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REGULAR ARTICLE

A 3D construct based on mesenchymal stromal cells, collagen microspheres and plasma clot supports the survival, proliferation and differentiation of hematopoietic cells in vivo

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Abstract

The hematopoietic niche is a specialized microenvironment that supports the survival, proliferation and differentiation of hematopoietic stem progenitor cells (HSPCs). Three-dimensional (3D) models mimicking hematopoiesis might allow in vitro and in vivo studies of the hematopoietic (HP) process. Here, we investigate the capacity of a 3D construct based on non-adherent murine bone marrow mononuclear cells (NA-BMMNCs), mesenchymal stromal cells (MSCs) and collagen microspheres (CMs), all embedded into plasma clot (PC) to support in vitro and in vivo hematopoiesis. Confocal analysis of the 3D hematopoietic construct (3D-HPC), cultured for 24 h, showed MSC lining the CM and the NA-BMMNCs closely associated with MSC. In vivo hematopoiesis was examined in 3D-HPC subcutaneously implanted in mice and harvested at different intervals. Hematopoiesis in the 3D-HPC was evaluated by histology, cell morphology, flow cytometry, confocal microscopy and hematopoietic colony formation assay. 3D-HPC implants were integrated and vascularized in the host tissue, after 3 months of implantation. Histological studies showed the presence of hematopoietic tissue with the presence of mature blood cells. Cells from 3D-HPC showed viability greater than 90%, expressed HSPCs markers, and formed hematopoietic colonies, in vitro. Confocal microscopy studies showed that MSCs adhered to the CM and NA-BMMNCs were scattered across the 3D-HPC area and in close association with MSC. In conclusion, the 3D-HPC mimics a hematopoietic niche supporting the survival, proliferation and differentiation of HSPCs, in vivo. 3D-HPC may allow evaluation of regulatory mechanisms involved in hematopoiesis.

Keywords 3D construct · Hematopoiesis · MSC · Collagen · Plasma clot

Introduction

The bone marrow hematopoietic niche is a complex biological microenvironment consisting of a heterogeneous cell population, extracellular matrix proteins and vascular endothelium (Scadden and Morrison 2014; Pinho and Frenette 2019; Domingues et al. 2017). It supports the survival, proliferation and differentiation of hematopoietic stem cells (HSCs). In the hematopoietic microenvironment, the HSCs reside in a three-dimensional framework that allows them to interact with stromal cells and proteins of the extracellular matrix (Scadden and Morrison 2014; Pinho and Frenette 2019; Domingues et al. 2017; Jones and Wagers 2009). Regarding the cellular microenvironment, it is well known that mesenchymal stromal cells (MSCs) constitute a fundamental population of the hematopoietic niche (Li and Wu 2011; Frenette et al. 2013; Majumdar et al. 2000; Méndez-Ferrer et al. 2010; Crane et al. 2017; Greenbaum et al. 2013). These cells regulate

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hematopoiesis through the production of hematopoietic cytokines and growth factors, which promote the survival, proliferation, and differentiation of HSC (Li and Wu 2011; Majumdar et al. 2000). Regarding the extracellular matrix, there is evidence showing that collagen represents one of the main constituents of the hematopoietic niche and provides a surface for cell adhesion (Klein et al. 1995; Klein et al. 1997; Çelebi et al. 2012; Probst et al. 2018).

Several in vitro models have been developed to mimic the hematopoietic niche and for supporting hematopoiesis. The first developed models of the hematopoietic niche used bidimensional (2D) culture systems, in which bone marrow cells (BMCs) grew on monolayers of stromal cells, which provide support for in vitro hematopoiesis (Dexter et al. 1977; Gartner and Kaplan 1980). More recently, new developments using 3D models have been reported to mimic the hematopoietic niche (Abarrategi et al. 2015; Di Maggio et al. 2011). In these 3D models, hematopoietic stem and progenitor cells (HSPCs) and stromal cells were co-cultured on different scaffolds, such as collagen and hydroxide apatite (Leisten et al. 2012; Nichols et al. 2009; Fujita et al. 2010; Ueda et al. 2014). Although these models showed the survival, proliferation and differentiation of HSPCs, it has been difficult to generate a 3D model of the hematopoietic niche in which HSPCs survive and maintain their biological functions in vitro and in vivo.

In this work, we investigate the capacity of a 3D construct based on non-adherent murine bone marrow mononuclear cells (NA-BMMNCs, which contain HSPCs), MSCs and collagen microspheres (CMs), all embedded into plasma clot (PC) to support in vitro and in vivo hematopoiesis.

Materials and methods

Reagents Fluorescein isothiocyanate (FITC) or phycoerythrin (PE) anti-mouse Sca-1, CD117 (c-Kit) and Gr-1; and PE anti-human CD90 were from Biolegend (USA). Collagen microspheres (CMs, collagen type I) were from Cosmo Bio (Japan). Alpha-MEM culture medium, MCDB and TrypLE Express were obtained from Invitrogen (USA). Chang medium was from Irvine Scientific (USA).

