THE DEVELOPMENT OF A NOVEL UNIVERSAL COATING FOR UROLOGICAL IMPLANTS

Submitted in fulfilment of the requirements of the degree of Master of Applied Science in Biotechnology in the Faculty of Applied Sciences at the Durban University of Technology

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REFERENCE DECLARATION

I, Mr M.H. Shongwe – 21439389, and Prof Tukayi Kudanga (Supervisor) do hereby declare that in respect of the following dissertation:

Title: **The development of a novel universal coating for urological implants**

1. As far as we ascertain:

a) no other similar dissertation exists;

b) the only similar dissertation(s) that exist(s) is/are referenced in my dissertation as follows:

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2. All references as detailed in the dissertation are complete in terms of all personal communication engaged in and published works consulted.

19/08/2024

Signature of student **Date**

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Signature of Supervisor **Date**

AUTHORS DECLARATION

This research presents original work carried out by the author and has not been submitted to another academic institution. The respective acknowledgement has been given in the text where external work was used. The research described in this dissertation was carried out in the Department of Biotechnology and Food Science, Durban University of Technology, South Africa and the Department of Chemical Engineering, Warsaw University of Technology, Poland.

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ABBREVIATIONS

AA	Ascorbic acid
AMF	Ames multi-well format
AST	Antimicrobial susceptibility testing
ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
BSLA	Brine shrimp lethality assay
CFU	Colony forming units
CHP	Cumene hydroperoxide
CPM	Continuous passive range of motion
DMEM	Dulbecco's modified Eagle medium
EC	European council
EGDMA	Ethylene glycol dimethyl acrylate
ETO	Ethylene oxide
EU	European union
EUCAST	European committee on antimicrobial susceptibility testing
FBS	Sterile foetal bovine serum
FDA	Food and drug administration
GHTF	Global Harmonization Task Force
GRRP	Good regulatory review practices
I	Iodine
IMDRF	International medical device regulators forum
ISO	International organisation for standardisation
IUD	Intrauterine device

IVD	In-vitro diagnostics devices
MIC	Minimum inhibitory concentration
MTT	3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide
NADH	Nicotinamide adenine dinucleotide
OECD	Organisation for Economic Co-operation and Development
PDA	Polydopamine
PDMS	Polydimethylsiloxane
PEG	Polyethylene glycol
PMA	Parts Manufacturer approval
PP	Polypropylene
PU	Polyurethane
PVP	Polyvinyl pyrrolidone
RHE	Reconstructed human epidermis
RSA	Republic of South Africa
SAHPRA	South Africa health products regulatory authority
TCC	Transitional cell carcinoma
TER	Transcutaneous Electrical Resistance
TFTC	Too few to count
UAS	Ureteral access sheath
UPEC	Uropathogenic *Escherichia coli*
USA	United States of America
UTI	Urinary tract infection
WG	The Working Group
XTT	2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide

ABSTRACT

Urology employs various implants, such as urinary catheters, ureteral stents, and ureteral access sheaths (UASs), to manage diseases associated with the urinary tract system. These implants serve different purposes. For example, urinary catheters are primarily used to drain urine from the bladder, ureteral stents are used to keep the ureteral cavity open for urine passage, and UASs are used in expanding the ureteral cavity to facilitate the introduction of other devices. However, these implants pose challenges due to their invasive nature and inadequate biocompatibility, which can lead to uroepithelial tissue damage (caused by friction) and an increase in the risk of urinary tract infections (UTIs). Urinary tract infections, in turn, can cause blockages that limit the effectiveness of these implants. Therefore, several strategies are currently employed to address these challenges, mainly the use of lubricious hydrophilic polymeric coatings and antimicrobial agents. While some success has been achieved, uroepithelial tissue damage and UTIs remain significant concerns. Furthermore, most of these strategies are tailored to specific urological fabricating materials, limiting their scope. In light of these challenges, this study developed a universal coating technology using polydopamine (PDA) as a versatile primer and polyvinyl pyrrolidone (PVP) as a hydrophilic polymeric top layer loaded with iodine (I_2) as an antibacterial agent to improve properties of urological implants.

Urological implant fabricating biopolymers including polyurethane (PU) and polydimethylsiloxane (PDMS) were modified with a PDA/PVP coating technology and loaded with various concentrations of I_2 (0.1%, 0.5% and 1% w/v). The ability of the coating technology to reduce the friction coefficient of urological biomaterials was evaluated by assessing its ability to introduce lubricity. Other biological effects that characterize the biocompatibility and antimicrobial activity of the coated samples were assessed using relevant standard ISO (International Organization for Standardization) and EUCAST (European Committee on Antimicrobial Susceptibility Testing) *In vitro* methods. The biocompatibility of the coating technology was assessed by determining their cytotoxicity with the XTT cytotoxicity assay using L292 cells while the brine shrimp lethality assay (BSLA) was carried out as a preliminary animal model study. Their genotoxicity was assessed with the Ames muta-chromo assay carried out with S*almonella typhimurium* T100 to detect base shift mutation. The antimicrobial activity of the coated technology was determined using the IS0 22196 assay with *Escherichia coli* (ATCC 25922), the EUCAST disk diffusion and broth microdilution assays

with *E. coli* (ATCC 25922), *Proteus mirabilis* and the urinary catheter bridge microbial migration assay with *E. coli* (ATCC 43888).

The novel coating technology was adaptable to urological implant fabricating biomaterials with different surface polarities including PDMS and PU. The polydopamine/polyvinyl pyrrolidone-coated biomaterials were observed to have improved lubricity (slipperiness rate of 5 s per 10 mm biomaterial-agar interaction) and the ability to absorb and trap fluids (0.088 ± 0.009 mg/cm^2). The concentration of iodine solution (0.1%, 0.5% and 1% w/v) loaded in the coatings significantly influenced their biocompatibility and antimicrobial efficacy. The biocompatibility observations made in the BSLA were mostly in line with the XTT cytotoxicity assay. When an iodine concentration of 0.1% and 0.5% w/v was incorporated, the PDA/PVP-coated biomaterials were not toxic towards L292 cells and not lethal towards brine shrimp larvae after a 24 h exposure period. However, potential lethality towards brine shrimp larvae was only observed in the biomaterial loaded with 0.5% w/v after an exposure period of 48 h. The coated biomaterials without iodine were not toxic while those that were loaded with a concentration of 1% w/v iodine were toxic according to both the XTT assay and BSLA. All the PDA/PVP-coated biomaterial loaded with iodine (0.1%, 0.5% and 1% w/v) did not demonstrate base shift mutations in Ames muta-chrome assay.

The ISO 22196 method demonstrated that the PDA/PVP-coated biomaterials loaded with at least 0.1% (w/v) iodine reduced *E. coli* (ATCC 43888) by at least 2 growth logs. The minimum inhibitory concentration (MIC) for the PU-PDA/PVP (0.1% I_2) surfaces was determined to be 40 mm^2/ml for *E. coli* (ATCC 2592) 0.5 McFarland standard. The broth microdilution showed that the MIC of extracts of the PU-PDA/PVP (0.5% I_2) to be 3 cm^2/ml and of PU-PDA/PVP (1% I_2) to be 1.5 cm^2/ml for *E. coli* (25922). However, only the extract of PU-PDA/PVP (1% I_2) was effective against *P. mirabilis* at 3 cm^2/ml. In the disk diffusion assay, the PU-PDA/PVP (0.1% I_2) was only effective against *E. coli* (ATCC 25922) with a 1 mm zone of inhibition. The PU-PDA/PVP (0.5% I_2) was effective against both *E. coli* (ATCC 25922) and *P. mirabilis* with 12.13 ± 1.53 mm and 8.5 ± 0.5 mm zones of inhibition, respectively. PU-PDA/PVP (1% I_2) showed the highest zone of inhibition (16.25 ± 1.77 mm) against *E. coli* (ATCC 25922) and 13 ± 1 mm against *P. mirabilis*. In the catheter bridge microbial migration assay, *E. coli* (ATCC 43888) growth across the bridge was observed after 24 h for PDMS and PDMS-PDA/PVP without iodine incorporation, after 48 h for PDMS-PDA/PVP (0.1% I_2) and PDMS-PDA/PVP (0.5% I_2), and after 72 h for PDMS-PDA/PVP (1% I_2). In addition, there was a positive correlation ($P<0.05$) between the concentration of iodine loaded in the PDA/PVP coated

biomaterials and antimicrobial activity (disk diffusion assay) while a negative correlation with biocompatibility (XTT cytotoxicity assay) was observed.

Overall, the research demonstrated that a PDA/PVP-coating technology is adaptable for urological biomaterials including PU and PDMS, introduces lubricity on the surface of the biomaterials, and when it is loaded with 0.5% w/v I_2, the coating can inhibit the growth of bacteria that cause UTI without causing toxicity in model eukaryotic cells. Therefore, the PDA/PVP (0.5% w/v I_2) coating is a promising strategy for improving urological implants.

CHAPTER 1: INTRODUCTION

Medical devices are used in urology to diagnose, monitor and give therapy for obstructions and diseases of the urinary tract system. Tube-like invasive implants including urinary catheters, ureteral stents and ureteral access sheaths (UASs) are commonly used during the management and therapy of patients who have lost their normal urinary function. Urinary catheters play a vital role in draining urine usually from the bladder, while ureteral stents are essential for maintaining an open ureteral cavity to enable urine passage. On the other hand, UASs are used for expanding the ureteral cavity, facilitating the introduction of other devices. However, the application of these urological implants is not without challenges, particularly when they are fabricated using biomaterials that have inadequate biocompatibility and lack sufficient antimicrobial activity to prevent infections (Yang et al., 2015, Denstedt et al., 1998, Awonusi et al., 2022).

Challenges caused by inadequate biocompatibility are the primary concern because they impact the device throughout therapy and mostly determine its safety profile and the comfort status of the patient (Teo et al., 2016). Common challenges caused by inadequate biocompatibility include uroepithelial tissue damage caused by friction when the implanted device rubs against the lumen of the urinary tract system. This can occur due to the movement of the implant during installation and therapy (Kazmierska et al., 2008, Niemczyk et al., 2015). Other related challenges can occur when the implants interact with urine salts and calcified debris resulting in encrustation, which may lead to blockage, especially for implantation cases lasting beyond 28 days (Dakkak et al., 2012, Awonusi et al., 2022).

Urinary tract infections (UTIs) are a major health problem worldwide, representing approximately 40% of nosocomial infections, of which urethral catheterisation accounts for at least 70%–80% of the infections (Lange et al., 2011). Any bacterial population at a minimum of 0.5 McFarland standard in the urinary tract system are considered a UTI. Infections are not only detrimental to the health of the patients but also may result in the implant losing its functionality. Most UTIs exist in mixed microbial communities often including uropathogenic *Escherichia coli* (UPEC) strains and *Proteus mirabilis* (Kazmierska et al., 2010). These microbes attach themselves to the surfaces of the implants and form biofilm. Microbes growing in biofilms are generally harder to eradicate since they are often encapsulated in a polymeric matrix that serves to protect them and aid adhesion. In addition, the sharing of drug resistance

is very common since members of the biofilm are in close proximity meaning they have higher chances of developing into serious infections which are difficult to eradicate (Ma and Bryers 2013, Soria et al., 2022). This is a particular concern for immunocompromised individuals that are often susceptible to developing drug resistance-related infections when relying on antibiotics.

Early approaches adopted to solve inadequate biocompatibility involved the search and development of better biomaterials. Thus, there was a shift from the use of metallic and unrefined materials such as natural latex to silicone and other more refined, synthetic polymers such as polyvinyl chloride and polyethene. Although these earlier versions of polymeric materials were better they still lacked specific desired qualities for some functions. For instance, earlier versions of silicone were too soft and thus difficult to introduce via a narrowed and tortuous ureter and thus unsuitable for stent fabrication (Denstedt et al., 1998). Regardless of their challenges, over the years polymers including latex rubber (polyisoprene), polyethylene, polyvinylchloride, polyurethane, silicone, and a myriad of specialised proprietary polymers have remained the choice materials for fabrication (Todd and Knudsen., 2017). However, these materials still do not address all the challenges caused by UTIs and inadequate biocompatibility (Khandwekar and Doble 2011).

Coating technologies are currently the most promising approach (Teo et al., 2016, Awonusi et al., 2022). They protect medical implants from infections and wear caused by the surrounding biological system and are thus crucial to the survivability of the implant (Yang et al., 2015). In some cases (including hydrogel coatings), the coating may serve to also improve the biocompatibility of invasive implants by reducing friction and enabling easy installation and removal (Niemczyk et al., 2015). Hydrogels including polyethylene glycol (PEG), polyvinyl pyrrolidone (PVP), hyaluronic acid and phosphorylcholine-containing materials are probably the most widely used coating agents for urological implants (Yang et al., 2015, Saul and Williams., 2011). They can increase the lubricity and elasticity of urological implants. These properties assist with implant handling during installation and removal which improve their comfort status. Hydrogel coating technologies achieve lubricity by absorbing and trapping water in their polyanionic structure, causing them to swell and form hydro layers on their surfaces which reduce friction. However, the swelling behaviour may result in a urologic implant having a bigger size and thus being more susceptible to blockage (Lange et al., 2011). Several studies have focussed on improving hydrogel coating technologies for urological devices by combining them with antimicrobial agents; for instance, hydrogel-coated stents were

soaked in antibiotics (John et al., 2007, Kazmierska et al., 2010). However, these strategies are inadequate since most patients using urological implants developed using the mentioned strategies still experience pain due to uroepithelial damage caused by friction, and cases of urinary tract infections (UTIs) remain high, especially among chronic and long-term users (Sali and Joshi., 2020). Furthermore, the application of these strategies also remains limited since they are not easily adaptable to other urological implants associated with similar problems (Singha et al,. 2017). Therefore, in this study, a novel adaptable coating technology was developed for urological implant biomaterials by using polydopamine (PDA) as a primer and polyvinyl pyrrolidone (PVP) as a lubricating top layer loaded with iodine as an antibacterial agent.

1.1 Research questions and hypotheses

The following questions regarding the study were set up to provide relevant hypotheses that were tested to provide significant findings required to develop an adaptable urological coating technology. This includes the applicability of PDA as a primer, PVP as a lubricious top layer and iodine as a biocompatible antimicrobial agent.

Questions

- Can PDA be used as a primer for a hydrogel coating technology to make it adaptable to different biomaterials with varying surface polarities?
- Will the use of PVP hydrogel as a top layering in the coating technology reduce the friction coefficient for urological implant polymers?
- What concentration of iodine can be loaded onto PDA/PVP-coated biomaterials without causing cytotoxicity towards mammalian cells and lethality towards brine shrimp larvae?
- What concentration of iodine can be loaded onto the PDA/PVP-coated biomaterials to cause antimicrobial activity that restricts bacterial population at 0.5 McFarland standard?

Hypotheses

A coating technology developed with PDA as a primer is adaptable to various materials, including those with different polarities.

PDA has been observed to easily attach to both polar and nonpolar materials (Ryu et al., 2018). This was tested by determining the applicability of adopting the coating technology to polar (PU) and nonpolar (PDMS) biomaterials.

Coating biomaterials with a PVP hydrogel will reduce their friction coefficient.
Numerous hydrogels including PVP have demonstrated the ability to introduce lubrication when hydrated, which reduces the friction coefficient of surfaces where they are applied (Khandwekar and Doble 2011,Butruk et al., 2012). This was tested by determining the lubricity of PVP-coated biomaterial versus its uncoated counterpart.

Iodine will improve the antimicrobial properties of PDA/PVP-coated biomaterials.
Iodine has been observed to be effective even at a concentration of 0.6% w/v solution. Therefore, an assumption can be made that a coating technology with at least a minimum concentration of 0.6% w/v will possess antimicrobial activity that restricts bacterial populations at 0.5 McFarland standard (Caruso et al., 2022). This was tested by determining the antimicrobial activity of the PDA/PVP-coated biomaterials loaded with various concentrations of iodine (0.1, % 0.5% and 1% w/v), against bacterial growth at 0.5 McFarland standard.

1.2 Aim and objectives

Aim

To develop a novel universal coating technology characterised by a low friction coefficient with urinary tract tissues and antibacterial properties.

Objectives

- To fabricate a universal coating technology characterised by a low friction coefficient for urological implants polymers using a primer and a hydrophilic polymeric material coupled with an antimicrobial agent.
- To evaluate the biocompatibility properties of biomaterials produced with the developed technology, by cytotoxic and mutagenicity analysis.
- To evaluate the antimicrobial properties of the biomaterials produced with the developed coating technology.

CHAPTER 2: LITERATURE REVIEW

Medical devices have always been a crucial part of human civilization and have evolved throughout the centuries to become the medical tools we know today. In general, they are tools specifically developed to be employed for medical applications. They are used for prevention, diagnosis, therapy and to reinforce the human body to prolong or improve the quality of life. They include simple items ranging from latex hand gloves used for protection, to complex machines such as embedded software pacemakers responsible for heart rate regulation. However, the exact definitions of medical devices are generally set up by regulatory bodies that oversee the development and enforcement of safety standards regarding medical devices in their jurisdictions. Any tool that fits the region-specific definition of a medical device is considered one within that region. Most definitions at least state that a medical device is a tool used for medical application which works without eliciting a chemical reaction.

2.1 Role of medical devices

All medical fields currently use medical devices for one purpose or another as summarised in Table 2.1. Depending on the situation, medical devices are used for different lengths of periods, at different sites, for different functions and use various energy sources. For instance, a medical vaccination syringe is used transiently, it pierces the skin and contact blood to deliver a vaccine and requires a pushing hand while an endoscopy capsule will go through the gastronomy system over a temporary period to observe the gastrointestinal tract and uses chemical energy source in the form of a battery (French and Collins 2016., Fireman and Kopelman., 2007). These differences mean that while both are medical devices, they are vastly different, hence they are highly specific and suitable for their duration, site and functions and have a sufficient energy source when required.

Table 2.1: Medical devices application in various medical fields

Medical field	Medical device	Application	Reference
Anaesthesiology	Epidural catheters	Tube-like implants used to administer anaesthetics via epidural space	Teo et al., 2016
Cardiovascular	Pacemaker	Heart implants for the heartbeat regulation	Wagner et al., 2020, Teo et al., 2016
	Mechanical heart valves	Used to replace defective natural heart valves.	Wagner et al., 2020, Teo et al., 2016
Dental	Dentures	Implants for replacing missing teeth	Wagner et al., 2020
	Dental braces	Used to reposition and strengthen teeth.	Teo et al., 2016
Ear, nose, and throat	Cochlear implants	Used to provide a sense of sound to patients suffering from moderate to profound sensorineural hearing loss.	Castiglione et al., 2016
	Nasal implants	The reconstruction of deformed nasal passages	Teo et al., 2016
Gastronomy	Endoscopy capsule	Administered for the diagnosis of the gastrointestinal tract.	Fireman and Kopelman., 2007
	Gastroscopes	Introduced through the mouth and oesophagus to inspect the stomach.	Teo et al., 2016
Urology	Urinary catheter	Used for the drainage of urine from the urinary bladder through the urethra	Diggery and Grint., 2012
	Ureteral stents	Used to keep the ureter expended to allow for the passage of urine.	Kaplan et al., 2016
General and Plastic Surgery	Cheek, jaw and chin implants	Used for facial reconstruction	Teo et al., 2016
	Synthetic blood vessels	Used in the replacement of damaged or diseased arteries or veins	Teo et al., 2016
	Medical syringes	Used to administer medicine via injection	French and Collins., 2016

Haematology	Central venous access devices	Serve as ports to draw blood and introduction of treatments	Teo et al., 2016
	Peripherally inserted central catheter	A peripherally inserted central catheter to give intravenous fluids	Dasgupta et al., 2016
Neurology	Implantable Pulse Generator for Deep Brain Stimulation	Used for the treatment of movement diseases by sending electrical impulses to specific Targets in the brain, through implanted electrodes.	Amon and Alesch., 2017
	Cognitive prostheses	Used to extend the capabilities of human cognition or sense perception.	Teo et al., 2016
Obstetrical and Gynaecological	Intrauterine device (IUD)	Used to prevent pregnancy.	Teo et al., 2016
	Intravaginal rings	Provide controlled release of drugs for intravaginal administration	Vargas et al., 2019
Ophthalmic	Fluocinolone ophthalmic implant	Used for the therapy of chronic non-infectious uveitis	Loewenstein et al., 2018
	Artificial Intraocular lens	Implanted in the eyes for the treatment of cataracts or myopia	Teo et al., 2016
Orthopaedic	Orthopaedic implants	Used to replace missing joints or bones or to support	Yamaguchi et al., 2021
Radiology	X-ray scanner	An imaging device used to diagnose chest-related diseases such as lung cancer	Ghobadi et al., 2017

The umbrella term “medical devices” include a wide range of technologies used in various medical fields as mentioned. Based on their application they can be grouped into the following:

Diagnostic devices: they are employed to determine diseases responsible for the symptoms exhibited by a patient or from a patient’s specimen. Well-established diagnostic device technologies include magnetic resonance apparatus, which are investigative tools for neurological-associated cancers. Chest X-ray imaging machines used to identify lung diseases such as lung cancer and pneumonia are also diagnostic devices (Ghobadi et al., 2017).

Monitoring medical devices: they show the status of required measurements over a set period and provide data used for medical purposes such as a thermoelectric transducer, and a body temperature monitoring device converting temperature readings into electrical signals over time (Dumciene and Sipaviciene 2018). The use of smart technology such as mobile phones and wearable watches to keep medical records including heat rate reports has increased the scope of monitor devices (Casselman et al., 2017).

Automated medical laboratory equipment: they are crucial for analysing data to give meaningful results. They have improved medical laboratory operations because they eliminate human errors, have high accuracy, save time and widen the scope of data processing to get medical insights (Hawker., 2017).

Therapeutic devices: they are for remediating diseases in various medical fields. They include most implants that are introduced into the human body to perform a therapeutic or corrective function (Kidd et al., 2015, Teo et al., 2016). Urological invasive implants including catheters and stents fall in this category since they are used to treat patients who have lost their natural urinary functions.

Rehabilitation devices: they mostly aid patients to heal after experiencing trauma. Physical therapy and sports medicine involve the use of rehabilitation devices such as continuous passive range of motion (CPM) and vibration machines to assist in healing after a patient has experienced an injury (Nehra et al., 1996,Peles., 2007).

Life support devices: they are used on critical patients who may have experienced failure in one or more vital organs. They function to maintain vital processes necessary for survival and are mostly used during intensive care and in emergencies. Widely used life support devices include medical ventilators incubators, used to provide mechanical ventilation by exchanging the air in and out of the lungs; incubators, to maintain suitable environmental conditions for infants and dialysis machines, to filter out toxins and excess salts and water from the blood (Badnjevic et al., 2017).

2.2 Urological devices

Urology is a subfield of medicine that focuses on diseases associated with the urinary tract system, which consists of the kidneys, ureters, urethra and urinary bladder. The urinary tract system is involved in the elimination of liquid waste from the body and homeostatic functions such as the regulation of blood, pH, electrolytes and other metabolites. Generally, diagnostic

devices including urological endoscopes are employed before the appropriate treatment is prescribed. The endoscope is a tube-like device with a camera at the end, inserted through an opening to view the lumen of organs. Endoscopes adopted for urological purposes include cystoscopes, cystourethroscopes and ureteroscopes, which are used to view the urinary bladder, urethra and ureter respectively.

Treatment urological medical devices include implants used to treat patients who have lost their natural urinary tract functions, possibly due to tissue or organ damage, infections, kidney stones and other impairment (Denstedt et al., 1998). They are used on transient, short-term and long-term bases. Transiently used implants include ureteral access sheath (UAS) which is implanted for less than an hour. Short-term implants including urinary catheters and stents are generally implanted for less than 30 days. However, in some cases, urinary catheters and stents have been implanted for longer periods including more than 30 days and can be implanted almost permanently. For this research, transient and short-term implants including the UAS, urinary catheters and ureteral stents will be looked at closely.

2.2.1 Urinary catheters

Modern catheters are thin tube-like medical devices fabricated from medical grade material and employed for a variety of medical purposes. They exist in various shapes, sizes and flexibilities depending on the intended function which usually involves the transportation of a fluid (Diggery and Grint., 2012). In urology, catheters are used for the drainage of urine via transurethral indwelling or suprapubic catheterisation. The more commonly prescribed transurethral indwelling catheterisation involves the passage of a catheter into the urinary bladder via the urethra for urine drainage while for suprapubic catheterisation, urine is drained from the urinary bladder or kidney via a surgically created outside connection (Kidd et al., 2015).

