Characterization of cellulose based sponges for wound dressings

by

Sandra Gustaite[a], J. Kazlauske[a], J. Bobokalonov[a], Stefano Perni[b], Victoria Dutschk[c], J. Liesiene[a], Polina Prokopovich[b,d*]

[a]*Kaunas University of Technology, Radvilenu pl. 19, LT-50254 Kaunas, Lithuania*

[b]*School of Pharmacy and Pharmaceutical Sciences, Cardiff University, King Edward VII Avenue, Cardiff, CF10 3NB, United Kingdom*

[c]*Twente University, Drienerlolaan 5, 7522 NB Enschede, Netherlands*

[d]*School of Engineering, Cardiff University, Cardiff, United Kingdom*

*Corresponding author..

Dr. Polina Prokopovich

School of Pharmacy and Pharmaceutical Sciences

Cardiff University

Redwood Building, King Edward VII Avenue

Cardiff, UK

CF10 3NB

E-mail address: ProkopovichP@cardiff.ac.uk

Tel: +44 (0)29208 75820

1. Introduction

Historically, the dressing of wounds was carried out through the use of cloth; recently, a new generation of wound dressings has been extensively developed. Functional dressings in different forms such as sponges, films and hydrocolloids have been developed. Modern dressing aims not only to prevent the wound infection, but also 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 (i) maintain the moisture in a wound, (ii) permit diffusion of gases, (iii) remove excess exudates but prevent saturation of the dressing on its outer surface. Other properties like wound protection from microorganisms, mechanical protection, control of local pH and temperature, stimulation of growth factors, easy and no painful removal from the wound, cost-effectiveness, lack of side effects and elasticity are also required [1,2] The dressings can also be used as drug delivery systems [3].

The wound dressing market has grown significantly over last 10 years, however fewer dressings have all the required properties for the purpose of wound protection and healing [1]. New and more effective materials for the production of appropriate dressings for chronic or burn wounds are still being searched with effort. Natural polymers are of special interest due to high absorption and swelling capacities, non-cytotoxicity [4–6]; moreover, various active compounds can be immobilized in these polymers. Natural polymers are also appropriate matrix for enzyme immobilization and functionalization.

Cellulose, being 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 gauzes are the simple, inexpensive and quite effective wound dressing. However, gauzes stick to the wound and could strip off newly formed epidermis when removed [7]. They also did not possess active healing properties. In order to give to cotton some healing activity Edwards *et al*. [8–10] have synthesized modified cotton-based wound dressing materials, which can selectively absorb neutrophil elastase or selectively lower the activity of cationic serine protease.

Another undesired risk posed by a healing wound is the offset of infections, the consequences of such events range from mild pain and discomfort to death. The outcome of infections depend on the nature of the microorganism and the host immunological system response. Antibiotics are generally used to prevent and treat infections; nonetheless their activity is gradually diminishing due to the resistance rise to these drugs among bacteria species. Novel antimicrobial agents are, therefore, urgently needed; among the alternatives to antibiotics, silver nanoparticles have shown great potential.

Cellulose based dressings were prepared in different forms, such as, mentioned above gauzes, lyophilized wafers [11], electrospuned cellulose acetate/gelatin membranes [12], hydrogels of cellulose nanowhiskers with polyvinyl alcohol [13] bacterial cellulose films [14,15] hydroxyethylcellulose nanofibers prepared by electrospinning [16], membranes from microcrystalline cellulose [17]. However, the porosity and absorption properties of cellulose materialss in such forms are not sufficient. In our work, dressings were prepared from cellulose based porous sponges which possess high sorption capacity. To promote wound healing process phenolic compounds from *Calendula officinalis* or C*hamomilla recutita* extracts were immobilized into the sponges and their release was studied. Additionally, to prevent bacterial contamination of wounds, silver nanoparticles were incorporated in the dressing and the antimicrobial activity tested against *Staphylococcus epidermidis*.

2. Materials and methods

2.1. Materials

Cellulose diacetate (DAC, 55% bond acetic acid) was obtained from Roshal (Russia). Hydrochloric acid (HCl) and formic acid (HCOOH) were obtained from Eurochemicals (ES). Ethyl acetate ($CH_3COOCH_2CH_3$) and sodium chloride (NaCl) were procured from Chempur (Poland). Potassium chloride (KCl), sodium hydrogen carbonate ($NaHCO_3$), sodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate dehydrate ($Na_2HPO_4 \cdot H_2O$), potassium dihydrogen phosphate (KH_2PO_4) and sodium carbonate (Na_2CO_3) were obtained from Merck (Germany). All other reagents were purchased from the Sigma-Aldrich company (Germany).

Calendula officinalis and *Chamomilla recutita* blossoms for polyphenols extraction were purchased from JSC "Acorus Calamus" (Lithuania).

Silver nanoparticles preparation

The Ag NPs synthesis was performed following a modified Tollens method proposed by [18]. In brief, Ag_2O was precipitated from a silver nitrate (0.1 M) 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 (UV lamp (λ=365 nm, 35 W) and continued for 8 h. The nanoparticles were characterised through transmission electron microscopy (TEM), a 4 µl droplet of nanoparticles suspension was placed on a plain carbon-coated copper TEM grid and allowed to evaporate in air under ambient laboratory conditions. Bright field TEM images were obtained using a JEOL-1010 microscope at 80 kV equipped with a Gatan digital camera. Typical magnification of the images was x 100 000. Images were analysed with the computer programme ImageJ and the diameters of at least 100 particles were determined.

Preparation of cellulose sponges

Cellulose based sponges were prepared by lyophilization of regeneration of cellulose obtained according to the method described in [19]. For this purpose, 25 g of cellulose diacetate was dissolved in 265 mL of an acetone-ammonia water mixture (1:0.5 v/v) and kept at room temperature until a solid gel was formed. The gel was rinsed with water until neutral pH was reached. Before lyophilization cellulose gel was cut mechanically to obtain rectangular samples of a length of 40 mm, a width of 20 mm and a thickness of about 5 mm. The samples were lyophilized using the Christ ALPHA 2-4 LSC freeze dryer, at -110ºC for a period of 12 hrs.

Preparation of pseudo extracellular fluid (PECF)

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 [20]. The pH of PECF was adjusted to 8 ± 0.2.

Preparation of buffer solutions PBS

Buffer solution of pH 7.4 was prepared by taking 0.137 g of sodium chloride (NaCl), 0.0025 g of potasium 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 reagents were dissolved in 800 mL of distilled water, pH was adjusted to 7.4 using 0.1 M HCl. Solutions were then diluted with distilled water to 1000 mL. The pH of PBS was measured with 744 pH Meter (Metrohom, USA).

Extraction of phenolic compounds

Phenolic compounds from dried *Calendula officinalis* (5 g) and *Chamomilla recutita* (5 g) blossoms were separately extracted by maceration with acetone:water:formic acid (80/20/0.2

v/v/v, 200.04 mL) mixture using shaker device at room temperature for 24 hrs. The solids of each medical plant were filtered off and acetone was completely evaporated under reduced pressure. The remaining solution was defatted with n-hexane (100 mL). In order to remove the oligomeric fraction, the remaining solution had been extracted with ethyl acetate (150 mL). Finally, phenolic compounds were derived by evaporating ethyl acetate and dissolving the residual in deionized water (50 mL).

