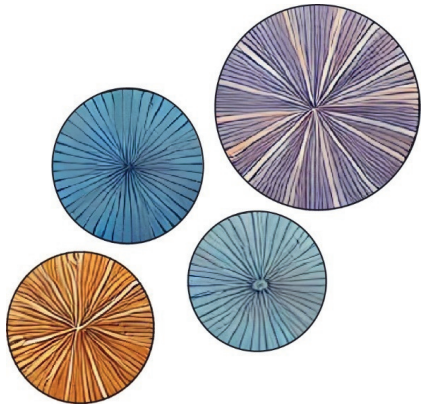
Making attachments

Chapter 7

Impact paragraph



In this chapter, we discuss the potential impact of the research described in this thesis. Specifically, we deliberate on how the miniaturized enthesis models developed here meet the societal needs and what the possible scientific applications are.

1. Societal/Clinical Relevance

Entheses are the sites where tendons and ligaments connect to the bone and as such experience high levels of stress. Entheses are susceptible to overuse injuries, also known as enthesopathies, which are frequently observed in athletes.[1] Studies indicate that up to 50% of injuries sustained by athletes engaged in daily exercise involve tendons and their attachment points to the bones.[2] The most common anatomical sites for sports-associated enthesopathies are the rotator cuff, the lower pole of the patella, and the Achilles tendon insertion.[1] Focusing on Achilles tendon insertion, tendon rupture is observed in 8.3% of competitive athletes and is caused by acute mechanical load, while the incidence of Achilles tendinopathy among competitive runners can be as high as 40–50%.[3] Such injuries are often caused by changes in the stiffness of the muscle-tendon unit resulting from exercise or muscle fatigue, which can lead to altered force transfer and enthesopathy.[4] Ground forces can also contribute to these injuries, particularly when training or landing heavily or repeatedly on hard surfaces, such as concrete.[5] Risk factors for Achilles tendon problems include age, male sex and obesity, with the peak age for rupture occurring between 30 and 40 years for both men and women. In addition, men are four to five times more likely than women to experience Achilles enthesopathies.[6] This has consequences on the quality of life of the patient, with a socio-economical impact estimated to be around 21,000,000 € in the Netherlands yearly.[7]

Enthesopathies related to sports are characterized by degenerative changes in the tendon, rather than acute inflammation.[8] The optimal treatment approach for such conditions is still being developed and the current methods include strengthening the affected tendon.[9] A novel approach for treating enthesopathies and tendinopathies involves the use of extracorporeal shock wave therapy (ESWT).[10] This treatment uses acoustic waves that carry energy and can pass through tissues, leading to neovascularization and short-term pain relief. The success rates of ESWT in treating Achilles tendon enthesopathies is 71.1% at immediate and 90.3% at 1-year follow-up.[11] In addition to treating injuries, ESWT can also be used as a preventive measure in athletes, since the treatment can increase the tensile strength of the tendon, which may help to prevent injuries.[12] The exposure of *in vitro* tendon and enthesis

models to acoustic stimulation offers the potential to improve the current understanding of tendopathies or enthesopathies, respectively and aid the development of effective treatment methods.

In addition to enthesopathies, inflammation of the enthesis (enthesitis) is a common manifestation of inflammatory musculoskeletal diseases. It can result from repeated mechanical overloads in healthy individuals.[13] Tennis elbow and golfer elbow are typical examples, affecting 1–3% of adults and reaching up to 50% of athletes each year.[14] In these cases, enthesitis affects only one enthesis in the body, and resolves spontaneously. However, enthesitis is a hallmark of psoriatic arthritis (PsA) and spondyloarthritis (SpA), showing an incidence of 30–50%, with the Achilles tendon being the most commonly involved site.[15] In such conditions, enthesitis affects more than one enthesis and shows a remarkable degree of chronicity, leading to higher levels of pain and disabilities that impede daily activities.[16] The current knowledge regarding the treatment of enthesitis is limited. Drugs currently used aim to prevent enthesis inflammation and relieve symptoms. For example, nonsteroidal anti-inflammatory drugs (NSAIDs) are used for acute enthesitis but are ineffective in the treatment of chronic diseases.[17] In such cases, apremilast inhibits the production of several cytokines involved in entheseal inflammation.[18] However, approximately half of the patients with psoriatic arthritis (PsA) treated with apremilast showed no resolution of the disease after 1 year of treatment.[19] Therefore, there is a need to develop treatments for enthesitis.

