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In vitro Effects of Mesenchymal Stem Cells and Their Niches in The Inhibition of *M. tuberculosis* Growth

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Abstract

Research Article

Mesenchymal stem cells (MSC) and their niches, which are the factors secreted by MSC in culture in vitro, are considered to have a balancing and also therapeutic effect in numerous diseases. The treatment of tuberculosis (TB) caused by multidrug-resistant or extensively resistant Mycobacterium tuberculosis (MTB) strains is a challenging concern and may not be achieved by conventional treatment options. In the present study, we aimed to investigate the effect of MSC and their niches on MTB growth in culture medium. Frozen adipose-derived MSC were grown in culture medium until they became confluent. MSC and their niches, which were the factors secreted in two-day medium, were used as the conditioned medium and their effects on MTB were evaluated. MTB was maintained in liquid medium until a positive growth signal appeared on the Bactec MGIT 960 device (Becton Dickinson, Sparks, MD, USA). The growth periods (in days) were recorded. In the latter stage, the effects of 104 MSC in 100 µl medium, of the MSC niche that had been maintained with MSC in 100 µl medium for 48 h, and of their combined use on MTB growth were analyzed. After the appearance of a positive growth signal, the MTB growth and cell morphology in the tubes were examined under light microscopy. It was revealed that both MSC and MTB sustained viability in liquid medium. In the tubes administered with niches, the MTT assay showed MSC proliferation. However, in the tubes administered with a combination of MSC and niches or niches, MTB growth was inhibited and delayed. The retardant effect of MSC and their niches on MTB proliferation in liquid medium implicates that MSC and their niches can be used as therapeutic products in TB treatment. Substantiating the contribution of this excellent effect, which was found in in vitro studies, to TB treatment via 3D biomedical supports and animal model studies will have a strong effect for phase studies. Accordingly, we consider that the investigation of stem cell and its secretion, which has high reliability and low cost, in clinical studies is of paramount importance for ensuring patients' quality of life.

INTRODUCTION

Tuberculosis (TB) remains a serious disease with an increasing prevalence in some regions around the world, leading to reduced quality of life and causing economic losses [1,2]. A recent problem reported by researchers regarding TB is Multidrug-resistant tuberculosis (MDR-TB) against combination drug therapies that target *Mycobacterium tuberculosis* (MTB), such as rifampicin and isoniazid. Although the initial diagnosis of pulmonary TB (PTB) and extrapulmonary TB (EPTB) is established by clinical findings, isolation of MTB from clinical samples by culture is the "gold standard" for a definitive diagnosis of both PTB and EPTB. The MTB complex (MTBC) grows slowly compared to other bacteria, with a generation time of 18-24 h. MTBC grows within 7-21 days in appropriate liquid and solid media at 35-37 °C. In advanced microbiology laboratories, cultures are considered negative if no growth is observed after 8 weeks' incubation. However, a number of fully automated systems have been developed for the detection of MTB proliferation in culture. Several large studies have demonstrated that liquid medium culture is superior to solid medium, returning results faster and yielding more MTB isolates. Growth of MTB can be easily followed by culture techniques[3,4].

Mesenchymal stem cells (MSC) are sustainable treatment products that can renew themselves and differentiate into any cell in the body. To date, no serious complications have been reported for adult MSC [5,6]. MSC and their niches which conditioned medium from 48 hours culture have a key role in reducing inflammation in areas of tissue damage and achieving tissue regeneration through immunomodulation. MSC can be easily obtained from various sources such as adipose tissue and can be rapidly processed for transplantation in high-quality standardized laboratories [7]. After the completion of clinical trials, MSC therapies have been used in numerous diseases including ischemia, Crohn's disease, ulcerative colitis, and liver diseases [8,9]. Similarly, various MSC therapies have been used for the treatment of TB in

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numerous clinical trials [10-12]. Despite these trials, however, there is little knowledge regarding MSC niches except for their being anatomically suitable spaces for dormant bacteria [13].

The aim of this study was to investigate the contribution of MSC niches to the known effects of MSC and the interaction between MSC and MTB with regard to cell behavior. The inhibition of MTB proliferation by MSC and their niches is of prime importance for their use in the treatment of TB.

