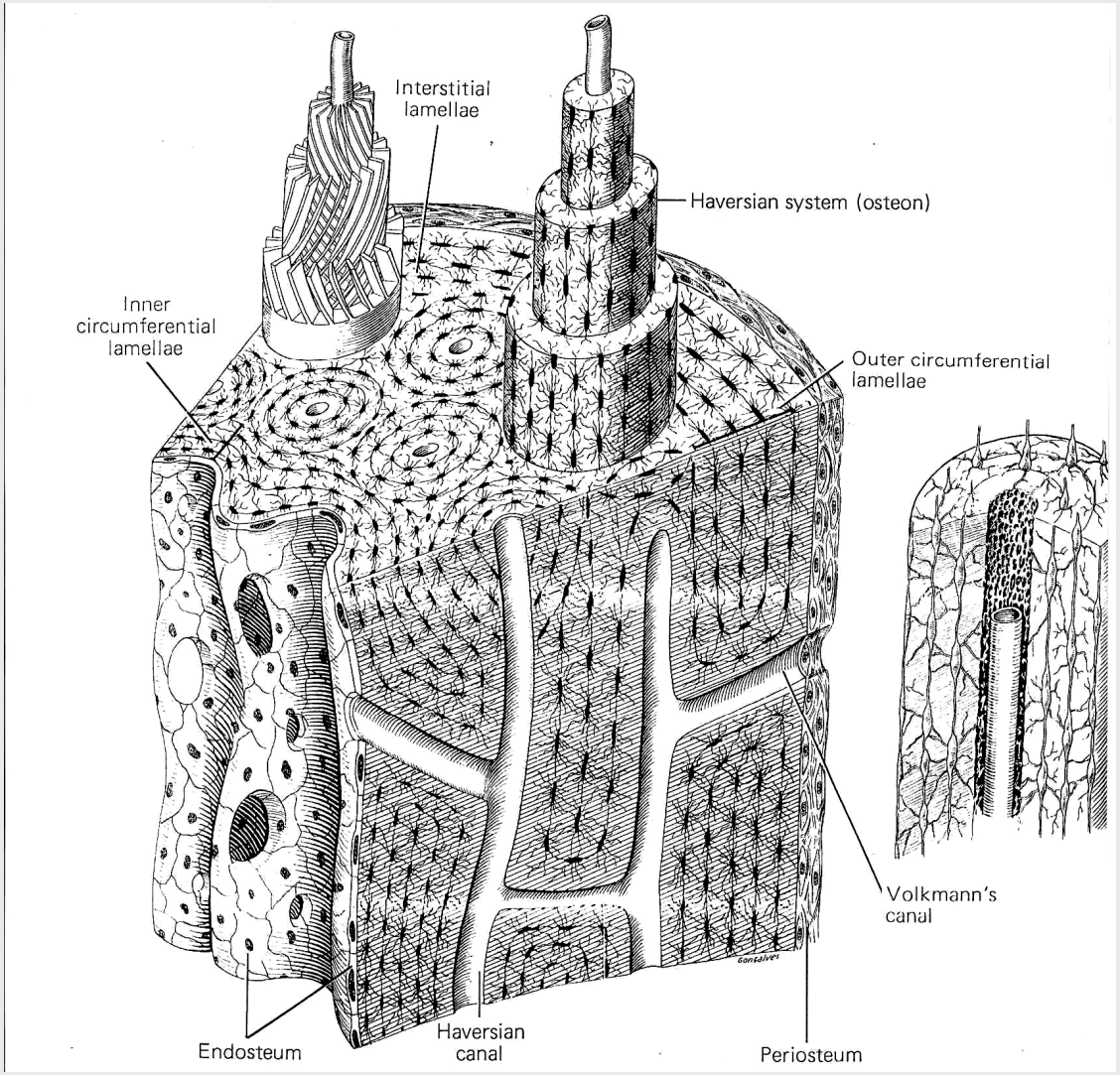
Bone

Different cells, scaffolds, bioreactors

Struttura dell’osso lamellare

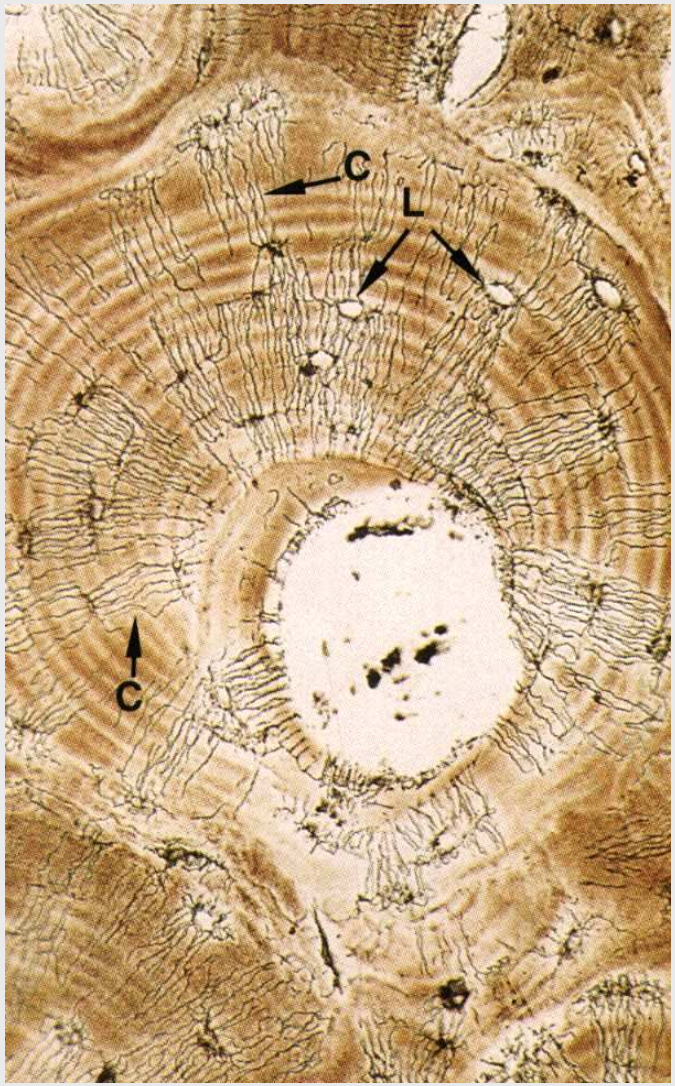


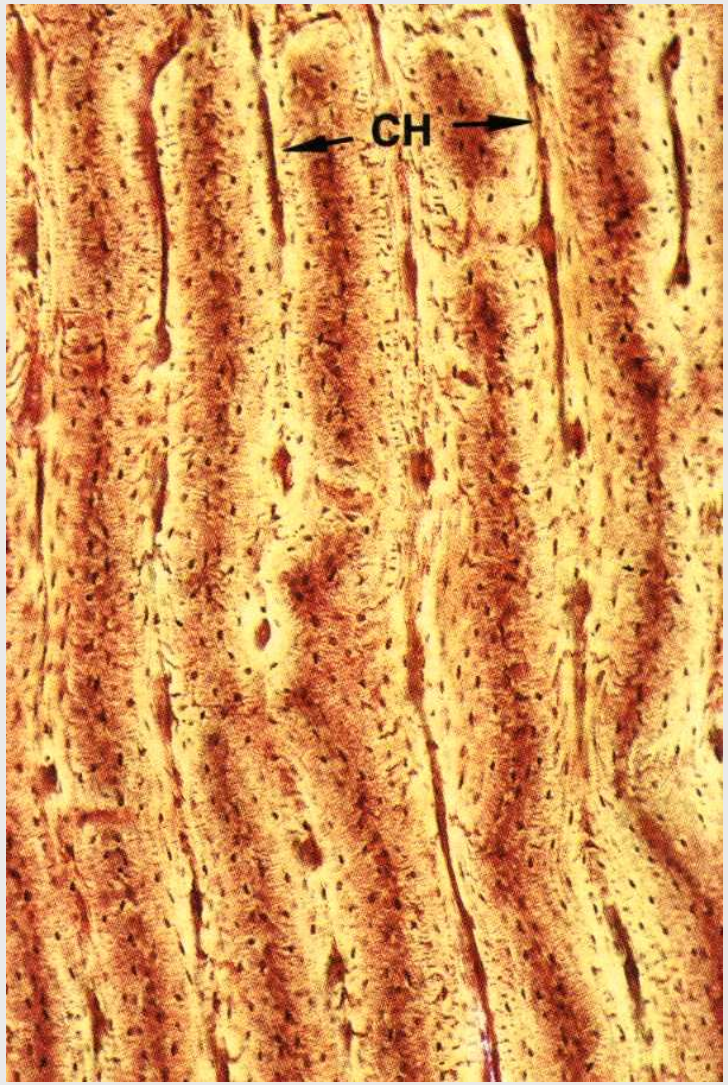
sistemi haversiani (canali di Havers)
sistemi interstiziali o breccia

con lacune e canalicoli

canali di Volkmann

Osso lamellare (per usura)





Tessuto Osseo

FUNZIONI

- SOSTEGNO MECCANICO (COSTE)
- PROTEZIONE (CRANIO)
- CONSENTE LA LOCOMOZIONE (OSSA LUNGHE)
- UNA RISERVA METABOLICA DI SALI MINERALI (CALCIO E FOSFATO)

PROPRIETA’

- VASCOLARIZZATO E METABOLICAMENTE MOLTO ATTIVO.
- TESSUTO DINAMICO IN QUANTO E’ DEPOSTO E RIASSORBITO DI CONTINUO SOTTO IL CONTROLLO DI FATTORI ORMONALI E FISICI

Tessuto osseo

MATRICE EXTRACELLULARE

sostanza organica (= osteoide) **(35%)**

- fibre collagene tipo I (90%), fibre elastiche,
- sostanza amorfa: glicoproteine: osteonectina, osteocalcina, osteopontina, proteine acide leganti il Ca++, BMP,proteoglicani **(CONDROITIN SOLFATO, KERATAN SOLFATO E ACIDO IALURONICO),**
- M-CSF e Rankl importanti per regolare il differenziamento degli osteoclasti

sostanza inorganica **(65%)**

- cristalli di idrossiapatite ($CaPO_4$, ...)

