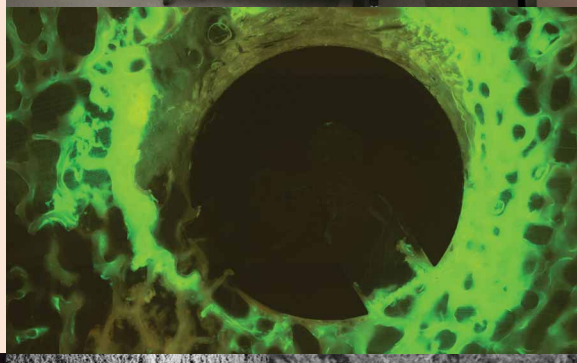
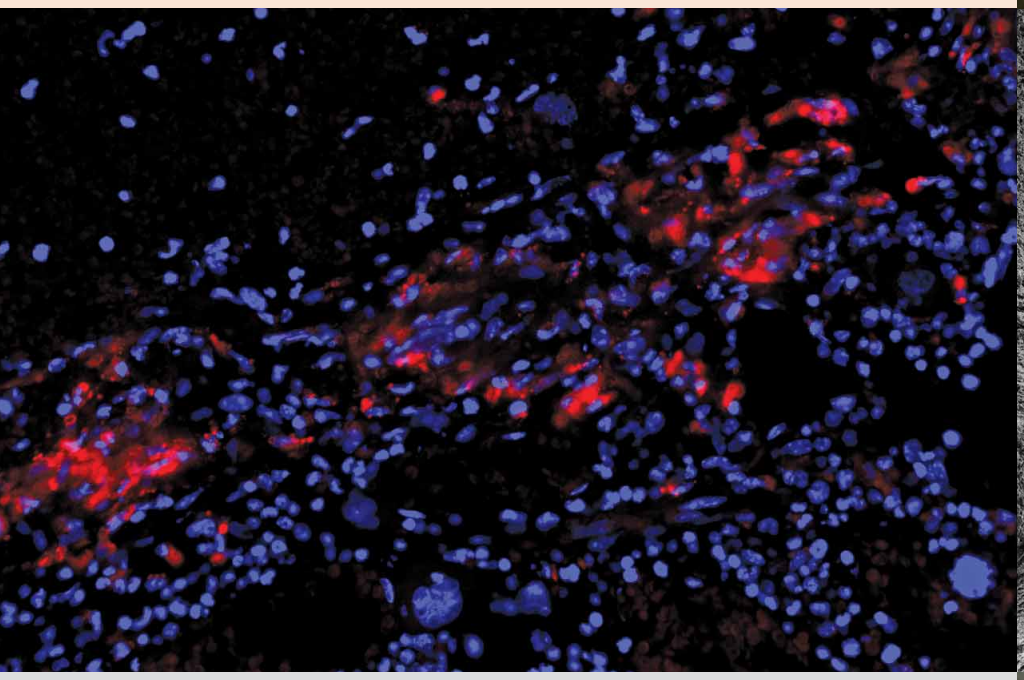
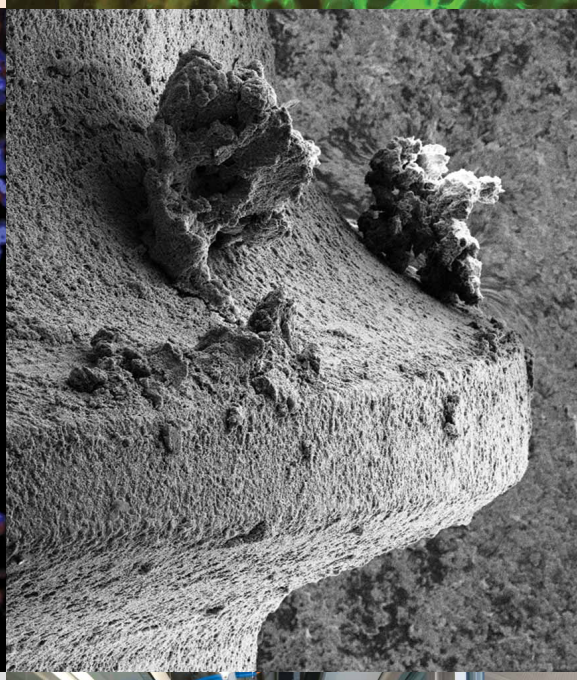
The Competence Center for Applied Biotechnology and Molecular Medicine







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From bench to bedside and back again

The Center for Applied Biotechnology and Molecular Medicine (CABMM)

The “Center for Applied Biotechnology and Molecular Medicine (CABMM)” is an official competence center of the University of Zurich with the objective to create a stimulating environment for interdisciplinary and translational research in order to promote scientific exchange and collaborations between basic and clinical researchers.

