Polyurethane Gel with Silver Nanoparticles for the Treatment of Skin Diseases

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Abstract: The aim of this study was focused on the bactericide test of Silver nanoparticles with an average diameter of 6 nm, over Escherichia coli, Staphylococcus aureus and Candida albicans and the treatment of a dermatosis of unknown etiology with a poly(hydroxy urethane-acrylate) gel (PHU) with a concentration of approximately 10 ppm Ag-NPs and a ratio-NHCOO-RGD (Arg-Gly-Asp) on the macromolecular chain of 200:1. The gel was obtained at the room temperature in sterile conditions, in a reactor with stirring facilities and a cushion of nitrogen purified by traces of oxygen, with several different silver (Ag⁺) concentrations (10.907, 15.96, 31.92, 39.5, respectively) and 56.88 ppm Ag-NPs ppm for the anti-germs tests, while for the treatment of a dermatosis of unknown etiology we used the gel with the smallest silver concentration. For the gel with the highest metallic silver concentration of 56.88 ppm, after 48 hours at 37°C, no one of the germs E. coli, S. aureus or C. albicans resisted. Candida albicans fungus showed an increased sensitivity to metallic silver, as it was eradicated after only 24 h. Probably, in the biological processes Ag⁺-cell, the poly(hydroxy urethane-acrylate), also intervenes, as this might facilitate the accumulation of nanometric silver on the extracellular matrix (ECM). Thus, the microorganism might accumulate faster the amount of silver, which stops its vital functions, such as the permeability and breathe. The developed gel with the smallest silver concentration, of 10.907 ppm Ag-NPs, lead to very good results in the treatment of a dermatosis of unknown etiology, after 78 days of treatment.

Keywords: Nanoparticles, dermatoses, signal tripeptides, keratolytic effects.

1. INTRODUCTION

Generally, the dermatological treatments is applied externally (through synthetic medicines, phytotherapy, physiotherapy or hydromineral approaches), or as a complement to an internal treatment. The treatment of several dermatoses of unknown etiology or resistance to the usual medication represents a serious issue in dermatology. Sometimes, one can obtain an apparent cure, after which the dermatosis manifests again, or the healing advances up to a certain level, and then it stands unchanged [1-5]. Several tests we performed for the biocompatibility and the antibacterial effect of some polyurethane membranes with Ag nanoparticles, linked to the urethane structure as urethane – silver – urethane [6], also emphasized an effect of regeneration of the hair follicle of tested rabbits and mice. One might assume that this effect is due to the stimulation of the stem cells in the skin [7]. The regenerative effect manifested for the Ag nanoparticles having average diameters under 10 nm, which best adhere to the extracellular matrix (ECM), with the best biocide effect [7, 8]. The skin is the only organ that comes in direct contact with the exterior, being exposed to a multitude of aggressive environmental factors: mechanical, physical, chemical, germs etc. In certain situations, these may lead to great chronic suffering, accompanied by a tremendous decay of the patient’s quality of life. On the other hand, several internal diseases manifest at the skin level, and the misunderstanding of the whole pathology, or the lack of knowledge concerning the links between the organism, the nervous system and that particular disease lead in most cases to the failure of treatments for the skin diseases [5].

The treatment of certain dermatosis of unknown etiology or resistant to the medication on the market represents severe issues in dermatology. One can sometimes perform an apparent cure, but the dermatosis reappears after a while, or the cure can be performed up to a certain extent, and then it stops. The skin treatments are generally applied externally (by means of chemicals, phytotherapy, physiotherapy or hydromineral treatment) or complementary to an internal treatment. One can obtain very good results in treating skin diseases by using preparations based on Silver compounds, the most common being silver sulfadiazine (Ag-SDA) [9]. On the other hand, where the antibiotics are no longer valid, one has proved that the treatment with silver nanoparticles represents an efficient cure. Most of the mechanisms, through which the silver nanoparticles manifest bactericide properties, consist of their anchoring on the bacteria’s cell wall and the tuning of the cellular signal, probably, by means of dephosphorylation of tyrosine groups in the peptide substrate [9]. Silver nanoparticles, especially those with a diameter of 1-10 nm [10, 11], attach themselves on the surface of the cell membrane, as it was demonstrated on HIV
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The measurement of Ag-NPs concentration was performed by UV-VIS (6505 UV/VIS spectrophotometer (UK)), and their diameter was estimated with a Zetasizer Nano System Malvern Ltd. (UK). From the histogram, one may notice that the nanoparticles’ diameter is within a range between 1.74-10.0 nm, most of them being between 4.0-5.0 nm. These values were also confirmed through electronic transmission microscopy (TEM).