Tessuto osseo

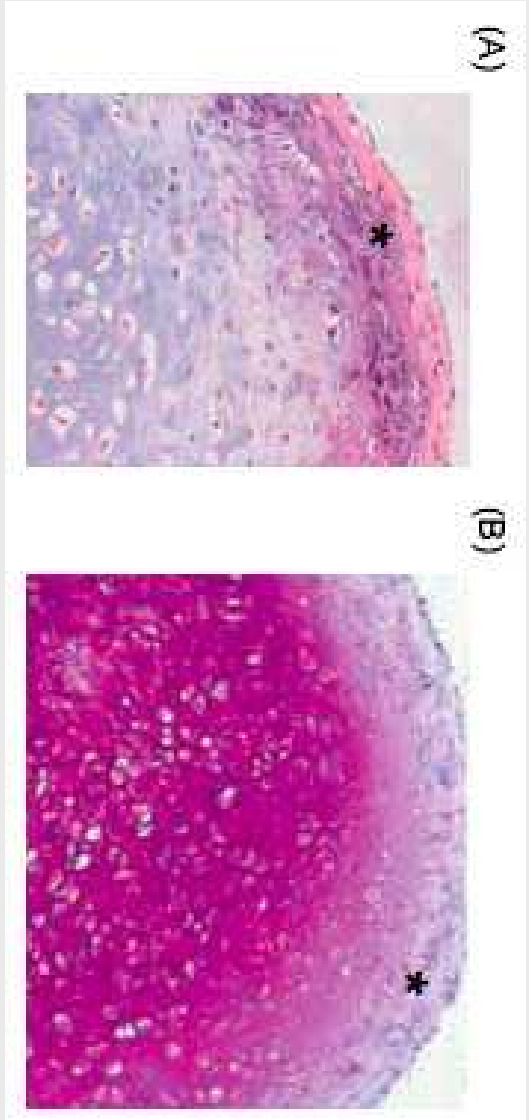
CELLULE

- Cellule osteoprogenitrici
- osteoblasti
- osteociti
- osteoclasti

3D

- micro-mass culture, based on the establishment of 3D cultures supported uniquely by the ECM produced by a pellet of cultured cells
- to investigate osteogenic cell differentiation
- → showed expression of alkaline phosphatase (ALP), bone sialoprotein (BSP), type I collagen, osteonectin (ON) and osteopontin (OPN) throughout the culture
- deposition of calcium even if not as a highly organized structure

- MSC + osteo-inductive medium
- → chondro-osseus organoid with a bony collar around hyaline cartilage formed in the centre of the pellet

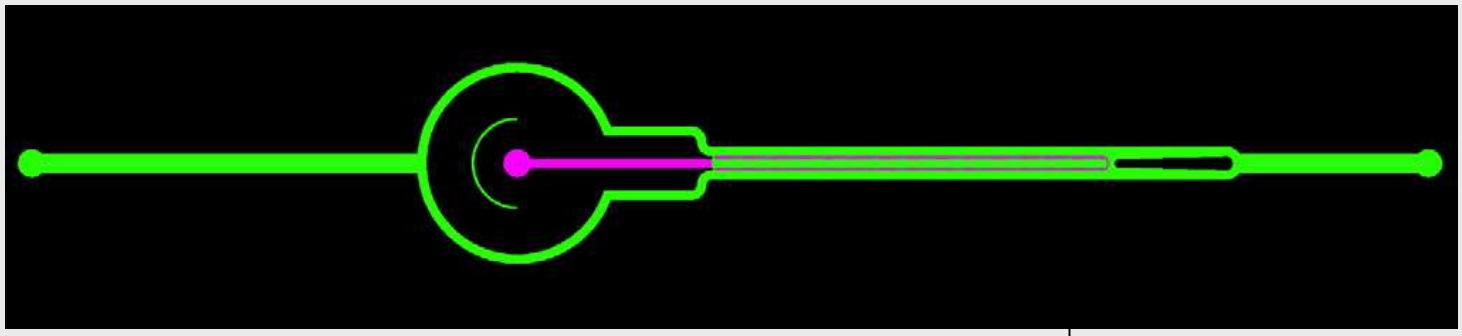


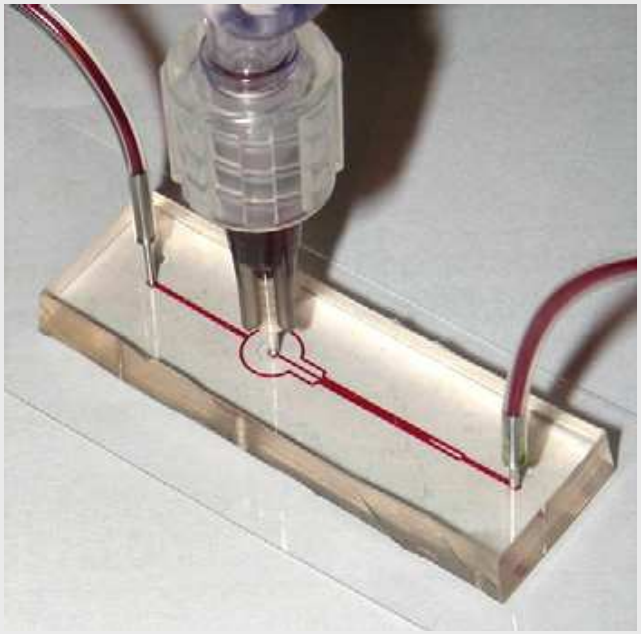
3D-μFCCS (a scaffold-free 3D microfluidic cell culture system)

- micro-mass culture does not allow high-throughput cultures and this represents a strong limitation for its use as model of bone for drug tests.
- →cells were chemically modified with an inter-cellular linker (polyethyleneimine-hydrazide) and introduced into the microfluidic channels
- the intercellular linker (transient) induced the formation of 3D multicellular aggregates
- subsequently cultured under perfusion
- → good cell viability, preserved 3D cell morphology
- produce a mineralized matrix as in the 2D counterpart

Ong et al, Biomaterials 2007; 29: 3237-3244

3D-μFCCS (a scaffold-free 3D microfluidic cell culture system)

