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## ORIGINAL ARTICLE

# TOPICALLY-USED GENTAMICIN ATTACHED TO NANOFIBRE MDOC™ COMPARED WITH GARAMYCIN SCHWAMM® IN AN ACUTE WOUND INFECTION MODEL. AN EXPERIMENTAL STUDY.

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**Summary**

The aim of this study was to examine the effect of topically-used gentamicin attached to a biodegradable carrier, formed by micro-dispersed oxidised cellulose (MDOC) in nanofibre form, in acute wound infection treatment and to compare it with Garamycin Schwamm®. Twelve domestic swines were used in a model of a full-thickness infected dermal wound. The effectiveness of both materials in wound infections caused by *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* was tested. The effectiveness of both gentamicin with MDOC and Garamycin Schwamm® was comparable in *Pseudomonas aeruginosa* and *Escherichia coli* infections according to microbiological cultures findings. With *Staphylococcus aureus* infections there was a higher percent of negative cultures when MDOC with gentamicin was administered, but without statistical significance ($p=0.069$). When macroscopically assesed, 100 % of infected wounds treated by gentamicin attached to MDOC were without signs of local infection compared to only 16.7 % when Garamycin Schwamm® was used and this was statistically significant ($p<0.01$). For statistical analysis we used a Fisher´s exact test. When combined with a nanofibre MDOC carrier, topically-used gentamicin seems to be rendered more for treatment of full-thickness skin infections. The resulting good haemostatic effect of MDOC was observed.

*Key words: gentamicin; micro-dispersed oxidised cellulose; wound infection; Garamycin Schwamm*

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## INTRODUCTION

Surgical site infections (SSI) are the second or third most frequent healthcare-associated infections and many researchers are involved in the development of new woundcare products and new technologies for wound healing [1].

Micro-dispersed oxidised cellulose (MDOC), a trademark of Alltracel company (Dublin, Ireland) which is a division of HemCon Medical Technologies, Inc. (U.S.A.), is a random copolymer of polyanhydroglucose and polyanhydroglucuronic acid. It has been produced as SEAL-ON™ for its haemostatic effect and its ability to facilitate blood clot formation. MDOC is also believed to have an influence on wound healing but no clear results have as yet been shown. This is why we decided to examine its effect in acute wound infection healing in connection with topically-used gentamicin and to compare its effect to that of Garamycin Schwamm® (Essex Chemie AG, Luzern, Switzerland). An experimental acute wound infection model in pigs was created and the effect on full-thickness dermal wound infections caused by *Staphylococcus aureus* (ATCC 14776), *Pseudomonas aeruginosa* (ATCC 19582) and *Escherichia coli* (ATCC 23539) was tested.

## MATERIALS AND METHODS

### Tested material:

MDOC in the form of sodium-calcium salt of polyanhydroglucuronic acid (CaNaPAGA) has several biologically-positive properties (active haemostasis, immunostimulation, quick absorption, anti-adhesive properties). From the physico-chemical point of view, this ionogenic polysaccharide, which functions as a carboxylate ion exchanger, is fully absorbed by the organism. But it is not a film-forming or fibre-forming substance itself. Depending on its concentration, it creates salts or gels in water as colloido-dispersed systems. However gels without any biocompatible non-toxic stabiliser prove syneretic (i.e. after some time the CaNaPAGA solution separates from the gel). Therefore, if we want to prepare fibres from MDOC, some modifying components (such as biocompatible carrier-polymers, softening agents, etc.) should be used.

MDOC as a carboxylate polymer (as well as hyaluronic or alginic acid) creates intermolecular complexes (IMC) with positively-charged low-molecular substances or polymers, i.e. it can work as a carrier of substances such as basic antibiotics including gentamicin.

For the production of MDOC nanofibres (Φ of fibres is 50 – 500nm) it is necessary to use biocompatible and absorbable fibre-forming polymers that would create nanofibres together with MDOC or would be used as a binding material for prepared nanofibres. Medicinal glycerine, which has an impact on the physical properties of micro or nanofibres, has been successfully used as a softening agent for these systems.

A typical composition of produced nanofibres is as follows:

| | |
|---|---|
| Polyvinylalcohol (PVA) | 50% w/w |
| MDOC (CaNaPAGA salt) | 33% w/w |
| Glycerol (in 100% form) | 17% w/w |
| | --- |
| Total | 100% w/w |

During experimental work, this composition was presented as a ratio (PVA/MDOC)/glycerine = (60/40)/20.