The current process of drug discovery is not only time-consuming but also expensive, with a high rate of failure in the clinical trial phase. Bringing a drug from discovery to approval can take up to 15 years and cost up to $ 1 billion, with only a small fraction of screened molecules making it to clinical trials.[20] Advanced technologies, particularly in the areas of chemical synthesis, compound screening, and preclinical testing, have the potential to improve and accelerate this process. Microfluidic tools offer an innovative approach that can provide new insights into biological systems and improve existing technologies. It has been estimated that microfluidics could reduce R&D costs by 10–26%.[21]

2. Scientific Impact

Over the past decade, tissue engineering has become a promising approach for the repair and regeneration of musculoskeletal tissues and their interfaces. However, regenerating the enthesis remains challenging because of its complexity in terms of structure, composition and

mechanical properties. Miniaturized models have emerged as a promising tool for studying the musculoskeletal system.[22] These models combine the benefits of traditional macroscale three-dimensional (3D) models with the use of a limited number of cells and reagents, real-time analysis through high-resolution imaging and the precise control of biochemical and biophysical stimuli. Microfluidic techniques have been utilized to create organ-on-chip systems that simulate organ function owing to the possibility of recreating molecular and cellular events in controlled microenvironments. Although the development of miniaturized models has increased in recent years, only a few studies have focused on musculoskeletal tissues. In contrast, significant progress has been made in developing and validating on-chip systems for major organs associated with high mortality or disease rates, such as heart, lung, intestine and liver (**Figure 1**). While these models reproduce key aspects of the physiological and pathological processes involved in the tissues, miniaturized musculoskeletal models mainly reproduce the physiological processes rather than pathological conditions. The slow adoption of microfluidics for musculoskeletal tissue can be attributed to conceptual and practical challenges in modeling the complex cellular composition and extracellular matrix structures, incorporating *in vivo*-like mechanical loading and integrating vascularization and innervation in healthy or diseased states.

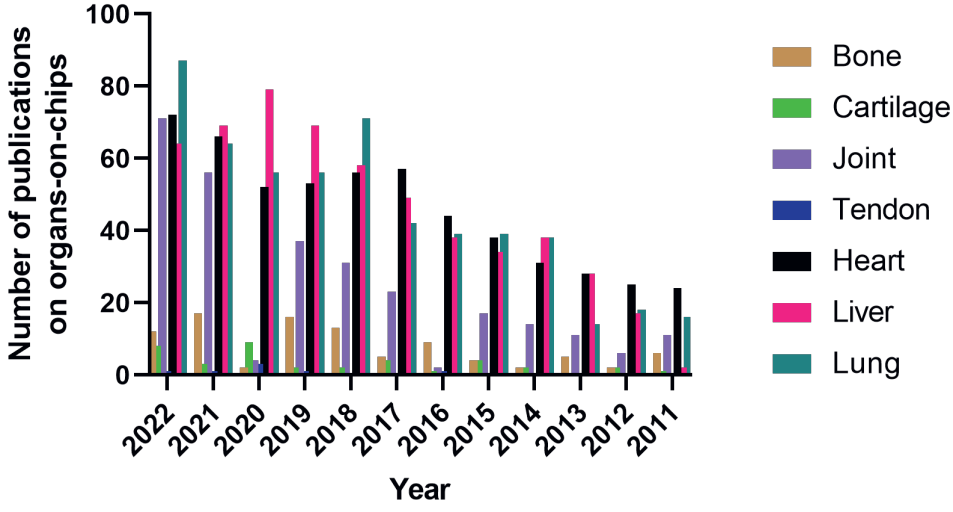


Figure 1. Scientific publications on organs-on-chips since 2011. PubMed search, February 2023.

In this thesis, we adapted established microtechnologies to engineer *in vitro* models of the enthesis, increasing our understanding of this tissue. We believe that this research provides a stepping stone for defining future applications of *in vitro* miniaturized models and can serve as a source of inspiration for future studies within the broader scientific community. In **chapter 2**, we propose a microfluidic piggyback platform to control the formation of micropatterns and alignment of collagen fibers in a culture dish through a simple and reproducible approach that can be easily implemented by other research groups studying tendon biology or regeneration. This method helps primary tenocytes to retain their differentiated phenotype, improving cell culture protocols for tissue engineering purposes. While this study focuses on 2D systems, in **chapter 3**, we present a novel method to introduce three-dimensionality in culture dishes. Collagen micropatterning of two opposing flat (microporous) substrates provides precise control of the microenvironment and organization of the cells in between, creating an *in vitro* model suited to mimic different connective tissue configurations. The scientific impact of this method relies on the fact that it helps to remove artificial polarization in cells introduced by flat standard culture substrates and recreates a physiological 3D microenvironment, thus improving the *in vitro* culturing conditions. Our platform could also open the door for further research on cell polarization in *in vitro* models.

In **chapter 4**, we describe the development of the first enthesis-on-a-chip model. The microfluidic chip is based on the layered co-culture of tendon- and fibrocartilage-like cells on opposite sides of a porous culture membrane. The microfluidic platform allows not only the establishment of a 'healthy' enthesis model but also the recapitulation of key aspects of acute and chronic enthesitis. This can facilitate the understanding of the impact of inflammation on enthesis cells and could speed up future research on the development of personalized, patient-specific treatments for clinical applications. Considering the limited efficacy of current drug treatments available, building personalized models including the patient's own MSCs in the chip, could facilitate the identification of chemical compounds that are more effective for the personalized treatment of enthesitis. In **chapter 5**, we take a step further by establishing a 3D enthesis model. Through the controlled fusion of ligament and bone spheroids, we formed heterotypic aggregates that express typical enthesis markers. These aggregates could be easily scaled up to a millimeter scale and shown to respond to external acoustic stimulation. This reproducible approach can be easily adapted to study other tissue-tissue

interfaces, representing an opportunity for scientists to engineer complex tissues in an easy and reproducible way.

