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Electrospun gelatin nanofibers functionalized with silver nanoparticles

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The present paper deals with gelatin nanofibres functionalized with silver nanoparticles, prepared by electrospinning using solutions of gelatin mixed with silver nitrate. As a common solvent for gelatin and $AgNO_3$ was selected a mixture of formic acid and acetic acid in volume ratio 4:1. In this system, formic acid was used as a solvent of gelatine, but also as reducing agent for silver ions in solution. Silver nanoparticles were stabilized through a mechanism that involves an interaction with oxygen atoms of carbonyl groups of gelatin. The gelatin nanofibres functionalised with silver nanoparticles were characterized by transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray diffraction (XRD) and antimicrobial test. The results of investigations by TEM and XRD confirmed the presence of silver nanoparticles with diameters less than 20 nm, uniformly distributed over the surface of smooth nanofibres with an average diameter of 70 nm. The tests demonstrated that gelatin/Ag nanofibers have a good antimicrobial activity against *Escherichia coli*.

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1. Introduction

One of the very important tissues for human health is the skin, which plays the role of an interface between body and environment, and acts as a protective barrier against physical, chemical and biological aggressions. The skin is actually a complex system involved in the processes of absorption and excretion, showing a selective permeability for some important substances in human metabolism [1].

The currently increasing incidence of skin diseases caused a significant demand for skin replacement materials which in many cases can not be assured from natural sources. It also increased the demand for skin regrowth substrates used in treating skin lesions, which stimulated tissue engineering to develop new biodegradable materials which can promote adhesion, development and migration of fibroblasts, necessary for a good quality reepithelization [2]. Skin substitutes used today are expensive and have limitations concerning the biocompatibility, making their use sometimes risky [3].

There have been developed various technologies to produce biomaterials used for skin lesions treatment of wound dressings. One of the very interesting nanotechnologies investigated now is polymer solutions spinning in electrostatic field (electrospinning), which can be applied to solutions of various natural and synthetic polymers such as gelatine, collagen, chitosan, fibrinogen, silk, polylactic acid, hyaluronic acid, obtaining non-woven nanofibres membrane with thicknesses in a range that goes from a few tens of nanometers to over one thousand nanometers [4-6]. This type of three-dimensional structure of nanofibres prepared by electrospinning resembles very well with the human extracellular matrix, which explains the great interest manifested today for this nano-technology.

The biopolymers have high viscosity and very low solubility in common organic solvents, which makes them difficult to process by solution spinning in electrostatic field. Natural polymers dissolve well in 1,1,1,3,3,3-hexafluoro-2-propanol and trifluoroacetic acid [7], which are toxic and expensive solvents, features that limit their use in applications on an industrial scale [8-11].

Gelatin is a biopolymer widely used in pharmaceutical industry and for medical device applications. As a result of the growing interest for regenerative medicine, in the recent past gelatin has begun to be studied also as a candidate material for obtaining scaffolds with similar qualities of natural media for a wide range of tissues [12-16].

In a previous study we reported the preparation of nanofibres with superior mechanical and biological characteristics, by electrospinning of gelatin solutions with concentrations up to 30% (w/v) [17]. In the present paper we investigated the possibility to improve the properties and extend the applicability of gelatin nanofibers by adding silver nanoparticles which are known to have a positive effect in wounds healing and skin regeneration.

2. Experimental

2.1. Gelatin/$AgNO_3$ solutions

The electrospinnable solutions used in our experiments were prepared using gelatin (Fisher HealthCare, MP Biomedicals), formic acid (98 +100%, Scharlau Chemie SA), acetic acid (99.5%, Chemical Company SA), silver nitrate (99.5% Chemical Company SA) and distilled deionised water. The reagents were used as received from the manufacturer.

In the beginning, a solution of 27% (w/v) gelatin in a mixture of formic acid with acetic acid in the volumetric ratio 4:1 was prepared. The solution was subjected to stirring at room temperature for 3h, after which the impurities and undissolved parts were eliminated using a stainless steel filter.

The silver precursor solution was prepared by dissolving 5% (w/v) $AgNO_3$ in 66% (v/v) acetic acid in distilled deionised water.

