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Effect of Silicone Rubber/Polyvinyl Alcohol /*Zirconium Oxide* Compound on Mesenchymal Stromal Cells

Efecto del Compuesto de Caucho De Silicona / Alcohol Polivinílico / Óxido de Circonio Sobre las Células Estromales Mesenquimales

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SUMMARY

Objective: *This study aimed to conduct a cytotoxicity test in determining the viability and proliferation profile for novel material of silicone rubber/polyvinyl alcohol (PVA) with or without Zirconium oxide on mesenchymal stromal cell culture.* **Methods:** *An in vitro study was carried out on adipose-derived mesenchymal stromal cell culture. Samples were divided into five groups: control, silicone rubber/polyvinyl alcohol (PVA) without Zirconium oxide, silicone rubber/polyvinyl alcohol (PVA) with Zirconium oxide 1 %, 3 %, and 5 %. Each group contained 2x10^5 seeded MSCs/well stained with MTT for its viability. For proliferation, MTT staining was performed on days 1, 3, and 5 to assess the trend of the percentage of the living cell. Statistical analysis was conducted using ANOVA, or the Kruskal-Walli's test with a CI of 95 %.* **Results***: After exposure to silicone rubber/PVA+ZrO_2 material, the viability of mesenchymal stromal cells was significantly lower in Silicone rubber/PVA+ZrO_2 3 % ($p< 0.05$), compared to Silicone rubber/PVA+ZrO_2 5 % (90,998 ± 3,970 vs. 107,762 ± 7,892). The percentage of living cells from mesenchymal stromal cell cultures after exposure to silicone rubber/PVA+ZrO_2 day-1 was not statistically significant, but silicone rubber/PVA had the maximum percentage (102.47 %). In contrast to day 1, the results of the ANOVA test on days -3 and -5 revealed a significant difference between the 5 groups ($p<0.001$). Similarly, the Tukey-Kramer post-hoc test on the group yielded comparable results. Decreased across all groups were observed on day 5 of observation with 3 % ZrO_2 group being the lowest.* **Conclusion:** *Silicone rubber/polyvinyl alcohol (PVA) compound with or without Zirconium oxide*

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(ZrO_2) exposure did not show a toxic effect on mesenchymal stroma cell culture. Further, in vivo studies are needed to confirm our findings.

Keywords: *Silicone rubber, Polyvinyl alcohol (PVA), Zirconium oxide (ZrO_2), Cytotoxicity, MTT assay.*

RESUMEN

Objetivo: *Este estudio tuvo como objetivo realizar una prueba de citotoxicidad para determinar la viabilidad y el perfil de proliferación de un nuevo material de caucho de silicona/alcohol polivinílico (PVA) con o sin óxido de circonio en cultivos de células estromales mesenquimales.* **Métodos:** *Se realizó un estudio in vitro en cultivo de células estromales mesenquimales derivadas del tejido adiposo. Las muestras se dividieron en cinco grupos: control, caucho de silicona/alcohol polivinílico (PVA) sin óxido de circonio, caucho de silicona/alcohol polivinílico (PVA) con óxido de circonio al 1 %, 3 % y 5 %. Cada grupo contenía $2x10^5$ MSC sembradas/pocillo teñido con MTT para determinar su viabilidad. Para la proliferación, se realizó tinción con MTT los días 1, 3 y 5 para evaluar la tendencia del porcentaje de células vivas. El análisis estadístico se realizó mediante ANOVA, la prueba de Kruskal-Walli con IC del 95 %.* **Resultados:** *Después de la exposición al material de caucho de silicona/PVA+ZrO_2, la viabilidad de las células estromales mesenquimales fue significativamente menor en caucho de silicona/PVA+ZrO_2 3 % ($p < 0,05$), en comparación con caucho de silicona/PVA+ZrO_2 5 % (90,998 ± 3.970 frente a 107.762 ± 7.892). El porcentaje de células vivas procedentes de cultivos de células estromales mesenquimales después de la exposición al caucho de silicona/PVA+ZrO_2 día-1 no fue estadísticamente significativo, pero el caucho de silicona/PVA tuvo el porcentaje máximo (102,47 %). A diferencia del día 1, los resultados de la prueba de comparación ANOVA de los días -3 y -5 revelaron una diferencia significativa entre los 5 grupos ($p <0,001$). De manera similar, la prueba post hoc de Tukey-Kramer en el grupo arrojó resultados comparables. Se observó una disminución en todos los grupos el día 5 de observación, siendo el grupo con 3 % de ZrO_2 el más bajo.* **Conclusión:** *El compuesto de caucho de silicona/alcohol polivinílico (PVA) con o sin exposición a óxido de circonio (ZrO_2) no mostró un efecto tóxico en el cultivo de células del estroma mesenquimatoso. Se necesitan más estudios in vivo para confirmar nuestros hallazgos.*

