

# Tissue engineering – nanomaterials in the musculoskeletal system

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## Summary

The musculoskeletal tissues bone, cartilage and ligament/tendon are highly structured nanocomposites consisting of nanofibres embedded in a matrix of different composition. Thus, it was a logical step that during the hype of *nano* in the last decade, nanotechnology and nanomaterials became a hot topic in the field of musculoskeletal repair. Especially the fact that using nanomaterials would encompass a biomimetic approach, thus copying nature, was promising. However, it became evident that using nanomaterials in the repair of musculoskeletal tissues had a longer history than initially thought and its way was paved with failures, which are important to remember when applying current ideas. This current opinion paper summarises some fundamental aspects of nanomaterials to be used for musculoskeletal application and discusses where this field might move to in the near future.

**Key words:** *nanomaterials; bone; cartilage; ligament; tendon; reconstruction; tissue engineering*

## Introduction

After introducing the concept – at that time a rather philosophical one – of how to manipulate atoms by the Nobel Prize-winner Richard Feynman in 1960 [1], it took more than 20 years until the atoms could be easily visualised by the scanning probe microscope [2]. In due course, nanoengineering became a hot topic for many research fields and the term *nano* was *en vogue* and used inflationarily for a myriad of techniques and applications. By definition, a nanomaterial comprises elements with structural dimensions of less than 100 nm. Thus many biological structures of musculoskeletal tissues, e.g., collagen fibrils or hydroxyapatite crystals, are natural nanomaterials by definition. As a consequence, it is not surprising that engineered nano concepts were also introduced to the field of the musculoskeletal system, as for general medicinal applications and medical devices [3, 4].

Most musculoskeletal lesions, especially traumatic bone lesions, heal after anatomical correction and stabilisation within a few weeks. However, if such lesions comprise large voids with critical distances to bridge or diseased tis-

ues, a more complex situation is present which may demand treatment strategies with autologous, allogenic or artificial biomaterials. Calcium based fillers, (bio)polymeric bone void fillers, or even porous metals are typically used as artificial biomaterials for bone repair and regeneration. In case of ruptured ligaments and tendons, it is critical to re-align the separated ends and guide them for regeneration. This can be attained by fibrous fabric material based on natural or synthetic polymeric or carbon materials. Defects of cartilaginous tissue in the diarthrodial joints do not or only barely regenerate and thus are frequently filled with naturally derived and synthetic polymeric materials. In all instances of skeletal repair, the biomaterials used are often combined with biological active agents and/or autologous cells and tissues to support healing. Nevertheless, the outcome does not always result in tissue regeneration and complete restoration of function.

As a consequence of the lack of reliable treatment options, new concepts of material engineering that integrate nano building blocks have been suggested and explored during the hype of *nano*. Today, nanomaterials are under consideration for many applications since they induce novel material properties, such as enhanced mechanics and improved material-tissue interaction that could not be engineered with traditional macro- and microscopic approaches. The subject of this paper is to review and discuss the implication of engineered nanomaterials when used as implantable devices for skeletal reconstruction. We restrict the review to the chances and risks of nano particles and fibres, and exclude, unless specifically mentioned, nanostructured micro and macroscopic surfaces. However, we include some micro and submicrometer features in our discussion as nano-like characteristics may be perceived already above the nano threshold of 100 nm.

## Nanomaterials and musculoskeletal tissues

### Musculoskeletal tissues – the prototypes of nanomaterials

The structural composition of bone, cartilage and ligaments/tendons is very similar, although these tissues have quite distinct appearances. In a simplified approach, a net-

work of collagen fibrils with diameters of approximately 100 nm is embedded within a characteristic tissue specific matrix. It is the matrix composition, the structural design, and the interaction of the fibers with these matrixes which defines the mechanical and biological properties. The cellular components, which are essential for maintenance and integrity of the tissues, are located within or on the surface of those composite materials.

