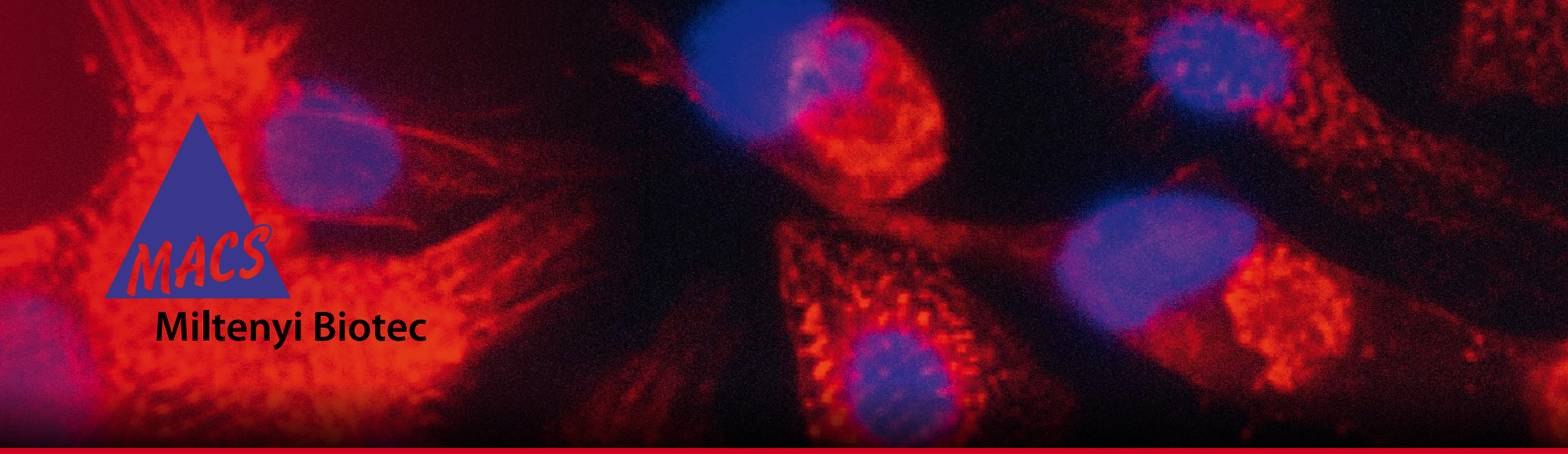

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Sample preparation

Neonatal mouse and rat hearts

Isolation of neonatal mouse and rat cardiomyocytes for the generation of functional engineered heart tissue using the gentleMACS™ Dissociator

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Background

Standardized preparation protocols are an important prerequisite to generate cardiomyocytes for in vitro studies. Isolation of cardiomyocytes from neonatal rat and mouse hearts by fractionated trypsin/DNAse digestions is a labor intensive and difficult to standardize procedure (6–24 hours[1,2]), and depends on experimentator skills and total amount of tissue. We hereby report on our experiences with the gentleMACS™ Dissociator to isolate cardiomyocytes from neonatal rat and mouse hearts.
This note exemplifies the use of the gentleMACS Dissociator for the dissociation of neonatal rat and mouse hearts into single cells.

Materials and methods

Materials

- Neonatal Wistar and Lewis rats and neonatal Black Swiss mice (postnatal day 0–3) were obtained from the animal facility of the University Medical Center Hamburg-Eppendorf. Experimental procedures were reviewed and approved by Ethics Committee, University Hamburg.
- Neonatal Heart Dissociation Kit, mouse and rat
- Phosphate-buffered saline (PBS), w/o calcium and magnesium, pH 7.4
- Cell culture medium without fetal bovine serum (FBS), e.g., DMEM
- Cell culture medium with FBS
- gentleMACS Dissociator or gentleMACS Octo Dissociator
- gentleMACS C Tubes

Additional for mouse heart dissociation:

- Pre-Separation Filter, 70 µm

Additional for rat heart dissociation:

- Filter, 100 µm mesh size
- Non-cardiomyocyte medium: Prepare DMEM with 10% inactivated FBS, 1% glutamine, and 1% penicillin/streptomycin

Methods

Neonatal mouse heart dissociation

1. Reconstitute and prepare the reagents from the Neonatal Heart Dissociation Kit according to the data sheet.
2. Harvest neonatal mouse hearts and transfer into a 10 cm dish containing ice-cold PBS. Utilizing forceps, carefully pump remaining blood out of the hearts. Cut vessels and remaining connective tissue away from the ventricles.
3. Preheat enzyme mix 1 for 5 minutes at 37 °C.
4. Add 2362.5 µL of enzyme mix 1 to 137.5 µL of enzyme mix 2.
5. Transfer harvested tissue into the gentleMACS C Tube. (Note: To reduce the volume of washing medium within the tube let tissue settle down by gravity and remove supernatant carefully.)
6. Add 2.5 mL of enzyme mix, tightly close the C Tube. (Note: Close C Tube tightly beyond the first resistance.)
7. Invert C Tube and place it with the cap down. To maximize cell recovery the C Tube should remain in this orientation until step 11.

8. Incubate sample without agitation for 15 minutes at 37 °C.
9. Attach C Tube onto the sleeve of the gentleMACS Dissociator.
10. Run the gentleMACS Program m_neoheart_01.
11. Repeat steps 8–10 two times.
12. After termination of the program, detach C Tube from the gentleMACS Dissociator and add 7.5 mL of cell culture medium.
13. Resuspend sample and apply the cell suspension to a Pre-Separation Filter, 70 µm, placed on a suitable tube.
14. Wash Pre-Separation Filter, 70 µm, with 3 mL of cell culture medium (DMEM with 10% active FBS).
15. Discard filter and centrifuge cell suspension at 300×g for 15 minutes. Aspirate supernatant completely.
16. Resuspend cells with appropriate buffer or medium to the required volume for further applications.
17. Calculate yield by cell count in a hemocytometer after trypan blue staining.

Neonatal rat heart dissociation

1. Reconstitute and prepare the reagents from the Neonatal Heart Dissociation Kit according to the data sheet.
2. Harvest neonatal rat hearts and transfer into a 10 cm dish containing ice-cold PBS. Remove atria and vascular pedicle. Cut isolated ventricles briefly (max. 10 minutes) to a homogenous mash using curved scissors.
3. Preheat enzyme mix 1 for 5 minutes at 37 °C.
4. Add 4727 µL of enzyme mix 1 to 275 µL of enzyme mix 2.
5. Transfer 10 or 20 minced hearts into one C Tube. (Note: To reduce the volume of washing medium within the tube let tissue settle down by gravity and remove supernatant carefully.)
6. Add 5 mL of enzyme mix per C Tube, tightly close the C Tube. (Note: Close C Tube tightly beyond the first resistance.)
7. Invert C Tube and place it with the cap down. To maximize cell recovery the C Tube should remain in this orientation until step 11.
8. Incubate sample without agitation for 15 minutes at 37 °C.
9. Attach C Tube onto the sleeve of the gentleMACS Dissociator.
10. Run the gentleMACS Program m_neoheart_01.
11. Repeat steps 8–10 two times.
12. After termination of the program, detach C Tube from the gentleMACS Dissociator and add 7.5 mL of cell culture medium (DMEM with 10% active FBS).
13. Resuspend sample and apply the cell suspension to a filter, 100 µm mesh size, placed on a suitable tube.
14. Wash filter with 3 mL of cell culture medium.
15. Discard filter and centrifuge cell suspension at 110×g for 15 minutes. Aspirate supernatant completely.
16. Resuspend cells with 40 mL non-cardiomyocyte medium.
17. Centrifuge cell suspension at 110×g for 15 minutes.
18. Resuspend cells with 10 mL non-cardiomyocyte medium.
19. Calculate yield by cell count in a hemocytometer after trypan blue staining.

