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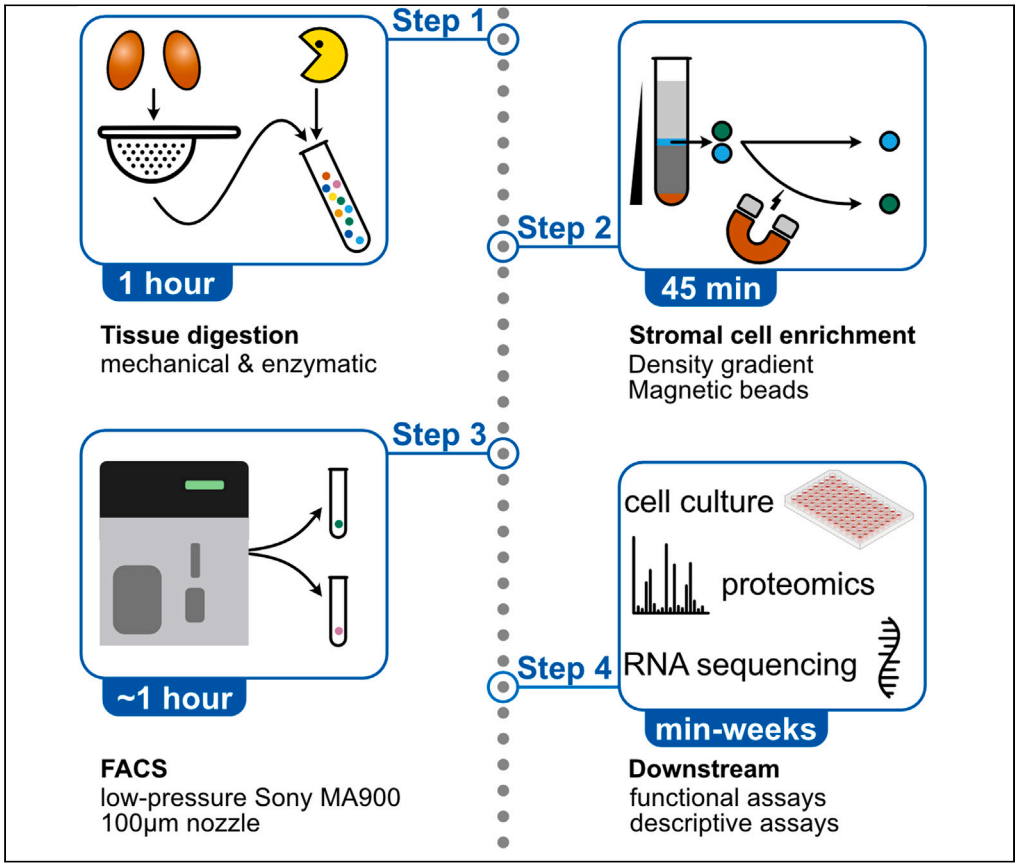
Protocol for the isolation and purification of human follicular dendritic cells for functional assays



In this protocol, we detail how to isolate and purify human follicular dendritic cells (FDCs) from lymphoid tissues. FDCs play a vital role in antibody development by presenting antigens to B cells in germinal centers. The assay involves enzymatic digestion and fluorescence-activated cell sorting and is successfully applied to various lymphoid tissues, including tonsils, lymph nodes, and tertiary lymphoid structures. Our robust technique enables the isolation of FDCs and facilitates downstream functional and descriptive assays.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

Human follicular dendritic cell isolation protocol

Isolation suitable for multiple downstream applications, including cell culture

FACS- and magnetic-bead-based isolation procedures

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Protocol for the isolation and purification of human follicular dendritic cells for functional assays

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SUMMARY

In this protocol, we detail how to isolate and purify human follicular dendritic cells (FDCs) from lymphoid tissues. FDCs play a vital role in antibody development by presenting antigens to B cells in germinal centers. The assay involves enzymatic digestion and fluorescence-activated cell sorting and is successfully applied to various lymphoid tissues, including tonsils, lymph nodes, and tertiary lymphoid structures. Our robust technique enables the isolation of FDCs and facilitates downstream functional and descriptive assays.

