and research. The multi-disciplinary nature of the speeches was highly appreciated and paved the way for synergic collaborations among the participants. The conference attracted the interest of numerous researchers as well as the media, who greatly contributed to the dissemination of Centro3R's mission and promoted it as a major reference point for research and teaching resources in Italian academia.

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Meeting Report

Swiss-Chinese Cooperation for Organs-on-a-Chip and Stem Cell Research

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Sino-Swiss Workshop for Tissue Engineering

Organ-on-a-chip (OOC) systems, or microphysiological systems (MPS), are a new biomedical research field that aims to recapitulate organ-level tissue structures and organ functions for drug evaluation and disease modelling. The research is gaining importance in the context of further advancements in the development and implementation of organotypic models for drug development. The Annual Meeting of the TEDD network (Tissue Engineering for Drug Development and Substance Testing) took place in October 2019 in Wädenswil, Switzerland. We hosted researchers from Southeast University (SEU), China, National Institutes of Health (NIH), USA, Chinese University of Hong Kong (CUHK), and Swiss academics and industrial research groups active in the field of tissue engineering to build a strategic research and implementation alliance. Speakers presented the latest findings on OOC, 3D cell culture, stem cells and bioprinting. On the podium, we discussed potential funding sources, programs and collaborations with European and international networks. The aim was to accelerate the translation and validation process of current tissue engineering technologies. Additionally, representatives of swissnex and Swiss National Science Foundation (SNSF) talked about how governmental institutions support research collaborations between China and Switzerland. Preceding the meeting, the international scientists visited leading research groups and companies in Switzerland.

The Chinese delegation included Prof. **Zhongze Gu** and Prof. **Ningping Huang** from the School of Biological Science and Medical Engineering, Southeast University, Nanjing and Dr **Zaozao Chen**, a technical director from the Institute of Biomedical Devices (IBMD), Suzhou. Prof Zhongze Gu's group has developed multiple OOC and MPS systems, including blood vessels, heart, kidney, and liver tissue. The miniature organs made with advanced microfabrication, 3D bioprinting, microfluidics, and tissue engineering techniques form tissue-specific structures and maintain desirable organ functions for more than four weeks. Current research is aimed at the development of an advanced 3D read-out system that can characterize OOC tissues and analyze their morphology and functional features automatically in a quantitative manner. For example, this system could offer a package solution to analyze a tumor spheroid's/organoid's viability and invasiveness, together with the prediction of drug classification and mechanism using deep-learning-based artificial intelligence (AI)-algorithms. Thus, this system could be useful for oncology drug screening and evalu-

ation. Additionally, the group is using 3D bioprinting combined with two-photon lasers to precisely reconstruct the ECM with hydrogels at an ultra-high resolution of less than 150 nm to provide an appropriate 3D microenvironment for OOCs. Prof Gu claimed that the application of these technologies and systems is ideal for high-throughput and high-content screening with OOC systems. Underpinned with big data from OOC and MPS testing, this SMART system aims to provide reliable and predictive results on drug efficacy and toxicity.

Dr **Danilo Tagle** from National Institutes of Health (NIH), Microphysiological Systems Program for Drug Development, National Center for Advancing Translational Sciences (NCATS), USA, presented his work on tissue chips. Approximately 30% of drugs fail in human clinical trials due to adverse reactions, and another 60% fail due to lack of efficacy. One of the major causes of the high attrition rate is the poor predictive value of current preclinical models used in drug development, i.e., 2D cell culture and animal models. The NIH Microphysiological Systems (Tissue Chips) Program led by NCATS is developing alternative approaches and tools for more reliable read-outs of toxicity and efficacy during drug development. Tissue chips are bioengineered microphysiological systems utilizing human primary or stem cells seeded on biomaterials manufactured with chip technology and microfluidics that mimic tissue cytoarchitecture and functional units of human tissues and organs. These platforms can be used for predictive toxicology and efficacy assessments of candidate therapeutics. Tissue chips can also contribute to studies in precision medicine, environmental exposures, reproduction and development, infectious diseases, microbiome and countermeasure agents. Dr Tagle presented examples of the utility of individual tissue chips and multi-organ integrated platforms in drug development and showed effective partnerships with stakeholders, such as regulatory agencies, pharmaceutical companies, patient groups, and other government agencies. In collaboration with the International Space Station – National Laboratory (ISS-NL), tissue chips are being used to model ageing-related diseases and for testing of potential new drugs for earth-based use. Dr Tagle summarized that the unique environment of the ISS-NL allows researchers to study cells in ways that are not possible on the ground, which is helping to advance the field of regenerative medicine.

