



Review Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Wafers for wound healing

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ABSTRACT

Wounds are subjected to infection of many microorganisms, so many antimicrobial agents are used to treat these chronic wounds. Wafers are being developed as drug delivery systems that can be applied directly to the surface of suppurating wounds. These wafers instantaneously adhered to the surfaces, absorbing water and transforming from glassy, porous solids to highly viscous gels. Wafers were characterized for morphology, mechanical and in vitro functional (swelling, adhesion, drug release in the presence of simulated wound fluid) characteristics. The optimized dressing has the potential to reduce bacterial infection and can also help to reduce swelling and pain associated with injury due to the anti-inflammatory action and help to achieve more rapid wound healing. Polymer-drug interactions and physical form were characterized by Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD), respectively, while morphological structure was examined using scanning electron microscopy (SEM). Functional characteristics (mechanical hardness and adhesion using texture analyzer, and swelling capacity) of blank wafers were determined in order to select the optimal formulations for drug loading. Finally, the in vitro drug dissolution properties of two selected drug loaded wafers were investigated. Many wafers are also used in cancer treatment and their toxicity is reported. The new innovative studies of the wafers gives an over view which is used to treat Corneal Cystinosis and also to treat dry eye in order to restore healthy corneal surface. This review helps to outline the novelty of the wafer in the future development of pharmaceutical dosage forms.

Keywords: Antimicrobials, wafers, glassy, porous, viscous gels, anti-inflammatory.

INTRODUCTION

A **wound** is a type of injury to the skin which happens very quick where the skin is cut, torn, or punctured (an *open* wound), where blunt force trauma causes a contusion (a *closed* wound). Wounds may also include diabetic ulcers and burns. Minor wounds are not very serious, but it is very important to clean it so that we can prevent them from infections. Infection in a wound delays healing and causes the wound to breakdown. [1]

It is widely documented that patients with chronic wounds are seen with problems such as leakages from dressings, malodour, and pain. [2]

Acute wounds: They are disruptions in the integrity of the skin and underlying tissues that progress through the healing process in a timely and uneventful manner.

Chronic wounds: These are those wounds which fail to proceed through an orderly process of healing (restoring the loss of tissue and skin function) in the expected period of time, usually within 30 days and for these wounds which do not respond properly for the initial treatment. [8][9]

A wound may fail to heal due to the use of corticosteroids leads to formation of squamous cell carcinoma and persistent infection. Chronic wounds may include Diabetic, Arterial, Venous, Pressure ulcers or “bed sores” related. [3]

It has been estimated that more than 23% of all hospital in-patients have a pressure ulcer and most pressure ulcers which occurs during hospitalization for an acute episode of illness/injury [4]

Types of wounds:[5]

1. **Lacerations:** It is caused due to cut or tearing of the tissue using local anesthetic is for the closure of the wounds. Sterile dressings are applied for the wound to heal. [Figure- 1]



[Figure- 1]

2. **Abrasions** –Here the superficial layer of tissue is removed. Local anesthetic can be used for pain, however the treatment of the wound is non-surgical, by using moist dressings and a topical antibiotic to protect the wound and aid healing. [Figure- 2]



[Figure-2]

CONTUSION

Injuries caused by the forceful blow to the skin and soft tissue, keeping the outer layer of the skin intact. These injuries require minimal care because there is no open wound [Figure- 3]



Contusions

[Figure- 3]

3. **Avulsions** – Injuries where a section of tissue is torn off, either partially or in total. In partial avulsions, the tissue is elevated and remains attached to the body. Total avulsion means that the tissue is completely torn from the body with no point of attachment [Figure- 4]



Avulsion

[Figure- 4]

➤ Miscellaneous wounds may include:[6]

- **Thermal wounds:** wounds which occur at extreme temperatures, it may be either hot or cold and can result in thermal injuries like(sunburns, burns and frostbite)
- **Chemical wounds:** They are formed in contact with or inhalation of chemical materials that cause lung or skin damage.
- **Stings and Bites:** Bites can be from humans, bat ,dogs, rodents, , snake tick and scorpions
- **Electrical wounds:** These are present with superficial burn-like or sting-like wounds secondary to the flow of high-voltage electrical currents are made to pass through the body, and may cause more severe internal damage.

Diabetic Foot Ulcers:

Many patients with diabetic foot ulcer are due to atherosclerosis that delays the blood flow [10]. It is a combination of vascular and neurological abnormalities of the disease. Diabetic to the extreme areas and peripheral neuropathy which prevents the sensation and discomfort associated with mechanical stress or injury to the feet. Patients develop an excessive thickness of the basement membranes in the muscles. When compared to the healthy people the basement membrane is 2 folds greater in diabetics. This may lead to further complications poor blood supply (hypoxia) to the blood vessels which lead to the formation of cell death and necrosis [11]. So as a result the breakdown of the skin after prolonged pressure and initiation of trauma leads to ulceration [12]

Diabetic foot ulcers is classified using the Wagner Classification System.[13] This system is based mainly on wound depth which consists of six wound grades.

- Grade 0 foot ulcers have dry keratinized skin that increases the potential for ulceration
- Grade 1 consists of ulceration of the dermis
- Grade 2 consists of ulceration of tendons and joints,
- Grade 3 extends to the bone and leads to osteomyelitis
- Grade 4 we can see localized gangrene
- Grade 5 consists of gangrene involving a major portion of the foot. [14]

Foot care can often help in healing foot ulcers caused by diabetic neuropathy, whereas in ischemic foot ulcers it is often difficult to heal, unless the underlying vascular problems have been corrected.[10 ,14]

Pressure ulcers:

Pressure ulcers are also called as “bed sores” or “Pressure sores” chronic ulcer is caused by constant pressure, due to a bony prominence which results in destruction to underlying tissue. [15] .These pressure ulcers result in ischemia leading to tissue necrosis .Ischemic cell death results in inflammation leading to blood clot further leading to damage of the bones and muscles some of the factors that increase the risk advance age , excessive exposure to moisture and impaired nutrition (i.e. wound drainage , incontinence).[16]

Generally Pressure ulcers are caused by various pathogens such as *P. aeruginosa*, staphylococci and enterococci and anaerobes such as *Clostridium* spp. which prolong the delay of wound healing. Further complications leads to osteomyelitis and cellulitis due to bacterial colonization. [17]

WOUND HEALING:

Wound healing is a complex process of replacing devitalized and missing cellular structures and tissue layers.[18]
Wound healing consists of different phases they include:[7]

INFLAMMATORY PHASE:

In this phase a clot is formed and the cells of inflammation debride the injured tissue. The inflammatory response causes the release of the plasma and neutrophils to the surrounding tissue and blood vessels to become leaky. Inflammation causes decrease in the host resistance and increase in the bacterial burden. It forms as a barrier to the bacterial infections. Inflammation phase is divided into two phases they are:

- Early inflammatory phase
- Late inflammatory phase [19]

