BONE MARROW-ON-A-CHIP

Yu-suke Torisawa[1], Catherine S. Spina[1,2], James J. Collins[1-3], and Donald E. Ingber[1,4,5]

[1]Wyss Institute for Biologically Inspired Engineering, Harvard University, USA, [2]Boston University School of Medicine, USA, [3]Howard Hughes Medical Institute, Department of Biomedical Engineering, Boston University, USA, [4]Vascular Biology Program, Departments of Pathology and Surgery Children's Hospital Boston and Harvard Medical School, USA, [5]School of Engineering and Applied Science, Harvard University, USA

ABSTRACT

This paper describes a bone marrow-on-a-chip technology in which new bone containing marrow is engineered by implanting in a polymer device containing a cylindrical hole filled with bone-inducing materials subcutaneously on the back of a mouse. The engineered bone containing marrow is then surgically removed intact and maintained viable under flow within a similarly shaped chamber of a microfluidic device *in vitro*. This approach results in formation of living bone filled with functional marrow that is virtually identical to bone marrow isolated from a mouse femur. The viability and hematopoietic cell distribution of the engineered bone marrow can be maintained in the microfluidic system *in vitro*.

KEYWORDS

Microfluidics, Tissue engineering, Organs-on-chips, Hematopoietic stem cells, Hematopoiesis, Blood formation

INTRODUCTION

Recent advances in microsystems technology development and tissue engineering have led to the development of organs-on-chips that can reconstitute organ-level function *in vitro* by mimicking natural tissue arrangements and microenvironmental cues within microfluidic devices created with fabrication techniques adapted from computer microchip manufacturing [1]. In this study, we describe a microfluidic bone marrow-on-a-chip technology created combining microsystems and tissue engineering strategies to produce bone *in vivo* that contains a complex bone marrow microenvironment with functional hematopoietic cells, and then explanting this whole bone and marrow and maintaining viability in a microfluidic system *in vitro*.

The bone marrow microenvironment is crucial for the maintenance of viability and function of hematopoietic stem cells (HSCs) that are responsible for producing all types of blood cells of the hematopoietic system [2]. However, it has been difficult to provide to maintain functional HSCs *in vitro* because the bone marrow microenvironment contains a complex set of cellular, chemical, structural, and physical cues [3]. Investigators have attempted to culture and expand HSCs *in vitro*, but long-term engraftment and host hematopoietic reconstitution from cultured HSCs has been very inefficient [4]. Thus, there is a great need to develop a system that can faithfully recapitulate the natural bone marrow microenvironment to study hematopoietic diseases, facilitate drug discovery research, and enable expansion of bone marrow for therapeutic transplantation.

Early studies of bone tissue engineering noted that bone marrow-like tissue formed within bone that was induced by implanting demineralized bone powder (DBP) and bone morphogenetic proteins (BMPs) subcutaneously in rodents [5, 6]. However, no method currently exists to maintain structurally and functionally sound engineered bone marrow *in vitro*. Therefore, we set out to explore whether we could induce formation of bone containing a fully functional engineered bone marrow (eBM) *in vivo*, surgically remove it, and cultured it *in vitro* to maintain hematopoietic function and study the living bone marrow microenvironment.