Palabras clave: *Caucho de silicona, alcohol polivinílico (PVA), óxido de circonio (ZrO_2), citotoxicidad, ensayo MTT.*

INTRODUCTION

Lumbar disc herniation (LDH) is an early and rather common sign of degeneration in the lumbar spine (1). Lumbar disc herniation results from several changes in the intervertebral disc including reduced water retention in the nucleus pulposus, increased type 1 collagen ratio in the nucleus pulposus and inner annulus fibrosus, destruction of collagen and extracellular material, and an upregulated activity of degrading systems such as matrix metalloproteinase expression, apoptosis, and inflammatory pathways. Ultimately, resulting in a local increase in inflammatory chemokines and mechanical compression applied by the protruding nucleus pulposus on the exiting nerve (1). The prevalence of LDH is estimated to be around 12 %, with a reported incidence of 2 % to 3 % (2). Patients with classical signs of motor deficit, cauda equina syndrome, and persistent pain will not benefit from conservative treatment and will require surgery to decompress the nerve involved (3). Numerous studies have compared conservative versus surgical treatment in lumbar disc herniation, observing faster pain relief and recovery in the early surgery groups, however, similar outcomes in the mid- and long-term were discovered (4,5). This phenomenon might be explained by the occurrence of substantial disc height reduction following discectomy which is proportional to the amount of nucleus removed (6). Disc height changes can have both local and global consequences. Reduced disc height and volume increase the stress on the remaining nucleus pulposus (NP), which can lead to a decrease in cell matrix synthesis and an increase in cell necrosis and apoptosis. Reduced disc height also causes major alterations in the spine's overall mechanical stability, which may lead to further spinal segment degeneration (7).

Several treatment options are currently available for LDH which focus on pain management, extruded disc tissue excision, and intervertebral disc (IVD) replacement or spinal fusion (8). The purpose of nucleus pulposus augmentation following disc removal is to prevent disc height decline and the associated biomechanical and biochemical changes (7). Clinical translation of implanted biomaterials cannot occur without evidence of durability, or

the ability to maintain physical support across millions of cycles of loading, as well as the generation of no or limited wear debris that could elicit a systemic immune response (9). Injectable biomaterials that can replace the disc nucleus pulposus after microdiscectomy have been developed. The novel injectable biomaterial was comprised of 40% PVA and 60% silicone rubber and the biomechanical compression test results revealed that the stress (MPa) and strain (%) values of the biomaterial resemble human nucleus pulposus properties (10,11). Although several NP augmentation biomaterials have been developed, only several have progressed beyond clinical trials to market approval (11,12).