The electrospinnable solution was prepared by adding dropwise the $AgNO_3$ solution to the gelatin solution, under continuous stirring for 20 minutes.

The solutions viscosity was determined at room temperature using a Nahita Rotary Viscometer, observing the rules ISO 3219/1993 [18].

2.2. Electrospinning

The equipment for spinning in electrostatic field was made of a 10 ml syringe filled with solution, actuated by a home-made syringe pump able to provide flow rates in the range 1.0-40.0 μL/min. The syringe was connected through a 40 cm Teflon tube of 0.5 mm inner diameter to a blunt needle placed 12 cm high above a collector covered with aluminium foil. Between the needle and collector high-voltages in the range 11.0-19.5 kV have been applied using a Brandenburg Alpha III Series HV Power Supply (30V-30kV). The optimum solution flow-rate for obtaining good quality nanofibers was 2.4μL/min.

2.3. Crosslinking with glutaraldehyde

The water stability of the nanofibers was improved by crosslinking with glutaraldehyde vapours (25% solution) for 24 h, drying at room temperature for 2 h and heating in a thermostat at 70°C for 2h. It is known that such a treatment promotes gelatin chains crosslinking by formation of Schiff bases type iminic groups [19,20].

2.3. Structural characterization

The morphology of the electrospun gelatin nanofibers was examined by scanning electron microscopy (SEM) using a Vega 2 Tescan (Czech Republic) microscope. The nanofibers diameter was estimated using Atlas Tescan software for image analysis.

The chemical structure of nanofibers was investigated by Fourier-transform infrared attenuated total reflectance spectroscopy (FTIR-ATR) using a DIGILAB – SCIMITAR Series FTS 2000 spectrometer with ZnSe crystal, working in the range 750-4000 cm^{-1}, with 4 cm^{-1} resolution.

Dimensional analysis and structural characterization of silver nanoparticles was carried out by transmission electron microscopy (TEM) using a Philips CM100 microscope, and by X-rays diffraction (XRD) using a DRON 2.0 diffractometer with Cu tube (Kα radiation).

2.4. Antimicrobial activity characterization

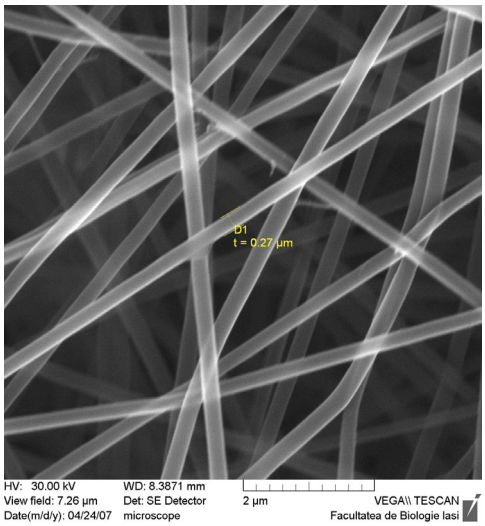
The antibacterial activity was tested on solid media by the disk diffusion method. The bacterium inocula were prepared from a culture of *Escherichia coli* ATCC 25922 overnight incubated at 37°C in nutrient broth, by diluting with peptoned water to a 0.5 McFarland standard. Petri plates containing Mueller Hinton agar were innoculated by inundation with prepared microbial suspension. Two samples of the investigated material, one containing Ag nanoparticles and one without Ag nanoparticles (used as control) were placed on the surface of the inoculated media. The plates were incubated at 37°C for 24 hours. Following incubation, plates were examined in order to identify zones of no growth (halos around the fragments) characteristic for antimicrobial activity.

3. Results and discussion

In case of electrospinning gelatine, its strong polar nature makes it difficult to solve in common solvents. The special solvents mentioned above are toxic and very expensive. Using the results of our previous study, in the present work we choosed as gelatin solvent a mixture of formic acid and acetic acid in volumetric ratio 4:1 that provided a better solution stability and a longer electrospinnability interval [17], due to the acetic acid which determined a dynamic rebuild of the hydrogen bonds involved in generating helicoidal structures of gelatin molecules.

