SURFACE BIOCOMPATIBILITY OF POROUS TITANIUM STRUCTURES WITH STEM CELLS

Marianna Trebuňová, Jana Demeterová, Darina Bačenková, Viktória Rajťuková, Jozef Živčák

Technical University of Košice, Košice, Slovakia

Abstract

Successful tissue regeneration requires scaffolds with mechanical stability or biodegradability, surface roughness, and porosity to provide a suitable microenvironment for sufficient cell interaction, migration, cell proliferation, and differentiation. This study features the design, fabrication, and biocompatibility testing of Ti-6Al-4V titanium alloy scaffolds. Cylindrical titanium samples were tested, where each sample had a porous structure with pore sizes of 0.4 mm, 0.8 mm, and 1.0 mm respectively, which were seeded with chorionic-derived mesenchymal stem cells (CMSCs). The viability of the seeded CMSCs was evaluated using the MTT test. The aim of the study was to evaluate the cytotoxic effect and biocompatibility of porous titanium scaffolds. CMSCs showed the highest viability, adhesion to surfaces, and good proliferation on samples with 0.4 mm pore size, on the other hand, the pore size of 1.0 mm showed relatively lowest compatibility with cells and their proliferation. However, the viability of cells on all tested sizes of porous titanium scaffolds showed sufficient viability for future use in regenerative medicine.

Keywords

Chorionic mesenchymal cells, scaffold, titanium, in vitro, cytotoxicity

Introduction

A scaffold is an artificial three-dimensional framework that mimics the extracellular matrix for cell adhesion, migration, proliferation, and tissue regeneration. An essential condition for the creation of a scaffold is its porous structure. Porosity mimics the natural structure of bone tissue with an interconnected network of pores specifically for cells, their growth and nutrient transport [1]. In 2016, Wang et al. published their findings on the effect of pore size on the acceleration of osseointegration, which should be in the range of 0.1–0.9 mm [2]. 3D scaffolds for tissue engineering applications face two main limitations—the scaffolds cannot be too porous (due to reduced mechanical strength), but neither can they have significantly low porosity (due to lack of cell infiltration, vascularization and signaling) [3]. For an improvement of incorporation of cells, a collagen film on the scaffold surface can be used to promote adhesion of fibroblast cell lines [4].

This work works with the cultivation of stem cells from fetal membranes—chorionic mesenchymal stromal cells (CMSC) on titanium Ti-6Al-4V scaffolds. Several studies have shown that these cells have higher immunosuppressive effects compared to adult mesenchymal stem cells [5, 6]. The advantage of these cells lies in the fact that mesenchymal stem cells from amniotic and chorionic membranes are pluripotent and differentiate not only into the mesodermal lineage, but also into ectodermal and endodermal cells [7]. The aim of the study was to evaluate the cytotoxic effect and biocompatibility of porous titanium scaffolds.

Materials and methods

Desing and implementation of 3D titanium scaffold

The design of the 3D CAD model was carried out in the modeling environment of the Solidworks software ver. PRO 2021 (Materialise, Belgium). As a reference sample, a sample with the same shape and dimensions was created, with a full structure—so-called "solid". Fifteen pieces of each type of test sample were produced. This was followed by semi-automatic generation of the porous structure of the test samples using the modeling software Autodesk Within Medical (Biomedical Engineering, s.r.o., Košice, Slovakia). When creating the porous structure, it was necessary to enter the necessary parameters listed in Table 1. Porous

Lekar a technika – Clinician and Technology 2023, vol. 53(1), pp. 15–18, DOI: 10.14311/CTJ.2023.1.03
ISSN 0301-5491 (Print), ISSN 2336-5552 (Online)

test samples were made using SLM (Selective Laser Melting) additive manufacturing. The result was cylindrical Ti-6Al-4V titanium test samples with an internal cubic structure in pore sizes of 0.4, 0.8 and 1.0 mm. Fig. 1 shows 3D CAD models of test samples with specific porous structures. A layer of collagen type I in buffered saline (PBS) (100 µg/ml) was applied to the samples. The titanium matrices were incubated in type I collagen solution at 7 °C for 24 hours. After aspirating in the collagen solution, the samples were let to air dry for approximately one hour.

Table 1: Basic parameters in creating a porous structure.

