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Araştırma Makalesi / Research Article

Synthesis and Characterization of TiO_2, Ag and TiO_2@Ag Nanoparticles via Assessing the Effects on Stem Cells in vitro

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Abstract

Stem cells and nanotechnology are two of the most talented research areas and a new area called stem cell nanotechnology has emerged. With knowledge of nanoparticles's inter- and intra-cellular transport abilities, developments on stem cell nanotechnology increases. The aim of this study is to investigate the effects of different featured nanoparticles silver, titaniumdioxide and titaniumdioxide-silver combination on human adipose tissue derived mesenchymal stem cells (ADMSCs), for the first time. Cell culture experiments and characterization parameters were practiced for ADMSCs. Moreover, nanoparticles were synthesized, Zeta and SEM characterization protocols were performed. MTT assay was performed for cytotoxicity. Cells were differentiated into adipocytes and osteocytes. The effects of nanoparticles on ADMSC differentiation were studied by staining protocols. Results showed effects of nanoparticles differ from each other. TiO_2 NPs were non-toxic up to 20 µg/mL. Ag NPs have a significant proliferative effect at 1 µg/mL. TiO_2@Ag NPs showed a high proliferative effect at all concentrations that applied. Evaluation of TiO_2@Ag NPs particularly showed that, effects of combination of TiO_2 and Ag NPs are different for same cell line. Results also support further investigation of nanoparticles solely and combinations on stem cell viability, cellular functions and cell fates, and also advantages for tissue engineering as an extra cellular matrix unit.

Keywords
Nanoparticle synthesis;
TiO2@Ag;
Scanning electron microscopy;
Stem cells;
Tissue engineering

TiO_2, Ag ve TiO_2@Ag Nanopartiküllerinin Sentezi, Karakterizasyonu ve Kök Hücreler Üzerindeki Etkilerinin in vitro Değerlendirilmesi

Öz

Kök hücre ve nanoteknoloji son yılların en hızlı gelişen araştırma alanlarındandır ve bu iki önemli alanın birleşmesi ile kök hücre nanoteknolojisi adı verilen yeni bir branş ortaya çıkmıştır. Yapılan çalışmalar ile nanoparçacıkların hücre içine girebildiği ve hücreler arası taşınabildiğinin belirlenmesinin ardından kök hücre nanoteknolojisindeki gelişmelerin arttığı görülmektedir. Bu çalışmanın amacı, ilk kez olarak, farklı karakteristik özelliklere sahip olduğu bilinen gümüş ve titanyumdioksit nanopartikülleri ile bu nanopartiküllerin kombinasyonu ile elde edilen titanyumdioksit-gümüş nanopartikülünün insan yağ dokusu kaynaklı mezenkimal kök hücreler (hADMKH'ler) üzerindeki etkilerini araştırmaktır. hADMKH'ler hücre kültürü yapılarak çoğaltılmıştır. Diğer taraftan nanopartiküller sentezlenmiş, elde edilen nanopartiküller için Zeta potansiyeli tayin edilip SEM ile görüntüleme yapılmıştır. Üç farklı nanopartikül türünün farklı konsantrasyonlarının hücreler üzerindeki toksisitesi MTT testi ile belirlenmiştir. Ayrıca nanopartiküllere maruz bırakılan hücrelerin adipojenik ve osteojenik farklılaşma potansiyelleri Oil Red O ve Alizarin Red S boyama ile incelenmiştir. Elde edilen sonuçlar aynı hücre hattı üzerinde her bir nanopartikül türünün farklı konsantrasyonlarının etkilerinin birbirinden farklı olduğunu göstermiştir. TiO_2 nanopartikülleri 20 µg/mL'ye kadar toksik değilken Ag nanopartiküllerinin 1 µg/mL'de hücreler üzerinde önemli bir proliferatif etkisi vardır. TiO_2@Ag nanopartikülleri ise tüm konsantrasyonlarda

Anahtar kelimeler
Nanopartikül sentezi;
TiO2@Ag;
Taramalı elektron mikroskopisi;
Kök hücreler;
Doku mühendisliği

proliferatif etkide artış göstermiştir. Sonuç olarak tek başına kullanılan nanopartiküllerin hücreler üzerinde gösterdikleri etkilerinin yanı sıra nanopartikül kombinasyonlarının da ayrıca incelenmesine ve nanopartiküllerin kök hücre canlılığı, hücresel fonksiyonlar üzerindeki etkileri ve hücrelerin akıbeti üzerinde araştırmalar yapılmasına ihtiyaç duyulmaktadır. Ayrıca nanopartiküllerin ve kombinasyonlarının özellikle doku mühendisliği uygulamaları için hücre dışı bir matris elemanı olarak etkilerinin belirlenmesine yönelik çalışmaların artırılması gerekliliği ortaya çıkmaktadır.

