KAUNAS UNIVERSITY OF TECHNOLOGY

SANDRA KISELIOVIENĖ

PREPARATION AND CHARACTERIZATION OF CELLULOSE-BASED HYDROGELS FOR WOUND DRESSINGS

Doctoral dissertation
Physical sciences, chemistry (03P)

2016, Kaunas

UDK 544.77.03 + 615.46](043.3).

Research for the doctoral dissertation was carried at Kaunas University of Technology, Faculty of Chemical Technology, Department of Polymer Chemistry and Technology in the period of 2011–2015.

Scientific supervisor:

Prof., Dr. Jolanta LIESIENĖ (Kaunas University of Technology, physical sciences, chemistry – 03P).

English Language Editor:

Brigita BRASIENĖ (Publishing house "Technologija").

Lithuanian Language Editor:

Ilona PETROVĖ (Publishing house "Technologija").

ISBN 978-609-02-1188-5

KAUNO TECHNOLOGIJOS UNIVERSITETAS

SANDRA KISELIOVIENĖ

CELIULIOZĖS HIDROGELIŲ, SKIRTŲ ŽAIZDŲ TVARSČIAMS, GAVIMAS IR TYRIMAS

Daktaro disertacija
Fiziniai mokslai, chemija (03P)

2016, Kaunas

UDK 544.77.03 + 615.46](043.3).

Tyrimai atlikti 2011–2015 metais Kauno technologijos universitete, Polimerų chemijos ir technologijos katedroje.

Mokslinis vadovas:

Prof. dr. Jolanta LIESIENĖ (Kauno technologijos universitetas, fiziniai mokslai, chemija, 03P).

Anglų kalbos redaktorė:

Brigita BRASIENĖ (Leidykla „Technologija“).

Lietuvių kalbos redaktorė:

Ilona PETROVĖ (Leidykla „Technologija“).

ISBN 978-609-02-1188-5

Abbreviations

BC – bacterial cellulose;
CH-CO_2 – cellulose hydrogel treated with supercritical carbon dioxide;
CHG – cellulose hydrogel in glycerol;
CS – cellulose hydrogel sponges;
CHW – cellulose hydrogel in water;
HEC – hydroxyethylcellulose;
HEMA – 2-hydroxyethyl methacrylate;
LDH – lactate dehydrogenase;
Micro-CT – the micro-computed tomography;
MMPs – matrix metalloproteases;
PBS – phosphate buffered saline;
PECF – pseudo extracellular fluid;
PEG – poly(ethylene glycol);
pHEMA – poly(hydroxyethyl methacrylate);
PVA – poly(vinyl alcohol);
PVP-I – polyvinylpyrrolidone iodine;
SC – *Stratum Corneum*;
SEM – scanning electron microscopy;
SPAH – sponges made from pectin, alginate and hydroxyethylcellulose;
TEM – transmission electron microscopy.

CONTENTS

INTRODUCTION

Skin lesions are inevitable processes, and many have used bandages or patches. Historically, the dressing of wounds was carried out through the use of cloth. Recently, in chemistry and material sciences, a new generation of wound dressings has been extensively developed. Dressings made of new forms such as sponges, films and hydrocolloids have been developed together with new functions. Modern bandages not only protect the wound from infection, but as well promote the healing process, irrigate the wound, absorb the wound secretions, achieve the desired pH of the wound medium, stimulate the necessary growth factors and carry active compounds into the wound. Therefore, it is very important to perform a variety of chemical investigations. In recent years, a number of new products adapted for different types of wound healing and the wound healing stages have been discovered. The new generation of bandages has developed taking into account the different biological mechanisms that promote the healing of the wound, as well as comfortable feeling for patient, good mechanical, fluid absorption and anti-microbial properties are being developed.

In the past, the bandages were made of the cotton fabric (cellulose) with a different degree of wound exudate absorbency. The primary function of these bandages was to keep the wound dry, let the exudate evaporate and protect the wound against the harmful bacteria. However, only several decades ago, it has been observed that faster and more successful wound healing is achieved in the wet environment. Wound dressing should create an optimal environment that allows unrestricted movement of epithelial cells in order to achieve a faster wound healing process. The optimal conditions for wound healing are a moist environment around the wound and an effective oxygen circulation, which promotes cell and tissue regeneration. The type of wound has a significant influence on the wound development process, as the variety of wounds is distinguished, for example, chronic, necrotic, sloughy and epithelializing.

Modern materials that are used to cover the wound can be divided into inert, interacting with skin and biologically active substances. Natural polymers (collagen, alginates, chitosan, cellulose derivatives, etc.) are widely used for the non-healing wounds. Moreover, the synthetic polymers due to their air, water vapour permeability, prevention of bacteria entry into wounds are used. Efficient wound healing depends on many factors, including the type of wound, the treatment process, the patient health status (e.g. diabetes), environmental and social conditions, physico-chemical properties of the bandages.

Cellulose as a natural, non-irritant, nontoxic, biocompatible, environmentally friendly material is widely used as a wound healing material. As a wound dressing, cellulose is used in various forms. In the early years, firstly, cellulose was used as gauzes. Nowadays, more improved cellulose forms for wound healing are investigated, for example, cellulose fibers, cellulose films, sponges. As a hydrogel form is becoming more and more popular, cellulose hydrogels are becoming a field of interest as well.

Aim and tasks of the study

The aim of this work was to prepare hydrogels from the regenerated cellulose, hydrogels from various compositions of sodium alginate, pectin, hydroxyethylcellulose, modify them and investigate their properties considering the possibility to use them as the wound dressings.

In order to achieve the aim set out above, the following tasks had to be carried out:

- ✓ to prepare four kinds of wound dressing materials, such as (i) regenerated cellulose-based hydrogel sheets, (ii) regenerated cellulose-based hydrogel treated with supercritical CO_2, (iii) regenerated cellulose-based sponges, (iv) sponges of different compositions made from water soluble polymers (pectin, sodium alginate, hydroxyethylcellulose);
- ✓ to investigate their properties: morphology, mechanical, bioadhesion, liquid absorption properties, water vapour permeability, transparency, porosity, blood clotting;
- ✓ to incorporate active compounds into wound dressing materials, investigate their release and anti-bacterial properties;
- ✓ to investigate the cytotoxicity of regenerated cellulose hydrogel sheets and sponges.

Scientific novelty and practical value of the work

Using natural, biocompatible, non-cytotoxic polymers, such as regenerated cellulose, sodium alginate, pectin, hydroxyethylcellulose, hydrogels were prepared, from which four different wound dressing materials were produced by using different drying techniques.

For the first time, the regenerated cellulose hydrogel was used for the preparation of wound dressing material. It has been shown that the structure and the properties of regenerated cellulose could be easily modified by the lyophilisation or treating with supercritical CO_2.

The analysis of mechanical properties has shown that the prepared samples for wound dressings would be appropriate to protect the wound from the external mechanical impact and withstand their original shape. Other additional advantages, such as low bioadhesion, transparency, blood clotting ability were disclosed during the research.

Bactericidal wound dressings with antimicrobial activity that was prepared by immobilizing silver nanoparticles or antibiotic neomycin were produced. In order to get an antioxidant effect on wound, plant extracts or extracted phenolic compounds from plants were immobilized.

Approbation of the research results

The results of the research were presented in 4 publications. Among them, 2 articles were published in the journals included in the list of Thomson Reuters Web of Knowledge: “Cellulose Chemistry and Technology” (accepted), “Colloids and Surfaces A: Physicochemical and Engineering Aspects”. Moreover, the results were reported in 2 scientific international conferences.

S**tructure and contents of the dissertation**

The dissertation consists of an introduction including research objectives, literature overview, experimental part, results and discussion, conclusions, list of references (174) and the list of publications on the dissertation topic. The material of the dissertation is presented in 114 pages, including 15 tables and 57 figures.

Contribution of the author

The author produced four different kinds of wound dressings, investigated their structure and morphology, mechanical, bioadhesion, liquid absorption properties, water vapour permeability, transparency, porosity, blood clotting, immobilization and release of the active substances, antibacterial and cytotoxicity properties.

Citotoxicity test *in vitro* was carried out at Porto University (Portugal) and Karazin Kharkiv National University (Ukraine). Antibacterial properties were examined at Cardiff University (the United Kingdom) by the author and at Vytautas Magnus University with the participation of the author.

The publications were prepared by the author after a discussion with the co-authors.

1. LITERATURE REVIEW

In order to create cellulose-based hydrogel for wound healing, it is necessary to know the wound healing processes and stages, wound types and conditions necessary for the proper healing. Moreover, it is important to know the requirements for different kinds of dressings, according to the wound type. It is important to consider which kind of a material is used as wound healing agents in order to investigate their advantages and disadvantages. These aforementioned issues are widely analysed below in the literature review.

1.1. Structure and functions of the skin

The skin is the largest organ of the body. Skin protects the organism against injury or damage, prevents passing of microorganisms and lets water vapour permeation. The skin has a complex composition that creates a barrier through enucleate corneocytes on the surface, production of intercellular materials, colonization with various microorganisms, intercellular communication and adhesion molecules. The skin structures have a variety of functions that preserve homeostasis of the body (cooling, electrolyte balance), formation and processing of vitamins, hormones, physical and thermal protection of the underlying muscles (Preedy, 2012).

The outermost layer of the skin is *Stratum Corneum* (SC). SC is a constantly renewing structure. SC is responsible for the protective functions. The structure of SC is created difficult for penetration in order to protect the deeper layers from the environmental injury: water loss (allowing normal water vapour) and penetration by outside agents, inside-outside barrier function.

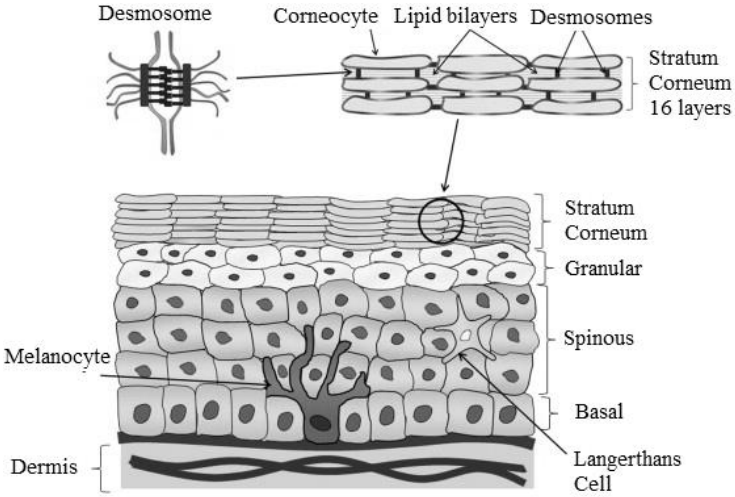


Fig. 1.1. Skin structure (Visscher and Narendran, 2014)

The SC has 16 layers of flattened cells joined together by the desmosomes (Fig. 1.1). Desmosomes are cell-cell junctions that are responsible for maintaining

the structural integrity of tissues (Stokes, 2007). The extracellular matrix of the *Stratum Granulosum* and *Stratum Corneum* contains lamellar layers of lipids released by the keratinosomes where ceramides, cholesterol and fatty acids create a hydrophobic barrier. Basal keratinocytes produce keratin, which attaches to the basal attachment plaque by providing the enhanced adhesion, strength and scaffolding of the keratinocytes. An extracellular matrix can be found in the epidermis and dermis. In the epidermis, the extracellular matrix becomes distinctively important in the *CS*, because the cells are actually no longer living; they do not maintain the normal intercellular adhesion seen in the spinal and basal layers (Preedy, 2012). The dermis is composed of collagen and elastin that is serving as the scaffold for the epidermis. The dermis layer as well contains glycoproteins, proteoglycans, glycosaminoglycans. Glycosaminoglycans precipitate in hydration and skin filtration processes. Glycosaminoglycans include hyaluronin, heparin sulphate, keratin sulphate and chondroitin/dermatan sulphate by providing a firm scaffold for the nutrients.

1.2. Wound healing stages

The wound is defined as a breakdown of the protective function of the skin, the loss of the continuity of epithelium with or without the loss of underlying connective tissue (i.e., bone, nerves, muscles). The wound is physical, chemical, mechanical or thermal injury and is accompanied by pain and bleeding. Wound healing is a dynamic and complex process. Five stages of wound healing are marked: hemostasis, inflammation, cellular migration, protein synthesis and wound contraction, remodelling (Monaco and Lawrence, 2003). These phases are shown in Fig. 1.2.

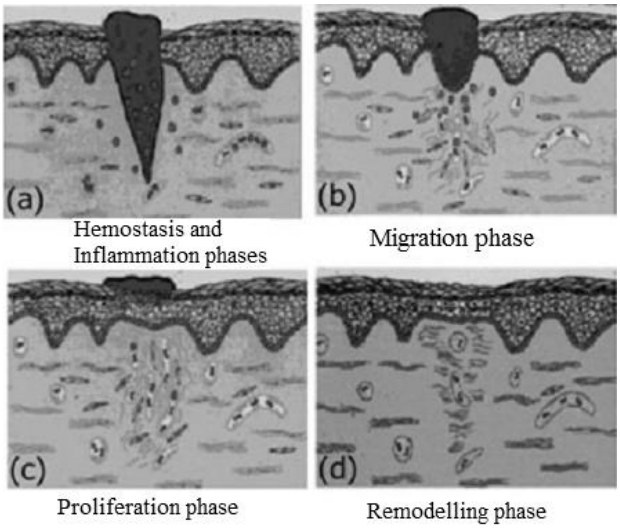


Fig. 1.2. Wound healing phases: (a) infiltration of neutrophils into the wound area, (b) invasion of wound area by epithelial cells, (c) epithelium completely covers the wound, (d) many of the capillaries and fibroblasts that formed at early stages have all disappeared (Boateng et al., 2008)

Inflammation, proliferation and remodelling are the main stages. Hemostasis and inflammation occur soon after the damage of the skin. The inflammatory phase is characterized by its cardinal signs: redness, warmth, swelling, pain and loss of function. Fibrinogen, which is one of the major components of the skin's connective tissues, elicits the clotting mechanism resulting in coagulation of exudates (blood without cells and platelets) and together with the formation of a fibrin network produces a clot and stops the bleeding. The clot dries; the scab is formed. In this way, strength and support are provided to the injured tissue. The inflammatory phase occurs almost simultaneously with hemostasis, sometimes a few minutes from the injury to 24 h and lasts for 3 days. At this stage, blood neutrophils followed by phagocytes enter the wound medium and penetrate inside the dead cells (Zahedi et al., 2010). In the migration phase, the newly synthesized collagen, which is deposited randomly in the granulation tissue, is typical for the newly formed granulation tissue. The collagen is remodelled into a more organized structure with increased tensile strength. Gradually, type I collagen replaces type III collagen until the normal skin ratio of 4:1 is achieved (Wild et al., 2010). The proliferation stage consists of the complete coverage of the wound by epithelium. These phases occur just after the migration phase. Basal cell proliferation lasts for 2 or 3 days. A granulation tissue is formed by the in-growth of capillaries and lymphatic vessels in the wound and the collagen is synthesized by fibroblasts giving the skin strength and form. Epithelial thickening takes place until the collagen bridges the wound. The fibroblast proliferation and collagen synthesis continues for up to 2 weeks, by which time the blood vessels decrease, and the oedema recedes. The remodelling phase involves the formation of cellular connective tissue and strengthening of the new epithelium, which determines the nature of the final scar. Cellular granular tissue is changed to a cellular mass from several months up to about 2 years (Boateng et al., 2008).

It is assumed that a wound is healing once the epithelium has closed the surface of the wound. The tensile strength of the wound may in fact take quite a considerable time to develop, and for some patients, it can take up to twelve months. The lack of tensile strength in a wound will increase the risk of breakdown that may be related to the tension of the tissue below the surface.

1.3. Wound classification

There are a few classifications of wounds. The main classification is based on the nature of the repair process, and two main types of the wounds are distinguished, i.e., acute and chronic. Acute wounds are tissue injuries that are practically completely cured, and the healing duration is about 8-12 weeks (Percival, 2002). Such wounds are caused by the external factors, such as bruises, abrasions, tears. Acute wounds involve burns and chemical injuries and can be caused by the mechanical injuries, including surgical wounds. Healing time of chronic wounds takes more than 12 weeks (Harding et al., 2002) and often recurs. Due to the repeated tissue insults or underlying physiological conditions (diabetes and malignancies, persistent infections, poor primary treatment and other patient related factors) such wounds fail to heal (Moore et al., 2006). Chronic wounds include

decubitis ulcers (bedsores or pressure sores) and leg ulcers (venous, ischaemic or of traumatic origin). Healing processes of such wounds are complicated because chronic wounds are often on the permanent inflammatory phase and have a high proteolytic activity. This will diminish the recognition and subsequent removal of cells by macrophages promoting a necrotic disintegration (Vasconcelos and Cavaco-Paulo, 2011).

Acute or chronic wounds are characterized by the production of exudate, which is a key component in all the stages of wound healing, keeping wound moist continuously. Chronic wounds usually have excessive amounts of exudate; therefore, maceration of a healthy tissue around the wound can be induced. Exudate supplies nutrients and leukocytes to the wound, which helps to control bacteria and infection (Boateng et al., 2008).

According to the depth of the wound, skin layers and the area of the skin affected, wounds are divided into three categories: superficial, partial thickness and full thickness wounds (Boateng et al., 2008). Injuries that affect only the epidermal layer of the skin are described as superficial wounds. Injuries that reach the epidermis and the deeper dermal layers of the skin, including blood vessels, sweat glands and hair follicles, are described as partial thickness wounds. Full thickness wounds occur when the underlying subcutaneous fat or deeper tissues are damaged in the epidermis and dermal layers.

Based on wound appearance and stage of healing, wounds can be classified into four categories:

- necrotic;
- sloughing;
- granulating;
- epithelializing.

Due to cell death, necrotic wounds usually have black or greenish-black colour. If necrotic wounds are infected, a sharp surgical debridement will be necessary in order to prevent systemic sepsis. In another case, if necrotic wounds are not infected, the necrotic tissue will eventually separate from the wound bed by autolysis. It is very important to maintain sufficient moisture in the local environment of the necrotic wound, because autolysis is inhibited if the wound is allowed to dry out.

The distinguishing feature of a sloughing wound is a glutinous yellow layer of tissue, which contains a mixture of leukocytes, wound exudate, dead bacteria and fibrin. The presence of slough provides a nutrient-rich environment for bacterial proliferation and may lead to the infection process. The successful healing of these wounds needs a debridement and clean wound bed, as the formation of a granulation tissue in a sloughing wound is delayed.

Granulating wounds are highly vascularized and have pink or red colour. The granulating tissue consists of a newly formed matrix of collagen, proteins and proteoglycans. For such kind of wounds, dressings with good absorbance capacity are required in order to absorb exudates. Granulating wounds may become overgranulating wounds. Overgranulating wounds have an excessive granulating tissue, when recurrent bleeding occurs, and epithelialization is delayed. In this case,

it is very important to control the excess tissue, and caustic pencils with silver nitrate or corticosteroids may be used.

Epithelializing wounds are formed by the migration of keratinocytes from the wound margins to the wound core, and a new epithelial tissue is formed. Epithelializing wound dressings should be non-adherent to avoid trauma during the changing dressing, should maintain a warm and moist environment around the wound (Casey, 2001).

Fig. 1.3 illustrates epithelizing, granulating, sloughing and necrotic wounds

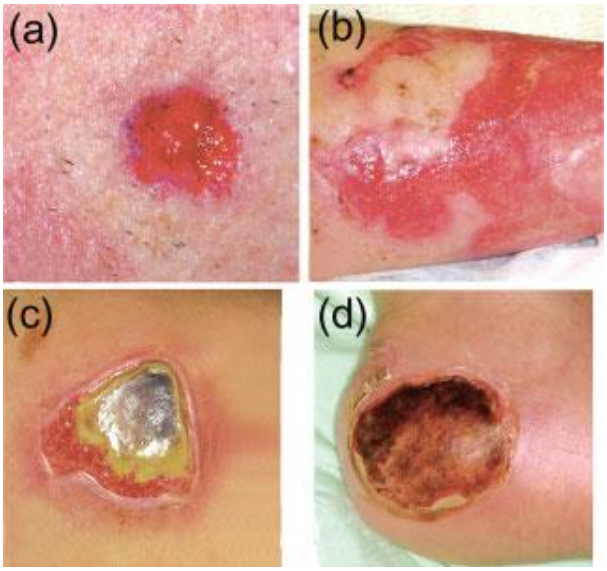


Fig. 1.3. Types of wounds based on their appearances: (a) epithelializing (clean, medium to high exudates), (b) granulating (clean, exudating), (c) sloughing and (d) necrotic (dry) (Zahedi et al., 2010)

The understanding of wound pathophysiology is the main goal of wound treatment. Wound evaluation combined with knowledge of basic wound dressing categories will provide guidance on product selection for different clinical cases.

1.4. Conditions for wound healing

Wounds differ from each other by the healing phase and the nature of the wound; thus, there are different requirements for the wound dressings as well. The basic requirements for all wounds to heal are a clean, adequately perfused wound environment free from an infection, necrotic tissue and foreign materials. In order to reach fast and proper healing of the wound, an ideal wound dressing should meet several requirements:

- maintain a moist environment around the wound;
- remove excess exudate, but prevent saturation of the dressing to its outer surface;
- permit gases exchange;
- provide mechanical protection;

- protect the wound from microorganisms;
- control local temperature and pH;
- minimize scar formation;
- be easily changeable and non-adherent to the wound bed;
- minimize the pain of the wound;
- control the wound odour;
- be non-allergenic;
- do not contaminate the wound with foreign particles;
- be cost effective.

The most important purpose of all the wound dressings is to maintain the wound moist and absorb the excess of exudates. It is important to maintain optimal physiological temperature of the site of injury; otherwise, it may disturb the oxygen flow to the wound and stop cell regeneration. An intact skin by acting as a barrier to the water vapour loss performs an important function of controlling the moisture balance.

More and more attention is paid to the treatment of wounds in a moist environment. Since Winter (1965) has proposed his classic hypothesis that the optimum environment for epithelialization is a moist environment, there is more evidence that good hydration is an important factor responsible for the optimal wound healing. In order to maintain moist tissues, it does not mean that the wound should be covered in fluid. Wound tissues should be physiologically moist, neither dry, nor wet. The inflammatory phase is often accompanied by the rich exudate environment; thus, a dressing should generate an optimal moisture level by absorbing the exudate and protecting the wound from maceration. However, decreasing the level of the exudates requires to minimize the absorption and maintain a natural moisture level for newly forming tissues. In the inflammatory phase, the wound may lose fluid through the evaporation as well as exudate production. Chronic or non-healing wounds are delayed in the inflammatory phase and produce exudate for long periods. Excessive wound exudate levels must be managed to prevent maceration. In such a case, the dressing must be able to absorb the exudate and establish optimal tissue moisture levels. If the wound tissues are adequately moist with minimal exudate production, then the dressing should be capable of maintaining the tissue hydration status without too much absorption that could desiccate the wound. If tissue moisture levels are already depleted, the dressing must be able to restore optimal tissue hydration by donating moisture to the wound (Ovington, 2007). Moist wound healing could be divided into three categories: healing with dressings that absorb excessive wound exudate, dressings that maintain existing levels of tissue moisture and those that add moisture to the tissues (Table 1.1).

Table 1.1. Examples of moist wound healing dressings (Ovington, 2007)

Dressing function	Dressing type	Dressing name	Manufacturer
Absorbs exudate	Alginate	Sorbsan,	Bertek Pharmaceuticals, Research Triangle Park, NC
	Hydrofiber	Aquacel Hydrofiber Wound Dressing	ConvaTec, Skillman, NJ, USA
	Foam	Optifoam Nonadhesive Dressing	Medline Industries, Inc., Mundelein, IL, USA
Maintains Moisture	Hydrocolloid	Duoderm CGF Sterile Dressing	Duoderm CGF Sterile Dressing
	Transparent film	Transeal	DeRoyal, Powell, TN, USA
Donates Moisture	Hydrogel—amorphous	Purilon Gel	Coloplast Corp, Marietta, GA, USA
	Hydrogel—wafer	FlexiGel Hydrogel Sheet Dressing	Smith and Nephew, Inc., Largo, FL, USA

Wound healing in a moist environment has several advantages, such as prevention of scab formation, faster epithelialization, reduced pH of the wound environment, which has a negative effect on bacteria (Stashak et al., 2004). Possible mechanisms explaining the improved healing include easier migration of epidermal cells over the moist wound surface compared with a dry scab, increased partial pressure of oxygen and the retention of growth factors, proteinases present in exudates that are allowed to exert their potentiating effect on wound healing (Svensjö et al., 2000). Dressings with controlled permeability provide a protective barrier, prevent eschar formation, reduce the dermal necrosis seen in wounds that have been allowed to dry and significantly accelerate wound re-epithelialization (Bolton et al., 2000). Healing under both wet and moist environments is significantly faster than under dry conditions. The speed of healing in moist or wet wounds may not be identical though not significantly different (Bernabei et al., 1999). Too high moisture content may cause the maceration of SC, cell swelling, increased permeability (Warner et al., 2003), irritation, inflammation, urticarial (Visscher and Narendran, 2014).

1.5. Wound exudates and microbial wound environment

An exudate describes the moisture emanating from the wound. Exudate is composed mainly of water with white blood cells, electrolytes, nutrients, inflammatory mediators, growth factors, protein digesting enzymes, such as proteases (Cutting, 2003). Exudate is a very important component in all the stages of wound healing as it helps to keep the wound continuously moist. Exudates supply nutrients and white blood cells to the wound, which helps to control bacterial

contamination and reduces the possibility of infection on the surface of the wound. The components of exudate, such as matrix metalloproteases (MMPs), can contribute to the breakdown of dead and devitalized tissue. Growth factors and cytokines can stimulate the formation of new tissue (Beldon, 2010).

Chronic wounds, due to the large amount of exudate, can lead to the maceration of healthy surrounding tissue of the wound. Excess of exudate can cause the edema (swelling caused by fluid retention), which occurs due to the inflammation, reduced mobility, venous or lymphatic insufficiency. The chronic wound exudate has a different composition. High levels of proteases can have a destructive impact on the tissue generation and the integrity of periwound tissues. Proliferation of keratinocytes, fibroblasts and endothelial cells is slowed or blocked; the growth factors can be denatured making them an ineffective stimulant for cell generation and healing, and high levels of proinflammatory cytokines promote a chronic inflammatory response. These factors contribute to the delayed healing (Cutting et al., 2010). As healing occurs, the amount of produced exudate usually decreases.

Wounds often give moist, warm and nutritious environment for bacterial colonization and proliferation. The quantity and diversity of microorganisms depend on the type of wound, depth, location and effectiveness of the immune system response. Wound colonization usually is polymicrobial, including potential pathogens, infection-causing bacteria (Bowler et al., 2001). Once infection has occurred, the wound fails to heal, the risk of trauma increases, and the cost of treatment increases as well. The emergence of the infection is accompanied by pyrexia, increased soreness and redness, lymphangitis and rapid increase in wound size. Chronic wounds can occur with no classic symptoms of infection, such as serous exudate plus concurrent inflammation, delayed healing, discoloration of granulation tissue, friable granulation tissue, foul odour (Gardner, 2001). Acute and chronic wound infections are primarily caused by aerobic pathogens, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *β-hemolytic streptococci* (Sehgal and Arunkumar, 1992). Therefore, in order to improve the treatment of chronic wounds, it is important to monitor and prevent infections, optimize wound exudate levels and remove foreign matter, which may cause complications.

1.6. pH of the skin and wound

In order to complete the regeneration process of the wound successfully, lots of biochemical reactions should occur, and for these processes, the pH value is a very important factor. To ensure the proper functions of SC enzymes, lipid metabolism, bilayer structure formation, ceramide synthesis and provide for bacterial homeostasis, skin colonization and inhibition of pathogenic bacteria, the epidermal barrier should maintain an acidic environment. The acidic milieu varies depending on the anatomical location and the age of the person between a pH of 4-6. Application of acidic treatments may assist in reducing the inflammation and normalizing a SC function. An increase in skin pH may reduce SC integrity and enhance susceptibility to mechanical trauma (Fluhr et al., 2001). The skin's acidic milieu is disturbed in wounds, where the underlying tissue with the body's internal

pH milieu of 7.4 becomes exposed. Most relevant human-pathogenic bacteria need pH values above 6, and their growth is inhibited by a lower pH value. Restoring the natural acidic milieu on the skin can help to reduce the microbial load on the body's surface, even if they are multiresistant to antibiotics. The application of acidic ointments in diabetics and stroke patients significantly reduced bacterial load on the skin surface (Kurabayashi et al., 2002). Essential bacterial enzymes like most of the *Staphylococci* derived proteases are diminished in their activity by an alkaline milieu, whereas the pH on normal skin promotes greater protease activity (Stewart et al., 2002). During the healing of acute wounds, a temporary physiological acidosis is observed. The generation of organic acids, e.g., lactic acid and the increased demand for O_2 during the healing processes, is combined with a stasis of tissue perfusion increasing the local amount of CO_2 in wounds (Roberts et al., 2015). This physiological acidosis might be helpful for the healing process, as an experiment with the human dermal fibroblasts showed a significant decrease in cell migration and DNA synthesis following an increase of the pH milieu (Lengheden and Jansson, 1995). In contrast to the acute wounds, the analysis of pH value in chronic venous leg ulcers and pressure sores has shown that the wound-milieu in these wounds remains shifted to an alkaline milieu for most of the time, except for the re-epithelisation phase, where it becomes acidic again (Sayegh et al., 1988). The pH value in chronic wounds is alkaline, and the ulcers are surrounded by the zone of skin with an acidic milieu where a barrier function is created. The use of modern dressings allows changing the pH values in the wound-milieu in various ways. Schneider et al. (2007) carried out the investigations with 39 patients with chronic wounds, and in total 247 pH measurements were done. The results showed that individual pH values in such wounds vary from person to person with a total range of 5.4–8.6. It was found that acute and chronic wounds treated by modern standards of wound care had a mean pH of about 7.4. A time course analysis of wound pH milieu in selected individual patients over 12 months revealed that the pH values over this period of time could vary by 1.73 units. Wound secretions of chronic wounds under the non-permeable dressings demonstrated a more acidic milieu than the secretions of wounds, where a permeable dressing is used (Fig. 1.4).

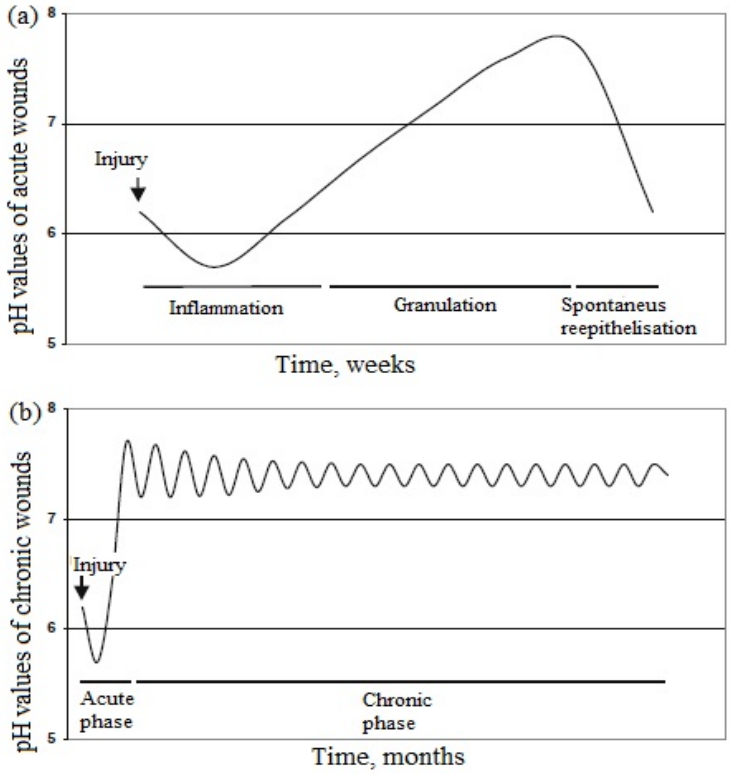


Fig. 1.4. pH values of (a) acute wounds and (b) chronic wounds (Schneider et al., 2007)

The pH value in wounds is a dynamic factor that can change rapidly with the therapeutic interventions. The pH value in wounds seems to be influenced by many different endogenous and exogenous factors. Investigations looking at various aspects of wound healing support the old view that an acidic pH in the wound supports the most aspects of the natural healing process. Acidic pH milieu suppresses bacterial growth, reduces proteolytic activity, enhances fibroblast growth in vitro, leads to more oxygen supply and is an indicator of successful self-healing of chronic wounds.

1.7. Wound dressings

Dressings can be classified in a number of ways. Firstly, dressings can be classified according to their functions (Purna and Babu, 2000) in the wounds and can be divided into the following sub-categories:

- debridement;
- antibacterial;
- occlusive;
- absorbent;
- adherence;
- odour-reducing dressings.

Secondly, wound dressings are classified according to the type of material applied to produce the dressings (Falabella, 2006). Such sub-categories can be singled out:

- hydrocolloid;
- hydrogel;
- alginate;
- collagen.

Thirdly, wound dressings can be classified according to the physical form (Falabella, 2006) of the dressing:

- film;
- foam;
- ointment;
- gel;
- sponge;
- gauze dressings.

Further dressings can be classified into primary, secondary and islands dressings (van Rijswijk, 2006). Dressings, which have a physical contact with the wound surface, are referred as primary dressings. Secondary dressings cover the primary dressings. Island dressings possess a central absorbent region that is surrounded by an adhesive portion.

The main classification of wound dressings includes three categories of wound dressings (Casey, 2001; Paul and Sharma, 2004):

- passive (gauze, tulle);
- interactive (semi-permeable films, semi-permeable foams, amorphous hydrogels);
- bioactive (hydrocolloids, alginates, collagen, chitosan, hydrofibers).

Passive products are ordinary dressings, i.e., gauze and tulle. These products act as a cover of a wound that the wound could rehabilitate underneath. These dressings do not perform any regulatory functions; they only absorb secretions. Gauze sticks to the wound surface and disrupts the balance of proper wound healing; thus, the gauze is suitable to treat small wounds. Tulle dressings consist of tulle gauze and petroleum jelly. This dressing does not stick to the wound surface and is suitable for a flat and shallow wound with minimal to moderate exudates.

The interactive wound dressings are made of materials containing polymeric films or foams, are transparent and permeable to water vapour and atmospheric oxygen. These types of wound dressings regulate wound healing processes and by maintaining a moist environment, is an excellent barrier against bacterial entering into the wound bed. It is an ideal dressing for abundantly exudating wounds. Semi-permeable films consist of a polyurethane membrane, which has an acrylic adhesive allowing wound check and are suitable for shallow wound with low exudates. Semi-permeable foams are soft, open hydrophobic, polyurethane foam sheets. These dressing are designed to absorb large amounts of exudates. They are not used for low exudating wounds, as they will cause dryness. Amorphous hydrogels are not

cross-linked. They are used for necrotic or sloughy wound beds to rehydrate and remove dead tissue. They are not used for moderate to heavily exudating wounds.