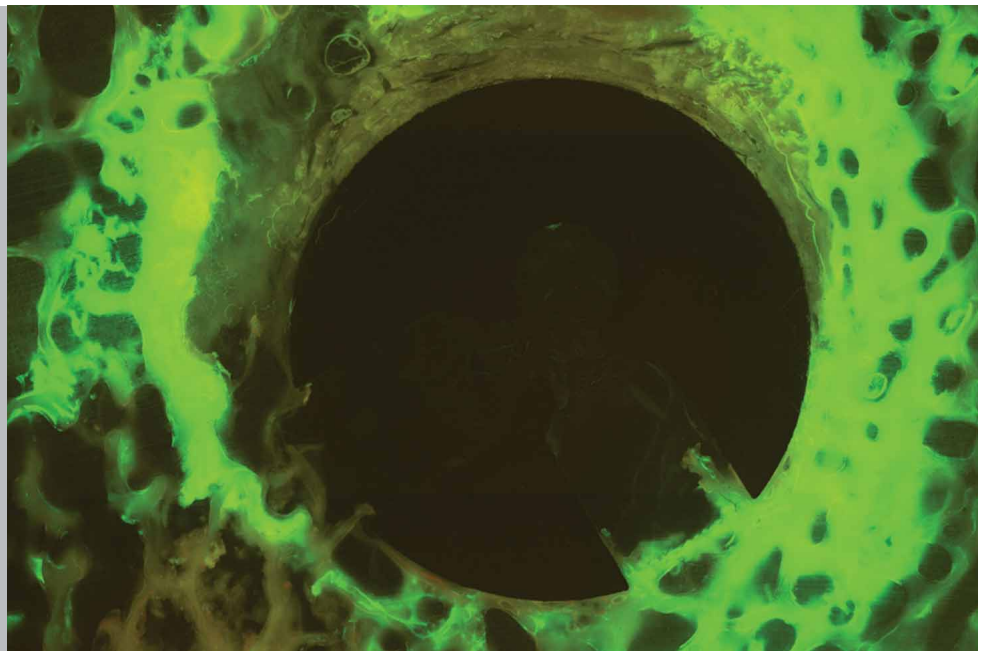
The CABMM shows a unique structure, combining (i) a network of existing research groups interested in scientific exchange and collaboration on interdisciplinary and translational research projects and (ii) a platform for collaborative research, where basic scientists, clinicians and veterinarians work shoulder to shoulder for the purpose of developing novel therapeutic approaches for the treatment of dysfunctional and diseased tissue.

Thereby, unlike other research centers, the CABMM is not focusing on one particular medical field, but on translational and interdisciplinary aspects. Thus, range and diversity or research being conducted within the CABMM is broad, but all research follows one aim: to facilitate the development of new treatment regimes by building a bridge between basic and clinical researchers.

Prof. Dr. Brigitte von Rechenberg
Center for Applied Biotechnology and Molecular Medicine (CABMM)
University of Zurich

tel +41 44 635 8410
bvonrechenberg@vetclinics.uzh.ch
www.cabmm.uzh.ch

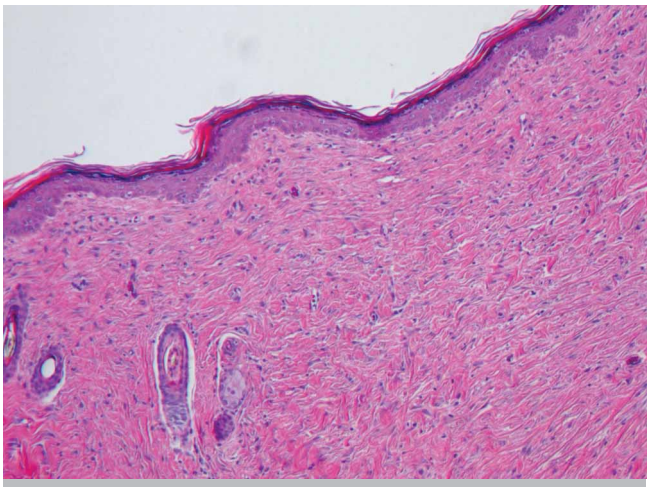
From bench to bedside and back



Bone apposition close to a ligament reconstruction with pressfit technique at three months after surgery. Green fluorescence shows the newly formed bone around the implant. The ligament is visible too close to the anchor

'The CABMM is the only network at a university in Europe that offers solutions for regulatory affairs under one roof.'

Translational research is part of the new framework programme Horizon 2020 from the European Union. There are not many centres that, independently from grant projects, have a translational research network set-up at their own universities, where basic scientists, clinicians, material scientists and industrial partners routinely work together to bring novel solutions and innovations in the field of biotechnology and molecular medicine to the medical market. The CABMM is such a network and an official competence centre at the University of Zürich (UZH), Switzerland.