Animals Female C57BL/6 mice (6–7 weeks old) were obtained from the IVIC Laboratory Animal Center and maintained on a standard laboratory diet and housed in a controlled environment. All animal experimentation was performed in accordance with institutional guidelines. This project was approved by the Bioethic Committee for Animal Research (Comisión de Bioética para Investigación en Animales, COBIANIM. No. 2014-15) of IVIC (Instituto Venezolano de Investigaciones Cientificas).

Isolation of murine hematopoietic cells Animals were sacrificed by cervical dislocation and the femurs were removed aseptically. BMCs were obtained by flushing the femurs with PBS. BM mononuclear cells (BMMNCs) were isolated by Ficoll-Hypaque density gradient (GE Healthcare Biosciences) and cultured in alpha-MEM/Chang medium. After 24 h of culture, the non-adherent fraction from BMMNCs (described here as NA-BMMNCs) was collected and resuspended in alpha-MEM/CHANG medium.

Isolation and culture of human MSC MSCs used in this work were from healthy individuals treated for bone regeneration due to seudoarthrosis, secondary to a fracture, who authorized the use of the cells by signing informed consent. These cells were from the bone marrow and isolated from the posterior iliac crest of patients (Wittig et al. 2016). MSCs were cultured, expanded and stored at − 70 °C until its use. For this study, MSCs were thawed and expanded until becoming near confluent. The MSCs were cultured with alpha-MEM-Chang medium supplemented with 20% fetal bovine serum (FBS).

Generation of a 3D hematopoietic construct (3D-HPC) MSCs (1.5×10^5 MSC) were cultured onto CMs (Fig. 1a). After 24 h of culture, NA-BMMNCs (1.5×10^5) were added to the MSC/CM complex and mixed with human platelet-rich plasma (PRP) (Fig. 1b). A mixture of 5% $CaCl_2$/thrombin was added to generate a plasma clot containing NA-BMMNCs/MSC/CM (3D hematopoietic construct, 3D-HPC) (Fig. 1c, d).

3D-HPC transplantation The 3D-HPC was subcutaneously (s.c.) implanted in the back of C57BL/6 mice (Fig. 1e). For this purpose, mice were anesthetized with a cocktail of ketamine/xylazine (100 mg/kg and 7.5 mg/kg body weight, respectively), hair was removed from the dorsal surface area and a small skin wound was made on the dorsal area. The 3D-HPC was s.c. implanted under the skin. The wound was closed and animals were maintained in the animal facility. Dipyrone was intraperitoneally administered for analgesia (5 mg/kg) every 12 h per 3 days. Animals were maintained in the animal facility. Groups of mice were sacrificed 15 days or 3 months after implantation and the 3D-HPCs were harvested from each mouse (Fig. 1f). Samples of 3D-HPC were evaluated by histology, confocal microscopy and phenotypical and functional assays for hematopoietic colony formation.

Histological analysis of 3D-HPC implanted in mice Samples from 3D-HPC were fixed, embedded in paraffin and stained with hematoxylin and eosin (H&E, Sigma) for histological analysis.

MSC migration from 3D-HP explants The presence of human MSC into 3D-HPC was examined by their capacity of migration and phenotypical characterization by flow cytometry. For