Materials used in medical devices, particularly those in which the device contacts or is temporarily inserted or permanently implanted in the body, must meet basic biocompatibility requirements, generally defined by the American Society for Testing and Materials (ASTM) F-748 and the International Standards Organization (ISO) 10993 standards, to be nontoxic, non-thrombogenic, noncarcinogenic, nonantigenic, and nonmutagenic (13). The cytotoxicity test is one of the biological evaluation and screening techniques that uses tissue cells *in vitro* to observe how medical devices affect cell growth, reproduction, and morphology (14). Because it is simple, fast, has a high sensitivity, and can rescue animals from poisoning, cytotoxicity is recommended as a pilot project test and an important signal for toxicity evaluation of medical devices (15). To examine the safety of our novel biomaterial of silicone rubber/ polyvinyl alcohol with additional *Zirconium oxide* (ZrO_2) compound usage against surrounding intervertebral disc (IVD) cells, this study aimed to conduct an *in vitro* cytotoxicity test against viability and proliferation on Mesenchymal Stromal Cells (MSCs) culture.

MATERIAL AND METHODS

Preparation of Silicone Rubber, PVA Material, and Zirconium oxide (ZrO_2)

PVA crosslinked with glutaraldehyde (GA) is obtained by mixing 20% wt. PVA in distilled water. This solution is added with H_2SO_4 (aq) solution at as much as 10% wt. to initiate crosslinking between PVA and GA. Room-temperature-vulcanizing (RTV) silicone rubber, RTV 585, was prepared with a variety of 5% catalysts. Silicone rubber RTV 585 was mixed with 40PVA60SR (40% PVA and 60% silicone rubber) and additional compositions of Zirconium oxide (ZrO_2) 1%, 3%, and 5% (10,11).

Radio opacity was assessed by the addition of ZrO_2 and was qualitatively assessed under conventional X-ray. The material was then soaked in culture medium for 24 hours of which 100 μL of the treated medium was placed into the well that had been seeded with mesenchymal stromal cells followed by an incubation period for 1 day, 3 days, and 5 days for each treatment groups.

Preparation of Mesenchymal Stromal Cells (MSCs) culture and MTT staining

Adipose-derived MSCs were taken from the CO_2 incubator in 80 % confluence for harvesting. Then, harvest the cells until they become single cells and homogenize them in the culture medium. Placed the cells into 3 pieces of 96 well culture plates with a concentration of $2x10^5$/well for evaluation on day 1, day 3, and day 5, then 2 rows of well were left on each plate for blanks. The cells were incubated in a 96-well plate by placing them into the incubator CO_2 for 24 hours until the cells adhered perfectly.

Cytotoxicity test: viability and proliferation

The evaluation of cytotoxicity was performed on two cytotoxicity parameters, cell viability and cell proliferation. The study material was introduced using intervention on four treatment groups which consisted of silicone rubber/PVA alone and silicone rubber/PVA+ZrO_2 1 %, 3 %, and 5 % as separate groups. The groups were comprised of control (n=6), silicon rubber/PVA (n=6), silicon rubber/PVA+ZrO_2 1 % (n=6), 3 % (n=6), and 5 % (n=6). Cell culture viability was estimated with a colorimetric assay using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) staining (Sigma-Aldrich Corp., St. Louis, MO, USA) on day-1 (24h). This assay measures the reduction of yellow MTT to an

insoluble blue formazan product by mitochondrial succinate dehydrogenase, and the amount of formazan produced is directly proportional to the number of livings, not dead cells, present during MTT exposure. Proliferations were evaluated with a colorimetric assay using subsequent MTT techniques on the first (24h), third (72h), and fifth days (120h) for the proliferation test.

In total 25 μL/well of 5 mg/mL MTT was used before the third incubation for 4h. The medium was discarded following the third incubation. *Dimethyl sulfoxide* (DMSO) 200 μL/well was added. MSCs absorbance was determined with the use of an ELISA reader (Multi Reader Promega GM35000) at 595 nm wavelength. The percentage of viable cells and IC_{50} value were calculated using linear regression of log concentration. All the samples were also evaluated under a microscope to assess the cell distribution using a Cell Culture Microscope (Olympus CKX53FL-DP27) with 100x magnification.