For complete details on the use and execution of this protocol, please refer to Heesters et al.[1]

BEFORE YOU BEGIN

The protocol below describes the isolation of FDCs from human tonsils using several enrichment steps. Isolation should be performed within hours of the tonsillectomy. Work fast but gently for the cells and keep the solutions on ice unless stated otherwise. For the digestion, a 37°C incubator is required. If the goal is to culture the FDCs, a culture plate should be coated in the days before the isolation. This protocol has been tested on human and mouse lymph nodes and spleen but was optimized for human tonsils.

Coat cell culture plate

Timing: 1–2 days

Collagen I, III, and laminin are the major constituents of the lymph node follicle. Therefore, a combination of Collagen I and laminin provides a more physiological coating for the culture of FDC. For long-term cultures, collagen I and laminin can be applied to culture plates by first coating the well with poly-d-lysine. This provides a more stable coating. The following steps will describe how to coat a 96-wells plate (for other formats the volume should be adjusted). All steps are carried out with sterile reagents in a laminar flow hood to prevent contamination of the plate.

1. Add 40 µL poly-D-lysine (100 µg/mL, Gibco) to the wells and spin down the plate (Cellvis P96-1.5P) at room temperature for 10 s at 200 g. It is important that the complete bottom of the well is covered in liquid. Take special attention to the middle as the meniscus might leave the center of the well dry.
2. Incubate for 2–24 h at room temperature (longer incubation is preferred).
3. Aspirate the poly-D-lysine from the wells and wash three times with milli-Q.

4. Add laminin (10–100 μg/mL) and type I collagen (100 μg/mL) in PBS to a total volume of 40 μL and briefly spin down the plate.
5. Incubate for 4–16 h at room temperature.
6. Rinse once with culture media and add cells or media. Do not allow the coating to dry.

Institutional permissions

Stromal cells were isolated from discarded palatine tonsils from routine tonsillectomies. The collection and use of all human samples was approved by the local Medical Ethical Testing Committee (METC). It is important to check your institutional requirements for working with discarded human tissues. A main aspect to keep in mind is patient privacy. As no patient information is needed for the isolation and functional experiments, we opted for total anonymous collection of samples. Therefore, informed consent is often required for studies that use any kind of sequencing.

Tonsils

This protocol has been applied to tonsils that were removed via tonsillectomy. Tonsils were collected by the surgeon in sterile beakers containing 10 mL PBS + 1% Pen/Strep. Transportation to the lab and the remainder of the protocol was done on ice unless stated otherwise. Processing of tonsils started within 2 h after the tonsillectomy.

FDC enrichment

In this protocol two methods for FDC enrichment are described. First, enrichment via magnetic beads with the MojoSort™ system from Biolegend. Second, enrichment with cell sorting using the Sony MA900 Cell Sorter.

Flow cytometry

It is recommended to take along an unstained control and single stain controls for all antibodies used in this protocol. For compensation we used compensation bead (Invitrogen). Alternative antibodies and fluorochromes may be used but might require titration before they can be used optimally in this protocol. Make sure that the machine is calibrated, and the proper quality controls suggested by the manufacturer have been performed before measuring to ensure optimal reproducibility.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Brilliant Violet 421™ anti-human CD31 Antibody	Biolegend	Cat# 303124; RRID: AB_2563810
FITC anti-human CD35 Antibody	Biolegend	Cat# 333404, RRID: AB_2085022
PE/Cyanine7 anti-human Podoplanin Antibody	Biolegend	Cat# 337014, RRID: AB_2563368
PerCP/Cyanine5.5 anti-human CD55 Antibody	Biolegend	Cat# 311316, RRID: AB_2565480
APC anti-human CD55 Antibody	Biolegend	Cat# 311311, RRID: AB_2075857
Helix NP™ NIR	Biolegend	Cat# 425301
Purified anti-human CD35	Sony	Cat# 2267010
MojoSort™ Streptavidin Nanobeads	Biolegend	Cat# 480016
UltraComp eBeads™ Compensation Beads	Invitrogen™	Cat# 01-2222-41
Biological samples		
Human tonsils (from infants, 2–16 years old)	Diakonessenhuis Utrecht	N/A
Chemicals, peptides, and recombinant proteins		
HBSS, no calcium, no magnesium, no phenol red	Gibco™	Cat# 14175095
IMDM	Gibco™	Cat# 12440053
FBS	Gibco™	Cat# 16000044