The nanofibres of this composition were always fully absorbable after their application. The formats of antimicrobial MDOC non-woven nanofabrics applied to contaminated wounds were the same as the basic nanofibre composition. Only gentamicin sulphate was added. This preparation is attached to PAGA during the process. Therefore, instead of the sulphuric acid in gentamicin sulphate, the anion (polyanhydroglucuronate of gentamicine) was created by PAGA with the following composition of nanofibres:

| | |
|---|---|
| PVA | 42.80 |
| MDOC (LOT No.72105) | 28.60 |
| Glycerol | 14.30 |
| Gentamicin sulphate LOT No.A7694, Product No. G-1067 (FLUKA) | 14.30 |
| | -------- |
| Total | 100% |

In the beginning of testing when nanofabrics were prepared, it was impossible to achieve a higher surface density than a max. amount of 15 g/m$^2$. This means that, upon a concentration of 14.3% w/w of gentamicin in the MDOC nanofabrics, the content of gentamicin sulphate per 100 cm$^2$ of the area was maximum 21.45 mg, which was insufficient for its intended effect (its brand name Garamycin Schwamm® contains 130 mg of gentamicin for the same area). Therefore, the microfibres from long-fibrous medicated cotton-wool were prepared. These microfibres were transferred to raw cellulose through nitrogen oxides in the $HNO^3$ medium followed by hydrolysis of the cellulose in the medium of $H^2O^2$ and Ca salts in the presence of assistant substances. After hydrolysis, gentamicin sulphate was added to the reaction mixture during homogenization at the temperature

of 20°C. The suspension of fibres was filtered through a filter divider. The fibres were created by being washed with hydrous alcohol and dewatered using concentrated ethylalcohol. Afterwards, they were dried in a laminar box to a constant weight.
The technology of the production procedure of MDOC nanofabrics with a surface density up to 150 g/m$^2$ was developed and controlled. Thus, a uniform concentration of gentamicin in an amount of up to 150 mg on the area of 100 cm$^2$ was achieved; i.e. the concentrations are comparable to Garamycin Schwamm®.

**Experiment description:**

There were 12 female domestic swines (35 – 45 kg of weight) used in our experimental study. Each experiment took seven days. After intramuscular premedication by ketamine 30 mg/ kg (Narkamon®, Zentiva, Czech Republic), azaperone 40 mg/ kg (Stressnil®, Janssen Pharmaceutica, Belgium) and atropine 0.5 mg (Atropin Biotika A.U.V.®, Biotika, Slovakia) the animal was put under general intravenous anesthesia and maintained with ketamine. After preparation of the operation field, eight full-thickness 5 cm long dermal defects with side incisions and a fascial injury were created in the paravertebral area (four wounds on each side – on the left marked L1 to L4, on the right marked R1 to R4) of the animal (Fig. 1). Contusion of skin margins using pean forceps was performed to imitate the most common wound type in routine practice. After that, 0.5 ml of microbiological agent suspension in a density of $10^8$ CFU/ml was innoculated into seven wounds, the last one left clean without infection (always marked R4) as a control. Generally, effectiveness to each microbiological agent infection was tested separately for MDOC in nanofibre form with gentamicin and Garamycin Schwamm® in two animals (in 12 treated infected wounds). In every animal there were 2 controls – one infected non-treated wound (marked L1) and one clean wound without infection (marked R4). After a 45 minute period, beds of MDOC with gentamicin or Garamycin Schwamm® (5 x 1.6 cm of size containing 10,83 mg of gentamicin) were always placed into six infected wounds (L2 to L4 and R1 to R3). The entire operation area was covered by gauze and a surgical towel. After 24, 48 and 168 hours, swabs were made (for cultivation on blood agar), macroscopic assessment (wounds divided in 3 groups according to the absorption of the carrier and the presence of signs of local infection) and photodocumentation was performed. Presence of pus in wound bed was considered to be a criteria of local infection. At the end of the experiment, some tissue samples of wound margins for histopathologic examination were taken, and the animal was sacrificed by intravenous application of T-61® (Intervet Canada Ltd., Canada). During the experiment, the animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Experimental Animals".