In early inflammatory phase it starts during the late coagulation where it activates the cascade and initiates the molecular events leading to the infiltration of the wound site with neutrophils whose main function is to prevent infections .the neutrophils starts with the phagocytosis in, order to destroy the bacteria and remove the foreign particles and damaged tissues .[20,21,19]

PROLIFERATIVE PHASE:

In this phase on the surface of the wound epidermal cells gets into the mitotic activity leading to proliferation (multiplication of the cells). Then haemostasis is achieved and an immunological functions [83, 84] is successfully set into place, the acute wound shifts toward tissue repairing [19,22]

Fibrin and fibronectin is formed, fibroblasts proliferate into the deeper parts of the wounds these fibroblast synthesizes small amount of collagen which acts as a scaffold for the migration and further fibroblast proliferation then abundant formation of granulating tissue is seen this diverse phase takes place in this proliferative process.[23,24]

Angiogenesis and granulation tissue formation leads to tissue remodeling and gives rise to new blood vessels during this process various neutrophils, macrophages and angiogenic factors helps to promote angiogenesis.[25,26, 27]

REMODELLING PHASE:

Remodeling phase is responsible for the formation of new epithelium and final scar tissue formation there's synthesis of extracellular matrix along with granulation tissue development in this phase this phase may take up to one or two years for a prolonged period of time.[28,29]

The remodeling of the acute wound leading to maintain a delicate balance between synthesis and degradation along with collagen bundles, intercellular matrix and where fibronectin and hyaluronic acid are degraded. Collagen fibers regains 80% of its original strength with unwounded tissues. [24,30,31]Metalloproteinase is an enzyme produced by neutrophils ,macrophages and fibroblasts in a wound which is responsible for degradation of collagen leading to formation of new matrix and this will help in normal wound healing.[31, 31, 33].

HEALING BY PRIMARY AND SECONDARY INTENTIONS:

In the healing process different cells dominate various phases of repair and the cellular patterns vary according to the extent of injury and tissue damage.[34,35,36]

During the normal wound healing the ,closed incisions and open wound tissues defects progress in a sires of molecular and cellular events resulting in tissue repair and regeneration healing of clean wounds uninfected surgical incisions and without loss of tissues this is referred to as primary intentions. [39, 37, 38]

Death due to limited number of epithelial cells and connective tissues occurs cause of small disruption of basement membranes, it is a fast process in deals with the open wound with extensive loss of tissue, here this reparative

process is more complicated because large tissue loss have to be filled which occurs due to healing by secondary intention. [34, 40, 43, 42, 44]

FACTORS AFFECTING WOUND HEALING:

There are many factors leads to impaired wound healing they may be categorized into

➤ Local factors that influence wound healing.

➤ Systemic factors that influence wound healing.

➤ Local Factors that Influence Wound Healing:

❖ **OXYGENATION:** Oxygen is very important for the cell metabolism where the energy is obtained by ATP and is very much needed in the wound healing process .It induces angiogenesis sand prevents the wound from infections helps in re- epithelialization, increases fibroblast proliferation ,collagen synthesis also promotes contraction of the wound. [45,46]

❖ **INFECTIONS:** Many microorganism obtain access into the underlying of the skin causing local infections these microorganisms replicate rapidly with the wound causing injury. [47]*P. aeruginosa* and *Staphylococcus* are some common microorganisms which cause bacterial infection in wounds. Many chronic ulcers do not heal because of the presence of biofilms containing *P. aeruginosa*, hence shielding the bacteria from the phagocytic activity of invading polymorph nuclear neutrophils (PMNs). This mechanism can explain why antibiotic failed to treat some chronic wounds. [48]

➤ Systemic Factors that Influence Wound Healing:

❖ **AGE :** Age is one of the unique that affects the wound healing process ,increase in the age results in the major risk in wound healing which is commonly seen in adults aging causes temporal delay in wound healing , not actual impairment in quality wound healing [49]

Delayed wound healing in aged persons is seen due to delayed T-cell infiltration into wound area causing the chemokine production which reduces phagocytic and macrophage capacity .[50]

❖ **STRESS:** Stress causes many cardiovascular disease[81,82], diabetes ,cancer are comprised wound healing .Many studies have shown that both humans and animals demonstrate physiological stress which causes delay in wound healing .[51,52]

Sex Hormones in Aged Individuals:

Sex hormones have effect on the wound healing process .Compared to females males have showed much delayed healing in the acute wounds.

A partial explanation is seen that the female estrogens (estrone and 17 β -estradiol) and male androgens (testosterone and 5 α -dihydrotestosterone, DHT) and their steroid precursor dehydroepiandrosterone(DHEA) which appear to have significant effects in wound-healing process.[53] It was found out that the differences in gene expression between young human and elderly male wounds are almost exclusively estrogen-regulated. Estrogen affects the wound healing by regulating various genes which is associated with regeneration .[54]. Many studies have shown that estrogen have the ability to improve the age related impairment of wound healing both in men and women. [53]

Diabetes

Diabetes causes impairment in wound healing .Moreover large number of people are suffering from Diabetic Foot Ulcer (DFU) it causes further complications like amputations [55], venous stasis disease ,DFUs and pressure-related chronic non-healing wounds, are always accompanied by hypoxia [56]. Hypoxia can increase the levels of oxygen radicals which leads to early inflammatory response [57], hyperglycemia and the interaction with their receptors(RAGE) is associated with impaired wound healing in diabetic patients.[58]

Medications

Large number of medications may interfere with the clot formation, anti-inflammatory responses and cell proliferation which has the capacity to affect wound healing process. Many drugs like, anti-inflammatory drugs, non-steroidal drugs, glucocorticoid steroids and chemotherapeutic drugs.[59,60,61]

Obesity

Obesity is the main cause for many diseases and health conditions like stroke, type 2 diabetes, hypertension, cancer, sleep apnea, dyslipidemia, respiratory problems and also impaired wound healing. Obese patients face wound complications like hematoma and skin infections, pressure ulcers [62] during surgery in the obese patients at the surgical site it leads to ischemia and hypo perfusion and that is seen in subcutaneous adipose tissue leading to decrease delivery of antibiotics causing slow wound healing.[62, 63]

Therefore obesity related changes is seen in the peripheral immune function which can be improved by weight loss.[64]

Alcohol Consumption

Many evidence have shown that alcohol consumption slows the wound healing process and increase the infection.[65] Ethanol toxicity increases the susceptibility to infections. [66] Alcohol levels of 100 mg/dL leads to angiogenesis, perturbed re-epithelialization, collagen production where in acute ethanol causes decrease in the angiogenic capacity leading to decrease in wound vascularity causing oxidative stress and hypoxia leading to impaired wound healing.[67]

Smoking

Smoking causes a various number of complications in human health it is well known to cause stroke, vascular diseases, chronic lung diseases the negative effect for wound healing is well known for a long time.[68]

Persons who smoke tend to show delayed wound healing with various complications like tissue rupture, infections, anastomatic leakage, flap necrosis is leading to decrease in the tensile strength of the wounds which causes impaired wound healing.[69,70]