The use of urinary catheters dates back to the 3rd century in Egypt, Greece and China. Primitive catheters made from plant matter and metals were used. Plant matter used include straws, hollow tops of onions and rolled-up palm leaves while metals included silver, copper, brass and gold (Bloom et al., 1994, Gonçalves et al., 2015). However, to the best of our knowledge, the earliest recorded use of malleable urinary catheters starts in 1779 (Elves and Feneley., 1997, Singha et al., 2017). The modern-day urinary catheter with a balloon (Figure 2.1) was later introduced in the mid-1930s by Dr Frederick B. Foley, originally manufactured from latex (Winson., 1997). However, latex is prone to bacterial adhesion making it unsuitable for catheter fabrication. Thus, silicone has over taken latex as the most preferred Foley catheter fabrication

biomaterial. Most Foley catheters available in the market are fabricated from synthetic polymeric biomaterial (Figure 2.1). With improvements, some modern urinary catheters can now remain inside patients for periods of up to 3 months (Lawrence and Turner., 2005).

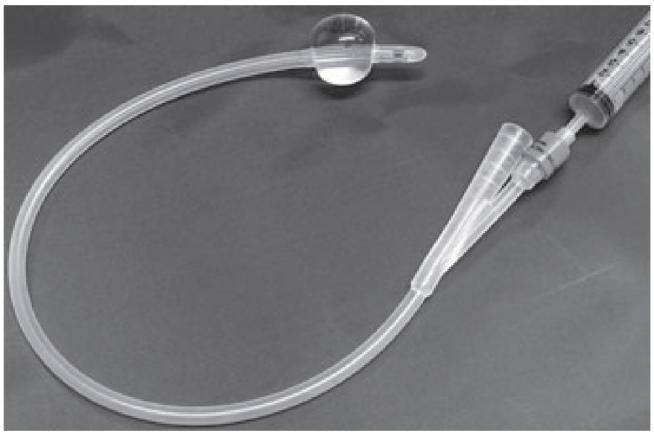


Figure 2.1: Urinary balloon catheter connected to a syringe (Stickler et al., 2003).

The advancement of material science yielded a range of natural, synthetic and semi-synthetic materials that are frequently used for catheter fabrication. These materials have been developed over the years to contain most of the desirable qualities for catheter efficiency including flexibility, high tensile strength, inertness, biocompatibility and the ability to meet flow requirements while maintaining a minimally invasive circumference or French profile. Due to the strengths and weaknesses of materials used for catheter fabrication, which have been realised by trial and error over the years, silicones including PDMS have emerged as the most suitable base material for catheter fabrication (Singha et al., 2017).

2.2.2 Ureteral stents

Morden stents are medical-grade manufactured tube-like medical devices available in different sizes, which are inserted inside into a specific lumen to keep the passageway open thus allowing movement of the intended fluids (Yang et al., 2015). In a case of a dysfunctional ureter due to a blockage caused by kidney stones or a surgical operation, a ureteral stent is inserted into the ureter lumen allowing for the flow of urine from the kidney to the bladder. The length and diameter of a ureteral stent to be used will depend on the size of the patient's ureter. Morden stents are typically in a form that enables them to stay in place post-installation such as possessing single or double J/coil-end. The coils may possess holes allowing for better drainage and release of urine (Gridley and Knudsen., 2017).

Early recordings of successful female and male urethral catheterisation were produced in the late 1800s. However, most of the advancement in ureteral stenting was accomplished during the 20th century. Early ureteral stents were fabricated with mostly plastic material and were designed as straight-line flexible tubes, which tended to move out of place post-installation. This led to the development of the Gibbons stent which prevents movement by possessing multiple sharp barbs along its shaft. However, the barbs increased the diameter of stents from a French scale of 7 to 11 Fr rendering insertion more difficult and decreasing the overall patient's comfort status. Thus, saw the introduction of a double J ureteral stent which had reduced diameters since they possessed double loops/J ends in opposite directions functioning to lock the stents in place post-installation. The double J stent was developed by Surgitek which still holds the patent for the original double J design, thus other companies have switched to pigtail/coiled stents. Pigtail stents rely on opposite preformed coils to stay in place post-installation, refer to Figure 2.2 (Alenezi and Denstedt., 2017).

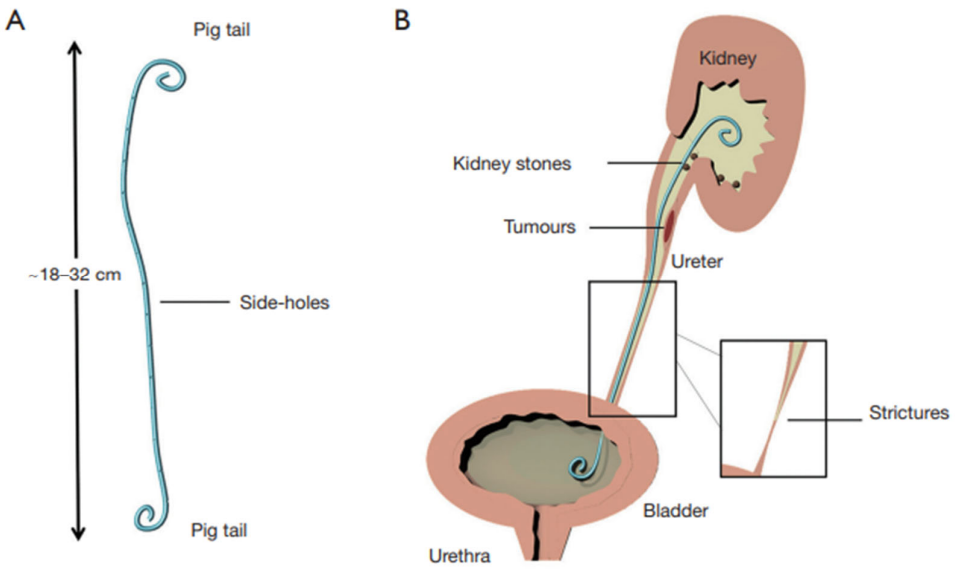


Figure 2.2: A) a pigtail stent B) diagram of an implanted double pigtail ureteral stent (De Grazia et al., 2019).

2.2.3 Ureteral access sheaths

A ureteral access sheath is a tubular instrument with a moveable outer shaft used to provide ureteral expansion allowing for the introduction of a ureteroscope and other medical devices into the ureter (Figure 2.3). The first UAS was introduced back in 1974 for the function of assisting the insertion of a flexible endoscope into the ureter for examination purposes and has since undergone numerous modifications (Kaplan et al., 2016).

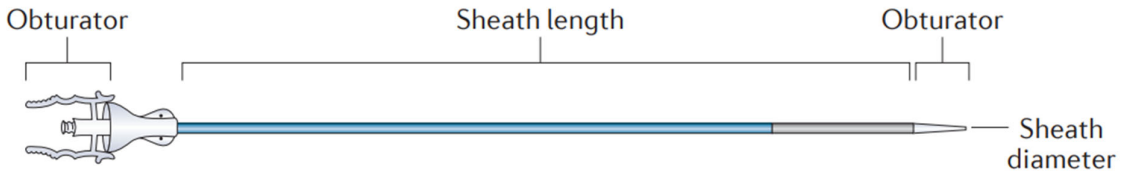


Figure 2.3: A ureteral access sheath (Kaplan et al., 2016).

Older models had a much higher risk of ureteral damage if the outer sheath was moved beyond the inner dilator while current devices are equipped with a impregnated wire (Kourambas et al., 2001). Other improvements include a hydrophilic coating that smoothens the passage of the sheath, a hub-locking mechanism allowing passage of the sheath and dilator together as a single unit, a kink-resistant design, the ability to perform a retrograde pyelogram through the dilator, and they are available in a variety of lengths and diameters to accommodate different sizes of both the patient and the device to be installed and the nature of the pathology (Monga et al., 2001). Thus, modern UAS have improved minimally invasive management of urinary tract-associated diseases and has become a crucial tool for most ureteral surgical procedures such as ureteroscopy, stone ablation, tumour ablation and stricture incision.

2.3 Complications associated with urological implants

As much as urological implants are beneficial to the healthcare sector, they are not without complications, which may outweigh the initial problem that necessitated their use. Therefore, the development of urological devices must negate possible complications that may arise due to device malfunction, the function of the implantation site, environmental changes, incompatibility and infections.

2.3.1 Urinary catheters

Catheterisation procedures are generally associated with some pain due to urothelial tissue damage caused by the physical rubbing of the device against the wall of the urethra. Patients undergoing urinary catheterisation are also very susceptible to UTIs due to partial implantation during catheterisation by providing access to the urinary bladder for infectious agents. Urinary catheterisation also negates the normal cycle of bladder filling and emptying which acts as a defence mechanism by flushing bacterial cells contaminating the urethra and bladder out of the system. Thus, instead of the bladder filling and flushing, urine trickles through the catheter into

the drainage bag facilitating the migration of bacteria along the urethra and bladder (Stickler et al., 2003).

Bacteria including *Escherichia coli, Staphylococcus epidermidis* and *Enterococcus faecalis* are generally responsible for most infections occurring during the early stages of catheterisation. These microbes can quickly form single species biofilm within 24 h of catheterisation. However, a variety of microorganisms including *Proteus mirabilis, Providencia stuartii, Pseudomonas aeruginosa, Klebsiella pneumoniae* and have also been regularly detected in patients undergoing long-term (>28 days) catheterisation. These mixed bacterial communities can result in encrustation which will eventually cause catheter blockage and thus loss of function (Singha et al., 2017).

During long-term catheterisation, catheters can be changed at 10–12-week intervals meaning urological pathogenic bacteria could be building up from point of colonisation to periods of up to 3 months hence long-term catheterised patients tend to develop a UTI. Bacteria also prefer to grow on surfaces rather than in planktonic suspensions, catheters thus provide an attractive site for attachment thus facilitating biofilm development. Biofilms are a major concern because are generally much harder to eradicate since the members of the communities forming them are protected by a self-develop encapsulating polysaccharide matrix. In addition, this polysaccharide matrix aids adhesion to the implant surface. The transfer of drug resistance is enabled via conjugation since bacterial cells are clumped together (Ma and Bryers., 2013). Hence large bacterial populations growing in biofilms on the surface of urinary catheters which were visible to the naked eye have been documented and communities of 5×10^9 viable cells per centimetre length of the catheter have been recovered from patients undergoing long-term (6 weeks) indwelling catheterisation (Ganderton et al., 1992). The most common organisms recovered from these biofilms are *E. coli*, *P. mirabilis*, *Enterococcus faecalis* and *Pseudomonas aeruginosa* (Singha et al., 2017). Infections growing in biofilms are much harder to eradicate since they are encapsulated in a polymeric matrix that protects them from antimicrobial agents and aid adhesion. Sharing of drug-resistance genes is very common since the bacteria pathogens are in close proximity and perform conjugation (Neal., 2008). Therefore, immunocompromised individuals who regularly rely on urological implants are at a higher risk of developing chronic UTIs.

2.3.2 Ureteral stents

Stents are implanted either for short or long-term depending on the nature of the urinary tract dysfunction. Most complications arising from stenting are to a certain degree similar to those

experienced with catheterisation, except for the fact that they are occur in the ureter. During stenting patients may experience side effects including epithelial damage, pain, blood in the urine and UTIs. The frequency of complications is mostly proportional to the duration of stenting (Auge and Preminger., 2002). Chronically stented patients experience the most hardship as they face all of the above complications continuously and often require new stents to be inserted immediately following the removal of a dysfunctional stent. This short turnaround time between stent replacement facilitates contamination of the newly installed stent. A high dosage of antimicrobial agents associated with their own cost is commonly prescribed for chronic or long-term stenting patients (Yang et al., 2015). Microorganisms commonly isolated during stenting include enterococci, coagulase-negative *Staphylococci* and *E. coli*, usually existing in biofilm communities, while *P. mirabilis* is rarely recovered from stents (Stickler et al., 2003).

2.3.3 Ureteral access sheaths

The use of a UAS is inherently risky as a strong shear force is required to place it over a stiff wire to a point just distal of the site of pathology and since sheath placement is conducted without direct visualisation, thus obstructions including stones, stone fragments or a tumour can be bypassed by the sheath, which can cause damage to both the ureter and the devices (Abrahams and Stoller., 2004). Furthermore, the UAS can damage the flexible ureteroscope if a small stone fragment becomes stuck between the shaft of the ureteroscope and the inner wall of the UAS (Kaplan et al., 2016).

Most UAS currently in the market are coated with hydrogels which tend to lose their lubricating properties over time thus causing tissue damage during insertion which can result in superficial mucosal damage, a deep injury involving the muscular layer, or the entire ureter can be perforated. The swelling effect of hydrogels as mentioned reduces the diameter of the UAS thus increasing the chances of the endoscopes or other urological devices to be introduced getting stuck and sticking to the walls of the UAS (Pedro et al., 2007).

2.4 Approaches to improve urological implants

The development of a urological device must take into account certain factors including its functionality, biocompatibility with the site of action, the intended period of use and the comfort status of the user (Teo et al., 2016). Thus, the selection of material for fabricating a urological device is dependent on how best it can fulfil those roles. Early advancements conducted to solve the complications associated with the use of urological implants mostly involved the

development of better materials for device fabrication. While recent improvement strategies mostly focus on developing coating technologies and the incorporation of antimicrobial agents.

2.4.1 Improved fabricating materials

In the past, pure latex was the choice material for the fabrication of urological implants such as catheters because it was inexpensive and contained desired physical properties such as good elasticity. However, the refining process where not yet efficient enough since some of the final products would contain potentially toxic agents. Natural latex is also prone to bacterial attachment and thus very susceptible to biofilm formation proving unsuitable for the fabrication of urological implants (Roberts et al., 1990, Singha et al., 2017).

Urological catheters fabricated from pure silicone substrate are expensive as compared to the former. They are non-reactive toward the body and thus non-toxic. Other desirable qualities include good surface properties allowing for smooth insertion, resistance to microbial adhesion and encrustation. Therefore, pure silicone was widely used for the fabrication of catheters since it overcame most of the challenges associated with latex. However, due to high flexibility, it is difficult to pass up both narrowed and tortuous ureters and elasticity makes it difficult to manage its passage over guide wires thus not suitable for stent fabrication. However, modified silicone was developed to overcome the challenges faced with pure silicone as a biomaterial for stent fabrication (Denstedt et al., 1998). Urological catheters have also been fabricated using plastic polymers including polyvinyl chloride and polyethene. The resulting catheters had better physical stability compared to those fabricated using the mentioned materials, but they tend to cause patient irritation and have been known to cause urethritis and stricture formation as a result of unreacted monomers on their surface (Lange et al., 2011).

Polyurethanes (PUs) and PDMS are some of the most widely used biomaterials for urological implants. In the case of catheters and stents, they are formed into thin layered tube-like devices of desired size and length. Polyurethanes are a class of polymers produced mainly from organic compounds in various forms depending on the desired qualities. Polyurethanes were developed in the 1930s and have been widely used as a biomaterial due to their desired characteristics including good mechanical properties, stability and overall good biocompatibility. Earlier PUs were similar to nylon 6,6 except that they had two additional oxygen per repeat unit, and thus have a lower melting point, lower water absorption, and better electrical and mechanical stability upon ageing (Wagner et al., 2020). Silicone-based materials on the other hand have been in use in the medical industry since the 1960s. They are available in various forms including fluids, emulsions, compounds, and resins which can be further formed into

elastomers, gels, and adhesives, and thus they have been widely used for various medical purposes. They are adopted as a biomaterial for fabrication and coating since they possess desirable characteristics including biocompatibility, good flexibility, durability and stability in a wide temperature range. Those that contain repeating units of one silicon atom bonded to one oxygen and two methyl groups termed siloxane are called polydimethylsiloxane, abbreviated as PDMS is the most adapted form of silicone for biomaterials (Wagner et al., 2020).

Polyurethanes and silicones have solved some of the challenges faced with prior urological implant fabricating materials and have thus achieved far more commercial success. This mainly includes better biocompatibility. However, uroepithelial damage caused by friction and UTIs remain major concerns thus prompting the need for further improvement strategies such as coating technologies and the addition of antimicrobial agents. Important differences between the two polymers include their surface polarity and mechanical strength. Polyurethane has a lower surface polarity and is mechanically more rigid compared to PDMS which has a higher surface polarity and is much more flexible. some difficulty is associated with binding coating technologies to some biomaterials.

The evolution of material science came with the development of new polymeric materials for device fabrication (Wagner et al., 2020). These new polymeric materials were designed to meet certain specifications. Most specialised polymers have enough flexibility and elasticity but are still mechanical and strong enough to perform their function. For instance, C-flex®, a PU developed by Consolidated Polymer Technologies is a mechanical strong but flexible and elastic specialised biomaterial for urological stent fabrication (Lange et al., 2011). Other specialised polymers used as urological biomaterial include specialised PUs such as Percuflex® and Tecoflex® and silicones such as Silitek® (Teo et al., 2016,Todd and Knudsen 2017).

2.4.2 Coating technologies and the addition of antimicrobial agents

Urological implants are required to maintain their function within the urinary tract system for the intended life span. Furthermore, essential requirements include biocompatibility and resistance to infections. These requirements are mostly influenced by the interaction of the biological system with the surface of the medical devices. Thus, coating technologies that modify the surfaces of devices are frequently employed to increase the lifespan and efficiency of device implants.

Several materials including metals, polymers, and polymer composites have been used for medical implant coating purposes. (Teo et al., 2016).Metallic coatings including silver, copper,

bismuth and other metals have been used as anti-microbial coating agents for medical implants. In recent times metallic coatings are fabricated in combination with other metals or with other materials, to improve coating properties and effectiveness against infections. A combination of silver and copper nanoparticles has been used to coat polyurethane catheters and observed to reduce infections (Gosau et al., 2016). The use of metallic material for anti-microbial coating is not associated with antibiotic resistance challenges because they do not depend on antibiotic action. Instead, their mechanism of action involves the release of antimicrobial metallic ions. However, the fast release of excessive ions can be cytotoxic to patients (Liu et al., 2011).

Polymers are a variety of macromolecules either naturally occurring, synthetic or semi-synthetic, made up of smaller repeating monomer subunits. They are the most used materials for fabricating and coating urological implants. They can be used in combination with different materials including metals to produce enhanced medical devices and they are available in a wide range of mechanical, electrical, chemical, and thermal properties (Teo et al., 2016). The diversity of polymers has facilitated several approaches used to improve medical devices. This includes priming and lubricating technologies to improve the attachment of other materials and reduce friction when it is not desired respectively.

2.4.2.1 Priming technologies

Priming is a process that involves the application of a primer layer on the surface of a material to improve adhesion to a subsequent layer. In the case of biomaterials, primers are often used to improve the adhesion of a target coating such as a hydrogel to the surface of a metallic, or polymeric material. The use of primers has several benefits. Primers can improve the wetting and spreading of the target coating on the surface of the material, which can lead to a more uniform and consistent coating. Additionally, primers can help to minimize the formation of defects or delamination of the target coating, which can compromise the performance and durability of the coated material (Ryu et al., 2018).The primer layer typically lacks functional properties, and its main role is to provide a stable and adhesive interface between the material surface and the target coating. The choice of primer material and the application method can vary depending on the specific biomaterial and the target coating being used. In general, the use of primers has enabled the development of coating technologies that can be applied to a wide range of biomaterials, including those used for the fabrication of urological implants. By improving the adhesion and durability of target coatings, primers can enhance the performance and functionality of biomaterials.

Polydopamine, a result of dopamine oxidation, is an organic polymer. The chemical structure of dopamine possesses open functional groups including HO and NH_2, which allows it to bind to various materials (Figure 2.4). It is widely adopted in surface coating technologies due to its simplicity, low cost, adaptability and biocompatible. It lacks any antimicrobial properties thus not suited for use as a UTI mitigation strategy. However, it can be deposited as a conformal thin layer onto almost any solid material (Ryu et al., 2018). PDA can be deposited in far simpler dip-coating processes compared to traditional surface modification methods such as self-assembled, layer-by-layer and plasma treatment. This suggests that using PDA as a primer may result in reduced production costs. PDA has successfully been used as a primer for several coating technologies as shown in Table 2.2.

Since its first reporting, PDA has been synthesized from dopamine by three methods including solution oxidation, enzymatic oxidation and electropolymerization. Solution oxidation is the most adopted method and is usually carried out for 24 h in an alkaline environment using atmospheric oxygen as an oxidizing agent. However, recent studies have investigated to use of other oxidants rather than atmospheric oxygen to make the process faster. This includes oxidants such as ammonium persulfate, copper sulphate, hydrogen peroxide, and sodium periodate. A PDA coating technology to improve cell adhesion in nanofibrous PU biomaterials for tissue engineering has successfully developed (Kopeć et al., 2020). The coating mechanism involved a fast (less than 30 min) and efficient solution oxidation process that used sodium periodate as an oxidative agent (Kopeć et al., 2020). This coating mechanism was adopted and modified for this study due to its reported quickness and efficiency.

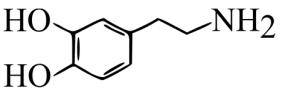


Figure 2.4: A dopamine monomer unit.

Table 2.2: Coating technologies that rely on polydopamine as a primer

Coating technology	References
PDA coating technology for PU nanofibrous biomaterials to enhance cell adhesion in tissue engineering	Kopeć et al., 2020
Peptides immobilized onto PD-coated PDMS urinary catheter	Lim et al., 2015
Heparin/poly-L-lysine-Copper nanoparticles coating mediated by PDA for PU ureteral stent	Awonusi et al., 2022
Hydrophobic polytetrafluoroethylene particle immobilized via a polydopamine anchor layer on a nitric oxide-releasing polymer	Mondal et al., 2021
A PVP/PDA polymer coating for the rapid sedimentation of Anionic silica nanoparticles nanomaterials in aqueous suspension	Lai et al., 2014

For evaluating the application of PDA as a primer, two different polymers were used including PU and PDMS. Polyurethanes are synthetic polymers that can be formulated with different chemical structures, resulting in variations in their surface properties. In general, polyurethane exhibits moderate to high surface polarity depending on its composition. The presence of polar groups, such as urethane linkages (-NHCOO-), in the polymer chain contributes to its polarity. This polar nature allows for better wetting and adhesion with polar liquids or surfaces. While PDMS a type of silicone, is a polymer composed of repeating units of dimethylsiloxane. PDMS is well-known for its low surface energy and hydrophobic properties. The methyl groups ($-CH_3$) on the polymer backbone contribute to its low surface polarity. PDMS surfaces are generally considered non-polar and exhibit low affinity for polar solvents or surfaces (Wagner et al., 2020). Therefore, by using these polymers, findings can be drawn about the coating technology applicability to different fabrication biomaterials with a wide surface polarity range.

2.4.2.2 Lubricating coating technologies

Hydrogels are highly versatile materials that have gained increasing attention in various biomedical applications due to their unique properties. These materials are composed of large, interconnected molecules made from natural or synthetic hydrophilic polymers, and they are capable of absorbing and retaining a significant amount of water without dissolving. Their ability to absorb water comes from the presence of hydrophilic functional groups located throughout the polymeric backbone chain. Because of the combination of solid-like and liquid-

like properties, hydrogels exhibit viscoelasticity that is similar to that of biological tissue. Hydrated hydrogels are characterised by having a slimy texture which indicates the presence of a hydro layer on their outer surface. This improves their smoothness and lubricity, hence they have been widely adopted for use as lubricating agents (Lawrence and Turner 2005). Hydrogels are suitable for a range of other applications because of their unique properties, including drug delivery, wound healing, tissue engineering. However, this study focuses on the use of hydrogels in coating technologies to improve urological implants by reducing friction between the implant and the lumen of the urethra or ureter. Hydrogels that have been used for this purpose include polyethylene glycol (PEG), PVP, hyaluronic acid and phosphorylcholine-containing materials (Driver., 2012; Todd and Knudsen., 2017b).