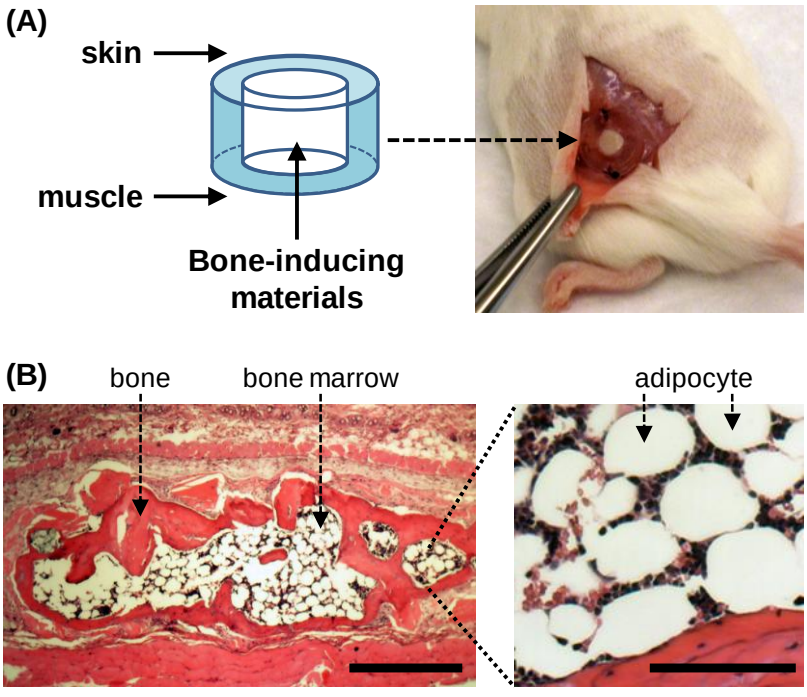

Figure 1. *(A) The PDMS device containing a central cylindrical cavity with two openings was used to form engineered bone marrow (eBM) in vivo. The cavity was filled with bone-inducing materials and implanted subcutaneously on the back of a mouse. Bone-inducing materials were exposed to both the underlying muscle and adipose tissue of the overlying skin. (B) Histological H&E-stained sections of the eBM formed in the devices 8 weeks following implantation. Scale bars, 500 μm (left), 100 μm (right).*

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EXPERIMENT

The bone-inducing materials consisted of a type I collagen gel (30 uL at 3 mg/mL), 3 mg DBP prepared from femurs harvested from mice [5], 100 ng BMP2 and 100 ng BMP4. The implanted polymer devices designed with a central cavity (1 mm high x 4 mm diameter) were fabricated from poly-dimethylsiloxane (PDMS). The PDMS devices filled with the bone-inducing materials were implanted subcutaneously on the backs of mice (Fig. 1A, 2A). Eight weeks after implantation, the eBM was removed from the PDMS device and cultured in a similarly shaped central chamber within a microfluidic device (Fig. 3) that was separated from overlying and underlying microfluidic channels (200 μm high) by porous PDMS membranes (20 μm thick with 100 μm pores). Culture medium (SFEM basal media) containing cytokines (50 ng/mL mouse SCF, 100 ng/mL human IL-11, 100 ng/mL human FLt-3, and 20 μg/mL human LDL) was perfused through the top and bottom channels (1 μL/min) after the eBM was inserted into the central chamber. To harvest bone marrow cells, the eBM was cut into small pieces and digested using 1 mg/mL collagenase for 30 min at 37 ^{o}C.

RESULTS AND DISCUSSION

To leverage bone tissue engineering techniques to form living marrow, we designed a PDMS device with a central cylindrical cavity with openings at its two ends (Fig. 1A). The cavity was filled with the bone-inducing materials and implanted subcutaneously on the back of a mouse. This system resulted in formation of bone containing marrow in the PDMS device within 8 weeks after implantation (Fig. 1B); however, the marrow exhibited an overall low level of cellularity and was dominated by adipocytes, which is consistent with past studies on bone tissue engineering [6]. Adipocytes also are known to have an inhibitory effect on the hematopoietic microenvironment [7]. Thus, to improve the quality of the engineered marrow, we designed the PDMS device to prevent infiltration of adipocytes by blocking the top of the central cavity of the device that exposed to the adipose tissue of the skin, while retaining accessibility to the underlying muscle tissue through the lower opening (Fig. 2A). Subcutaneous implantation of this improved PDMS device resulted in the formation of a cylindrical disk of white bone containing a central blood-containing marrow over a period of 8 weeks (Fig. 3A). Histological analysis revealed that a thick shell of well-formed bone surrounding healthy appearing marrow that was dominated by hematopoietic cells, with only rare adipocytes visible (Fig. 2B, C). Comparison of histological sections of the eBM to sections from an intact mouse femur (Fig. 2D) confirmed that the morphology of the eBM was nearly identical to that of natural bone marrow. These data suggest that we have successfully engineered a cylindrical disk of bone containing marrow that is nearly identical to natural bone marrow.