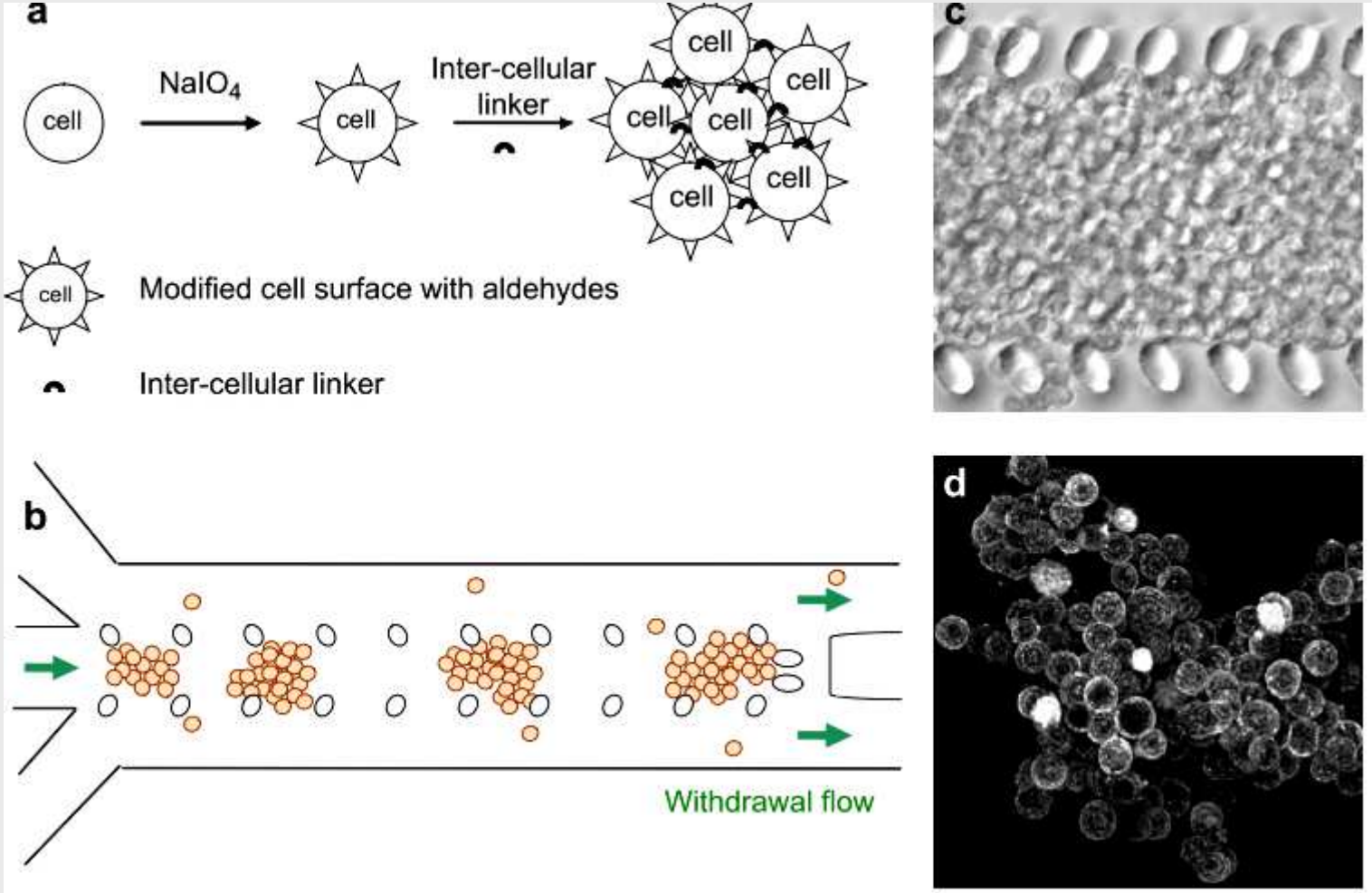




Central cell culture compartment and two side channels for perfusion of culture medium

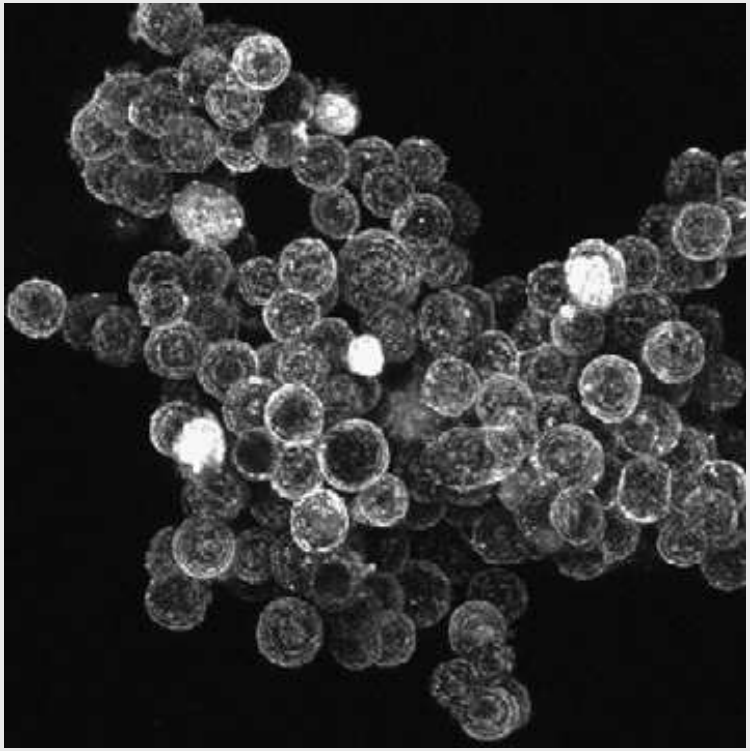
two inlets (one for culture medium infusion, one as cell reservoir) and one outlet