1. Introduction

Nanotechnology is one of the most popular research areas of recent years and has a working area with talented particles of 1-100 nm size. Nanoparticles may have several shapes and their unique and small size give these particles the properties of both bulk materials and molecular structures (Donaldson *et al*. 2004, Radomska *et al*. 2016, Sauer 2009). Nanoparticles enable to develop particular physical and chemical characteristics and enhance their performance. Their small size also provides them one of their most attractive intrinsic properties such as a high surface to volume ratio and high mobility when they are in a free state. All these properties gain them benefits for a wide range of applications (Hasan *et al*. 2018). Thus, the importance of nanotechnology increases day by day (Donaldson *et al*. 2004, Sauer 2009).

As reported in some studies, there are several kinds of metallic nanoparticles used in research areas. Especially metal nanoparticles, Ag, Cr, Fe, Mo, Ni, Ta, Cr, Co and Sb and were seemed to be applied in biomedical engineering and bioengineering (Hasan *et al*. 2018). Reviewing literature has revealed that Ag and TiO_2 nanoparticles are used commonly in bioengineering applications such as healing wounds, contraceptives, surgery materials, and bone prosthesis (Donaldson *et al*. 2004, Kuppusamy *et al*. 2016, Sauer 2009, Zou *et al*. 2014). Ag NPs, with the antimicrobial effects, are used in medical, surgical and dental products (Oberdorster *et al*. 2005), and also started to be used as a drug carrier for targeted cells and organs by their optic diversity properties for imaging (Moss and Wong 2006). TiO_2 NPs takes places in personal care products (Greish *et al*. 2012). While single metal formed nanoparticles are synthesized for nanotechnology applications, in recent years', in addition to existing NPs, composites (two or more kind of materials) are started to be produced / synthesized. Effectiveness of nanoscale composites are started to be investigated (Khang *et al*. 2007). These nano compositions show different physico-chemical characteristics and effect with a different way from solely of the nanoparticles (El-Sadik *et al*. 2010). In our previous study, we showed that TiO_2@Ag, (a combination of Ag and TiO_2 NPs) is a highly effective antimicrobial agent (Allahverdiyev *et al*. 2013). Therefore, the use of nanocomposites can have an important place in similar areas.

With the current knowledge of nanoparticles, new and functional nanomaterials are generated each day. Developments in nanoparticles' science lead their use at medical fields as nanoparticle based transport-systems, cancer treatments and vaccine developments. However, the intracellular localization of nanoparticles, in a living cell, is not cleared and fate of stem cells after NP internalization is unpredictable. While using NPs as a daily product ingredient, it should be in mind that exposure to new nanomaterials and their combinations are the point in question (Chithrani *et al*. 2009, Schafer 2002).

Stem cells and nanotechnology are two of the most talented research areas and a new area called *stem cell nanotechnology* was raised. Stem cells are undifferentiated and renewable cells and they are used successfully in regenerative medicine to repair damaged or non-functional tissues and organs (Freitas 2005, Hu *et al*. 2011, Hyung *et al*. 2008, Larsen *et al*. 2010, Medintz *et al*. 2008), in immunotherapies for anti-inflammatory effects, (Boverhof *et al*. 2015, Swai *et al*. 2009, Ullah and Dutta 2008), at targeted systems (Pan *et al*. 2009) and cellular therapies (Sauer 2009, Xu *et al*. 2007). In regenerative medicine, it is known that stem cells are in a relationship with the niche. Cell-cell interaction, secretion factors, extracellular matrix

(ECM) and mechanic conditions are essential as the micro components of tissue engineering. In recent years, it is detected that nanoparticles may be useful for the enrichment of microenvironment for cell proliferation and differentiation.

