Hindawi
Journal of Nanomaterials
Volume 2017, Article ID 8706921, 11 pages
https://doi.org/10.1155/2017/8706921

Research Article

The Effect of PEI and PVP-Stabilized Gold Nanoparticles on Equine Platelets Activation: Potential Application in Equine Regenerative Medicine

Mateusz Hecold,[1] Roma Buczkowska,[1] Aleksandra Mucha,[2] Jakub Grzesiak,[3] Olga Rac-Rumijowska,[4] Helena Teterycz,[4] and Krzysztof Marycz[2,3]

[1] *Warsaw University of Life Sciences-SGGW, Nowoursynowska 100, 02-797 Warszawa, Poland*
[2] *Wroclaw University of Environmental and Life Sciences, Kozuchowska 5b, 51-631 Wroclaw, Poland*
[3] *Wroclaw Research Centre EIT+, Stablowicka 147, 54-066 Wroclaw, Poland*
[4] *Wroclaw University of Science and Technology, Wybrzeze Wyspianskiego 27, 50-370 Wroclaw, Poland*

Correspondence should be addressed to Krzysztof Marycz; krzysztofmarycz@interia.pl

Received 30 August 2016; Accepted 31 October 2016; Published 24 January 2017

Academic Editor: Md Nurunnabi

Copyright © 2017 Mateusz Hecold et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The aim of this work was to assess the effect of different stabilizing agents, for example, polyethylenimine (PEI) and polyvinylpyrrolidone (PVP), on gold nanoparticles (AuNPs) and their influence on equine platelet activation and release of particular growth factors. The gold nanoparticles were produced by chemical reduction of chloroauric acid. UV-Vis spectroscopy confirmed the presence of gold nanoparticles in investigated solutions. The AuNPs were incubated with whole blood at various concentrations. The morphology of platelets in PRP prepared from the blood incubated with AuNPs was characterized by scanning transmission electron microscopy, whereas the concentrations of growth factors and cytokines were evaluated by ELISA assays. The most promising results were obtained with equine platelets incubated with 5% AuNPs stabilized by PEI, which lead to secretion of bone morphogenetic protein 2 (BMP-2), vascular endothelial growth factor (VEGF), and fibroblast growth factor 1 (FGF-1) and simultaneously cause decrease in concentration of interleukin-1 alpha (IL-1α). The qRT-PCR confirmed ELISA test results. The incubation with 5% AuNPs stabilized by PEI leads to upregulation of BMP-2 and VEGF transcripts of mRNA level and to downregulating expression of interleukin-6 (IL-6). Obtained data shed a promising light on gold nanoparticle application for future regenerative medicine application.

1. Introduction

Nanotechnology has a significant and steadily increasing role in the current biotechnological sciences and industry. One of the branches within this interdisciplinary field of science is nanomaterials. Nanomaterial is defined as a material having at least one dimension less than 100 nm. Nanomaterials can have different forms, such as nanofibers, nanolayers, and nanoparticles. Nanoparticles possess a numerous different properties than conventional bulk materials. They have very high surface area-to-volume ratio, which leads to very high reactivity of these materials. This reactivity leads to the formation of clusters and agglomerate, which is often unwelcome phenomenon [1–5].

There are two approaches for obtaining nanoparticles: *top-down*, which is focused on removing nanosized particles from microstructural material, and *bottom-up*, which is the opposite of the first approach, because the base of this method is to synthesize nanoparticles from single atoms and molecules. Several *bottom-up* synthesis methods are available to produce nanoparticles like chemical vacuum deposition (CVD), physical vacuum deposition (PVD), self-assembly, and, the most popular, the chemical reduction. The latter of these methods relies on chemical reduction of precursor salts by the reducing agent in the presence of a stabilizing agent [1, 2, 4–8]. The main purpose of the use of stabilizers is to control the growth of the nanoparticles and impede agglomeration. A wide range of stabilizing agents is available, for example,

donor ligands, surfactants, and polymers [1, 7, 9]. There are two mechanisms of stabilization of nanoparticles. The first, electrostatic stabilization, is based on electrostatic repulsive forces resulting from the electrical double layer between the nanoparticles. The second one, called steric stabilization, is achieved through steric repulsions between the molecules, which act as protective shields on metallic surface. As a result, the nanoparticles are isolated from each other and may not agglomerate; thereby they cannot form micrometer particles and clusters. Polyelectrolytes are examples of popular stabilizing agents due to their properties, which lead to connecting steric and electrostatic stabilization. Among all polyelectrolytes as stabilizing agents, polyethylenimine (PEI) and polyvinylpyrrolidone (PVP) are well known, which work through the mentioned mechanisms [1–3, 7, 10, 11].

Nanoparticles of noble metals are in the spotlight because of their incomparable properties and wide applicability. The gold nanoparticles as well as other precious metal nanoparticles possess unique optical and physicochemical properties, such as surface plasmon resonance, which make them very attractive material for imaging and medical diagnostics [2, 4, 6, 7, 10]. Next to this there is a possibility to implement them as markers for cancer diagnostics, antitumor therapy, and drug delivery system. Currently, they have been already used, for example, in the cosmetic and pharmaceutical industry [3, 10].

Nanomaterials offer a growing range of applications, particularly in human and veterinary medicine. Tissue engineering and regenerative medicine are increasingly popular fields of current trend in science. The main aim of regenerative medicine is to regenerate damage tissues and organs and restore their functions [12]. In recent years, the importance of veterinary regenerative medicine has significantly increased due to the development of nanotechnology. It is particularly visible in equine veterinary medicine, where the majority of horses, especially racehorses, suffer from acute and chronic injuries. Equine regenerative medicine allows for quicker, more efficient, and more effective treatment of joint, ligament, and tendon injuries, compared to conventional treatments [13]. Interesting solutions, which could combine tissue engineering with regenerative medicine, are peripheral blood derived platelets. They are rich source of growth factors which can enhance injured tissue regeneration. The use of autologous platelets eliminates risk of transplant rejection or various diseases derived from the donor. Platelet-rich plasma (PRP) is very popular in equine medicine, since the cost of its production is low and the preparation in the clinic is relatively simple and not time-consuming. Platelets play one of the most important roles in homeostasis, wound healing, and inflammation. Thrombocytes commence homeostasis; they form platelet plug as they become activated. Platelet activation is a complex process involving a number of proteins. Activated thrombocytes change their structure and aggregate, thereby forming platelet plug [14–16]. Previous studies have shown that nanoparticles have an influence on the activation process [17–19]. However, they have concentrated mainly on the process of platelet activation and not on the effect of stabilization of gold nanoparticles. Platelets from platelet-rich plasma are the source of growth factors: signaling molecules involved in cells proliferation, synthesis of extracellular matrix, and vascularization. Growth factors include bone morphogenetic protein 2 (BMP-2), fibroblast growth factor 1 (FGF-1), and vascular endothelial growth factor (VEGF). BMP-2 regulates growth and induces osteoblast and chondroblast differentiation, while FGF-1 controls cell migration and proliferation. In addition, VEGF is responsible for vascularization [20]. Platelet-derived cytokines also control immune response of an organism. Interleukin-1 alpha (IL-1α) and interleukin-1 beta (IL-1β) are classified as proinflammatory cytokines. They are responsible for the induction of inflammation, fever, tissue, and organ damage [21]. Therefore, the optimal preparation of PRP should reduce the release of these proinflammatory cytokines, while increasing the release of growth factors. The incubation of platelets with gold nanoparticles has been shown as selective inducer of cytokine release from platelets [19]. Obtained gold nanoparticles might become a valuable factor, which might be used as an agent promoting platelets secretion of particular growth factors, that is, BMP-2, VEGF, and FGF-1, which are all crucial from equine regenerative medicine perspective. Our previous research showed that gold nanoparticles stimulate equine platelets for secretion of particular growth factors, which are crucial from regenerative medicine point of view [18]. The present study addressed the question whether PEI or PVP-stabilized AuNPs stimulate platelets to secrete same quantity of chosen growth factors. Moreover, the mRNA analysis of platelets was performed to show if the genes responsible for particular growth factors synthesis were upregulated.