Wound healing with biotechnological product after 28 days. Experiment in rats, note fully reconstituted epithelium and regular arrangement of collagen fibres in the dermis after such a short time period. Reduced scar tissue formation due to internal up-regulation of TGF-beta3

The location in Zürich is ideal for this network, where leading research institutions at the medical faculty and Vetsuisse Faculty of the UZH, as well as the Federal Institute of Technology (ETHZ), are within walking distance and where scientific exchange is facilitated by close and daily interactions on all levels. All three schools belong to the leading institutions in Europe and again being unique, there is no other location in Europe (where the expertise of the medical and veterinary school with facilities for preclinical studies and researchers from the technology side at the ETHZ) that can encounter this daily exchange. An ultramodern and vast infrastructure at all institutions guarantees access to the most modern technologies in the field of biotechnology.

Unique approach

Normally, research networks centre on a research topic. However, the CABMM has a different approach, making it unique, since translation in applied biotechnology and molecular medicine is not centred on a research topic, but includes many different areas in the medical, veterinary and materials field that finally make translation possible. Four major fields are represented in the CABMM: experimental medicine and surgery; molecular medicine; regenerative medicine; and applied biotechnology.

Regulatory affairs are cornerstones in translational medicine for bringing novel solutions to the patient and to the market. The CABMM is the only network at a university in Europe that offers solutions for regulatory affairs under one roof. Accreditations for good manufacturing practice (GMP), good clinical practice (GCP) and good laboratory practice (GLP) that are required for the registration of novel medical products at the Food and Drug Administration (FDA) are available at the CABMM, and make it possible to keep translation from the very beginning of an innovation in focus, and also get there effectively for industrial partners.

Lessons from the womb

Professors Zenobi-Wong (ETHZ) and Laurent-Applegate (CHUV/UNIL) describe biologically-driven mechanisms with bioactive scaffolds combined with clinical grade chondroprogenitors to achieve scar-free tissue regeneration

The Centre for Applied Biotechnology and Molecular Medicine (CABMM) at the University of Zürich has a mission to promote collaborative research projects in the fields of regenerative medicine, experimental medicine and surgery, applied biotechnology, and molecular medicine. Under this umbrella, CHUV/UNIL Professor Lee Ann Laurent-Applegate and ETH Zürich Professor Marcy Zenobi-Wong have joined forces to address the age-old problem of cartilage regeneration.

Using seed money provided by the CABMM, Professors Laurent-Applegate and Zenobi-Wong are exploring how novel biomaterials could be used to deliver chondroprogenitor cells to cartilage defects, with the ultimate aim of recapitulating the formation of hyaline cartilage which take place during development.

Scar-free tissue repair

The majority of adult tissues subjected to injury, inflammation, and pathogens will react by forming scar tissue. One notable exception to this rule is seen with foetal tissues which can repair injuries in a scar-free manner. What it is exactly that enables a foetus to heal without fibrosis is not fully understood, but likely involves a reduced inflammatory response to injury, an ECM environment which is high in hyaluronic acid (HA), and also the superior (re)generation potential of foetal progenitor cells themselves.

Cell choice and cell banking

The Laurent-Applegate Laboratory (www.chuv.ch/cpr; cbt.epfl.ch; and www.cabmm.uzh.ch) has developed a transplantation programme for foetal progenitor cells that has been registered with the Department of Public Health and SwissMedic since 1993. Research over the last 20 years, studying the fundamental mechanisms of foetal progenitor cells, has helped to identify their virtues in tissue repair and early clinical studies using these cells in burn and wound management, show promising results. Clinical grade cells for musculoskeletal tissues have been prepared for specific cell banking procedures to optimise tissue from one organ donation. Master and working cell banks (MCB and WCB) developed for muscle, bone, cartilage, tendon and skin tissue have been produced to date and skin progenitor cells have even been produced under full current Good Manufacturing Practices (cGMP).

One of the major challenges of assuring increasing patients benefitting from tissue engineering in the future is the optimisation of the choice of cell type and their isolation and proliferation. A major aspect contributing to the facility of using foetal progenitor cells for tissue repair is their extensive and rapid expansion possibilities (cGMP within 14 days) with no additional growth factors or feeder layers. It requires only one organ donation (1-2cm^2 of tissue) to create enough frozen cells to produce a bank of cells capable of making millions of bioengineered constructs. For clinical application, all processes need to be easily adapted to current Good Manufacturing Practices (cGMP).