2.2. **Morphological and structural characterization of the sponges**

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

The micro-computed tomography (micro-CT) analysis was performed 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 – air, the energy – 45 kVp, the integration time – 600 ms, frame averaging – 2x, the nominal resolution – 10 μm. The data was filtered using a constrained 3D Gaussian filter to partially suppress the noise in the images (σ=0.8, support=1).

2.3. **Absorption of PECF**

This experiment imitates an open exuding wound dressed with cellulose sponges. The initial weight of rectangular samples of the sponges (15×15×5 mm) was determined (m_0). Samples were incubated in PECF (pH 8.0) at 37ºC for 24 hrs. The swollen weights of the samples were determined by draining the sample surface with filter paper and weighing the sample (m_t). The weights of swollen samples were recorded after 1hr, 2hrs, 3hrs, 5 hrs and 24 hrs.

PECF absorption (I_A, %) was calculated from Eq (1):

$$I_A = \frac{m_t - m_0}{m_0} \times 100,\ \% \quad (1)$$

Triplicate experiments were carried out.

2.4. Water vapor transmission rate (WVTR)

Water vapor transmission rate (WVTR) was determined according to the ASTM method E96-90, procedure D [21]. Water vapor transmission rate was determined by fixing the cellulose sponge's disc of 30 mm diameter to the cap of 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 saturated magnesium chloride solution was used. A desiccator was kept in a drying oven at 35ºC. The relative humidity in the desiccator after equilibration was approximately 40%. Then the cups were placed in desiccator and re-weighed at determined intervals (1, 3, 5, 7, 9, 12, 24 hrs). The weight loss corresponds to evaporated water content. The experiments were triplicate and the average values were calculated. Water vapour transmission rate (WVTR) was calculated using the Eq (2):

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

where m_l is mass loss during the time t (measured in hours), S is the area of cup mouth.

2.5. Mechanical properties

Tensile strength of the samples and Young's modulus were evaluated using material testing device Zwick/Roell BDO-FB0.5TH (Zwick GmbH & Co, Ulm, Germany) with a crosshead speed of 2 mm/min. Prior mechanical testing dry cellulose samples were kept in PBS solution (pH 7.4) for 30 minutes at room temperature, then samples were removed and gently wiped with filter paper. Strip-shaped sponges (40 × 10 × 5 mm^3) were used for test.

Triplicate experiments were carried out.

2.6. Determination of total content of phenolic compounds using spectrophotometric method

Total phenolic compounds content was determined using Folin-Ciocalteu reagent as described by Singlaton et al [22]. Briefly, the samples of 0.2 mL were mixed with 1 mL of Folin-Ciocalteu reagent, which was diluted with 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 room temperature. The absorbance of the reaction mixture was measured at 765 nm on a spectrophotometer *Varian Cary 50 UV-VIS* (Varian, Australia). The calibration curve was obtained measuring the absorbance of gallic acid solutions of different concentrations (5, 10, 20, 30, 40 and 50 ppm).

2.7. Immobilization of phenolic compounds in cellulose sponges

In ordinary experiment, phenolic compounds (derived from *Calendula officinalis* or C*hamomilla recutita* were immobilized in cellulose matrix by immersing the sample of sponge (about 1 g) in 10 mL of 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 4ºC temperature. The initial weight of cellulose sponge was recorded as m_1. After 24 hrs the sponge was removed from active substances solutions and dried with filter paper, in order to remove the extra free water. The soaked samples was weighed directly, the weight was recorded as m_2. The amount of absorbed phenolic compounds in sample X_{Abs} (μg) was determined comparing the difference between m_1 and m_2 by Eq (3):

$$X_{Abs} = (m_2 - m_1) \times C \quad (3)$$

Triplicate experiments were carried out.

2.8. Release kinetics of phenolic compounds from cellulose sponges

To study phenolics release kinetic PBS buffer, pH 7.4, as a medium was chosen. One piece of cellulose sponge 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 the 0.2 μL of liquid was removed from the incubator and released total phenolics content was determined using the spectrophotometric method. Triplicate experiments were carried out.

2.9. Incorporation and quantification of silver nanoparticles into cellulose matrix

For silver incorporation into the cellulose matrix, 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 into 2 mL of the silver nanoparticle solution for 1, 2, and 4 days for diffusion experiments. Afterward 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 means of the atomic absorption spectroscopy using a Varian atomic absorption spectroscope AAS SpectraAA 20 Plus. The samples were introduced into the air-acetylene flame and silver recorded at the wavelength of 328.1 nm. The calibration curve was created using a solution of $AgNO_3$ in 10 % HNO_3 (v/v).

2.10. Silver release from cellulose sponge

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

2.11. Antibacterial activity

Antibacterial activity of the cellulose sponges loaded with silver nanoparticles were studied using *Staphylococcus epidermidis RP62a*. Before testing, bacteria were grown

statically overnight in 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 BHI broth (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 BHI broth diluted in sterile PBS (1:10) was transferred onto the sponges and incubated at 37°C for 24 hrs. Afterwards bacteria cells were counted through the Miles and Misra method on 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.

3. Results and discussion

3.1. Characterization of silver nanoparticles and cellulose gel

The nanopartivles prepared rounded (Fig. 1) and they had diameters normally distributed with average of 5.4 nm and standard deviation of 1.2 nm; the suspension appered pale yellow. 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} NP/L, because of the dilution employed (1:99) the working solution of silver nanoparticles had a concentration ~10^{17} NP/L.

Cellulose based sponges were prepared by lyophilization of regenerated cellulose gel. According to micro-computed tomography analysis, 75% of porosity was reached. Fig. 2 and Fig. 3. show two-dimentional (2D) and three-dimentional (3D) images of the cellulose sponge, respectively. Micro-CT images revealed interconnected porous structures. Mean pore diameter was 750 μm (Table 1). The majority of 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. 4.). Only 1% of pores were greater than 1200 µm. Table 1 gives a summary of the structural parameters of the sponge. Specific surface area 14.5 mm^2/mm^3 mm^{-1} thus be promising to be a good exudate adsorbent. After mechanical properties evaluation tensile strength of the samples was found to be approx. 140 kPa and Young's modulus approx. 2.7 N/mm^2. According to the literature [23] mechanical properties meets the requirements for wound bandages.

3.2. PECF 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 healing process or causing complications. A good fluid absorption capacity of wound dressing helps to keep wound environment dry and promotes healing processes.

In this research, regenerated cellulose sponges after 24 hrs absorbed 210% (±15%) (mass percentage) of PECF buffer (Fig. 5.). Equilibrium was reached after 3 hrs, but even after 10 min cellulose sponges were able to absorb up to 132% (±14%) of PECF buffer. Fast absorption of exudates is an important factor in order to use this material for festering wound healing, because too much exudates slow down cell proliferation. There is a big variety of sponges with varying liquid absorption characteristics available in the literature . N. Chiaoprakobkij et al. [24] presented bacterial cellulose/alginate composite sponges with human kerotinocytes and gingival fibroblast for wound healing, where depending on modification water uptake differs from 1170% to 1390%. P. Ang-antikarnkul et al. [25] prepared cellulose nanofiber/chitin whisker/silk sericin bionanocomposite sponges, where water uptake was reached up to 3000%. In spite of different liquid absorption capacity, each adsorbent can find application depending on the type of wound and the amount of secretions. It is also important to prevent the wound from drying out too much.

