Comparative Cartilage Biology

24th - 26th June 2019

Banyuls-sur-mer, France

PROGRAMME

Welcome from the organizers

Welcome to Banyuls and thanks for your help in making this conference a reality!

CCB was designed to take a uniquely broad approach to cartilage rarely explored in skeletal biology conferences, combining researchers with expertise in developmental genetics, biomaterials & biomedicine, and evolution of cartilage. We organized the meeting in response to the recent increase in groups recognizing the need for wider perspectives in skeletal biology and the potential of non-mammal systems as models. By bringing together diverse cartilage research, we hope the meeting will help to integrate the full community of cartilage biologists, framing common ground and potential axes of cooperation, accelerating research innovation and community-building.

We are excited by the range of topics in the program and we hope you will be too! To build wider concepts in cartilage biology, we have included work on cartilage structure and mechanics, but also paid particular attention to work on less studied cartilage types, on evolutionary perspectives of skeletal diversity and on less traditional models for skeletal biology (e.g. sharks and rays), and how these can enhance modern medical understandings of cartilage. Please take advantage of the integrative and intimate meeting format to approach researchers with whom you might not otherwise cross paths, to ask the oddest most cartilage-y questions you can think of, and to make new friends.

We are excited to share this meeting with you, thanks for coming!

Mason, Mélanie, Júlia & Fidji

Follow the conference on Twitter! #ccb_banyuls




Conference information

- The workshop will take place at the Observatoire Ocanologique de Banyuls-sur-mer, Avenue Pierre Fabre, 66650, Banyuls-sur-mer.
- Speeches will take place in amphitheater of **building B** (2nd floor).
- Coffee breaks and posters will be in meeting room of **building A** (3rd floor).
- Lunch will be served at the restaurant of the **accommodation center**.
- An internet access will be provided to the attendees in the conference and accommodation buildings.

Detailed program

Monday, 24 June

Time	Event	Speaker
9:00 - 9:15	Introduction to the workshop	Mason DEAN
9:15 - 10:00	**Plenary 1** - Early cartilage evolution	Philippe JANVIER
10:00 - 10:30	*COFFEE BREAK*	
10:30 - 11:45	**Morning session**	Chair : Sidney OMELON
10:30 - 10:45	Vertebrate cartilage diversity revealed by iodine staining and propagation phase-contrast synchrotron microtomography	Sophie SANCHEZ
10:45 - 11:00	A peculiar vertebrate skeleton the cartilaginous endoskeleton in sharks and rays	Ronald SEIDEL
11:00 - 11:15	Tesserae in shark cartilage: Evidence of developmental patterning?	John MAISEY
11:15 - 11:30	The cartilaginous skeleton of Chimaera (Chondrichthyes) is tessellated	Júlia CHAUMEL
11:30 - 11:45	An unusual mineralized tissue in *Astraspis*	Damien GERMAIN
12:00 - 14:00	*LUNCH*	
14:00 - 14:45	**Afternoon session**	Chair : Nicolas GOUDEMAND
14:00 - 14:15	The interplay between collagen organisation and biomineralisation in the spine	Eve NEBBIOLO
14:15 - 14:30	The evolutionary origins of tessellated calcified cartilage in Chondrichthyans	Alan PRADEL
14:30 - 14:45	Skeletal development and axial regionalization of *Centroscyllium fabricii* (Selachii)	Margot ANGIBAUD
14:45 - 15:15	*COFFEE BREAK*	
15:15 - 16:00	**Afternoon session**	Chair : Ronald SEIDEL
15:15 - 15:30	Mineralisation in the synarcual of the elephant shark (*Callorhinchus milii*)	Jacob PEARS
15:30 - 15:45	Histological description of cell types involved in the chondrichthyan skeletogenesis	Fidji BERIO
15:45 - 16:00	Assessment of the 3D microstructure of cartilage using high resolution X-ray tomography	Julien LESSEUR
16:00 - 16:30	*BREAK*	
16:30 - 18:00	**Round table** - How to observe cartilage mineralization?	

Tuesday, 25 June

Time	Event	Speaker
9:00 - 9:30	Introduction to the Banyuls-sur-mer facility	Vincent LAUDET
9:30 - 10:15	**Plenary 2** - Development of the cartilaginous skeleton: tracing the evolutionary history of chondrogenesis	Martin COHN
10:15 - 10:45	*COFFEE BREAK*	
10:45 - 12:00	**Morning session**	Chair : Martin COHN
10:45 - 11:00	Mechanobiology of the chondrocyte primary cilium	Sue McGLASHAN
11:00 - 11:15	Defining limits of plasticity; can joint cartilage recover following embryonic paralysis?	Rebecca ROLFE
11:15 - 11:30	Cartilage canals are common in mammals, birds, fish however, their role is not yet understood	Michael BLUMER
11:30 - 11:45	Strong biomechanical fixation of osteochondral scaffold enhanced cartilage healthy formation	Chaozong LIU
11:45 - 12:00	Exploring molecular fingerprints of chondrichthyan skeletal cells to reaffirm conservation of bone in vertebrates: do skates have osteoblasts?	Oghenevwogaga ATAKE
12:00 - 14:00	*LUNCH*	
14:00 - 14:45	**Afternoon session**	Chair : Eckhard WITTEN
14:00 - 14:15	Biomimetic, biofunctionalised polymer implants to promote *in situ* repair of traumatic and early osteoarthritic cartilage defects	Caroline TAYLOR
14:15 - 14:30	Skeletal mineralisation in association with type X collagen expression is an ancestral feature for jawed vertebrates	Mélanie DEBIAIS-THIBAUD
14:30 - 14:45	The genetic actors of a potential estrogen-signaling regulation of cartilage mineralization in the catshark	Nicolas LEURS
14:45 - 15:15	*COFFEE BREAK*	
15:15 - 16:00	**Afternoon session**	Chair : Sophie SANCHEZ
15:15 - 15:30	Cartilage development of the dual origin stapes	Nahdrah ALI
15:30 - 15:45	Study of cartilage regeneration in zebrafish	Dora SAPEDE
15:45 - 16:00	Pelvic fin chondrogenesis in the catshark *Scyliorhinus canicula* embryo: a test for sex hormone regulation	Camille MARTINAND-MARI
16:00 - 16:30	*BREAK*	
16:30 - 18:00	**Round table** - How to sample, experiment and compare?	
19:00	**Social event** - Evening wine tasting	