Nutrition

Nutrition is one of the very important factor for wound healing, malnutrition or nutrition deficiency have an impact on wound healing nutrients like carbohydrates, fats and proteins are the primary source for the wound healing process where the glucose is the major fuel to create cellular ATP which helps in deposition of the new tissues and source of energy for the Angiogenesis and use of ATP is also prevents the depilation of amino acids and protein substrates. [71,72]

Protein

Protein is one of the major component for the wound repair deficiency of protein can impair capillary formation, proteoglycan synthesis, collagen synthesis, fibroblast proliferation and wound remodeling. It also results in affecting the immune system and decrease in phagocytosis and increases the susceptibility for microbial infections.[73]

Collagen

Collagen synthesis requires certain co-factors like ferrous iron and vitamin C along with hydroxylation of lysine and proline hence the deficiency in any of these co factors results in impaired wound healing. [74]

Arginine

Arginine is one of the component which improves immune function, and helps in the wound healing in healthy and ill patients.[75]

Glutamine

Glutamine is one of the most abundant amino acid present in plasma and it is a major source of metabolic energy for rapidly proliferating cells such as lymphocytes, epithelial cells, macrophages and fibroblasts.[72, 74]

Oral glutamine supplementation has shown to Improve the wound breaking strength and also increase levels of mature collagen.[76]

Lipids

Lipids are used for nutritional support for critically ill patients in order to meet the energy requirements which is the building blocks for tissue repair and wound healing, omega-3 fatty acids on wound healing are said to be not conclusive. It is said to affect cell metabolism, angiogenesis and gene expression at the site of the wound.[77]

Vitamin C

Vitamin C is very potent anti - oxidant and also helps in wound healing, deficiency in this leads to impairment in the tissue repair and also leads to wound infections. [72,74]

Vitamin E

Vitamin E is an antioxidant and maintains cell integrity it also has anti - inflammatory response [85] and decreases scar [86] formation in chronic wounds, Vitamin E when used topically acts as an anti scarring agent in chronic wounds, hence Vitamin E supplements is useful for wound healing. [78]

Many micronutrient supplements have shown their role in wound repair like zinc is a cofactor for DNA and RNA polymerase and deficiency of this leads to impaired wound healing Hence zinc is very much important for wound healing. [71, 72, 74, 89] Therefore high energy supplements containing arginine, protein enriched supplements, vitamin E, vitamin C can improve healing of chronic pressure ulcer [70] Multiple factors can cause impaired wound healing in different phases therefore each factors have a influence on the wound healing process. [80]

RATIONALE FOR SELECTING:

Some dressings, such as hydrocolloids can cause infections, and open wounds have a high risk of infection (i.e. up to 6.4%) when it is treated with primary gauze, which lead to an average hospital stay of 10.6 days [95]. Many commercial dressings are available that contain silver, such as Aquacel[®] hydrofiber, Acticot[®] absorbent, Urgocell[®] silver and Poly Mem[®] silver [96]. Therefore, incorporation of antimicrobials in lyophilised wafers is essential not only to promote wound healing but also to prevent infection from microorganisms. Antimicrobials, such as neomycin and silver nitrate and sulphacetamide sodium are broad spectrum antimicrobials and commonly used to treat infected wounds. [97]

NEED FOR STUDY

The objective of the invention was therefore to provide thin sheet like pharmaceutical preparations (wafers) for the administration of active substance which are having an unpleasant taste containing at least one matrix forming polymer. The pharmaceutical preparation according to the present invention are suitable for a plurality of different active substance. [98]

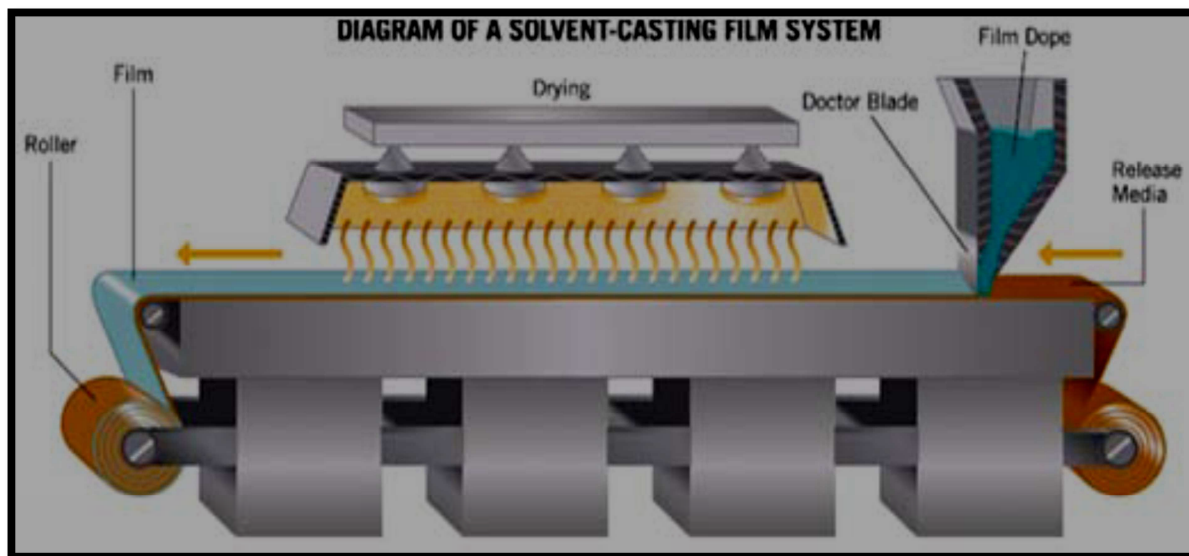
MANUFACTURING METHODS:

1. Solvent casting method: [99,100]
2. Semisolid casting
3. Solid dispersion extrusion [101]
4. Hot melt extrusion [102,103]
5. Rolling Method

1. Solvent casting method :

In solvent casting method the water soluble polymers is dissolved in water and drug and other excipients are dissolved in suitable solvents and these both the solutions are mixed and stirred dwell then finally they are casted in Petri plate and then dried after drying they are cut into desired shapes.