MATERIAL and METHODS

Ethical approval

All procedures were approved by the Ethics Committee on Human Research of Celal Bayar University, Manisa, Turkey (15.01.2014, 20478486-25)

M. tuberculosis culture method

Bactec Mycobacterium Growth Indicator Tube (MGIT 960, BD Biosciences, Sparks, MD), also known as MGIT tube, is designed for rapid detection, high-capacity, fully automated, continuous-monitoring instrument that can test up to 960 MGIT vials for the presence of MTB using nonradiometric fluorescence technology of mycobacterial growth in culture. Middlebrook 7H9 is another system primarily used for the identification of growth of mycobacterial cultures in liquid media. Liquid medium is the primary medium used in rapid culture systems. In MGIT 960 tubes, there is silicon that contains oxygen-sensitive ruthenium metal complex in Middlebrook 7H9 liquid medium and its bottom sections. Following the growth of mycobacterium or other microorganisms, fluorescence occurs against UV rays as a result of the use of oxygen, and the amount of resultant fluorescence is evaluated as the growth index. In the absence of growth, however, no fluorescence occurs against the UV rays directed to the silicon layer due to the presence of oxygen. The instrument's photodetectors measure the fluorescence in each vial every 60 min. When a certain level of fluorescence is reached, the instrument indicates that the vial is positive. The purpose of this study was to evaluate the fully automated 7-ml BACTEC MGIT 960 system for the detection of mycobacteria in specimens (14,15). In the present study, the standard strain of MTB H37Rv (ATCC 27294) stock (5×10^7 CFU/ml) was inoculated in an MGIT growth media tube with BACTEC™ MGIT 960 growth. For isolates initially grown in MGIT medium, inoculum was prepared from the positive culture on the 1st day of positivity as detected by the BACTEC MGIT 960 instrument. After being mixed well, 0.5 ml of the positive broth cultures was used for BACTEC MGIT 960 growth. For isolates grown on, a suspension of the microorganism was prepared in 7H9 medium at a density of 0.5 McFarland. One-half milliliter of this dilution was used for BACTEC MGIT 960 AST Inoculations were performed in accordance with the manufacturer's guidelines.

Mesenchymal stem cell culture

Adipose-derived MSC at passage 4 that had been frozen at -80 °C were used for the culture analysis. A vial of frozen cells was placed into a 37 °C water bath to allow the vial to sit at room temperature until the cells achieved normal growth and confluency. Subsequently, the vial was maintained in an α-MEM culture containing 15% fetal bovine serum (FBS), 50 µgr/ml gentamycin, 100 UI/ml penicillin, and 100 UI/ml amphotericin. The cells were incubated at 37 °C in an atmosphere of 5% CO_2 in air. The cells in each well was photographed and analyzed morphologically using an inverted phase-contrast microscope [16,17,18].

Experimental groups in culture medium

Inoculation: In the first stage of the experiment, nine MGIT tubes were used for each group and nine MGIT tubes were used as control (Table 1).

Table 1. Groups for invitro experiments.

Control groups	Experimental groups
MTB (n=3)	MTB + MSC (n=6)
MSC (n=3)	MTB + Niche (n=6)
	MTB + MSC + Niche (n=6)

Each tube was labeled with specimen number and then subjected to a testing protocol during the preparation phase. All the procedures were performed in a Class III biological safety cabinet. Using a sterile pipette, 15 ml of MGIT Growth Supplement was added into the lyophilized MGIT PANTA (Polimiksin B, Amfoterisin B, Nalidiksik asit, Trimetoprim laktat, Azlosilin) vial and dissolved completely by proper mixing. Each MGIT tubes was uncapped and was added with 0.8 ml of the Growth Supplement/MGIT PANTA mixture. MGIT inocula were prepared from the newly grown *MTB* H37Rv ATCC 27294 reference strain with McFarland 0.5

turbidity. To evaluate bacterial contamination, the mixture obtained from each third tube was inoculated onto a blood agar plate and was incubated at 35 °C. The plates were evaluated for bacterial growth 48 h after inoculation. Each MGIT tubes was added with 0.5 ml of the mixture. In the latter stage, 10^4 MSC counted with hemocytometry in 100 µl medium and MSC niche that had been maintained in 100 µl medium for 48 h were combined in separate tubes. Subsequently, the 27 MGIT tubes were added with combinations of MTB+MSC, MTB+Niche, and MTB+MSC+Niche. The 27 MGIT tubes were added with materials as per the study protocol and were placed in the MGIT 960 device in the order of their specimen numbers. After obtaining a positive signal, the samples with a value above the culture positivity threshold of the device were removed from the device. The specimens obtained from tubes were evaluated for the presence of acid-resistant bacilli (ARB) on Erlich Ziehl Neelson -stained smears. In the specimens that were positive for ARB on staining, MTB identification was performed using a rapid card test (BD MGIT TB Identification Test)