3.3. Water vapour transmission rate (WVTR)

An important factor for the wound healing process is an appropriate exchange of 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. Evaporative water loss for normal skin is 204 ± 12 g/m^2/day, for injured skin 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 [26]. To ensure an adequate level of moisture, avoiding the risk of wound dehydration and build up of exudates on the wound, water vapour transmission rate from injured wound should be in the range 2000-2500 g/m^2/day [27]. The prepared cellulose based sponges showed WVTR of value 2656 ± 30 g/m^2/day, 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 (Fig. 6.).

3.4. Immobilization of phenolic compounds and their release

The polyphenols have a broad range of biological effects for animal cells: antitumor, antitrombogenic, antioxidative and antiinflammatory effects, free radical neutralization [28]. The bandages for wound healing saturated with polyphenols from plants, could help avoid inflammation, induce faster healing and are suitable for people with allergies to synthetic products. For this purpose, phenolic compounds extracted from *Calendula officinalis* or *Chamomilla recutita* were immobilized into the sponges by simple 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 imitating physiological conditions in phosphate buffer solution (PBS), pH 7.4, at 37ºC temperature. In both cases equilibrium was reached after 1 hr (Fig. 7.), however, just 38% of phenols from *Calendula officinalis* extract

was released before the equilibrium reached, while 68% of phenols was released from *Chamomilla recutita.* Such difference between released amounts of phenols may be received due to different structure of phenolic compounds as well as due to different absorbed amount of total phenols. In case of *Calendula officinalis* 2.6 mg/g for dry sponge ± 0.2 mg of total phenols were absorbed, while in 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, cellulose sponges incorporated with phenolic compounds could be active more than 5 hrs. Comparing 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 bigger amount of phenolic compounds than *Calendula officinalis* blossoms.

3.5. Silver incorporation and release

One of the main problems in wound healing is the offset of infections. This undesirable effect may lead to prolonged healing process or cause a number of complications up to death. To prevent bacterial contamination of wounds, silver nanoparticles are often incorporated into dressing material [3,29–31] Silver is effective against a broad range of microorganisms including *Pseudomonas aeruginosa* and *Staphylococcus aureus* which are common in chronic wounds [1]. It is a wide range biocide that kills over 650 disease-causing bacteria, fungi, viruses and moulds [32]. The typical minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles are 0.78–6.25 μg/mL and 12.5 μg/mL, respectively [33].

In this study, silver nanoparticles stabilized in oleic acid were incorporated in cellulose sponges by diffusion method. One day absorption, two days absorption and four days absorption experiments were carried out; the amount of silver embedded in the sponges increased with time, after one day diffusion was 42±6 μg/cm^2, after two days of diffusion

53±8 μg/cm^2 of silver were incorporated and after four days absorption experiment 93±5 μg/cm^2 of silver was found (Fig. 8.).

Silver release kinetics (Fig. 9.) showed prolonged release, equilibrium was not reached even after 24 hrs. As in four days absorption experiment adsorbed silver content was the highest, the cumulative released amount of silver content, was also highest (after 24 hrs – 51 μg of silver).

3.6. Antibacterial activity

Antibacterial activity experiment for *Staphylococcus epidermidis* culture showed excellent results. Even cellulose sponges after one day silver incorporation experiment 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 detection limit (100 CFU/ml) and the corresponding reduction was >99.9999% or 6 $\log_{10}$ for 4 days of diffusion.

4. Conclusions

Cellulose based sponges were developed by freeze-drying of regenerated cellulose gel. Due to hydrophylicity of cellulose and porous structure of the sponges with large surface area, high PECF absorption capacity and water vapour transmission rate were reached. Different active compounds, such as polyphenols from *Calendula officinalis* or C*hamomilla recutita* extracts, silver nanoparticles, were immobilized into the sponges. The sponges incorporated with silver, showed antibacterial resistance for *Staphylococcus epidermidis* culture. The results revealed that prepared cellulose based sponges have a high potential to be used as a wound dressing material.

Acknowledgements

The studies were performed in a frame of COST CM1101 project Colloidal Aspects of Nanoscience for Innovative Processes and Materials. Postdoctoral fellowship is being funded by Europe Union Structural Funds project ”Postdoctoral Fellowship Implementation in Lithuania”.

References

[1] T. Abdelrahman, H. Newton, Wound dressings: principles and practice, Surg. 29 (2011) 491–495. doi:10.1016/j.mpsur.2011.06.007.

[2] L.A. Schneider, A. Korber, S. Grabbe, J. Dissemond, Influence of pH on wound-healing: a new perspective for wound-therapy?, Arch. Dermatol. Res. 298 (2007) 413–20. doi:10.1007/s00403-006-0713-x.

[3] H. V Pawar, J. Tetteh, J.S. Boateng, Preparation, optimisation and characterisation of novel wound healing film dressings loaded with streptomycin and diclofenac., Colloids Surf. B. Biointerfaces. 102 (2013) 102–10. doi:10.1016/j.colsurfb.2012.08.014.

[4] A. Vasconcelos, A. Cavaco-Paulo, Wound dressings for a proteolytic-rich environment., Appl. Microbiol. Biotechnol. 90 (2011) 445–60. doi:10.1007/s00253-011-3135-4.

[5] G.D. Mogoşanu, A.M. Grumezescu, Natural and synthetic polymers for wounds and burns dressing., Int. J. Pharm. 463 (2014) 127–36. doi:10.1016/j.ijpharm.2013.12.015.

[6] A.D.S. and E. Cevher, Biomaterials Applications for Nanomedicine, InTech, 2011. doi:10.5772/1957.

[7] T.S. Stashak, E. Farstvedt, A. Othic, Update on wound dressings: Indications and best use, Clin. Tech. Equine Pract. 3 (2004) 148–163. doi:10.1053/j.ctep.2004.08.006.

[8] J. V. Edwards, P. Howley, N. Prevost, B. Condon, J. Arnold, R. Diegelmann, Positively and negatively charged ionic modifications to cellulose assessed as cotton-based protease-lowering and hemostatic wound agents, Cellulose. 16 (2009) 911–921. doi:10.1007/s10570-009-9302-4.

[9] J.V. Edwards, P. Howley, R. Davis, A. Mashchak, S.C. Goheen, Protease inhibition by oleic acid transfer from chronic wound dressings to albumin., Int. J. Pharm. 340 (2007) 42–51. doi:10.1016/j.ijpharm.2007.03.018.

[10] J. V Edwards, D.R. Yager, I.K. Cohen, R.F. Diegelmann, S. Montante, N. Bertoniere, et al., Modified cotton gauze dressings that selectively absorb neutrophil elastase activity in solution., Wound Repair Regen. 9 (n.d.) 50–8.

