



OHSU

Location-specific differentiation potential of clonal articular cartilage progenitor cells

Leah Snyder, B.S.^{1,2}, Phillip Lam, M.D.^{1,2}, Ajit Elhance, B.S.²,
Kenneth Weekes, B.S.^{2,3}, Jonas Kruckel, B.S.², Brian
Johnstone, Ph.D.

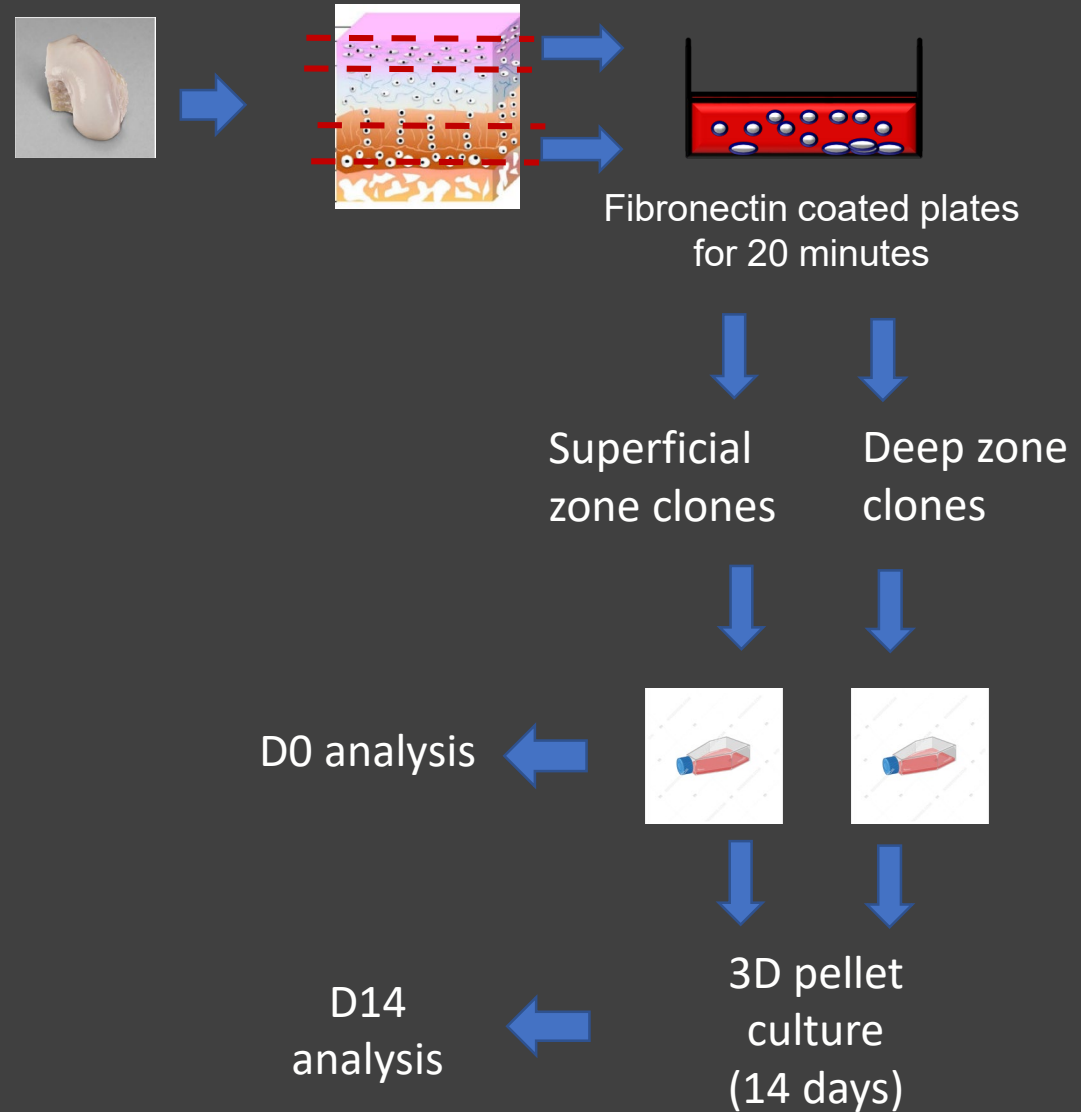
Department of Orthopaedics and Rehabilitation