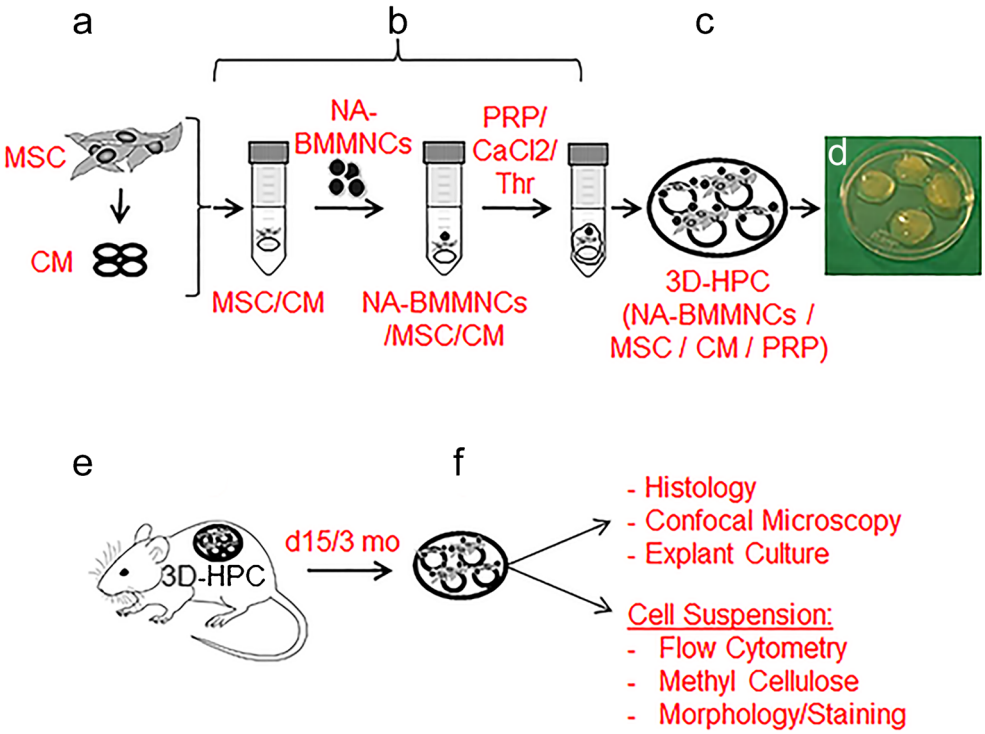

Fig. 1 Schematic illustration of the experimental design. MSCs from human BM were seeded for 24 h on CM (a). NA-BMMNCs were added to the MSC/CM preparation and included into PRP clots (b, c). The 3D-HPC (c, d) was subcutaneously implanted in mice (e). After 15 days and 3 months, the 3D-HPC was extracted and divided for analysis of migration assay, histology and confocal microscopy. The rest of the samples were digested with collagenase for phenotypic and morphologic analysis and for colony-forming assay (f)

this purpose, samples of 3D-HPC were cultured as explants and cell migration from them was evaluated by microscopy. Adherent cells were harvested and examined for human CD90 expression by flow cytometry.

Confocal microscopy The cell organization within the 3D-HPC was examined by confocal microscopy. For this purpose, MSCs and NA-BMMNCs were stained with PKH Green and PKH Red (MSC-PKH^{G} and NA-BMMNCs-PKH^{R}, respectively) (Sigma). 3D-HPCs were prepared with both stained cells, as described above, and cultured in vitro or subcutaneously implanted in mice. 3D-HPCs were harvested after 24 h of culture or after 7 and 15 days of implantation and examined by confocal microscopy using a laser scanning confocal system (Nikon C1, Japan). Data were acquired and processed with the Nikon EZ-C1 and Free Viewer 3.2 software, respectively.

Flow cytometric analysis for detection of HSPCs and MSCs Phenotypical characterization of cells was performed by flow cytometry. For this purpose, cells from 3D-HPCs were analyzed for the expression of hematopoietic (Sca-1, c-Kit, Gr-1) and human MSC (CD90) markers. Data collection and cell analysis were made using a FACSCalibur and Accuri flow cytometry (Becton Dickinson).

Cell morphology analysis Cells obtained from 3D-HPCs were stained with May-Grünwald/Giemsa and examined for cell morphology under a microscope.

Hematopoietic progenitor cell assay (colony-forming unit) Cells from 3D-HPCs were assayed for their capacity to form hematopoietic colonies in a methylcellulose-based medium supplemented with recombinant hematopoietic cytokines (Methocult GF H4034, Stem Cells Technologies, Vancouver, Canada). CFU were observed at day 7 under an inverted microscope (Zeiss-Axiovert, Germany). Single colonies were collected and stained with May-Grünwald/Giemsa for cell morphology analysis.

Results

Morphological and phenotypical characterization of MSC Cryopreserved human MSCs were thawed and cultured in alpha-MEM-Chang medium. They showed a fibroblast-like morphology (Fig. 2a) and expressed the typical MSC markers CD73, CD90 and CD105 (Fig. 2b, b′, b″, respectively). These cells showed their multipotential capacity of differentiation to osteogenic, chondrogenic and adipogenic cells (Fig. 2c–e).

In vitro evaluation of the 3D-HPC by confocal microscopy We used confocal microscopy for topographical characterization of the 3D-HPC and for assessment of NA-BMMNCs, MSCs, and CM organization into the construct. Flow cytometric studies allow the identification of NA-BMMNCs and MSCs based on their differences of size and granularity (FSC and SSC, respectively). NA-BMMNCs showed a lower FSC and SSC than MSC (R1 and R2, respectively, Fig. 3a, a′, a″). Flow