(*Continued on next page*)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
DNase I	Roche	Cat# 10104159001
Collagenase, Type IV, powder	Gibco™	Cat# 17104019
HEPES (1 M)	Gibco™	Cat# 15630080
Penicillin-Streptomycin (10,000 U/mL)	Gibco™	Cat# 15140122
MEM Non-Essential Amino Acids Solution (100×)	Gibco™	Cat# 11140035
Sodium pyruvate (100 mM)	Gibco™	Cat# 11360070
Gentamycin	Sigma-Aldrich	Cat# G1397
Percoll™	Cytiva	Cat# 17-0891-01
Phosphate buffered saline	Sigma	Cat# 806552-500ML
Poly-D-Lysine	Gibco™	Cat# A3890401
Collagen, Type I solution from rat tail	Sigma-Aldrich	Cat# C3867-1VL
Laminin	Roche	Cat# 11243217001
EZ-Link™ Sulfo-NHS-LC-Biotinylation Kit	Thermo Scientific™	Cat# 21435
Software and algorithms		
FlowJo™ Software	BD Biosciences	N/A
Other		
96-well plate for microscopy and culture	Cellvis	Cat# P96-1.5P
RVS sieve 18 cm	Blokker	Cat# 0722559
RVS mini sieve 7,5 cm	Blokker	Cat# 0722551
Pipette Filler, S1, Thermo Scientific (Pipetboy)	Thermo Scientific™	Cat# 89204-754
CELLSTAR® Centrifuge Tubes	Greiner Bio-One	Cat# 227261
Disposable serological pipettes 10 mL	Greiner Bio-One	Cat# 607180
Disposable serological pipettes 25 mL	Greiner Bio-One	Cat# 760180
Scissors	Fine Science Tools	Cat# 14060-11
Petri dish	Falcon	Cat# 351029
MojoSort™ Magnet	Biolegend	Cat# 480019
Round-bottom polystyrene tubes	Corning	Cat# 734-0442
Sony MA900 Multi-Application Cell Sorter	Sony	N/A
Cell strainer 70 μm	VWR	Cat# 76327-100
Pasteur pipettes	Kartell	Cat# KART334

MATERIALS AND EQUIPMENT

Digestion mix (per tonsil pair)		
Reagent	Final concentration	Amount
HBSS (supplemented with 3 mM $CaCl_2$ + 5 mM $MgCl_2$)	1×	7.75 mL
HEPES	20 mM	200 μL
FCS	0.5%	50 μL
DNase I	50 U/mL	1 mL
Collagenase D (IV)	200 U/mL	1 mL

Note: DNase I and Collagenase D (IV) 10× stocks preparation described below and can be stored at −20°C for up to 6 months.

FDC medium		
Reagent	Final concentration	Amount
IMDM	1×	500 mL
HEPES	20 mM	10 mL
NEAA	1×	5 mL
Sodium Pyruvate	1×	5 mL
FCS	5%	25 mL
Pen/Strep	1×	5 mL

Note: store at 4°C for up to 30 days.

Sort buffer		
Reagent	Final concentration	Amount
HBSS (w/o calcium and magnesium)	1×	500 mL
HEPES	20 mM	10 mL
EDTA (1 M stock)	2 mM	1 mL
FCS	5%	25 mL
Gentamycin (5000× stock)	1×	100 µL

Note: store at 4 °Cfor up to 30 days.