The aim of this study was to evaluate the effect of stabilization of gold nanoparticles with two different stabilizing agents on platelets activation and secretion of particular growth factors, which are important activators of tissue regeneration processes.

2. Materials and Methods

2.1. Synthesis of Gold Nanoparticles. Gold nanoparticles (AuNPs) were synthesized by chemical reduction of aqueous solution of chloroauric acid, $HAuCl_4$ (0.04 M, 16000 ppm, POCH, Poland) [19]. Two different polymers were used as stabilizers, polyethylenimine (PEI, Sigma-Aldrich, Germany) and polyvinylpyrrolidone (PVP, Sigma-Aldrich, Germany), at a concentration of 1 M. Aqueous solution of ascorbic acid (0.2 M, POCH, Poland) was used as a reducing agent. The solutions of $HAuCl_4$ and ascorbic acid were prepared directly before synthesis. Both syntheses were carried out in the same way at room temperature. First 0.5 mL of PEI and PVP was dissolved in distilled water (250 mL) under magnetic stirring for 5 min and then 0.625 mL of chloroauric acid and 0.5 mL of ascorbic acid were added. The final concentration of AuNPs in solutions was 50 ppm [8].

2.2. Characterization of AuNPs. The presence of AuNPs in solutions was verified using the spectrophotometer UV-Vis (QE6500, Ocean Optics). The absorption spectrum was measured at a wavelength of 300 to 800 nm in quartz cuvette.

Dynamic Light Scattering (DLS) was used to determine the size and the distribution profile of gold particles in dispersions. The Particle Sizing System NICOMP 380, a product of Nicomp, (Santa Barbara, USA), was used. This technique can be used to determine the size distribution profile of small particles in colloid solution.

The analysis of gold nanoparticles was performed using scanning electron microscope (Auriga 60, Zeiss) at 2 kV. Before SEM observation, each of the solutions of gold nanoparticles was applied on the aluminum stubs and dried at room temperature.

2.3. Incubation of Equine Whole Blood with Gold Nanoparticles. Equine peripheral blood was collected to the blood containers with CDPA anticoagulant. Equine blood was incubated in centrifuge tubes with 0.1 mL, 0.5 mL, and 1 mL of gold nanoparticles-stabilized PEI solutions and 0.1 mL, 0.5 mL, and 1 mL of gold nanoparticles-stabilized PVP solutions. The final concentrations of AuNPs in equine blood were 1%, 5%, and 10%. The tubes with a blood were incubated at 37°C for 24 h to activate platelets. Blood cells were separated from plasma by centrifugation for 10 minutes at 300 ×g. Received fractions were transferred to new tubes. Plasma was centrifuged at 640 ×g for 10 minutes to separate platelet-rich plasma (PRP) from platelet-poor plasma (PPP).

2.4. Morphological Analysis of Platelets. The platelets from PRP were fixed with 2.5% glutaraldehyde for 1 hour at room temperature; then they were divided into two sets. First group of thrombocytes were centrifuged at 640 ×g for 10 minutes and glutaraldehyde was removed from the tubes. The pellets were washed two times using Sorensen's buffer (pH = 7.3) and also two times with ultrapure distilled water. Subsequently, the pellets were dehydrated in a graded series of ethyl alcohol. The pellets were centrifuged at 640 ×g for 10 minutes before each washing. After dehydration, the solutions of platelets suspended in 99.8% of ethyl alcohol were applied on microscope slide, dried at room temperature, and sputtered with film of gold. The morphology of specimens was analyzed using scanning electron microscopy (EVO LS15, Zeiss) at 10 kV.

To analyze the ultrastructure of platelets from each group, samples fixed with glutaraldehyde were incubated in 2% buffered aqueous solution of osmium tetroxide (pH = 7.3) for one hour. Next, samples were washed three times with buffer and two times with water, followed by the incubation of platelets in 1% of uranyl acetate aqueous solution overnight at 4°C. After triple washing in water, platelets were centrifuged at 1200 ×g for 10 min and embedded in Epon 812 resin. The ultrathin sections were obtained using ultramicrotome (Leica UC7) and observed with scanning electron microscope (Auriga 60, Zeiss) using the scanning transmission electron microscopy (STEM) detector at 20 kV.

2.5. Detection of BMP-2, VEGF, FGF-1, IL-1α, and IL-1β in Equine PRP. ELISA assays were performed for the detection and quantification of bone morphogenetic protein 2 (BMP-2), vascular endothelial growth factor (VEGF), fibroblast growth factor 1 (FGF-1), interleukin-1 alpha (IL-1α), and interleukin-1 beta (IL-1β) released from platelets during their incubation with AuNPs. ELISA kits were purchased from MyBioSource Inc., San Diego, CA, USA. The procedures were performed according to the instructions provided by the vendor. ELISA tests were performed on the PRP incubated with different concentration of gold nanoparticles stabilized with PEI and PVP and on a control sample incubated at same conditions but without addition of nanoparticles.

3. Results and Discussion

3.1. Characterization of AuNPs. The results of spectrophotometric analysis confirmed the presence of gold nanoparticles stabilized by PEI and PVP. The characteristic peak for AuNPs at 530 nm wavelength was observed for each solution (Figures 1(a) and 1(b)).

Kinetic of formation of nanoparticles and their stabilization over time was evaluated. The differences in rate and mechanism of gold nanoparticles formation are the results of the use of different stabilizers. During the stabilization of gold nanoparticles by PVP, ascorbic acid was also added as a reducing agent. The UV-Vis spectra show a peak at 520 nm wavelength, which does not change during the synthesis. This means that the total reduction of gold occurs immediately after adding chloroauric acid to mixture comprising stabilizing and reducing agents. Peak at 250 nm is visible, which after 135 min significantly decreases (Figure 2). It may indicate the formation of coordination complexes between ions of gold and polymer particles [22]. These interactions cause nanoparticles to be synthesized in polymeric micelle and not agglomerate.

AuNPs stabilized by PEI do not require additional reducing agent, because this polymer may have dual role during the synthesis of gold nanoparticles [23]. In this case, nanoparticles are formed slowly; the characteristic peak for gold nanoparticles was visible after 1 hour and increased for next hour until a constant level. The absorption spectrum showed that both types of polymeric stabilizers ensured long-time stability of gold nanoparticles.

The morphology of the generated nanoparticles using PEI and PVP was investigated by DLS and SEM. Figure 2(a) displays the diameter of 6.5 nm (36.5%) and 49.2 nm (3.5%) for PEI-stabilized AU nanoparticles, whereas it is 8.2 nm in case of PVP. The discrepancies between DLS results (20–50 nm) and higher AuNPs diameters measured in SEM might result from the air-drying process of particles prior to SEM analysis, though only the freeze-drying of nanoparticles prior to SEM observations preserves their size and prevents them from agglomeration (Figure 3) [24]. The analysis of EDX showed that in addition to the atoms of the precursor there are no other substances in the solution (Figure 4). Copper appears in the spectrum from the substrate on which the nanoparticles are applied in the preparation of the sample.

Chemical reduction of chloroauric acid is relatively quick and common method for obtaining gold nanoparticles [25].

