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Hierarchically Structured Biomaterials for Tissue Engineering

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Tissue engineering is generally conceived as a tool to re-establish lost functionality and morphology to a previously damaged tissue. In this regard, several strategies combine scientific advances in medical treatment and therapeutics but also in the field of engineering and biomaterials. Many important aspects in tissue engineering science have been addressed in a previous editorial by Horch [1] - nevertheless, the role of structure and hierarchy in biomaterials design should be emphasized in greater detail.

Structural design in biological tissues often relates to a specific mechanical function besides fulfilling other functional requirements. This is obvious for example for cartilage, tendon, bone and dentine. The properties of both articular cartilage and tendon are largely determined by the arrangement of their main building blocks, the collagen fibrils. However, while articular cartilage is optimized for an almost frictionless movement of joints combined with a high load bearing capacity, tendon is adapted to optimized spring-like stress-strain behaviour with high elastic energy absorbing capacity. The mineralised tissues bone and dentine are composites of the inorganic mineral hydroxyapatite and - similar to cartilage and tendon - the protein collagen. While bone combines light weight with flexibility and strength, allowing and repairing damage, dentine is being characterized as material sustaining high cyclic loads virtually without failure over the lifetime of the organism.

These specific mechanical adaptations seem to be achieved by structural hierarchy comprising several levels from the macroscopic tissue arrangement down to the molecular arrangement of proteins as detailed in the overview by Fratzl and Weinkamer [2]. Regarding the transfer of structure-property-relationships from nature to engineering, the author states that "It is not evident at all that the lessons learned from hierarchical biological materials will be applicable immediately to the design of new engineering materials. The reason arises from striking differences between the design strategies common in engineering and those used by nature, which are contributed by the different sets of elements used by nature and the engineer".

As already outlined above, biological tissue is mostly based on complex hydrocarbons like proteins and specifically in bone tissue, a non-engineering ceramic: hydroxyapatite. Furthermore, while engineering strategies can be regarded "top down" being based on the design of the final product, nature depends on a "bottom up" approach by merging educts via complex biochemical processes. Therefore, in engineering the "structure" is a design principle of the product, while in nature it is achieved through growth processes. An adaptation of the natural pathway to a synthetic process is often referred to as biomimetics after Schmitt [3,4], while yet another common term used in tissue engineering is the word "generation". In this regard, usage of *in vitro* cultivated autologous cells in a defect area is considered as 1st generation tissue engineering, whereas the application of biomaterials together with *in vitro* cultivation of cells is termed 2nd generation. Finally, so called 3rd generation approaches try to mimic or even enhance tissue regeneration via specialized signalling molecules [5-7]. The combination of hierarchical engineering principles with 2nd or 3rd generation tissue engineering methodology is highly useful in regenerative medicine. Following, some examples from our own research in the field of cartilage, bone and dental engineering are presented, demonstrating concepts of "hierarchically structured biomaterials".

Cartilage Tissue Engineering

This work on tissue engineered articular cartilage was intended to achieve an integrated biomaterial design focussing on three main properties: a) biological and biochemical, b) structural and c) mechanical similarity to native cartilage tissue. Furthermore, the material should be extendible - offering the possibility to integrate signalling molecules or bone like structures.

The biochemical similarity was achieved through usage of the collagen derivative gelatine which was dissolved in water and processed via freeze structuring coupled with an electrolysis process [8]. This process allows the defined growth of ice crystals in the direction of an applied temperature gradient yielding directional porosity and a directional arrangement of collagen chains after subsequent freeze-drying. This first level of structural hierarchy was extended by incorporation of other components, allowing us to create composite scaffolds or to achieve different pore morphologies. The incorporation of hydroxyapatite as a bottom layer to mimic subchondral bone (Figure 1a) or the introduction of drug-loaded polymeric poly-lactide-co-glycolide microspheres (Figure 1b) can be thought of yet another level of structural hierarchy [9].

In particular, the impact of the structure on the mechanical properties was investigated, showing similarity to the native tissue [10]. By X-ray tomography we were able to determine the changes in morphology under mechanical loading conditions. As indicated in figure 1c, the directional pore network collapses under compressive stresses showing bulging of the pore-channel structure in three dimensions [11].

In summary, the developed material features several levels of hierarchy with aligned molecules and a distinctive and directional pore channel structure complemented with an optional hydroxyapatite layer and polymeric microparticles as drug release system. While the directional pore structure adapts the arched collagen network of the native tissue to give improved mechanical behaviour, the hydroxyapatite layer not only mimics the subchondral bone, but further introduces a stiff component to which the ordered gelatine structure is attached.

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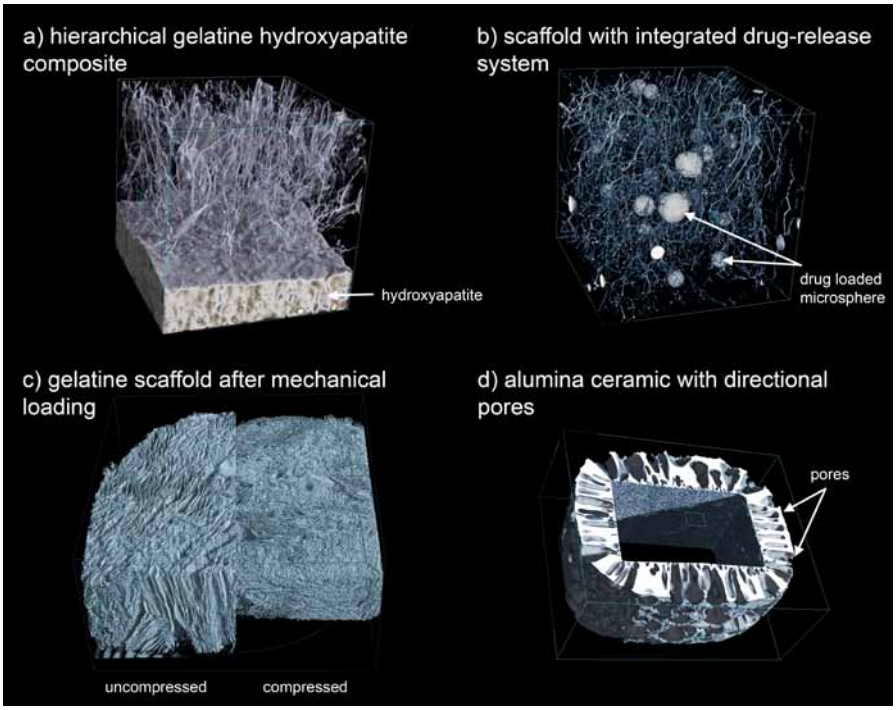

