A MICROFLUIDIC MODEL FOR CANCER METASTASES TO BONE
Simone Bersini1,2, J. Jeon3, C. Arrigoni2, G. Dubini4, M. Moretti5, Roger D. Kamm3

1Dipartimento di Elettronica, Informazione e Bioingegneria, Politecnico di Milano, Italy
2Gruppo Ospedaliero San Donato Foundation, Milano, Italy
3Department of Mechanical Engineering, Massachusetts Institute of Technology, USA
4Dipartimento di Chimica, Materiali e Ingegneria Chimica, Politecnico di Milano, Italy
5IRCCS Istituto Ortopedico Galeazzi, Milano, Italy

Introduction
Metastases arise following the spread of tumors from a primary site to target organs where circulating cancer cells can extravasate [Chambers et al., 2002]. Bone represents an attractive site of metastasis for various tumors including breast cancer. Although regulation of bone metastases was intensively investigated little is known about how osteoblasts influence the tissue specificity of metastases and which molecules are involved in their cross-talk with cancer cells [Hsu et al., 2012]. Understanding the mechanism of translocation of breast tumor cells could be extremely valuable in the search for methods to prevent metastases [Valastyan and Weinberg, 2011]. Microfluidic systems provide a controllable micro-environment that cannot be achieved by conventional tissue culture methods. The aim of the project is the generation of a microfluidic in-vitro model to study circulating breast cancer cells transmigration into a bone micro-environment, thus providing new insights into the biology underlying bone metastases.

Methods
A polydimethylsiloxane (PDMS) microfluidic system composed of three channels and four gel regions (Figure 1a) was developed using soft lithographic methods [Shin et al., 2012]. Human micro vascular endothelial cells (hMVECs) were seeded in the central channel. Then, MDA-MB-231 breast cancer cells were injected to study tumor cell extravasation through an endothelial monolayer mimicking the blood vessel, whose permeability was quantified by means of a 70 kDa fluorescent dextran. A normal epithelial cell line (MCF-10A) was used as a control. Preliminary studies were performed to recreate a bone micro-environment by means of osteo-differentiated mesenchymal stem cells (MSCs) seeded within the gel, thus generating a tri-culture system. The model was characterized through confocal microscopy to analyze gel structure and cancer cell extravasation.

Results
The bone environment was successfully generated combining differentiated MSCs and collagen type I gel high concentration [6mg/ml] and calcium production visualized as red spots within the cell matrix (Figure 1b). MDA-MB-231 extravasation (Figure 1c) was higher (38.8±7.9%) compared to control (23.8±4.7%). Correspondingly, hMVEC barrier permeability increased after tumor cells were introduced from (3.70±0.59)⋅10^{-6} to (14.2±2.6)⋅10^{-6} cm/s.

Discussion
MDA-MB-231 show an increased tendency to extravasate relative to normal epithelial cells and, in doing so, compromise endothelial barrier. Preliminary experiments to mimic the bone micro-environment highlight calcium production, thus demonstrating that MSCs cooperate for the generation of a functional matrix. System optimization could allow us to characterize the complex cell interactions leading to bone metastases and to quantify the effect of chemo-attractive molecules or drugs.

References
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