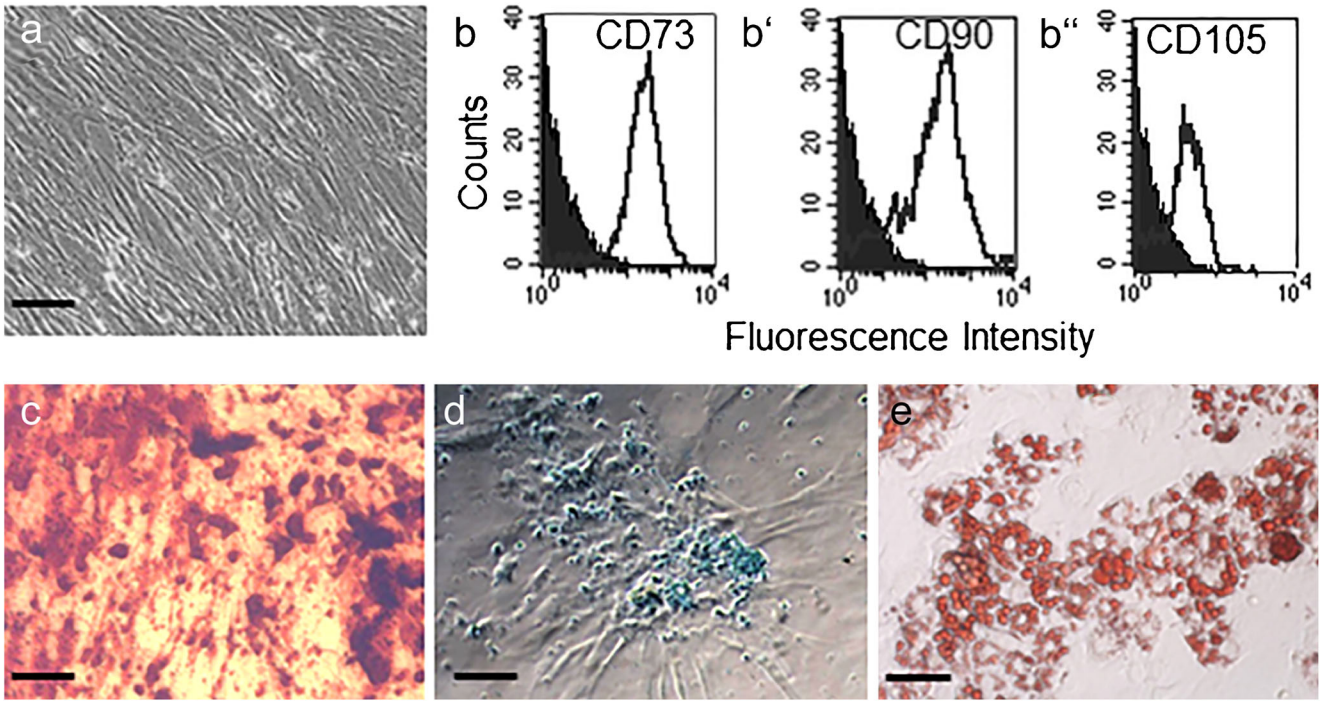

Fig. 2 Culture and characterization of human MSCs. Adherent cells from BM show fibroblast-like cell morphology (a) and express MSC markers (b, CD73; b′, CD90; b″, CD105). Osteogenic (c), chondrogenic (d) and adipocytic (e) differentiation of MSC is shown by alizarin, alcian blue, and oil red staining. Bar: 10 μm

cytometric analysis showed fluorescent staining of NA-BMMNCs (HSPCs)-PKH^R (Fig. 3b) and MSC-PKH^G (Fig. 3b′). The 3D-HPC (NA-BMMNCs-PKH^R/MSC-PKH^G/CM and embedded into a PRP clot) was cultured at 37 °C and 5% CO_2. After 24 h, confocal analysis of the 3D-HPCs showed MSC-PKH^G lining the CM and NA-BMMNCs-PKH^R scattered through the construct and closely associated with MSC (Fig. 3c).

Hematopoiesis in the 3D-HPCs subcutaneously implanted in mice for 15 days We examined whether HSPCs (contained in the NA-BMMNCs) might survive, proliferate, differentiate and maintain their functionality into the 3D-HPCs, in vivo. For this purpose, 3D-HPC was subcutaneously implanted in mice (Fig. 1e). After 15 days, macroscopic observation showed that the 3D-HPC was completely integrated to the host tissue (Fig. 4a). Vascularization was observed around the 3D-HPC implant (Fig. 4a, b). Histological studies showed fibrous tissue along the 3D-HPC (Fig. 4c). Cells of mesenchymal appearance were in close contact with CMs (Fig. 4c). Clusters of cells with hematopoietic morphology were found close to the sites at which MSCs and CMs were observed (Fig. 4c). Cells obtained from 3D-HPCs were evaluated for viability, morphology and phenotype. Cell viability, assayed by trypan blue staining, was greater than 90% (not shown). Cytological evaluation showed the presence of cells with myeloid morphology (Fig. 4d). Flow cytometric analysis showed the presence of cells expressing HSPCs (Fig. 4e, Sca-1$^+$; Fig. 4e′, c-Kit$^+$) and mature blood cell markers (Fig. 4e″, Gr-1$^+$ cells). Additionally, there were cells expressing the human MSC marker CD90 (Fig. 4e‴). Functional hematopoietic evaluation of cells from 3D-HPCs was performed by colony formation assays. After 7 days, hematopoietic colonies were observed in the methylcellulose assay (Fig. 4f). Cytological evaluation of cells harvested from these colonies showed the presence of cells with myeloid morphology (Fig. 4g). Fibroblastoid-like cells migrating from 3D-HPC explant were observed after 5 days of culture (Fig. 4h).

Hematopoiesis into the 3D-HPC subcutaneously implanted in mice for 3 months 3D-HPC was integrated to the host tissue and rounded by blood vessels, after 3 months of implantation (Fig. 5a, b). Histological analysis showed the fibrous tissue around the 3D-HPC. There were fusiform cells of mesenchymal appearance in close contact with CM (Fig. 5c). Cells with myeloid morphology were observed in the 3D-HPC tissue (Fig. 5c). Cells obtained from the 3D-HPC showed a viability greater than 90% (not shown). Cytological studies confirmed the presence of cells of hematopoietic origin (Fig. 5d). Flow cytometry analysis showed the presence of cells expressing hematopoietic (Fig. 5e, Sca-1$^+$; Fig. 5e′, c-Kit$^+$ cells) and myeloid (Gr-1$^+$ cells) markers (Fig. 5e″). These studies indicate the presence of active hematopoiesis in the 3D-HPC. Cells expressing the human MSC marker CD90 were also detected (Fig. 5e‴). Colony formation assays showed the presence of hematopoietic colonies after 7 days of culture (Fig. 5f).