Water soluble polymers are used in this method e.g. hydroxyl propyl cellulose (HPC), hydroxyl propyl methyl cellulose (HPMC), polyvinyl alcohol (PVA), carboxy methyl cellulose (CMC). Solvents used are from the solvent list of ICH class 3. Equipment used in large scale production is shown below; [Figure- 5]



[Figure –5]

2. Semisolid casting:

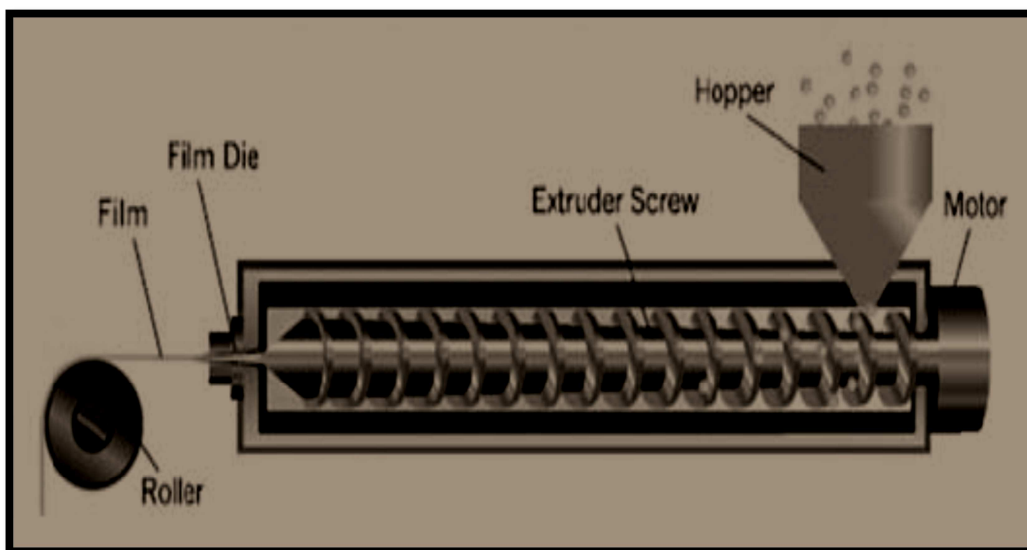
In this technique water soluble hydrocolloids are dissolved in water in order to form a viscous homogeneous solution, then later active ingredients are added and both solutions are mixed together and degassed under vacuum. This bubble-free solution is then coated on a non-treated casting wafer. The coated wafer is then sent into the oven for drying. The wafers are then cut into desired shapes and sizes.

3. Solid dispersion extrusion:

In this technique the immiscible components are extruded along with the drug, then a solid dispersion is prepared. The obtained solid dispersions are then shaped into wafers by using dies.

4. Hot melt extrusion:

In hot melt extrusion technique [Figure- 6] initially the drug is mixed with suitable carriers in solid form. Then the extruder, having heaters, helps to melt the mixture. Finally, the melt is then shaped into wafers by the use of dies.



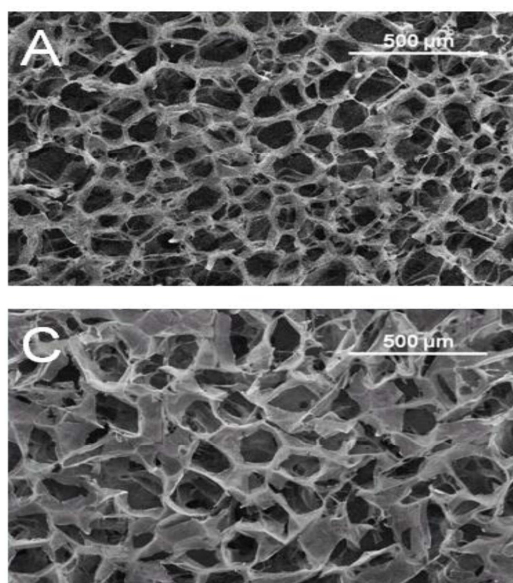
[Figure – 6]

5. Rolling Method:

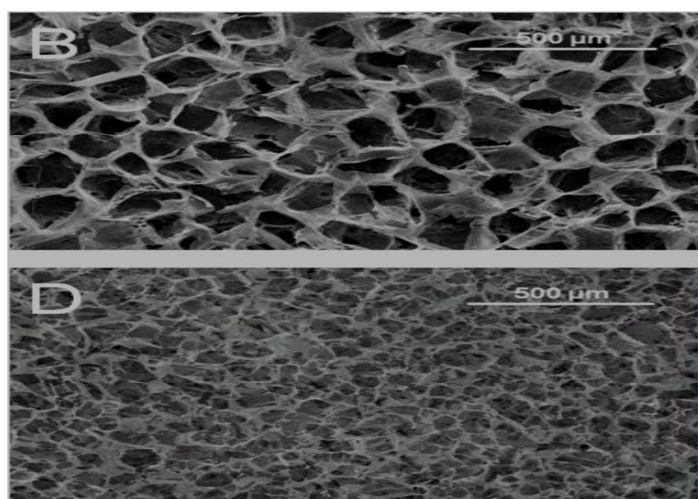
In this method a solution or suspension containing a drug is rolled on a suitable carrier. The solvent used is mainly water or mixture of water and alcohol. The active agents dissolved in small portion of aqueous solvent using a high shear processor. The Wafer obtained is dried on the rollers and cut in to desired shapes and sizes.

CHARACTERIZATION:**1. Scanning electron microscopy (S.E.M)**

A *Leo S430* digital scanning electron microscope is used to get the image of the microstructure of the drug-loaded wafers. Samples that are for imaging are coated with gold and palladium for 4 minutes. Images of the wafer surfaces (Top and Bottom) were taken at 200x and 500x magnifications. [104,105].



Scanning electron microscopy (S.E.M) images of wafer matrices top surfaces taken at 200x magnification. [Figure – 7]



Scanning electron microscopy (S.E.M) images of wafer matrices top surfaces taken at 500x magnification. [Figure - 8]

2. X-ray diffraction (XRD):[105]

XRD analyses of the wafers were performed using D8 Advantage X-Ray diffractometer (Bruker AXS GmbH, Karlsruhe, Germany). The lyophilised wafers were compressed to a width size of 0.5 mm using a clean pair of

compression glasses and was mounted on the sample holder. The transmission diffractograms were acquired using DIFFRAC plus XRD Commander over a start to end the diffraction angle of 2θ from 5° to 45° step size of 0.02 and a scan speed of 0.4 sec. X-ray patterns of the wafers and the starting materials were obtained with DIFFRAC plus (Bruker Coventry, UK) having an XRD commander programme. A Goebbel mirror was used as a monochromator which produced a focused monochromatic $\text{CuK}\alpha 1\&2$ primary beam ($\lambda=1.54184 \text{ \AA}$) with an exit slit of 0.6 mm. The detector used for performing the experiment was Lynx Eye. The operating condition during the experiment was 40 kV and 40 mA.

3. Differential scanning calorimetry (DSC):

DSC analysis of the POL-CAR and POL-SA (BLK and DL) wafers and starting materials (drugs) was undertaken on a DSC1 Mettler Toledo instrument (Leicester, 211 UK) calibrated with indium (based on heating range). Wafers are cut into small pieces and 3-5 mg of sample was placed into 40 μl aluminum pans with lids (Mettler Toledo, Leicester, UK) and sealed using crucible sealing press (Metler Toledo Leicester, UK). An empty aluminum pan sealed with lid was used as reference. A *STAR*[®] software program was used to run the samples by initially cooling from 25°C to -50°C and then heated from $(-50^\circ\text{C}$ to $350^\circ\text{C})$ at the rate of $10^\circ\text{C}/\text{min}$ under constant purge of nitrogen (100 ml/min) to evaluate the thermal behavior of the polymers and drugs present in the wafers.[105]

4. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR):

A FTIR spectrophotometer is used in combination with (Thermo Nicolet, Thermoscientific, UK), ZnSe attenuated total reflectance (ATR) accessory to characterize the wafers. The FTIR was equipped with potassium bromide (KBr) beam splitter and MCT detector. The wafers is placed on ZnSe ATR crystal (45°) and maximum pressure was applied by using a pressure clamp accessory in order to allow intimate contact of the wafers with the ATR crystal. Similarly the pure starting materials (drugs) was analyzed as controls.