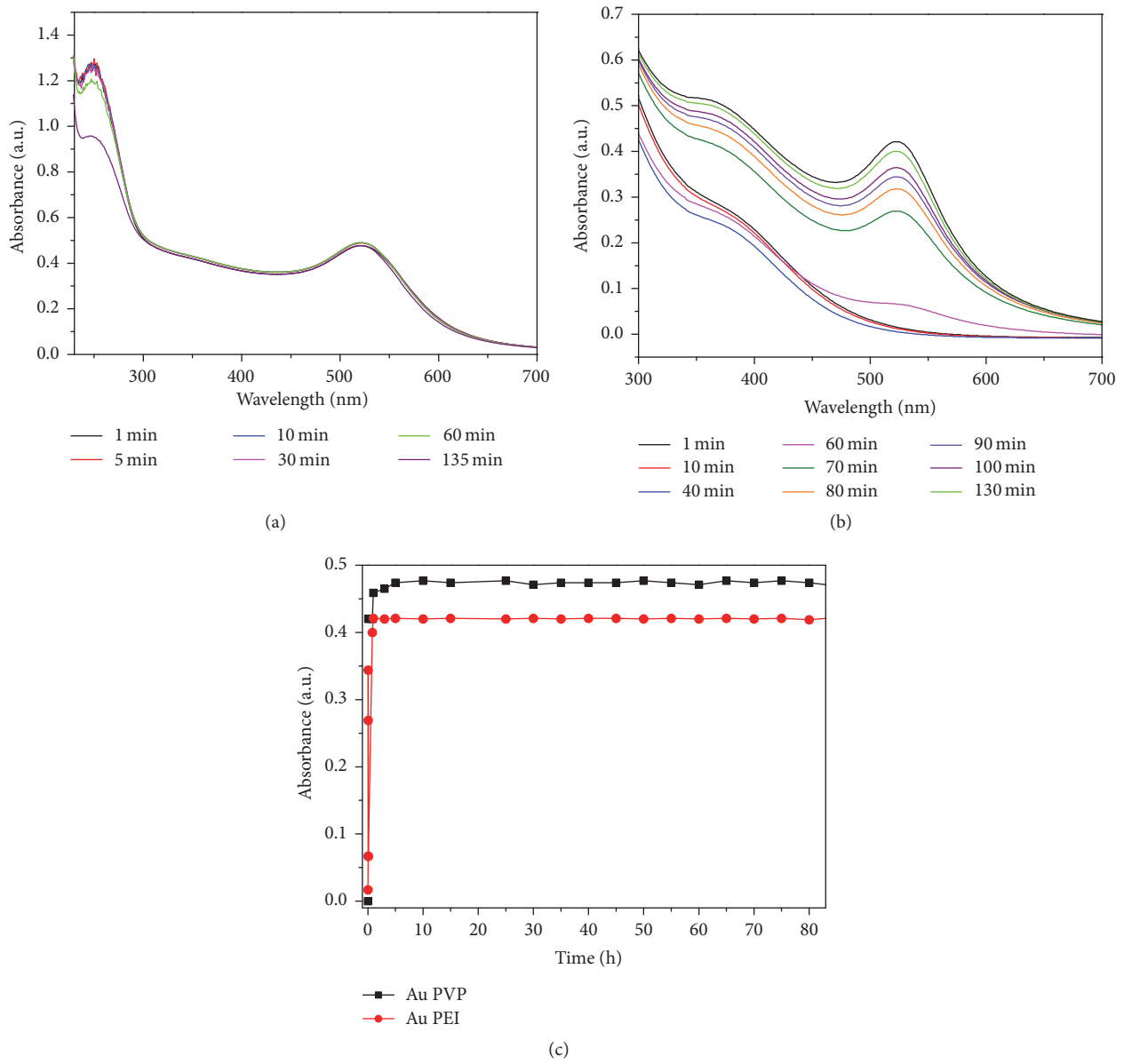


Figure 1: UV-Vis spectra of gold nanoparticles: (a) formation kinetics of AuNPs with PVP, (b) formation kinetics of AuNPs with PEI, and (c) test time-dependent stability.

The microphotography of gold nanoparticles showed difference between size and agglomeration of AuNPs according to stabilization agent. Diameter of AuNPs stabilized by PEI was approximately 20 nm, when diameter of AuNPs stabilized by PVP was higher and amounted to 50 nm. Moreover, agglomeration of AuNPs stabilized by PVP was bigger. It might be related to the length of polymer molecules. Polymers of low molecular weight cause weaker stabilization of the nanoparticles and consequently increase their size. Polymers of high molecular weight effectively surround the nanoparticles and inhibit their agglomeration [1, 26]. PEI (M_w = 750000) is characterized by higher average molecular weight than PVP (M_w = 58000). According to previous studies, the polymer with higher molecular weight has better protective properties and is a better stabilizer. This is due to the fact that the chemical bond is formed between one end of the polymer chain and nanoparticle, whereas other portions of the chain create a loose layer, which surrounds the nanoparticle. If the length of the polymer is too short, it cannot be a fully efficient stabilizing agent; in other words, the nanoparticles are not completely covered with the polymer layer, which leads to agglomeration. The agglomeration of nanoparticles is the main problem in production of these unconventional materials, because it negatively affects their properties by increasing the size of particles and simultaneously decreasing the surface-to-volume ratio. The size of agglomerated nanoparticles accrues to several micrometers, which definitely is undesired effect, because nanoparticles

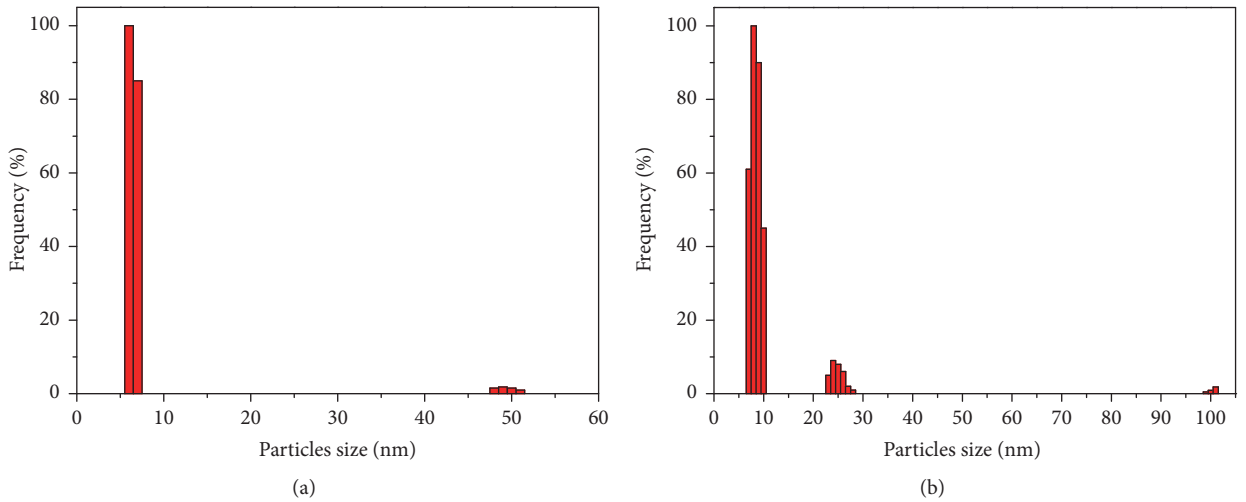


Figure 2: DLS results of the diameter distribution of gold nanoparticles stabilized by PEI (a) and PVP (b).

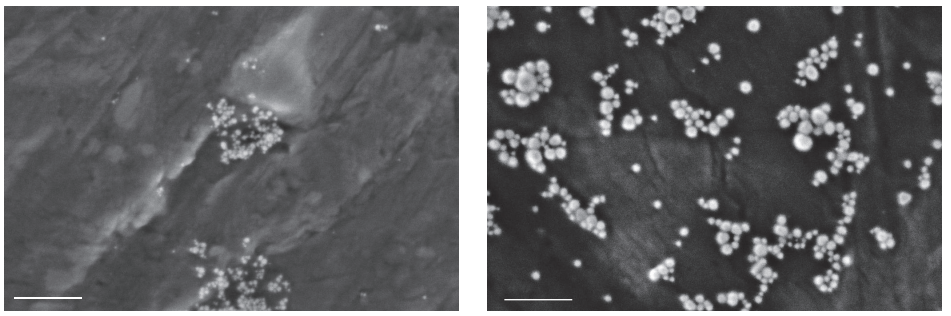


Figure 3: SEM images of AuNPs stabilized by (a) PEI and (b) PVP.