In the following session, Swiss academics presented their work. Prof. **Mark Tibbitt**, Assistant Professor of Macromolecular Engineering in the Department of Mechanical and Process Engineering at ETH Zürich, Switzerland talked about responsive hydrogels for tissue engineering applications. He described recent efforts in the engineering of hydrogels and development of additive manufacturing technologies for the design of functional tissues. Prof. Tibbitt described a surface tension-assisted additive manufacturing method where a lattice structure, generated by conventional additive manufacturing, is coated with a hydrogel, filling the void spaces of the original lattice. In this approach, the lattice structure defines the mechanical properties and geometry, whereas the hydrogel coating provides biofunctionality and direct inclusion of cells. Surface tension-assisted additive manufacturing has been applied to the design of replacement trachea and can be used broadly in the design of tubular tissue constructs. In the second part of the talk, a universal nano-carrier ink for direct ink writing of biomaterials was presented. The nano-carrier exploits polymer–nanoparticle assembly to engineer a gel with rheology suitable for extrusion-based printing. Uniquely, the nano-carrier is combined with a range of secondary polymers to formulate printable inks that enable post-printing stabilization. The nano-carrier inks were used for the printing of acellular vascular structures as well as for direct bioprinting of living tissue constructs. In both approaches, these technologies are being applied to create tissue phantoms for disease modelling and drug screening and in the future may aid in the design of implants for regenerative medicine.

Prof. **Olivier Guenat** from the University of Bern, OOC Technologies Laboratory, ARTORG presented OOC models of the lung parenchyma. The group aims to develop OOC, focusing on lung and its diseases, in collaboration with the Pulmonary Medicine and the Thoracic Surgery Clinics of the Inselspital, Bern University Hospital. The group combines engineering, particularly microfluidics and microfabrication, tissue engineering methods and material sciences. They have developed a breathing lung-on-chip, an advanced lung alveolar model that emulates the ultra-thin air-blood barrier, including the three-dimensional cyclic mechanical strain generated by breathing motions. This system is currently being further developed in collaboration with the start-up AlveoliX for the preclinical market. Another aspect under focus is the rebuilding of the lung alveolar environment represented by a functional lung microvasculature. Lung cells seeded in a micro-engineered environment self-assemble to build a network of perfusable and contractile microvessels. Next to the pharmaceutical applications, OOC models have the potential to be used in precision medicine using the patient's own cells to tailor the therapy. Such systems have significant potential to reduce the use of animal models in medical and life science research.

Prof. **Andreas Hierlemann** from ETH Zürich, Department of Biosystems Science and Engineering (D-BSSE) and his team are working on interdisciplinary projects targeted at questions in systems biology, drug testing, personalized medicine, and neuroscience. The group has developed versatile microfluidic platforms for formation, cultivation and analysis of fluidically interconnected organotypic spherical 3D microtissues, so-called spheroids. Sensor modules were designed as small plug-ins, which allow convenient functionalization and calibration of the sensors and do not interfere with microfluidic functions. Several variants of sensors and microfluidic device architectures were shown. These include hanging-drop networks and well-plate-based devices, both of which were designed for long-term culturing. Applications include multi-tissue-interaction studies and comprehensive characterization of potential drug candidates, including testing of their efficacy and toxicity as well as of potential metabolic processes. Moreover, Prof Hierlemann presented high-density microelectrode arrays (HD-MEAs) for high-resolution extracellular

electrophysiology. Complementary metal-oxide-semiconductor (CMOS)-technology is used to batch-produce such HD-MEAs with thousands of densely packed microelectrodes. The complex microsystems feature logic and circuitry units for signal conditioning and stimulation on the same chip. HD-MEAs feature a very high spatial density (> 3000 electrodes per mm^2) of comparably small electrodes (diameters of 5-7 µm and a center-to-center pitch of < 15 µm) and can be used for the electrophysiological analysis of networks of, e.g., brain cells at a cellular or subcellular resolution in dissociated cell cultures, organotypic tissue or slice cultures, and acute tissue slices.