Chondroprogenitor cells

Human foetal chondroprogenitors form homogeneous cell populations that have remarkable expansion potential with a steady proliferative potential averaging three population doublings

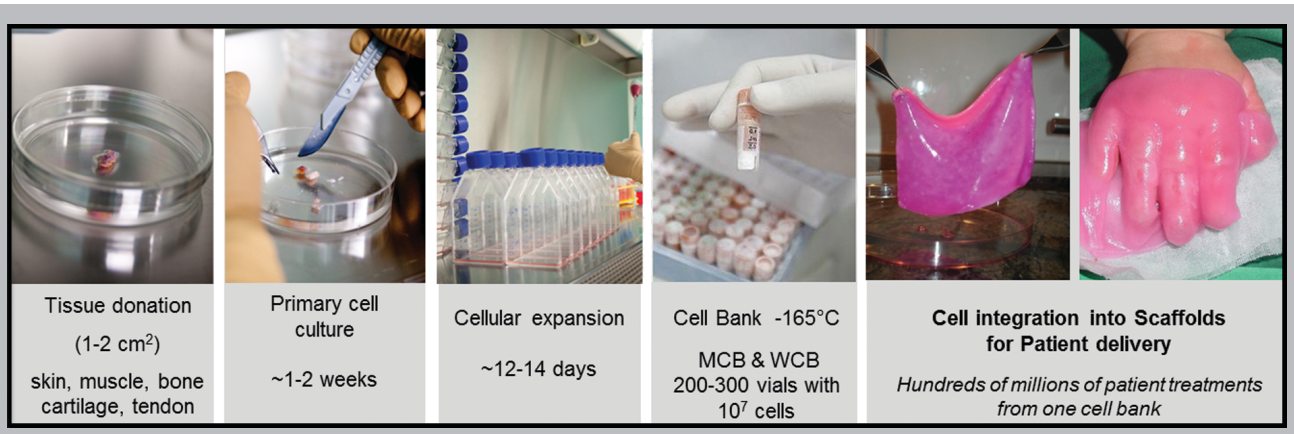


Fig. 1 Foetal progenitor cell banks can be produced from 1-2cm^2 of skin, muscle, bone or cartilage (14 week gestation organ donation) which can adapt to cell culture and storage under cGMP conditions to rapidly create master and working cell banks (MCB & WCB, ~12-14 days production run), that are stable (liquid nitrogen standardised freezing) with high live cell recovery (~98%), and enough cells to seed biocompatible scaffolds and prepare hundreds of millions of patient treatments from one cell bank

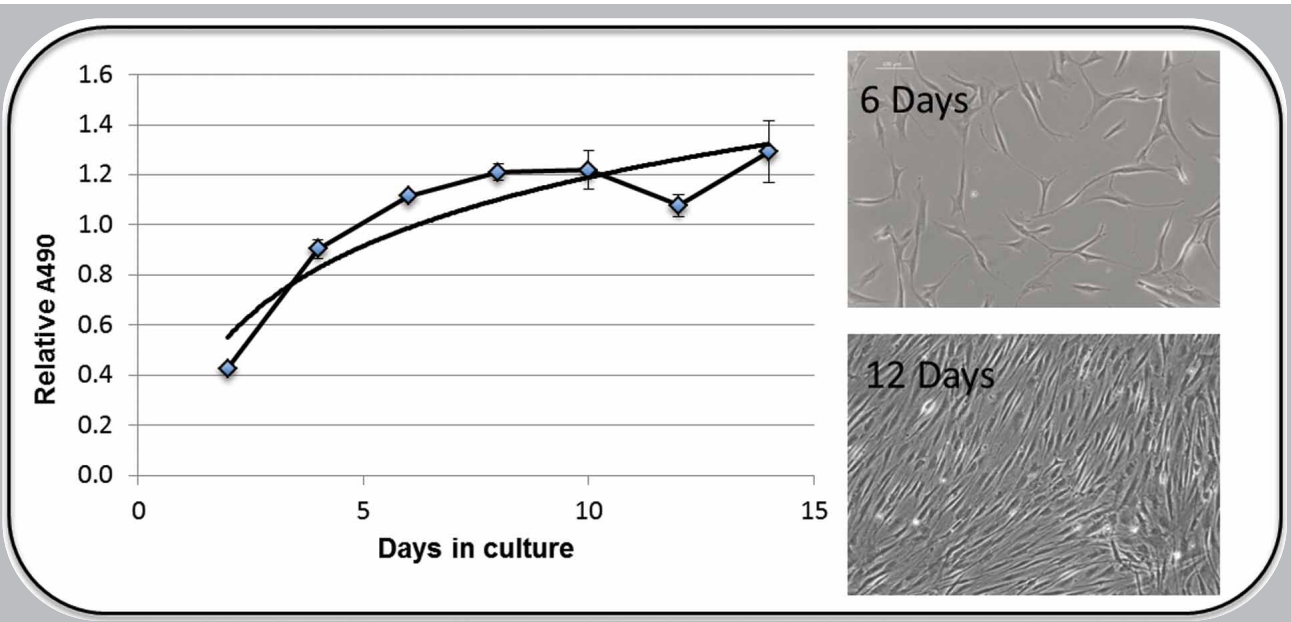


Fig. 2 Chondroprogenitor proliferation kinetics and morphology using CellTiter96 aqueous one solution cell proliferation assay (Promega, Fitchburg, WI, USA). Proliferation kinetics during a time course from 2-14 days were assessed using the Wallac VICTOR2™ 1420 multi-label plate reader (PerkinElmer, Waltham, MA, USA) with 590nm absorbance values (correlating with the amount of metabolically active cells)

