Alzheimer's Disease in 3D: Characterization of Plaques and Neuroinflammation within AD Mouse Models Using Innovative Tissue Clearing and Imaging Techniques

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INTRODUCTION

- Transgenic mouse models are crucial to understanding mechanisms and evaluating new therapeutics of Alzheimer's disease (AD)
- Identifying detailed spatial distribution and density of β-amyloid plaques and pathological markers of neuroinflammation is critical to further our understanding of AD
- Goal: Demonstrate proof of concept *ex vivo* 3D imaging by characterizing 3D distribution and densities of β-amyloid plaques and other pathological markers in brains of male and female ARTE10 (APP-PS1) and APPSWE (Tg2576) mice across different ages

METHODS

- Homozygous male and female transgenic ARTE10 (Taconic model #16347) and control C57BL/6NTac (Taconic model #B6) animals were assessed at 10 wks, 6 mths, and 10 mths
- Male and female transgenic APPSWE (Taconic model #1349, tg/wt) and control wild type APPSWE (Taconic model #1349, wt/wt) animals were assessed at 10 wks, 10 mths, and 14 mths
- Mice were transcardially perfused with 4% PFA and brain samples dissected. Intact fixed samples were post-fixed with SHIELD, delipidated, stained with SYTO™, anti-IBA-1 (microglia) or anti-GFAP (astrocytes) and anti-β-amyloid (plaques) antibodies, index-matched and imaged using a SmartSPIM light sheet microscope. Whole brain images were acquired with a 3.6x objective, registered to the Allen Brain Atlas and regional cell counts/plaque densities quantified using SmartAnalytics (Fig 1)

LifeCanvas Technologies: 3D Histology Pipeline

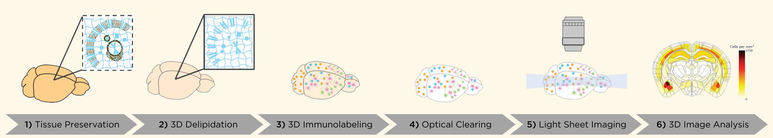


Figure 1. In the LifeCanvas processing pipeline, PFA-fixed brain samples are:

1) Post-fixed with SHIELD reagent for tissue preservation;
2) Delipidated in SmartClear Pro, a device that can delipidate mouse brain samples in 1 day;
3) Immunolabeled with primary and secondary antibodies using SmartBatch+;
4) Optically cleared using EasyIndex refractive index-matching solution (RI= 1.52);
5) Imaged using the SmartSPIM lightsheet microscope with a 3.6x objective; and
6) Analyzed using SmartAnalytics for atlas registration and segmentation analyses of amyloid deposition.

RESULTS

Widespread β-amyloid Plaque Deposition Observed Earlier in ARTE10 Transgenic Model

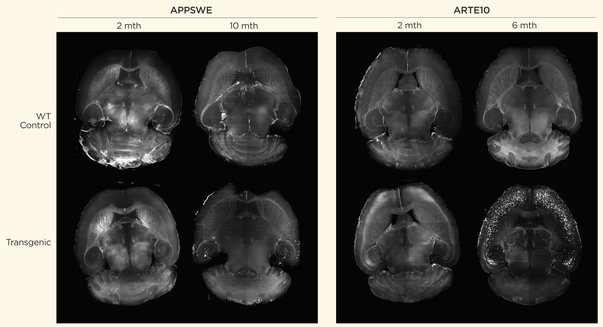


Figure 2. Acquired transverse SmartSpim images (600 μm MIPs) from immunolabeled whole brain samples of transgenic APPSWE and ARTE10 mice compared to WT control. Plaques are visible in cortical and hippocampal regions of ARTE10 and APPSWE mice at >6 and >10 mths, respectively with significantly more pronounced beta-amyloid plaque deposition evident in the ARTE10 transgenic model.

Heatmaps with Allen Brain Atlas Coronal Overlay Highlight Age-Dependent Aβ Increase

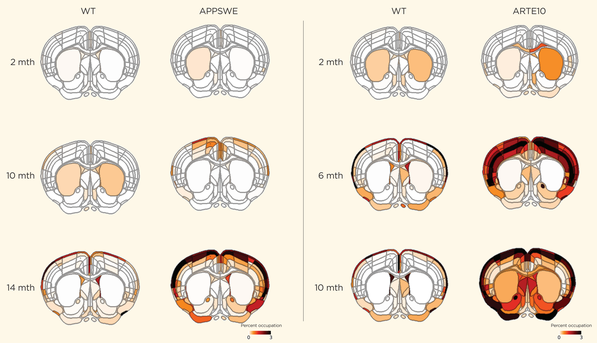


Figure 3. Heatmaps (25 μm sections) generated from image registration to the Allen Brain Atlas and segmentation analyses of plaque deposition (SmartAnalytics). Coronal views shown for control wild type (WT) and for APPSWE & ARTE10 transgenic mice highlight increased plaque deposition in cortical regions in ARTE10 mice >6 mths and at >14 mths for the APPSWE model.