2.4.2.2.1 Polyvinyl pyrrolidone as a hydrogel

Hydrogel coatings are effective in enhancing the lubricity of urological implants, such as catheters, by reducing friction between the implant and the surrounding tissue. Hydrogels are composed of a network of cross-linked hydrophilic polymer chains that are capable of absorbing large amounts of water (Wagner et al., 2020). This property makes them highly lubricious and enables them to form a thin layer of lubricating fluids between the implant and the uroepithelial tissues. The lubricating fluids thus reduce friction between the implants and the uroepithelial tissues and facilitate smooth insertion and removal of the implant which improves their biocompatibility.

PVP is a water-soluble polymer that is composed of repeating units of vinyl pyrrolidone monomer (Figure 2.5). It is FDA-approved for human use and is widely adopted in the pharmaceutical, cosmetic, and food industries. PVP is available in different grades, each with different molecular weights and physical properties, making it suitable for a wide range of applications. One of the most significant properties of PVP is its high solubility in water and other polar solvents. This makes it an excellent excipient in the pharmaceutical industry, where it is used as a binder, disintegrant, and solubilizer in tablets, capsules, and other dosage forms. PVP is also used as a stabilizer in emulsions, suspensions, and ointments. In addition to its use in the pharmaceutical and cosmetic industries, PVP has been widely adopted in the medical industry as a drug delivery system and in numerous lubricating technologies (Wang et al., 2016). Numerous PVP coating technologies have been developed to improve the biocompatibility of urological biomaterials by introducing lubrication and thus reducing their friction coefficient. Most of these technologies generally rely on macromolecule polymer chain crosslinking to bind the PVP to biomaterials as opposed to monomer polymerization which may

result in unreacted monomers that are often toxic to human tissues. However, macromolecule polymer chain crosslinking produces PVP hydrogels that are not stable and tend to easily detach after a few days (Butruk et al., 2012).

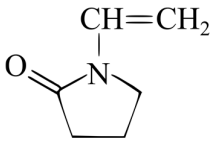


Figure 2.5: A vinyl pyrrolidone monomer unit.

A free-radical macromolecular grafting–crosslinking method that relied on a Fenton-type reaction presented by Butruk. *et.al* demonstrated to ability to improve the stability of PVP-coated PU biomaterials by ensuring the formation of covalent bonds between them (Butruk et al., 2012). This formulation and coating mechanism was adopted in this study to functionalised PDA-coated biomaterials to improve their biocompatibility by reducing their surface friction coefficient.

2.4.2.3 Antimicrobial agents in coatings

Urological implants, such as catheters and stents commonly used in medical procedures are prone to infections (Denstedt et al., 1998). Urinary tract infections (UTIs) are one of the most common complications associated with these devices. Therefore, it has become essential to incorporate antimicrobial properties in these implants to prevent infections and ensure better patient outcomes.

Most modern urological implants are designed to possess some antimicrobial agent or an intrinsic antimicrobial factor, which makes them unsuitable for microbial colonization. Intrinsic factors refer to the inherent properties of a material that make it difficult for bacterial adherence and growth (Braun et al., 2017). For instance, materials including silver or copper have intrinsic antimicrobial properties that can kill bacteria on contact. However, in most cases, the antimicrobial properties of urological implants are improved by the addition of antimicrobial agents such as antibiotics, triclosan, and iodine. These agents are incorporated into the implant coating or the fabricating biomaterial itself to provide a sustained release of the antimicrobial agent over time, reducing the risk of infection (Percival et al., 2016).

Urinary tract infections are commonly caused by bacterial pathogens and can be treated with antibiotics. However, the overuse and misuse of antibiotics have led to the emergence of antibiotic-resistant bacteria, which pose a major threat to public health (Singha et al., 2017). To combat this issue, researchers have explored alternative antimicrobial agents, such as silver nanoparticles and quaternary ammonium compounds that can be incorporated into coatings on medical devices.

Coating technologies have been developed to apply antimicrobial agents onto the surfaces of medical devices, such as urinary catheters, to prevent bacterial colonization and biofilm formation. The effectiveness of these coatings depends on the method of combining the antimicrobial agents with the coatings. The two main methods are physical entrapment and chemical bonding. Physical entrapment involves incorporating the antimicrobial agent into the coating material, which can then slowly release the agent over time. The release rate of the agent can be controlled by modifying the properties of the coating material, such as its porosity and thickness. This can influence the concentration and duration of exposure of the antimicrobial agent to the bacteria, and thus affect its effectiveness. While chemical bonding involves covalently attaching the antimicrobial agent to the coating material, which can provide more stable and long-lasting antimicrobial properties. However, this method may also reduce the release rate and concentration of the antimicrobial agent, which could affect its effectiveness against bacteria (Khandwekar and Doble., 2011, Wagner et al., 2020).

Overall, the combination of antibiotic and non-antibiotic antimicrobial agents with coating technologies can provide an effective defence against UTIs. The method of combining the agents with the coatings can influence their effectiveness, and careful consideration must be given to the release rate, concentration, and duration of exposure of the antimicrobial agent to the bacteria. Physical entrapment methods have been successfully applied to combine iodine with PVP coatings. This has been demonstrated in a study were iodine was trapped in a PU/PVP hydrogel coating technology, the coating was observed through preliminary investigations to be effective against *Micrococcus luteus*, as evident by the presence of zones of growth inhibition (Paradowska et al., 2010).

2.4.2.3.1 Iodine as an antimicrobial agent

Iodine is a well-known antimicrobial agent that has been used for many years to prevent infections in medical procedures. It is a broad-spectrum antimicrobial agent that targets a wide range of microorganisms, including bacteria, viruses, and fungi. In urological coatings, iodine is often incorporated into the coating material or added to the coating in the form of a solution.

The iodine is released slowly over time, providing a sustained antimicrobial effect that can prevent bacterial adhesion and growth on the surface of the implant (Percival et al., 2016).

Iodine inhibits cellular growth by oxidizing the nucleotides fatty/amino acids in their cell membranes and denaturing enzymes involved in their mitochondrial respiratory chain. In most cases, elemental iodide is avoided, instead a drug delivery system is employed. For example, PVP and iodine combinations are heavily used for antisepsis and wound healing due to their proven antimicrobial efficacy and ability to target a broad spectrum of microbes (Percival et al., 2016). Generally, iodine is considered safe to use in low concentrations. Several *In vivo* studies have observed the use of iodine up to 10% w/v without inhibiting the granulation and epithelialization processes during wound healing (Bigliardi et al., 2017).

2.4.3 Polyvinyl pyrrolidone-iodine complexes

Povidone-iodine (PVP-I), a mixture of PVP and iodine has numerous medical applications due to its hydrogel and antimicrobial properties. In urology, it is generally used in different forms including solutions, gels and coatings to treat or prevent UTIs. It can be composed of varying concentration PVP and iodine. In principle, the PVP is used as a highly biocompatibility drug delivery system to release the iodine over a prolonged period. Therefore, PVP-I complexes tend to provide longer protection as opposed to the use of iodine directly. The release rate of the iodine is mostly influenced by the concentration PVP owing to PVP hydrophilic functional groups and the hydrogel overall structure and porousness.

The toxicity profile of PVP-I has been a subject of research, and a consensus has not been reached. Toxicity in implant coatings may depend on a variety of factors, including the specific application of the implants, the fabricating materials and their concentration used, and the individual patient's needs and medical history. Several studies have been conducted to evaluate the safety and efficacy of povidone-iodine in implant coatings. One study that examined the cytotoxicity of povidone-iodine on human fibroblast cells, which are important for wound healing and tissue repair found that povidone-iodine concentrations higher than 1% were cytotoxic, thus indicating a potential risk of tissue damage and impaired healing (Balin and Pratt 2002). However, PVP-I solutions with concentrations of iodine up to 10% w/v are generally administered in urology as antiseptics (Bigliardi et al., 2017). While povidone-iodine can be an effective antimicrobial agent in implant coatings, the risks and benefits of using it must be carefully evaluated for signs of toxicity.

Several studies have demonstrated the effectiveness of iodine in preventing infections in urological implants. One such study modified Tecoflex®, a biomaterial commonly used for the fabrication of urological implants, by incorporating 5% w/v of PVP-I. This study showed that the PVP-I entrapment process can be applied to polyurethane to reduce complications associated with bacterial adhesion and deposition of encrustation (Khandwekar and Doble., 2011). Another study compared povidone-iodine to a placebo gel as a urinary catheter lubricant and found that povidone-iodine gel significantly reduced bacterial counts compared to the placebo (Harrison., 1980). Therefore, coating technologies that incorporate a sufficient amount of iodine are expected to demonstrate some antimicrobial activity. To confirm that the antimicrobial activity of novel coating technologies that incorporate iodine is sufficient to restrict UTIs, appropriate antimicrobial evaluation methods are employed.

2.5 Regulation of medical devices

Even though medical devices have been used since ancient times, for the majority of human history they were not regulated. Only in the mid-20th century did nations start setting up laws that addressed medicinal products (Gupta., 2016). In most cases, such laws did not properly cover most medical devices since they do not rely on metabolic activity thus different from most generic medicinal products. However, by the beginning of this century most industrialised nations have already set up regulatory bodies to oversee the development and enforcement of safety standards regarding medical devices in their jurisdictions, which involves defining them, and their classification into different categories with each having a set of requirements (De Maria et al., 2018). Thus, medical device regulations differ according to authorities having jurisdiction per region as evidenced by the differing regulations in the United States of America (USA), the European Union (EU) and the Republic of South Africa (RSA). However, several national regulatory bodies of developed countries have formed other bodies such as the Global Harmonization Task Force (GHTF), and subsequently the International Medical Device Regulators Forum (IMDRF) whose function is to drive global harmonisation (Geremia., 2018).

The regulation of medical devices is the responsibility of the state; therefore, the development, regulation and enforcement of medical device standards will be an extension of how a state operates (Wilkinson., 2021). However, most countries often partially outsource regulation and adopt standards developed by reputable standardization bodies such as the International Organization for Standardization (ISO). This is done intentionally to enhance global harmonization (Imagawa et al., 2018, Melvin and Torre., 2019).

For a novel medical device technology to gain market entry, premarket approval must be obtained from the relevant authorities. The premarket approval pathway often dictates the conformity assessment methods that will be used to provide evidence. Therefore, this study also looks closely at the regulatory controls that this novel technology will need to satisfy to be accepted in the USA, EU and RSA. The standards adopted for the regulation of medical devices in the mentioned regions are thus used as a guideline for the selection of in-vitro evaluation methods for the novel coating technology.

2.5.1 Medical devices regulations in the United States of America

The Food and Drug Administration (FDA) is responsible for regulating medical devices in the USA. It provides a medical device definition and list that categorizes them into 16 specific medical fields, referred to as panels. Each panel consists of a sub-list of all generic types of medical devices available in that medical field. These panels collectively have approximately 1,700 different generic types of medical devices. Each generic type of medical device has a regulatory number and is assigned to one of three regulatory classes based on the level of risk associated with it (Sorenson and Drummond., 2014). Class I cover generic types of medical devices with the least risks and thus have the least controls. They are generally exempt from premarket approval (PMA). Class II have more controls than class I and less generic types of devices in this group are exempt from premarket approval, a 501k application can also be used to also gain clearance for class II medical devices. It is based on determining the risk equivalence of medical devices seeking clearance to already cleared similar types of class II medical devices. While class III generic types of medical devices are associated with the highest risks and required premarket approval. All devices exempted from premarket approval are subject to restrictions set up by the FDA for each generic type (Sorenson and Drummond., 2014). Therefore, device classification is the most determining factor for the type of premarketing submission/application required to gain FDA clearance to operate in the USA. Most urological implants are assigned to class II and either require a 501k application or proof of conformity by adopting USA-accepted standards.

Understanding these FDA regulations is crucial for the development of coatings for urological devices, as it ensures that the new products meet safety and efficacy standards set by the FDA for the USA market. This knowledge forms the foundation for successful product development by providing the appropriate assessment criteria. For instance, standards such as ISO 10993 and ASTM F623-19 are widely accepted for urological catheters and stents. ISO 10993 provides a framework for assessing the biocompatibility of medical devices, ensuring that they are safe for

patient contact. ASTM F623 sets specific performance criteria for Foley catheters, including requirements for tensile strength, balloon integrity, and drainage efficiency.

Table 2.3: The regulation of urological implants in the United States of America

Type of urological implants (regulatory number)	Classification category (special condition)	Adopted standards for conformity
Urinary Catheters (RN 876.5130)	Class II Class I (accessories)	● ISO 10993:2020 - Biological evaluation of medical devices ● ASTM F623-19 Standard Performance Specification for Foley Catheter
Ureteral Stents (RN 876.4620)	Class II	● ISO 10993:2020 - Biological evaluation of medical devices
Ureteral Access Sheaths	Class II	● ISO 10993:2020 - Biological evaluation of medical devices

ISO- international standardisation organisation and RN- regulatory number

2.5.2 Medical devices regulations in the European Union

According to the EU regulation, (MDR 2017/745) published by the European Council (EC), the EU's regulatory authority, medical devices will be categorised into one of four classes. From the lowest to highest risk; Class I, Class IIa, Class IIb, and Class III (Union., 2017)). The risk assessment for each medical device is based on 22 rules, set out in the MDR of 2017 which take into account invasiveness, intended purpose, period of use, energy source and overall inherent risk (Union., 2017, Thunborg and Österberg., 2021). Class I devices are exempted from seeking conformance assessment; manufacturers can self-certify conformity according to the EC regulations and affix the CE mark, which allows them to be used in the EU's markets. For Class IIa, IIb, and III devices, proof of device conformance to safety and performance standards is required for market entry thus manufacturers must seek relevant conformance. Unlike the USA, the classification of urological implants is heavily influenced by their contact time during implantation (Table 2.4). This also means that the evaluation methods adopted to prove conformance should also take use into account their intended application duration.

Table 2.4: The regulation of urological implants in the European Union

Type of Urological implants	Classification category (special condition)	Adopted standards for conformity
Urinary Catheters	Class I (used for < 1 h) Class IIa (used for < 30 d) Class IIb (used for > 30 d) Class III (contains balloon)	● ISO 10993:2020 - Biological evaluation of medical devices
Ureteral Stents	Class IIb	● ISO 10993:2020 - Biological evaluation of medical devices
Ureteral Access Sheaths	Class III	● ISO 10993:2020 - Biological evaluation of medical devices

ISO- international standardisation organisation

2.5.3 Medical devices regulations in the Republic of South Africa

Unlike the previous regions, countries in Southern Africa are not fully industrialized and lack the resources to develop stringent regulatory systems (Saidi and Douglas., 2018). Countries that have established compressive medical device regulatory systems in this region have done so within the last 10 years, while others have yet to do so. However, while some of these countries lack proper controls, medical devices are often considered medical products and thus regulated as such. RSA, the most developed country in the region, recently updated their regulatory controls in 2015 to incorporate a classification system. The South African Health Products Regulatory Authority (SAHPRA) has published guidelines which set out 16 and 7 rules for the classification of medical devices and In-vitro diagnostics devices (IVDs) respectively to one of the four classes. From the least to highest risk A, B, C and D. Similar to the EU, these rules take into account the intended use, invasiveness, period of use, implant device energy source, their inherent risks and the technology the devices utilize. In addition, IVD rules also consider risks to public health as a whole (8.05 Classification Medical Devices IVDs Nov19). The classification of urological implants in RSA is also influenced by their contact time during implantation. SAHPRA also accepts standards published by the IMDRF (Table 2.5).

Table 2.5: The regulation of urological implants in the Republic of South Africa

Type of urological implants	Classification category (special condition)	Adopted standards for conformity
Urinary Catheters	Class A (if used for < 1 h) Class B (if used for < 30 d) Class C (if used for > 30 d) Class D (if it contains drug)	● IMDRF/GRRP WG/N66FINAL:2021 ● ISO 10993:2020- Biological evaluation of medical devices
Ureteral Stents	Class A (if used for < 1 h) Class B (if used for < 30 d) Class C (if used for > 30 d) Class D (if it contains drug)	● IMDRF/GRRP WG/N66FINAL:2021 ● ISO 10993:2020 - Biological evaluation of medical devices
Ureteral Access Sheaths	Class B	● IMDRF/GRRP WG/N66FINAL:2021 ● ISO 10993:2020 - Biological evaluation of medical devices

ISO- international standardisation organisation, IMDRF-international medical device Regulators Forum, GRRP- good regulatory review practices and WG- charter of the Working Group (WG)

2.5.4 Standards adopted for the regulation of urological implants

Most governments work together with international standardization bodies with ISO being the most notable. In addition, they also work with regional ones, for instance, the EU may require (European Committee on Antimicrobial Susceptibility Testing) EUCAST standards for proof of antimicrobial activity. These institutions are focused on technical matters such as developing and certifying administrative practices and scientific methodologies to follow when conducting conformity assessments via *in-vitro* tests and *in vivo* studies.

ISO 10993 is the most widely adopted and accepted standard for proving the biocompatibility of a medical device hence it is accepted by the FDA, EC, SAHPRA and IMDRF. The standard layouts are both in-vitro and in-vivo assessment methods for evaluating the biological effects of medical devices intended to contact the human body. This includes cytotoxicity, sensitization, irritation, systemic toxicity (acute), subchronic toxicity (subacute toxicity), genotoxicity, implantation and haemocompatibility (Table 2.6). In addition, the standards also give guidelines on how to prepare samples and assess possible ethylene oxide sterilization residuals and potential degradation products.

In vitro evaluation methods are conducted first and must prove that the medical device is safe before *In vivo* methods are carried out excluding cases where the former is not available. The specific assays carried out on the samples are selected based on the type of human body contact and duration. The type of human-body contact is mainly classified into surface contact, external communicating and implant devices, with each having two or more subheadings. While the contact duration is separated into limited (< 24 h), prolonged (> 24 h to 30 d) and permanent (> 30 d). Medical devices that have any contact with the human body must be evaluated for cytotoxicity, sensitization and irritation including all urological implants. However, permeant external communicating devices that have indirect contact with the blood path are the exception for irritation assessments. While the remaining four biological effects that can be evaluated including systemic toxicity (acute), subchronic toxicity (subacute toxicity), genotoxicity, implantation and hemocompatibility are also recommended for most permeant surface devices, external communicating devices and other invasive implant devices. In cases where urological implants are intended to be used for more than 30 days, subchronic toxicity (subacute toxicity) and genotoxicity are also recommended.

ISO 10993 is updated regularly to keep up with newer technologies, however, the chapters of the standard are often updated independently. For instance, chapter 12 has been recently updated in 2021 while Chapter 4 was last updated in 2017. These updates negate the previous versions, thus medical device developers are required to purchase the newer versions (Heise et al., 2022).

Table 2.6: Biological evaluation methods suggested by International Standardisation Organisation 10993 standard

Biological effect evaluated	*In vitro* methods	*In vivo* methods
Cytotoxicity	• MTT assay • XTT assay • Agar diffusion • Filter diffusion • Colony formation • Neutral red uptake	• N/A
Sensitization	• N/A	• Local lymph node assay in rodent • Occluded patch test in guinea pigs • Guinea pig maximization test • Non-invasive human clinical trials
Irritation or intracutaneous reactivity	• Transcutaneous Electrical Resistance (TER) tests • Reconstructed human epidermis (RHE) Model for irritation studies	• Animal skin irritation test • Animal intracutaneous (intradermal) reactivity test • Human skin irritation test
Systemic toxicity (acute)	• N/A	• Animal studies
Subchronic toxicity (Subacute toxicity)	• N/A	• Animal studies
Genotoxicity	• Gene mutations in bacteria (OECD 471) • Gene mutations in mammalian cells (OECD 476) • Clastogenicity in mammalian cells	• Micronucleus test in rodents (OECD 474) • Metaphase analysis in rodent bone marrow (OECD 475) • C) unscheduled DNA synthesis test with mammalian liver cells (OECD 486)
Implantation	• N/A	• Animal studies

		• wabnClinical trials
Hemocompatibility	• Thrombosis • Coagulation • Platelets and Platelet Functions • Haematology • Complement System	• Thrombosis • Coagulation • Platelets and Platelet Functions • Haematology • Complement System

N/A- Not available, OECD- Organisation for Economic Co-operation and Development

Most invasive medical devices, particularly those used for extended periods tend to be prone to microbial invasion. Therefore, to mitigate this challenge, numerous studies have explored strategies to improve their antimicrobial properties including incorporating them with antimicrobial agents. For invasive medical devices intended to have improved antimicrobial properties, the relevant standards are thus adopted.

2.6 *In vitro* evaluation methods adopted for urological implant coating technologies

In the assessment of novel urological coating technologies, a combination of standardized and non-standardized evaluation methods is typically employed. These methods serve to determine the efficacy of these technologies in fulfilling their intended purpose and ensuring adequate biocompatibility. Specifically, for urological coated biomaterials designed to exhibit a low friction coefficient with urinary tract tissues and possess antibacterial properties sufficient to prevent uroepithelial damage and UTIs, relevant evaluation methods are employed. These methods mainly evaluate lubricity, biocompatibility, and UTI prevention (Wagner et al., 2020).

2.6.1 The lubricity evaluation of hydrogel-coated urological biomaterial

The friction coefficient of a biomaterial is a measure of the degree of resistance to sliding that the material offers when it contacts another surface. While lubricity refers to the ability of a surface to reduce friction and allow for smooth movement (Chang et al., 2001). Therefore, a highly lubricous biomaterial will have a low friction coefficient. Therefore, factors influencing lubricity including slipperiness and the swelling behaviour of hydrogel coating technology are generally evaluated.

The friction properties of biomaterials have historically been studied using drag-type devices. To quantitatively characterize the lubricity of catheter surfaces, a non-standardized evaluation method first described by Marmieri has been adopted. This method is based on the principle

that when two objects of the same mass and surface area are subjected to the same applied force and slide over different surfaces, the time taken to cover the same distance can reflect the friction coefficient of each surface. If one object takes longer to slide the same distance, it suggests a higher friction coefficient, as the object is experiencing greater resistance. On the other hand, if an object slides faster, it indicates a lower coefficient of friction, as there is less resistance to its motion (Marmieri et al., 1996,Tunney and Gorman., 2002).

Hydrogels are characterised by their ability to absorb hydrofluids. When absorption occurs, they will increase in size and demonstrate swelling behaviour. This results in the formation of a thin water film on the contacting surface, which improves surface smoothness and produces lubricity (Lawrence and Turner., 2005). Therefore, the swelling effect of hydrogel is associated with lubricity. The swelling effect of the coating technology was evaluated to confirm the expected hydrogel swelling behaviour.

2.6.2 The biocompatibility evaluation of urological biomaterial

Non-standardized evaluation methods, such as the brine shrimp lethality assay (BSLA), are commonly used in the preliminary developmental stages of medical products. However, standardized methods are more widely adopted for critical assessments. For example, the ISO 10993 series is the most widely used standard to demonstrate compatibility in the medical device industry, offering a selection of in vitro evaluation methods and guidelines for sample preparation. In investigating the biocompatibility of urological biomaterials, standardized MTT and XTT cytotoxicity assays are frequently employed (Podgórski et al., 2022). For genotoxicity, the Ames mutagenicity assay has been historically adopted (Wagner et al., 2020).

The BSLA was first introduced in 1956 and is a low-cost simple preliminary benchtop test employed to evaluate the cytotoxic effects of bioactive compounds (Michael et al., 1956). It involves the exposure of several larvae (*nauplii*) to the test sample for more than 24 h. The *nauplii* are large enough about 10 mm long, to observe with the naked eye but small enough for handling in multi-well plates (Michael et al., 1956). Due to its simplicity, numerous studies have also relied on it. In most cases, the testing parameters including sample size, type of media used, positive control and incubation conditions are often adjusted for optimisation. These adjustments thus further complicate the adoption of the assay as a reputable standard. However, the genetic variation of brine shrimps is probably responsible for the majority of challenges associated with it. Therefore, this test does not produce the most accurate results. However, a correlation between BLSA and other more precise toxicity evaluation methods including

cytotoxic assays has been proven. Thus, the assay has retained its relevance and is adopted on a preliminary basis. Several studies have observed a positive correlation between BSLA and cytotoxic studies. The BSLA showed consistent toxicity results when 1000 µg/ml of samples was assessed with two human cell lines: lung carcinoma (A-549), and colon carcinoma (HT-29) (Carballo et al., 2002).

