3D-imaging fusion models in orthodontics
Frits Rangel, Anne Marie Kuijpers-Jagtman, Stefaan Bergé, Thomas Maal, Rania Nada

With the introduction of Cone Beam Computed Tomography (CBCT), it became possible to obtain an accurate three-dimensional (3D) representation of the patient's head with a much lower radiation exposure, compared to Multi Slice Computed Tomography (MSCT), and a much higher information content, compared to two-dimensional (2D) X-rays. With the introduction of digital dental models development of three-dimensional digital datasets, combining the triad bone, soft tissues and dentition, have regained interest. If it would be possible to add the dentition to the 3D-stereophotographic image and/or to the CBCT dataset, this would give a 3D dataset of the face of the patient, with the dentition in an anatomical correct position. This research focuses on the development of fusion models of the head that are useful for orthodontic purposes.

Heme oxygenase as a novel target in the prevention of vascular complications in type 2 diabetes mellitus
Douwe Dekker, Paul Smits, Frank Wagener

The prognosis of type 2 diabetes mellitus (DM) is largely determined by the development of micro- and macrovascular complications. In this project, we will investigate whether the enzyme Heme Oxygenase (HO) is a relevant molecular target for the prevention and treatment of the macrovascular complications. In DM, the production of radical oxygen species (ROS), the expression of inflammatory adhesion molecules and the resulting leukocyte binding and vascular injury have been attributed to DM. One of the protective mechanisms is the activity of the enzyme HO, which breaks down heme into the strong anti-oxidants biliverdin/bilirubin and the potent vasodilator molecule carbon monoxide. In general, the versatile HO-products strongly protect against these vascular insults by reducing oxidative stress, inflammation, proliferation and apoptosis. However, hyperglycemia itself has been observed to inhibit HO-activity, thereby impairing protective mechanisms against vascular dysfunction in DM. The present study will focus on "proof of concept" studies in the human in vivo setting. We postulate that induction of HO-activity or administration of its effector molecules will ameliorate vascular function in DM patients. Parallel to the clinical studies, cellular in vitro and animal experiments will be performed to fill in particular gaps in the pathophysiological concept, and to control for confounding mechanisms.

3D-stereophotographic study into facial growth of CLP babies and unaffected children
Sander Brons, Jeneé Meulstee, Anne Marie Kuijpers-Jagtman, Stefaan Bergé, Thomas Maal

Stereophotogrammetry is a noninvasive technique based on the principle of photographing a 3D object with two pairs of identical cameras separated by a known base distance. The result is a stereo pair of photographs of the face taken from two different positions at the same time. These two photo images are then combined to form a 3D model. To date, a few cross-sectional studies have used stereophogrammetry to characterize facial soft tissue features in babies. There is very little data regarding 3D facial growth for very young Caucasian children in the age range of 0–6 y with or without orofacial clefts. For longitudinal studies, facial growth can be evaluated with stereophotogrammetry by measuring changes in 3D facial volume and changes in anatomical landmark positions over time and then superimposing 3D stereophotogrammetry pictures from different time points.

Heme oxygenase and stem cells as novel strategy to prevent fibrosis and excessive scar formation
Niels Cremers, Frank Wagener, Carine Carels

Aberrant wound healing, oxidative and inflammatory stress, are important down-stream complications in CLP patients and contribute to the progression towards excessive or hypertrophic scarring, which interrupts normal midfacial growth and development. Bone marrow stem cells are thought to mediate tissue repair. Following injury, stem cells can migrate towards the injury, where these normally quiescent cells start to multiply, and differentiate into the cells that are needed. However, stem cell functioning is sometimes impaired in an oxidative environment. Therefore we aim to overexpress cytoprotective proteins to protect the stem cell, and in addition, to create an environment that prevents exacerbation of injury, fibrosis, and hypertrophic scarring. *Heme Oxygenase* (HO) is an important cytoprotective protein and functions as an adaptive response against injurious processes, including oxidative stress, inflammation, apoptosis, and fibrosis. We postulate here that stem cell therapy alone and in combination with induction of the cytoprotective gene HO provides protection in CLP against oxidative and inflammatory insults, fibrosis, and hypertrophic scarring. Therefore, we are confident

that this study will give us a better understanding in the mechanisms involved in wound healing and may provide novel therapeutic strategies for CLP patients.

