Hollow Fibre Membrane Bioreactors for Bone Tissue Engineering: 
Effect of Mass Transport on Cell Distribution and Proliferation

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Introduction
Development of engineered grafts to repair large bone defects is limited by poor cell distribution and O2 and nutrients supply to cells in large 3D scaffolds. New bioreactor designs may allow for proper nutrients and oxygen supply to cells in large 3D scaffolds. Bioreactors equipped with hollow fibre membranes (HF) present structural analogy to natural bone tissue, where the HF membranes simulate the Havemian channels bringing nutrients to the cells. In the present study, the effect of geometry and operating conditions on cell distribution and mass transport towards cells cultured inside HF membrane bioreactors was investigated with tracer experiments and by analyzing model cell distribution and proliferation.

Materials and Methods
HF membrane bioreactors were used consisting of a bundle of HF membranes laid in a cylindrical housing parallel to its axis. Cells are cultured in the extracapillary space (ECS) outside and among the HF membranes while medium flows in the membrane lumen. Mathematical models were set-up describing mass and momentum transport into the HF membranes and in the ECS. Dimensional analysis yielded the following non-dimensional parameters determining bioreactor performance: Re, the Reynolds number at the lumen inlet; alpha, the membrane pressure modulus; q, the Thiele modulus for the cellular compartment; and Per, the radial Peclet number at the wall. The Darcy permeability of the cell compartment plays also an important role. A positive radial Per number indicates a net mass flow from the lumen to the ECS. Alpha values around 0.1 or lower indicate pure diffusive solute transport; values higher than 1 indicate the occurrence of Starling flows in the ECS. Bioreactors were designed and operated under conditions at which solutes are transported towards the ECS: in pure diffusive mode; in the presence of high Starling flows; and in the presence of pulsed radial net perfusive flows caused by pulsed stopping the flow of culture medium leaving the bioreactor. The effectiveness of solute transport towards the ECS was characterized by challenging cell-free bioreactors with a step of an inert tracer, by measuring spectrophotometrically its outlet concentration in time, and by evaluating the bioreactor residence time distribution (RTD). Models consisting of one or two CSRTs with mass interchange were used to analyze the data. Proliferation and distribution of model cells (luciferase + or -) cultured inside the HF membrane bioreactors for a short time was analysed by a bioluminescence non-invasive technique and by HE stain of histologic sections.

Results e Discussion
Transport models generally described well tracer distribution in bioreactors. Bioreactors operated in the pure diffusion mode yielded a small bioreactor mean residence time suggesting that large solutes are transported only in the lumen, and a small interchange flow rate suggesting that small solutes are transported to the ECS on ly to a small extent. The absence of convective flows determined a uniform axial cell distribution in close proximity of the membrane surface. Increasing alpha from 0.1 to ca. 1 and Per from 0.08 to ca. 8 increased both mean residence time and interchange flow rate suggesting a more effective solute transport towards the ECS. The high net convective flux in the axial direction in the ECS caused cell accumulation at the bioreactor outlet due to drag. The pulsed stopped flow mode yielded the highest solute transport effectiveness and a better radial cell distribution.

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Disclosures
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