Spectra obtained were recorded at 4 cm^{-1} resolution within a range of 650-4000 cm^{-1} using OMNIC[®] software. True absorbance of each of the sample was obtained by background subtracting spectral information for the ATR crystal.[105,106]

5. Water uptake:

The water uptake capacity of different lyophilised controls and drug loaded wafers when placed on top of a constantly hydrated cellulose membrane for 24 hours, as presented in Figure 4.1, was determined. Wafer weights were recorded prior to being placed on the membrane (W_0) and after 24 hour of water uptake (W_t), using an analytical balance, Mettler AE 50. Water uptake (WU) was calculated in percentage terms (%) using the simple formula: $W_U = (W_t / W_0) \times 100$, where W_t = weight of swollen wafer after 24 hours of hydration and W_0 = weight of lyophilised wafer at time zero.[104]

6. Water loss:

Wafer lyophilisation involved cooling the gel to -80°C and then heating them in a series of thermal ramps to room temperature (20°C) under reduced pressure from 1 atm to 0.001 mBar (Scanlaf A/S). The entire freeze-drying process took place over 26 h. Once the freeze-drying process was completed, the lyophilized wafers were removed from the jar, weighed and placed in polyethylene bags. After that, the wafers were stored in glass desiccators containing silica gel beads prior to testing. The gel and wafer were weighed before and after the lyophilisation process, and the percentage of water loss was calculated using:

$$\text{Water loss\%} = (W_g - W_w) / W_g \times 100$$

- W_g is the weight of the hydrogel (before freeze-drying) and
- W_w is the weight of the wafer (after freeze drying).[97]

7. Microscopic examination:

The morphology of the wafer was observed using a microscope (Olympus BX41) and captured with a camera. The wafer was cut into thin layers using a razor blade, placed onto the glass slide and carefully covered with a cover slip. The porous structures of Wafers were observed under 10x magnification.[97]

8. Thermogravimetric (TGA) analysis of the lyophilised wafers

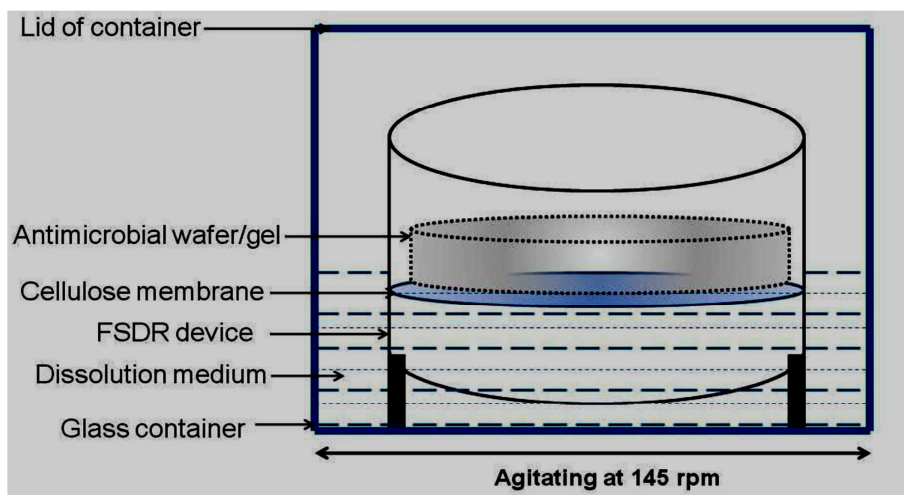
Thermogravimetric analysis was undertaken to determine the water content of the lyophilized wafers. All wafer samples were manually compressed to fit on the pre-tarred platinum sample pan. Powder-free, latex gloves were worn to avoid external contamination from skin moisture/sweat. Mean sample weights of 9.8 ± 2.4 mg were used. The residual water content of lyophilized wafers was analyzed as the weight loss of the sample during a heating ramp from 25°C – 200°C at a heating rate of $10^{\circ}\text{C}/\text{min}$. A *TA Q500 Instrument* was used for all measurements.[104]

EVALUATION TESTS:

1. In vitro release of Drug from lyophilised wafers and gels:

Six identical free standing dissolution rafts (FSDR) was constructed the lyophilised wafers and freshly prepared gels (1.5 ± 0.2 g) was placed on top of the cellulose membrane (12-14 kDa) kept in contact with the dissolution medium for 24 hours at $36 \pm 0.5^{\circ}\text{C}$. The FSDR was placed in 20 ± 2 ml of dissolution medium (NaCl-CaCl₂) in a glass container topped with a lid in order to avoid evaporation during the experiment. The enclosed dissolution system was placed in an agitating water bath (Fischer Scientific) at 145 rpm. Samples (3 ml) was taken, analyzed and returned to the glass container at hourly intervals for eight hours and after 24 hours, in order to maintain a constant volume of dissolution medium with time. The presence of drug was detected by using UV analysis at 254 nm and the concentration determined from the regression equation generated from the calibration curve in NaCl-CaCl₂ solution ($y = 0.0306x$, $R^2 = 0.9995$).

Two controls containing known amounts of the known drugs in the dissolution medium were included. The first contained known concentration of drug and was placed under the same conditions with the tested drug-polymeric carriers ($36 \pm 0.5^{\circ}\text{C}$). The second control contained known drug, and a dissolution raft with no drug-wafers/gel was placed at $20 \pm 0.5^{\circ}\text{C}$. Samples (3 ml) were taken every hour for 8 hours and at 24 hours and analyzed as described above. The absorbance indicated the amount of drug in the solution over 24 hours. [104]