Statistical analysis

The results of data collection were presented as mean ± standard deviation (SD), median minimum-maximum, and percentage (%). Statistical analysis was performed using the IBM SPSS Statistics software version 23.0 for Mac (IBM Corp., Armonk, NY, USA). Data distributions were calculated using the Saphiro-Wilk test, while data variance was calculated using Levene's test. ANOVA test followed by Tukey-Kramer test a post-hoc statistical test used to determine whether the means of two sets of data are statistically different from each other. This test is based on the studentized range distribution. Kruskal-Walli's test, a nonparametric method, was used to test whether samples originated from the same distribution. A p-value of 0.05 was considered a significant difference between the means, and correlation was determined within 95 % CI with $p < 0.05$.

RESULTS

The radio-opacity of a mixture of Silicon Rubber/PVA+ZrO_2 (1 %, 3 %, and 5 %) was qualitatively evaluated using conventional X-ray. The result showed that the addition of ZrO_2 produces a radiopacity which corresponded to an increase in ZrO_2 concentrations (Figure 1).

Viability of MSCs exposed with Novel compound

MSCs data evaluation showed normal data distribution (p=0.896) and homogeneous data (p=0.056). The mean percentage (%) of living cells is 100 ± 8.843 for the control group, 93.867 ± 12.283 for the Silicone rubber/PVA group, 97.605 ± 6.524 for the Silicone rubber/PVA+1 % ZrO_2 group, 90.998 ± 3.970 for the Silicone rubber/PVA+ group ZrO_2 3 %, and 107.762 ± 7.892 for the Silicon rubber/PVA+ZrO_2 5 % group. A

Figure 1. Conventional X-ray image of the composite material Silicon Rubber/PVA + ZrO_2 [1 %(11-12E/D), 3 %(1-2E/D), and 5 %(21-22E/D)].

significant difference ($p < 0.05$) was observed between the Silicon rubber/PVA+ZrO_2 3 % group when compared with the Silicon rubber/PVA+ZrO_2 5 % group (Table 1).

Table 1

ANOVA test for MSCs viability following exposure to the treated culture medium

Group	**Viable cell (%)**		
	Mean	**SD**	**p**
Control	100	8.843	
Silicone rubber/PVA	93.867	12.283	
Silicon rubber/PVA+ZrO_2 1%	97.605	6.524	0.02
Silicon rubber/PVA+ZrO_2 3%	90.998	3.970	
Silicon rubber/PVA+ZrO_2 5%	107.762	7.892	

Proliferation of MSCs

On days 3 and 5, the live cell counts with MTT staining in each well (n=30) were read by colorimetric assay. Cell confluences in all groups were captured under the microscope and are shown in Figures 2 and 3. ANOVA test showed a significant difference ($p<0.01$) between groups for each day. The mean percentage value can be seen in Table 2.

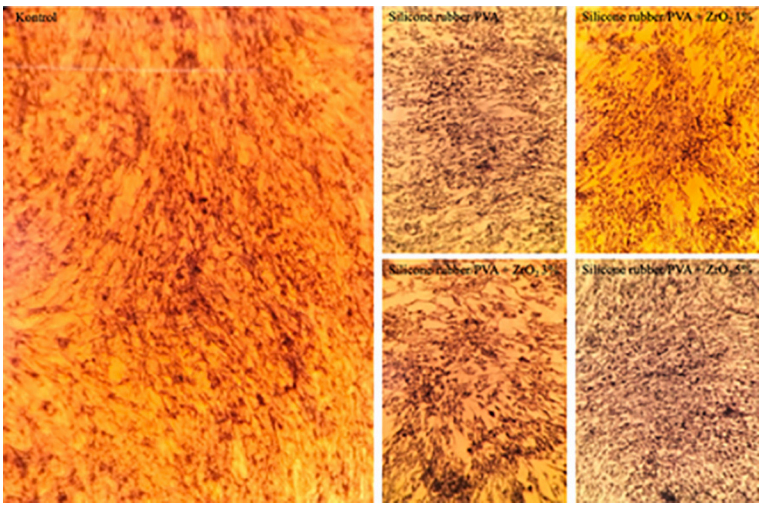

Figure 2. Mesenchymal Stromal cell culture for examination on day 3 with MTT staining for the five groups: control, silicone rubber/PVA, silicone rubber/PVA+ZrO_2 1 %, 3 %, and 5 %. (Olympus Microscope CKX53FL-DP27, 100x magnification).

