제 출 문

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요 약 문

Ⅰ. 제 목

과제명: 미세유체칩을 활용한 완보동물의 특성 연구

보고서명: 미세유체칩을 활용한 완보동물의 분리기술 연구

Ⅱ. 연구개발의 목적 및 필요성

지금까지 온도, 압력, 방사능, 화학물질 등 다양한 극한환경에서 완보동물의 휴면상태 전환에 대한 연구가 수행되었다. 하지만 자연계에서 생명체가 경험하는 대표적인 기계적 자극인 음파 자극에 대한 완보동물의 거동에 대한 연구는 미비한 실정이다. 또한 배양된 완보동물로 실험을 하기 위해서는 배양액에서 핀셋(tweezer) 등을 이용한 접촉식 방법으로 완보동물을 분리해야 한다. 1 ㎜ 이내의 몸길이를 갖는 완보동물을 핀셋을 이용해 분리하는 것은 숙련된 연구자에게도 매우 까다로운 작업이다. 또한 접촉식 방법으로 분리한 완보동물을 대상으로 한 연구는 샘플 오염이 빈번히 발생해 정확한 완보동물 연구가 어려운 실정이다. 따라서 완보동물 배양 샘플에서 비접촉식/고속/자동화된 방법으로 완보동물을 분리할 수 있는 방법이 필요하다.

Ⅲ. 연구개발의 내용 및 범위

본 연구는 생명체가 자연계에서 경험하는 대표적인 기계적 자극인 음파 자극에 대한 완보동물의 거동을 분석하고 마이크로필터 어레이 구조와 음향미세유체역학 기술을 이용해 완보동물 배양 샘플 내에서 완보동물을 선택적/비접촉식/고속/자동화된 형태로 분리하는 기술을 개발하는 것을 목표로 한다.

Ⅳ. 연구개발결과

고주파수 표면탄성파에서 유래한 음향방사력이 완보동물에 가해졌을 때 미세유세칩 내 완보동물의 위치를 정교하게 제어할 수 있으며, 음향방사력에 의해 완보동물의 운동능력과 번식능력이 저해되지 않음을 확인하였다. 아울러 마이크로필터 어레이 구조를 이용해 완보동물 배양 샘플 내 미세조류 군집을 효율적으로 제거할 수 있음을 확인하였으며, optical density 값이 약 77% 감소함을 확인하였다. 미세조류 군집이 제거된 완보동물 배

양 샘플은 음향미세유체칩 내 음향방사력에 의해 완보동물만 선택적으로 분리할 수 있음을 확인하였다. 분리된 완보동물은 약 95% 이상의 샘플회수율과 약 4% 이내의 불순물도를 가짐을 확인하였다.

Ⅴ. 연구개발결과의 활용계획

본 연구는 지금까지 완보동물 연구의 걸림돌이었던 비접촉식 고속 분리를 자동화하는 초소형 미세유체칩을 개발하여 완보동물 연구의 기폭제가 될 것으로 기대된다. 또한 개발된 미세유체칩과 관련 유체역학기술은 완보동물 뿐만 아니라 다른 극지 미소동물 연구에도 활용될 수 있다.

Summary

Tardigrades are capable of surviving in extreme physical conditions (e.g., temperature, pressure, vacuum, UV and Gamma ray exposure, desiccation, starvation) based on the property known as cryptobiosis. A further insight into tardigrade behavioral characteristics could play a vital role in developing novel bio-technologies, biological markers to study the evolution and improving human health care technologies. Therefore, it is important to prepare laboratory-based culturing of tardigrade, which can survive extreme polar environmental conditions to perform further biological studies.

The proposed work is a very fundamental yet novel study that integrates microfluidics into polar biology research, especially on tardigrades. Microfluidic chips will be used to filter the unwanted content from the sample and thus to separate tardigrades. The microfluidic chip can generate high frequency acoustic waves that can be used to actively and continuously filter tardigrades based on their size, density and shape, while a passive microfluidic channel based filter can be used to capture algal clusters based on their size and morphology.

In an effort to explore the response of tardigrades to high frequency (10~200 MHz) acoustic waves and thermal stresses, the proposed microfluidic chip is used. Previously, various other physical stimuli, such as thermal, radiative, desiccative, have been used to actuate different gene expressions in tardigrades. However, the effect of a mechanical stimulus, in the form of a vibration or acoustic wave, on the tardigrade gene expression has never been scrutinized. We hope to obtain a further insight into the tardigrade response to acoustic waves and in turn their genomics. In addition, migration behavior in reesponse to non-uniform food distribution will be first examined using the proposed microfluidic chip.

The proposed microfluidic chip for non-contact separation of tardigrades can serve as a next-generation research tool for tardigrades genomics and proteomics studies, and it can be also applied to other polar microscopic animals. In the industrial perspective, commercial-scale culturing of tardigrades can be used for research purposes and development of futuristic technologies such as dry vaccines in tardigrades.

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제 1 장 서론

제 1 절. 완보동물(tardigrade)

흔히 물곰이라고 알려져 있는 완보동물(tardigrade)은 곤충, 거미가 포함된 절지동물로서, 현존 동물 중 가장 뛰어난 생존력을 바탕으로 극지는 물론 적도, 심해, 고산지대에 이르기까지 지구 전체에 걸쳐 서식한다(그림 1). 완보동물의 강력한 생존력은 극한 환경에 노출되면 신진대사를 멈추고 휴면상태(cryptobiosis)로 전환하는 것에 기인한다. 신진대사가 멈춰진 휴면상태의 완보동물은 먹지도 호흡하지도 않으며 생명을 유지한다. 휴면상태에서 완보생물은 단백질, 체내 수분 등을 트레할로스(trehalose)라는 당분으로 감싸 세포 파괴를 방지한다. 또한 항산화제를 다량으로 생산하여 DNA 파괴를 막고 손상된 DNA를 보수한다. 학계에 보고된 바에 따르면, 완보동물은 절대영도에 가까운 -272°C의 극저온, 150°C 이상의 고온, 대기압의 1000배 이상의 고압, 진공상태에서 생존할 수 있고, 570000 rad 이상의 방사능(인간 치사량 약 500 rad)과 각종 독성물질에도 강한 내성을 갖고 있다(그림 2).

그림 1. 완보동물(Tardigrade)

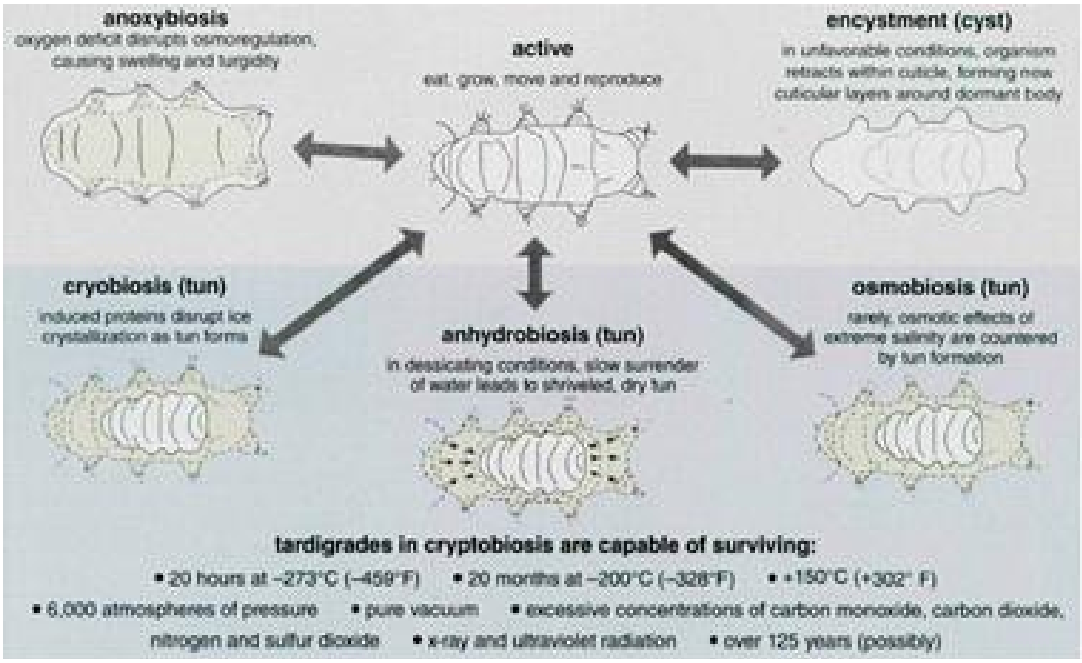

그림 2. 다양한 극한환경에 대한 완보동물의 휴면상태 전환

제 2 장 국내외 기술개발 현황

제 1 절. 완보동물 관련 연구

1773년 독일인 동물학자 Johann August Ephraim Goeze에 의해 처음 발견된 완보동물은 휴면상태 전환을 통한 뛰어난 생존력이 주목받기 시작한 2000년대 이후 활발히 연구되어지고 있다. 완보동물 연구는 크게 1) 발생생물학적 연구, 2) 다양한 극한환경에 대한 휴면상태 전환 연구, 3) 휴면상태 전환에 연관된 유전체, 단백질체, 대사체 분석 연구로 나눌 수 있으며 각각의 연구동향은 아래와 같다.

1. 발생생물학적 연구

모델생물(model animal)은 생물학 전반의 영역에 걸쳐 생물학적 현상을 연구하고 해석하기 위한 생물로서, 그 대표적인 예로는 예쁜꼬마선충(C.elegans)과 초파리(Drosophila)가 있다. 두 모델생물은 크기가 작고 실험실 환경에서 키우기 용이하며, 번식력이 우수하여 사람을 포함한 많은 동물에 대한 발생생물학적 연구를 수행하는 데 큰 공헌을 하였다. 하지만 이들 모델생물은 고/저온, 고/저압, 고방사능 등 극한 환경에서 생존할 수 없어 관련 연구를 위한 모델생물로 적합지 않다. 따라서 다양한 극한환경에서 생존 가능한 완보동물이 새로운 모델생물로서 주목받아 관련 연구가 활발히 진행 중이다. Gabriel et al. (2007)에 의해 모델생물로서의 완보동물의 가능성이 처음으로 제시된 이후, 현재 관련 연구는 미국 노스캐롤라이나 대학의 Goldstein 교수 연구팀이 주도하고 있다. 최근 Goldstein 연구팀은 완보생물의 한 종류인 H.dujardini의 차세대 모델생물로서의 가능성을 입증하였으며 (Boothby et al. (2017)), 영국 랭커셔 주 한 연못의 저생(benthic) 샘플에서 H.dujardini 완보동물을 채집하여 연구실 내 배양을 위한 프로토콜을 보고하였고 Sciento 사를 설립해 전 세계 완보동물 연구자들에게 공급하고 있다. 또한 Horikawa et al. (2008)은 완보동물을 포함한 극지적응 미소생물을 극지에서 채집한 후 연구실 환경에서 배양하는 방법을 보고하였다.