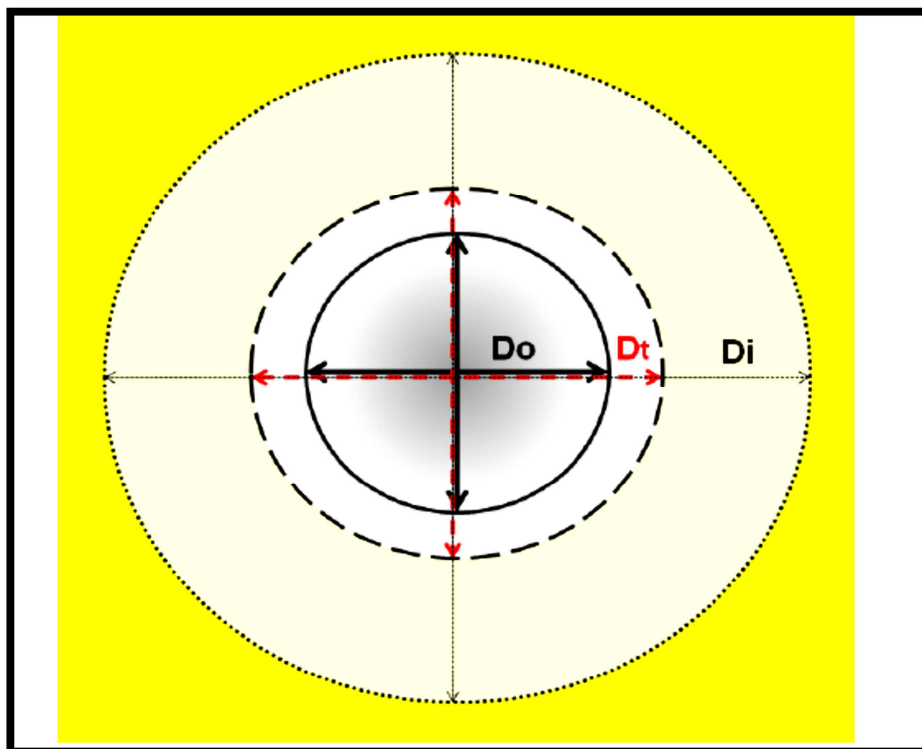
Configuration of the free standing dissolution raft (FSDR)
[Figure – 9]

2. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of antimicrobials

Antimicrobial susceptibility of MRSA, MSSA, *E. coli* and *P. aeruginosa* were determined by establishing the MIC using a standard 2-fold macro-broth dilution method in 10 ml volumes of nutrient broth inoculated with 5×10^5 cfu/ml. Suitable controls incorporating a non-inoculated bottle of nutrient broth (negative) and an inoculated bottle of nutrient broth (positive) were included in all assays. After 24 hours incubation at 37°C , the presence or not of bacterial growth was determined optically comparing each dilution to the negative and positive controls.

The MBC values were determined by plating duplicate samples of $100\ \mu\text{l}$ ($20\ \mu\text{l} \times 5$ volumes) taken from each MIC dilution on agar plates and incubated at 37°C for further 24 hours. The absence of bacterial colonies was determined

as the MBC value of the tested antimicrobial against the bacterial strains. All MIC/MBC determinations for each antimicrobial were performed twice in duplicate.[104]



Aerial view of disc diffusion measurements
[Figure -10]

3. Modified disc diffusion assay as a simple model of an exuding surface :

A modified disc diffusion assay was used to determine initially the bacterial susceptibility to the antimicrobial compounds incorporated within the matrix structure of lyophilised wafers. The method was optimized according to the protocol of the National Committee of Clinical Laboratory Standards (NCCLS) and Barry (1986). Triple vented sterile Petri dishes (90 mm) were prepared by pouring 20 ml of molten nutrient agar (1.5 %) containing yeast extract (20 g/L), peptone (5.0 g/L), sodium chloride (5.0 g/L). Each plate was inoculated with 5×10^5 cfu/ml of either MRSA, MSSA, *E. coli* or *P. aeruginosa*. Individual circular (diameter ≈ 20 mm) antimicrobial wafers, including controls, were placed onto solidified, seeded agar plates and incubated at 37°C for 24 hours. The diameter of inhibition of the antimicrobial wafers was measured from one edge of the circular zone of inhibition to the other zones of inhibition (calculated from the average of two measurements of the diameter of the inhibition zone taken at intervals of 00 and 900) and the swollen diameter of wafers (D_t) were measured after incubation using a rigid steel engineering rule (Mitutoyo). The initial diameters of the wafers (D_0) were measured with a digital caliper (Mitutoyo) prior to being placed on the centre of the seeded agar plate. Wafer expansion was calculated as the ratio of D_t/D_0 [92]. Antimicrobial activity was determined as an inhibition ratio $IR = D_i/D_0$, where D_i = mean diameter of inhibition zone.[104]

4. In vitro assessment of the efficacy of antimicrobial wafers using a diffusion cell:

The use of a Franz diffusion apparatus was altered in order to assess the antimicrobial activity of loaded wafers as they slowly rehydrated in contact with the dissolution medium. In order to perform the experiment the modified and optimized method is referred to as an antimicrobial diffusion cell (ADC). This original term is used to characterize the precise use of the diffusion apparatus, as both chambers (donor and receptor) were filled with media inoculated with known concentrations of MRSA.

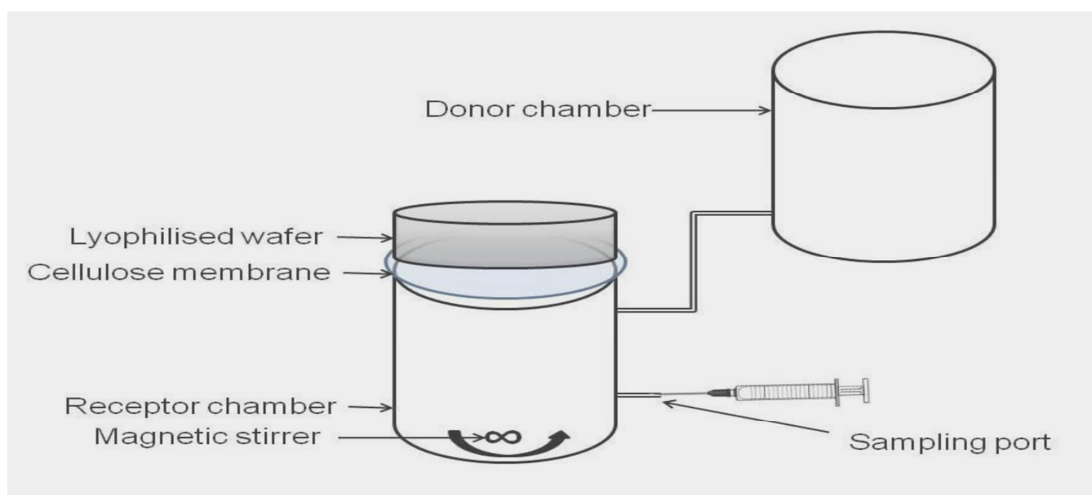
The effective diffusion area of the receptor chamber was 3.46 cm^2 , matching perfectly with the surfaces of lyophilized formulations and had a total volume of 20 mL. The receptor chamber kept at $25 \pm 0.5^\circ\text{C}$ was inoculated

to yield a final bacterial cell density of 5×10^5 cfu/ml of MRSA. An antimicrobial wafer was placed on top of a cellulose membrane (12 - 14 kDa) in contact with the inoculated medium. The system was sealed in order to maintain a continuous volume of medium in the receptor chamber (20 mL) and to keep the membrane and wafer constantly hydrated. A maximal volume of 200 μ L was withdrawn at each time point in order to minimize the introduction of untreated bacterial cells into the receptor chamber.

The sample was withdrawn using a sterile syringe and needle. Samples (200 μ L) of the dissolution medium were taken at 0, 2, 4, 6, 8 and 24 hour intervals and each diluted to sub-inhibitory levels, ten-fold down to 10^{-5} , in 0.9% (w/v) NaCl solution. Dilutions were plated in duplicate on nutrient agar plates (5 μ L \times 20 volumes) and incubated at 37°C for 24 hours. After incubation, bacterial colonies were counted and the original cell count (cfu/ml) calculated. The lowest detectable limit of the method was 100 cfu/mL.