Introduction

- Damage to articular cartilage poses significant morbidity and leads to osteoarthritis^{2-4, 7}
 - Articular cartilage has poor regenerative potential
 - Significant limitations of all existing treatment options^{1,5-6,8}
 - Formerly believed that articular cartilage lacks progenitor populations
- Articular cartilage progenitors (ACPs) that form stable cartilage have been isolated and cloned⁹
- Recently found a subset that undergoes hypertrophy (endochondral phenotype)
- Aim: to determine the location of ACPs that form the two distinct phenotypes
- Relevance: tissue engineering to address relative lack of effective treatment for articular cartilage damage

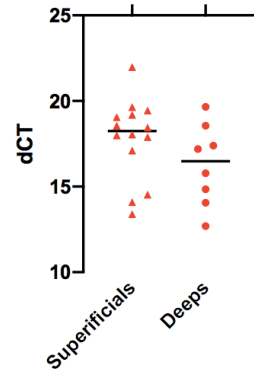
Methods

- Human articular cartilage from OCAs
- Punch biopsy, separation of superficial vs deep zones
- Selection for progenitor cells on fibronectin⁹
- Isolate and expand clones
- Collect for day 0 analysis
- Pellet and grow in 3D culture for 14 days
- Analysis: gene expression, collagen content and activity, extracellular matrix production, population doubling time