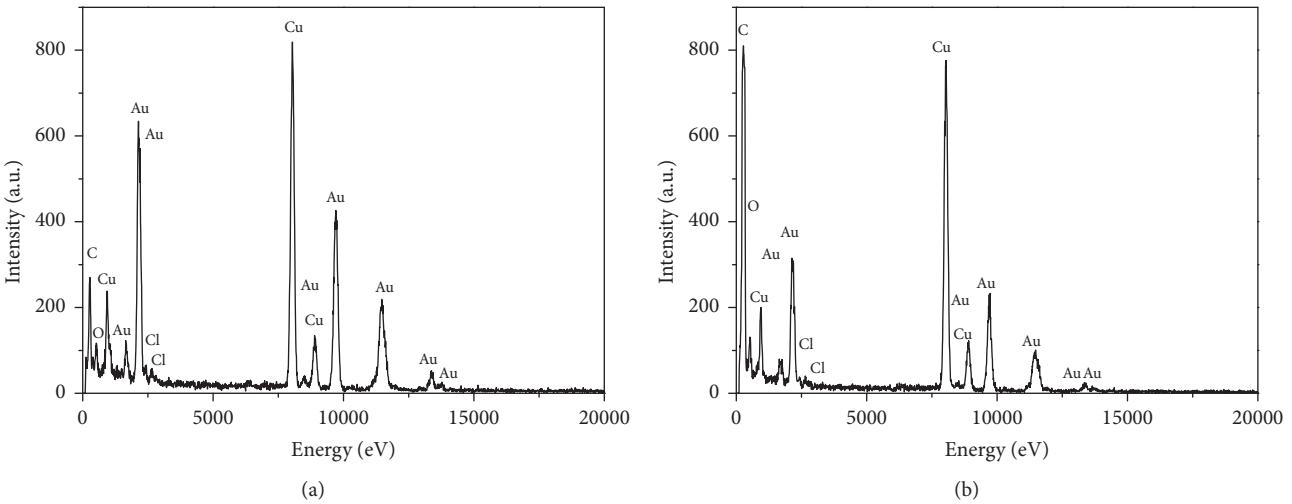


Figure 4: EDX results of gold nanoparticles stabilized by PEI (a) and PVP (b).

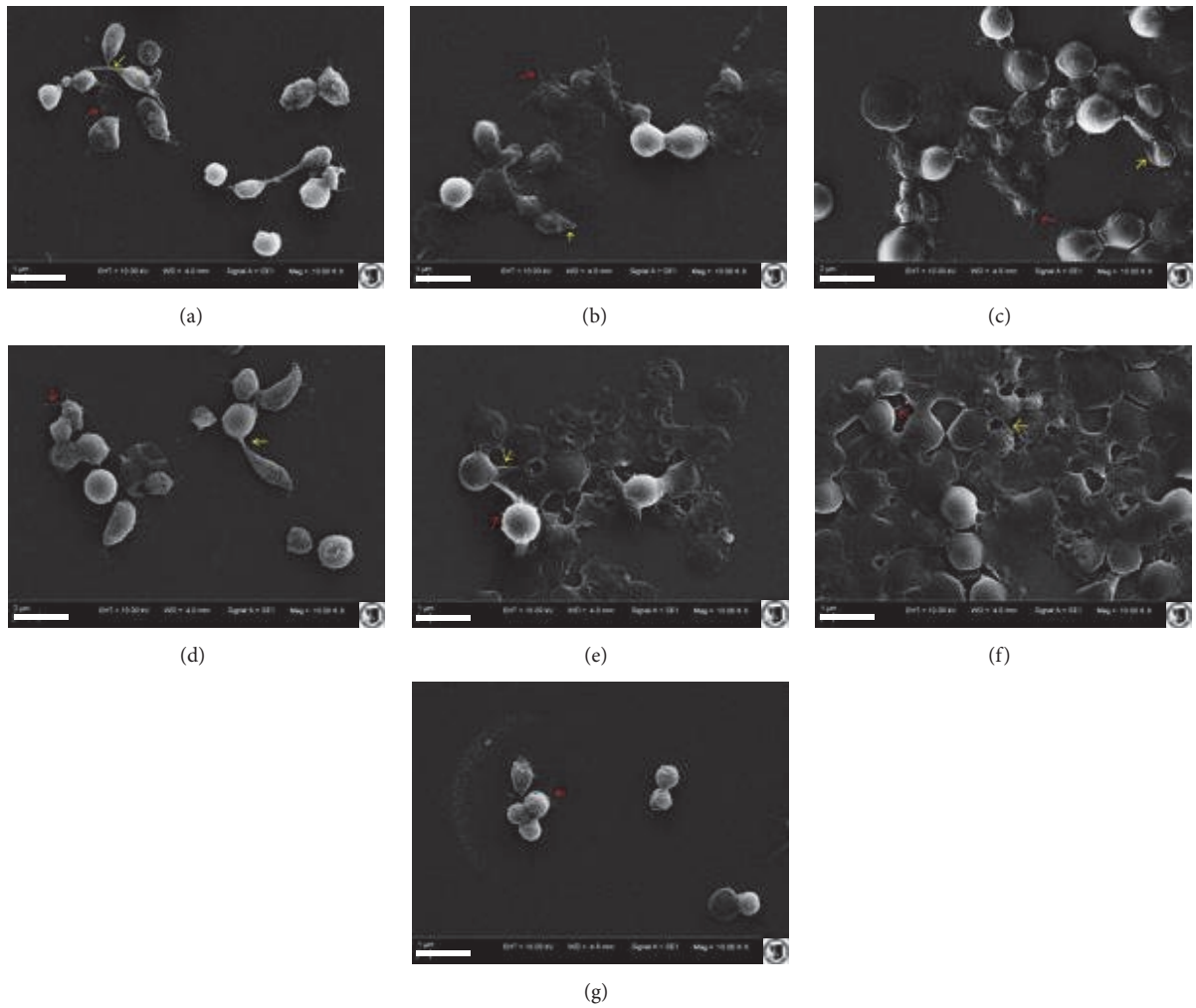


Figure 5: SEM images of platelets morphology incubated with different concentrations of AuNPs and stabilized by different polymers agent: (a) 1% AuNPs stabilized by PEI, (b) 5% AuNPs stabilized by PEI, (c) 10% AuNPs stabilized by PEI, (d) 1% AuNPs stabilized by PVP, (e) 5% AuNPs stabilized by PVP, (f) 10% AuNPs stabilized by PVP, and (g) control sample. Filopodia are marked with red arrows; lamellipodia are marked with yellow arrows. Magnifications, 10000x; scale bars = 5 μm.

lose their unique properties. However, the agglomeration does not occur in the solution, for example, during the incubation with whole blood.

3.2. Influence of AuNPs on Platelets' Morphology and Ultrastructure. SEM images of platelets showed the impact of gold nanoparticles on platelets activation and aggregation. The platelets incubated at 37°C without nanoadditive had discoidal shape and they did not form agglomerate; also only single filopodia were formed (Figure 5(g)).

STEM analysis of thrombocytes from control group showed the presence of microtubules and cell organelles, especially alpha granules and dense granules (Figures 6(g) and 7(g)). The α-granules are characterized by the heterogeneous electron density, with more dense central region and less dense peripheral area. They contain mostly the growth factors. The δ-granules are characterized by the homogeneous high electron density and contain mostly the proinflammatory cytokines. Incubation of thrombocytes with 1% AuNPs resulted in changes of platelets morphology, compared with morphology of control sample. Shape of platelets was more irregular, more filopodia were observed, and lamellipodia were also noticeable. Similar changes were observed for the two types of stabilized gold nanoparticles (Figures 5(a) and 5(d)). Ultrastructural investigation showed that the most of organelles remained in the platelets, with some of α-granules released outside the cell membrane. Moreover, the changes in platelets, which were visible on SEM images, have been confirmed. The shape of platelets was more irregular and thrombocytes aggregated (Figures 6(a) and 6(d)). The higher aggregation of platelets was observed in thrombocytes incubated with 5% AuNPs. The ultrastructural analysis of platelets morphology revealed presence of numerous filopodia and lamellipodia. Stage of activation was more advanced than in incubation with 1% of gold nanoparticles; numerous filopodia and lamellipodia were visible (Figures 5(b) and 5(e)). Ultrastructural