[11] S.-F. Ng, N. Jumaat, Carboxymethyl cellulose wafers containing antimicrobials: a modern drug delivery system for wound infections., Eur. J. Pharm. Sci. 51 (2014) 173–9. doi:10.1016/j.ejps.2013.09.015.

[12] E. Vatankhah, M.P. Prabhakaran, G. Jin, L.G. Mobarakeh, S. Ramakrishna, Development of nanofibrous cellulose acetate/gelatin skin substitutes for variety wound treatment applications., J. Biomater. Appl. 28 (2014) 909–21. doi:10.1177/0885328213486527.

[13] J.S. Gonzalez, L.N. Ludueña, A. Ponce, V.A. Alvarez, Poly(vinyl alcohol)/cellulose nanowhiskers nanocomposite hydrogels for potential wound dressings., Mater. Sci. Eng. C. Mater. Biol. Appl. 34 (2014) 54–61. doi:10.1016/j.msec.2013.10.006.

[14] B. Wei, G. Yang, F. Hong, Preparation and evaluation of a kind of bacterial cellulose dry films with antibacterial properties, Carbohydr. Polym. 84 (2011) 533–538. doi:10.1016/j.carbpol.2010.12.017.

[15] L. Fu, P. Zhou, S. Zhang, G. Yang, Evaluation of bacterial nanocellulose-based uniform wound dressing for large area skin transplantation., Mater. Sci. Eng. C. Mater. Biol. Appl. 33 (2013) 2995–3000. doi:10.1016/j.msec.2013.03.026.

[16] R. ROSIC, P. KOCBEK, S. BAUMGARTNER, J. KRISTL, Electro-spun hydroxyethyl cellulose nanofibers: the relationship between structure and process, J. Drug Deliv. Sci. Technol. 21 (n.d.) 229–236.

[17] E.B. Ricci, R. Cassino, C. Di Campli, Microcrystalline cellulose membrane for re-epithelisation of chronic leg wounds: a prospective open study., Int. Wound J. 7 (2010) 438–47. doi:10.1111/j.1742-481X.2010.00707.x.

[18] A.-T. Le, L.T. Tam, P.D. Tam, P.. Huy, T.Q. Huy, N. Van Hieu, et al., Synthesis of oleic acid-stabilized silver nanoparticles and analysis of their antibacterial activity, Mater. Sci. Eng. C. 30 (2010) 910–916. doi:10.1016/j.msec.2010.04.009.

[19] O. Petrauskaite, P.D.S. Gomes, M.H. Fernandes, G. Juodzbalys, A. Stumbras, J. Maminskas, et al., Biomimetic mineralization on a macroporous cellulose-based matrix for bone regeneration, Biomed Res. Int. 2013 (2013).

[20] B. Singh, L. Pal, Development of sterculia gum based wound dressings for use in drug delivery, Eur. Polym. J. 44 (2008) 3222–3230. doi:10.1016/j.eurpolymj.2008.07.013.

[21] L. Ruiz-Cardona, Y.D. Sanzgiri, L.M. Benedetti, V.J. Stella, E.M. Topp, Application of benzyl hyaluronate membranes as potential wound dressings: evaluation of water vapour and gas permeabilities., Biomaterials. 17 (1996) 1639–43.

[22] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós, Oxidants and Antioxidants Part A, Elsevier, 1999. doi:10.1016/S0076-6879(99)99017-1.

[23] H.W. Sung, Y. Chang, C.T. Chiu, C.N. Chen, H.C. Liang, Crosslinking characteristics and mechanical properties of a bovine pericardium fixed with a naturally occurring crosslinking agent., J. Biomed. Mater. Res. 47 (1999) 116–26.

[24] N. Chiaoprakobkij, N. Sanchavanakit, K. Subbalekha, P. Pavasant, M. Phisalaphong, Characterization and biocompatibility of bacterial cellulose/alginate composite sponges with human keratinocytes and gingival fibroblasts, Carbohydr. Polym. 85 (2011) 548–553. doi:10.1016/j.carbpol.2011.03.011.

[25] P. Ang-atikarnkul, A. Watthanaphanit, R. Rujiravanit, Fabrication of cellulose nanofiber/chitin whisker/silk sericin bionanocomposite sponges and characterizations of their physical and biological properties, Compos. Sci. Technol. 96 (2014) 88–96. doi:10.1016/j.compscitech.2014.03.006.

[26] L.-O. Lamke, G.E. Nilsson, H.L. Reithner, The evaporative water loss from burns and the water-vapour permeability of grafts and artificial membranes used in the treatment of burns, Burns. 3 (1977) 159–165. doi:10.1016/0305-4179(77)90004-3.

[27] D. Queen, J.D.. Gaylor, J.. Evans, J.. Courtney, W.. Reid, The preclinical evaluation of the water vapour transmission rate through burn wound dressings, Biomaterials. 8 (1987) 367–371. doi:10.1016/0142-9612(87)90007-X.

[28] R.J. Nijveldt, E. van Nood, D.E. van Hoorn, P.G. Boelens, K. van Norren, P.A. van Leeuwen, Flavonoids: a review of probable mechanisms of action and potential applications., Am. J. Clin. Nutr. 74 (2001) 418–25.

[29] S. Berndt, F. Wesarg, C. Wiegand, D. Kralisch, F.A. Müller, Antimicrobial porous hybrids consisting of bacterial nanocellulose and silver nanoparticles, Cellulose. 20 (2013) 771–783. doi:10.1007/s10570-013-9870-1.

[30] C. Lipp, K. Kirker, A. Agostinho, G. James, P. Stewart, Testing wound dressings using an in vitro wound model., J. Wound Care. 19 (2010) 220–6.

[31] K. Madhumathi, P.T. Sudheesh Kumar, S. Abhilash, V. Sreeja, H. Tamura, K. Manzoor, et al., Development of novel chitin/nanosilver composite scaffolds for wound dressing applications., J. Mater. Sci. Mater. Med. 21 (2010) 807–13. doi:10.1007/s10856-009-3877-z.

[32] Hoon Joo Lee, Sung Hoon Jeong, Bacteriostasis of Nanosized Colloidal Silver on Polyester Nonwovens, Text. Res. J. 74 (2004) 442–447. doi:10.1177/004051750407400511.

[33] J. Jain, S. Arora, J.M. Rajwade, P. Omray, S. Khandelwal, K.M. Paknikar, Silver nanoparticles in therapeutics: development of an antimicrobial gel formulation for topical use., Mol. Pharm. 6 (2009) 1388–401. doi:10.1021/mp900056g.

