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HIGH-FIDELITY SIMULATION OF FLOWS IN BONE-LIKE ENVIRONMENT TO INVESTIGATE THE GROWTH OF CANCER CELLS

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ABSTRACT

Metastatic cancer in bones is incurable, which causes significant mobility and mortality to the patients. In this work, we investigate the role of interstitial fluid flow on cancer cells' growth within the interconnected pores of human bone. In-vitro experiments were carried out in a bio-reactor which includes bone-like scaffold specimens. A pump is used to maintain a laminar flow condition inside the bioreactor to resemble fluid flow in bones. The scaffold specimens are harvested after 23 days in the bioreactor. The scaffold specimen is scanned with Micro-CT under the resolution of 70 micrometers. We created a full-scale 3D computational model of the scaffold based on the micro-CT data using the open-source software Seg3D and Meshmixer. Based on the geometrical models, we generated the computational grids using the commercial software Gridgen. We performed Computational Fluid Dynamics (CFD) simulations with the immersed boundary method (Gilmanov, Le, Sotiropoulos, JCP 300, 1, 2015) to investigate the flow patterns inside the pores of the scaffolds. The results reveal a non-uniform flow distribution in the vicinity of the scaffold. The flow velocity and the shear stress distributions inside the scaffold are shown to be convoluted and very sensitive to the pore sizes. Our future work will further quantify these distributions and correlate them to cancer cells' growth observed in the experiments.

Keywords: Bone, Scaffold, CFD.

NOMENCLATURE

Micro-CT	Micro Computed Tomography
hMSCs	human mesenchymal stem cells
Na-MMT	sodium montmorillonite
HAP	hydroxyapatite
PCL	polycaprolactone
DAPI	6-diamidino-2-phenylindole

1. INTRODUCTION

Prostate cancer is the most common cancer in men with a high malignancy rate. The American cancer society has estimated 191,930 new cases of prostate cancer and 33,330 deaths due to prostate cancer in 2020. Among them, about 80 percent of advanced prostate cancer patients are believed to develop bone metastases. The metastasized prostate cancer cells mainly spread to the axial skeleton, such as the spine and pelvic bones, leading to skeletal-related defects. Previous studies suggest a strong connection between mechanical cues generated by interstitial fluid flow around the bone with alteration in the bone remodeling process and increased cancer cell migration.

Numerous experimental studies were deployed to well-document the mechanical stimuli effect on cancer cells' growth [1]. Fluid flow is the key to characterize these mechanical signals and the migratory behavior of the cell's population out of the tumor [2,3,4]. However, the critical mechanisms are still poorly understood, such as the shear stresses role.

In the present study, we designed a customized perfusion bioreactor that facilitates cell growth on nanoclay based scaffolds under dynamic culturing conditions. We have divided this study into two parts. We aimed to predict the rate of shear stress generated by fluid flow under dynamic conditions at different regions of a scaffold that influence human mesenchymal stem cells (hMSCs) behavior in growth rate, osteogenesis, cellular distribution, and orientation compared to static culture. In the second part of the study, we will understand the metastatic behavior of two different prostate cancer cell lines. To achieve this goal, we planned to correlate CFD with experimental data. In this work, we created a full-scale 3D computational model of a physical bone-mimicking scaffold based on the micro-CT scans to relate the patterns of the flow inside the scaffold with the cancer cells' dynamics resulting from shear stress and velocity of the flow.

2. METHODOLOGY

Scaffolds were prepared by the freeze-drying method, as shown in our prior studies [6]. First, we modified sodium montmorillonite (Na-MMT) clay with 5-aminovaleric acid, then hydroxyapatite (HAP) was intercalated into the clay mixture to form in situ HAP Clay. Finally, polycaprolactone (PCL) polymer was added into the reaction mixture to form PCL/in-situ HAP Clay composite. The dimensions of scaffolds used during the study are 12 mm in diameter and 3 mm in thickness.

The bioreactor chambers and their components were designed using Solid Works and fabricated using 3D printing instrument, Formlabs Form 2. The chambers were connected to the pump and media reservoir with silicone tubing. The whole assembly was set up inside the incubator to maintain optimum conditions for cell survival. The perfusion flow rate through the bioreactor chamber was maintained at 0.2 ml/min.

Human bone marrow-derived mesenchymal stem cells (hMSCs) were cultured under standard incubation conditions, i.e., 37 °C, 5% CO2, and high moisture level. 5x105 hMSCs were seeded on each scaffold and allowed to attach on the surface for 24 hours. Among seeded scaffolds, half of the scaffold samples were transferred under continuous perfusion culture. Samples were maintained under both static and dynamic conditions for 23 days. Media was changed every 2 days and 3 days for static and dynamic cultures, respectively.

Scaffolds were harvested at day 23 from static and dynamic cultures and fixed with 4% formaldehyde for 45 minutes. The samples were permeabilized with 0.2% Triton-X solution and incubated with actin-phalloidin stain for 45 mins. The samples were further stained with 4′,6-diamidino-2-phenylindole (DAPI) for 5 minutes to stain nuclei and observed under a confocal microscope.

Scaffolds were retrieved from the bioreactor and static conditions at day 23 and washed twice with PBS. The washed samples were fixed with 2.5% glutaraldehyde and were dehydrated with a series of ethanol concentrations. The dried samples were finally utilized for Micro CT scanning. Micro CT was carried out using GE Phoenix vltomel xs X-ray computed tomography system with an 80 kV X-ray energy source and 350 μA current intensity with a molybdenum target. Scans were performed at multiple detector exposure times 200 msec, 500 msec, 1000 msec, and 2000 msec, and the final image was reconstructed using a 500 msec detector timing. Sample magnification was carried out with a voxel size of 15.510 microns. The recognized scan area includes a complete cylindrical scaffold with the volume of interest, 12 mm wide × 3 mm depth.