After the academics group, Swiss entrepreneurs presented real-life applications of science. Dr **Olivier Frey** from InSphero talked about complex 3D *in vitro* models for fast, automation-compatible and translational drug discovery. His focus was on the liver and pancreas, which communicate via different endocrine factors to maintain glucose homeostasis in the human body. An impaired function of one of the organs can lead to several metabolic diseases such as diabetes or non-alcoholic steatohepatitis (NASH). The study of these diseases requires a systemic model that is able to reconstitute organ-organ-interaction. The practical implementation of human *in vitro* multi-tissue systems in a scalable format faces several challenges: Practicability including biological and technical reproducibility, the free availability of the tissue model, on-demand production, amenability to meaningful treatment windows, ease of access to clinically relevant read-outs, and compatibility with standard lab processes. At InSphero, a primary human liver and a primary human islet model are combined in a microfluidic device. The liver model consists of a hepatocyte/Kupffer cell co-culture with preserved metabolic and inflammatory function over at least two weeks when cultured with the islet model. Islet microtissues comprise all endocrine cells at a physiological ratio and remain glucose-responsive over the complete duration of an experiment. The read-outs include secreted molecules in the medium, lytic assays and histology. The microtissues are assembled on-chip using large-scale pipetting systems, which can set up more than fifty functional multi-tissue conditions in less than two hours, enabling an adequate number of replicates at minimal operational complexity for compound testing. Both tissue models can be induced to become diseased – fibrosis, steatosis/NAFLD, NASH for liver and T1D, T2D for pancreatic islets – and thus allow straightforward access to higher-order disease modelling.

Dr **Janick Stucki**, Co-CEO of AlveoliX, a spinoff from Prof Olivier Guenat's lab at ARTORG, University of Bern, works on OOC models of the lung parenchyma. The AlveoliX lung-on-chip system is based on a two-part design and equipped with a passive medium exchange mechanism. This allows user-friendly handling and the precise control of cell seeding on the ultra-thin, elastic and porous membrane. The standard well-plate footprint of the lung-on-chip includes an array of twelve independent wells, which allows simultaneous sample processing and medium experimental throughput. Additionally, the breathing lung-on-chip is compatible with customary laboratory equipment and is tested for various molecular biological read-outs and assays. Primary alveolar epithelial cells from patients cultured at the air-liquid-interface and exposed to physiological cyclic mechanical stress preserve their typical alveolar epithelial phenotype. Long-term co-culturing of lung epithelial and endothelial cells leads to increased barrier functionality and improved tissue integrity. Breathing motion also changes the permeability of specific low and high permeable molecules. In cooperation with industrial and academic partners, AlveoliX in working on different alveolar disease models using inflammatory modulators such as endotoxins, cytokines and immune cells. Furthermore, they are testing the applicability and performance of the lung-on-chip system in the fields of next genome sequencing, sensor integrations and robotization.

Dr **Vincent Ronfard** from CUTISS, talked about their approach to bio-engineer large quantities of individually customized human skin grafts, denovoSkin™, starting from a very small piece of healthy patient's skin. Because of its intrinsic characteristics, the product is expected to result in a minimal scarring outcome after transplantation. denovoSkin™ has received a Swissmedic, EMA and FDA Orphan Drug Designation for the treatment of skin burns.

To accelerate the collaboration between partners, representatives from Swiss science and innovation support presented collaboration opportunities. **Malin Borg**, Head of Unit, swissnex Network at Secretariat for Education, Research and Innovation (SERI) showed how to connect the dots in education, research, and innovation between China and Switzerland using swissnex China. Their mission is to support outreach and active engagement of partners in the international exchange of knowledge, ideas and talents. swissnex is involved in an initiative of the SERI in collaboration with the Federal Department for Foreign Affairs. The long-standing collaboration between Switzerland and China is based on several agreements such as the S&T bilateral agreement (1989) as well as the Innovative Partnership (2016). swissnex China offers services in the area of academic relations and start-up engagement. It also connects students from Swiss higher education institutions with alumni in Shanghai and prepares Chinese students for studies in Switzerland. In the start-up realm, swissnex China organizes Innosuisse internationalization camps, which offer tailor-made support for market validation or market entry of Innosuisse accredited start-ups. Events such as CES Asia offer an excellent platform for start-ups to showcase their products and seek investors as affiliates to the Swiss stand, one of the biggest at the fair. Moreover, swissnex China offers individual support to SERI stakeholders to identify potential and relevant Chinese partners.