MTT cell viability and proliferation assay

The MTT (3-(4,5-dimethyl-2-yl)-2,5-diphenyltetrazolium) assay is a colorimetric assay for measuring the number of viable cells in culture by determining the activity of mitochondrial enzymes such as succinate dehydrogenase. In this assay, the light-yellow tetrazolium is reduced to purple formazan in living cells as a result of the cleavage of the tetrazolium ring. Accordingly, viable cells with intact mitochondrial function are dyed purple while dead cells with impaired mitochondrial function are not dyed. The MTT assay consists of three steps: (1) incubation of cells with MTT dye, (2), conversion of the resultant reaction product into water-soluble formazan, and (3) colorimetric measurement of the reaction product.

For the MTT assay, the cells were inoculated into 96-well microplates at a density of 10^4 cells/well and were maintained in a climate-controlled incubator (5% CO_2/95% air and 60-80% humidity) set at 37 °C for 24 h to allow cells to form colonies. The following day, the growth medium was removed from each microplate and then 250 µl of the MTT working solution (1 ml MTT + 9 ml growth medium) prepared from previously refrigerated MTT stock solution (50 g MTT + 10 ml phosphate buffered saline (PBS) was added into each well and was incubated for 4 h. Following incubation, the MTT solution was removed and 100 µL of dimethyl sulfoxide (DMSO) was added to each microplate, and then spectrophotometric measurements were performed using a microplate reader at 570 nm with a 690 nm reference filter [19].

Statistical analysis

The data are expressed as means ± SD and were analyzed using repeated-measures ANOVA. The Tukey-Kramer multiple comparisons test was used to determine differences among means. Values for $p < 0.05$ were considered significant.

RESULTS

The liquid medium was added to the culture medium to analyze the effect of TB liquid medium on stem cell growth. The results indicated that stem cells became confluent in the culture medium that was not added with liquid medium and remained semi-confluent in the culture medium that was added with liquid medium, implicating that MSC survived after the addition of liquid medium (Figure 1).

MSC was characterized by CD45 negative and CD90 positive labelling. To evaluate MSC behavior in a culture medium added with TB liquid medium, smears were obtained from the tubes with a positive signal and were examined under light

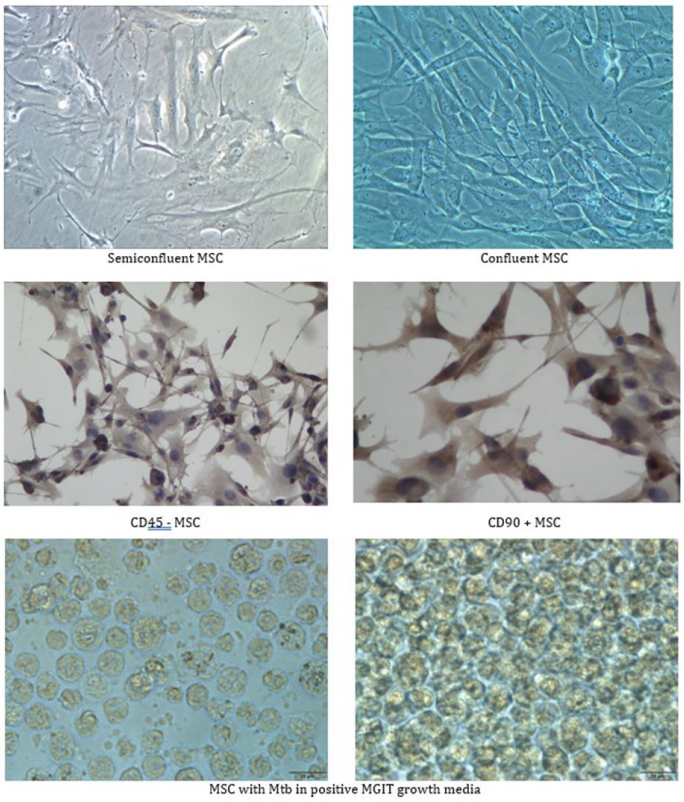

Figure 1. Adipose-derived stem cells in culture media added with (right) and not added with liquid medium (left). As seen in these images, cell growth is viable and proliferative, albeit reduced.

microscopy. The examination showed round viable cells with no adherence capability and MSC infected with MTB (Figure 2).