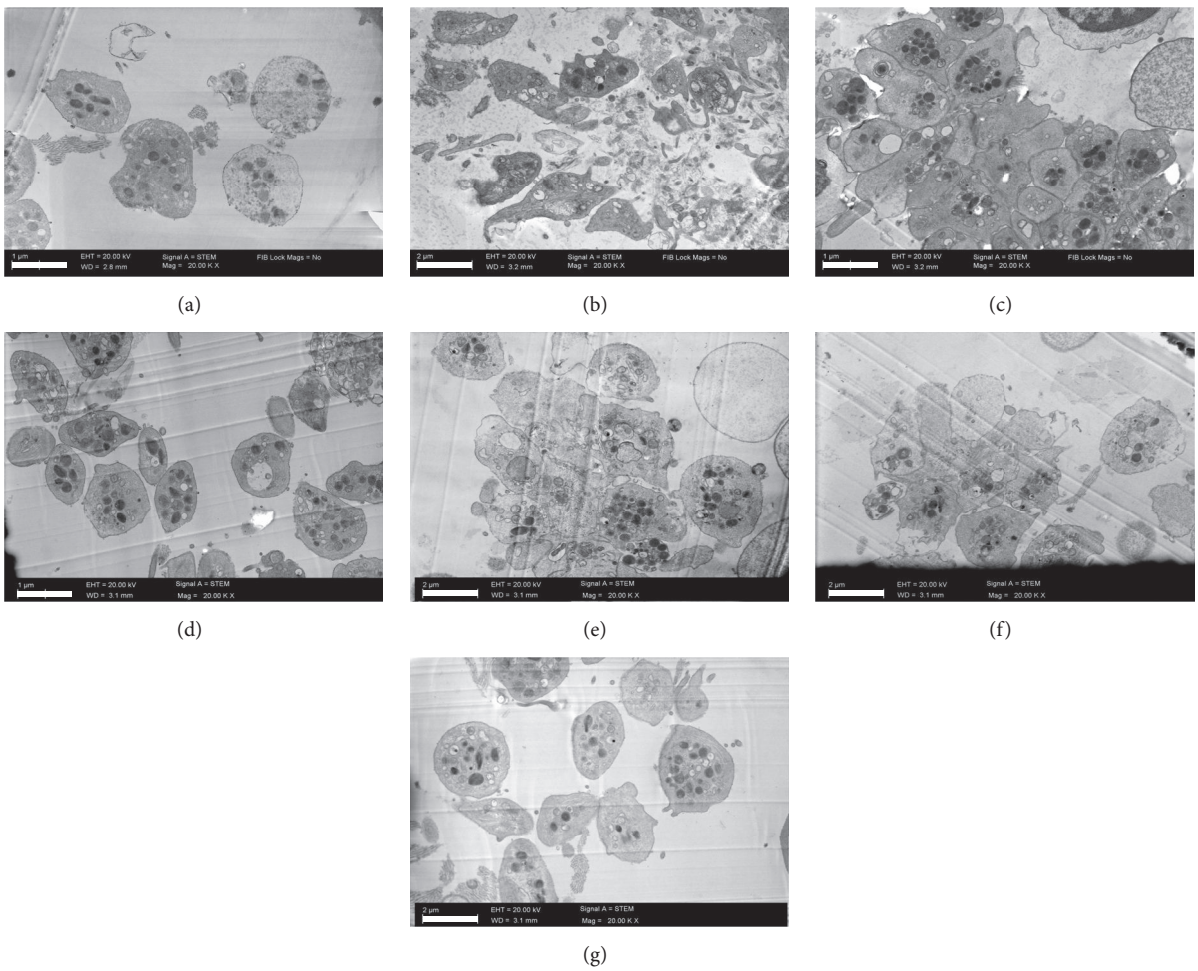


Figure 6: STEM images of platelets incubated with different concentrations of AuNPs and stabilized by different polymers agent: (a) 1% AuNPs stabilized by PEI, (b) 5% AuNPs stabilized by PEI, (c) 10% AuNPs stabilized by PEI, (d) 1% AuNPs stabilized by PVP, (e) 5% AuNPs stabilized by PVP, (f) 10% AuNPs stabilized by PVP, and (g) control sample. Magnifications, 20000x; scale bar = 2 μm.

investigation confirmed morphological changes in platelets. Merging of cell membranes, disorganization of microtubules, and released granules were observed; however, AuNPs stabilized by PVP did not induce the release of δ-granules (Figures 6(b) and 7(b)). Platelets incubated with 10% AuNPs had the most developed microstructure, numerous pseudopodia were observed, and the level of aggregations was the highest (Figures 5(c) and 5(f)). The microtubules inside thrombocytes were not visible. Cell membranes were severed and merged, while α-granules were released in both stabilizers. Additionally, δ-granules were released in the sample with AuNPs stabilized by PEI. In platelets with AuNPs stabilized by PVP, δ-granules moved in the platelets' center but were not released (Figures 6(c), 6(f), 7(c), and 7(f)).

The morphology and ultrastructure of platelets are important features, which might provide valuable information concerning their secretory activity. Observations revealed that the morphology of platelets changed compared to a control sample. The platelets activation occurred in each of the incubated samples. The number of morphological changes in thrombocytes was directly proportional to the concentration of nanoparticles, which were used for incubation with equine blood. The platelets changed their discoidal shape irregularly; also numerous pseudopodia were visible. Granules were released outside the membrane cells and additionally platelets aggregation was observed. Morphological changes of thrombocytes confirm the beginning of activation process. 1% concentration of AuNPs was too low for making significant changes in the platelets morphology. However, the blood incubated with 5% of AuNPs stabilized with PEI had most flattened morphology and was degranulated. In turn, the 10% of AuNPs stabilized with PEI led to the most effective platelets degranulation and morphological changes. Both stabilizers allowed for the release of the α-granules; however, only the PVP inhibited the release of δ-granules content. Bearing in mind the fact that the benefits for regenerative medicine growth factors localize in the α-granules, while the serotonin and histamine are localized within the δ-granules [26], the PVP-stabilized AuNPs seem to be more suitable for regenerative medicine purpose, since the proinflammatory granules were not released. This could probably result from the higher diameter of PVP-stabilized