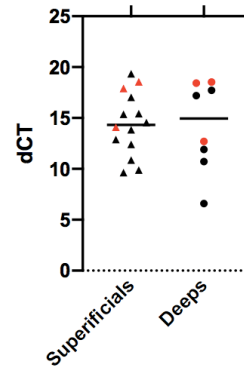




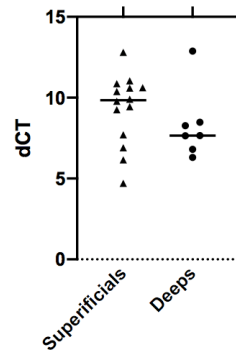
COL2A1 d0



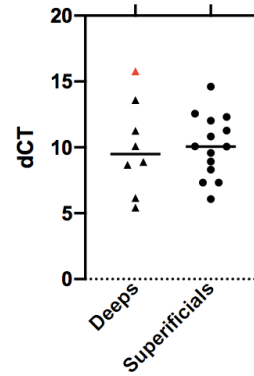
COL10A1 d0



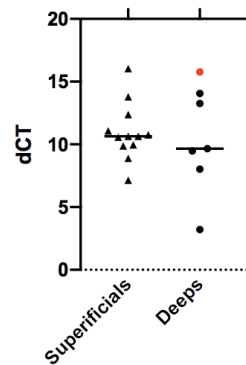
SOX9 d0



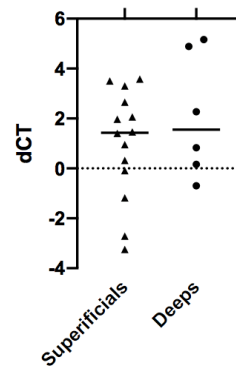
ACAN d0



PRG4 d0

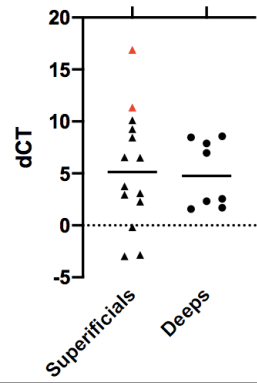


COL1A1 d0

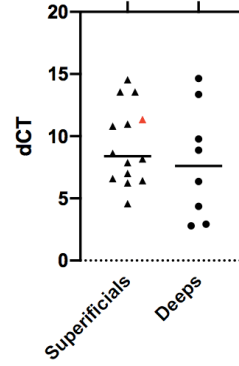


Results— d0 Gene Expression

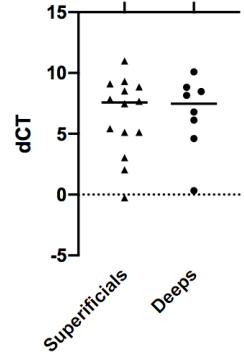
COL2A1 d14



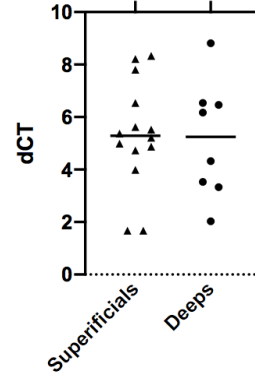
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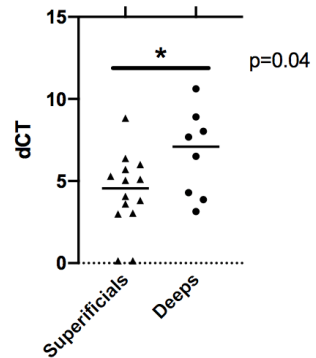
SOX9 d14



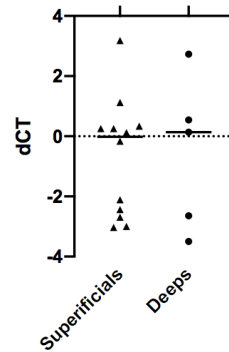
ACAN d14



PRG4 d14

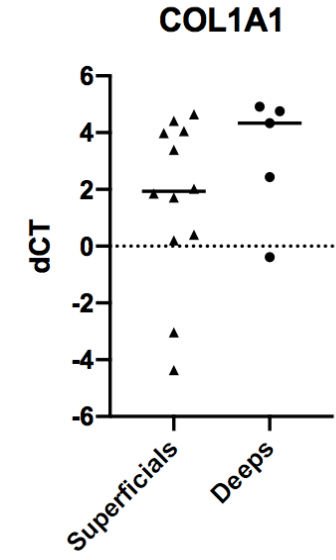
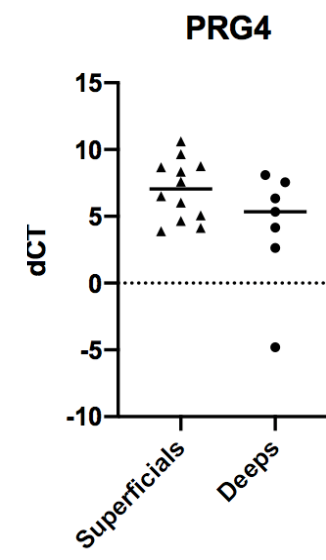
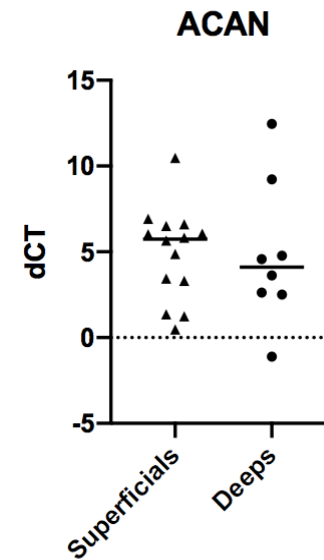
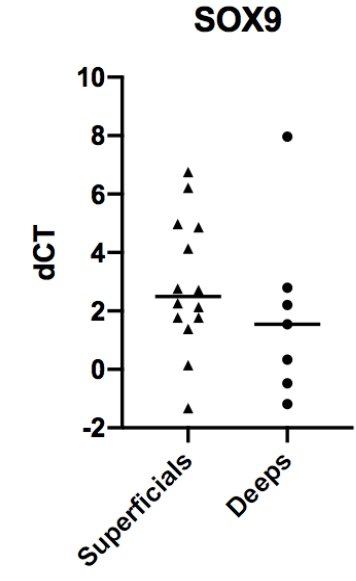
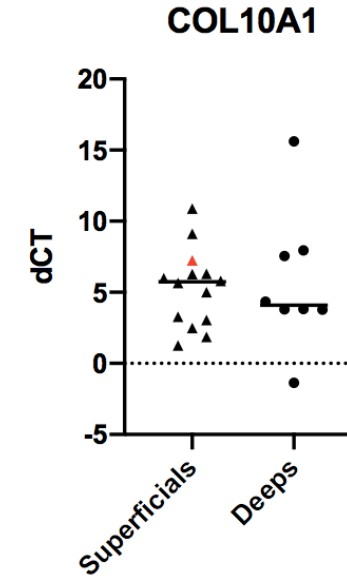
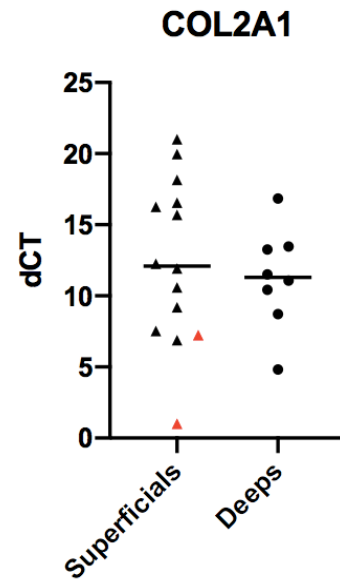