2. 다양한 극한환경에 대한 휴명상태 전환 연구

극저온, 극저압, 강한 자외선과 방사선으로 대표되는 우주환경은 극한환경의 대표적인 예다. 우주

생물학 연구를 위해서는 이러한 극한환경에서 생존할 수 있는 모델생물로서 완보동물이 많이 활용되어 왔다. 2007년 9월 유럽우주국(ESA) 무인우주선 포톤-M3호와 2011년 미항공우주국(NASA) 우주왕복선 엔데버호에서 실시된 실험에서 완보동물은 물과 산소가 없는 우주의 진공상태에서 생존은 물론 번식까지 가능함이 밝혀졌다. 이를 바탕으로 완보동물은 2013년 미항공우주국(NASA)에 의해 '지구에 사는 동물 중 외계 생명체로 가장 적합한 후보'로 선정되었으며, 이탈리아 Modena 대학 Rizzo et al. (2015)은 우주연구를 위한 최적의 모델생물은 완보동물이라고 주장하였다. 다양한 극한환경에서 완보동물의 생존 메커니즘을 규명하기위한 지난 수년간 많은 연구가 수행되어 왔으며 대표적 연구는 아래와 같다.

	선행 연구	극한 환경 조건
1	Horikawa *et al.* (2006)	UV ray
2	Hengherr, Worland, Reuner, Brümmer, & Schill (2009)	thermal stress
3	Møbjerg *et al.* (2011)	outer space
4	Halberg, Jørgensen, & Møbjerg (2013)	anhydrobiosis (desiccation)
5	Beltrán-Pardo, Jönsson, Harms-Ringdahl, Haghdoost, & Wojcik (2015)	gamma ray
6	Tsujimoto, Imura, & Kanda (2016)	cryogenic temperature
7	Hashimoto *et al.* (2016)	radiation

3. 휴면상태 전환에 연관된 유전체, 단백질체, 대사체 분석 연구

완보동물 연구의 의의는 완보동물의 뛰어난 생존 메커니즘 규명을 통해 인간을 포함한 유기체의 생명력을 증진시키고, 우주생물학 연구를 통해 지구 밖 환경을 밝히는 데 있다. 이를 위해 다양한 극한환경에 대해 완보동물의 휴면상태 전환 메커니즘을 규명하기 위해서는 관련 유전체, 단백질체, 대사체 연구가 필수적이며, 완보동물과 관련된 연구 중 가장 활발히 수행되고 있는 연구 분야다 (Arakawa et al. (2016)). Schokraie et al. (2012)는 완보동물의 각 발달단계마다 유전체와 단백질체 분석을 수행하였다. Boothby et al. (2015)는 완보생물이 수평적 유전자 이동을 통해 유기체로부터 많은 유전자를 획득한다고 주장하였다. 하지만 Koutsovoulos et al. (2016)은 기존의 완보생물에 대한 연구 성과의 대부분이 샘플 오염에 의한 오류라고 주장하였다.

제 3 장 연구개발수행 내용 및 결과

제 1 절. 연구의 필요성, 독창성, 중요성

1. 연구의 필요성

앞서 서술한 바와 같이 완보동물 연구는 다각도로 활발히 수행되어 왔다. 하지만 기존의 연구는 아래의 한계점을 지닌다. 지금까지 온도, 압력, 방사능, 화학물질 등 다양한 극한환경에서 완보동물의 휴면상태 전환에 대한 연구가 수행되었다. 하지만 자연계에서 생명체가 경험하는 대표적인 기계적 자극인 음파 자극에 대한 완보동물의 거동에 대한 연구는 미비한 실정이다. 또한 배양된 완보동물로 실험을 하기 위해서는 배양액에서 핀셋(tweezer) 등을 이용한 접촉식 방법으로 완보동물을 분리해야 한다. 1 mm 이내의 몸길이를 갖는 완보동물을 핀셋을 이용해 분리하는 것은 숙련된 연구자에게도 매우 까다로운 작업이다. 또한 접촉식 방법으로 분리한 완보동물을 대상으로 한 연구는 Koutsovoulos et al. (2016)이 지적한 바와 같이 샘플 오염이 빈번히 발생해 정확한 완보동물 연구가 어려운 실정이다.

2. 연구의 독창성

본 연구는 KAIST 성형진 교수 연구팀과 KRIBB 윤태성 교수 연구팀의 공동연구이자, 각 연구팀의 연구 분야인 미세유체역학과 극지생물학의 융합연구이다. KAIST 성형진 교수 연구팀은 미세유체역학 연구를 필두로 유체역학 전반에 걸쳐 SCI급 국제학술지 320편 이상 출판이라는 탁월한 연구성과를 거둔 우리나라 대표적 유체공학자로서, 성형진 교수 연구팀은 미세유체역학 분야에 세계적 수준의 기술을 보유하고 있다. 개발된 미세유체칩을 바탕으로 다양한 생화학적 응용 연구를 수행하왔으며, 특히 2016년에는 대표적인 극지적응 미소생물인 남세균(cyanobacteria)을 미세유체칩 내에서 광력을 이용해 대량으로 배양하는 기술을 개발해 보고하는 등 미세유체역학을 기반으로 극지생물학을 융합하는 연구 경험을 보유하고 있다. KRIBB 윤태성 교수 연구팀은 현재 국내에서 유일하게 완보동물 연구를 수행하는 연구팀으로서 완보동물 배양에 대한 노하우와 휴면상태 전환 메커니즘 규명을 위한 오랜 선행연구를 수행해왔다. 두 연구팀은 2016년 KAIST K-Valley RED&B 사업의 지원으로 6개월 간 선행연구를 진행한 바 있다. 본 연구의 목표 중 하나는 KAIST 성형진 교수 연구

팀이 그동안 축적한 미세유체역학 기술을 바탕으로 완보동물 연구를 위한 미세유체칩을 개발하는 것이다. 이를 통해 기존 완보동물 연구에서 불가능했던 완보동물의 비접촉식/고속 분리가 가능해 완보동물 연구의 새로운 성장 동력을 제공하고자 한다.

3. 연구의 중요성

완보동물은 휴면상태 전환을 통해 지구상 현존하는 가장 뛰어난 생존력을 지닌 생명체이다. 이런 완보동물의 생존 메커니즘을 규명함으로써 인간을 포함한 유기체의 생명력을 증진시킬 수 있다. 더 나아가 완보동물은 뛰어난 생존력을 바탕으로 기존의 초파리, 예쁜꼬마선충을 대체할 수 있는 차세대 모델생물로서, 우주생물학과 극지생물학을 비롯해 생물학 연구 전반에 막대한 기여를 할 수 있을 것으로 평가된다. 본 연구의 중요성을 요약하면 아래와 같다. 지난 10년 여간 완보동물에 대한 활발한 연구가 다각도로 수행되었다. 하지만 기존 연구에서 배양된 완보동물을 핀셋 등을 이용한 접촉적인 방법을 통해 분리함으로써 샘플오염에 노출되었다. 이를 해결하기 위해 본 연구는 미세유체역학 기술을 통해 소형 미세유체칩 내 통제된 환경에서 완보동물을 대량배양하고 비접촉적인 방법으로 완보동물을 고속으로 분리하는 기술을 개발하고자 한다. 따라서 개발되는 미세유체칩은 완보동물의 연구의 새로운 지평을 열 수 있는 도구로서 큰 기여를 할 수 있을 것으로 판단된다. 뿐만 아니라 개발되는 미세유체칩은 마이크로 스케일의 다양한 극지적응 미소생물에도 적용할 수 있어 극지생물학 연구에도 그 활용가능성이 매우 높다.

제 2 절. 연구수행 방법

구분	연구개발 목표	연구수행방법 (이론적・실험적 접근방법)	구체적인 내용*
2017년도	음파/온도 자극에 대한 완보동물 거동 연구	완보동물과 미세조류 배양	완보동물(*Hypsibius dujardini*)과 먹이로 사용되는 미세조류(*Chlorococcum*) 배양
		음파 자극에 대한 완보동물 거동 연구	리튬니오베이트 압전기판 위 증착된 빗살무늬전극에서 생성되는 고주파수 표면탄성파에 노출된 완보

			동물의 거동 분석
		온도 자극에 대한 완보동물 거동 연구	리튬니오베이트 압전기판 위 증착된 빗살무늬전극에서 생성되는 고주파수 표면탄성파에 노출되어 음향열적가열을 받는 완보동물의 거동 분석
2018년도	완보동물 분리를 위한 미세유체칩 개발	미세유체칩 설계 및 제작	Soft lithography 기법을 이용해 제작된 PDMS 미세유체칩과 리튬니오베이트 압전기판 위 증착된 빗살무늬전극과 결합된 음향미세유체칩 설계 및 제작
		미세유세칩 성능 검증	마이크로필터 어레이 기반 완보동물 수동적 분리 실험 및 음향미세유체역학적 분리 실험 수행
		완보동물의 선택적 분리	미세조류 제거 비율은 optical density 측정으로 수행하고 완보동물의 분리 후 영상처리를 통해 분리 효율 분석

제 3 절. 연구내용 및 연구결과

1. 음파/온도 자극에 대한 완보동물의 거동 연구

다양한 극한환경에 대한 완보동물의 휴면상태 전환 메커니즘 규명 연구를 통해 인간을 포함한 유기체의 생명력을 증진시킬 수 있다. 또한 뛰어난 생존력을 바탕으로 완보동물은 우주생물학, 극지생물학을 비롯한 생물학 연구 분야 전반에서 차세대 모델생물로서 큰 기여를 할 수 있다. 이를 위해는 다양한 자극에 대한 완보동물의 거동 연구가 필수적이다. 그동안 관련 연구는 온도, 압력, 방사능, 화학물질 등의 자극에 국한되어있어 자연계에서 생명체가 쉽게 경험할 수 있는 대표적인 기계적 자극인 음파 자극에 대한 연구는 수행되지 않았다. 본 연구는 음향미세유체칩과 관련 기술을 바탕으로 고주파(10~200 MHz) 음파와 음향열적가열에 의한 온도 자극에 노출됐을 때 완보동물의 거동에 대한 연구를 수행하였다.

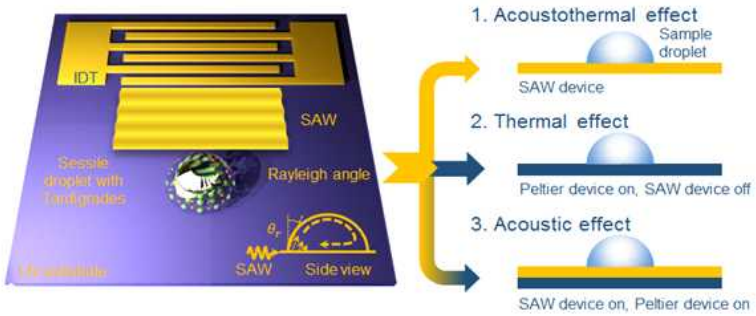

그림 3 음파/온도/음향열적가열 자극 인가를 위한 실험 구성

아래 그림과 같이 1) 음파의 감쇠로 인해 발생하는 열을 기반으로 하는 음향열적 자극을 통해 음파와 열 자극이 공존할 때, 2) 열 자극만 가해질 때, 3) 음파 자극만 가해질 때 총 세 가지 유형의 자극에 대해 완보동물의 반응을 살펴보았다. 그 결과 그림 10과 같이, 고주파수 음파와 이에 수반되는 열 자극이 공존할 때와 열 자극만 가해질 때 완보동물은 휴면상태로 전환되어 신진대사를 멈추는 것을 현미경을 통해 확인하였다. 이와 달리 음파 자극만 가해질 때 완보동물은 휴면상태로 전환되지 않고 보통 상태의 신진대사를 유지하는 것을 확인하였다.

