# Patterning of Multiple Cell Lineages from a Single Stem Cell Population

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**Abstract:** Program #327

Muscle-derived stem cells (MDSCs) have the capacity to regenerate bone and muscle *in vivo*. To refine therapeutic options for the use of MDSCs in regenerative medicine, the response of these progenitor cells should be tailored for each specific application. The purpose of this study was to engineer patterned cell differentiation of a single population of mouse MDSCs towards the osteogenic and myogenic lineages simultaneously *in vitro* in the same well. We based our strategy on the assumption that morphogens exert tissue-specific differentiative effects as solid-phase patterns *in vivo* during development and regeneration of tissues. To engineer cell response to solid-phase morphogen patterns, we used our established ink-jet printing technology to deposit patterned arrays (750 µm x 750 µm) of bone morphogenetic protein (BMP)-2. Induction of ALP activity was used to detect differentiation of MDSCs towards the osteogenic lineage. To demonstrate differentiation towards the myogenic lineage (default lineage of MDSCs), immunocytochemical staining for myosin heavy chain was performed. MDSCs differentiated towards the osteogenic lineage in direct register to square patterns of bioprinted BMP-2 in a dose-dependent manner. Cells off pattern do not express substantial ALP activity. MDSCs cultured on BMP-2 patterns under serum deprivation resulted in differentiation towards the osteogenic lineage in register to BMP-2 printed patterns and towards the myogenic lineage off pattern. This work provides proof-of-concept for engineered patterning of multiple cell lineages from a single population of MDSCs. This technology will enable spatial control over cell lineage determination using a high-throughput approach to pattern multiple morphogens that regulate stemness and tissue-specific differentiation. Researchers could use this approach to tailor tissue-engineered therapies for specific regenerative applications for multiple tissues.

**Methods:**

• Our team has custom-built an inkjet bio-printer capable of printing solid-phase 2-D and 3-D patterns and gradients of growth factors as "bio-inks" immobilized to ECM substrates such as fibrin and collagen (Fig 1). [1, 2]

• Four immobilized BMP-2 square patterns were printed onto fibrin-coated glass slides in various surface concentrations (6.25-50 ng/cm$^2$) using an overprinting strategy to control surface concentration of deposited BMP-2 "bio-inks". The slides with printed BMP-2 patterns were sterilized in 70% ethanol and incubated for 24 hr in DMEM, 10% FBS and 1% pen/strep at 37°C to release unbound BMP-2.

• Mouse muscle-derived stem cells (MDSCs) were isolated by a modified "pre-plate technique" [3]. MDSCs were cultured on BMP-2 printed patterns for 3 days under normal culture conditions (20% serum). To promote differentiation towards the myogenic lineage, cells were cultured in medium containing 2% serum.

• Cell differentiation towards the osteogenic lineage was detected by staining for alkaline phosphatase activity (Sigma).

• Cell differentiation towards the myogenic lineage was detected by immunocytochemical staining for myosin heavy chain (fast) (MHC-f) and time-lapse microscopy for cell fusion forming multi-nucleated myotubes.


Figure 1

[1] Campbell, et al., *Biomaterials*. 2005 Nov;26(33):6762-70.
[2] Miller, et al., *Biomaterials*. 2006 Apr;27(10):2213-21.
[3] Qu-Peterson, et al., *J Cell Biol*. 2002 May 27;157(5):851-64.

**Results:**

**Ink-jet printing was used to create persistent two dimensional square patterns of Cy-3-labeled BMP-2 immobilized to fibrin-coated glass slides**

Figure 2

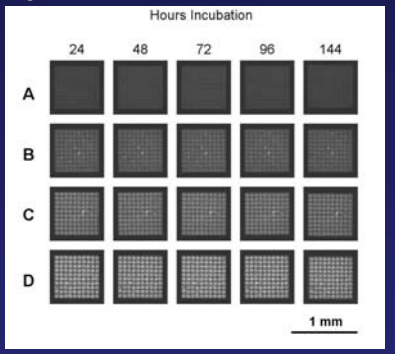


**Figure 2**: Printed patterns of Cy3-labeled BMP-2 are persistent. A) 2 overprints B) 12 overprints C) 22 overprints D) 32 overprints.

Figure 3

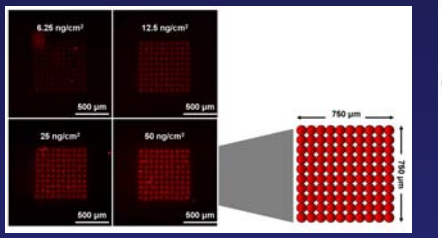


**Figure 3**: Array of 4 squares of various doses of ink-jet printed Cy3-labeled BMP-2 immobilized to fibrin. Each square is 750 µm x 750 µm consisting of 100 "splats" of BMP-2.