In control groups, both MTB and MSC showed a posi-

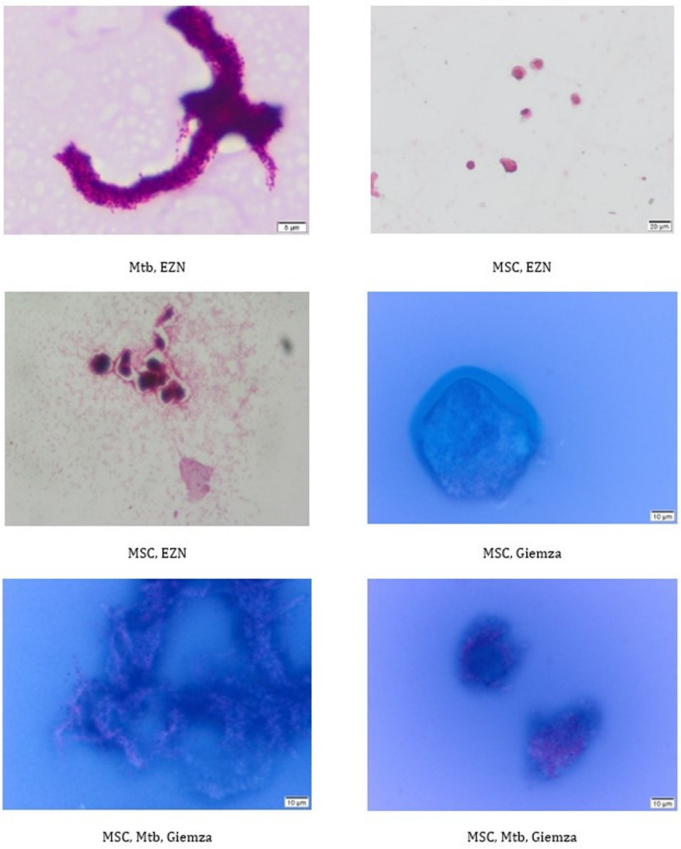

Figure 2. Viable stem cells in culture medium added with TB liquid medium

tive growth signal, which was delayed in the MSC group compared to the MTB group. The MTT findings, however, showed no cell proliferation in the MTB and MSC groups. In the MTB + MSC group, the cells showed a positive but delayed growth signal and the MTT findings showed no cell proliferation. The MTB + Niche group showed the longest delay in cell growth and the administration of Niche in this group led to cell proliferation on the MTT assay. In the MTB + MSC + Niche group, MTB led to delayed cell growth and no cell proliferation was detected on the MTT assay. All the cell growth and proliferation results were significantly ($p < 0.05$) altered (Table 2).

Table 2. Results with mean plus standard deviation for cell growth in the groups

Group (MTT)	Number	SM	SD	Mean	Median
MSC	9	0.04389	0.007044	0.002348	0.04200
MTB	9	0.03844	0.006106	0.002035	0.03800
MTB+MSC	9	0.1007	0.1502	0.05005	0.02900
MTB+NC	9	0.2056	0.1183	0.03942	0.2650
MTB+MSC+NC	9	0.03878	0.01656	0.005520	0.03800
Group (GI)	Number	SM	SD	Mean	Median
MSC	9	26039	2730.7	910.23	26688
MTB	9	8546.1	3056.5	1018.8	8495.3
MTB+MSC	9	3940.7	2633.3	877.78	3359.5
MTB+NC	9	28057	5702.0	1900.7	28309
MTB+MSC+NC	9	28309	7150.0	2383.3	22948
Group (ATR)	Number	SM	SD	Mean	Median
MSC	9	14.778	1.563	0.5212	14.000
MTB	9	8.000	1.871	0.6236	8.000
MTB+MSC	9	11.111	6.353	2.118	9.000
MTB+NC	9	23.667	8.972	2.991	18.000
MTB+MSC+NC	9	18.000	3.296	1.099	21.000