In the present work, by electrospinning a solution of AgNO3 in gelatin, good quality nanofibers with smooth surface and without beads were obtained, as proved by the SEM images presented in Figs. 1, 2.

The morphology of the nanofibers shown in Fig. 2(a) suggest that adding 5% (w/w) silver nitrate to gelatin resulted in an increased solution conductivity and caused a slight decrease of fibers diameter. Moreover, the increased conductivity and decreased viscosity of the electrospun solution determined occasionally the formation of some auxiliary microjets which formed nanofibres with diameters less than 30 nm, intercalated among majority nanofibres with an average diameter of 190 nm.



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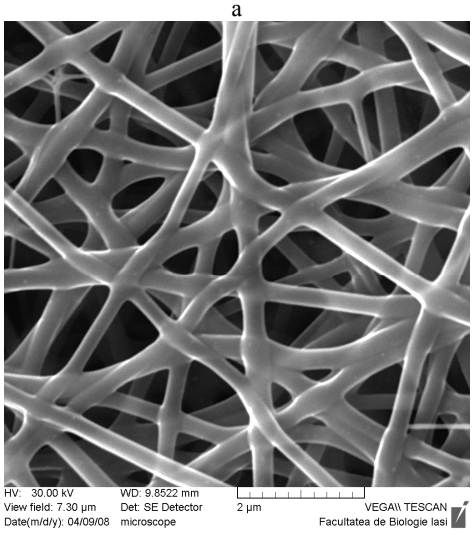
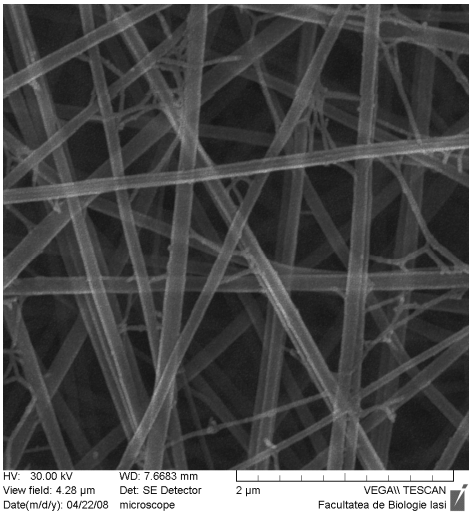


b

Fig. 1. SEM images of gelatin nanofibres prepared by electrospinning a solution of gelatin in formic acid and acetic acid: (a) as electrospun, (b)crosslinked in vapours of glutaraldehyde.

Further treatment of gelatin or gelatin/Ag nanofibers with glutaraldehye vapours resulted in a slight increase of nanofibers diameter due to water removal and increase of material density (Figs. 1b, 2b). The water removed during the crosslinking process accumulated on the nanofibers surface and caused a superficial dissolution and bonding of nanofibers at the contact points.



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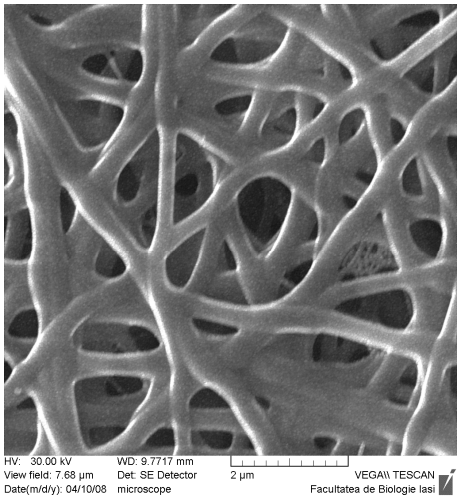
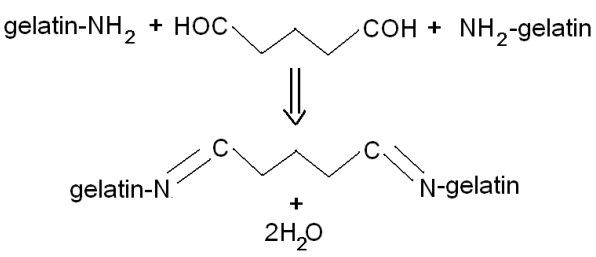