In vitro cytotoxicity assays including the MTT and XTT are unusually the first step in evaluating any potential toxic effects of novel technologies before animal studies. These methods are similar since they involve the preparation of their respect reagents in physiologically balanced solutions which are introduced to cell culture pre-exposed to the sample and measuring the absorbance using a plate reading spectrophotometer. The quantity of formazan product formed represents the number of viable cells (Mosmann., 1983). In addition, they are both recommended by ISO 10993.

The MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) tetrazolium reduction assay was the first homogeneous cell viability assay developed for a 96-well format suitable for high throughput screening. During the assay, only metabolically active viable cells convert MTT into a purple-coloured formazan product which has an absorbance maximum close to 570 nm. The purple colour formation is thus used as a positive marker for viable cells. The cellular mechanism of MTT reduction into formazan involves the reaction of MTT with NADH or similar reducing molecules. Furthermore, it is been speculated that MTT measures mitochondrial activity which is directly proportional to metabolic active viable cells. The formazan product will accumulate as an insoluble precipitate both intracellular and extracellular and thus must be solubilised by homogenisation with a suitable solvent to allow absorbance reading. Solvents that have been used to solubilise formazan product precipitate include acidified isopropanol, dimethyl formamide, and a combination of detergent and an organic solvent (Riss et al., 2016). While the XTT (2, 3-bis-(2-methoxy-4-nitro-5 sulfophenyl)-2H-tetrazolium-5- carboxanilide assay is an improved version of the MTT since it still relies on colourimetric measures to quantify cellular growth. However, unlike the latter, the XTT reagent is reduced to water-soluble orange formazan. Therefore, extra regents are not required to dissolve the formazan. This feature also means that this assay is more accurate with the elimination of undissolved formazan. The formazan resulting from the XTT has a maximum absorbance of around 450 nm.

Mutagenicity assays determine the probability of a substance's capacity to cause mutation. They depend on the ability to quantify mutagenic changes caused by exposure to the substance in question under defined parameters to determine the level of mutagenicity. The Ames mutagenicity assay is an *In vitro* mutagenicity evaluation procedure developed back in the 1970s by Dr Bruce Ames. It involves the culturing of defective bacterial strains that have lost the ability to take up and metabolise specific nutrients necessary for growth. The defective bacterial strain is exposed to the sample and grown in solid media. The solid media is composed of specific nutrients which only allow the growth of revertants, and bacterial cells that have recovered their normal metabolic function. It assumed that exposure of defective bacterial strains to the sample leads to reversion mutation thus recovery of normal function. Therefore, the growth of revertant bacterial cells is assumed to be dependent on the mutagenic level of the sample and thus reflects mutagenicity (Dorothy and Bruce., 1983). For samples that are ingested by humans, the incorporation of a mammalian exogenous activation system in conjunction with the sample and testers strains. This is done to enable the detection of substances that become mutagenic post-metabolism in the liver.

The most frequently used strains including *Salmonella typhimurium* strains are TA97, TA98, TA100, TA102, TA104, TA1535, and TA1537. *E. coli* strains include WP2uvrA & pKM101. They all are defective bacterial strains that possess mutant genes that prevent the synthesis of the essential amino acids, histidine and tryptophan in particular. However, each strain reflects a particular type of mutation. For instance, T100 and TA1537 reflect base pair mutagenic changes while T97 and T98 reflect frameshift. T100 which was used in this study specifically detects mutation in the HisG46 gene. Rat liver enzymes or hamster S9 microsomal fractions are generally used to promote the metabolic conversion of the sample (Barbezan et al., 2017).

Ames multi-well format (AMF), Ames II™ and the Ames muta-chrome mutagenicity assay are recent modifications of the traditional test (Rao and Lifshitz., 1995). They involve growing the tester strain in multi-well plates using liquid media that consist of the sample, specific nutrients and a PH indicator. The plates are incubated for more than 48 h before positive revertant wells are scored against a negative control, the background plate.

2.6.3 The antimicrobial activity evaluation of urological biomaterial

Several in-vitro standardised evaluation methods that are commonly used for antimicrobial analysis include ISO 22196, disk diffusion and the dilution method. These methods rely on determining and quantifying a microbe’s susceptibility to a particular agent. The disk diffusion

and broth microdilution assays are one of the most adopted methods for quality purposes and thus have more information available regarding them. This enables better comparison among previous studies. The disk diffusion and the dilution methods have also been published by numerous standardization organizations including ISO, EUCAST and CSI. In addition, EUCAST and CSI also regularly release the MIC of standard cultures against standard antimicrobial agents.

ISO 22196 is an *In vitro* antimicrobial efficacy test used to evaluate the effectiveness of antimicrobial coatings on non-porous surfaces. The test method involves exposing a tester strain in a suspension with diluted media to the surface of the sample. The surface is incubated under controlled conditions to allow for microbial growth. Post-incubation, the number of viable bacteria present is quantified. This method is also commonly adopted for coatings, where a comparison is made between the coated and uncoated samples. By comparing the bacterial counts on the coated and uncoated samples, the efficacy of the coating in preventing microbial growth can be determined (Yamaguchi et al., 2021).

The broth dilution test is the oldest antimicrobial susceptibility testing method. It involves the preparation of a series of dilutions of an antimicrobial agent in a liquid growth medium. The antimicrobial agent-containing media are inoculated with a standardised bacterial suspension. Post incubation, the tubes are examined for turbidity to determine bacterial growth. The lowest concentration of the antimicrobial agent that prevented growth represented the minimal inhibitory concentration (MIC) (Jorgensen and Turnidge., 2015). The broth microdilution method is an updated standardised version of the traditional broth dilution method. As the name suggests, it is conducted in small amounts, usually a total volume of less than 200 µl consisting of the agent being tested, media and the tester strain per U-shaped bottom well. A microorganism that is not susceptible to the concentration of the agent being tested will thus be able to grow. Microbes growing in the sample will form a pellet at the bottom of the well due to the gravitational pull. The limited amount of nutrition also means the microbe completes its growth curve faster facilitating the formation of the pellet as cells reach their dead phase sooner. Therefore, the presence or absence of the pellet indicates the tested microbe's susceptibility to the agent.

The disk diffusion assay also called the Kirby–Bauer test is used to assess the antimicrobial effectiveness of an agent or the susceptibility of a particular microbial culture to an agent. The method was developed by W. Kirby and A. Bauer back in the 1960s hence the name and has been widely adopted by most laboratories. It involves growing bacteria on solid media and then

placing disks usually made from paper containing the agent to be tested. Ideally, the agent will diffuse from the paper disk into the surrounding agar and if the agent is effective against the growth of the tester microbe, it forms a circular zone of growth inhibition. The effectiveness of the agent against the microbe is positively correlated to the size of the zone of inhibition (Jorgensen and Turnidge., 2015).

Clinical studies have indicated that bacteria can initiate a UTI by migrating from the surrounding environment. Bacteria from the urethral meatus-catheter junction migrating along the external surface of the catheter into the urinary bladder is such a case (Stamm., 1991). Thus, the quality of the catheter's external surface to aid or restrict bacterial migration will facilitate or inhibit bacterial infection. The bridge model microbial migration assay is used to investigate microbial migration over a solid surface. The test involves creating a slit on solid agar, forming two separate islands and placing the sample over them to act as a bridge. Only one side of the bridge is inoculated with the tester microbe. Growth in the inoculated part post-incubation indicates microbial migration on samples (Sabbuba et al., 2002). This method has been used to evaluate the antimicrobial properties of implant fabricating material. Suitable microbial strains with a proven ability to migrate on solid surfaces must be selected for this test. This includes *Proteus mirabilis* and *E. coli* (43888).

2.7 The potential of a polydopamine/polyvinyl pyrrolidone and iodine-loaded coating technology to improve urological biomaterial

As highlighted in the literature review, the use of urological devices is still associated with uroepithelial damage and UTIs. Current strategies employed to mitigate these issues are still lacking. Recently, a PVP coating technology was developed for biomaterials and observed to reduce their friction coefficient. However, this technology was limited since it is poorly adaptable to biomaterial with lower surface polarity. Therefore, this study put forward the use of a double-layered coating technology consisting of a primer, and a hydrogel loaded with iodine to improve biomaterials used for fabricating urological implants (Figure 2.6). The primer functions to widen the adaptability of the technology, the hydrogel improves biocompatibility by reducing the friction coefficient between the surface of the implants and uroepithelial tissues and the iodine serves to mitigate UTIs.

For mitigating UTIs, other studies have investigated the use of numerous antimicrobial agents for urological biomaterial coating technologies. In this study, iodine was chosen due to its reported broad spectrum and efficacy. However, it should be noted that iodine cytotoxicity has

mostly been demonstrated in in-vitro studies at higher concentrations. Therefore, the use of iodine at lower concentrations most likely does not cause toxicity. Additionally, since the iodine is loaded to PVP, it may be further released in limited quantities over a longer period, which has been shown to be more effective compared to direct exposure which tends to be more vulnerable to the flush cycle of the urinary tract system.

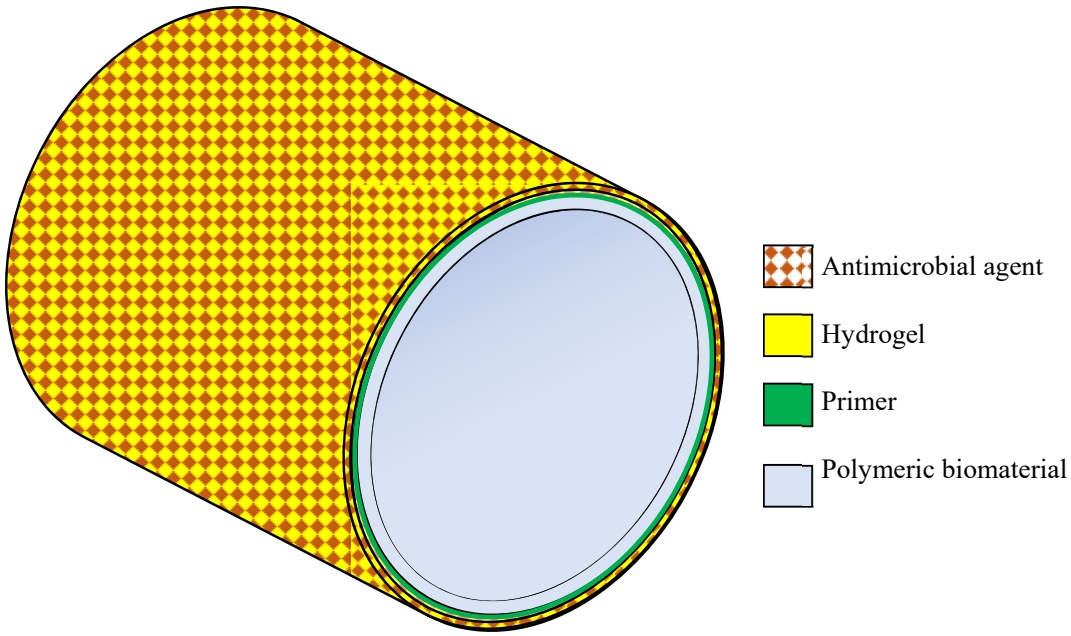


Figure 2.6: A urological implant polymer tube coated with a primer and hydrogel loaded with an antimicrobial agent.

2.8 The scope of this study

In this study, a coating technology was produced with a primer (PDA), and a hydrogel (PVP) loaded with varying concentrations of iodine (0.1,0.5 and 1%) as an antimicrobial agent. The coating technology was applied to two different biomaterials commonly used for fabricating urological implants (PU and PDMS). The capacity of the hydrogel top layer to introduce lubricity when hydrated was confirmed by evaluating its swelling behaviour and slipperiness. To confirm the safety of the coating technology, its biocompatibility was evaluated by using BSLA and in-vitro methods as recommended by ISO 10993 to assess cytotoxicity and genotoxicity. The XTT assay was used to evaluate cytotoxicity while the Ames assay was used to determine genotoxicity. To evaluate antimicrobial activity, assays including ISO 22196 and ECUAST standards (disk diffusion and broth microdilutions) were used. In addition, the urinary

catheter bridge microbial migration assay was used to evaluate the coating technology's ability to inhibit microbial migration, a common occurrence during the use of partially implanted catheters exposed to the outside environment. Figure 2.7 shows the full scope of this study.

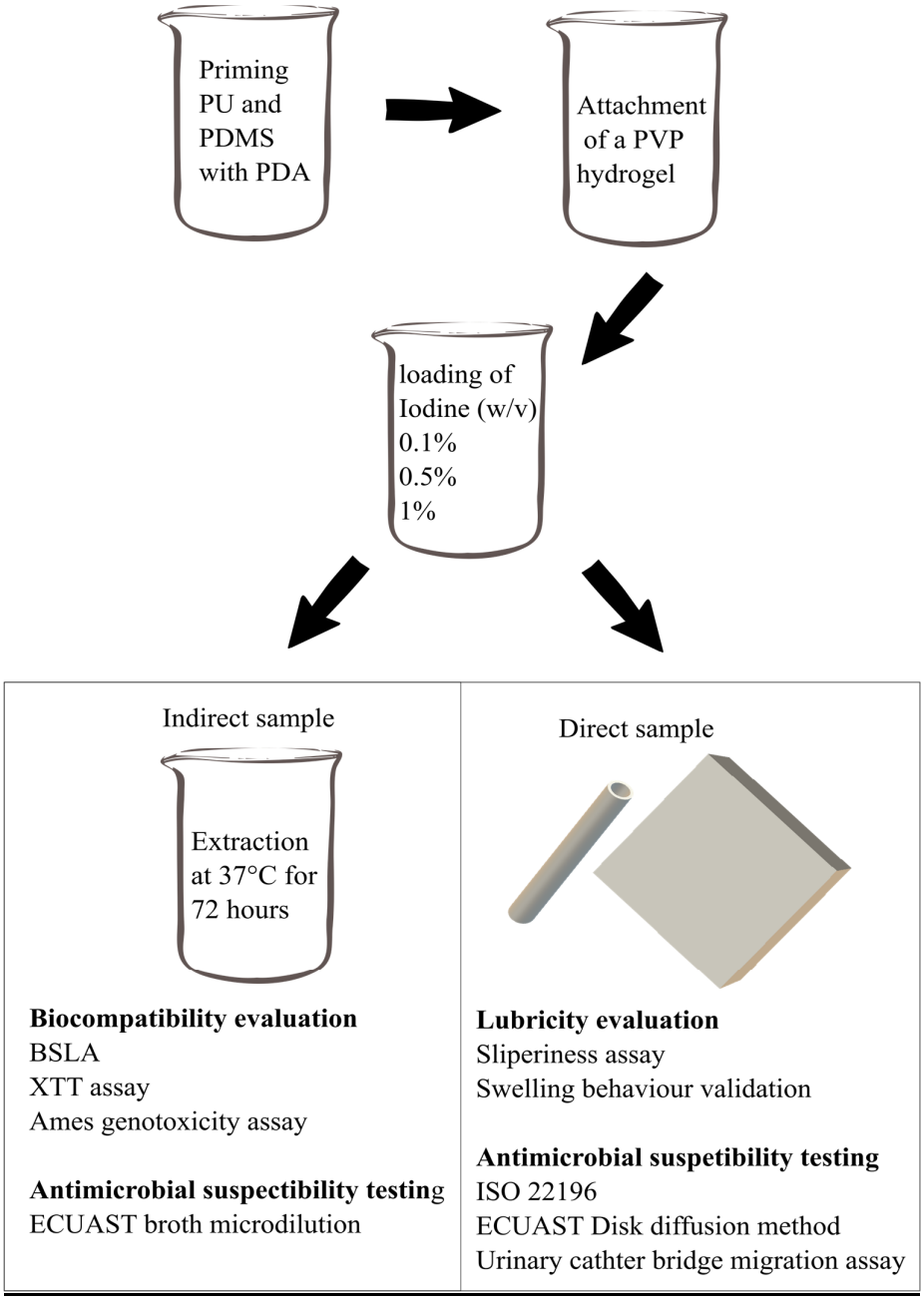


Figure 2.7: Flow diagram describing the scope of this study.

CHAPTER 3: MATERIALS AND METHODS

3.1 Materials

The polymeric biomaterials, polyurethane (PU) and polydimethylsiloxane (PDMS) and the reagents for fabrication of the coating were sourced from Dow Chemical Company, USA and Thermo Fisher Scientific, Poland, respectively. The brine shrimp eggs were sourced from a Berea pet shop in Durban, South Africa. The fibroblasts used for cytotoxic assays were sourced from American Type Culture Collection and the reagents for the assays were purchased from Thermo Fisher Scientific, Poland. The culture and reagents for the Ames mutagenic assay were purchased from Environmental Bio-detection Products Incorporated, Canada. The *E. coli* cultures for conducting the antimicrobial susceptibility testing (AST) were sourced from the American Type Culture Collection and the *P. mirabilis* was obtained from a culture collection at the Department of Biotechnology and Food Science, Durban University of Technology, South Africa. The media and other associated reagents were sourced from Thermo Fisher Scientific, South Africa.

All other chemicals were purchased from Merck, South Africa.

3.2 Fabrication of polydopamine/polyvinyl pyrrolidone-coated and iodine-loaded biomaterials

The coating of the biomaterial samples was carried out in three steps, which include primer attachment, hydrogel attachment and antimicrobial agent loading. There were intervals of 20 ± 2 h between each step to give the biomaterial samples enough time to dry. All the steps were carried out under aseptic conditions

Urological biomaterials (PU and PDA) were cut into samples of manageable sizes including sheets (50×50 mm), disks (10 mm diameter) and tubes (100 mm height). All the biomaterial samples were then washed in deionized water and dried at room temperature for 15 min. The biomaterial samples were then dipped in a vessel with absolute acetone and swirled for 10 s before being removed and dried again at room temperature for 10 min.

3.2.1 Priming of polymeric biomaterials (polyurethane and polydimethylsiloxane) by attachment of polydopamine

A 2 mg/ml dopamine hydrochloride solution was prepared with 1 g of dopamine hydrochloride (99% w/w) in 500 ml sodium acetate buffer (10 mM; pH 5.5). The dopamine/buffer solution

was mixed with sodium periodate 99% v/v in a ratio of 1:1. The mixture was added to a vessel containing the prepared biomaterial samples (PU and PDMS), thoroughly shaken to mix and left standing for 30 min. The biomaterial samples were then removed from the vessels, washed thoroughly with deionised water and dried overnight at room temperature (Kopeć et al., 2020).

3.2.2 Attachment of a polyvinyl pyrrolidone hydrogel to polydopamine-coated biomaterials

The addition of the PVP hydrogel was carried out in a two-step dipping process as shown in Figure 3.1. The PVP attachment process involved submerging the PDA-coated biomaterials first in a nonpolar, organic solution containing 5% v/v cumene hydroperoxide (CHP) and 5% v/v ethylene glycol dimethyl acrylate (EGDMA) in absolute hexane for 5 s followed by air-drying in a fume hood at room temperature. In the second step, the PDA-coated biomaterials were then submerged in a polar solution consisting of 5% w/v PVP 360 kDa, 0.05% $FeCl_2$ w/v and 0.1% w/v ascorbic acid (AA) in deionised water for 15 min before being rigorously washed using running distilled water to remove unattached hydrogel. The PDA/PVP coated samples were then placed in a beaker containing 96% ethanol for 1 min before rinsing with water.

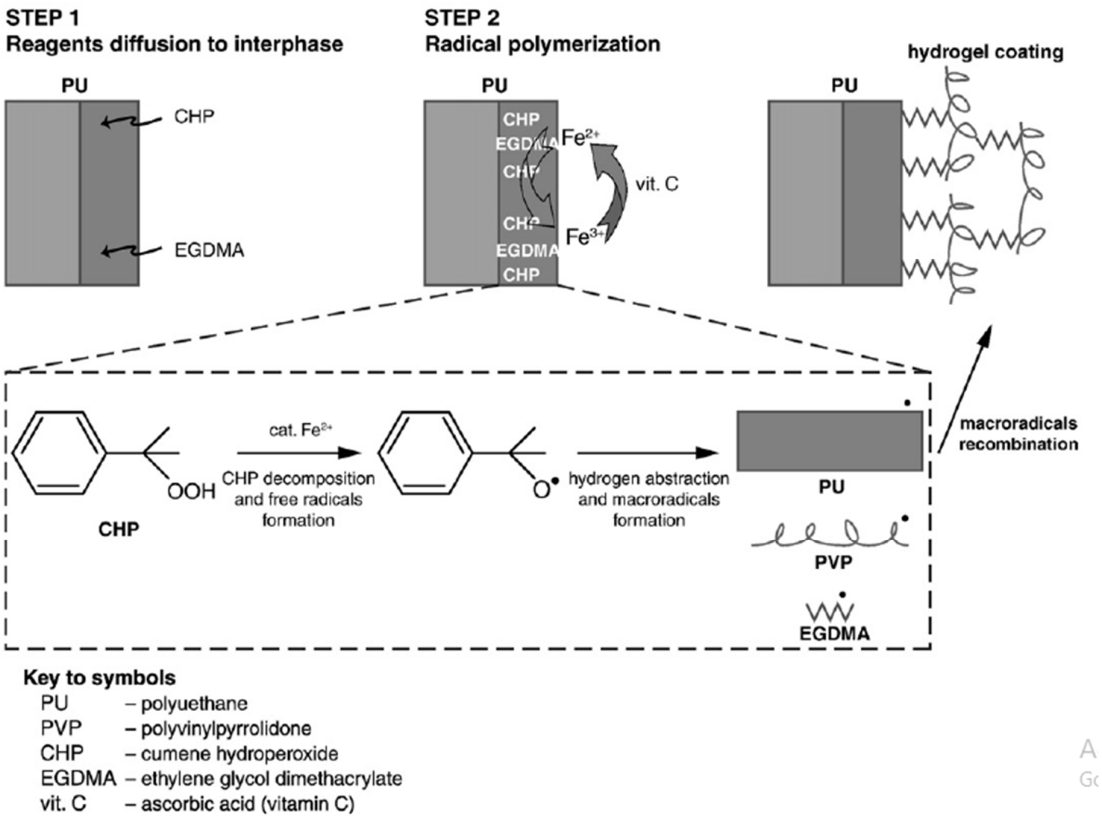


Figure 3.1: Flow diagram describing the binding of a polyvinyl pyrrolidone hydrogel to polymeric biomaterial (Butruk et al., 2012).

3.2.3 Loading antimicrobial agent, iodine, to polydopamine/polyvinyl pyrrolidone-coated biomaterials

The addition of iodine was carried out under sterile conditions at room temperature. Three iodine solutions at different concentrations (0.1%, 0.5% and 1% w/v) were prepared in absolute ethanol. The PDA/PVP-coated samples were then divided into four sets. Three sets were assigned and submerged in their respective iodine solution (for 30 s) and the fourth set was submerged in absolute ethanol. The produced PDA/PVP-coated biomaterial samples (Table 3.1) with and without iodine were dried under a biosafety cabinet

Table 3.1: Biomaterial samples produced with the polydopamine/polyvinyl pyrrolidone coating technology and loaded with iodine

Type of biomaterial	Polyurethane	Polydimethylsiloxane
Name of sample	PU-PDA/PVP	PDMS-PDA/PVP
	PU-PDA/PVP (0.1% I_2)	PDMS-PDA/PVP (0.1% I_2)
	PU-PDA/PVP (0.5% I_2)	PDMS-PDA/PVP (0.5% I_2)
	PU-PDA/PVP (1% I_2)	PDMS-PDA/PVP (1% I_2)

PU-polyurethane, PDMS-polydimethylsiloxane, PDA-polydopamine, PVP-polyvinyl pyrrolidone and I_2-iodine

3.3 Evaluation of the lubricity of polydopamine/polyvinyl pyrrolidone-coated biomaterials

Building upon the fabrication process detailed earlier, the assessment of coating lubricity was conducted through two key methodologies: biomaterial slipperiness and swelling behaviour analysis.

3.3.1 Evaluation of slipperiness of the polydopamine/polyvinyl pyrrolidone-coated biomaterials with a catheter slipperiness model

A coated piece of catheter tube with a length of 100 mm and diameter of 16.7 mm was used as a sample for this test. One end of the sample was immersed 70 mm deep into de-ionised water and hydrated for 10 min. The other end was attached to a string. The hydrated section of the sample was inserted parallel to the long axis of a McCartney bottle containing 1% w/v agar. After 10 min, the sample was pulled out of the agar using a nylon thread attached to a 20 g weight. The time taken to pull the sample out of the agar was subsequently recorded as the experimentally measurable variable; the longer the time, the greater the friction at the sample/agar interface (Marmieri et al., 1996,Tunney and Gorman., 2002).

