

Evaluation of Beta Amyloid Loss to CSF Sample Collection and Storage Containers

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Abstract

Background:. The accurate quantification of CSF biomarkers is paramount in the development of AD drugs but measurement has varied due to handling and storage material characteristics. The purpose of this study was to systematically evaluate an assortment of CSF collection/storage materials in hopes of identifying those associated with the least amount of CSF beta amyloid (Aβ) loss.

Methods: Nine separate types of collection/storage tubes were compared to an aliquotted control pipetted directly from the source polypropylene conical vial for three isoforms of Aβ (38, 40, 42). Coefficient of variations determined sample accuracy based on two collections corresponding to regular Rainin pipet tips and Rainin Low-Retention pipet tips. Additionally, two common long tubing types (Tygon and Silastic) were compared across various cumulative CSF quantities of 2, 4, 6, 8, 10 and 12 mLs.

Results: Grand mean differences in storage/collection tubes were evident (- 28.4, -29.4 and - 34.8% for Aβ40, 38, and 42, respectively). ANOVA results for differences among types of tubes was highly significant p = 0.005. Tukey post-hoc comparisons suggested that the Sarstedt 10mL SC Tube (15x92 RB PP GWB Str cat# 62.610.201) had the lowest mean loss across Aβ species (-9.7) and differed significantly from all other tube types but one, even after adjustment for multiplicity. For tubing types a regression analysis with percent difference as the response, volume of CSF an explanatory variable, and tubing type as the factor of interest was performed with a quadratic term added to the model. Aβ40, 38 and 42 were analyzed separately and for each analysis the quadratic term was significant (p < 0.05). For both Aβ40 and 38 there was a significant difference due to tube type (p = 0.037) with Silastic having a lower response consistently across all CSF volumes. For Aβ42 there was an interaction between volume and tubing type (p = 0.049) suggesting that for lower CSF volumes (under 6mL) Silastic is superior but for larger volumes Tygon is better.

Conclusions: This study supports the use of specific collection/storage and tubing types when conducting CSF biomarker studies in order to reduce the loss of Aβ due to nonspecific binding.

Background

- In order to determine if various amyloid-targeting drugs/vaccines have been successfully able to impact their intended targets in their predicted manner more data is needed regarding the variability of CSF measurement due to collection techniques and materials.
- This study was designed to assess the nonspecific loss of CSF due to storage and handling materials commonly used in CSF procedures at various clinical sites.
- The goal of this research was to suggest materials associated with the lowest and most stable levels of loss and attempt to standardize CSF collection and storage procedures.

Methods

- All experiments were performed in the lab using pooled CSF from Bioreclamation.
- Sample analyses were done through modifications to the MSD® 96-Well MULTI-SPOT® Human (6E10) Abeta Triplex Assay (cat# K15148E-2) kit designed to improve assay sensitivity.
- Nine separate types of collection/storage tubes were compared to an aliquotted control.
- These were chosen based on their common use and representation in the literature.

Methods (cont)

- For the larger collection tubes (1st three listed in table below) 1 mL pooled CSF was added, for the smaller tubes 200μL of the pooled CSF was added to each tube. A control was aliquotted directly from the pooled CSF unto the assay plate and ran with the samples.
- Coefficient of variations determined sample accuracy based on two collections corresponding to regular Rainin pipet tips and Rainin Low-Retention pipet tips
- Means were compared across tube types via ANOVA and post-host tests.
- Six feet of each tubing type (Tygon and Silastic) were used to assess differences in tubing type. A 60mL syringe with pooled CSF was filled, attached to the appropriate tubing and ~2mL increments of CSF were allowed to flow through.
- Collections were made for each time point and 2 replicates were analyzed from each (6 time points total). Results were compared to the mean of 4 results pipetted directly from syringe with no tubing attached.
- For tubing types a regression analysis was performed with the percent differences as the response, the size of the tube as the continuous explanatory variable and tube type as the factor of interest.
- As confirmed by the plots of the observed data there was curvature in the data. Therefore, a log transformation was not able to linearize this relationship so a quadratic term was added to the regression model as shown below.

$$Y = \text{intercept} + a \cdot \text{type} + b \cdot \text{size} + c \cdot \text{size}^2 + d \cdot \text{size} \cdot \text{type}$$

- Each AB class (40, 38 and 42) was analyzed separately.
- For each analysis the quadratic term was significant (p < 0.05).