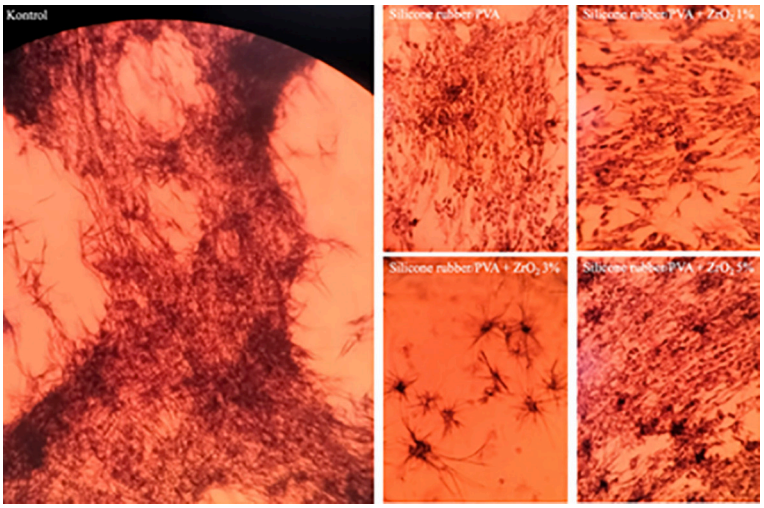

Figure 3. Mesenchymal Stromal Cells culture for examination on day 5 with MTT staining for the five groups: control, silicone rubber/PVA, silicone rubber/PVA+ZrO_2 1 %, 3 %, and 5 %. (Olympus Microscope CKX53FL-DP27, 100x magnification).

Table 2

ANOVA Test for Day 3 and 5 observations of MSCs following treated culture medium

Group		Viable cell (%)		
Day 3		Mean	SD	p
	Control	100	5.761	
	Silicone rubber/PVA	76.430	9.051	
	Silicon rubber/PVA+ZrO_2 1 %	72.173	2.761	< 0.001
	Silicon rubber/PVA+ZrO_2 3 %	66.693	6.933	
	Silicon rubber/PVA+ZrO_2 5 %	92.762	5.705	
Day 5				
	Control	100	20.437	
	Silicone rubber/PVA	57.945	16.408	
	Silicon rubber/PVA+ZrO_2 1 %	58.077	7.941	< 0.001
	Silicon rubber/PVA+ZrO_2 3 %	10.345	4.799	
	Silicon rubber/PVA+ZrO_2 5 %	86.688	7.254	

We further analysed for significant differences between each observation's days (1,3,5) using the Mann-Whitney test, which revealed a statistically significant difference in the percentage of living cells between the groups treated. Day 1 and 3 comparison of silicone rubber/PVA, silicone rubber/PVA+ZrO_2 1 %, and silicone rubber/PVA+ZrO_2 3 % showed a significant difference ($p<0.05$), whereas day 1 and 5 comparison showed statistically significant difference ($p< 0.05$) between the percentage of living cells observed in the groups treated with silicone rubber/PVA, silicone rubber/PVA+ZrO_2 1 %, and silicone rubber/PVA+ZrO_2 3 %. We also found statistically significant differences ($p< 0.05$) between the percentage of viable cells when comparing days 3 and 5 in the treatment group between 1 % silicone rubber/PVA+ZrO_2 and 3 % silicone rubber/PVA+ZrO_2. Figure 4 illustrates

the trend of the average percentage of living cells in each treatment group for five days. The percentage decreased, which is generally stable. From day 3 to day 5, the Rubber / PVA + ZrO_2 3 % group showed a significant decrease.