3.3.2 Evaluation of swelling behaviour of polydopamine/polyvinyl pyrrolidone-coated biomaterials

Coated biomaterial samples were dried overnight by placement in an incubator at 30°C before weighing them on an analytic balance (Excellence XPE205DR/M, Mettler Toledo, Columbus, OH, USA). This was then taken as their initial mass before being placed in excess amounts of phosphate-buffered saline (PBS) at room temperature for a minute. Then excess unabsorbed PBS was removed from the samples by dabbing on filter paper before weighing the samples

again and this was taken as mass at 1 minute. After weighing, the samples were again exposed to excess amounts of PBS for another 1 minute and then the process was repeated until no changes were observed in the mass gained by the hydrated PDA/PVP-coated biomaterial samples (Butruk et al., 2012).

3.4 The biocompatibility evaluation of polydopamine/polyvinyl pyrrolidone-coated and iodine-loaded biomaterials

The safety profile and compatibility of the coated biomaterials with biological systems was evaluated utilizing the brine shrimp lethality assay, XTT cytotoxicity assay, and Ames multi-well format mutagenicity assay. Each assay provided unique insights into the biomaterials' potential toxicity, cytotoxicity, and mutagenicity, respectively. Biomaterial extracts were prepared using standardized methodologies to ensure consistency and reproducibility across experiments. These extracts served as the test samples in each assay, enabling the assessment of their impact on a living organism, mammalian cell culture, and bacterial strain. Integration of these diverse assays into the evaluation framework, facilitated a holistic understanding of the biocompatibility of the polydopamine/polyvinyl pyrrolidone-coated biomaterials loaded with iodine.

3.4.1 The biocompatibility evaluation of polydopamine/polyvinyl pyrrolidone-coated and iodine-loaded biomaterials with the brine shrimp lethality assay

For the Brine shrimp lethality assay, it was carried out with *Artemia salinai* as a test organism to assess the impact of the polydopamine/polyvinyl pyrrolidone-coated biomaterials a living organism.

Artificial seawater was prepared by mixing 28.15 g of sodium chloride (NaCl), 0.67 g of potassium chloride (KCl), 5.51 g of magnesium chloride hexahydrate ($MaCl_2.6H_2O$), 1.45 g of calcium chloride dihydrate ($CaCl_2.2H_2O$) and 6.92 g of magnesium sulphate heptahydrate ($MgSO_4.7H_2O$) in 1000 ml deionised water (Xu et al., 2011). Extract samples were prepared by exposing each coated sheet (50×50 mm) with 13 ml ASW in a petri dish for 72 h at 37°C, then the extracts were topped up with ASW to a volume of 16 ml and sterilized using a 0.22 μm membrane filter (Heise et al., 2022).

Cysts of *Artemia salina* (200 mg) were allowed to hatch in 200 ml ASW in a semi-closed system. This was done to avoid the interference of contaminants. The system was incubated for

48 h in a water bath at 30°C under continuous illumination (100 watts light bulb) and good aeration (peristaltic pump), refer to Figure 3.2 A (Ismail et al., 2018).

The freshly hatched nauplii were then separated into a petri dish with a dropper under a laminar flow. A volume of 5 ml of each sample extract and the controls were transferred into separate wells of a 12-well plate. Artificial seawater was used as the negative control while potassium dichromate ($K_2Cr_2O_7$ 100 µg/ml) was used as the positive control. Then 10 nauplii were carefully transferred from the petri dish into each well using a pipette. The plates were then incubated for 48 h at 30°C under continuous illumination (Figure 3.2 B). The samples were tested in triplicates and viewed at 24 and 48 h. Nauplii that did not show any movement within 30 s were designated as dead (Ismail et al., 2018). The toxicity of the coating was expressed using the following formula:

% Lethality = ((A-B) ×100)/A **Equation 1**

Were

A- number of live nauplii in the negative control

B- number of alive nauplii in the sample

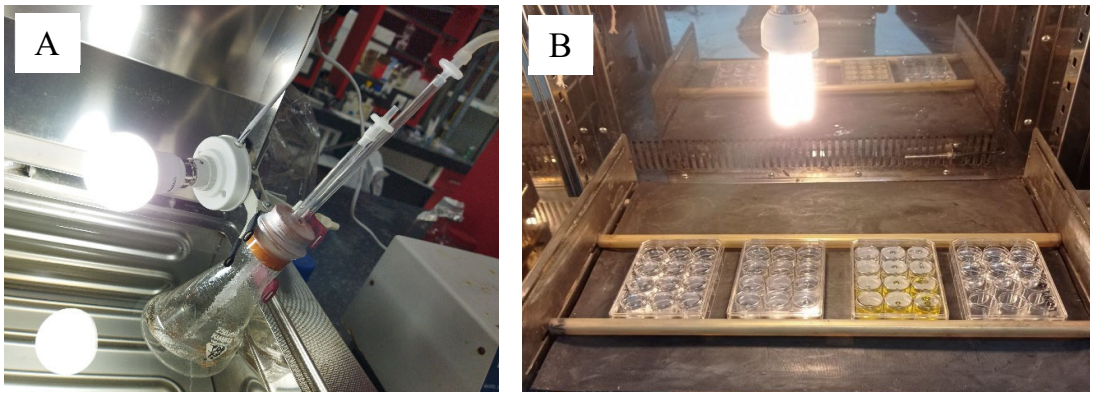


Figure 3.2: A) Apparatus for hatching of *Artemia salina* cyst and B) incubation during the brine shrimp lethality assay.

The data obtained from the BSLA was processed with Microsoft Excel 2016 and IBM SPSS Statistics (ver. 19). The data were checked for normal distribution based on skewness and kurtosis before the T-Test was used to compare each mean of samples to the negative control to determine whether or not the difference is significant at $p<0.05$.

3.4.2 The biocompatibility evaluation of polydopamine/polyvinyl pyrrolidone-coated and iodine-loaded biomaterials with the XTT cytotoxicity assay

Fibroblast cells grown under in-vitro conditions were exposed to the extracts of the PDA/PVP-coated biomaterials loaded with varying concentrations of iodine. Any negative changes in viability compared to the negative control were thus credited to the sample. Each variant concentration of the iodine sample was tested in 6 replicates to improve the reliability of the results.

A sterile medium mixture was prepared by adding 450 ml of Dulbecco's modified Eagle medium (DMEM), 50 ml of sterile foetal bovine serum (FBS), 500 U of sterile penicillin, and 500 µg sterile streptomycin under aseptic conditions (Podgórski et al., 2022). Extract samples were prepared by exposing each 50×50 mm coated sheet with 13 ml medium mixture in a petri dish for 72 h at 37°C, then the extracts were topped up with the medium mixture to a volume of 16 ml (Heise et al., 2022).

L929 (ATCC CCL-1) cells stored in a vial were recovered from storage and thawed in a water bath at 37ºC for 2 min. The contents were then transferred to a T-75 flask with a 10 ml of the previously prepared medium mixture (see previous subsection). The flask was kept at standard cell culture conditions (37ºC in an incubator with 5% CO_2). After 24 h, the cells were viewed under a microscope to confirm adherence, then the media was changed by tilting the flask sideways and using a pipette to exchange with a fresh medium mixture. The flask was then incubated again under the standard cell culture conditions for 48 h and monitored under the microscope every 24 h until a near 100% confluent was observed. The media was then removed and PBS at 37ºC was used to wash the cells twice by using a pipette to add and remove 4 ml of the PBS each time. Thereafter, 2 ml of 0.25% Trypsin/EDTA solution was introduced and the cell culture was incubated for 2 min under the standard cell culture conditions. After incubation, the culture was viewed under the microscope to confirm cell detachment. Subsequently, 4 ml of the medium mixture was added to stop the enzymatic activity of the trypsin-EDTA solution. The complete cell culture suspension was then transferred using a pipette to a 15 ml tube and centrifuged at 276.705 x g for 5 min. The liquid was then carefully removed from the tube without disturbing the cells' pellet. Thereafter, 10 ml of the medium mixture was introduced and mixed with the pellet using a pipette before transferring them to a T-75 flask and incubating them at the set standard cell culture conditions.

For the XTT viability assay, 100 µl of DMEM, without phenol red and supplementation, and 70 µl of XTT with electron-coupling reagent solution were added to each culture well and incubated for 4 h. When XTT was reduced to formazan pigment by living cells, the 100 µl of assay medium from each well was transferred to a new 96-well plate, and the absorbance was measured at 475 nm. In all cases, the relative cell viability was defined as the ratio of absorbance of the sample to the absorbance of the negative control as shown in Equation 2, and represented as a mean ± standard deviation (Podgórski et al., 2022).

Cell viability was calculated using the following formula:

Viability % =S/ N×100 **Equation 2**

Were

N - Negative control absorbance

S - Normalised absorbance of the sample

3.4.3 The biocompatibility evaluation of polydopamine/polyvinyl pyrrolidone-coated and iodine-loaded biomaterials with the Ames muta chrome mutagenicity assay

Extract samples were prepared by exposing each 50×50 mm coated sheet with 13 ml deionized water in a petri dish for 72 h at 37°C, then the extracts were topped up with deionised water to a volume of 16.0 ml and sterilized using a 0.22 µm membrane filter (Heise et al., 2022).

The Ames assay was conducted using *Salmonella typhimurium* TA 100 as test organism. The inoculum was prepared by culturing lyophilized TA 100 cells overnight in 5 ml nutrient broth at 37°C to a population of 0.5 McFarland standard.

Davis-Mingioli salt solution was prepared by mixing 38.5 g of potassium sulphate (K_2SO_4), 11 g of monopotassium phosphate (KH_2PO_4), 5.5 g of ammonium sulphate [$(NH_4)_2SO_4$], 1.375 g of trisodium citrate ($Na_3C_6H_5O_7$), and 0.55 g of $MgSO_4.7H_2O$ in 1000 ml deionised water. A D-glucose solution was prepared by mixing 40 g of D-glucose was mixed with 100 ml; a bromocresol purple solution was prepared by mixing 0.2 g of bromocresol purple with 100 ml deionised water; a D-biotin solution was prepared by mixing 0.01g of D-biotin with 100 ml deionised water, while an L-histidine solution was prepared by mixing 0.1 g L-histidine with 100 ml deionised water. All the solutions were sterilized by autoclaving at 121°C for 15 min.

A reaction mixture was prepared by mixing Davis-Mingioli salts solution 71.02 ml, D-glucose solution 15.6 ml, bromocresol purple solution 7.82 ml, D-biotin solution 3.91 % v/v and L-

histidine solution 1.64% v/v in a test tube. Then 8.75 ml of each sample extract, 1.3 ml of the reaction mixture, and 5 µl of T100 inoculum at more than 0.5 McFarland standard were transferred to a test tube and mixed using a vortex mixer. Thereafter, 200 µl were pipetted from the test tube to each well in a 96-well plate, and 48 wells were filled per sample. Negative control was prepared by putting water instead of an extract sample and sodium azide solution was used as a positive control. The plates were incubated at 37°C for 96 h. After incubation, the plates were viewed to determine reverent wells. A baseline was formulated by adding the mean of the negative control and negative control standard deviation. Table 3.1 shows the criteria used to determine mutagenicity toxicity (Rao and Lifshitz., 1995, Shafaei et al., 2015, Fateh et al., 2018).

Table 3.2: Evaluation criteria for Ames multi-well format mutagenicity assay

No. of wells >2× baseline	No. of wells >4× baseline	Compound label
0	0	Negative
1	0	Negative
0	1	EQ/possibly positive
1	+1, not adjacent	EQ/possibly positive
1	+1 adjacent	Positive
2, adjacent	0	Weak positive
2, adjacent	>0, any	Positive
2, non-adjacent	0	EQ
2, non-adjacent	non-adjacent	Weak positive
2, any	>0, adjacent	Positive
3+, any	0	Weak positive
3+, any	>0, any	Positive

EQ, equivocal, possibly positive if >4× baseline at the highest concentration (Rao and Lifshitz 1995)

3.5 The antimicrobial activity evaluation of polydopamine/polyvinyl pyrrolidone-coated and iodine-loaded biomaterials

The antimicrobial properties of the polydopamine/polyvinyl pyrrolidone-coated and iodine-loaded biomaterials were assessed with standardized assays to determine their effectiveness against bacterial growth indicative of a UTI. These include the 22196 assay, broth microdilution assay, disk diffusion assay, and the catheter bridge model microbial migration method.

3.5.1 The antimicrobial activity evaluation of polydopamine/polyvinyl pyrrolidone-coated and iodine-loaded with the ISOI 22196 assay

Sterile PDA/PVP-coated biomaterials samples loaded with varying iodine concentrations (0.1, 0.5, and 1 % w/v with a surface area of 50×50 mm were produced as previously described in 3.1.5.

An *E. coli* (ATCC 25922) inoculum suspension of ± 6 $\times 10^5$ cells/ml was prepared from a fresh culture grown overnight on nutrient agar with 1/500 nutrient broth.

An inoculum of 0.4 ml was aseptically suspended on top of each hydrated biomaterial sample and then covered with 40×40 mm sterile sheets of parafilm (sterilised by submersion in 70% ethanol) to prevent evaporation**.** The suspension was then recovered in the 1st batch of controls but the rest of the samples including the 2nd batch of uncoated samples were incubated for a period of 24 h at 36°C. Recovery was then carried out by transferring 10 ml of maximum recovery diluent (Thermo Fisher Scientific, South Africa) into each sample and gently mixing for 2 min. The recovered cells were serially diluted in saline phosphate buffer and pour-plated on plate count agar. The plates were then incubated for 24 h at 36°C. After incubation, the colonies were counted to determine the number of viable bacteria recovered per sample. The log of recovered CFU was compared between the negative control and samples to determine antimicrobial activity.

The number of viable bacteria recovered for each sample was determined according to the following equation:

$$N = (100 \times CDV)\ A \qquad \text{Equation 3}$$

Where:

N is the number of viable bacteria recovered per cm^2 per test sample

C is the average plate count

D is the dilution factor for the plates count

V is the volume, in ml, of maximum recovery diluent, added to the sample

A is the surface area, in mm^2, of the cover film.

Then the log of recovered CFU was compared between the negative control and samples to determine antimicrobial activity. A log difference of more than two indicates antimicrobial activity. The following equation was used to determine if the sample showed antimicrobial activity.

Antibacterial activity = log (recovered CFU on uncoated samples after inoculation /recovered CFU in samples after 24 h incubation). Equation 4

3.5.2 The antimicrobial activity evaluation of polydopamine/polyvinyl pyrrolidone-coated and iodine-loaded with the broth microdilution assay

Extract samples were prepared by exposing each coated sheet (50×50 mm) with 13 ml of deionised water in a petri dish for 72 h at 37°C, then the extracts were topped up with deionised water to a volume of 16 ml and sterilized using a 0.22 µm membrane filter.

Inoculum suspensions of *E. coli* (ATCC 25922) and *Proteus mirabilis* at above 0.5 McFarland standard were prepared from fresh cultures grown overnight on nutrient agar with cation-adjusted Mueller-Hinton broth.

Dilutions were performed on the extracts of the samples and the positive control using deionised water. The diluted extracts were inoculated with the prepared inoculum to 0.5 MacFarland standard. A volume of 200 µl of each dilution was aseptically transferred to a well of a 96-well U-bottom plate, before incubation at 36°C for 20 h (Dydak et al., 2021). The absence of a bacterial pellet post-incubation at the bottom of the well was used as an indicator of growth inhibition (Figure 3.4).

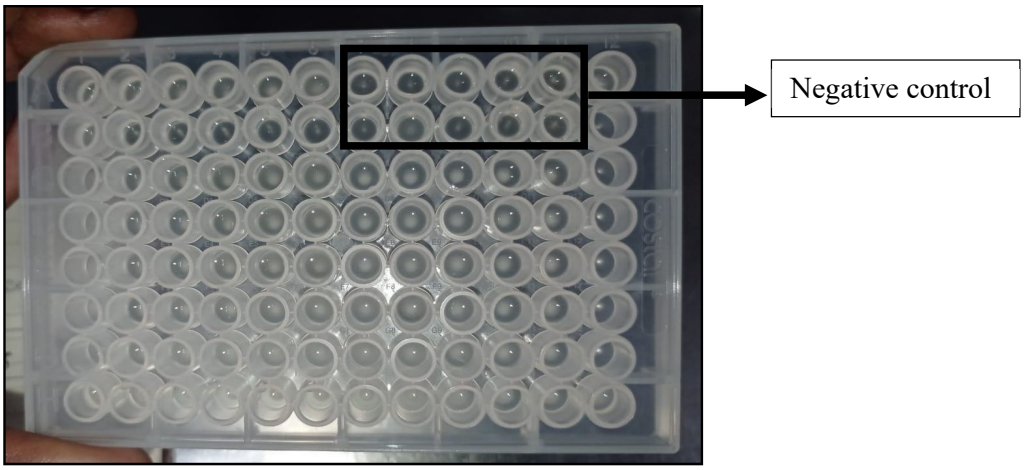


Figure 3.3: A U-shaped bottom 96 microplate to determine bacterial pallet growth inhibition.

3.6 The antimicrobial activity evaluation of polydopamine/polyvinyl pyrrolidone-coated and iodine-loaded materials with the disk diffusion assay

Sterile PDA/PVP-coated disks (10 mm diameter) samples loaded with varying iodine concentrations (0.1, 0.5, and 1 % w/v) were produced as previously described in 3.1.5.

Inoculum suspensions of *E. coli* (ATCC 25922) and *Proteus mirabilis* at 0.5 McFarland standard were prepared from fresh cultures grown overnight on nutrient agar with nutrient broth.

Test cultures were spread on Mueller-Hinton agar plates and disks (10 mm diameter) of the samples, negative control (plain disk) and positive control (5 µg ofloxacin) were aseptically placed on each plate. The plates were incubated for 20 h at 36°C. Post incubation, clear zones around the disks were measured and their size (mm) was used as a measure of antimicrobial activity (Dydak et al., 2021).

3.6.1 The antimicrobial migration activity evaluation of polydopamine/polyvinyl pyrrolidone-coated and iodine-loaded with the catheter bridge model method

Sterile PDA/PVP-coated catheter tubes (10 mm diameter) samples loaded with varying iodine concentrations (0.1, 0.5, and 1 % w/v) were produced as previously described in 3.1.5.

Inoculum suspensions of *E. coli* (ATCC 43888) at 0.5 McFarland standard were prepared from a fresh culture grown overnight on nutrient agar with nutrient broth.

The test involves creating a 1 cm slit on solid agar forming two separate parts and placing the test substance over the two-part to form a bridge as shown in Figure 3.5. Only one side of the

bridge is inoculated with a target microbe and growth in the un-inoculated part post-incubation indicates microbial migration on the test substance (Kazmierska et al., 2010).

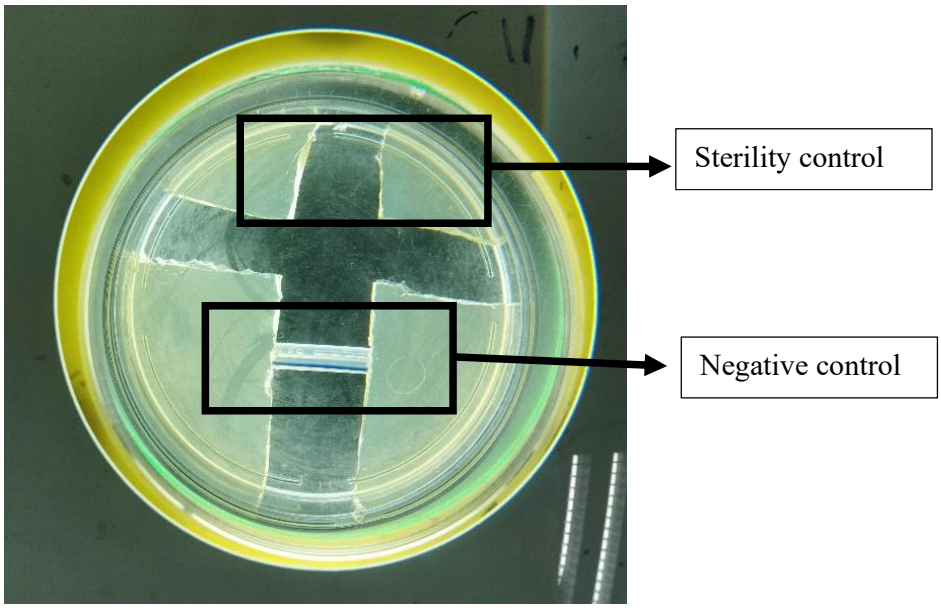


Figure 3.4: A catheter bridge model constructed with an uncoated polydimethylsiloxane urinary catheter tube sample.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Fabrication of polydopamine/polyvinyl pyrrolidone-coated biomaterials loaded

Dip-coating processes were used, including oxidation solubilisation and a free-radical macromolecular grafting method involving a Fenton-type reaction. The former was used to attach polydopamine (PDA) as a primer, while the latter was used for the additional attachment of polyvinyl pyrrolidone (PVP) as a hydrogel top layer, to urological biomaterials including polyurethane (PU) and polydimethylsiloxane (PDMS). In addition, iodine (I_2) was incorporated into the polydopamine/polyvinyl pyrrolidone-coated biomaterials as an antimicrobial agent by solvent evaporation. These methods are simpler than other surface modification methods that require sophisticated tools. For example, plasma treatment requires a laser-producing instrument and energy, which adds to the production cost and environmental burden associated with power generation. Therefore, the use of this coating technology in the production of urological implants may reduce production costs and be environmentally friendly compared to other priming processes. In addition, the oxidation solubilization PDA-coating process adopted in this study was quicker compared to those that use atmospheric oxygen as an oxidizing agent as described by Kopec. et al. (2019). The process of priming biomaterials (PU and PDMS) was completed in less than 1 h. The attachment of the PVP hydrogel by the free-radical macromolecular grafting-crosslinking method that involves a Fenton-type reaction ensured the formation of covalent bonds between PDA-coated biomaterials and the PVP, which most likely improved the stability of the coating technology, as previously described by Paradowska et al. (2010).

4.1.1 Attachment of polydopamine as a primer to polymeric biomaterials (polyurethane and polydimethylsiloxane)

After the addition of PDA, the biomaterial samples showed a noticeable colour change, from transparent to a dark greyish colour (Figure 4.1). This indicated that a PDA layer had been successfully attached to the biomaterial surfaces (PU and PDMS). The darkening of the PU biomaterial post the addition of PDA has also been observed in the study where PDA was coated on PU nanofibers using a similar coating mechanism (Kopeć et al., 2020). Since PU and PDMA are biomaterials with different surface polarities, this suggests that the PDA primer is adaptable to the different biomaterials.

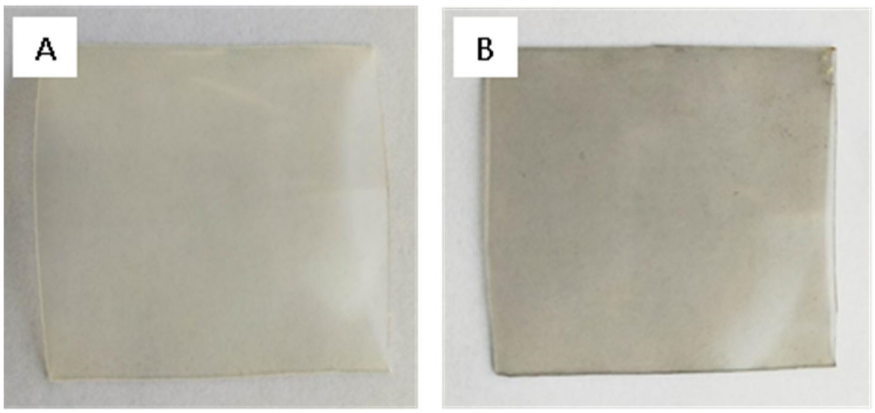


Figure 4.1: A) An uncoated polyurethane biomaterial sheet and B) A polyurethane biomaterial sheet coated with polydopamine.