Wednesday, 26 June

Time	Event	Speaker
9:15 - 10:00	**Plenary 3** - Too efficient to evolve? Similarities between the chemistry of cartilage calcification in elasmobranch fish and mice	Sidney OMELON
10:00 - 10:30	*COFFEE BREAK*	
10:30 - 11:45	**Morning session**	Chair : Brian EAMES
10:30 - 10:45	Tooth-cartilage relationships in vertebrates	Ann HUYSSEUNE
10:45 - 11:00	Ocean acidification and warming affect skeletal mineralization in a marine fish	Valentina DI SANTO
11:00 - 11:15	Mechanisms of jaw strengthening in the skates and rays (Batoidea; Chondrichthyes)	Zerina JOHANSON
11:15 - 11:30	Characterization of the jaw joint formation in zebrafish controlled by the evolutionary conserved cis-regulatory element	Tatjana HAITINA
11:30 - 11:45	Notochord and bone precursor cells as source for cartilage	Eckhard WITTEN
12:00 - 14:00	*LUNCH*	
14:00 - 14:45	**Afternoon session**	Chair : Valentina DI SANTO
14:00 - 14:15	Using 3D-bioprinted hybrid constructs to engineer mechanical and biological properties of articular cartilage	Brian EAMES
14:15 - 14:30	Testing jaw cartilages as a scaffolding constraint on the dental lamina in elasmobranchs	Admin CORDOBA de LEON
14:30 - 14:45	EmbryoMaker - a multiscale model uniting biological and mechanical aspects of hard tissue development	Roland ZIMM
14:45 - 15:00	Timing of jaw shape remodeling in sharks development to establish species-specific feeding mechanisms	Faviel LOPEZ-ROMERO
15:00 - 15:30	*COFFEE BREAK*	
15:30 - 17:00	**Afternoon session**	Chair : Sue McGLASHAN
15:30 - 17:00	**Round table** - Comparative Cartilage Biology - Moving forward!	
19:00	**Social event** - Conference farewell dinner @ Restaurant Le Miradou	

Contents

Abstracts

Cartilage development of the dual origin stapes

25 Jun
3:15pm

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When it comes to embryonic origin, most cartilages of the body are derived from mesoderm. In contrast, many facial cartilages are derived from neural crest cells, a migratory population of cells from the neuroectoderm. The middle ear of mammals is made of three small bones that begin as cartilage. The malleus and incus are neural crest derived but the third bone in the ear, the stapes, is of dual origin. Lineage tracing studies have shown that whilst most of the stapes is composed of neural crest tissue, a small ring around the base of the stapes is mesodermal in origin. We explore whether these two tissues have different developmental trajectories. We achieve this using transgenic reporter mice for neural crest (Wnt1cre) and mesoderm (Mesp1cre) derived tissues. The reporter proteins are combined with immunofluorescence against matrix proteins defining different cartilage maturation states (e.g. ColX) to ascertain whether the timing of cartilage development is different between two tissues that contribute to a single, unified structure. Preliminary data suggests the mesoderm develops faster than the neural crest component, indicating that developmental origin may influence the timing of differentiation.

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24 Jun
2:30pm

Skeletal development and axial regionalization of *Centroscyllium fabricii* (Selachii)

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For more than a century, the axial skeleton (vertebral column and ribs) has been recognized to have played an important structural role during vertebrate evolution. The vertebral column consists of serial vertebrae that are cartilaginous, mineralized or ossified, and ligamentous elements around the notochord. Anatomical similarities among vertebral units define vertebral regions or domains (e.g., occipital, cervical, thoracic, lumbar, sacral, precaudal, caudal) along the anteroposterior axis. The presence of five axial regions (i.e., cervical, thoracic, lumbar, sacral, caudal) was first recognized in tetrapods, and subsequently reported in basal osteichthyans but knowledge on chondrichthyans is needed. We cleared and stained 110 specimens of Black dogfish, *Centroscyllium fabricii*, measuring from 2.9 to 28.2 cm in total length, to characterize the chondrification and mineralization of their vertebral column. For the first time, the complete development of cartilaginous and mineralized structures of the vertebral column of a shark is described. Qualitative and quantitative morphological differences were found among vertebral segments along the body axis, and during ontogeny, forming five distinct anatomical and developmental regions. Morphological abnormalities (e.g., fusion of hemal arches, addition of neural arch) are found in transitional zones delimiting each region; and primarily between the thoracic and sacral regions. Transitional zones seem to match the overlap areas found for *Hox* genes expression. The presence of five anatomical and developmental axial regions is most likely a gnathostome novelty than an osteichthyan synapomorphy.

Exploring molecular fingerprints of chondrichthyan skeletal cells to reaffirm conservation of bone in vertebrates: do skates have osteoblasts?

25 Jun
11:30am

OJ Atake[1], P Gómez-Picos[1], K Ovens[1], I McQuillan[1], BF Eames[1]

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Only vertebrates can make bone, and despite the fact that chondrichthyans (e.g., sharks, skates) clearly evolved from an ancestral bony vertebrate, the chondrichthyan skeleton is believed to lack bone. Multiple reports of mineralized bone-like tissues in tesserae, neural arches, and centra of sharks, however, challenge this belief. Recently, we reported the same bone-like features in mineralized tissues of skates, suggesting a novel shared trait among elasmobranchs. Relying upon histological staining, candidate gene expression, and cell morphology, these reports failed to classify chondrichthyan mineralized tissues conclusively as bone, due to largely overlapping characteristics of bone and mineralized cartilage. Just like other cell types, however, osteoblasts and chondrocytes express characteristic sets of genes, termed molecular fingerprints. Using laser capture microdissection coupled to RNA-sequencing, we identified molecular fingerprints of osteoblasts in bone and chondrocytes in mineralizing cartilage of mouse and chick. Osteoblasts in mouse and chick had similar gene expression profiles. Conversely, molecular fingerprints of osteoblasts and mineralizing chondrocytes were distinguishable in both of these terrestrial vertebrates. Expanding this approach to skeletal cells of aquatic vertebrates, such as gar and skate, we are testing the hypothesis that skate bone-like tissue is homologous to bone. Although osteoblasts of aquatic vertebrates, such as gar and zebrafish, express chondrogenic markers (e.g., col2), an unbiased comparative transcriptomic approach can discriminate more easily than candidate approaches. These studies can unravel homology between skeletal tissue types, and can help to rewrite current knowledge about bone in chondrichthyans.

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24 Jun
3:30pm

Histological description of cell types involved in the chondrichthyan skeletogenesis

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The chondrichthyan -sharks, batoids, chimeras- skeleton is made of various morphological structures that can be taxa-specific (synarcual, ribs) or broadly distributed among taxa (spines, claspers). These skeletal elements are made of an unmineralized core of cartilaginous extracellular matrix (CECM) that undergoes outer mineralizing processes during chondrichthyan growth. The mineralization type differs depending on the skeletal elements: the tesserae are made of prismatic and globular mineralizations and cover most of the skeletal elements (e. g. vertebral arches and spines, fin rays, cranium), whereas areolar mineralization covers the vertebral centra. The organization of the cell types involved in these various mineralization patterns remains, however, largely unknown. The layering of the CECM-secreting cells, as well as the CECM itself presumably also differ according to the skeletal elements. Here, we aim at describing the skeletal cellular and CECM organization in (i) different skeletal elements, (ii) and in various chondrichthyan species. For that, we made several histological stainings of vertebrae, jaw cartilages, and claspers of embryonic and mature sharks, skates and chimeras. We compared their tissue and cell type nature and spatial organization to infer evolutionary patterns on chondrichthyan skeletogenesis.