Bioactive dressings either deliver bioactive compounds or are constructed from the material having endogenous activity. Such dressings contain hydrocolloids, alginates, collagens and chitosan. Hydrocolloid dressings, semi-permeable polyurethane films, are in the form of solid wafers; they contain hydroactive particles as sodium carboxymethylcellulose that swells with exudates or forms a gel. Depending on the chosen hydrocolloid dressing, they can be used in wounds with light to heavy exudate, sloughing or granulating wounds (Ousey et al., 2012). Hydrofibers are soft nonwoven pad or ribbon dressings made from sodium carboxymethylcellulose fibers. They absorb exudates and provide a moist environment in a deep wound that needs packing. The classification of the wound dressings used for different wound treatment is presented in Fig. 1.5.

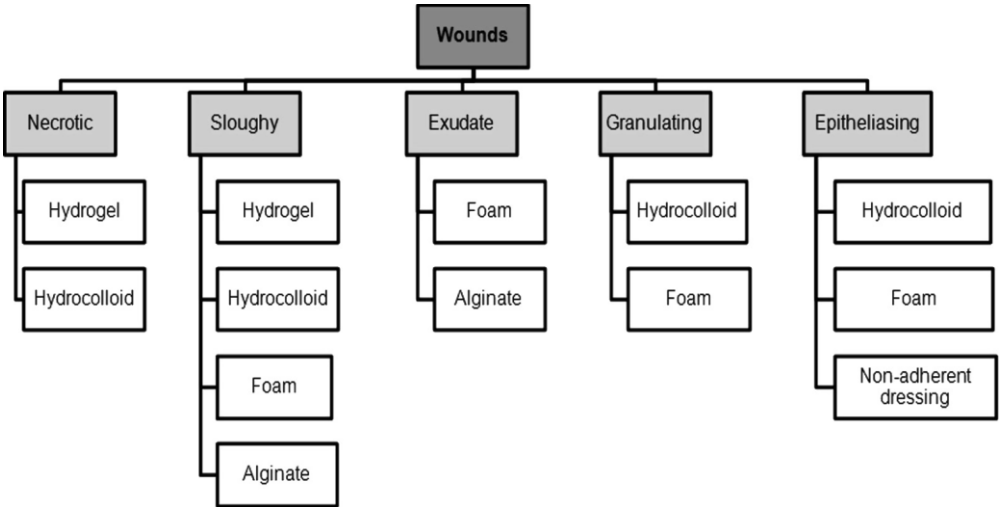


Fig. 1.5. Classification of the different dressing types used in the wound treatment (Moura et al., 2013)

Bioactive wound dressing materials can be modified in order to create an environment, which could be similar to the physiological conditions, and create a more rapid healing as compared to the conventional wound dressing materials. The bioactive wound dressings could be produced from a variety of biopolymers such as collagen, hyaluronic acid, alginate, elastin, etc. Active ingredients against contaminations and infections such as antimicrobials and antibiotics could be incorporated into the dressing materials. Various grow factors, such as epithelia growth factor, platelet derived growth factor, fibroblasts growth factor, transforming growth factor, insulin-like growth factor, and human growth hormone and granulocyte-macrophage colony-stimulating factors are as well used for accelerating the inflammation stage of a wound. These ingredients have a direct influence on the proliferation stage and the activation of fibroblasts (Komarcević, 2000). The other important active compounds in the healing of a wound are vitamins A, C, E, zinc, and copper minerals. Vitamin A is required for the epithelial and bone formation,

cellular differentiation and the immune function. Vitamin C is necessary for the collagen formation, a proper immune function and as a tissue antioxidant. Vitamin E is the major lipid-soluble antioxidant in the skin (MacKay and Miller, 2003). Zinc is a cofactor for many enzymatic reactions that are involved in the biosynthesis of RNA, DNA and proteins. Zinc is essential for all the proliferating cells, and the low zinc status decreases the closure and draft pressure of the wound and suppresses the inflammatory process (Wild et al., 2010). Iron is a cofactor of prolyl and lysyl hydrolysis enzymes, which are essential for the synthesis of collagen (Harris and Fraser, 2004).

1.7.1. Traditional dressings

Gauze dressings are made from woven and nonwoven fibers of cotton, rayon polyester or a combination of both. Such dressings are dry and do not supply moisture to the wound. They can be used as the primary or secondary dressings that are providing specific functions. For example, cotton-wool is used as a primary wound dressing in order to soak wound exudate. Cotton-wool can be used together with the roll bandage in order to prevent wound contamination. Gauze bandages are made from natural (cotton, wool and cellulose) and synthetic (e.g. polyamide) materials, which perform different functions. Sterile gauze bandage is used since ancient times to protect the open wounds, as dressings absorb the wound exudate. Gauze bandages must be changed regularly in order to protect the healthy and intact skin layers from the maceration. In economic view, these bandages are cheaper compared to the prices of modern dressings. Gauzes could protect from bacteria entering into the wound, but this feature is temporary, because the outer surface of the dressing may become moistened either by the wound exudate or by external fluids. In addition, gauze dressings tend to become more adherent to the wounds as fluid production diminishes and are painful to remove. As the dressing is peeled off the wound, fibrin, debris and necrotic tissue are removed. Gauze dressings as well provide little occlusion and allow evaporation of moisture resulting in the wound bed dehydration (Stashak et al., 2004). In addition, one of the most significant problems encountered in this material is a foreign body reaction in the wound caused by the cotton fibres. The biggest advantage of these materials is their low cost (Lim et al., 2000).

1.7.2. Hydrogel dressings

Hydrogels are three-dimensional polymer networks that are able to retain a large amount of water in their swollen state. Hydrogels are composed of 80-99% of water and are available in amorphous (dispensed from a tube) or sheet forms. Depending on the source of the constituting polymers, hydrogels may be classified as natural, synthetic or hybrid. Hydrogels can be chemically crosslinked by covalent bonds, physically crosslinked by non-covalent interactions or crosslinked by a combination of both (Hoffman, 2012).

Methods for synthesizing the physical hydrogels:

- warm the polymer solution to form a gel (PEO-PPO-PEO block copolymers in water);

- cool the polymer solution to form a gel (agarose or gelatin in water)
- crosslink a polymer in aqueous solution by using freeze–thaw cycles to form a polymer microcrystal (freeze–thaw PVA in aqueous solution);
- lower pH to form an H-bonded gel between two different polymers in the same aqueous solution (PEO and PAAc);
- mix solutions of a polyanion and a polycation to form a complex coacervate gel (sodium alginate plus polylysine);
- gel the polyelectrolyte solution with a multivalent ion of the opposite charge (Na^+alginate$^-$+Ca^{2+}+ 2Cl^-).

Methods for synthesizing the chemical hydrogels, crosslink polymers in the solid state or in solution with:

- radiation (e.g., irradiate PEO in water);
- chemical crosslinkers (treat collagen with glutaraldehyde or bis-epoxide);
- multi-functional reactive compounds (PEG + diisocyanate PU hydrogel);
- copolymerize a monomer + crosslinker in solution (HEMA + EGDMA);
- copolymerize a monomer + a multifunctional macromer (bis-methacrylate terminated PLA-PEO-PLA+photosensitizer+visible light radiation);
- polymerize a monomer within a different solid polymer to form an IPN gel (e.g., AN + starch);
- chemically convert a hydrophobic polymer to a hydrogel (e.g., partially hydrolyse PVAc to PVA or PAN to PAN/PAAm/PAAc).

Hydrogels are among the most frequently used dressing materials in the treatment of the wounds and burns. As hydrogels are capable to absorb much more water than their weight, they act as a dressing reducing the potential irritation when in contact with the tissue. They keep moisture at the application site and permit oxygen penetration (Hoffman, 2012). Because of the ability to absorb a large amount of water, hydrogels flexibility is similar to the living tissue. Hydrogel structure perfectly swells as well in the biological fluids (Siegel and Federation, 2004). It is considered that the hydrogels have the most properties of an ideal wound dressing. Usually, hydrogels are transparent that the wound could be observed without removing the dressing; they are soft, and their rubbery nature minimizes the inflammatory reactions of the surrounding cells (Buwalda et al., 2014). The three-dimensional structure of hydrogels allows them to be used as a drug delivery system (Lee et al., 2013). Hydrogels provide the moisture and at the same time are nonadherent materials. Due to the high water content, hydrogels allow vapour and oxygen transmission and fits for pressure sores, leg ulcers, burns, necrotic and surgical wounds (Caló and Khutoryanskiy, 2014). Hydrogels are inactive with the biological tissues, are permeable to metabolites, do not disintegrate in the wound and create a barrier against bacteria (Kokabi et al., 2007).

Aqueous solutions of the hydrophilic polymers at low concentrations, where no substantial entanglement of chains occurs, normally show Newtonian behaviour, but once crosslinks between the different polymer chains are introduced, the obtained networks show viscoelastic and sometimes pure elastic behaviour (Hennink and van Nostrum, 2012). The cross-linking density, the number of chemical or

physical cross-links in a given volume controls many fundamental hydrogel properties that are important in the healthcare applications (Metters et al., 2000). These properties are volumetric swelling ratio, shear modulus and the diffusion coefficient of entrapped molecules. The volumetric swelling ratio is representative of the water content of the swollen hydrogel and is based on the structure of the gel and the polymer–solvent interaction parameter. According to the rubber elasticity theory, the shear modulus of the gel is directly related to the network cross-linking density and defines many aspects of the mechanical properties of the gel. The diffusion coefficient of the entrapped molecules is directly related to the network mesh size, which is a measure of the space that is available between the macromolecular chains for the diffusion of cell-secreted molecules for tissue regeneration or drugs intended for the delivery (Peppas et al., 2006).

1.7.2.1. Natural hydrogels for the wound dressings

Natural hydrogels are typically composed of extracellular matrix components, such as polysaccharides or proteins. These hydrogels can be derived from polymers such chitosan, gelatine, silk fibrin, collagen, alginate, hyaluronic acid, agarose. The advantages of natural polymers are low toxicity and biocompatibility. Inducing and stimulating the wound healing process, natural polymers are involved in the repair of damaged tissues and consequently in the skin regeneration (Huang and Fu, 2010).

Natural polymers used to synthesize hydrogels:

- anionic polymers (hyaluronan, alginic acid, pectin, carrageenan, chondroitin sulphate, dextran sulphate);
- cationic polymers (chitosan, polylysine);
- amphipathic polymers (collagen and gelatin, carboxymethyl chitin, fibrin);
- neutral polymers (dextran, agarose, pullulan).

Hyaluronan is widely used in the treatment of wounds. It is non-toxic, non-immunogenic and has very good resorption characteristics in biomedical applications. Hyaluronan has a function of a regulator of tissue hydrodynamics, comprising cell dynamics, inflammation and tissue repair (Petrey and de la Motte, 2014). Wang (2006) prepared cross-linked glycol chitosan/hyaluronan hydrogels and found that the hydrogel displayed the characteristics required of an ideal wound dressing material. Catanzano et al. (2015) prepared an alginate/hyaluronan hydrogel with enhanced wound healing activity, where hyaluronan incorporation into alginate hydrogels promoted cell migration without affecting the viability.

Alginate is an anionic polysaccharide, which is capable to form hydrogels under very mild conditions, at the room temperature and in the absence of toxic solvents. Alginate has been widely used and investigated due to its biocompatibility, biodegradability, relative low cost, low toxicity and gelling properties. Alginate hydrogels are mostly prepared by the external gelation by using calcium ions as cross-linking agents. Due to high water content, elasticity, permeability and the ability to create a moist environment in the wound bed, alginate gels have been widely used (Balakrishnan et al., 2012; Pereira et al., 2013; Murakami et al., 2010; Wang et al., 2002).

Chitosan has several advantages, such as hemostasis, wound healing, bacteriostatic, biocompatibility and biodegradability properties. Chitosan have no adverse effects after implantation in tissues, and for this reason, it has been used for a wide range of biomedical applications. Chitosan was as well used to inhibit fibroplasia in wound healing and promote tissue growth and differentiation in culture (Alsarra, 2009).

Collagen is considered a biocompatible scaffold because it is the major protein component of the extracellular matrix, and the multiple scaffolds based on collagen are currently available for the clinical use as the wound dressings (Chen et al., 2013; Dell et al., 2011). Collagen has good biocompatibility and low antigenicity and can be tailored to capitalize on its mechanical, degradation and water uptake properties (Yang et al., 2004). The collagen that has derived from the animal tissues may be unsafe because of the potential for viral and prior contamination (Lynn et al., 2004).

Gelatin has relatively low antigenicity because of it being denatured and is in contrast to collagen, which is known to have antigenicity owing to its animal origin (Tabata et al., 1994). The isoelectric point of gelatin can be modified during the fabrication process to yield a negatively charged acidic gelatin or a positively charged basic gelatin at the physiological pH. This process theoretically allows electrostatic interactions to take place between a charged biomolecule and the gelatin of the opposite charge to form the polyion complexes (Djagny et al., 2001).

Fibrin hydrogels have been used widely in tissue engineering applications due to their high tissue-like water content, high biocompatibility, mechanical properties that are similar to soft tissues, efficient transport of nutrients, ability to uniformly encapsulate cells and ability to be injected as a liquid that gels (Huang and Fu, 2010). The major drawback of fibrin is that it is susceptible to rapid degradation in vivo and has difficulty in maintaining structural integrity.

Natural polymers can mimic many features of an extracellular matrix and can direct the migration, growth and organization of cells during the tissue regeneration and wound healing. The natural polymers provide a natural environment for cell proliferation and differentiation or morphogenesis, which contributes to the tissue regeneration and organogenesis (Xiao-Dong et al., 2002).

1.7.2.2. Synthetic hydrogels for the wound dressings

Polymer networks can be synthesized by using various chemical methods. Hydrogels can be synthesised from monomers, prepolymers and polymers. The polymer engineer can design and synthesize polymer networks with molecular-scale control over the structure such as crosslinking density and with tailored properties, such as biodegradation, mechanical strength and chemical and biological response to stimuli (Peppas et al., 2006).

Neutral synthetic polymers that are intended for wound dressings can be generated from derivatives of poly(hydroxyethyl methacrylate) (pHEMA), poly(ethylene glycol) (PEG) and poly(vinyl alcohol) (PVA). In Fig. 1.6, the chemical structure of pHEMA, PEG and PVA can be seen.

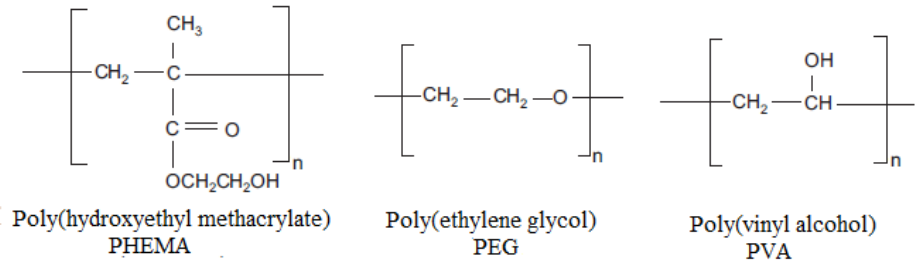


Fig. 1.6. Chemical structure of pHEMA, PEG and PVA polymers

pHEMA networks are usually prepared by the free radical copolymerization of 2-hydroxyethyl methacrylate (HEMA) and a crosslinking agent in the presence of water. Monomer HEMA is soluble in water, while pHEMA is not soluble and has limited compatibility with the solvent, and the structure of pHEMA networks is highly dependent upon the water content of the polymerization mixture. There are relatively few studies on HEMA hydrogel synthesis in hydro-organic media (mixtures of water and organic solvents). In those studies, it has been observed that the addition of various organic compounds, those that are a solvent for both the monomer and polymer, to the reaction mixture can lead to the formation of a clear hydrogel (Kwok et al., 2004). Various modifications can be made to pHEMA derivatives to modify its properties. For example, chitosan modified pHEMA gels have been synthesized via gamma irradiation (Casimiro et al., 2007).

PEG is a polyether which as well could be named as poly(ethylene oxide) (PEO) or as poly(oxyethylene) (POE) depending on its molecular weight (Saul and Williams, 2011). This polymer is a hydrophilic, flexible, biocompatible, non-toxic and non-immunogenic. It is resistant to protein adsorption and can be synthesized by anionic or cationic polymerization of ethylene oxide. As PEG macromeres have low toxicity, they can be coupled with growth factors or peptides (Zhu, 2010). Through the copolymerization with other macromolecules, the multiple functional fragments are introduced to suppress or promote cell survival and function. For example, the integrin binding peptide Arg-Gly-Asp is often introduced as a pendant functional group within otherwise bio-inert PEG hydrogels to promote the survival of adherent-dependent cells, such as osteoblasts (Lin and Anseth, 2009). Several cross-linking methods have been used to prepare PEG-based hydrogels: radiation of linear or branched PEG polymers, free radical polymerization of PEG acrylates and other chemical reactions, such as condensation reactions (Moura et al., 2013). PEG can be as well blended with other polymers such as chitosan, poly(lactic-*co*-glycolic acid) and poly(propylenefumarate) in order to improve its inherent solubility, erosion, mechanical and thermal properties and its crystallinity and viscosity (Zhu, 2010).

PVA hydrogel is one of the well-known polymer hydrogel with good biocompatibility. PVA has several advantages, including non-toxicity, non-carcinogenicity, biocompatibility, bio-adhesive characteristics, excellent film-forming, excellent transparency and additionally ease of processing (Kenawy et al., 2014). This polymer due to its simple chemical structure can be easily modified by

the chemical reactions. PVA hydrogel has a high degree of swelling in aqueous medium, which may lead to a chemical structural collapse and impact the membrane's performance of PVA (Pourjafar et al., 2012). In order to create a balance, the hydrophilic and hydrophobic properties of such membranes should be created, which might be achieved by cross-linking. PVA can be cross-linked by using the multifunctional compounds, such as dialdehydes, dicarboxylic acids and dianhydrides, which are capable of reacting with the PVA hydroxyl groups (Drury and Mooney, 2003). PVA hydrogel dressings were limited because of poor mechanical properties. This problem was solved by blending PVA with natural materials. The composite system represents better mechanical properties than PVA alone (Silva et al., 2013). Most investigators focus on modifying PVA by introducing natural molecules such as alginate (Kim et al., 2008; Levi et al., 2011), dextran (Hwang et al., 2010; Fathi et al., 2011; Liu et al., 2010), gelatin (Hago and Li, 2013; Pal et al., 2007), chitosan (Don et al., 2006; Yang et al., 2010).

1.7.2.3. Cellulose and cellulose hydrogel in wound dressing application

Cellulose is the primary structural component of plant cell walls and is the most abundant organic polymer on Earth. It is a linear polymer constituted by β-1,4 linked D-glucose units (Fig. 1.7), which are joined to form cellobiose repeating units (Mano et al., 2007). The possibility of inter- and intra-hydrogen bonding formation, due to the presence of the hydroxyl groups in cellulose unit, allows the interaction between the different linear chains and their aggregation into chain domains, i.e., the cellulose network (Baccaro et al., 2013). This highly cohesive hydrogen-bonded structure provides cellulose fibers of great stability, rigidity and tensile strength and makes them water insoluble.

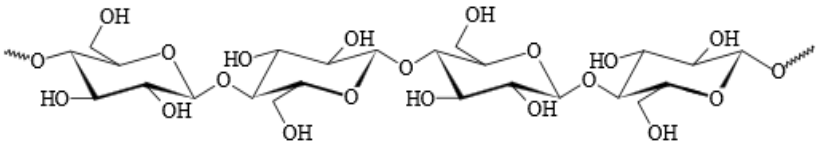


Fig. 1.7. Chemical structure of cellulose

Some cellulose derivatives such as methylcellulose, hydroxypropylcellulose, hydroxyethylcellulose, hydroxyethylmethylcellulose, hydroxypropylmethylcellulose and carboxymethylcellulose are water-soluble and present other interesting properties such as organic-solvent solubility, thermoplastic behaviour and biosurface activity. Cellulose acetate, cellulose triacetate and cellulose sulphate are fiber and film forming materials (Du and Hsieh, 2009).

Cellulose, being the most abundant renewable biocompatible natural polymer, has been widely used for the preparation of various types of wound dressings. One of the most popular cellulose based dressings is the traditional various types of gauzes. Fine and wide meshed cotton gauze has commonly been used in clinical practice for many years. If the wound fluids are of low viscosity, the gauze is

applied as a dry dressing; for the wounds of dehydrated surfaces, the gauze can be wetted with a salt solution. The gauze is a simple, inexpensive and quite effective wound dressing. However, gauze sticks to the wound and could strip off the newly formed epidermis when removed. They as well did not possess active healing properties. In order to give to cotton some healing activity, Edwards et al. (2009; 2007) have synthesized the modified cotton-based wound dressing materials, which can selectively absorb neutrophil elastase or selectively lower the activity of cationic serine protease.

As a wound dressing, cellulose is used in various forms. In the early years, firstly, cellulose was used as gauzes. Nowadays, more improved cellulose forms for wound healing are being investigated, for example, cellulose fibers (Waring and Parsons, 2001; Miao et al., 2011; Sun et al., 2015), cellulose films (Saibuatong and Phisalaphong, 2010; Wong and Ramli, 2014; Wu et al., 2014), sponges (Ang-atikarnkul et al., 2014; Chiaoprakobkij et al., 2011). As a hydrogel form is becoming more and more popular, cellulose hydrogels are as well becoming a field of interest. Until now, some cellulose hydrogels have been investigated (Hashem et al., 2013; Laçin, 2014; Mohamad et al., 2014).

Some studies have shown that cellulose stimulates wound healing through the release and maintenance of several growth factors at the wound site and promotes dermal fibroblast migration and proliferation, inhibits bacterial proliferation in wounds (Moura et al., 2013). Cellulose stimulates the granulation and epithelialization process of the partial- and full-thickness wounds and is used especially as healing scaffold/matrix for chronic wound dressings (Mogoşanu and Grumezescu, 2014). Wound dressings made from modified cellulose can be loaded with different active molecules such as enzymes, antioxidants, hormones, vitamins, antimicrobial drugs (Medusheva et al., 2007).

Bacterial cellulose (BC) studies have shown that this cellulose that is synthesized by *Acetobacter xylinum* has a potential for use in the wound dressings and artificial skin (Czaja et al., 2006). BC is a hydrogel that can take water up to 99% of its own weight, mainly due to its amorphous structure (Schrecker and Gostomski, 2005). BC has high water retention, high mechanical strength, biocompatibility, and due to its similarity to the extracellular matrix, it is used in regenerative medicine as a wound-healing scaffold for severely damaged skin and small-diameter blood vessel replacement (Czaja et al., 2007).

Cellulose-based hydrogels can be obtained by physical or chemical stabilization of aqueous solutions. Natural or synthetic polymers might be combined with cellulose to obtain composite hydrogels with specific properties. Physical, thermoreversible gels are usually prepared from water solutions of methylcellulose and/or hydroxypropylmethylcellulose (Arboleya and Wilde, 2005). Physically crosslinked hydrogels are reversible (might flow under mechanical loading) and might degrade in an uncontrollable manner. Due to such drawbacks, physical hydrogels based on methylcellulose and hydroxypropylmethylcellulose are not recommended for use *in vivo* (Chen et al., 2006). Stable and stiff hydrogels of cellulose can be prepared by inducing the formation of chemical, irreversible crosslinks among the cellulose chains. Chemical agents or physical treatments can

be used to form stable cellulose-based networks. The degree of crosslinking affects the mechanical and degradation properties of the hydrogel and can be controlled during the synthesis. Depending on the used cellulose derivatives, a number of crosslinking agents and catalysts can be employed to form hydrogels, for example, epichlorhydrin, aldehydes and aldehyde-based reagents, urea derivatives, carbodiimides and multifunctional carboxylic acids are the most widely used crosslinkers for cellulose. Some of these reagents are toxic in their unreacted state. Unreacted chemicals are usually eliminated through washing in distilled water, but toxic crosslinkers should be avoided in order to preserve the biocompatibility of the final hydrogel as well as to ensure the environmentally sustainable production process (Sannino et al., 2009). Cellulose-based hydrogels made without toxic crosslinking agents are biocompatible, non-toxic and have wide applications in tissue engineering and controllable delivery systems. The high water absorption ability of the hydrogels is very important for wound dressing to absorb blood and tissue fluid on acute traumas and promote wound healing (Fu et al., 2013). Burn wounds have responded well to the application of hydrogels; the cooling effect of hydrogels is both soothing and healing (Jandera et al., 2000).

However, most articles about cellulose hydrogels describe hydrogels made from BC.

1.7.3. Alginate dressings

Alginate or alginic acid, a polysaccharide from seaweed, is a linear biological copolymer consisting of (1-4) linked α-L-galuronate (G) and β-D-mannuronate (M) monomeric units (Fig. 1.8). Alginate forms gel when it comes in contact with the divalent cations (Ca^{2+}, Ba^{2+}, Mg^{2+}), and the resulted hydrogel has been widely investigated for the use in many biotechnological and medical applications (Lee and Mooney, 2012).

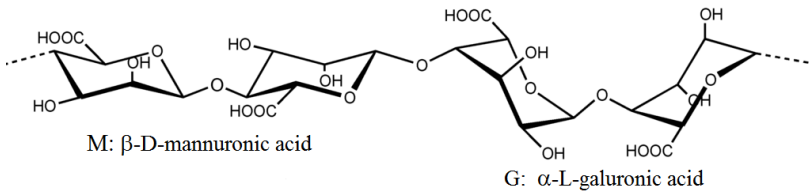


Fig. 1.8. Chemical structure of alginic acid

Divalent cations interact with the carboxyl groups in the alginate galuronic acid residues. An alginate hydrogel is non-toxic but like most of the polysaccharide hydrogels has weak mechanical properties. When in contact with wound exudates, a hydrophilic gel is formed, which makes the dressing removal easier (Guo et al., 2013). Gel formation prevents the wound surface from drying out, providing moist wound treatment, which favours wound healing. The calcium component within the dressing acts as a haemostatic agent and is therefore useful in bleeding wounds. Because of its soft nature, alginate gel dressings fit the contours of wound beds and provide a moist environment that accelerates wound healing (Balakrishnan et al.,

2012). Alginate positively affects the wound healing by the activation of macrophages, ensuring elevation of cytokine levels, including cytokines TNF-α and IL-6, the appearance of which results in a pro-inflammatory stimulation advantageous for the wound healing (Balakrishnan and Jayakrishnan, 2005; Passaglia et al., 2008).

Alginate-based hydrogels have several disadvantages. Unpredictable and uncontrollable degradation and dissolution may appear after the loss of the divalent cation cross-linkers (D'Ayala et al., 2008). In order to overcome this issue, cross-linking can be employed with other biopolymers such as gelatin, heparin, polyvinyl alcohol or chitosan. Crosslinking alginate with selected polymers increases the mechanical strength and decreases the degradation rate of the alginate-based dressing (Chiu et al., 2008). Another disadvantage of alginate-based materials is their inability to undergo efficient and rapid enzymatic degradation in mammals. Alginates are as well very hydrophilic and hinders its interactions with skin proteins (Moura et al., 2013).

Alginates were reported to dissolve at higher pH values than an acidic medium. As the pH environment of the wounds had been recorded to be within the range of 7.15–8.9, it can be considered as a convenient medium for the formulation of alginate dressings with controlled drug release (Sarheed et al., 2014). Alginate gel dressings are characterised by a good permeability for oxygen, other gases and fluids, which is similar to that of a natural skin (Thomas, 2000). Alginate dressings are useful for moderate to heavily exuding wounds. As alginates are highly absorbent, they should not be used with dry wounds or wounds with minimal drainage. Wound dressings will require a secondary dressing; foams or hydrocolloids will secure the alginate and keep it from drying out.

1.7.4. Pectin dressings

Pectin is a natural heterogeneous anionic polysaccharide constituting the cell walls of most plants, especially in apple pomace, citrus fruits and sugar beet root. Chemically, pectin is poly α 1–4-galacturonic acids with varying degree of methylation of carboxylic acid residues (Kaya et al., 2014), see Fig. 1.9.

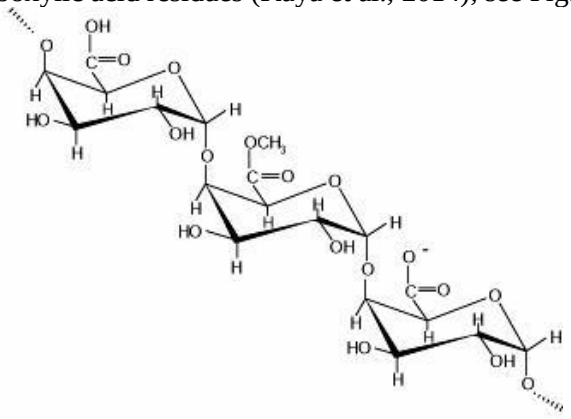


Fig. 1.9. Chemical structure of pectin

Pectins with low degree of methylation form a gel in the presence of multivalent ions, while the pectins with a higher degree of methylation form a gel in the acidic media with the addition of different sugars (sucrose or glucose) (Videcoq et al., 2011). Due to the biocompatibility, biodegradability and nontoxicity of pectin, it is used in pharmaceutical and biomedical applications, particularly, in engineering drug carriers for oral drug delivery. Moreover, pectin has been used and investigated in the field of wound healing (Jáuregui et al., 2009; Farris et al., 2011; Mishra et al., 2011). Several advantages of using pectin as wound dressing should be mentioned: it has hydrophilicity, which permits removal of exudates, the retention of an acid environment, which may act as the barrier against bacteria, and the ability of binding active molecules as drugs or growth factors to heal the wounds. Then the healing process of the wound begins, the cells around the wound are stimulated by growth factors to proliferate and grow into the wound. When pectin is in contact with the wound, it serves as a binding agent protecting growth factors from degradation (Munarin et al., 2012). Cipriani et al. (2009) suggested that chemically sulphated citrus pectin fractions possess good antithrombogenic properties. Hydrophilic parts of pectin with the wound fluids form a soft gel over the wound bed and remove the exudates excess from the wound. The acid environment obtained with pectin solubilisation act as a bacterial or viral barrier. Pectin hydrogels provide improved systems of loading and releasing drugs, i.e., antibiotics, pain relievers and/or tissue repair factors at the site of action (Mishra et al., 2008). However, pectin films have low thermal stability and poor mechanical properties; therefore, it has been blended with different polymers to improve its thermal and mechanical stability (Mishra et al., 2011).

Functionalization of pectin offers many possibilities to transform this material into a wide range of interesting products. A variety of hydrocolloid pectin-based wound dressings has been patented, and nowadays, they are commercially available.

1.7.5. Hydrocolloid dressings

Hydrocolloid dressings are products obtained from the colloidal (gel forming agents) materials combined with the other materials such as elastomers and adhesives. Typically, the gel forming agents are carboxymethylcellulose, pectin or gelatine. Hydrocolloid dressings usually are in the form of thin films and sheets or as composite dressings in combination with the other materials such as alginates. These dressings are indicated for wounds with low to moderate exudates. Hydrocolloid dressings are conformable to the patient's body and adhere well to high-friction areas, they as well adhere to both moist and dry sites. Minimal to moderate amounts of exudate can be absorbed by the hydrocolloids dressings and that makes these dressings suitable for partial- or full-thickness acute and chronic wounds (McIntosh, 2007). During the contact with wound exudate, these dressings will absorb wound fluids and create a moist environment. The moisture retentive properties of hydrocolloids help to gently soften and rehydrate necrotic tissue and slough by aiding autolytic debridement. As they do not cause pain on removal, they are particularly useful in paediatric wound care for management of both acute and chronic wounds (Koksal and Bozkurt, 2003). Hydrocolloid dressings are semi-

permeable to water and oxygen: in their intact state, hydrocolloid dressings are impermeable to water vapour, but after the absorption of wound exudate, they become more permeable to water and air as the gel forms (Vowden and Vowden, 2014). Hydrocolloids help to maintain an acidic environment; the acidic environment can inhibit bacteria growth. In addition, more alkaline wounds promote infection (Jones et al., 2007). Hydrocolloids can help a wound to granulate or epithelialize and encourage autolytic debridement in wounds with necrotic or sloughy tissue present (Dumville et al., 2013). The moist, oxygen depleted, environment produced by the dressing is thought to protect the nerve endings and thus help to reduce pain in the wound bed. Hydrocolloids should not be used if the wound or surrounding skin is infected and are not recommended for use in diabetic foot ulceration, because of the occlusive nature of these dressings. Hydrocolloid dressings generally have an occlusive outer cover that prevents water vapour exchange between the wound and its surroundings. This can be disadvantageous for the infected wounds that require a certain amount of oxygen to heal rapidly. Another disadvantage applies to the dressings containing fibres that are deposited in the wound and often have to be removed during the dressing change.

1.7.6. Antibacterial dressings

Antimicrobials are commonly applied in order to prevent infection. Infection and bacterial colonization remain very important factors in delayed wound healing. Exudate supplies nutrients and leukocytes to the wound, which helps to control bacteria and infection. Otherwise, the wound provides a moist, warm and nutritious environment to microbial colonization and proliferation. The number and diversity of microorganisms depend on the type of wound, depth, location and the antimicrobial efficacy of the host immune response. Wound colonization is most frequently polymicrobial, where some can be pathogenic and may lead to infection (Bowler et al., 2001). Microbial pathogens delay the healing process by producing the inflammatory mediators, toxins, because of maintenance of neutrophils and because of competition for oxygen and nutrients with the host cells (Rucigaj, 2014). Wound contamination by bacteria usually means more pain to the patient, the increase of exudate, unpleasant odour and delayed healing.

Nowadays, various antiseptics are used. The most popular among them are antibiotics, such as gentamicin, ofloxacin, bacitracin, neomycin. Local delivery of a drug accelerates the wound healing and usually has no side effects (Kataria et al., 2014). The use of dressings to deliver antibiotics to wound sites can provide tissue compatibility, low occurrence of bacterial resistance and reduced interference with wound healing. Moreover, silver and iodine compounds are popular and effective. Local delivery from dressings can overcome the problem of ineffective systemic antibiotic therapy resulting from the poor blood circulation at the extremities in diabetic foot ulcers.

1.7.6.1. Silver containing dressings

Silver has been used for many years, and a silver ion (Ag^+) has proven broad antimicrobial activity against bacteria, fungi and viruses. It has broad spectrum and

inactivates almost all known bacteria. Silver is believed to interact with thiol groups of key respiratory enzymes as well as interrupting the hydrogen bonding of microbial DNA, and it has been shown to damage bacterial cell membranes (Boonkaew et al., 2014). Silver has been used in the treatment of burns as a silver sulfadiazine cream. The difficulty in applying a cream to a mucous surface is that it will cause the development of mucilaginous slough. The development of a range of modern silver dressings overcomes this difficulty. Silver particles or silver compounds can be incorporated in various forms of wound dressing. Some dressings release silver into the wound, and some maintain the silver within the dressing and kill bacteria, as they are absorbed into the dressing. Dressings with silver are often used in burn wounds and are as well indicated for infected and heavily colonized wounds. The effective antimicrobial actions of silver nanoparticles have been reported by Burrell (2003). The decrease in size and increase in surface area of silver nanoparticles increases their antimicrobial efficacy, but the mechanism of action is unclear due to the difficulty in detection of the unstable silver nano form. The balance between the cytotoxicity and antimicrobial properties of each dressing should always be considered for each wound environment (Boonkaew et al., 2014). The typical minimum inhibitory concentration and minimum bactericidal concentration of silver nanoparticles are 0.78-6.25 μg/mL and 12.5 μg/mL, respectively (Jain et al., 2009).

