it didn’t absorb SBF solution as much as the uncoated UHMWPE. Also, it is worth to mention that the presence of these cracks will allow solutions to diffuse through them into the polymer allowing for mechanical instabilities (Ayre et al., 2014). This would indicate that the nylon coating acts as a shield on the surface of the UHMWPE polymer to avoid these cracks.

4.6 Energy Dispersive X-ray Spectroscopy (EDX)

EDX elemental analysis is usually used for unveiling compositional aspects of a specific material; however it cannot be considered as a sufficient tool (Drouet, 2013). So, herein we use EDX as a confirmatory experiment to prove the absence of the apatite layer on the surface of nylon coated and uncoated UHMWPE. The observation of both calcium and phosphorus peaks are supporting information for the formation of calcium phosphate phase (hydroxyl apatite layer). A strong calcium peak was seen with the nylon coated UHMWPE and a short calcium peak was seen with the uncoated UHMWPE that were not immersed in SBF solution. The high calcium peak in the nylon coated UHMWPE is suggested to be due to the usage of anhydrous calcium chloride that was used during the sample preparation to dissolve the nylon pellet (Firouzi, Youssef, et al., 2014). Even though the authors have mentioned that washing of the material was done, we expect that residues of calcium might have remained within the matrix. Moreover, a sharp chlorine peak was seen in all samples at 0 KeV, this peak is mainly due to the noise of the detector.

However, the calcium peaks ceased after the immersion in SBF with both samples which might be due to the ionic interaction of Ca$^{2+}$ with the H$^+$ and HCO$_3^-$ in the SBF solution leading to a decrease in the pH of the solution as indicated in section 4.4 and as reported earlier (Duta, A. C. Popa, Miculescu, & Mihaiescu, 2014). Also, over time some turbidity was seen in the test tube indicating that there might have been some precipitation of calcium carbonate CaCO$_3$ due to the previously mentioned reaction.

EDX analysis showed no phosphorus peak in all the samples, confirming the absence of the apatite layer. It is suggested that the hydrophobic nature of the polymer decreases its ability to promote for osteoconductivity, this supports the results of the EDX analysis. Actually, this suggestion is in agreement with a previously reported result that has indicated that there is a direct relationship between the hydrophobicity of the material and the osteoconductivity (Kuroda & Okido, 2017).

Moreover, it has pointed out that the samples were poorly washed due to the presence of chloride peaks. This also indicated the presence of NaCl crystals on the surface of the sample (Drouet,
Moreover, it is suggested that the chloride peaks might be due to the release of Cl⁻ ions onto the polymer, which was produced by the interaction of CaCl₂ with water H₂O leading to the release of Ca²⁺(aq) and 2Cl⁻(aq). In fact, the Cl⁻ ions produced from this reaction might be one of the major causes of an increased chlorine peak. Also, it is suggested that the increase in the chloride ions (as salts) may affect the diffusion of solutions into the polymer. Leading to an increased salt concentration within the polymer, herby reducing the polymer’s water concentration, and eventually causing swelling of the material. These results are in agreement with the results obtained from the SBF absorption experiment (figure10 b), which might be the reason for the increase in the weight of the material over time as previously mentioned in the SBF absorption experiment.

4.7 Fourier-Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of nylon coated UHMWPE before and after immersion in SBF solution is shown in figure (15). The FTIR spectrum is a plot of absorbance vs. wave number, the bond vibrational energies changes as we move horizontally on the graph. Both the functional group region between 1500-4000 cm⁻¹ and the frequency region between 500-1500 cm⁻¹ which resemble the complex finger print regions were assessed. We aimed at checking certain finger print regions which signify the presence of P-O stretching band, representing the presence of hydroxyl apatite formation on the surface of the nylon coated UHMWPE when immersed in SBF. Also, we wanted to compare the band frequencies obtained from nylon 6,6 UHMWPE immersed and unimmersed in SBF. This comparison should give an insight about the chemical reactions that may take place inside the human body due to the presence of the polymeric biomaterial implant.

