Culture of haematopoietic cells in a 3-D bioreactor made of Al_2O_3 or apatite foam

H. SCHUBERT, I. GARRN, A. BERTHOLD
Institut für Werkstoffwissenschaften und-technologien, Technische Universität Berlin, Englische Strasse 20, D 10587 Berlin, Germany

W. U. KNAUF, B. REUFI, T. FIETZ
Medizinische Klinik III (Hämatologie, Onkologie und Transfusionsmedizin), Charité-Campus Benjamin Franklin, Hindenburgdamm 30, D 12200 Berlin, Germany

U. M. GROSS
Labor für Biomaterialforschung, Abteilung für Experimentelle Zahn-, Mund- und Kieferheilkunde, Charité-Campus Benjamin Franklin, Freie Universität Berlin, Aßmannshauser Strasse 4–6, D 14197 Berlin, Germany
E-mail: ugross@zedat.fu-berlin.de

Foams of Al_2O_3 and apatite ceramics with interconnecting pores were produced using a new technique. The surfaces of the ceramics served as substrates for the culture of human peripheral and bone marrow derived stem cells. Up to 27 days the cells were kept in culture where they proliferated and developed into different morphologies consistent with bone marrow cell lines.

1. Introduction

The culture of haematopoietic cells allows for a limited expansion of cells when conventional cell culture plates are used. This result can be attributed to some extent to the unphysiological arrangement of explanted cells on flat surfaces. Attempts to use three-dimensional (3-D) models for the culture of haematopoietic progenitor cells were published [1–4]. The arrangement of the 3-D surfaces in ceramic foams with interconnecting pores is thought to be in some way comparable to the trabeculae of bone and their interconnecting pores. It can be assumed that the growth of cells in small compartments of the ceramic foams provide a more favourable microenvironment than the flat surfaces of culture dishes. In this feasibility study the foams of Al_2O_3-ceramics and apatite were tested in a cell culture system in using haematopoietic progenitor cells from different human donors. The surfaces were studied morphologically in using conventional staining of total specimens and scanning electron microscopy (SEM).

2. Materials and methods

2.1. Materials

Two powders were used: Al_2O_3-powder AKP 50 (Sumitomo Chemicals Co., Japan) with a purity of 99.99% and a particle size in between 0.1 and 0.3 μm, specific surface $10\,m^2/g$ (measured with BET), and hydroxyapatite (HA) powder (CFB Budenheim, Germany) type B powder NF pretreated by heat, particle size 0.5–4 μm, specific surface was $0.5\,m^2/g$. Al_2O_3- or HA-powder, protein bovine serum albumin (BSA) (ICN Biomedicals Inc., USA), an organic dispersion substance Dispex A 40 (Ciba Speciality Chemicals, Basel, Switzerland) – for stabilisation of the suspension – and water were mixed in a planetary ball mill (with Al_2O_3-balls and walls covered by polyethylene) for 15 min with 1200 rpm. During this mixing procedure a substance with the consistency of whipped cream developed. This substance was put into PTFE (polytetrafluorethylene) moulds and solidified using an industry microwave apparatus (type μwaveVac0140, Püschner Company, Germany). For Al_2O_3-ceramic 31 vol % Al_2O_3 (AKP50), 6 wt % BSA related to the solids, 0.5 wt % Dispex A40 (Ciba Speciality Chemicals, Basel, Switzerland) as substance to stabilise dispersion were used. For HA-ceramic 25 vol % HA, 8 wt % BSA related to the solids, and 0.25 wt % Dispex A40 were used. The protein contained in the creamy status of the material was denatured by heating in the microwave apparatus making the green bodies. Thereafter the organic components were burned out under air at 600 °C. The sintering of the green bodies was under air at 1600 °C for Al_2O_3 and 1200 °C for HA. The pores had a diameter of up to 500 μm in the Al_2O_3 and of up to 1 mm in the HA foams, they were for the most part interconnecting (Figs. 1 and 2). The process parameters for the microwave treatment were for Type I Al_2O_3-foam 1000 mbar, 120 W, 30 min, for Type II Al_2O_3-foam 500 mbar, 200 W, 25 min, for HA foam 700 mbar, 200 W and 25 min. From the solids pieces with a diameter of up to 8 × 8 × 2 mm were sawn and sterilised by dry heat at 120 °C for 30 min.

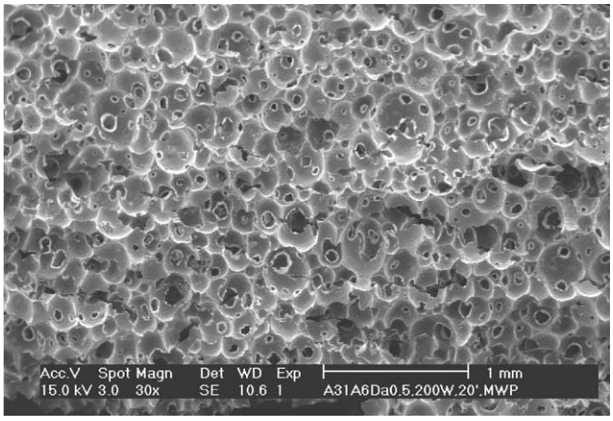

Figure 1 Foam of Al_2O_3 ceramics with interconnecting pores in SEM.