Various silver-containing dressings act as both absorbing the exudates and releasing the silver onto the wound bed. Chitosan-PVP-nano silver oxide films for wound dressing were created by Archana et al. (2015), where non-cytotoxic products with good antibacterial properties were created. Lin et al. (2014) investigated various silver-containing polyacrylonitrile activated carbon fibers. All the tested wound dressings showed high biocompatibility with sufficient antimicrobial activity *in vitro*. Silver-doped self-assembling di-phenylalanine hydrogels were investigated by Paladini et al. (2013). Successful porous hybrids consisting of bacterial nanocellulose and silver nanoparticles were created by Berndt et al. (2013) by using a gentle synthesis route.

1.7.6.2. Iodine containing dressings

Iodine is one of the oldest known skin antiseptics. However, the use of pure iodine causes local pain and tissue rejection, i.e., allergies. Povidone iodine (polyvinylpyrrolidone)-iodine – PVP-I) has begun to be used as softer effect giving iodine antiseptic. In this complex, iodine is sealed with a polymer film of polyvinylpyrrolidone. The chemical structure of PVP-I is given in Fig. 1.10. PVP-I is a water soluble complex. PVP-I is in fact an iodophor. Iodophors are compounds of iodine linked to the surfactants that act as carriers or solubilizing agents. As such, a small amount of free iodine is released into the solution, thereby minimizing the toxicity while preserving the moderate germicidal activity of the element (Heiner et al., 2010).

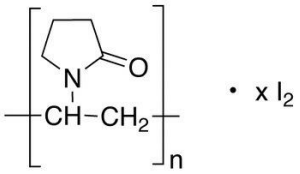


Fig. 1.10. Chemical structure of PVP-I

It is known that PVP-I has a broad anti-microbial spectrum, including putative periodontal pathogens, fungi, mycobacteria, viruses and protozoa; it fails to initiate sensitivity reactions or allows the development of bacterial resistance (Demir et al., 2009). However, there is a confusion concerning the toxicity of povidone–iodine. The most common form of PVP-I complex is its aqueous solution containing from 10% available iodine. These PVP-I solutions are directly applied to open wounds or infected regions. Slow release of iodine from the PVP-I complex minimizes iodine toxicity to the mammalian cells; its sudden absorption by some open wounds during irrigation can cause toxicity problems and sever skin burn limiting its surgical applications, particularly under the repeated applications (Wong et al., 2011). PVP-I can be used straight on the wound, but it can be easily washed away, and the antibacterial effect will be drastically reduced. In order to avoid this effect, PVP-I can be immobilized into various wound dressing materials (Liakos et al., 2013; Mishra and Chaudhary, 2010). The use of iodine in wound treatment is still defendable because the best available evidence supports neither the harmful effects, nor a delay of the wound-healing process, particularly in chronic and burn wounds (Vermeulen et al., 2010).

1.8. Summary of literature data and the justification of the work aim

Wound dressings have significantly changed over the years based on the knowledge of the biochemical processes occurring in the organism. Modern dressing aims not only to prevent the wound infection but as well to improve wound healing. Depending on the wound type and the phase of wound healing, the dressing requires different therapeutic and pharmacological functions. The dressings should maintain moisture in a wound, permit diffusion of gases, remove excess exudates but prevent saturation of the dressing on its outer surface, protect the wound from microorganisms, control the local temperature and pH, minimize scar formation, should be easily changeable and non-adherent to the wound bed, minimize the pain of the wound, should be hypoallergenic, should provide mechanical protection. The development of bandages is significantly influenced by the type of a wound, the condition of the wound and the patient, the stage of wound healing, since the wound healing process consists of several stages **(**hemostasis, inflammation, cellular migration, protein synthesis, and wound contraction).

Variety of wound dressings with different properties are distinguished: low or non-adherent contact layer dressings, hydrogel dressings, hydrofiber dressings, semipermeable film dressings, alginate dressings, foam dressings, hydrocolloid dressings, antimicrobial dressings, deodorizing dressings. Traditional dressings, which are natural, or synthetic bandages and gauzes are cheap but do not provide

moisture for the wound, are adherent to the wound surface, need to be changed regularly and can dehydrate the wound. It is considered that hydrogels have the most properties of an ideal wound dressing. Usually, hydrogels are transparent that the wound could be observed without removing the dressing; they are soft, and their rubbery nature minimizes inflammatory reactions of the surrounding cells. The three-dimensional structure of hydrogels allows them to be used as a drug delivery system. Hydrogels provide the moisture and at the same time are nonadherent materials. Due to high water content, hydrogels allow vapour and oxygen transmission and fit for pressure sores, leg ulcers, burns, necrotic and surgical wounds.

Based on the literature analysis, the objective of this work was to create new wound dressings based on the natural polymers, such as regenerated cellulose, hydroxyethylcellulose, pectin and sodium alginate. Natural polymers are of a special interest due to the high absorption and swelling capacities, non-cytotoxicity. Moreover, various active compounds can be immobilized in these polymers; they are as well an appropriate matrix for the enzyme immobilization and functionalization.

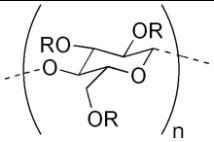
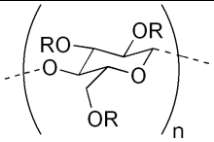
2. MATERIALS AND METHODS

2.1. Materials

Materials used in this study are provided in Table 2.1. Additional purification of materials was not used.

Table. 2.1. Materials used for the research and their characteristics

Material	Chemical formula	Manufacturer and the purity class
Acetic acid	CH_3COOH	SIGMA-ALDRICH, Germany, assay ≥99.7%
Acetone	CH_3COCH_3	Stanlab, Poland, chemically clean
Acetylcellulose	$[C_6H_7O_2(OCOCH_3)_2OH]_n$	SIGMA-ALDRICH (USA), 55% of bonded acetic acid
Aqueous ammonia	$NH_3 \cdot H_2O$	Stanlab, Poland, chemically clean, conc. 25%
Brain Heart Infusion broth	-	Sigma-Aldrich, Germany
Calcium chloride	$CaCl_2$	REACHEM, Slovakia, assay 99.7%
Calendula officinalis blossoms	-	JSC "Acorus Calamus", Lithuania
Chamomilla recutita blossoms	-	JSC "Acorus Calamus" Lithuania
Caspase-3 Assay Kit Z-DE VD- R110	$C_{72}H_{78}N_{10}O_{27}$	Thermo Fisher Scientific, the Netherlands
Citric acid, anhydrous	$HOC(COOH)(CH_2COOH)_2$	REACHEM, Slovakia, assay 99.7%
Disodium phosphate dihydrate	$Na_2HPO_4 \cdot 2H_2O$	MERCK, Germany, assay 99.9%
Dry *Betula pendula* extract		Made in Lithuanian University of Health Sciences
Dry *Perilla frutescens* extract		Made in Lithuanian University of Health Sciences
Ethyl acetate	$CH_3COOCH_2CH_3$	CHEMPUR, Poland, assay 99.4%
Ethyl alcohol	C_2H_5OH	Stumbras, Lithuania, rectified, assay 96.3%
Fetal bovine serum	-	Gibco, South America

Material	Chemical formula	Manufacturer and the purity class
Folin-Ciocalteu reagent	$H_3PMo_{12}O_{40}$ $H_3PW_{12}O_{40}$	SIGMA-ALDRICH, Germany, assay 95%
Formic acid	HCOOH	SIGMA-ALDRICH, Germany, assay ≥95%
Gallic acid	$(HO)_3C_6H_2CO_2H$	SIGMA-ALDRICH, German, assay 97.5%
Glucose anhydrous	$C_6H_{12}O_6$	SIGMA-ALDRICH, German, chemically pure
Glycerol	$C_3H_8O_3$	Eurochemicals, ES, assay 99.5%
Hydroxyethylcellulose HEC (1)	 R = H or CH_2CH_2OH	SIGMA-ALDRICH, Germany, Wm~1300000
Hydroxyethylcellulose HEC (2)	 R = H or CH_2CH_2OH	SIGMA-ALDRICH, Germany, Wm~720000
HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)	$C_8H_{18}N_2O_4S$	Gibco, South America
Hydrochloric acid	HCl	REACHEM, Slovakia, chemically pure, assay 37.0%
Iglo culture medium	-	Sigma-Aldrich, Germany
Magnesium chloride	$MgCl_2$	Sigma Aldrich, Germany, assay 98%
Magnesium nitrate	$Mg(NO_3)_2$	Sigma Aldrich, Germany, assay 99%
Monochloroacetic acid	$ClCH_2COOH$	Sigma-Aldrich, Germany, assay 99%
n-Hexane	$CH_3(CH_2)_4CH_3$	SIGMA-ALDRICH, Germany, assay 99.5%
Neomycin	$C_{23}H_{46}N_6O_{13}$	BELA-PHARM GmbH and Co. KG, Germany

Material	Chemical formula	Manufacturer and the purity class
Ninhydrin	$C_9HO_3 \cdot H_2O$	CHEMAPOL, the Czech Republic, assay 99.8%
Nitric acid	HNO_3	REACHEM, Slovakia, chemically pure, assay 66.0%
Oleic acid	$C_{18}H_{34}O_2$	SIGMA-ALDRICH, Germany, chemically pure
Pectin from citrus peel	$(C_6H_{10}O_7)_n$	SIGMA-ALDRICH, Denmark, galacturonic acid ≥ 74% (dried basis)
Potassium chloride	KCl	MERCK, Germany, assay 99.5%
Potassium dihydrogen phosphate	KH_2PO_4	MERCK, Germany, assay 99.5%
Povidone iodine	$(C_6H_9O)_n \cdot xI$	"Betadine" EGIS PHARMACEUTICALS PLC, Hungary
Quanti-iT™ PicoGreen® dsDNA	-	Invitrogen, USA
Silver nitrate	$AgNO_3$	SIGMA-ALDRICH, Germany, chemically pure
Sodium alginate	$(C_6H_7NaO_6)_n$	SIGMA-ALDRICH, Germany
Sodium carbonate	Na_2CO_3	MERCK, German, assay 99.9%
Sodium chloride	NaCl	REACHEM, Slovakia, assay 99.7%
Sodium dihydrogen phosphate	NaH_2PO_4	MERCK, Germany, assay 99.5%
Sodium hydrogen carbonate	$NaHCO_3$	MERCK, Germany, assay 99.9%
Sodium hydroxide	NaOH	MERCK, Germany, chemically pure
Sodium thiosulphate pentahydrate (fixanal)	$Na_2S_2O_3 \cdot 5H_2O$	Cherkask, Ukraine, chemically pure
Starch (soluble)	$(C_6H_{10}O_5)n$	SIGMA-ALDRICH, Germany
Triton X-100	$C_{14}H_{22}O(C_2H_4O)_{9\text{-}10}$	Sigma Aldrich, Germany
Trypsin-EDTA solution (0.05% trypsin, 0.25% EDTA)	-	Gibco, South America

2.2. Preparation of cellulose hydrogel sheets

Regenerated cellulose was obtained by the hydrolysis of cellulose acetate in an acetone solution according to the patent (Maruska, 1993). The obtained regenerated cellulose solution was poured into the plastic forms and kept at the room temperature until the solid gel was formed. After 10 days, the gel was rinsed with water until the neutral pH was reached. The obtained sheets were marked as a cellulose hydrogel in water, i.e., CHW. For further modifications, in order to obtain glycerol saturated sheets, the initial hydrogel sheets were immersed into glycerol for 24 hrs. The obtained samples were marked as a cellulose hydrogel in glycerol, i.e., CHG.

2.3. Preparation of cellulose hydrogel sponges

In order to obtain cellulose sponges, the lyophilisation process was used. Before lyophilisation, the cellulose gel was cut mechanically to obtain rectangular samples of 40 mm in length, 20 mm in width and about 5 mm in thickness. The obtained pieces of the hydrogel were soaked in the 20% ethanol solution for 24 hours. Subsequently, the samples were removed from the ethanol solution, placed in a tightly sealed container and kept in a freezer at -18 °C temperature for 24 hours. Thereafter, the samples were lyophilised by using the *ALPHA 2-4 LSC* (Martin Christ Gefriertrocknungsanlagen GmbH, Germany) freeze dryer at -110 ºC for a period of 12 hrs. The samples were abbreviated as CS.

2.4. Carboxylation of cellulose hydrogel

Ten grams of regenerated cellulose (~1 g of dry cellulose) are poured with a mixture consisting of 2.75 mL of NaOH (40%) and 30 mL of ethyl alcohol (96.3%). The reaction mixture was heated up to 50 ºC temperature, and 1.29 g monochloroacetic acid was added. The reaction was carried out for 2 hrs at 60 ºC temperature. After the reaction, the hydrogel was rinsed with water till neutral pH.

The amount of carboxyl groups is determined in the following way: wet carboxymethylated hydrogel is poured with 0.1 M HCl and kept for 24 hrs. Hydrogel is washed with water; 0.60 g of hydrogel is poured with 10 mL of 0.1 M NaOH solution. After 5 hrs, hydrogel was sonicated for 20 minutes, filtered and washed with 10 mL of water. The filtrate was titrated with 0.1 M solution of HCl. In parallel, the moisture of the sample was determined. The amount of carboxyl groups was calculated by the formula:

$$A = \frac{(V_t - V_p) \cdot C_N}{m_{abs}}; mmol/g \qquad (2.1)$$

where V_t – 0.1 M HCl amount used to titrate 10 mL of 0.1 M NaOH solution, mL;
V_p – 0.1 M HCl amount used for titration of filtrate, mL;
C_N – HCl molar concentration, mol/L;
m_{abs} –weight of absolutely dry sample, g.

2.5. Modification of the cellulose hydrogel structure by using supercritical carbon dioxide

The cellulose hydrogel obtained as described in chapter 2.2 was modified by using supercritical carbon dioxide. This modification was accomplished by using a supercritical fluid extraction system *SFT-150* (Supercritical Fluid Technologies, USA). The samples were marked as CH-CO_2. Four different groups of the samples were prepared: (1) saturated with water and exposed to supercritical carbon dioxide at the temperature of 50 °C and the pressure at 4000 psi; (2) saturated with water, exposed to supercritical carbon dioxide at the temperature of 80 °C and the pressure at 8000 psi; (3) soaked for 48 hours in the 20% ethyl alcohol solution and exposed to supercritical carbon dioxide at the temperature of 50 °C and the pressure at 4000 psi; (4) soaked for 48 hours in the 50% ethyl alcohol solution and exposed to supercritical carbon dioxide at the temperature of 50 °C and the pressure at 4000 psi.

2.6. Preparation of sodium alginate-pectin-hydroxyethylcellulose hydrogel sponges

The aqueous solutions of 2% w/w sodium alginate, 2% w/w pectin and 1% w/w hydroxyethylcellulose (HEC) were prepared. The obtained solutions were mixed in different ratios. The prepared sponges from pectin, sodium alginate and HEC were marked as SPAH samples.

A povidone iodinated solution was poured in the prepared mixtures of polymers. The final concentration of povidone iodine in all the mixtures was 12 mg/mL. The obtained mixtures were filled into the moulds and stored in a freezer at -21 °C temperature for 12 hours. Subsequently, the samples were freeze-dried for 12 hours in a lyophilizer *ALPHA 2-4 LSC* (Martin Christ Gefriertrocknungsanlagen GmbH, Germany) by using a condenser under -85 °C temperature.

Further, the obtained sponges were cross-linked with $CaCl_2$. Sponges were immersed into the 1% ethanolic solution of $CaCl_2$ for 30 seconds and dried by using a dryer *Binder ED Series 53* (BINDER GmbH, USA) at 37 °C temperature.

2.7. Preparation of silver nanoparticles

The synthesis of silver nanoparticles was performed following the modified Tollens method proposed by Le et al. (2010). Ag_2O was precipitated from a silver nitrate (0.1 mM) solution with NaOH, and the obtained precipitate was filtered and redissolved in the same volume of aqueous ammonia to form $[Ag(NH_3)_2]^+_{(aq)}$. Oleic acid was added under vigorous stirring followed by glucose. The reduction process of the silver complex solution was initiated with UV irradiation (λ=365 nm, 35 W) and continued for 8 h. Silver nanoparticles were prepared in Cardiff University (the United Kingdom).

2.8. Incorporation of silver nanoparticles into the cellulose sponges

For silver incorporation into the samples CS, a diffusion method was used. The initial solution of silver nanoparticles was diluted with water in a ratio 1:99 v/v. Cellulose based sponges were mechanically cut into pieces of 1×1 cm and immersed in 2 mL of the silver nanoparticle solution for 1, 2 and 4 days for diffusion

experiments. Afterwards, the samples were removed from the silver solutions and digested in 4 mL of aqua regia (a mixture of concentrated hydrochloric and nitric acids 3:1 v/v) and diluted with water up to 25 mL. The absorbed silver content was determined by the means of the atomic absorption spectroscopy by using a Varian atomic absorption spectroscope *AAS SpectraAA 20 Plus* (Varian Mulgrave Victoria, Australia). The samples were introduced into the air-acetylene flame and silver recorded at the wavelength of 328.1 nm. The calibration curve was created by using a solution of $AgNO_3$ in 10% HNO_3 (v/v). A calibration curve of absorbance ($A_{328.1}$) *vs* concentration (Ag) was plotted (Fig. 2.1).

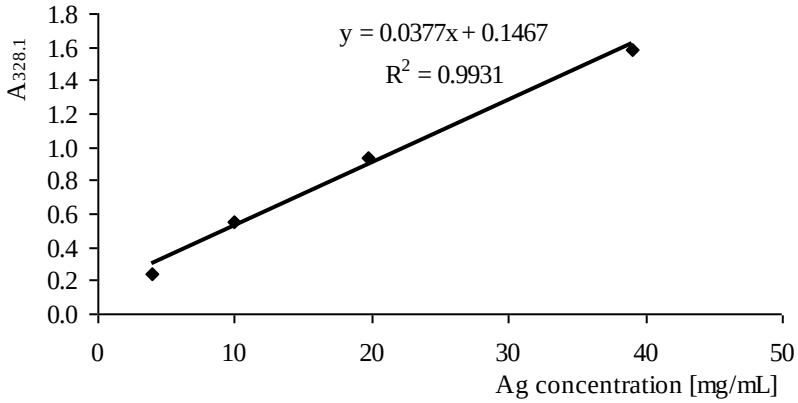


Fig. 2.1. Calibration curve of silver

The silver concentrations in samples were calculated by using the calibration curve.

2.9. Silver release from the cellulose sponge

The analysis of the silver release from the sample CS was performed by keeping the sample in 10 mL of PBS (the phosphate buffer solution, pH 7.4) at 37 ºC temperature. At discrete time intervals (0.5, 1, 2, 3, 6, 12, 24 hrs), 1 mL of the liquid was removed, diluted up to 25 mL with water, and the released silver content was determined by using the atomic absorption spectroscopy method. The accumulative released silver content was calculated.

2.10. Extraction of phenolic compounds from *Callendula officinalis* and *Chamomilla recutita*

Phenolic compounds from dried *Calendula officinalis* (5 g) and *Chamomilla recutita* (5 g) blossoms were separately extracted by maceration with the acetone:water:formic acid (80/20/0.2 v/v/v, total volume 200.04 mL) mixture by using a shaker device at the room temperature for 24 hrs. The solids of each medical plant were filtered off, and acetone completely evaporated under the reduced pressure. The remaining solution was defatted with n-Hexane (100 mL). In order to remove the oligomeric fraction, the remaining solution has been extracted with ethyl

acetate (150 mL). Finally, phenolic compounds derived by evaporating ethyl acetate and dissolving the residual in the deionized water (50 mL).

2.11. Determination of the total content of phenolic compounds by using the spectrophotometric method

The content of total phenolic compounds was determined by using the Folin-Ciocalteu reagent as described by Singleton et al. 1999 (see Fig. 2.2). Briefly, the samples of 0.2 mL were mixed with 1 mL of Folin-Ciocalteu reagent which was diluted with the distilled water (1:10) and 1 mL of 7.5% sodium carbonate (Na_2CO_3). The samples were left to react for 2 hrs at the room temperature. The absorbance of the reaction mixture was measured at 765 nm on a spectrophotometer *Varian Cary 50 UV-VIS* (Varian, Australia).

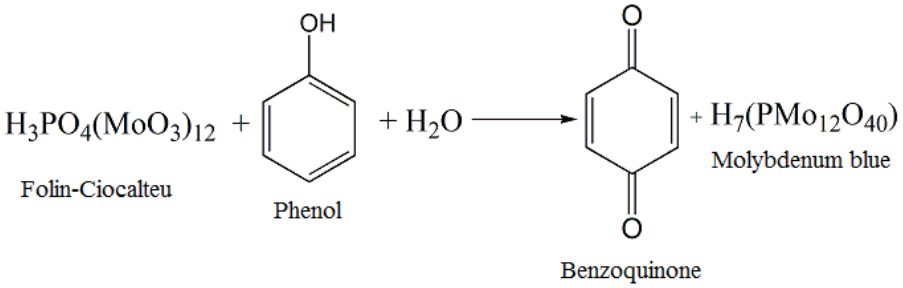


Fig. 2.2. The demonstrative Folin-Ciocalteu reagent reaction with phenolic compounds

The calibration curve was obtained by measuring the absorbance of gallic acid in water solutions and glycerol:water (50:50, % v/v) solutions of different concentrations (Fig. 2.3 and Fig. 2.4).

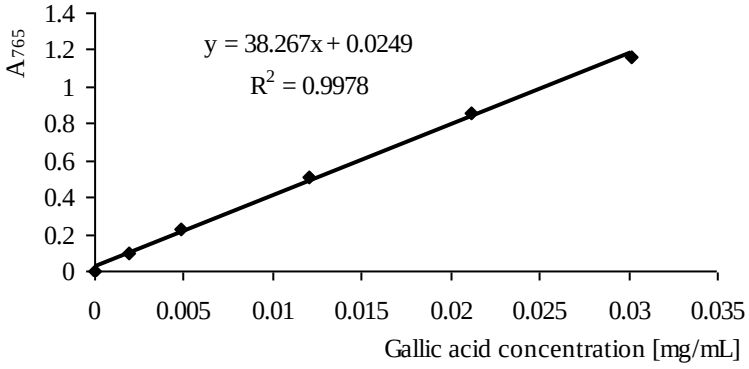


Fig. 2.3. The calibration curve of gallic acid in water solutions

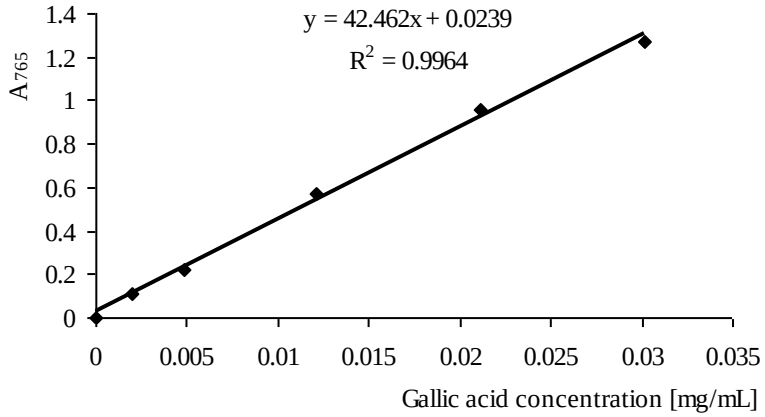


Fig. 2.4. The calibration curve of gallic acid in glycerol:water (50:50, % v/v) solutions

The total concentration of phenolic compounds in samples was calculated by using the calibration curves.

2.12. Immobilization of phenolic compounds from *Callendula officinalis, Chamomilla recutita, Perilla frutescens* and *Betula pendula* extracts

In an ordinary experiment, phenolic compounds (derived from *Calendula officinalis* or *Chamomilla recutita*) were immobilized in CS samples by immersing the CS samples (about 1 g) in 10 mL of the aqueous solution of phenolic compounds (of known phenolic concentration as *C*: 0.38 mg/mL for *Calendula officinalis* and 0.45 mg/mL for *Chamomilla recutita*) for 24 hrs at 4 ºC temperature. The initial weight of CS samples was recorded as m_1. After 24 hrs, the sponge was removed from the active substance solution and dried with a filter paper in order to remove the extra free water. The soaked samples were weighed directly; the weight was recorded as m_2. The amount of absorbed phenolic compounds in sample X_{Abs} (mg) was determined by comparing the difference between m_1 and m_2 by equation:

$$X_{Abs} = (m_2 - m_1) \times C \,. \tag{2.2}$$

Triplicate experiments were carried out.

Phenolic compounds derived from *Perilla frutescens* and *Betula pendula* extracts were immobilized in CH-CO_2 samples (sample size 1×1 cm) by immersing the samples in 5 mL of water-based and water-glycerol (1:1, v/v) based solutions for 48 hours at 4 ºC temperature. Active substance solutions of dry extracts from *Perilla frutescens* and *Betula pendula* were prepared by dissolving them in water and the water-glycerol (1:1, v/v) mixture. The concentration of water based *Perilla frutescens* extract was 0.0115 mg/mL, which of the water-glycerol extract was 0.0098 mg/mL. The concentration of the water based *Betula pendula* extract was 0.0204 mg/mL, which of the water-glycerol extract was 0.0169 mg/mL. The total

amount of phenolic compounds was determined by using the spectrophotometric method described in the chapter 2.11.

Perilla frutescens and *Betula pendula* dry extracts were prepared at the Lithuanian Health Sciences University.

2.13. Release kinetics of the phenolic compounds from cellulose sponges and hydrogels

In order to study phenolic release kinetics, PBS buffer (pH 7.4) was chosen as a medium. One piece of CS sample saturated with phenolic compounds was placed in a beaker containing 10 mL of PBS. The beaker was kept in an incubator for 5 hrs at a constant temperature of 37 °C. At discrete time intervals, an aliquot of 0.2 μL of the liquid was removed from the incubator and the content of released total phenolic compounds was determined by using the spectrophotometric method. Triplicate experiments were carried out.

Tests of the release of phenols from CH-CO_2 samples were made at the room temperature by using water as a model medium. Samples with immobilized *Perilla frutescens* and *Betula pendula* extracts were immersed in a beaker with 5 mL of water. The beaker was kept in an incubator for 24 hrs at a constant temperature of 37 °C. At discrete time intervals, the aliquot of 0.2 μL of the liquid was removed from the incubator, and the total amount of released phenols was determined by using the spectrophotometric method. Triplicate experiments were carried out.

2.14. Immobilization of neomycin in cellulose sponges and cellulose hydrogels

The CS samples were immobilised with water-based neomycin solution. Samples were cut into 15×15 mm pieces, weighted (m_1) and immersed for 24 hrs at 4 ºC temperature into the neomycin solution with the concentration of 40 mg/mL (C). After 24 hrs, the sponges were removed from the active substances solutions, dried with a filter paper in order to remove the extra free water and directly weighed (m_2). The amount of absorbed neomycin in sample X_{Abs} (mg) was determined by the equation (2.2).

Water-based and glycerol-based solutions of concentrations 40 mg/mL were used for the immobilization into CHW and CHG samples. Samples of hydrogel sheets were cut into 15×15 mm pieces and immersed for 24 hrs at the 4 ºC temperature into water-based or glycerol-based neomycin solutions with the concentration mentioned above. The amount of absorbed neomycin in a sample X_{Abs} (mg) was determined by the equation (2.2).

2.15. Determination of neomycin by using the spectrophotometric method

For the determination of neomycin, the reaction with ninhydrin was carried out (Fig. 2.5).

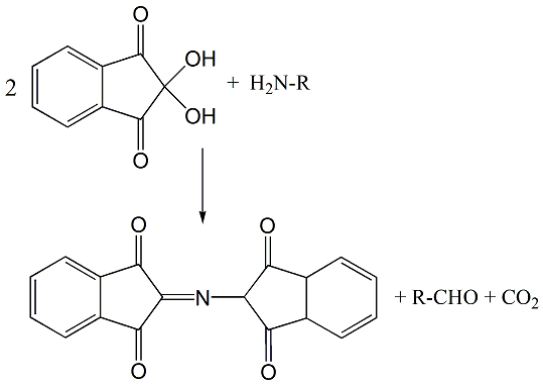


Fig. 2.5. The ninhydrin reaction with amino groups where R is the residue of neomycin

The 0.1% solution of ninhydrin is used in the 1:1 proportion with the sample. In order to accelerate the reaction, the heating of samples in a boiling water bath for 6 minutes was used. Further, the samples were cooled under the stream of cold water, and UV absorbance measurements at 320 nm were carried out by using a spectrophotometer *Varian Cary 50 UV-VIS* (Varian, Australia). The calibration curve was obtained by measuring the absorbance of water-based and glycerol-based neomycin solutions of known concentrations (Fig. 2.6 and Fig. 2.7).

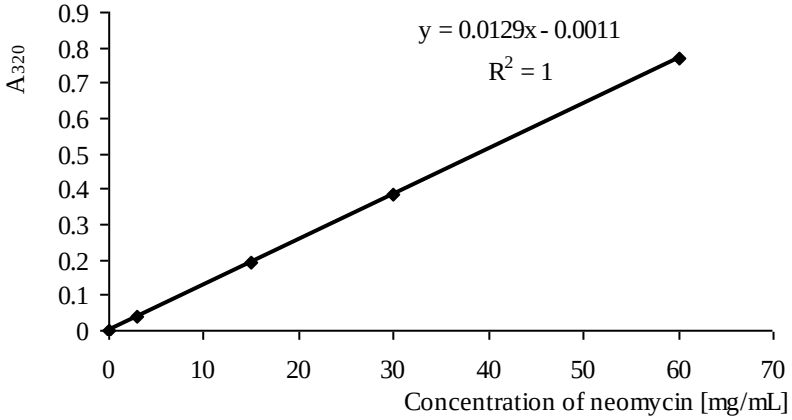


Fig. 2.6. The calibration curve of neomycin in water

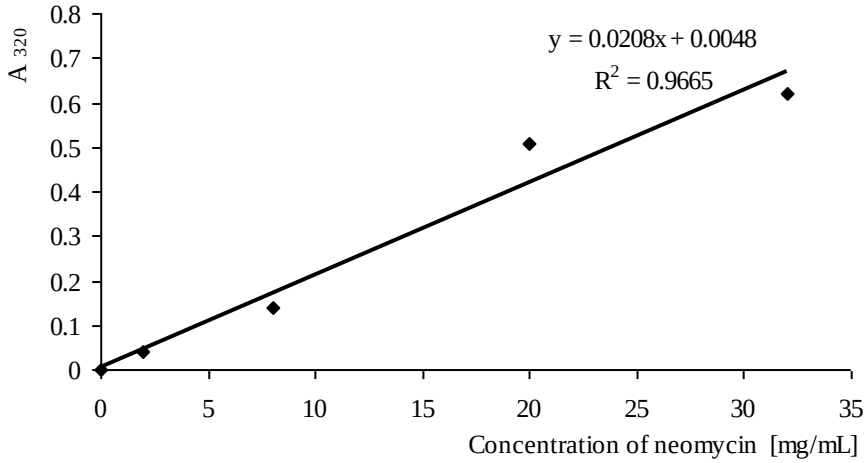


Fig. 2.7. The calibration curve of neomycin in glycerol

Neomycin concentrations in samples were calculated by using the calibration curves.

2.16. Kinetics of neomycin release from the cellulose sponges and cellulose hydrogels

The tests of the neomycin release from CS, CHW and CHG samples were made at the room temperature by using PBS (pH 7.4) as a model medium. Samples with immobilized neomycin were immersed in a beaker with 5 mL of the phosphate-buffered saline (pH 7.4) solution. The beaker was kept in an incubator for 24 hrs at a constant temperature of 37 °C. At discrete time intervals, the aliquot of 0.2 μL of the liquid was removed from the incubator, and the released amount of neomycin was determined by using the spectrophotometric method. Triplicate experiments were carried out.

2.17. Determination of iodine release by using the titration method

The tests of iodine release from sodium alginate-pectin-hydroxyethylcellulose sponges were made according to the European Pharmacopoeia 8.0 article 01/2012:2416 (Povidone iodine). The sponges with immobilised povidone iodine were immersed in a beaker with 20 mL of distilled water, closed tightly, mixed by using the magnetic stirrer protecting the samples from the light. At discrete time intervals, an aliquot of 1 mL of the liquid was removed from the beaker; 0.1 mL of dilute acetic acid and a few drops of starch indicator were added. Sample titration was carried on with 0.01 M sodium thiosulphate solution, until the blue colour of the solution disappeared; 1 mL of used sodium thiosulphate is equal for 1.269 mg of released iodine. The triplicate experiments were carried out.

Dilute acetic acid was prepared according to the European Pharmacopoeia 8.0 article 01/2011:40 101 (Reagents); 12 g of glacial acetic acid was diluted to 100 mL with water.

Starch indicator was prepared according to the European Pharmacopoeia 8.0 article 01/2011:40 101 (Reagents); 1.0 g of soluble starch was triturated with 5 mL of water and whilst stirring the mixture was poured into 100 mL of boiling water.

2.18. Morphological and structural characterization

A high resolution field emission scanning electron microscope (SEM) *Quanta 200 FEG* (FEI Company, the Netherlands) with the *Schottky* type electron gun was chosen to observe the morphology of the sponges and hydrogels. Approximate sizes of pores were measured directly. The micrographs were done at a magnification of 100.

The micro-computed tomography (micro-CT) analysis was performed by using a *μCT40* micro-CT system (Scanco Medical AG, Switzerland). For the analysis, a digital cylinder with a diameter of 1000 voxels and a height of 800 voxels was extracted. The scanning settings were set as follows: the scanning medium was the air, the energy 45 kVp, the integration time 600 ms, frame averaging 2x, the nominal resolution 10 μm. The data were filtered by using a constrained 3D Gaussian filter to partially suppress the noise in the images (σ=0.8, support=1).

The silver nanoparticles were characterized by the transmission electron microscopy (TEM); a 4 μL droplet of the nanoparticle suspension was placed on a plain carbon-coated copper TEM grid and allowed to evaporate in the air under the ambient laboratory conditions. Bright field TEM images were obtained by using a JEOL-1010 microscope at 80 kV, equipped with a Gatan digital camera. Typical magnification of the images was 100000x. The images were analysed with the computer programme *ImageJ*, and the diameters of at least 100 particles were determined.

2.19. Preparation of the pseudo extracellular fluid

PECF, which simulates the wound fluids, was prepared by dissolving 0.68 g of sodium chloride (NaCl), 0.22 g of potassium chloride (KCl), 2.5 g of sodium hydrogen carbonate ($NaHCO_3$) and 0.35 g of sodium dihydrogen phosphate (NaH_2PO_4) in 100 mL of distilled water (Singh and Pal, 2008). The pH of PECF was adjusted to 8 ± 0.2. The pH was measured with *744 pH Meter* (Metrohom, USA).

2.20. Preparation of the buffer solution PBS

The phosphate buffered saline (PBS) solution of pH 7.4 was prepared by taking 0.137 g of sodium chloride (NaCl), 0.0025 g of potassium chloride (KCl), 0.01 g disodium phosphate dihydrate ($Na_2HPO_4{\cdot}2H_2O$) and 0.002 g potassium dihydrogen phosphate (KH_2PO_4). All the reagents were dissolved in 800 mL of distilled water, and the pH was adjusted to 7.4 by using 0.1 M HCl. Then, the solutions were diluted with the distilled water up to 1000 mL.