Results

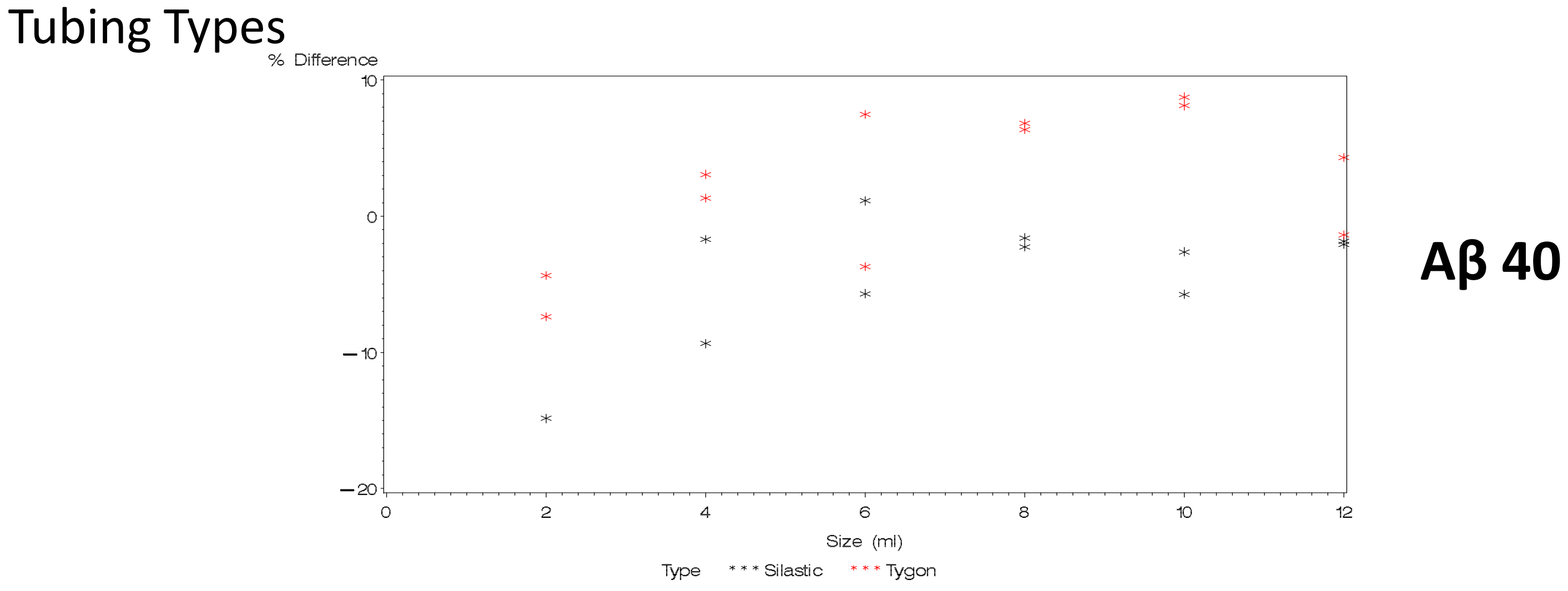
Tube Description	AB40	AB38	AB42
Nalgene Cryogenic 5mL vial	-21.8	-21.8	-25.4
Sarstedt 10mL SC Tube 16x79 RBS PP cat# 60.551	-32.1	-26.8	-28.9
Sarstedt 10mL SC Tube 15x92 RB PP GWB Str cat# 62.610.201	-7.0	-11.7	-10.4
Bio Plas 0.5mL siliconized screw cap micro tube cat# 4200SLS	-28.4	-27.9	-37.8
Sarstedt micro tube 0.5mL PP cat# 72.730	-33.9	-33.2	-39.5
Fisherbrand microcentrifuge tubes 0.5mL natural cat# 02681370	-39.7	-38.8	-45.9
Fisherbrand microcentrifuge tubes 1.5mL Low Retention Natural cat# 02681320	-30.8	-34.6	-39.7
Sarstedt micro tube 1.5mL Low Binding cat#72706600	-28.9	-34.2	-40.8
Sorenson microcentrifuge tubes 1.7mL natural low binding cat# 39640T	-32.8	-35.8	-45.2