Parameter	Value
Sample diameter (mm)	10.0
Sample height (mm)	10.0
Grid size (mm)	0.4 / 0.8 / 1.0
Lamella thickness (mm)	0.2
Internal topology	grid
Profile of the internal structure (pore)	square
Trimming of the surface	advanced

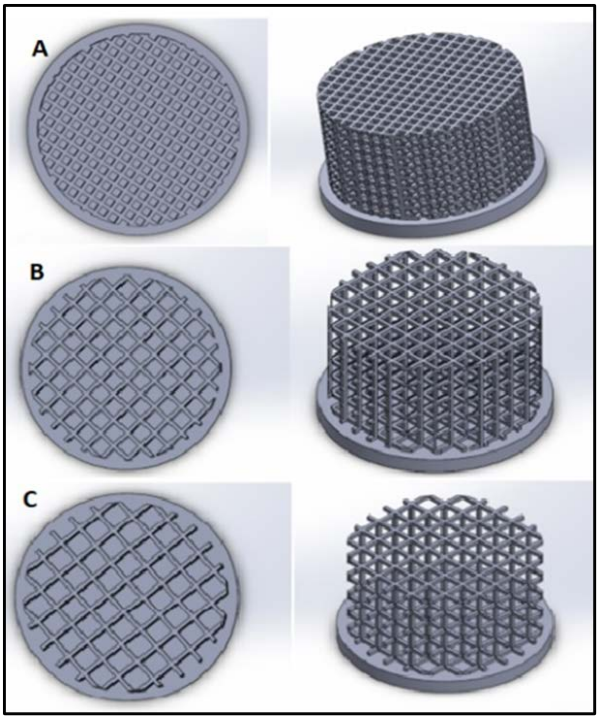

Fig. 1: 3D CAD model of test sample with internal cubic structure, pore size: 0.4 mm (A), 0.8 mm (B), 1.0 mm (C).

Isolation and cultivation of chorionic mesenchymal stem cells

In cooperation with the gynecology-obstetrics clinic of the AGEL Košice-Šaca hospital, after the planned termination of the pregnancy, with the consent of the obstetricians, the fetal envelopes were preserved. The hospital has written informed consent from the donor according to the hospital's internal standards and according to the national ethical guidelines for stem cell research and regenerative medicine. CMSCs were isolated enzymatically with dispase followed by type II collagenase (1 mg/mL, Gibco) in Dulbecco's Modified Eagle Medium (DMEM) with antibiotics. Enzyme solutions were sterilized by filtration before use. A microfilter (0.2 µm) was used. The amnion was manually separated from the chorion and subsequently washed in DMEM with the antibiotics penicillin and streptomycin. Then the amnion and chorion were cut into small pieces 2×2 cm. Pieces of chorion were incubated for 15 min at a temperature of 37 °C in 20–40 ml of dispase solution 2.4 U/ml in DMEM. After the dispase enzyme, the chorionic tissue was washed with DMEM medium and centrifuged for 10 minutes at 1300 rpm. After washing, the fragments were digested with the enzyme collagenase (1.0 mg/ml) for 90 minutes at a temperature of 37 °C. The digested chorion fragments—cell suspension with tissue remnants were filtered through a 40 µm cell strainer and the cells were centrifuged again for 10 minutes at 1300 rpm. The isolated washed cells were seeded at a concentration of 4000 cells/cm^2 in a plastic culture bottle with a culture area of 75 cm^2. This was followed by in vitro cultivation of chorionic mesenchymal cells in a 5% CO_2 atmosphere and 37 °C in an incubator. DMEM with 10% fetal bovine serum (FCS) and 1% antibiotics/antimycotics was used as a complete culture medium. The complete culture medium was changed 2 to 3 times a week. After the formation of a monolayer of adherent cells for a period of approximately 10 to 14 days, CMSCs are passaged with the enzyme trypsin-EDTA 0.25%. Trypsin was left to act for 5 minutes at 37 °C in an incubator. During this time the cells were released and after washing the cells formed a suspension suitable for further cytotoxicity testing.

Determination of biocompatibility of titanium scaffold

Scaffolds made of titanium Ti-6Al-4V in the form of a grid in three pore size modifications were used for cytotoxicity testing: 0.4 mm, 0.8 mm and 1.0 mm. The titanium scaffolds were washed in distilled water and sterilized in a UV box (Opsytec, Germany) for 1 hour. They were tested on a 24-well plate using seeded CMSCs. CMSCs together with scaffolds were cultured in vitro at 5% CO_2 atmosphere, 37 °C using DMEM culture medium with 10% FCS and 1% antibiotic solution, penicillin-streptomycin-amphotericin B (Pen/Strep/Amph). The culture medium was changed 2–3 times a week. In the comparative experiment, the grid material was coated with bovine collagen type I in all modifications of 0.4 mm, 0.8 mm and 1.0 mm. CMSCs cells were trypsinized after 14 days of culture with titanium matrices with the enzyme trypsin-EDTA

Lekar a technika – Clinician and Technology 2023, vol. 53(1), pp. 15–18, DOI: 10.14311/CTJ.2023.1.03
ISSN 0301-5491 (Print), ISSN 2336-5552 (Online)

0.25%. After approx. 4–5 min after enzyme action, cells were detached and aspirated after adding DMEM medium with 5% FCS to stop enzyme action. The cell suspension was washed by centrifugation for 13 minutes at 1300 rpm at 4 °C. The number of cells was determined by staining with trypan blue. Subsequently, the MTT test was performed. 100 µl of cell suspension with the number of 10 000 cells per well was pipetted onto a 96-well plate, and 20 µl of MTT (2.5 mg of MTT per 1 ml of PBS) was added the next day [8]. After 4 hours, 100 µl of prepared SDS (1 g SDS per 10 ml PBS) was added. The plate was left to incubate for the next day in the dark at room temperature. Finally, the absorbance was measured with a spectrophotometer (PG instruments, Great Britain) at a wavelength of 490 nm.

