P1-2

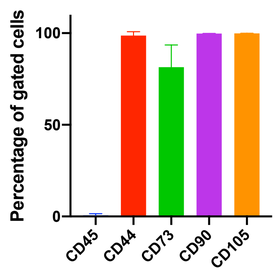
Extracellular Matrix Derived from Dental Pulp Stem Cells Promotes Mineralization

Nunthawan Nowwarote[1], Stephane Petit[2], Francois Come Ferre[2], F Dingli[3], D Loew[3], Thanaphum Osathanon[1,4], Benjamin P.J. Fournier[2,5]

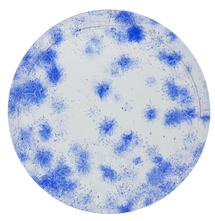
[1] Center of Excellence for Regenerative Dentistry, Faculty of Dentistry, Chulalongkorn University, Bangkok 10330 THAILAND
[2] Centre de Recherche des Cordeliers, Universite de Paris, Sorbonne Universite, INSERM UMRS 1138, Molecular Oral Pathophysiology; Paris, F-75006, France
[3] Institut Curie, PSL Research University, Laboratoire de Spectrométrie de Masse Protéomique, 26 rue d'Ulm, Paris 75248, France
[4] Genomics and Precision Dentistry Research Unit and Department of Anatomy, Faculty of Dentistry, Chulalongkorn University, Bangkok 10330 THAILAND
[5] Universite de Paris, Dental Faculty Garanciere. Paris, F-75006, France