β-amyloid Plaque Deposition in Cerebral Cortex and Hippocampus Increase More Progressively in the ARTE10 Model

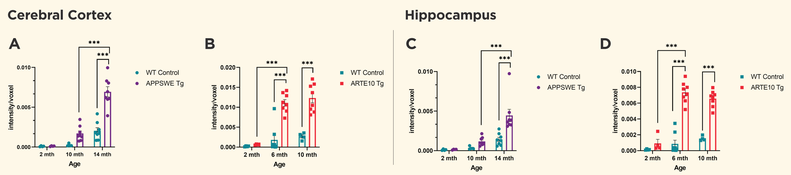
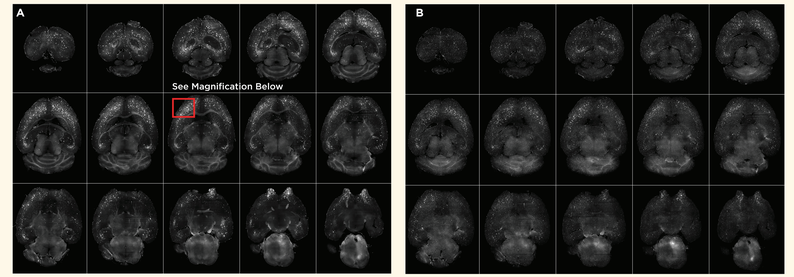


Figure 4. Intensity of plaques per voxel calculated for entire cerebral cortex (A and B) and hippocampus (C and D) per animal (n=4-8 per group) for the APPSWE transgenic (Tg) model (A and C) and ARTE10 Tg model (B and D). Each data point represents the average of both left and right hemispheres. Males and females (n=2-4 each) did not differ. Two way ANOVAs yielded significant interactions ($p<0.0001$) for all. Plaque deposition was significantly increased relative to wild type (WT) controls by 6 months of age in both the cortex (B) and hippocampus (D) for ARTE10 mice, whereas they were not significantly increased in the APPSWE mice until 14 months of age in both regions (A and C). Plaque deposition in the ARTE10 mice was consistent by 6 months of age, yet took time to develop in the APPSWE mice. *** $p<0.0001$ (Bonferroni post hoc analyses)

Clear Plaque Deposition and Microglial Clustering Throughout Brain of ARTE10 Transgenic Mouse by 6 Months of Age



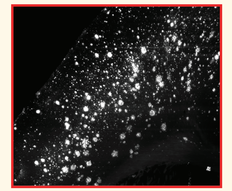


Figure 5. Image montage depicting plaque deposition (A) and microglial clustering (B) throughout the complete volume of a 6 mth old ARTE10 mouse brain. Ventral to dorsal stack of selected 400 μm MIPS depicting immunolabeled plaque deposits and IBA-1 highlighting microglia in the brain of a male ARTE10 mouse brain at 6 mths of age. Plaques and microglia are evident throughout the brain with pronounced deposition in cortical, hippocampal structures and in the olfactory bulbs.

Magnification of indicated area in image A

Neuroinflammatory Markers Cluster Around Plaque Deposits in ARTE10 Mice

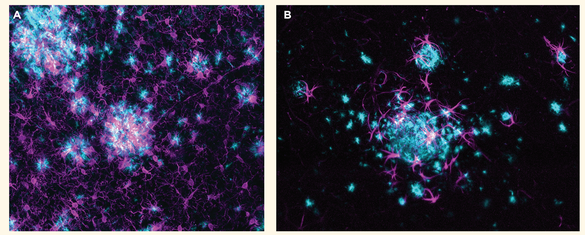


Figure 6. Transverse images from the hippocampus of ARTE10 6 mth old mice. IBA-1 staining for microglia is in magenta and beta-amyloid staining is depicted in cyan (A). GFAP staining for astrocytes is magenta and beta-amyloid staining is depicted in cyan (B). Clustering of activated microglia and astrocytes are in the proximity of the beta-amyloid plaque deposits.

CONCLUSION

- This study resulted in more robust, unbiased, and high-resolution data with improved characterization than would be possible with traditional 2D immunohistochemistry
- Increased understanding of disease progression across different models, ages, and sexes
- Plaques within the cerebral cortex and hippocampus increased over time in both models
- Plaques developed more aggressively in the ARTE10 animals relative to the APPSWE animals in both brain regions, forming at least by 6 months in ARTE10 and 14 months in APPSWE
- This data showed for the first time brain-wide neuroinflammation involvement via both microglia and astrocytes in both ARTE10 and APPSWE transgenic models
- There were no clear sex differences in plaque deposition for any groups, although this may be due to low sample size (n=4 per sex)

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