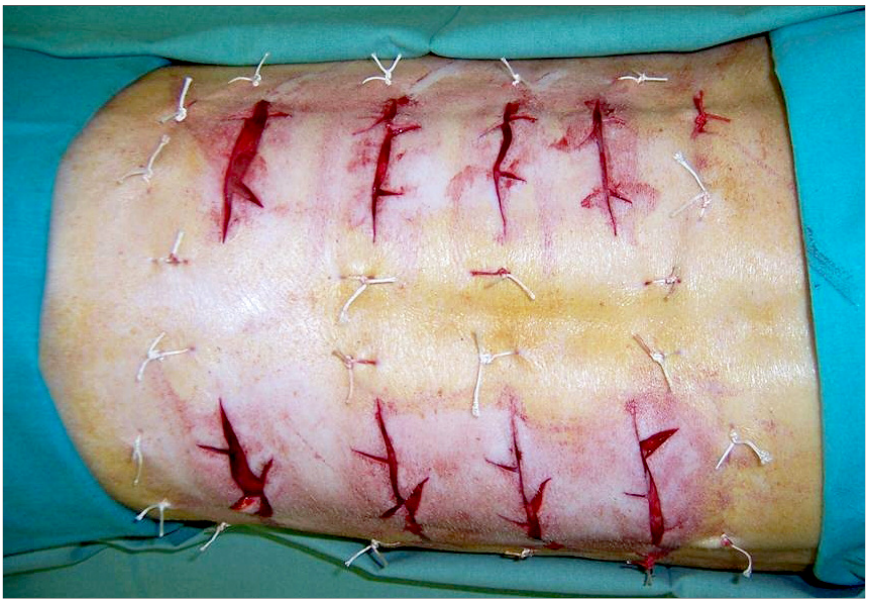

**Figure 1.** View on paravertebral area of an animal with 8 full-thickness dermal wounds.

## RESULTS

The results of cultivations (on blood agar) of microbiological swabs from the wounds taken after 24, 48 and 168 hours were compared. No significant differences in *Escherichia coli* and *Pseudomonas aeruginosa* - infected wounds were noted. In *Staphylococcus aureus* infections, 50 % of the wounds treated by nanofibre MDOC with gentamicin were negative compared to only 8.3 % treated by Garamycin Schwamm, but it was not statistically significant (p=0.069). (Tab. 1)

**Table 1.** Microbiological cultivations - counts and percent of treated wounds with negative cultivation findings.

| | E. coli | | | St. aureus | | | Ps. aeruginosa | | |
|---|---|---|---|---|---|---|---|---|---|
| | **24 h** | **48 h** | **168 h** | **24 h** | **48 h** | **168 h** | **24 h** | **48 h** | **168 h** |
| **Garamycin Schwamm** | 12/12 | 12/12 | 12/12 | 12/12 | 0/12 | 1/12 | 12/12 | 12/12 | 8/12 |
| | 100% | 100% | 100% | 100% | 0% | 8.30% | 100% | 100% | 66.70% |
| **nanoMDOC** | 12/12 | 12/12 | 12/12 | 12/12 | 12/12 | 6/12 | 12/12 | 12/12 | 6/12 |
| | 100% | 100% | 100% | 100% | 100% | 50% | 100% | 100% | 50% |

All the infected controls did have a positive cultivation finding at the end of the experiment, half of the clean ones too, mostly with a secondary contamination.

Although the microbial results, with the exception of the Staphylococcus aureus infections, are similar, macroscopic assessment showed big differences between both tested materials. Nanofibre MDOC with gentamicin was fully absorbed in 94.4 % of treated wounds after 48 hours and in 100 % of those in 168 hours. All the wounds were macroscopically clean, healed with a crust and did not show signs of local infection (presence of pus) after 48 hours and at the end of the experiment as well. Garamycin Schwamm was fully absorbed in 5.6 % of wounds after 48 hours and in 16.7 % after 168 hours. Additionally, 25 % of the wounds after 48 hours and 83.3 % after 168 hours showed local signs of infection, especially if the collagen carrier of Garamycin Schwamm was not absorbed. All these results were of statistical significance with $p<0.01$. Counts and percentages of wounds were compared by a Fisher´s exact test. Probability values were 2-tailed and were considered significant if < 0.01. (Tab. 2)

In all cases macroscopic assessment of both infected and non-infected controls showed similar results to those treated with MDOC, it means without signs of local infection, or pus respectively.