Dental pulp cells exhibited the characteristic of mesenchymal stem cells

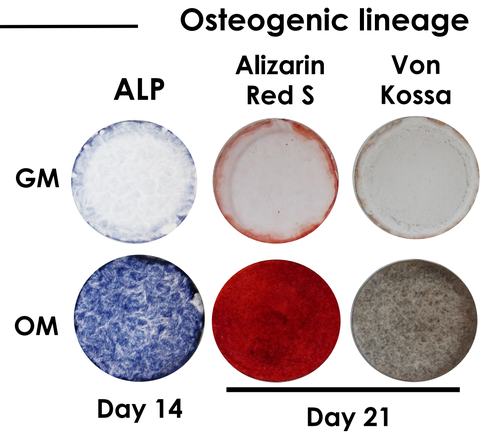
Surface Marker

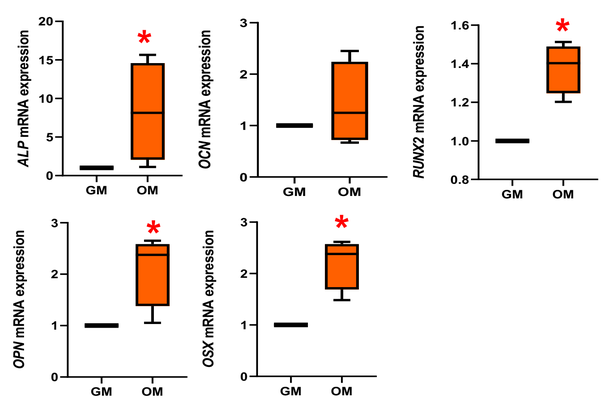


Self-renewal

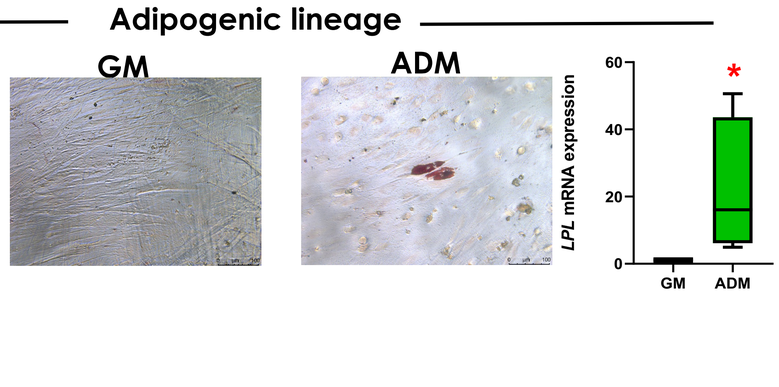


Osteogenic lineage





Adipogenic lineage



Extracellular matrix production and decellularization

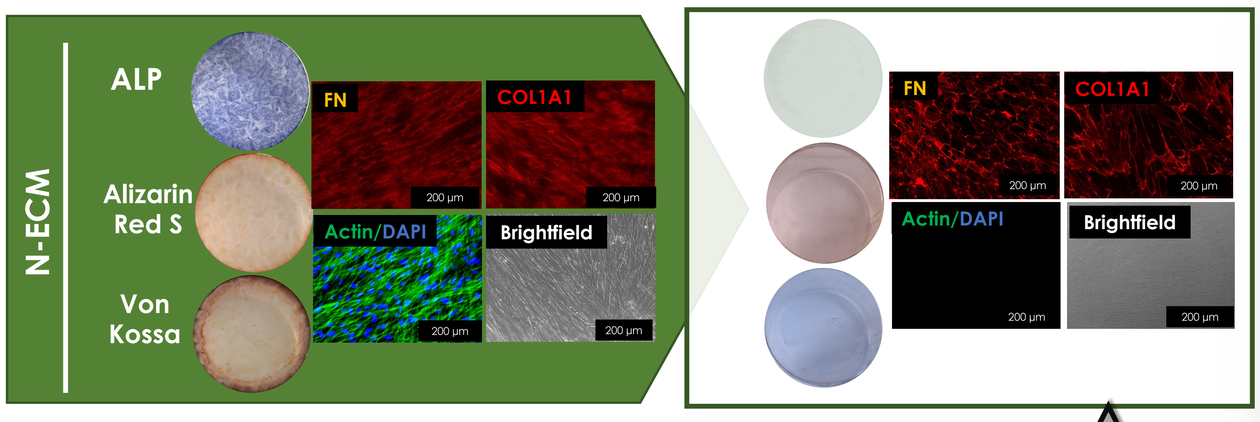
ECM were extracted from DPSCs cultured in normal growth medium supplemented with L-ascorbic acid (N- ECM) or in osteogenic induction medium (OM-ECM). ECM decellularization (dECM) was performed using 0.5% triton X-100 in 20mM ammonium hydroxide after 21 days. Mass spectrometry and proteomic analysis identified and quantified matrisome proteins.

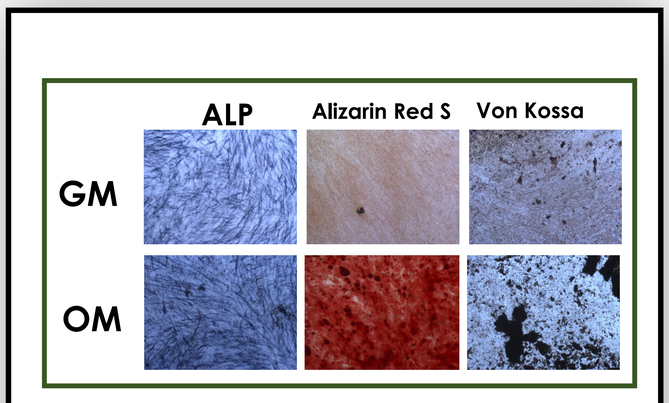
Decellularized extracellular matrix enhanced osteogenic differentiation and mineralization

