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Modeling the fluid-dynamics and oxygen consumption in a porous scaffold stimulated by cyclic squeeze pressure

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ABSTRACT

The architecture and dynamic physical environment of tissues can be recreated in-vitro by combining 3D porous scaffolds and bioreactors able to apply controlled mechanical stimuli on cells. In such systems, the entity of the stimuli and the distribution of nutrients within the engineered construct depend on the micro-structure of the scaffolds. In this work, we present a new approach for optimizing computational fluid-dynamics (CFD) models for the investigation of fluid-induced forces generated by cyclic squeeze pressure within a porous construct, coupled with oxygen consumption of cardiomyocytes. A 2D axial symmetric macro-scaled model of a squeeze pressure bioreactor chamber was used as starting point for generating time dependent pressure profiles. Subsequently the fluid movement generated by the pressure fields was coupled with a complete 3D micro-scaled model of a porous protein cryogel. Oxygen transport and consumption inside the scaffold was evaluated considering a homogeneous distribution of cardiomyocytes throughout the structure, as confirmed by preliminary cell culture experiments. The results show that a 3D description of the system, coupling a porous geometry and time dependent pressure driven flow with fluid–structure-interaction provides an accurate and meaningful description of the microenvironment in terms of shear stress and oxygen distribution than simple stationary 2D models.

1. Introduction

Current tissue engineering strategies are aimed at in-vitro recapitulation of the mechanical and the biochemical cues of the physiological environment in terms of both tissue architecture and physical stimuli [1]. In this context, porous 3D constructs are considered as excellent substitutes for cell culture, as they are able to provide structural, mechanical and architectural templates for cell attachment and tissue formation while maintaining acceptable mass transport [2–4]. Moreover, the combined use of 3D structures in bioreactors is fundamental for recreating the physiological milieu, improving the exchange of nutrients and metabolites as well as providing dynamic molecular and physical regulatory signals [5,6]. This is particularly important for load-bearing or actuating tissues like bone or cardiac tissue, which require specific stimuli in order to maintain their differentiated state in-vitro.

Focusing on cardiac tissue engineering, several studies have demonstrated the role of mechanical forces and fluid motion on the organization and function of cardiomyocytes as well as on cardiovascular development [7–9]. In particular, fluid motion increases oxygen and nutrient transport [10], promoting homogenous cell growth in the scaffold as well as the maintenance of cell function and contractility [11,12], whereas mechanical forces can activate mechanotransduction pathways and induce cell alignment and cytoskeletal re-organization [13]. In a previous study, we reported that a cyclic hydrodynamic squeeze pressure can be used for inducing phenotypic differentiation in cardiac cells seeded on 2D collagen sandwiches [14]. However, 3D scaffolds as far more appropriate for regenerative applications as 2D constructs do not adequately replicate the structure and mechanical properties of the native tissue [6].

Abbreviations: CDF, computational fluid-dynamics; microCT, micro-computed tomography; FSI, fluid–structure interactions; S^2PR, sensorized squeeze pressure bioreactor; SQPR, squeeze pressure bioreactor; GTA, glutaraldehyde; ALE, arbitrary Lagrangian–Eulerian; OC, oxygen consumption; OC-S, oxygen consumption in static conditions; OC-D, oxygen consumption in dynamic conditions; LF, laminar flow; BDF, backward differentiation formula.

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When bioreactors are used in combination with porous scaffolds, the entity and the distribution of fluid-induced forces inside the structure are very complex, as they not only depend on the working conditions of the system but also on 3D microstructural parameters of the scaffold, like pore diameter, porosity, and pore interconnectivity [15]. Computational methods are powerful tools able to estimate local fluid-induced shear, pressure and velocity fields in bioreactor environments. Previous studies have shown that the fluid-dynamic environment within a porous structure can be represented using time-invariant computational fluid-dynamics (CFD) models, in order to quantify the fluid–structure interactions and the mechanical stimuli applied to cells. These CFD models follow two different approaches: i) 3D solid models of the real scaffold microstructure, reconstructed from micro-computed tomography (microCT) images [15–17]; ii) a simplified and idealized geometry obtained from the macro-characteristics of the scaffold (pore size, interconnected porosity) [18–20]. Moreover, fluid movement also plays a fundamental role in the transport of nutrients within the construct, which is a critical factor for modulating cell growth and functions. Several mathematical and computational models have been implemented to combine the diffusion of nutrients, like oxygen or glucose, with cell consumption rates, both in static [21–23] and dynamic conditions [24–27]. However, very few studies have attempted to combine fluid perfusion through the interconnected pores and cell consumption of oxygen considering the effective micro-architecture of the scaffold [15,16], instead of its global characteristics (i.e. porosity, permeability) [25–27]. This is mainly due to the extremely high computational costs required for such analyses, particularly if further combined with fluid–structure interactions (FSI) during the mechanical stimulation.

The aim of this study was thus to implement a series of computationally optimized 2D axial symmetric macro-scaled and 3D micro-scaled models of porous cryogels in the presence of a complex fluid-dynamic microenvironment. In particular the investigation was focused on the evaluation of the physical and biochemical stimuli imposed by cyclic pressure waves and pulsatile media flow generated in a sensorized squeeze pressure bioreactor (S^2PR) [28]. Our preliminary cell culture experiments in the bioreactor confirmed that cardiac cells in gelatin cryogel scaffolds subject to a 24 h long stimulation remain highly viable and are able to proliferate.

