AAO Foundation Final Report Form
(a/o 2/9/2021)

In an attempt to make things a little easier for the reviewer who will read this report, please consider these two questions before this is sent for review:
- *Is this an example of your very best work, in that it provides sufficient explanation and justification, and is something otherwise worthy of publication? (We do publish the Final Report on our website, so this does need to be complete and polished.)*
- *Does this Final Report provide the level of detail, etc. that you would expect, if you were the reviewer?*

Please prepare a report that addresses the following:

Type of Award
Orthodontic Faculty Development Fellowship Award

Name(s) of Principal Investigator(s)
Chenshuang Li

Institution
University of Pennsylvania School of Dental Medicine

Title of Project
Establishing a rat model for experimental studies of craniofacial growth following cleft lip and palate

Period of AAOF Support (e.g. 07-01-2021 to 06-30-2022):
07-01-2021 to 06-30-2022

Amount of Funding
$ 20,000
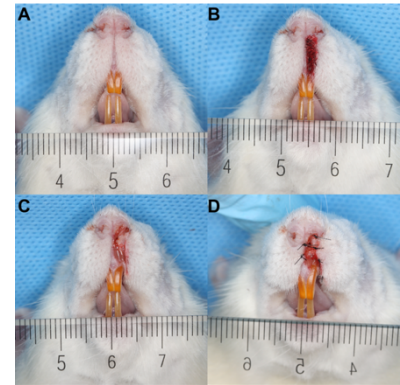
Summary/Abstract
Cleft lip and palate (CLP) is the second most common congenital malformation, affecting one in every 600 births worldwide. Due to the significant lack of local tissue, extensive scarring is a common complication after early repair of the cleft lip that vitally impacts patients' maxilla growth
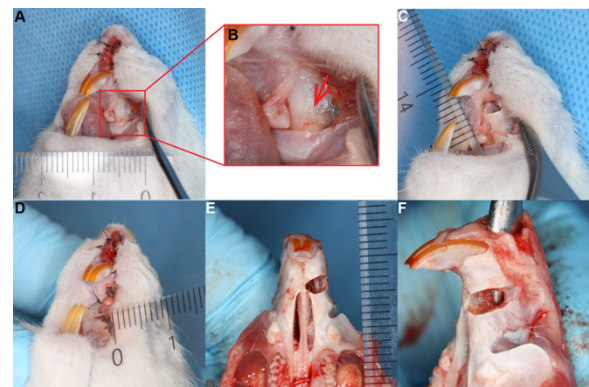
and development, while an urgent need exists for seeking effective craniofacial tissue regenerative approaches in early CLP revision. Unfortunately, the disadvantages of the currently available animal models hurdle them from properly mimicking human CLP development, particularly the CLP revision outcome assessment. For instance, in utero congenitally induced models require immense technical expertise and relate to multiple fetal malformations, increased intrauterine fetal death and abortions, and large variation of the cleft severity. Meanwhile, only dogs and monkeys were used as surgical-induced models representing the CLP conditions in young patients. Not only being expensive, but these models are hardly used for cell-based regenerative approaches since immunosuppression has to be applied to permit heterogeneous cell usage, which may lead to an intricate argument in data interpretation. Moreover, no published surgical small animal models are accompanied by defect development before puberty, and thus the impact of early cleft lip repair on craniofacial growth and development has not been fully elucidated. To conquer these questions, in the current proposal, we intend to establish a novel CLP model in young rodents to mimic the craniofacial growth deformation observed in CLP patients. Among the commonly used small experimental animals, rats are extremely useful for conducting basic research involving the skeleton based on the bone mass and structure measurements, and thus represent reliable and affordable alternatives to large animals. Notably, the craniofacial growth pattern of rats has been deeply documented and correlated to that of humans, making rats more advantageous for representing human CLP development than other small animals. With the support of AAOF, we have successfully established two rat models:

Fig. 1: Surgical creation of cleft lip in rats. (A) The submental view of a rat before surgery. **(B)** To ensure the size of the cleft lip, the soft tissue to be removed is marked with ink (the red line on the left half of the upper lip). **(C)** After soft tissue removal, an even 2-mm soft tissue gap is created from the left nasal base to the vemillion border. **(D)** The left side skin and mucosa are sutured together and the right side skin and mucosa are sutured together to close the wound edge on each side of the created cleft lip.

First model - cleft lip model: A whole thickness of the soft tissue block containing the skin to the oral mucosa has been removed lateral to the fusion site, from the edge of the upper lip margin up to the base of the nose, forming a 2-mm width defect (Fig. 1). On each side of the defect, the skin layer was sutured with the oral mucosa layer by 3-0 nylon threads to close the open wound.