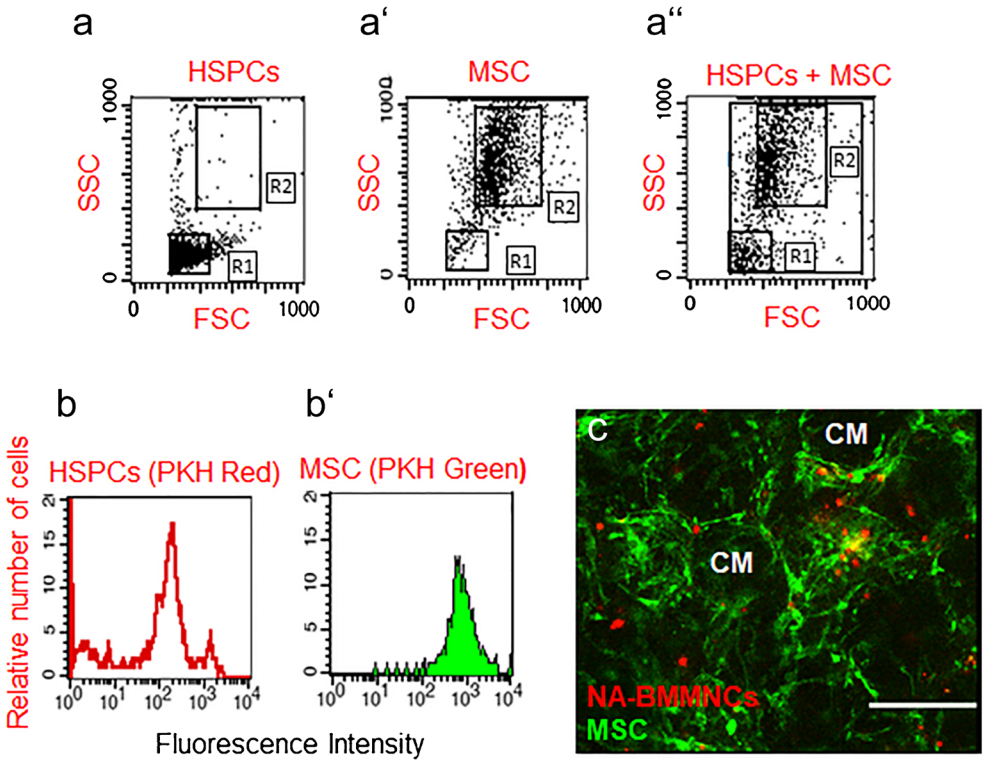

Fig. 3 Analysis of the 3D-HPC by flow cytometry and confocal microscopy. NA-BMMNCs and MSC population were characterized based on the differences in their size (FSC) and granularity (SSC) (a). Analysis of NA-BMMNCs shows that they are located in a cytometric region of low FSC and SSC (region R1) (a). MSCs are in a high FSC and SSC region (R2) (a′). Flow cytometric analysis of both populations (NA-BMMNCs + MSC) shows that NA-BMMNCs have a lower FSC and SSC than MSCs (a″). Fluorescence analysis of NA-BMMNCs stained with PKH red (b, HSPCS-PKH^R) and MSCs with PKH green (b′, MSC-PKH^G) was performed by flow cytometry. Confocal analysis of the 3D-HPC shows the presence of NA-BMMNCs-PKH^R and MSC-PKH^G around the surface of CM (c). Results are representative of at least three experiments. Bar: 200 μm

Cytological evaluation of cells from these colonies showed the presence of cells with myeloid morphology (Fig. 5g). Fibroblastoid-like cells migrating from the 3D-HPC explant were observed after 5 days of culture (Fig. 5h). These cells were harvested and analyzed by flow cytometry showing the expression of the human MSC marker CD90 (Fig. 5i), indicating the survival of MSC in the 3D-HPC, after 3 months of implantation.

Evaluation of the cellular niche in the 3D-HPC implanted in mice Based on the results showing that the 3D-HPC has a structure that not only mimics the hematopoietic microenvironment (Fig. 3c) but also supports hematopoiesis (Figs. 4 and 5), we examined the 3D-HPC microenvironment after being subcutaneously implanted in mice for 7 and 15 days. For this purpose, 3D-HPCs (NA-BMMNCs-PKH^R/MSC-PKH^G/CM/PRP) subcutaneously implanted in mice were analyzed by confocal microscopy. After 7 and 15 days of implantation, the 3D-HPC was integrated to the host tissue (Fig. 6a) and numerous vessels were observed on the surface of the tissue construct (Fig. 6a, b). Confocal analysis of the 3D-HPC showed clusters of HSCP-PKH^R and MSC-PKH^G lining the CM (Fig. 6c). NA-BMMNCs were located in direct association to MSC. After 15 days of implantation, more clusters of HSCP-PKH^R cells were observed in the 3D-HPC (Fig. 6d).

Discussion

Based on the knowledge that the hematopoietic niche supports the survival, proliferation and differentiation of HSC, it is of great interest to develop 3D models that mimic the hematopoietic process. Here, we report a 3D-HPC constituted by NA-BMMNCs (containing HSPCs) and MSCs (as cellular elements), collagen microspheres (as extracellular matrix scaffold) and plasma clot (as the 3D framework), which support hematopoiesis in vivo.