2.21. Absorption of liquids imitating the body fluids

This experiment imitates an open exuding wound dressed with a wound dressing. The initial weight of rectangular samples of the sponges (approximately 15×15 mm) was determined (m_0). Samples were incubated in the PECF solution (pH

8.0) or in the PBS solution (pH 7.4) at 37 ºC. The weights of the swollen samples were determined by draining the sample surface with a filter paper and weighing the sample (m_t). The weights of swollen samples were recorded after the predetermined time intervals.

PECF, PBS absorption (I_A, %) was calculated from the equation:

$$I_A = \frac{m_t - m_0}{m_0} \times 100, \%. \quad (2.3)$$

Triplicate experiments were carried out.

2.22. The rate water evaporation from a hydrogel

In order to prevent the variations of humidity, a desiccator filled with a saturated magnesium nitrate solution was used. A desiccator was kept in a drying oven at 35 ºC. After equilibration, the relative humidity in the desiccator was approximately 50%. The hydrogels prepared as described earlier were slightly dried up with a filter paper and weighted (W_0), then placed in a desiccator, and after certain intervals of time, the samples were weighted again (W_t). The evaporated water content was calculated by the equation:

$$W_E = \frac{W_0 - W_t}{W_0} \times 100, \%. \quad (2.4)$$

Triplicate experiments were carried out.

2.23. The water vapour transmission rate

The water vapour transmission rate (WVTR) was determined according to the procedure of the ASTM method E96-90 (Ruiz-Cardona et al., 1996). The water vapour transmission rate was determined by fixing the cellulose sponge's disc of 30 mm diameter to the cap of a permeability cup containing 20 mL of distilled water. An open cup was used as a control. In order to prevent variations of humidity, a desiccator filled with the saturated magnesium chloride solution was used. A desiccator was kept in a drying oven at 35 ºC. After the equilibration, the relative humidity in the desiccator was approximately 40%. Then, the cups were placed in the desiccator and re-weighed at determined intervals (1, 3, 5, 7, 9, 12, 24 hrs). The weight loss corresponds to the evaporated water content. The experiments were triplicated, and the average values were calculated. The water vapour transmission rate (WVTR) was calculated by using the equation:

$$WVTR = \frac{m_l \times 24}{t\,S}, \; (g / m^2 / day); \quad (2.5)$$

where m_l is the mass loss during the time t (measured in hours), and S is the area of the cup mouth. Triplicate experiments were carried out.

2.24. Mechanical properties (a tensile test)

The tensile strength of the samples and Young's modulus were evaluated by using a material testing device *Zwick/Roell BDO-FB0.5TH* (Zwick GmbH and Co, Ulm, Germany). Prior mechanical testing dry CS samples were kept in the PBS solution (pH 7.4) for 30 minutes at the room temperature, and then the samples were removed and gently wiped with a filter paper. Strip-shaped sponges (40×10×5 mm^3) were used for the test. Dumbbell shape specimens with the dimensions 35×5 mm were punched out from the cellulose hydrogel sheets. The thickness of each sample was measured with a digital calliper. Samples were fixed with two clamps in a vertical position and preloaded at 0.02 N. After the pre-load, the force was set to zero, and a constant crosshead speed of 2 mm/min was determined. Data were collected until the sample's rupture. Strain-stress curves were recorded. The computer Software *V11.02 TestXpert* provided the tensile strength (MPa), the percentage elongation at the break moment (%) and the Young's modulus (MPa). Triplicate experiments were carried out.

2.25. Mechanical properties (a rupture test)

The standard pharmaceutical drug release film test was performed with a *TA.TX Plus Texture Analyser* (Stable Micro Systems, United Kingdom) device. Samples were cut into 30×30 mm diameter pieces. The stainless steel spherical ball probe and 5 kg load cell were used for the test. The film support rig was fitted to the heavy duty platform and positioned loosely on the machine base. The ball probe was aligned with the film support rig that the probe could move through the aperture centre. Then, the probe reached the sample, the force increased until the film broke. The test speed was 1 mm/s. The force needed to rupture the films (N) and the distance to burst (mm) as the indication of flexibility was estimated. Triplicate experiments were carried out.

2.26. Mechanical properties (a compressive test)

A compression test was carried out with a *Zwick/Roell BDO-FB0.5TH* (Zwick GmbH and Co, Ulm, Germany) dynamometer, where samples can be pressed at different speeds and power. Cylindrical specimens were used for the test. The samples were placed between the compression machine brick, centred under the direction of the applied force (1N), and a pressure of 2 mm/min. The relative deformation ε was calculated by the following equation:

$$\varepsilon = \frac{x}{l}, \%; \tag{2.6}$$

where χ is sample deformation, shortening, m; l is the length of a sample, m.

Young's modulus is a physical parameter that describes the resistance to compression or stretching. It is defined as the stress and elongation (deformation) ratio:

$$E = \frac{P}{x \cdot S}, \text{MPa}; \quad (2.7)$$

where P is a stretch ratio of χ (m) which satisfies the input force, N; S is the cross-sectional area of the sample, m^2.

The modulus of elasticity multiplied by the cross-sectional area of the sample is called a tension-compression rod stiffness ζ. It quantifies the ability of the rod to resist for the deformable influence:

$$\xi = S \cdot E, \ m^2, \text{Pa}; \quad (2.8)$$

where S is the cross-sectional area of the specimen, m^2; E is the Young's modulus, Pa.

Triplicate experiments were carried out.

2.27. Hardness and resiliency investigation

The hardness and resiliency test was performed with a *TA.XT Plus Texture Analyser* (Stable Micro Systems, UK) machine. Cylindrical shaped samples were used for the analysis. The tests were performed at the room temperature. The specimens were placed between the parallel flat plates with 100 mm diameter and compressed twice at 1.0 mm/s. Then, the plate compresses the sample to half height of the sample and returns to its original position. After 10 seconds, the test is repeated. The force-time curve is recorded; hardness and resiliency values are provided by computer software. Triplicate experiments were carried out.

2.28. Dynamic-thermomechanical analysis

The dynamic-thermomechanical analysis was performed by using a *Modular Compact Rheometer MCR 302* (Anton Paar GmbH, Austria) device. The research was conducted by using 50 mm length, 7 mm wide and 0.5 mm thick specimens. During the experiment, the temperature gradient was used. The measurements of the extensional storage modulus and extensional loss modulus were performed in a temperature range from 20 ºC to 40 ºC (heating rate 1 ºC/min). The specimens were tested in an air atmosphere and treated with a constant mechanical vibration under the tensile mode at a frequency of 0.1 Hz (oscillation amplitude 2 mm).

2.29. *In vitro* bioadhesion measurements

Bioadhesion properties were evaluated with a *Modular Compact Rheometer MCR 302* (Anton Paar GmbH, Austria). In order to imitate the wound surface, an egg shell membrane was used (Sandri et al., 2013). A cylinder probe of the diameter 10 mm was used, the sample size was 1×1 cm. In this analysis, the force of 10 N was applied. In order to fix the eggshell membrane, a double-sided adhesive tape was used. The hydrogel sheets were fixed to the upper probe and the eggshell membrane to the bottom probe. The sample and the biological substrate were put into contact for 60 s. After this period, the upper probe was raised up at 0.05 mm/s speed until the eggshell membrane, and the sample became completely unstuck. Before each measurement, the eggshell membrane was moistened with 100 μL of 0.9% sodium

chloride solution. The adhesive properties were evaluated by the maximum force (F_{max}) necessary to detach the eggshell membrane from the sample; the total work of adhesion (TWA) was represented by the area under the force versus distance curve and cohesiveness (d) was expressed as the sample distance travelled until the detachment moment from the biological membrane. Triplicate experiments were carried out.

2.30. Transparency/chromaticity test

Transparency/chromaticity of cellulose hydrogel sheets was analysed with a *MiniScan XE Plus colorimeter* (Hunter Associates Laboratory, Inc, Reston, VA, USA). Before each series of measurements, the spectrophotometer was calibrated on a standard white tile (X = 81.3, Y = 86.2, Z = 92.7). The colorimeter was set to measure the total reflectance with an illuminant C and at the 10° observation angle. The parameters L*, a* and b*, that mean lightness, red value and yellow value within the CIE (Commission Internationale l'Eclairage) Lab three-dimensional colour space, were measured, converted into the hue angle ($h° = \arctan(b^*/a^*)$) and chroma ($C = (a^{*2}+b^{*2})^{1/2}$) (Mcguire, 1992). The hue angle is expressed on a 360º grid, where 0º = red, 90º = yellow, 180º = green and 270º = blue. The coordinates of the colour were processed with the *Universal Application Software V.4-10*. The white background was used for measurements. Triplicate experiments were carried out.

2.31. Antibacterial activity of the regenerated cellulose sponges loaded with silver nanoparticles

The antibacterial activity of the CS samples loaded with silver nanoparticles was studied by using *Staphylococcus epidermidis RP62a*. Before testing, bacteria were grown statically overnight in the Brain Heart Infusion (BHI) broth at 37 °C. The sponges containing silver nanoparticles were placed in a sterile 24 wells plates, and 1 mL of *Staphylococcus epidermidis* bacteria in the BHI broth (the concentration of cells was 10^9 CFU mL^{-1}) was added. Plates were incubated at 37 °C for one hour; then, the bacteria cultures were removed, and the samples were washed with 1 mL of PBS three times; 1 mL of the BHI broth diluted with the sterile PBS (1:10) was transferred onto the sponges and incubated at 37 °C for 24 hrs. Afterwards, bacteria cells were counted by the Miles and Misra method on a BHI Agar after serial dilutions in PBS. Experiments were performed in triplicate. Petri dishes were incubated for 24 hours at 37 °C. After that, *Staphylococcus epidermidis* colonies were counted, and the average result was evaluated.

2.32. Antibacterial activity of the regenerated cellulose hydrogel loaded with neomycin

Bacteria strains ATCC (American Type Culture Collection) were used for the experiment. Gram-negative *Escherichia coli* (DH5 α) and Gram-positive *Bacillus subtilus* (ATCC 6051) bacteria strains were used for the test; 250 μL ($0.5\text{-}2.5 \times 10^{-8}$ cells/mL) of each bacteria suspension were flooded on a Petri dish with the LB agar (Lennox, USA). The cellulose samples were cut to 15 mm side squares, placed on

Petri dishes with agar and incubated for 18 hrs at 37 °C temperature. Antibacterial activity was assessed by measuring the inhibition zone, measuring from the edge of the disk to the end of the clear zone.

2.33. Cytotoxicity test

Cytotoxicity was tested by two different methods.

This part of cytotoxicity studies was carried out in collaboration with scientists from the National University of Karazin Kharkiv, Department of Physiology of Ontogenesis (Ukraine). The cytotoxicity test was carried out for rat hepatocytes. The hepatocyte stock from a healthy 3 month old rat was isolated. The primary cell counting was performed: the cell number in stock was about 10^8 cells/mL, viability ~ 95%. Further, hepatocyte cells were resuspended in a culture medium; 2×10^7 cells/mL hepatocytes were resuspended in 199 mL of a buffered medium with HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) at pH 7.4, where cells can maintain high viability and normal metabolic rates for a long time, up to 24 hours. After resuspension, the cells were dispensed on Petri dishes. Milled samples at a concentration of 50 mg/mL were added to Petri dishes with hepatocytes and incubated for 90 minutes at 37 °C. After incubation, the cells were transferred to centrifuge tubes. Aliquots for the cell count and viability testing were dispensed. Cell viability was evaluated by staining with the trypan blue; triplicate experiments were carried; ≥600 cells for every test were used. The cells were counted in two chambers of a standard hemocytometer 50 squares each time for every sample. Moreover, in order to determine if the hepatocyte membrane can be damaged by the CHG and CHW samples, lactate dehydrogenase (LDH) activity of the samples was measured in the incubation medium by the means of a bioassay kit for LDH determination.

The next part of cytotoxicity studies was carried out in collaboration with scientists from the University of Porto, Faculty of Dentistry (Portugal). Cytotoxicity studies were performed with CS samples and CS samples immobilized with the antibiotic neomycin. For the research, the human L-929 fibroblast line cells culture was used. L-929 line cells were grown in a modified α Iglo culture medium (α-MEM) supplemented with 10% fetal bovine serum, 1% of vitamin C, 1% of penicillin plus 1% of fungicide in plastic flacons at 37 °C in a humidified atmosphere of 5% CO_2 in the air. For a subculture, the cell monolayer was washed twice with a phosphate-buffered saline (PBS) and incubated with a trypsin-EDTA solution (0.05% trypsin, 0.25% EDTA) for 5 minutes at 37 °C to ensure cell detachment.

Before seeding cells, samples were sterilized by ionizing UV radiation for 24 hours. The samples were soaked for 1 hour in α-MEM medium containing 10% of fetal bovine serum, 1% of vitamin C, 1% of penicillin and 1% of a fungicide. Samples of cellulose hydrogels were placed on the bottom of the wells of 48-well culture plates (one sample/well) and were seeded with L-929 fibroblast cells ($10^4 \text{cells}\,\text{cm}^2$). Seeded samples were cultured for 1, 4 and 8 days at 37 °C in a humidified atmosphere of 5% CO_2 in the air. Colonized materials were evaluated throughout the culture time by CLSM (confocal laser scanning microscopy) *Leica*

SP2 AOBS (Leica Microsystems) and SEM (*Quanta 400 FEG ESEM*) in order to address the cells morphology and the cells proliferation.

The determination of cell apoptosis was done in the following way. The cells culture grown on samples were collected after 1, 4 and 8 days and washed twice with the PBS solution, where 50 μL of the prepared samples were used further. The working buffer is prepared from 10 μL of the 5 mM Z-DE VD- R110 (Caspase-3 Assay Kit) substrate and 990 μL of the reaction buffer; 50 μL of this mixture was placed in a dish containing the samples. The prepared specimens were kept in the dark for 30 minutes, and then the fluorescence was measured at 496-520 nm *Elisa reader* (Synergy HT, Biotek).

DNA was analysed by the PicoGreen DNA quantification assay (Quant-iT PicoGreen dsDNA Assay Kit, Molecular Probes Inc., Eugene) according to manufacturer's instructions. Cultures were treated with Triton X-100 (0.1%), and fluorescence was measured on an *Elisa reader* (Synergy HT, Biotek) at wavelengths of 485-528 nm excitation and emission, respectively, and corrected for fluorescence of reagent blanks. The amount of DNA was calculated by extrapolating a standard curve obtained by running the assay with the given DNA standard. Triplicate experiments were carried out.

2.34. Porosity test

The porosity test for sponges was carried out by the liquid displacement method (Yang et al., 2002). Ethanol (96.3%) was used as a displacement liquid because it penetrates easily into the pores and does not cause shrinking or swelling of the sample. Rectangular samples of 10 mm in length, 10 mm in width and about 5 mm in thickness were cut out. Using a Vernier calliper, the samples were measured accurately and the volume of the sample (V_s) was calculated. The samples were placed in a graduated test-tube and poured with 2.5 mL of ethanol that was tightly closed. Then, the samples were exposed to ultrasound in an ultrasonic bath for 5 minutes, kept until the sample cooled-down and diluted with ethanol up to the 4 mL mark. The test-tube was tightly closed and weighted (W_t). The samples saturated with ethanol were taken out carefully, and the test-tubes were weighed (W_k). After weighing, the remaining quantity of ethanol was diluted with the same solvent up to the 4 mL mark of the test-tube. The test-tubes were clogged with the same stoppers and weighed again (W_w). The density of 96.3% ethanol solution was measured with an aerometer (the determined density was 0.810 g/cm^3). The actual volume of the sample was calculated according to the formula (2.9):

$$V_a = \frac{(W_w - W_t + W_m)}{0.81},\ g/cm^3. \tag{2.9}$$

The porosity of the sample was calculated according to the formula (2.10):

$$P = \frac{V_S - V_a}{V_S} \cdot 100,\ \%. \tag{2.10}$$

Triplicate experiments were carried out.

2.35. The blood clotting test

A blood coagulation study was carried out by the spectrophotometric method according to the modified Pei-Leun Kang method (Kang et al., 2011). Blood was obtained from the vein of a volunteer and mixed with the blood anticoagulant (ACD) in a ratio 6:1. The ACD solution was prepared according to the European Pharmacopoeia 7.0 article 01/2008:0209 (Anticoagulant and preservative solutions for human blood). The ACD solution was prepared by mixing substances presented below:

- sodium citrate 22.0 g;
- citric acid, anhydrous 7.3 g;
- glucose, anhydrous 22.3 g;
- water for injections up to 1000 mL.

The samples of the 1×1 cm size were cut out from the sponges. The weight of all samples was around 0.02 g. The samples were placed in the plastic test tubes, closed and kept in a thermostat *Binder ED series 53* (BINDER GmbH, USA) at 37 °C for 15 minutes. After the set period of time, 200 μL of blood with ACD was slowly spread onto the surface of the sample. In order to initiate the blood clotting process, 20 μL of the 0.2 M $CaCl_2$ solution was spread on the surface of the sponge. Then, the tubes containing the samples were incubated at 37 °C. After 15min., 20 mL of distilled water was carefully added by dripping water down inside the wall of the tubes, preventing disruption of the clotted blood. Red blood cells that were not entrapped in the clot were haemolysed with distilled water, and the absorbance of the resultant haemoglobin solution was measured at 540 nm *Cary 50 UV– VIS* (Varian, Inc., Netherlands). The absorbance of 200 μL of the whole blood in 20 mL of distilled water was used as a reference value. Triplicate experiments were carried out.

2.36. Statistical data analysis

The mean values of experiments and standard deviation values were calculated by using MS Excel software package.

$\bar{x}$ arithmetic average was calculated as follows:

$$\overline{x_n} = \frac{1}{n}\sum_{i=1}^{n} x_i \ ; \tag{2.11}$$

where n is the number of measurements; x_i is the measuring value obtained by i experiment.

The standard deviation (SD) was calculated by using the following formula:

$$SD = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}} \tag{2.12}$$

where n is the number of measurements; x_i is the measuring value obtained by i experiment; $\bar{x}$ is the arithmetic average of the measurement values.

3. RESULTS AND DISCUSSION

There are different kinds of wounds with different requirements. In a case of burn wounds, the dressing should be compliant and durable, non-adherent, allow a gaseous exchange, provide a high humidity level to the wound and allow a maximum activity for the wound to heal without retarding or inhibiting any stage of healing processes. Chronic wounds are highly inflammatory, occur because of tissue destruction and usually have the excessive production of exudate. Exudate of chronic wounds slows down the proliferation of keratinocytes, fibroblasts and endothelial cells, which are important in the healing processes, which is why it is very important to absorb the exudate from chronic wounds. In a case of highly bleeding wounds, it is important to initiate blood clotting, absorb the exudate and prevent the wound from infection. Overall, a wound dressing should protect the wound from the physical damage and micro-organisms, be compatible with topical therapeutic agents, should be non-toxic and non-irritant; therefore, it should be preferably made from natural materials. A variety of wound dressing forms are necessary as well, as wounds differ in their size and depth.

In this work, four different kinds of wound dressings, which could be applied for the different wound treatment, were made. In order to make it easier to understand from what are the samples made, there a scheme (Fig. 3.1) of the manufactured samples with their abbreviations and illustrations of a sample look is presented below.

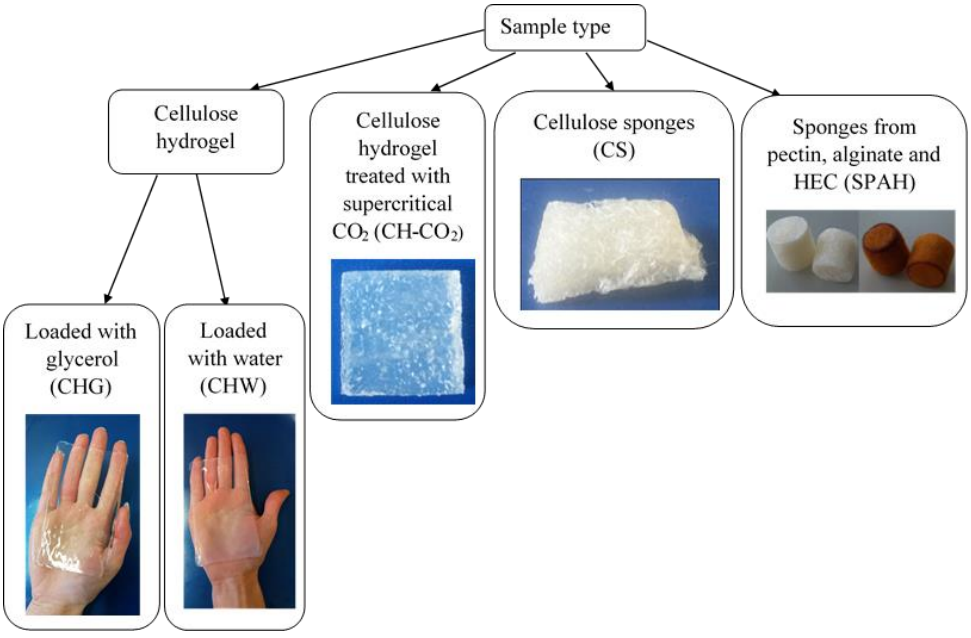


Fig. 3.1. Scheme of the manufactured samples for the wound dressings

3.1. Preparation and characterization of regenerated cellulose hydrogel sheets

Hydrogel sheets, as inert matrices with the three-dimensional structure, were prepared by the regeneration of cellulose from cellulose diacetate. The shape of cellulose hydrogel sheets could be easily modified as it could be poured in the desired size and desired thickness. After selecting the desired size of the vessel, the sheets can be poured out according to the wound size or body part, where the dressing will be used. It is worth to mention that the obtained cellulose hydrogel sheets are transparent, and the wound could be monitored without unnecessary ligation and interfering with the healing process. Hydrogels are weakly adherent; thus, they do not irritate the wound and cause less pain for the patient.

The initial cellulose hydrogel is water-based (CHW). In order to improve mechanical, bioadhesion properties, the initial cellulose hydrogel sheets were loaded with glycerol (CHG). Cooling and hydrating effects are very important for burn wound treatment, and various additives are used to get the moisturizing effect, for example, glycerol. Glycerol is nontoxic, colourless, odourless, able to attract moisture from the air and does not oxidize in the air. Concentrated glycerol is antibacterial and antiviral.

Due to a high water content and flexibility, the hydrogels are very similar to natural tissue. The sheets could be loaded with different active compounds. Due to high porosity, the transport of various active substances can be easily achieved. These and many other features make hydrogel sheets to be promising as a burn wound dressing. Hydrogels are highly moisturizing, it could be suitable as well for cut scabs and abrasion wounds, as the moist environment prevents from the formation of hard scabs, which act as a barrier to the development of new tissue.

In the previous works where the inverse size exclusion chromatography was used, it was determined that the pores of the cellulose gel prepared according the same method were available for molecules with a molecular weight of up to 500.000 Da (Aniulytė et al., 2006). Such porosity should permeate the gases, while the hydrogel nature will keep the wound environment moist. The fluid balance, especially for the burn injury, is a very important factor, since an abundant loss of water from the wound by exudation and evaporation may lead to the fall in body temperature and an increase in the metabolic rate.

3.1.1. Transparency/chromaticity test

Nowadays, transparency of wound dressings gets more and more attention. Transparency in wound healing has many advantages, as the wound can be observed externally during the healing period, and unnecessary ligation of wound dressings is avoided; thus, a patient feels less pain and more comfort.

The index L* refers to the white and black colour ratio (brightness) and ranges from 0 (pure black) to 100 (pure white). In this case, the CHW sample has closer value to pure white (88.61 ± 0.77) than the CHG samples (79.54 ± 0.10). The negative values of the index a* refer to the intensity of a green colour opposite to red as well as the positive b* index to the intensity of a yellow colour opposite to blue. The CHG samples have a more intense yellow colour compared with CHW as well as the chromaticity index C* that increases in the CHG samples (Table 3.1).

Table 3.1. Transparency/chromaticity of CHW and CHG samples

Sample	L*±SD	a*±SD	b*±SD	C*±SD	h°±SD
CHW	88.61±0.77	-1.19±0.01	2.57±0.20	2.84±0.18	114.99±1.63
CHG	79.54±0.10	-0.43±0.02	12.76±0.07	12.76±0.0	91.95±0.09

As the colour becomes more intense, the C* value increases. The hue angle in the CHG samples confirms that the samples saturated with glycerol appear more yellow, and the colour gets more pure. As it is seen from Fig. 3.2, both films have very good transparency.

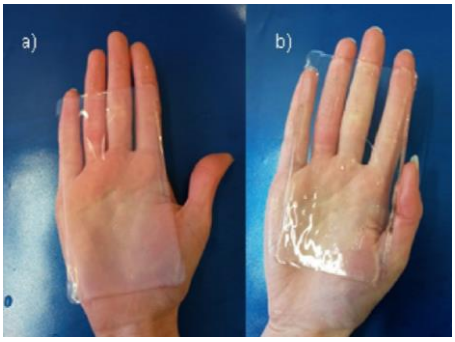


Fig. 3.2. Demonstrative transparency of CHW (a) and CHG (b) samples

The transparency of these sheets permits constant observation of the wound during the healing process that the dressing would not be prematurely removed.

3.1.2. *In vitro* bioadhesion measurements

Table 3.2 shows the results of bioadhesion measurements. Impregnation with glycerol had a positive effect on bioadhesion properties. The maximum force necessary to detach cellulose sheets from a biological membrane, the total work of adhesion and cohesiveness decreased significantly. Values of this test are close or even better than those mentioned in the literature (Boateng et al., 2013; Khan et al., 2000), where polyox with carrageenan based composite films and chitosan-based films for wound dressing were prepared.

Table 3.2. Bioadhesion measurements (maximum force (F_{max}) necessary to detach the egg shell membrane from the sample, the total work of adhesion (TWA) and cohesiveness (d))

Sample	F_{max}, N ± SD	TWA, mJ ± SD	d, mm ± SD
CHW	1.6 ± 0.39	0.35 ± 0.10	2.8 ± 0.27
CHG	0.7 ± 0.10	0.2 ± 0.01	1.5 ± 0.02

The typical wound dressings after the contact with the wound become soft and could stick to the wound. The removing of such wound dressing causes discomfort for a patient and becomes painful. In order to keep the wound clean, traditional wound dressings as gauzes should be changed once a day or twice a day, while hydrocolloids need to be less frequently changed; thus, they cause less pain and are

as well less painful during the removal process (Lagana and Anderson, 2010). The less adhesiveness has a wound dressing, the less damage is made to a newly formed wound bed.

3.1.3. Mechanical properties

In order to achieve successful wound treatment, elasticity and resilience are necessary properties for dressings. The elasticity helps to retain the shape of a dressing, and its use is extended, as it does not need to be changed often. The Young modulus specifies the stiffness or rigidity of the film, the strain at break indicates the tensile strength of the film to resist breaking, and the elongation at break describes the flexibility or extensibility of the films.

The tensile strength, elongation at break and Young's modulus of cellulose sheets are listed in Table 3.3.

Table 3.3. Mechanical properties of CHW and CHG samples obtained from the tensile test and rupture test

Sample	Tensile strength, MPa ± SD	Elongation at break, % ± SD	Young's modulus, MPa ± SD
CHW	2.73 ± 0.66	13.81 ± 0.58	5.76 ± 0.61
CHG	6.33 ± 0.98	16.00 ± 0.57	7.50 ± 0.55

The samples saturated with glycerol showed better mechanical properties than the initial cellulose hydrogel sheets. They demonstrated more than twice higher tensile strength. Moreover, the elongation at break and the Young's modulus increased significantly. Sheets designed to be used as wound dressings should be enough robust and fictile, although too high value of Young's modulus could bring rigidity and stiffness thus giving an unpleasant sensation to the patient. Skin is a viscoelastic material, which constantly undergoes slight stress. The tensile strength of skin depends on gender, age, side as well as loading force and direction of the stress. The tensile strength of the skin is reported to be about 1.8 MPa (Chellamani et al., 2014). Thus, the wound dressing should have a tensile strength higher than this value.

CHG sheets as wound dressings could be enough robust for a long time and at the same time could have sufficiently good stretching properties comparing with CHW. Higher values of the mechanical parameters in the case of cellulose sheets loaded with glycerol suggest that this kind of sheets would be strong enough to withstand the pressure from a compressive or tensile force and would be easy to handle as a wound dressing. Fig. 3.3 demonstrates the flexibility of the cellulose hydrogel sheet.

Fig. 3.3. Demonstrative flexibility of cellulose hydrogel

The soft, rubbery and flexible texture of such wound dressings resembles human tissues, and the wound healing could be accelerated.

3.1.4. Dynamic-thermomechanical analysis

The dynamic properties of CHW and CHG samples are expressed in terms of the storage modulus and the loss modulus. Fig. 3.4 and Fig. 3.5 show the storage and loss modulus *vs.* the temperature. Increasing the temperature, the storage modulus as well as the loss modulus of CHW samples increases at the beginning (Fig. 3.4), showing the loss of elastic properties; thus, a sample is becoming more rigid. Meanwhile, CHG samples maintain a constant storage and loss modulus during the whole experiment (Fig. 3.5).

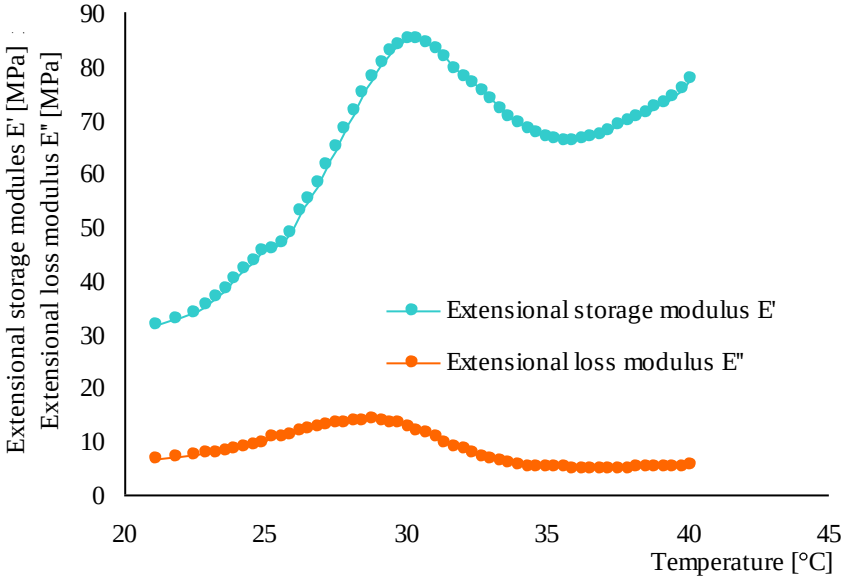


Fig. 3.4. The dynamic thermomechanical analysis of storage and loss modulus *vs* temperature of CHW samples

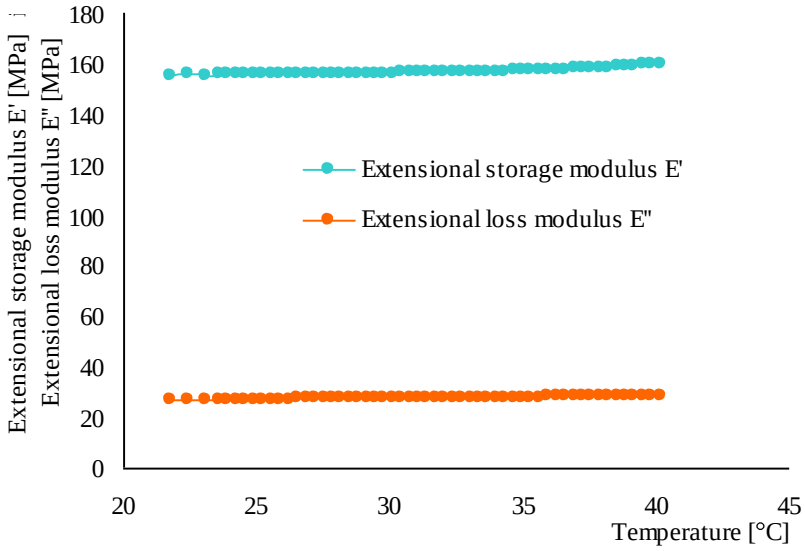


Fig. 3.5. The dynamic thermomechanical analysis of storage and loss modulus *vs* temperature of CHG samples

CHG samples comparing with CHW have higher values of the loss and storage modulus that shows the bigger stiffness of CHG samples. CHG samples used as a wound dressing, despite an increased temperature and motion, could retain their shape and mechanical properties for a longer period of time than CHW samples (as well see moisture retention ratio test, chapter 3.1.5.), and the frequent change of a dressing would be unnecessary.

3.1.5. Moisture retention ratio

Wound dressings that can retain moisture generate a protective barrier help to reduce the scar formation, accelerate wound re-epithelialization, which results in a faster healing process compared to the treatment in a dry environment (Straccia et al., 2014). Fig. 3.6 shows the amount of mass loss of the CHW and CHG samples with time.

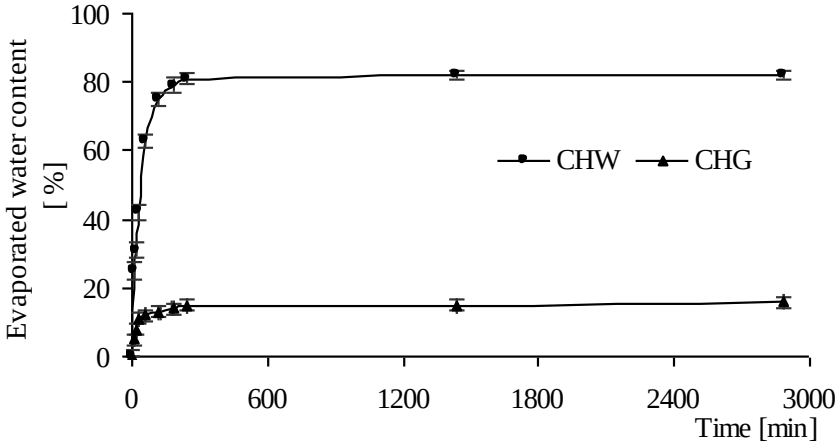


Fig. 3.6. Kinetic curves of water amounts evaporated from the CHW and CHG samples at the room temperature

The results of the study show that the regenerated cellulose sheets loaded with glycerol would be appropriate to be used not only due to the improved mechanical properties but as well because of the low mass loss from the wound dressing even after two days. After 4 hrs, the CHW samples retain only 18% of the initial sample weight; 82% of the hydrogel mass is lost, while the samples loaded with glycerol even after 2 days loose only 17% of the initial hydrogel mass. As CHG samples are transparent and can retain moisture for a long time, frequent dressing replacement could be avoided.

3.1.6. Fluid uptake ability of hydrogels

The fluid absorption capacity of a wound dressing is a very important parameter for maintaining the moist environment over the wound. The swelling kinetics of the hydrogel sheet samples in the PECF buffer is shown in Fig. 3.7.

