# Impact of Digestion Time and Tissue Size on the Isolation of TIE2+ Nucleus Pulposus Progenitor Cells

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## Introduction

- Low back pain (and associated intervertebral disc (IVD) degeneration) is the most common health problem worldwide and leads to an immense social and financial burden. [1,2]
- Recently discovered progenitor cells in the IVD, the so-called Nucleus pulposus (NP) progenitor cells (NPPC), might be used for cell-based therapies. [3]
- These NPPCs are positive for the receptor TIE2 (aka. Angiopoietin-1 receptor/ CD202b).
- Isolation protocols vary widely, yielding <1% to >80% NPPCsTIE2+ across studies. Protocols differ in digestion enzyme type, concentration, and incubation time, antibody use, whether tissues undergo culture before cell isolation, and whether cells undergo a recovery period prior to antibody staining. The size of digested NP fragments is often unreported.

## Study Aims

**Investigate the effect of…**

**1. NP Tissue Size before Digestion**

**2. Digestion Time in Collagenase Type II**

**… on the yield of TIE2+ NP cells isolated from bovine NP tissue**

## Materials and Methods

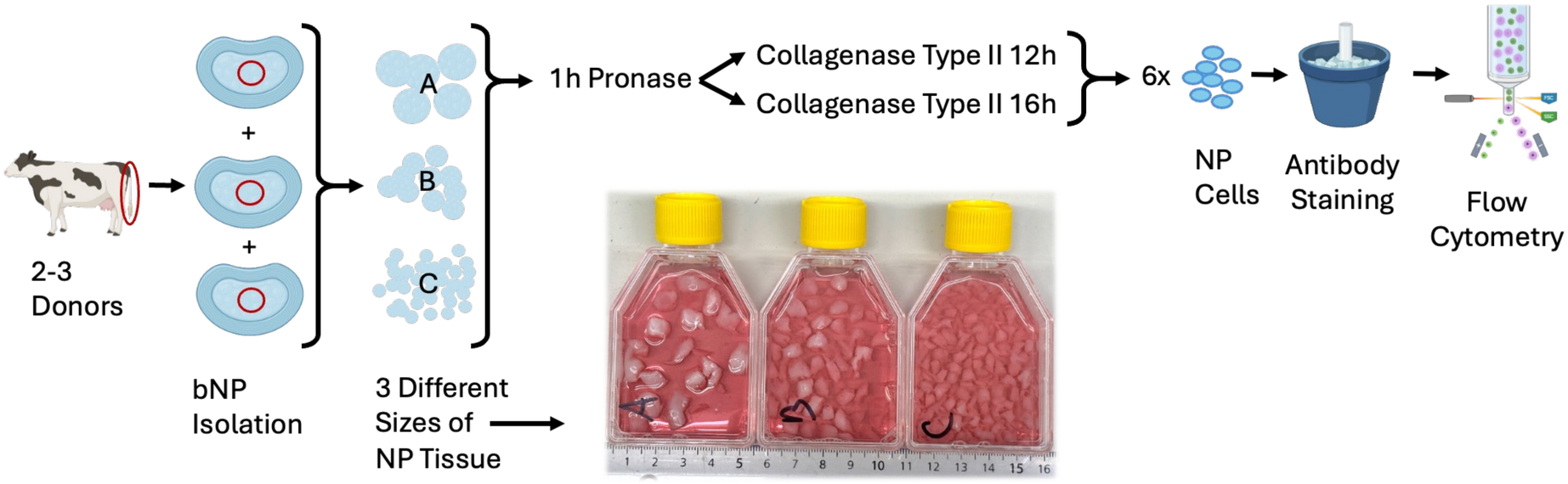



**Figure 1: Graphical study design**: For every replicate, 10-12 coccygeal bovine NPs from two to three animals were pooled and manually minced using a scalpel into small (~2-4mm), medium (~4-6mm) or large (~6-10mm) fragments. Cells were isolated by sequential pronase (1h) and collagenase type II (12h or 16h) digestion, stained with an anti-TIE2 antibody (Bioss, bs-1300R-BF488), and analysed by flow cytometry using the BD FACSDiscover™ S8 Cell Sorter or BD FACSDiscover™ A8 Cell Analyzer (Becton & Dickinson, Brussels, Belgium). Figure contains elements from BioRender.com