Cartilage canals are common in mammals, birds, fish however, their role is not yet understood

25 Jun
11:00am

M Blumer[1], H Fritsch[1], R Seidel[2], J Chaumel[3], Kady Lyons[4], M Dean[3]

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Mammalian and birds long bones develop from a cartilaginous model. Once an osseous collar has formed endochondral bone formation starts within the model, leading to the establishment of a primary and a secondary ossification center (SOC). A crucial event preceding the development of the SOC is the initial formation of cartilage canals in the epiphysis of long bones. For example, in developing long bones of chicken and mice, skeletal cartilage exhibits canals, which originate from the perichondrium and contain loose connective tissue and blood vessels. Their formation is stimulated by vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs), which disintegrate and resorb the cartilage matrix, leading to further invasion of the canals, excavation of the epiphysis and the establishment of a marrow cavity. During proceeding development, cells within the canals synthesize Runx2, alkaline phosphatase, periostin and type-I collagen all together typical features of bone forming cells. Type-I collagen, the major protein of bone, is laid down onto non-resorbed cartilage and initiates the formation of the SOC. Eventually, the entire epiphyseal cartilage is replaced by bone, and only the articular cartilage remains at the end of the long bones lacking canals. In contrast to the development of the skeleton described above, the endoskeleton of cartilaginous fish (rays and sharks) are largely made of cartilage and never turned into bone. Cartilage canals, however, are also found in their skeleton, originate from the perichondrium, contain blood vessels embedded in loose connective tissue and penetrate deep into the uncalcified cartilage matrix as well. We think that the canals are involved in cartilage nourishment, and the lack of bone formation in cartilaginous fish leads to their persistence within adult animal skeletons, hinting at the fact that bone formation depends on but is likely not the cause of the formation of cartilage canals in vertebrates.

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24 Jun
11:15am

The cartilaginous skeleton of Chimaera (Chondrichthyes) is tessellated

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An accepted uniting character of cartilaginous fishes (sharks, rays and chimaera) is the presence of mineralized tiles (tesserae) on the outside of the cartilage skeleton. This is contradictory since tesserae have never been demonstrated in modern chimaera and it is debated whether the skeleton mineralizes at all. Using materials and biological tissue characterization techniques we present, for the first time, a detailed characterization of the mineralized and tessellated cartilage in several skeletal elements (jaws, cranium and vertebral column) in three genera of chimaeroid fish. The mineralized tiles are irregular and not uniformly distributed, unlike most shark and ray tesserae, yet share several features with tesserae. The mineralized layer is peripheral in the unmineralized cartilage and seems to grow by periodic accretion of mineral at edges, forming laminated patterns of mineral density variation similar to those in shark and ray tesserae (e.g. in Liesegang lines, hypermineralized spokes). Chimaeroid mineralized cartilage, however, appears to lack the network of cell spaces that characterize tesserae, although we observe occasionally mineralized regions appearing to be infilled cell spaces. Significant is the apparent absence of the cell- and fiber-rich joints that link shark and ray tesserae, suggesting that cells and true intertesseral joints may be vital to the development of more geometric tessellations. Our data indicate that skeletal mineralization is more widespread and diverse in extant cartilaginous fishes than previously thought; developmental studies of chimaeroid mineralization are necessary to determine the mechanisms underlying skeletal patterning and their conservation across cartilaginous fishes.

Development of the cartilaginous skeleton: tracing the evolutionary history of chondrogenesis

25 Jun
9:30am

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A defining feature of vertebrates is a rigid endoskeleton, the primary components of which are cartilage and bone. Advances in the molecular genetics, paleobiology, and evolutionary developmental biology of vertebrate skeletogenesis have improved our understanding of the early evolution and development of the vertebrate skeleton. Insights have come from a variety of approaches, including genetic experiments in model organisms, comparative developmental analyses within a phylogenetic framework, human genetics, and highly detailed investigations of fossil vertebrates. In addition, comparative genomic studies have revealed that gene duplication has played an important role in the evolutionary diversification of skeletal tissues. Integration of these disparate lines of investigation is a challenge, but has the potential to shed new light onto the evolutionary history of cartilage. Among the findings that have emerged from interdisciplinary approaches is the discovery of an unexpectedly deep origin of the gene network that regulates chondrogenesis. This raises new questions about the origin of the chondrocyte, the number of times that cartilage has evolved in animals, the developmental genetic mechanisms of parallel evolution. In this talk, I will review recent progress in a number of these areas and will discuss some key gaps in our knowledge of skeletal evolution.

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26 Jun
2:30pm

Testing jaw cartilages as a scaffolding force on the dental lamina in elasmobranches

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Individuals of elasmobranch species can display an incredibly high diversity of tooth shapes that usually follow a continuous gradient from the symphyseal to commissural ends of their jaw. Several factors acting during tooth development determine the shape of teeth in mature specimens. Although the broad genetic machinery underlying the regulation of tooth shape in elasmobranches have been partially characterized before, the physical factors affecting this shape remain largely understudied. Therefore, in this study, we aim to test if the local curvature of the cartilaginous jaw elements can be taken as an explanatory variable for the proximo-distal tooth shape changes along the jaw of the lesser spotted catshark Scyliorhinus canicula. To this end, we extracted the virtual surfaces of the mineralized cartilage of the right jaw in 9 different individuals (4 males, 5 females). By means of topographic analyses on the cartilage surfaces, we will obtain the global and local curvature parameters of each jaw in these individuals. Additionally, we will perform virtual sections of the soft tissues in order to visualize the dental lamina conformation along the jaw and compare these results to the cartilage curvature parameters found in the previous step. With these data, we will be able to test the putative correlation between tooth shape variation along the jaw and the metric defined by our curvature parameters.

Skeletal mineralisation in association with type X collagen expression is an ancestral feature for jawed vertebrates

25 Jun
2:15pm

M Debiais-Thibaud[1], P Simion[2], S Vento[1], D Muñoz[3], S Marcellini[3], S Mazan[4], T Haitina[5]

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Endoskeletal mineralisation first evolved in early jawless vertebrates, but developmental genetics has focused on endochondral bone, which is a derived feature of bony vertebrates only. To better characterize the molecular bases of mineralising cell evolution, we studied type X collagen, encoded by the *Col10a1* gene. It is known to be involved in the process of endochondral ossification in bony fishes, but until now unknown in cartilaginous fishes. We show that holocephalans and elasmobranchs have respectively five and six tandemly duplicated *Col10a1* gene copies that display conserved genomic synteny with osteichthyan *Col10a1* genes. All *Col10a1* genes in the catshark *Scyliorhinus canicula* are expressed in mineralizing cells of teeth and scales, but only one duplicate is expressed in the endoskeletal (vertebral) mineralising tissues. Our findings demonstrate the ancestral association of *Col10a1* with mineralisation of both the odontode skeleton and the endoskeleton.

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26 Jun
10:45am

Ocean acidification and warming affect skeletal mineralization in a marine fish

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Ocean acidification and warming often decrease calcification rates of shell and exoskeleton of marine invertebrates. However, to date, there are no datasets on the combined effect of ocean pH and temperature on skeletal mineralization of water-breathing marine vertebrates, i.e. fishes. Here, the embryos of an oviparous marine fish, the little skate (*Leucoraja erinacea*), were developmentally acclimatized to current and increased temperature and CO2 conditions as expected by the year 2100 (15 and 20C, approx. 400 and 1100 atm, respectively), in a fully crossed experimental design. Using micro-computed tomography, hydroxyapatite density was estimated in the mineralized portion of the cartilage in jaws, crura, vertebrae, denticles and pectoral fins of juvenile skates. Mineralization increased as a consequence of high CO2 in the cartilage of crura and jaws, while temperature decreased mineralization in the pectoral fins. Mineralization affects stiffness and strength of skeletal elements linearly, with implications for feeding and locomotion performance and efficiency. This study shows a significant change in mineralization in the skeleton of a fish and that directional shifts in temperature and pH of the oceans have complex consequences on fish skeletal morphology.