While stem cells have secured an important place in regenerative medicine and tissue engineering, nanoscale structures have recently become prominent in tissue engineering, as well as various industrial and medical areas (such as cosmetics, texture, food, pharmacy and microbiology) (Donaldson *et al*. 2004). With the knowledge of nanoparticles' inter- and intra-cellular transport abilities because of their ultra-small size and wide surface area, developments on stem cell nanotechnology increases (Chen *et al*. 2007, Lansdown 2002, Muller *et al*. 2010, Nepple *et al*. 2012, Oberdorster 2010, Taylor *et al*. 2005). And, investigation of nanoparticle interaction on stem cells gains an extreme importance (Accomasso *et al*. 2016, Allahverdiyev *et al*. 2013, Asharani *et al*. 2008, Morones *et al*. 2005, Panacek *et al*. 2006, Sakthivel *et al*. 2004). Nanoparticles are highly mobile when they are in a free state (Hasan *et al*. 2018), and the effect of NPs in stem cell metabolism and the futurity of cells are not clearly known. Thus, while the interaction between stem cells and NPs is a mystery, studies about this subject are vital.

While concerns about the nanoparticle exposure for the human raised in public and scientific society, cytotoxicology researches gained a great importance (also for Ag and TiO_2). Some studies on nanotoxicology warn about exposure. It is reported that Ag NPs, used in cosmetics and textile, get through into the cells by perspiration (Khare *et al*. 2015). In another study, keratinocytes and fibroblasts are exposed by NPs used in healing materials and their toxic effects were observed. In a study, some concentrations (≤0,5 µg/ml) of Ag NPs are reported as non-toxic for human hematomas cells (HepG2), but these concentrations cause oxidative stress in these cells and also they are toxic for mesenchymal stem cells. Thus it is understood that non-toxic concentrations of Ag also pose a threat for some somatic cell types (Angeli *et al*. 2008, Iavicoli *et al*. 2011). On the other hand, studies on TiO_2 NPs used in food, pharmacy and cosmetics stated the ultrafine structure of this NP causes cytotoxic effects such as inflammation, fibrosis and DNA damages (Asharani *et al*. 2009) for cells by dermal contact and inhalation (Nielsen *et al*. 2008). Jin *et al*. (2008) reported that fibroblast cells showed different characteristics according to dose and time (this is a time and dose depended reaction) (Braydich-Stolle *et al*. 2005). Also effects of TiO_2 NPs depends on type of cells such as inflammation on endothelial cells (Asharani *et al*. 2009), DNA damage on lymphoblastic cells (Greulich *et al*. 2011) and apoptosis in hamster embryo fibroblasts (Berry *et al*. 2004). TiO_2 NPs synthesized for tissue engineering applications were reported that NPs showed negative effects on mesenchymal stem cell proliferation, migration and differentiation according to dose, size and conditions (de la Fuente *et al*. 2006).

Adipose tissue derived stem cells are one of the most talented stem cells used in researches because of easily obtaining and large number of MSC amount. As mentioned above nano compositions show different physico-chemical characteristics and effect with a different way from solely of the nanoparticles (El-Sadik *et al*. 2010).

Considering our previous results, we hypothesize that mesenchymal stem cells can be also directed by silver-doped TiO_2 nanoparticles since the differentiation mechanism of action of TiO_2@Ag nanoparticles. It can be also estimated that nanoparticle combinations may further provide more effective applications for tissue engineering based on stem cell nanotechnology. However, to the best of our knowledge, there is no study on the proliferation and differentiation effects of TiO_2@Ag nanoparticles in literature. Therefore, the aim of the present study was to explore the cytotoxicity effects of Ti@Ag NPs on the biological properties of hADMSCs, and to suggest their potential role in tissue engineering as an extracellular matrix unit.

