Comparison of Hydroxyapatite Deposits in Primate and Human sub-RPE Deposits #A0250

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Purpose: While sub-RPE deposits such as drusen are widely accepted as precursors to age-related macular degeneration (AMD), the molecular events that initiate deposit formation have not yet been fully elucidated. We recently discovered that sub-RPE deposits in human cadaver eyes have microscopic spherules of hydroxyapatite (HAP, also called hydroxylapatite), the hard, insoluble form of calcium phosphate found predominantly in bones and teeth. These spherules are distinct from the well-known calcification of the Bruch's membrane itself. In this study we compared the morphology and incidence of HAP deposition in human and macaque retinas.

Methods: HAP deposition was visualized by the use of LiCor BoneTag 680RD or Alizarin Red S, two bone stains that fluoresce when bound to HAP. We labelled unfixed and fixed macaque and human flatmounts of Bruch's membrane/choroid complexes after the removal of the neurosensory retina and the RPE or sections from whole eyes. We examined eyes from ages ranging 16 to 38 years (comparable to 48 to 114 years in humans) for primates and 34 to 95 years for humans. In some cases fundus photographs of the macaques were available. HAP deposition was imaged by scanning the flatmount using the Odyssey system from LiCor and bright field and fluorescence confocal microscopy.

Conclusions: Our results show that HAP deposition is present in both primate and human eyes with similar distribution. The reduced numbers of spherules and particularly hollow spherules in primates compared to fresh human tissue suggest that the molecular events involved in deposit formation in primate and human eyes are similar but not identical. Our results clearly indicate that fresh tissues with short post-mortem time are preferable when analyzing HAP deposition in the eye..

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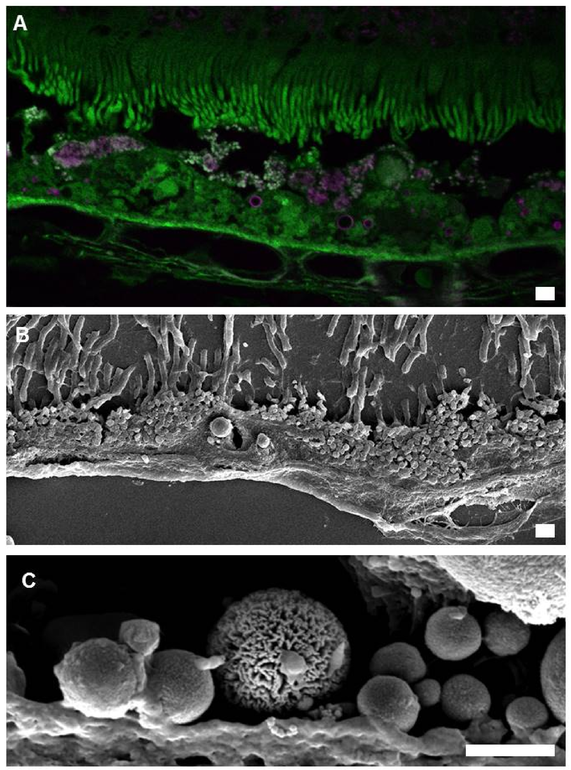
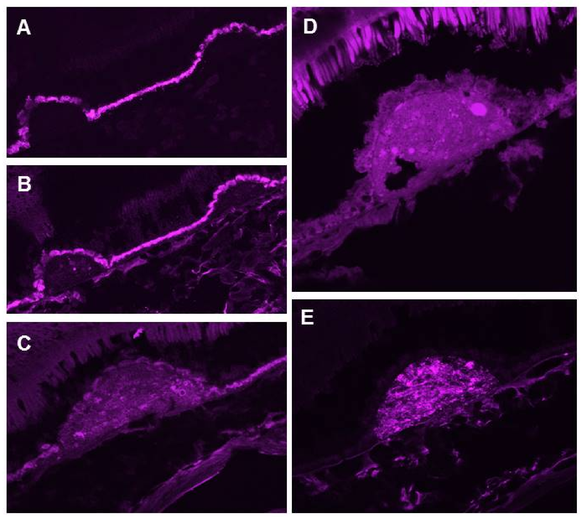
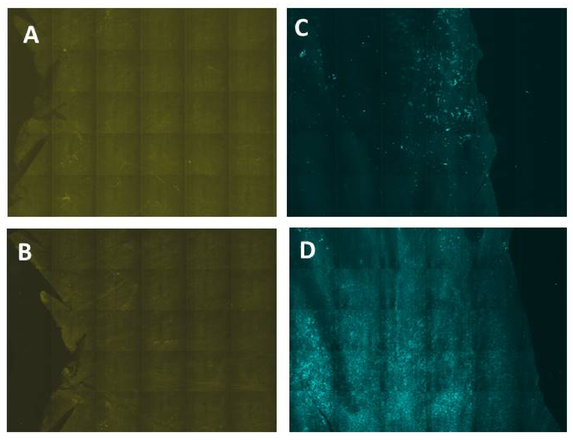
The authors all declare no competing financial interest and certify that all research was conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.