Role of microRNAs in palatogenesis and orofacial clefts
Christian Schoen, Armaz Aschrafi, Hans von den Hoff, Hans van Bokhoven, Carine Carels, Geert Poelmans

Palatogenesis requires a precise spatiotemporal regulation of gene expression, which is controlled by an intricate network of transcription factors and their corresponding DNA motifs. Even slight perturbations of this network may cause cleft palate, the most common congenital craniofacial defect in humans. MicroRNAs (miRNAs), a class of small non-coding RNAs, have elicited strong interest as key regulators of embryological development, and as etiological factors in disease. MiRNAs function as post-transcriptional repressors of gene expression and are therefore able to fine-tune gene regulatory networks. Several miRNAs are already known to be involved in congenital diseases. In this project,current research into the role of miRNAs in cleft palate will first be reviewed. Secondly a small RNA sequencing study will be undertaken to identify specific differential miRNA expression between CLP patients and age matched controls. Thirdly and following Michelle Thonissen's article, I will perform an experimental validation study of a possible nsCLP associated novel miRNA. Lastly I will analyze the possible link between miR-17-92, vitamin A and cleft palate.

Function of CLP genes in mouse palatogenesis
Laury Roa Fuentes, Hans Von den Hoff, Frank Wagener, Carine Carels

Several signaling pathways are known to be involved in palatogenesis and cleft palate. One of the main pathways is the wnt-signaling. The wnt pathway can be divided into a canonical pathway -catenin, and at least two other non-canonical pathways. Initially, this project will focus on finding marker genes for these three wnt pathways in cell lines, organ culture of mouse embryo palate, and ex-vivo embryo palates. The project will then investigate the interactions between known cleft palate genes and wnt signalling in cell lines and mouse embryo palates. Gene function will be inhibited by siRNA or by pharmacological agents. These experiments will involve known genes discovered in human studies as well as in animal studies (mouse). In addition, candidate genes from our own research will be studied such as LRP6.

Cone-beam Computed Tomography Dentition Improvement and Application
Olivier de Waard,Thomas Maal, Edwin Ongkosuwito, Hero Breuning

Integration of accurate 3D information of dental structures with CBCT radiographs is challenging. CBCT imaging is insufficient for accurate visualization of the dentition due to inaccurate rendering of the teeth and streak artifacts caused by dental restorations or metal orthodontic appliances. For orthognathic treatment, accurate dental information is essential for the planning/ assessment of the final dental and skeletal positions.
First the accuracy of two digital dental model types derived from CBCT information will be assessed. As the accuracy of the dentition on intra-oral scans is much more accurate, superimposition techniques can be used to improve the digital dental models on CBCT without increasing the radiation dose. With the use of digital dental models it is now possible to construct digital orthodontic setups. These digital setups can be used for planning of the pre-surgical orthodontic treatment phase. With the introduction of digital setups it is now possible to integrate orthodontic setups in the virtual orthognatic planning. Instead of just before the surgery the whole planning can be performed ahead of the start of orthodontic treatment. In the final step the application of such set-ups will be tested.

Biomimetic Hydrogels for smart wound dressings
Roel op 't Veld, Frank Wagener, Frank Walboomers, John Jansen

Imagine spraying-on a smart liquid wound dressing, which immediately gelates, covering the wound, filling even deep ulcer wounds. A dressing which has nanosized pores which allows water to escape yet prevents bacteria from getting in, yet is smart and triggers rapid healing, stimulating the cells to grow in an ordered fashion preventing scarring. A smart material, which mimics the extracellular matrix found in your body aiding wound repair, yet can be easily applied and removed. This ideal dream material is at hand!
This proposal uses the state of the art design and synthesis of a unique tunable biomechanical hydrogel, which can not only cover wounds but also controls and facilitates cell fate. It combines chemistry, biochemistry, and

cell physiology, to deliver the next generation of biomimetic active wound dressings for direct application by the clinicians in burns and deep ulcer wounds.