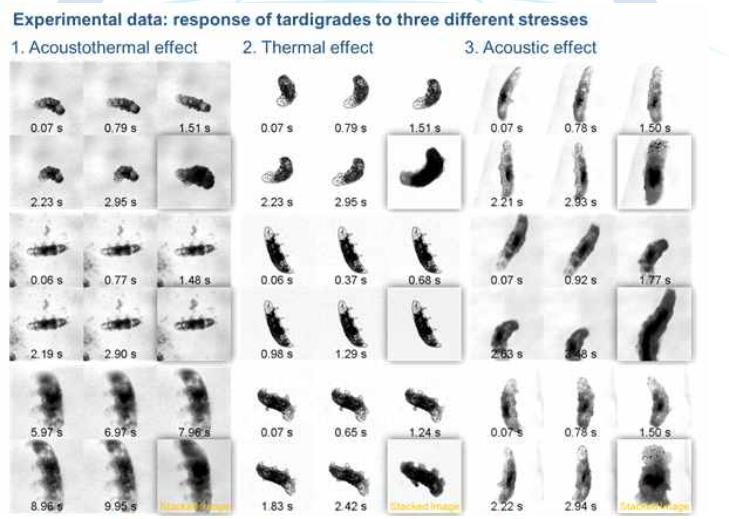

그림 4 기계적 자극 하의 완보동물 거동: 1) 음향열적 자극, 2) 열적 자극, 3) 음파 자극

2. 완보동물 연구를 위한 미세유체칩 개발

가. 요약

지난 10년 여간 완보동물에 대한 연구가 활발히 수행되어 왔지만 아직까지 기초연구단계이다. 특히 완보동물 연구를 위해서는 배양된 개체를 비접촉식으로 분리하여 샘플오염으로 인한 실험오차를 줄이는 것이 필요하다. 이를 위해 본 연구는 미세유체역학을 기반으로 완보동물의 비접촉식 고속 분리가 가능한 초소형 미세유체칩을 개발하였다. 기존 연구에서 배양된 완보동물은 핀셋(tweezer)를 이용해 배양액에서 분리되었다. 1 mm 이내의 몸길이를 갖는 완보동물을 핀셋을 이용해 분리하는 것은 매우 까다로운 작업이다. 또한 접촉식 방법으로 분리한 완보동물을 대상으로 한 연구는 Koutsovoulos et al. (2016)이 지적한 바와 같이 샘플 오염이 빈번히 발생해 정확한 완보동물 연구가 어려운 실정이다. 이를 해결하기 위해 본 연구는 미세유체역학 분리기술을 도입하여 비접촉식으로 완보동물을 고속으로 분리하는 기술을 개발하였다. 본 연구는 두 단계로 구성되어 있으며, 이는 마이크로필터 어레이를 기반으로 유체역학적 힘을 이용한 수동적 미세조류 군집 제거와 음향미세유체칩 내에서 음향력을 이용한 완보동물의 능동적 분리이다. 마이크로필터 기반 수동적 분리방법은 유동 분석 통해 완보동물 분리에 최적 설계된 반원 형상의 마이크로필터 수백 여개를 이용한다. 마이크로필터의 미세구멍(pore) 크기를 조절하여 연구자가 원하는 발육상태의 완보동물을 배양액으로부터 분리한다. 음향력 기반 능동적 분리방법은 음향미세유체칩에서 발생된 고주파수 표면탄성파(음파)가 유체영역 내 완보동물을 만나 음향방사력을 가해 완보동물의 횡방향 위치를 제어하여 원하는 때에 선택적으로 완보동물을 고속으로 분리한다. 완보동물 뿐만 아니라 다양한 극지동물 연구에 활용하기 위한 범용 플랫폼을 개발하기 위해 낮은 온도 조건을 구현하였다.

나. 완보동물 연구를 위한 미세유체칩 개발 연구 논문
(아래 연구 논문은 현재 국제학술지에 투고되어 심사 중임)

(1) Abstract

Tardigrades are microscopic animals well known for their survival capabilities under extreme conditions. This microorganism is the focus of current research in the fields of taxonomy, biogeography, genomics, proteomics, development, space biology, evolution, and ecology. Tardigrades, such as Hypsibius exemplaris, are being advocated as a next-generation model organism for genomic and developmental studies. The raw culture of Hypsibius exemplaris usually contains tardigrades themselves, their eggs, and algal food. Experimentation with tardigrades requires the demanding and laborious separation of tardigrades from raw samples to prepare pure and contamination-free tardigrade samples. In this paper, we propose a two-step

acousto-microfluidic separation method to isolate tardigrades from raw samples. In the first step, a passive microfluidic filter composed of an array of traps is used to remove large algal clusters in the raw sample. In the second step, a surface acoustic wave-based active microfluidic separation device is used to continuously deflect tardigrades from their original streamlines inside the microchannel and thus selectively isolate them from algae and eggs. The experimental results demonstrated the efficient tardigrade separation with a recovery rate of 95.76% and an algae impurity of 3.96% on average in a continuous, contactless, automated, rapid, biocompatible manner.

(2) Introduction

Tardigrades, microscopic animals found on all continents, are well known for their cryptobiotic abilities to survive under extreme physical conditions (Guidetti, Altiero, & Rebecchi, 2011; Møbjerg et al., 2011) such as temperature (Hengherr et al., 2009a,b), pressure (Seki & Toyoshima, 1998; Ono et al., 2008, 2016), space vacuum (Jönsson et al., 2008), UV (Altiero et al., 2011; Horikawa et al., 2013; Giovannini et al., 2018), and Gamma ray exposure (Beltrán-Pardo et al., 2015). Much effort has been devoted to characterizing the taxonomy (Jørgensen, Kristensen, & Møbjerg, 2018), biogeography (Pilato & Binda, 2001), evolution and ecology (Nelson, 2002), genomics (Yoshida et al., 2017), proteomics (Schokraie et al., 2012), development (Smith et al., 2016; Gross, Minich, & Mayer, 2017), and space biology (Rebecchi et al., 2009; Weronika & Łukasz, 2017) of tardigrades in an effort to better understand this microscopic organism. Recently, the recovery and reproduction of the tardigrade that had been frozen for over 30 years attracted a great deal of attention (Tsujimoto, Imura, & Kanda, 2016). Tardigrades have been found to produce specific proteins (e.g., CAHS, SAHS, MAHS, RvLEAM) under external stresses to enable survival (Yamaguchi et al., 2012; Tanaka et al., 2015). A unique DNA-associated protein (Dsup) from the tardigrade has been expressed in the human cultured cells to improve human radiation tolerance and reduce X-ray-induced damage of the cellular DNA by 40% (Hashimoto et al., 2016).

With the aforementioned characteristics, the tardigrade (Tardigrada clade) has been suggested as one of the potential model organisms for space research (Jönsson, 2007) as well as for developmental and genomic studies to be investigated along with two commonly used model

organisms: Caenorhabditis elegans (Nematoda) and Drosophila melanogaster (Arthropoda) (Tenlen, McCaskill, & Goldstein, 2013; Goldstein & King, 2016; Martin et al., 2017). The Hypsibius exemplaris tardigrade has been specifically investigated as a new model animal for evolutionary developmental research due to its characteristics shared with the two model animals mentioned above, short generation time and ease to culture continuously for decades (Gabriel et al., 2007). The genome of Hypsibius exemplaris has been sequenced to investigate the evolution of molecular and developmental mechanisms, and it was erroneously suggested that a horizontal gene transfer from other animals has undesirably impacted the genome composition of the tardigrade Hypsibius exemplaris (Boothby et al., 2015). However, some follow-up studies by other researchers refuted this earlier claim and contend that the false finding was attributed to bacterial contamination derived from the uncontrolled sample preparation (Bemm et al., 2016; Koutsovoulos et al., 2016; Arakawa, 2016). To prevent false-positive results in the experimental data, it is imperative to develop effective separation methods for selective isolation of tardigrades from raw cultures.

Increased research interest in tardigrades has demanded advancements in supportive technologies to facilitate experimentation and the development of standard protocols. Several studies on the laboratory-scale culture methods of tardigrades have been reported (Altiero & Rebecchi, 2001; Suzuki, 2003; Horikawa et al., 2008; Tsujimoto, Suzuki, & Imura, 2015; Altiero et al., 2015). The growth cultures of tardigrades contain different age groups of tardigrades (adults, newborns, and juveniles of variable sizes), eggs, exuviae, and algal food. It is important to prepare homogenous samples of tardigrades having a uniform age or size distribution for further experimentation (Gabriel et al., 2007). Separating tardigrades from food, eggs, exuviae, or unwanted debris from a raw sample is laborious and time-consuming work, in which individual tardigrades must be manually picked using a wire Irwin loop (Sands et al., 2008), a micropipette (Degma, Katina, & Sabatovičová, 2011), or a needle (Gross et al., 2017) under a microscope. These labor-intensive methods require a skilled person to dexterously locate and capture a single tardigrade within a sample under a microscope, provide a limited separation throughput, and are susceptible to contamination. On the other hand, tardigrades can also be isolated in batches from their algal food using the Baermann filtration technique, in which raw cultures are repeatedly passed through a filter paper (Koutsovoulos et al., 2016). Despite the simple operation and low cost of the filtration method, it has been reported to have inherent limitations such as

long processing time and its dependency on sample volume (Van Bezooijen, 2006). The manual and filtration methods described above do not offer continuous, automated, non-contact, on-demand control over the separated constituents.

In recent years, microfluidic approaches to separate microscale objects have been proven to be effective and shown great potential for many medical, biological, chemical and industrial applications (Sajeesh & Sen, 2014; Wyatt Shields IV, Reyes, & López, 2015). Microscale flows within a microchannel allows precise control over the flow and suspended objects and continuous sample processing. A variety of microfluidic separation techniques (Bhagat et al., 2010), including passive methods that utilize hydrodynamics forces arising from the microchannel geometry and active methods that rely on external force fields, have been reported to offer rapid, continuous, automated, biocompatible sample processing. Inertial microfluidics (Hur, Tse, & Di Carlo, 2010), hydrodynamic filtration (Choi et al., 2008), magnetophoresis (Alnaimat et al., 2018), optofluidics (Jung et al., 2014), dielectrophoresis (Mathew et al., 2016), and acoustofluidics (Ding et al., 2013; Destgeer & Sung, 2015) are common techniques for isolating micro-objects based on size, magnetic, optical, electrical, or mechanical properties. In particular, acoustofluidic platforms are widely used for numerous applications, including cell sorting (Ma et al., 2017), microparticle separation (Ahmed et al., 2017a, 2018), microparticle patterning (Destgeer et al., 2016, 2019; Collins et al., 2018), microscale flow mixing (Nam, Jang, & Lim, 2018; Ahmed et al., 2019), chemical and thermal gradient generation (Destgeer et al., 2014b; Ha et al., 2015), protein isolation (Ahmad et al., 2017), droplet handling (Park et al., 2017a, 2018b), in-droplet microparticle manipulation (Park et al., 2017b, 2018a), nebulization (Winkler et al., 2017), and microorganism manipulation (Ding et al., 2012). Microfluidic platforms can be utilized for selective isolation of tardigrades from complex samples to address the limitations of the conventional tardigrade separation techniques based on filter papers, tweezers, or Irwin loops.