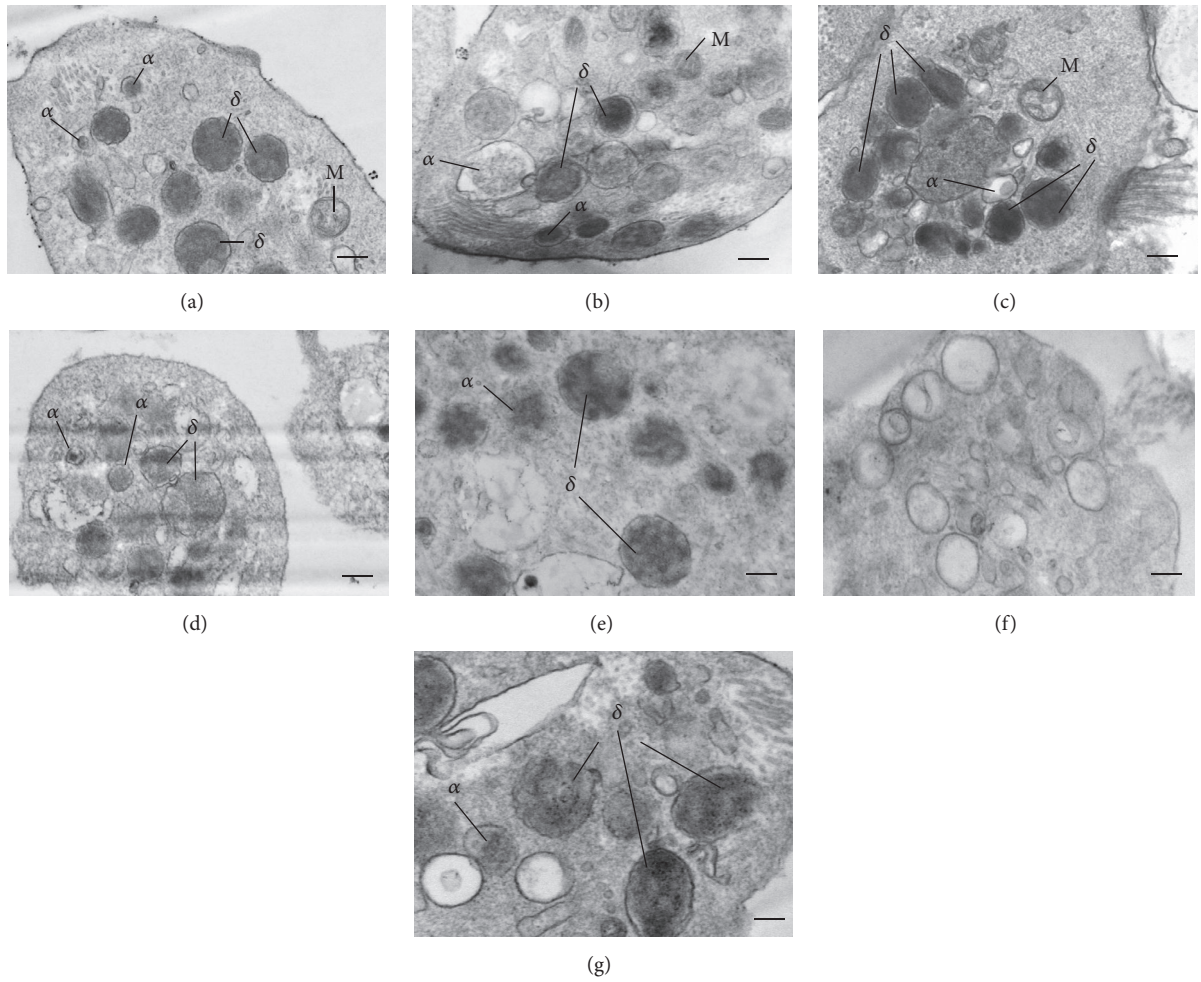


Figure 7: Ultrastructure of platelets incubated with different concentrations of AuNPs and stabilized by different polymers agent: (a) 1% AuNPs stabilized by PEI, (b) 5% AuNPs stabilized by PEI, (c) 10% AuNPs stabilized by PEI, (d) 1% AuNPs stabilized by PVP, (e) 5% AuNPs stabilized by PVP, (f) 10% AuNPs stabilized by PVP, and (g) control sample. α, alpha granules; δ, delta granules; M, mitochondria. Scale bars = 200 nm.

AuNPs, while the smaller nanoparticles stabilized with PEI induced the release of proinflammatory granules. Interestingly, although the release of alpha granules was similar in all investigated groups, the levels of growth factors differed between them. While the distribution and activity of alpha-granule-derived growth factor moieties are dependent on the interactions between them and various adhesive proteins and other molecules, the AuNPs probably influenced this mechanism, leading to different amplification of functional protein expression [27]. However, the exact mechanism of this interaction has yet to be explained.

There is an important question whether the AuNPs might be transferred to the patient during the administration of prepared PRP due to the safety concerns. However, the microscopic ultrastructural analysis revealed the absence of AuNPs within the analyzed platelets. Therefore, they are not internalized to platelets but are probably removed from the whole blood by subsequent centrifugations during preparation of PRP due to high density of AuNPs in relation to the blood components. Therefore, the risk of transferring them to the patient together with PRP is very low.

3.3. ELISA Assays for Growth Factors BMP-2, VEGF, and FGF-1 and Proinflammatory Cytokines IL-1α and IL-1β. The utilization of growth factors derived from various cellular sources is one of the main goals of applied regenerative medicine in humans and animals [28]. Therefore, the main focus was put on the influence of AuNPs on the concentrations of certain growth factors in the equine platelet-rich-plasma. The highest concentration of BMP-2 was observed in the sample containing 5% of AuNPs stabilized with PEI ($p < 0.01$; Figure 8(a)). The lowest concentration of BMP-2 was observed in samples containing 1% of AuNPs stabilized with both PEI and PVP. Interestingly, the concentration of 10% AuNPs stabilized with PVP resulted in lower concentration of BMP-2 than 5% PEI. When the concentration of VEGF is considered, 5% AuNPs stabilized by PEI stimulated the

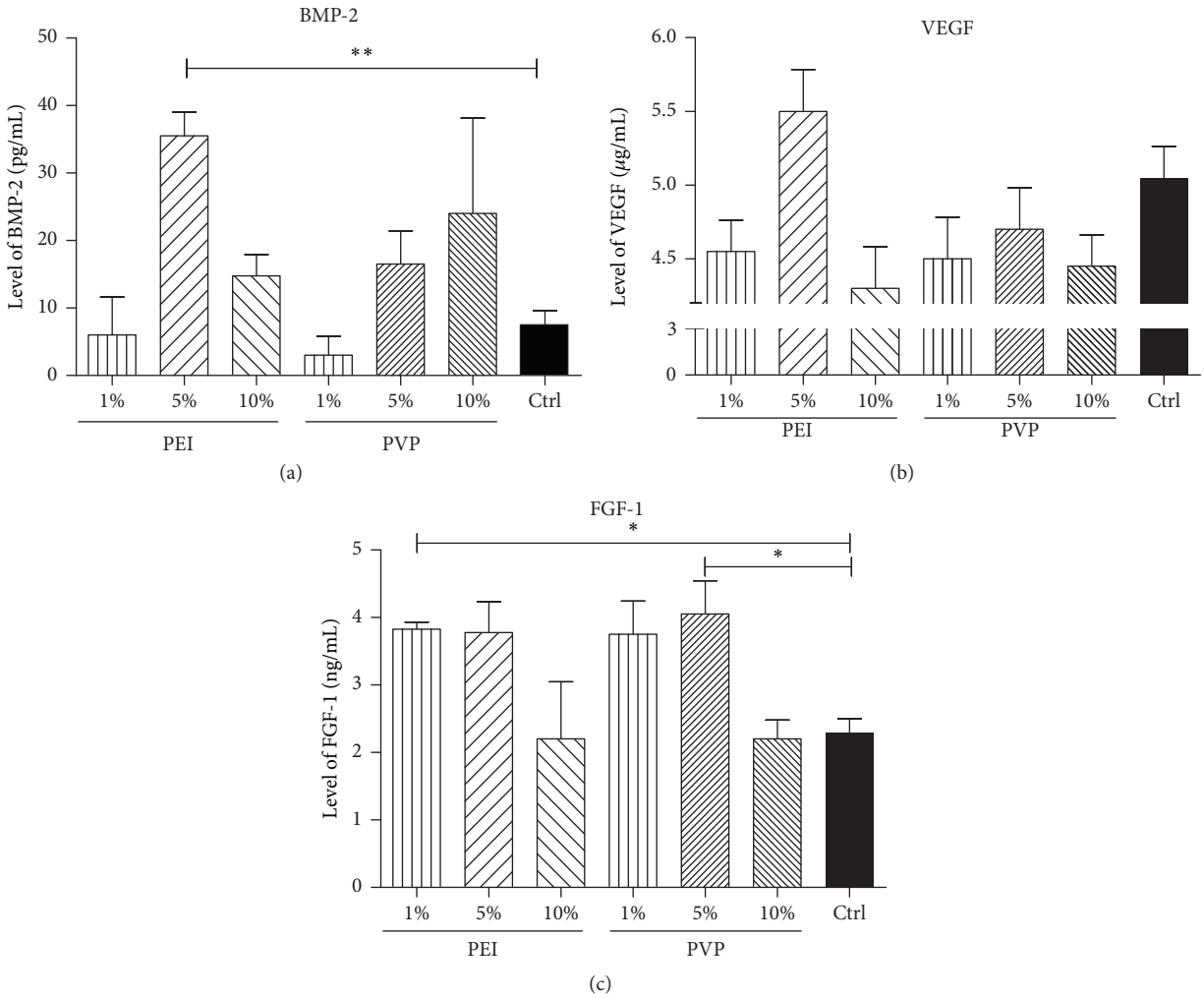


Figure 8: The concentration of growth factors (a) BMP-2, (b) VEGF, and (c) FGF-1 in PRP incubated with different concentrations of AuNPs; $^{*}p < 0.05$ and $^{**}p < 0.01$.

platelets to realizing the highest concentration of this growth factor (Figure 8(b)). On the other hand, 1% and 10% of AuNPs stabilized by both polymers inhibited secretion of VEGF. Also, 5% AuNPs stabilized by PVP brought on a decrease of VEGF concentrations, in relation to not-treated-with-AuNPs control. Contrary to VEGF, AuNPs did not adversely affect the release of FGF-1. The concentrations of FGF-1 in the plasmas with 10% AuNPs stabilized by PEI and by PVP were approximate to FGF-1 concentration in the control sample (Figure 8(c)). Furthermore, about 1.6 times higher concentration was observed in the samples with 1% AuNPs and 5% AuNPs stabilized by PEI and by PVP. Since all these growth factors are localized in the alpha granules, the influence of AuNPs on their release is probably connected not only with the inhibition or activation of degranulation but also with more complex interaction between adhesion molecules and receptors [27]. All investigated growth factors play substantial role in the regeneration processes: BMP-2 is a pleiotropic molecule that plays a broad role not only in the development of cartilage and bone tissues but also in cardiac differentiation and other processes [29] and VEGF is the molecule crucial for angiogenesis and vascularization [30], while FGF-1 is a protein involved in angiogenesis and wound healing [31].