In order to predict the levels of shear stress and hydrodynamic pressure acting on cells within the construct and quantify oxygen transport and consumption inside the scaffold, the hydrodynamic stimuli generated by the bioreactor was modeled using a new computational approach. The stepwise approach can be used to investigate and understand the effects of complex stimuli imposed by dynamic cell culture devices on 3D tissue constructs.

2. Materials and methods

2.1. The S^2PR bioreactor

The S^2PR is a sensorized version of the SQPR (squeeze pressure bioreactor), a stimulation chamber designed to impose a cyclic, hydrodynamic and contactless overpressure on cell cultures using a simple vertical piston movement [28]. The entity of the stimulus depends principally on the piston velocity and the vertical space in the meatus between the two approaching surfaces. The SQPR is able to generate local overpressures between 0.5 and 8 kPa, as well as a pulsatile flow of cell culture media through the scaffold. As a result, cells seeded in the 3D constructs are subjected to mechanical forces, which enhance the diffusion of oxygen and nutrients through the structure.

The architecture of the bioreactor has been described in our previous publication [28]. In this work, it has been modified to incorporate a force and a position sensor, assuring high precision and control of the piston movement with an accuracy of 5 μm. (Fig. 1). The force sensor (Flexiforce A201, Tekscan, Inc. MA, USA) is placed under the sample brace so as to detect any contact between the piston and the scaffold (Fig. 1.A). To improve the sensitivity of the Flexiforce, the base of the S^2PR is designed to maximize the resolution and the sensitivity of the sensor: the bottom of the base is provided with a 5 mm diameter puck to optimize the contact surface with the sensing area of the sensor. This configuration significantly increases its sensitivity (230 ± 20 bits/N with the puck; 128 ± 6.2 bits/N without the puck).

In addition, the stepper motor (L4118S144, Nanotec Electronic GmbH & Co, Munich, Germany) driving the piston is provided with

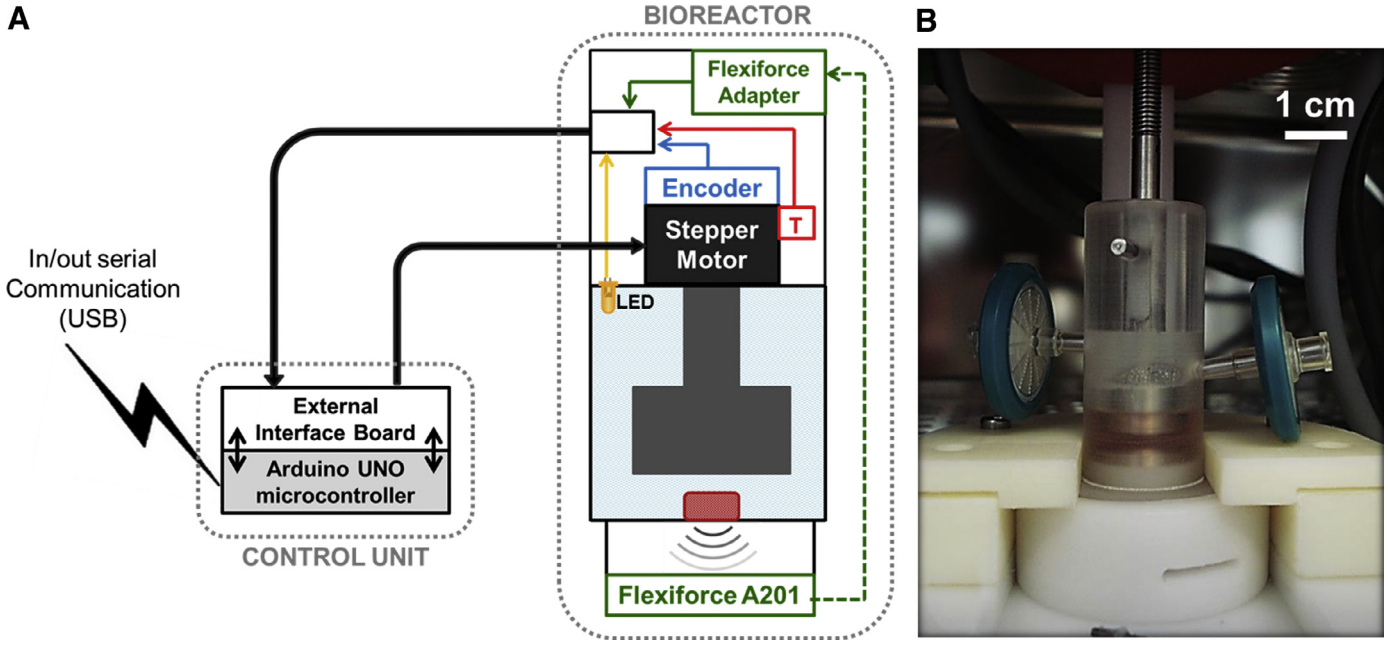

Fig. 1. (A) Functional block diagram showing the architecture of the S^2PR bioreactor. (B) A detail of the S^2PR bioreactor chamber and support base.

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