Rethinking articular cartilage regeneration based on a 250-year-old statement

Jos Malda[1,2]*, *Jürgen Groll*[3] *and P. René van Weeren*[2]

Cartilage has a limited healing capacity; however, studies into the basic biological characteristics, formation and structural maintenance of the cartilage collagen network are providing explanations for the failure of current therapeutic approaches, urging us to rethink our approach to the regeneration of articular cartilage.

> "we cannot restore the biomechanical properties of cartilage via traditional regenerative medicine approaches"

The musculoskeletal system provides shape and stability to the body and enables motion. As an avascular and aneural component of this system, articular cartilage has an almost exclusively biomechanical function. The word 'biomechanics' comes from the Ancient Greek terms for 'life' and 'mechanics' and refers to the study of the mechanical principles of living organisms; in other words, how living tissues deal with mechanical demands. In mechanical terms, the strength or carrying capacity of any structure is determined by the mechanical characteristics of the components of the structure and the spatial architecture of these components. This principle is of particular importance for articular cartilage, given its biomechanical function in the body.

In 1743, William Hunter stated "If we consult the standard Chirurgical Writers from Hippocrates down to the present Age, we shall find, that an ulcerated Cartilage is universally allowed to be a very troublesome Disease; that it admits of a Cure with more Difficulty than a carious Bone; and that, when destroyed, it is never recovered"[1]. This centuries-old observation is as true today as it was in Hunter's time, unlike many other medical observations made in the mid-18th century. Clinically, the introduction of metal implants in the middle of the last century has had an enormous effect on the quality of life of many individuals with joint disease, as these devices can usually restore biomechanical function to the joint for up to 20 years. However, such treatment does not result in the restoration of articular cartilage.

In the past few decades, extensive efforts have been made to achieve functional repair or even complete regeneration of articular cartilage. However, these attempts have consistently failed, despite many of them initially resulting in the gradual formation of a cartilage-like tissue. The reason for the lack of progress in cartilage regeneration might, at least in part, be attributable to a focus on the cell biology aspects, rather than on the mechanical aspects, of the problem. Additionally, a lack of knowledge about the basic biology, formation and maintenance of the biomechanically decisive features of articular cartilage — the components and the architecture of its extracellular matrix — is an important issue.

In 1925, Alfred Benninghoff discovered that the collagen in hyaline cartilage is organized into an arcade-like structure[2]. The 'pillars' of these arcades are firmly anchored in a layer of calcified cartilage and their actual arches are linked to tangential collagen fibres running parallel to the joint surface in the lamina splendens. This knowledge enabled a better understanding of how the entire composite structure of hydrophilic proteoglycans interspersed in a tough collagen network provides the desired combination of strength and resilience needed for the proper function of articular cartilage through the interaction of mechanical and electrostatic forces[3].

Many attempts at regenerating cartilage have produced hyaline-like tissue in vitro; in these techniques a variety of cells were able to produce copious amounts of proteoglycans and type II collagen[4]. However, when tested in vivo in large animal models, none of these techniques could restore the architecture of the collagen network, and instead formed fibrocartilaginous repair tissue[5], which explains their functional failure.

In the early 1990s, important work on collagen metabolism[6] showed that type II collagen from healthy mature individuals had extremely long turnover times, in the order of hundreds of years. Another elegant study[7] based on carbon dating that used the fact that the level of radioactive carbon in the atmosphere has fluctuated considerably as a result of man-made nuclear activity since the Second World War produced irrefutable evidence that the metabolic turnover of the collagen network in cartilage is indeed nil in mature individuals, irrespective of whether or not a person is affected by articular disease, such as osteoarthritis.

This inherent incapacity of the network of type II collagen fibrils to repair or re-form within any biologically relevant timeframe and, hence, the inability to restore the architecture of articular cartilage, must be

[1]*Department of Orthopaedics, University Medical Centre Utrecht, Utrecht, Netherlands.*

[2]*Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands.*

[3]*Department of Functional Materials in Medicine and Dentistry and the Bavarian Polymer Institute, University of Würzburg, Würzburg, Germany.*

**e-mail: j.malda@umcutrecht.nl*

https://doi.org/10.1038/s41584-019-0278-7

> a shift in focus is urgently needed regarding the development of regenerative medicine approaches for cartilage

considered. This incapacity means that the proven ability of cells to produce and secrete the correct matrix components is not enough for long-term functionality, as biomechanically indispensable architectural structures are not formed. Hence, the prevailing paradigm of regenerative medicine, the aim of which is to use our body's own resources to regenerate, rather than to replace or to repair tissue[8], does not apply to articular cartilage in mature individuals.

Accepting this insight means accepting that we cannot restore the biomechanical properties of cartilage via traditional regenerative medicine approaches and explains why we have thus far not been able to reproduce the healthy native tissue in vivo, either anatomically or functionally. This situation, which is still largely ignored in the field, implies that the classic tissue engineering approach[9] that has been pursued for cartilage for the past 25 years will never be able to provide a long-term functional solution and must be abandoned. A radical change in focus for the regeneration of articular cartilage is, therefore, required if we want to improve on Hunter's sombre prognosis.