FDC antibody cocktail		
Reagent	Final concentration	Amount
Brilliant Violet 421™ anti-human CD31 antibody	1 µL per 1 × 10^7 cells	N/A
FITC anti-human CD35 antibody	1 µL per 1 × 10^7 cells	N/A
PE/Cyanine7 anti-human Podoplanin antibody	1 µL per 1 × 10^7 cells	N/A
PerCP/Cyanine5.5 anti-human CD55 antibody	1 µL per 1 × 10^7 cells	N/A

Reagents and solutions	Preparation
Stock isotonic Percoll (SIP)	Take 9 parts Percoll™ and add 1-part 10× PBS.
DNase I 10×	For a 500 U/mL 10× stock solution dissolve the DNase I powder in 1 mL milliQ. Transfer to 19 mL HBSS supplemented with 3 mM $CaCl_2$ + 5 mM $MgCl_2$. Filter sterilized through 20 µm filter. Make 1 mL aliquots and store at −20°C.
Collagenase D (Type IV) 10×	Our product came with 310 U/mL. The amount needed must be adjusted according to the Units in the batch. For 2000 U/mL stock solution: Weigh 64,5 mg dissolve in 10 mL HBSS supplemented with 3 mM CaCL2 + 5 mM $MgCl_2$. Filter sterilized through 20 µm filter. Make 1 mL aliquots and store at −20°C.
Percoll™ work solutions	Work solutions can be prepared prior to the experiment. The percentage required is taken from the SIP solution and supplemented to 100% with PBS 1×.
Transport media	PBS supplemented with 1% Pen/Strep.
Biotinylated anti-human CD35	Purified anti-human CD35 (Sony, cat# 2267010) was biotinylated using EZ-Link™ Sulfo-NHS-LC-Biotinylation Kit (Thermo Scientific™) according to manufacturer's protocol.

⚠ CRITICAL: No harmful chemicals or reagents were used.

STEP-BY-STEP METHOD DETAILS

Stromal cell isolation

Timing: 3 h

Stromal cells in lymphoid tissue are bound to the extracellular matrix and form a mesh like network that act as scaffolds for antigen presentation. Lymphocytes are either bound to the stromal cells or loosely interact with this mesh network. This protocol is optimized for the isolation of stromal cells from human tonsils but can also be used on other (human) lymphoid tissue. Here we describe the steps needed to isolate stromal cells from tonsils via enzymatic digestion and enrichment using Percoll™ (Cytiva).

1. Discard the liquid from beaker containing the tonsils.

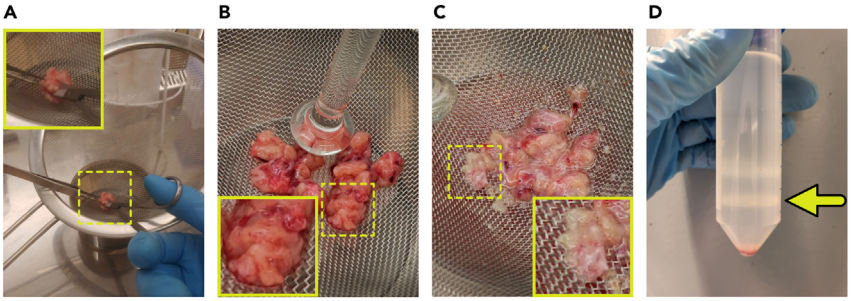

Figure 1. Tonsil processing and Percoll gradient

(A) Tonsils are cut into smaller pieces with scissors on top of the sieve.

(B) Glass plunger is used to push the tissue fragments through the metal mesh and lymphocytes and smaller tissue fragments are collected in PBS.

(C and D) (C) White connective tissue and some blood cloths that remain after the brown tissue has been forced through the metal sieve (D) After underlaying 21% and 34% Percoll and centrifugation of 300 g for 25 min a faint band containing the stromal cells can be seen on the interface between 21% and 34% Percoll.

2. Add ice cold PBS to the beaker and remove any blood cloths from the outside of the tonsils using tweezers and scissors.
3. Pour ice cold PBS (20–30 mL) in a Petri dish or equivalent container.

   Note: If mass spectroscopy is one of the end goals make sure that the container is not made from Polyethylene. This greatly increases noise in the mass spectrometer. To avoid this completely, a stainless-steel container can be used.