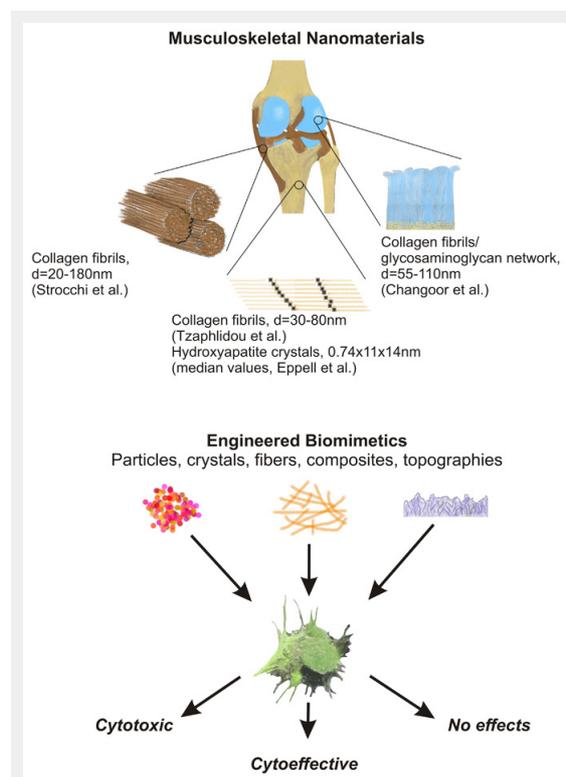
Bone tissue has a highly nano hierarchical structure consisting mainly of collagen type I fibres and nano hydroxyapatite crystals as the matrix. That combination yields in a material with unique mechanical properties such as high compressive and tensional strength [5]. It exhibits a high biological plasticity reacting to stress and strain by remodeling with bone apposition and resorption. Ligaments and tendons have a similar building pattern with fibres basically oriented parallel to the stress axis and consisting mainly of collagen type III embedded in a proteoglycan matrix. It is that fibre orientation which is responsible for the strength of the tissue still yielding elastic behaviour [6]. Ligaments and tendons have some plasticity

to adapt to changing stresses and for regeneration. Mature articular cartilage has a highly hierarchical structure with stratified collagen fibrils, mainly of collagen type II, embedded in a hydrogel formed by glycosaminoglycans and proteoglycans [7, 8]. In concert, these structures mediate the compressive and tensile strength of the cartilage tissue [9]. Although articular cartilage requires a continued but cycling stress for survival, the potential to adapt to changing stresses and to regenerate is very limited, if not absent. This biologically separates the cartilage from the bone and ligaments/ tendons.

### Nanomaterials for musculoskeletal reconstruction

Whenever endogenous repair of skeletal defects is not sufficient, more or less simple therapy approaches are applied, i.e., that defects are bridged or filled with autologous tissue or with biomaterial based implants which stay in place or are absorbed over time. Regardless whether bone, ligaments/ tendons, or cartilage repair is approached, lack of mechanical stability of the implant and the repair tissue is a major cause for failure. Thus, it is not surprising that it has been the aim of many research groups to integrate nano sized components into their materials with the expectation to improve the clinical outcome. These expectations have been nourished mainly by two facts. First, the so-called biomimetic approach aims to copy the nano dimensional natural architecture and thus to generate a micro-environment which instructs invading cells to appropriately differentiate and to form a competent tissue [10]. Second, the mechanical properties can be highly improved by integrating nano components and engineering nano-composite-materials as compared to micro- and macroscopic materials. In the field of musculoskeletal regeneration, various types of nanomaterials are experimentally used including nano crystals and particles, nano fibres and tubes, or nano structured composites and surfaces, some of which will be detailed below [4, 11].