In this paper, we have combined passive and active microfluidic separation techniques to isolate Hypsibius exemplaris tardigrades from raw samples. In the first step, a laboratory culture of tardigrades was passed through a passive microfluidic filter to capture large algal clusters. The passive filter was composed of an array of micro-pillars that acted as traps and were built inside a polydimethylsiloxane (PDMS) microchannel to allow passage of micro-objects (i.e., tardigrades, eggs, algae) smaller than the trap pore size while trapping larger objects (i.e., algal clusters). The sample collected from the outlet of the passive filter contained tardigrades,

exuviae, eggs, and algal food, and it was then passed through an active separation device for the second step. The active microfluidic platform was composed of an interdigital transducer (IDT) mounted on top of a piezoelectric substrate and a PDMS microfluidic chip. Surface acoustic waves (SAWs) produced from the IDT were transformed into compressional waves (CWs) inside the microchannel and exerted an acoustic radiation force (ARF) on the tardigrades in the direction of wave propagation. The deflected tardigrades were thereby isolated from any remaining algae, exuviae, and eggs with the achievement of more than 95% of recovery rate and less than 4% of algae impurity. The experimental demonstration has proven that the proposed method is a promising technique for tardigrade sample preparation in a continuous, automated, rapid, biocompatible, non-contact, on-demand manner.

(3) Material and methods

(3-1) Taridgrade culture

Cultures containing the tardigrade Hypsibius exemplaris and Chlorococcum algae were purchased and grown according to the protocol provided by the supplier (Sciento). The Hypsibius exemplaris tardigrades were grown in Chalkley's medium plus soil extract at 16°C, with light and dark periods of 14:10 hours (Arakawa, Yoshida, & Tomita, 2016). The Chlorococcum algae, used as tardigrade food, were prepared in Bold's Basal medium plus soil extract at 25°C under 16:8 hours of light and dark periods. Every 4 to 6 weeks, subcultures of the tardigrades were prepared, as they had consumed their algal food and were looking for a spacious new habitat. A new generation of tardigrades were produced every two weeks. Tardigrades laid eggs inside their exuviae shells. The exuviae of a healthy tardigrade can contain 3 - 5 eggs that hatched within 4 - 5 days. The juveniles matured into adult tardigrades within 2 weeks, at which point they were ready to lay eggs (Gabriel et al., 2007).

(3-2) Tardigrade separation process

그림 5 shows the workflow of the two-step tardigrade separation process composed of the passive filtration of algal clusters and the active separation of tardigrades from eggs and algae.

Unicellular Chlorococcum algae tend to flocculate to form large clusters in the tardigrade growth cultures. These aggregated clusters even exceed tardigrades in size; thus, they often cause microchannel clogging and produce pulsating fluctuations that significantly disturb the laminar flow inside the microfluidic channel. For these reasons, the algal clusters present in the growth cultures should be removed for the effective separation of tardigrades prior to the acoustofluidic tardigrade separation. In the first step, the raw sample was passively filtered in the passive microfluidic device so that the large clusters of algae were removed by an array of algal cluster traps, each of which consisted of four micropillars. As a result, the amount of algae present in the raw tardigrade sample could be significantly decreased. The micropillars of the traps also broke the flocculated algal clusters into smaller units to facilitate the active microfluidic tardigrade separation. In the second step, the filtered sample was continuously pumped into an active separation device to selectively separate the tardigrades from the remaining algae and eggs. The SAW-induced ARF acting on the tardigrades deflected them from their original laminar streamlines inside a microchannel and thus sorted them into a desired outlet while the algae and eggs were collected at a different outlet without being affected by the acoustic field. The detailed underlying physics of selective separation of tardigrades will be discussed in detail in a following section.

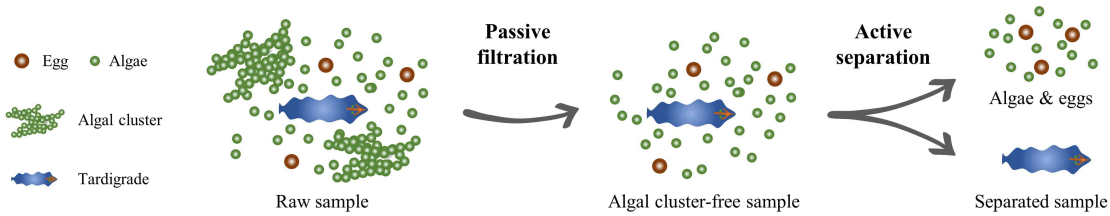

그림 5. A schematic diagram showing the overall workflow of the two-step acousto-microfluidic separation of tardigrades. In the first step, algal clusters in the tardigrade growth culture were filtered by an array of traps composed of micropillars. In the second step, tardigrades were selectively isolated from any remaining algae and eggs for pure sample preparation in an active separation device.

(3-3) Device design and fabrication

Passive and active microfluidic devices were separately designed and fabricated in parallel

prior to conducting the experiments in series. A passive filtration device was designed to have a total of 218 algal cluster traps composed of four micropillars of 200 μm in diameter. The pillars were arranged in a polar array having a 500 μm radius, 40° angle, and 142 μm distance between adjoining pillars, which determined the pore size of the traps, as shown in 그림 6. A soft lithography technique was used to fabricate PDMS microfluidic chips on which a microchannel is patterned (Friend & Yeo, 2010; Destgeer et al., 2013). PDMS has been chosen as the microchannel material because it is biocompatible, optically transparent, gas-permeable and chemically stable (Weibel et al., 2006). A negative photoresist (SU-8 2150, MicroChem), coated on a silicon wafer using a spin coater (Midas System) and sequentially baked on hot plates (65°C and 95°C, respectively), was selectively exposed to UV through a chrome/glass mask (NEPCO) mounted on the stage of a mask aligner (Midas System). The height of the algal cluster traps in the passive microfluidic filter was defined as the thickness of the photoresist layer. After baking, the wafer was placed in a SU-8 developer solution to remove the uncured photoresist and thus obtain the desired microchannel pattern. The PDMS base and curing agent (Sylgard 184A and 184B, Dow Corning) were thoroughly mixed with a weight ratio of 10:1, and the PDMS mixture was poured onto the patterned SU-8 mold on the silicon wafer placed inside a square petri dish. The petri dish was placed in a vacuum chamber and degassed for bubble removal. After the PDMS solution was cured in an oven at 65°C for two hours, PDMS microfluidic chips were peeled off the Si substrate and cut into appropriate sizes. The inlet and outlet ports were punched (Harris Uni-Core) through the microchannels for sample injection and collection. The surfaces of the PDMS microchannels and a glass slide were treated with oxygen plasma for 2 minutes (Covance, Femto Science) and gently placed in contact to achieve irreversible bonding.

The active tardigrade separation device consisted of an IDT deposited on a piezoelectric substrate and a PDMS microfluidic chip, on which a desired microchannel was patterned. The IDTs were fabricated by spin-coating a photoresist layer onto a 128° rotated Y-cut, X-propagating LiNbO3 substrate (MTI Korea) and patterning the resist in the reverse shape of comb-like transducers. A bimetallic conductive layer composed of a 300 Å thick layer of chrome that promoted adhesion of a 1000 Å thick gold layer was deposited by the e-beam evaporation process onto the piezoelectric substrate, followed by a lift-off process to remove the excess metals and photoresist. Two IDTs with uniform electrode width and spacing were formed on the

substrate. Electrodes having width and spacing of 20 μm and 12.5 μm were designed to obtain IDTs with resonant frequencies at 45 MHz and 72 MHz, respectively. A 200 nm-thick SiO2 layer was deposited to cover the electrodes to protect them from mechanical damage (Destgeer et al., 2015). PDMS microfluidic chips were fabricated using the soft lithography process described above. A thin PDMS membrane was used to seal the PDMS microfluidic chip to investigate the effects of the SAW-induced ARF on tardigrades, as shown in 그림 7 (Park et al., 2018b). The 38 μm-thick PDMS membrane was prepared by spin-coating the base and curing agent mixture on top of the saline-treated silicon wafer. After oxygen plasma treatment, the PDMS microchannel and membrane were permanently bonded. The height and width of the PDMS microfluidic channel were 190 μm and 500 μm, respectively. The PDMS microchannel was placed directly on top of the IDT (72 MHz) to reversibly bond it to the LiNbO3 substrate covered with a SiO2 layer. For tardigrade separation, a PDMS microfluidic chip with 163 μm in height and 500 μm in width was used; this microchannel was not sealed using a PDMS membrane but permanently bonded to a SiO2-covered LiNbO3 substrate such that the IDT (45 MHz) was positioned outside of the microchannel (see 그림 8).

(3-4) Experimental setup

Initially, the raw tardigrade sample was homogenized by repeatedly (8 times) pumped back and forth inside a 15 mL conical centrifuge tube (Falcon). The 2.5 mL tardigrade sample was then injected into the passive microfluidic filtration device using PTFE tubing (0.56 mm ID x 1.07 mm OD, Cole Parmer Company) attached to a gas-tight glass syringe (Hamilton Company) connected with a 27G stainless steel hypodermic needle. After repeated passive filtration, the processed sample was loaded into a separate syringe and injected into the active microfluidic tardigrade separation device using a syringe pump (neMESYS, CETONI GmbH) along with two sheath flows (pure DI water). The SAW-based active separation device was mounted on top of a microscope stage (BX53, Olympus), and a 10-bit high-speed COMS camera (pco.1200 hs, PCO) was used to capture the images. An RF signal generator (N5181A, Agilent Technologies) and an amplifier (LZT-22+, Mini-Circuits) were used to produce high-frequency (45 MHz and 72 MHz) AC signals with varying amplitude to actuate the IDTs. ImageJ software (http://imagej.nih.gov/ij/) was used to analyze experimental images, as shown in 그림 7 and 8.

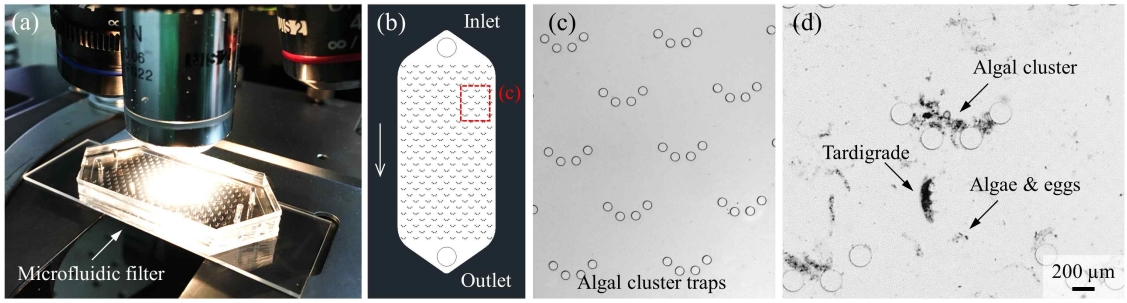

그림 6. A passive filtration process for algal cluster removal from the raw tardigrade sample prior to active tardigrade separation. (a) A PDMS microfluidic filtration device placed on a microscope. (b) The microchannel layout. (c) A magnified view of algal cluster traps composed of four micropillars. (d) A microscopic snapshot in which algal clusters were filtered by the trap whereas a tardigrade passed through it along with smaller algae and eggs.