Cell seeding into the gel-free 3D-μFCCS



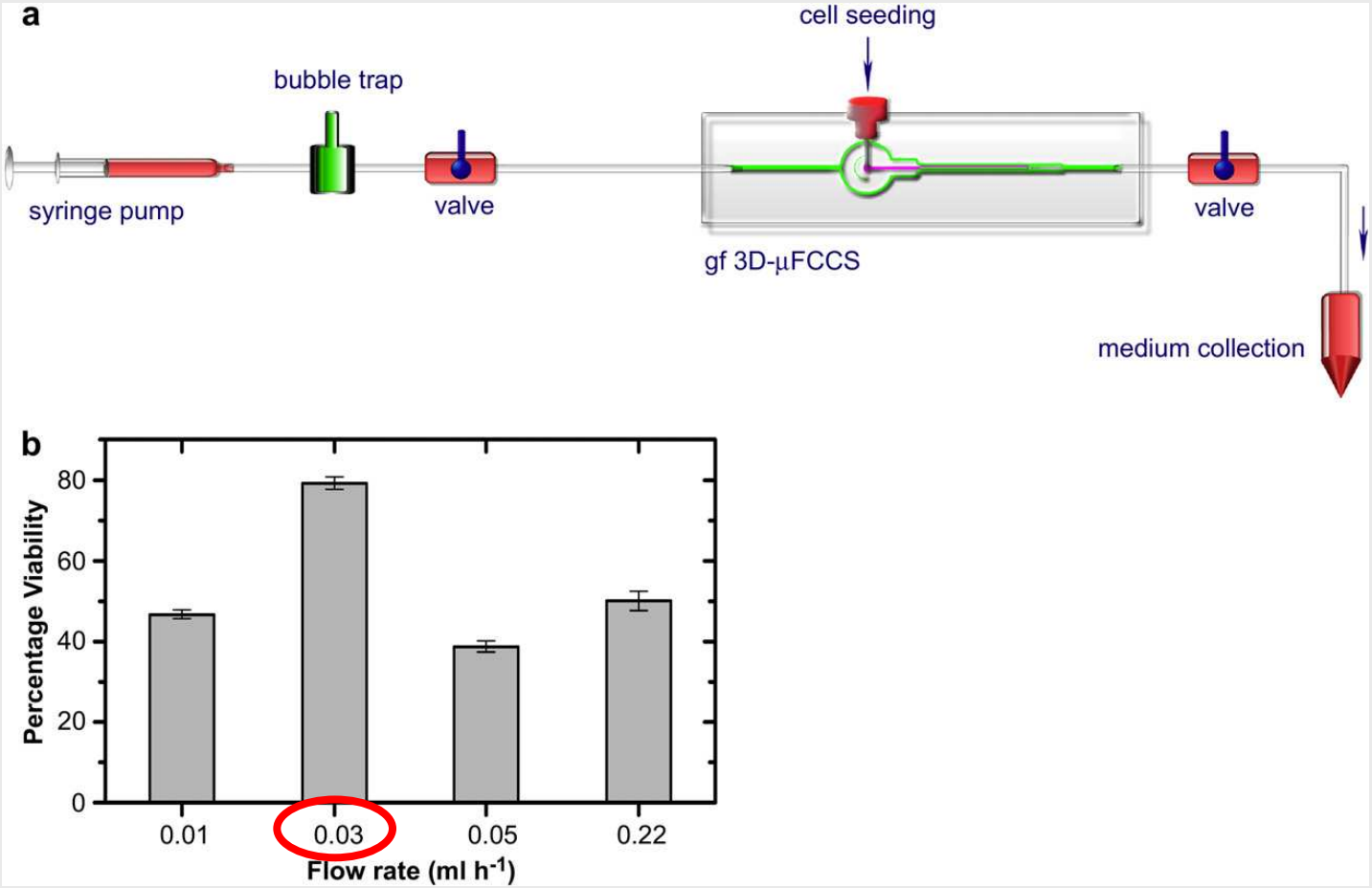
3D-μFCCS

- Cell surfaces modified by sodium periodate ($NaIO_4$) display aldehyde groups which react with the hydrazides on the inter-cellular linker to form multi-cellular aggregates
- Cells seeded into the microfluidic channel



Confocal image

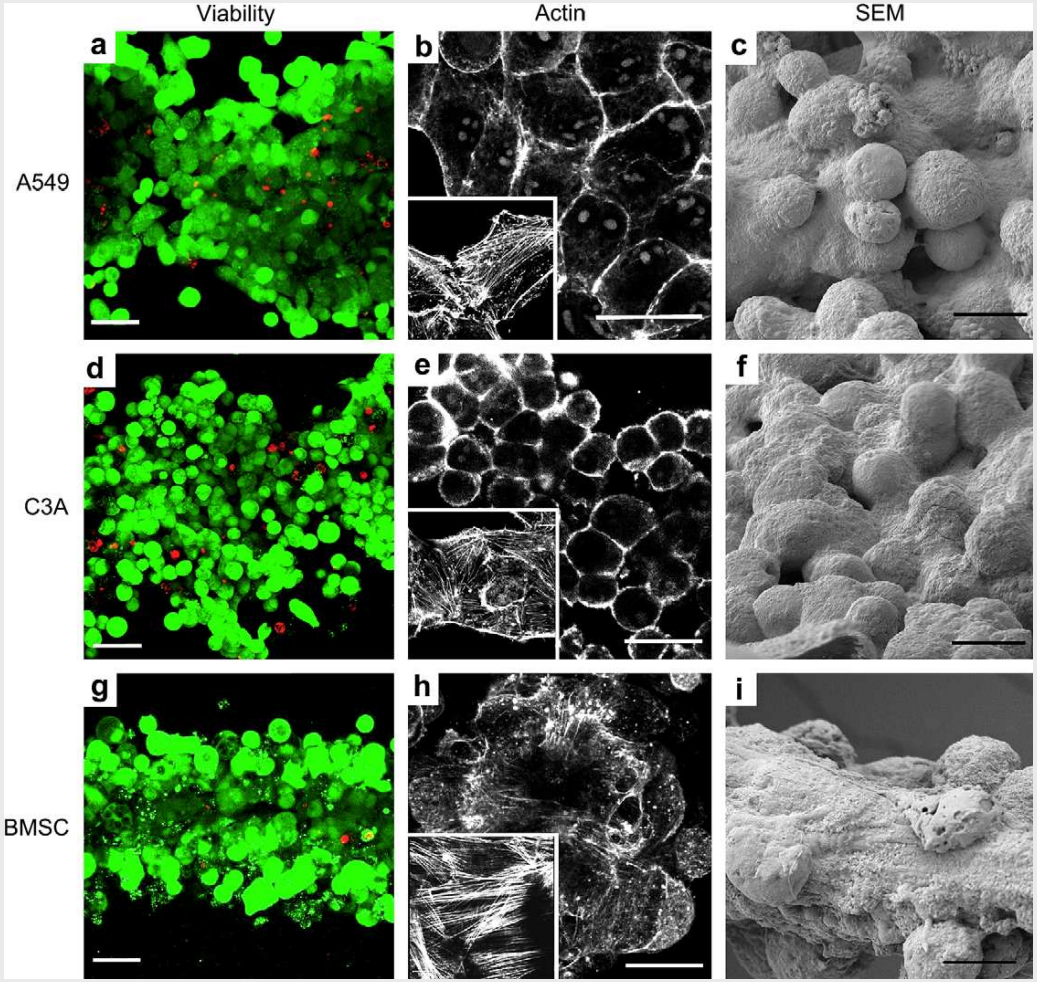
Optimization of perfusion culture flow rate for cell viability



