MICRO-MECHANICAL TESTING OF MECHANO-ACTIVE SCAFFOLDS FOR SKELETAL TISSUE REGENERATION

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Abstract: Many tissue engineering strategies rely on using combined cells and scaffold approaches. Bone is thought to use local mechanical strain as a cue for bone production, thus ensuring bone is laid down where it is needed most. It is reported that an agonist to strain-operated membrane channels, Bay K8466, enhances the sensitivity of bone cells to strain and increases matrix production [1]. Encapsulating this agonist into the scaffold may make bone cells more responsive to local mechanical strains in the scaffold. If so, mechanically loading the scaffold will lead to increased bone production at locations of apposite strain.

To test this strategy of enhancing bone regeneration, the relation between strain at the cellular level and micro-level bone matrix formation must be investigated. Because the scaffolds consist of random pores, we propose to derive the inhomogeneous surface strain distribution numerically by combining micro-compression experiments with micro-Finite Element (FE) models, both based on micro-Computed Tomography (µCT) images. µCT imaging of polymeric scaffolds under physiological conditions identical to those in the bioreactor is however difficult. The overlapping attenuation coefficients of scaffold material and water make it difficult to separate the two in the image.

Introduction

A main question in the application of science and technology in medicine is if human tissue can be regenerated *ex vivo*, generating functional tissue or even whole organs as implants. The general approach is to extract stem cells from a patient, which are cultured to further proliferate. When enough cells are obtained they are seeded onto a carefully engineered scaffold that is then placed in a bioreactor, which controls the environment during tissue regeneration. The scaffold has to be biocompatible at the least or biodegradable into non-hazardous compounds. In case of skeletal tissue regeneration the scaffold must also provide a suitable mechanical environment. The entire concept is referred to as tissue engineering.

The process of skeletal tissue regeneration is not yet understood to a great extend. Most of the acquired knowledge concerns the molecular biology of single cells or two-dimensional cell cultures ex vivo and the remodelling behaviour of whole bones in vivo. Little is known yet about how these processes connect with each other and, more important, how bone growth and remodelling at the macro level is regulated at the micro- or even molecular level, both in vivo and ex vivo. This study is focussed at part of this area, to investigate the influence of the micro-mechanical environment on the development of regenerating bone tissue.

In this study ±90% porous poly (L-Lactide) acid (PLLA) scaffolds of 4 mm height and 9 mm diameter are used [2]. A salt leaching method is used to produce the scaffolds. Randomly shaped and oriented salt grains of 250-350 µm in diameter form the pores of the construct. The hydrophobic scaffold is coated with collagen and seeded with primary bone cells. Regeneration of bone tissue takes place in a perfusion-compression bioreactor over 4 weeks time. In the last week cyclic compression is applied to the cell-scaffold constructs at 1 Hz for 1 hour per day, by pneumatic compression of approximately 2% strain at the macro-level.

To investigate the effect of the micro-mechanical signal that is thus applied to the tissue, Bay K8466 (Bay) is incorporated in the scaffold to enhance the sensitivity of the bone cells. Bay is an agonist to strain-operated membrane channels, which are related to an increase of bone matrix production in response to mechanical cues.

The aim of this study is to develop a method to estimate the local surface strain in the pores of a wet scaffold before cell seeding. This distribution in space of the local strains can then be compared to the distribution in space of the mineralised bone matrix that is laid down during cultivation in the bioreactor. The latter can be determined using µCT.

Materials and Methods

A micro-level strain estimation method is presented in Figure 1, based on µCT assessment of scaffolds, in combination with FE simulation

Because of the random nature of the scaffolds, a method is needed by which each individual scaffold can be easily studied. Using µCT to provide for this requirement, a three-dimensional reconstruction of the geometry of a scaffold is obtained with a resolution of 8µm. The voxels of the reconstruction are directly converted to 8-node brick elements as the basis for a FE model.