The exact composition of the coating technology developed in this study has never been reported as a strategy to improve urological implants. However, several coating technologies developed with at least PDA as a primer or PVP as the hydrogel with and without an antimicrobial agent or agents have been conducted (Ryu et al., 2018). Coating technologies with a similar composition to the one developed in this study are at least consistent regarding some characteristics. A study by Jiang. et.al demonstrated that a PDA could be used to prime a hydrophobic polypropylene (PP) porous membrane before the application of a PVP hydrogel coat (2013).

4.1.2 Attachment of polyvinyl pyrrolidone as a hydrogel to polydopamine-coated polymeric biomaterials

The PU-PDA coated with PVP showed a visually translucent surface appearance (Figure 4.2). This translucent characteristic is consistent with observations made in other PVP hydrogels (Saha et al., 2019, Wagner et al., 2020). The translucent appearance is attributed to the refractive index of PVP, which measures 1.53 at 25°C (Slistan-Grijalva et al., 2008). This refractive index is close to that of water and enables the passage of light without significant scattering or absorption resulting in the observed translucency associated with PVP hydrogels.

The binding mechanism of action involves the attachment of cumene hydroperoxide (CHP) and ethylene glycol dimethacrylate (EGDMA) on the outer surface of PDA-coated biomaterials during the first dipping step. This was possible due to the formation of hydrogen bonds facilitated by −NH, −OOH and −CO groups in all three molecules (PDA, CHP and EGDMA). In the second dipping step, ferrous ion (Fe^{2+}) in the water solution meets with some of the CHP

on the outer layer of the PDA-coated biomaterial and quickly reacts, forming cumene radicals. Due to the presence of water, some of them are transformed into hydroxide radicals. The cumene and hydroxide radicals reacted with EGDMA, starting radical polymerization with the PDA-coated biomaterials and PVP chains producing macroradicals by hydrogen abstraction. The recombination of these macroradicals leads to PVP grafting on PDA-coated biomaterials. Since EGMA is soluble in both water and organic phases, it thus acts as a bridge for both sides of the interface and improves the bond between the PDA-coated biomaterials and the PVP. Fe^{2+} ions consumed during the Fenton reaction are regenerated by the added ascorbic acid.

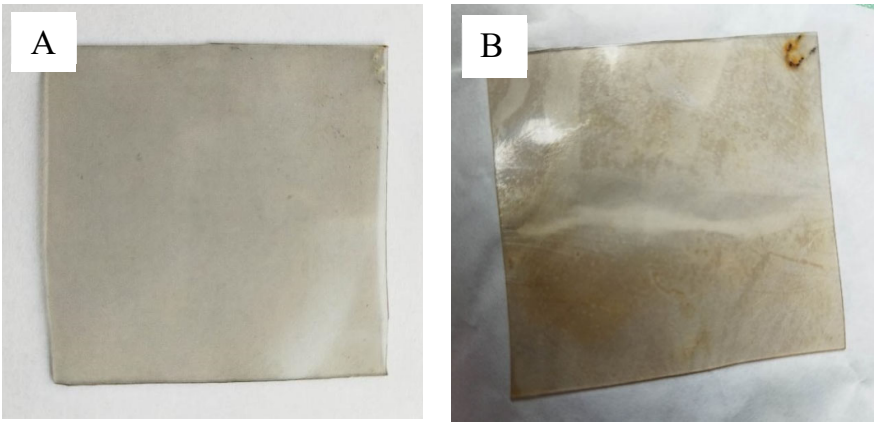


Figure 4.2: A) A polyurethane biomaterial sheet coated with polydopamine and B) A polyurethane biomaterial sheet coated with polydopamine and a polyvinyl pyrrolidone hydrogel top layer.

4.1.3 Effect of loading iodine as an antimicrobial agent to polydopamine/polyvinyl pyrrolidone-coated biomaterials

Absolute ethanol via solvent evaporation was used to load the iodine into the PDA/PVP-coated biomaterials. A colour ranging from light to dark yellow was observed among the produced PDA/PVP-coated biomaterials samples loaded with varying iodine concentrations (Figure 4.3). As expected, the intensity of the deep yellow colour increased with the concentration of iodine since iodine is characterised by this yellow colour. Therefore, Sample A had the lightest shade of yellow and lowest iodine concentration (0.1% w/v), Sample B was darker than A and had a higher iodine concentration (0.5% w/v) and Sample C had the darkest shade among all the samples and the highest iodine concentration (1% w/v). This suggests that the iodine was successfully entrapped within the hydrogel of the PDA/PVP-coated biomaterials. Similar results have been observed with a PDA/PEG/PVP-coated PP biomaterial loaded with 5% (w/v) iodine (Jiang. et.al., 2013).

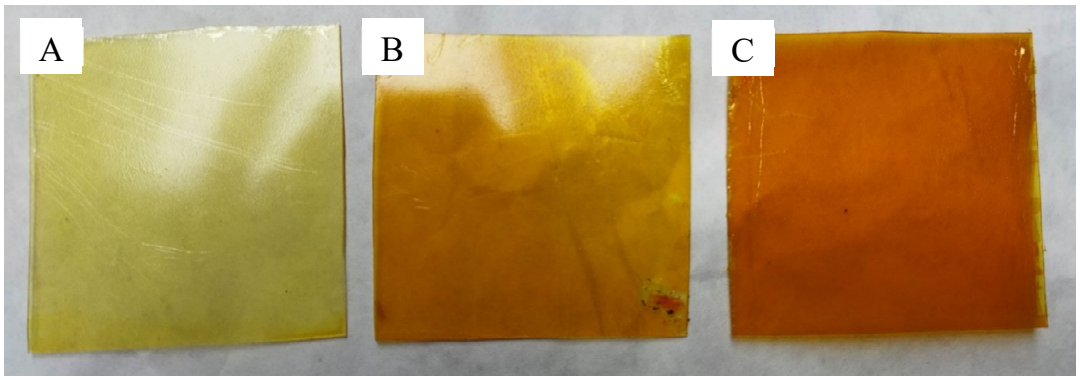


Figure 4.3: Polydopamine/polyvinyl pyrrolidone-coated polyurethane biomaterial sheets loaded with varying concentrations of iodine A) PU-PDA/PVP (0.1% I_2), B) PU-PDA/PVP (0.5% I_2) and C) PU-PDA/PVP (1% I_2).

4.2 Lubricity of polydopamine/polyvinyl pyrrolidone-coated biomaterials

The slipperiness and swelling behaviour of the polydopamine/polyvinyl pyrrolidone-coated biomaterials were used as indicators of lubricity.

4.2.1 Slipperiness of polydopamine/polyvinyl pyrrolidone-coated biomaterials

The observed time required to remove the biomaterial sections from agar indicated significant differences in lubricity between the uncoated and the PDA/PVP-coated biomaterials (P-value < 0.05). A PDA/PVP-coated PU biomaterial sample took 16.55 ± 2.07 s, while the uncoated PU biomaterial sample took a longer period of 22.26 ± 1.93 s. Since friction is an external force that opposes the relative motion between two contacting bodies. By comparing the time required for objects of the same mass and surface area to slide over a specified distance under the same applied force (pulled by a 20 g weight), the amount of friction each surface must overcome can be inferred. Objects experiencing a higher friction force will require more time to overcome it. Therefore, the mean reduction in time (5.71 s) required to remove the PDA/PVP-coated PU biomaterial from agar compared to the uncoated ones suggests that they experienced a lower amount of friction force. This suggests that the coated sample had a lower friction coefficient than the uncoated ones. This observation was expected since hydrogel coatings have been well-documented to improve lubricity by introducing a hydro layer in the interface of interacting surfaces, a hydro layer facilitates the flowing of interacting surfaces against each other. Similar observations were made in a study where a PVP coating technology reduced the time (by ~12 s) required to remove a PVP-coated PU biomaterial from agar (Tunney and Gorman 2002).

Using skewness 0 ± 2 and a normal distribution curve as parameters, the data was considered normal distribution. To analyse the data, the One-way ANOVA (P-value < 0.05) test compared the mean time required for dragging and removing uncoated-PU and PDA/PVP-coated biomaterial samples from agar. The null hypothesis, assuming no significant difference, was rejected with a determined P-value of 0.025, indicating a statistically significant difference in the time taken to drag out uncoated PU and PDA/PVP-coated biomaterial samples from agar.

4.2.2 Swelling behaviour of polydopamine/polyvinyl pyrrolidone-coated biomaterials

The PDA/PVP coating was observed to absorb 0.088 ± 0.009 mg/cm^2 of PBS after an exposure period of 1 minute and an additional 0.0148 ± 0.01 mg/cm^2 when further exposed for another minute. From the second minute until the tenth minute, no further changes were observed. This indicates that a) the coating technology can absorb and trap a limited amount of fluid and b) the majority of the absorption occurs within the first minute, up to 85%. PVP is hydrophilic and traps water within its polymeric structure. Therefore, as soon as the PDA/PVP-coated biomaterials samples were exposed to PBS, absorption occurred and the fluid remained trapped in the PVP hence the observed swelling behaviour. On other hand, the untreated biomaterial retained 0.0026 ±0.003 mg/cm^2 from the first minute until the tenth minute. This suggests that the untreated biomaterials cannot absorb water, the slight increase in mass per surface indicates that the surface area of the biomaterial was most likely still covered by some negligence amount of PBS. This finding suggests that the PDA/PVP-coated biomaterials will be able to form a hydro layer on their surface with the trapped fluid, unlike the uncoated biomaterials. This swelling behaviour will thus be able to introduce smoothness and lubricity (Wagner et al., 2020). This could also explain the lower friction coefficient compared to the uncoated ones. This hydrogel swelling behaviour has also been reported in numerous coating technologies that relied on PVP (Paradowska et al., 2010).

4.3 Biocompatibility of polydopamine/polyvinyl pyrrolidone-coated and iodine-loaded biomaterials

This study was able to determine the biocompatibility of the novel PDA/PVP-I_2 coating technology for the first time by relying on the XTT cytotoxicity assay, BSLA and Ames mutachrome mutagenicity assay.

4.3.1 Biocompatibility of polydopamine/polyvinyl pyrrolidone-coated and iodine-loaded biomaterials based on the brine shrimp lethality assay

Over 50% of the larvae survived in the negative control throughout the test indicating that the larvae can survive under the test parameters. However, as time progressed during the assay (>24 h) some larvae naturally died in the negative control, this indicated that the assay became less accurate beyond 24 h. The positive control, 100 µg/ml of $K_2Cr_2O_7$ showed high lethality, above 90% (95.65%) at 24 h and 100% lethality at 48 h (Figure 4.4). Similar positive control values for the BSLA have been reported in other studies, suggesting that the assay had good sensitivity to detect toxicity.

The PU-PDA/PVP and PDMS-PDA/PVP extract samples showed less than 50% lethality at 24 h until the end of the assay at 48 h. This was expected since the constituents of this coating technology (PDA and PVP) have previously been demonstrated to be biocompatible and are thus commonly used separately or in combination with other substances in the fabrication of invasive medical devices (Paradowska et al., 2010). The BSLA further suggests that the PDA primer and a PVP hydrogel do not produce a synergic toxic effect.

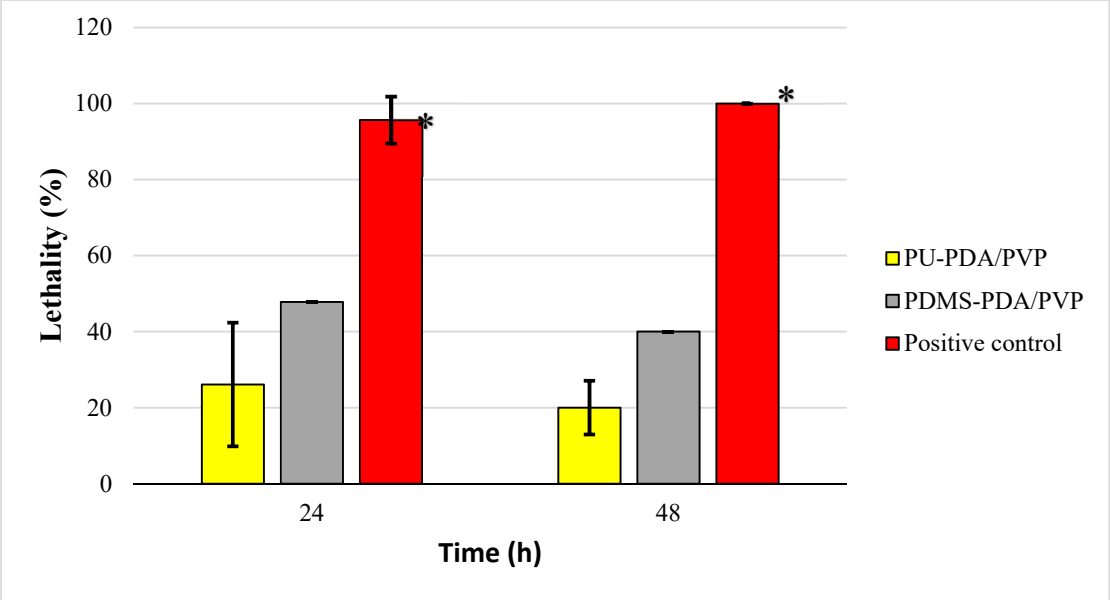


Figure 4.4: Lethality of the extracts of the polydopamine/polyvinyl pyrrolidone-coated polyurethane biomaterial samples. Lethality was evaluated against *Artemia salina* over 48 h, * denotes a statistically significant difference compared to the negative control at $P<0.05$.

The extracts of the PDA/PVP-coated biomaterial loaded with 0.1% and 0.5% w/v iodine showed less than 50% lethality towards brine shrimp larvae at 24 h. On the other hand, the

PDA/PVP-coated biomaterial loaded with 1% (w/v) demonstrated 100% lethality at 24 h. The lethality of the extracts of the PU-PDA/PVP (0.1% I_2) biomaterial remained relatively the same, around 10% for up to 48 h (Figure 4.5). However, the lethality of the PU-PDA/PVP (0.5% I_2) extract increased to 75% after 48 h which is considered toxic since it is more than 50%. This suggests that i) exposure to a PDA/PVP-coated biomaterial loaded with 0.1% (w/v) iodine does not cause toxicity, ii) if the biomaterial is loaded with 0.5% (w/v) iodine, potential toxicity may only be observed after an exposure period of more than 24 h and iii) PDA/PVP-coated biomaterials loaded with ≥1% (w/v) iodine are most likely toxic. Therefore, this suggests that toxicity is influenced by the concentration of the antimicrobial agent loaded and the exposure period. This is most likely due to the higher concentration of iodine translating to a higher number of iodine molecules available to react with the cells. At the same time, toxicity after a long time (more than 24 h) can be attributed to more iodine molecules being accumulated by brine shrimp larvae as time progresses. However, it should be noted that these findings do not directly translate to clinical settings. For instance, urological implants are exposed to a wash cycle when urine flows or is drained through the lumen possibly causing dilution. This is not the case in the BSLA since this assay measures the effect at a constant concentration over the run of the assay (48 h). In addition, since an indirect sample was used, only the leached iodine that was released during extraction was evaluated.

Other PVP-I hydrogel with low iodine concentrations have demonstrated their biocompatibility and are thus commonly used in the fabrication of invasive medical devices. Similar observations have been made in a study where a biodegradable chitosan-based crosslinked hydrogel was developed and loaded with PVP-I (Gull et al., 2020).

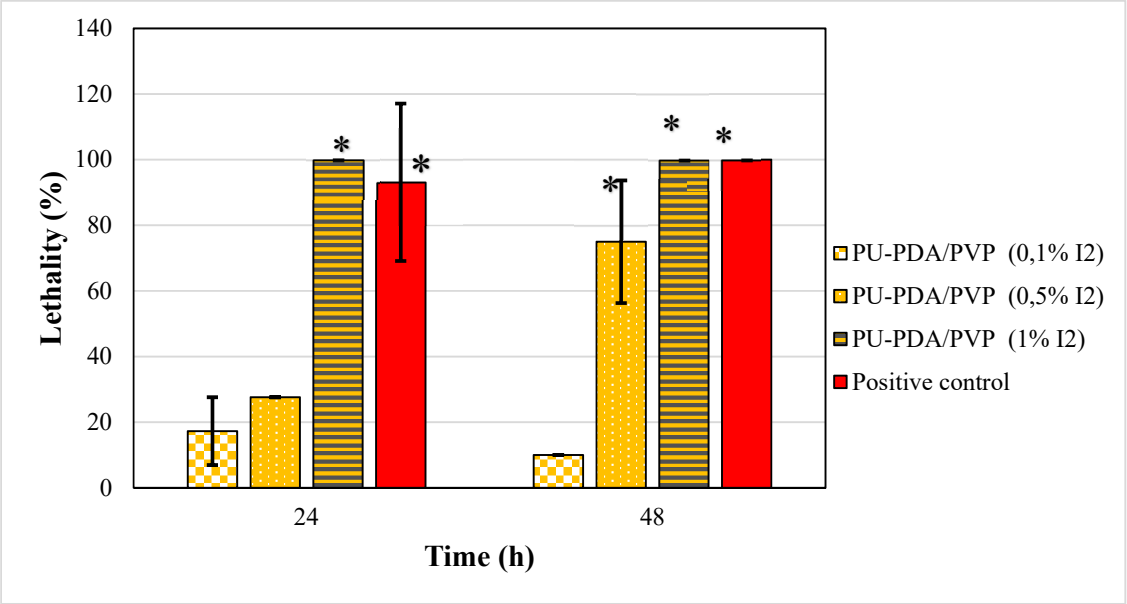


Figure 4.5: Lethality of the extracts of polydopamine/polyvinyl pyrrolidone-coated polyurethane biomaterials loaded with varying iodine concentrations. Lethality was evaluated against *Artemia salina* over 48 h. * Denotes statistical significance difference of $P<0.05$ compared to the negative control.

For the BSLA, the variables had a Skewness that ranged from -1.73 to 1.73, however, it should be noted that the variables that did not have 0 also had higher standard deviations. A skewness close to 0 indicates a strong possibility of normal distributions. The skewness values were between -2 and 2 were thus considered normally distributed (George and Mallery., 2020). The T-Test was thus used to test the null hypothesis that stated that the mean between the negative control and each sample is = 0 (Table 4.1), with P-value <0.05 indicating a statistically significant difference.

The null hypothesis for the paired T-Test of the BSLA between the negative control and the iodine-loaded samples at 24 h stated that there is no significant difference between the mean number of surviving nauplii in the negative control and each sample if the P-value is more than 0.05. Therefore, for PU-PDA/PVP (0.1% I_2) and PU-PDA/PVP (0.5% I_2), the null hypothesis is accepted (Table 4.1). This means that there is no significant difference between the mean number of surviving nauplii observed in the negative control, sample PU-PDA/PVP (0.1% I_2) and PU-PDA/PVP (0.5% I_2) at 24 h of exposure. Therefore, it assumed that these samples are as safe as the negative control. The null hypothesis is rejected for the sample PU-PDA/PVP (1% I_2) which means that the iodine is potentially toxic when applied at 1%.

Table 4.1: Paired T-Test of the brine shrimp lethality assay between the negative control and the iodine-loaded samples at 24 h.

Samples paired with the negative Control	Paired differences	Significance
	Mean	One-sided P value
PU-PDA/PVP (0.1% I_2)	1.000	0.239
PU-PDA/PVP (0.5% I_2)	0.667	0.317
PU-PDA/PVP (1 % I_2)	9.667	<0.001*
Positive Control	9.000	0.002*

* Denotes statistically significant difference at P<0.05 compared to the negative control

After 48 h, the null hypothesis is accepted only for PU-PDA/PVP (0.1% I_2) (Table 4.2). Thus, PU-PDA/PVP (0.1% I_2) is the only sample that is non-toxic at 48 h.

Table 4.2: Paired T-Test of the brine shrimp lethality assay between the negative control and the iodine-loaded samples at 48 h.

Samples paired with the negative control	Paired differences	Significance
	Mean	One-sided P value
PU-PDA/PVP (0.1% I_2)	0.667	0.092
PU-PDA/PVP (0.5% I_2)	5.000	0.025*
PU-PDA/PVP (1% I_2)	6.667	0.001*
Positive Control	6.667	0.001*

* Denotes statistically significant difference at P<0.05 compared to the negative control

4.3.2 Biocompatibility of polydopamine/polyvinyl pyrrolidone-coated and iodine-loaded biomaterials based on the XTT cytotoxicity assay

Viable L292 cells can reduce XTT to an orange formazan with a peak absorbance at 450 nm. In line with expectation, the positive control was observed to have the lowest absorbance (0 at

450 nm) while the negative control had the highest absorbance (2.77 ±0.03) at 450 nm. All the extracts of the samples had at least more than half the absorbance of the negative control, ranging from 1.53 ± 0.39 to 2.6 ± 0.66 at 450 nm (Table 4.3). This suggests that at least more than half of the L292 cells exposed to the extract samples for a period of 24 h remained viable. The absorbance of XTT formazan in the PU-PDA/PVP (0.1% I_2) and PU-PDA/PVP (0.5% I_2) where almost similar at 2.03 ± 0.51 and 2.06 ± 0.39, respectively. This suggests that the samples of PU-PDA/PVP loaded with iodine concentration of 0.5% or lower have a similar effect on the viability of L292 cells.

Table 4.3: The normalised absorbance of XTT at 450 nm and viability of L292 cells after 24 h exposed to extract samples as determined by the XTT assay.

Samples	Normalised absorbance of XTT at 450 nm	Viability (%)
Negative Control	2.77 ± 0.03	100
PU	2.6 ± 0.66	94 ± 22.02
PU-PDA/PVP	2.34 ± 0.61	85 ± 18.38*
PU-PDA/PVP (0.1% I_2)	2.03 ± 0.51	73 ± 14.03*
PU-PDA/PVP (0.5% I_2)	2.06 ± 0.39	74 ± 14.03*
PU-PDA/PVP (1% I_2)	1.53 ± 0.39	55 ± 8.54*
Positive Control	0 ± 0.24	0 *

* Denotes statistically significant difference at $P<0.05$ compared to the negative control.

Apart from sample PU-PDA/PVP (1% I_2) and the negative control, more than 70% viability was observed for the samples as shown by the red line in Figure 4.6. According to ISO 10993.5, more than 70% viability demonstrates non-cytotoxicity. Thus, the rest of the extract samples were non-cytotoxic towards the L929 cells. This suggests that the use of PDA as a primer and PVP as the hydrogel top layer for the coating technology does not introduce any detectable *in vitro*-cytotoxic effects. Other reported coatings that have separately incorporated PDA or PVP have also not shown any cytotoxicity. A PVP coating with a similar formulation (5% PVP 360k w/v) was reported as non-cytotoxic when assessed via the MTT assay (Butruk et al., 2012). However, iodine-incorporation has at times been observed to be toxic, especially when a high iodine concentration is used (Percival et al., 2016). Similarly, in this study the sample with the highest iodine concentration, PU-PDA/PVP (1% I_2), showed the lowest viability (55%) which indicates potential toxicity. However, it should be noted that the sample had over 50% viability, which indicates that 1% I_2 is still below the minimum inhibitory concentration. Therefore, the PDA/PVP (I_2)-coating technology most likely has good biocompatibility when loaded with 0.5% w/v iodine or less.