4. Place the metal sieve on top of the petri dish and transfer the tonsils to the sieve. We recommend a regular kitchen sieve.
5. Cut the tonsils into 1–2 mm slices using tweezers and scissors. (Figure 1A).
6. Using a flattened glass rod, you can push the tissue through the metal sieve (Figure 1B). Making sure that the light brown colored tissue makes it through. This contains the cells we need. If done correctly, mainly white tissue remains (Figure 1C).

   ⚠ CRITICAL: The brown tissue passes through the metal sieve when moderate force is applied. Overworking the tissue in this step will result in a lot of the connective tissue to be carried over to the subsequent digestion steps. This should be avoided as much as possible as this will lower yield.

7. Discard the residual (white) tissue in the metal sieve.
8. Using a 10 mL serological pipet, you can use some of the cell suspension in the petri dish to wash any remaining tissue that is stuck to the underside of the sieve and transfer it to the Petri dish.
9. Transfer the cell suspension to a 50 mL Falcon tube (Greiner Bio-One). Make sure you transfer all the tiny debris as this contains the stromal cells. You can add some fresh PBS to acquire all the tiny fragments.
10. Spin the suspension in a centrifuge (10 s up to 200 g) to pellet all the tissue fragments. The pellet contains the tissue with the stromal cells. The supernatant will contain all the free-floating lymphocytes.
11. Remove the supernatant containing the lymphocytes. The supernatant can be used to isolate lymphocytes if required.
    a. *Optional:* Underlay lymphocytes with 21% and 80% Percoll™ (Cytiva) and continue at step 20.
12. Add the 3 mL digestion mix (HBSS, 20 mM HEPES, 0.5% FCS, Collagenase type IV (200 U/mL), DNase I (50 μg/mL)) gently mix by pipetting and incubate for 15 min at 37°C.

13. Transfer the supernatant (2–3 mL) to a 50 mL Falcon tube on ice containing 20 mL HBSS w/o calcium/magnesium + 20 mM HEPES + 5% FCS + 2 mM EDTA + gentamycin. This should stop any activity of the enzymes in the digestion mix.
14. Repeat steps 12 and 13 three times until the tonsil is fully digested, up to 1 h. The last two times 2 mL digestion mix can be added.

⚠ CRITICAL: Be gentle while mixing the tissue fragments and the digestion mix. The mixing is used to add some mechanical stress to the collagen fibers aiding the enzymes. Pipetting to rough will result in killing the cells.

15. Filter the cell suspension through a final metal sieve. We recommend a tea sieve containing a fine metal mesh. This will filter out the bigger undigested fragments and spin down at 200 g at 4°C for 6 min.
16. Aspirate the supernatant and resuspend the pellet in 25 mL PBS.
17. Filter through a cell strainer (70 μM).
18. Carefully underlay with 10–15 mL of 21% Percoll™ [1.03 g/mL] using gravity mode on a pipetboy.
19. Carefully underlay with 10–15 mL of 34% Percoll™ [1.045 g/mL] using gravity mode on a pipetboy.
    a. *Optional*: Underlay an additional 80% Percoll™ layer to isolate lymphocytes as well.

⚠ CRITICAL: The densities used in this protocol do not differ much therefore they mix easily. Take your time when underlaying Percoll density solutions. Underlaying at a slower rate will result in better separation. Slightly angling at the start of underlaying in the 50 mL falcon tube gives more control over the flow rate. When there is no clear (cell) band visible between the 21% and 34% fractions after centrifugation, this generally means the underlaying was performed too fast which mixes the fractions.

20. Spin w/o breaks in a swinging bucket centrifuge at 300 g for 25 min at room temperature.
21. Collect the stromal cells at the interface between 21% and 34% Percoll™ (Figure 1D) using a Pasteur pipette and transfer to a 50 mL falcon tube with 20 mL HBSS w/o calcium/magnesium + 20 mM HEPES + 2 mM EDTA + 5% FCS + gentamycin.
22. Spin down at 200 g for 7 min at 4°C.
23. Aspirate the supernatant to remove any residual Percoll™.
24. Resuspend the pellet in 5 mL FDC medium and count the cells.
25. Cells are now ready for FDC enrichment (MojoSort or cell sorter).