Interleukin-1α and interleukin-1β are proinflammatory molecules with pleiotropic character. They are involved in the initiation of inflammatory reaction and can be produced and released by various cell types [32]. Experiment revealed that the level of IL-1α (Figure 9(a)) significantly increased in samples incubated with 5% AuNPs, 10% AuNPs stabilized by PEI, and also 10% AuNPs stabilized by PVP, notwithstanding 1% AuNPs stabilized by PEI and 5% stabilized by PVP inhibited release of IL-1α. Moreover, the concentration of IL-1β (Figure 9(b)) decreased in all of the experimental samples stabilized by both polymers. Interleukins are localized within the cytoplasm of platelets, not in the granules, as it was determined by Dinarello [33]. Moreover, the release of these two interleukins is dependent on the platelet disintegration process during their activation: in disintegrated platelets, their release is facilitated. While these molecules are naturally

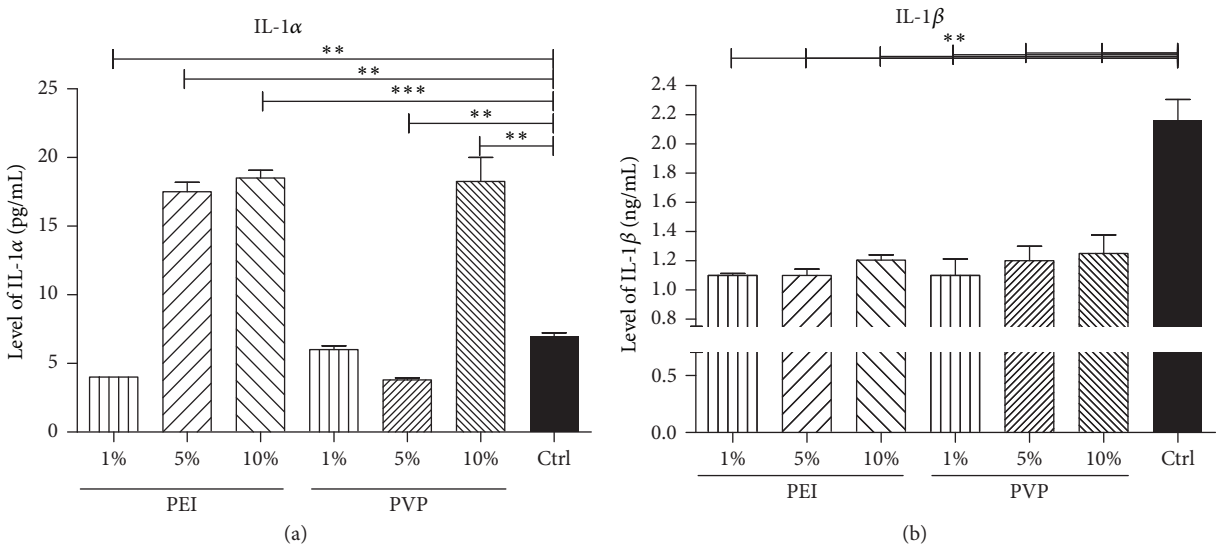


Figure 9: The concentration of cytokines (a) IL-1α and (b) IL-1β in PRP incubated with different concentrations of AuNPs; $^{**}p < 0.01$ and $^{***}p < 0.005$.

adsorbed on the certain proteins (e.g., fibrin), IL-1β might be adsorbed on AuNPs, while its level was similar in all investigated samples and significantly lower than that in control. On the other hand, the level of IL-1α increased with the concentration of AuNPs, suggesting the dependence of its concentration on the platelet activation status. These assumptions, however, need more analysis for confirmation.

4. Conclusion

This present research has shown that size and shape of the nanoparticles are strongly influenced by the stabilizing agents. Development on stabilization allows controlling the production of tailored nanoparticles. Stabilized gold nanoparticles catalyze the activation of platelets and selective release of growth factors; however, the mechanism of this action has not been explained yet. Nevertheless, these results could be particularly valuable for equine regenerative medicine applications.

Competing Interests

The authors declare that they have no competing interests.

References

[1] X. Sun, S. Dong, and E. Wang, "One-step preparation of highly concentrated well-stable gold colloids by direct mix of polyelectrolyte and $HAuCl_4$ aqueous solutions at room temperature," *Journal of Colloid and Interface Science*, vol. 288, no. 1, pp. 301–303, 2005.

[2] H. Wang, X. Qiao, J. Chen, and S. Ding, "Preparation of silver nanoparticles by chemical reduction method," *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, vol. 256, no. 2-3, pp. 111–115, 2005.

[3] S. Wen, F. Zheng, M. Shen, and X. Shi, "Synthesis of polyethyleneimine-stabilized gold nanoparticles for colorimetric sensing of heparin," *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, vol. 419, pp. 80–86, 2013.

[4] K. Zarschler, L. Rocks, N. Licciardello et al., "Ultrasmall inorganic nanoparticles: state-of-the-art and perspectives for biomedical applications," *Nanomedicine: Nanotechnology, Biology and Medicine*, vol. 12, no. 6, pp. 1663–1701, 2016.

[5] P. Chen, S. Mwakwari, and A. Oyelere, "Gold nanoparticles: from nanomedicine to nanosensing," *Journal Nanotechnology, Science and Applications*, vol. 1, pp. 45–66, 2008.

[6] H. S. Shin, H. J. Yang, S. B. Kim, and M. S. Lee, "Mechanism of growth of colloidal silver nanoparticles stabilized by polyvinyl pyrrolidone in γ-irradiated silver nitrate solution," *Journal of Colloid and Interface Science*, vol. 274, no. 1, pp. 89–94, 2004.

[7] H. Wang, X. Qiao, J. Chen, X. Wang, and S. Ding, "Mechanisms of PVP in the preparation of silver nanoparticles," *Materials Chemistry and Physics*, vol. 94, no. 2-3, pp. 449–453, 2005.

[8] M. Singh, S. Manikandan, and A. Kumaraguru, "Nanoparticles: a new technology with wide applications," *Research Journal of Nanoscience and Nanotechnology*, vol. 1, no. 1, pp. 1–11, 2011.

[9] N. A. Cortez-Lemus, A. Licea-Claverie, F. Paraguay-Delgado, and G. Alonso-Nuñez, "Gold nanoparticles size design and control by poly(N,N′-diethylaminoethyl methacrylate)," *Journal of Nanomaterials*, vol. 2015, Article ID 273814, 10 pages, 2015.

[10] A. Zielinska, E. Skwarek, A. Zaleska et al., "Preparation of silver nanoparticles with controlled particle size," *Procedia Chemistry*, vol. 1, no. 2, pp. 1560–1566, 2009.

[11] P. Everts, J. Knappe, G. Weibrich et al., "Platelet-rich plasma and platelet gel: a review," *The Journal of The American Society of Extra-Corporeal Technology*, vol. 38, pp. 174–187, 2006.

[12] C. Mason and P. Dunnil, "A brief definition of regenerative medicine," *Regenerative Medicine*, vol. 3, no. 1, pp. 1–5, 2008.