Timothy Ryan from the Swiss National Science Foundation (SNSF) presented bilateral collaboration opportunities with China. The SNSF has several funding instruments in order to foster international collaborations. The Sino-Swiss Science and Technology Co-operation (SSSTC) is the largest funding scheme that promotes joint research activities between researchers based in Switzerland and China. Within the SSSTC, the SNSF supports Joint Research Projects (JRPs) together with the National Natural Science Foundation of China (NSFC). The JRPs enable research-

Participants of the Sino-Swiss Workshop for Tissue Engineering

ers in Switzerland to conduct a project together with a Chinese partner. These projects generally have a duration of three to four years, with funding on the Swiss side amounting to a maximum of CHF 350,000 per project. In addition to various mobility programs, the SNSF also provides the following collaboration opportunities with China:

- *Scientific Exchanges:* This funding scheme is aimed at researchers who want to host their scientific event in Switzerland, invite colleagues from abroad for a research visit to Switzerland, or visit their colleagues in another country[1].
- *Sinergia:* This scheme promotes the interdisciplinary collaboration of up to four research groups that propose breakthrough research. If there are three or more applicants, one applicant may be based at a research institution abroad, provided their expertise is essential and not available in Switzerland[2].
- *Project partner:* They are responsible for a small contribution to a research project. They are not the driving force behind the project and are not responsible for its progress. Project partners may be academic researchers or individuals working in the public or non-profit sector. They may receive funding through the project but cannot be employed as project staff.

Further information regarding all relevant eligibility criteria can be found on websites or in consultation with the SNSF Funding Regulations[3] and the General Implementation Regulations for the Funding Regulations[4].

TEDD Annual Meeting 2019

The main symposium on October 24, 2019 was focused on primary and stem cells, which are relevant sources for the generation of organotypic tissue models used in many biopharmaceutical applications, regenerative medicine, disease modelling and drug discovery. Stem cells promise to revolutionize the drug discovery process at all stages, from target identification to toxicology studies. While primary cells would represent native tissue most accurately, they usually have limited capacity to divide and thus need to be freshly isolated for each assay, limiting their application and decreasing their robustness. In contrast, the ability of stem cells to generate physiologically relevant cells in limitless supply makes them an attractive alternative to currently used recombinant cell lines or primary cells. Emerging technologies involve the production of organoids from human pluripotent stem cells (hPSCs) and the use of OOC devices. These approaches show great promise for developing a more reliable, rapid and cost-effective process when compared with the current use of animal models. The current challenges include directing stem cell differentiation towards pure specific lineages in a reproducible, robust and cost-effective way.

Dr **Markus Rimann** and Prof. **Christian Hinderling** opened the meeting with a report on TEDD activities in 2019. International speakers presented different aspects of stem cell research for tissue engineering and drug development applications.

[1] http://www.snf.ch/en/funding/science-communication/scientific-exchanges/Pages/default.aspx
[2] http://www.snf.ch/de/foerderung/programme/sinergia/Seiten/default.aspx
[3] http://www.snf.ch/SiteCollectionDocuments/allg_reglement_16_e.pdf
[4] http://www.snf.ch/SiteCollectionDocuments/snsf-general-implementation-regulations-for-the-funding-regulations-e.pdf

