Micro-Deposit of Hydroxyapatite for *Bone-on-a-Chip* Microfluidic Devices

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Statement of Purpose

- Microfluidic devices that manipulate fluids in channels at the micron scale have been used widely to mimic the physiological environment and dynamic interactions within organs[1,2,3].
- *Bone-on-a-chip* platforms are a relatively recent development in the field[4,5,6].
- Devices commonly have a poly-dimethyl-siloxane (PDMS)-on-glass configuration.
- Strategies to improve the chemical similarity of *bone-on-a-chip* microchannels to bone involve the insertion of loose particles/scaffolds after PDMS bonding to glass slide[7,8,9,10].
- There is opportunity to incorporate a contiguous layer of carbonate-rich hydroxyapatite in the microchannels of *bone-on-a-chip* platforms to enhance similarity to bone mineral[11,12].

Objective

Develop a low-temperature method to incorporate a micro-deposit of hydroxyapatite in a microchannel of a microfluidic device

Methods

Fabrication

- **Device fabrication:** A microfluidic device designed by Middleton et *al.*[6], which consists of three parallel and interconnected cell culture channels (Width: 1 mm, Height: 60 µm), was used in the present work. PDMS was poured onto a silicon wafer with the device design, degassed in a vacuum chamber, and cured in an oven at 60°C.
- **$CaCO_3$ deposition[13,14] (Fig.1):** A glass slide was covered/masked with polypropylene-based tape and $CaCO_3$ nanoparticles ($CaCO_3$-NP) were deposited on exposed areas by convective self-assembly.
- **Conversion to hydroxyapatite[14] (Fig.1):** The $CaCO_3$ was converted to carbonate-rich hydroxyapatite by dissolution-recrystallization in phosphate buffered saline (PBS).
- **Mineralized microfluidic device:** The micro-deposit was aligned/incorporated within a microchannel when the underlying glass was bonded to a PDMS structure with the device layout.

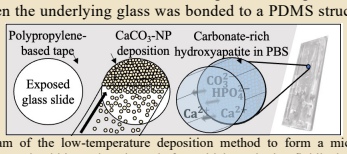

Fig. 1 Schematic diagram of the low-temperature deposition method to form a micro-layer of carbonate-rich hydroxyapatite and incorporate it within a microchannel of a multichannel microfluidic device by Middleton et *al.*[6].

- **Advantages: 1);** Low temperature method that enable incorporation of biologics to further enhance similarity to bone; **2)** Ability to be tailored to different design layouts/areas; and **3)** Can form carbonate-rich hydroxyapatite or hydroxyapatite.

Characterization

- Thickness was characterized by 3D laser confocal microscopy (**3D-microscopy**).
- Morphology was characterized by scanning electron microscopy (**SEM**)
- Crystallography was characterized by X-ray diffraction (**XRD**)
- Chemical composition was characterized by laser Raman micro-spectroscopy (**Raman**) and Fourier-transform Infrared Spectroscopy (**FTIR**)
- ***In vitro* characterization** with monocultures and co-cultures of osteoblast-lineage (MC3T3-E1 (P7) or MG63 (P118)) and preosteoclast-lineage (RAW 264.7 (P16)) cell lines was performed to determine the effect of the micro-deposit on: cell chemotaxis/proliferation (crystal violet); growth patterns (optical microscopy and SEM); and mineralization (alizarin red). Seeding density: 4×10^5 cells/mL.

Results and Discussion

- 3D-microscopy indicates that the micro-deposit was 19 ± 1.4 µm thick (**Fig. 2a**).
- SEM shows the micro-deposit consist of randomly oriented rod-like nanoparticles (**Fig. 2b**). The XRD pattern indicates that the micro-deposit is comprised of nanocrystalline carbonate-rich hydroxyapatite (**Fig. 2c**). Raman and FTIR spectra (**Fig. 2d,e**) exhibit the key phosphate and carbonate peaks characteristic of carbonate-rich hydroxyapatite.
- Materials characterization results indicate that the micro-deposits were comprised of nanocrystalline Type B carbonate-rich hydroxyapatite. The phase change from precursor $CaCO_3$ nanoparticles was through dissolution-recrystallization in PBS.
- The cell chemotaxis/proliferation assay (**Fig. 3**) showed that there was no statistical difference between the groups with and without the micro-deposit.
- The mineralization assay (**Fig. 4a**) showed that there was significant Alizarin red staining was observed on the micro-deposit and inlet wells of samples, but limited mineralization elsewhere in the microchannels.
- SEM micrographs show that cells are distributed across the micro-layer relatively uniformly (**Fig. 4b**), there is a high level of cell-matrix interaction on the mineral layer may (**Fig. 4c**), and a dense network of nanofibrils on cells (Fig. 4d), suggesting deposition of an extracellular matrix.