Using 3D-bioprinted hybrid constructs to engineer mechanical and biological properties of articular cartilage

26 Jun
2:00pm

BF Eames[1], Z Izadifar[1], F You[1], A Olabamiji[1], X Chan[1]
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In Tissue engineering aims to provide a permanent solution for defects in articular cartilage (AC), such as those underlying osteoarthritis, by producing constructs that mimic the native tissue. However, AC is extremely complex, having four zones, each with different mechanical and biological properties. Our hybrid 3D printing approach overcomes the major limitations of current AC engineering strategies. We use two printing heads to juxtapose strands of biodegradable polymer (PCL), which provide mechanical strength, and strands of chondrocyte-impregnated hydrogel, which provide uniform distribution of chondrocytes with desired biological properties. We demonstrated that this hybrid bioprinting approach has good cell viability and cartilage production using two different cell lines: ATDC5 cells and primary chick chondrocytes. Printing layer-by-layer also permits us to mimic the four AC zones. For example, we have altered the geometry of PCL strands to mimic each zones mechanical properties. The interface of AC with underlying subchondral bone is critical for AC function, and a zone of calcified cartilage mediates this interaction. To mimic biological properties of the calcified zone of AC, we developed a technique to emulsify hydroxyapatite (the mineral in skeletal tissues) in hydrogel, and chondrocytes embedded in this new biomaterial were stimulated to secrete calcified cartilage. Finally, we are developing novel synchrotron techniques to monitor engineered AC constructs non-invasively both in vitro and in vivo.

Otlet: an online platform for sourcing biological samples

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Research teams collect more than 94 million biological samples annually, the majority of which are subsampled for targeted analysis. The remaining sample is then able to be repurposed for additional studies by collaborators around the world. However, the absence of a systematic way to source these unused samples results in wasted tissues, organisms and opportunities for research as scientists undertake redundant sampling regimes. As such, Otlet, a global online database, was set up to overcome the challenges of sourcing scientific research samples from colleagues. The platform allows the users to 1) upload a record of their unused samples, 2) search the database of existing samples from other users and request them directly from the contributor and, 3) post a request for samples onto a searchable community board. The platform facilitates communication between research teams across different locations, taxa and expertise to foster novel collaborations while accelerating scientific output. Otlets newly constructed platform is an important tool for biological scientists of all disciplines to efficiently communicate and source research material. Currently there are over 300 active researchers signed up and 16,000+ biological sample listings from 197 species (over 8,000 of these samples are from sharks and rays and the number of cartilage samples listed is increasing rapidly). Membership is freely available for scientific use by researchers from universities, government agencies, museums, private consulting and NGOs.

An unusual mineralized tissue in *Astraspis*

24 Jun
11:30am

D Germain[1] and A Lemierre[1]

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The dermoskeleton of the earliest vertebrates is well known but their endoskeleton is thought to have been largely cartilaginous until the Late Silurian. We confirm that the dermal plates of *Astraspis* are three-layered, with a superficial layer of enameloid and orthodentine, a middle layer of aspidin and a basal layer of lamellar acellular bone. This dermoskeleton is found in association with globular calcified cartilage, indicating the presence of a partially mineralized endoskeleton. In addition to the classical threelayered organization, some dermal plates exhibit alignments of chondrocyte-like lacunae, very similar to a pattern typical of chondroid metaplastic bone, previously unknown in early vertebrates. This discovery implies the presence of a proliferative cartilage, hitherto only known in Osteichthyans. This discovery indicates that a pattern similar to the first step of endochondral ossification was already present in the earliest vertebrates.

Characterization of the jaw joint formation in zebrafish controlled by the evolutionary conserved cis-regulatory element

26 Jun
11:15am

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The establishment of the articulated jaws was one of the major events that promoted the evolutionary transition from jawless to jawed vertebrates. Jaw joint enables the movement of the jaws and its development requires the establishment of the interzone between cartilage elements. Transcription factor *Nkx3.2* is expressed in the primary jaw joint of non-mammalian vertebrates where it plays a central role in inhibiting chondrocyte maturation and promoting joint formation. However little is known about the precise regulation of interzone forming cells by *Nkx3.2*. Here we present the identification and characterization of the evolutionary conserved cis-regulatory element of *Nkx3.2*, which labels the developing jaw joint cells in zebrafish *Danio rerio* larvae. We used bioinformatic pipeline to search for cis-regulatory elements in the proximity of *Nkx3.2* gene and have generated novel transgenic lines for in vivo functional characterization of identified sequences in the zebrafish. By applying live imaging of forming jaw joint cells with active cisregulatory element of Nkx3.2 we are able to follow the establishment of the jaw joint interzone and describe the morphology of the cells involved in this process. We are also able to distinguish between cartilage cells and other cell types involved in the interzone formation. Together our data contribute to the understanding the role of joint progenitor cells during the establishment of the primary jaw joint interzone.

Tooth-cartilage relationships in vertebrates

26 Jun
10:30am

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It is now widely accepted that oral teeth in jawed vertebrates are homologous to odontodes, or skin denticles. We have previously hypothesized that during evolution the competence of epithelia to form odontodes in the skin may have spread into the pharynx via different openings (the mouth, the spiraculum and the gill slits) to generate oral and pharyngeal teeth. This transfer of odontogenic competence does not depend on the presence of jaws and may thus even have preceded the origin of jaws. This is in line with the idea that the close developmental association between teeth and jaws may be a derived phenomenon that evolved later in jawed vertebrate phylogeny. Eventually however, as teeth came to pave the elements lining the buccopharyngeal cavity, they became associated with the viscerocranial cartilages of the endoskeleton and the bones of endo-and dermal skeleton. In this presentation, we will survey how tooth-cartilage relationships are established developmentally and morphologically, taking examples from extant chondrichthyans and osteichthyans. Tooth-cartilage interactions range from purely connective tissue connections in extant chondrichthyans, teeth attaching directly to perichondral bone, to tooth plates composed of attachment bone and fusing secondarily to perichondral bone and/or membrane bone apolamellae. In extreme cases, cartilage undergoes resorption to allow teeth to become settled within the confines of the cartilage element, as shown in cichlid pharyngeal jaws. Together, the examples demonstrate a large diversity in the association between cartilage and elements of the dermal skeleton.