Note: Yield will be highly dependent on the size of the tonsils. In general, we get between 10^5 and 10^7 cells with a cell viability >80%. Both yield and viability will be highly dependent on the time between surgery and the start of processing. Also, the speed at which the steps above have been performed will influence total yield and viability. Minimizing the time the intact tonsils spend at room temperature will have the biggest effect on yield and viability.

FDC enrichment using MojoSort

Timing: 1 h

FDCs are only a small subset of all stromal cells present in lymphoid tissues. CD35 is highly abundant on FDCs but not on other cells in the stromal cell fraction. To further enrich the FDCs, the stromal cell fraction is stained with biotinylated anti-CD35 antibodies. This can then be bound by streptavidin nanobeads (Biolegend) and separated using a strong magnet (MojoSort magnet). This method will suffice for most experiments when culturing FDCs is the goal. All steps are performed at 4°C.

26. Transfer cells to a Round-Bottom Polystyrene Tubes (FACS tube) and adjust the total cell amount to a maximum of 1 × 10^8 cells.
27. Wash the cells with sort buffer and spin down at 200 g for 5 min.
28. Aspirate supernatant and resuspend the cell suspension in 100 μL sort buffer.
29. For every 1 × 10^7 cells add 1 μL biotinylated CD35 antibody and incubate on ice for 15 min.
30. Wash the cells with 2.5 mL sort buffer spin down at 200 g for 5 min.
31. Aspirate supernatant and resuspend cells in 100 μL sort buffer.
32. Resuspend the streptavidin nanobeads (Biolegend) by vortexing at max speed with 5 touches.
33. Add 10 μL of streptavidin nanobeads per 1 × 10^7 cells of sample.
    a. If < 1 × 10^7 cells, use of 10 μL of nanobeads
    b. Adjust the volume so that the nanobeads do not exceed 20% of the total volume.
34. Wash the cells with 2.5 mL sort buffer spin down at 200 g for 5 min.
35. Resuspend the cells in 2.5 mL sort buffer and place in the MojoSort magnet for 5 min.
36. After 5 min pour off the unlabeled cells. This fraction can be stored to check the efficiency of the enrichment.
37. Resuspend the labeled (selected) cells in 2.5 mL sort buffer and place in the MojoSort magnet for 5 min.
38. Repeat step 35–36 two more times to a total of three separations.
39. Resuspend the cells in FDC medium to the desired concentration.
    a. Plate the cells in collagen and laminin coated wells.
    b. Incubate at 37°C in a 5% CO_2 incubator.

Note: Yield should be between 10^2–10^4 cells. Some lymphocytes will still be attached to the FDCs at this point hence the purity is hard to monitor. The most important part will be that the FRCs and MRCs are not labeled by CD35 and will be removed in step 35–37.

Note: If you opt for separation with a cell sorter the magnet sort step should be skipped.

⚠ CRITICAL: The streptavidin nanobeads sediment very quickly, it is therefore essential to properly resuspend and vortex right before use. If this is not done properly you will add too few nanobeads and the cell separation will fail.

FDC enrichment using Sony MA900 cell sorter

Timing: 1–3 h

FDC enrichment via flow cytometric cell sorting. This method allows for more precise separation of FDCs from all the other cell types that are present in the stromal cell fractions but results in lower yields. Continue the protocol from step 24.

40. Wash the cells with 2.5 mL sort buffer and spin down 200 g for 5 min.
41. Resuspend the cells in 100 μL sort buffer add the FDC antibody cocktail and incubate on ice for 30 min in the dark. A viability dye will be added later to exclude dead cells.
42. Wash the cells with 2.5 mL sort buffer and spin down 200 g for 5 min.
43. Resuspend the cells in sort buffer to a concentration of 3 × 10^7 cells per mL.
44. Just before starting the sort add 2.5 μL helix-NP per 1 mL of cell suspension to discriminate live and dead cells.
45. Sort the cells using a 100 μm nozzle on the Sony MA900 cell sorter (Figure 2A for gating strategy). Yield should be around 10^3–10^4 FDCs per tonsil pair.
46. Collect the FDCs in a tube with 1 mL FDC medium at 4°C.
47. Spin down the cells at 200 g for 5 min.
48. Resuspend the cells in FDC medium to the desired concentration. The cells can then be plated in collagen and laminin coated wells and incubate at 37°Cin a 5% CO_2 incubator.