Prof. **Marisa Jaconi**, Faculty of Medicine of Geneva University, Switzerland and vice-director and founder of the Swiss Institute of Cell Therapies, started the scientific session with a keynote presentation entitled: "Heart Sweetheart: from cardiogenesis to cardiac engineering." In the past years, her group has addressed critical questions related to cardiac differentiation of human embryonic stem cells (hESC) by investigating 1) the molecular mechanisms of cardiogenesis and functional cardiac maturation in hESC, 2) the genes and signaling pathways underlying congenital heart defects in patients with Down Syndrome, 3) the optimal use of hESC-derived cardiac progenitors for cardiac tissue modelling *in vitro* and regeneration via tissue engineering strategies applied to animal models of myocardial infarction. Using trisomy 21 sibling hESC models of Down Syndrome, they showed that T21-hESC display many significant differences in expression of genes and cell populations associated with mesodermal and, more specifically, secondary heart field (SHF) development, including a reduced number of progenitor cells positive for the transcription factor gene ISL1+. They have provided evidence that overexpression during cardiac commitment of two genes located on chromosome 21, ETS2 and ERG, likely accounts for the disruption of SHF development, as revealed by downregulation or overexpression experiments. They have also discovered an abnormal electrophysiological phenotype in functional T21-cardiomyocytes, a result further supported by mRNA expression data acquired using RNA-Seq. In combination, these data revealed a cardiomyocyte-specific phenotype in T21-cardiomyocytes likely due to the overexpression of genes such as RYR2, NCX and L-type Ca^{2+}-channel. These results contribute to the understanding of the mechanisms involved in the development of congenital heart disease in complex genetic diseases. Further, a beneficial functional effect of cardiopatches seeded with ESC-derived cardiac progenitors for the targeted deep engraftment in the infarcted rat myocardium was shown. Presently, the group is working on 3D microtissues composed of different cardiogenic human cell sources as a format of addressing fundamental questions of tissue generation and for regeneration purposes.

Prof. **Cornelia Kasper** from University of Natural Resources and Life Sciences in Vienna, Austria started her talk by showing that the regenerative potential and immunomodulatory effects of mesenchymal stem cells (MSC) make them prime candidates for use in tissue engineering and stem cell therapies. In traditional cell culture techniques, most adherent cells are grown in 2D monolayers on plastic surfaces at atmospheric O_2 concentration (21%) in static conditions. These parameters differ immensely from the *in vivo* environment. Depending on the tissue, oxygen levels between 18% (lungs) and 1% (cartilage, bone marrow) are found; the higher oxygen levels in culture may thus change metabolic activity and increase the risk of oxidative damage. Secondly, already during isolation, plastic labware favors selection of certain cell populations and long-term cultivation in this foreign environment, in the absence of cell-to-cell contacts and 3D architecture, leads to changes in morphology and regenerative capacity. Lastly, next to biochemical signals, a plethora of physical stimuli such as shear stress, compression, uniaxial or radial strain are lacking in traditional cell culture. The group is focussing on transitioning MSC cultures from standard 2D set-ups to a more physiological environment. Temperature, humidity and CO_2-levels are already standardized in most culture systems; the next step is tight control of the oxygen levels the cells are exposed to. Already at isolation, hypoxic conditions and a 3D-to-3D approach, using different biomaterials, is implemented. Further control of external stimuli during expansion in 3D aggregates is applied using different bioreactors, cultivation units that allow regulation of mechanical stimulation by perfusion, and shear stress or compression.

Dr **Robert Knight** from the lab of Prof Phil Stephens from the College of Biomedical and Life Sciences at Cardiff University, UK spoke on soft tissue wound healing, which includes chronic wounds that fail to heal, adult scarring wounds, and wounds in the oral cavity that heal in a scarless fashion. This spectrum of response is reflected by distinct cellular genotypes/phenotypes of mesenchymal cells isolated from these tissues. Oral mucosal fibroblasts exhibit preferential wound healing properties and resemble regenerative fetal cells. The group has demonstrated that patient-matched oral mucosal (OMFs) and skin (SFs) fibroblasts have distinct wound reparative differences. For example, OMFs demonstrate an increased ability to migrate into/repopulate a monolayer wound space and to reorganize their surrounding extracellular matrix by increased production of matrix metalloproteinases and growth factors such as hepatocyte growth factor. This is linked to intrinsic differences in the ageing profiles of OMFs, with such cells being able to undergo many more population doublings than patient-matched SFs and thereby senescing later. Their more recent work, however, has demonstrated that an oral progenitor cell population within this general fibroblast population is potentially key to the successful tissue repair process. Such oral progenitors are highly clonally expandable (greater than 50 population doublings), multipotent (bone, fat, cartilage and neuronal-like cells) and potently immunosuppressive (many hundreds of fold times better than mesenchymal stromal cells). They also have the added benefit of being anti-bacterial (gram-negative and gram-positive) in nature. On-going work is investigating small extracellular vesicle (exosome) secretions from these oral progenitor cells to analyze if these are the critical factors involved in the scarless wound repair process. With the tissue containing these oral progenitor cells being easy to access and healing without a scar, these oral progenitor cells may be useful for future tissue repair strategies.