Cell viability and morphology

Green
Calcein

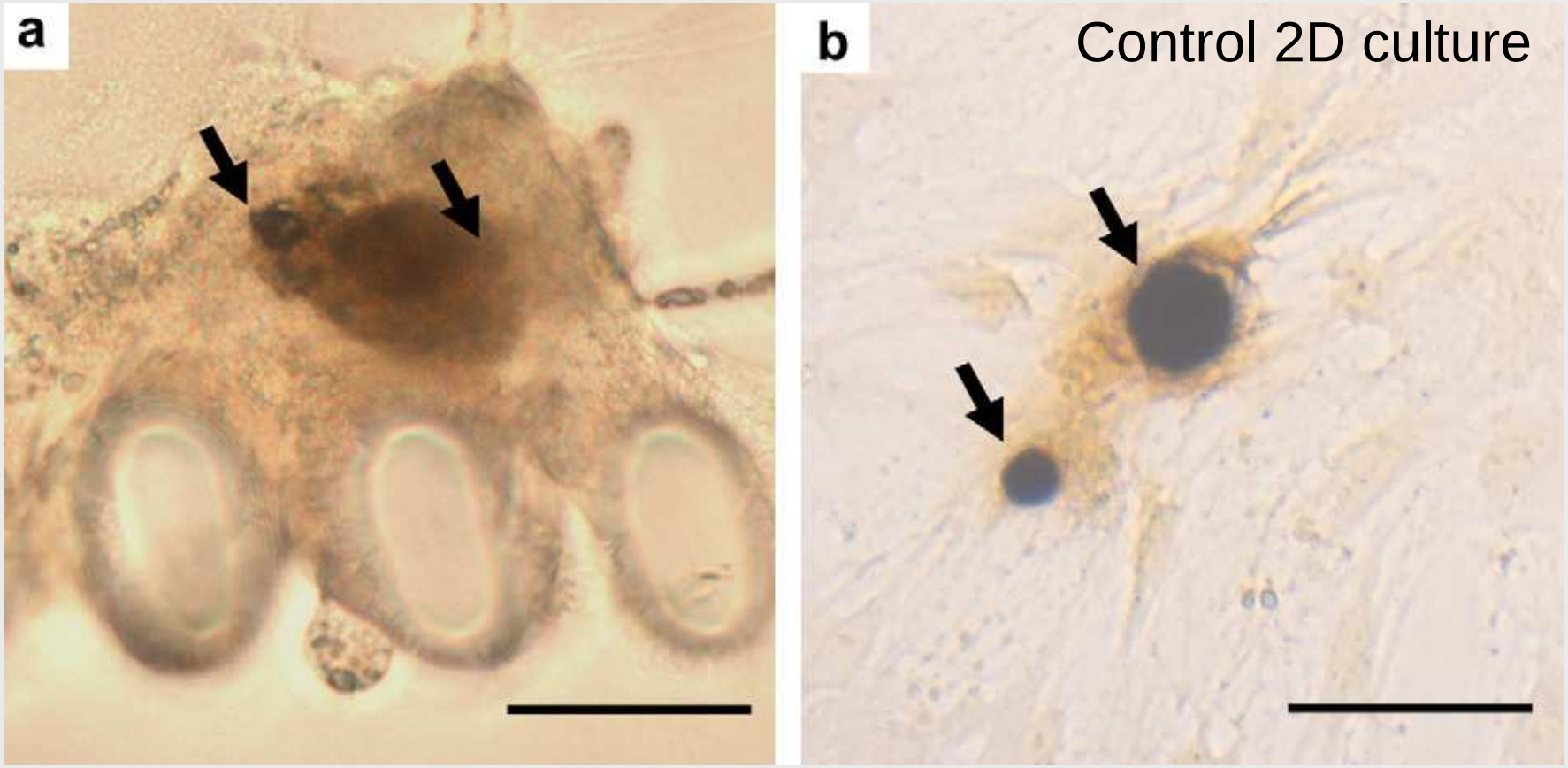
Red
PI =
dead cells



Actin

3D vs
2D <8insert)

BMSCs in gel-free 3D-mFCCS (1 week of osteogenic induction)



von Kossa staining showed calcium salt deposition

Synthetic-based polymers as a scaffold for cultures of osteogenic cells

- saturated poly-α-hydroxy esters, including poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(ε-caprolactone), poly(lactic-coglycolide) (PLGA) copolymers
- PLGA the best
- poly(ε-caprolactone) biocompatible, but highly hydrophobic and low degradable
- dynamic flow positively affected cell distribution through the scaffolds and further enhanced cell phenotypic expression and mineralized matrix synthesis within PLGA constructs compared to the static condition counterpart

- PLA fibers reinforced with poly(ε-caprolactone) allowed a high proliferation of human MSCs and human osteoblasts, as well as the expression of ALP
- → important role of the micro and nanoscale structure of the scaffold
- Increase of ALP, OC and OPN expression was observed, as well as a good mineralization

Composite scaffolds

- hydroxyapatite, biphase calcium phosphate or tricalcium phosphate
- Poly(DL-lactic acid), (PDLLA)/Bioglass©, PLA/calcium metaphosphate, PLGA/bioactive glass
- → increase of cell adhesion, but necessary to evaluate the expression of other key genes of osteogenesis (osteocalcin, type I collagen, BSP osteopontin)
- nanofibrous scaffolds (mixture of PLA and nanocrystal demineralized bone powders) supported bone formation after 12 weeks of implantation, but no bettere effect on in vitro MSC differentiation compared to the PLA scaffolds

type I collagen for bone TE

- can be used intact or after proteolytic removal of the small nonhelical telopeptides, which reduces possible antigenicity
- Two forms for native collagen (swollen hydrogels or sparse fibres in a lattice-like organization)
- Bovine type I collagen biomaterial forming the basis of several commercial products such as CollapatII© (Biomet Inc.), Healos© (Depoy Spine Inc.), Collagraft© (Nuecoll Inc., Zimmer Inc.) and Biostite (Vebas S.r.l.)