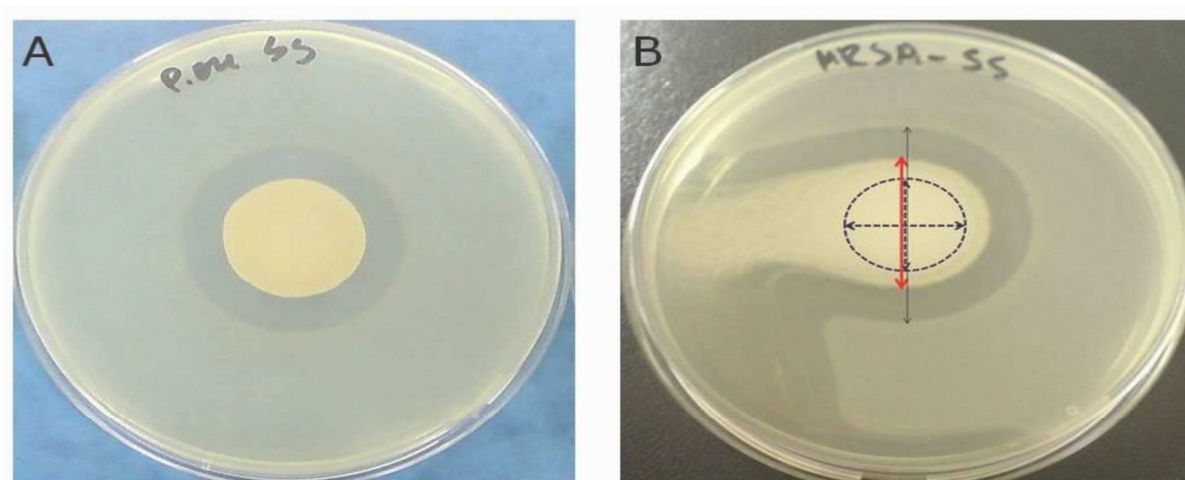
Different dissolution media were used and inoculated with the same MRSA densities, in order to compare the activity of released antimicrobials from the re-hydrated matrices of antimicrobial wafers. The dissolution media of the chambers were stirred at 1200 rpm and consisted of either:

- Phosphate buffer solution (PBS) containing 0.2 % potassium chloride (KCl) and 0.8 % sodium chloride (NaCl) (pH 7.4).
- Sodium/calcium chloride solution (pH 6.5) containing 142 mol/litre of sodium ions (0.82 %) and 2.5 mol/litre of calcium ions (0.027 %), typical of those found in wound exudate Thomas (2007),
- Pseudo-exudate containing sodium/calcium chloride solution enriched with 3 % (w/v) BSA (pH 7.4), similar to protein levels found in wound exudate (Clought and Noble 2003).[104]



Schematic of antimicrobial diffusion cell (ADC) assembly used for in vitro determination of antimicrobial measurements
[Figure -11]

5. A modified disc diffusion method was used to investigate the performance and antimicrobial Properties of irradiated wafers. Measurements of the of initial diameter of the wafers (D_0), the swollen diameter of the wafers (D_t) and diameter of the inhibition zone (D_i) were taken at intervals of 00 and 900 using a digital caliper and steel engineering ruler (*Mitutojo*). The wafers was taken (with and without antimicrobial), was the swollen diameter (D_t) and diameter of the inhibition zone (D_i) measured in one direction as indicated in Figure, due to a total erosion of the disc shape of wafers after 24 hours incubation at 37 °C. Expansion and inhibition ratios were calculated as $ER = D_t/D_0$ and $IR = D_i/D_0$, respectively.



Typical images taken after 24 hours incubation
 A - Non-irradiated and B - irradiated antimicrobial wafer.
 [Figure -12]

PROPERTIES THAT HELPS IN WOUND HEALING ACTIVITY:

The wound healing process is a complex phenomenon and involves different phases such as haemostasis, inflammation, proliferation, remodeling and scar maturation. [107,108] based on the nature of the repair process, wounds are classified as acute and chronic. Compared with acute wounds, chronic wounds represent a medical challenge due to various complicating factors including diabetes and malignancies, chronic systemic inflammation, persistent infection, destruction of neighboring tissues, poor primary treatment and other patient related factors such as poor nutrition.[109]

Lyophilised wafers can be suitable formulations for topical drug delivery on every moist biological membrane as they can be self-adhesive. Adhesion at the site of delivery can provide targeted delivery of the appropriate therapeutic compounds. Porosity and hydrophilicity have been considered as the most dominant parameters which govern the swelling behavior of drug loaded, hydrophilic polymers as the drug is being released as the polymer swells. [110]

Lyophilised wafers are produced by freeze-drying polymer solutions and gels to yield solid porous structures that can easily be applied to exuding wound surfaces. [90]

It is anticipated that a lyophilised polymer matrix would preserve the size, shape and form of contained compounds unlike a conventional gel suspension, where crystal ripening, agglomeration and polymorphic changes may occur.[111] Their physical architecture resembles those of foam dressings which are made of porous polyurethane drug stability is better in a lyophilised dosage form compared to a semi-solid hydrogel based formulation. [112]

Lyophilized wafers provide a potential means of delivering pharmacological agents to wound surfaces to aid healing they have the ability to incorporate soluble and insoluble antimicrobial compounds greater than their minimum bactericidal concentration for antibacterial activity against pathogenic bacteria. The wafer absorbs wound exudates and transforms into a gel, thus providing a moist environment which is essential for wound healing.[97,104]

Lyophilised wafers can be considered as ideal carriers of therapeutic agents, including antimicrobials and therefore are thought to be efficient systems to deliver antimicrobial treatment on a wide range of suppurating chronic wounds.[104]

WAFERS USED IN PLASMID DELIVERY:

Plasmid DNA delivery by physical methods generally results in low but sustained expression in vivo, which is limited by poor uptake due to factors such as degradation and clearance.[113,114] Physical (e.g., ultrasound, hydrodynamic injection) methods are continually being improved to enhance cellular uptake of DNA by altering cell permeability. [113,115] Plasmid uptake may involve intrinsic cellular processes, but the processes governing

intracellular transport remain elusive. Following delivery to the nucleus, expression can typically occur over time scales of days to weeks or months.[115]

Plasmid DNA interacts weakly with many polymers, leading to in vitro release from the vehicle with rates modulated by the polymer properties. Many synthetic and natural polymers are negatively charged, and thus the weak interactions likely result from repulsive charge interactions between plasmid and polymer. Collagen based materials release plasmid DNA for times ranging from hours to days. [116,117]

Carmustine wafer:

Wafer implants are a way of giving chemotherapy for brain tumours into the area of the tumour. The wafer is made of gel that contains a chemotherapy drug. During brain surgery to remove some or all of tumors, the doctor puts up to 8 wafers in the space where the tumour was over the next few days, the wafers slowly release a chemotherapy drug called carmustine (BCNU) into this area. The wafers dissolve over 2 to 3 weeks.