Results

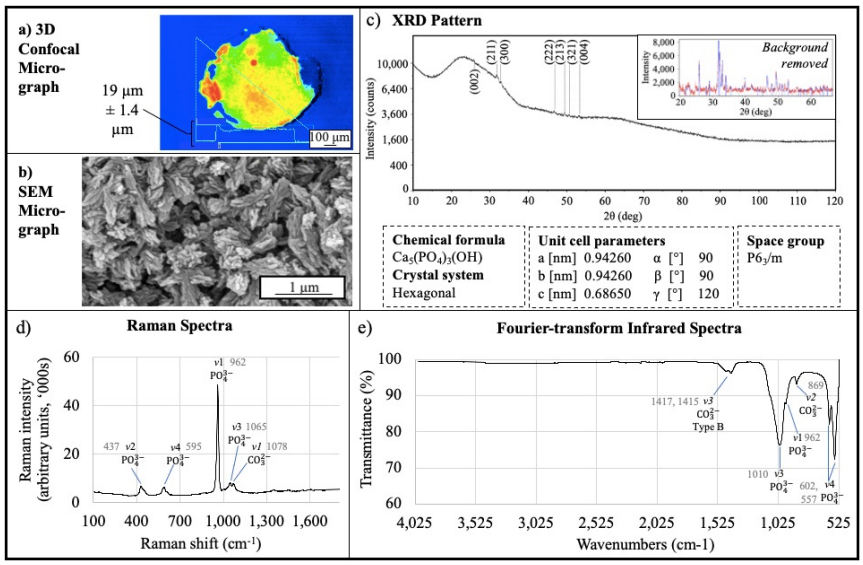

Fig. 2 Materials characterization of the micro-deposit; **a)** 3D laser confocal micrograph; **b)** SEM micrograph; **c)** XRD pattern (Top right: XRD pattern after subtraction of the amorphous glass background; reference used for phase identification: 04-007-2837); **d)** Raman spectra; **e)** Fourier-transform infrared spectra.

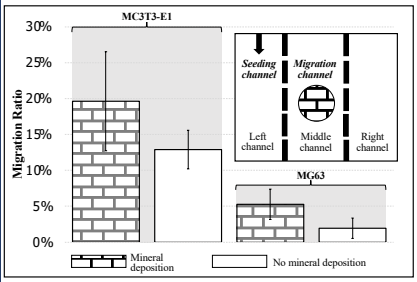

Fig. 3 Chemotactic and proliferative effect of the micro-deposit on osteoblast-lineage cells. Bars represent the mean of n = 3 replicates with standard error. However, the mean migration ratio for samples with and without a micro-layer was not statistically different ($p = 0.21$).

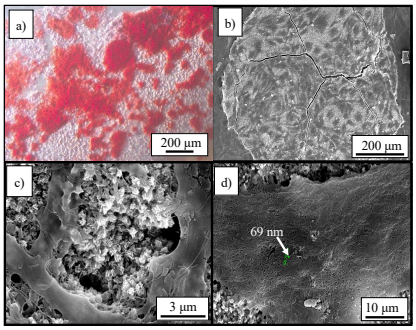

Fig. 4 a) Significant Alizarin red staining in the MG63 inlet well; **b)** MC3T3-E1 on the micro-layer - dark regions represent cells; **c)** High magnification image of MG63 cell-matrix interaction; **d)** High magnification image of a MC3T3-E1 cell on the micro-layer

Conclusions and Future Work

- A contiguous/conformal micro-layer of carbonate-rich hydroxyapatite was incorporated in a microfluidic device.
- The results provided preliminary indication of suitability for further development for *bone-on-a-chip* and *tissue-engineering-on-a-chip* platforms.
- There is opportunity to build on the present work by forming micro-deposits that correspond to larger areas of a microchannel and different device layouts. Specific phases and thicknesses of the micro-deposit could be achieved by altering CSA parameters and reagents in the dissolution-recrystallization conversion process.

Significance

- Application to *bone-on-a-chip* microfluidic platforms used to study bone biology and skeletal diseases as the micro-deposit has much closer similarity to bone mineral than glass (chemical composition and crystallography)
- *Medical-devices-on-a-chip* are in the nascent stages of development and the micro-deposit represents orthopedic implant coatings for a model *implant-on-a-chip*.

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