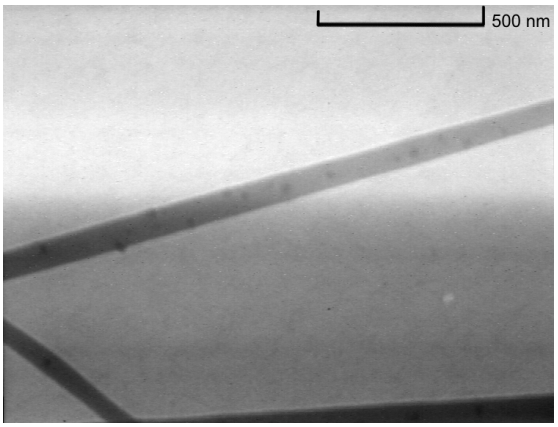
b

Fig. 2. SEM images of gelatin nanofibers functionalised with silver nanoparticles: (a) as electrospun, (b) crosslinked in vapours of glutaraldehyde.



As compared with pure gelatin nanofibers in Fig. 1b, the crosslinked gelatin/Ag nanofibers shown in Fig. 2b appear to be more affected by water, probably because an increased hydrophylicity as a consequence of structural changes induced by silver nanoparticles surfaces.

Images obtained by TEM (Fig. 3) show the presence of some nanoparticles on the surface and in the volume of gelatin nanofibers. The shape of nanoparticles is spherical, with diameters less than 20 nm.



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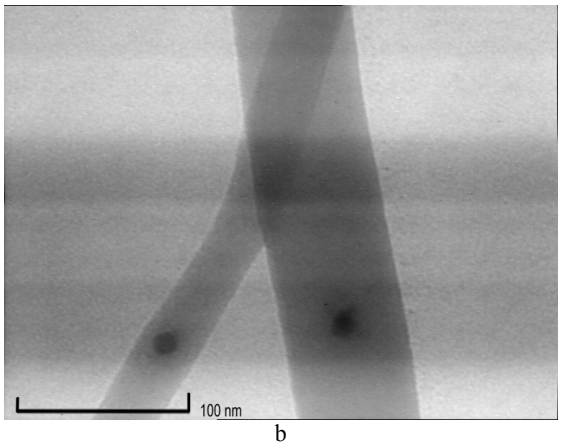
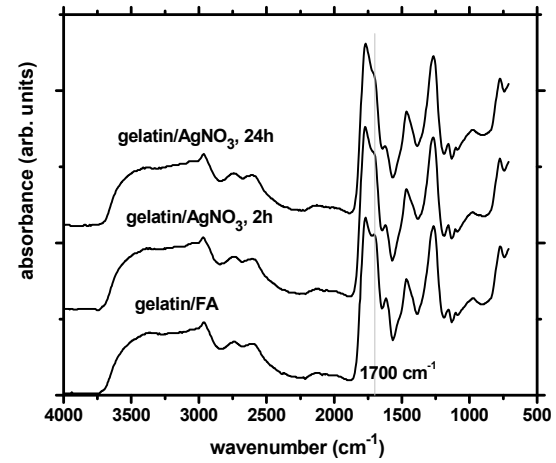


b

Fig. 3. TEM images of silver nanoparticles on the surface (a), and inside (b) of electrospun gelatin nanofibers.

3.3. Structural characterization by FTIR and XRD

The infrared spectra showed in Fig. 4 show that the spectrum of gelatine nanofibers is almost identical with the spectrum of gelatin/Ag nanofibers, with just two small differences. First, it can be observed a more intense absorption in the band at 1406 cm^{-1}, assigned to symmetrical stretching of the carboxyl group, -COOH. Second, the absorption peaks at 2922 cm^{-1} and 2848 cm^{-1}, suggest that the silver nanoparticles caused a reordering of gelatin chains by promoting the formation of peptide CH_2 links [21]. This restructuring effect is very similar to the effect reported in our previous paper [17], where the bands at 2922 cm^{-1} and 2848 cm^{-1} appeared in infrared spectra of films prepared from aqueous gelatin solutions and were not present in spectra of gelatin nanofibers. The high relative elongation of electrospun nanofibers and the fast solvent evaporation freezes an amorphous structure in pure gelatin nanofibers. By contrast, as the spectra from Fig.4 prove, the presence of silver nanoparticles stimulates the restoration of local order of molecular chains and facilitates the formation of peptide links between them.