Traditionally, calcium phosphates have been used as bioactive materials since the early 20<sup>th</sup> century to treat bone defects [12, 13]. Together with other mineral materials and surface precipitations, they are *per se* nanomaterials since they are typically agglomerates based on nano crystals or amorphous particles if they are not sintered. The hypothesis that a high specific surface area, an inherent property of nanomaterials, of bone substitutes results in enhanced osteoinduction has been commonly accepted and proven in several studies [14, 15]. Upon sintering the nano scale is lost and micro and meso scale features are obtained which are typically osteoconductive. However, it is probably less the nano structured surfaces which affects bone formation, but rather the availability of calcium and phosphate ions for osteoblasts and osteoclasts due to a higher solubility of nanocrystallites [16]. On the downside, pure calcium phosphate compounds exhibit very limited mechanical properties as they are brittle and break easily under load. Therefore, there have been many efforts to enhance their strength and fracture toughness, specifically by mimicking the hierarchical structure of bone or nacre [17–19]. Supporting evidence that architectural organisation is indeed a key factor was given experimentally in a study with human cortical bone tissue revealing that fracture en-



**Figure 1**

*Musculoskeletal tissues – the prototypes of nanomaterials.* The structural composition of ligaments/ tendons, bone and cartilage is very similar, although these tissues have quite a distinct appearance. In a simplified approach they consist of a characteristic network of collagen fibrils with diameters of approximately 100 nm embedded within a tissue specific matrix. Appropriate engineering of nanobiomaterials making use of particles, crystals, fibres, composites or surface topographies may lead to biomimetic constructs that exhibit a favourable inductive interplay with the host cells and tissues. Detailed and standardised investigations are necessary to predict the cellular and organ reaction upon exposition to those nanomaterials. (References: Strocchi R et al. Ital J Anat Embryol. 1996;101(4):213–20. Changoor A et al. Osteoarthritis Cartilage. 2011;19(12):1458–68. Tzaphlidou M et al. Micron. 2005;36:593–601. Eppell S et al. J Orthop Res. 2001;19:1027–34.)

ergy absorption varies by two orders of magnitude in dependence of the orientation of the collagen fibrils [20]. Similarly, in nacre, the highly oriented arrangement of relatively weak components, i.e., calcium carbonate nanocrystals and proteins, results in excellent stiffness, strength and toughness [21]. A very simple way to mimic the structure and to engineer a synthetic nacre like material was achieved by dip-coating technology with sequential layer-by-layer deposition of a polycationic compound (poly-diallyldimethylammonium chloride, PDDA) and an anionic montmorillonite clay [18]. This resulted in thin films with a Young's modulus close to lamellar bone. Apart from this hybrid composite with a highly organised structure, there have been many more studies on non-ordered composite materials as bone biomimetics. In particular, fibre-reinforced composite (FRC) materials have been studied in recent years at macro-, micro-, and nano-scale. There are commercial composite bone substitute materials available which are all based on collagen and calcium phosphates like Healos<sup>®</sup> (DePuy Spine), Collapat<sup>®</sup> II (Biomet Inc.), or Collagraft<sup>®</sup> (Zimmer Inc.). These products were, however, not developed to address mechanical issues, but to enhance tissue integration through the combination of collagen and hydroxyapatite. If a mechanical more competent material is anticipated, a tight and stable fiber-matrix interaction is required, otherwise the FRCs may be even weaker than the pure matrix [22, 23]. This can be achieved by chemical interaction as shown above for nacre mimics or by sintering. In case of a robust fiber-matrix interaction it has been found that length, volume fraction, and strength of the fibres are key microstructural parameters that control the mechanical properties of the FRCs [24, 25]. A broad variety of natural versus synthetic and resorbable versus non-resorbable materials have been used to engineer FRCs. A similar but still different approach encompassed engineering of porous  $\beta$ -TCP based scaffolds reinforced with hydroxyapatite nanowhiskers [26]. Nanowhiskers with dimensions of 20 nm diameter and 200 nm length prevent straight crack propagation and promote cracking along a wavier path which increases the toughness of the material and achieving mechanical properties close to cancellous bone. While engineering FRCs for tissue applications, it is important to select an engineering-material design which is absorbed in a timed manner, so that the FRC-tissue complex does not lose its mechanical strength. For example, when using fast absorbable fibres in FRCs, it weakens the whole FRC and channel-like porous structures are left behind [25, 27]. Therefore, not only concerted absorption of the FRC and *de novo* tissue formation is required, but also timed or delayed absorption of the reinforcing component within the FRCs.