Second model - Cleft lip + alveolar cleft model: The cleft lip was created as described above. At the same time, a mucosal incision was made perpendicular to the maxillary left alveolar ridge in the midpoint between the maxillary left central incisor and maxillary left first molar. The incision was connected with the cleft lip on the buccal side and stop at the midline on the palatal side. The gingivae and soft tissue were elevated to expose the left premaxillary-maxillary suture. The alveolar bone was removed along the suture by a slow handpiece to create a linear bony defect with a width of 2 mm. Next, an incision was created on the nasal mucosa and then sutured with gingivae by 3-0 nylon threads on both the medial and lateral sides of the alveolar bone defect to form the oronasal fistula (Fig. 2).

Fig. 2: Surgical creation of alveolar cleft in rats. (A) After cleft lip creation, the oral mocusa covering the left alveolar process is flapped. **(B)** Enlarged photo to show the left premaxillary-maxillary suture (red arrow). **(C)** An even 2-mm hard tissue gap is created at the alveolar process. **(D)** The oral mocusa is sutured with the nasal mocusa to mimic the oronasal fistula. **(E)** To clearly demonstrate the location of the created alveolar cleft, soft tissue is removed. **(F)** A stainless steel surgical instrument is inserted into the nasal cavity to show the connection between the oral cavity and nasal cavity after creation of the alveolar cleft.

The prelimiary data generated from this AAOF supported projects have led to a NIH grant submission (Application ID: 1 R03 DE030400-01A1). In addition, through the generous support from this AAOF Orthodontic Faculty Development Fellowship Award, several publications have been accepted in peer-reviewed journals. Please see the publication listed below.

Respond to the following questions:

1. Were the original, specific aims of the proposal realized?
    Yes, The prelimiary data generated from this AAOF supported projects have led to a NIH grant submission

2. Were the results published?
    a. If so, cite reference/s for publication/s including titles, dates, author or co-authors, journal, issue and page numbers
    With the support of AAOF following peer-reviewed articles were published as part of my early career development:
    1) **Li, C.**, Zheng, Z.: Male and Females Have Distinct Molecular Events in the Articular Cartilage during Knee Osteoarthritis. International Journal of Molecular Science, 22(15):7876, 2021. PMID: 34360640
    2) **Li, C. (co-first and co-corresponding author)**, Teixeira, H., Tanna, N., Zheng, Z., Chen, S. H., Zou, M., Chung, C.-H.: The Reliability of Two- and Three-Dimensional Cephalometric Measurements: A CBCT Study. Diagnostics, 11(12): 2292, 2021. PMID: 3493528
    3) Syverson, A., **Li, C. (co-first and co-corresponding author)**, Zheng, Z., Proskurnin, E., chung, C.-H., Zou, M.: Maxillary Sinus Dimensions in Skeletal Class II Population with Different Vertical Skeletal Patterns. Clinical Oral Investigations, 2022 Mar 31. Online ahead of print. PMID: 35359186
    4) Gershater, E., **Li, C. (co-first and co-corresponding author)**, Ha, P., Chung, C.-H., Tanna, N., Zou, M., Zheng, Z.: Genes and Pathways Associated With Skeletal Sagittal Malocclusions: A Systematic Review. International Journal of Molecular Science, 22(23): 13037, 2021. PMID: 34884839
    5) Huang, G., Yang, M., Qali, M., Wang, T.-J., **Li, C. (co-corresponding author)**, Chang, Y.-C.: Clinical Considerations in Orthodontically Forced Eruption for Restorative Purposes. Journal of Clinical Medicine. 10(24): 5950, 2021. PMID: 34945246
    6) **Li, C. (first and co-corresponding author)**, Jiang, W., Chen, S.-C., Borenstein, K., Tanna, N., Chung, C-H., Moon, W.: En-Mass Retraction of Maxillary Anterior Teeth with Severe Proclination and Root Resorption – A Case Report. Diagnostics, 12(5), 1055, 2022. PMID: 35626211
    7) Xu, X., Ha, P., Yen, E., **Li, C. (co-corresponding author)**, Zheng, Z.: Small Leucine-Rich Proteoglycans in Tendon Wound Healing. Adv Wound Care (New Rochelle) 11(4): 202-214. 2022. PMID: 34978952
    8) Ha, P., Liu, T., **Li, C. (co-corresponding author)**, Zheng, Z.: Novel Strategies for Orofacial Soft Tissue Regeneration. Adv Wound Care (New Rochelle), 2022 Jun 1. doi: 10.1089/wound.2022.0037. Online ahead of print. PMID: 35651274.