The percent differences were calculated as 100 (value – control average)/control average

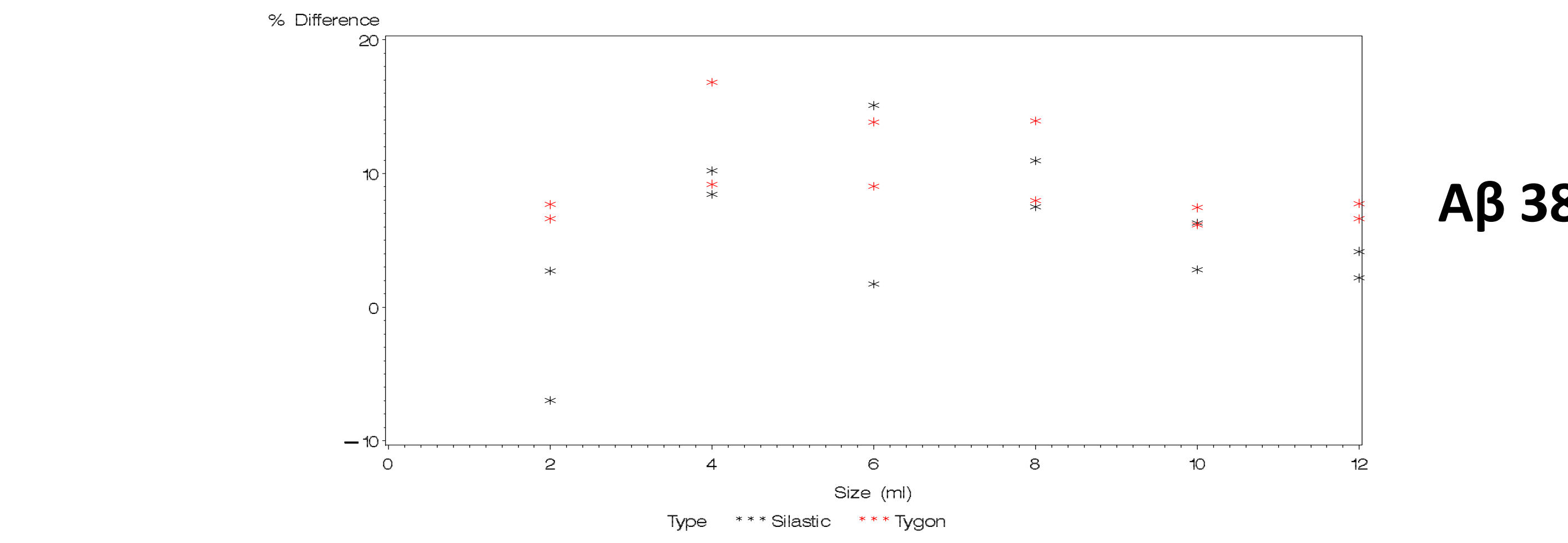
•ANOVA results for differences among types of tubes was highly significant p = 0.005.