Results

Isolation and cultivation of cells

At 24 hours after cell seeding, cell adherence was observed on a transparent plate of a 24-well culture plate with a Ti-6Al-4V scaffold. For 14 days, proliferation was visually analyzed in all compared groups. Fig. 2 shows proliferation in the 1st, 6th and 14th day of cultivation. Cells were typically spindle-shaped in all monitored wells with scaffolds compared to the control: CMSCs without the presence of a scaffold. No significant difference was observed between individual groups.

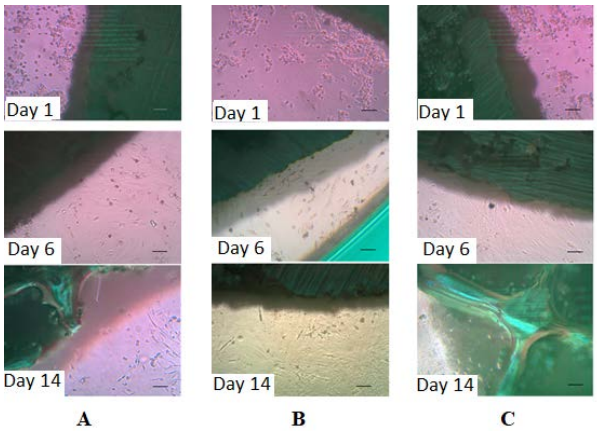

Fig. 2: Titanium matrix, pore size: 0.4 mm (A), 0.8 mm (B), and 1.0 mm (C) on the first, sixth and fourteenth day of culture of CMSCs. A gradual change in cell shape to vital fibroblasts is visible. Scale bar 50 µm.

MTT test

The percentage degree of compatibility and the minimal cytotoxicity effect of the titanium scaffold were recorded by the MTT test. Cell proliferation and viability were visually confirmed in all samples. Graph in Fig. 3 shows the percentage values of the viability of individual samples, where the values were in the range of 60 to 80%, while in the group with treatment of the titanium surface with collagen, the percentage of viability was higher compared to the group without treatment with collagen.

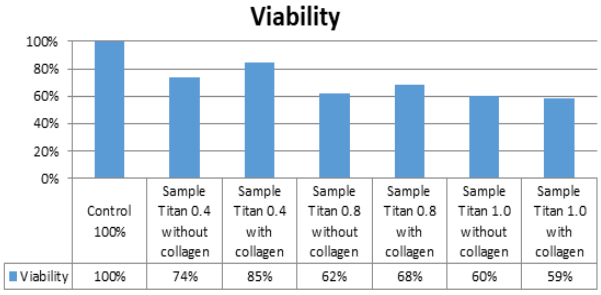

Fig. 3: Comparison of the viability of CMSCs on Ti-6Al-4V titanium scaffold samples.

Discussion and Conclusion

Titanium biomaterials are still widely used for the manufacture of implants. Provided they are properly processed, they enable reliable long-term implant performance even in stressful situations [1, 9]. Wang et al. in his work pointed out scaffolds made of titanium and its alloys as suitable for the production of implants and the repair of bone defects, used primarily in orthopedics, due to their advanced mechanical properties, corrosion resistance and favorable osseointegration [2]. In this work, we prepared scaffolds from Ti-6Al-4V titanium in different pore sizes—0.4 mm, 0.8 mm, and 1.0 mm by SLM 3D printing method. *In vitro* testing using CMSCs was used to assess the biocompatibility status of the scaffold. The result of the experiment was the evaluation of the influence of titanium scaffolds on the proliferation of CMSCs by means of the proliferation colorimetric MTT test. The MTT test determined a relatively high percentage of compatibility of the titanium scaffold and minimal cytotoxicity. Visual confirmation of cell proliferation and vitality was obtained in all samples. The lifespan of individual samples ranged from 60 to 80%, with the percentage of lifespan being higher in the group treated with collagen on a titanium surface compared to the group not treated with collagen. The exception was the scaffold with a pore size of 1.0 mm, where the pore size probably had a more significant effect on cell viability than the collagen film on the surface. Chen et al. tested porous scaffolds with different pore size and observed a creation of cell clusters in scaffolds with bigger pore sizes [10]. On the other hand, limitation of smaller pores was observed by Yao et al., where they discovered, that with the increasing porosity protein absorption rises, which is an unwanted characteristic in implantology

Lekar a technika – Clinician and Technology 2023, vol. 53(1), pp. 15–18, DOI: 10.14311/CTJ.2023.1.03
ISSN 0301-5491 (Print), ISSN 2336-5552 (Online)

[11]. Therefore, the optimal pore size for transplantation and tissue regeneration is still in question for future research.