2. Materials and Methods

2.1 Preparation of Adipose Derived Mesenchymal Cell Culture

Cells, isolated, characterized and cryopreserved from previous study (Allahverdiyev *et al*. 2011b) were thawed and cultured in 10% FBS (Biochrom AG, 1038K), anti-anti (Gibco Invitrogen, 100X, 15240), and Glutamax (Gibco Invitrogen 35050) containing DMEM (Allahverdiyev *et al*. 2011b, Allahverdiyev *et al*. 2012). Cells were cultured at 37 ^{0}C in %5 CO_2 humidified incubator for 3-4 days. During incubation period, proliferated cells were observed and it was understood that cells have adapted to cell culture conditions. By the time cell amount is enough for characterization repetition and experiments, cell culture continued. After cells in culture flasks reached 70-80% confluence, cells were washed with PBS. Cells were deattached using Trypsin/EDTA (Gibco Invitrogen, 1X, %0.05, 25300) into several and passaged flasks and continued incubation.

2.2 Characterization of ADMSC

Histochemical analysis for adipogenic and osteogenic differentiation period, confluent ADMSC culture were passaged and harvested into 6-well plates with an amount of 50.000 cells/well. Plates were grouped as adipogenic, osteogenic and control groups. Adipogenic and osteogenic groups were induced to undergo adipogenesis (ThermoFisher Scientific, StemPro™ Adipogenesis Differentiation Kit, A1007001, USA) and osteogenesis (ThermoFisher Scientific, StemPro™ Osteogenesis Differentiation Kit, A1007201, USA) by replacing the stromal media with adipocyte induction medium and osteogenic induction medium respectively according to manufacturers'. Control group was treated with stromal media (Allahverdiyev *et al*. 2012). Cells were maintained in culture for 14 days for adipogenesis, 21 days for osteogenic and control groups. Each group's media was replaced every 3 days. After adipogenic incubation, cells were washed with PBS for three times, fixed in a 10 % solution of formaldehyde for 1 hour. Following fixation, cells washed with 60 % isopropanol and stained with Oil Red O solution for 10 minutes. Stained cells were washed with tap water, and finally destined in 100 % isopropanol for 15 min (Allahverdiyev *et al*. 2011b, Ogawa *et al*. 2004). Osteogenic differentiation has been performed Alizarin Red S stain according to previously published protocols (Allahverdiyev *et al*. 2011b, Kroeze *et al*. 2011). After washing the cells with PBS, Alizarin Red S stain was added onto cells and reaction between 30s - 5min was performed at room temperature. Cells were washed and covered with acetone. After that, acetone was removed, acetone/xylene (1/1) mixture was added. Finally, cells were covered with xylene. After removing the xylene, cells were dried in RT and observed under microscope with glycerol.

2.3 Synthesis of Nanoparticles

Synthesis of Ag Nanoparticles. 300 mL of sodium hydroxide (NaOH) solution (1 mol/L, Merck, Darmstadt, Germany) and 500 mL of silver nitrate ($AgNO_3$) solution (0.5 mol/L) were mixed using magnetic stirring. After mixing, a brown precipitate (pH 10) was observed. Following this, 5 mL of sodium dodecyl sulphate solution (Merck, Darmstadt, Germany) 1 mol/L was added and mixed until a homogeneous solution was obtained. Consequently, 3 mL of hydrazine hydrate (Merck, Hohenbrunn, Germany) was added drop-wise. After the washing step with distilled water, the solution was dried at 80 °C for 48 h and finally was calcined at 350 °C for 2 h. The solution of Ag NPs (1 mg.mL^{-1}) was prepared in distilled water. The solution was mixed by vortexing for 1–2 min and sonicated for 30 min in order to disperse aggregates. After sonication, the solution was autoclaved and was again mixed by vortexing before each use.

Synthesis of TiO_2 Nanoparticles. 20 mL of 2-propanol ($CH_3CH_2CH_2OH$) and 10 mL of TTIP ($C_{12}H_{28}O_4Ti$ - Titanium (IV) isopropoxide) were mixed. On the other hand, pure water and nitric acid were mixed in a container. 2-proponal-TTIP is added dropwise to the mixture until the pH reaches 1.5. The mixture was stirred with magnetic stirrer for 24 h. Obtained transparent solution is washed with pure water and powders were precipitated. Powders were dried and calcined at 500 °C for 1 h. Synthesis of TiO_2@Ag Nanoparticles. Synthesis of TiO_2@Ag NPs were

