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A "sweet-spot" for fluid-induced oscillations in the conditioning of stem cell-based engineered heart valve tissues

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ABSTRACT

Fluid-induced shear stresses are involved in the development of cardiovascular tissues. In a tissue engineering framework, this stimulus has also been considered as a mechanical regulator of stem cell differentiation. We recently demonstrated that the fluid-oscillating effect in combination with a physiologically-relevant shear stress magnitude contributes to the formation of stem cell-derived *de novo* heart valve tissues. However, the range of oscillations necessary to induce favorable gene expression and engineered tissue formation is unknown. In this study, we took a computational approach to establish a range of oscillatory shear stresses that may optimize *in vitro* valvular tissue growth. Taking a biomimetic approach, three physiologically-relevant flow waveforms from the human: (i) aorta, (ii) pulmonary artery and (iii) superior vena cava were utilized to simulate pulsatile flow conditions within a bioreactor that housed 3 tissue specimens. Results were compared to non-physiological pulsatile flow (NPPF) and cyclic flexure-steady flow (Flex-Flow) conditions. The oscillatory shear index (OSI) was used to quantify the fluid-induced oscillations occurring on the specimen surfaces. The range of mean OSI under the physiological conditions investigated was found to be $0.18 \leq \text{OSI} \leq 0.23$. On the other hand, NPPF and Flex-Flow environments yielded a mean OSI of 0.37 and 0.11 respectively, which were 46% higher and 45% lower than physiological conditions. Moreover, we subsequently conducted OSI-based human bone marrow stem cell (HBMSC) culture experiments which resulted in preferential valvular gene expression and phenotype (significant upregulation of BMP, KLF2A, CD31 and α-SMA using an OSI of 0.23 in comparison to a lower OSI of 0.10 or a higher OSI of 0.38; $p < .05$). These findings suggest that a distinct range or a "sweet-spot" for physiological OSI exists in the mechanical conditioning of tissue engineered heart valves grown from stem cell sources. We conclude that *in vitro* heart valve matrix development could be further enhanced by simultaneous exposure of the engineered tissues to physiologically-relevant magnitudes of *both* fluid-induced oscillations and shear stresses.

1. Introduction

Young patients born with critical congenital valve diseases are faced with a grim prognosis. Prosthetic valves while a mature technology do not cater to the small size of these patients, nor can they enable somatic growth. Towards the development of functional tissue engineered heart valves (TEHVs) several protocols have been investigated in an effort to optimize physical, mechanical and biochemical properties (Schmidt et al., 2010, Rabkin et al., 2002; Hasan et al., 2014). Regardless of the specific approaches, gene, cellular and tissue-level responses of the TEHV are intimately tied to the mechanical environment native to the cardiovascular system (Nejad et al., 2016; Roman and Pekkan 2012, Hasan et al., 2014; Salinas et al., 2016). Recently, the directionality of fluid-induced shear stress, i.e., oscillatory shear stresses have been shown to be essential in heart valve tissue development including cellular proliferation, extracellular matrix (ECM) production and the overall preservation of the valvular phenotype (Adamo and García-Cardeña, 2011; Li et al., 2004; Riddle et al., 2006). In an *in vivo* model, Vermot et al. established a link between OSS and its direct regulatory role on the critical gene KLF2A, whose absence results in heart valve defects (Vermot et al., 2009). In our laboratory, as well as elsewhere, *in vitro* mechanical environments that elicit OSS,

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such as Flex-Flow or pulsatile flow demonstrated clear merits of engineered extracellular matrix (ECM) collagen production and valvular phenotype derived from human bone marrow mesenchymal stem cells (HBMSCs) (Rath et al., 2015; Salinas et al., 2016; Engelmayr et al., 2006). In particular when the mechanical conditions were in the physiological range (fluid shear stresses of 4–5 dynes/cm^2 and cyclic flexure frequency of 1 Hz), *de novo* tissue content was further augmented, with relatively-high presence of genes supporting the cardiovascular phenotype, including robust KLF2A expression (Rath et al., 2015). On the other hand, use of a NPPF condition adversely impacted KLF2A expression, although other pertinent genes (such as FZD2) and overall ECM collagen content remained unaffected. Regardless of the specific mechanical conditions used, the common underlying thread in studies where construct biological properties were substantially improved in comparison to static culture, was the exposure of BMSC-derived engineered tissues to OSS. Indeed, we previously established that OSS plays a critical role in the formation of engineered heart valve tissues when the magnitude of fluid-induced shear stresses is physiologically-relevant (Salinas et al., 2016). In this investigation, we wanted to identify the specific value(s) of fluid oscillations rather than the shear stresses that led to these enhanced properties. He and Ku (Ku et al., 1985) have shown that the fluid oscillations can be quantified using the oscillatory shear index (OSI) which is defined as follows:

$$OSI = 0.5 \cdot \left(1 - \frac{\left|\int_0^T WSS\ dt\right|}{\int_0^T |WSS|\ dt}\right) \quad (1)$$

Utilizing physiologically-relevant hemodynamic conditions from different vascular sources, here, we took a computational approach towards determining a range of OSI that may be important in enhancing gene, cell and tissue-level properties in TEHVs. Subsequently the effect of physiologically-relevant OSI versus non-physiological OSI, either too high or too low was evaluated experimentally in HBMSC culture experiments.