While bone substitutes are mainly mineral based materials, scaffolds used for cartilage and ligament/ tendon applications are typically based on polymeric materials. It is the correct choice of structure where further potential may reside to improve current repair strategies. Electrospinning of nano fibres has been approached on many occasions with a variety of materials and setups [28]. Of particular importance appears to be a tight control of fibre diameter, arrangement and orientation, since they affect basic cell function, cell differentiation and immunologic response

within a recipient. Culturing fibroblasts on uniform polycaprolactone fibres revealed that the initial response of cells, in particular cell adhesion and growth, decreased with increasing fiber diameters [29]. Furthermore, fibroblasts seeded on electrospun polyester nanofibre scaffolds assembled and produced well-defined fibronectin fibrils early on, while on microfibers the matrix assembly was delayed and less organised [30]. It is not only adhesion and proliferation which is affected by the structural features, but also phenotype and differentiation. Chondrocytes seeded on polylactic acid based scaffolds engineered with electrospun fibres with a diameter of 500-900 nm produced a matrix with a more cartilage-like phenotype, i.e. a chondrocytic cell morphology and the production of cartilage specific matrix, as compared to cells seeded on scaffolds based on 15  $\mu$ m thick fibres [31]. Whether it is a pure fibre diameter effect or a combination of fibre diameter, the resulting pore dimensions, as well as the integral structures of the scaffolds could not be concluded. That issue was picked up recently in a study revealing that fiber diameters of less than the size of a chondrocyte were beneficial in terms of chondrocytic differentiation, but that the scaffold design, i.e., scaffold density, pore size and architecture, influenced as well the chondrocytic phenotype [32]. It is important to note, however, that the outcome may be strongly dependent on the cell type used for assessing the effects of fibre diameter and geometry. For example, when working with mesenchymal stem cells, scaffolds with micrometer sized fibres have proven more favourable as compared to nano sized fibers with regard to chondrogenic differentiation [33]. A systematic *in vivo* study on the effect of fibre diameter on host response after subcutaneous implantation of polypropylene fibres revealed that around six micrometers a critical threshold exists below which tissue capsule formation is significantly reduced [34]. Importantly, capsule formation is a sign of a prolonged inflammatory phase (see below), which may interfere with the formation of an organised tissue like cartilage or ligament/ tendon. The reduced capsule formation was attributed to a change in cell-material contact surface area or to a curvature threshold effect that triggers a distinct signaling in cells of the innate immune system affecting the host's response to an implant. Such results as presented above have important implications on the design of fibro-porous mesh implants to be used in tissue repair and regeneration. Furthermore, it is important to realise that the nano sized elements are in the same morphological size regime as the components of the natural extracellular matrix and that those potentially affect the interaction with cells. Recent reviews have discussed this relationship and the effect on tissue engineering extensively [35, 36].

## Biological response to nanomaterials

Implantation of a material into a living body can be considered as an injury and likewise evokes a cascade of host reactions including blood-material interactions with formation of a fibrin matrix, inflammation, cellular infiltration, new tissue formation and remodeling with or without absorption of the biomaterial [37, 38]. The initial material-blood interaction may define the further course of the bio-