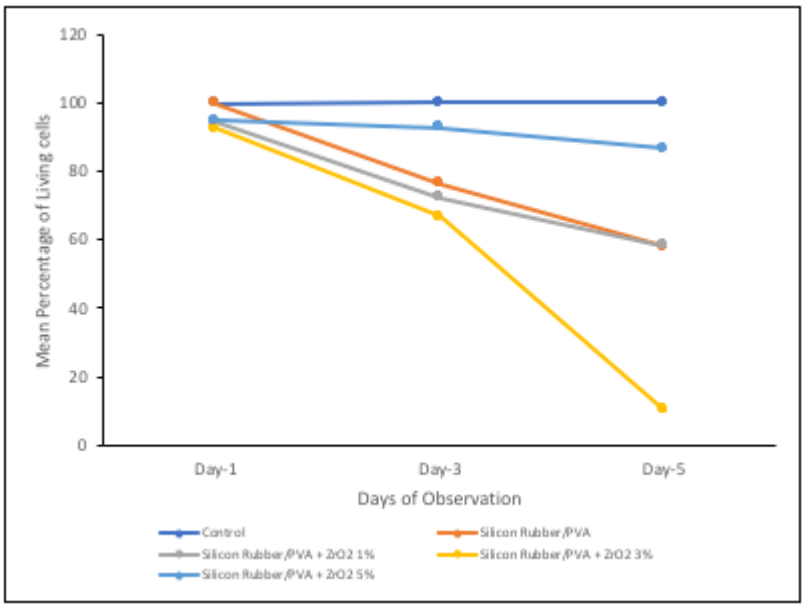

Figure 4. Percentage of surviving cells in the treatment group.

DISCUSSION

American Standards and Test Methods (ASTM) International developed the first standards for testing cytotoxicity in the early 1980s. This standard has then been adapted by various countries and by the International Organization for Standardization (ISO), where cytotoxicity testing is specifically addressed in ISO 10993-5 (14). Types of cytotoxicity tests are stated in ISO 10993-5: Extract, direct contact, and indirect contact tests (including agar overlay assay and filter diffusion). In general, the extracted test is suitable for detecting the toxicity of soluble substances of medical devices and is usually consistent with the results of animal toxicity tests (10,13,15). Applications for silicones extend to extracorporeal devices, catheters, drains, shunts, various long-term implants, orthopaedic implants, and aesthetic implants (16). An ideal NP implant should have the same biomechanical properties and bioavailability as human NP (17,18) A composite of silicon rubber and polyvinyl alcohol (PVA) is a promising material for artificial disc replacement (15,19-22).

Cytotoxicity is one of the many parameters of compatibility and should be one of the principal parameters assessed in biocompatibility testing (23). Cytotoxicity can be evaluated by various tests, including the Cytotoxicity elution test (MEM elution), MTT assay, Agar overlay assay, and other means. Among these tests, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay is considered the gold standard for *in vitro* cytotoxicity testing (24). The direct contact assay is the most sensitive for testing the cytotoxicity of medical devices; the medical devices can be measured even with weak cytotoxicity. It does however have a prerequisite that the sample should cover only 10 % of the culture dish surface. Therefore, the dimension