2. Methods

The overall approach of this study is summarized in Fig. 1.

2.1. Computational model Set-up

All simulations in this study were carried out using commercially available software (ANSYS® Workbench 2016, Ansys Inc., Canonsburg, PA) within a Windows® 7 64-bit Operating System environment (Microsoft Inc., Redmond, WA). A workstation with dual processors was used to facilitate an efficient and accurate solution convergence (Intel Xeon® X5550, 2.66 GHz processor speed, Intel Inc., Santa Clara, CA). The simulation comprised a geometry depicting a flow-stretch-flexure (FSF) bioreactor (Fig. 2a) which has been used to conduct several tissue engineering experiments in our laboratory (Ramaswamy et al., 2014; Rath et al., 2015; Salinas and Ramaswamy 2014, Salinas et al., 2014; Salinas et al., 2016). The bioreactor chamber consists of a u-shaped flow path, with a diameter of 13 mm.

For each physiologically-relevant pulsatile flow simulation that was run, a no slip boundary condition was assigned to the walls of the bioreactor while a porous fluid interface was prescribed to the surfaces of each rectangular-shaped scaffold (n = 3 specimens per flow chamber; specimen dimensions: 17 mm × 6.5 mm × 1 mm). Three pulsatile flow simulations were conducted; specifically, the inlet pulsatile velocity boundary conditions from two arteries

Figure 1

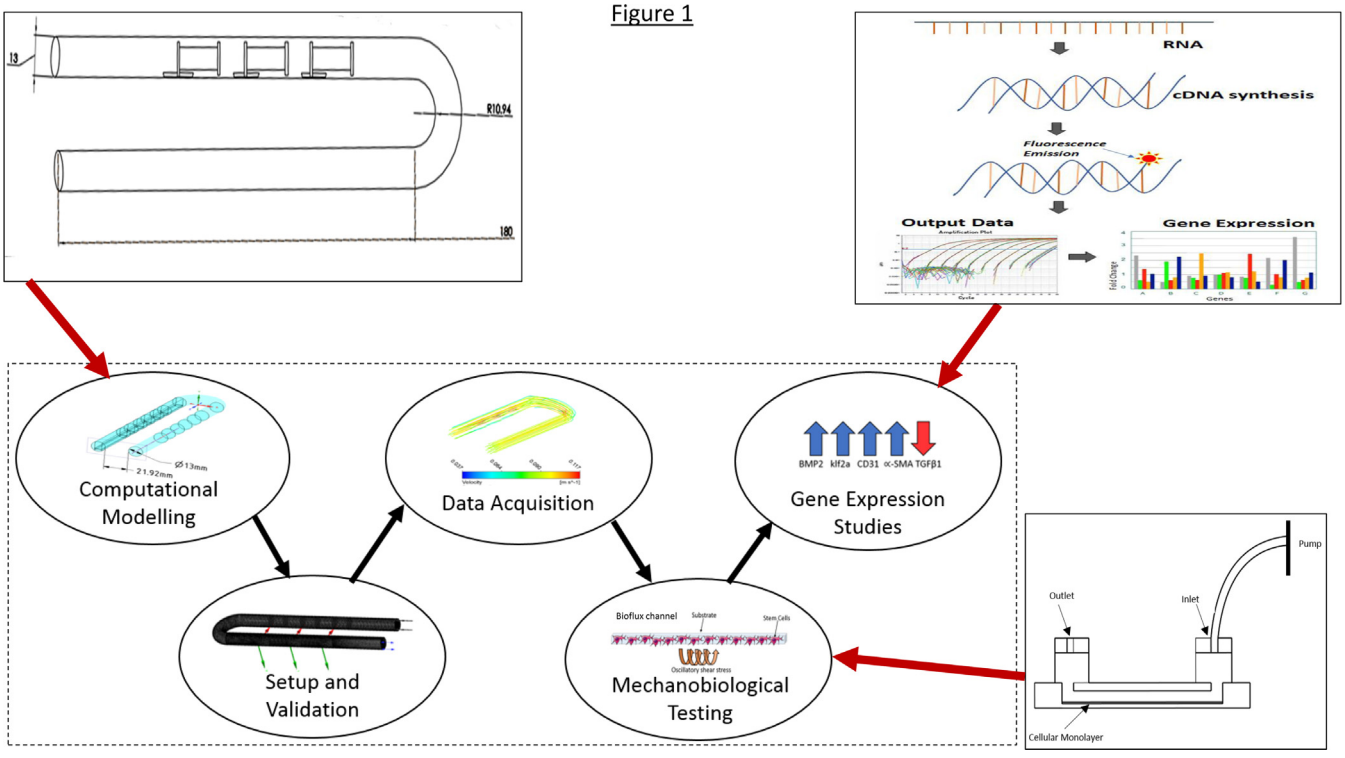

Fig. 1. Summary of Study Design: As the flowchart illustrates, computational fluid dynamic simulations were first used to quantify the OSI within a bioreactor device which emulates the shear stress patterns on the surfaces of native aortic heart valve leaflets (Ramaswamy et al. 2014; Ramaswamy et al. 2010). Subsequently a shear stress cell assay system was utilized to apply low, physiologically-relevant ("sweet-spot") and high OSI environments to HBMSCs being cultured in a monolayer. Finally, valve-relevant gene expression of the cells was evaluated via real-time polymerase chain reaction.

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