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24 Jun
9:15am

Introduction to early cartilage evolution

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Current vertebrate phylogenies suggest that the earliest known fossil vertebrates, dated to about 535 Million years (Myr), only possessed an entirely cartilaginous endoskeleton which is retained in living cyclostomes, and that bone arose later, first in the dermal skeleton. All extinct jawless vertebrates that possess a bony dermoskeleton (ostracoderms), be it cellular or acellular, now appear as more closely related to jawed vertebrates (gnathostomes) than to cyclostomes. They are thus stem gnathostomes. Cartilage can sometimes be exceptionally preserved in these early jawless vertebrates, either when it was calcified *in vivo* or when it underwent *post-mortem*, bacterially-induced calcification. A spectacular case is the cyclostome-related, 370 Myr-old) *Euphanerops*, whose endoskeleton displays calcified chondrocyte nests that resemble lamprey chondrocytes. Many ostracoderms show no preserved cartilage, and appear as empty nutshells, but it is hypothesized that bone cells have been later recruited from the basal layer of the dermoskeleton to form the perichondral bone layer of the endoskeleton. Shortly after the rise of perichondral bone, a clade of vertebrate, the chondrichthyans, underwent an extensive reduction of the perichondral bone, which became soon replaced by prismatic calcified cartilage. By contrast, its sister clade, the osteichthyans, rapidly developed, in addition to perichondral bone, extensive endochondral bone. By providing a flexible, but calcified support for the musculature, tessellate prismatic calcified cartilage ensured the evolutionary success of chondrichthyans, which now appear as the most derived vertebrates.

Mechanisms of jaw strengthening in the skates and rays (Batoidea; Chondrichthyes)

26 Jun
11:00am

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In batoid chondrichthyans (skates, rays), species crushing hard prey (durophagy) exhibit morphological features such as a flat, pavement-like dentition and strengthening of the jaw by thickening or addition of extra layers of the mineralized blocks (tesserae) forming the cortex, and by the addition of reinforcing struts within the jaw itself. These struts (trabeculae) have been described in two disparate batoid groups (Torpediniformes, Myliobatiformes), and so were thought to have evolved independently. Here we describe jaw strengthening mechanisms in the sister taxon to the Myliobatiformes, the Rhinopristiformes, including *Rhina ancyclostoma* (Bowmouth Guitarfish; Rhinidae) and *Rhynchobatus australiae* (White spotted Wedgefish; Rhynchobatidae). Micro-CT scanning shows that both species possess trabeculae, particularly numerous below the dentition of *Rhina*, which is not observed in *Rhynchobatus*. Trabeculae in Rhinopristiformes expands the distribution of this feature to all major batoid groups except the phylogenetically basal Rajidae. Volumetric quantification of the teeth and jaw tesserae show that *Rhina* and *Rhynchobatus* also exhibit cortical thickening, with enlarged, less mineralized and morphologically irregular tesserae particularly in regions where the dentition is thinnest, being a predominant feature of *Rhynchobatus*. These tesserae may remain in a developmentally flexible state to better to respond to pressures associated with a crushing dentition. Thus, although *Rhynchobatus* and *Rhina* possess similar durophagous dentitions (but different in size), there are distinct differences jaw strengthening, with trabeculae playing a greater role in *Rhina* and modified/enlarged, less mineralized tesserae more significant in *Rhynchobatus*.

24 Jun
3:45pm

Assessment of the 3D microstructure of cartilage using high resolution X-ray tomography

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Cartilage brings flexibility, elasticity and lubrication to joints, minimizes mechanical contact stresses, and forms the embryonic (and, in some cases, the adult) skeleton of vertebrates. The exceptional properties of cartilage are of key interest to biomedicine, but also the design of new artificial materials (e.g. with high performance, but low friction and low density), and demand fine-scale characterization of complex 3D microgeometry structured at different scales (in the range of micrometers down to hundreds of nanometers). High Resolution X-ray Tomography (HRXT) is a 3D-imaging method able to provide a complete description of the internal microstructure for a wide range of materials. Recent developments offer a spatial resolution down to 0.3 m for the best laboratory systems. Achieving sub-micron resolutions can be challenging with sensitive specimens, especially polymeric samples and soft biological tissues, like cartilage. High-stability of the object is necessary during measurement to avoid blurring effect due to motion, and such movements can be induced by long X-ray exposure time. In this presentation, we will focus on the capabilities, limitations and good practices of the HRXT imaging technique applied to cartilage samples. Through different examples, we demonstrate that HRXT can provide unique insights into 3D architecture of cartilage microstructure, while also generating high-resolution datasets for quantitative analysis and mechanical simulations.

The genetic actors of a potential estrogen-signaling regulation of cartilage mineralization in the catshark

25 Jun
2:30pm

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In living vertebrates, bone is only present in bony fishes, in contrast to the widespread distribution of cartilage. Indeed, cartilaginous fishes have no bone tissue and rely on mineralized cartilage for structural support. This has been shown to be a secondary loss through their evolutionary history (Min Zhu, 2014). Sex hormones such as estrogen and androgens can bind to specific receptors and act as transcription factors as to upregulate or downregulate the expression of specific genes. It has been shown, at least in mammals, that some mechanisms of cartilage and bone formation and turnover are regulated by sex hormone signaling (Frenkel et al., 2010; Vanderschueren et al., 2014). In this project, we raise the question of the conservation in all jawed vertebrates of (i) the genetic actors behind skeletal mineralization processes and of (ii) their regulation by an estrogen signaling mechanism homologous to the one in mammals. Phylogenetic analyses were performed to identify a set of homologous skeletal genes in bony and cartilaginous fishes as well as genes known to be involved in estrogen signaling in mammals. RT-qPCR were carried out to validate expression of these candidate during skeletal development, in particular during the embryonic onset of cartilage mineralisation in the catshark *Scyliorhinus canicula*. Furthermore, an analysis of the upstream region of each genetic actor in the catshark should show if there are specific estrogen-receptor elements (EREs) that might regulate the expression of these skeletal genes. Further functional tests will then be needed to validate these candidate estrogen-responsive skeletal genes.

25 Jun
11:30pm

Strong biomechanical fixation of osteochondral scaffold enhanced cartilage healthy formation

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The treatment of cartilage lesions has become a major concern in Orthopaedics because predominantly these defects do not heal spontaneously which make the joints susceptible to early onset secondary osteoarthritis [1]. Tissue engineering approaches have emerged for the repair of cartilage defects and damages to the subchondral bones and have shown potential in restoring joints function. However, tissue engineering scaffolds often fail to satisfactorily regenerate the bone and the native hyaline cartilage and result in the formation of inferior (in terms of mechanical properties) fibrocartilage [2], affecting the durability of the regenerated tissue. We have developed a functionally graded multi-layered scaffold to address large osteochondral defects, with focus on improving bone ingrowth and cartilage quality. This study investigated the efficacy of this scaffold in vivo following implantation in sheep knee. The scaffold was fabricated using a combination of additive manufacturing and freeze-drying/critical drying techniques. Three layers of Ti matrix, PLA and collagen-PLGA were created to mimic subchondral bone, calcified cartilage and cartilage in native tissue, respectively. Multi-layered collagen/hydroxyapatite scaffolds were used as control. Ten sheep were operated on and either the scaffold (n=6) or the control (n=4) was implanted in the left medial condyle. The tissue was retrieved 12 weeks post-operation. Bone ingrowth into the titanium matrix and quality of the cartilage was assessed macroscopically, histologically and with the use of uCT.