over eight days (Fig. 2). Because of this remarkable expansion potential, master and working cell banks can be easily derived from the one original tissue sample. MCB and WCB can be optimised to obtain a maximum quantity of ready-to-use clinical-grade cells. As the stability of these cells has been shown to be at least to passage 13 under specific growth conditions, it would be acceptable to use the cells easily up to passage 6-8 (Fig. 3). General characteristics of these cells have shown, with surface marker analysis, no detectable contaminating subpopulations or population enrichment during prolonged culture periods. When cells are grown in micro-tissues, these cells are capable of depositing glycosaminoglycans, producing aggrecan, collagen I and collagen II after low and high population doublings indicating a stable spontaneous chondrogenic potential. De-differentiation of these cells was restricted and only observed by placing these cells in severe osteogenic-inducing conditions (observed by von Kossa staining of calcified matrix, with a notable collagen X, MMP13 and ADAMTS5 down-regulation) or adipogenic-inducing conditions (evidenced by cytoplasmic lipid accumulation detectable by Oil Red O staining). These findings highlight the reliability, stability and responsiveness of foetal chondroprogenitor cells over prolonged culture, making them ideal candidates in defining novel strategies for cartilage regeneration.

Human foetal chondroprogenitors therefore present distinct advantages due to their tissue-specific origin and capacity to adapt to rapidly changing environmental factors during the course of natural tissue growth, differentiation and development. Importantly, these cells also maintain a commitment to their differentiation programme with only mild plasticity, and therefore could allow them to modulate their responses to varying micro-environments and adapt well to scaffolds.

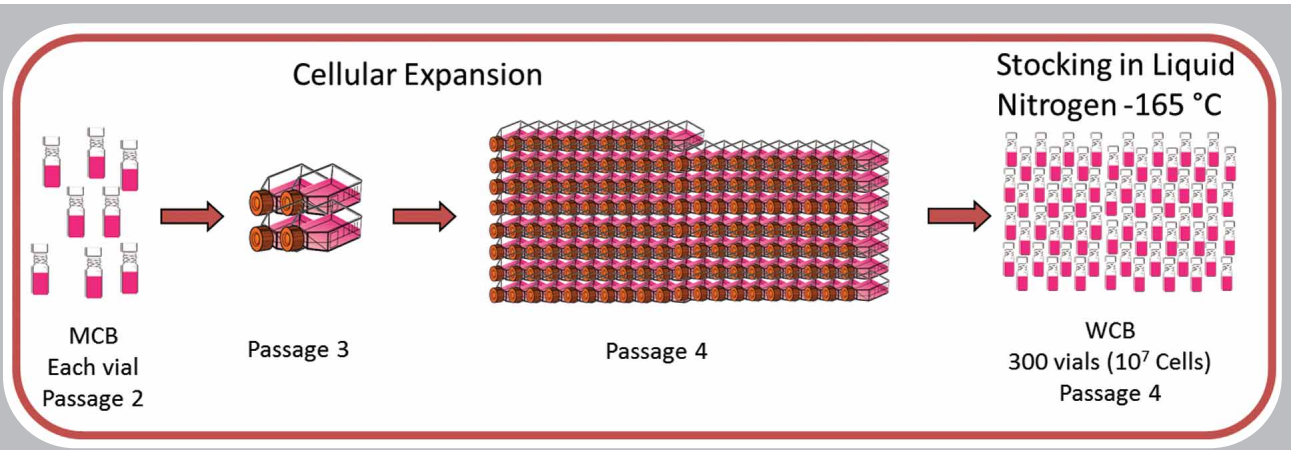


Fig. 3 Schematic for the maximisation of master cell banks (MCB) in developing working cell banks. Each of the frozen vials of cells that have been developed for the MCB can be expanded to make working cell banks (WCB) with high numbers of frozen ready-for-use clinical-grade cells at low passage (Passage 4). Each of the WCB vials can also be expanded in the same pathway to make a secondary WCB (Passage 6)

