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Mezoporlu Silika Nanoparçacıklarının Kanser Mikrodokusuna Nüfuz Etmesinin Fizibilite Çalışması

Feasibility Study of Mesoporous Silica Nanoparticles Permeability through the Cancer Microtissues

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***Özetçe*— Mikrodokular, hücre hattının (i.e.MCF-7) gerçek ortamının taklit edilmesini sağlamak amacıyla oluşturulan 3 boyutlu yapılardır. Bu çalışmada; meme kanseri hücre hattıyla oluşturulan mikrodokuya eklenen farklı yüzey modifikasyonuna sahip mezoporlu silika nanoparçacıkların (MSN, MSN-PEI ve MSN-PEI-SUCC) 3 boyutlu mikrodokuya nüfuz etmesi ve hazırlanmış olan dokunun morfolojik yapısına olan etkisi araştırılmış, MSN yüzey modifikasyonuna ve artan konsantrasyona bağlı etkiler gözlemlenmiştir.**

Anahtar Kelimeler — Kanser; MCF-7 Mikrodokusu; Mezoporlu silika Nanoparçaçık.

***Abstract*— Microtissues are 3D structures created to stimulate the real environment of the cell line (i.e.MCF-7). In this study, mesoporous silica with different surface modifications nanopartciles (MSN, MSN-PEI and MSN-PEI-SUCC) were added to breast cell line-generated microtissue. Thereafter, the penetration of MSNs in microtissue and the effect on the morphological tissue was investigated, and MSN-surface functinalization and increasing concentration dependent effects were observed.**

I. INTRODUCTION

Tumor microenvironment has a crucial role in cancer progression. In oncology related treatments i*n vitro* culture models are essential to peredict the therapeutic efficacy in *in vivo*, by providing insight about the microenvironment of tumors. Especially, drug delivery, vasculogenesis and cancer modelling use microtissues to find response to cell or tissue needed. Human Breast Adenocarcinoma Cell (MCF-7) microtissue mimics natural breast cancer microenviroments by self-producing matrix in 3D scaffold free conditions [1]. MCF-7 has been developed to repeat the complexity of the tumor microenvironment and to analyze its efficacy for drug delivery systems by this model. Contrary to monolayer systems, 3D culture presents matrix interaction and cellular connection effect just as *in vivo*. Thus, effect of 3D systems is different from 2D tecniques in terms of cellular responses, relation between cell to matrix and physiological pathways [2]. Therefore, microtissues and organoids have a trend model for understanding of natural environments of tumors.

In oncology research, Mesoporous silica nanoparticles (MSN) have been proposed as a promising class of versatile drug/DNA delivery vehicles, as well as efficient tools for fluorescent cell tracking. MSN can interact with cells, penetrate through the extracellular matrix and interfere with the cell communication machinery with the aid of their tunable size and surface area in addition to composition and surface functionalization. It affects tumor and it can regulate microenvironments.

II. MATERIAL AND METHODS

A. CELL CULTURE

MCF-7 line was taken from Ege University, Bioengineering Department. All cell culture materials were taken from Sigma (Taufkirchen, Germany) and all protocols are used as in previous studies [3]. Cells were used between passage numbers 27-28. To growth cells, DMEM (Dulbecco's Modified Eagle Medium) was used as media with 1% L-Glutamine and 1% Penicillin, 10% FBS (Fetal Bovine Serum) and refreshed per 2 days.

After monolayer culture, agarose mold was done by commercial 3D Petri Dish (Sigma, Taufkirchen, Germany). 3D microtissue was constructed with 100,000 cells / 75 µl.

B. PREPARATION and CHARACTERIZATION OF NANOPARTICLES

Mesoporous silica nanoparticle (MSN) synthesis was carried out by using a similar protocol as in our previous study [4]. Briefly, cetyltrimethylammonium bromide (CTAB) as structure-directing agent (SDA) was dissolved in the ethanolic basic aqueous reaction solution. In this reaction, tetraethylorthosilicate (TEOS) was used as silica source. The reaction was kept stirring for overnight. The molar composition of the synthesis solution was 1TEOS:0.122 CTAB:0.31NaOH:72.3EtOH :946 H_2O. Fluorescent labeling of particles were carried out by fluorescein isothiocyanate (FITC) with the pre-reaction of the fluorophores with aminopropyl triethoxysilane (APTES) The pre-reaction solution was added to the reaction solution right before adding the TEOS to the