It was observed that gross morphological appearance of regenerated cartilage was superior in osteochondral scaffold group compared to the control group. Collagen 2 and Safranin-O stainings confirmed formation of a hyaline-like cartilage. The pQCT examination revealed that the BV/TV ratio in the surrounding subchondral bone was significantly higher (p=0.01) in the osteochondral scaffold group (40%) than that in the control group (15%). The bone-scaffold contact analysis revealed the bone-implant contact achieved 61%. It is believed that the new bone growth into the Ti matrix at bone section provided a stable mechanical fixation providing a strong support to the overlying cartilage layer leading to an improved cartilage formation.

References: 1. Bentley, G., et al., Repair of osteochondral defects in joints - how to achieve success. Injury, 2013. 44(S1): p. S3-S10. 2. Christensen, B.B., et al., Poor osteochondral repair by a biomimetic collagen scaffold: 1- to 3-year clinical and radiological follow-up. Knee surgery, sports traumatology, arthroscopy, 2015: p. 1-8.
Acknowledgements: This work was financially supported by ARUK (grant no: 21160) & Innovate UK (grant no 102470).

Timing of jaw shape remodeling in sharks development to establish species-specific feeding mechanisms

26 Jun
3:30pm

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Despite elasmobranch sharks represent a rather small part of vertebrate diversity they display a wide array of life styles, reproduction modes, and morphological adaptations throughout their evolutionary history. The elements of their mandibular apparatus might appear simple at first, however it can show several modifications for specific functions. Regarding the feeding mechanisms, distinct modes are present like ram, suction, grasping, crushing and cutting. These mechanisms evolved in different groups of sharks independently, however the morphological changes to achieve the same feeding mechanisms did not lead to similarities in the skeletal elements. Modifications during the development can be traced to follow the changes for a specific functional morphology. The main goal of this study is to determine the specific changes in the shape of the Meckels cartilage occurring during the development that lead to specialized morphologies in an orectolobiform shark, *Chiloscyllium punctatum* (Bamboo shark) - a specialized suction feeder, and a carcharhiniform shark, *Scyliorhinus canicula* (Catshark) - a generalist ram feeder. We also intend to understand how other elements in the craniofacial morphology are related to the changes in the Meckels cartilage. In this way we will understand if these elements have become integrated as a functional unit within the jaws or if the elements function as separated modules. We employed geometric morphometric approaches to quantify the shape changes in the cartilage elements of the jaws through comparable embryonic stages to observe the patterns of divergence in their developmental trajectories that can lead to specific jaws morphology for feeding mechanisms.

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24 Jun
11:00am

Tesserae in shark cartilage; Evidence of developmental patterning?

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Tessellated calcified cartilage (TCC) is a hallmark chondrichthyan hard tissue that probably arose after the group diverged from osteichthyans. Tesserae do not typically display an ordered arrangement; nevertheless, the ontogenetically earliest tesserae are formed at specific sites in the cranium and visceral skeleton. Thus, initial patterning of TCC in elasmobranchs can be highly organized, but this transient stage largely disappears secondarily. However, rows of large tesserae are sometimes observed along cartilage edges and around foramina even after TCC is fully formed, defining borders to fields of smaller random tesserae. Such site-specific tesserae often exhibit distinct morphologies related to their position on the cartilage. In addition, tesserae never form in certain areas (e.g., on the retinal surface of scleral cartilage and on articulation surfaces). Collectively, these observations suggest the existence of a regulatory mechanism for tesserae that may be locally inhibited, perhaps comparable to inhibitory field and odontode regulation mechanisms in the dermal skeleton. Thus, there is evidence suggesting the existence of underlying developmental patterning of TCC, possibly analogous to that proposed elsewhere for the elasmobranch dermal skeleton, and individual tesseral development may be regulated by diffusion gradients of an inhibiting signal, possibly through the perichondrium.

Pelvic fin chondrogenesis in the catshark *Scyliorhinus canicula* embryo: a test for sex hormone regulation

25 Jun
3:45pm

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First identified as sexual hormones, androgens and estrogens were shown to have a key role in the development of gonads but also to be involved in the development and homeostasis of the skeleton. In mammals, androgen and estrogen receptors (AR, ERa and ERb) are expressed in osteoblasts, osteoclasts, osteocytes as well as chondrocytes and chondroblasts, regulating their activity to make up and maintain cartilage and bone. Previous works has demonstrated a sexual dimorphism in the AR, ERa and ERb expression in chondrichthyan embryos, particularly in pelvic fin development. These differences were associated to the initial growth of claspers (intromittent organs derived from pelvic fin skeleton) in males. Using the chondrichthyan model *Scyliorhinus canicula*, we studied the chondrogenesis of the pelvic fin during male and female embryo development, from stage 32 onward. We followed the developing cartilaginous claspers and pelvic fins by alcian blue staining. In addition, we performed qPCR experiments at similar stages to analyze the expression of various genes known to be involved in the formation of cartilage and bone in bony vertebrates. In this context, we aimed at testing the potential for sex-hormone regulation of cartilage specific genes.

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25 Jun
10:30am

Mechanobiology of the chondrocyte primary cilium

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Chondrocyte primary cilia are microtubule-based organelles (1-3m long) that are formed from the centriolar anchor known as the basal body. In other tissues, primary cilia act as cellular sensors that receive diverse signals from the extracellular environment including light, growth factors and mechanical stimuli. In development, signalling through the primary cilium is essential for patterning and endochondral ossification. However, the role of the chondrocyte primary cilium in adult cartilages still remains unclear. Structurally, chondrocyte cilia are associated with the collagen fibres, they express integrins and are mechanically deflected through ECM interactions. In maturing articular cartilage, cilia length and position relative to the articular surface changes with zone. Functionally, chondrocyte primary cilia are mechanosensitive, whereby cilia incidence and length are modulated by compressive or tensile forces, and removal of cilia results in reduced mechanotransduction, and alterations in cartilage matrix gene expression. Due to the highly robust mechanical properties of cartilage and the heterogeneity of chondrocyte cilia length and position within articular cartilage, we are interested in how the mechanics of the local microenvironment influences cilia function. Using a gradient hydrogel system with tunable stiffness, we have shown that substrate stiffness strongly influences cilia length, whereby primary cilia are shorter in stiffer microenvironments. Our data suggest an intricate relationship between substrate stiffness, F-actin and cilia length and more work is essential to understand the critical role of the mechanical microenvironment on ciliary signalling.

The interplay between collagen organisation and biomineralisation in the spine

24 Jun
2:00pm

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The vertebral end plate is a transitional structure between the intervertebral disc and the vertebral body, comprising of two distinct regions of non-mineralised cartilage and calcified cartilage adjacent to the disc and bone respectively. Whilst the endplate shares structural similarities to the articular cartilage, it differs in the anchorage mechanisms to the subchondral bone. The endplate has important roles in providing appropriate coupling between the two mechanically very different tissues and in allowing the exchange of nutrients, metabolites and water between the disc and the bone microcirculation. With ageing, the endplate calcifies to a level higher than that in the underlying bone, but whether this or other structural changes are associated with the degeneration of the intervertebral disc remains unknown. This project uses a combination of nonlinear optical microscopies, including Second Harmonic Generation (SHG) to detect collagen, two photon fluorescence microscopy (TPF) to detect elastin and other endogenous fluorophores and Raman spectroscopic imaging to detect biochemical composition. It explores the relationships between changes in mineralisation, collagen organisation and markers of cellular activities. The immediate aim is to characterise variations through the depth of the end plate and differences between the regions beneath the disc nucleus and annulus, which are presumed to have different physiological roles. Thereafter, we shall investigate changes with ages, with the long-term aim of determining factors associated with disc degeneration as targets for diagnosis or therapy.