practiced according to Allahverdiyev and Abamor's studies (Abamor and Allahverdiyev 2016, Abamor *et al*. 2017, Allahverdiyev *et al*. 2013). 10 mL Titanium isopropoxide (TTIP) was mixed with 20 mL 2-propanol (C_3H_8O) and the mixture was dropped into a 400 mL distilled water. This mixture was stirred with magnetic stirrer for 24 hours. On the other hand, at the same time $AgNO_3$ solution consisted of 10% Ag and 6% PVP by weight were prepared. Afterwards this solution was put into the TiO_2 including suspension, drop by drop. The hydrothermal synthesis was carried out by autoclaving the prepared solution for 2 h. Then the solution was rinsed with the deionized water and dried at 80 ^{0}C for 72 h. Finally obtained powder was calcined at 500^{0}C for 1 hour.

2.4 Characterization of Nanoparticles

Characterization of synthesized Ag, TiO_2 and TiO_2@Ag nano-powders were maintained by using Transmission Electron Microscope (TEM), Scanning Electron Microscope (SEM) and Zeta-Sizer Analysis which were mentioned in details in previous paper (Abamor *et al*. 2017, Allahverdiyev *et al*. 2013, Allahverdiyev *et al*. 2011a). SEM analysis were verified with Zeta sizer Nano ZS (Malvern, ZEN3600) in Yildiz Technical University Science and Technology Application and Investigation Laboratory. Collected Ag, TiO_2 and TiO_2@Ag NPs were characterized by transmission electron microscopy and their size and morphology were assessed. For characterization assays, nanoparticle powders were diluted in pure acetone and the prepared suspension was ultrasonicated. The suspension was put drop-wise on carbon-coated copper grids and dried. Transmission electron microscopy characterization of the Ag, TiO_2 and TiO_2@Ag were performed using a JEOL-1010 microscope (Jeol Korea Ltd, Korea). To measure the hydrodynamic size of the nanoparticles in dynamic light scattering (DLS), 100 µL of each particle solution were diluted with 1.5 mL of water and placed into the cuvette of a Zetasizer Nano instrument (Malvern Instruments Ltd, Philadelphia, PA). Experiments were conducted in triplicate to obtain an average number-size distribution. The same device was used to measure zeta potentials.

2.5 Determination of the Effect of Nanoparticles on Stem Cell Proliferation

ADMSC culture was harvested into microplates with 20.000 cells in each well and incubated in 37 ^{0}C, %5 CO_2 for 24 hours. After incubation period, different concentrations of TiO_2, Ag and TiO_2@Ag NPs ranging from 1 µg/mL to 250 µg/mL were put into each wells of the microplate. The control group was not exposed to any nanoparticles. After 72 hours of incubation of the cells with nanoparticles, MTT assay was conducted on ADMSCs to previous study (Allahverdiyev *et al*. 2013) and optical density was read at 570 nm by using ELISA-reader.

2.6 Determination of the Effect of Nanoparticles on Stem Cell Differentiation

Continuous cell culture was exposed to chemically synthesized Ag, TiO_2 and TiO_2@Ag nanoparticles (1 µg/mL– 100 µg/mL) for adipogenic and osteogenic differentiation periods were observed.

2.7 Statistical Analysis

Experiments were triplicated. GraphPad Prism 5 program was used. Homogeneity of variants was determined by ANOVA one-way. Values were $p<0.005$ and they were statistically meaningful.

3. Results

3.1 Stem Cell Culture

ADMSCs were taken from cryobank where isolated, characterized and cryopreserved cells were kept, in previous study (Allahverdiyev *et al*. 2012), thawed and incubated. Adhered cells showed intact and healthy morphologies, continued proliferation and passaged every 3-4 days when they get 70-80 % of cell confluence. The shape and adherence of cells conformed to fibroblast like cells as mesenchymal stem cells characteristic (Figure 1).