logical reaction. In special cases as in cartilage repair, there is no initial contact with blood, however, the synovial fluid components will take over this role and prime the surface with the adsorption of biomacromolecules. Initially, the material will be covered mainly with albumin and fibrin in the case of blood, while in case of synovial fluids the highly charged glycosaminoglycans may also tightly adsorb onto the surface. The composition of the adsorption coatings depends on the chemical and physical properties of the nanomaterial and on the biological fluid they are exposed to, and it is important to note that it must not necessarily follow macro and microscopic characteristics of the surface. Consequently, cells at later stages will primarily see the proteins presented by the surface and react accordingly. These initial mechanisms of the interactions at the nano-bio-interface were excellently reviewed recently [39, 40]. That type of interaction is well-defined for macroscopic surfaces with micro- and nanosized features. The biomacromolecule interaction with nanoparticles and nanofibres, however, is less studied. It is important to realise that the adsorption processes and the subsequent denaturation of the biomacromolecules is different from nanomaterials due to the high aspect ratio of the curved surface, which requires more energy for these events to occur [41]. In cases where the nanoparticles are very small or the energy needed for adsorption and denaturation exceeds the capability of the biologic system, the proteins are adsorbed to the surface by point contact only and thus can be more easily recognised by cells. Consequently, a nanomaterial-biomacromolecule complex may mimic a pure biological aggregate and can be absorbed and metabolised by host cells. It is this fact, which is a chance for many therapeutic applications, but also a risk if the nanomaterial elicits an unwanted host response.

As part of the injury response as described above, an implanted biomaterial interacts with monocytes of the host, triggering an inflammatory reaction, which is usually resolved within two weeks [37]. If nanomaterial-protein aggregates are involved, the initial response to the biomaterial will be similar, but non-metabolisable nanomaterials may reside as a continued irritation to the monocytic cells and result in a persistent chronic inflammation. Furthermore, nanomaterials are able to cross cellular membranes by non-phagocytic mechanisms [42], and might intracellularly elicit further adverse reactions, especially when the cell has no mean for elimination. Since the discovery of the harmful effects of asbestos fibres, it is known that submicron- and nanoparticles can lead to a sustained, uninterrupted activation of monocytic cells and thus lead to chronic inflammation and even tumour formation through persistent release of inflammatory cytokines. Another example are carbon nanotubes, which are controversially discussed. Some studies suggest that carbon nanotubes are excellent substrates for cellular growth while others describe negative influence on cellular function up to cytotoxic effects after the nanotubes penetrated the cells [43, 44]. These controversies arise as there is nothing such as a commercial pure primal carbon nanotube type; rather they differ in their production methods, functionalisation, trace contamination etc. Thus, most studies are not comparable for the raw materials and since typically, no comparison and normalisation to stand-

ard reference materials is done, it is also difficult to compare these results. However, most *in vivo* results demonstrate that the long and stiff nanotube bundles have adverse effects, whereas short tubes or those, which go into suspension, have no biological impact on cells or organs [45].

After the initial inflammatory response, blood vessels and mesenchymal cells will start to approach and eventually invade an implant. Hereby, the proteins initially adsorbed on the surface of the materials will define the attachment, spreading and migration of the cells through ligand-receptor mediated signals. The kind of tissue which is finally formed depends on the microenvironment the migrated cells will be exposed to [10]. Herby, the topographical and structural organisation and the physico-chemical properties will further contribute to those steps [46–48] as discussed above.