We are aware of many methods for cartilage repair that give good, or even excellent, clinical results. For example, allograft transplantation has produced promising results because the required collagen structure is maintained in the transplanted material, as have bioartificial implants that provide this structure; however, integration of grafts and implants into the surrounding tissue remains a challenge[4]. From an engineering point of view, it is the increasingly sophisticated techniques available to researchers (such as bioprinting) that have contributed to progress in many aspects of cartilage regeneration[4]. However, to date, none of these techniques addresses the important aspect of reconstruction of the collagen architecture, which might be owing to an insufficient ability to replicate the orientation and fibre diameter of native collagen. The interaction of biology and mechanics to determine the function of articular cartilage conceptually leads to two distinct avenues that might be explored. We hypothesize that exploring these avenues, either separately or in a combined approach, might break through the current deadlock.

First, acknowledging the fact that the body lays down a definitive and life-long immutable structural element of cartilage in the juvenile phase of life that, unlike almost any other tissue, does not renew itself at regular intervals, could lead to the concept of manufacturing constructs that also contain an immutable part. In those constructs, long-term (non-degradable) structure-giving materials could be combined with regenerative components, such as cell-loaded or cell-instructive biodegradable hydrogels, thereby forming a favourable environment for the formation of articular cartilage tissue. The long-lasting structural element would provide sufficient biomechanical resistance to guarantee functionality from the onset of implantation, thereby enabling the optimal formation of neo-tissue that would, as in native cartilage, lubricate the joint and protect the structural element against wear and tear.

A second approach relies on the observation that the natural arcade-shaped collagen structures that provide the mechanical resilience of the cartilage are formed during the late fetal and early juvenile phases of life[10]. Partial restoration of the microenvironment prevalent in these stages of life (which includes the appropriate cytokine and growth factor profile and targeted mechanical loading) might be achieved by the use of rejuvenated or induced pluripotent stem cells, which have the potential to mimic this juvenile milieu. This process could be supported and accompanied by biomaterials that transiently mimic the structural features of cartilage.

Taken together, we propose that a shift in focus is urgently needed regarding the development of regenerative medicine approaches for cartilage. Unravelling the mechanisms by which the collagen structure of cartilage is initially formed will undoubtedly be a decisive breakthrough in attempts to restore it at later stages, and might have implications beyond articular cartilage (for example, for regeneration of intervertebral discs and the meniscus). We hypothesize that evolving fabrication and printing approaches that enable researchers to functionally mimic cartilage architecture will facilitate advances in our endeavour to achieve true regeneration of articular cartilage.

1. Hunter, W. Of the structure and disease of the articulating cartilages. *Roy. Soc. Lond. Phil. Trans.* **9**, 514-521 (1743).
2. Benninghoff, A. Form und Bau der Gelenkknorpel in Ihren Beziehungen zur Funktion. *Z. Zellforsch* **2**, 783–862 (1925).
3. MacConaill, M. A. The movement of bones and joints; the mechanical structure of articulating cartilage. *J. Bone Joint Surg. Br.* **33**, 251–257 (1951).
4. Armiento, A. R. et al. Biomaterials for articular cartilage tissue engineering: Learning from biology. *Acta Biomater.* **65**, 1–20 (2018).
5. Vindas Bolanos, R. A. et al. The use of a cartilage decellularized matrix scaffold for the repair of osteochondral defects: the importance of long-term studies in a large animal model. *Osteoarthritis Cartilage* **25**, 413–420 (2017).
6. Maroudas, A., Palla, G. & Gilav, E. Racemization of aspartic acid in human articular cartilage. *Connect. Tissue Res.* **28**, 161–169 (1992).
7. Heinemeier, K. M. et al. Radiocarbon dating reveals minimal collagen turnover in both healthy and osteoarthritic human cartilage. *Sci. Transl. Med.* **8**, 346ra390 (2016).
8. Mao, A. S. & Mooney, D. J. Regenerative medicine: Current therapies and future directions. *Proc. Natl Acad. Sci. USA* **112**, 14452–14459 (2015).
9. Langer, R. & Vacanti, J. Tissue engineering. *Science* **260**, 920–926 (1993).
10. van Turnhout, M. C. et al. Quantitative description of collagen structure in the articular cartilage of the young and adult equine distal metacarpus. *Animal Biol.* **58**, 353–370 (2008).

Acknowledgements
The authors acknowledge the Dutch Arthritis Foundation (LLP-12 (to J.M.) and LLP-22 (to P.R.v.W.)), and the Netherlands Organization for Scientific Research (Materials Driven Regeneration, 024.003.013 (to J.M.)) for financial support.

Competing interests
The authors declare no competing interests.