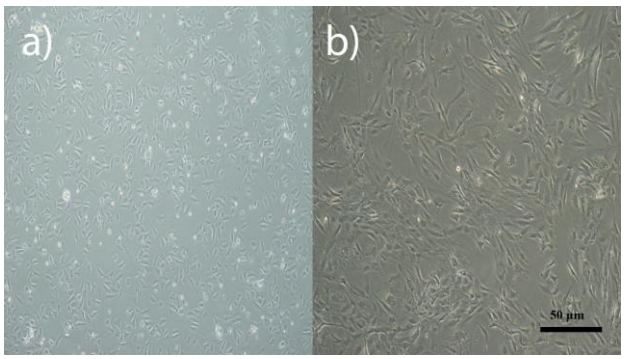

Figure 1. ADMSC culture at day a) 7, b) 10 (x40)

3.2 Stem Cell Characterization

Characterization repetition studies were performed for thawed ADMSCs to see if cryopreservation period affected stem cell properties. Cells showed a healthy morphology in culture and after that, characterization assays were applied. On the other hand, differentiation characterization of ADMSCs were performed by using differentiation media for adipogenesis and osteogenesis using StemPro™ Differentiation Kit, (USA) according to manufacturer's recommendations at passage 5. After 10 days of adipogenic differentiation, lipid-rich cells were observed and stained by Oil Red O (Figure 2).

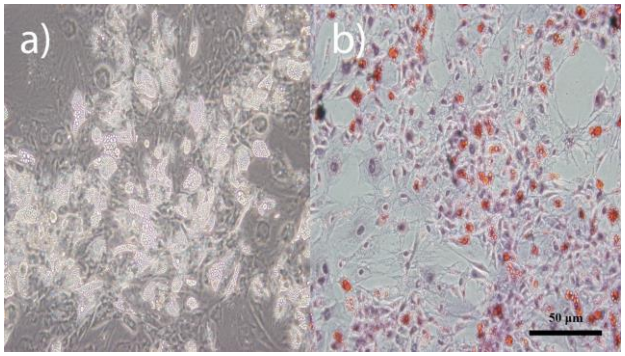

Figure 2. Adipogenic differentiation of hADMSCs (a) culture (b) Oil Red O staining

3.3 Nanoparticle Characterization

The characterization of synthetized TiO_2, Ag and TiO_2@Ag nano-powder species were maintained by using Scanning Electron Microscope (SEM) (Figures 3 and 4), Dynamic Light Scattering (DLS) and Zeta-Sizer Analysis. Size measurement analysis results were detailed at Table. Zeta potency and average size of nanoparticles by DLS and SEM were also showed in the Table. Distribution of nanoparticles SEM pictures are mentioned below.

Table 1. SEM and characterization of TiO_2, Ag and TiO_2@Ag nanoparticles.

Nanoparticles	DLS / Zeta Potency	SEM Analysis
TiO2 NPs	-34,2 mV	40-86 nm
Ag NPs	-24 mV	76-220 nm
TiO_2@Ag NPs	-31,2 mV	35-65 nm

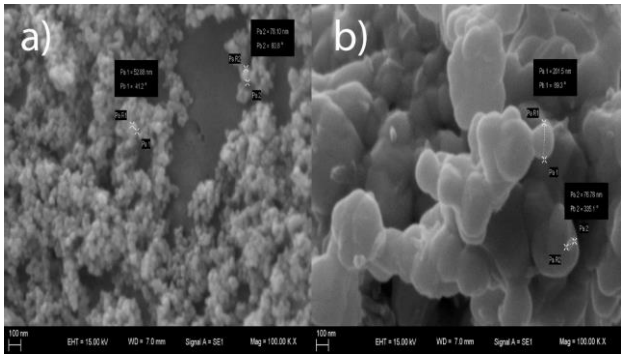

Figure 3. Scanning electron microscopy of (a) TiO_2 and (b) Ag NPs (100,00K X)

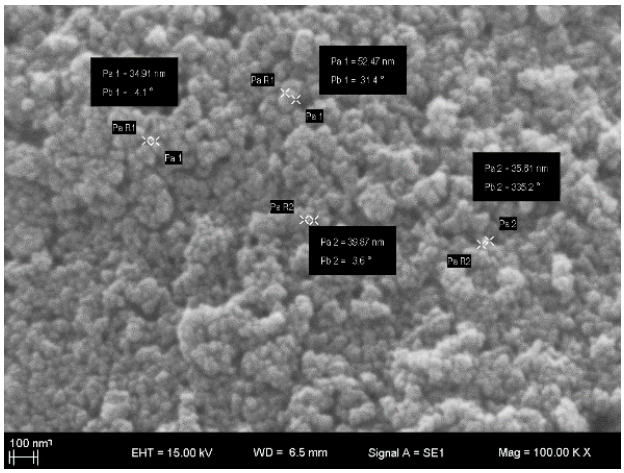

Figure 4. Scanning electron microscopy observation of TiO_2@Ag NPs (100,00K X)

According to nanoparticle characterization results, TiO_2, Ag and TiO_2@Ag are all negatively charged. Nanoparticles suspension of each type is seen that they have a size of less than 100 nm. Thus, synthesis of nanoparticles was successful as we expected.