(4) Results

(4-1) Passive filtration

Raw cultures of tardigrades contain numerous algal clusters along with the tardigrades, their eggs, and algae. For effective and efficient acoustofluidic separation of tardigrades, the algal clusters should be removed by passive microfluidic filtration so that the amount of algae present in the sample is significantly decreased. As shown in 그림 6(a-c), the passive microfluidic filter was designed to have an array of 218 traps composed of four micropillars to capture the large algal clusters whereas the tardigrades, their eggs, and algal food readily passed through the gaps between the pillars. The raw tardigrade sample including algal clusters, tardigrades, eggs and algae was injected into the passive microfluidic device at a flow rate of 15-20 mL min-1 (7.5-10 s per round). The interdistance between the adjoining pillars was 142 μm, so the tardigrades (50-100 μm in length), eggs (40-50 μm in diameter), and algae (10-20 μm in diameter) were allowed to pass through the algal cluster traps, as shown in 그림 6(d). Once a trap was filled with an algal cluster, the increased flow resistance caused the following fluid streams carrying large algal clusters and tardigrades to be diverted away from the occupied trap toward the empty traps downstream of the device. For validation on the algal cluster removal,

we measured the optical density (OD) of the tardigrade sample before and after the passive microfluidic filtration by a spectrophotometer (GeneQuant 1300) at 600 nm; the measured OD value of the tardigrade sample was significantly decreased by 76.53% ± 2.88% from 2.198 ± 0.177 to 0.512 ± 0.037 after the algal cluster filtration on average for ten independent repeated experiments.

(4-2) Acoustic radiation force on tardigardes

For effective acoustofluidic tardigrade isolation, the tardigrades should be selectively affected by SAW-based ARF while the eggs and algae remain unaffected. We investigated the effects of the SAW-induced ARF on the filtered tardigrade sample using a parallel-type acoustofluidic device (Park et al., 2017, 2018b), where the wave propagation direction was opposite to the flow direction. 그림 7(a) shows the top and side views of the device, in which the filtered sample was injected through the inlet of the microchannel. The bottom of the microchannel was sealed by a thin PDMS membrane to prevent the tardigrade sample was not in direct contact with the IDT, which may induce undesired side effects by the electric field formed around the electrodes. When AC signals were applied to the IDT at its resonant frequency, SAWs were produced from the electrodes and immediately transformed into CWs. 그림 7(b) shows a series of experimental images where the passively filtered tardigrade sample fluid was continuously passing through the microchannel at a flow rate of 100 μL h-1 when 72 MHz SAWs at 17.2 Vpp were produced from the IDT placed underneath the microchannel. The experiments were conducted in the parallel-type device to confirm that the SAW-based ARF was selectively effective to the tardigrades for isolation them from the eggs and algae. As the tardigrade approached the IDT, the magnitude of the ARF acting on the tardigrade was increased until they were located at the equilibrium position of two counter-acting forces: the flow-induced drag force (FD) and ARF (FAR), as shown in the figure (t = 7.5 s). The vertical component of the ARF continuously pushed the tardigrade toward the ceiling of the microchannel while the horizontal component of the ARF matched the drag force (Ahmed et al., 2017a,b, 2018). In contrast to the tardigrade trapped at the equilibrium position right next to the IDT, the eggs and algae present in the sample passed freely through the microchannel, verifying our hypothesis that the SAW-induced ARF can solely separate the tardigrades from the eggs and algae (see also Supplementary

Movie 1). We observed that the tardigrades collected after being exposed to the acoustic field were actively moving (see also Supplementary Movie 2) and succeeded in reproduction.

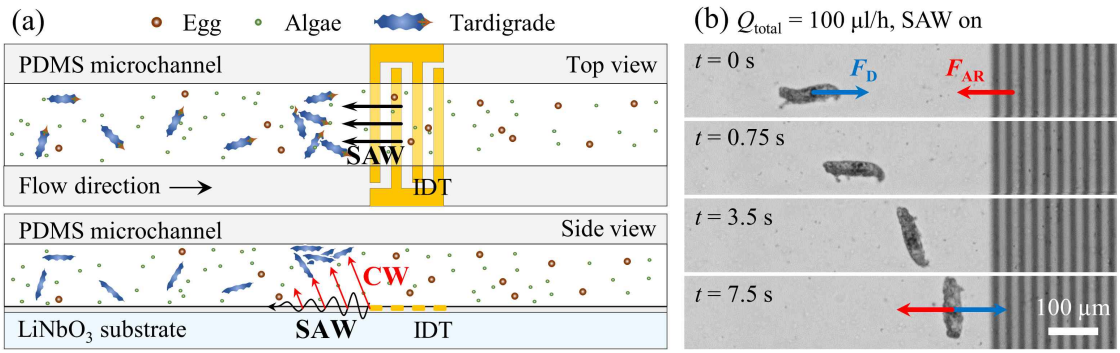

그림 7. (a) A schematic diagram showing the top and side views of the active acoustofluidic platform in which incoming tardigrades were trapped besides the IDT by the SAW-derived ARF (FAR). (b) A flow rate of 100 µL h-1 exerted a drag force (FD) on the tardigrade, which was balanced by the SAW-based ARF at an equilibrium position.

(4-3) Acoustofluidic separation of tardigrades

그림 8(a) shows a schematic diagram of the cross-type acoustofluidic device, where the wave propagation direction was perpendicular to the flow direction, used to prepare pure samples of tardigrades from raw cultures. The filtered tardigrade sample fluid was injected into the central inlet, along with the sheath flows 1 (left) and 2 (right) of DI water from the two other inlets, to hydrodynamically focus the sample flow at the desired location. The flow rates were 2000 µL h-1 (sample flow), 1000 µL h-1 (sheath 1), and 3000 µL h-1 (sheath 2). The sheath flow 2 from the right-hand inlet was used to pinch the sample flow close to the microchannel sidewall while the sheath flow 1 was introduced to prevent the suspended constituents in the tardigrade sample from being located in the anechoic region, where the effective acoustic field was not formed (Destgeer et al., 2015). The IDT was placed beside the microchannel within an acoustic window to minimize acoustic wave damping so that the acoustic waves could be coupled with the fluid inside the microchannel in an energy-efficient manner (Shi et al., 2009; Destgeer et al., 2013). The SAWs radiating from the IDT propagated along the surface of the piezoelectric substrate and penetrated into the microchannel passed through the narrow PDMS wall with a thickness of

100 μm. 그림 8(b) shows the stacked experimental image in which the hydrodynamically focused sample fluid was collected at the outlet 1 (left) without the acoustic field applied to the microchannel. The microscopic images of the samples collected from the outlets 1 and 2 clearly show the presence (그림 8(b1)) and absence (그림 8(b2)) of the tardigrade sample including the tardigrades, eggs and algae. The average OD values of the samples collected at both outlets were measured to be 0.1948 (outlet 1) and 0.0162 (outlet 2), confirming our experimental observation of all the components of the tardigrade sample flowing into the outlet 1. On the other hand, 그림 8(c) shows the stacked microscopic image when 45 MHz SAWs at 17.2 Vpp were applied to the microchannel. As clearly seen in the figure, the SAW-induced ARF acting on the tardigrades exclusively deflected them from the original streamlines so that the tardigrades were transferred from the filtered sample to the sheath flow 2. Consequently, the tardigrades could be separated into the outlet 2 whereas the algae and eggs followed their original streamlines and thus were collected at the outlet 1 (see also Supplementary Movie 3). In five independent repeated experiments with more than 100 tardigrades, the average recovery rate was measured to be 95.76% ± 2.42% with an average throughput of 400 tardigrades per hour. The average OD values of the samples at the outlet 1 and 2 were 0.1736 (그림 8(c1)) and 0.0203 (그림 8(c2)), respectively. Considering the average OD value of the filtered tardigrade sample was 0.512, the algae impurity was 3.96% ± 1.67% in the sample collected at the outlet 2 after the acoustofluidic tardigrade separation.

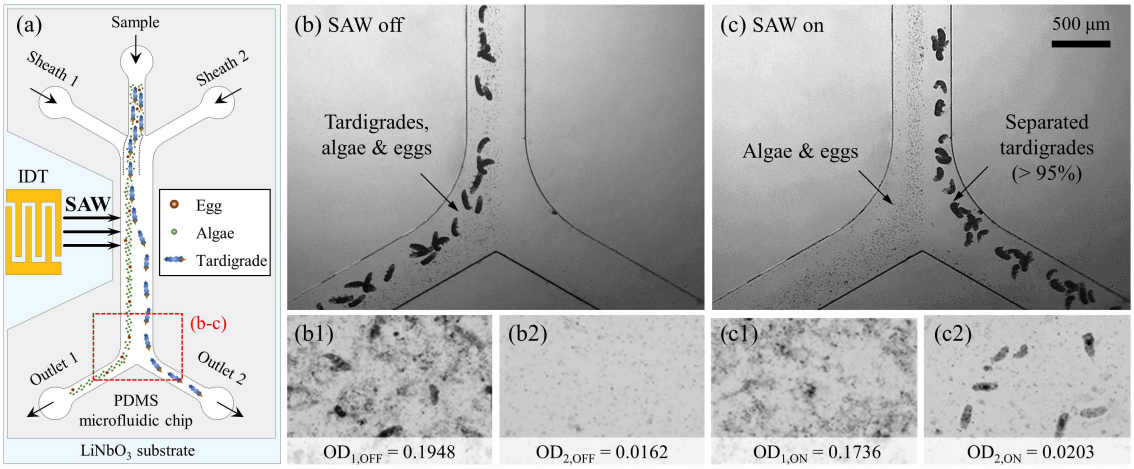

그림 8. (a) A schematic diagram illustrating the continuous separation of tardigrades from algal food and eggs based on SAW-induced ARF. The sample fluid containing tardigrades, their eggs, and algal food was pumped through a central inlet and focused by two sheath flows. The SAW-based ARF deflected the tardigrades away from their original path to separate into a desired outlet port. The eggs and algae were unaffected by the force and thus move along with their original streamlines. (b) When the SAWs were not applied, a focused culture sample fluid passed through the microchannel and was collected at outlet 1 without deflection of the tardigrades. Images (b1) and (b2) of the collected samples at outlets 1 and 2 revealed the presence and absence of tardigrades as well as optical density (OD) values for algae quantification at respective outlets. (c) The ARF produced by 45 MHz SAWs originating from the IDT pushed the tardigrades from their original path to sort them into outlet 2 with a recovery rate of more than 95%. The algae and eggs were mostly unaffected by the force and thus collected at outlet 1. Images (c1) and (c2) of the outlet samples revealed the absence and presence of tardigrades and OD values for algae impurity quantification at outlets 1 and 2, respectively.