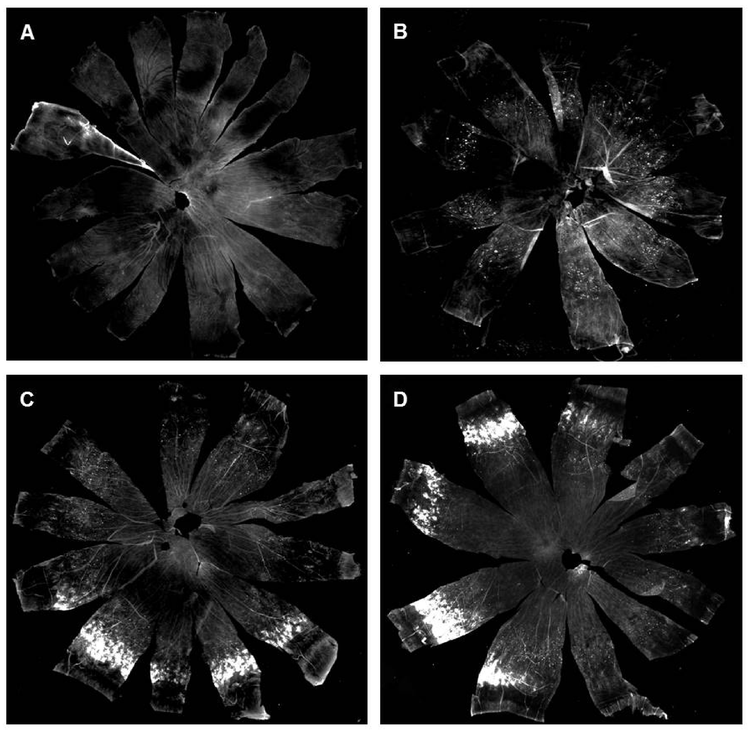
HAP spherules in human retinal sections from an AMD donor. A: autofluorescence (green) and BoneTag hydroxyapatite staining (magenta). B: SEM showing HAP spherule embedded in drusen. C: High magnification SEM images of spherules in a druse. D: immunolabeling of CFH (red) and Amyloid beta (green) on HAP (blue) surfaces. E: SEM image of potential protein deposition on HAP surfaces. Scale bar represent 2 µm



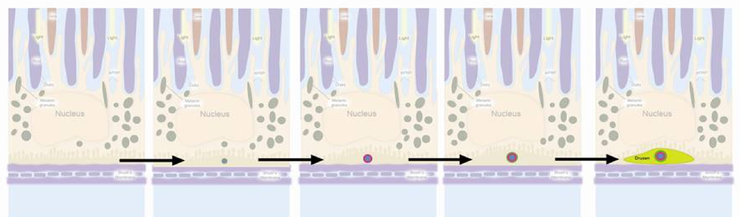
30 year Old (~ 90 in human years) female Rhesus Macaque fixed tissue slices stained with LiCor BoneTag 680RD (panels B-D) and Osteosense 680EX (panel E). Panels A and B show same drusen before (A) and after (B) staining; panels C-E show different drusen after staining. Excitation 633 nm, em 670-710 nm, 20x /0.85 (panels A-C, E); 40x/1.3 (panel D)



Tiled fluorescence confocal micrographs of flat-mounted fixed human tissues with neural retina and RPE removed, before (A, C) and after (B, D) staining with Alizarin Red S. Panels A and B: 34 year old; panels C and D: 94 year old female. All panels: 10x/0.45, exc. 561 nm, emiss. 575+ nm, field = 5.6 mm^2, pixel size 2.2 um^2 ; gain: panels A,B,C = 50, panel D = 10.



Whole-mount human Bruch's membrane labelled with the HAP selective BoneTag 680 fluorescent dye. A: 70 year old donor samples without significant HAP deposition. B: 68 year old donor samples with extensive HAP deposition especially in mid periphery. C-D: 79 years old donor with extensive HAP deposition throughout labelled with BoneTag680 (C) and BoneTag800 (D). Images were generated by the Li-cor Bioscience Odyssey 9120 Infrared Imaging System.



A schematic diagram of the initiation of sub-RPE deposit formation. Deposit formation is initiated by the deposition of HAP (magenta) onto lipid (cholesterol) droplets (blue). Consequently different proteins (green) bind to the HAP surface, which facilitate further deposition in a self-driven oligomerisation process ultimately to form the visible deposit (yellow-green).