In this work, we show that the 3D-HPC resembles the organization of the components of the hematopoietic niche (BMCs, stromal cells and ECM). In our model, the NA-BMMNCs/MSC/CM complex is entrapped in a 3D framework formed by a PRP clot, which may not only increase the interaction between NA-BMMNCs, MSCs and CMs but also support several biological functions of HSPCs. There is evidence showing that collagen and fibrin may enhance

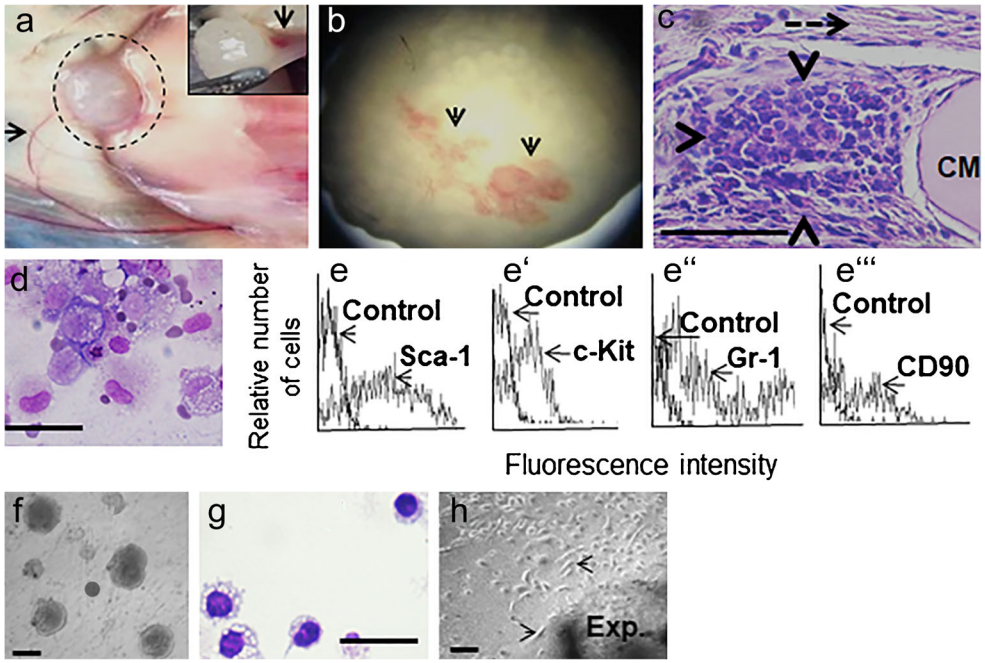

Fig. 4 Hematopoiesis in the 3D-HPC implanted in mice for 15 days. After 15 days of implantation, the animals were sacrificed and 3D-HPCs were extracted. The 3D-HPC (circle) is surrounded by blood vessels (arrows) and integrated to the tissue of the animals (small square and arrow) (a, b). Histological studies of samples of the 3D-HPC show the presence of fibrous tissue (broken arrow) and hematopoietic cells (head arrow and small square) around the collagen microspheres (CMs) (c). Cytospin and May-Grünwald/Giemsa staining of cells obtained from 3D-HPC samples show the presence of polymorphonuclear monocytes and lymphoid cells (d). Cells collected from 3D-HPC express hematopoietic (e, Sca-1; e′, c-kit), myeloid (e″, Gr-1) and human MSC (e‴, CD90) markers. Negative controls were stained with the respective isotype (control). Colony-forming assay shows that these cells form hematopoietic colonies after 7 days of culture (f). Cytological evaluation of cells harvested from these colonies shows the presence of cells with myeloid morphology (g). Fibroblastoid-like cells (arrow) migrating from 3D-HPC explants (Exp) are observed, after 5 days of culture. (h) Results are representative of at least three animals. Bar: 50 μm (c), 25 μm (d, g), 200 μm (f, h)

hematopoiesis of HSPCs (Leisten et al. 2012; Ventura Ferreira et al. 2012). Previously, we have shown that CMs and PRP clot constitute optimal biological scaffolds for adhesion, survival and preserving the functional capacity of MSCs (Wittig et al. 2016, 2018). Here, we show that in the 3D-HPC, the MSCs have their typical elongated morphology lining the CMs and the NA-BMMNCs not only closely associated with them but also formed a cluster in the construct. It is possible that in the 3D-HPC, MSC may constitute a layer of stromal cells on CMs providing a hematopoietic-like niche for the expansion of HSPCs.