3.4 Effect of Nanoparticles on Stem Cells

Metabolic activities of ADMSCs that were treated with all the concentrations of three NPs, were first determined by microscopic observation after formazan crystal formation. It is the absolute signature of cell viability and metabolic activity. Effects of each nanoparticle on ADMSCs were measured by spectrophotometer with MTT assay.

Results were showed in Figure 5. As seen in the figure, each concentration of TiO_2 NP was effective on cell proliferation. There is no decrease on viability of cells. But the most significant effect on cell proliferation was at the concentration of 10 µg/mL of TiO_2. But while the concentration of NPs increases, viability of cells decreases to thereabouts the control group. Cells showed a healthy morphology in culture and after.

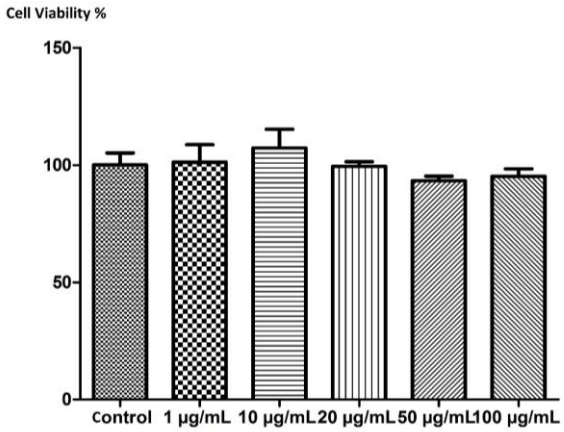

Figure 5. MTT results of TiO_2 NP on ADMSC proliferation

Ag nanoparticles caused differently viability effects on ADMSC according to concentration. As seen in the Figure 6 two concentrations are effective on cell viability (1 and 20 µg/mL ($p<0,005$) at a rate of average 15%), and other concentrations (10, 50 and 100 µg/mL) were non-effective, in fact they show similarity to control's.

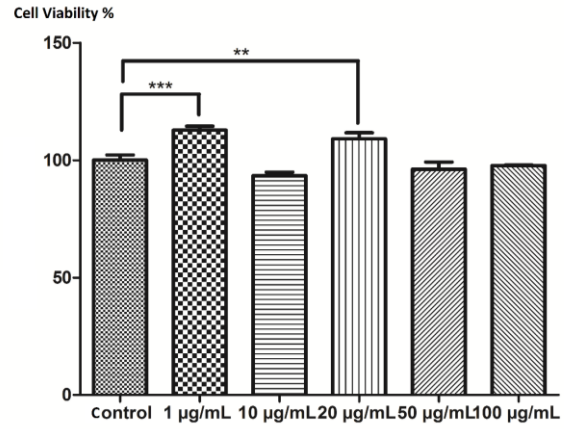

Figure 6. MTT results of Ag NP on ADMSC proliferation

TiO_2@Ag nanoparticles stimulate the proliferation of ADMSC at any concentrations. The most significant increase was seen at lower concentrations for 1 and 10 µg/mL (≈25 %) respectively. Other higher concentrations proliferated cell division about 15% higher than control's (Figure 7).