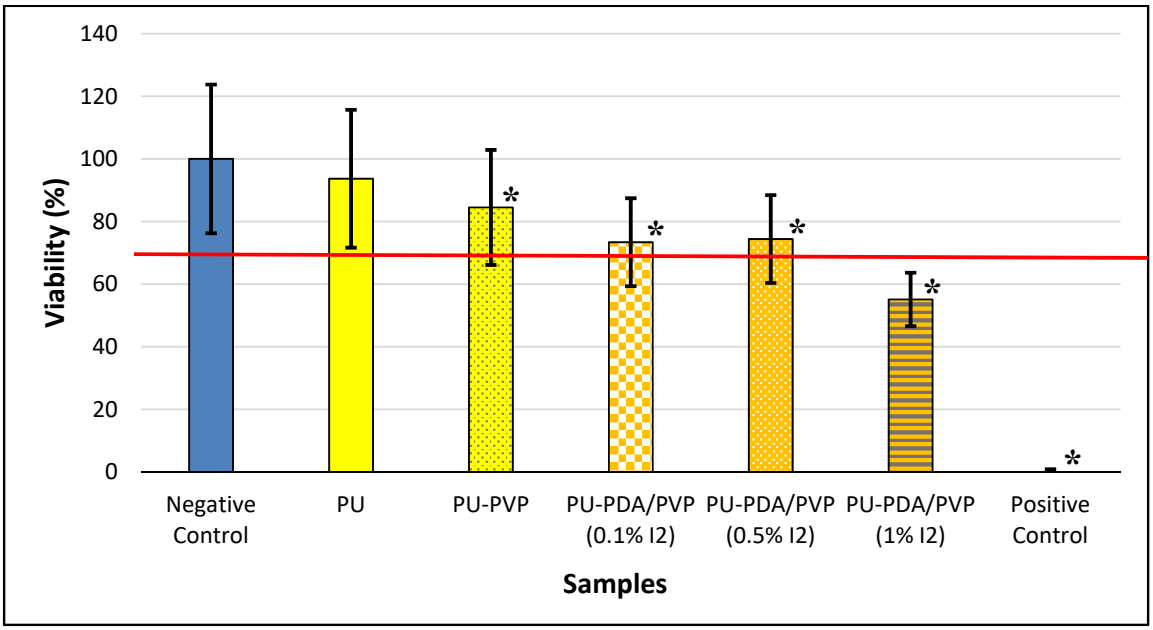


Figure 4.6: Viability of L292 cells after 24 h exposure to the extracts of polydopamine/polyvinyl pyrrolidone-coated polyurethane biomaterial samples loaded with varying concentrations of iodine, as measured by the XTT cytotoxicity assay. * Denotes statistically significant difference at $P<0.05$ compared to the negative control.

The cytotoxicity of three wound dressing products, Betaisodona® solution, Betaisodona® ointment and Repithel® which contain around 10%, 11% and 3% (w/v) of available iodine, respectively were evaluated with the MTT assay using L929 cells (Müller and Kramer 2006). The products were diluted to an iodine range of 0.3% to around 1%. It was observed that iodine concentrations higher than 0.53% in the Betaisodona® solution, 0.96% in the Betaisodona® ointment and 0.63% in the Repithel® were cytotoxic since they caused more than a 70% decrease in the viability of L292 cells (Müller and Kramer, 2006). However, another study that relied on a 3-dimensional human skin model (Vitrolife-Skin®) did not detect significant cytotoxicity of an antiseptic formulation with a concentration of 10% (w/v) PVP-I (Nagasawa et al., 2002). Therefore, the cytotoxicity profile of the iodine-loaded samples including PU-PDA/PVP (0.1% I_2), PU-PDA/PVP (<0.5% I_2) and PU-PDA/PVP (1% I_2) are in line with some previous studies (Müller and Kramer., 2006, Nagasawa et al., 2002).

Using a skewness and kurtosis of 0 ± 2 as parameters, the data was considered normal distribution, therefore the t-test was adopted to compare each sample to the negative control.

The null hypothesis for the paired T-Test between the normalised mean absorbance of the negative control and the iodine-loaded samples at 24 h stated that there is no significant difference between them if the P-value is more than 0.05. Therefore, the null hypothesis was accepted for the uncoated sample (PU) while it was rejected for the rest of the samples (Table 4.4). This means that the untreated PU samples do not cause significant changes in the proliferation of L292 cells compared to the negative control. Even though the null hypothesis was rejected for the rest of the sample, this does not necessarily mean they are toxic. Rather, it indicates that they are significantly different compared to the negative control. However, since the viability is above the 70% threshold accepted by ISO 10993.5, they can be considered non-toxic.

Table 4.4: Paired T-Test for normalised absorbance for XTT assay

Samples paired with the negative control	Significance
	One-sided P-value
PU	0.058
PU-PVP	0.020*
PU-PDA/PVP (0.1% I2)	0.012*
PU-PDA/PVP (0.5% I2)	0.006*
PU-PDA/PVP (1% I2)	0.001*
Positive Control	<0.001*

* denotes statistically significant difference at P-value <0.05 compared to the negative control.

The null hypothesis states that there is no correlation between the iodine concentration and viability (%) as measured using the XTT assay. The P-value (1-Tailed) value was less than 0.05. Therefore, the null hypothesis was rejected and thus there is a statistically significant correlation between the iodine concentration and viability (%) measured via XTT assay. Since the value of the Pearson Correlation is negative (Table 4.5), an increase in the concentration of iodine in the loaded samples will result in a decrease in the viability of the exposed cells.

Table 4.5: Pearson correlation between iodine concentration and viability (%) measured via XTT assay

		Iodine concentration
Viability (%)	Pearson correlation	-0.561
	Sig. (2-tailed)	0.004*
	N	24

* denotes statistically significant correlation at P-value <0.05 compared to the negative control

4.3.3 Biocompatibility of polydopamine/polyvinyl pyrrolidone-coated and iodine-loaded biomaterials based on the Ames mutachrome mutagenicity assay

The Ames mutachrome mutagenicity assay was executed utilizing *S. typhimurium* TA 100 to assess the potential of PDA/PVP-coated biomaterials to induce base pair mutations. *S. typhimurium TA* 100 cells have mutation in their histidine biosynthesis genes, rendering them incapable of synthesizing histidine, an essential amino acid for growth. Consequently, *S. typhimurium* TA 100 cells rely on external sources of histidine for their growth. If a substance transforms histidine-requiring S. *typhimurium TA 100* cells into a histidine-independent state, this indicates that it is potential mutagenic. Histidine-independent *S. typhimurium* TA 100 cells can grow and undergo fermentation without requiring external histidine, unlike unreverted cells. During this fermentation process, these cells release acid into the growth medium, leading to a reduction in pH. As the growth medium is equipped with a pH colour indicator (bromocresol purple), a shift from purple to yellow indicates mutagenic reversion (Figure 4.7). Consequently, wells displaying this colour transition from purple to yellow are classified as revertant wells.

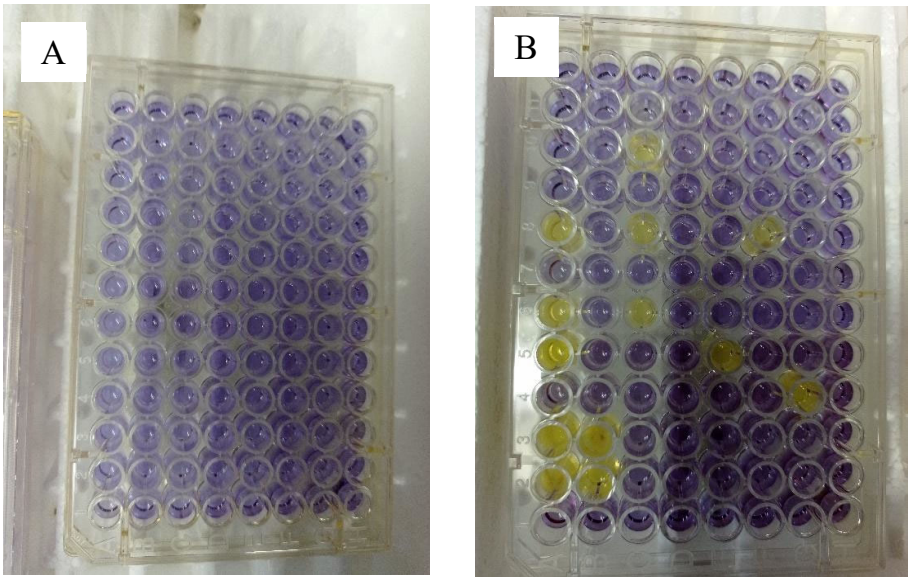


Figure 4.7: The Ames assay carried out with the Ames muta-chromo assay A) pre-incubation and B) post 72 h incubation with observable yellow revertant wells.

Samples extracts of PDA/PVP-coated biomaterials were subjected to evaluation using the criteria described by Rao and Lifshitz (1995). This evaluation process entailed the establishment of a baseline value based on the number of reventant wells in the negative control, which is subsequently compared to each individual sample. Samples that generated more than twice the number of revertant wells compared to the baseline value were considered potential mutagens.

The negative control displayed the lowest number of revertant wells among the evaluated samples, with 7 ± 1.14 at 72 h and 8.5 ± 0.71 at 96 h. The baseline values for this assay were determined using the formula (Baseline value = mean number of revertants in the negative control + standard deviation), resulting in values of 8.14 at 72 h and 9.21 at 96 h. Therefore, since the minimum requirement for classifying a substance as a potential mutagen is twice the baseline value, only samples with more than 16.28 revertant wells at 96 h and 18.42 revertant wells at 96 h were deemed potential mutagens, as indicated by the two dotted- lines in Figure 4.8. The number of revertant wells observed in the PDMS-PDA/PVP coated biomaterials without iodine at 72 h was 9.5 ± 4.95, and this increased to 13 ± 1.41 at 96 h. Similarly, the PU-PDA/PVP sample without iodine showed 7.5 ± 0.71 revertant wells at 72 h, which slightly increased to 11 ± 1.41 at 96 h. However, both of these samples displayed less than twice the number of revertant wells in their respective baseline values, as indicated by the red line in Figure 4.8. In contrast, the positive control exhibited the highest number of revertant wells, with 26.5 ± 4.95 at 72 h and 40.5 ± 4.95 at 96 h, both exceeding their respective minimum

requirement. These observations suggest that PDMS-PDA/PVP and PU-PDA/PVP biomaterials are unlikely to induce base mutations after an exposure period of 72 and 96 h, whereas the positive control sodium azide as expected induced such mutations.

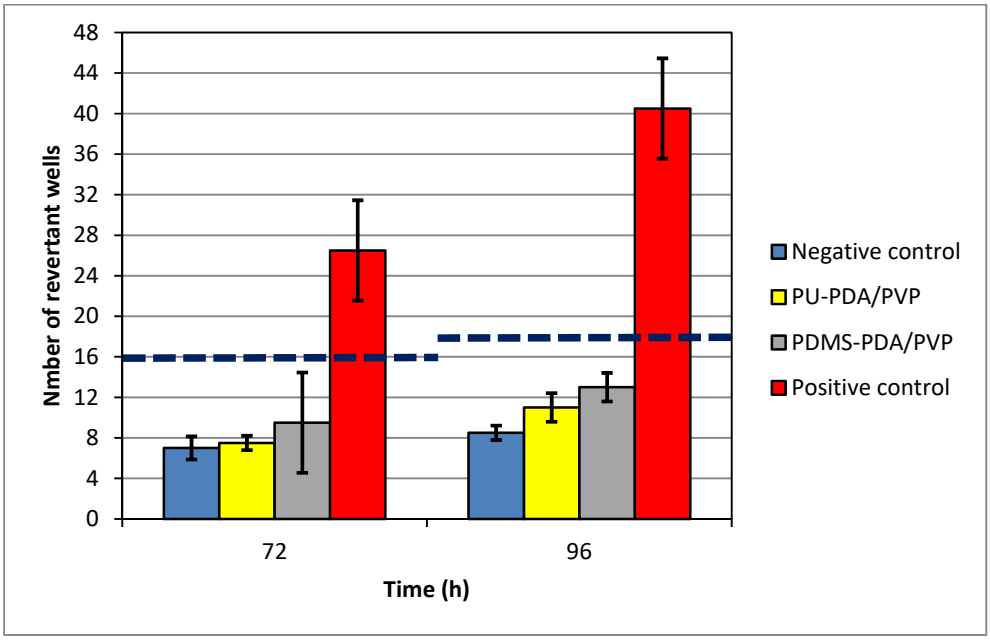


Figure 4.8: The revertant wells of *Salmonella typhimurium* T100 after 72 and 96 h of exposure to the extracts of polydopamine/polyvinyl pyrrolidone-coated polyurethane biomaterial samples as measured by the Ames muta-chromo assay.

In the PDA/PVP-coated biomaterial samples loaded with iodine, the negative control displayed the lowest number of revertant wells among the evaluated samples, with 5 ± 1 at 72 h and 7 ± 2 at 96 h. This resulted in baseline values of 6 at 72 h and 9 at 96 h. Therefore, a minimum of 12 revetant wells at 72 h and 18 revertant wells at 96 h were required to classify a sample as a potential mutagen as indicated by the two dotted- lines in Figure 4.9. Among the samples loaded with iodine, PU-PDA/PVP (1% I_2) had the highest number of revertant wells, with 7.67 ± 3.06 at 72 h and 10.67 ± 1.53 at 96 h. PU-PDA/PVP (0.5% I_2) exhibited 7.67 ± 1.53 revertant wells at 72 h and 8.33 ± 1.53 at 96 h. PU-PDA/PVP (0.1% I_2) showed 5.33 ± 2.52 revertant wells at 72 h and 7 ± 1.00 at 96 h. The positive control, sodium azide, also had the highest number of revertant wells, with 29.33 ± 4.16 at 72 h and 38.67 ± 3.06 at 96 h. With the exception of the positive control, all the PU-PDA/PVP biomaterials loaded with different concentrations of iodine (0.1%, 0.5%, and 1% w/v) displayed a lower number than the minimum requirement to classifying them as mutagenic after an exposure period of 72 and 96 h as shown in Figure4.9. On the other hand, the number of revertant wells in the positive control exceeded the minimum

requirement, indicating its potential mutagenic effect. A similar findings were made in a study that relied on the L5178Y mouse (TK+/-) lymphoma assay, it was concluded that PVP, PVP-I, KI, and I_2 did not possess any biologically significant mutagenic or cell transforming ability (Kessler et al., 1980).

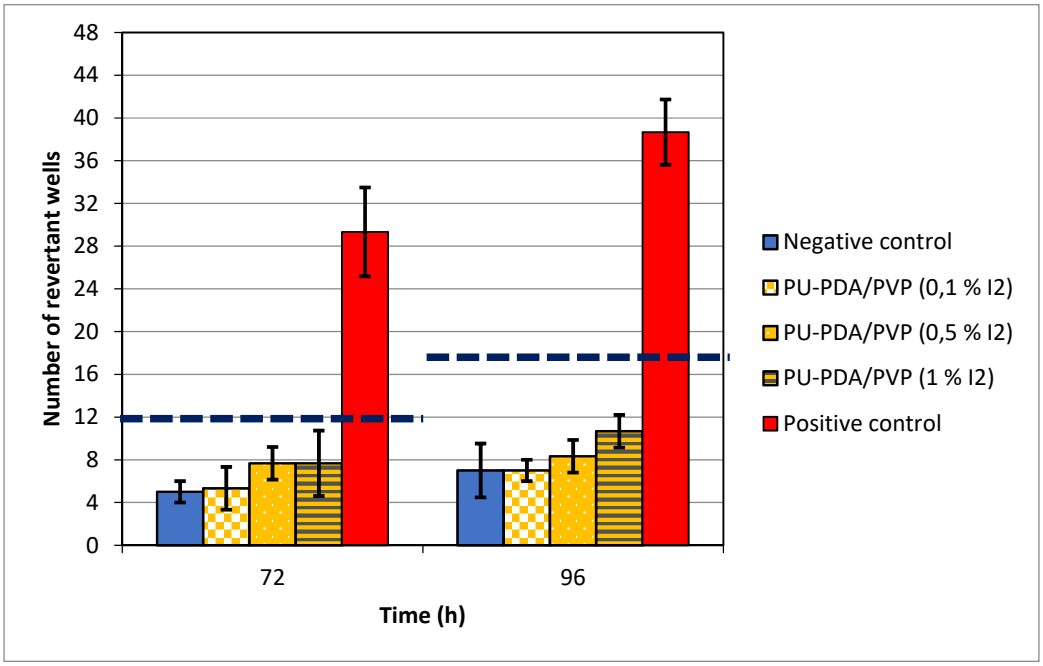


Figure 4.9: The revertant wells of *Salmonella typhimurium* T100 after 72 and 96 h of exposure to the extracts of polydopamine/polyvinyl pyrrolidone-coated polyurethane biomaterial samples loaded with a varying concentration of iodine as measured by the Ames muta-chromo assay.

4.4 Antimicrobial activity of polydopamine/polyvinyl pyrrolidone-coated and iodine-loaded biomaterials

All the biomaterial samples (PU, PDMS, PU-PDA/PVP and PDMS-PDA/PVP) without iodine did not show any antimicrobial efficacy. Similar observations have also been made with PDA/PVP-coated on PP biomaterial (Wang et al., 2016). This suggests that the PDA/PVP coating by itself does not possess any antimicrobial properties and the overall antimicrobial efficacy of the coating is mostly determined by the antimicrobial agent incorporated. Therefore, the coating technology is also most likely having broad spectrum antimicrobial activity owing to the nature of iodine (Bigliardi et al., 2017). The samples with iodine showed a varying antimicrobial efficacy influenced by the concentration of the iodine loaded and the assessment

method employed. In general, the direct samples demonstrated better results compared to the extraction samples. This suggests that the biomaterials did not release all the iodine loaded during the extraction process and thus had a higher concentration than the latter. This is desirable since PDA/PVP-coated biomaterial loaded with iodine will release it over a long duration. This suggests that the coating technology will be effective post a wash cycle, which is a critical requirement for urological implants since the urinary tract system generally involves a wash cycle when urine flows or is drained.

The ISO 22196 method showed that the PDA/PVP coated sample loaded with a minimum of concentration 0.1% w/v iodine reduced bacterial growth by at least 2 logs. The Disk diffusion assay, broth microdilution and Catheter bridge model assay showed that antimicrobial efficacy was positively correlated to the concentration of the iodine loaded per sample. Therefore, the evidence suggests that when the PDA/PVP coating is loaded with an appropriate concentration of iodine solution, it will inhibit bacterial colonisation. Most of the observations from the antimicrobial susceptibility testing methods were consistent with other published works (Müller and Kramer 2006). For most hydrogels, antimicrobial activity has mostly been observed when a concentration of 0.1% w/v or more iodine has been incorporated (Müller and Kramer 2006). However, the degree of antimicrobial activity for most iodine-loaded hydrogel varies, most likely due to the specific drug release rate of each hydrogel (Müller and Kramer 2006).

4.4.1 The antimicrobial activity of polydopamine/polyvinyl pyrrolidone-coated and iodine-loaded biomaterials based on the ISO 22196 assay

Before incubation, $2.25 \pm 0.23 \times 10^4$ and $1.61 \pm 0.16 \times 10^4$ (CFU/cm^2) were recovered for PU and PDMS, respectively. After a 24-h incubation period, the bacterial counts were reduced to $7.48 \pm 1.35 \times 10^3$ and $6.39 \pm 1.9 \times 10^3$, respectively. When PDA and PVP were incorporated into the PU and PDMS, the *E. coli* (CFU/cm^2) recovered after 24 h was further reduced to $4.92 \pm 1.33 \times 10^2$ and $7.27 \pm 1.47 \times 10^2$, respectively (Table 4.6). According to ISO 22196 standard, samples that do not reduce the recovered bacterial (CFU/cm^2) by at least 2 bacterial growth logs do not possess sufficient antimicrobial activity to prevent infections by 99.9% (bactericidal effect). Therefore, this suggests that all the samples without iodine do not have any antibacterial activity.

Table 4.6: Viable *Escherichia coli* (ATCC 25922) recovered from polydopamine/polyvinyl pyrrolidone-coated biomaterial samples

Time (h)	Sample sheets (50×50 mm)	*E. coli* (CFU/cm^2)	Antibacterial activity (bacterial log growth reduction)
0 (before incubation)	PU	$2.25 \pm 0.23 \times 10^4$	N/A
	PDMS	$1.61 \pm 0.16 \times 10^4$	N/A
24 (after incubation)	PU	$7.48 \pm 1.35 \times 10^3$	0.00
	PDMS	$6.39 \pm 1.9 \times 10^3$	0.00
	PU-PDA/PVP	$4.92 \pm 1.33 \times 10^2$	1.18
	PDMS-PDA/PVP	$7.27 \pm 1.47 \times 10^2$	0.94

The PU-PDA/PVP samples loaded with a varying concentration of iodine (0.1, 0.5 and 1% w/v) were able to reduce the number of recovered bacteria by more than 2 growth logs (Table 4.7). PU-PDA/PVP samples loaded with 0.1%, 0.5% and 1% w/v of iodine demonstrated the same antimicrobial activity (2.75 log growth reduction). This suggests that when the coating technology is loaded with 0.1% w/v of iodine it will be able to prevent bacterial growth at 0.5 McFarland standards. Therefore, even if a higher concentration of iodine is incorporated, it would not be detected within the test parameters. In essence, PDA/PVP samples loaded with 0.1% w/v meet the minimum inhibitory concentration for *E. coli* (ATCC 2592) at 0.5 McFarland. This was determined to be 40 mm^2/ml for *E. coli* (ATCC 2592). Other studies have also observed similar findings. This includes a titanium implant treated with 8.6% iodine, it was demonstrated via ISO 22196 to be able to reduce *E. coli* growth by up to 6 logs (Yamaguchi et al., 2021). This observed higher antimicrobial activity in the iodine-treated titanium implant compared to the PDA/PVP (I_2) coating technology developed in this study can be explained by the difference in iodine concentration.

Table 4.7: Viable *Escherichia coli* (ATCC 25922) cells recovered from polydopamine/polyvinyl pyrrolidone-coated biomaterial samples loaded with iodine (0.1, 0.5 or 1% w/v)

Time (h)	Sample sheets (50×50 mm)	Average CFU (cells/cm^2)	Antibacterial activity (bacterial log growth reduction)
0 (after inoculation)	PU	$1.6 \pm 1 \times 10^5$	N/A
24 h (after incubation)	PU	$5.72 \pm 1.15 \times 10^3$	0.00
	PU-PDA/PVP (0.1% I_2)	$1 \pm 0.2 \times 10^1$ (TFTC)	2.75
	PU-PDA/PVP (0.5% I_2)	$1 \pm 0.2 \times 10^1$ (TFTC)	2.75
	PU-PDA/PVP (1% I_2)	$1 \pm 0.2 \times 10^1$ (TFTC)	2.75

TFTC- Too few to count

4.4.2 The antimicrobial activity of polydopamine/polyvinyl pyrrolidone-coated and iodine-loaded biomaterials based on the broth microdilution assay

The broth microdilution depends on the presence of bacterial pellets at the bottom of the U-bottom wells of a microplate post-incubation to determine bacterial growth. Therefore, the absence of such a pellet post-incubation thus suggests the absence of viable bacteria in that well. The absence of pellets post-incubation in the presence of the extract samples was observed only in four samples and the positive control (Table 4.8). These include PU-PDA/PVP (0.5% I_2) at a concentration of 3 cm^2/ml and PU-PDA/PVP (1% I_2) at 1.5 and 3 cm^2/ml for *E. coli* 25922. For *P. mirabilis* only PU-PDA/PVP (1% I_2) at 3 cm^2/ml. Since the pellets indicate bacterial growth, it can be thus deduced that only these extract samples possessed enough antibacterial activity to inhibit cultures at 0.5 McFarland standards. Thus, the MICs of the PU-PDA/PVP (0.5% I_2) and PU-PDA/PVP (1% I_2) extracts towards *E. coli* 25922 are 3 and 1.5 cm^2/ml, respectively, while for *P. mirabilis* only the extract of PU-PDA/PVP (1% I_2) was effective at a MIC of 3 cm^2/ml. These observations suggest that the antimicrobial activity produced by the extracts of the PDA/PVP coating technology is effective when it is loaded with an iodine concentration of ≥0.5% w/v. An ophthalmic solution containing 0.6% PVP-I has been shown to inhibit the growth of *E. coli* 25922 when diluted ten times (Caruso et al., 2022).

Table 4.8: Bacterial growth pellet at the bottom of a 96 U-well for *Escherichia coli* (ATCC 25922) and *Proteus mirabilis*.

Extract samples	Bacterial growth at different concentrations of the extract samples (cm^2/ml)					
	E. coli ATCC 25922			*P. mirabilis*		
	3	1.5	0.75	3	1.5	0.75
Negative Control	+	+	+	+	+	+
PU- PDA/PVP	+	+	+	+	+	+
PDMS- PDA/PVP	+	+	+	+	+	+
PU-PDA/PVP (0.1% I_2)	+	+	+	+	+	+
PU-PDA/PVP (0.5% I_2)	-	+	+	+	+	+
PU-PDA/PVP (1% I_2)	-	-	+	-	+	+
Positive control	Negative (ofloxacin > 5 µg)					

+ positive bacterial growth: - negative bacterial growth

The samples for conducting the broth microdilution were prepared via extraction and thus only the iodine that was released during the 72-h extraction period was measured. Therefore, these observations made with the broth microdilution suggest that the PDA/PVP-coated biomaterials loaded with an iodine concentration of ≥0.5% w/v will release iodine molecules sufficient to inhibit bacteria into their surrounding environment when exposed to an access polar solvent such as urine. This may help reduce contamination since urine exposed to the implants developed with this technology will possess iodine, which will go through the urinary tract system possibly sanitising it, as opposed to just preventing bacterial invasion of the surface of the coated biomaterials. This is of particular importance since most UTIs develop when bacteria migrate from the outside environment into the urinary tract system. For instance, to invade urinary stents, most UTIs need to migrate from the urethra to the ureter. Therefore, if urine contains sufficient iodine molecules to inhibit bacterial growth, it can sanitise the urethra and slow down or prevent the bacteria from reaching the ureter.