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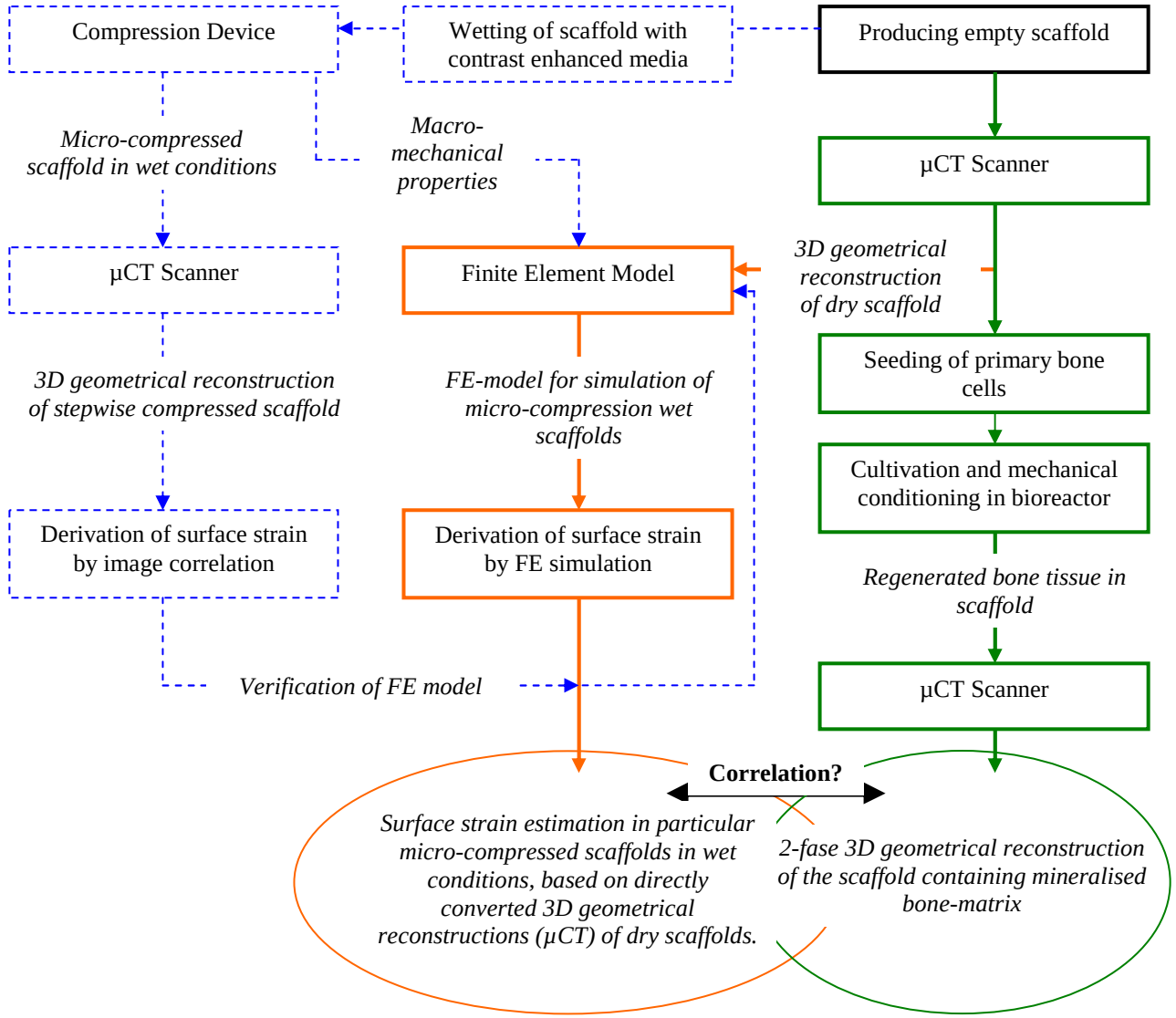

Figure 1: An outline of the method to estimate the local surface strain in the pores of a wet scaffold to compare with the mineralised bone matrix that is laid down during cultivation in the bioreactor. The development of the FE model is presented in blue, the application of the FE model in orange and the biological experiment in green.

To generate and verify the FE model, a computer-controlled device was developed to apply stepwise compression to the scaffold (Figure 2). A stepper motor with a resolution of 1.25 µm applies the compression.

The displacement is measured with a sensor to reduce errors due to backlash of the motor. A load cell is incorporated in the micro-compression device and attached to the computer. Hence, the macroscopic mechanical properties of the scaffold and appropriate boundary conditions for the FE model can be determined. The scaffold is placed in a small container to enable wet conditions, in which a piston that can be uncoupled from the stepper motor compresses the scaffold. To hold the applied compression the piston is fixed in its position relative to the scaffold by four brass-padded grub screws. After decoupling, the whole container including piston (Figure 3) can be inserted in the µCT scanner to obtain a reconstruction of the compressed scaffold in wet conditions.

This is repeated for different levels of compression, resulting in a sequence of three-dimensional images of an increasingly compressed scaffold. The surface strain can be derived from the images using digital image correlation techniques. With this protocol the simulation of scaffold compression by the FE model can be verified (Figure 1).