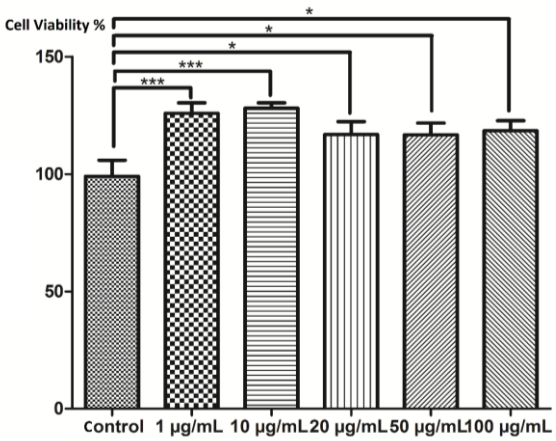

Figure 7. MTT results of TiO2@Ag NP on ADMSC proliferation

3.5 Stem Cell Characterization

Differentiation capacity of stem cells is the main property that is needed to be investigated after any interaction with materials. Not only proliferation, differentiation potency is also a key for stem cell fate for identification and characterization.

Adipogenic and osteogenic differentiation capacity of nanoparticle-effected ADMSCs were performed. All results were observed and estimated under microscopy. Effects of TiO_2 NPs on ADMSCs were observed. After adipogenic differentiation period, at the 14^{th} day, it is observed that 20 µg/mL concentrations of TiO_2 NPs shows same signs like the control group (non-treated ADMSCs) and this concentration has the most simulative effect by comparison of others. On the other hand, it is noted that 10 µg/mL of the concentration inhibits adipogenic differentiation rate (Figure 8).

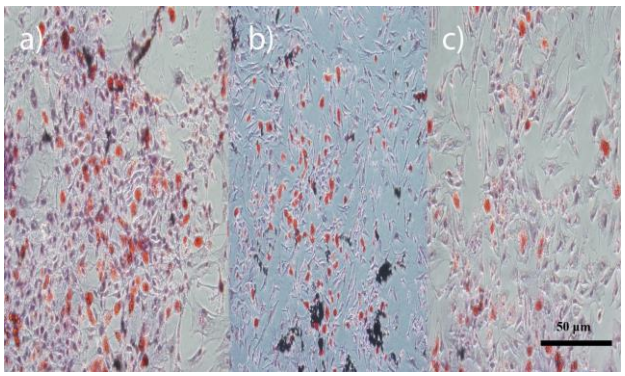

Figure 8. Adipogenic differentiation of a) TiO_2 b) Ag and c) TiO_2@Ag exposed hADMSCs (Oil Red O staining, 20 µg/mL)

Adipogenic and osteogenic differentiation capacity of nanoparticle Osteogenic effect of TiO_2 for cell groups at the 21st day, concentration of 1 µg/mL and 10 µg/mL of NPs showed similar differentiation properties with control group. As the concentration of nanoparticle increase, degeneration of cells occurs. (Data not shown).

While observing the adipogenic differentiation effect of Ag NPs on ADMSCs, it is determined that lower concentrations show similar differentiation with comparison of control although concentrations of 50 µg/mL and 100 µg/mL inhibited differentiation substantially (Figure 8).

Osteogenic differentiation of ADMSCs exposed to Ag NPs followed a leisurely period than the control group at the concentration of 100 µg/mLbut at lower concentrations cell death occurred (Data not shown).

On the other side, it is determined that TiO_2@Ag NPs stimulated the adipogenic differentiation at 1 µg/mL, 10 µg/mL, 20 µg/mL and 50 µg/mL concentrations a little bit much than the control group, but inhibited cell viability at concentration of 100 µg/mL as seen at the Figure 10a and b. Osteogenic differentiation was observed just for the concentrations of 20 µg/mL and 50 µg/mL. Because at the 14th day of the culture, cell death was observed in the wells. Group at the 50 µg/mL concentration showed similar morphology with the control group during osteogenic differentiation (Data not shown).

4. Conclusion

Since nanoparticles are started to be used in medical fields such as an alternative for tissue engineering agents, it is important to reveal their properties. Thus, effects of nanoparticles (type, size, concentration and surface charge) and their combinations are needed to be examined, because they are physicochemical different from each other. There is restricted knowledge about usage of nanoparticles for tissue engineering and also less about TiO_2@Ag NPs.

For the first time in this study we investigated the effects of Ag, TiO_2 and TiO_2@Ag nanoparticles on hADMSCs comparing. The results support further investigation of nanoparticles solely and their combinations on stem cell viability, cellular functions and cell fates such as differentiation, and also advantages for tissue engineering as an extra cellular matrix unit.

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5. Kaynaklar

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