9) Orr, J.C., **Li, C. (co-first and co-corresponding author)**, Shah, S., Backstrand, M.R., Chung, C.-H., Boucher, N.S.: Mandibular Transverse Dentoalveolar and Skeletal Changes Associated with Lip Bumper and Rapid Maxillary Expander: CBCT Study. American Journal of Orthodontics and Dentofacial Orthopedics, accepted on 12/27/2021.

10) Miao, Y., Chang, Y.-C., Tanna, N., Almer, N., Chung, C.-H., Zou, M., Zheng, Z., **Li, C. (co-corresponding author)**: Impact of Frontier Development of Alveolar Bone Grafting on Orthodontic Tooth Movement. Frontiers in Bioengineering and Biotechnology, accpeted on 6/13/2022.

   b. Was AAOF support acknowledged?
     Yes, AAOF support was acknowledged in all of the manuscripts listed above.

   c. If not, are there plans to publish? If not, why not?
     N/A

3. Have the results of this proposal been presented?
   a. If so, list titles, author or co-authors of these presentation/s, year and locations

1) Syverson, A., Proskurnin, E., Boucher, N., Mupparapu, M., Chung, C.-H., Zou, M., **Li, C.**: Maxillary Sinus Differs Among Vertical Patterns of Class II Populations. (Presented at the 2021 Penn Dental Medicine Research Day, 2021).

2) Kilgman, S., Ren, Z., Chung, C.-H., Perillo, M.A., Chang, Y.-C., Koo, H., Zheng, Z., **Li, C.**: The Impact of Dental Implant Surface Modifications on Osseointegration and Biofilm Formation. (Presented at the 2021 Penn Dental Medicine Research Day, 2021).

3) Syverson, A., Proskurnin, E., Boucher, N., Mupparapu, M., Chung, C., Zou, M., **Li, C.**: Maxillary Sinus Differs Among Vertical Patterns of Class II Populations. (Presented at the 2021 General Session and Exhibition of International/American/Canadian Association for Dental Research, 2021).

4) Al Abdullah, U., Lin, J-H., **Li, C.**: Transverse Asymmetric Expansion Following Surgically Assisted Rapid Palatal Expansion (SARPE): A Systematic Review. (Presented at the Greater New York Dental Meeting 97th Annual Session, New York City, 2021).

5) **Li, C.**, Zheng, Z.. Genes and Pathways Associated With Skeletal Malocclusions. (Presented as table clinic at the AAO 2022 Annual Meeting, Miami Beach, Florida, 2022).

6) Shin, M. K., Liu. Y., **Li, C.**: Oral And Oronasal Biofilm In Cleft Lip And Palate Patients: A Comprehensive Review. (Presented at the 2022 Penn Dental Medicine Advances in Clinical Care and Education (ACCE) Day, 2022).

7) Miao, Y., Chang, Y.-C., Tanna, N., Almer, N.J., Chung, C.-H., Zou, M., Zheng, Z., **Li, C.**: The Impact Of Novel Alveolar Bone Graft Materials On Orthodontic Tooth Movement: A Review. (Presented at the 2022 Penn Dental Medicine Advances in Clinical Care and Education (ACCE) Day, 2022).

8) Yang, M.S., Zhong, Z., Tanna, N., Chung, C.-H., **Li, C.**: Gingival Soft Tissue Analysis In Orthodontic Treatment Planning - The Segmentation Method Study Using Pig Jaw. (Presented at the 2022 Penn Dental Medicine Advances in Clinical Care and Education (ACCE) Day, 2022).

9) **Li, C.**, Orr, J.C., Shah, S., Backstrand, M.R., Chung, C.-H., Boucher, N.S.. Lip Bumper and Rapid Maxillary Expander Induced Mandibular Transverse Changes. (Presented at 2022 IADR/APR General Session, 2022)

b. Was AAOF support acknowledged?
   Yes, AAOF support was acknowledged in all of the conference abstract listed above.

c. If not, are there plans to do so? If not, why not?
   N/A

4. To what extent have you used, or how do you intend to use, AAOF funding to further your career?
   The support from the AAOF is critical for me to produce the preliminary data and develop a competitive research program aimed at obtaining NIH grants; as well as to follow a well-structured education, teaching, and clinical practice plan to pursue my academic career in orthodontics.

Accounting for Project; (i.e.), any leftover funds, etc.
Compensation: $1,436.86
Travel: $ 190
Supplies: $ 10,685.94
Professional Services: 7,687.20
Leftover funds: $0