[13] L. A. Fortier and R. K. W. Smith, "Regenerative medicine for tendinous and ligamentous injuries of sport horses," *Veterinary Clinics of North America: Equine Practice*, vol. 24, no. 1, pp. 191–201, 2008.

[14] T. Kawase, "Platelet-rich plasma and its derivatives as promising bioactive materials for regenerative medicine: basic principles and concepts underlying recent advances," *Odontology*, vol. 103, no. 2, pp. 126–135, 2015.

[15] D. Segura, L. Monreal, S. Pérez-Pujol et al., "Assessment of platelet function in horses: ultrastructure, flow cytometry, and perfusion techniques," *Journal of Veterinary Internal Medicine*, vol. 20, no. 3, pp. 581–588, 2006.

[16] J. W. M. Heemskerk, E. M. Bevers, and T. Lindhout, "Platelet activation and blood coagulation," *Thrombosis and Haemostasis*, vol. 88, no. 2, pp. 186–193, 2002.

[17] G. F. Guidetti, A. Consonni, L. Cipolla, P. Mustarelli, C. Balduini, and M. Torti, "Nanoparticles induce platelet activation in vitro through stimulation of canonical signalling pathways," *Nanomedicine: Nanotechnology, Biology, and Medicine*, vol. 8, no. 8, pp. 1329–1336, 2012.

[18] H. N. Verma, P. Singh, and R. M. Chavan, "Gold nanoparticle: synthesis and characterization," *Veterinary World*, vol. 7, no. 2, pp. 72–77, 2014.

[19] K. Marycz, J. Kolankowski, J. Grzesiak, M. Hecold, O. Rac, and H. Teterycz, "Application of gold nanoparticles of different concentrations to improve the therapeutic potential of autologous conditioned serum: potential implications for equine regenerative medicine," *Journal of Nanomaterials*, vol. 2015, Article ID 521207, 9 pages, 2015.

[20] M. Rodrigues, L. G. Griffith, and A. Wells, "Growth factor regulation of proliferation and survival of multipotential stromal cells," *Stem Cell Research and Therapy*, vol. 1, no. 4, article 32, 2010.

[21] C. A. Dinarello, "Proinflammatory cytokines," *Chest*, vol. 118, no. 2, pp. 503–508, 2000.

[22] L. Mercado, W. Castro, E. Vicuna et al., "Real time kinetic measurements of silver nanocluster growth," in *Proceedings of the International Conference on Computational Nanoscience and Nanotechnology (ICCN '02)*, pp. 439–442, San Juan, Puerto Rico, USA, April 2002.

[23] C. Note, S. Kosmella, and J. Koetz, "Poly(ethyleneimine) as reducing and stabilizing agent for the formation of gold nanoparticles in w/o microemulsions," *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, vol. 290, no. 1–3, pp. 150–156, 2006.

[24] A. Bootz, V. Vogel, D. Schubert, and J. Kreuter, "Comparison of scanning electron microscopy, dynamic light scattering and analytical ultracentrifugation for the sizing of poly(butyl cyanoacrylate) nanoparticles," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 57, no. 2, pp. 369–375, 2004.

[25] P. R. Selvakannan, S. Mandal, S. Phadtare et al., "Water-dispersible tryptophan-protected gold nanoparticles prepared by the spontaneous reduction of aqueous chloroaurate ions by the amino acid," *Journal of Colloid and Interface Science*, vol. 269, no. 1, pp. 97–102, 2004.

[26] O. Rac, P. Suchorska-Woźniak, M. Fiedot, and H. Teterycz, "Influence of stabilizing agents and pH on the size of SnO_2 nanoparticles," *Beilstein Journal of Nanotechnology*, vol. 5, pp. 2192–2201, 2014.

[27] F. Rendu and B. Brohard-Bohn, "The platelet release reaction: granules' constituents, secretion and functions," *Platelets*, vol. 12, no. 5, pp. 261–273, 2001.

[28] P. Harrison, G. F. Savidge, and E. M. Cramer, "The origin and physiological relevance of alpha-granule adhesive proteins," *British Journal of Haematology*, vol. 74, no. 2, pp. 125–130, 1990.

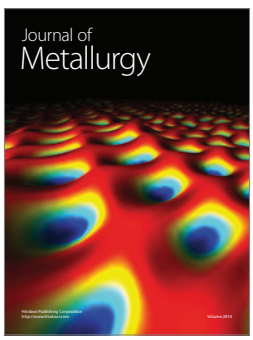
[29] J. Ramos-Torrecillas, E. De Luna-Bertos, O. García-Martínez, and C. Ruiz, "Clinical utility of growth factors and platelet-rich plasma in tissue regeneration: a review," *Wounds*, vol. 26, no. 7, pp. 207–213, 2014.

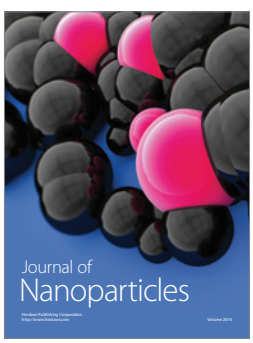
[30] P. J. Marie, F. Debiais, and E. Haÿ, "Regulation of human cranial osteoblast phenotype by FGF-2, FGFR-2 and BMP-2 signaling," *Histology and Histopathology*, vol. 17, no. 3, pp. 877–885, 2002.

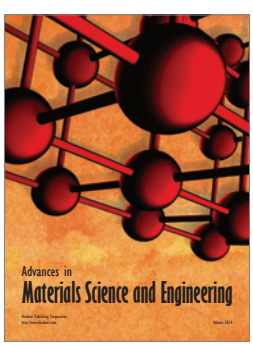
[31] A. J. B. O. G. Salgado, R. L. G. Reis, N. J. C. Sousa, and J. M. Gimble, "Adipose tissue derived stem cells secretome: soluble factors and their roles in regenerative medicine," *Current Stem Cell Research & Therapy*, vol. 5, no. 2, pp. 103–110, 2010.

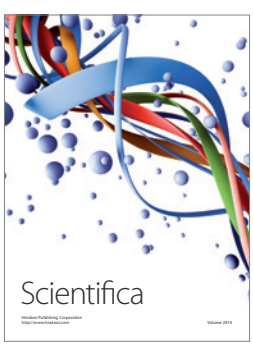
[32] D. Gospodarowicz, G. Neufeld, and L. Schweigerer, "Fibroblast growth factor," *Molecular and Cellular Endocrinology*, vol. 46, no. 3, pp. 187–204, 1986.

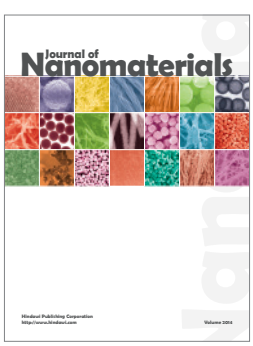
[33] C. A. Dinarello, "Interleukin-1," in *The Cytokine Handbook*, A. Thomson, Ed., pp. 47–82, Academic Press, London, UK, 1991.

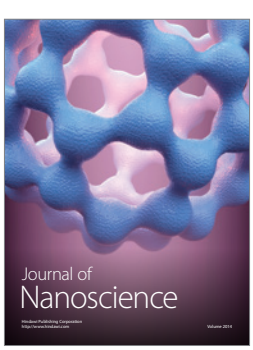


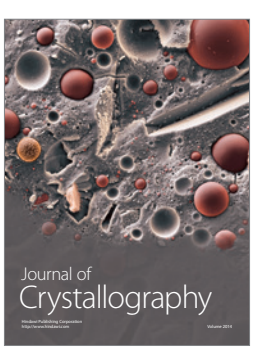


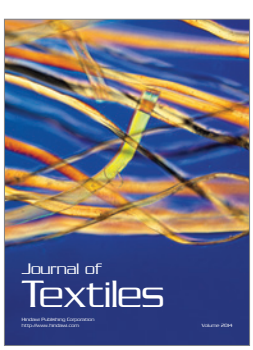












Hindawi

Submit your manuscripts at
https://www.hindawi.com