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Deformation simulation of cells seeded on a collagen-GAG scaffold in a flow perfusion bioreactor using a sequential 3D CFD-elastostatics model

C. Jungreuthmayer[a,b,c], M.J. Jaasma[a,b], A.A. Al-Munajjed[a,b], J. Zanghellini[d], D.J. Kelly[b], F.J. O'Brien[a,b,*]

[a] Department of Anatomy, Royal College of Surgeons in Ireland, Dublin, Ireland
[b] Trinity Centre for Bioengineering, Department of Mechanical and Manufacturing Engineering, Trinity College Dublin, Dublin, Ireland
[c] Siemens Medical Ireland, Leeson Close, Dublin 2, Ireland
[d] Institute of Chemistry, Karl Franzens University Graz, Graz, Austria

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ABSTRACT

Tissue-engineered bone shows promise in meeting the huge demand for bone grafts caused by up to 4 million bone replacement procedures per year, worldwide. State-of-the-art bone tissue engineering strategies use flow perfusion bioreactors to apply biophysical stimuli to cells seeded on scaffolds and to grow tissue suitable for implantation into the patient's body. The aim of this study was to quantify the deformation of cells seeded on a collagen-GAG scaffold which was perfused by culture medium inside a flow perfusion bioreactor. Using a μCT scan of an unseeded collagen-GAG scaffold, a sequential 3D CFD-deformation model was developed. The wall shear stress and the hydrostatic wall pressure acting on the cells were computed through the use of a CFD simulation and fed into a linear elastostatics model in order to calculate the deformation of the cells. The model used numerically seeded cells of two common morphologies where cells are either attached flatly on the scaffold wall or bridging two struts of the scaffold. Our study showed that the displacement of the cells is primarily determined by the cell morphology. Although cells of both attachment profiles were subjected to the same mechanical load, cells bridging two struts experienced a deformation up to 500 times higher than cells only attached to one strut. As the scaffold's pore size determines both the mechanical load and the type of attachment, the design of an optimal scaffold must take into account the interplay of these two features and requires a design process that optimizes both parameters at the same time.

1. Introduction

Every year, up to 4 million bone replacement procedures are performed worldwide which require the use of a bone graft [1]. However, both of the most common treatments show substantial drawbacks. Autografts, where bone is taken from the patient's own body and then re-implanted, has only limited availability and an additional invasive surgery is necessary which raises the possibility of donor site morbidity. Allograft, where bone is removed from an organ donor, possesses a small risk of disease transmission and again has limited availability. Therefore, the recent focus of bone graft research has switched to bone tissue engineering, where cells (taken from the patient's bone marrow) are seeded onto a biological scaffold. These cells produce bone tissue *in vitro* [2] using chemical and biological growth factors or by responding to biophysical stimuli applied by a bioreactor.

Scaffolds play a key role in tissue engineering and must meet various demands. The scaffold material has to be biodegradable and the products of degradation should be non-toxic [3]. The scaffold structure has to be highly porous with a high interconnectivity and a surface area [4] which allows nutrient flow throughout the scaffold and the surrounding host tissue. Collagen-glycosaminoglycan (GAG) scaffolds developed by Yannas et al. [5] and adapted for bone tissue engineering by O'Brien et al. [6] fulfil all these key criteria and show promising results *in vitro* [7] and *in vivo* [8].

The only disadvantage of the collagen-GAG scaffold for bone tissue engineering is that it has relatively poor mechanical properties. However, the mechanical properties of the cell-seeded collagen-GAG scaffold can be improved through *in vitro* matrix production and mineralisation. Biophysical stimuli can be used to increase this matrix production and improve the levels of mineralisation. One way of applying biophysical stimuli is through the use of a flow perfusion bioreactor as shown in Fig. 1, where culture medium is pumped through the cell-seeded scaffold [9–11] exposing the cells to a shear stress.

Although several biophysical stimuli have been proposed such as deviatoric stress, hydrostatic stress, and principal strain [12,13], it has been established that shear stress is the main biophysical

* Corresponding author at: Department of Anatomy, Royal College of Surgeons in Ireland, 123 St. Stephen's Green, Dublin 2, Ireland. Tel.: +353 1 402 2149; fax: +353 1 402 2355.
E-mail address: fjobrien@rcsi.ie (F.J. O'Brien).