Here, we show that subcutaneously implanted 3D-HPCs were not only integrated to the host tissues but were also vascularized. The presence of angiogenesis around the 3D-HPC could be related to the production of angiogenic factors (i.e., VEGF and FGF) by the human MSC, which may attract host endothelial cells and promote neovascularization (Chen et al. 2008; Wu et al. 2007). This possibility is based on the complete cross-reactivity of some human and murine angiogenic factors, such as VEGF and FGF. In fact, previous results have shown the angiogenic effect induced by human MSC in immunocompetent mice (Chen et al. 2008). Importantly, vascularization may provide factors to support the survival of HSPCs and MSCs into the 3D-HPC. This possibility is supported by the high viability observed in cells collected from the 3D-HPC (more than 95%). Additionally, vascularization of the 3D-HPC may allow the entry of host cells to the implant or the exit of cells from the implant to the host. Previous evidence has shown that subcutaneously transplanted human MSCs generate a hematopoietic niche, which recruits HSPCs from the circulation of the host (Serafini et al. 2014; Sacchetti et al. 2007). Ongoing studies are being developed to demonstrate these possibilities.

For hematopoiesis research, it was very important to investigate whether the 3D-HPC may support hematopoiesis in vivo. Histological studies of samples from implanted 3D-HPC showed the presence of cells with myeloid and mononuclear morphology. These results were confirmed by morphological analysis of cells harvested from the 3D-HPC, which showed the presence of cells of hematopoietic origin. Together, these results indicate the presence of hematopoietic activity in the 3D-HPC. Our result supports previous in vitro evidence showing 3D models that support the expansion and differentiation of hematopoietic cells (Leisten et al. 2012; Nichols et al. 2009; Fujita et al. 2010; Ueda et al. 2014). However, our model differs from these studies because we show hematopoietic activity in the 3D-HPC in vivo.

Gr-1 is expressed on granulocytes and macrophages. In the bone marrow, the expression levels of Gr-1 correlate with

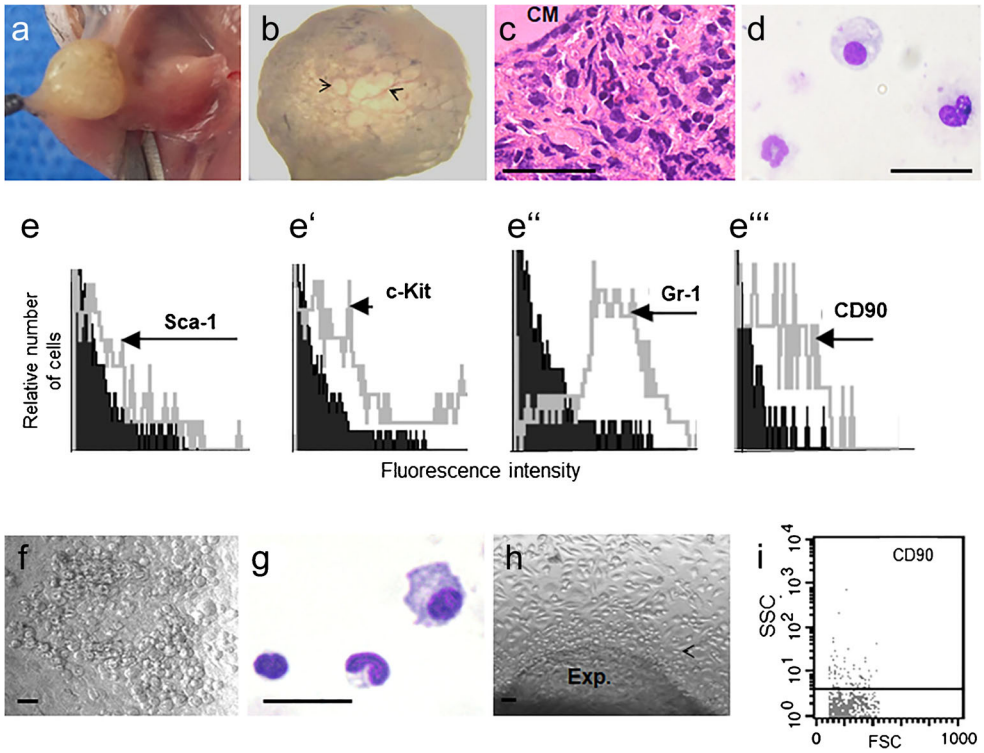

Fig. 5 Hematopoiesis in the 3D-HPC implanted in mice for 3 months. Mice were sacrificed after 3 months of 3D-HPC implantation. Macroscopical observations show that the 3D-HPC is surrounded by blood vessels (arrows) and integrated to the tissue of the animals (a, b). Histological studies of samples of the 3D-HPC show the presence of fibrous tissue (broken arrow) and fusiform cells attached to the surface of CMs (head arrows and small square) (c). Cytospin and May-Grünwald/Giemsa staining of cells obtained from 3D-HPC samples show the presence of cells of hematopoietic origin (d). Cells collected from the 3D-HPC express hematopoietic (e′, Sca-1; e′, c-kit) myeloid (e″, Gr-1), and human MSC (e‴, CD90) markers. Negative controls were stained with the respective isotype (control, black histogram). Colony-forming assay shows that these cells form hematopoietic colonies after 7 days of culture (f). Cytological evaluation of cells harvested from these hematopoietic colonies showed the presence of cells with myeloid morphology (g). Fibroblastoid-like cells (arrow) migrating from 3D-HPC explants (Exp) are observed after 5 days of culture (h). Flow cytometry analysis of migrating cells expressed the human MSC marker CD90 (i). Results are representative of at least three animals. Bar: 50 μm (c), 25 μm (d, g), 200 μm (f, h)