of the test subject might not allow direct testing in this study. Furthermore, direct testing is dependent on the contact regime affected by the material density and adherence to cell culture. A certain material with a higher density might potentially crush the cells, while a less dense material might float in the medium. Adherent synthetic material might cause rupture of the underlying cell during removal and might cause a falsely perceived reduction in cell count. Extract testing through a conditioned medium is thought to have an advantage in this study, providing a uniform concentration exposure to the cell culture. This concentration is reflective of the constituents released from the initial contact, as opposed to the direct method, where the maximal concentration is achieved only at the end of the treatment period, normally 24 h (14). This standard recommends Phosphate Buffer saline (PBS) as a buffer solution which is isotonic and non-toxic and aims to maintain cell osmolarity and not interfere with tissue viability (25). MTT undergoes enzymatic reduction to purple formazan in metabolically active cells Assay is then done by comparing cell exposure to a substance and measuring the decrease in optical density. Studies also compare optical density at different times to determine exposure duration. According to previous studies, a cell viability decrease of over 50 % indicates positive cytotoxicity (26,27). The biocompatibility standards conform regarding material–tissue contact duration, which is differentiated into three time periods: (i)<24 h, intra-operative contact, (ii) 24 h to 30 days, defined as short-term implantation, and (iii)>30 days, which is called permanent or chronic implantation (28). This study uses MSCs *in vitro*, as stroma cells exist in embryos and adult cells with multiple differentiation stages (29). MSCs are easy to isolate, culture, and manipulate in ex vivo culture. The cell populations could represent different points of a hierarchy or a continuum of differentiation, for example, the intervertebral disc tissue (30). Preliminary assessment of the material *in vitro* can already provide insight into the applicability of the biomaterial *in vivo* (31). Determining *in vivo* cytotoxicity is found to be more expensive and needs a longer duration of observation, therefore *in vitro* experiments are chosen in the study. The study found that the Silicon rubber/PVA group had the maximum viability, with a median percentage of living cells of 102.47. The Silicon rubber/PVA+ZrO_2 3 % group had the lowest viability, with a median percentage of living cells of 89.06 which was in conjunction with the study by Mirzadeh, showing silicone having the highest viability for MSCs (32). Silicone's use as a biomaterial necessitates consideration of its surface properties, including surface charge, water-binding ability, chemistry, topography, electrical conductivity, critical surface tension, morphology, roughness, and rigidity (32). Zirconium, which can generate reactive oxygen species, affects cytotoxicity (33). Due to its biocompatibility and corrosion resistance, zirconium is still utilized in bioceramics and implants despite the lack of information regarding its toxicity (34). Despite Ye and Shi (35) assertion that the addition of zirconium in a certain proportion increased toxicity, additional research is required to determine the cytotoxicity of the zirconium component.

Biomimetic scaffold is one of the most promising strategies in the field of bone tissue engineering. Zirconium oxide (ZrO_2), as a kind of bioceramic material, has attracted much attention in biomimetic scaffolds due to its excellent biocompatibility, high mechanical strength, and great chemical stability. ZrO_2 is widely used in industry, biomedicine, and dentistry, for example as ceramic dental prostheses, dental implant coatings, and bone restorative materials. A lot of work has been carried out to investigate the characteristics and applications of zirconia-based biomimetic scaffolds. However, few works can provide a systematic comparison and overview of the research progress of zirconia-based biomimetic scaffolds. It was proposed the use of ZrO_2 as the basis for the scaffold and the use of bioactive materials as layers to achieve a combination of mechanical properties and bioactivity (36). However, it was suggested that ZrO_2-NPs have negative impacts on the liver and exhibit potential risks for non-alcoholic fatty liver disease. In this regard, Sun et al. investigated the hepatic biodistribution and toxicological effects of ZrO_2-NPs after intravenous administration (20 mg/kg, bw) *in vivo* and the toxicological mechanism toward hepatocytes *in vitro*. They demonstrated that the liver showed continuous ZrO_2-NP accumulations associated with oxidative stress, increased inflammatory responses, and

functional injury. Meanwhile, the results of the *in vitro* studies demonstrated that ZrO_2-NPs exposure resulted in cytotoxicity in Hepg2 cells in a dose- and time-dependent manner. RNA-sequence from the spleen and brain of mice injected with ZrO_2 nanoparticles showed significant changes in gene expression (37). Alzahrani et al. reported the apoptotic and DNA-damaging effects of Yttria-stabilized ZrO_2-NPs also known as Yttria Zirconia, Yttria Stabilized Zirconium Oxide, on human skin epithelial cells (38). In addition, it was studied the effects of ZrO_2NPs on early life stages of the zebrafish (Danio rerio) to examine such effects on embryonic development in this species. ZrO_2NPs instigated developmental acute toxicity in these embryos, causing mortality, hatching delay, and malformation. Developmental toxicity of zebrafish embryos caused by zirconium oxide nanoparticles in aquatic environments shows that exposure to zirconium oxide nanoparticles is toxic to embryonic zebrafish (39). However, Yang et al. (40) stated that mice injected with ZrO_2 had remained material in lysosomal vesicles, in the liver and spleen macrophages, without any abnormal ultrastructural changes up to a dose of 500 mg/kg. Our study showed a decrease in the number of living mesenchymal stroma cells, which was found below 50 % in the group with 3 % ZrO_2 levels, suggesting that at concentration used does not present cytotoxic effects.