To maintain functional hematopoietic cells *in vitro*, the eBM formed *in vivo* was cultured in a microfluidic device (Fig. 3). The cylindrical eBM that formed within the single opening PDMS device for 8 weeks was surgically removed from the mouse, placed into a similarly shaped central chamber of the microfluidic device, and cultured while perfused culture medium through the top and bottom channels. After 4 days of culture within the microfluidic bone marrow-on-a-chip, cells in the eBM maintained their viability. Importantly, preliminary results suggest that the distribution of HSCs and hematopoietic progenitor cells in the eBM cultured for 4 days on-chip remained very similar to the proportions in mouse femur bone marrow, whereas the composition of the blood cells in conventional dish cultures was strikingly different, as indicated by flow

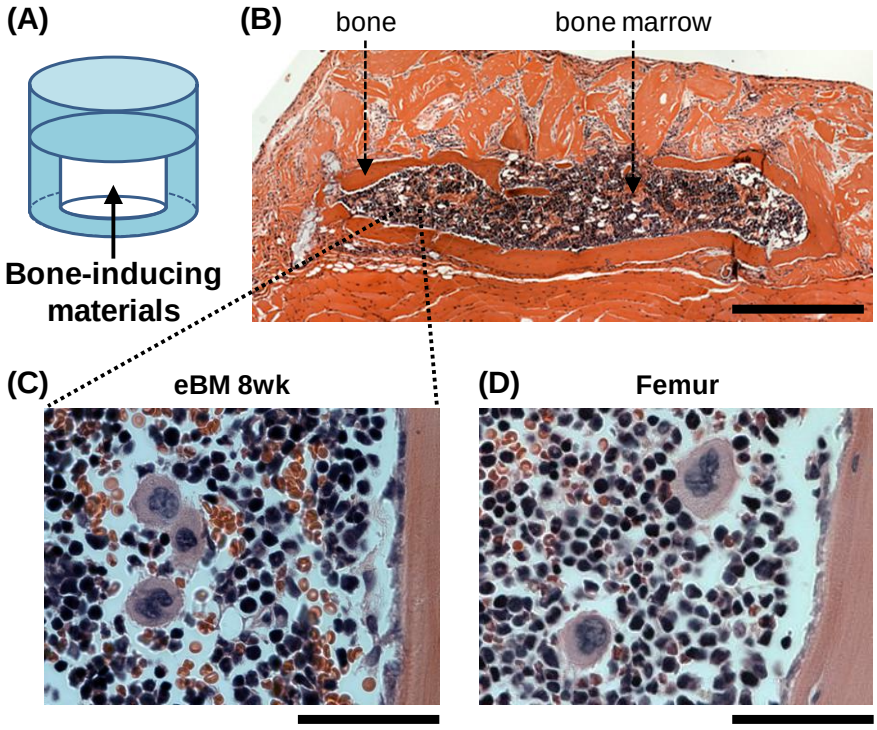

Figure 2. *(A) The PDMS device with only one lower opening was filled with bone-inducing materials and implanted subcutaneously on the back of a mouse. The PDMS device was improved by covering the top opening with a solid PDMS layer so that the bone-inducing materials only contacted the underlying muscle. (B-D) Histological H&E-stained sections of the eBM formed in the improved PDMS device 8 weeks following implantation (B, C) compared with a cross-section of bone marrow within a mouse femur (D). Scale bars, 500 μm (top), 50 μm (bottom).*