Reference preparations of 30–50 neonatal rat and mouse heart cells were performed by an fractionated trypsin/DNase digestion as published previously.[3,4,5]

Generation of engineered heart tissue (EHT)

Cardiomyocytes were used to generate fibrin-based EHT and characterized by video-optical recording and analysis as previously described.[3]

Results

Cell yield of isolation procedures

Heart cell isolation from neonatal rat hearts using the gentleMACS Dissociator and the Neonatal Heart Dissociation Kit, mouse and rat resulted in a mean of $2.66\times10^6\pm0.08\times10^6$ viable cells per heart. The cell number did not depend on the number of hearts per C Tube (10 versus 20) if the volume of the enzyme mix was adjusted accordingly (figure 1). The cell number per heart was similar to manual cell dissociation technique with 30–50 hearts ($2.64\times10^6\pm0.08\times10^6$ cells per heart; figure 2). Neonatal mouse heart cell preparation from 20 hearts resulted in $0.65\times10^6\pm0.05\times10^6$ cells per heart, similar to manual dissociation of 30–50 hearts ($0.59\times10^6\pm0.09\times10^6$ cells per heart; figure 3).

Contractile development of EHTs

Cell quality was assessed by analysis of the contractile force of EHTs. Generation of EHTs from rat and mouse heart cells isolated with the gentleMACS Dissociator resulted in spontaneously and coherently beating tissues. No differences in force of contraction or EHT morphology were seen between neonatal mouse heart cell – EHTs from gentleMACS Dissociator – and manual cell preparations (figure 4). Rat EHTs were monitored during four weeks of culture and contractions were evaluated via video-optical analysis. Cell preparations from 20 hearts per C Tube performed slightly better than preparations from 10 hearts per tube (figure 5). Smaller numbers of hearts per preparation were not studied since manual fractionated trypsin/DNAse dissociation is known to be notoriously difficult from small numbers (<30) of input hearts. Similar to the results with mouse heart cells, there was no deleterious effect of the new cell isolation protocol on the contraction force of the rat EHTs. These EHTs showed higher contraction forces at early stages in tissue development and were similar to EHT from manual dissociation at later time points (figure 6). Morphologically based EHTs appeared slightly thinner than EHTs based on manual dissociation (figure 7).

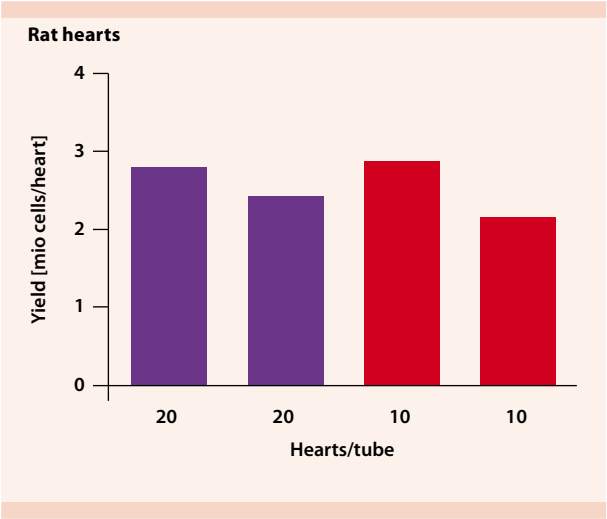

Figure 1: Shown are the results from 2 independently performed experiments utilizing 10 and 20 rat hearts per C Tube, respectively.