The present study examined the proliferation of mesenchymal stroma cell cultures exposed to a silicone rubber/PVA mixture on days 1, 3, and 5 using the MTT method (41). In this study, we used a period of 1, 3, and 5 days which is the midterm category for this analytical test, a long-term evaluation is needed to find out more about the effect of silicon rubber/PVA exposure on mesenchymal stroma cell culture after day 5. Another classification regarding the period is mentioned in ISO 10993 by observing the interventions in a certain period. They divided the tests into systemic toxicity (acute toxicity), subacute toxicity, and subchronic toxicity. Acute toxicity is observed within the first 24 hours, while subacute and subchronic toxicity is observed for a period not less than 24 hours and <10 % of the total lifespan of the mesenchymal stroma cell. The subacute period is chosen in this research to evaluate the progressivity of cytotoxic effects on the short-term implantation period, as mentioned above, within 24 hours to 30 days (34). The IC_{50} is the concentration of the biomaterial that causes 50 % cell mortality when tested. Consistent with research by Ye and Shi demonstrating zirconium's apoptotic effect, our study showed a decrease in cell proliferation proportional to exposure duration (35). In addition to the concentration of zirconium, exposure duration also impacts proliferation. Silicon, despite its widespread use, is toxic, as demonstrated by Onnekink et al. and Chen et al., who demonstrated that nano silicon carbide had a toxic effect on human mesenchymal stroma cells, but not on cancer cell lines at a concentration of 0.1 mg/mL (42,43). It can be assumed that tissue integration of material is correlated with optimal cell proliferation. The scaffold creates tissue with cells, factors, or a bioreactor. Factors for choosing a scaffold in tissue engineering. A biocompatible scaffold is necessary for cells to attach, function, migrate, and proliferate without an immune response. It should be biodegradable, non-toxic, and easily expelled to support cells in creating their extracellular matrix. The perfect implant scaffold must be site-specific, and strong yet allow cell infiltration. The scaffold for tissue engineering should have a porous structure and high porosity for cell penetration, nutrient diffusion, and waste removal without harming nearby organs or tissues. The pore size is vital for cell-scaffold interaction (44). Since our data are not conclusive, further research is required to determine the optimal concentration and changes in bonding or morphology resulting from the mixture of the experimental constituents and their effects on proliferation.

Study Limitations

The limitations of this study include (1) The immunocytochemical assessment is not directly analysed by evaluating cell morphology; (2) Neither inflammation nor morphology was evaluated; (3) No animal experimental investigations (*in vivo*) were conducted.

CONCLUSION

The cytotoxicity profile of novel silicone rubber/PVA compound with or without Zirconium oxide (ZrO_2) was found as a biomaterial for nucleus pulposus replacement on a mesenchymal stromal cell culture. The mixture of silicone rubber/PVA treated with or without ZrO_2 concentrations of 1, 3, and 5 % did not decrease cell viability in mesenchymal stroma cell cultures. Comparing the four components of the silicone rubber/PVA compound with or without Zirconium oxide (ZrO_2), it was determined that the mixture containing 5 % ZrO_2 had the greatest cell proliferation results. The silicone rubber/PVA compound with or without Zirconium oxide (ZrO_2) was not toxic for up to 5 days of exposure, except the SR/PVA+ ZrO_2 3 %.

Ethical approval

All procedures performed and materials included in the study are by the ethical standards of Dr. Soetomo Hospital, Surabaya, Indonesia (Ethical number 0869/111/2/VIII/2021).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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