### Risk of nanomaterials in musculoskeletal applications

Are nanomaterials the Holy Grail in regenerative medicine of the musculoskeletal system? Can they solve what has been unsolved so far by adding distinctive chemical and mechanical properties? The history of nanomaterials teaches us to be reluctant, especially when thinking of asbestos, aramid and carbon fibres. In addition to the beneficial properties of nanomaterials mentioned above, they can adversely, but unpredictably, affect normal function of cells and tissues. More than thirty years ago, without having the knowledge of using nanomaterials, carbon fibre constructs and composites were developed for tissue engineering [49] and promoted specifically for use as tendon and ligament replacement (e.g., Proplast – Vitex Inc, Houston, Tx; Intergraft – Osteonics Biomaterials, Livermore, CA). In many instances the outcome of experimental application of carbon fibres as tendon, cartilage or bone devices were promising and therefore the materials were used for clinical studies [50–52]. Although there had been some clinical success in the short term, long term observations revealed insufficient incorporation of the carbon fibres and disintegration resulting in carbon fragments distributed in the knee joint [53, 54]. Though chemically inert, these fibres were found to elicit substantial foreign body giant cell reactions and immunological responses, leading to synovitis and bone resorption around articular joints. Moreover, carbon deposits in- and outside of cellular components were not only found in perimplant tissues, but also in lymph nodes and in the liver [54, 55]. A further argument to carefully study the effect and fate of nano-materials in musculoskeletal applications arose from studies on wear particles generated by the articulating components of total hip and total knee arthroplasties. Up to  $10^{10}$  micro- and nanometer particles ( $<10\ \mu\text{m}$ ) per gram tissue were isolated from tissues around aseptically loosened implants. These particles stimulate cellular secretion of inflammatory cytokines (i.e., IL-1 $\alpha/\beta$ , IL-6, TNF $\alpha$ ), leading to a persistent inflammatory reaction and eventually tissue degradation and bone loss. Moreover, the submicrometer wear particles are also absorbed by the lymphatic system and transported to the lymph nodes and organs such as liver and spleen [56].

## Conclusions and future

Engineered nanomaterials have been widely studied in recent years regarding novel or enhanced material properties but also regarding their effect on biological systems. One of the major issues arising when using nanomaterials in medical applications is their variability regarding performance and biocompatibility. It is well-known from carbon nanotubes that their production method and the resulting dimensions influence their behaviour and performance. Thus, for the prediction of the biological outcome, each combination has to be measured separately and current cell culture approaches will have to be refined. The nanomaterials will have to be evaluated in 2D and 3D cell culture system to allow cell-material interactions [57]. Even more important will be for predictive *in vitro* assessment that mixed cell cultures with niche specific cell types are used and a broad battery of inflammatory and immunological markers are assessed. Furthermore, it is essential that for cross study comparison well-defined standardised reference materials and test protocols are included. Nevertheless, a vast number of studies have been published describing hundreds of novel nanomaterials showing clearly the chances to engineer biomaterials with advanced properties. Saito and co-workers state in a recent review on the past and future as carbon micro- and nano-fibres that “carbon fibres also show high cell adhesion and specific and interesting biological reactions. Investigating the correct usage and use site of carbon fibres could lead to big breakthroughs in the biomaterial field” [58].

Indeed, nanomaterials may portray a piece of the Holy Grail in regenerative medicine of the musculoskeletal system. Engineered nanomaterials are on the same structural level as these natural tissues. They allow for inducing material effects that are not possible with macroscopic designs. Appropriate engineering of nanobiomaterials may lead to biomimetic constructs that exhibit a favourable inductive interplay with the host cells and tissues. Successful engineering of biomaterials applying nano-technology may lead to next generation bone, cartilage and ligament/ tendon substitute materials relevant to biomedical device industries, patient benefit and health care economics.

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Figures (large format)