When chemotherapy wafer implants are used:

Using chemotherapy wafers as well as surgery and radiotherapy can help some people with glioma to live longer. At the moment wafers are licensed for people with either;

- High grade malignant glioma
- Glioblastoma multiform which has come back after treatment.[118]

Malignant gliomas are the most aggressive type of brain tumor, with poor prognosis after recurrence. The most common site of recurrence is within two centimeters of the resection margin. BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea) wafers ("BCNU wafers") are used to deliver local high dose chemotherapy around the resection cavity, and serve as a treatment option for treating malignant gliomas in an effort to delay tumor recurrence. [119]

Gliadel Wafer:

Two unique features set the Gliadel wafer apart from other drug delivery systems. First, it is a localized drug delivery system that delivers high concentrations of the chemotherapeutic agent directly to the tumor cavity, thereby overcoming the adverse side effects associated with conventional systemic administration. Secondly, Gliadel wafers are biodegradable and dissolve slowly over time releasing high concentrations of the drug in a sustained fashion over an extended time interval.

Carmustine is a nitrosourea that is commonly used as a palliative therapy. It is also an antineoplastic agent. It is used in combination with other approved chemotherapeutic agents for treating brain tumors, multiple myeloma, Hodgkin's disease, and non-Hodgkin's lymphomas. It is homogeneously distributed in the copolymer matrix. Carmustine is highly lipophilic, and thus crosses the blood-brain barrier readily. Carmustine leads to the inhibition of DNA synthesis, RNA production and protein synthesis by cross linking with DNA and RNA. It also carbamoylates proteins, including DNA repair enzymes, resulting in an enhanced cytotoxic effect. [120]

TOXICITY REPORTED:

Gliadel wafer treatment cannot be used on patients who are allergic to carmustine. Animal studies have shown that carmustine in other dosage forms causes birth defects and other problems, hence can cause fetal harm when administered to a pregnant woman. The presence of other medical problems may also affect the use of carmustine.[121]

Carmustine wafers have been reported to increase an aggressive sarcoma developed in the patient's bone flap which eroded rapidly through the scalp and leads to death. [119]

Antiseptics are antimicrobial compounds usually limited to external application which possess a broad spectrum of activity against pathogens and often demonstrate cytotoxicity against main cell mediators of the healing process.[104]

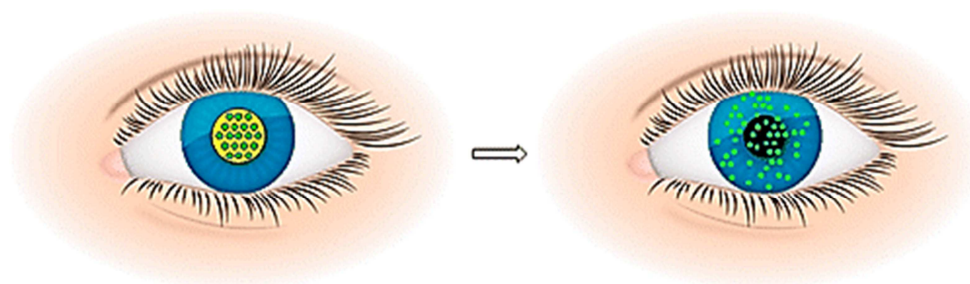
Nanowafer Drug Delivery for Corneal Cystinosis:

Alternative drug delivery have mainly focused on topical formulations, with the negative impact on effectiveness. To overcome these issues, the development of ananofabricated drug delivery system that can deliver the drug in a controlled release fashion for prolonged periods, thereby enhancing the drug efficacy and patient compliance.

Current nanofabrication technologies is to create an controlled drug delivery technology to develop a programmable *nanowafer* for sustained-release delivery of cysteamine, using materials like polyvinyl alcohol (PVA), cysteamine, PLGA, and collagen that are already in clinical use.

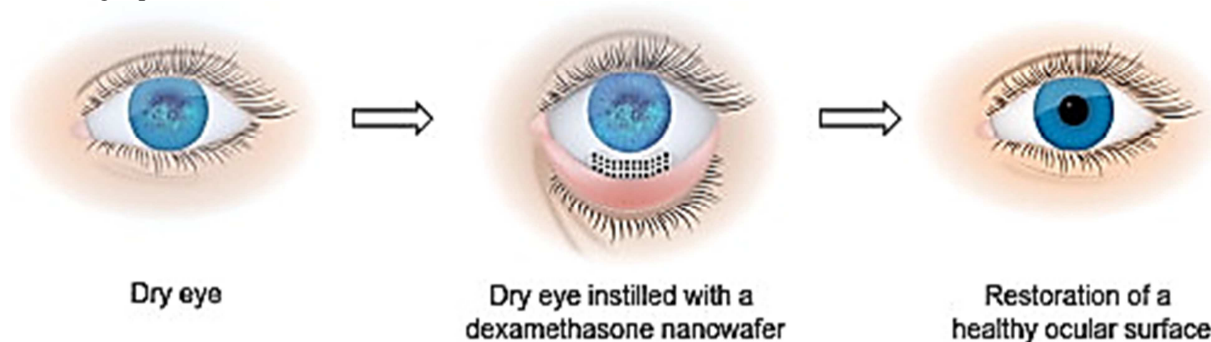
The nanowafers are fabricated into hydrogel wafers which is filled with cysteamine-PLGA matrix which is fabricated using a hydrogel template which are designed to deliver the drug in controlled release form. [122]

Eye injuries are treated with topical eye drop therapy due to ocular surface barrier eye drops must be applied frequently which cause side effects like cataract, glaucoma. Therefore development of nanowafer contains drug loaded Nano reservoirs slowly the drug release from the nanowafer increases the drug residence time on the ocular surface and its absorption into the surrounding ocular tissue. At the end of the stipulated period of drug release, the nanowafer will dissolve and fade away. [122]



[Figure-13]

Dry eye disease is one of the major public health problem that affects many of people. It is presently treated with artificial tear and anti-inflammatory eye drops that are administered several times a day which may have limited therapeutic efficacy. Hence Dexamethasone nanowafer is developed for the release of the drug on the ocular surface for a longer period of time [124]



[Figure-14]

CONCLUSION

With the development of wafer by incorporating many antimicrobial agents which helps in wound healing process, now many innovative ideas have been used in order to cure brain tumors like Malignant gliomas using Gliadel wafer, wafers are also used in nanaoforms which are used in various ocular preparations for the Corneal Cystinosis and also for the treatment of dry eyes using Dexamethasone which prevents dry eyes and healthy restoration of the ocular surface. Therefore the wafers are made of natural materials showing most promising compatibility. This review aimed at providing an insight to the development of an idealized wound healing device which is having capacity to overcome all the current challenges.

Acknowledgement

The authors express their gratitude to the JSS University and JSS College of Pharmacy for providing necessary support in due course of the work.

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