Prof. **Kenneth Lee** from the Chinese University of Hong Kong (CUHK), Developmental and Regenerative Biology Thematic Research Program, School of Biomedical Sciences and director of the Key Laboratory for Regenerative Medicine, Ministry of Education China, and Director of the Joint CUHK-University of Southampton Laboratory Stem Cell and Regenerative Medicine reported that spheroid formation primes somatic and senescent cells to reprogramming by small molecules. Cells will form spheroids when cultured on super-hydrophobic culture plates since they cannot adhere to the plate surface. 3D spheroids respond to small molecules differently than cells maintained on flat adhesive culture dishes in a 2D culture. Prof. Lee reported that

human fibroblasts maintained as spheroids become primed to respond to small molecules. Specifically, human fibroblasts could be reprogramed into stem cells strongly expressing Oct4, Sox3 and Nanog using three small molecules. The same approach was used to rejuvenate senescent human mesenchymal stem cells and fibroblasts. Senescent cells, cultured as spheroids and treated with a single small molecule, began to proliferate and regained their multipotency and differentiation efficiency into bone cells. These observations have important implications for pharmaceutical companies on how they should conduct cell-based screening for small bioactive molecules.

The afternoon session started with the keynote of Prof **Zhongze Gu**, in which he presented the activities of Southeast University (SEU) in China for the TEDD community similarly as the day before at the workshop. Then, Dr **Wing Chang**, director of research and development at STEMCELL Technologies, Cambridge, UK talked about robust and efficient tools for pluripotent stem cell and organoid research.

Dr **Parto Toofan** from REPROCELL Europe Limited, presented the company's efforts to produce GMP-grade iPSCs for drug screening and translational medicine using a safe mRNA strategy. iPSCs produced in GMP-grade for drug screening and translational medicine have the potential to provide patient-specific scalable biologic material of various tissue types, useful for investigating the pathophysiology of rare disorders. iPSCs not only serve as excellent stem cell models, but they also can differentiate into a wide variety of cell types for preclinical studies, providing an unlimited source for the development of healthy or diseased cell models in which to study the effectiveness and toxicity of pharmaceuticals.

After the industry session, the physical Competence Centre TEDD embedded in the Centre for Cell Biology and Tissue Engineering at the ZHAW presented its core competencies, including:

- Cell biology with a focus on stem cell research, cell-based assays, cell differentiation, glycobiology, and cellular engineering
- Applied matrix biology and biophysics (macromolecular crowding, supramolecular aggregates) to develop metabolically active human models such as fatty tissue and skeletal muscle
- Establishment and analysis of tissue equivalents on scaffolds used in the fields of regenerative medicine and substance testing
- 3D cell culture model development that is suitable for the pharma industry and personalized medicine, including technologies such as bioprinting

Dr Markus Rimann concluded meeting with an outlook for the TEDD network. The goal of the network is to grow further and to closely collaborate with other networks and associations to integrate new stakeholders from different industry sectors.

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Meeting Report

Virtual Summer School: Alternative Methods and Models in Science: A Multidisciplinary *In Vitro* Approach

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The international Virtual Summer School: Alternative Methods and Models in Science: A Multidisciplinary *in vitro* Approach[1], targeted at PhD students and young scientists was focused on *in vitro* advanced and innovative methodologies, their application in different disciplines, from toxicology to microbiology, and future perspectives. The two-day event held on June 3-4, 2020 was chaired by Francesca Caloni, Università degli Studi di Milano, Department of Environmental Science and Policy, and was attended by 26 international participants with different scientific backgrounds.

Giulio Casati, director of the Lake Como School of Advanced Studies, opened the event by presenting the activities and the mission of the school.

Francesca Caloni gave an overview on "*Alternative models and* in vitro *strategy*", stressing the importance of a predictive interdisciplinary science for risks related to the environment, hu-

[1] https://amms.lakecomoschool.org/