By comparing the band frequencies of nylon 6,6 UHMWPE immersed and unimmersed in SBF, it was clearly observed that there were changes in the band frequencies between both samples. The unimmersed sample has shown results that are comparable to nylon6,6 FTIR spectrum that was previously published in the literature (Pramanik et al., 2015). Whereas, the sample immersed in SBF has shown some shifts in the peak positions, meaning that there is a change in the bond strength. Also, it has shown absence of certain absorption band which reflects that there might be a scission or break in certain bonding structures.

The results have shown that no peak was seen at 1045 cm⁻¹ in the FTIR spectrum for both samples. It was previously reported that the hydroxyapatite is characterized by a peak at 1045 cm⁻¹ (Gaharwar et al., 2014), that corresponds to P-O stretching band, which confirms that the nylon6,6 UHMWPE has no osteoconductive ability. Moreover, we noticed the absence of a band at 3330 cm⁻¹ that represents the N-H stretching in the unimmersed sample only, this indicates that an ionic
interaction of NH$_2$ and SBF solution has taken place. The amide I (C=O) band is a characteristic band for nylon 6,6 (Pramanik et al., 2015), the band was only seen in the unimmersed sample in SBF at 1696 cm$^{-1}$. This peak was substituted by two shoulder peaks at 1630 cm$^{-1}$ and 1650 cm$^{-1}$ and a peak at 1899 cm$^{-1}$ of the carboxylic carbonyl in the sample immersed in SBF. This reflects that hydrolysis of amide by water has taken place, which is indicated by the carboxylic acid formation and the two shoulder peaks indicate that not all the amide molecules were exposed to hydrolysis. Another peak at 1143 cm$^{-1}$ that was only detected with the sample immersed in SBF, which usually arises due to chain defects. Moreover, the crystalline structure of nylon 6,6 was kept intact even after immersion in SBF solution due the presence of two important peaks which resemble the crystalline band of nylon in both samples (Pramanik, Alam, Kh, & al, 2015). The degree of crystallinity plays an important role in the properties of semi crystalline polymers.

The intensity of the peaks in the finger print region was higher in case of the un-immersed nylon coated UHMWPE, when compared to the immersed Nylon coated UHMWPE in SBF. Usually, two peaks may have different intensities because there are molecules present in different concentrations. This might indicate that the SBF solution has caused changes in the concentration of the components of the nylon coated polymer.

4.8 Alizarin Red Staining

Alizarin red is a widely known stain for evaluating calcium-rich deposits by cells in culture. The mechanism of action of Alizarin red depends on the complex formation between the stain and calcium to produce a red color (Juraski et al., 2017). We aimed at assessing the ability of the released wear debris from UHMWPE sheets to promote the calcification process when incubated with U2-OS osteosarcoma cells. The Alizarin red staining after 21 days, showed no red color indicating that no calcification has taken place.

4.9 MTT Cell Viability Assay

One of the most common in vitro tests to assess the cell viability is methyl thiazolyl tetrazolium (MTT). MTT assay is usually used in many cell culture systems (Macuvele et al., 2017). They are intended to determine the effect of a material or a test compound on the viability of the cells. This is done through the identification of detrimental intracellular effects on mitochondria and metabolic activity, according to the selective ability of viable cells to reduce tetrazolium bromide into purple formazan crystals that are only soluble in organic solvents.