The material titanium Ti-6Al-4V appears to be a suitable material for scaffolds in combination with autologous stem cells, without negative effects on the human body and with the ability to fill connective tissue defects.

Acknowledgement

This work was supported by the project KEGA 018TUKE-4/2023, APVV-22-0340, and ITMS2014+: 313011BWQ1 supported by the Operational Programme Integrated Infrastructure funded by the European Regional Development Fund.

References

[1] Xiong YZ, Gao RN, Zhang H, Dong LL, Li JT, Li X. Rationally designed functionally graded porous Ti6Al4V scaffolds with high strength and toughness built via selective laser melting for load-bearing orthopedic applications. Journal of the mechanical behavior of biomedical materials. 2020 Apr 1;104:103673. DOI: 10.1016/j.jmbbm.2020.103673

[2] Wang X, Xu S, Zhou S, Xu W, Leary M, Choong P, et al. Topological design and additive manufacturing of porous metals for bone scaffolds and orthopaedic implants: A review. Biomaterials. 2016 Mar 1;83:127–41. DOI: 10.1016/j.biomaterials.2016.01.012

[3] Khodaei M, Valanezhad A, Watanabe I, Yousefi R. Surface and mechanical properties of modified porous titanium scaffold. Surface and Coatings Technology. 2017 Apr 15;315:61–6. DOI: 10.1016/j.surfcoat.2017.02.032

[4] He Y, Liu W, Guan L, Chen J, Duan L, Jia Z, et al. A 3D-printed PLCL scaffold coated with collagen type I and its biocompatibility. BioMed Research International. 2018 Feb 28;2018:5147156. DOI: 10.1155/2018/5147156

[5] Bačenková D, Trebuňová M, Čížková D, Hudák R, Dosedla E, Findrik-Balogová A, et al. In vitro model of human trophoblast in early placentation. Biomedicines. 2022 Apr 15;10(4):904. DOI: 10.3390/biomedicines10040904

[6] Kadiyala S, Young RG, Thiede MA, Bruder SP. Culture expanded canine mesenchymal stem cells possess osteochondrogenic potential in vivo and in vitro. Cell transplantation. 1997 Mar 1;6(2):125–34. DOI: 10.1016/S0963-6897(96)00279-5

[7] Bačenková D, Trebuňová M, Zachar L, Hudák R, Ižaríková G, Šurínová K, et al. Analysis of same selected immunomodulatory properties of chorionic mesenchymal stem cells. Applied Sciences. 2020 Dec 17;10(24):9040. DOI: 10.3390/app10249040

[8] Ciapetti G, Cenni E, Pratelli L, Pizzoferrato A. In vitro evaluation of cell/biomaterial interaction by MTT assay. Biomaterials. 1993 Apr 1;14(5):359–64. DOI: 10.1016/0142-9612(93)90055-7

[9] Modrák M, Trebuňová M, Balogová AF, Hudák R, Živčák J. Biodegradable Materials for Tissue Engineering: Development, Classification and Current Applications. Journal of Functional Biomaterials. 2023 Mar 16;14(3):159. DOI: 10.3390/jfb14030159

[10] Chen Y, Jessica F, Ali DM, Hooyar A, Damon K, Soro N, et al. Mechanical properties and biocompatibility of porous titanium scaffolds for bone tissue engineering. Journal of the mechanical behavior of biomedical materials, 2017 Nov 1;75:169–74. DOI: 10.1016/j.jmbbm.2017.07.015

[11] Yao YT, Yang Y, Ye Q, Cao SS, Zhang XP, Zhao K, et al. Effects of pore size and porosity on cytocompatibility and osteogenic differentiation of porous titanium. Journal of Materials Science: Materials in Medicine. 2021 Jun 14;32:72. DOI: 10.1007/s10856-021-06548-0

doc. RNDr. Marianna Trebuňová PhD.
Department of Biomedical Engineering and Measurement
Faculty of Mechanical Engineering
Technical University of Kosice
Letná 1/9, 040 01 Košice
E-mail: marianna.trebunova@tuke.sk
Phone: + 421 556 022 380

Lekar a technika – Clinician and Technology 2023, vol. 53(1), pp. 15–18, DOI: 10.14311/CTJ.2023.1.03
ISSN 0301-5491 (Print), ISSN 2336-5552 (Online)