(5) Discussion and conclusion

When the SAWs interacted with the fluid inside the microchannel, these waves were transformed into CWs and propagated through the fluid at a Rayleigh angle ΘR = $\sin^{-1}(cf/cs) \approx 22°$, where cf and cs are the sound speeds inside the fluid and the substrate, respectively (Collins, Alan, & Neild, 2014; Fakhfouri et al., 2018). When micro-objects are suspended in the microchannel and exposed to the CWs in the path of the wave propagation, these waves are scattered in an inhomogeneous manner at the interface between the liquid and the object; as a result, the suspended microscale objects experience the ARF in the wave propagation direction. The magnitude of the ARF acting on the micro-objects strongly depends on the dimensionless

Helmholtz number, $\kappa = \pi dm/\lambda f$, where dm is the representative length of the objects and λf is the acoustic wavelength. If $dm > \lambda f$, asymmetric wave scattering off the micro-object results in a significant ARF in the direction of the wave propagation whereas the ARF acting on small objects compared to the acoustic wavelength ($dm < \lambda f$) is negligible because of homogeneous wave scattering (Skowronek et al., 2013; Skowronek, Rambach, & Franke, 2015; Destgeer et al., 2014a; Devendran et al., 2016). Considering that the average length of the tardigrades that we targeted to manipulate was approximately 100 μm in our experiments, we used IDTs with their resonant frequencies above 36 MHz such that the tardigrades experienced the ARF strong enough to overcome the flow-induced drag force. The 72 MHz SAWs were used in the experiments above to prevent standing SAWs formed in the vertical direction within the parallel-type acoustofluidic device. As shown in 그림 7(b), 72 MHz SAWs, whose wavelength was smaller than the tardigrades but larger than the eggs and algae, imparted the ARF selectively to the tardigrades. As a consequence, only the tardigrade remained standstill while the other components of the tardigrade sample after microfluidic filtration passed freely through the acoustic field. As previously reported (Wiklund, 2012; Li et al., 2015), the ARF-based acoustofluidic manipulation was found to be biocompatible and imparted no harmful stresses to the tardigrades as they moved actively (see also Supplementary Movie 2) and successfully reproduced.

The average recovery rate of the acoustofluidic tardigrade separation was above 95%. A few tardigrades did not experience the ARF enough to be deflected toward the outlet 2 for the following reasons. First, a few tardigrades may have passed through the acoustic anechoic region, where the acoustic field was not effective, due to unintended flow disturbance induced by pressure fluctuations and clogging within the microchannel. Second, the characteristic length of some tardigrades was smaller than the acoustic wavelength (λf = 80 μm for 45 MHz SAWs), so insignificant acoustic radiation occurred around the tardigrades. Third, when multiple tardigrades were passing through the acoustic field close to each other, insufficient magnitude of the acoustic field was applied to the tardigrade located farther from the IDT, leading to the insignificant wave scattering. In the acoustofluidic tardigrade separation, the average time required to collect 100 tardigrades was measured to be 15 min (approximately 400 tardigrades per hour). However, the throughput can be varied depending on the number of tardigrades per unit sample volume injected into the active separation device. In addition, the algae impurity of

the tardigrade sample after acoustofluidic separation was measured to be slightly less than 4% based on the OD measurement. A very small amount of the algae and eggs in the sample flow were unintendedly separated along with the tardigrades for the following reasons. First, the acoustic streaming flow-induced drag force was imparted to the eggs and algae. Second, unintended flow disturbance caused the tardigrade sample flow to be imperfectly guided to the outlet 1 by the sheath flows. Third, some algae attached to the tardigrades were separated together with the tardigrades that were affected by the SAW-based ARF. The acoustofluidic tardigrade separation device can be easily switched on and off by simply controlling the electrical signal applied to the IDT. The on-demand control of the device can be utilized to prepare the tardigrade samples with the desired amount of the tardigrades.

We developed a two-step tardigrade separation method to separate tardigrades from raw culture samples in a continuous, automated, contactless, on-demand manner. In the passive microfluidic filtration step, large algal clusters were removed by an array of 218 traps composed of four micropillars, with a significantly decreased OD value by 77% on average, to facilitate the acoustofluidic tardigrade separation. The effects of the acoustic waves on the tardigrade sample were examined in a parallel acoustofluidic device. In our experiments, tardigrades were found to experience the SAW-based ARF that was significant enough to overcome the flow-induced drag force acting on the tardigrades whereas the eggs and algae remained unaffected by the acoustic field. Biocompatibility of the acoustic waves applied to the tardigrades was confirmed by active movement and reproduction of the tardigrades after being exposed to the acoustic field. In the active tardigrade separation device, the passively filtered tardigrade sample was injected to the cross-type acoustofluidic device. We demonstrated that the tardigrades could be selectively deflected by the SAW-induced ARF and thus collected at a separated outlet at a recovery rate of 95.76% and an algae impurity of 3.96%. The proposed acousto-microfluidic approach to tardigrade separation is expected to play a vital role in tardigrade research and may be further extended to the selective isolation of the tardigrades with different age groups.

제 4 장 연구개발목표 달성도 및 대외기여도

제 1 절. 연구개발목표 달성도

1. 정성적 목표달성도

가. 1차년도 정성적 성과

연구개발목표	달성내용	달성도
○ 음파 자극에 대한 완보동물 거동 분석	○ 고주파수 음파 자극에 대한 완보동물 거동 분석 완료	100 %
○ 온도 자극에 대한 완보동물 거동 분석	○ 음향열적가열에 의한 온도 자극이 있을 때 완보동물 거동 분석 완료	100 %

나. 2차년도 정성적 성과

연구개발목표	달성내용	달성도
○ 완보동물 연구를 위한 수동적 미세유체칩 개발	○ 마이크로필터 어레이 기반 완보동물의 수동적 분리를 위한 미세유체칩 및 구동 기술 개발 완료	100 %
○ 완보동물 연구를 위한 음향미세유체칩 개발	○ 표면탄성파 기반 음향방사력을 이용한 음향미세유체칩 및 구동 기술 개발 완료.	100 %

2. 정량적 목표달성도

가. 1차년도 정량적 성과

구 분		목표(건)	달성 실적(건)	주저자 실적	달성도		비고
국외 논문	SCI	3	7	참여연구원 주저자 및 교신저자 7건	233	%	-
	SCIE						
국내 논문	SCI	2	0		0	%	국외논문(SCI)로 대체
	SCIE						
특 허 출 원		2	0		0	%	특허 출원 준비
기 타							

나. 2차년도 정량적 성과

구 분		목표(건)	달성 실적(건)	주저자 실적	달성도		비고
국외 논문	SCI	3	4	참여연구원 주저자 및 교신저자 4건	133	%	-
	SCIE						
국내 논문	SCI	2	0		0	%	국외논문(SCI)로 대체
	SCIE						
특 허 출 원		2	1		50	%	특허 출원 준비중
기 타							

제 2 절. 대외기여도

1. 인력양성

가. 1차년도 인력양성 추진실적

(명)

구 분	석사(석사과정)		박사(박사과정)		계	
	목표	달성	목표	달성	목표	달성
인 원	1	2	4	3	5	5

나. 2차년도 인력양성 추진실적

(명)

구 분	석사(석사과정)		박사(박사과정)		계	
	목표	달성	목표	달성	목표	달성
인 원			1	1	1	1

다. 인력 구성 및 활용실적

과제 참여연구원				주요 활용실적
소속	이름	전공	학위	
KAIST	성형진	기계공학	박사	① 과제책임자로서 연구 총괄

KAIST	박진수	기계공학	박사	① 과제수행을 통해 음향미세유체역학 연구분야, 연구로 극지생물학 연구 플랫폼 개발 ② 해당연구인력의 KAIST 박사학위 취득, 미세유체역학 분야 자기 계발
KRIBB/ KAIST	Muhammad Afzal	생명과학	박사	① 과제수행을 통해 음향미세유체역학 연구분야, 연구로 극지생물학 연구 플랫폼 개발 ② 해당연구인력의 UST-KRIBB 박사학위 취득 후 KAIST 박사후연구원, 극지생물학 분야 자기계발
KAIST	Ghulam Destgeer	기계공학	박사	① 과제수행을 통해 음향미세유체역학 연구분야, 연구로 극지생물학 연구 플랫폼 개발 ② 해당연구인력의 KAIST 박사학위 취득 후 KAIST 박사후연구원, 미세유체역학 분야 자기계발
KAIST	Husnain Ahmed	기계공학	석사	① 과제수행을 통해 음향미세유체역학 연구분야, 연구로 극지생물학 연구 플랫폼 개발 ② 해당연구인력의 KAIST 석사학위 취득 후 KAIST 연구원, 미세유체역학 분야 자기계발
KAIST	정진호	기계공학	박사	① 과제수행을 통해 음향미세유체역학 연구분야, 연구로 극지생물학 연구 플랫폼 개발 ② 해당연구인력의 KAIST 박사학위 취득 후 기술보증기금 취직, 미세유체역학 분야 자기계발
KAIST	박광석	기계공학	석사	① 과제수행을 통해 음향미세유체역학 연구분야, 연구로 극지생물학 연구 플랫폼 개발 ② 해당연구인력의 KAIST 석사학위 취득 후 KAIST 박사과정 진학, 미세유체역학 분야 자기계발

2. 연구개발의 우수성

가. 음파/온도/음향열적 자극에 대한 완보동물 거동 분석

자연계에서 생명체가 쉽게 경험할 수 있는 대표적인 기계적 자극인 음파 자극에 대한 연구를 수행하였다. 본 연구는 음향미세유체칩과 관련 기술을 바탕으로 고주파(10~200 MHz) 음파와 음향열적

가열에 의한 온도 자극에 노출됐을 때 완보동물의 거동에 대한 연구를 수행하였다.

나. 미세유체역학과 완보동물 융합 연구

KAIST의 미세유체역학 연구와 KRIBB의 극지생물학 연구가 융합된 다학제 연구를 수행함으로써 극지생물학과 미세유체역학의 융합연구의 기초를 다졌으며 본 과제에 참여하는 학생들이 미래 극지 연구 연구자로 성장할 수 있는 초석을 마련하였다.

다. 마이크로필터 어레이를 이용한 완보동물 샘플 내 미세조류 군집 제거

기존의 수작업 기반의 완보동물 분리 기법에서 벗어나 비접촉/비표지/고속/자동화 방식으로 완보동물을 분리하기 위해 선행되어야 하는 완보동물 샘플 내 미세조류 군집을 마이크로필터 어레이 기반의 수동적 미세유체역학 기술을 이용하여 효율적으로 제거하였다.

라. 음향방사력을 이용한 미세유체칩 내 완보동물의 선택적 분리

기존의 수작업 기반의 완보동물 분리 기법에서 벗어나 비접촉/비표지/고속/자동화 방식으로 완보동물을 분리하기 위해 고주파수 표면탄성파에서 기인하는 음향방사력을 이용해 완보동물을 미세조류, 알 등 완보동물 샘플 내 다른 구성성분으로부터 선택적으로 분리할 수 있음을 보였다.