Properties of collagen

- Biocompatibility
- Ability to sustain proliferation and differentiation of osteogenic cells in the 3D provided by collagen sponges
- SAOS 2 cells able to colonize the collagen sponges and to synthesize osteocalcin
- Perfusion better than static culture
- poor mechanical strength → combination with other materials (PGA)

collagen sponges reinforced with **HA**

- Cells cultured for 28 days in both basal and osteogenic conditions revealed the penetration of ALP positive cells throughout the constructs as well as the synthesis of new matrix

  but

- Osteocalcin was localized only in the periphery of the constructs, thus suggesting a limited diffusion of nutrient factors that does not allow for the formation of ECM in the centre of the scaffolds
- → required appropriate perfusion

Mineralized type I collagen - based scaffolds

- osteoclast-like cells were able to invade and to degrade the scaffolds while osteoblasts proliferated, differentiated and produced mineralized ECM

Titanium based scaffolds

- traditional inert biomaterial
- elicit a minimal immune response
- extensively employed as a fibre mesh based scaffold for three-dimensional culture
- Rat MSCs cultured on titanium fibre meshes under dynamic conditions increased their proliferation, differentiation and mineralized matrix production (positive effect with greater perfused medium viscosity, coupled with a constant flow)

Organoapatites (OA) of hydroxyapatite (HA) and organic macromolecules

- **Zinc**, an important trace element found in bone, is known to increase in vitro biomineralization
- increase of the collagen I expression and ALP activity in a rotating bioreactor culture

  still
- extreme difficulty of performing histological evaluations of the constructs remains a big drawback that seems to be difficult to solve and can only in part be overcome with different techniques, such as confocal laser and scanning electron microscopy.

bioceramic based scaffolds

- Should be superior to the previous materials
- the majority of the studies have been focused on solving the difficulties of homogeneously supplying **oxygen** and nutrients to cells within a large scaffold and to increase **seeding efficacy**, two of the biggest problems in bone tissue engineering.

- adequate studies about the effects of the three-dimensional mineralized environment provided by the bioceramic-based scaffolds on cell differentiation
- medium **perfusion** through the scaffolds seems to be an absolute requirement
- low-pressure and oscillatory cell seeding led to an increased seeding efficiency

- Flow perfusion bioreactor helps to culture cells within three-dimensional bioceramic scaffolds.
- effect of both porosity and surface microstructure on the bioceramics' osteoinductive properties
- not only chemistry but also geometry of the biomaterial in contact with the cells is a critical factor

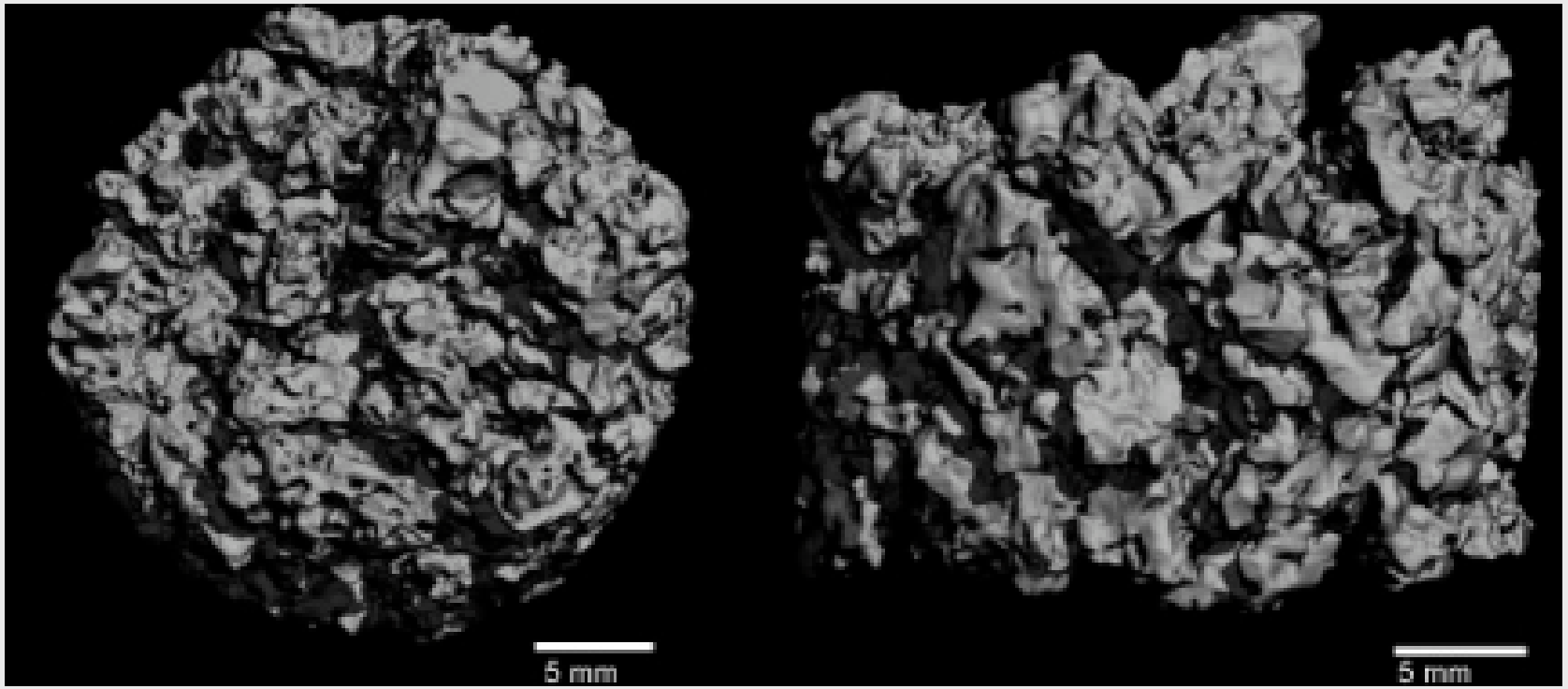
- Opacity of the scaffold is a problem for the analysis of the experiments