2.2. The Gel

The Gel was obtained in sterile conditions in a stirring reactor with nitrogen cushion purified by oxygen traces, at the room temperature, from PHU with different Silver concentrations, respectively 10.907, 15.96, 31.92, 39.50 ppm for anti-germs tests, while for the treatment of a dermatosis with unknown etiology we used the formula: - Ag-NPs: 0.0010907 % (10,907 ppm); - PHU: 2.6 %; -glycerin: 8.0 %; -silica gel: 0.02 %; -water: up to 100.

2.3. Cultivation and Analysis of the E. coli, S. aureus and C. albicans Micro Organisms

The micro organisms were inoculated in the cultures support sterilized at 37°C, for 20 hours, in a stirring hot chamber. The microorganisms’ suspensions for the tests contain from 1.2*10^5 up to 4.2*10^5 unities of formed colonies (CFU)/ml. For the kinetic test, for each sample, we introduced in three sterilized test tubes of 2 ml an amount of 4 mg de gel Ag-NPs-PHU per each, and one ml of inoculated sample of the suspension of every organism (E. coli, S. aureus and C. albicans). This operation was done for every Silver nanoparticles concentration, respectively, 10.907, 15.96, 31.92, 39.50 ppm. The number of colonies was determined by the spread plate method where every microorganism is plated on nutrient agar and incubated at 37°C for 24 and 48 hours respectively, and then the colonies were counted.

2.4. Human Study

Informed written consent was obtained for the skin diseases investigations from the adult patients involved in this study on which were performed following diagnostic protocols were performed, which were approved by the local Bioethical Committee and which were in accordance with the Helsinki Declaration.

Where: PU - Polyurethane; P - Protein chain

Fig. (1). Silver nanoparticles with urethane and peptide groups interaction mechanism.
3. RESULTS

The structure of polyhydroxy-urethan-acrylate (PHU) with RGD (Arg-Gly-Asp) sequences is presented in Fig. (2).

![Structure of PHU with RGD sequences.](image)

where: - molar ratio n/m = 200 : 1
- A = -CONH-(CH$_2$)$_2$-NHCOO-(CH$_2$)$_2$-OH;
- B = -CONH-CH$_2$-CONH-RGD.

Fig. (2). Structure of PHU with RGD sequences.

The RGD sequence is by far the most used peptide sequence for the cellular adhesion at the synthetic polymers surface. In the multi-cellular organisms, having neighboring cells around the extra cellular matrix (ECM) are mediated through the adhesion receptive cells, such as the integrins [22, 23]. Thus, PHU is not merely an excipient but it also helps curing the wounds, as the polyurethanes are often used in the treatment of several skin diseases [24], especially for burnt skin [25].

The gel composition (gram/100 grams) is:
- silver nanoparticles of: G ppm;
- PHU: 2,6 %
- glycerin: 8,0 %,
- silica gel: 0.02 %,
- Water: rest of water up to 100, and G = 10.907, 15.96, 31.92, 39.5, 56.88 ppm Ag-NPs.

The sequence Arg-Gly-Asp as the signal tripeptide, which helps to the growth and proliferation of the cells, also presents a synergic effect with respect to Ag-NPs which is the biologically active principle.

Fig. (3). The anti-germ activity of the Silver nanoparticles over (a) Escherichia coli ATCC 25992, (b) Staphylococcus aureus ATCC 6538, and (c) Candida albicans 10231.
The anti-germ activity of the silver nanoparticles against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* appears in Fig. (3a-c).

Fig. (3) shows that *E. coli* is very sensitive to Ag-NPs with respect to the duration, while *S. aureus* strain is totally destroyed after 48 hours. *Candida albicans* strain is also totally destroyed also after 24, and 48 hours respectively for concentrations of 39.5 and 56.88 ppm Ag-NPs, respectively. These results suggest that in the biological processes Ag-cell the structure poly(hydroxy urethane-acrylate), also intervenes, as this might adhere to the extra cellular matrix (ECM), facilitating the accumulation of nanometric silver into the micro-organisms a certain amount of this blocks its vital functions, such as the permeability and breath.