* Tukey post-hoc comparisons suggested that the Sarstedt 10mL SC Tube (15x92 RB PP GWB Str cat# 62.610.201) had the lowest mean loss across Aβ species (-9.7) and differed significantly from all other tube types but one, even after adjustment for multiplicity

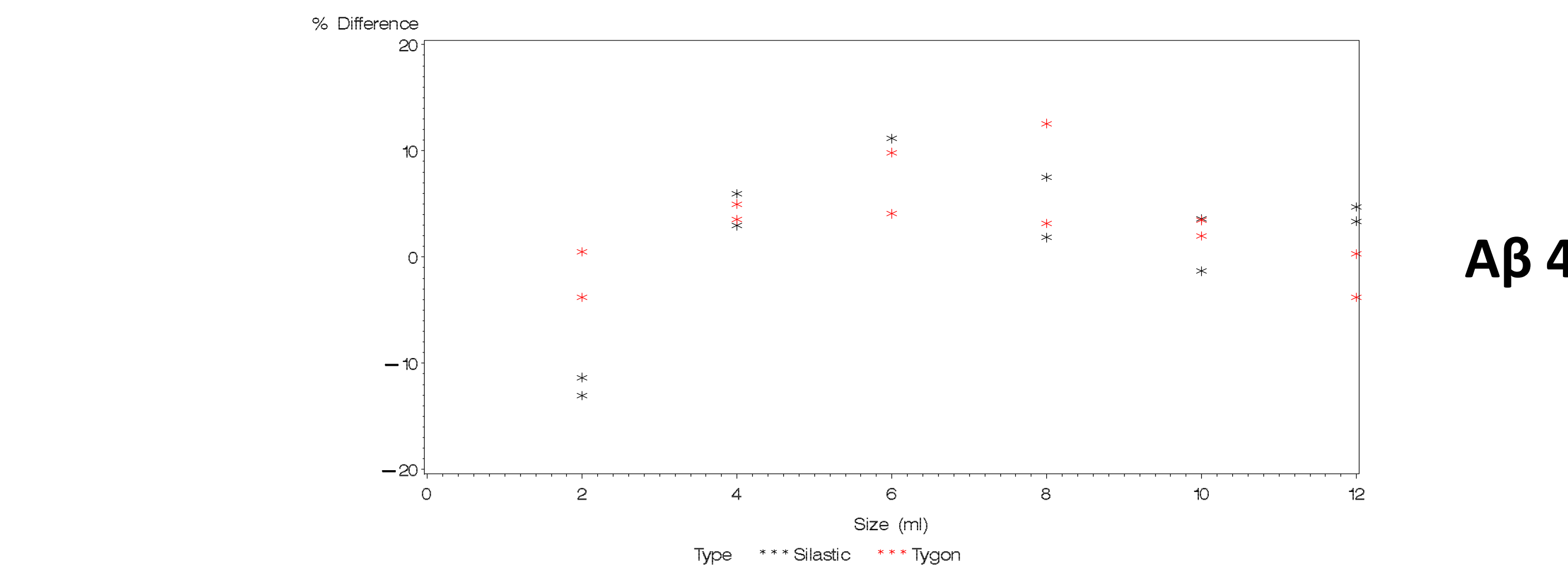
Results (cont.)



For Aβ40 the size by type interaction was not significant (p = 0.8824) and was dropped from the model. There was a significant difference due to tube type (p = 0.0372) with Silastic having a lower response consistently across volume.



Similar results were found for Aβ38. The interaction of size by type was not significant (p = 0.3553). There was a significant difference due to type (p = 0.0245) with Silastic having a consistently lower loss.



For Aβ42 there was an interaction between size and type (p = 0.0486). This implies that the effect of type depends on the volume of CSF. A plot of the predicted values shows that for low volumes Silastic is better but for larger volumes the mean response for Tygon is better.

Conclusions

- This study highlights the large variability in non-specific binding of CSF amongst various collection/storage and tubing types with differences often exceeding changes associated with drugs targeting Aβ.
- Careful selection and control of CSF handling and storage apparatus is necessary in order to more accurately assess changes in CSF associated with drug and vaccine treatment.