PROCORE - FRANCE/HONG KONG JOINT RESEARCH SCHEME
COMPLETION REPORT

Project Reference Number

F-HK37/10T

Project Title

Investigation of the interplay of polyphosphates and matrix vesicles in bone mineralization

Particulars

	Hong Kong team	French team
Name and title of Principal Investigator (PI))	English: Dr. Julian A. Tanner Chinese: N/A	Professor Rene Buchet
Position	Assistant Professor	Professor
Name of Co-Investigator (if any)	English: Dr. Lina Li Chinese:李丽娜	Dr. Saida Mebarek
Position	Post-doctoral Research Associate	Assistant Professor
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Name(s) of other project team members (if any)	N/A	Prof. David Magne, University of Lyon 1

Funding Period

	1st year	2nd year (if applicable)
Start Date	1/1/11	N/A
Completion Date	31/12/11	N/A

Objective(s) as per original application

1. Identify and quantify the levels of inorganic polyphosphate (polyP) in matrix vesicles (MVs) relative to total cellular levels in osteoblasts and chondrocytes.

2. Clarify the functional involvement of polyP within MVs in the first step of MV-mediated bone matrix mineralization, as well as within cells (osteoblast-like Saos-2 and chondrocytes) during mineral formation.

3. Investigate the metabolism of polyP in MVs and in cells, and identify the enzymes implicated in the process of polyP metabolism within MVs and cells.

recorded (Revision 06/11)

[Please attach relevant document(s)]

i) Outline of proposed research and results obtained

We have completed each of the original objectives as outlined for each objective below:

Objective 1. Identify and quantify the levels of inorganic polyphosphate (polyP) in matrix vesicles (MVs) relative to total cellular levels in osteoblasts and chondrocytes.

Dr. Lina Li developed an innovative approach to measure the levels of polyP using fluorescent techniques with DAPI. Working on osteoblast cell cultures Dr. Lina Li observed the highest concentration of polyP anywhere in the human body within matrix vesicles. In collaboration with the University of Lyon with the help of this grant, we conducted similar experiments in parallel using matrix vesicles isolated from chicken embryo femurs collected and analysed at the University of Lyon. These data also showed extraordinarily high concentrations of matrix vesicles. Polyphosphate is enriched in matrix vesicles approximately 10-fold over the concentrations within the cells.

Objective 2. Clarify the functional involvement of polyP within MVs in the first step of MV-mediated bone matrix mineralization, as well as within cells (osteoblast-like Saos-2 and chondrocytes) during mineral formation.

Hydroxyapatite mineralization has long thought to be controlled by the balance between orthophosphate and pyrophosphate. In this collaboration, we also investigated the impact of polyphosphate on rates of mineralization and on cell gene expression. Two directions were performed – in Lyon the co-investigators studied the effects of polyP directly on hydroxyapatite formation in vitro, and observed that polyP potently inhibits mineralization. In Hong Kong, we studied the effect of polyP on SaOS-2 gene expression using microarrays and observed a number of genes including interleukin 11 upregulated. This is the first example of a direct effect of polyphosphate on cytokines and carries implications for polyP roles in cellular signaling.

Objective 3. Investigate the metabolism of polyP in MVs and in cells, and identify the enzymes implicated in the process of polyP metabolism within MVs and cells.

Using the newly developed assays of polyP observation in matrix vesicles, we were able to observe direct synthesis of polyP by purified matrix vesicles. Matrix vesicles were purified by differential centrifugation, and their purity was confirmed by electron-microscopy. When incubated with orthophosphate and calcium at appropriate concentrations then polyP synthesis could be observed with a polyP product approximately 300-500 phosphates synthesized. If the membranes were disrupted then the polyphosphate synthesis was also disrupted. Our results imply that there is a polyP synthesis mechanism in matrix vesicles themselves, which is possibly membrane-related. Our newly developing hypothesis is that ion pumping across the membrane drives polyP synthesis by an as-yet-undetermined membrane protein in matrix vesicles.

ii) Significance of research results

Whilst major progress has been made in the understanding of polyP function in prokaryotes, polyP function in higher eukaryotes, particularly humans, remain unclear. We have been the first research group internationally to investigate polyphosphates in matrix vesicles specifically, and have observed polyphosphates to be particularly highly enriched in matrix vesicles, to an extent that this is the highest concentration anywhere in the human body. Furthermore, we have observed a direct link between polyphosphate and the cytokine interleukin 11. As interleukin 11 has been implicated in the control of remodeling then this may be an important link between mineralization and control of osteoblast functioning. Finally, observing synthesis of polyP by a matrix vesicle is particularly exciting as we still do not know how polyphosphates are synthesized in higher eukaryotes, and if the protein is present in matrix vesicle this narrows down the candidate for polyP synthesis considerably. Taken together, the research which has been performed as a result of this France Procore grant has been highly significant.

iii) Research output

Research Papers – Submitted and in Preparation.

Lui, E.L.H., Li, L., Shum, K.T. & Tanner, J.A. Microarray analysis reveals interleukin 11 is upregulated in response to inorganic polyphosphate in osteoblasts (submitted to Journal of Bone and Mineral Research in June 2012, under review). (Appendix A)

Li, L., Lui, E.L.H., Ao, C.K.L., Magne, D., Mebarek, S., Buchet, R. & Tanner J.A. Enrichment and synthesis of long-chain polyphosphate by matrix vesicles from osteoblasts and chondrocytes (prepared and to be submitted to JBC July 2012). (Appendix B)

Research Seminars

Lina, L., Lui, E. & Tanner, J.A. Inorganic polyphosphate and bone - new roles for an ancient molecule. Procore Collaboration Meeting, University of Lyon, France (May 2011)

R. Buchet. Phosphate and Bone Mineralization. Procore Collaboration Meeting, University of Hong Kong (September 2011)

iv) Potential for or impact on further research collaboration

Two major grants were submitted as a result of this collaboration which will build to strengthen and reinforce this research collaboration:

Hong Kong RGC GRF 2012/2013: Defining the Roles of the Fundamental Macromolecule Inorganic Polyphosphate in Osteoblasts. PI: Dr. J.A. Tanner, CoIs: Prof. R. Buchet, Prof. D. Magne, Dr. R.M.Watt

France Premise Programme Blanc Consortium Grant Proposal 2012/2013: Phosphate, pyrophosphate and polyphosphate : Their regulation mechanisms at mineralization, enzyme and osseous-cell levels. PI: Prof R. Buchet, CoI includes Dr. J.A. Tanner.