제 5 장 연구개발결과의 활용계획

제 1 절. 활용방안

기존의 완보동물 연구에서 완보동물은 소량씩 배양된 후 숙련된 연구자가 핀셋(tweezer)으로 분리하여 연구에 사용되었다. 따라서 많은 개체수에 대한 연구가 어려워 연구를 위해 많은 시간과 노력이 필요했다. 이러한 걸림돌은 미세유체역학 기술을 기반으로 본 연구에서 개발되는 미세유체칩을 통해 해결될 수 있다. 자동화된 미세유체칩 내에서 완보동물을 대량배양하고 음파와 마이크로 필터를 통해 분리함으로써 미래 완보동물 연구의 기폭제가 될 것으로 기대된다.

제 2 절. 기대효과

본 연구에서 개발된 완보동물 비접촉식 고속 분리를 위한 미세유체칩과 관련 미세유체역학 기술은 완보동물 이외에도 다른 극지적응 미소생물 연구에도 활용될 수 있다. 특히 현재 극지연구소에서 수행 중인 '극지유전체 101 프로젝트: 극지생물 유전체 정보 분석 및 활용기반 구축' 과제와 '극지적응 고유생물 유래 대사체의 상용화 구축사업' 과제 등과 같이 극지생물학 연구에 있어 본 연구에서 개발된 미세유체칩과 관련 미세유체역학 기술이 유용하게 사용될 수 있을 것으로 판단된다. 본 연구에서 개발되는 미세유체칩과 미세유체역학 기술들은 그 자체로 완보동물을 비롯한 극지적응 미소생물 연구를 위한 기술로 산업화될 잠재력을 지니고 있다. 뿐만 아니라 현재 전량 해외수입에 의존하고 있는 완보동물을 단기간에 적은 비용으로 대량 배양함으로써 부수적인 경제적 효과를 창출해낼 수 있다. 본 연구팀은 KAIST-KRIBB의 공동연구팀으로서 KAIST의 미세유체역학 연구와 KRIBB의 극지생물학 연구가 융합된 다학제 연구를 수행함으로써, 본 과제에 참여하는 학생들이 미래 극지연구 연구자로 성장할 수 있는 초석을 마련할 수 있을 것으로 기대된다.

제 3 절. 연구종료 후 성과창출 계획

구분	주요내용	의의(시사점)	비고
논문	연구 종료 후 3년 이내 SCI급 논문 2편	완보동물을 비롯한 미소동물 연구를 위한 미세유체칩 및 플랫폼 최초 개발	현재 1편 심사 단계
지적재산권	연구 종류 후 3년 이내 특허출원 2건	완보동물을 비롯한 미소동물 연구를 위한 미세유체칩 관련 원천기술 특허 확보	현재 공동출원 절차 진행 중
인력양성	극지생물학-미세유체 융합분야 전문가로 양성	현재 극지연에 없는 연구인력임.	
기타	추가 국가연구개발사업으로 발전		

제 6 장 참고문헌

Ahmad R, Destgeer G, Afzal M, Park J, Ahmed H, Jung JH, Park K, Yoon TS, Sung HJ. 2017. Acoustic wave-driven functionalized particles for aptamer-based target biomolecule separation. Analytical Chemistry 89: 13313 - 13319.

Ahmed H, Destgeer G, Park J, Afzal M, Sung HJ. 2018. Sheathless focusing and separation of microparticles using tilted-angle traveling surface acoustic waves. Analytical Chemistry 90: 8546 - 8552.

Ahmed H, Destgeer G, Park J, Jung JH, Ahmad R, Park K, Sung HJ. 2017a. A pumpless acoustofluidic platform for size-selective concentration and separation of microparticles. Analytical Chemistry 89: 13575 - 13581.

Ahmed H, Destgeer G, Park J, Jung JH, Sung HJ. 2017b. Vertical hydrodynamic focusing and continuous acoustofluidic separation of particles via upward migration. Advanced Science 5: 1700285.

Ahmed H, Park J, Destgeer G, Afzal M, Sung HJ. 2019. Surface acoustic wave-based micromixing enhancement using a single interdigital transducer. Applied Physics Letters 114: 043702.

Alnaimat F, Dagher S, Mathew B, Hilal-Alnqbi A, Khashan S. 2018. Microfluidics based magnetophoresis: a review. The Chemical Record 18: 1596 - 1612.

Altiero T, Giovannini I, Guidetti R, Rebecchi L. 2015. Life history traits and reproductive mode of the tardigrade Acutuncus antarcticus under laboratory conditions: strategies to colonize the Antarctic environment. Hydrobiologia 761: 277 - 291.

Altiero T, Guidetti R, Caselli V, Cesari M, Rebecchi L. 2011. Ultraviolet radiation tolerance in hydrated and desiccated eutardigrades. Journal of Zoological Systematics and Evolutionary Research 49: 104 - 110.

Altiero T, Rebecchi L. 2001. Rearing tardigrades: results and problems. Zoologischer Anzeiger - A Journal of Comparative Zoology 240: 217 - 221.

Arakawa K. 2016. No evidence for extensive horizontal gene transfer from the draft genome of a tardigrade. Proceedings of the National Academy of Sciences 113: E3057.

Arakawa K, Yoshida Y, Tomita M. 2016. Genome sequencing of a single tardigrade Hypsibius

dujardini individual. Scientific Data 3: 160063.

Beltrán-Pardo E, Jönsson KI, Harms-Ringdahl M, Haghdoost S, Wojcik A. 2015. Tolerance to gamma radiation in the tardigrade Hypsibius dujardini from embryo to adult correlate inversely with cellular proliferation. PLoS One 10: e0133658.

Bemm F, Weiß CL, Schultz J, Förster F. 2016. Genome of a tardigrade: horizontal gene transfer or bacterial contamination? Proceedings of the National Academy of Sciences 113: E3054-6.

Van Bezooijen J. 2006. Methods and techniques for nematology. Wageningen, The Netherlands: Wageningen University.

Bhagat AAS, Bow H, Hou HW, Tan SJ, Han J, Lim CT. 2010. Microfluidics for cell separation. Medical & Biological Engineering & Computing 48: 999 - 1014.

Boothby TC, Tenlen JR, Smith FW, Wang JR, Patanella KA, Osborne Nishimura E, Tintori SC, Li Q, Jones CD, Yandell M, Messina DN, Glasscock J, Goldstein B. 2015. Evidence for extensive horizontal gene transfer from the draft genome of a tardigrade. Proceedings of the National Academy of Sciences 112: 15976 - 15981.

Choi S, Song S, Choi C, Park JK. 2008. Sheathless focusing of microbeads and blood cells based on hydrophoresis. Small 4: 634 - 641.

Collins DJ, Alan T, Neild A. 2014. The particle valve: on-demand particle trapping, filtering, and release from a microfabricated polydimethylsiloxane membrane using surface acoustic waves. Applied Physics Letters 105: 033509.

Collins DJ, O'Rorke R, Devendran C, Ma Z, Han J, Neild A, Ai Y. 2018. Self-aligned acoustofluidic particle focusing and patterning in microfluidic channels from channel-based acoustic waveguides. Physical Review Letters 120: 074502.

Degma P, Katina S, Sabatovičová L. 2011. Horizontal distribution of moisture and Tardigrada in a single moss cushion. Journal of Zoological Systematics and Evolutionary Research 49: 71 - 77.

Destgeer G, Cho H, Ha BH, Jung JH, Park J, Sung HJ. 2016. Acoustofluidic particle manipulation inside a sessile droplet: four distinct regimes of particle concentration. Lab on a Chip 16: 660 - 667.

Destgeer G, Ha BH, Jung JH, Sung HJ. 2014a. Submicron separation of microspheres via travelling surface acoustic waves. Lab on a Chip 14: 4665 - 4672.

Destgeer G, Ha BH, Park J, Jung JH, Alazzam A, Sung HJ. 2015. Microchannel anechoic corner for size-selective separation and medium exchange via traveling surface acoustic waves.

Analytical Chemistry 87: 4627 – 4632.

Destgeer G, Hashmi A, Park J, Ahmed H, Afzal M, Sung HJ. 2019. Microparticle self-assembly induced by travelling surface acoustic waves. RSC Advances 9: 7916 – 7921.

Destgeer G, Im S, Ha BH, Jung JH, Ansari MA, Sung HJ. 2014b. Adjustable, rapidly switching microfluidic gradient generation using focused travelling surface acoustic waves. Applied Physics Letters 104: 023506.

Destgeer G, Lee KH, Jung JH, Alazzam A, Sung HJ. 2013. Continuous separation of particles in a PDMS microfluidic channel via travelling surface acoustic waves (TSAW). Lab on a Chip 13: 4210 – 6.

Destgeer G, Sung HJ. 2015. Recent advances in microfluidic actuation and micro-object manipulation via surface acoustic waves. Lab on a Chip 15: 2722 – 2738.

Devendran C, Albrecht T, Brenker J, Alan T, Neild A. 2016. The importance of travelling wave components in standing surface acoustic wave (SSAW) systems. Lab on a Chip 16: 3756 – 3766.

Ding X, Li P, Lin SCS, Stratton ZS, Nama N, Guo F, Slotcavage D, Mao X, Shi J, Costanzo F, Huang TJ. 2013. Surface acoustic wave microfluidics. Lab on a Chip 13: 3626 – 49.

Ding X, Lin SCS, Kiraly B, Yue H, Li S, Chiang IK, Shi J, Benkovic SJ, Huang TJ. 2012. On-chip manipulation of single microparticles, cells, and organisms using surface acoustic waves. Proceedings of the National Academy of Sciences 109: 11105 – 9.

Fakhfouri A, Devendran C, Albrecht T, Collins DJ, Winkler A, Schmidt H, Neild A. 2018. Surface acoustic wave diffraction driven mechanisms in microfluidic systems. Lab on a Chip 18: 2214 – 2224.

Friend J, Yeo L. 2010. Fabrication of microfluidic devices using polydimethylsiloxane. Biomicrofluidics 4: 026502.

Gabriel WN, McNuff R, Patel SK, Gregory TR, Jeck WR, Jones CD, Goldstein B. 2007. The tardigrade Hypsibius dujardini, a new model for studying the evolution of development. Developmental Biology 312: 545 – 59.

Giovannini I, Altiero T, Guidetti R, Rebecchi L. 2018. Will the Antarctic tardigrade Acutuncus antarcticus be able to withstand environmental stresses related to global climate change? Journal of Experimental Biology 221: 160622.

Goldstein B, King N. 2016. The future of cell biology: emerging model organisms. Trends in Cell Biology 26: 818 – 824.

Gross V, Minich I, Mayer G. 2017. External morphogenesis of the tardigrade Hypsibius dujardini as revealed by scanning electron microscopy. Journal of Morphology 278: 563 – 573.

Guidetti R, Altiero T, Rebecchi L. 2011. On dormancy strategies in tardigrades. Journal of Insect Physiology 57: 567 – 576.

Ha BH, Park J, Destgeer G, Jung JH, Sung HJ. 2015. Generation of dynamic free-form temperature gradients in a disposable microchip. Analytical Chemistry 87: 11568 – 11574.