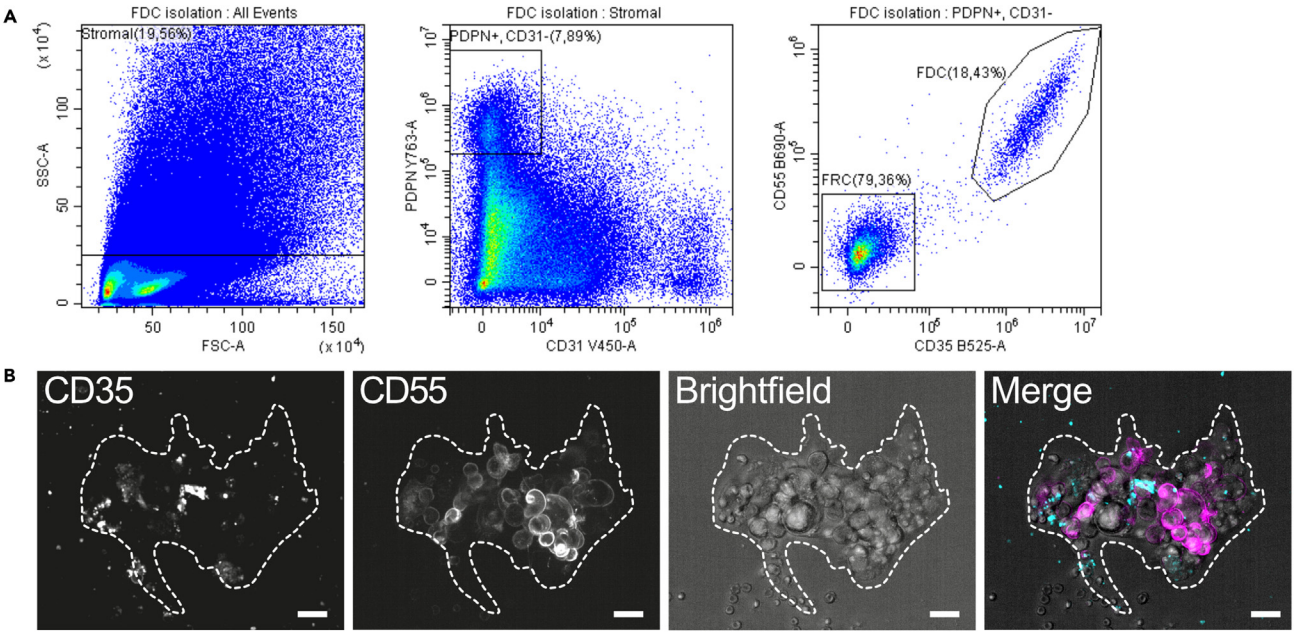

Figure 2. Expected outcomes for stromal cell FACS and FDC culture

(A) Gating strategy for stromal cells. This sample was measured after step 24 of the protocol before MojoSort beads enrichment. SSC-A^{high} were selected, then PDPN^{+}, CD31^{-} cells. FDCs were identified with CD35^{+}, CD55^{+}. Data acquisition on Cytoflex LX.

(B) FDCs isolated using MojoSort magnetic bead isolation after 1 week of culture at 37°Cat 5% CO_2. Cells were stained for CD35 and CD55 to identify FDCs. GC B cells are attached to cultured FDC. Dotted line indicates the FDC. Images were taken using a spinning disk confocal (Yokogawa CV7000S) with a 40× objective at 37°C and 5% CO2. CD35-FITC, 488-laser on 30% and 525/50 filter. CD55-APC, 647-laser on 30%, 676/29. Confocal-Brightfield path was used for brightfield acquisition. Scale bar = 20 μm.