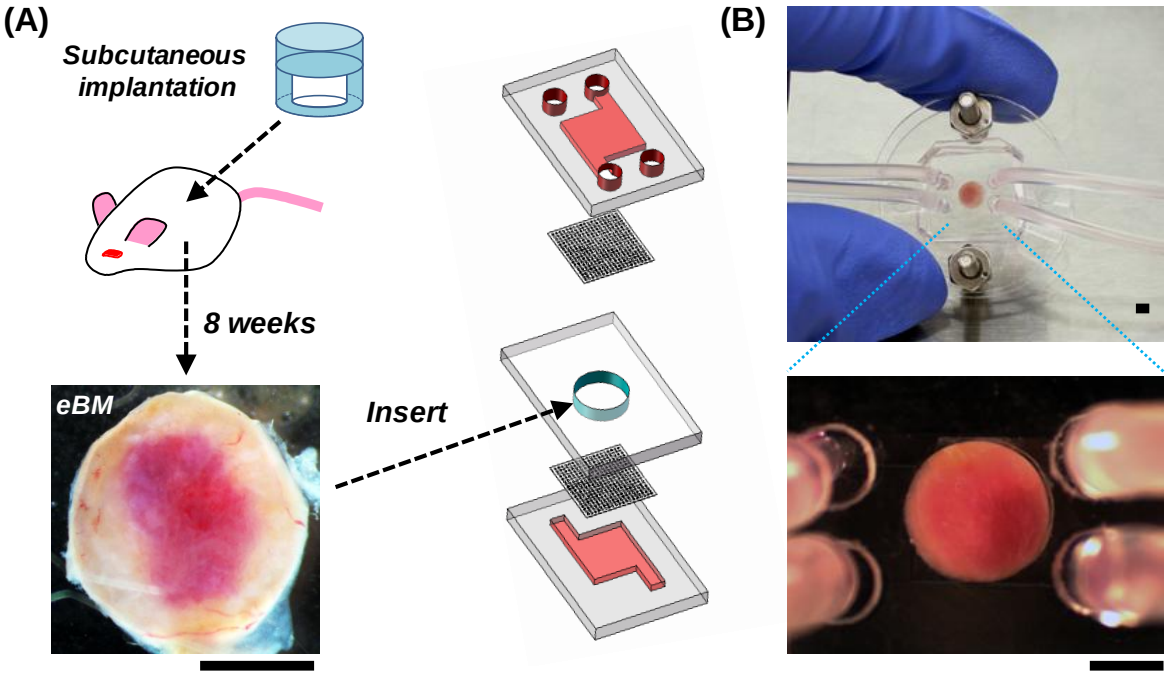

Figure 3. *Diagrammatic (A) and photographical representation (B) of the microfluidic culture of the eBM in vitro. The cylindrically shaped eBM that formed within the single opening PDMS device was surgically removed 8 weeks after implantation, placed into the similarly shaped central chamber of the microfluidic device, and then maintained in culture in vitro. The microfluidic bone marrow-on-a-chip device consists of three PDMS layers separated by PDMS porous membranes. Scale bars, 2 mm.*

cytometric analysis (not shown). Therefore, the microengineered bone marrow-on-a-chip system appears to create and maintain a functional hematopoietic microenvironment capable of supporting viable hematopoietic stem and progenitor cells *in vitro*.

This microengineered bone marrow-on-a-chip approach differs from conventional tissue engineering approaches in which newly formed tissue will structurally and functionally integrate with existing organs. In contrast, our approach was designed to engineer bone containing functional marrow in a pre-specified polymer device so that it can be removed intact from the body. This ability to engineer and maintain a complex three-dimensional bone marrow microenvironment with functional hematopoietic cells *in vitro* opens up entirely new avenues to explore drug efficacy and toxicities and study hematopoietic diseases.

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CONTACT

Donald Ingber, MD, PhD (don.ingber@wyss.harvard.edu)