Table 1. Structural parameters of cellulose sponges obtained by 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}

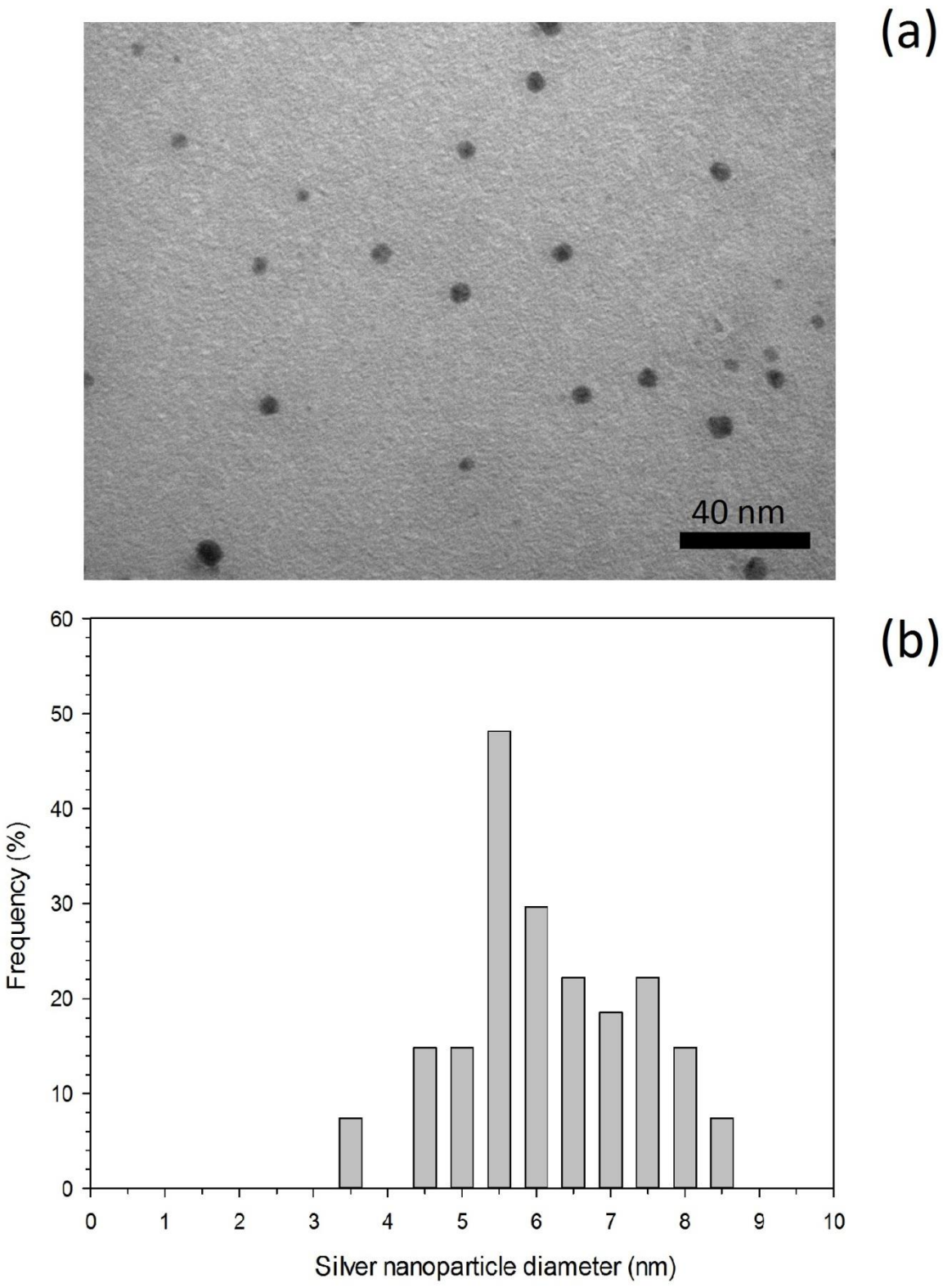

Fig. 1.
Example of TEM image of silver nanoparticles (a) and distribution of the diameters (b).

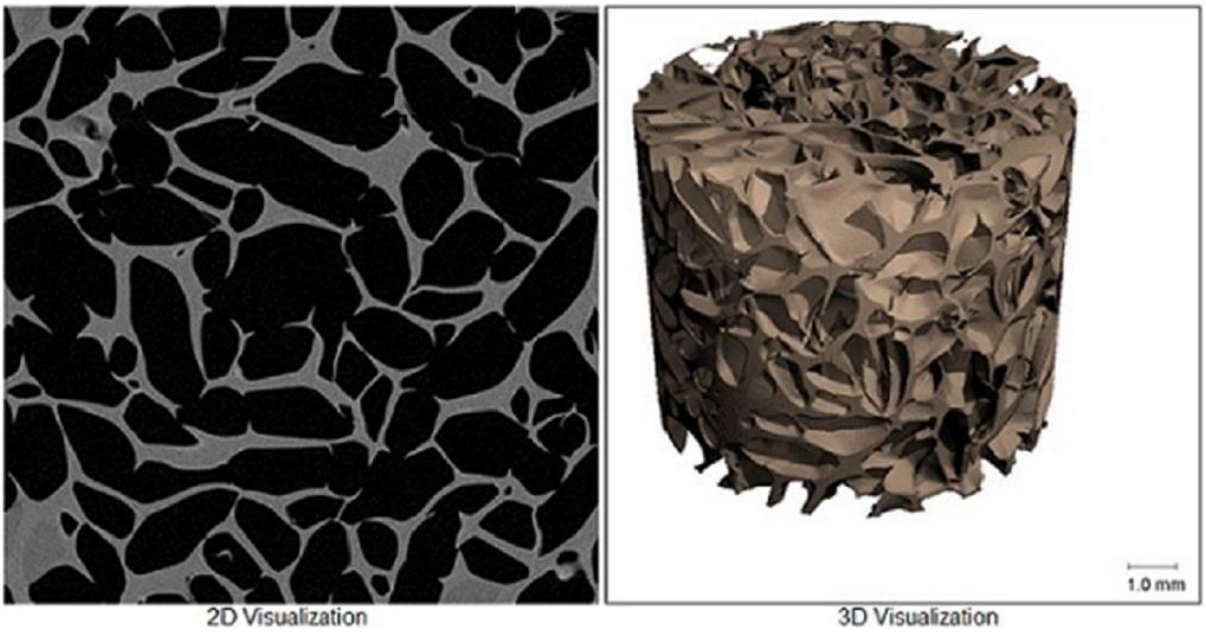

Fig. 2.
Micro-CT image of regenerated cellulose sponge.