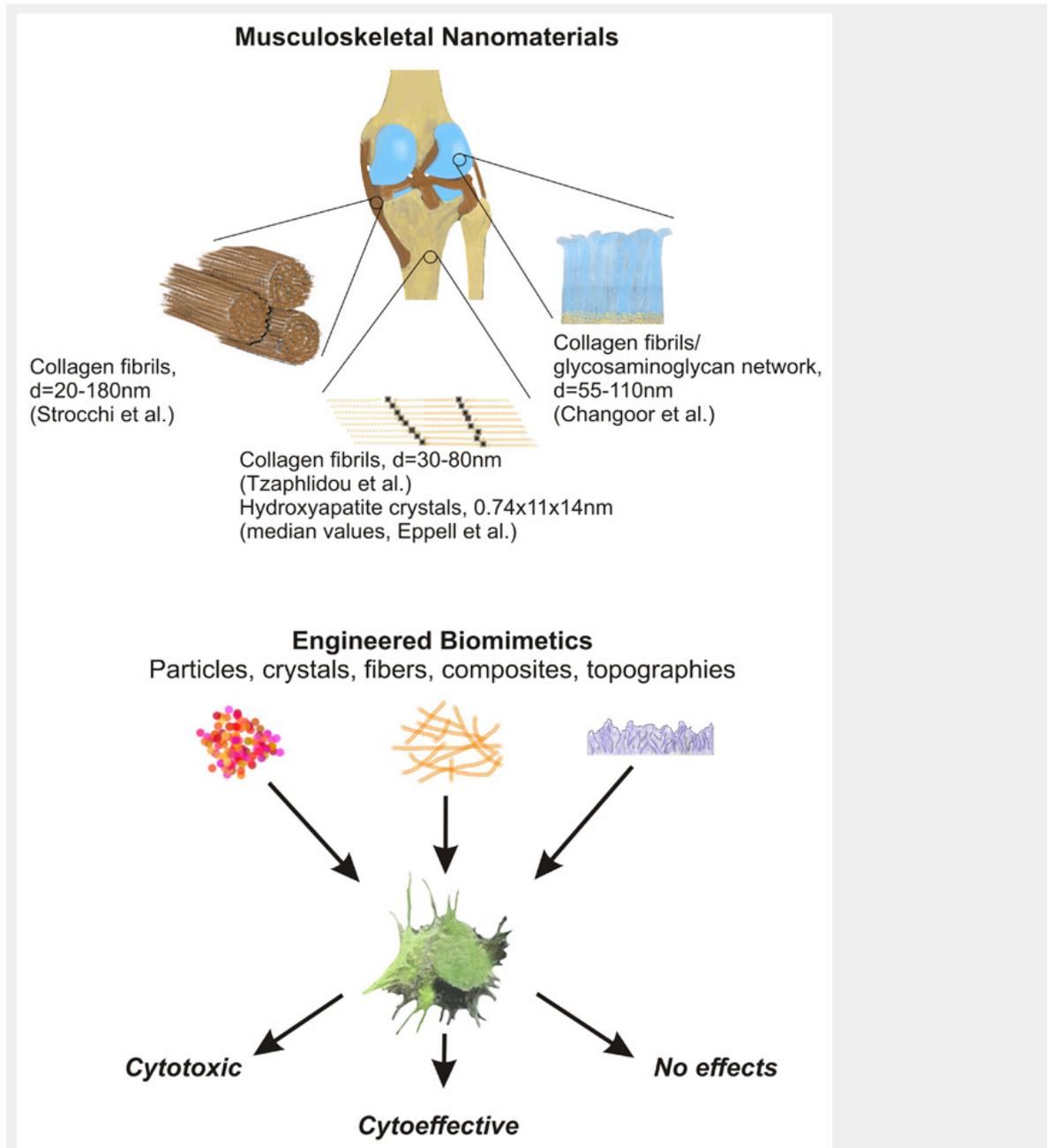


Figure 1

*Musculoskeletal tissues – the prototypes of nanomaterials.* The structural composition of ligaments/ tendons, bone and cartilage is very similar, although these tissues have quite a distinct appearance. In a simplified approach they consist of a characteristic network of collagen fibrils with diameters of approximately 100 nm embedded within a tissue specific matrix. Appropriate engineering of nanobiomaterials making use of particles, crystals, fibres, composites or surface topographies may lead to biomimetic constructs that exhibit a favourable inductive interplay with the host cells and tissues. Detailed and standardised investigations are necessary to predict the cellular and organ reaction upon exposition to those nanomaterials. (References: Strocchi R et al. Ital J Anat Embryol. 1996;101(4):213–20. Changoor A et al. Osteoarthritis Cartilage. 2011;19(12):1458–68. Tzaphlidou M et al. Micron. 2005;36:593–601. Eppell S et al. J Orthop Res. 2001;19:1027–34.)