Optional: Cells can be sorted directly in lysis buffer for sequencing or mass spectrometry.

Note: Other cell sorters can be used when the nozzle is > 100 μm and the pressure below 30psi.

EXPECTED OUTCOMES

The goal of this experiment is to isolate FDCs from tonsils. After digestion and Percoll enrichment of the stromal cell fraction the yield on average is somewhere between 10^5–10^7 cells mainly depending on the size of the tonsils. Figure 2A shows what FACS analysis of the stromal cell fraction should look like.

After MojoSort enrichment the FDCs yield should be around 10^2–10^4 cells. Figure 2B shows an FDC after 1 week of culture.

Using the SONY MA900 sorter enrichment the FDC yield should be between 10^3–10^4 cells. Yield will be highly variable on the size and quality of the tonsil.

LIMITATIONS

The primary limitation of this protocol is getting infections in long term cultures. Tonsillectomies are generally only performed when patients sustain persistent infections most often caused by bacteria. On top of this, the mouth is far from sterile, hence there will most likely be a lot of bacteria at the start of this protocol. The protocol is also quite lengthy and contains a lot of steps increasing the likelihood of introducing contamination. To mitigate the risk, different broad-spectrum antibiotics are used throughout the protocol. The initial bacterial content should gradually decrease as FDCs are

enriched and any contaminants should be filtered out. There is always the change of resistant microorganisms to the antibiotics used in this protocol however we found that in trained hands the infection rates should be below 10%. We found infection rates to be around 30% when only pen/strep was used.

Due to the potential contamination, it is important to follow the recommended cleaning procedure after running these samples on a flow cytometer or sorter.

TROUBLESHOOTING

Problem 1

Related to Step 24. Low amount of (living) stromal cells left after the isolation.

Potential solution

Adding mechanical stress by pipetting after adding the digestion mix improves the destruction of the collagen fibers. It is very easy to add too much mechanical stress which rips the cells apart rather than the collagen fibers. Alternatively, one can choose to perform the digestion in a rocking incubator at 37°C. This is gentler for the cells and can greatly improve yield. It is not advised to digest for much more than 1 h due to the cells being in a stressful environment with relatively little nutrients. The enzyme mixture used is relatively mild and should not have a big effect on cell viability.

Problem 2

Related to Step 38. High amount of debris after magnetic bead isolation.

Potential solution

When pushing the tonsil pieces through the metal sieve you should only push through the light brown colored tissue. This contains the lymphoid structures containing FDCs and lymphocytes. The leftover tissue consists mainly of white connective tissue which you want to discard. When you overwork the tissue in this step you risk transferring the connective tissue into the suspension. Since this also contains a lot of collagens you lower the release of FDCs into suspension by collagenase during the digestion step and ultimately this will result in a lot of collagen fragments in your cell suspension.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Balthasar Heesters, b.a.heesters@uu.nl.

Materials availability

This protocol requires no unique reagents.

Data and code availability

This protocol does not require any unique data or code.

ACKNOWLEDGMENTS

We acknowledge Rick van den Langenberg and all other ENT (ear, nose, and throat) surgeons and the assisting personnel at the Diakonessenhuis for performing the tonsillectomies and kindly providing the tonsils used in this protocol. We acknowledge the Core Flow cytometry Facility of the UMC Utrecht for access to the Sony MA900 Sorter.

AUTHOR CONTRIBUTIONS

Conceptualization, B.A.H.; methodology, B.A.H.; investigation, M.B.; writing – original draft, M.B.; writing – review & editing, B.A.H.; funding acquisition, B.A.H.; supervision, B.A.H.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Heesters, B.A., van Megesen, K., Tomris, I., de Vries, R.P., Magri, G., and Spits, H. (2021). Characterization of human FDCs reveals regulation of T cells and antigen presentation to B cells. J. Exp. Med. *218*, e20210790. https://doi.org/10.1084/jem.20210790.