The samples were retrieved at day 23 and treated with 2.5 % glutaraldehyde and ethanol series similar to microCT samples. The dried samples were sputter-coated with gold target and mounted on SEM stubs for imaging using a scanning electron microscope.

The scanned images in form of DICOM files (70 micrometers) were processed using the open-source imaging software Osirix to create the 3D mesh of the scaffold by thresholding to capture the maximum possible interconnected pores. Using the open-source software Meshmixer, the resulting surface mesh is split into four separate solids (quarters). Each solid is re-meshed, smoothed, and triangulated to approximately 250 thousand vertices and 500 thousand elements.

Simulations were performed using our in-house CFD code. The governing equations for the fluid are the three-dimensional, unsteady incompressible continuity and Navier-Stokes equations The fluid was assumed to be incompressible with constant density ($\rho = 1000\, kg/\mathrm{m}^3$) and kinematic viscosity ($\nu = 1.1 \times 10^{-6}\, m^2/s$). The continuity and Navier-Stokes equations are solved using curvilinear-immersed boundary (CURVIB) method [5]. The discrete equations are integrated in time using a fractional step method. A Newton-Krylov solver is used to solve the momentum equations in the momentum step and a GMRES solver with multigrid pre-conditioner is employed for the Poisson equation.

At the inlet we apply uniform velocity $U_0 = 16.57\, \mu m/s$ based on the volumetric flow rate $Q = 0.2\, mL/min$ and diameter of the bioreactor chamber $D = 16\, mm$. The length scale is chosen to be $D = 1\, mm$. These flow conditions led to Reynolds number $R_e = 0.0151$.

The computational domain is discretized using a structured grid of size 301x301x321 (approximately 30 million grid points) with a rectangular geometry and average spacing of 0.054 mm, 0.054 mm and 0.0143mm defining the resolution of the domain in the directions x, y, and z respectively.

3. RESULTS AND DISCUSSION

Our immunostaining data indicates that human mesenchymal stem cells (hMSCs) were heterogeneously

distributed over the scaffold surface on day 23. On the contrary, hMSCs cultured under perfusion bioreactor showed uniform distribution of cells on the scaffold surface. The SEM data is in good agreement with immunostaining data in which cells cultured under static conditions were appeared to form a cluster while hMSCs grown under dynamic conditions represent a specific directional orientation of cells

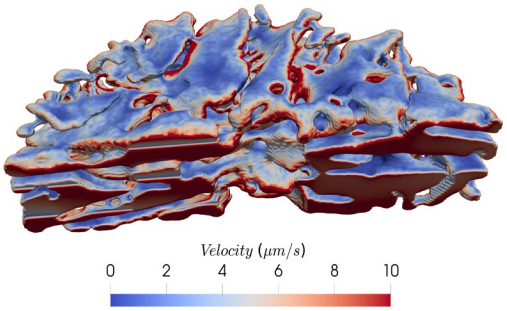

FIGURE 1: Velocity distribution of the three-dimensional scaffold. The complex porous topology leads to a wide range of flow velocity.

The porous geometry of the scaffold is very complex, as shown in Figure 1. This complex topology can be seen on both the surfaces as well as the interior part of the scaffold. The velocity distribution on the scaffold shows that the flow at the vicinity and within the scaffold is highly complex and non-uniform. The velocity contour in the scaffold is shown in Figure 1, the results reveal that the flow at the vicinity and within the scaffold in highly complex and non-uniform.

To investigate the pore sizes' effect on the flow velocity, we took a cross-sectional plane, as shown in Figure 2, showing that the flow velocity is very sensitive to the pore size. Flow velocity varies from 0 to 10 micrometers/seconds. The pore size correlates well with the velocity magnitude. In particular, the large pore in the center of the scaffold drives the most significant portion of the flow. There exist patches of very low flow with stagnant fluid within the pores. A wide range of fluid velocity indicates that the cancer cells are subjected to a wide range of shear stresses.

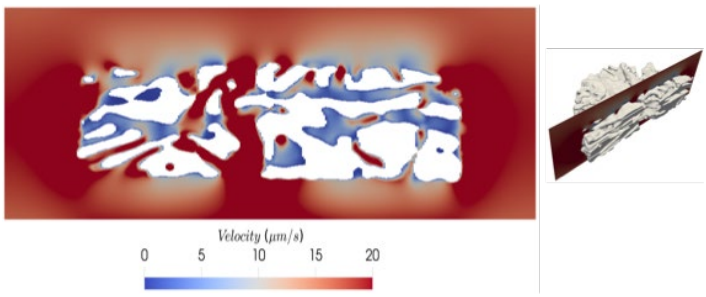

FIGURE 2: Velocity distribution on a plane. The right inset shows the location of the plane. Only the fluid area is shown. The blank space indicates the solid part of the scaffold.

4. CONCLUSION

Metastatic cancer in bones was and still a very active research area because of the lack of a detailed model on how the growth and migration of cells are really effected by the fluid flow within human bone. In this work, we investigate the profile of the fluid flow in the scaffold. We perform a high resolution CFD simulation on a full-scale 3D computational model of the scaffold based on the micro-CT scans. Our results showed the degree of complexity the flow distribution follows inside the scaffold. Also, the Immersed Boundary Method can tackle this kind of complex geometry and produce accurate results, which is promising for future works where we plan to simulate multiple full scaffolds inside a bioreactor.

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