The study was done on an untreated skin wound un pruriginosa, which associates the hyperkeratosis and peripheral papule wounds on a discrete erythema foundation. This dermatosis was extended as follows: a) on the posterior face of the left forearm, on an approximately ellipsoidal surface with $D = 8.0$ cm and $d = 4.0$ cm, b) on the posterior face of the right forearm, on an approximately ellipsoidal surface, with $D = 7.0$ cm and $d = 3.7$ cm. Figs. (4-7) show the evolution of the treatment of the dermatosis during 78 days of application of PHU gel, with Ag-NPs.

4. DISCUSSION

Considering the photos and daily observations of the dermatosis evolution during the treatment over 78 days, we concluded the following aspects have been concluded: During the first 8 days of treatment, one may observe a strong keratolytic effect of the gel, with the remission of the hyperkeratosis on a surface of approximately 80% of the initial surface. Between the 8th and the 19th days, one may observe a complete disappearance of the hyperkeratosis, but also the persistence of the skin papule and hyperpigmentation wounds. In the 24th day, one can observe a restriction of the colored zone surface, of ~60% at the left forearm, and of ~50% at the right fore arm. This restriction was made from the exterior to the interior of the zone. Both arms showed visible papule, especially the right one. Between the 24th and the 65th days, one can observe a gradual decrease of the hyperpigmentary zone and a gradual regeneration of the skin from the outside, up to the image previously showed in Fig. (6). On the 65th day, the dimensions of the zones still hyperpigmentary were for the left forearm, $D = 4.0$ cm and $d = 2.0$ cm, and for the right forearm, $D = 3.5$ cm and $d = 1.5$ cm. Between the 57th-78th days, one can notice a visible regeneration of the skin, especially of the hairs (Fig. 7). This observation leads to the idea that a stimulation of the stem cells took place, which lead to a regeneration of the hair folliculi. Meanwhile, at the right forearm, one cannot observe this kind of spectacular regeneration of the hairs, the reason being sustained by the affection of this arm. The same reason probably stands the persistence of the papule on this forearm. Between the 57th-78th days, one can notice a visible regeneration of the skin, especially of the hairs (Fig. 7). This observation leads to the idea that a stimulation of the stem cells took place, which lead to a regeneration of the hair folliculi. Meanwhile, at the right forearm, one cannot observe this kind of spectacular regeneration of the hairs, the reason being sustained by the affection of this arm. The same reason probably stands the persistence of the papule on this forearm. Thus, it is well known that the wounds healing takes place in a differentiated way for the skin covered with hairs or uncovered with hairs, respectively [7, 25], while during the healing process, the folliculi of hairs contribute to the epidermic wounds healing. If these do not exist, the mesenchymal stem cells derived from the derma can differentiate in epidermic cells and can contribute to the healing, but with less efficiency [26, 27]. Thus, for the mutant mice, without hairs, one cannot observe the hair regeneration, as it is the case for the normal mice [7]. In this case, it is also possible that the PHU structure, through the RGD peptide on the macromolecular chain, substantially contributed to the healing of the dermatosis. When the skin wounds are healed, the derma cells can appear from mesenchymal stem cells or from fibroblasts [25, 28, 29], while from the stem cells of the folliculi bulbs one may differentiate *in vivo* keratinocytes and new hair folliculi which constitute new resources of stem cells [25], which can contribute to the recovery of the superficial skin layer.

The research on rabbits, within the project CEEX C26-2005 revealed that Ag delivered from a polyurethane implant
doped with Ag nanoparticles determines an accelerated healing, qualitatively superior (the hair follicle are also regenerated) of the hair covered skin wounds, produced by the stimulation of the multiplication of the hair follicle [7, 30, 31]. In this case, there is also a possibility that the PHU structure, through the RGD signal tripeptide on the macromolecular chain, might have substantially contributed to the recovery of the healthy tissue and the healing of the dermatosis.

5. CONCLUSIONS

The Ag-NPs gel tested in vitro has a powerful biological activity upon the germs E. coli, S. aureus and C. albicans, according to the concentration in Ag-NPs, as well as in the PHU structure. The Ag-NPs gel presents a visible healing action on the treated dermatosis. The healing process started with an intense keratolysis during the first 8 days, which was completed after 19 days of treatment, and then the papule disappearance and the skin regeneration could be observed. On the 78th day, the skin was beautifully regenerated, with no scars. The hyperpigmentation is specific to a wound during the healing process, thus it is not generated by argyria.

ABBREVIATIONS

Ag-NPs = Silver nanoparticles
Ag-SDA = Silver sulfadiazine
ECM = Extracellular matrix
PHU = Poly(hydroxy urethane-acrylate)
RGD = Arg-Gly-Asp tripeptide

REFERENCES


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