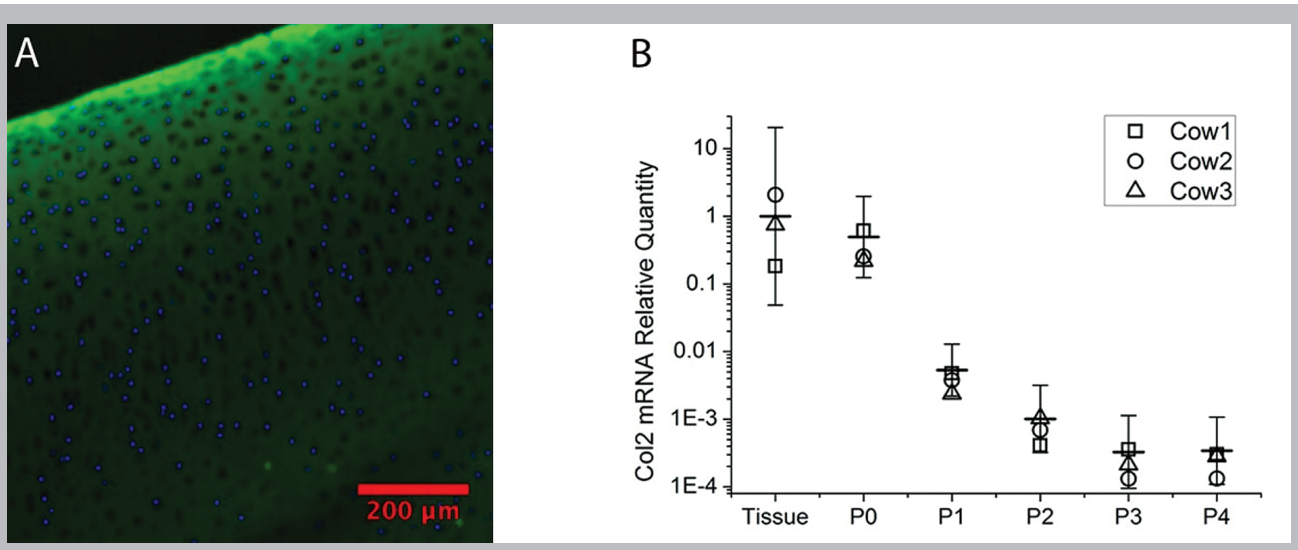


Fig. 4 (A) Bovine articular cartilage stained with antibody against collagen type 2 (green) and DAPI nuclear stain (blue). From Queralt Vallmajo-Martin. (B) Expression of cartilage phenotypic markers like collagen type 2 are strongly depressed with increasing passaging on 2D substrates[1]

Scaffolds and biomechanics

Equally important is the delivery system of chosen cells and their interaction with scaffolds or hydrogels. Physical characteristics of scaffolds such as porosity and mechanical stability are important for withstanding cell contraction forces, and assuring a homogenous distribution of cells throughout the scaffold. Long term collaboration with Professor Dominique Pioletti of EPFL, who leads the Laboratory of Biomechanics in Orthopedics (LBO), has addressed these essential biomechanical criteria for tissue engineering.

Our combined laboratories at the CHUV/EPFL have an extensive technical platform for characterisation and biocompatibility of human cells which helps to provide necessary criteria for defining cell types and associated delivery systems that are adapted for clinical applications.

"In tissue engineering, the cellular choice is of utmost importance in developing clinical applications. Overall stability, safety and capacity for biocompatibility with materials are determining factors for success". Professor Lee Ann Laurent-Applegate

Clinical problem and novel treatments

At least since the time of Hunter (1743) it has been noted that cartilage is a tissue with very low self-regeneration potential. In the absence of blood vessels there is no built-in method for progenitor cells to populate the site of the defect. The two most common treatments for cartilage repair are both designed to do this. In microfracture, the subchondral plate is mechanically punctured, allowing stromal cells from the underlying bone marrow and blood products to populate the defect in the form of a clot. In autologous chondrocyte implantation (ACI), passaged autologous chondrocytes are injected into the lesion through a syringe or via a matrix scaffold. More recent developments of marrow stimulation techniques involve a mix of bone marrow aspirate and autologous platelet-rich plasma (PRP) to fill the defect. Despite these improvements, the regeneration of the cartilage lesion is a mix of hyaline and fibrous cartilage which does not lead to full regeneration of the organ. A reliable treatment for cartilage lesions which allows the patients to return to full activity in a pain-free manner is still lacking.

Biomimetic material science

The Zenobi-Wong Laboratory (www.cartilage.ethz.ch; and www.cabmm.uzh.ch) develops biomaterial scaffolds to control the fate of the resident cells. Articular chondrocytes produce an extracellular matrix which is dominated by the synthesis of type 2 collagen (Fig. 4, left). One of the biggest problems with the clinical use of autologous chondrocytes to treat cartilage defects is their de-differentiation upon expansion on 2D tissue culture plastic (Fig. 4, right). Certain hydrogel materials, such as highly sulfated ones, allow chondrocytes and progenitor cells to proliferate within a 3D environment without the precipitous loss of collagen 2 expression.