Cellular signaling and migration shaping the face
Maarten Suttorp, Frank Wagener

In this study we investigate the role of cell migratory pathways and cellular signalling molecules during craniofacial development. Hereto we study cell migration in relation to palatogenesis, wound repair, and orthodontics in vitro and in vivo. Cranial neural crest cells, macrophages, osteoclasts and osteoblasts are important for regeneration, remodelling, and shaping the craniofacial area. Although we know these cells have crucial functions, the decisive signalling machinery that modulates migration and recruitment of these cells to the oral area still remains elusive. During palatogenesis the midline epithelial seam disappears allowing fusion of the mesenchym resulting in osteoblastic activity in the hard palatum and myogenesis in the soft palatum. Some questions that we wish to answer: What are the decisive signaling pathways that determine the recruitment of osteoblast and muscle cell precursors? How do the cells understand where to go? What are the effects of mechanical stress and inflammatory and oxidative networks?

Optimization of the orthognathic trajectory using emerging 3D techniques
Frank Baan, Thomas Maal, Edwin Ongkosuwito, Stefaan Bergé

Intra-oral scan in CBCT
Integration of accurate 3D information of dental structures with CBCT radiographs is challenging. CBCT imaging is insufficient for accurate visualization of the dentition due to streak artifacts caused by dental restorations or metal orthodontic appliances. As the accuracy of the dentition on intra-oral scans is much more accurate, superimposition techniques can be used to improve the digital dental models on CBCT without increasing the radiation dose
Virtual setup in pre-surgical orthodontics
The workup for orthognathic surgery often starts with presurgical orthodontics which aims to arch alignment, arch co-coordination and arch decompensation. The incisor decompensation has a high influence on the surgical outcome. When incisor decompensation fails, the surgical advancement is limited by the existing overjet at the time of surgery. Currently there is no virtual planning involved in the orthodontic treatment but with the introduction of digital setups this is now possible. The whole orthodontic treatment can be virtually planned before the actual orthodontics takes place. Theoretically this should lead to less treatment plan changes and a more predictable outcome of the orthognathic surgery. The accuracy of the virtual orthodontic planning is unknown and therefore needs to be assessed before it can be implemented in the orthognathic trajectory.
Assessing the accuracy of bimaxillary surgery compared to the virtual 3D planning
Once the point of orthognathic surgery is reached, a 3D reconstruction of the skull is made and the surgery is virtually prepared by means of a 3D virtual treatment planning. One of the key issues in obtaining a favorable postoperative outcome is an accurate transfer of the 3D planned bony movements to the patient in the operating theatre. For assessing the accuracy of the postoperative outcome with regard to the 3D surgical planning this PhD presents a new approach to quantify the accuracy of the 3D virtual orthognathic planning.

Three-Dimensional Imaging in Cleft Palate Patients
Robin Bruggink, Edwin Ongkosuwito, Thomas Maal

Standardization of the techniques for surgical repair of the clefts can help in patient care, as they all have they own advantages. It is theorized that late closure of the palate will minimize the deformation of the maxilla, as early surgery will help in speech development. As not every patient is the same, individualization of the patients can help choosing the right treatment protocol for optimal results. A better understanding in the growth processes, and more important the differences in growth processes, are key for better patient care. In recent literature there are almost no articles which describe the alveolar and palatal changes during the development of a child with CP. What is often seen in literature is the use of 2D techniques like cephalograms and describing growth in two dimensions. Plaster casts are made at fixed times during the development of the child with CP. These can be used to perform 3D measurements to compare the differences between each time interval, creating a growth model. Rousseau et al. [2] and Fernandez et al. [3] already performed the first steps doing this. However only a few landmarks were used to determine the maxillary width. Increasing the amount

of landmarks will increase the representation of the shape of the maxillary arch, and the measurements can be seen on each section of the maxilla (shown in figure 1 and 2).
Better understanding of the maxillary growth in CP patients can help in the decision making during the child's CP trajectory. Therefore the ultimate goal is to create a three dimensional growth model based on plaster cast models which were obtained during multiple timestamps in the child's development. This model then can be used to achieve the best outcome in new CP patients, creating a more evidence based decision.