MSC: Mesenchymal Stem cells, MTB: *Mycobacterium tuberculosis*, MTT: (3-(4,5-dimethyl-2-yl)-2,5-diphenyltetrazolium), ATR: Avarage Time of Recovering (Detection of positive group), GI: Growth index

DISCUSSION

Tuberculosis remains a serious bacterial infection affecting large numbers of people across the world. Viral infections and diabetic conditions are serious problems leading to drug resistance in the treatment of TB. MTB has been associated with numerous cells, particularly including immune cells, and cellular interaction has been shown to be a significant factor in TB. MSC are long known to have an immunomodulatory effect against TB bacilli [1] which was discovered through investigations focused on IL2 and Th2 cells (i.e. propathogenic T cells). The present study evaluated the effect of the administration of MSC and their niches on TB bacilli by using different combinations of MTB, MSC and Niche (MTB+MSC, MTB+Niche, and MTB+MSC+Niche) which were added into the culture medium prepared in MGIT tubes. It was revealed that MTB growth was delayed especially by the application of niche in the MTB+Niche and MTB+MSC+Niche groups. These findings could be promising for clinical trials when confirmed by future experimental animal studies.

Literature indicates that MTB evades host defenses and leads to a persistent infection. During this stage, MSC suppress response of T-lymphocyte to MTB. Some previous rat studies detected T lymphocytes and MSC in the same milieu in tissues with MSC infiltration. The MTB strains accumulate in the form of aerosol droplets, and inhalation of these droplets results in pulmonary infection. Fast growth of MTB leads to the recruitment of macrophages, epithelioid cells, and lymphocytes, thereby leading to the formation granulomas which promote permanent residence of MTB [20]. Cellular immune response plays a key role in host resistance to MTB infection. T lymphocytes generate IFN-gamma that plays a role in immune response against MTB and have also been shown to promote the production of reactive nitric oxide (NO) by

activating macrophages. MSC have been shown to suppress T-cell response in the infection site, and the administration of stem cell therapy in animals has been shown to suppress MTB infection. On the other hand, in rats administered with MSC infusion, FoxP3 regulatory T cells (Tregs) are generated from the peripheral pool of $CD4^+$ Tregs. This generation is considered to be associated with NO and to reduce MTB production. MSC are histologically localized peripheral to granulomas and they do not only keep MTB at a distant location but also provide a safe milieu for MTB. In a previous study, an experimental TB model was induced by infecting C57BL/6 rats with virulent H37Rv through the aerosol route. The results indicated that splenocytes showed significantly reduced proliferation in response to the T-cell mitogen Con A, thus implicating immune suppression. Moreover, the authors also found that the proliferative responses of both CD4+ and CD8+ T cells from infected and uninfected rats were similar [21]. The inhibition of cell proliferation is associated with other cells, which is directly related to MTB. Previous studies showed that in spleens infected with MTB, bone marrow cells exhibit the characteristics of MSC and are marked with Sca-1 in the suppression of the immune system. On the other hand, it has been reported that lungs infected with MTB harbor large numbers of MSC which are also marked with Sca-1 and the number of MSC in MTB-infected organs has been shown to be twice higher in spleen than in lung at day 60 post-infection [26]. Additionally, MSC have been used as a therapeutic tool in patients with chronic obstructive pulmonary disease (COPD) and pulmonary hypertension [27]. A previous study induced a rat lung infection model and showed that MSC suppressed the inflammation and exhibited an immunomodulatory effect. The study proposed that lung injury may result in more substantial engraftment of type II alveolar epithelial cells and of interstitial and pulmonary vascular cells during MSC transplantation. The MSC transplanted via local (intratracheal) or systemic (intravenous) routes are considered to be replaced with injured cells via infusion or to promote cell rescue or cell survival. These interactions are mediated by β-catenin pathway, exosomes, horizontal transfer of microRNAs, and proteins. A previous study induced an experimental rat lung injury model and showed that MSC reached the lung tissue 4 h after the administration of systemic stem cell therapy and exhibited their effects in epithelial and vascular endothelial cells. Subsequently, MSC established an interaction with interstitial cells and exhibited a contributory effect on fibrosis [28].