3. Conclusion

Overall, this thesis presents new efforts and provides new insights that may lead to the development of novel miniaturized models of tissue interfaces, for a better understanding of the underlying biological mechanisms, but also for the rationale of respective advanced platforms for diagnosis, drug screening and disease treatment.

References

[1] M. Benjamin, H. Toumi, J. R. Ralphs, G. Bydder, T. M. Best, S. Milz, *J Anat* **2006**, 208, 471.

[2] M. Paavola, P. Kannus, M. Järvinen, in Tendon Injuries. *Epidemiology of Tendon Problems in Sport, Springer, London* **2005**, pp. 50-85.

[3] N. J. Lemme, N. Y. Li, S. F. DeFroda, J. Kleiner, B. D. Owens, *Orthop J Sports Med* **2018**, 6, 2325967118808238.

[4] T. Horita, P. V. Komi, I. Hamalainen, J. Avela, *Eur J Appl Physiol* **2003**, 88, 527.

[5] H. Moksnes, L. Snyder-Mackler, M. A. Risberg, *J Orthop Sports Phys Ther* **2008**, 38, 586.

[6] a)A. C. van der Vlist, S. J. Breda, E. H. G. Oei, J. A. N. Verhaar, R. J. de Vos, *Br J Sports Med* **2019**, 53, 1352; b)B. Magnan, M. Bondi, S. Pierantoni, E. Samaila, *Foot and Ankle Surgery* **2014**, 20, 154.

[7] T. S. O. Sleeswijk Visser, A. C. van der Vlist, R. F. van Oosterom, P. van Veldhoven, J. A. N. Verhaar, R. J. de Vos, *BMJ Open Sport Exerc Med* **2021**, 7, e001023.

[8] a)L. Jozsa, P. Kannus, *Scand J Med Sci Sports* **1997**, 7, 113; b) K.M. Khan, P. Kannus, N. Maffulli, S.F. Bonar, *BMJ* **2002**, 16, 324.

[9] M. A. Young, J. L. Cook, C. R. Purdam, Z. S. Kiss, H. Alfredson, *Br J Sports Med* **2005**, 39, 102.

[10] a)C. J. Wang, F. S. Wang, K. D. Yang, L. H. Weng, C. C. Hsu, C. S. Huang, L. C. Yang, J *Orthop Res* **2003**, 21, 984; b)M. Maier, B. Averbeck, S. Milz, H. J. Refior, C. Schmitz, *Clin Orthop Relat Res* **2003**, 237.

[11] J. Y. Lee, K. Yoon, Y. Yi, C. H. Park, J. S. Lee, K. H. Seo, Y. S. Park, Y. T. Lee, *Ann Rehabil Med* **2017**, 41, 42.

[12] C. L. Simplicio, J. Purita, W. Murrell, G. S. Santos, R. G. Dos Santos, J. Lana, *J Clin Orthop Trauma* **2020**, 11, S309.

[13] G. Schett, R. J. Lories, M. A. D'Agostino, D. Elewaut, B. Kirkham, E. R. Soriano, D. McGonagle, *Nat Rev Rheumatol* **2017**, 13, 731.

[14] T. L. Sanders, Jr., H. Maradit Kremers, A. J. Bryan, J. E. Ransom, J. Smith, B. F. Morrey, *Am J Sports Med* **2015**, 43, 1066.

[15] M. Benjamin, D. McGonagle, *J Anat* **2001**, 199, 503.

[16] D. S. Fahmi, W. K. Makarm, R. S. Zaghlol, *Egyptian Rheumatology and Rehabilitation* **2022**, 49.

[17] G. Mouterde, P. Aegerter, J. M. Correas, M. Breban, M. A. D'Agostino, *Arthritis Care Res (Hoboken)* **2014**, 66, 131.

[18] M. Ariga, B. Neitzert, S. Nakae, G. Mottin, C. Bertrand, M. P. Pruniaux, S. L. Jin, M. Conti, *J Immunol* **2004**, 173, 7531.

[19] A. Kavanaugh, P. J. Mease, J. J. Gomez-Reino, A. O. Adebajo, J. Wollenhaupt, D. D. Gladman, M. Hochfeld, L. L. Teng, G. Schett, E. Lespessailles, S. Hall*, J Rheumatol* **2015**, 42, 479.

[20] R. Sonawane, R. Wagh, J. R. Dhumane, A. B. Deore, A*sian Journal of Pharmaceutical Research and Development* **2019**, 7, 62.

[21] N. Franzen, W. H. van Harten, V. P. Retel, P. Loskill, J. van den Eijnden-van Raaij, I. J. M, *Drug Discov Today* **2019**, 24, 1720.

[22] S. Bersini, C. Arrigoni, S. Lopa, M. Bongio, I. Martin, M. Moretti, *Drug Discov Today* **2016**, 21, 1429.