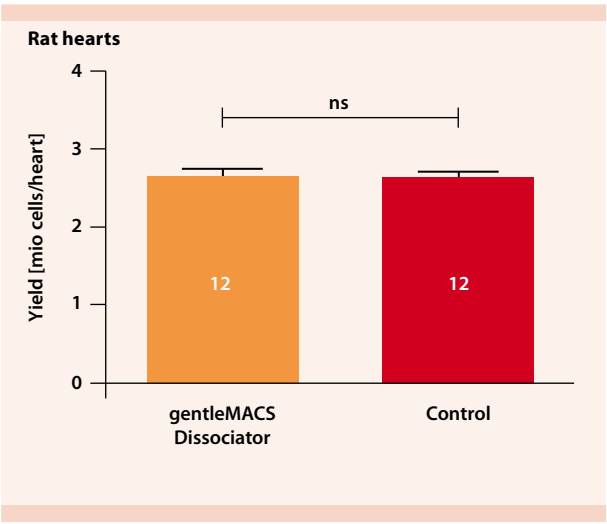

Figure 2: Cell yield after isolation with the gentleMACS Dissociator with 20 rat hearts per C Tube and the usual trypsin-DNase-digestion, respectively. (n=12; Student's t test).

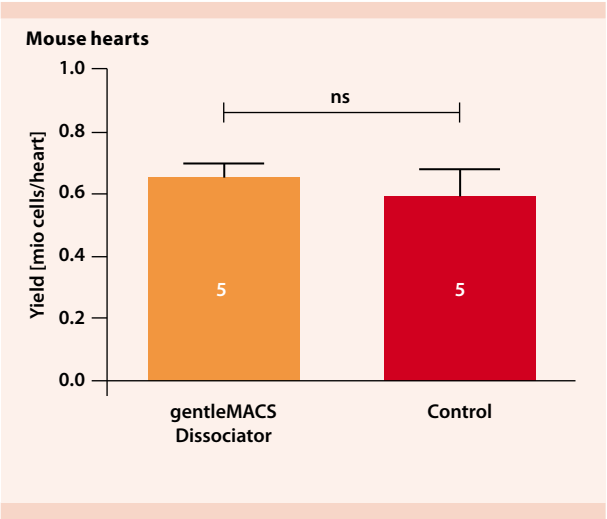

Figure 3: Cell yield after isolation with the gentleMACS Dissociator with 20 mouse hearts per C Tube and the usual trypsin-DNase-digestion, respectively. (n=5; Student's t-test)

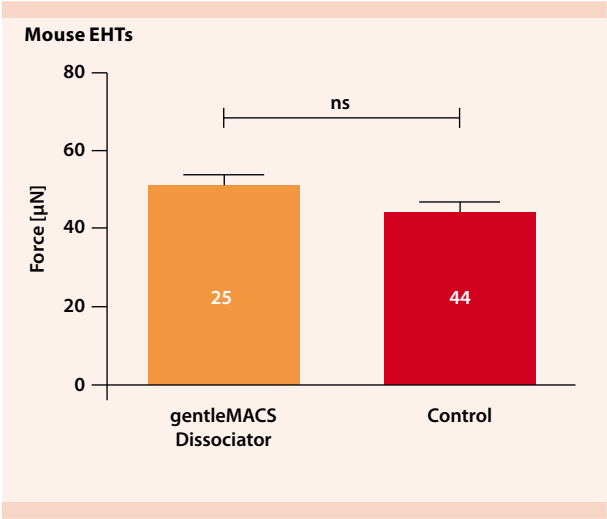

Figure 4: Contraction force of engineered heart tissue (mouse) at day 14 in culture. (n=25–44; Student's t-test).