## Results

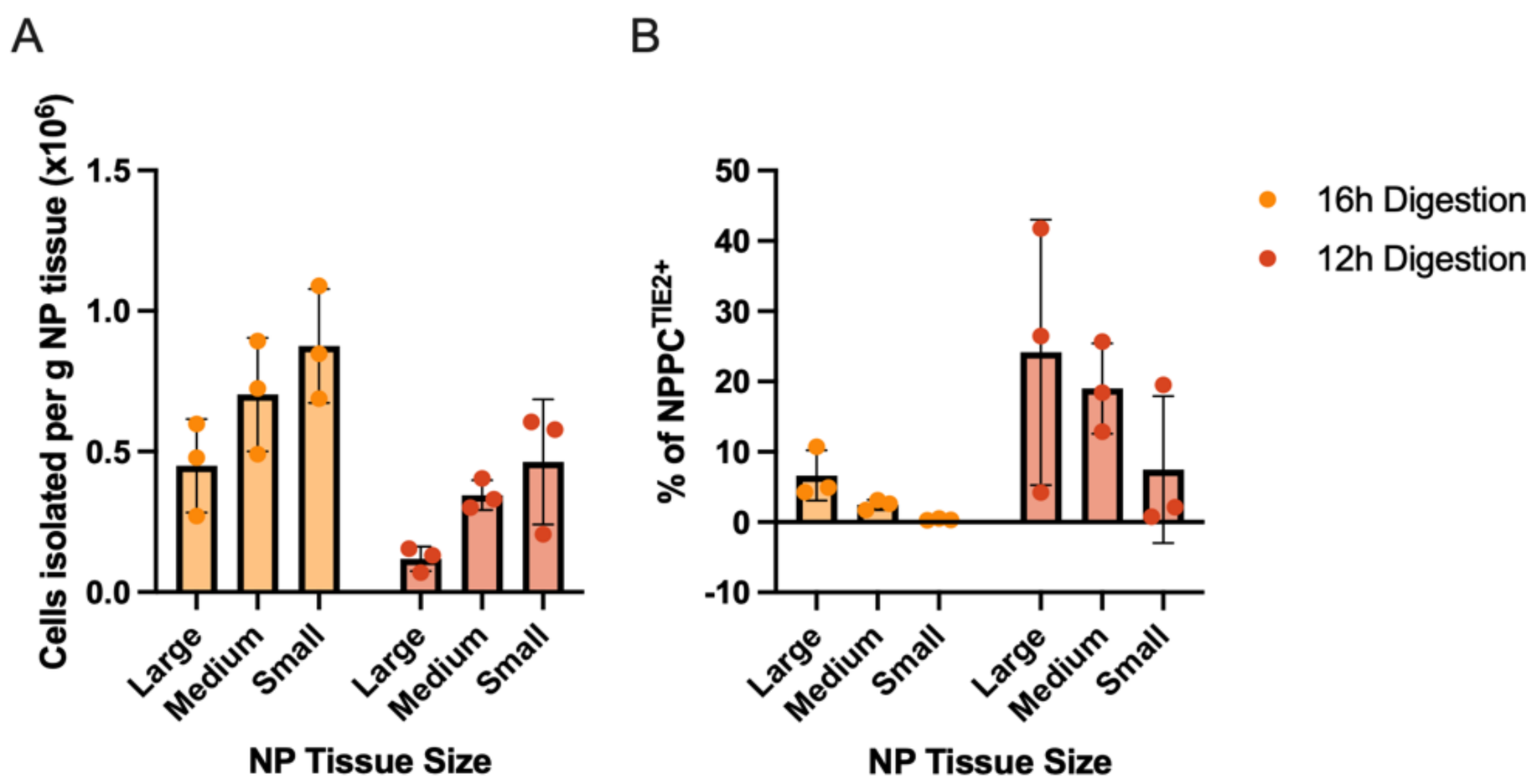



**Figure 2:** A) Number of cells ($x10^6$) per gram tissue isolated after enzymatic digestion of NP tissue: overall cell yield increased with smaller tissue size and longer digestion time B) Percentage of $NPPCs^{TIE2+}$ after FCM analysis: $NPPCs^{TIE2+}$ percentages increased with bigger tissue size and shorter digestion time. $NPPCs^{TIE2+}$ percentages varied between <1% and >40%. Abbreviations: Angiopoietin receptor-1 positive nucleus pulposus progenitor cells ($NPPCs^{TIE2+}$), nucleus pulposus (NP), flow cytometry (FCM).

## Discussion

- NP tissue size influences overall cell yield. Consistent with findings from Lee et al. [4]
- NP tissue size impacted overall cell yield and $NPPCs^{TIE2+}$ percentages.
- This highlights the need of further standardization of the basic $NPPCs^{TIE2+}$ isolation protocols
- Our results suggest that the high variance in cell number of NPPCsTIE2+ across labs might arise through unfixed sensitive parameters in cell isolation.

## Outlook

- Further replicates are needed for robust statistical analysis
- Investigation of the effect of recovery for the staining of TIE2
- Test different concentrations of the digestive enzymes
- Investigate different antibodies
- Eventually standardize $NPPC^{TIE2+}$ cell isolation as it has been done for NP cell culture [4],[5].

## References

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[5] Basatvat et al. 2023 JOR Spine. 2023;6(1):e1238.

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Online poster

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