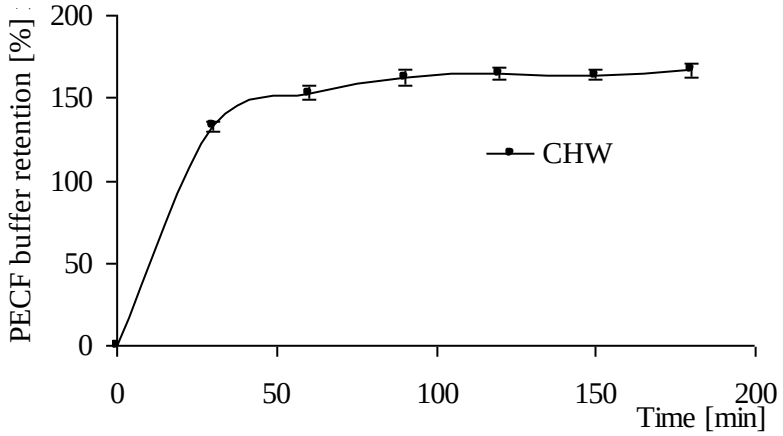


Fig. 3.7. A kinetic curve of the PECF buffer retention by cellulose hydrogel sheets

The dried cellulose sheets reached the swelling equilibrium approximately after one and a half hour. These samples were able to absorb up to 167% (±4%) of the PECF buffer, but after 30 min, the hydrogel sheets were able to absorb up to 133% (±3%) of the buffer. Such liquid absorption of a wound dressing is efficient, effective and would encourage the wound to heal more successfully by moving quicker to the scar maturation stage.

3.1.7. Absorption and release of the antibiotic neomycin

Antimicrobial agents are one of the most commonly used therapeutic classes. The success of an antimicrobial therapy is related to the biological activity of the used antibiotic. Among the antimicrobial agents, aminoglycosides are one of the most used antibiotics in treating an infectious disease due to their broad-spectrum (Butler and Cooper, 2011). Neomycin is an aminoglycoside antibiotic that is produced naturally by the actinomycete bacterium *Streptomyces fradiae* via the fermentation process. Aminoglycoside antibiotics inhibit the protein synthesis by interacting with the 30S subunit of bacterial ribosomes, and like others aminoglycosides, neomycin displays an excellent activity against Gram negative bacteria and a partial activity against Gram positive bacteria (Huidobro et al., 2009).

The absorption test indicated that by using an aqueous solution of neomycin, cellulose sheets absorbed 231 ± 4.6 mg/g of neomycin counting for the dry substance, while using the glycerol-based neomycin solution 190 ± 6.8 mg/g, the counting for the dry gel were absorbed. The release of neomycin was studied by imitating the physiological conditions in PBS (pH 7.4) at 37 ºC temperature (Fig. 3.8).

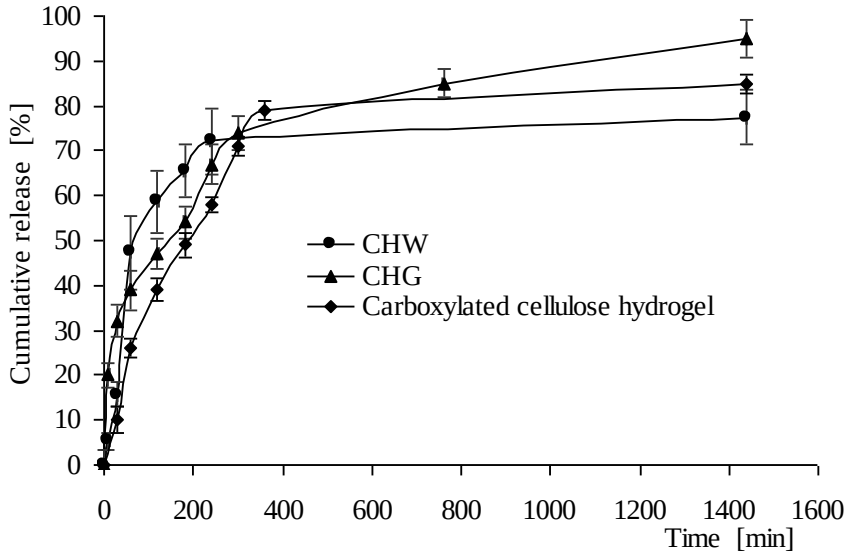


Fig. 3.8. Cumulative release of neomycin from carboxylated cellulose sheets, from cellulose sheets saturated with the water based neomycin solution (CHW), with the glycerol based neomycin solution (CHG)

For the cellulose sheets loaded with the water based neomycin solution, the release study of the active compound showed that after 4 hrs, 72% of neomycin was released to the PBS solution. After 24 hrs, 77% of neomycin was released. Such wound dressing would have a prolonged exposure of the antibiotic and could be used longer than 24 hrs.

For the cellulose sheets loaded with the glycerol based neomycin solution, the release study of neomycin showed slower release of the active compound. After 4 hrs, 67% of neomycin was released. After 24 hrs, 95% of neomycin was released from the cellulose sheets. Therefore, such wound dressing would show even release of the active compound and could be used longer than 24 hrs.

In order to prolong the release of neomycin carboxylation of cellulose hydrogel by using monochloroacetic acid was carried out according to the following formula:

$$Cel\text{-}OH + ClCH_2COOH + 2NaOH \rightarrow Cel\text{-}OCH_2COONa + NaCl + H_2O.$$

The determined amount of carboxyl groups was 1.3 mmol/g.

Carboxylated cellulose hydrogel absorbed 309±5.3 mg/g of neomycin counting for the dry substance. Carboxylated cellulose hydrogel is able to absorb more neomycin due to the electrostatic interaction between the amino groups in neomycin and carboxyl groups in cellulose hydrogel. It is appropriate to carboxylate cellulose hydrogel in order to create a slightly acidic medium for the wound, which requires an acidic medium, usually wounds at the end of healing.

According to neomycin release study, carboxylated cellulose hydrogel has the prolonged release of neomycin. Neomycin releases slower and uniformly. As mentioned above, due to the electrostatic interaction, noemycin has stronger absorbtion forces with carboxylated hydrogel, which is why neomycin release is as well slower comparing with the non-carboxylated hydrogel. The longer it takes to release the active compound from the dressing, the less frequent wound dressing should be changed.

3.1.8. Antibacterial activity

The healing of open wounds is a complex process, which is often interfered with infections by various microorganisms. Such complications may prolong the healing process, which is why the treating with antibiotics may be required. An aminoglycoside antibiotic neomycin has a strong activity against Gram-negative bacteria and a partial activity against Gram-positive bacteria (Nitanan et al., 2013). It is known that glycerol as well possesses bacteriostatic properties (Vloemans et al., 2002). The antibacterial activity of cellulose sheets saturated with water or glycerol based solutions of the antibiotic neomycin was tested. The bacterial growth inhibition zone in mm was measured for the antibacterial activity evaluation. The results are shown in Table 3.4.

Table 3.4. Results of bacterial growth inhibitory zone measurements

Sample	Bacterial growth inhibitory zone diameter, mm	
	Gram-negative bacteria *Escherichia coli (DH5α)*	Gram-positive bacteria *Bacillus subtilus (ATCC6051)*
CHW	-	-
CHG	7	4
CHW with neomycin	20	18
CHG with neomycin	25	21

Antimicrobial activity results show that the cellulose hydrogel sheets loaded with neomycin have good antibacterial properties. CHW samples without active ingredients demonstrated no antibacterial activity, while cellulose sheets with glycerol showed antibacterial activity against both gram-negative and gram-positive bacteria. The best results were obtained for CHG samples loaded with the glycerol based neomycin solution. The inhibitory zone of the bacterial growth for gram-positive bacteria was 21 mm, while against gram-negative ones, it was larger and reached 25 mm. CHW samples loaded with neomycin demonstrated a slightly lower antibacterial activity compared with the CHG samples loaded with neomycin.

3.1.9. Cytotoxicity study

The viability of hepatocytes incubated with milled CHW and CHG samples was compared with the control, i.e., cells viability without the samples. Control cells viability reached over 92%. The viability of the control hepatocytes did not reduce significantly through the incubation time. The high viability in the control group indicated that all the necessary conditions for the hepatocyte incubation, such as pH, temperature, cultural medium type, were satisfied in the best way. Cells viability for samples CHW and CHG as well showed the excellent viability of hepatocytes and had values close to those of the control group (Fig. 3.9).

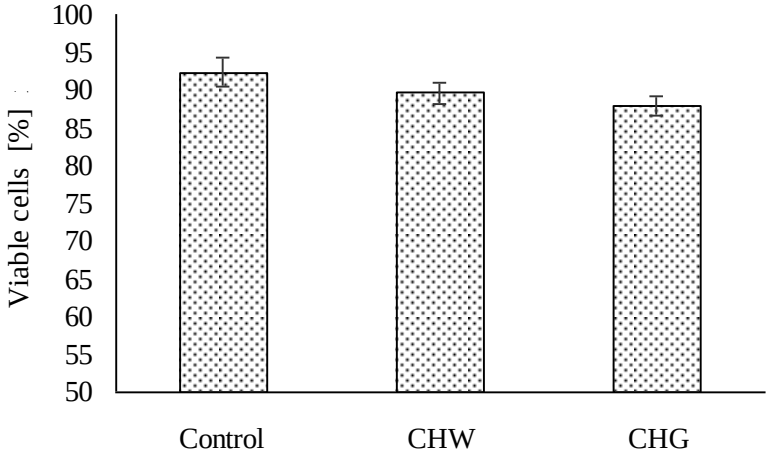


Fig. 3.9. The viability of isolated hepatocytes after incubation with samples CHW and CHG

Moreover, the lactate dehydrogenase (LDH) activity in the culture medium of control and CHW and CHG treated hepatocytes was studied. LDH is found in many body tissues, including the liver. Elevated levels of LDH in the blood serum may indicate the liver damage. The LDH test is a common clinical procedure to estimate the liver damage. If the hepatocyte membrane is damaged, LDH can come out through the membrane to the incubation medium and stay in the supernatant, where it can be detected by the biochemical assay.

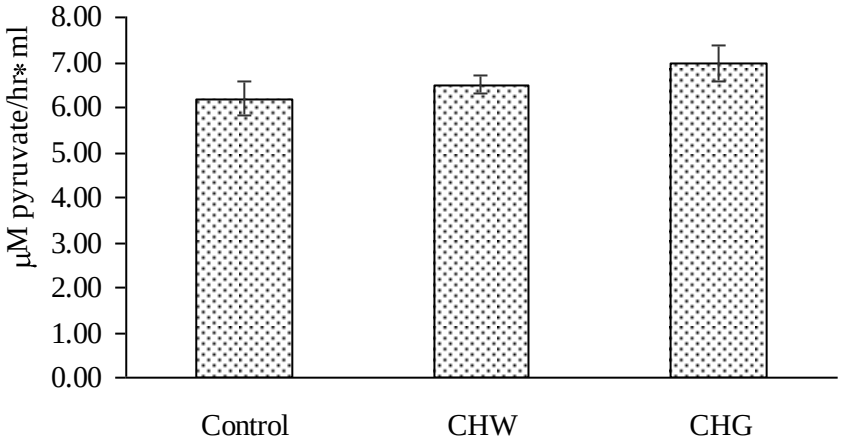


Fig. 3.10. LDH activity in a culture medium of hepatocytes after the incubation with CHW and CHG samples

The obtained results demonstrated that the pre-treatment of cells by the CHW or CHG did not increase the LDH release from the hepatocytes, in respect to control cells (Fig. 3.10). The results revealed that both hydrogels are not cytotoxic.

3.2. Preparation and characterization of cellulose hydrogels treated with supercritical carbon dioxide

In order to obtain a porous structure; the samples were treated with supercritical CO_2. In order to find out how the morphology and other physical properties are influenced by various conditions, 4 different groups of the samples have been prepared. The numeration of the samples is as follows: the first group of the samples (1) was saturated with water and exposed to supercritical carbon dioxide at 50 °C temperature and 4000 psi pressure. The second group of the samples (2) was saturated with water, exposed to supercritical carbon dioxide at a temperature of 80 °C and a pressure of 8000 psi. The third group of the samples (3) was soaked in the 20% ethyl alcohol solution for 48 hours and exposed to the supercritical carbon dioxide at a temperature of 50 °C and a pressure of 4000 psi. The fourth group of the samples (4) was soaked in the 50% ethyl alcohol solution for 48 hours and exposed to the supercritical carbon dioxide at a temperature of 50 °C and a pressure of 4000 psi. This numeration is used continuously.

The microstructure of the material, such as its porosity, pore shape, size and distribution, has significant effects on the cell proliferation and proper healing of the

wound. In order to modify the structure of the prepared cellulose hydrogel, it was treated with supercritical CO_2. The hydrogel with micropores was obtained for the better gas exchange between the air and wound surface. The continuous supply of oxygen to the tissue through the microcirculation is vital to the healing process. The gas exchange through a wound dressing is important, because high CO_2 content increases the acidity and slows down the healing process, and the higher oxygen concentration increases the regeneration of tissue cells. The vapour and oxygen transmission is very important for the pressure sores, leg ulcers, burns, necrotic and surgical wounds.

Fig. 3.11 represents the SEM image of CHW hydrogel, while Fig. 3.12 represents the SEM image of all four groups of CH-CO_2 samples. In Fig. 3.11, it is seen that the initial dried cellulose hydrogel has a homogeneous structure; there are no micropores, which could be seen because of the gel shrinking. Cellulose hydrogels treated with supercritical CO_2 have micropores even after the drying of a hydrogel (Fig. 3.12).

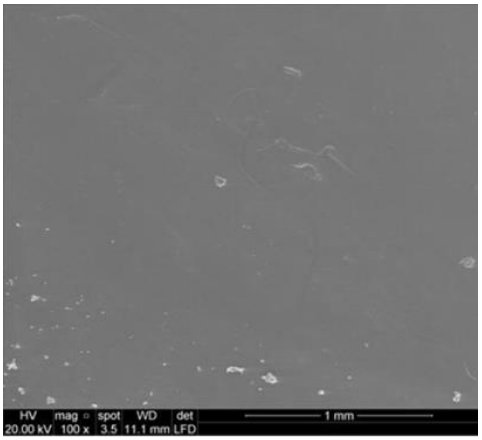


Fig. 3.11. SEM image of the cellulose hydrogel (mag. 100×)

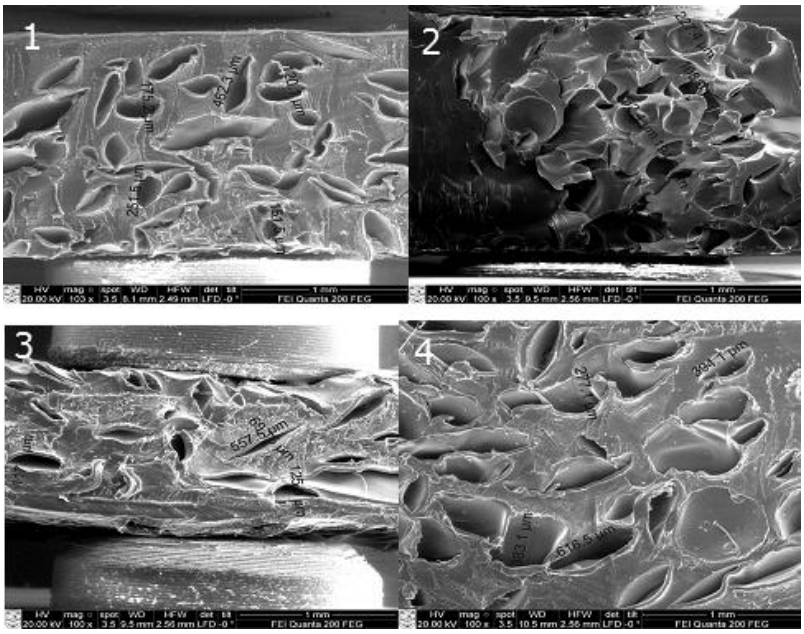


Fig. 3.12. SEM image of CH-CO_2 samples (mag. 100×). Sample 1 saturated with water and exposed to supercritical carbon dioxide at a temperature of 50 °C and a pressure of 4000 psi. Sample 2 saturated with water, exposed to supercritical carbon dioxide at a temperature of 80 °C and a pressure of 8000 psi. Sample 3 soaked in the 20% ethyl alcohol solution for 48 hours and exposed to supercritical carbon dioxide at a temperature of 50 °C and a pressure of 4000 psi. Sample 4 soaked in the 50% ethyl alcohol solution for 48 hours and exposed to supercritical carbon dioxide at a temperature of 50 °C and a pressure of 4000 psi.

The pore sizes, their arrangement and shapes are not uniform in all samples. The most uniform pore shape is seen in the second one sample. The sample No. 2 has almost round pores, while other samples have elongated shape pores. The pore size ranges approx. from 100 μm to 600 μm.

3.2.1. Mechanical properties of hydrogels treated with supercritical carbon dioxide

The mechanical properties are very important in the application of wound dressings. Bandages should be elastic, resilient and pliable. This extends their use because they do not need to be often replaced due to their change of shape or position. A series of tests of the mechanical properties were done. In order to compare the mechanical properties with those of the initial cellulose hydrogel, the samples with the same thickness, length and width were cut for the experiments.

Table 3.5 represents the results obtained in a rupture test.

Table 3.5. The rupture test results of samples treated with supercritical CO_2

Sample	Rupture force, N ± SD	Distance to burst, mm ± SD
1	864 ± 1.7	6.53 ± 0.55
2	924 ± 1.6	4.15 ± 0.41
3	1039 ± 2.3	4.18 ± 0.32
4	765 ± 1.6	3.86 ± 0.32
Cellulose hydrogel	654 ± 1.3	2.68 ± 0.25

The results show that by treating hydrogels with supercritical CO_2 has a positive impact on the elasticity of the cellulose hydrogels as hydrogels become more elastic. After the treatment with supercritical CO_2, not only micropores are formed, but at the same time, the macromolecules of the polymer are thickened, and the result is a more elastic polymer. The highest elasticity was obtained for the sample No. 3. Moreover, the sample No.1 showed good elasticity properties, as the distance to burst was the greatest and comparing with the untreated cellulose hydrogel, was bigger more than twice. The lowest elasticity of the samples treated with supercritical CO_2 was shown by the sample No. 4. The strongest influence probably could be attributed to the pore size, as the SEM analysis showed that the sample No. 4 had the biggest pores.

The results of the compressive test are presented in Fig 3.13 and Fig. 3.14.

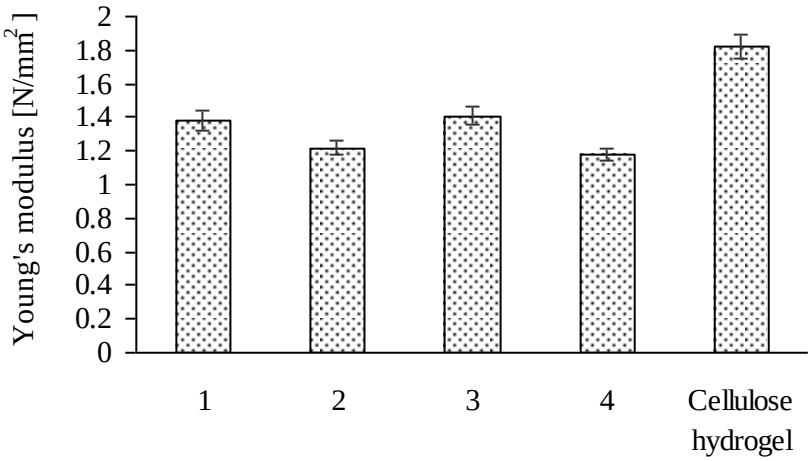


Fig. 3.13. Young's modulus values of the compressive test. Sample 1 saturated with water and exposed to supercritical carbon dioxide at a temperature of 50 °C and a pressure of 4000 psi. Sample 2 saturated with water, exposed to supercritical carbon dioxide at a temperature of 80 °C and a pressure of 8000 psi. Sample 3 soaked in the 20% ethyl alcohol solution for 48 hours and exposed to supercritical carbon dioxide at a temperature of 50 °C and a pressure of 4000 psi. Sample 4 soaked in the 50% ethyl alcohol solution for 48 hours and exposed to supercritical carbon dioxide at a temperature of 50 °C and a pressure of 4000 psi.

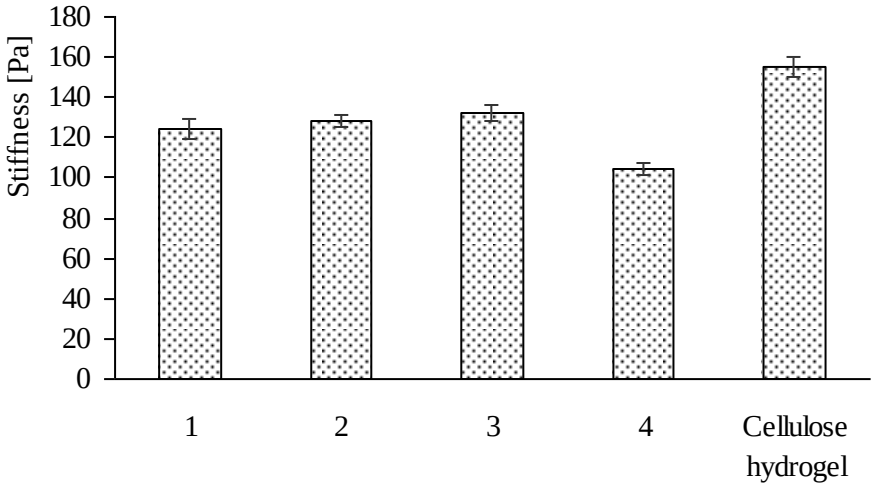


Fig. 3.14. Stiffness values of the compressive test. Sample 1 saturated with water and exposed to supercritical carbon dioxide at a temperature of 50 °C and a pressure of 4000 psi. Sample 2 saturated with water, exposed to supercritical carbon dioxide at a temperature of 80 °C and a pressure of 8000 psi. Sample 3 soaked in the 20% ethyl alcohol solution for 48 hours and exposed to supercritical carbon dioxide at a temperature of 50 °C and a pressure of 4000 psi. Sample 4 soaked in the 50% ethyl alcohol solution for 48 hours and exposed to supercritical carbon dioxide at a temperature of 50 °C and a pressure of 4000 psi.

When having the Young's modulus and stiffness calculations, it can be seen that the most resistant to compression is the initial cellulose hydrogel. The least resistant to compression is the sample No. 4, as the sample No. 4 had the biggest pores. Other samples treated with supercritical CO_2 show similar values of the stiffness and Young's modulus.

3.2.2. Pseudo extracellular fluid retention test

One of the key features of a wound dressing is the ability to absorb liquids and at the same time irrigate the wound, as wounds heal best in a moist environment.

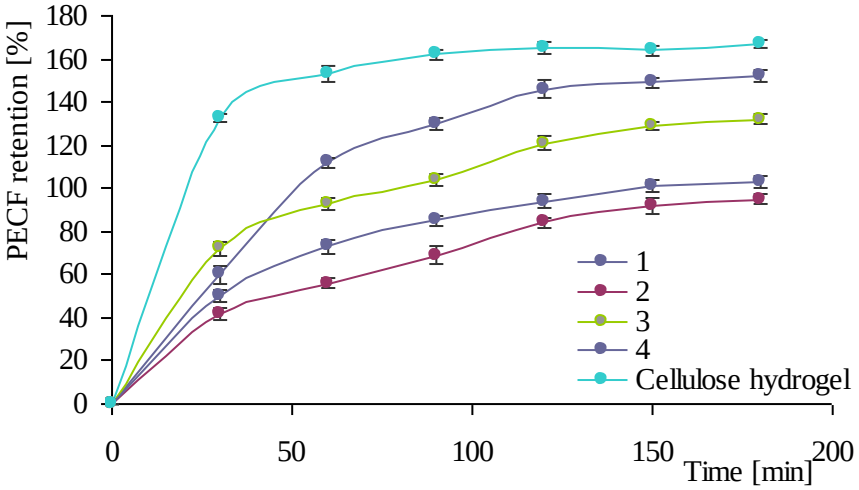


Fig. 3.15. PECF retention test. Sample 1 saturated with water and exposed to supercritical carbon dioxide at a temperature of 50 °C and a pressure of 4000 psi. Sample 2 saturated with water, exposed to supercritical carbon dioxide at a temperature of 80 °C and a pressure of 8000 psi. Sample 3 soaked in the 20% ethyl alcohol solution for 48 hours and exposed to supercritical carbon dioxide at a temperature of 50 °C and a pressure of 4000 psi. Sample 4 soaked in the 50% ethyl alcohol solution for 48 hours and exposed to supercritical carbon dioxide at a temperature of 50 °C and a pressure of 4000 psi.

Fig. 3.15 demonstrates the PECF retention kinetics of the prepared samples. It can be seen that the initial cellulose hydrogel has the best PECF retention. The sample No. 1 treated with supercritical CO_2 has values similar to the initial cellulose hydrogel, and after equilibrium, it can retain 152% of PECF. The sample No. 2 has the lowest PECF retention. All the samples treated with supercritical CO_2 reach the equilibrium slower, in comparison to the initial cellulose hydrogel sheet.

Taking into account the morphology, rupture, compressive tests and PECF retention, for the further investigations, the sample No. 1 was chosen.

3.2.3. Hardness and resilience test

In this test, the samples underwent the exposure twice. The hardness of the samples was determined from the first compression. The resilience is the ability of a material to return to its original shape after removing the pressing force. The resilience was determined from the second compression. As mentioned above, for the experiment, the sample No. 1 was used. This sample was further modified by loading it with a water:glycerol (1:1, v/v) mixture. For the comparison, the initial cellulose hydrogel and the initial cellulose hydrogel loaded with the water:glycerol (1:1, v/v) mixture were used.

Table 3.6. Hardness and resilience values

Sample	Hardness, $N/mm^2 \pm SD$	Resilience, % ± SD
Cellulose hydrogel loaded with water	1.26 ± 0.03	44.5 ± 1.2
Cellulose hydrogel treated with supercritical CO_2 and loaded with water	0.46 ± 0.02	13.9 ± 1.0
Cellulose hydrogel loaded with water:glycerol mixture	1.84 ± 0.04	46.6 ± 1.1
Cellulose hydrogel treated with supercritical CO_2 and loaded with the water:glycerol mixture	0.68 ± 0.03	14.9 ± 0.9

Results from Table 3.6 show that the samples treated with supercritical CO_2 have lower hardness and lower resilience values; thus, the porosity of samples makes them weaker. The supplement of glycerol improves both hardness and resilience; the resilience increases by 1%. Thus, the cellulose hydrogels treated with supercritical CO_2 are deformed easier. Moreover, the material should not be too soft; otherwise, the residues of the wound dressing could remain, which could affect the healing process.

3.2.4. Immobilization of the phenolic compounds from *Perilla frutescens* and *Betula pendula* extracts and their release

Phenolic compounds found in plants, such as phenolic acids, flavonoids, stilbenes, lignans, have excellent antioxidant, anti-allergic, anti-virus, anti-inflammatory characteristics, and phenolic compounds can be used to promote the wound healing.

Rosmarinic acid and α-linolenic acid have been reported as anti-inflammatory and antiallergic substances, and luteolin as an anti-inflammatory and antitumor-promoting substance in *Perilla* leaves and seeds (Banno et al., 2005). *Betula* buds and leaves are enriched with phenol compounds (flavonoids, lignans, caffeine acid), essential oils (betulinol, betuleno naphthalene, sesquiterpenes), ascorbic acid, vitamin C, E, B_3, B_5, saponins, sterols, α-hydroxy acids, organic acids, fatty acids (linoleic, linolenic), tannins, quercetin, minerals (Cu, Ca, Mn, Fe, Sr). *Betula* species exhibits a wide range of biological activities, such as anticancer, anti-inflammatory and immunomodulatory and others like antioxidant, antidiabetic, antiviral and antiarthritic activities (Rastogi et al., 2015).

Fig. 3.16 demonstrates the content of absorbed total phenols from solutions made of the *Perilla frutescens* dry extract.

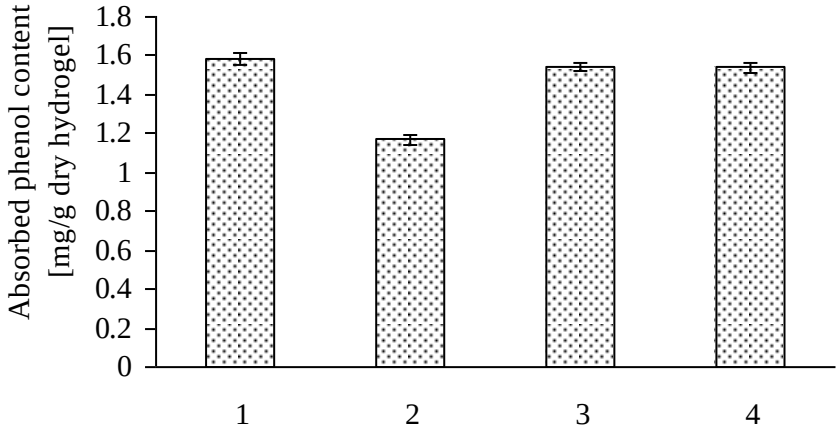


Fig. 3.16. The absorbed total content of phenols from the *Perilla frutescens* dry extract: 1 – CHW loaded with the aqueous extract, 2 – CH-CO_2 loaded with the aqueous extract, 3 – CHW loaded with the water:glycerol based extract, 4 – CH-CO_2 loaded with the water:glycerol based extract

The initial cellulose hydrogel was capable to absorb more phenols than the cellulose hydrogel sheets treated with supercritical CO_2. The greatest absorbed amount of total phenols was reached with the water based *Perilla frutescens* extract by using the initial cellulose hydrogel sheet (1.6 mg/g for the dry hydrogel). The lowest absorbed amount of total phenols was reached with the water based *Perilla frutescens* extract by using the cellulose hydrogel treated with supercritical CO_2 (1.2 mg/g for dry hydrogel). Overall, all the samples showed similar results of the absorbed total content of phenols.

Fig. 3.17 demonstrates the content of absorbed total phenols from solutions made of the *Betula pendula* dry extract.

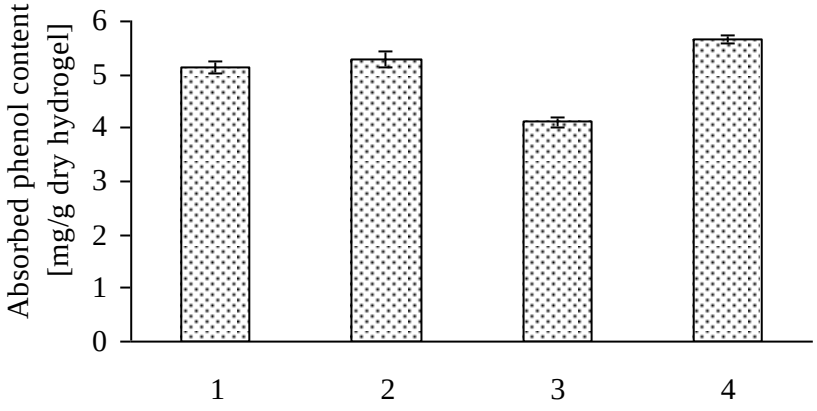


Fig. 3.17. The absorbed total content of phenols from the *Betula pendula* dry extract: 1 – CHW loaded with the aqueous extract, 2 – CH-CO_2 l loaded with the aqueous extract, 3 – CHW loaded with the water:glycerol based extract, 4 – CH-CO_2 loaded with the water:glycerol based extract

From Fig. 3.16 and Fig. 3.17, it can be seen that the cellulose samples are capable to absorb 3 times more phenols from the *Betula Pendula* dry extract than from the *Perilla frutescens* dry extract. Thus, the *Betula pendula* dry extract can enrich the hydrogels with phenolic compounds to a higher extent comparing with the *Perilla frutescens* dry extract. The greatest absorbed amount of total phenols was reached by the water:glycerol based *Betula Pendula* extract by using the cellulose hydrogel sheet treated with supercritical CO_2 (5.7 mg/g for the dry hydrogel). Overall, like in a case of the *Perilla frutescens dry extract*, the samples showed very similar results for the total content of absorbed phenols.

Fig 3.18 demonstrates the release of total phenols of the *Perilla frutescens* dry extract from the hydrogels. It can be seen that the cellulose hydrogels loaded with the aqueous and water:glycerol based *Perilla frutescens* extract solutions act similarly; the equilibrium of phenol release is reached after 6 hrs, and about 80% of absorbed phenolic compounds, according to the total phenol content, are released. The cellulose hydrogels treated with supercritical CO_2 as well act similarly; the equilibrium of the phenol release is reached after 6 hrs, and about 90% of absorbed phenolic compounds are released.

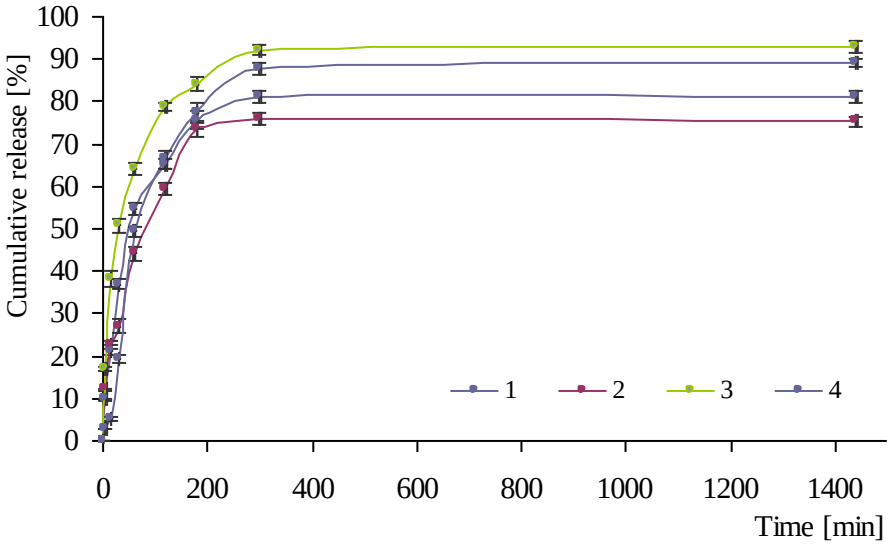


Fig. 3.18. The release of total phenols from the hydrogels loaded with the extracts of *Perilla frutescens*: 1 – CHW loaded with the aqueous extract, 2 – CHW loaded with the water:glycerol based extract, 3 – CH-CO_2 loaded with the aqueous extract, 4 – CH-CO_2 with the water:glycerol based extract

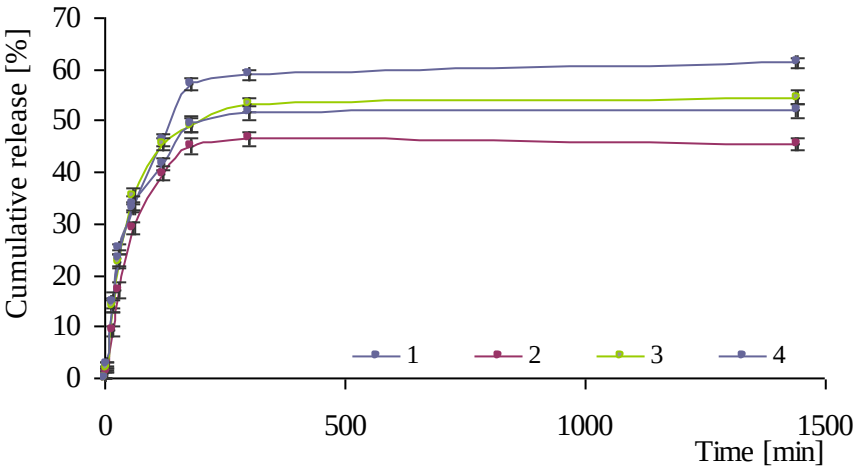


Fig. 3.19. The release of total phenols from the hydrogels loaded with the extracts of *Betula pendula*: 1 – CHW loaded with the aqueous extract, 2 – CHW loaded with the water:glycerol based extract, 3 – CH-CO_2 loaded with the aqueous extract, 4 – CH-CO_2 with the water:glycerol based extract

Fig. 3.19 demonstrates that the cellulose hydrogel treated with supercritical CO_2 in both cases, saturated with water based and water:glycerol based *Betula Pendula* dry extract solutions, releases about 50% of phenolic compounds, and the equilibrium is reached after 6 hrs.