**MDSCs differentiate towards the osteogenic lineage dose-dependently in register to BMP-2 patterns**

Figure 4

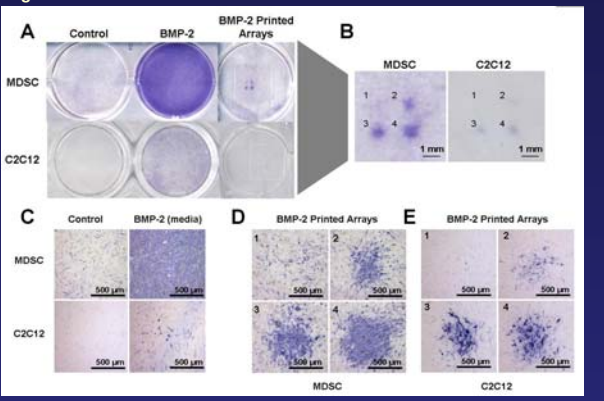


**Figure 4**: Patterned control of osteogenic lineage progression for MDSCs and C2C12 shown by ALP activity (purple). A) Macroscopic view of patterned cell differentiation. B) Increased magnification of (A) for cells seeded on BMP-2 printed patterns. ALP activity increased dose-dependently within 3 days of culture for MDSC and C2C12 cells. C) BMP-2 delivered to the culture media resulted in increased ALP activity uniformly across the entire population of cells. Cells on BMP-2 patterns exhibit ALP activity dose-dependently for MDSC (D) and C2C12 (E). Images were captured at 10X.

**Time-lapse microscopy focused on BMP-2 printed patterns and immediate surrounding area**

Figure 5

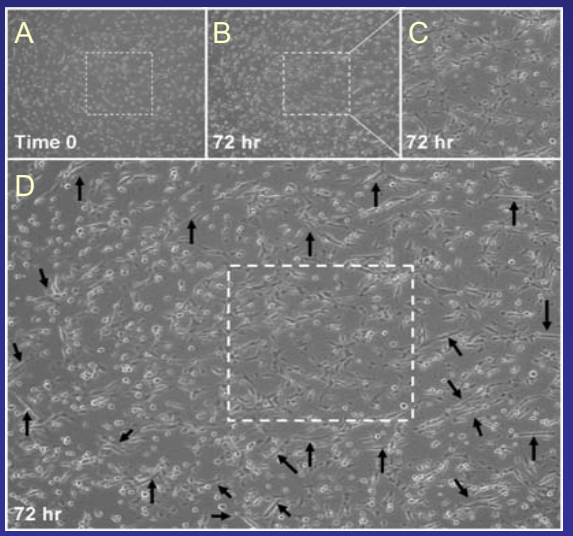


**Figure 5**: Time-lapse microscopy of MDSCs on ink-jet printed BMP-2 pattern (~25 ng/cm$^2$). Cells were cultured in media containing 2% serum to promote progression of the myogenic lineage (default lineage for MDSCs). Images were captured every 10 min and representative images are shown for time 0 (A) and 72 hr (B). BMP-2 patterns are indicated by the dashed box. C) Increased magnification of cells on BMP-2 pattern in (B) at 72 hr. D) Enlarged view of (B). Arrows indicate myotube formation. No myotubes were visualized on BMP-2 pattern.

**MDSCs differentiate towards multiple lineages (osteogenic and myogenic) in register to ink-jet printed patterns**

Figure 6

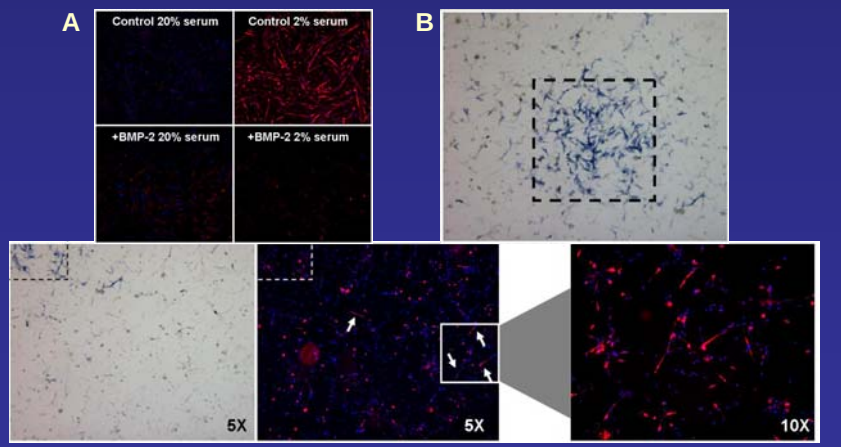


**Figure 6**: Patterning of multiple subpopulations by ink-jet printing. A) MDSCs differentiate towards the myogenic lineage under 2% serum conditions and without BMP-2. MDSCs form multinucleated (blue) myotubes shown by expression of MHC-f (red). B) ALP positive cells on BMP-2 pattern indicate progression of the osteogenic lineage. Bottom right corner of square pattern of ALP activity is shown in (C-left) Myotubes form only outside of the BMP-2 pattern as indicated by arrows (C-middle) and increased magnification (C-right). BMP-2 pattern (ALP+, blue cells) are depicted by the dashed line box.

**Conclusions and Future Directions:**

• Ink-jet printing can be used to spatially control differentiation of multiple lineages from a single stem cell population simultaneously ***within the same well***.

• Ongoing studies include real-time tracking of cell differentiation towards multiple lineages using fluorescent biomarkers as well as printing of multiple factors in combinatorial arrays and opposing linear gradients.

• Future directions may include clinical applications for tissue-engineering therapies.

**Funding Sources:**
• Pittsburgh Tissue Engineering Initiative (JAP)
• Pennsylvania Infrastructure Technology Alliance (PGC)
• NIH RO1: DE13420-01(JH)
• NIH National Technology Center for Networks and Pathways: 1-U54-RR022241-01 (AW)