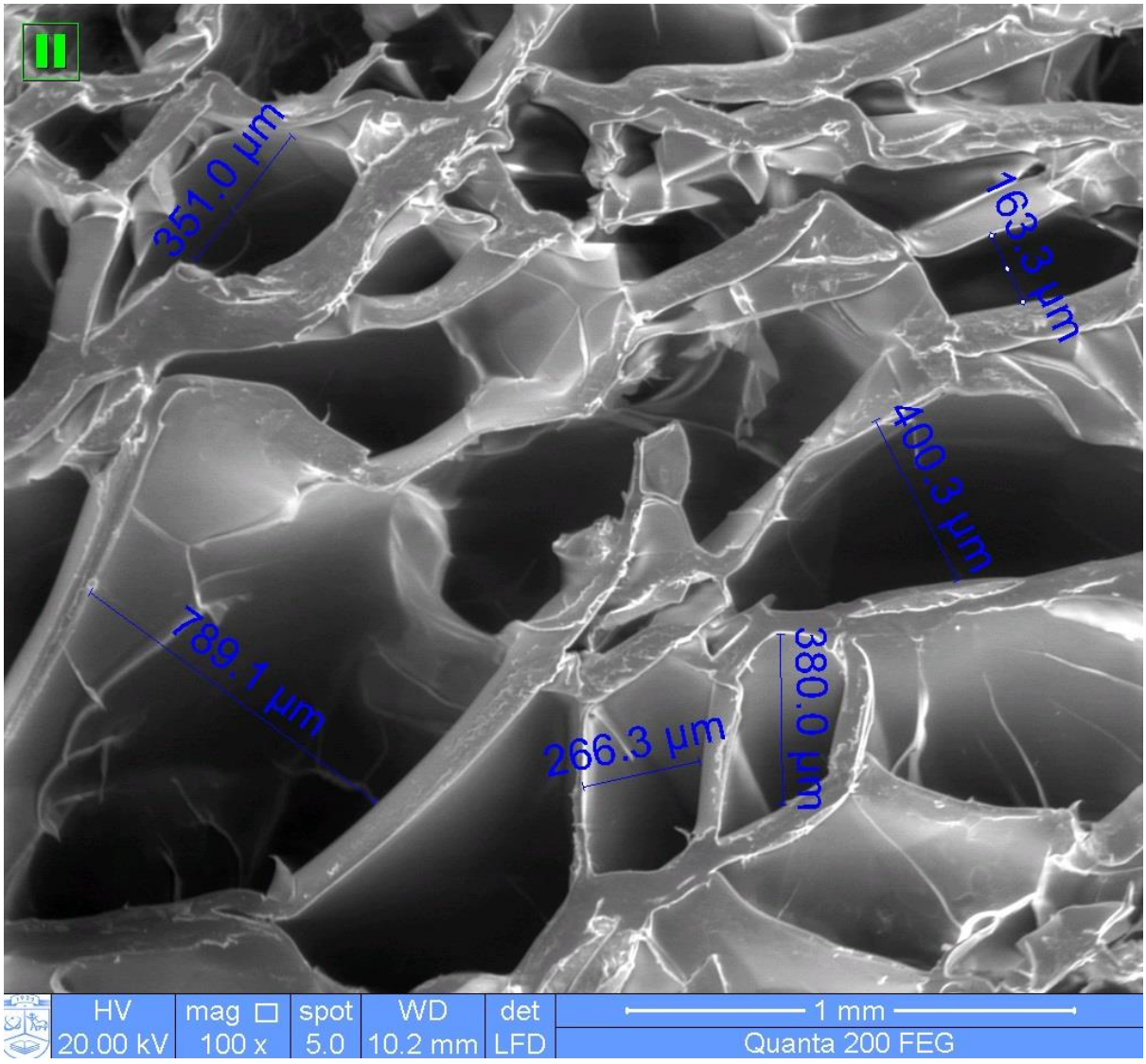

Fig. 3.
SEM image of regenerated cellulose sponge.

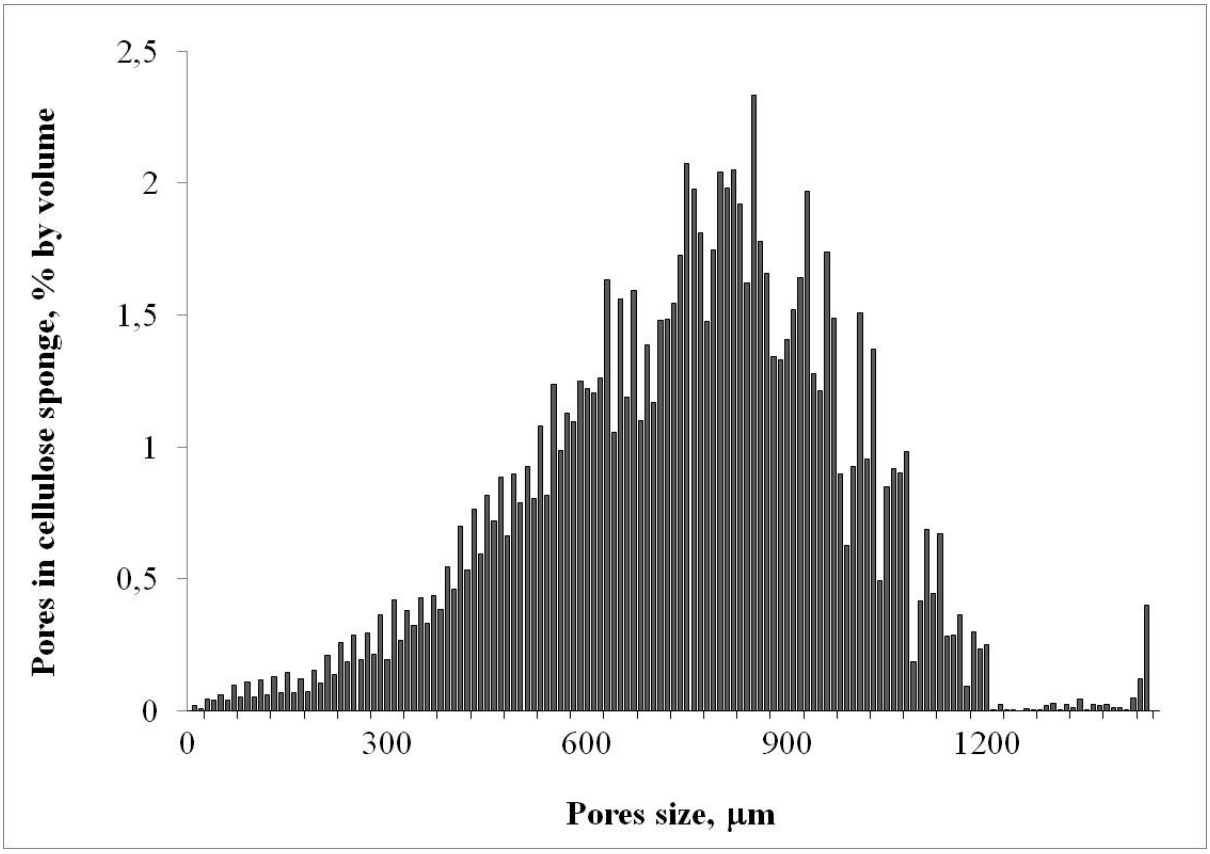

Fig. 4.
Pore size distribution in cellulose sponge estimated by micro-CT.