The release of phenols from the cellulose hydrogel sheet loaded with the aqueous *Petula pendula* dry extract solution till the equilibrium takes 6 hrs, and 60% of total phenols are released. The cellulose hydrogel loaded with the water:glycerol based *Petula pendula* dry extract solution after the equilibrium releases about 47% of the total phenols.

Cellulose hydrogel sheets loaded with a greater amount of phenols could be used for the wound dressings with prolonged exposure of phenolic compounds, as the more phenolic compounds are loaded, the more time it takes to release them.

3.3. Preparation and characterization of the cellulose sponges

The second type of wound dressings in this work was prepared from CHW samples by the lyophilisation process. The parameters of regenerated cellulose sponges, the length, width and height are adjustable, as the initial CHW hydrogel parameters can be regulated. Sponge type wound dressings can be used as primary or secondary dressings on a variety of wound types, where exudate is a problem. Sponge type dressings absorb destructive components of the chronic wound fluid.

Morphological characteristics were analysed by the means of a micro-computed tomography. Due to hydrophilicity of cellulose and a high specific surface area, the sponges possess a high sorption capacity for simulated wound fluids and a high water vapour transmission ability. Different active compounds, such as polyphenols from *Calendula officinalis* or *Chamomilla recutita* extracts, silver nanoparticles were immobilized into the sponges in order to obtain an antimicrobial

and antioxidative effect. The sponges incorporated with silver showed an antibacterial activity against *Staphylococcus epidermidis.* Thus, these cellulose-based sponges are promising wound dressing materials for fester and infected wounds.

According to the micro-computed tomography analysis, 75% of porosity was reached. Fig. 3.20 shows two-dimensional (2D) images of the cellulose sponge.

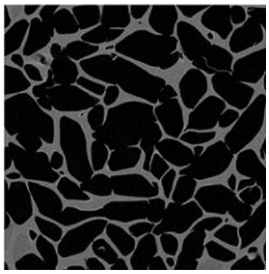


Fig. 3.20. Micro-CT image (2D) of regenerated cellulose sponge

Micro-CT images revealed interconnected porous structures. The mean pore diameter was 750 μm (Table 3.7).

Table 3.7. Structural parameters of the cellulose sponges obtained by the micro-CT

Structural parameters	Value	Units
Pore volume	470	mm^3
Mean pore diameter	0.75	mm
Mean polymer thickness	0.21	mm
Porosity	75	%
Surface area	14.5	mm^{-1}

The majority of the pores were from 600 to 900 μm in size (48% of all pores). The pores from 10 to 300 μm constituted only 4%. The pores in the range of 300–600 μm and 900–1200 μm comprised 25% and 22%, respectively (Fig. 3.21).

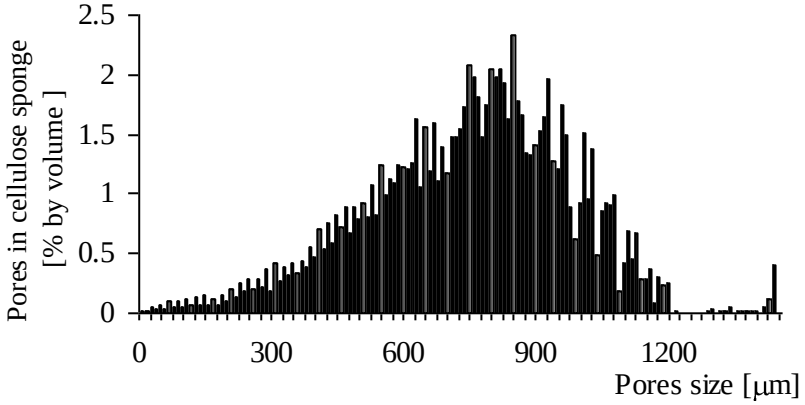


Fig. 3.21. The pore size distribution in cellulose sponge estimated by the micro-CT

Only 1% of pores were greater than 1200 μm. Table 3.7 gives a summary of the structural parameters of the sponge. The specific surface area was 14.5 $mm^2/mm^3\ mm^{-1}$, thus promising to be a good exudate adsorbent.

In the mechanical properties evaluation, the tensile strength of the samples was found to be approx. 140 kPa and the Young's modulus approx. 2.7 N/mm^2. According to the literature (Sung et al., 1999), the mechanical properties meet the requirements for the wound bandages. As wound dressings, these materials would be sufficiently elastic and resistant to wear.

3.3.1. Pseudo extracellular fluid buffer retention

It is very important to protect an open wound from accumulation of exudates, because too much fluid can cause skin maceration, thus prolonging the healing process or causing complications. A good fluid absorption capacity of wound dressing helps to keep the wound environment dry and promotes the healing processes.

In this research, the cellulose sponges after 24 hrs absorbed 210% (±15%) of the PECF buffer (Fig 3.22).

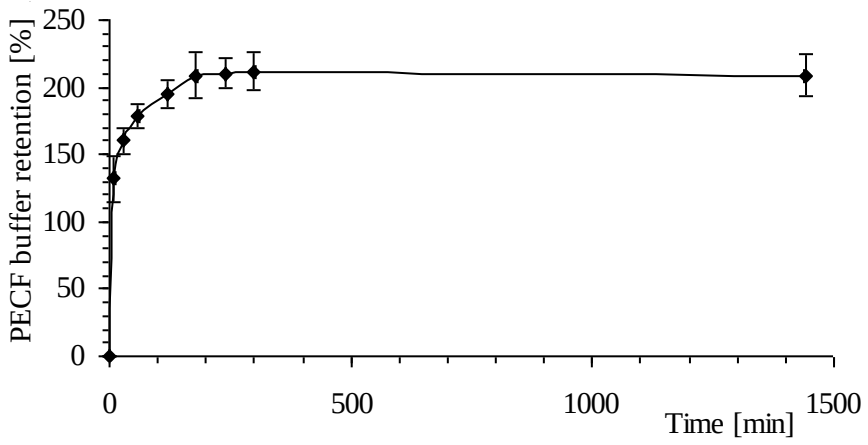


Fig. 3.22. PECF buffer retention by the cellulose sponge at 37 °C

The equilibrium was reached after 3 hrs, but even after 10 min, the cellulose sponges were able to absorb up to 132% (±14%) of the PECF buffer. Fast absorption of exudates is an important factor in order to use this material for festering wound healing, because too much exudate slows down the cell proliferation. There is a big variety of sponges with varying liquid absorption characteristics available in the literature. Chiaoprakobkij et al. (2011) presented bacterial cellulose/alginate composite sponges with human keratinocytes and the gingival fibroblast for wound healing, where depending on a modification; water uptake differs from 1170% to 1390%. Angatikarnkul et al. (2014) prepared cellulose nanofiber/chitin whisker/silk sericin bionanocomposite sponges, where water uptake reached up to 3000%. In spite of different liquid absorption capacity, each adsorbent can find its application

depending on the type of the wound and the amount of secretions. It is as well important to prevent the wound from dewatering drying too much.

3.3.2. The water vapour transmission rate (WVTR)

An important factor in the wound healing process is an appropriate exchange of the moisture content. Letting the wound to become too dry can delay the healing process; otherwise, an accumulation of exudate may influence the occurrence of the infection or maceration. The evaporative water loss for a normal skin is 204 ± 12 g/m^2/day for the injured skin; the water loss can range from 279 ± 26 g/m^2/day for a first degree burn to 5138 ± 202 g/m^2/day for a granulating wound (Lamke et al., 1977). In order to ensure an adequate level of moisture, avoiding the risk of wound dehydration and build-up of exudates on the wound, the water vapour transmission rate from the injured wound should be in the range of 2000-2500 g/m^2/day (Queen et al., 1987).

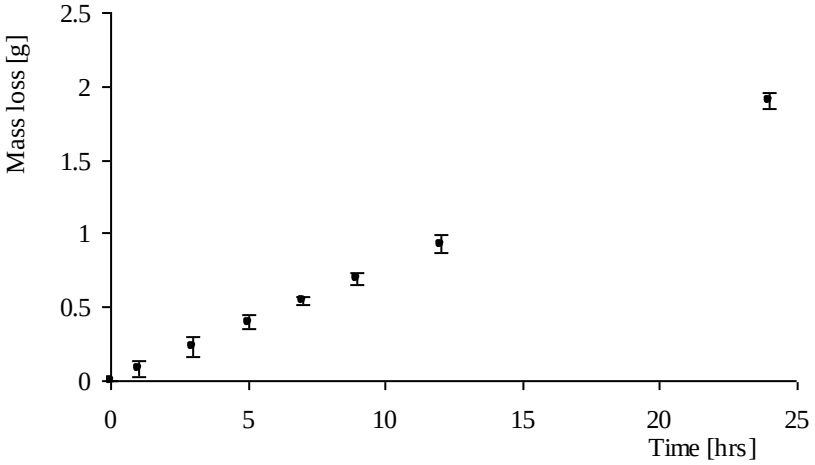


Fig. 3.23. The water loss in WVTR experiments *versus* time plots

The prepared cellulose based sponges showed WVTR of the value 2656 ± 30 g/m^2/day (Fig. 3.23), thus being close to the adequate range of the ideal wound dressing. Such WVTR value can strengthen the cellular re-epithelialization. WVTR was calculated from the water loss.

3.3.3. Immobilization and release of the phenolic compounds and neomycin

Polyphenols have a broad range of biological effects for animal cells: antitumor, anti-trombogenic, anti-oxidative and anti-inflammatory effects, free radical neutralization. The bandages for wound healing saturated with the polyphenols from plants could help avoiding inflammation, induce faster healing and be suitable for people with allergies to synthetic products. For this purpose, the phenolic compounds extracted from *Calendula officinalis* or *Chamomilla recutita* were immobilized into the sponges by absorption. The composition of phenolic compounds of *Calendula officinalis* and *Chamomilla recutita* extracts is different.

Flavonoids of isorhamnetin group predominate in *Calendula officinalis* and apigenin, luteolin and quercetin in *Chamomilla recutita.*

The release of phenolic compounds was studied by imitating the physiological conditions in PBS (pH 7.4) at 37 ºC temperature. In both cases, the equilibrium was reached after 1 hr (Fig. 3.24); however, just 38% of phenols of the *Calendula officinalis* extract were released before the equilibrium was reached, while 68% of phenols of *Chamomilla recutita* were released.

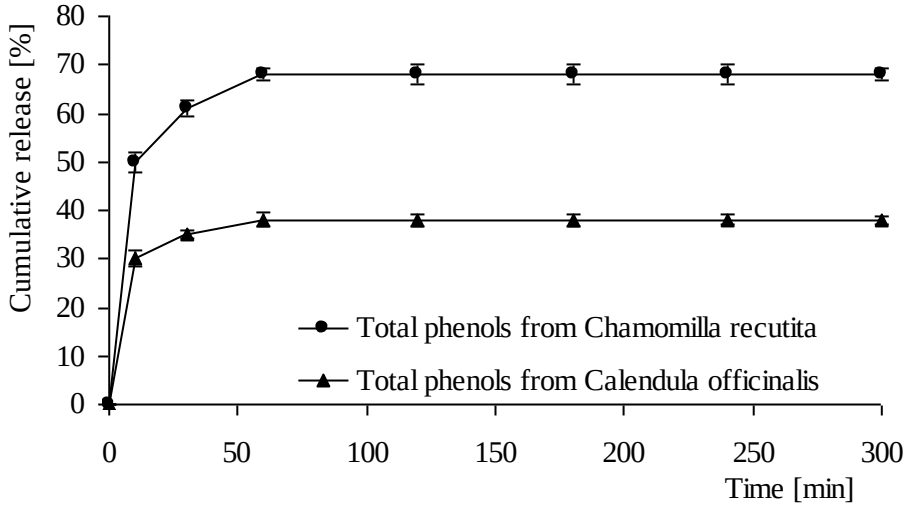


Fig. 3.24. Cumulative release of the total phenols of *Calendula officinalis* or *Chamomilla recutita* from cellulose sponges

Such difference between released amounts of phenols may be received due to the different structure of phenolic compounds as well as due to the different absorbed amount of total phenols. In the case of *Calendula officinalis*, 2.6 mg/g for dry sponge ± 0.2 mg of total phenols were absorbed, while in the case of *Chamomilla recutita*, 3.9 mg/g for dry gel ± 0.3 mg of total phenols were absorbed; respectively, more phenolic compounds were released. Regarding the results, the cellulose sponges incorporated with phenolic compounds could be active more than 5 hrs. Comparing the initial concentrations, which were 0.38 mg/mL for *Calendula officinalis* and 0.45 mg/mL for C*hamomilla recutita,* it is obvious that C*hamomilla recutita* blossoms were enriched with a bigger amount of phenolic compounds than *Calendula officinalis* blossoms.

The cumulative data of the release of antibiotic neomycin from CS samples are shown in Fig 3.25. The release of neomycin was studied by imitating the physiological conditions in PBS (pH 7.4) at 37 ºC temperature. The equilibrium of neomycin release is reached quite fast, i.e., after 3 hrs, and is around 50%. It is important that not all of the immobilized active compound is released. In this way, the antibacterial effect of wound dressing lasts longer, and 3.1 mg/g for dry sponge ± 0.3 mg of neomycin were absorbed by CS samples.

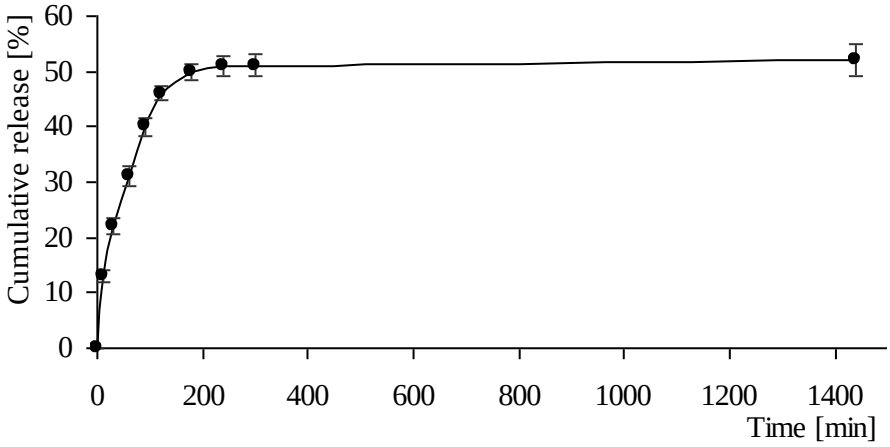


Fig. 3.25. Cumulative release of neomycin

Comparing the absorption and release results with total phenols, it can be predicted that a greater absorbed amount of neomycin could result in faster and greater release of neomycin and vice versa, a lower amount of absorbed neomycin could result in slower and lower release of neomycin. In such a way, the release of active compounds could be controlled.

3.3.4. Characterization of the silver nanoparticles

Silver nanoparticles were precipitated from a silver nitrate solution with NaOH. The obtained precipitate was filtered and redissolved in the aqueous ammonia. Silver nanoparticles were stabilized in oleic acid under vigorous stirring followed by the glucose addition. The prepared silver nanoparticles appeared to be rounded (Fig. 3.26), and they had diameters normally distributed with the average of 5.4 nm and the standard deviation of 1.2 nm (Fig. 3.27).

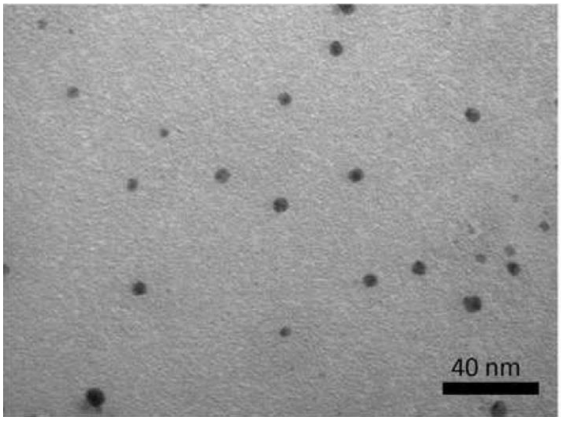


Fig. 3.26. TEM image of silver nanoparticles

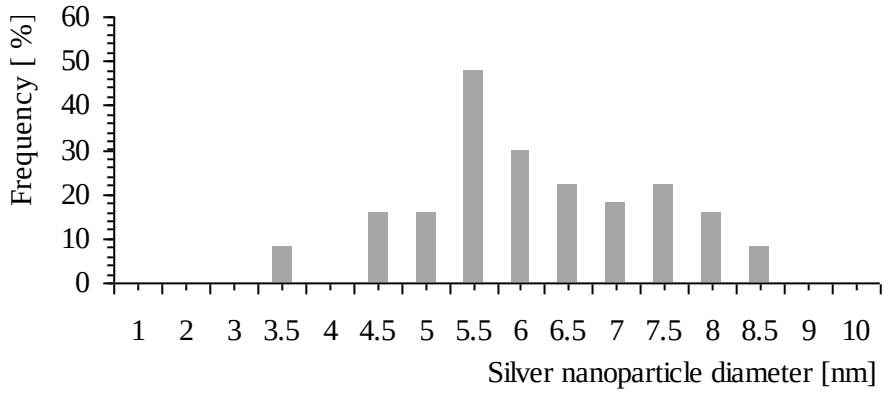


Fig. **3.27.** The distribution of silver nanoparticles diameters

From the initial concentration of silver (0.1 M), the density of metal silver (10.5 g/cm^3) and the average radius, it was estimated that the concentration of nanoparticles was ∼10^{19} nanoparticles/L, because of the employed dilution (1:99), the working solution of silver nanoparticles had a concentration of ∼10^{17} nanoparticles/L.

3.3.5. Silver incorporation and release

One of the main problems in wound healing is the onset of infections. This undesirable effect may lead to a prolonged healing process or cause a number of complications up to death. In order to prevent bacterial contamination of wounds, the silver nanoparticles are often incorporated into a dressing material (Berndt et al., 2013; Queen et al., 1987). Silver is effective against a broad range of microorganisms, including *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which are common in chronic wounds (Abdelrahman and Newton, 2011). It is a wide range biocide that kills over 650 types of disease-causing bacteria, fungi, viruses and moulds (Hoon and Sung, 2004). The typical minimum inhibitory concentration and minimum bactericidal concentration of silver nanoparticles are 0.78–6.25 μg/mL and 12.5 μg/mL, respectively (Jain et al., 2009).

In this study, silver nanoparticles stabilized in oleic acid were incorporated in cellulose sponges by a diffusion method. One-day absorption, two-day absorption and four-day absorption experiments were carried out (Fig. 3.28).

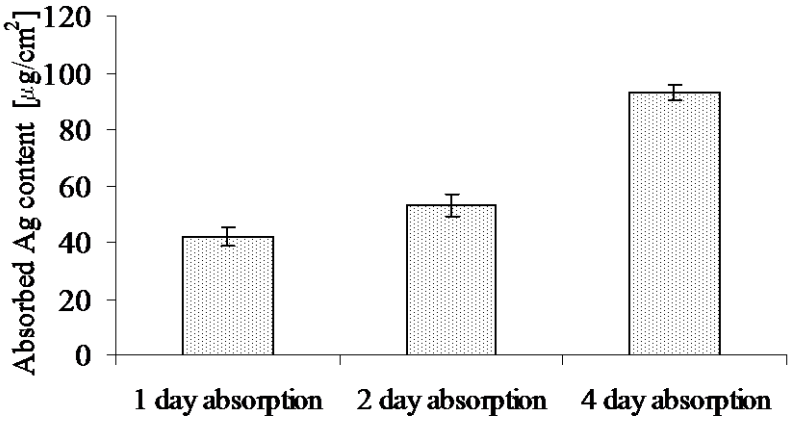


Fig. 3.28. Silver absorption by cellulose based sponges from a silver nanoparticle solution after one, two and four days of experiments

The amount of silver embedded in the sponges increased with time: after one-day diffusion, it was 42±6 μg/cm^2; after two-day diffusion, 53±8 μg/cm^2 of silver were incorporated, and after four-day absorption experiment, 93±5 μg/cm^2 of silver was found.

Silver release kinetics (Fig. 3.29) showed a prolonged release; the equilibrium was not reached even after 24 hrs.

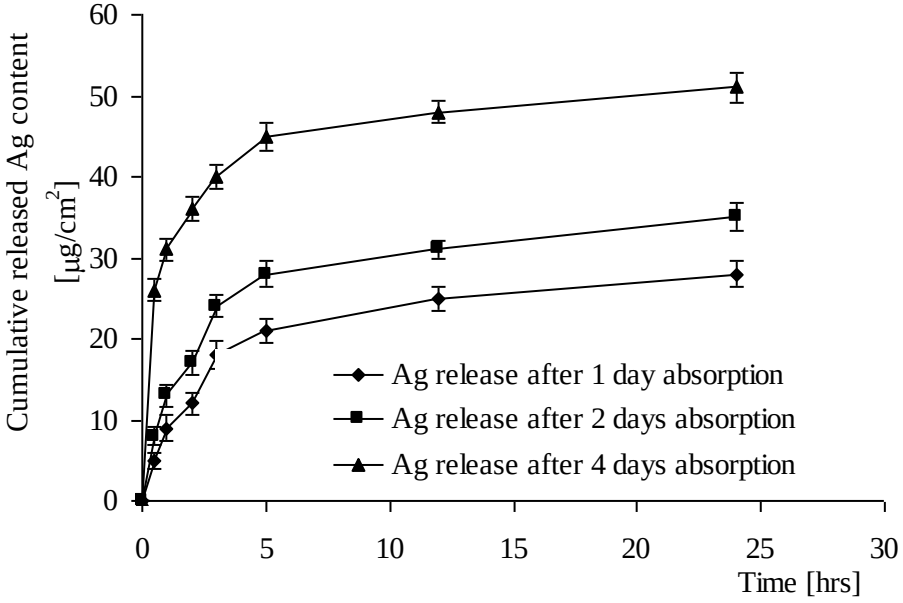


Fig. 3.29. Cumulative silver release kinetics from cellulose based sponges

As in four-day absorption experiment, the adsorbed silver content was the highest; the cumulative released amount of silver content was as well the highest (after 24 hrs – 51 μg/cm^2 of silver).

3.3.6. Antibacterial activity

The antibacterial activity experiment for the *Staphylococcus epidermidis* culture showed excellent results. Even after the one-day silver incorporation experiment, the cellulose sponges showed good antibacterial resistance. For sponges exposed to the silver nanoparticles solution for 1 day, the reduction of *S. epidermidis* was about 99.8% (or ~3 $\log_{10}$). When the amount of silver was increased prolonging the exposure of the sponges to the silver nanoparticles, the antimicrobial activity increased with a reduction of 99.999% (5 $\log_{10}$) of cells after 2 days. The number of cells was below the detection limit (100 CFU/ml), and the corresponding reduction was >99.9999% or 6 $\log_{10}$ for 4 days of diffusion.

3.3.7. Cytotoxicity study *in vitro*

L-929 line cells seeded on the cellulose sponges showed good growth. Cell growth was observed through a microscope (Scientific Microscope, Moris Technology). As cells grew well, the investigation was continuing.

Apoptosis is as well known as programmed cell death, which plays a key role in many different stages of the disease. This process is different from necrosis biochemically and morphologically. Unlike necrotic cells, the apoptotic cells are morphologically characterized by thickening the chromatin in the cell nucleus, shrinkaging of cytoplasm and apoptosis connection to the membrane. Biochemically, apoptosis is characterized by the fragmentation of the genome and degradation or destruction of some cell proteins.

During apoptosis, the proteolytic proteins caspases (cysteine proteases) are irreversibly activated. The activated caspase proteolitically activates other procaspases. Some caspases destroy structural proteins of the cells, such as nuclear shell proteins, the proteins of the cytoskeleton and intercellular connections, and activate the endonuclease that decompose the DNA.

The apoptosis was evaluated by the caspase-3 activity (Fig. 3.30) that determines the cytotoxicity of samples. For the analysis, the cellulose sponges and cellulose sponges loaded with neomycin were used. The experiment was carried out for 8 days.

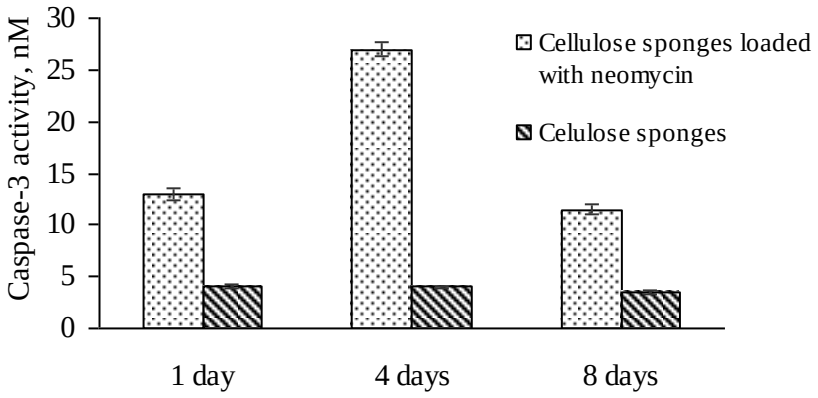


Fig. 3.30. Caspase-3 activity after the interaction with the cellulose sponges and the cellulose sponges loaded with neomycin

During 8 days experiment, the cellulose sponges showed similar and very low caspase-3 activity. The biggest caspase-3 activity was observed after 4 days for the cellulose sponges loaded with neomycin, apparently because there existed the most cells. It can be said that neomycin increases the cytotoxicity of cellulose sponges. The highest activity is observed after 4 days, and after 8 days, the activity decreases.

The determination of the DNA concentration allows to figure out how well the cells are able to grow on the samples. The PicoGreen reagent was used in the study, which is particularly sensitive to the nucleic acids.

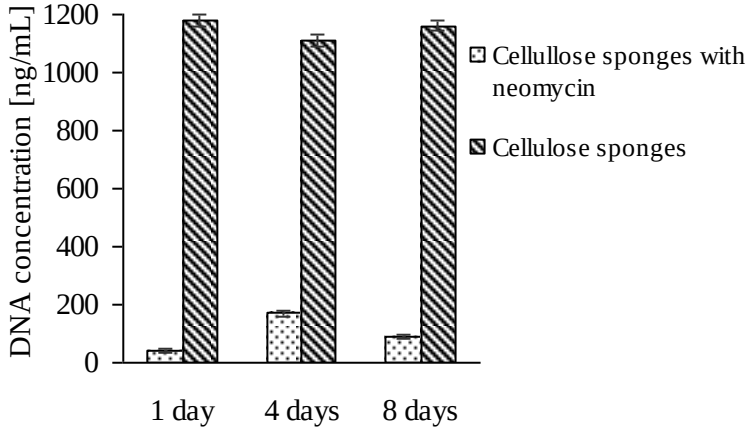


Fig. 3.31. The DNA concentration on cellulose sponges and cellulose sponges with neomycin after 1, 4 and 8 days of experiment

Fig. 3.31 shows that cellulose sponges create good conditions for cell survival, as during all 8 days, the DNA concentration remains very similar. In the case of cellulose sponges loaded with neomycin, the DNA concentration after 4 days decreases. A dressing with neomycin could be used for 4 days, because later, the cells under the dressing will not be able to renew.

3.4. Preparation and characterization of sponges made from the compositions of pectin, sodium alginate and hydroxyethylcellulose

The third group of wound dressings were sponges made from polymers of pectin, sodium alginate and different molecular weight HEC (SPAH) obtained by a freeze-drying process. Polymers were mixed in various ratios to get the optimal result. The active compound of povidone iodine was immobilized. Povidone iodine is antiseptic with a broad anti-microbial spectrum, including putative periodontal pathogens, fungi, mycobacteria, viruses. SPAH sponges were prepared in a cylindrical shape that could be used to initiate blood clotting in deep wounds, for example, in cavities after unrooting a tooth. At this time, for such cases, collagen sponges are used widely, but collagens are not used in infected or contaminated wounds and may initiate the bacterial grow.

3D image from micro-CT analysis of sample No.12 are represented in Fig. 3.32.

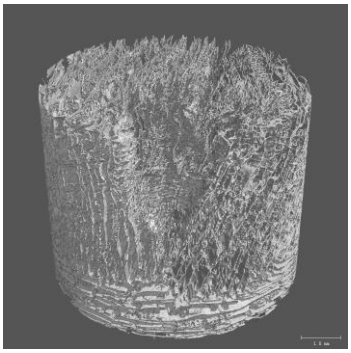


Fig. 3.32. Micro-CT image of SPAH sponge No.12, where image demonstrates the sponge in 3D view

Micro-CT analysis revealed that SPAH sponges have interconnected pores structure; thus, the air circulation between the atmosphere and the wound surface would not be complicated.

Alginate acts as a haemostatic agent and was selected due to its wide use for bleeding wounds. Alginate could absorb a large amount of liquids and at the same time has a gel forming property providing a moist environment for the wound. Thus, alginate can fit the contours of the wound bed. The removing of such dressing causes minimal pain, minimal trauma and reduces the painful experience for the patient during the dressing change. Pectin as well possesses gelling properties. Pectin creates an acid environment, which may act as a barrier against bacteria and possesses the ability of binding active molecules. One of the main drawbacks is poor mechanical properties of pectin. In order to improve mechanical properties, HEC was chosen. In the market, there is no selection of alginate:pectin:HEC wound dressings. Moreover, there are just a few alginate/pectin based products.

The porous structure of sponges was obtained by using water-based sodium alginate (2%), pectin (2%), HEC 1 with a molecular weight 1300000 (1%) or HEC 2 with a molecular weight 720 000 (1%) solutions. The prepared sponges are soluble in water and physiological fluids. In order to make them insoluble, the sponges were

cross-linked with Ca^{2+} ions (Fig. 3.33). When sodium alginate is put into a solution of calcium ions, the calcium ions replace the sodium ions in the polymer. Each calcium ion can attach to two of the polymer strands.

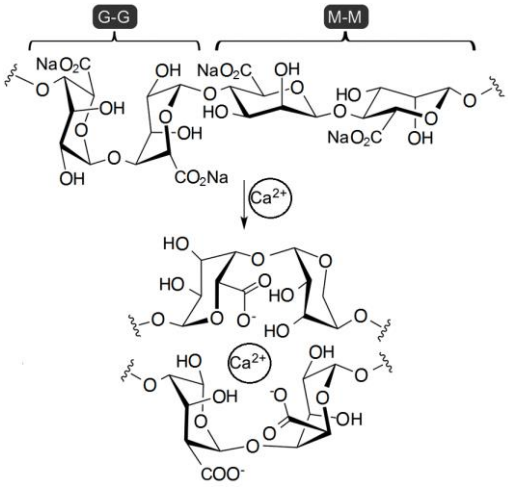


Fig. 3.33. Crosslinking of sodium alginate with calcium ions

The numbers of samples with ratios of mixed polymers are listed in Table 3.8 and Table 3.9.

Table 3.8. Composition of polymers for the preparation of samples

Sample number	Sodium alginate 2%, volume ratio in the mixture	Pectin 2%, volume ratio in the mixture	HEC (1) 1%, volume ratio in the mixture	Povidone iodinated
1	1	2	2	-
2	2	1	2	-
3	2	2	1	-
4	1	2	2	+
5	2	1	2	+
6	2	2	1	+

Table 3.9. Composition of polymers for the preparation of samples

Sample number	Sodium alginate 2%, volume ratio in the mixture	Pectin 2%, volume ratio in the mixture	HEC (2) 1%, volume ratio in the mixture	Povidone iodinated
7	1	2	2	-
8	2	1	2	-
9	2	2	1	-
10	1	2	2	+
11	2	1	2	+
12	2	2	1	+

The numbering of the samples will be continuously used in describing the next experiments.

The morphology of sponges was studied by using the SEM method. The obtained images are presented in Fig. 3.34 and Fig. 3.35.