A previous study showed that MTB may sustain long-term intracellular viability in a human bone marrow-derived CD271+/CD45− MSC population *in vitro*. The study also suggested that MTB sustained viability in a similar population of bone marrow-derived MSC in a rat model of dormant TB infection and also in bone marrow-derived CD271+/CD45− MSC isolated from individuals who had completed months of anti-MTB drug treatment [13]. Based on the findings of our study, it is wise to consider that the findings presented in this study[13].It could be associated with MTB proliferation. Both macrophages and dendritic cells are known to act as host cells for MTB growth. However, to our knowledge, there is no information regarding their niches. In a previous study, both MSC and hematopoietic cells were shown to act as host cells for MTB and this condition was attributed to the low levels of endogenous reactive oxygen species (ROS) produced by MSC. Similarly, an animal study also showed that both MSC and hematopoietic cells acted as host cells for MTB in infected rats. The study also noted that MTB was detected in these cells in both in vivo and in vitro model. The MSC in bone marrow and in rat lung were positive for Sca-1, CD105, CD44, and CD271. It was also revealed that MSC niches exerted their therapeutic effects on replicating MTB while they provided a protective intracellular niche for MTB to maintain its viability [13, 30].

The retardant effect of MSC and their niches on MTB proliferation detected in our study could be explained by various mechanisms. In necrotic MTB lesions, the hydrolytic enzymes, metalloproteinases, and serine proteases released by macrophages and neutrophils initiate fibrosis. Meaningfully, the administration of MSC and their niches is likely to promote the effect of MSC therapy through these factors. Moreover, the effects of IF-1, IL-13, and macrophage-mediate TNF-α in the infection site could also be exerted by the effect of MSC and their niches. The therapeutic effects of MSC and their niches could be explained by the activities of NO and related molecules, iNOS and Arginase-1 protein, and M1 and M2 macrophages. High concentrations of molecules that modulate macrophage death in the infected site, such as Prostaglandin E2, lipoxin A4, and TNF-α, may reverse the effect of MSC and their niches. Factors causing macrophage death, such as Early Secreted Antigenic Target (ESAT)-6, cytotoxin, Culture Filtrate Protein (CFP)-10, and TB10.7, can be inhibited by

MSC and their niches. The antioxidant and anti-inflammatory effects of neutrophils recruited in the infected site may be further activated by MSC and their niches. Moreover, capillary thrombosis that occurs following cell death might be prevented by MSC and their niches. MSC and their niches may have a role in the reduction of bacterial burden resulting from the interaction between neutrophils and other cells. Meaningfully, the chromosomal abnormalities associated with neutrophils and macrophages in animal models might be prevented by MSC and their niches, which in turn could eliminate the challenges encountered in the treatment of granulomas [31,32]. Literature indicates that MSC and their niches may have a role in cell proliferation and differentiation, response of cells to signals derived from tissues and organisms, future behaviors of divided cells, and mutational errors [33]. MSC and their niches have been shown to produce signals that affect both immunomodulatory cells and the cells present in the infection site MTB infection models both *in vivo* and *in vitro* [34].

A previous clinical trial reported that the systemic transplantation of autologous MSC resulted in resolution of sustained lung tissue cavities in almost half of the patients and the therapy provided effective outcomes in most patients. Moreover, significantly favorable bacteriological and chemical alterations were observed in 6 patients [10,11].

The incidence of multidrug-resistant TB has been found to be significantly higher in organ transplant recipients compared to the general population. However, the recipients of allogenic bone marrow, lung, and heart transplant have been shown to exhibit good response to second-line therapy [35]. Bone TB is a rarely known form of TB affecting the spine, long bones and joints. In a previous study, the authors used 3D printing technology to fabricate a programmed release multi-drug implant for bone TB therapy. The implant consisted of a concentric cylinder divided into four layers from the center to the periphery. Isoniazid and rifampicin were distributed individually into the different layers in a rotating sequence and the drug release assays indicated that both drugs were released orderly. Moreover, no negative effect was detected regarding the proliferation of rabbit bone marrow MSC. The authors proposed that this implant represents an ideal solution for the elimination of problems in the treatment of bone TB and for an effective local treatment of the disease [36,37]. We consider that this implant could become further effective by the implementation of the findings of our study regarding MSC and their niches.