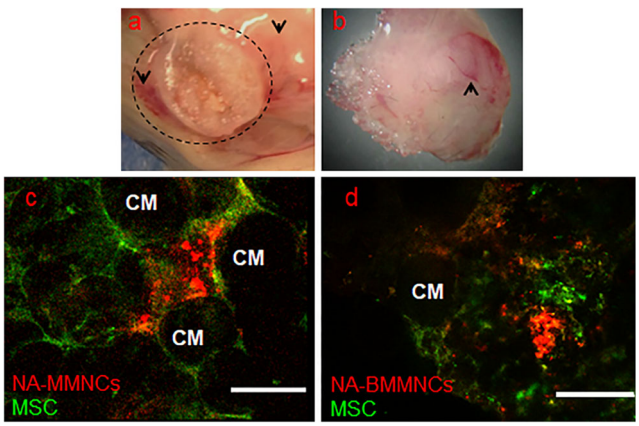

Fig. 6 Analysis of the hematopoietic niche inside the 3D-HPC. 3D-HPCs containing NA-BMMNCs-PKH^{R} and MSC-PKH^{G} were subcutaneously implanted in mice. After 7 and 15 days of implantation, the 3D-HPCs were extracted. Macroscopical observation of the 3D-HPC (circle), after 7 days of implantation, shows that it is integrated to the host tissue and surrounded by several blood vessels (head arrows) (a, b). Confocal analysis of the 3D-HPC shows clusters of NA-BMMNCs-PKH^{R} cells and MSC-PKH^{G} surrounding the CM after 7 and 15 days of implantation (c and d, respectively). Results are representative of at least three animals. Bar: 200 μm (c, d)

granulocyte differentiation and maturation; Gr-1 is also transiently expressed on bone marrow cells in the monocyte lineage.

It was also very important in this work to examine whether HSPCs maintained their functionality in the implanted 3D-HPC. Flow cytometry analysis of cells from the 3D-HPC showed the presence of HSPCs (i.e., Sca-1 and c-Kit), which may explain the active hematopoiesis observed in the 3D-HPC implants. Moreover, cells expressing the myeloid marker Gr-1 were also detected, indicating hematopoietic differentiation from HSPCs (contained in the NA-BMMNC population) included in the 3D-HPC. HSPCs from the 3D-HPC maintained their capacity to form hematopoietic colonies in semisolid cultures supplemented with hematopoietic cytokines. These results indicate that the 3D-HPCs support the proliferation and differentiation and maintain the clonogenic capacity of HSPCs. Together, our results show that HSPCs not only survive but also maintain their functionality in the 3D-HPC implanted in mice. It may be due to the effects of hematopoietic cytokines produced by MSCs in the

niche of the 3D-HPC (Li and Wu 2011). A 3D model of a hematopoietic niche that retains HSPCs within the formed hematopoietic-like niche for up to 28 days in culture has been reported (Torisawa et al. 2014). We considered that our 3D-HPC constitutes a more physiological system for evaluating hematopoiesis, because its cellular and matrix elements were included in a tridimensional matrix of a plasma clot. In fact, active hematopoiesis in the 3D-HPC implant was demonstrated at short and long terms in mice (15 days and 3 months), as was shown by histological studies. Hematopoietic stem cell transplantation using cells from the 3D-HPC may allow determining the capacity of this hematopoietic construct to support in vivo hematopoietic differentiation.

In this work, we show that human MSCs not only survive but also maintain their migration capacity in the 3D-HPC. Likewise, because it is well known that MSCs support hematopoiesis in vivo and in vitro (Li and Wu 2011; Frenette et al. 2013; Majumdar et al. 2000; Méndez-Ferrer et al. 2010; Crane et al. 2017; Dexter et al. 1977), the persistence of these cells for long-term periods (3 months) may explain the active hematopoiesis observed in the 3D-HPC implant. MSCs may provide adhesion and hematopoietic cytokines, which may explain the survival, proliferation and differentiation of HSPCs in the 3D-HPC. Importantly, due to the low expression of MHC class I and class II and no expression of costimulatory molecules, the possibility of rejection in allogeneic or xenogeneic models of transplantation is very low (Caliari-Oliveira et al. 2016; Chen et al. 2009; Dai et al. 2005; Wang et al. 2018). In fact, in our study, there was no evidence of tissue rejection or inflammatory signs around the 3D-HPC implant.

In conclusion, our 3D-HPC mimics a hematopoietic niche by maintaining the survival and functionality of HSPCs. Our 3D-HPC model may allow evaluation of HSPC interaction with MSC and extracellular matrix, and it may constitute a system for engineering bone marrow tissue.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals All animal experimentation was performed in accordance with institutional guidelines. MSCs used in this work were from healthy individuals treated for bone regeneration due to seudoarthrosis, secondary to a fracture, who authorized its use by signing informed consent.

Ethical approval All applicable international, national and/or institutional guidelines for the care and use of animals were followed. This project was approved by the Bioethic Committee for Animal Research (Comisión de Bioética para Investigación en Animales, COBIANIM. No. 2014-15) of IVIC (Instituto Venezolano de Investigaciones Cientificas).

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