Hyaluronic acid chemistry

One of the most common three-dimensional culture systems for chondrocytes makes use of the marine polysaccharide alginate. As seen in Fig. 5, alginate causes chondrocytes to acquire a rounded morphology similar to their *in vivo* morphology. Alginate however is a chemically inert material which in the unmodified state cells cannot interact with. Chondrocytes on the other hand have CD44 receptors which allow them to interact with hyaluronic acid (HA). HA-based hydrogels in addition recreate a foetal-like, anti-inflammatory environment for regeneration to take place. HA hydrogels for example can biologically

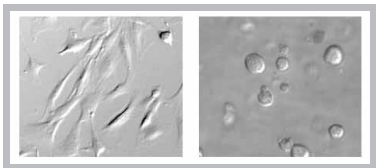


Fig. 5 Left: Chondroprogenitors cultured on 2D tissue culture polystyrene attain a spread proliferative, morphology. Right: In alginate hydrogel, the cells become round and slows their proliferation

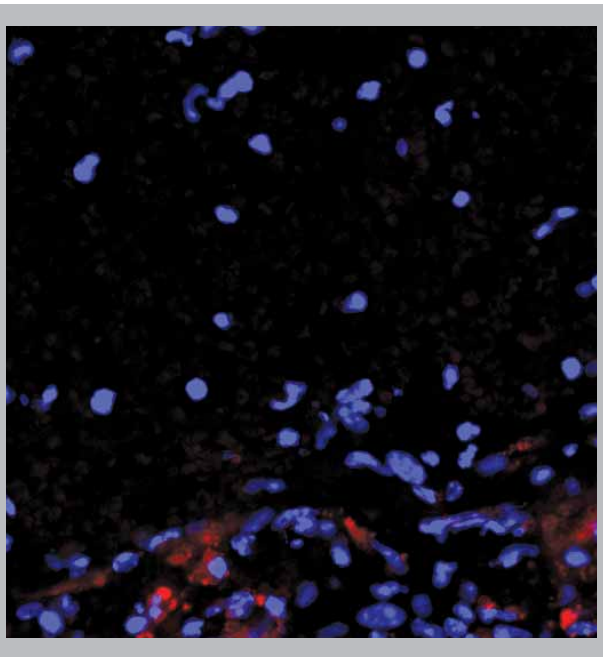


Fig. 6 (A) Hyaluronic acid is a linear carbohydrate consisting of disaccharide of D-glucuronic acid and D-N-acetylglucosamine. The hydroxyl groups can be substituted by acrylates and sulfates, enhancing its cross-linking properties and biofunctionality. (B) A stiffness range from 1-80kPa can be made by tailoring the macromere content of the gels. These materials were characterised by Ece Oztürk and synthesised in collaboration with Innovent e.V., Jena, Germany, Drs Jana Becher and Matthias Schnabelrauch

'Firstly, as already differentiated cells, they are potentially safer than more multi-potent cells which can differentiate along unanticipated pathways. The cells are less prone to de-differentiation during 2D expansion and are less dependent on the proper growth factor cocktails.'

and physically keep large amounts of invading inflammatory cells out of the lesion. HA can be cross-linked into hydrogels using a number of cell compatible cross-linking chemistries including free radical polymerisation, Michael addition reactions and enzymatic cross-linking. In Fig. 6 we show two possible modifications of HA, namely addition of acrylate and sulfate groups. Here it is possible to create a wide range of stiffness of materials allowing us to study how stiffness and charge affect the proliferation of the encapsulated cells.

'"The fate of the chondrocyte is exquisitely tuned to their 3D environment. Engineering 'biomimetic' materials can powerfully prime cells for regenerative medicine applications". Professor Marcy Zenobi-Wong '

Conclusions and outlook

The cell choice of foetal progenitor cells is, in many aspects, ideal for regeneration applications. Firstly, as already differentiated cells, they are potentially safer than more multi-potent cells which can differentiate along unanticipated pathways. The cells are less prone to de-differentiation during 2D expansion and are less dependent on the proper growth factor cocktails. Their rapid expansion allows for cell banking easily under full cGMP compliance, and stability has been shown to be exceptional compared to other cell types such as stem cells from bone marrow and adipose tissue. Finally, foetal progenitor cells have an immune-protected status that would allow allogeneic transplantation, something which would only be possible with adult cells when immunosuppressive drugs were used. Rapid developments in the field of biomimetic materials provide a second, powerful arm to direct the differentiation/proliferation balance of the cells. Combining these cells with materials which mimic the foetal environment could offer the possibility of achieving true scar-free regeneration for a number of tissue injuries and diseases and also in stabilising permanent medical devices by consolidating the tissue-implant interface.