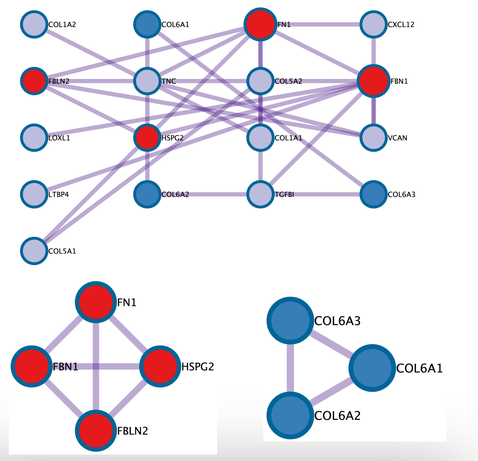
GF were reseeded on N-dECM and OM-dECM and cultured in normal or osteogenic medium. GF were able to attach and proliferate on N-dECM and OM-dECM. Both dECM enhanced mineralization of GF at day 14 compared to tissue culture plate (TCP). In addition, OM-dECM promoted higher mineralization of GF than N-dECM although cultured in growth medium.

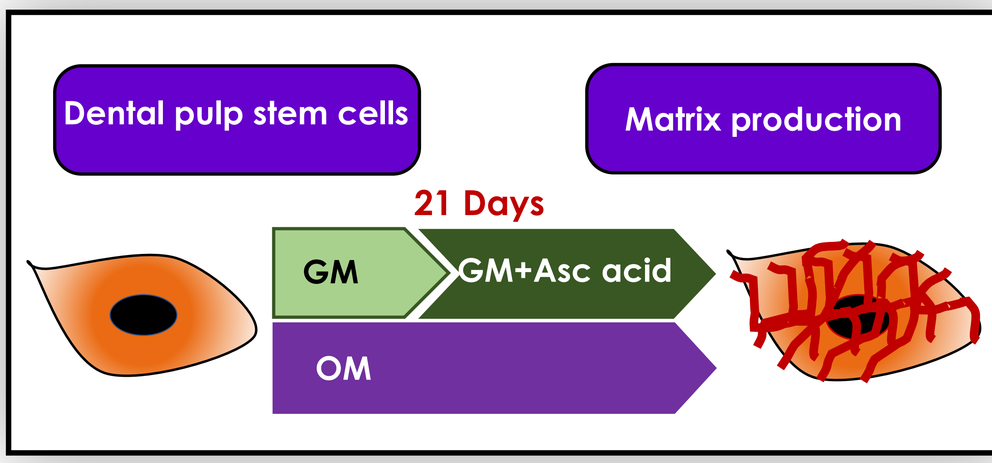
Matrisome analysis of extracellular matrix

Extracellular matrix derived from dental pulp stem cells exhibited specific matrisome protein of elastic fiber in N-ECM and affiliated proteins in OM-ECM.