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26 Jun
9:30pm

Too efficient to evolve? Similarities between the chemistry of cartilage calcification in elasmobranch fish and mice

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The mineral produced within the cartilaginous elasmobranch skeleton is a poorly crystalline calcium carbonate phosphate apatite. This mineral is similar in chemical composition to bone and calcified terrestrial cartilage. However, the elasmobranch skeletal apatite crystals are smaller and/or less crystalline than terrestrial skeletal apatite minerals. One theory of the genesis of these minerals may apply to both elasmobranch and murine growth plate cartilage. This theory builds on a 1923 discovery of the correlation between skeletal mineralization and alkaline phosphatase activity. Alkaline phosphatase cleaves phosphate ions from organic and inorganic sources. One source for skeletal mineral nucleation is hypothesized to be inorganic polyphosphates. Inorganic polyphosphates are lines chains of phosphate ions bonded together through P-O-P bonds. In general, polyphosphate chains range in size from three to several hundred phosphate units. Inorganic polyphosphates are produced by mitochondria, and are theorized to be an ancient metabolic energy storage molecule, among other roles. It is hypothesized that inorganic polyphosphates could be packaged by cartilage cells, and transported to the extracellular matrix. Once within the extracellular matrix, alkaline phosphatase activity cleaves phosphate ions from polyphosphate. This locally increases the phosphate ion concentration, favouring the nucleation of calcium carbonate apatite in in the presence of adequate available calcium and carbonate ions. This presentation will survey cartilage calcification chemistry, recent work on the possible roles of polyphosphate in controlled calcium carbonate phosphate mineral production within cartilaginous fish and murine calcified cartilage, and possible biochemical methods of controlling calcium carbonate apatite crystallite size.

Mineralisation in the synarcual of the elephant shark (*Callorhinchus milii*)

24 Jun
3:15pm

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Over the last decade, mineralised skeletal tissues of elasmobranchs (sharks, rays) have been described in ever-increasing detail, particularly the cortical mineralised tiles that overlay the cartilaginous skeleton, known as tesserae. In contrast, the examination of mineralised skeletal tissues in holocephalans seems to be largely limited to historical studies that describe mineralised platelets that correspond to elasmobranch tesserae, disagreeing with claims in the scarce modern literature which instead identify mineralization as continuous calcified cartilage. Both holocephalans and batoids experience cervical vertebral fusion during their embryonic development that produces a structure known as the synarcual. Through the use of histology, complemented by micro-CT and micro MRI scanning, we examined the mineralised tissues and development of the synarcual in three stage 36 elephant shark (Holocephali: *Callorhinchus milii*) embryos. Our examinations reveal the presence of surficial units of mineralisation in vertebral components composed of hyaline cartilage (basidorsal, basiventrals and neural arches) that seem distinct from the tesserae described in elasmobranchs. During vertebral development, we observe that the synarcual forms from the gradual growth of distinct cartilages that eventually fuse together, and mineralization begins anteriorly, progressing posteriorly. This corroborates the results of previous studies of the development of the synarcual in *C. milii*, further emphasising the non-homology of the holocephalan and batoid synarcua and their development.

24 Jun
2:15pm

The evolutionary origins of tessellated calcified cartilage in chondrichthyans

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Uniquely, chondrichthyans represent a monophyletic group of organisms, customarily defined by a skeletal hard tissue rather than by morphology! Modern chondrichthyans possess a unique endoskeletal hard tissue; tessellated calcified cartilage (TCC). Similar hard tissue was present in many Paleozoic shark-like fishes. Some extinct jawed fishes such as Climatius, Ischnacanthus, and Doliodus (formerly classified as acanthodians) possessed TCC-like hard tissues, blurring the distinction between conventionally-defined and putative chondrichthyans. TCC is thus a plesiomorphic feature of the chondrichthyan crown (= elasmobranchs + chimaeroids), and was probably lost/modified in the lineage leading to modern chimaeroids. TCC is an apomorphic feature that evolved at least 400 MYA within the chondrichthyan total group after its divergence from osteichthyans. Paleozoic TCC exhibits a range of ultrastructural diversity not found in later (post-Permian) chondrichthyan fossils. Unfortunately, current phylogenetic relationships among stem chondrichthyans are unstable, obscuring the early evolutionary history of TCC.

Defining limits of plasticity; can joint cartilage recover following embryonic paralysis?

25 Jun
10:45am

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During development, decreased *in utero* movements result in skeletal abnormalities including joint fusions and fragile bones prone to fracture. We have studied the cellular and molecular basis of the impact of reduced movement on joint formation and have shown that the cell territories that give rise to stable articular cartilage are lost, with concomitant loss of *Wnt* signalling. While fetal activity is a normal part of human development, indicating that all is well, it remains unclear when sensitivity of the skeletal system is greatest and if there can be recovery if movement is restored. The aim of this work is to explore the effect of short-term immobility on articular cartilage and examine potential for joint recovery post paralysis. Varying the duration and onset of immobilisation and resumption of movement, both natural movement and the effect of hyperactivity is being tested in chick embryos. Initial histological analysis indicates that short term immobilisation early in development (E4-E6) with natural resumption of movement (E7-E9) still results in joint cavity loss and absence of articular cartilage definition, i.e. does not reverse the outcome for the joint. Further analysis will determine if there is any change in the molecular disturbances that we previously described. This work will further define the periods of sensitivity during articular cartilage development, asking if there are periods when the effects of reduced movement are permanent and periods when the effects are more plastic. This could translate to the prescription of therapeutic regimes to ameliorate the effects of human fetal immobility.

Vertebrate cartilage diversity revealed by iodine staining and propagation phase-contrast synchrotron microtomography

24 Jun
10:30pm

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Extant vertebrates produce several types of cartilage that differ greatly in their developmental origins, growth dynamics and mineralisation processes. Osteichthyans (including actinopterygians, lungfishes and amphibians) calcify their cartilage as part of an evolved process called *endochondral ossification*, where cartilage cells become hypertrophic as blood vessels invade to transport cells and growth factors responsible for new, internal bone deposition. Chondrichthyans however produce different types of calcified cartilage in the absence of blood vessel invasion. Three types of cartilage are present in sharks: *areolar cartilage* in the vertebrae, *globular cartilage* peripherally and *prismatic cartilage* that forms the superficial tessellated skeleton. Here we present a protocol based on iodine staining and propagation phase contrast synchrotron microtomography. Scans were made at the beamline ID19 of the European Synchrotron Radiation Facility with a submicron resolution and an energy varying between 26.5 keV and 70 keV to highlight the 3D arrangement of the cells, the cellular volumes, the sphericity of the cells, the relative density of the calcifying region of the cartilage and the presence/absence of blood vessels in a large range of vertebrates (including the chimaera *Callorhinchus*, the shark *Scyliorhinus*, the ray *Raja*, the ray-finned fish *Polypterus* and *Danio*, the lungfish *Neoceratodus*, and the amphibian *Xenopus*). Our data reveal a large variety of cartilage configurations which not only differ between species but largely evolve over the development within species. This review will constitute a fundamental database for investigating fossilized cartilaginous tissues and understand their evolution.