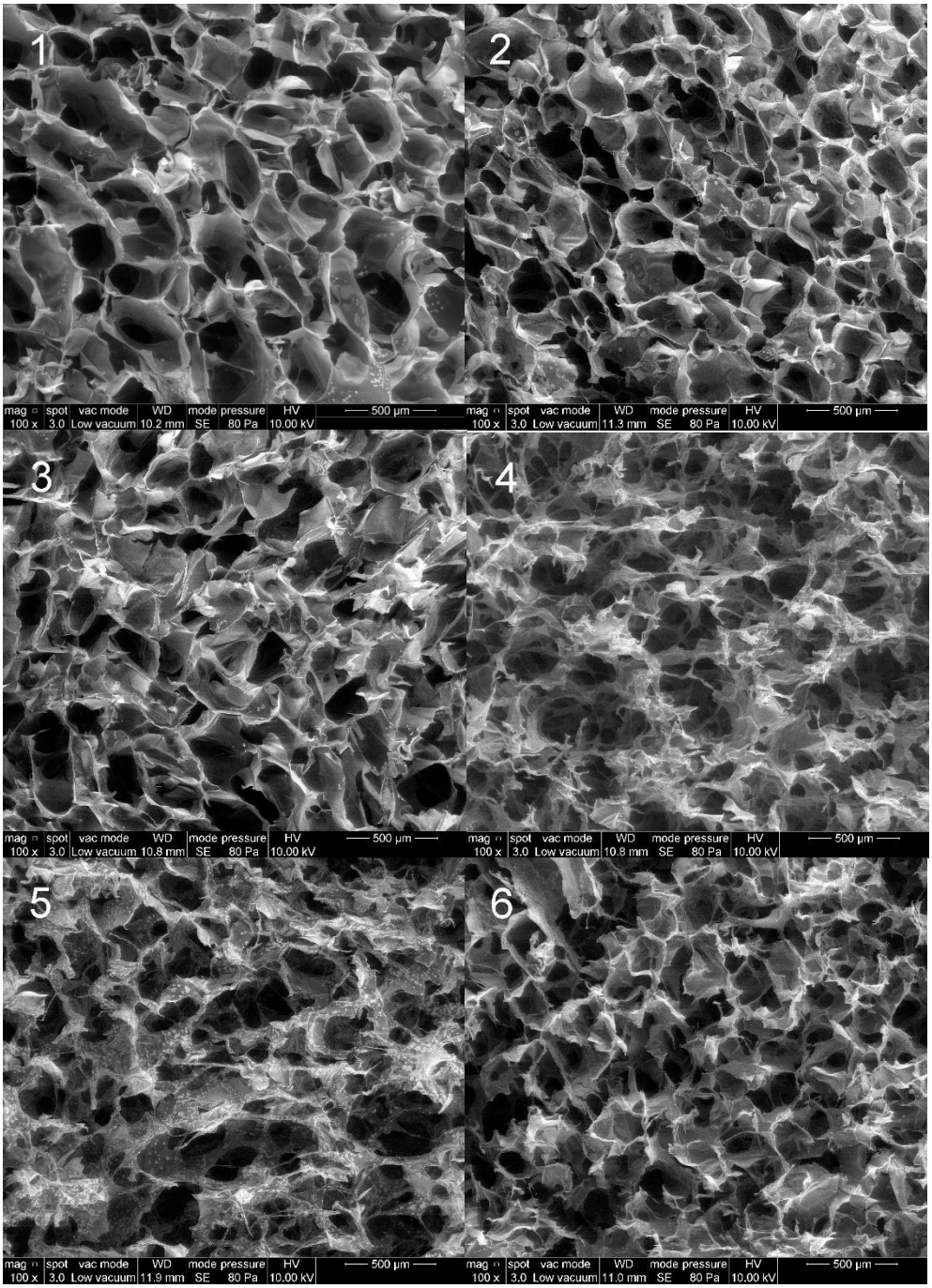


Fig. 3.34. SEM images of sponges No. 1-6

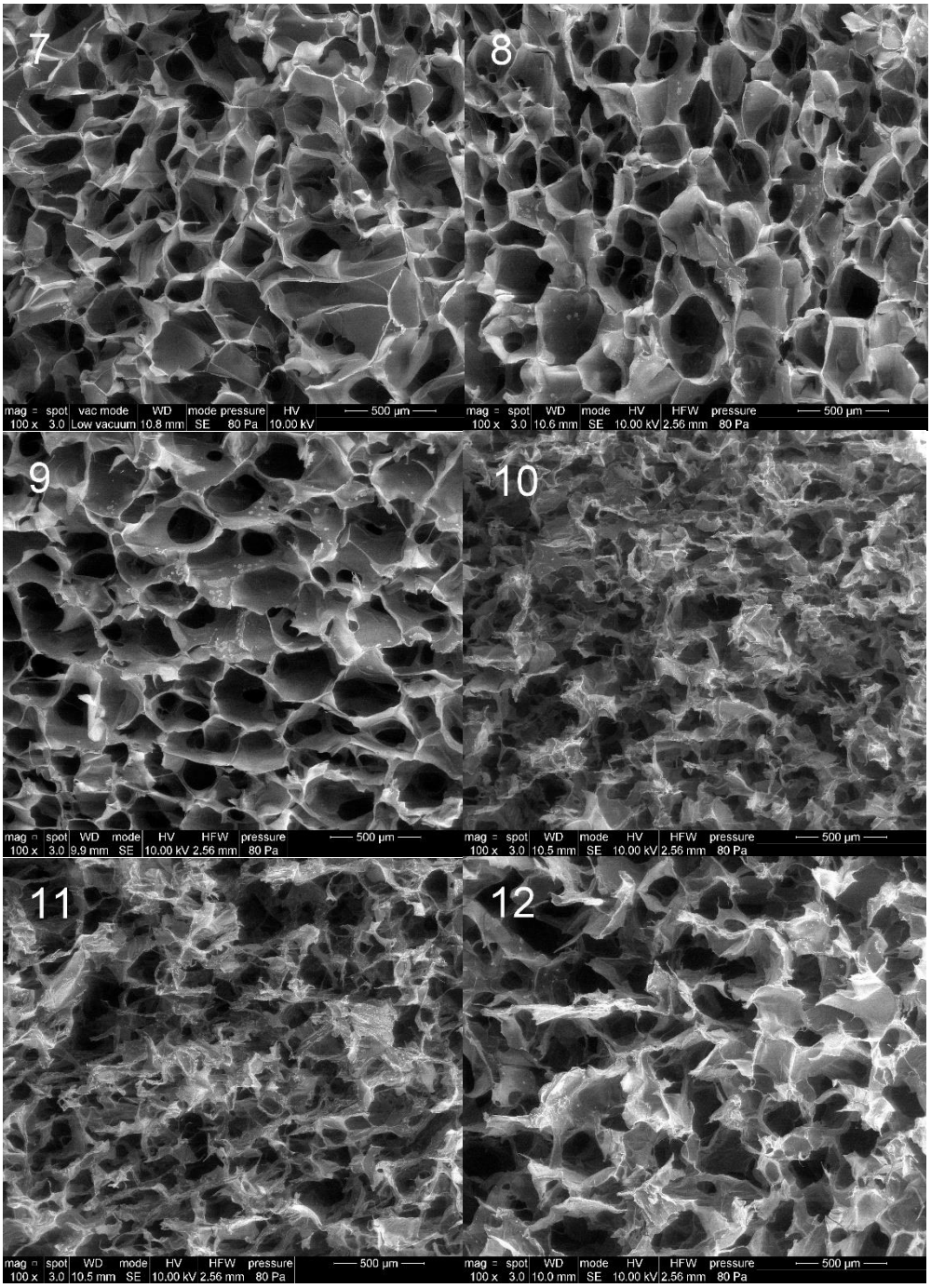


Fig. 3.35. SEM images of sponges No. 7-12

The SEM images showed that all the samples had a porous structure. The samples No. 1, 2, 3 had oval shape pores with similar diameters. The samples No. 4,

5, 6 were mixed in the same ratios of polymers as samples No. 1, 2, 3 plus the supplement of the povidone-iodine solution. From the SEM images and pore size measurements (Table 3.10), it can be seen that iodinated povidone decreases the diameter of pores, and they become more flattened.

Table 3.10. Pore sizes of the samples No. 1-12

Sample No.	Pore size, μm ± SD
1	315 ± 6.2
2	294 ± 8.4
3	255 ± 9.3
4	150 ± 10.2
5	187 ± 6.2
6	192 ± 7.7
7	307 ± 6.1
8	275 ± 8.3
9	267 ± 5.4
10	133 ± 5.6
11	196 ± 8.2
12	228 ± 7.5

The same applies as well to the samples made of the smaller molecular weight HEC (sample No. 7, 8, 9, 10, 11, 12). The supplement of the povidone-iodine solution in the samples produced of polymers sodium alginate:pectin:HEC in a ratio 2:2:1 did not change the pore form that much, and did not reduce the pore size that significantly.

3.4.1. The pseudo extracellular fluid retention test

The role of wound sponges is to absorb the blood and wound exudate, promote blood clotting. The prepared sponges are soluble in water and physiological fluids. The results of the absorbed PBS after soaking sponges in this solution for 15 minutes are represented in Table 3.11.

Table 3.11. PBS retention of samples No. 1-12

Sample No.	PBS retention, % ± SD
1	465 ± 8
2	424 ± 7
3	407 ± 10
4	294 ± 9
5	351 ± 7
6	395 ± 9
7	385 ± 11
8	406 ± 9
9	414 ± 9
10	292 ± 10
11	320 ± 8
12	385 ± 8

The PBS retention test shows that mixing polymers sodium alginate:pectin:HEC in a ratio 2:2:1 without the supplement of povidone-iodine and with the supplement of this active compound shows similar values of the PBS retention. The supplement of povidone-iodine in this case decreases the PBS retention slightly. The addition of the active substance to the mixed polymers in other ratios has a greater impact on the PBS retention. In all cases, the addition of PBS decreases the PBS retention. Comparing two groups of the samples from the point of view of the HEC molecular weight, it is seen that a larger molecular weight of HEC gives better absorption characteristics. The samples containing higher amounts of HEC show lower absorption values; thus, the higher amounts of pectin and sodium alginate gives higher liquid absorption values.

3.4.2. Porosity of sponges

The porosity of a material is important for the water vapour, oxygen permeability. It is important to ensure the sufficient permeability of these materials through the dressing for proper breathing of the wound. Moreover, the porosity has an impact on the liquid absorption ability. The results of porosity measurements are submitted in Table 3.12.

Table 3.12. Porosity measurements of samples No. 1-12

Sample No.	Porosity P, % ± SD
1	82.8 ± 4.8
2	81.4 ± 4.1
3	79.9 ± 4.2
4	56.3 ± 2.5
5	70.0 ± 2.4
6	72.4 ± 5.6
7	84.3 ± 1.7
8	88.4 ± 2.3
9	91.9 ± 2.4
10	52.7 ± 1.6
11	80.5 ± 0.6
12	83.4 ± 1.6

Comparing the porosity of the sponges without and with the active substance, it can be seen that the addition of the active substance decreases the porosity as the pore size decreases as well. The active substance immobilization most significantly reduces the porosity of the samples composed of sodium alginate:pectin:HEC polymers in the ratio 1:2:2. In this case, the addition of the active compound with both HECs lowers the porosity by 20% comparing with the samples without the active compound. The addition of the active substance in other samples reduces the porosity by around 10%. The samples prepared from the higher molecular weight HEC have reduced the porosity comparing with the samples prepared from HEC with the lower molecular weight.

3.4.3. The compression test

During this search, the Young's modulus, which describes the material resistance to external forces, was determined. Young's modulus values of PSAH samples are presented in Fig. 3.36.

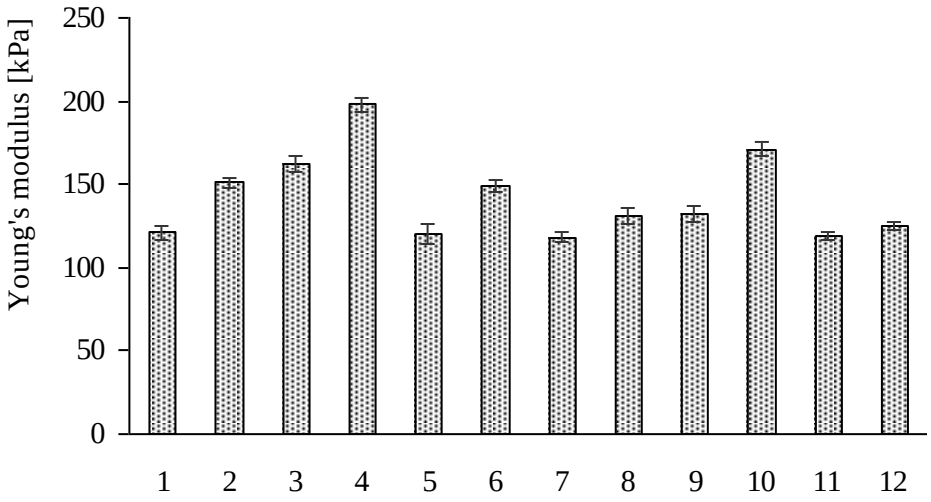


Fig. 3.36. The Young's modulus of PSAH samples

The best resistance to compression and the highest values of the Young's modulus has the sample No. 4 (198 kPa) and the sample No. 10 (171 kPa). Such values of the Young's modulus may be due to the porosity of the samples, which is the lowest for the samples No. 4 and No. 10. Greater porosity gives lower values of the Young's modulus. Comparing two groups of the samples with different molecular weight HEC, it can be seen that the samples with the higher molecular weight (the samples No. 1-6) have higher values of the Young's modulus than the samples with the lower molecular weight HEC (the samples No. 7-12). For the samples that are made from polymers sodium alginate:pectin:HEC in the ratio 1:2:2, the compressive strength increases when the active compound is immobilized: value of Young's modulus of sample No. 1 is 121 kPa, while the sample with povidone-iodine of the sample No. 4 has the Young's value of 198 kPa. The same applies for the samples made with lower molecular weight HEC, the sample No. 7 without the active substance has a lower Young's modulus value 118 kPa than the samples with the active substance, where the Young's modulus reaches 171 kPa (the sample No. 10). The hardness of a wound dressing should not be very high; otherwise, the newly formed tissues may be damaged. The hardness of the wound dressings, however, should not be too low, because a wound dressing may stick to the wound surface and disrupt the healing process.

3.3.4. The hardness and resilience test

The hardness and resilience values are submitted in Table 3.13.

Table 3.13. Results of the hardness and resilience test

Sample No.	Hardness, N ± SD	Resilience, % ± SD
1	5.6 ± 0.8	12.0 ± 1.2
2	8.9 ± 2.6	12.0 ± 1.6
3	11.1 ± 0.1	16.3 ± 2.7
4	7.9 ± 2.3	16.3 ± 2.7
5	6.8 ± 1.6	17.3 ± 1.5
6	9.3 ± 1.1	15.9 ± 1.5
7	9.0 ± 1.3	10.6 ± 0.8
8	7.3 ± 1.5	11.4 ± 2.2
9	9.5 ± 2.7	10.4 ± 1.3
10	9.8 ± 2.4	14.8 ± 2.8
11	5.6 ± 2.3	18.3 ± 2.1
12	9.2 ± 0.5	16.1 ± 0.8

The analysis of the data shows that the HEC amount in the mixture has a negative impact on hardness. The lesser amount of HEC gives better mechanical properties. The hardest samples were obtained by mixing polymers sodium alginate:pectin:HEC in the ratio 2:2:1. The hardness of the samples made of sodium alginate, pectin and HEC polymers in the ratio 1:2:2 increased when povidone-iodine is added. For the polymers mixed in other ratios, the addition of the active substance reduces the hardness. In order to obtain the strongest sponges, it would be appropriate to use sodium alginate:pectin:HEC polymers mixed in the ratio 2:2:1.

As mentioned above, the resilience of a material shows the ability to return to its original shape after removing the applied force. The higher is the resilience value, the more elastic is the obtained material. The obtained resilience value shows that the samples No. 5 and 11 have the greatest resilience, and those samples contain less pectin and the immobilized active substance. The lowest resilience values are shown by the samples No. 7 and 9, i.e., the samples containing sodium alginate:pectin:HEC in the ratio 1:2:2 and 2:2:1. Thus, a greater amount of pectin makes a sample less resilient. The results indicate that the immobilization of povidone-iodine increases the resilience together with the elasticity by about 4-7% comparing to the samples without the active substance. The samples without the active substance with higher molecular weight HEC show higher resilience values than the samples with lower molecular weight HEC. The resilience values of the samples with the active substance do not differ significantly and varies within the range of 15-18%.

3.4.5. Effects on blood coagulation

In order to use a wound dressing for haemostatic properties, the main function would be to stop bleeding and promote wound healing. When making sure that the sponges are able to stop the bleeding, a blood clotting test was performed. The data of the blood coagulation test are presented in Fig. 3.37.

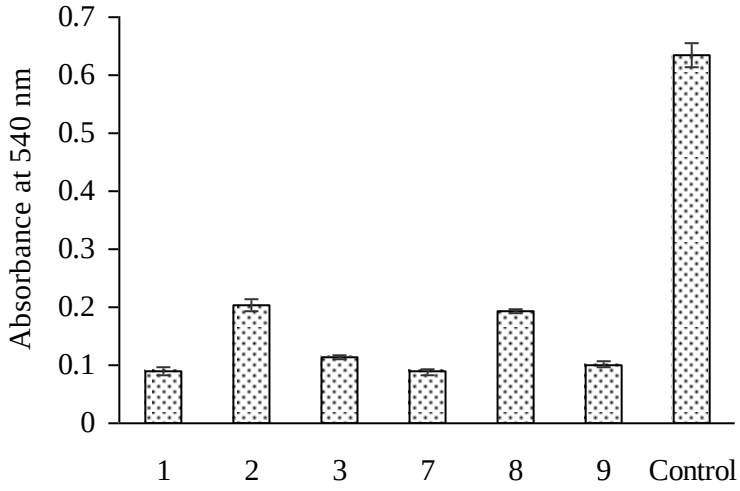


Fig. 3.37. Hemoglobin absorbance of PSAH samples

The study revealed that the sponges fairly quick absorbed the blood spread on the surface. The blood clotting process was initiated with the solution of $CaCl_2$. During the test, the measurements of not absorbed red blood cells were carried out. The measurement of the control samples, i.e., blood absorption without the sponge was done as well.

The lower the light absorption results are obtained, the less red blood cells are remaining in the solution, the better blood absorption properties have the sponges. The highest amount of untrapped red blood cells is found in the samples No. 2 and No. 8. These samples have the lowest content of pectin; thus, it can be concluded that a higher content of pectin improves the haemostatic properties. The values of the hemoglobin absorption of the samples No. 1, 3, 7 and 9 are quite similar and are about 0.1. The obtained values are optimal comparing the results with a control sample, where hemoglobin absorption reaches 0.6. The prepared sponges are good at a blood coagulation function and could be applied in medicine for haemostatic purposes.

Fig. 3.38 shows sponges after the blood absorption test.

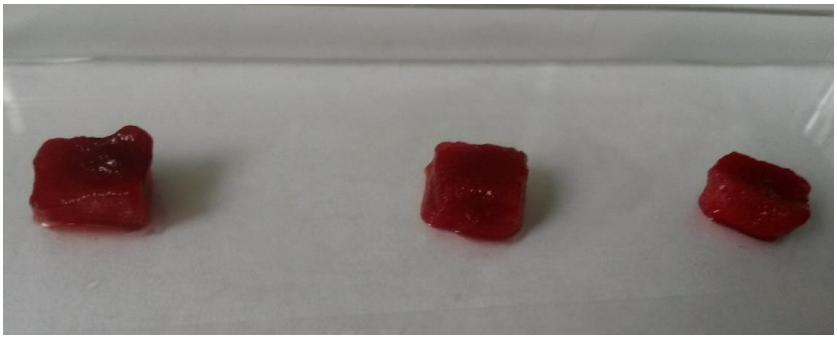


Fig. 3.38. Sponges No. 1-3 after the blood absorption test

Fig. 3.38 demonstrates that the blood was absorbed at a full volume of the sponges; after blood absorption, the sponges are able to maintain their shape.

3.4.6. Release of povidone-iodine

Povidone-iodine immobilization helps to protect wound from the bacterial contamination and damage. During this study, the tests of povidone-iodine release with time were carried out. The results obtained from the samples made with higher molecular weight HEC are presented in Fig. 3.39.

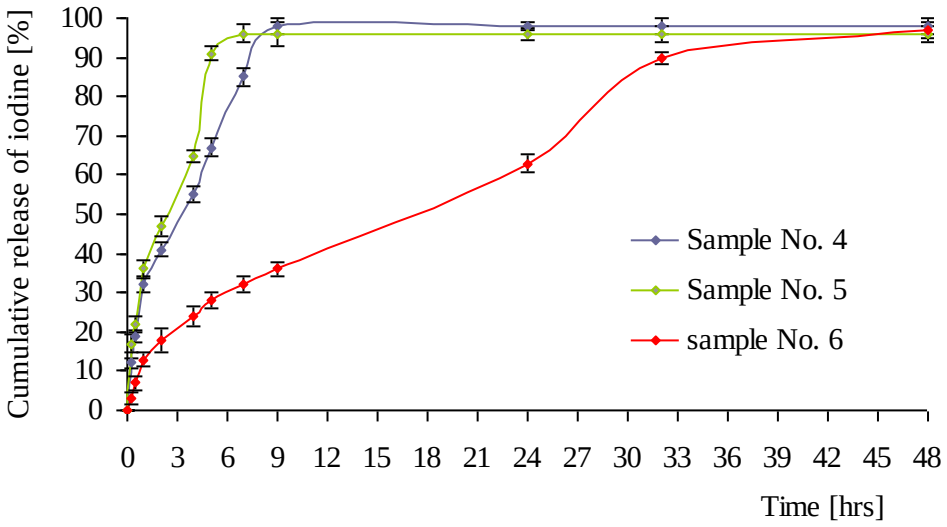


Fig. 3.39. Cumulative release of povidone-iodine from samples No. 4-6

In Fig. 3.38, it can be seen that the sample No. 5 releases the active compound faster, while the sample No. 6 releases the iodine the slowest. The sample No. 5 has a lower amount of pectin comparing with the sample No. 6. The sample No. 4 reaches the equilibrium after 9 hours, the sample No. 5 after 7 hours, while the sample No. 6 reaches the equilibrium after about 34 hours.

The obtained results for the samples made with lower molecular weight HEC are presented in Fig. 3.40.

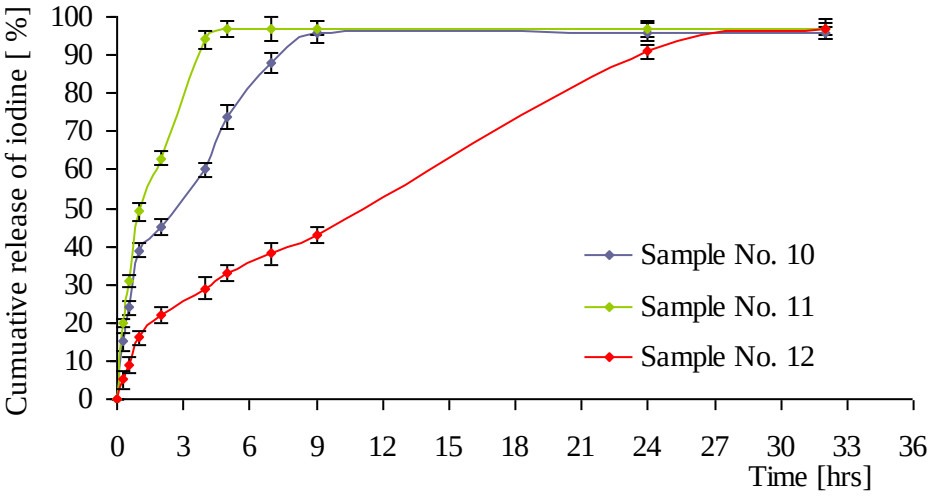


Fig. 3.40. Cumulative release of povidone-iodine from the samples No. 10-12

Fig. 3.40 shows that the fastest iodine release is observed for the sample No. 11 and the slowest for the sample No. 12. The sample No. 11 has a lower amount of pectin than the sample No. 12. The sample No. 10 reaches the equilibrium after 9 hours, the sample No. 11 after 5 hours, while the sample No. 12 reaches the equilibrium after 27 hours.

From Fig. 3.39 and Fig. 3.40, it can be seen that after reaching the equilibrium, almost all immobilised iodine is released. The results show that the rate of iodine release is influenced by the molecular weight of HEC. Iodine from the samples with higher molecular weight HEC (sample No. 4-6) is released slower. Swelling of a polymer with a higher molecular weight takes longer. The ratio of mixed polymers as well has influence on the iodine release rate. A greater amount of pectin results in prolonged release of iodine. In order to prolong the release of iodine, the samples should be prepared in the ratio 2:2:1 of polymers alginate:pectin:HEC. In order to prolong the iodine release even more, it would be appropriate to use HEC with a higher molecular weight.

CONCLUSIONS

1. Three new wound dressing materials for different wound treatment were created from the regenerated cellulose.

1.1 Transparent cellulose hydrogel sheets were prepared, where liquid phase is water or water-glycerol solution. These hydrogel sheets possess low bioadhesion and a high moisture content; thus, they are promising wound dressings for burn wounds, cuts, scratches and abrasions. It has been found that the saturation of hydrogel sheets with glycerol has a positive impact for the mechanical properties. Cellulose hydrogels loaded with glycerol have more than twice higher tensile strength (6.33 MPa) comparing with the same hydrogel (2,73 MPa). Moreover, the elongation at break and the Young's modulus increases significantly after loading the hydrogel with glycerol. The dynamic-termomechanical analysis showed that cellulose hydrogels loaded with glycerol, despite an increasing temperature and motion, could retain their shape.

1.2. It has been shown that treating the cellulose hydrogel with supercritical carbon dioxide, a macro porous structure is obtained (pore size 100-600 μm) by improving the vapour and oxygen transmission, which is very important for pressure sores, leg ulcers, burns, necrotic and surgical wounds.

1.3. Cellulose-based sponges prepared from the regenerated cellulose hydrogel by a lyophilization process showed a high liquid absorption capacity (210%) and the ability to be loaded with antibacterial agents; consequently, these sponges could be applied for necrotic, abundantly exuding wounds. Water vapour transmission rate of regenerated cellulose sponges was 2656 ± 30 g/m^2/day and almost met an ideally requirements which are 2000-2500 g/m^2/day.

2. Sponges from water soluble polymers, such as pectin, sodium alginate and hydroxyethylcellulose, were prepared by a lyophilization process. These sponges possess a high liquid absorption capacity (~300-450%) and good blood clotting ability; thus, these sponges could be applied as wound dressings for exuding and bleeding wounds. Mechanical properties, porosity, liquid absorption and blood clotting of the sponges made from pectin, sodium alginate and hydroxyethylcellulose can be regulated by changing ratios of the constituting polymers.

3. Active compounds were immobilized into the wound dressing material by the diffusion method, and the release of active compounds was analysed.

3.1. The release of antibiotic neomycin from cellulose hydrogel sheets takes more than 24 hours. Carboxylated cellulose hydrogel releases the active compound slower due to the ionic bonding between the carboxy groups in cellulose hydrogel and amino groups which are in neomycin. Hydrogel sheets loaded with neomycin showed good antimicrobial resistance against *Escherichia coli* and *Bacillus subtilus.*

3.2. Immobilization of *Perilla frutescens* and *Betula pendula* extracts into cellulose hydrogels treated with supercritical carbon dioxide showed that the equilibrium of total phenols release is reached after 6 hrs, after which, a part of the material remains in a hydrogel. In a case of *Perilla frutescens*, 90% of immobilized phenolic compounds are released, while in a case of *Betula pendula*, 50% of immobilized phenolic compounds are released.

3.3. Silver nanoparticles were incorporated into cellulose sponges by diffusion method and even cellulose sponges after one-day silver incorporation experiment showed good antibacterial resistance to the *Staphylococcus epidermidis* culture, where bacterial amount reduction was 99.8% (or ~3 $\log_{10}$). The release studies of immobilized phenolic compounds from *Chamomilla recutita* showed that after the equilibrium, 68% of phenolic compounds are released. In a case of *Calendula officinalis*, after the equilibrium, only 38% of phenolic compounds are released. Not completely released active compound, results in longer antioxidant effect of the wound dressing material.

3.4. The release of povidone-iodine from sponges made of pectin, sodium alginate and hydroxyethylcellulose could be controlled by changing the ratio of these polymers.

4. *In vitro* studies with human fibroblast confirmed that cellulose sponges are non cytotoxic. The cytotoxicity test *in vitro* with rat hepatocytes confirmed that the cellulose hydrogel, and the cellulose hydrogel loaded with glycerol were non-cytotoxic as well.

Bibliography

1. Abdelrahman, T. and Newton, H., 2011. Wound dressings: Principles and practice. *Surgery (Oxford)*, 29(10), pp. 491–495. Available at: http://www.sciencedirect.com/science/article/pii/S0263931911001281 [Accessed May 9, 2014].
2. Alsarra, I. A., 2009. Chitosan topical gel formulation in the management of burn wounds. *International Journal of Biological Macromolecules*, 45(1), pp. 16–21.
3. Ang-atikarnkul, P., Watthanaphanit, A. and Rujiravanit, R., 2014. Fabrication of cellulose nanofiber/chitin whisker/silk sericin bionanocomposite sponges and characterizations of their physical and biological properties. *Composites Science and Technology*, 96, pp. 88–96. Available at: http://www.deepdyve.com/lp/elsevier/fabrication-of-cellulose-nanofiber-chitin-whisker-silk-sericin-DEY0l02L0F [Accessed July 13, 2014].
4. Aniulyte, J., Liesiene, J. and Niemeyer, B., 2006. Evaluation of cellulose-based biospecific adsorbents as a stationary phase for lectin affinity chromatography. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 831(1-2), pp. 24–30.
5. Arboleya, J. and Wilde, P., 2005. Competitive adsorption of proteins with methylcellulose and hydroxypropyl methylcellulose. *Food Hydrocolloids*, 19(3), pp. 485–491.
6. Archana, D. et al., 2015. Chitosan-PVP-nano silver oxide wound dressing: In vitro and in vivo evaluation. *International Journal of Biological Macromolecules*, 73, pp. 49–57. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0141813014007351.
7. Baccaro, S. et al., 2013. Structure modifications and interaction with moisture in γ-irradiated pure cellulose by thermal analysis and infrared spectroscopy. *Polymer Degradation and Stability*, 98(10), pp. 2005–2010. Available at: http://dx.doi.org/10.1016/j.polymdegradstab.2013.07.011.
8. Balakrishnan, B. et al., 2012. Anti-bacterial properties of an in situ forming hydrogel based on oxidized alginate and gelatin loaded with gentamycin. *Trends in Biomaterials and Artificial Organs*, 26(3), pp. 139–145.
9. Balakrishnan, B. and Jayakrishnan, A., 2005. Self-cross-linking biopolymers as injectable in situ forming biodegradable scaffolds. *Biomaterials*, 26(18), pp. 3941–3951.
10. Banno, N. et al., 2005. Anti-inflammatory and antitumor-promoting effects of the triterpene acids from the leaves of Eriobotrya japonica. *Biological and Pharmaceutical Bulletin*, 28(10), pp. 1995–1999.
11. Beldon, P., 2010. Basic science of wound healing. *Surgery (Oxford)*, 28(9), pp. 409–412.
12. Bernabei, R. et al., 1999. Effect of topical application of nerve-growth factor on pressure ulcers. *Lancet*, 354(9175), p. 307. Available at: http://www.sciencedirect.com/science/article/pii/S0140673699027841 [Accessed May 24, 2015].
13. Berndt, S. et al., 2013. Antimicrobial porous hybrids consisting of bacterial nanocellulose and silver nanoparticles. *Cellulose*, 20(2), pp. 771–783. Available at: http://link.springer.com/10.1007/s10570-013-9870-1 [Accessed May 12, 2014].

14. Boateng, J. S. et al., 2008. Wound healing dressings and drug delivery systems: A review. *Journal of Pharmaceutical Sciences*, 97(8), pp. 2892–923. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17963217 [Accessed June 4, 2015].
15. Boateng, J. S., Pawar, H. V. and Tetteh, J., 2013. Polyox and carrageenan based composite film dressing containing anti-microbial and anti-inflammatory drugs for effective wound healing. *International Journal of Pharmaceutics*, 441(1-2), pp. 181–191. Available at: http://dx.doi.org/10.1016/j.ijpharm.2012.11.045.
16. Bolton, L. L., Monte, K. and Pirone, L. A., 2000. Moisture and healing: Beyond the jargon. *Ostomy/wound management*, 46(1A Suppl), pp. 51S–62S; quiz 63S–64S. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10732640 [Accessed May 24, 2015].
17. Boonkaew, B. et al., 2014. Antimicrobial efficacy of a novel silver hydrogel dressing compared to two common silver burn wound dressings: Acticoat™ and PolyMem Silver ®. *Burns*, 40(1), pp. 89–96. Available at: http://dx.doi.org/10.1016/j.burns.2013.05.011.
18. Bowler, P. G., Duerden, B. I. and Armstrong, D. G., 2001. Wound microbiology and associated approaches to wound management. *Clinical Microbiology Reviews*, 14(2), pp. 244–269.
19. Burrell, R. E., 2003. A scientific perspective on the use of topical silver preparations. *Ostomy/Wound Management*, 49(5A Suppl), pp. 19–24. Available at: http://www.researchgate.net/publication/10642958_A_scientific_perspective_on_the_use_of_topical_silver_preparations [Accessed July 1, 2015].
20. Butler, M. S. and Cooper, M. A, 2011. Antibiotics in the clinical pipeline in 2011. *The Journal of Antibiotics*, 64(6), pp. 413–425. Available at: http://dx.doi.org/10.1038/ja.2011.44.
21. Buwalda, S. J. et al., 2014. Hydrogels in a historical perspective: From simple networks to smart materials. *Journal of Controlled Release: Official Journal of the Controlled Release Society*, 190, pp. 254–273. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24746623 [Accessed July 13, 2014].
22. Caló, E. and Khutoryanskiy, V. V., 2014. Biomedical applications of hydrogels: A review of patents and commercial products. *European Polymer Journal*. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0014305714004091.
23. Casey, G., 2001. Wound dressings. *Paediatric Nursing*, 13(4), pp. 39–42; quiz 43.
24. Casimiro, M. H., Gil, M. H. and Leal, J. P., 2007. Drug release assays from new chitosan/pHEMA membranes obtained by gamma irradiation. *Nuclear Instruments and Methods in Physics Research, Section B: Beam Interactions with Materials and Atoms*, 265(1), pp. 406–409.
25. Catanzano, O. et al., 2015. Alginate-hyaluronan composite hydrogels accelerate wound healing process. *Carbohydrate Polymers*, 131, pp. 407-414.
26. Chellamani, K. P., Balaji, R. S. V. and Veerasubramanian, D., 2014. Quality evaluation methods for textile substrates based wound dressings. *International Journal of Emerging Technology and Advanced Engineering*, 4(6), pp. 811–817.
27. Chen, C. H. et al., 2006. Novel living cell sheet harvest system composed of thermoreversible methylcellulose hydrogels. *Biomacromolecules*, 7(3), pp. 736–743.
28. Chen, J., Chen, J. and Song, J., 2013. Collagen–PVP hybrid based anti-inflammatory hydrogel for wound repairing. *Journal of Controlled Release*, 172(1), pp. e129–e130. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0168365913006767.

29. Chiaoprakobkij, N. et al., 2011. Characterization and biocompatibility of bacterial cellulose/alginate composite sponges with human keratinocytes and gingival fibroblasts. *Carbohydrate Polymers*, 85(3), pp. 548–553. Available at: http://dx.doi.org/10.1016/j.carbpol.2011.03.011.
30. Chiu, C. T. et al., 2008. Development of two alginate-based wound dressings. *Journal of Materials Science: Materials in Medicine*, 19(6), pp. 2503–2513.
31. Cipriani, T. R. et al., 2009. Influence of molecular weight of chemically sulfated citrus pectin fractions on their antithrombotic and bleeding effects. *Thrombosis and Haemostasis*, 101(5), pp. 860–866. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19404539 [Accessed June 30, 2015].
32. Cutting, K. F., 2003. Wound exudate: composition and functions. *British Journal of Community Nursing*, 8(Sup3), pp. S4–S9. Available at: http://www.researchgate.net/publication/8950463_Wound_exudate_composition_and_functions [Accessed June 2, 2015].
33. Cutting, K. F., White, R. J. and Butcher, M., 2010. Wound dressing performance: Meeting 21st century requirements. *Journal Of Wound Care Sorbion Supplement 2010*, pp. 1–20.
34. Czaja, W. et al., 2006. Microbial cellulose - The natural power to heal wounds. *Biomaterials*, 27(2), pp. 145–151.
35. Czaja, W. K. et al., 2007. The future prospects of microbial cellulose in biomedical applications. *Biomacromolecules*, 8(1), pp. 1–12.
36. D'Ayala, G. G., Malinconico, M. and Laurienzo, P., 2008. Marine derived polysaccharides for biomedical applications: Chemical modification approaches. *Molecules*, 13(9), pp. 2069–2106.
37. Dell, S. J. et al., 2011. Randomized comparison of postoperative use of hydrogel ocular bandage and collagen corneal shield for wound protection and patient tolerability after cataract surgery. *Journal of Cataract and Refractive Surgery*, 37(1), pp. 113–21. Available at: http://www.sciencedirect.com/science/article/pii/S0886335010015828 [Accessed June 17, 2015].
38. Demir, A. et al., 2009. Effects of Chlorhexidine and Povidone-Iodine Mouth Rinses on the Bond Strength of an Orthodontic Composite. Available at: http://www.angle.org/doi/10.1043/0003-3219(2005)75[392:EOCAPM]2.0.CO;2?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub%3Dpubmed& [Accessed July 1, 2015].
39. Djagny, V. B., Wang, Z. and Xu, S., 2001. Gelatin: A valuable protein for food and pharmaceutical industries: Review. *Critical Reviews in Food Science and Nutrition*, 41(6), pp. 481–492.
40. Don, T. M. et al., 2006. Preparation and characterization of chitosan-g-poly(vinyl alcohol)/poly(vinyl alcohol) blends used for the evaluation of blood-contacting compatibility. *Carbohydrate Polymers*, 63(3), pp. 331–339.
41. Drury, J. L. and Mooney, D. J., 2003. Hydrogels for tissue engineering: Scaffold design variables and applications. *Biomaterials*, 24(24), pp. 4337–4351.
42. Du, J. and Hsieh, Y. L., 2009. Cellulose/chitosan hybrid nanofibers from electrospinning of their ester derivatives. *Cellulose*, 16(2), pp. 247–260.