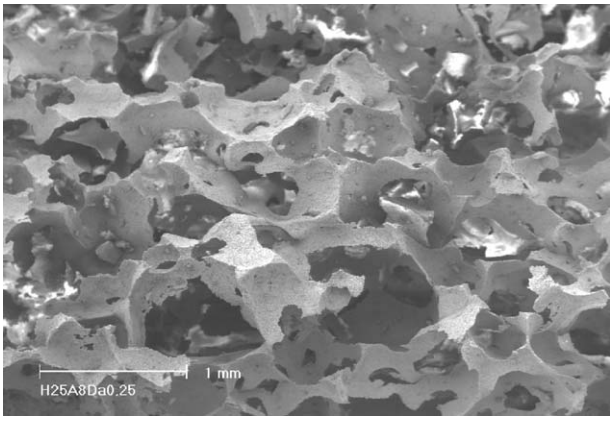

Figure 2 Foam of HA ceramics with interconnecting pores in SEM.

2.2. Cell culture

Cell donation was from healthy allogeneic donors involved in therapeutic programs for the treatment of tumour patients undergoing a haematopoietic cell transplantation. The donors underwent leukapheresis using a Cobe Spectra (Gambro BCT, Planegg, Germany) following standard procedures [4]. Informed consent was obtained from all donors according to institutional guidelines. Cells of four donors were used. For example: The smear of donor R.S. contained in % myelocytes 7, band 3, neutrophils 27, lymphocytes 42 and monocytes 21. 0.2% of all cells in the leukapheresis product were CD34/CD45 positive. There were three experiments with peripheral stem cells prepared by leukapheresis and one experiment with bone marrow derived cells from an aspirate which underwent density gradient centrifugation using Ficoll (Pharmacia, Sweden) 30 min with 1600 rpm. Number of specimens are presented in Table I. Culture medium was Iscove's modified Dulbeccos's medium (Gibco, Paisley, UK) containing 10% human AB serum (Sigma Taufkirchen, Germany), 100 ng/ml of SCF, 50 ng/ml of flt-3L, 8 ng/ml of IL-3, 20 ng/ml of IL-6, 13.5 ng/ml of G-CSF (all cytokines: Pepro Tech/Tebu, Offenbach, Germany) and 0.4 U of EPO (Cilag, Schaffhausen, Switzerland).

The ceramic specimens were placed in six well plates (Nunc, Naperville, IL, USA). Cells were seeded at a concentration of 1×10^6 cells/ml, i.e. 5 ml/well containing the specimens. The cell suspension was either poured directly onto the ceramic block or added into the well beneath the ceramic. Since seeding directly onto the ceramic surface was best, this procedure was used in the following experiments. The medium was changed one or two times per week.

2.3. Cell analysis after culture

After 12–27 days in culture the specimens were fixed in buffered formaldehyde solution pH 7.4 or in cold aceton, air dried and stained using Giemsa staining solution. One specimen (number 32) was stained, dried, sputtered with gold target and analysed in a SEM (CamScan MaXim 2040, England, UK). Light microscopy used transmitted and reflected light including fluorescence.

TABLE I

Specimens number	Material	Cell preparation	Time (days)	Cells on ceramics
1	Al_2O_3 type1	pstc R.S.	12	Few cells
2	Al_2O_3 type1	pstc R.S.	12	Medium cell density
3	Al_2O_3 type1	pstc R.S.	12	Medium cell density
4	Al_2O_3 type2	pstc R.S.	12	Few cells
5	Al_2O_3 type2	pstc R.S.	12	Medium cell density
6	Al_2O_3 type2	pstc R.S.	12	Medium cell density
7	HA	pstc R.S.	12	Few to medium cell density
8	HA	pstc R.S.	12	Few to medium cell density
24	Al_2O_3 type1	KM-MNC R.M.	15	Few cells in clusters
25	Al_2O_3 type2	KM-MNC R.M.	15	Flat cell clusters, stellate cells
26	HA	KM-MNC R.M.	15	Scattered few cells
29	Al_2O_3 type1	pstc Z.T.	15	Cells and cell remnants, autolysis
30	Al_2O_3 type2	pstc Z.T.	15	Cells, cell remnants, stellate cells, autolysis
31	HA	pstc Z.T.	15	Cell remnants, autolysis
32	Al_2O_3 type1	pstc DKMS14763	27	Many cells, different size, clusters, erythropoiesis, myelopoesis, Metachromatic granula in cells, stellate cells of matrix
33	Al_2O_3 type2	pstc DKMS14763	27	Many cells, different size, clusters, erythropoiesis, myelopoesis, Metachromatic granula in cells, stellate cells of matrix
34	HA	pstc DKMS14763	27	Surface covered by cells, stellate forms, round cells Differentiating, rare clusters