CONCLUSION

The promising results obtained in the present study and the relationship between MSC and their niches and MTB will pave the way for significant developments when confirmed by future biomedical studies. A 3D examination of the effects of MSC and their niches on MTB behavior and its confirmation by clinical trials will provide significant contributions to the eradication of MTB and the improvement of patients' quality of life.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Khan A, Hunter RL, Jagannath C. Emerging role of mesenchymal stem cells during tuberculosis: The fifth element in cell mediated immunity. T*uberculosis* 2016; 101:45-52.
2. Altice FL, Azbel L, Stone J et al. The perfect storm: incarceration and the high-risk environment perpetuating transmission of HIV, hepatitis C virus, and tuberculosis in Eastern Europe and Central Asia. *Lancet* 2016; 388:p 1228-1248
3. Zumla A, Rao M, Dodoo E, et al. Potential of immunomodulatory agents as adjunct host-directed therapies for multidrug-resistant tuberculosis. *BMC Med*. 2016; 14: 89
4. Mitnick CD, Rodriguez CA, Hatton ML, et al; RESIST-TB (Research Excellence to Stop TB Resistance) and GDI (Global Drug Resistant TB Initiative) Programmatic Management of Drug-Resistant Tuberculosis: *An Updated Research Agenda. PLoS One* 2016; 25: 11
5. Körbling M, Freireich EJ. Twenty-five years of peripheral blood stem cell transplantation. *Blood*, 2011;24: p6411-6417
6. Michael S, Achilleos C, Panayiotou T, Strati K. Inflammation Shapes Stem Cells and Stemness during Infection and Beyond. *Front Cell Dev Biol*. 2016; 4: 118
7. Erbey F, Atay D, Akcay A, ve ark. Mesenchymal Stem Cell Treatment for Steroid Refractory Graft-versus-Host Disease in Children: A Pilot and First Study from Turkey. *Stem Cells Int* 2016;2016: 1641402