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synthesis. The molar ratio between APTES and TEOS was kept as 1:100. After overnight reaction of the MSNs, the SDA was removed by the solvent extraction method after the partciles are obtained two diffrent surace modifications(i.e surface graftings with PEI and succinic acid groups)were carried out according to literature protocols [5] in order to investigate the impact of MSN surface functionalization on the penetration through the tumor microtissue. Thermogravimetrical analysis was used to determine the amount of PEI added at temperature intervals of 170°C to 770°C. Successful modification of PEI and further derivatization with succinic acid were further confirmed by zeta potential measurements. Full redispersibility of dried, extracted and surface-functionalized particles was con- firmed by redispersion of dry particles in HEPES buffer at pH 7.2 and subsequent dynamic light scattering (DLS) measurements (Malvern ZetaSizer NanoZS).

C. *VIABILITY ASSAY , CHARACTERIZATION AND PENETRATION*

MCF-7 lines seeded 24 well plate with 2 different nanoparticles. Nanoparticles were preared as 10 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml in MSN-PEI, MSNexposure time. and MSN-PEI-SUCC nanoparticles and 0 mg/ml used as negative control. MTT (3-(4,5- Dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) (Biotium, California, USA) assay was done at at 24 and 48 hours. %90 media without serum and %10 MTT solution were used cells after 4 hours, DMSO (Dimethyl sulfoxide) was added into cells as descripted in Biotium MTT assay protocols. Then absorbance values was run in 596 nm. The diameters of microtissues in different concentration was measured by Image-J software (NIH). Before and after screen were compared. The penetration of nanopaticles was analyzed by confocal microscopy analysis.

III. RESULTS AND DISCUSSIONS

The produced MSN net surface charge, hydrodynamic Radius and PDI values are presented in the table below.

Table 1. Hydrodynamic Radius, Polydispersity Index (PDI) and ζ-Potential Values of prepared MSN (0,25 mg/mL)in HEPES buffer solution.

Type	Hydrodynamic Radius (nm)	PDI	ζ-Potential (mV)
MSN	493.5 ±8.9	0.28±0.06	-20.4±0.6
MSN-PEI	253.5±1.4	0.15±0.03	46.9±0.7
MSN-PEI-SUCC	228.3±3.2	0.08±0.02	-40.4±1.5

Viability of MCF-7 cells was evaluated by MTT assay for 24th and 48th hours as presented in Figure 1.When the cells were exposed to prepared MSN and MSN-PEI and MSN-PEI-SUCC for 24 hours the cell viability was almost the same with the control samples. Only slight differnecs wre observed with the incerased consantraions of MSN-PEI. At the end of 48 hours, statistically significant (p*<0,05 and p**<0,01) results were observed for MSN-PEI-SUCC with the increased concentrations from 10 µg/ml to 100 µg/ml. 50 mg/ml and 100 mg/ml only MSN groups had higher viability compared to control samples after 48h. Lowre viability values were observed for MSN-PEI samples compared to conbtrol. At teh same time points. (p**<0,01).

Diameter of microtissues including MSN and SUC nanoparticles was calculated by ImageJ. The shrinkage of microstiisues was oberved for all tytpe of MSNs after their exposure to 50 mg/ml concentration of MSN-PEI and MSN-PEI-SUC.Even distribution of MSNs through the microtissue were evaluated by using confocal microscopy imaging to demonstrate the penetration ability of MSNs..

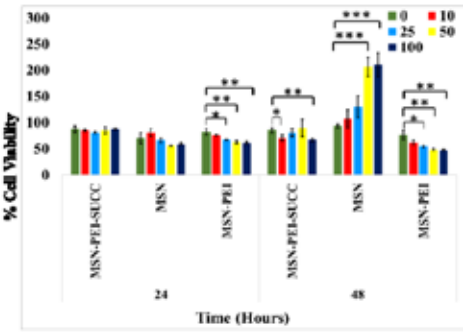

Figure 1. The effect of different nanoparticles on MCF-7 cell viability

IV. CONCLUSION

In this study we have investigated the penetration of MSN through the formed MCF-7 microtissues in order to predict their biological fate in MSN-aided oncology treatments. Our results revealed that the bare MSN and their surface modified counterparts do not affect the viability of MCF-7. Whereas, the MCF-7 microtissues morphology shrinked down to 20% compared to control experiments when MSN net surface charge was positive. Dominant effect of concentration as a parameter is also observed for the same type of MSN. In the case of net negatively surface charged MSN-PEI-SUCC the morphology of microtissue has shrinked down to 15% with the highest concentration and the impact of concentration increments stayed as insignificant.

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