[1] Mhanna R, Kashap A, Palazzolo G, Vallmajo-Martin Q, Becher J, Moller S, *et al.* Chondrocyte Culture in 3D Alginate Sulfate Hydrogels Promotes Proliferation While Maintaining Expression of Chondrogenic Markers. *Tissue Eng Part A* 2013.

Academic history

Both Profs Zenobi-Wong and Laurent-Applegate are American citizens who came to work in Switzerland initially because of their Swiss-born husbands.

Marcy Zenobi-Wong has a PhD from Stanford University in mechanical engineering. She was a postdoc and then group leader at the M. E. Müller Institute for Biomechanics at the University of Bern. Her primary focus there was mechanobiology of cartilage and tissue mechanics. In 2003 she was called to ETH Zürich as a senior scientist in tissue engineering and to establish a master's programme in biomedical engineering which she directed until 2011. In 2012, she was elected assistant professor of cartilage engineering and regeneration. Her research group has focuses on tissue engineering, biofabrication, additive manufacturing, drug delivery, and biomaterial control of cell fate.

Lee Ann Laurent-Applegate acquired her BS at South Dakota State University, and PhD at the University of New Mexico with postdoctoral appointments at Lovelace Medical Center, M.D. Anderson Cancer Center and Baylor College of Medicine. She moved to Switzerland for an International Fellowship for Cancer Research Award (IARC) in 1989 and is the Director of the Regenerative Therapy Unit in the Department of Musculoskeletal Medicine at the University Hospital (CHUV) in Lausanne, Switzerland. She has developed cellular therapies for different clinical applications since 1993 using progenitor cells from musculoskeletal tissues and particularly for the treatment of burn patients. She is responsible for the Department of Musculoskeletal Medicine Biobank and the Transplantation Program for Foetal Progenitor Cells.

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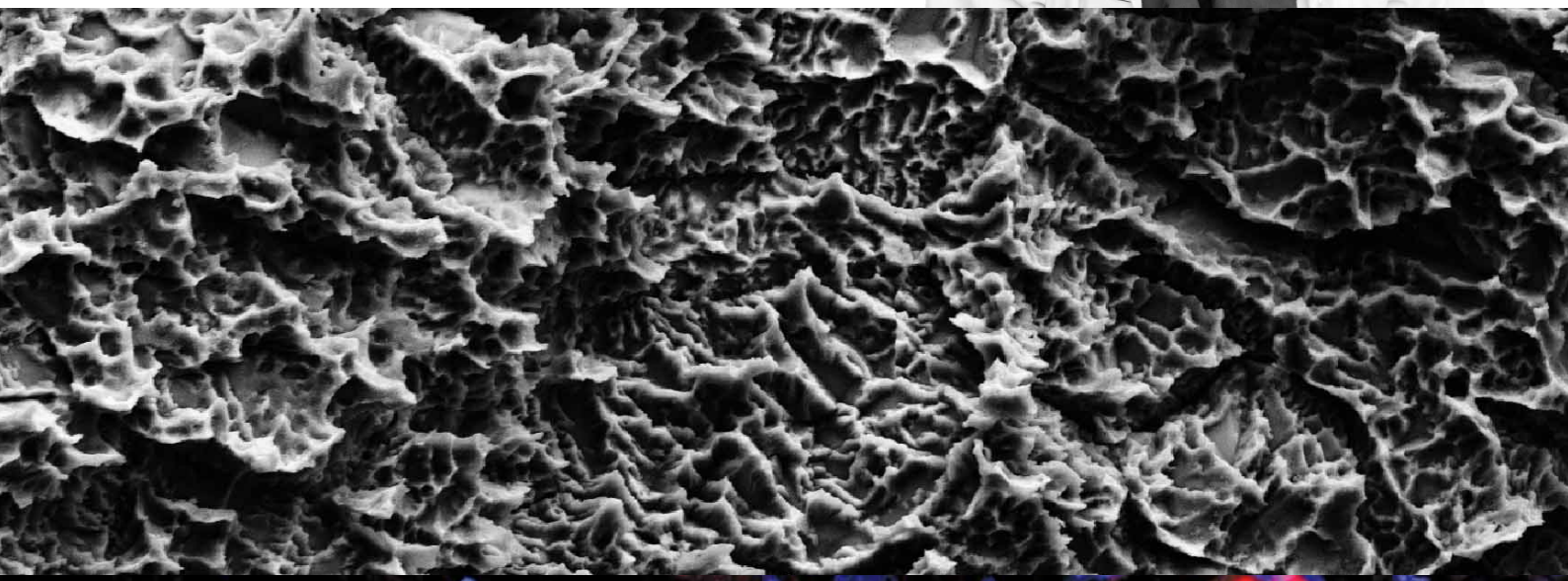

Prof Dr Brigitte von Rechenberg
Chair
Competence Center for Applied Biology & Molecular Medicine

tel: +41 446 358 410

bvonrechenberg@vetclinics.uzh.ch