**Table 2.** Macroscopic assessment of the wounds at the end of experiment (after 168 hours) – counts and percent of wounds.

| | E. coli | | | St. aureus | | | Ps. aeruginosa | | |
|---|---|---|---|---|---|---|---|---|---|
| | **I** | **II** | **III** | **I** | **II** | **III** | **I** | **II** | **III** |
| **Garamycin Schwamm** | 0/12 | 0/12 | 12/12 | 3/12 | 0/12 | 9/12 | 2/12 | 0/12 | 10/12 |
| | 0% | 0% | 100% | 25% | 0% | 75% | 16.70% | 0% | 83.30% |
| **nanoMDOC** | 12/12 | 0/12 | 0/12 | 12/12 | 0/12 | 0/12 | 12/12 | 0/12 | 0/12 |
| | 100% | 0% | 0% | 100% | 0% | 0% | 100% | 0% | 0% |

group I - clean wound, tested material fully absorbed
group II - clean wound, tested material not fully absorbed
group III - wound with signs of infection, tested material not absorbed

## DISCUSSION

Surgical site infections (SSI) lead to increased morbidity, mortality, longer hospital stay and higher hospital costs. Antibiotic prophylaxis is generally recommended for all clean-contaminated, contaminated and dirty procedures, mostly by systemic administration. Topical administration of antibiotics for prophylaxis is supported by some studies, as well as in wounds that are clean [2]. Topical antimicrobials can be used in the treatment of secondarily- infected wounds, and the result of the treatment was found to be equivalent to systemic administration of antibiotics in the case of minor

infected wounds [3]. The more important role of topical antibiotics is in the treatment of chronic wounds [4].

One of the most common topically-used antibiotics in surgery is gentamicin. This is probably due to its effectiveness on a broad spectrum of gram positive and gram negative bacteria and, when it is topically-used, it is also effective against methicillin-resistant Staphylococcus epidermidis (MRSE) and methicillin-resistant Staphylococcus aureus (MRSA) [5, 6]. The other advantages of topically-administered gentamicin are its high concentration in wound fluid and minimum concentration in serum. High local gentamicin levels, about 75-200 times higher than minimum inhibition concentration (MIC), 4mg/l, were observed in wound fluid compared to that in serum (1-4 mg/l after 24 hours): both levels being safely below the toxic threshold of 10 mg/l [5, 7-9].

Most published articles involving topically-administered gentamicin with a collagen carrier describe results of the prophylaxis of SSI in a cardiac surgery [5-11]. Gentamicin-collagen implants of various brand names (Collatamp-G®, Gentacoll®, Sulmycin Implant®) that contain 130 mg of gentamicin and 280 mg of collagen were put underneath the sternum or between its edges before sternotomy closure. In some studies, more than one implant of gentamicin-collagen was applied [9, 10, 12].

In the first randomized controlled study published by Friberg et al. in 2005, the sternal wound infection rate was 4 % in the gentamicin group and 5.9 % in the control group. This was not statistically significant. Presented data showed a slight reduction in infection rate in gentamicin-collagen groups, but study population was too small to make a valid conclusion [7].

In Friberg´s next randomized control study, the incidence of SSI in 2000 patients undergoing an open-heart surgery was significantly reduced from 9 % to 4.3 % in the gentamicin group, but without an effect to the occurence of osteitis or mediastinitis [12]. The difference in all groups involving deep SSI was not of statistical significance, except in groups of patients with a BMI>25 kg/m$^2$ and/or diabetes mellitus [10, 12]. On the other hand, there were significantly more reoperations for bleeding in the gentamicin-collagen group (4.0 % vs. 2.3 %). This result was not explained, but it can be argued that the difference may have occurred due to bleeding from the bone marrow from a gap between both sternum halves when two gentamicin-collagen layers were inserted [12]. A similar increase in the number of cases with postoperative bleeding with dehiscence was noted by Leyh et al. [9]. In general, topically-administered gentamicin-collagen implants are recommended for antibiotic prophylaxis in a cardiac surgery.

The use of topical gentamicin as a prophylaxis is also recommended in some clean procedures in orthopedic and general surgery. Eveillard et al. proved the effectiveness of gentamicin-impregnated cement in the prevention of deep wound infection after a primary total knee arthroplasty. The infection rates were 1.21 % for patients who had antimicrobial cement and 9.52 % for those who had not [13]. Musella et al. recommended the use of gentamicin-laced collagen tampons in groin hernia patients if polypropylene mesh is inserted under aponeurosis of the external oblique muscle. In the gentamicin group, 1/301 patients (0.3 %) developed a postoperative wound infection compared to 6/294 in the control group (2.0 %) [14].