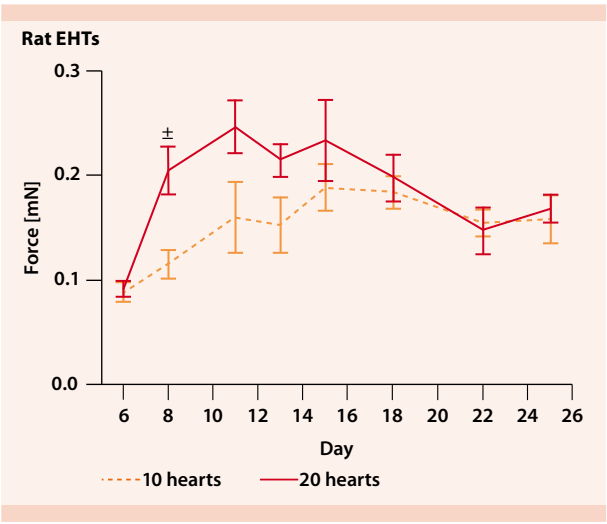

Figure 5: Contractile development of engineered heart tissue (rat) during four weeks in culture. (n=8; 2 wayANOVA)

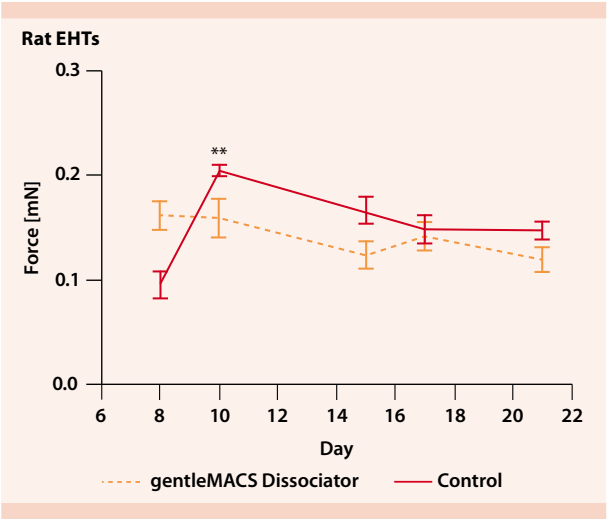

Figure 6: Contractile development of engineered heart tissue (rat) during four weeks in culture. (n=20-30; 2 wayANOVA)

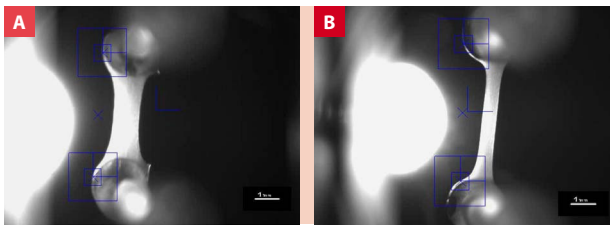

Figure 7: Live-image of engineered heart tissue (rat) during contraction analysis at day 7 in culture. Engineered heart tissue (EHT) based on traditional cell isolation protocol (A) and cells isolated with gentleMACS Dissociator (B).

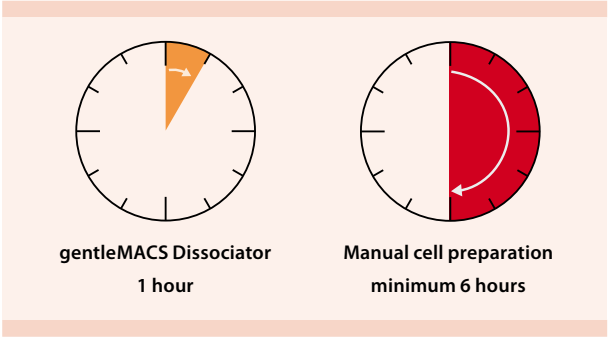

Figure 8: Comparison of labor time. Using the gentleMACS Dissociator saves a minimum of about 5 hours of working time compared to manual cell preparation (6–24 hours).

Conclusion

The gentleMACS Dissociator is an alternative to manual fractionated trypsin/DNAse dissociation for neonatal rat and mouse heart cells. The protocol is faster and easier to standardize (figure 8). Cell quantity (cell count) and quality (contractile force in EHT technology) were similar between both cell isolation techniques. The study was performed with low numbers of input hearts which are generally too low for manual dissociation techniques.

References

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