However, a PLLA scaffold is invisible in wet conditions, when scanned with the X-ray based µCT. Therefore contrast enhancement is needed to distinguish the PLLA phase from the liquid phase. The contrast is only needed for the verification of the FE-model, so the contrast agent does not have to be compatible with bone cells. The requirement then is that the contrast agent does not have a significant additional effect on the mechanical properties of the scaffold. This rules out the

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option to coat the scaffold with a more radiopaque material. Instead, the fluid is stained with a heavy element, to raise its attenuation coefficient (**μ**). Two different agents were tested; Iodine in solution at concentrations of 1.25%, 2.5%, 5% and 10%; and gold colloid suspensions of 2 nm and 250 nm diameter and respectively $15*10^{13}$ particle/ml and $3.6*10^{8}$ particle/ml.

Figure 2: Micro-compression device, showing the displacement sensor on top, the stepper motor and its interface suspended from the platform and the container for the scaffold with the piston and load cell at the bottom. The coupling between the piston and the stepper motor axis stands next to the container.

Figure 3: Container holding the scaffold and its assembly that is inserted into the μCT scanner. Left: **a** Base containing the load cell **b** Watertight sample holder **c** Body with radiolucent window and grub screws for fixation of piston **d** Piston. Middle and right: Complete assembly.

Results

The feasibility of scanning a compressed dry scaffold with the aid of the micro-compression device is demonstrated in Figure 4. The resolution of the obtained reconstruction was lowered to 15 μm due to the wider geometry of the device holding the scaffold, somewhat limiting the precision of the verification of the FE model.

The results of the contrast enhancement with gold colloid suspensions were both negative. During scanning the sample warmed up to slightly above room temperature. The particles settled mostly at the bottom of the suspension and some were found adhering to the scaffold. Higher concentrations of suspended particles did not solve this problem.

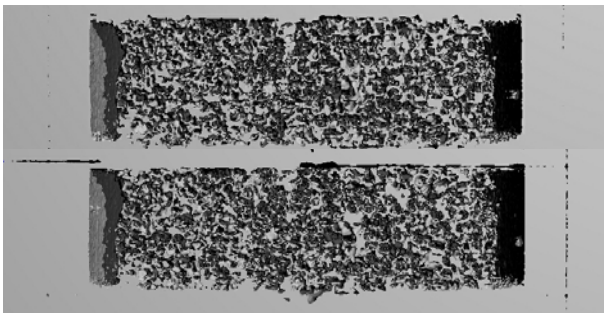

Figure 4: Two cross-sections of three-dimensional μCT reconstructions of the same scaffold under a compression of 7% (top) and 2% (bottom).

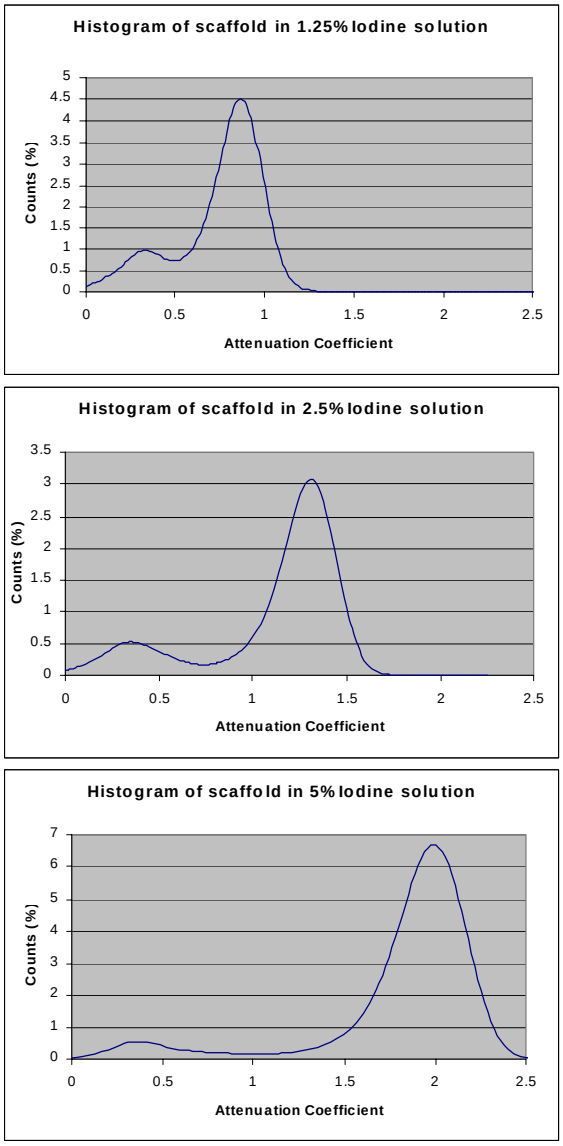

Figure 5: Histograms of a scaffold in a 1.25%, 2.5% and 5% Iodine solution. The peak of the scaffold (left) is well separated from the Iodine peak (right) in the 5% Iodine solution.