4.4.3 Antimicrobial activity of polydopamine/polyvinyl pyrrolidone-coated and iodine-loaded biomaterials based on the disk diffusion assay

In the disk diffusion assay, leaching samples are placed directly on the top surface of inoculated agar plates. If the leaching contents of the sample are polar, they will diffuse into the surrounding agar. This can result in the formation of zones of bacterial inhibition around the samples if their leached contents have antimicrobial properties (Figure 4.10). The sizes of the

formed zones of inhibition are reflective of the bacterial susceptibility to the leached contents that diffused into the agar.

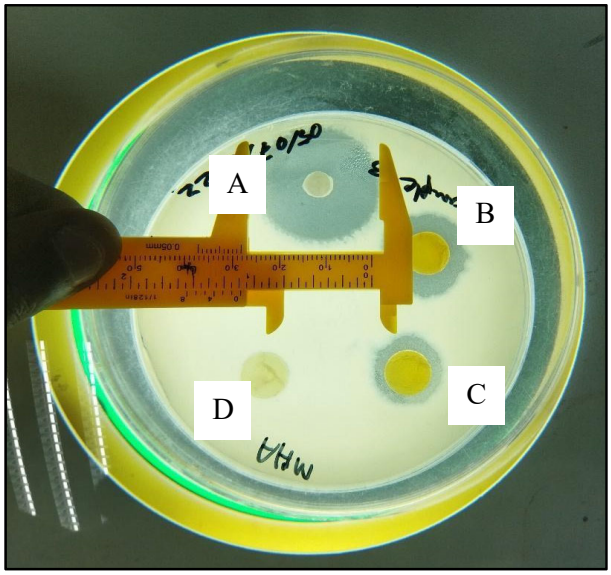


Figure 4.10: Measuring zones of growth inhibition against *Escherichia coli* (ATCC 25922) based on the disk diffusion assay. A) Positive control (ofloxacin 5 µg), B) PU-PDA/PVP (1% I_2), C) PU-PDA/PVP (0.5% I_2) and D) PU-PDA/PVP (0.1% I_2)

Zones of inhibition against *E. coli* (ATCC 25922) and *P. mirabilis* at 0.5 McFarland standard, were not observed in PU, PDMS, PU-PDA/PVP, and PDMS-PDA/PVP biomaterial samples. For samples loaded with iodine, including PU-PDA/PVP (0.1% I_2), PU-PDA/PVP (0.5% I_2), and PU-PDA/PVP (1% I_2), minimal zones of inhibition were observed for *E. coli* (ATCC 25922). Specifically, PU-PDA/PVP loaded with 0.1% I_2 (w/v) exhibited the smallest zones of inhibition (1.00 mm) against *E. coli* (ATCC 25922). However, when the PU-PDA/PVP biomaterial was loaded with 0.5% and 1% I_2 (w/v), larger zones of growth inhibition (12.13 ± 1.53 and 16.25 ± 1.77 mm, respectively) were observed against *E. coli* (ATCC 25922). For *P. mirabilis*, zones of growth inhibition (8.5 ± 0.50 mm and 13 ± 1.00 mm) were only observed from the PU-PDA/PVP samples loaded with 0.5% and 1% w/v iodine, respectively (see Figure 4.10). These findings suggest that only PDA/PVP-coated samples loaded with 0.5% w/v or more iodine possess sufficient antibacterial activity to inhibit cultures at the 0.5 McFarland standard. Additionally, the size of the zones of growth inhibition produced by the PDA/PVP iodine-loaded samples varied in an increasing gradient respective to their iodine concentration. This implies that their antimicrobial activity is due to the action of iodine. Furthermore, this observation supports the correlation between antimicrobial activity and iodine concentration,

as PDA/PVP samples loaded with a higher concentration have a greater number of iodine molecules to react with the tester strain. This was expected since the antimicrobial activity of iodine has been documented in numerous studies (Percival et al., 2016). In a related study, 10 µl of an ophthalmic solution that contained 0.6% PVP-I also showed zones of inhibition against *E. coli* (ATCC 25922), although the size was not reported (Caruso et al., 2022).

The positive control: ofloxacin at a mass of 5 µg had the highest antimicrobial efficacy with a zone in inhibition of 27.17 ± 0.76 for *E. coli* (ATCC 25922) and 25 ± 0.58 for *P. mirabilis*. These results are in line with the current zones of inhibition breakpoints published by EUCAST which reports Enterobacterales susceptibility to ofloxacin to have zones of inhibitions with more than 24 mm and not less than 22 mm for resistant strains. *Escherichia coli* (ATCC 25922) also demonstrated a higher level of susceptibility compared to *P. mirabilis* (Figure 4.11). This was expected since *P. mirabilis* is characterised by swarm motility which affects the size of the zone of growth inhibition. Based on the EUCAST zones of inhibition breakpoints, the test conditions were sufficient to produce acceptable results.

In contrast to the broth microdilution assay, the disk diffusion assay demonstrated that PU-PDA/PVP (0.5% w/v) is capable of inhibiting *P. mirabilis* growth at 0.5 McFarland standard. This is most likely because, unlike the later essay, the disk diffusion measures the direct sample and thus it probably has a higher iodine concentration. This suggests that during extraction some iodine molecules were not released into the extraction solution supporting the notion that the PVP hydrogel coating does not release all the iodine at the same time rather it is released at a slower rate over a longer period.

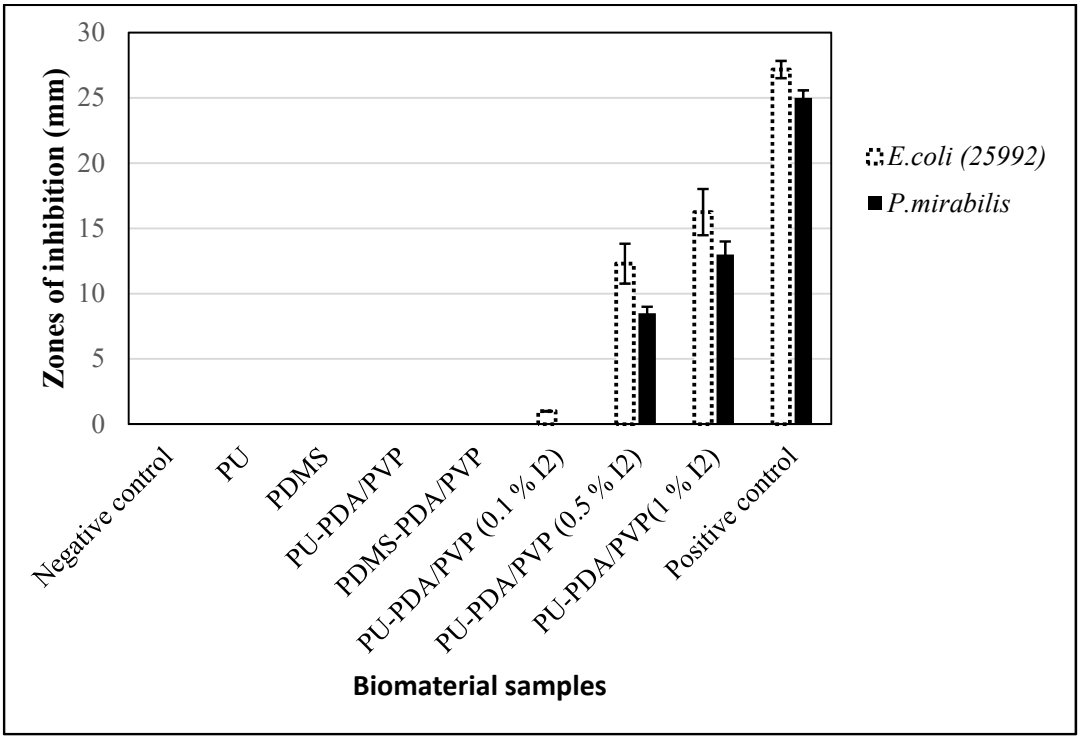


Figure 4.11: The zones of growth inhibitions against *Escherichia coli* (ATCC 25922) and *Proteus mirabilis* at 0.5 MacFarland standard for uncoated and coated biomaterials

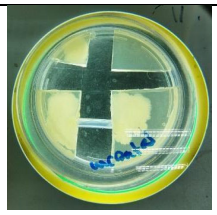
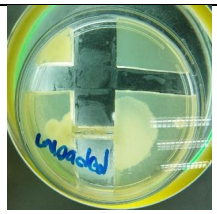
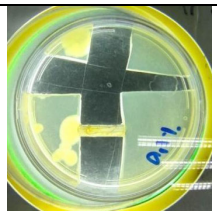
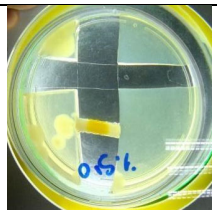
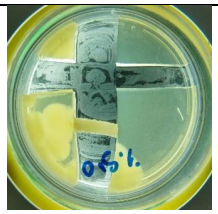
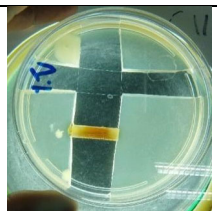
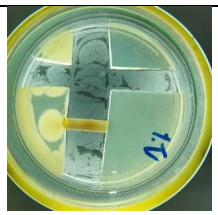
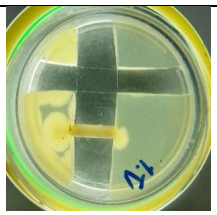
4.4.4 Antimicrobial activity of polydopamine/polyvinyl pyrrolidone-coated and iodine-loaded biomaterials based on the catheter bridge model

Growth across the bridge was observed at 24 h for PDMS and PDMS-PDA/PVP without iodine incorporation, at 48 h for PDA/PVP (0.1% I_2) and PDA/PVP (0.5% I_2), and at 72 h for PDA/PVP (1% I_2) as shown in Table 4.9. Since the biomaterials were the only objects with direct contact with the inoculated islands and the un-inoculated ones, growth in the latter is credited to microbial migration on the surface of the biomaterials. Therefore, microbial growth across the bridge indicates the inability of a biomaterial to prevent microbial migration. The PDMS and PDMS-PDA/PVP samples are thus unable to prevent microbial migration. This was expected since both biomaterials were not loaded with any antimicrobial agents. The PDA/PVP (0.1% I_2) and PDA/PVP (0.5% I_2) samples had some antimicrobial activity since they were able to prevent microbial migration for 24 h and thus PD PVP (1% I_2) had the highest capacity to prevent microbial migration from the tested samples. This suggests that the capacity of the PDA/PVP (I_2)-coated biomaterials to prevent microbial migration is influenced by the iodine concentration, in line with the disk diffusion assay. A study conducted with a lower iodine concentration (0.07%) on silicone biomaterial (PVP (0.07% I_2 w/v) showed that the material

was unable to prevent migration of *P. mirabilis* for a distance of 10 mm within 24 h (Kazmierska et al., 2010), unlike the iodine loaded materials tested in this study. This further supports the notion that the effectiveness of coating technologies is influenced by their iodine concentration.

E. coli 43888 is a clinically important food- and waterborne pathogenic O157:H7 serotype that can colonise the human colon epithelium. This serotype possesses the $Stx1^-$ and $Stx2^+$ virulence genes that felicitate its adherence to epithelial cells. (Medellin-Peña et al., 2007). Therefore, the ability of the PDA/PVP-coated biomaterials loaded with iodine to mitigate microbial migration of this serotype with its virulence features suggests that it may be effective against other similar pathogens. This is of particular importance since UTIs tend to invade the urinary tract system by migrating on newly installed implants. Single-species biofilms have been observed as soon as 24 h after the installation of a new implant. This is often due to most pathogenic microbes preferring to grow on solid surfaces, thus implant surfaces provide an ideal site of invasion. The mechanism of action in which the coating technology prevents microbial migration involves the interaction of iodine molecules immobilised in the hydrogel with the invading pathogens. Iodine molecules will disrupt the cell membrane and the internal systems of these pathogens, resulting in their death (Percival et al., 2016).

Table 4.9: *Escherichia coli* (ATCC 43888) growth across the catheter bridge model for PDA/PVP-coated biomaterials with and without iodine over a 72-h duration.

Sample	Microbial growth time (h)		
	24	48	72
PDMS	 Positive	Positive	Positive
PDMS-PDA/PVP	 Positive	Positive	Positive
PDMS-PDA/PVP (0.1% I_2)	 Negative	 Positive	Positive
PDMS-PDA/PVP (0.5% I_2)	 Negative	 Positive	Positive
PDMS-PDA/PVP (1% I_2)	 Negative	 Negative	 Positive

PU-polyurethane, PDMS-polydimethylsiloxane, PDA-polydopamine, PVP-polyvinyl pyrrolidone and I_2-iodine

4.5 Impact of iodine concentration loaded in the polydopamine/polyvinyl pyrrolidone-coated biomaterials

By considering the observations made from the biocompatibility and AST assays, particularly the XTT cytotoxicity, BSLA, disk diffusion, broth microdilution assays and catheter bridge model, an insight can be drawn that describes the relationship between the biocompatibility and antimicrobial efficacy of the PDA/PVP(I_2) coating technology. The study demonstrated that an increase in the concentration of iodine loaded in the PDA/PVP-coated biomaterials will reduce biocompatibility while improving antimicrobial activity or vice versa. This is expected since iodine is a broad-spectrum cell-killing agent, therefore at a higher concentration, it will even be toxic towards eukaryotic cells by disrupting their cell membrane. Similar findings have been observed with iodine-incorporated PVP hydrogels (Müller and Kramer 2006). For this instance, the coating technology was observed to be potentially toxic when loaded with a concentration of 1% w/v iodine with the XTT and BSLA. However, the same concentration demonstrated

the highest degree of antimicrobial activity with the disk diffusion, broth microdilution and catheter bridge model assays.

Based on the XTT and disk diffusion assays, Figure 4.12 can be used to summarise the impact of the concentrations of iodine loaded in the produced biomaterials. A trend line based on disk diffusion assay suggests that an increase in the concentration of iodine correlates with an increase in the size of zones of inhibition of *E coli* produced by the coated samples. While the former assay was used to produce 3 regions on the chart, with region A showing the concentration of iodine (≥0.5% w/v) considered safe (non-cytotoxic according to ISO 10993 since it caused less than a 70% decrease in the viability of L292 cells). Region C showed potential toxicity since it decreased the viability of L292 cells by more than 70% while region B shows the concentration of iodine that is untested. Figure 4.12 also demonstrated that region B, PDA/PVP-coated biomaterials loaded with more than 0.5% but less than 1% iodine w/v may be safe but still possess improved antimicrobial activity compared to samples loaded with 0.5% w/v or less iodine. These findings suggest that the iodine concentration loaded on the PVP hydrogel coating can be further optimised.

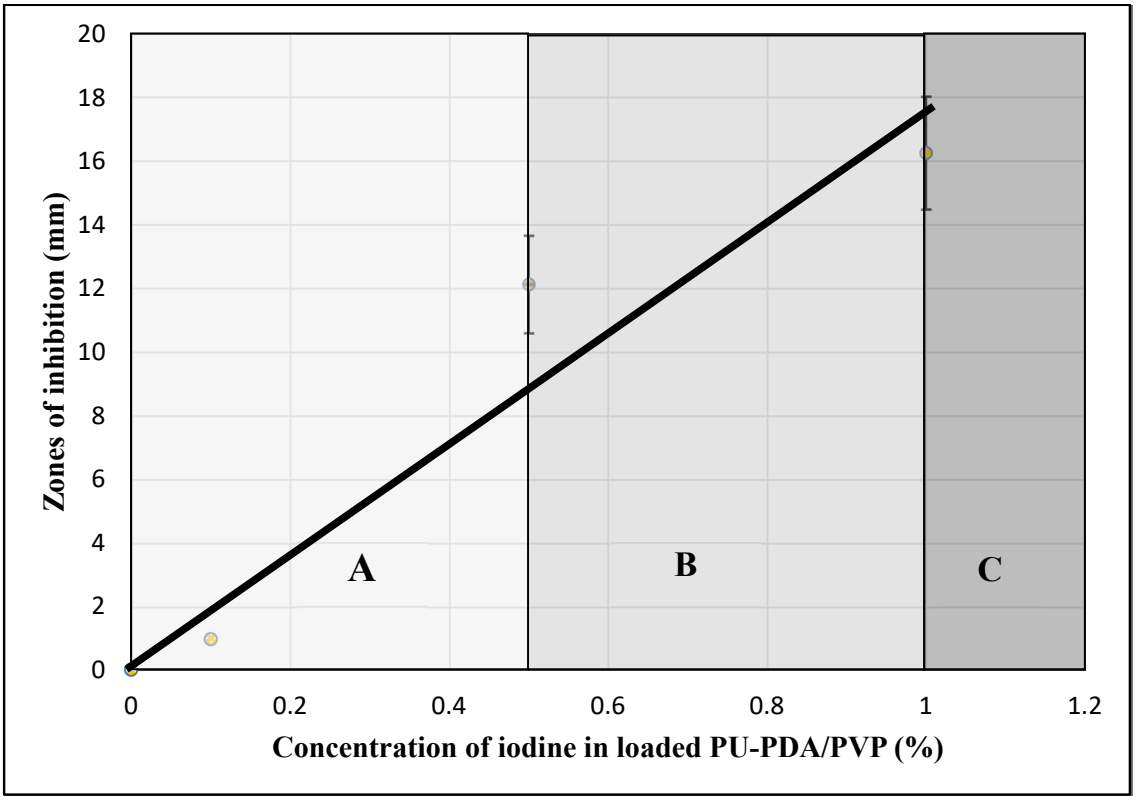


Figure 4.12: Effect of the concentrations of iodine (% w/v) loaded in the polydopamine/polyvinyl pyrrolidone-coated polyurethane biomaterials, on their observed zones of inhibition and cytotoxicity represented by A) safe region, B) unknown region and C) toxic region.

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Coating technologies remain an attractive and viable improvement strategy for urological implants since they can be tailored to meet specific demands by incorporating various materials. These include hydrogels and antimicrobial agents which possess desirable characteristics such as lubricity and mitigation of UTIs, respectively. In addition, a combination of these materials can be used to develop coating technologies which possess multiple desirable qualities. This study aimed to develop a novel universal coating technology characterised by a low friction coefficient with urinary tract tissues and antibacterial properties and is thus suitable for urological devices, by using a primer and a hydrophilic polymeric material coupled with an antimicrobial agent. The objectives set to accomplish the aim include the following:

- To fabricate a universal technology for urological implant polymers, characterised by a low friction coefficient using a primer and a hydrophilic polymeric material coupled with an antimicrobial agent.
- To evaluate the biocompatibility properties of biomaterials produced with the developed technology, by cytotoxic and mutagenicity analysis.
- To evaluate the antimicrobial properties of the biomaterials produced with the developed coating technology.

The objectives were achieved and the following are the key findings

- A coating technology adaptable to polymeric surfaces with varying polarity can be successfully fabricated with a polydopamine (PDA) primer and a polyvinylpyrrolidone (PVP) hydrogel as a top layer loaded with iodine (I_2). The produced PDA/PVP (I_2)-coated biomaterial demonstrates improved slipperiness and swelling when hydrated, a character of hydrogel surfaces with a low friction coefficient.
- PDA/PVP (I_2)-coated biomaterials are biocompatible according to the XTT cytotoxicity and brine shrimp lethality assays when the hydrogel is loaded with an iodine concentration of 0.5% w/v or less. Potential toxicity can be expected when an iodine concentration ≥1% w/v is incorporated. PDA/PVP-coated biomaterial loaded with an iodine concentration of ≤1% w/v does not demonstrate base shift genotoxicity according to the Ames mutachrome mutagenicity assay.

– PDA/PVP-coated biomaterials loaded with an iodine concentration of ≥0.5% w/v demonstrated antibacterial efficacy against *Escherichia coli* and *Proteus mirabilis* including a uropathogenic strain (ATCC 4388) at 0.5 McFarland standard.

Overall, this study demonstrated that a coating technology developed with PDA as a primer and a PVP hydrogel top layer loaded with a concentration of 0.5% w/v iodine is a promising approach for improving the properties of urological implants. The coating is adaptable to various polymeric biomaterials commonly employed to fabricate urological implants, reduces the friction coefficient of implant surfaces, and demonstrates good biocompatibility and a sufficient level of antimicrobial activity to mitigate UTIs.

5.2 Recommendations and directions for future work

Future work could include *in-vivo* biocompatibility studies and investigating the impact of the coated biomaterials when used over an extended implantation period. It is also recommended to investigate appropriate device sterilisation methods and the shelf-life of the developed materials.

- Further validation of the biocompatibility of the coating technology

Although the novel universal coating technology is a promising strategy for improving urological implants, in-vivo studies as recommended by standardisation bodies such as ISO 10993 are still required to further confirm biocompatibility. ISO 10993 provides a list of animal studies that can be performed to prove that the coating technology does not cause biological effects when in contact with the human body during its intended application. Furthermore, specific evaluation methods that enable a wash cycle similar to clinical settings should be adopted. For instance, a urinary bladder model which allows the flow of artificial urine has been adopted to evaluate the amicrobial properties of a treated urinary catheter (Kazmierska et al., 2010). This is particularly important since this study demonstrated that the concentration of iodine loaded in the coating technology impacts biocompatibility and antimicrobial activity. During a wash cycle when urine flows or is drained through the urinary tract system, the concentration of iodine loaded in the coating technology may be diluted.

- Impact of intended implantation period

While many evaluation methods employed in this study are designed to run for around 24 h, it is important to recognize that the effects of the coating technology may continue to develop

beyond this initial period. Therefore, it is necessary to develop and employ evaluation methods that can detect the effects of the coating technology over a longer duration.

Regulatory bodies such as the EC and IMDRF may classify the same implants differently based on the intended duration of use. For example, implants designed for more than 30 days (longer-term) use may be subject to more stringent regulations. Therefore, to ensure that the evaluation methods used are appropriate for the intended period of use, it may be necessary to conduct studies that extend beyond 24 h. Ideally, the evaluation methods used should match the intended period of use. This may involve longer-term *in vitro* and animal studies that can provide insight into the long-term effects of the coating. Additionally, it may be necessary to develop new evaluation methods that can simulate the long-term conditions that the implant coating will experience.

- Sterilisation investigation

Invasive medical devices need to be sterile before they are used to prevent infections. As a result, sterilization methods are routinely used in the medical devices industry. There are several sterilization methods approved for medical devices, including steam sterilization (autoclaving), ethylene oxide (ETO) sterilization, gamma irradiation, electron beam (E-beam) sterilization, hydrogen peroxide sterilization, and other chemical sterilization methods. It is important to choose the appropriate sterilization method to ensure that the coating technology is not modified or degraded and that no toxic residues are left behind (Harrington et al., 2020). In addition, regulatory bodies require proof of a medical implant's sterility and its maintenance until the period of intended use. Therefore, standards including 10993-7 are adopted to prove that medical devices are sterile and do not harbour any toxic residues after sterilization. It is crucial to follow such standards to ensure that medical devices are safe for use. Thus, an appropriate sterilization method should be prescribed, taking into account other factors such as the type and size of material used. Therefore, it is still necessary to carry out an investigation to evaluate the most appropriate sterilisation method for this coating technology.

- A shelf-life investigation

A shelf-life study also needs to be conducted to determine the appropriate storage conditions and for how long the technology remains effective and safe. The storage condition describes the temperature range, pressure and oxygen levels which do not affect the stability and thus efficacy of coating technology. In addition to determining the appropriate storage conditions, a

shelf-life study also determines the expiry date. This can be achieved by monitoring the efficacy and safety of the coating over an extended period (Rathinam et al., 2021).

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