Dr Angelo Leone's report

Research carried out at the Craniofacial Development and Stem cell Biolofy of King's College London from January the 2nd 2015 to October the 4th 2015.

Research Topics:
1)Adult cell sources for biological tooth replacement

2) Histological and immunohistochemistry investigation in replanted teeth.

3) New frontiers in teaching Histology and Embryology online.

Research Background On Topic 1
This project forms pat of an ongoing research program in the laboratory of Professor Pal Sharpe aimed at developing cell-based methods of tooth repair and regeneration. This program was initiated following the landmark publication showing transplantation into adult jaw formed teeth that erupt and formed roots (Ohazama et al 2004; Sharpe and Young 2005).

Theoretical review
Tooth loss is common to a large proportion of the population and leads to the requirement for implantation of non-biological implants that have a limited lifespan. Therefore, production of a biological tooth using stem cell approaches would be a favourable alternative. Tooth development is a complex process that results from multiple signaling events between the dental epithelial and mesenchymal compartments. These signaling events have been well documented thanksto gene knockout approaches in the mouse. Tooth germs have been shown to develp successfully following their dissociation and re-association of the cellular components even without a scaffold. Recently, it has become

apparent that de novo tooth regeneration can be achieved using stem cells in process that mimics developmental odontogenesis (Sharpe and Young 2005)

Research goals

Adult human mesenchymal neural crest-drived stem cells lack the capacity to induce tooth formation that is present in embryonic tooth cells that are also derived from neural crest (Volponi et al 2010). Since adult cells are a more practical source than embryonic for therapeutic applications, this project aims to identify methods of altering adult human cells to become tooth inducing. By utilizing cell and molecular approaches, this project will lead towards the novo generation of teeth for implantation in patients lacking full dentition. This will have a major positive impact on health status and quality of life and will provide a viable therapy for trauma patients as well as those experiencing age –related tooth loss.

Research Methods

Microarray gene identification screens will be used to identify the key tooth inducing transcription factor gene expressed in embryonic cells by analysis of gene profiles of human embryonic tooth cells at different stages of development. Following verification using qPCR and in situ hybridization, the genes identified will be used in an iPS-like process to reprogram adult tooth cells to an embryonic tooth cell state that has tooth inducing capacity (Induced Odontogenic Capacity-iOC). In a parallel approach, conventional iPS of adult dental cells will be used to partially reprogram them to an embryonic neural crest-like state which can then be induced to form odontogenic mesenchyme cells.

A third line of investigation will involve an analysis of the effects of signaling pathway stimulation of non-odontogenic mesenchyme cells to induce odontogenic capacity. This will

involve treatment of stem cell cultures with signaling pathway agonists and antagonists and gene expression analysis using qPCR.

Cell testing will involve combination of iOC cells with adult odontogenic epithelial cells in a scaffold matrix to promote tooth formation in vitro and in vivo using methods already developed.

Time Plan

This project accommodated in Department of Craniofacial Development, where Dr Angelo Leone is visiting Professor since 2009.

It was planned a 4 years research studies to put in place the above described project.

At the end of this dissertation results obtained in the first 10 months from Doctor's Leone work will be presented.

Along the mentioned project Dr Leone carried out a histological and immunohistochemistry research on dental pulp coming from replanted human teeth.

An alternative method to replaced missing teeth can be the replantation of the lost tooth, for example after accident, where in general those teeth are intact. The aim was to understand the roots sprouting mechanisms in the alveolar bone and if the structural changes of the dental pulp and odontoblasts, due to the interruption of its blood supply, can be reversible and therefore the possibility of the tooth to regain full vitality. A series of molecules iNOS, BCL-2, MMP9, MMP2, Annexin V were studied immunohistochemically to help us to elucidate those events.

Beside laboratory work, Dr Leone completed a teaching research about Feedback and assessment in Histolgy and Embryology for Dental Students.

Histology online teaching is one of Dr Leone's interests in teaching and an experiment was carried out with first year program dental student, Result of this experiment will be

available in January 2016, The submitted paper was accepted and it is in PRESS.

Results.
1) The results of the qPCR stem cells from Dental Bud are described in the following histogram.
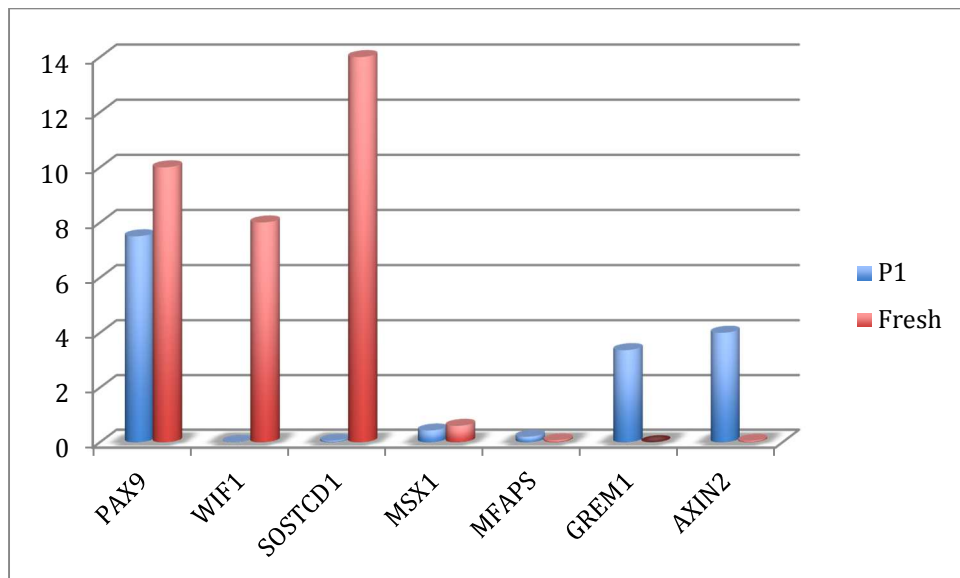
This represent the first phase of the project. The difference in expession of odontogenic genes between fresh and coltured cells .

2) Dental Pulp in mature replanted human teeth: Morphological alteration and metalloproteinases-2 and -9, Annexin-5, BCL-2 and iNOS modulation. **A.Leone**et al Paper accepted October 5, 2015

3) Assessment and Feedback: essential for an Effective Online Learning of Histology.   Published on JADE journal , August 2015
Article 2 pages 21-31 available online.

Proofs are attached to this report.
Dr Angelo Leone inform the Bionec that he will continue the project with the Kings College during the 2016.

Angelo Leone   Palermo November the 3rd 2015