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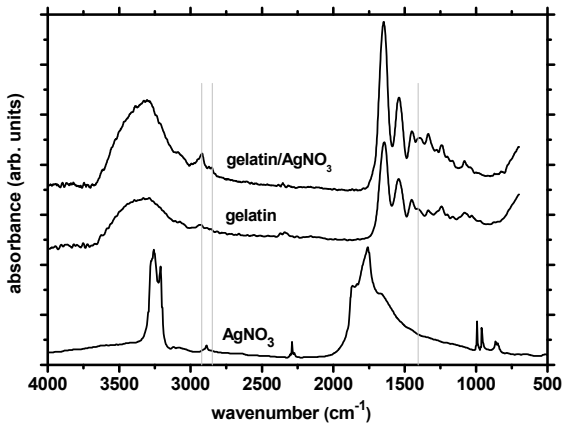

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Fig. 4. FT-IR spectra: (a) gelatin/AgNO3 solution, (b) electrospun gelatin nanofibers doped with silver nanoparticles.

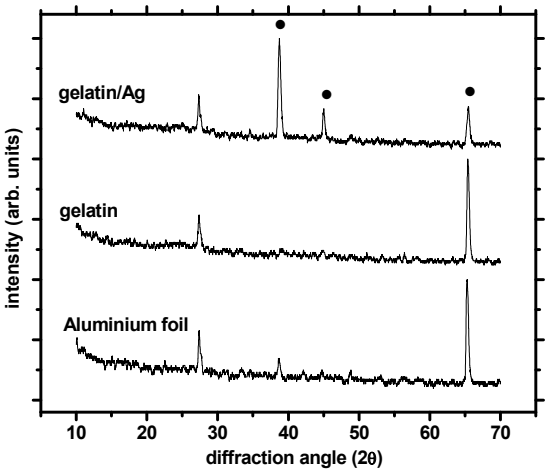

Fig. 5. X-ray diffractograms of electrospun gelatin nanofibers functionalised with silver nanoparticles, crosslinked with glutaraldehyde.

In the FTIR spectrum of the gelatin/$AgNO_3$ solution in Fig. 4a, one can see a slight increase in the intensity of the absorption bands amide I and II, but without any new peaks that could be assigned to a possible unreacted phase of $AgNO_3$. On the basis of Fig. 4a it can be assumed that the entire amount of silver nitrate reacted with the formic acid and silver is present in the gelatin/Ag nanofibers only in metal form. The presence of silver only in the form of well crystallised metal nanoparticles is certified by the X-ray difractogramme shown in Fig. 5, where the peaks due metallic silver are clearly present.

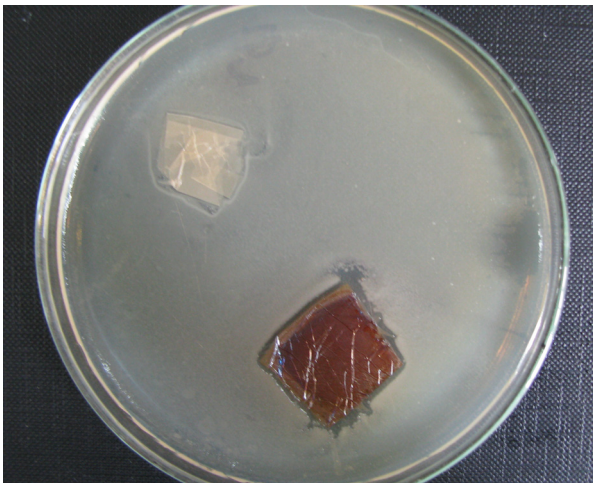

Fig. 6. Antimicrobial activity of gelatin nanofibers against Escherichia coli ATCC 25922. Upper left – gelatin, bottom right - gelatin with silver nanoparticles.