Hashimoto T, Horikawa DD, Saito Y, Kuwahara H, Kozuka-Hata H, Shin-I T, Minakuchi Y, Ohishi K, Motoyama A, Aizu T, Enomoto A, Kondo K, Tanaka S, Hara Y, Koshikawa S, Sagara H, Miura T, Yokobori S ichi, Miyagawa K, Suzuki Y, Kubo T, Oyama M, Kohara Y, Fujiyama A, Arakawa K, Katayama T, Toyoda A, Kunieda T. 2016. Extremotolerant tardigrade genome and improved radiotolerance of human cultured cells by tardigrade-unique protein. Nature Communications 7: 12808.

Hengherr S, Worland MR, Reuner A, Brümmer F, Schill RO. 2009a. High temperature tolerance in anhydrobiotic tardigrades is limited by glass transition. Physiological and Biochemical Zoology 82: 749 – 755.

Hengherr S, Worland MR, Reuner A, Brümmer F, Schill RO. 2009b. Freeze tolerance, supercooling points and ice formation: comparative studies on the subzero temperature survival of limno-terrestrial tardigrades. Journal of Experimental Biology 212: 802 – 7.

Horikawa DD, Cumbers J, Sakakibara I, Rogoff D, Leuko S, Harnoto R, Arakawa K, Katayama T, Kunieda T, Toyoda A, Fujiyama A, Rothschild LJ. 2013. Analysis of DNA repair and protection in the tardigrade Ramazzottius varieornatus and Hypsibius dujardini after exposure to UVC radiation. PLoS One 8: e64793.

Horikawa DD, Kunieda T, Abe W, Watanabe M, Nakahara Y, Yukuhiro F, Sakashita T, Hamada N, Wada S, Funayama T, Katagiri C, Kobayashi Y, Higashi S, Okuda T. 2008. Establishment of a rearing system of the extremotolerant tardigrade Ramazzottius varieornatus: a new model animal for astrobiology. Astrobiology 8: 549 – 556.

Hur SC, Tse HTK, Di Carlo D. 2010. Sheathless inertial cell ordering for extreme throughput flow cytometry. Lab on a Chip 10: 274 – 280.

Jönsson KI. 2007. Tardigrades as a potential model organism in space research. Astrobiology 7: 757 – 766.

Jönsson KI, Rabbow E, Schill RO, Harms-Ringdahl M, Rettberg P. 2008. Tardigrades survive

exposure to space in low earth orbit. Current Biology 18: R729 – R731.

Jørgensen A, Kristensen RM, Møbjerg N. 2018. Phylogeny and integrative taxonomy of Tardigrada. In: Schill RO, ed. Water Bears: The Biology of Tardigrades. Zoological Monographs vol. 2. Cham, Switzerland: Springer, .

Jung JH, Lee KH, Lee KS, Ha BH, Oh YS, Sung HJ. 2014. Optical separation of droplets on a microfluidic platform. Microfluidics and Nanofluidics 16: 635 – 644.

Koutsovoulos G, Kumar S, Laetsch DR, Stevens L, Daub J, Conlon C, Maroon H, Thomas F, Aboobaker AA, Blaxter M. 2016. No evidence for extensive horizontal gene transfer in the genome of the tardigrade Hypsibius dujardini. Proceedings of the National Academy of Sciences 113: 5053 – 5058.

Li P, Mao Z, Peng Z, Zhou L, Chen Y, Huang PH, Truica CI, Drabick JJ, El-Deiry WS, Dao M, Suresh S, Huang TJ. 2015. Acoustic separation of circulating tumor cells. Proceedings of the National Academy of Sciences 112: 4970 – 4975.

Ma Z, Zhou Y, Collins DJ, Ai Y. 2017. Fluorescence activated cell sorting via a focused traveling surface acoustic beam. Lab on a Chip 17: 3176 – 3185.

Martin C, Gross V, Hering L, Tepper B, Jahn H, de Sena Oliveira I, Stevenson PA, Mayer G. 2017. The nervous and visual systems of onychophorans and tardigrades: learning about arthropod evolution from their closest relatives. Journal of Comparative Physiology A 203: 565 – 590.

Mathew B, Alazzam A, Destgeer G, Sung HJ. 2016. Dielectrophoresis based cell switching in continuous flow microfluidic devices. Journal of Electrostatics 84: 63 – 72.

Møbjerg N, Halberg KA, Jørgensen A, Persson D, Bjørn M, Ramløv H, Kristensen RM. 2011. Survival in extreme environments – on the current knowledge of adaptations in tardigrades. Acta Physiologica 202: 409 – 420.

Nam J, Jang WS, Lim CS. 2018. Micromixing using a conductive liquid-based focused surface acoustic wave (CL-FSAW). Sensors and Actuators B: Chemical 258: 991 – 997.

Nelson DR. 2002. Current status of the Tardigrada: evolution and ecology. Integrative and Comparative Biology 42: 652 – 659.

Ono F, Mori Y, Takarabe K, Fujii A, Saigusa M, Matsushima Y, Yamazaki D, Ito E, Galas S, Saini NL. 2016. Effect of ultra-high pressure on small animals, tardigrades and Artemia. Cogent Physics 3: 1167575.

Ono F, Saigusa M, Uozumi T, Matsushima Y, Ikeda H, Saini NL, Yamashita M. 2008. Effect of high hydrostatic pressure on to life of the tiny animal tardigrade. Journal of Physics and Chemistry of Solids 69: 2297 – 2300.

Park J, Destgeer G, Kim H, Cho Y, Sung HJ. 2018a. In-droplet microparticle washing and enrichment using surface acoustic wave-driven acoustic radiation force. Lab on a Chip 18: 2936 – 2945.

Park J, Jung JH, Destgeer G, Ahmed H, Park K, Sung HJ. 2017a. Acoustothermal tweezer for droplet sorting in a disposable microfluidic chip. Lab on a Chip 17: 1031 – 1040.

Park J, Jung JH, Park K, Destgeer G, Ahmed H, Ahmad R, Sung HJ. 2018b. On-demand acoustic droplet splitting and steering in a disposable microfluidic chip. Lab on a Chip 18: 422 – 432.

Park K, Park J, Jung JH, Destgeer G, Ahmed H, Sung HJ. 2017b. In-droplet microparticle separation using travelling surface acoustic wave. Biomicrofluidics 11: 064112.

Pilato G, Binda MG. 2001. Biogeography and limno-terrestrial tardigrades: are they truly incompatible binomials? Zoologischer Anzeiger – A Journal of Comparative Zoology 240: 511 – 516.

Rebecchi L, Altiero T, Guidetti R, Cesari M, Bertolani R, Negroni M, Rizzo AM. 2009. Tardigrade resistance to space effects: first results of experiments on the LIFE-TARSE mission on FOTON-M3 (September 2007). Astrobiology 9: 581 – 591.

Sajeesh P, Sen AK. 2014. Particle separation and sorting in microfluidic devices: a review. Microfluidics and Nanofluidics 17: 1 – 52.

Sands CJ, Convey P, Linse K, McInnes SJ. 2008. Assessing meiofaunal variation among individuals utilising morphological and molecular approaches: an example using the Tardigrada. BMC Ecology 8: 7.

Schokraie E, Warnken U, Hotz-Wagenblatt A, Grohme MA, Hengherr S, Forster F, Schill RO, Frohme M, Dandekar T, Schnolzer M. 2012. Comparative proteome analysis of Milnesium tardigradum in early embryonic state versus adults in active and anhydrobiotic state. PLoS One 7: e45682.

Seki K, Toyoshima M. 1998. Preserving tardigrades under pressure. Nature 395: 853 – 854.

Shi J, Huang H, Stratton Z, Huang Y, Huang TJ. 2009. Continuous particle separation in a microfluidic channel via standing surface acoustic waves (SSAW). Lab on a Chip 9: 3354 – 3359.

Skowronek V, Rambach RW, Franke T. 2015. Surface acoustic wave controlled integrated band-pass filter. Microfluidics and Nanofluidics 19: 335 - 341.

Skowronek V, Rambach RW, Schmid L, Haase K, Franke T. 2013. Particle deflection in a poly(dimethylsiloxane) microchannel using a propagating surface acoustic wave: size and frequency dependence. Analytical Chemistry 85: 9955 - 9.

Smith FW, Boothby TC, Giovannini I, Rebecchi L, Jockusch EL, Goldstein B. 2016. The compact body plan of tardigrades evolved by the loss of a large body region. Current Biology 26: 224 - 229.

Suzuki AC. 2003. Life history of Milnesium tardigradum Doyère (tardigrada) under a rearing environment. Zoological Science 20: 49 - 57.

Tanaka S, Tanaka J, Miwa Y, Horikawa DD, Katayama T, Arakawa K, Toyoda A, Kubo T, Kunieda T. 2015. Novel mitochondria-targeted heat-soluble proteins identified in the anhydrobiotic tardigrade improve osmotic tolerance of human cells (JR Battista, Ed.). PLoS One 10: e0118272.

Tenlen JR, McCaskill S, Goldstein B. 2013. RNA interference can be used to disrupt gene function in tardigrades. Development Genes and Evolution 223: 171 - 181.

Tsujimoto M, Imura S, Kanda H. 2016. Recovery and reproduction of an Antarctic tardigrade retrieved from a moss sample frozen for over 30 years. Cryobiology 72: 78 - 81.

Tsujimoto M, Suzuki AC, Imura S. 2015. Life history of the Antarctic tardigrade, Acutuncus antarcticus, under a constant laboratory environment. Polar Biology 38: 1575 - 1581.

Weibel DB, Whitesides GM, Basu A, Schneider J. 2006. Applications of microfluidics in chemical biology. Current Opinion in Chemical Biology 10: 584 - 591.

Weronika E, Łukasz K. 2017. Tardigrades in space research - past and future. Origins of Life and Evolution of Biospheres 47: 545 - 553.

Wiklund M. 2012. Acoustofluidics 12: biocompatibility and cell viability in microfluidic acoustic resonators. Lab on a Chip 12: 2018 - 2028.

Winkler A, Harazim S, Collins DJ, Brünig R, Schmidt H, Menzel SB. 2017. Compact SAW aerosol generator. Biomedical Microdevices 19: 9.

Wyatt Shields IV C, Reyes CD, López GP. 2015. Microfluidic cell sorting: a review of the advances in the separation of cells from debulking to rare cell isolation. Lab on a Chip 15: 1230 - 1249.

Yamaguchi A, Tanaka S, Yamaguchi S, Kuwahara H, Takamura C, Imajoh-Ohmi S, Horikawa DD, Toyoda A, Katayama T, Arakawa K, Fujiyama A, Kubo T, Kunieda T. 2012. Two novel heat-soluble protein families abundantly expressed in an anhydrobiotic tardigrade. PLoS One 7: e44209.

Yoshida Y, Koutsovoulos G, Laetsch DR, Stevens L, Kumar S, Horikawa DD, Ishino K, Komine S, Kunieda T, Tomita M, Blaxter M, Arakawa K. 2017. Comparative genomics of the tardigrades Hypsibius dujardini and Ramazzottius varieornatus. PLoS Biology 15: e2002266.

주 의

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