8. Lazow SP, Fauza DO. Transamniotic Stem Cell Therapy. *Adv Exp Med Biol*. 2020; 1237p61-74.
9. Saudemont A, Madrigal JA. Immunotherapy after hematopoietic stem cell transplantation using umbilical cord blood-derived products. *Cancer Immunol Immunother* 2016; 6: 215-221
10. Erokhin VV, Vasil'eva IA, Konopliannikov AG, et al. Systemic transplantation of autologous mesenchymal stem cells of the bone marrow in the treatment of patients with multidrug-resistant pulmonary tuberculosis. *Probl Tuberk Bolezn Legk* 2008;10: 3-6.
11. Skrahin A, Ahmed RK, Ferrara G, et al. Autologous mesenchymal stromal cell infusion as adjunct treatment in patients with multidrug and extensively drug-resistant tuberculosis: an open-label phase 1 safety trial. *Lancet Respir Med*, 2014; 2:108-122
12. Ahmad T, Mukherjee S, Pattnaik B, et al. *EMBO J* 2014;9:994-1010
13. Das B, Kashino SS, Pulu I, et al. CD271(+) bone marrow mesenchymal stem cells may provide a niche for dormant *MTB*. *Sci Transl Med*, 2013;5:170
14. Bektöre B, Haznedaroğlu T, Baylan O, ve ark. Investigation of extensive drug resistance in multidrug resistance tuberculosis isolates. *Mikrobiyol Bul J* 2013; 47: 59-70.
15. Mirza IA, Khan FA, Khan KA, et al. Extensively and pre-extensively drug resistant tuberculosis in clinical isolates of multi-drug resistant tuberculosis using classical second line drugs (levofloxacin and amikacin). *J Coll Physicians Surg Pak* 2015; 25 (5):337-341.
16. Vatansever HS, Gumus B, Aydogdu O, ve ark. The role of stem/ progenitor cells and Wnt/β-catenin signaling pathway in the patients with prostate cancer. *Minerva Urol Nefrol* 2014;66(4): 249-255.
17. Akpinar G, Kasap M, Aksoy A, ve ark. Phenotypic and proteomic characteristics of human dental pulp derived mesenchymal stem cells from a natal, an exfoliated deciduous, and an impacted third molar tooth. Stem Cells Int 2014;2014; 457059,
18. Karaoz E, Cetinalp Demircan P, Erman G, ve ark Comparative Analyses of Immune-Suppressive Characteristics of Bone-Marrow, Wharton's Jelly and Adipose-Tissue Derived Human MSC. *Turk J Haematol*. 2017;34(3):213–225.
19. Gorgun C, Ozturk S, Gokalp S, ve ark. AS. Synergistic role of threedimensional niche and hypoxia on conservation of cancer stem cell phenotype. *Int J Biol Macromol* 2016; 90:20-26.
20. Kalscheuer R, Palacios A, Anso I, et all. The *MTB* capsule: a cell structure with key implications in pathogenesis. *Biochem J* 2019;14: 1995-2016.
21. Sia JK, Georgieva M, Rengarajan J. Innate Immune Defenses in Human Tuberculosis: An Overview of the Interactions between *MTB* and Innate Immune Cells. *J Immunol* 2015; 747543.
22. Upadhyay S, Mittal E, Philips JA. Tuberculosis and the art of macrophage manipulation. *Pathog DisJun* 2018; 1:76
23. Goodwin M, Sueblinvong V, Eisenhauer P, et al. Bone marrow-derived mesenchymal stromal cells inhibit Th2-mediated allergic airways inflammation in mice. *Stem Cells* 2011;29: 71137-1148.
24. Raghuvanshi S, Sharma P, Singh S, et al. *MTB* evades host immunity by recruiting mesenchymal stem cells. *Proc Natl Acad Sci* U S A ,2010; 50: 21653-8.
25. Zhang T, Li SY, Williams KN, et al. Short-course chemotherapy with TMC207 and rifapentine in a murine model of latent tuberculosis infection. *Am J Respir Crit Care Med* 2011;6:p732-9.
26. Sueblinvong V, Weiss DJ. Stem cells and cell therapy approaches in lung biology and diseases. *Transl Res* 2010; 3:188-205.
27. Bethune MT, Gee MH, Bunse M, et al. Domain-swapped T cell receptors improve the safety of TCR gene therapy. *Elife* 2016; 8:5.
28. Sueblinvong V, Loi R, Eisenhauer PL, et al. Derivation of lung epithelium from human cord blood-derived mesenchymal stem cells. *Am J Respir Crit Care Med* 2008,7, 701-711.
29. Sueblinvong V, Weiss DJ. Cell therapy approaches for lung diseases: current status. *Curr Opin Pharmacol* 2009; 3: 268-273.
30. da Silva MV, Tiburcio MG, Machado JR, et al. Complexity and Controversies over the Cytokine Profiles of T Helper Cell Subpopulations in Tuberculosis. *J Immunol Res* 2015; 2015:639107.
31. Garhyan J, Bhuyan S, Pulu I. et al. Preclinical and Clinical Evidence of *MTB* Persistence in the Hypoxic Niche of Bone Marrow Mesenchymal Stem Cells after Therapy. *Am J Pathol* 2015;7: 1924-1934.
32. Kramnik I, Beamer G. Mouse models of human TB pathology: roles in the analysis of necrosis and the development of host-directed therapies. *Semin Immunopathol* 2016; 2: 221-37.
33. Gautam US, Mehra S, Kaushal D. In-Vivo Gene Signatures of *MTB* in C3HeB/FeJ Mice. PLoS One 2015;10: 8.
34. Scadden DT. Nice neighborhood: emerging concepts of the stem cell niche. *Cell. Mar* 2014; 1: 41-50.

Abad CLR, Razonable RR. An update on MTB infection after hematopoietic stem cell transplantation in adults. *Clin Transplant*. 2018; 32: 12: e13430.
36. Wu W, Zheng Q, Guo X et al. A programmed release multi-drug implant fabricated by three-dimensional printing technology for bone tuberculosis therapy. *Biomed Mater* 2009; 4: 6.
37. Wu Z, Zhang ZH, Xu JZ. Therapeutic efficacy of drug susceptibility test-guided individualized anti-tuberculosis chemotherapy for spinal tuberculosis. 2010;48(15) p1141-1144.