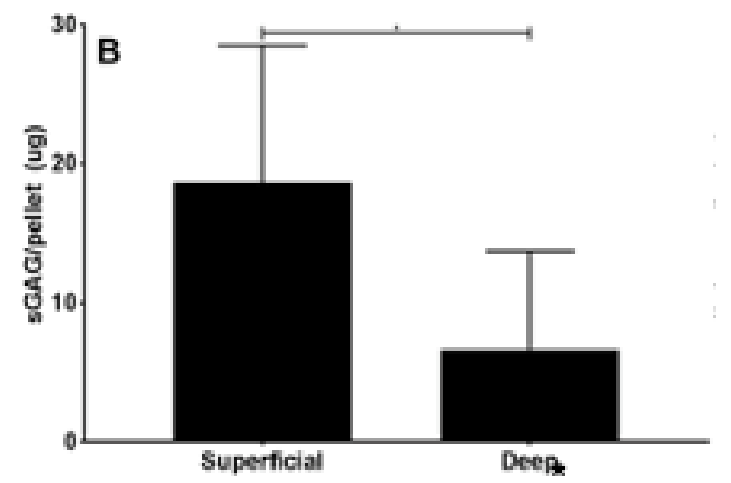
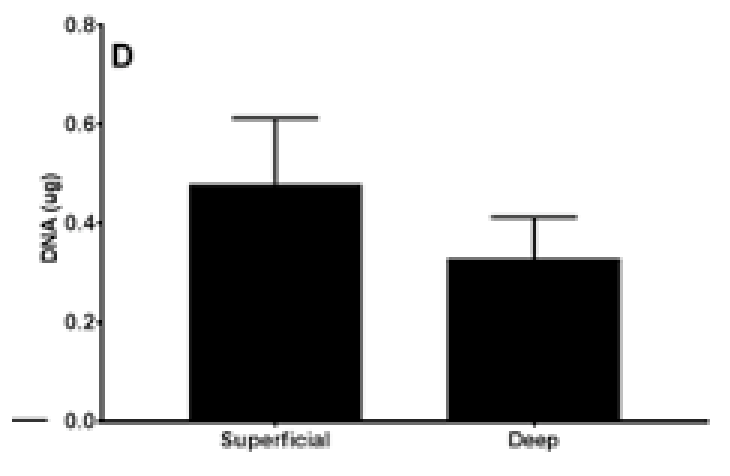
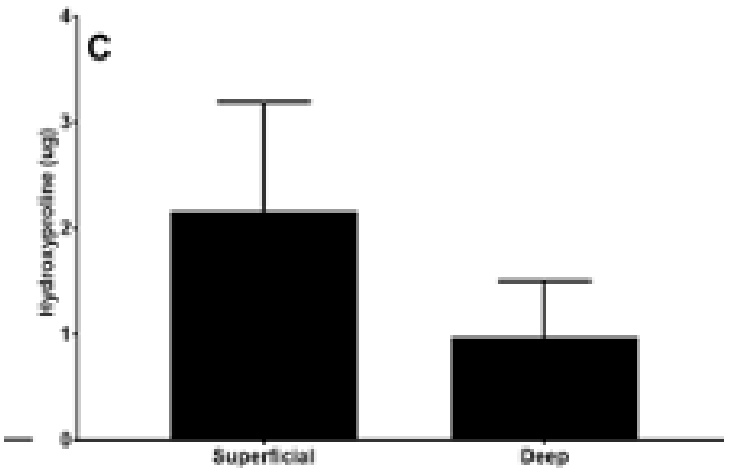
COL1A1 d14