The results obtained from MTT assay showed no significant differences for either the coated or uncoated UHMWPE, when compared to the control condition after 48 hours of incubating the cells
with conditioned media. However, after 72 hours a significant increase in the cell viability of both coated UHMWPE and uncoated UHMWPE in comparison to the control condition was seen. This indicates that the presence of the wear debris from the material is not causing cell cytotoxicity and might be accelerating the cellular proliferation. We correlated the MTT results with the pH measurements of the SBF solution. In our case in the MTT assay, we used conditioned media that was incubated with the biomaterial for 72 hours. By referring to (figure 11) we can notice a decrease in the pH of the SBF solution after 72 hours. For the nylon coated UHMWPE, the change in the pH was from 7.3 to 7.1, whereas for the uncoated UHMWPE, the change in the pH was from 7.4 to 6.5. These changes in the pH will not affect the cells negatively. It was previously reported that the pH range of 6.5-8.0 in the medium will still allow the cells to maintain an optimal intracellular pH value that is close to pH 8.0 (Haveman, 1979). In addition, these results are in agreement with the results of MTT assay obtained from nano-hydroxyapatite re-enforced ultra-high molecular weight polyethylene composite (Mirsalehi, Sattari, Khavandi, Mirdamadi, & Naimi-Jamal, 2016). They reported that the composites used in the experiments have shown a higher rate of proliferation of MG-63 osteosarcoma cells by 11.5 % when compared to the neat UHMWPE (positive control).

4.10 Wound Healing

It was previously reported that UHMWPE is used in orthopedic sutures (Barber et al., 2009), due to its strength which is considered as a critical factor in characterizing the performance of sutures (Kurtz, 2015). Also, it is important to mention that UHMWPE sutures are already commercially used (Kurtz, 2015). Therefore, we are testing the nylon coating on UHMWPE, to see its effect on wound closure. This was done by evaluating the migration of U2-OS cells by wound healing assay. The results indicated that the cells incubated with nylon coated UHMWPE conditioned media showed an increasing healing ability when compared to uncoated UHMWPE and control. The healing process took about 24 hours in total which agrees with the previously published results which stated that the wound in U2-OS healed after approx. 28 hours (Tschon, Incerti-Parenti, Cepollaro, Checchi, & Fini, 2015). These results support that nylon might be enhancing the cell migration process, however further in vivo experiments must be conducted to confirm these results.
5 Conclusion

In conclusion, nylon 6,6 coated UHMWPE appears to enhance the load bearing capacity of the neat UHMWPE as demonstrated by absorption of less moisture, avoiding pH fluctuations in SBF solution. The morphological properties of the material after the immersion in SBF, showed the presence of pores and cracks on the surface of the nylon coated and uncoated UHMWPE, respectively. Moreover, changes in the chemical structure of the nylon coated polymer was seen to be changed after the immersion in SBF solution. Also the absence of hydroxyapatite layer on the surface of the nylon coated UHMWPE after immersion in SBF confirmed that the polymer is not osteoconductive. Nylon 6,6 coated UHMWPE shows an enhanced antibacterial activity against S.aureus and E.coli bacteria. Also, both materials show an increased cell viability of U2-OS cells after 72 hours of incubation with the conditioned media. Finally, nylon coated UHMWPE might be considered as a possible candidate in wound healing applications. To the best of our knowledge, this study is the first to investigate the enhanced characteristics of UHMWPE with the addition of nylon 6,6 using U2-OS cell line.

6 Future recommendations

The work that has been conducted for this thesis has highlighted several topics to which further research would be beneficial. Nylon 6,6 UHMWPE was seen to have antibacterial activity against gram-negative and gram-positive bacteria. To further enhance this activity, the addition of antibacterial agents might be useful; for instance, an addition of quaternary ammonium salts to the nylon which could be achieved by reacting carboxylic end groups with quaternary ammonium salts. Alternatively, the addition of silver ions to nylon 6,6 might also enhance the antibacterial activity. It is also important to assess the minimum inhibitory concentration (MIC) using different sizes of the polymer and incubating the samples with the bacteria for a longer period of time such as 48 or 72 hours.
References


Appendix

1. Approval for a part of figure 2 (osteolysis image) and figure 5 (bacterial adhesion image)

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   Envoyé : jeudi 25 octobre 2018 17:33
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   Objet : Permission

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