Study of cartilage regeneration in zebrafish

25 Jun
3:30pm

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Cartilage, as the majority of adult mammalian tissues, lacks the capacity to self-repair often leading to joint diseases. In contrast, some vertebrate species have the remarkable capacity to spontaneously regenerate complex structures even after severe injuries. In zebrafish, we recently showed that mandibular cartilage (Meckels cartilage; MC) regenerates in few days upon mechanical injury. Our main objective is to elucidate the cellular molecular mechanisms allowing cartilage regeneration in this model. We first demonstrated that early events of this process consists in an increased number of MC chondrocytes re-entering the cell cycle, and the activation of Erk signaling in chondrocytes at the wound site that start to proliferate. Expression analyses revealed that neuregulin 1 (NRG1), a growth factor of the EGF family, is specifically induced in the lesioned tissue, while the corresponding erbB receptors are upregulated. We hypothesized that NRG1/ErbB signaling triggered by the injury activates chondrocyte dedifferentiation / proliferation and controls cartilage regeneration via Erk activation. Consistent with this, pharmacological inhibition of ErbB signaling resulted in the abolition of Erk phosphorylation in chondrocytes and impaired MC regeneration. In conclusion, we identified the NRG1/ErbB pathway as crucial for spontaneous cartilage regeneration in zebrafish. We are currently investigating whether this pathway could have similar functions in mammalian chondrocytes, and could be targeted to induce the formation of new chondrocytes in patients.

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24 Jun
10:45am

A peculiar vertebrate skeleton - the cartilaginous endoskeleton in sharks and rays

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Besides bone, cartilage is one of the major constituents of vertebrate skeletons. However, the majority of data available on the biology of cartilage is rooted in studies of mammals, ignoring the tissues diversity within vertebrates. In order to understand the breadth of natural variation in cartilage, our workgroup focuses on the development, ultrastructure and mechanics of the endoskeleton of sharks and rays (elasmobranchs). Their skeletons are almost entirely made of cartilage, similar to our hyaline cartilage, yet are never replaced by bone and bear a unique superficial mineralization. In order to understand the distinct characteristics of this cartilage, we use a variety of biological tissue and materials characterization techniques, outlining distinct skeletal mineralization phases, defining features of mineral density variation related to growth and mechanics, and characterizing tissue compositions, allowing for comparison with other vertebrate skeletal tissues. Despite large variation in mineralization pattern, we observe commonalities in ultrastructural features among species of all major elasmobranch groups. This suggests universal principles of skeletal growth and form across species, and reveals that elasmobranch cartilage lacks some features believed to be associated with vertebrate cartilage mineralization (e.g. chondrocyte hypertrophy and death, type X-collagen expression). In this way, our data create new reference points for the comparison of vertebrate cartilages and their underpinning mechanisms of homeostasis and mineralization.

Biomimetic, biofunctionalised polymer implants to promote *in situ* repair of traumatic and early osteoarthritic cartilage defects

25 Jun
2:00pm

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Introduction: We are developing a biomimetic, implantable medical device which is bio-functionalised in a similar conformational and biochemical context to articular cartilage, to promote stem cell homing and retention in the implant and subsequent chondrogenesis and de novo cartilage formation. The aim of this study was to assess the efficacy of this technology on the regeneration of articular cartilage *in vitro* and *in vivo*.

Methods: Poly-L-lactic acid (PLLA) random-fibre scaffolds were electrospun and surfaces modified by plasma polymerisation. Scaffolds were treated with heparin and pmol amounts of chondrogenic and stem-cell homing factors. *In vitro* analysis of scaffolds was performed by seeding bone-marrow MSCs and primary chondrocytes onto scaffolds and staining for PrestoBlue and measuring glycosaminoglycan content. Bio-functionalised scaffolds were assessed for *in vivo* activity by implantation into surgically created full-thickness chondral lesions including subchondral bone micro-fracture. 6mm chondral lesions in the medial condyles of sheep, with microfracture, were surgically created. At 4 and 16 weeks, cartilage regeneration was assessed macroscopically and histologically after joint retrieval.

Results: Functionalisation of the PLLA scaffold with a combination of TGF3 and CXCL12 promoted MSC attachment and chondrogenic differentiation. MSCs underwent chondrogenesis and produced significantly more ECM (P0.05) than non-functionalised. Scaffolds promoted chondrocyte attachment, long-term cell viability and ECM formation. *In vivo* implantation of TGF3 and CXCL12 functionalised implants showed biological efficacy with regeneration of neocartilage with hyaline features occurring at 4 weeks with the bio-functionalised implants but not with empty defects or control non-functionalised implants.

Conclusions: Bio-functionalisation of PLLA scaffolds with pmol quantities of TGF3 and CXCL12 promoted MSC migration and retention in the scaffolds and chondrogenesis with neocartilage tissue regeneration *in vitro* and *in vivo*.

26 Jun
11:30pm

Notochord and bone precursor cells as source for cartilage

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Cartilage is commonly considered as deriving from mesenchymal condensations, and constituting the embryonic precursor of the bony endoskeleton in osteichthyans. As such it is replaced by bone and persists only in limited areas, such as the diarthrodial joints. Less acknowledged are other sources of cartilage. Cartilage also derives from notochord and from bone tissue. The notochord not only induces cartilage development, it also gives rise to cartilage as regular part of vertebral centrum development in urodeles, lissamphibians and birds. In mammals, the notochord contributes to the fibrocartilage in the outer part of the intervertebral disk, the annulus fibrosus (1). In teleosts, injuries of the notochord, including regenerative processes, turn the notochord into cartilage. Likewise, damage of the intervertebral disk induces cartilage development. Another sources of cartilage is the bone periostum. Teleosts provide several examples for secondary cartilage. Cartilage that derives from the periostum can be hyaline cartilage, zellknorpel, a mixture of bone and cartilage or a type of chondroid bone. Skeletal injuries in teleosts, similar to the notochord injuries, trigger cartilage development. A newly described trigger for secondary cartilage development is non-mineralised bone. A switch from notochord to cartilage or from bone to cartilage is best understood if we consider skeletal tissue types as continuum, rather than as discrete entities.

(1) Risbud MV, Shapiro IM (2011) Notochordal Cells in the Adult Intervertebral Disc

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EmbryoMaker - a multiscale model uniting biological and mechanical aspects of hard tissue development

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Morphogenesis of animal tissues involves genetic, biomechanical and cell biological mechanisms that interact in a non-linear manner. In order to test developmental hypotheses, in-silico modelling approaches can be a valuable method, although usually restricted to a specific subset of developmental mechanisms. The EmbryoMaker software combines gene regulatory networks, cell-cell signalling, tissue biomechanics, and all main cell behaviours known to play a role in early animal development, including cell proliferation, condensation and secretion of extracellular matrix. Thus, it can be a useful tool to understand the development of hard animal tissues such as tooth and cartilage, and provide insight into the morphological variation produced by changes in the developmental program.

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