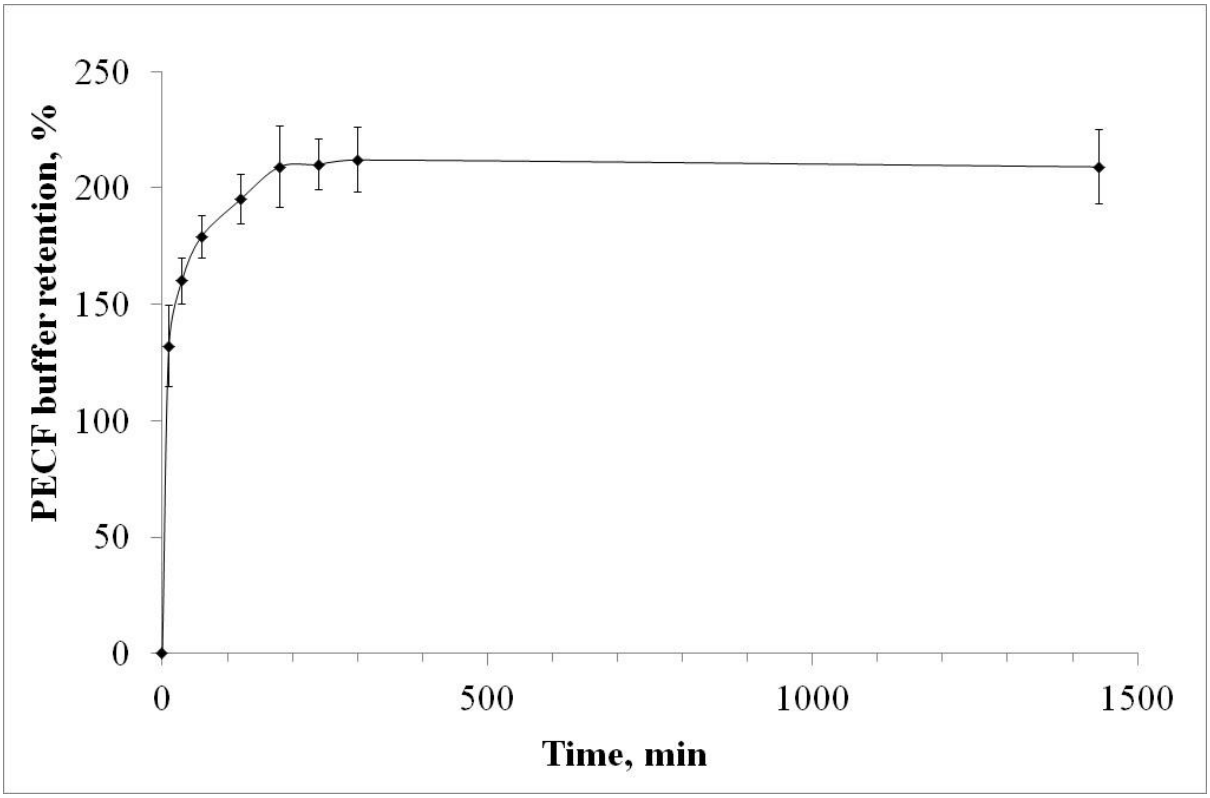

Fig. 5.
PECF buffer (pH 8.0) retention by cellulose sponge at 37°C.

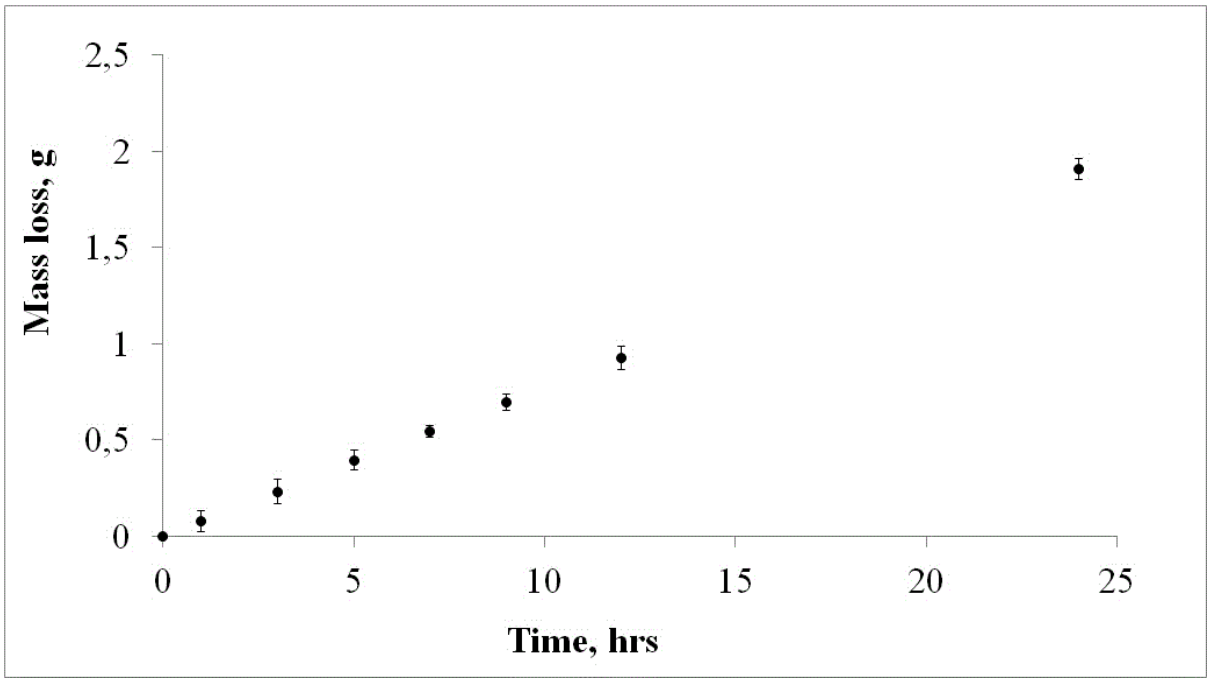

Fig. 6.
Time dependednt water loss in WVTR experiments. Experiments were performed at humidity approx. 40%.