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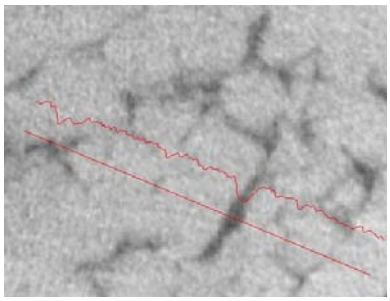

Figure 6: Two-dimensional cross-section of the µCT reconstruction of a scaffold in 5% Iodine solution. A plot of attenuation coefficient **µ** along a straight line demonstrates the ill-defined boundary of the scaffold.

The results of the contrast enhancement with Iodine solutions were more positive. The histograms (Figure 5) show two distinctive peaks representing the distribution of the scaffold attenuation coefficient μ_s and the contrasted media attenuation coefficient μ_m, using an Iodine concentration of 5%. However, at closer examination of a cross-section of the un-segmented reconstruction, the boundary of the scaffold material was difficult to discern from the media (Figures 6 and 7a). Increasing the Iodine concentration to 10% resolved this problem (Figure 7b).

Discussion

In this paper, we developed a method to determine specimen-specific local mechanical strains in a compressed porous scaffold. The method is based on a combination of micro-CT scanning and numerical models.

As part of the method, porous scaffold need to be scanned while submerged in fluid. We tested several methods to visualise the submerged scaffold radiographically, but found none satisfactory. The most promising of these, enhancing fluid attenuation coefficient by adding Iodine, suffered from indistinctness of the scaffold boundary. A possible explanation for this indistinctness is that the Iodine will diffuse into the scaffold, thus causing a **µ**-gradient, with Iodine concentration slowly decreasing with the distance from the boundary of the scaffold. The gradient made it difficult to set a proper threshold for segmentation of the scaffold geometry and generates flaws in the continuity of the segmented structure.

These problems could be solved partly with prior information of the same scaffold scanned dry, but when the scaffold is compressed the reliability of this method rapidly decreases. The problem was overcome by using an Iodine concentration of 10%. This made the gradient more profound, enabling proper thresholding (Figure 7b). However, Iodine in higher concentrations could chemically interact with the PLLA scaffold material and influence the mechanical properties of the scaffold. Hence, a lower concentration of Iodine would be preferable to minimise such interactions.

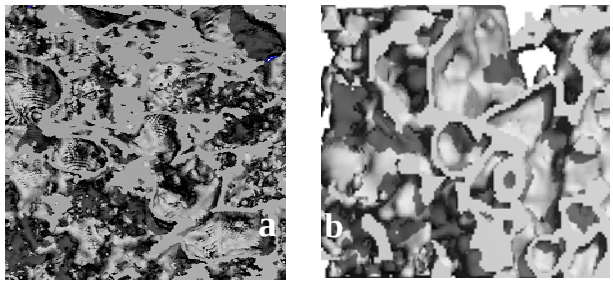

Figure 7: The segmented image of a scaffold in 5% Iodine solution (a) and 10% Iodine solution (b).

Conclusions

A method was presented to investigate the relationship between strain at the micro-level in compressed scaffolds and the deposit of calcified bone matrix by cells in the scaffold. The method consists of two main experiments. The first experiment consists of seeding strain sensitive bone cells onto a PLLA scaffold and conditioning it mechanically with cyclic compression. Secondly, a FE model is generated for the same scaffold, using a dry µCT scan made before the first experiment. The strain at the surface of the pores in the scaffold is derived from simulation of the compression of the scaffold with the FE model.

To verify the FE model a third experiment is required. An empty scaffold in wet conditions is stepwise compressed and scanned by µCT. For this purpose a micro-compression device is made and the contrast of the scaffold in media is enhanced using a 10% Iodine solution. Once verified, the surface strain inside the scaffold when compressed in wet conditions can be estimated with the FE model, for any particular dry PLLA scaffold, as long as the macro-level properties of the random porous scaffolds remain constant.

With the results presented in this paper the method in Figure 1 can be technically applied to study the relationship between micro-level strain and bone tissue regeneration. However, there are a few assumptions made that need to be addressed.

First, the assumption was made that a direct relation exists between strain at the surface of the scaffold and mechanical signals transmitted to the cells adhering to the scaffold. This assumption is needed to study the effect of the mechanical environment at the micro-level.

Secondly, the mechanical environment will change due to the regeneration of bone tissue, both by tissue filling the pores and by strengthening of the tissue by the formation of load bearing bone matrix. This is not accounted for in this method. More precisely, therefore, this method will allow to study the relationship between the *micro-level strain induced to cells* attached to the surface of the scaffold *at the start* of cyclic compression of the whole scaffold, *and micro-level bone matrix formation at the end* of the biological experiment.

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