Results— Gene Expression (d14)

Results— Gene Expression (d14:d0)





Results—Extracellular Matrix

Discussion

Conclusions:

- Genetic and extracellular differences between sACPs and eACPs
 - Increased PRG4 expression and sGAG production in sACPs

Future Directions:

- Finish gathering data
 - GAG
 - HyPro
 - ColX
 - Population doubling times
- Epigenetics of ACPs
- Characterization of 40 & 60 population doublings

References

1. Anderson JM, Brown SA, Hoffman AS, Kowalski JB, Merric K, Morrissey RF, et al. Implants, Devices, and Biomaterials: Issues Unique to this Field. In: Ratner BD, Hoffman AS, Schoen FJ, Lemons KE, editors. Biomaterials Science. San Diego, CA: Elsevier; 2004.
2. Brown TD, Johnston RC, Saltzman CL, Marsh JL, Buckwalter JA. Posttraumatic Osteoarthritis: A First Estimate of Incidence, Prevalence, and Burden of Disease. *J Orthop Trauma*. 2006;20(10): 739-44.
3. Carbone A, Rodeo S. A review of current understanding of post-traumatic osteoarthritis resulting from sports injuries. *J Orthop Res*. 2016;In Press:1-25.
4. Hootman JM, Brault MW, Helmick CG, Theis KA, Armour BS. Prevalence and Most Common Cause of Disability Among Adults. *CDC Morbidity and Mortality Weekly Report*. 2009;58(16): 421-6.
5. Makris EA, Gomoll AH, Malizos KN, Hu JC, Athanasiou KA. Repair and tissue engineering techniques for articular cartilage. *Nat Rev Rheumatol*. 2014;11(1):21-34.
6. Martín, A.R., Patel, J.M., Zlotnick, H.M. *et al*. Emerging therapies for cartilage regeneration in currently excluded 'red knee' populations. *npj Regen Med* 4, 12 (2019). <https://doi.org/10.1038/s41536-019-0074-7>
7. Murphy L, Schwartz TA, Helmick CG, Renner JB, Tudor G, Koch GG, et al. Lifetime risk of symptomatic knee osteoarthritis. *Arthritis Rheum*. 2008;59(9):1207-13.
8. Peterson L, Vasiliadis HS, Briceburg M, Lindahl A. Autologous Chondrocyte Implantation: A Long-term Follow-up. *Am J Sport Med*. 2010;38(6):1117-24.
9. Williams R, Khan IM, Richardson K, Nelson L, McCarthy HE, Anabalsi T, et al. Identification and Clonal Characterisation of a Progenitor Cell Sub-Population in Normal Human Articular Cartilage. *PLoS ONE*. 2010;5(10):e13246.
10. <https://jrfortho.org/products> (OCA pictures)