- Human tissue-engineered bone produced in clinically relevant amounts using a semi-automated perfusion bioreactor system: a preliminary study

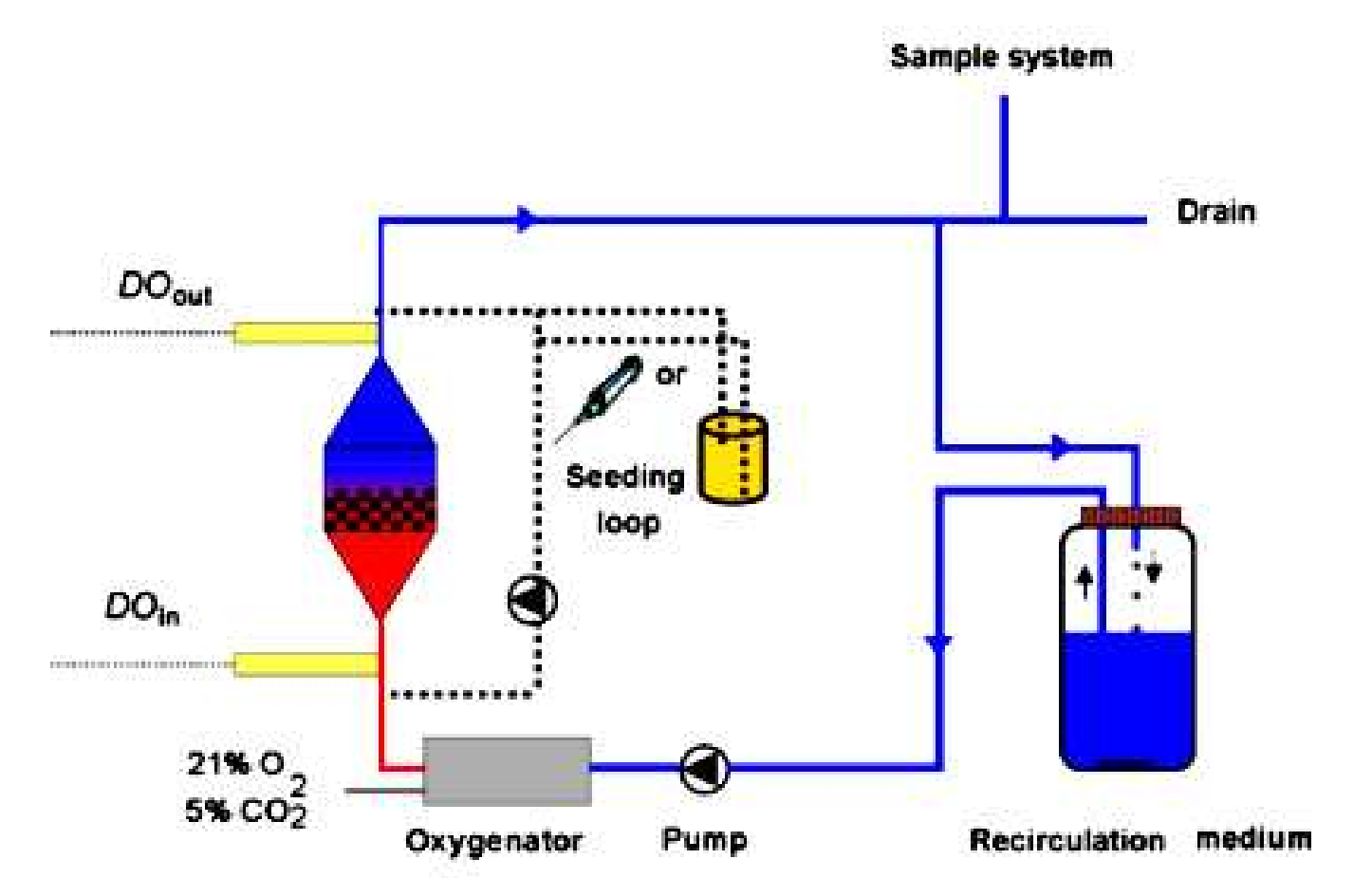
J Tissue Eng Regen Med 2010; **4**: 12–24.

- Human bone marrow stromal cells (hBMSCs) of eight donors dynamically seeded and proliferated in a perfusion bioreactor system in clinically relevant volumes (10 cm3) of macroporous biphasic calcium phosphate scaffolds
- methylene blue staining to follow cells
- MTT staining → viability of the present cells. After 20 days of cultivation: layer of cells differentiated towards the osteogenic lineage (ALP, BMP2, Id1, Id2, Smad6, collagen type I, osteocalcin, osteonectin and S100A4)
- bone-forming potential after implantation in nude mice

Micro CT images of the packed scaffold bed inside the bioreactor



The bioreactor system



Subcutaneous implantation of hybrid constructs

Run #	Cultivation time in vitro (days)	Number of cultured hybrid constructs implanted per mouse	Number of mice used	Total number of implanted cultured hybrid constructs
1-8	30	6	2	12
9	40	6	6	36
10-15	7	3	10	30

- Scaffolds acellulari (con GF)
- Scaffolds cellularizzati (limite # cells)