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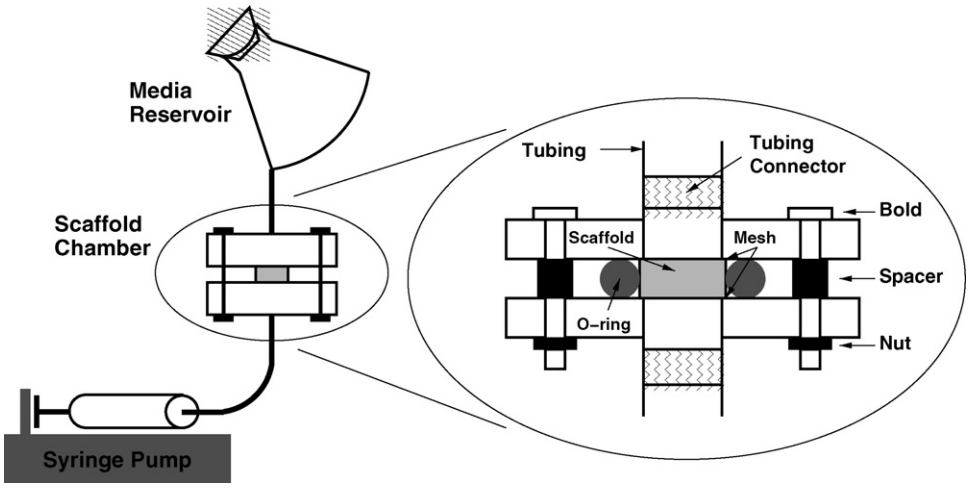

Fig. 1. Flow perfusion bioreactor used in our laboratory to apply biophysical stimuli and thereby increase the mechanical properties of cell-seeded collagen-GAG scaffolds [11,20].

stimulus which causes cells to activate matrix production and mineralisation [14–16]. The applied wall shear stress should be in a physiologically relevant range. *In vivo*, bone cells experience estimated shear stresses of 0.8–3.0 Pa during routine physical activity [17]. Shear stress values outside the physiologically relevant range might lead to a lack of osteogenic stimulation or a stimulation of osteogenic activity that is sub-optimal. A number of studies have estimated the levels of shear stress required to stimulate osteoblasts to upregulate osteogenic markers and produce extracellular matrix. Smalt et al. [18] reported that wall shear stress values of 3 Pa caused osteoblastic cells seeded on a substrate to release the early bone formation marker prostaglandin E_2 but that levels of shear stress as low as 30 mPa did not result in a significant increase of prostaglandin E_2. A calcium phosphate scaffold (pore size ~350 μm) seeded with MC3T3 cells were used by Vance et al. [19] to stimulate the cells to release prostaglandin E_2 by applying wall shear stress values of up to 1.2 Pa using a flow perfusion bioreactor. However, Jaasma and O'Brien [20] reported that much lower wall shear stress values (~20 mPa) are sufficient to stimulate osteoblastic cells seeded on collagen-GAG scaffolds (pore size ~96 μm) to release prostaglandin E_2. Besides the fluid flow, the cell morphology and the cell's position within the scaffold also affect the mechanical response [21]. This indicates that the applied wall shear stress alone is an inadequate measure to determine the optimal biophysical stimulus. We hypothesize that the cell deformation is more suitable to characterize the applied biophysical stimulus, because it not only takes into account the acting forces caused by the fluid flow, but also the cell morphology.

Computational fluid-dynamics models (CFD) have been successfully used to quantify the shear stresses acting inside microstructures [22–24], whereas finite element deformation simulations have been used to quantify the deformation of cells caused by a fluid flow [15]. Little work has been done in combining these two methods to determine the exact fluid conditions inside a scaffold and calculate its mechanical effect on the seeded cells. The objective of this study was to determine the cell-level biophysical stimuli within collagen-GAG scaffolds subjected to externally applied fluid flow using a bioreactor by quantifying the mechanical deformation of the cells. We hypothesize that the different cell attachment profiles to the scaffold struts that are observed experimentally [25] will determine the levels of cell deformation within the scaffold. To investigate this hypothesis, a novel 3D CFD-elastostatics model of a cell-seeded collagen-GAG scaffold was developed to (i) quantify the velocity, the shear stress, and the hydrostatic pressure of the fluid inside the scaffold; to (ii) determine the wall shear stress and the hydrostatic wall pressure, that cells seeded on the scaffold are exposed to; and to (iii) analyse the deformation of cells of two common cell attachment profiles.

2. Methods

A computational model was designed to characterize the deformation of osteoblastic cells seeded on a collagen-GAG scaffold [6] exposed to flow perfusion in a bioreactor. The scaffolds were fabricated in our laboratory [26] and had an average pore size of 96 μm [6]. 2×10^6 cells were seeded on the scaffold (diameter = ~12.0 mm, thickness = ~3.5 mm) as described in [11].

The development of the model required a five-step procedure: geometry reconstruction, numerical cell seeding, mesh creation, CFD simulation, and elastostatic simulation.

2.1. Step 1

A micro-computed tomography (μCT) scan of an unseeded collagen-GAG scaffold was used to obtain a numerical model of the scaffold. The μCT scan was performed by SCANCO Medical AG (Bassersdorf, Switzerland). The scan comprised a volume of 10,240 μm × 10,240 μm × 520 μm (Fig. 2). The pixel size was 5 μm × 5 μm × 5 μm. In order to reduce the computational costs, three randomly chosen sub-volumes with dimensions of

Fig. 2. μCT scan of the unseeded collagen-GAG scaffold used to reconstruct the 3D geometry for the sequential CFD-deformation simulation.