In clean-contaminated and contaminated procedures, the results are ambivalent. After one week of treatment, Buimer et al. describe a significant reduction in postoperative complications (dehiscence, infection) in a group of patients treated with enclosure of gentamicin after primary excision in case of hidradenitis suppurativa (35 %) compared with patients treated with primary excision only (52 %). However, after 3 months, complications in both groups were comparable [15].

In case of pilonidal sinus treatment, bacteriologic culture findings were not statistically different when treated by excision, gentamicin-collagen implantation and primary closure, or open treatment alone [16]. The study indicates that the use of gentamicin-collagen shortens the time of wound healing in patients with a primary closure.

In case of a repair of an anal fistula, using advancement flap gentamicin-collagen did not decrease the infection rate, nor was a different reccurence rate observed [17].

A number of studies tried to evaluate the effectiveness of topical administration of gentamicin in colorectal surgery procedures. Wounds after stoma closure and perineal wounds after abdominoperineal excisions were contaminated, and the incidence of SSI was high. Haase et al. did not show any differential effect between topically-subcutaneously-used gentamicin implants with regard to the prevention of wound infections after loop-ileostomy closure [18]. However, very good results with topical administration of gentamicin were achieved in more randomized controlled studies [19-21]. Grüssner et al. found a reduction of pathogens in cultures and a

lower infection rate in perineal wounds after abdominoperineal excisions. In total, bacteriologic efficacy amounted to 83.7 % in the treated group vs. 60.4 % in controls. The difference in infection rates was significant as well, 6 % vs. 20.8 % [19]. Similar results were reported in the studies of Rosen et al. and Gomez et al. – though with a significantly higher percent of primary wound healing in the gentamicin group, 87 % vs. control 46 %, and rate of infections in 9 % vs. 44 %, respectively [20-21].

In our study, we decided to use a new biodegradable carrier formed by micro-dispersed oxidised cellulose in nanofibre form. This cellulose has good hemostatic effect and facilitates blood clot formation, proven prior to our study, and is produced as SEAL-ON™ (HemCon Medical Technologies, Inc., U.S.A.) which is determined to stop bleeding [22-23]. Because it is very difficult to create a model of a chronic infected wound in a healthy laboratory animal we used the one with acute deep incisional infection. We wanted to test the effectiveness of MDOC on three most cultivated bacteria from acute wound swabs taken in our hospital and also wanted to compare it to one of the degradable carriers of topical antibiotics which has been already used in many products – collagen. In our hospital, Garamycin Schwamm® (gentamicin attached to collagen) is widely used for prophylaxis of SSI. This was the next reason why gentamicin was chosen as an antimicrobial, although it is known that it is not acceptable as a monotherapy especially in Pseudomonas aeruginosa infections where silver-based products are the method of choice [24-25]. Silver compared to topically used antibiotics has the advantage of having broad antimicrobial activities and there is also minimum development of bacterial resistance and only limited side effects [26]. Bacterial resistance is probably the biggest fear when topical antibiotic such as gentamicin is administered, but in doses used in our study it is not necessary to be afraid of any adverse effects. We did not observe any side effects in accordance to previously published results. On the other hand we observed an advantage of MDOC related to its better resorption.

Collagen matrix is fully biodegradable and resorbed within 1 to 8 weeks, depending on the vascularization of the tissue [15, 17]. In contrast, micro-dispersed oxidised cellulose is fully resorbed within the first 48 hours in 94 % of wounds.

So, when gentamicin is attached to the carrier formed by MDOC in comparable amount (130 mg/100 $cm^2$), we found this superior for its topical administration. In our study, Garamycin Schwamm® was not fully absorbed in 83.3 % at the end of the experiment and, like a foreign body in the wound bed, could increase the rate of SSI.

## CONCLUSIONS

Topically-used gentamicin attached to micro-dispersed oxidised cellulose in nanofibre form seems to be effective in soft tissue infections treatment thanks to its antimicrobial effect, excellent resorption of the carrier compared to collagen, as well as due to its influence on blood clot formation. In our opinion, this combination could be successfully used as a prophylaxis of SSI in contaminated or dirty procedures or in traumatic wounds prior to surgical debridement and also for topical treatment of secondarily infected wounds in absence of general signs of inflammation or sepsis.

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