4. Conclusions

In the present work, there were prepared non-woven membranes made of gelatin nanofibers functionalized with silver nanoparticles with diameters less than 20 nm. After crosslinking with glutaraldehyde vapours, the nanofibers gained good water stability.

The structural analysis by infrared spectroscopy and X-ray diffractometry have shown that in the final nanofibers silver is present only in the form of well crystallised metal nanoparticles.

The presence of growth inhibition zones, i.e. halos around the samples of gelatin/Ag nanofibers proved the antimicrobial activity of the investigated material against *Escherichia coli* ATCC 25922.

The superior physical and chemical characteristics, along with a good biocompatibility and other improved properties as a lack of inflammatory reaction and relatively fast resorbtion, recommend the gelatin nanofibers functionalized with Ag nanoparticles as a good candidate material for the manufacture of wound dressings skin tissue scaffolds.

References

[1] D. N. Shier, J. L. Butler, R. Lewis, Hole's Human Anatomy & Physiology, 11th edition, McGraw-Hill Higher Education (2006).
[2] R. Langer, J. P.Vacanti, Science **260**, 920 (1993).
[3] G. Damodaran, M. Syed, I. Leigh, S. Myers, H. Navsaria, Expert Review of Dermatology **3**(3), 345 (2008).
[4] X. Wen, D. Shi, N. Zhang, Applications of Nanotechnology in Tissue Engineering, in Handbook of Nanostructured Biomaterials and Their Applications in Nanobiotechnology, Ed. H. S. Nalwa, American Scientific Publishers, 1 (2005).
[5] Y. Zhang, H. Ouyang, C. T. Lim, S. Ramarkrishina Z. M. Huang, J. Biomed. Mater. Res. Part B: Appl. Biomater. **72B**, 156 (2005).
[6] H. W. Kim, J. H. Song, H. E. Kim, Adv. Funct. Mater. **15**, 1988, (2005).
[7] R. Murugan, S. Ramakrishna, Tissue Engineering **12**(3), 435 (2006).
[8] M. Li, M. J. Mondrinos, M. R. Gandhi, F. K. Ko, A. S. Weiss, P. I. Lelkes, Biomaterials **26**, 5999 (2005).
[9] G. E. Wnek, M. E. Carr, D. G. Simpson, G. L. Bowlin, Nano Lett. **3**, 213 (2003).
[10] K. Ohkawa, D. Cha, H. Kim, A. Nishida, H. Yamamoto, Macromol. Rapid Commun. **25**, 1600 (2004).
[11] J. A. Matthews, G. E. Wnek, D. G. Simpson, G. L. Bowlin, Biomacromolecules **3**, 232 (2002).
[12] M. Li, M. J. Mondrinos, M. R. Gandhi, F. K. Ko, A. S. Weiss, P. I. Lelkes, Biomaterials **26**, 5999 (2005).
[13] S. Liao, L. Bojun, M. Zuwei, H. Wei, C. Chan, S. Ramakrishna, Biomed. Mater. **1**, R45 (2006).
[14] R. Langer and D. A. Tirrell, Nature **428**, 487 (2004).
[15] P. Y. W. Dankers, M. C. Harmsen, L. A. Brouwer, M. J. van Luyn, E. W. Meijer, Nature Mater. **4**, 568 (2005).
[16] Z. M. Huang, Y. Z. Zhang, M. Kotaki, S. Ramarkrishna, Compos. Sci. Technol. **63**, 2223 (2005).
[17] T. Balau Mindru, I. Balau Mindru, T. Malutan, V. Tura, J. Optoelectron. Adv. Mater. **9**(11), 3633 (2007).
[18] ISO 3219:1993. Plastics - Polymers/resins in the liquid state or as emulsions or dispersions – Determination of viscosity using rotational viscometer with defined shear rate.
[19] Y. Koyama, A. Taniguchi, J. Appl. Polym. Sci., **31(6)**, 1951 (1986).
[20] C. Tual, E. Espuche, M. Escoubes, A. J. Domard, Polym. Sci., Part B: Polym. Phys., **38(11)**, 1521 (2000).
[21] D. A. Prystupa and A. M. Donald, Polymer Gels and Networks **4**, 87 (1996).

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