43. Dumville, J. C. et al., 2013. Hydrocolloid dressings for healing diabetic foot ulcers. *The Cochrane Database of Systematic Reviews*, 8, p. CD009099. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23922167 [Accessed August 5, 2015].
44. Edwards, J. V et al., 2001. Modified cotton gauze dressings that selectively absorb neutrophil elastase activity in solution. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society*, 9(1), pp. 50–58. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11350640 [Accessed May 11, 2014].
45. Edwards, J. V. et al., 2009. Positively and negatively charged ionic modifications to cellulose assessed as cotton-based protease-lowering and hemostatic wound agents. *Cellulose*, 16(5), pp. 911–921. Available at: http://link.springer.com/10.1007/s10570-009-9302-4 [Accessed May 11, 2014].
46. Edwards, J. V. et al., 2007. Protease inhibition by oleic acid transfer from chronic wound dressings to albumin. *International Journal of Pharmaceutics*, 340(1-2), pp. 42–51. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17570626 [Accessed May 11, 2014].
47. Falabella, A. F., 2006. Debridement and wound bed preparation. *Dermatologic Therapy*, 19(6), pp. 317–325.
48. Farris, S. et al., 2011. Gelatin-pectin composite films from polyion-complex hydrogels. *Food Hydrocolloids*, 25(1), pp. 61–70. Available at: http://dx.doi.org/10.1016/j.foodhyd.2010.05.006.
49. Fathi, E. et al., 2011. Physically crosslinked polyvinyl alcohol-dextran blend xerogels: Morphology and thermal behavior. *Carbohydrate Polymers*, 84(1), pp. 145–152. Available at: http://dx.doi.org/10.1016/j.carbpol.2010.11.018.
50. Fluhr, J. W. et al., 2001. Generation of free fatty acids from phospholipids regulates stratum corneum acidification and integrity. *Journal of Investigative Dermatology*, 117(1), pp. 44–51.
51. Fu, L., Zhang, J. and Yang, G., 2013. Present status and applications of bacterial cellulose-based materials for skin tissue repair. *Carbohydrate Polymers*, 92(2), pp. 1432–1442. Available at: http://dx.doi.org/10.1016/j.carbpol.2012.10.071.
52. Gardner, S., 2001. The validity of the clinical signs and symptoms used to identify localized chronic wound infection. *Wound Repair and Regeneration*, 9(3), pp. 178–186. Available at: http://onlinelibrary.wiley.com/doi/10.1046/j.1524-475x.2001.00178.x/full.
53. Guo, C., Zhou, L. and Lv, J., 2013. Effects of expandable graphite and modified ammonium polyphosphate on the flame-retardant and mechanical properties of wood flour-polypropylene composites. *Polymers and Polymer Composites*, 21(7), pp. 449–456.
54. Hago, E. E. and Li, X., 2013. Interpenetrating polymer network hydrogels based on gelatin and PVA by biocompatible approaches: Synthesis and characterization. *Advances in Materials Science and Engineering*, 2013(1), pp. 1-8. Available at: http://www.hindawi.com/journals/amse/2013/328763/.
55. Harding, K. G., Morris, H. L. and Patel, G. K., 2002. Science, medicine and the future: Healing chronic wounds. *BMJ (Clinical Research ed.)*, 324(7330), pp. 160–163.
56. Harris, C. L. and Fraser, C., 2004. Malnutrition in the institutionalized elderly: The effects on wound healing. *Ostomy/Wound Management*, 50(10), pp. 54–63. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15509882 [Accessed June 8, 2015].

57. Hashem, M. et al., 2013. Synthesis and characterization of novel carboxymethylcellulose hydrogels and carboxymethylcellulolse-hydrogel-ZnO-nanocomposites. *Carbohydrate Polymers*, 95(1), pp. 421–427. Available at: http://dx.doi.org/10.1016/j.carbpol.2013.03.013.

58. Heiner, J. D. et al., 2010. 10% Povidone-iodine may be a practical field water disinfectant. *Wilderness and Environmental Medicine*, 21(4), pp. 332–336. Available at: http://dx.doi.org/10.1016/j.wem.2010.09.008.

59. Hennink, W. E. and van Nostrum, C. F., 2012. Novel crosslinking methods to design hydrogels. *Advanced Drug Delivery Reviews*, 64(SUPPL.), pp. 223–236. Available at: http://dx.doi.org/10.1016/j.addr.2012.09.009.

60. Hoffman, A. S., 2012. Hydrogels for biomedical applications. *Advanced Drug Delivery Reviews*, 64(SUPPL.), pp. 18–23.

61. Hoon, J. L. and Sung, H. J., 2004. Bacteriostasis of Nanosized Colloidal Silver on Polyester Nonwovens. *Textile Research Journal*, 74(5), pp. 442–447.

62. Huang, S. and Fu, X., 2010. Naturally derived materials-based cell and drug delivery systems in skin regeneration. *Journal of Controlled Release*, 142(2), pp. 149–159. Available at: http://dx.doi.org/10.1016/j.jconrel.2009.10.018.

63. Huidobro, A. L., García, A. and Barbas, C., 2009. Rapid analytical procedure for neomycin determination in ointments by CE with direct UV detection. *Journal of Pharmaceutical and Biomedical Analysis*, 49(5), pp. 1303–1307.

64. Hwang, M. R. et al., 2010. Gentamicin-loaded wound dressing with polyvinyl alcohol/dextran hydrogel: Gel characterization and in vivo healing evaluation. *AAPS PharmSciTech*, 11(3), pp. 1092–1103.

65. Yang, C. et al., 2004. The application of recombinant human collagen in tissue engineering. *BioDrugs*, 18(2), pp. 103–119. Available at: http://link.springer.com/10.2165/00063030-200418020-00004 [Accessed June 17, 2015].

66. Yang, J. et al., 2002. Fabrication and surface modification of macroporous poly (L-lactic acid) and poly (L-lactic-co-glycolic acid)(70/30) cell scaffolds for human skin fibroblast cell culture. *Journal of Biomedical Materials Research*, 62(3), pp. 438–446.

67. Yang, X. et al., 2010. Cytotoxicity and wound healing properties of PVA/ws-chitosan/glycerol hydrogels made by irradiation followed by freeze–thawing. *Radiation Physics and Chemistry*, 79(5), pp. 606–611. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0969806X09005763 [Accessed March 27, 2015].

68. Jain, J., et al., 2009. Silver nanoparticles in therapeutics: development of an antimicrobial gel formulation for topical use. *Molecular Pharmaceutics*, 6(5), pp. 1388–401. Available at: http://dx.doi.org/10.1021/mp900056g [Accessed May 8, 2014].

69. Jandera, V. et al., 2000. Cooling the burn wound: Evaluation of different modalites. *Burns*, 26(3), pp. 265–270.

70. Jáuregui, K. M. G. et al., 2009. A new formulated stable papin-pectin aerosol spray for skin wound healing. *Biotechnology and Bioprocess Engineering*, 14(4), pp. 450–456.

71. Jones, K. R., Fennie, K. and Lenihan, A., 2007. Chronic wounds: Factors influencing healing within 3 months and nonhealing after 5-6 months of care. *Wounds: A Compendium of Clinical Research and Practice*, 19(3), pp. 51–63. Available at: http://europepmc.org/abstract/med/26111416 [Accessed August 5, 2015].

72. Kaya, M. et al., 2014. Characterization of citrus pectin samples extracted under different conditions: Influence of acid type and pH of extraction. *Annals of Botany*, (OCTOBER). Available at: http://aob.oxfordjournals.org/content/early/2014/07/31/aob.mcu150.abstract.

73. Kang, P. L. et al., 2011. Development and assessment of hemostasis chitosan dressings. *Carbohydrate Polymers*, 85(3), pp. 565–570. Available at: http://dx.doi.org/10.1016/j.carbpol.2011.03.015.

74. Kataria, K. et al., 2014. In vivo wound healing performance of drug loaded electrospun composite nanofibers transdermal patch. *International Journal of Pharmaceutics*, 469(1), pp. 102–110. Available at: http://dx.doi.org/10.1016/j.ijpharm.2014.04.047.

75. Kenawy, E. R. et al., 2014. Physically crosslinked poly(vinyl alcohol)-hydroxyethyl starch blend hydrogel membranes: Synthesis and characterization for biomedical applications. *Arabian Journal of Chemistry*, 7(3), pp. 372–380. Available at: http://dx.doi.org/10.1016/j.arabjc.2013.05.026.

76. Khan, T. A., Peh, K. K. and Seng, H., 2000. Mechanical, bioadhesive strength and biological evaluations of chitosan films for wound dressing. *J. Pharm. Pharmaceut. Sci.*, 3(3), pp. 303-311

77. Kim, J. O. et al., 2008. Development of polyvinyl alcohol-sodium alginate gel-matrix-based wound dressing system containing nitrofurazone. *International Journal of Pharmaceutics*, 359(1-2), pp. 79–86.

78. Kokabi, M., Sirousazar, M. and Hassan, Z. M., 2007. PVA-clay nanocomposite hydrogels for wound dressing. *European Polymer Journal*, 43(3), pp. 773–781.

79. Koksal, C. and Bozkurt, A. K., 2003. Combination of hydrocolloid dressing and medical compression stocking versus Unna's boot for the treatment of venous leg ulcers. *Swiss Medical Weekly*, 133(25-26), pp. 364–368.

80. Komarcević, A., 2000. The modern approach to wound treatment. *Medicinski pregled*, 53(7-8), pp. 363–368. Available at: http://europepmc.org/abstract/med/11214479 [Accessed August 5, 2015].

81. Kurabayashi, H. et al., 2002. Inhibiting bacteria and skin pH in hemiplegia: Effects of washing hands with acidic mineral water. *American Journal of Physical Medicine and Rehabilitation / Association of Academic Physiatrists*, 81(1), pp. 40–46. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11807331 [Accessed June 7, 2015].

82. Kwok, A. Y., Qiao, G. G. and Solomon, D. H., 2004. Synthetic hydrogels 3. Solvent effects on poly(2-hydroxyethyl methacrylate) networks. *Polymer*, 45(12), pp. 4017–4027.

83. Laçin, N. T., 2014. Development of biodegradable antibacterial cellulose based hydrogel membranes for wound healing. *International Journal of Biological Macromolecules*, 67, pp. 22–27.

84. Lagana, G. and Anderson, E. H., 2010. Moisture dressings: The new standard in wound care. *Journal for Nurse Practitioners*, 6(5), pp. 366–370. Available at: http://dx.doi.org/10.1016/j.nurpra.2010.01.025.

85. Lamke, L. O., Nilsson, G. E. and Reithner, H. L., 1977. The evaporative water loss from burns and the water-vapour permeability of grafts and artificial membranes used in the treatment of burns. *Burns*, 3(3), pp. 159–165. Available at: http://www.sciencedirect.com/science/article/pii/0305417977900043 [Accessed May 8, 2014].

86. Le, A.T. et al., 2010. Synthesis of oleic acid-stabilized silver nanoparticles and analysis of their antibacterial activity. *Materials Science and Engineering: C*, 30(6), pp. 910–916. Available at: http://www.sciencedirect.com/science/article/pii/S0928493110001050 [Accessed May 15, 2014].

87. Lee, K. Y. and Mooney, D. J., 2012. Alginate: Properties and biomedical applications. *Progress in Polymer Science (Oxford)*, 37(1), pp. 106–126. Available at: http://dx.doi.org/10.1016/j.progpolymsci.2011.06.003.

88. Lee, S. C., Kwon, I. K. and Park, K., 2013. Hydrogels for delivery of bioactive agents: A historical perspective. *Advanced Drug Delivery Reviews*, 65(1), pp. 17–20. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22906864 [Accessed March 15, 2015].

89. Lengheden, A. and Jansson, L., 1995. pH effects on experimental wound healing of human fibroblasts in vitro. *European Journal of Oral Sciences*, 103(3), pp. 148–55. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7634130 [Accessed June 7, 2015].

90. Levi, S. et al., 2011. Limonene encapsulation in alginate/poly (vinyl alcohol). *Procedia Food Science*, 1, pp. 1816–1820. Available at: http://dx.doi.org/10.1016/j.profoo.2011.09.266.

91. Liakos, I. et al., 2013. Controlled antiseptic release by alginate polymer films and beads. *Carbohydrate Polymers*, 92(1), pp. 176–183. Available at: http://dx.doi.org/10.1016/j.carbpol.2012.09.034.

92. Lim, J. K. et al., 2000. Normal saline wound dressing--Is it really normal? *British Journal of Plastic Surgery*, 53(1), pp. 42–45. Available at: http://www.sciencedirect.com/science/article/pii/S0007122699932460 [Accessed August 5, 2015].

93. Lin, C. C. and Anseth, K. S., 2009. PEG hydrogels for the controlled release of biomolecules in regenerative medicine. *Pharmaceutical Research*, 26(3), pp. 631–643.

94. Lin, Y. H. et al., 2014. Evaluation of various silver-containing dressing on infected excision wound healing study. *Journal of Materials Science: Materials in Medicine*, 25(5), pp. 1375-1386.

95. Lynn, A. K., Yannas, I. V. and Bonfield, W., 2004. Antigenicity and immunogenicity of collagen. *Journal of Biomedical Materials Research - Part B Applied Biomaterials*, 71(2), pp. 343–354.

96. Liu, Y. et al., 2010. Thermal behavior and mechanical properties of physically crosslinked PVA/Gelatin hydrogels. *Journal of the Mechanical Behavior of Biomedical Materials*, 3(2), pp. 203–209. Available at: http://dx.doi.org/10.1016/j.jmbbm.2009.07.001.

97. MacKay, D. and Miller, A. L., 2003. Nutritional support for wound healing. *Alternative Medicine Review: A Journal of Clinical Therapeutic*, 8(4), pp. 359–377. Available at: http://europepmc.org/abstract/med/14653765 [Accessed June 8, 2015].

98. Mano, J. F. et al., 2007. Natural origin biodegradable systems in tissue engineering and regenerative medicine: Present status and some moving trends. *Journal of the Royal Society, Interface/The Royal Society*, 4(17), pp. 999–1030.

99. Maruška, A., Liesienė, J. and Šerys, A. *Lithuanian Patent*. 1993, no. 2299.

100. Mcguire, R. G., 1992. Reporting of Objective Color Measurements. *Feature*, 27(12), pp. 1254–1255.

101. McIntosh, C., 2007. Are hydrocolloid dressings suitable for diabetic foot ulcers? *Wound Essentials*, 2, pp. 170-172. Available at: http://eprints.hud.ac.uk/1245/ [Accessed August 5, 2015].

102. Medusheva, E. O. et al., 2007. New medical materials with an integral lasting effect based on fibre-forming polymers. *Fibre Chemistry*, 39(4), pp. 268–271.

103. Metters, A. T., Bowman, C. N. and Anseth, K. S., 2000. A statistical kinetic model for the bulk degradation of PLA- b -PEG- b -PLA hydrogel networks. *The Journal of Physical Chemistry B*, 104(30), pp. 7043–7049. Available at: http://pubs.acs.org/doi/abs/10.1021/jp000523t.

104. Miao, J. et al., 2011. Lysostaphin-functionalized cellulose fibers with antistaphylococcal activity for wound healing applications. *Biomaterials*, 32(36), pp. 9557–9567. Available at: http://dx.doi.org/10.1016/j.biomaterials.2011.08.080.

105. Mishra, A. and Chaudhary, N., 2010. Study of povidone iodine loaded hydrogels as wound dressing material. *Trends in Biomaterials and Artificial Organs*, 23(3), pp. 122–128.

106. Mishra, R. K. et al., 2008. Preparation and characterization of amidated pectin based hydrogels for drug delivery system. *Journal of Materials Science: Materials in Medicine*, 19(6), pp. 2275–2280.

107. Mishra, R. K., Majeed, A. B. A. and Banthia, A. K., 2011. Development and characterization of pectin/gelatin hydrogel membranes for wound dressing. *International Journal of Plastics Technology*, 15(1), pp. 82–95.

108. Mogoşanu, G. D. and Grumezescu, A. M., 2014. Natural and synthetic polymers for wounds and burns dressing. *International Journal of Pharmaceutics*, 463(2), pp. 127–136.

109. Mohamad, N. et al., 2014. Bacterial cellulose/acrylic acid hydrogel synthesized via electron beam irradiation: Accelerated burn wound healing in an animal model. *Carbohydrate Polymers*, 114, pp. 312–320. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0144861714007942.

110. Monaco, J. L. and Lawrence, W. T., 2003. Acute wound healing: An overview. *Clinics in Plastic Surgery*, 30(1), pp. 1–12.

111. Moore, K. et al., 2006. Prediction and monitoring the therapeutic response of chronic dermal wounds. *International Wound Journal*, 3(2), pp. 89–96.

112. Moura, L. I. F. et al., 2013. Recent advances on the development of wound dressings for diabetic foot ulcer treatment - A review. *Acta Biomaterialia*, 9(7), pp. 7093–7114. Available at: http://dx.doi.org/10.1016/j.actbio.2013.03.033.

113. Munarin, F., Tanzi, M. C. and Petrini, P., 2012. Advances in biomedical applications of pectin gels. *International Journal of Biological Macromolecules*, 51(4), pp. 681–689. Available at: http://dx.doi.org/10.1016/j.ijbiomac.2012.07.002.

114. Murakami, K. et al., 2010. Hydrogel blends of chitin/chitosan, fucoidan and alginate as healing-impaired wound dressings. *Biomaterials*, 31(1), pp. 83–90. Available at: http://dx.doi.org/10.1016/j.biomaterials.2009.09.031.

115. Nitanan, T. et al., 2013. Neomycin-loaded poly(styrene sulfonic acid-co-maleic acid) (PSSA-MA)/polyvinyl alcohol (PVA) ion exchange nanofibers for wound dressing materials. *International Journal of Pharmaceutics*, 448(1), pp. 71–78. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23510799 [Accessed March 27, 2015].

116. Ousey, K. et al., 2012. Hydrocolloids in practice. *Wounds UK*, 8(1), pp. 1-6.

117. Ovington, L. G., 2007. Advances in wound dressings. *Clinics in Dermatology*, 25(1), pp. 33–38.

118. Pal, K., Banthia, A. K. and Majumdar, D. K., 2007. Preparation and characterization of polyvinyl alcohol-gelatin hydrogel membranes for biomedical applications. *AAPS PharmSciTech*, 8(1), p. 21.

119. Paladini, F. et al., 2013. Silver-doped self-assembling di-phenylalanine hydrogels as wound dressing biomaterials. *Journal of Materials Science: Materials in Medicine*, 24(10), pp. 2461–2472.

120. Passaglia, E. et al., 2008. Nanostructured polyolefins/clay composites: Role of the molecular interaction at the interface. *Polymers for Advanced Technologies*, 19(April), pp. 560–568.

121. Paul, W. and Sharma, C., 2004. Chitosan and alginate wound dressings: A short review. *Trends Biomater Artif Organs*, 18(1), pp. 18–23. Available at: http://medind.nic.in/taa/t04/i1/taat04i1p18.pdf.

122. Peppas, N. A. et al., 2006. Hydrogels in biology and medicine: From molecular principles to bionanotechnology. *Advanced Materials*, 18(11), pp. 1345–1360.

123. Percival, N. J., 2002. Classification of wounds and their management. *Surgery (Oxford)*, 20(5), pp. 114–117.

124. Pereira, R. et al., 2013. Development of novel alginate based hydrogel films for wound healing applications. *International Journal of Biological Macromolecules*, 52(1), pp. 221–230. Available at: http://dx.doi.org/10.1016/j.ijbiomac.2012.09.031.

125. Petrey, A. C. and de la Motte, C. A., 2014. Hyaluronan, a crucial regulator of inflammation. *Frontiers in Immunology*, 5(MAR), pp. 1–13.

126. Pourjafar, S., Rahimpour, A. and Jahanshahi, M., 2012. Synthesis and characterization of PVA/PES thin film composite nanofiltration membrane modified with TiO 2 nanoparticles for better performance and surface properties. *Journal of Industrial and Engineering Chemistry*, 18(4), pp. 1398–1405. Available at: http://dx.doi.org/10.1016/j.jiec.2012.01.041.

127. Preedy, V., 2012. *Handbook of Diet, Nutrition and the Skin*, Wageningen: Wageningen Academic Publishers.

128. Purna, S. K. and Babu, M., 2000. Collagen based dressings--A review. *Burns: Journal of the International Society for Burn Injuries*, 26(1), pp. 54–62.

129. Queen, D. et al., 1987. The preclinical evaluation of the water vapour transmission rate through burn wound dressings. *Biomaterials*, 8(5), pp. 367–371. Available at: http://www.sciencedirect.com/science/article/pii/014296128790007X [Accessed May 8, 2014].

130. Rastogi, S., Pandey, M. M. and Kumar Singh Rawat, A., 2015. Medicinal plants of the genus Betula—Traditional uses and a phytochemical–Pharmacological review. *Journal of Ethnopharmacology*, 159, pp. 62–83. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0378874114007934.

131. Van Rijswijk, L., 2006. Ingredient-based wound dressing classification: A paradigm that is passé and in need of replacement. *Journal of Wound Care*, 15(1), pp. 11–14. Available at: http://www.magonlinelibrary.com/doi/abs/10.12968/jowc.2006.15.1.26859 [Accessed June 8, 2015].

132. Roberts, G. et al., 2002. Some effects of sustained compression on ulcerated tissues. *Angiology*, 53(4), pp. 451–456.

133. Rucigaj, T. P., 2014. How to choose the proper dressing in venous leg ulcers care. *Reviews in Vascular Medicine*, 2(2), pp. 58–61. Available at: http://dx.doi.org/10.1016/j.rvm.2013.12.001.

134. Ruiz-Cardona, L. et al., 1996. Application of benzyl hyaluronate membranes as potential wound dressings: Evaluation of water vapour and gas permeabilities. *Biomaterials*, 17(16), pp. 1639–1643. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8842370 [Accessed July 12, 2014].

135. Saibuatong, O. A. and Phisalaphong, M., 2010. Novo aloe vera-bacterial cellulose composite film from biosynthesis. *Carbohydrate Polymers*, 79(2), pp. 455–460. Available at: http://dx.doi.org/10.1016/j.carbpol.2009.08.039.

136. Sayegh, N. et al., 1988. Wound pH as a predictor of skin graft survival. *Current Surgery*, 45(1), pp. 23–24. Available at: http://www.researchgate.net/publication/19877173_Wound_pH_as_a_predictor_of_skin_graft_survival [Accessed June 7, 2015].

137. Sandri, G. et al., 2013. Wound dressings based on silver sulfadiazine solid lipid nanoparticles for tissue repairing. *European Journal of Pharmaceutics and Biopharmaceutics*, 84(1), pp. 84–90. Available at: http://dx.doi.org/10.1016/j.ejpb.2012.11.022.

138. Sannino, A., Demitri, C. and Madaghiele, M., 2009. Biodegradable cellulose-based hydrogels: Design and applications. *Materials*, 2(2), pp. 353–373.

139. Sarheed, O. et al., 2014. An investigation and characterization on alginate hydrogel dressing loaded with metronidazole prepared by combined inotropic gelation and freeze-thawing cycles for controlled release. *AAPS PharmSciTech*, 16(3), pp. 601-609 Available at: http://link.springer.com/10.1208/s12249-014-0237-1.

140. Saul, J. M. and Williams, D. F., 2011. Hydrogels in regenerative medicine. *Principles of Regenerative Medicine.* 2nd ed. San Diego (CA): Academic Press, pp. 637-661.

141. Schneider, L. A. et al., 2007. Influence of pH on wound-healing: A new perspective for wound-therapy? *Archives of Dermatological Research*, 298(9), pp. 413–420.

142. Schrecker, S. T. and Gostomski, P. A., 2005. Determining the water holding capacity of microbial cellulose. *Biotechnology Letters*, 27(19), pp. 1435–1438.

143. Sehgal, S. C. and Arunkumar, B. K., 1992. Microbial flora and its significance in pathology of sickle cell disease leg ulcers. *Infection*, 20(2), pp. 86–88.

144. Siegel, R. A. and Federation, P. P., 2004. *Fundamentals and Applications of Polymer Gels: PPF-7 Microsymposium, Oaxaca City (Mexico), 2001*, WILEY-VCH Verlag. Available at: https://books.google.co.in/books/about/Fundamentals_and_applications_of_polymer.html?id=PmIvAQAAIAAJ&pgis=1 [Accessed August 5, 2015].

145. Silva, F. E. F. et al., 2013. PVA/polysaccharides blended films: Mechanical properties. *Journal of Materials*, 2013(1), pp. 1-6 Available at: http://www.hindawi.com/journals/jma/2013/413578.

146. Singh, B. and Pal, L., 2008. Development of sterculia gum based wound dressings for use in drug delivery. *European Polymer Journal*, 44(10), pp. 3222–3230. Available at: http://www.sciencedirect.com/science/article/pii/S0014305708003418 [Accessed July 13, 2014].

147. Singleton, V. L., Orthofer, R. and Lamuela-Raventós, R. M., 1999. *Oxidants and Antioxidants Part A*, Elsevier. Available at:

http://www.researchgate.net/publication/231213516_Analysis_of_total_phenols_and_other_oxidation_substrates_and_antioxidants_by_means_of_Folin-Ciocalteu_reagent [Accessed December 18, 2014].

148. Stashak, T. S., Farstvedt, E. and Othic, A., 2004. Update on wound dressings: Indications and best use. *Clinical Techniques in Equine Practice*, 3(2 SPEC. ISS.), pp. 148–163.

149. Stewart, C. M. et al., 2002. Staphylococcus aureus growth boundaries: Moving towards mechanistic predictive models based on solute-specific effects staphylococcus aureus growth boundaries: Moving towards mechanistic predictive models based on solute-specific effects. *Applied and Environmental Microbiology*, 68(4), pp. 1864–1871.

150. Stokes, D. L., 2007. Desmosomes from a structural perspective. *Current Opinion in Cell Biology*, 19(5), pp. 565–571.

151. Straccia, M. C. et al., 2014. Crosslinker effects on functional properties of alginate/N-succinylchitosan based hydrogels. *Carbohydrate Polymers*, 108, pp. 321–330. Available at: http://dx.doi.org/10.1016/j.carbpol.2014.02.054.

152. Sun, L. et al., 2015. Crystalline characteristics of cellulose fiber and film regenerated from ionic liquid solution. *Carbohydrate Polymers*, 118, pp. 150–155. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0144861714011060.

153. Sung, H. W. et al., 1999. Crosslinking characteristics and mechanical properties of a bovine pericardium fixed with a naturally occurring crosslinking agent. *Journal of Biomedical Materials Research*, 47(2), pp. 116–26. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10449623 [Accessed May 12, 2014].

154. Svensjö, T. et al., 2000. Accelerated healing of full-thickness skin wounds in a wet environment. *Plastic and Reconstructive Surgery*, 106(3), pp. 602–12; discussion 613–614. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10987467 [Accessed May 13, 2015].

155. Tabata, Y., Hijikata, S. and Ikada, Y., 1994. Enhanced vascularization and tissue granulation by basic fibroblast growth factor impregnated in gelatin hydrogels. *Journal of Controlled Release*, 31(2), pp. 189–199. Available at: http://www.sciencedirect.com/science/article/pii/0168365994000352 [Accessed June 17, 2015].

156. Thomas, S., 2000. Alginate dressings in surgery and wound management-Part 1. *Journal of Wound Care*, 9(2), pp. 56–60. Available at: http://www.magonlinelibrary.com/doi/abs/10.12968/jowc.2000.9.2.26338 [Accessed June 29, 2015].

157. Vasconcelos, A. and Cavaco-Paulo, A., 2011. Wound dressings for a proteolytic-rich environment. *Applied Microbiology and Biotechnology*, 90(2), pp. 445–60. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21360151 [Accessed May 11, 2014].

158. Vermeulen, H., Westerbos, S. J. and Ubbink, D. T., 2010. Benefit and harm of iodine in wound care: A systematic review. *Journal of Hospital Infection*, 76(3), pp. 191–199. Available at: http://dx.doi.org/10.1016/j.jhin.2010.04.026.

159. Videcoq, P. et al., 2011. Influence of calcium on pectin methylesterase behaviour in the presence of medium methylated pectins. *Carbohydrate Polymers*, 86(4), pp. 1657–1664. Available at: http://dx.doi.org/10.1016/j.carbpol.2011.06.081.

160. Visscher, M. and Narendran, V., 2014. Neonatal infant skin: Development, structure and function. *Newborn and Infant Nursing Reviews*, 14(4), pp. 135–141. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1527336914000907.

161. Vloemans, A. F. P. M. et al., 2002. The use of glycerol-preserved allografts in the Beverwijk Burn Centre: A retrospective study. *Burns: Journal of the International Society for Burn Injuries*, 28 Suppl 1, pp. S2–S9.

162. Vowden, K. and Vowden, P., 2014. Wound dressings: Principles and practice. *Surgery (Oxford)*, 32(9), pp. 462–467. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0263931914001410.

163. Wang, L. et al., 2002. Chitosan-alginate PEC membrane as a wound dressing: Assessment of incisional wound healing. *Journal of Biomedical Materials Research*, 63(5), pp. 610–618. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12209908 [Accessed June 7, 2015].

164. Wang, W., 2006. A novel hydrogel crosslinked hyaluronan with glycol chitosan. *Journal of Materials Science: Materials in Medicine*, 17(12), pp.1259–1265.

165. Waring, M. J. and Parsons, D., 2001. Physico-chemical characterisation of carboxymethylated spun cellulose fibres. *Biomaterials*, 22(9), pp. 903–912.

166. Warner, R. R., Stone, K. J. and Boissy, Y. L., 2003. Hydration disrupts human stratum corneum ultrastructure. *Journal of Investigative Dermatology*, 120(2), pp. 275–284.

167. Wild, T. et al., 2010. Basics in nutrition and wound healing. *Nutrition*, 26(9), pp. 862–866.

168. Winter, G. D., 1965. A note on wound healing under dressings with special reference to perforated-film dressings. *The Journal of Investigative Dermatology*, 45(4), pp. 299–302.

169. Wong, R. H. L. et al., 2011. Topical application of povidone-iodine before wound closure is associated with significant increase in serum iodine level. *Surgical Practice*, 15(3), pp. 79–82. Available at: http://doi.wiley.com/10.1111/j.1744-1633.2011.00547.x.

170. Wong, T. W. and Ramli, N. A., 2014. Carboxymethylcellulose film for bacterial wound infection control and healing. *Carbohydrate Polymers*, 112, pp. 367–375. Available at: http://dx.doi.org/10.1016/j.carbpol.2014.06.002.

171. Wu, T. et al., 2014. Mechanical behavior of transparent nanofibrillar cellulose-chitosan nanocomposite films in dry and wet conditions. *Journal of the Mechanical Behavior of Biomedical Materials*, 32, pp. 279–286. Available at: http://dx.doi.org/10.1016/j.jmbbm.2014.01.014.

172. Xiao-Dong, G. et al., 2002. Molecular tissue engineering: Concepts, status and challenge. *Journal of Wuhan University of Technology-Mater. Sci. Ed.*, 17(3), pp. 30–34.

173. Zahedi, P. et al., 2010. A review on wound dressings with an emphasis on electrospun nanofibrous polymeric bandages. *Polymers for Advanced Technologies*, 21(2), pp. 77–95.

174. Zhu, J., 2010. Bioactive modification of poly(ethylene glycol) hydrogels for tissue engineering. *Biomaterials*, 31(17), pp. 4639–4656. Available at: http://dx.doi.org/10.1016/j.biomaterials.2010.02.044.

LIST OF SCIENTIFIC PUBLICATIONS ON THE TOP OF THE DISSERTATION

Articles in the journals included in the list of Thomson Reuters Web of Knowledge

1. Sandra Kiseliovienė; Odeta Baniukaitienė; Volodymyr Harkavenko; Nataliya Babenko; Jolanta Liesienė. Cellulose hydrogels sheets for wound dressing // Cellulose Chemistry and Technology. 2015. [Thomson Reuters Web of Knowledge] [Impact factor: 0.833] (accepted).
2. Sandra Gustaite; Jurgita Kazlauskė, Jamshed Bobokalonov; Stefano Perni; Victoria Dutschk; Jolanta Liesienė, Polina Prokopovich. Characterization of cellulose based sponges for wound dressing // Colloids and Surfaces A: Physicochemical and Engineering Aspects. ISSN:0927-7757. 2015, pp. 336-342. [Thomson Reuters Web of Knowledge] [Impact factor: 2.752].

Papers of scientific conferences

1. Gustaitė, Sandra; Grybauskaitė, Gintarė; Liesienė, Jolanta; Prokopovich, Polina. Nanosilver loaded cellulose-based gel for antibacterial wound treatment // Chemistry and Chemical Technology: Proceedings of the International Conference, 25 April, 2014, Kaunas University of Technology. Kaunas. ISSN 2351-5643. 2014, pp. 260-263.
2. Gustaitė, Sandra; Kazlauskė, Jurgita; Liesienė, Jolanta; Nasiri Boroumand, M.; Dutschk, V. Cellulose-based hydrogels for wound dressing // Baltic Polymer Symposium 2013: Trakai, Lithuania, September 18-21, 2013: Programme and Abstracts. Vilnius University, Kaunas University of Technology. Vilnius. ISBN 9786094592270. p. 133.

LIST OF OTHER SCIENTIFIC PUBLICATIONS

Articles in the journals included in the list of Thomson Reuters Web of Knowledge

1. Jolita Baranauskienė; Jurgita Kazlauskė; Sandra Gustaitė; Bernd Niemeyer; Jolanta Liesienė. Comparative study of macroporous silica-and cellulose-based sorbents for lectin affinity chromatography // Journal of Liquid Chromatography and Related Technologies. ISSN 1082-6076. 2014, pp. 1847-1861. [Thomson Reuters Web of Knowledge] [Impact factor: 0.606].

Articles in the other reviewed Thomson Reuters Web of Knowledge Database [Proceedings and others]

1. Gustaitė, Sandra; Survilaitė, Indrė; Liesienė, Jolanta. Comparison of adsorbents based on silica coated with different PVA derivatives // Polymer Chemistry and Technology: Proceedings of Scientific Conference Chemistry and Chemical Technology, Kaunas University of Technology, 25 April, 2012, Kaunas University of Technology. Kaunas: Technologija. ISSN 2029-2457. 2012, pp. 88-92.

Papers of scientific conferences

1. Gustaitė, Sandra; Survilaitė, Indrė; Liesienė, Jolanta. Coating of macroporous silica with poly(vinyl alcohol) derivatives // EUPOC 2012: Popous Polymer-Based Systems: From Design to Application, Europolymer Conference, 3-7 June, 2012, Gargnano, Italy: Booklet of Abstracts. Gargnano: European Polymer Federation, 2012. p. 83.
2. Kazlauskė, Jurgita; Gustaitė, Sandra; Liesienė, Jolanta. Comparison of different adsorbents for binding of bile acids // Baltic Polymer Symposium 2012: Liepaja, Latvia, September 19-22: Program and Abstracts. Riga: Riga Technical University, 2012. p. 25.

UDK 544.77.03 + 615.46](043.3).
SL344. 2016-01-21, 14,25 leidyb. apsk. l. Tiražas 10 egz. Užsakymas 15.
Išleido Kauno technologijos universitetas, K. Donelaičio g. 73, 44249 Kaunas
Spausdino leidyklos „Technologija“ spaustuvė, Studentų g. 54, 51424 Kaunas