Decellularization

Reseeding

Gingival fibroblast

Osteogenic induction

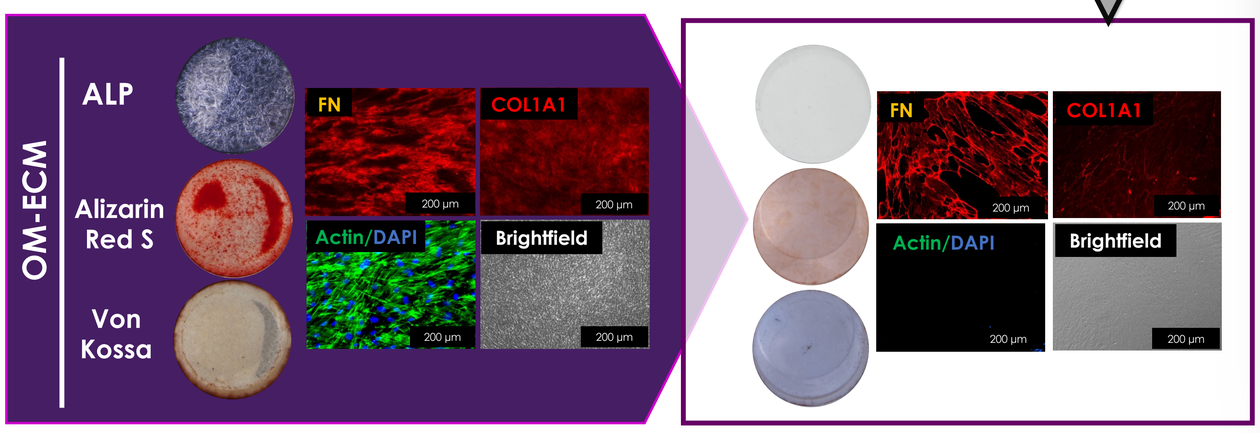
14 Days

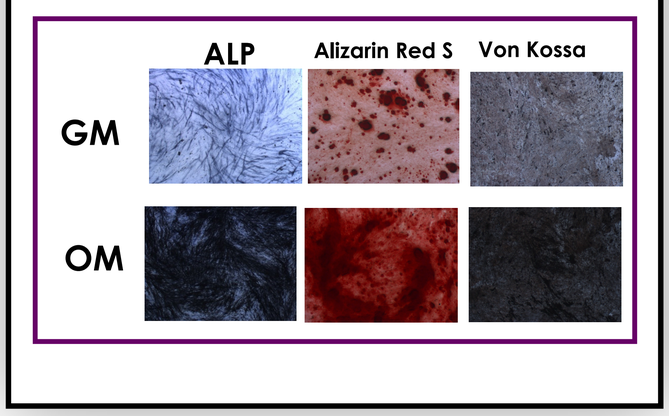
Promote mineralization

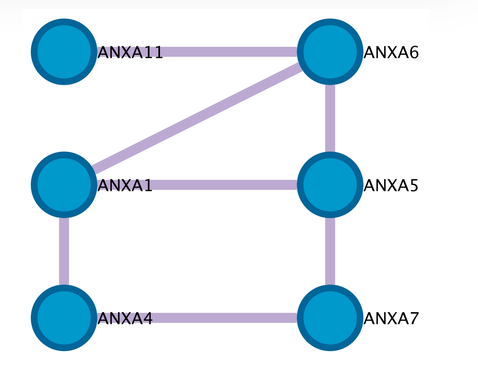
Collagens and Elastic fibers in N-ECM

Matrisome Analysis

Affiliated proteins in OM-ECM







Conclusion

Matrisome proteins of DPSCs derived ECM differ according to culture conditions, with increased core matrisome proteins (collagens, elastic fibres associated) in N-ECM while matrisome associated proteins (annexins) were enriched in OM-ECM. We demonstrated that ECM impacts cell behavior and differentiation. DPSCs ECM proved to be mineralization inductive. Our identified ECM proteins can be used as a marker for osteogenic differentiation. Further study will investigate the patterns of those proteins during osteogenic differentiation. This *in vitro* ECM or some of its proteins may be helpful for tissue engineering and could be used for mineralized tissue therapy or to decorate biomaterials.

Acknowledgements

- Ratchadapisek Sompote Fund for Postdoctoral Fellowship, Chulalongkorn University and Junior Research Fellowship Program, French Embassy Junior Research Fellowship Program, French Embassy, Thailand.
- Franco-Thai Mobility Programme/PHC SIAM
- Thailand Science Research and Innovation Fund Chulalongkorn University (CU_FRB65_hea(2_008_32_03)).
- INSERM/APHP INTERFACE, "La Fondation des Gueules Cassées, Union des Blessés de la Face et de la Tête (FR)
- "Région Ile-de-France" and Fondation pour la Recherche Médicale grants

Reference

- Naba, H. Ding, C.A. Whittaker, R.O. Hynes, http://matrisomeproject.mit.edu.
- Osathanon, T., N. Nowwarote and P. Pavasant (2011). "Basic fibroblast growth factor inhibits mineralization but induces neuronal differentiation by human dental pulp stem cells through a FGFR and PLCgamma signaling pathway." J Cell Biochem 112(7): 1807-1816.