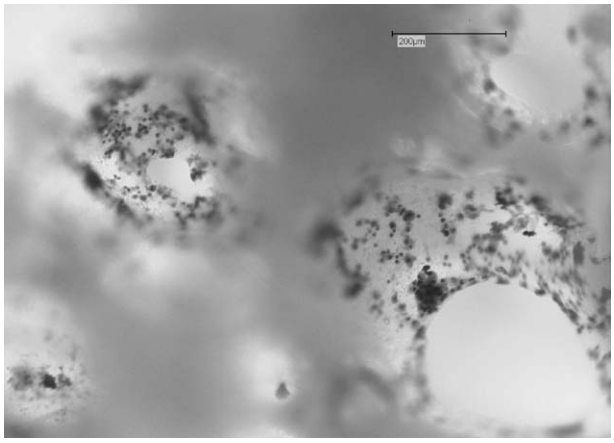

Figure 3 Surface of Al_2O_3 ceramics with human peripheral stem cells 27 days in culture. Giemsa stain reveals scattered cells with different size of cytoplasm, nuclei and cell clusters. In few cells metachromatic (red) staining of cytoplasmic granula speak for mast cell differentiation. (Only some areas are in the same plane and in focus due to the structure of the pores.)

3. Results

The surfaces of Al_2O_3 and apatite ceramics were in most specimens covered by cells and cell clusters as summarised in Table I. Generally, there were more cells on Al_2O_3 than on HA ceramics. The cells displayed different morphologies indicating differentiation into different cell lines and different stages of maturation. Some specimens displayed many stellate cell forms. In others there were clusters of cells morphologically consistent with erythropoietic and leucopoietic cell lines. One aspect is shown in Fig. 3, where clusters and scattered cells of variable diameter, content and stainability of cytoplasma can be recognised. There were rare cells with metachromatically stained granula indicating mast cell differentiation. In specimens 29–31 (Table I) abundant cells with signs of autolysis were present which is interpreted as an insufficient maintenance of the cell metabolism combined with features of the donor. Judging from the outlines and morphology of the proliferating cells there were also stellate cells indicating relationship to stromal cells. This aspect was underlined by the findings in SEM of the already Giemsa stained specimen (Fig. 4). The surface demonstrated different cell sizes, different expression of surface morphologies, i.e. some cells displayed filo- and lamellopodia consistent with the assumption of phagocytosing cells. In some areas the cell clusters were speaking for precursors of erythrocytes. Therefore, cells of at least three different haematopoietic cell lines are indicating the potential of cell proliferation and differentiation. Quantitative analysis is still not possible due to the limited number of specimens, however, there are arguments for the development of differentiating cells in the described 3-D ceramic scaffolds. Immunohistological staining using the PAAP- method [5] and antibodies that are used in routine pathology did not function sufficiently at the studied surfaces. The number of cells at the surface of HA was clearly smaller than on the surface of Al_2O_3 ceramics.

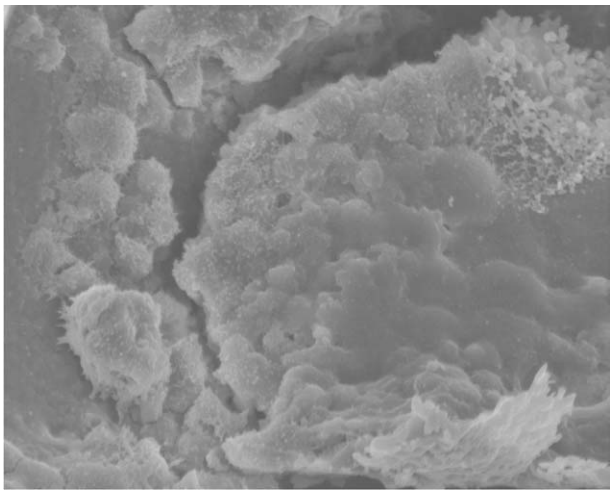

Figure 4 Surface of different cells in SEM display different structures, e.g. lamellopodia or rather flat surface. Field width 80 μm.

4. Discussion

The morphology and staining properties of the cells grown on surfaces of Al_2O_3 and apatite ceramics indicate a potential for growth and differentiation of bone marrow cells into lines of blood cells. Since the number of specimens were limited there was in general a need for selective staining. More immunohistological data are needed for quantitative evaluation. The detailed analysis of cell growth and differentiation can only be realised using more than four donors and more than the mentioned cell markers. Conditions for SEM were not optimal, nevertheless there was important information considering morphology of the cell surfaces. The investigated models with 3-D surfaces of Al_2O_3-ceramics and apatite were able to serve as substrate for the proliferation and differentiation of human peripheral stem cells and human bone marrow derived cells. The cultures were maintained only for less than four weeks. Therefore, it is unknown how long the cells can survive the artificial conditions. Additionally, nothing can be said about the functionallity of the described cells, i.e. do they work within the limits of known bone marrow educated and released cells.

5. Conclusion

Surfaces of Al_2O_3 and apatite ceramics were able to provide support for human peripheral stem cells under cell culture conditions for up to 27 days. The cells showed proliferation and differentiation into more than three different cell lines.

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