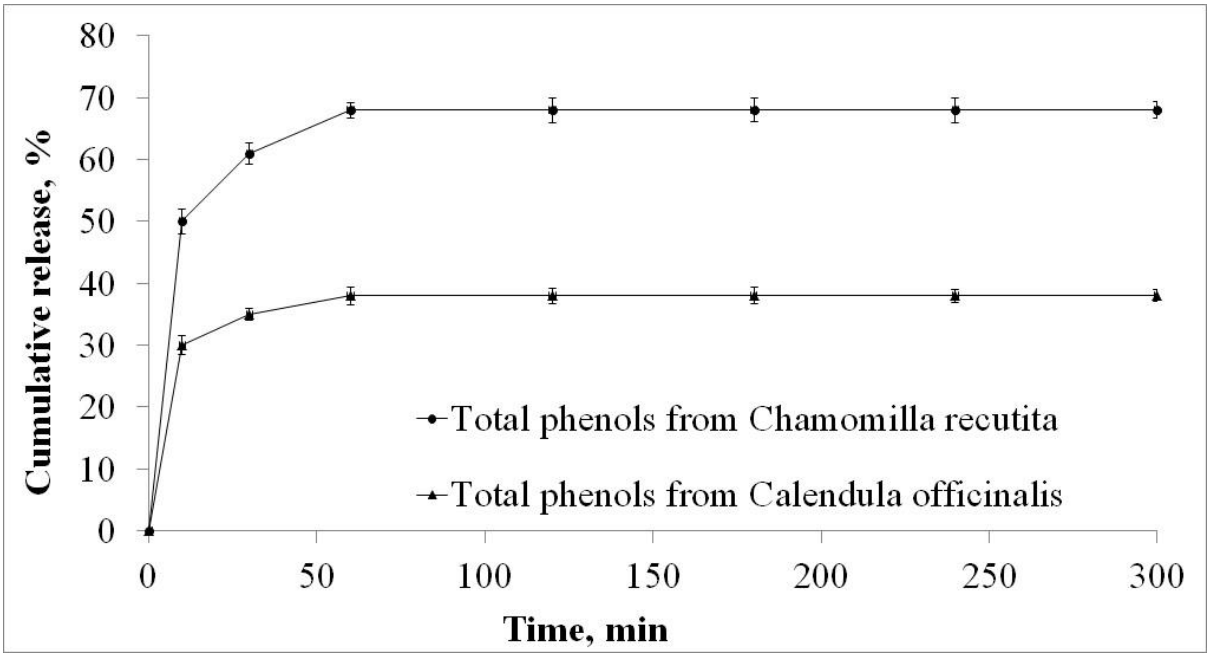

Fig. 7.
Cumulative release of total phenols from *Calendula officinalis* or *Chamomilla recutita.*

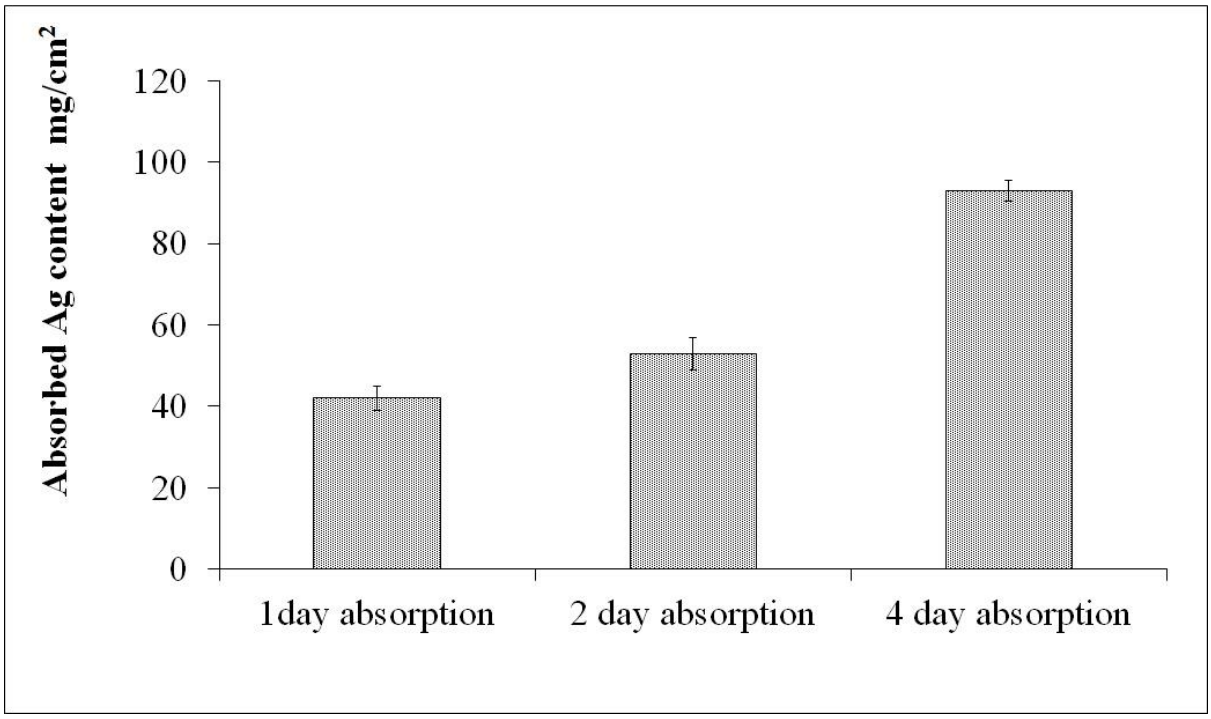

Fig. 8.

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

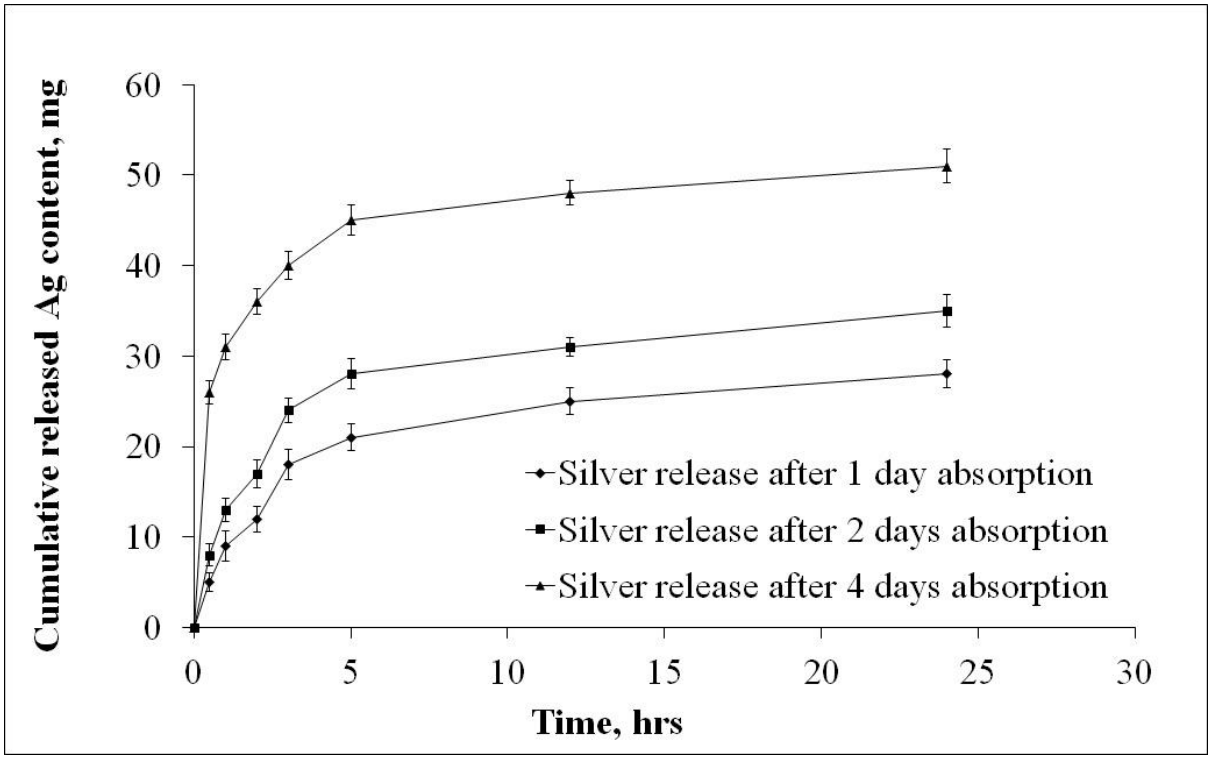

Fig. 9.
Cumulative silver release kinetics from cellulose based sponges.