Functional analysis of constructs for soft palate repair
Doris Rosero, Paola Carvajal Monroy, Hans Von den Hoff

Background: Cleft lip and/or palate (CLP) is a common congenital facial malformation occurring in 1/500 to 1/1000 newborns. About 45% of all children with CLP have a cleft of the soft palate. After surgical repair, velopharyngeal dysfunction persists in about 30% of these children resulting in speech abnormalities. Fibrosis and defective muscle regeneration are key causative factors.
Clinical relevance: Surgical repair of the soft palate in CLP patients needs to be improved to enhance speech and quality of life of these children.
Hypothesis: We hypothesize that the function of the soft palate will improve by the application of muscle constructs during surgery.
Research questions: The main research question is: Can we improve soft palate function in our rat model for soft palate repair by the application of a muscle construct? Our sub questions are:
1. How can we measure soft palate function in our rat model?
2. How can we develop muscle constructs for repair of the soft palate?
3. Can we improve muscle function with these muscle constructs?
Methodology: The function of the soft palate will be measured by electromyography in vivo or by electro stimulation of the isolated soft palate ex vivo. Fibrin-based constructs will be prepared with two-component fibrin glue, isolated satellite cells and/or growth factors. The rat model and techniques for the isolation of satellite cells were developed in a previous project supported by the Osteology Foundation (12-008).

Patiënt perception of Mini-screw Assisted Rapid Palatal Expansion in adults
Aldin Kapetanovic, Jan Schols, Rene Noverraz,Tong Xi

MARPE or Mini-screw Assisted Rapid Palatal Expansion is a treatment to achieve widening of the upper jaw in adults aged 16 years or older (adolescents and adults) without a previous surgical procedure in which a number of maxillary sutures are released (Surgically- Assisted Rapid Maxillary Expansion). The MARPE treatment of adult patients with maxillary constriction is currently the standard clinical treatment within the orthodontics department at Radboudumc.With the help of 4 mini screws in the palate, the expansion forces engage in the center of the resistance. By turning the expansion screw daily, the midpalatal suture can be opened and the maxilla can be widened. In the initial phase of expansion, a relatively large force is exerted on the midpalatal suture, in which usually some pain is experienced by the patients (Brunetto et al., 2017). With a view to continuous improvement of our treatments, in this case MARPE in adults, we want to investigate the experience of our patients, in particular their impact and pain. It is known that pain can have a negative influence on the (motivation for) treatment and is therefore an important factor that needs to be taken into account (Banerjee et al., 2018, Greene, 2009, Krishnan, 2007). Nevertheless, this has never been investigated before MARPE. Understanding patient perception is an essential step towards managing pain and impact on the patient and thus also on the treatment itself.

Investigating craniofacial development by targeting candidate genes in zebrafish models

Liesbeth Gebuijs, Hans Vonden Hoff, Frank Wagener, Carine Carels

Using exome sequencing of patient DNA it is possible to decipher what genes are involved in disturbed palatogenesis and orofacial clefting. Candidate genes found with this technique in our laboratory including LRP6. The zebrafish model enables investigation to putative genetic and environmental factors involved in craniofacial development. In the present project, we aim to better understand the molecular mechanisms involved in palatogenesis and clefting by translating human craniofacial malformations to zebrafish models. Hereto, we will pharmacologically or genetically (using e.g. morpholinos) target zebrafish embryos and investigate its' effects on craniofacial development. We expect that establishing craniofacial zebrafish models in our laboratory allows development of preventive and/or therapeutic strategies.