Figure 1

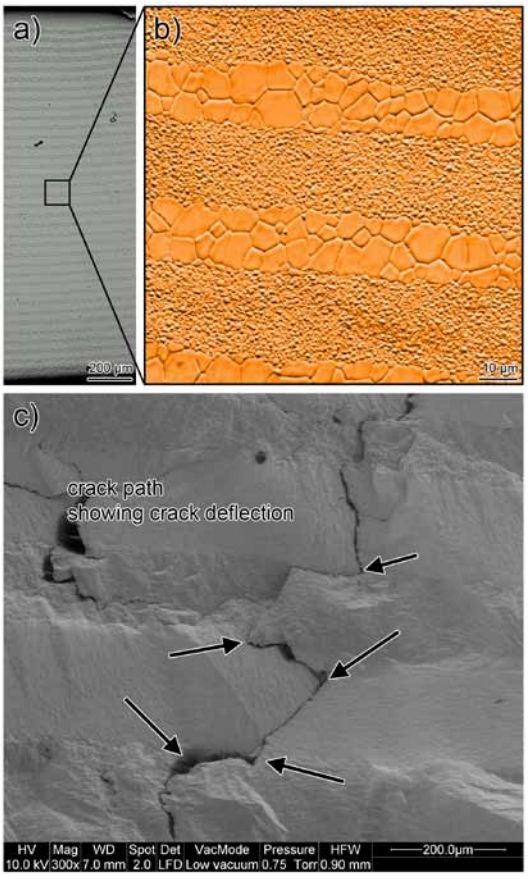

Figure 2

Bone Tissue Engineering

Similar to the previously described material design, the objective of this ongoing work is to develop a biomaterial for bone tissue engineering with a porous polymer-ceramic structure in which a strontium compound is immobilized as a so called dual action bone agent - stimulating bone growth and inhibiting bone resorption [12,13]. The material is based on a paste-like mixture of polyester (e.g. poly-ε-caprolactone) dissolved in an organic solvent and calcium hydroxide and strontium hydroxide. The organic solvent is removed via supercritical drying with carbon dioxide yielding a porous dry body, while the hydroxides are simultaneously transformed into the more biocompatible carbonates [14]. Depending on the processing parameters of the drying process, the pore morphology can be changed, yielding pore gradients and hierarchical pore channel structures. The reason behind this structural and functional material design is similar to the above described material for cartilage tissue engineering.

Hierarchically Structured Ceramics for Dental Applications

Another recent approach is directed at biomaterials for dental applications. Dental ceramics are usually only weakly interacting with the surrounding tissue and are optimized for improved hardness. Nevertheless, biomaterials concepts which offer this tissue integration or which enhance the mechanical properties is highly relevant.

In this regard, we have developed a shaping technology, combining a protein consolidation mechanism with an electrophoretic deposition process [15]. Here, the water soluble fibrinogen is electrochemically transformed into fibrin while alumina particles are attracted towards the electrode and trapped in the simultaneously forming fibrin network. This process can yield hierarchical porous structures as displayed in figure 1d. Currently we are investigating, if the protein or other polymeric substances can be integrated permanently in the ceramic in a more biomimetic approach as the protein is currently completely removed via thermal sintering. Nevertheless, the burn out of the organic matter leaves yet another level of hierarchy by creating a microporous structure (Figure 1).

Within another current project we characterise the influence of structural hierarchy on the mechanical properties of ceramic constructs [16]. Our focus is on increasing the fracture toughness, while our co-workers have improved the corresponding electrophoretic deposition process to produce multilayers of two different zirconia types (tetragonal and cubic zirconia), thereby improving the resistance against crack growth [17]. In addition, the tetragonal zirconia undergoes a phase transformation which is well known as transformation toughening. Figure 2 displays the repeating sequence of cubic and tetragonal zirconia layers in light microscopy (Figure 2a) and in higher resolution using AFM imaging (Figure 2b). Here, AFM imaging shows different grain sizes, which acts as another level of hierarchy at the microscopic scale and which presumably influence crack propagation, although possibly to a lesser extend than the different zirconia types. Finally, figure 2c shows the deflection of a propagating crack at the layer interfaces, which is a toughening mechanism unrelated to the well known transformation toughening (Figure 2).

Discussion

Adapting hierarchical structures to biomaterials is a promising methodology to further enhance the innate materials properties. Of these, the mechanical properties are highly important and relevant to several tissue engineering areas including cartilage tissue engineering, bone engineering and dental biomaterials.

Overall, the structural design of a biomaterial should closely related to the mechanical loading conditions of the corresponding native tissue, while several levels of hierarchy (e.g. at macroscopic, and microscopic lengths scales) can further improve the material properties through different toughening or strengthening mechanisms. Here, a composite of two different materials, ordered structures or size effects play a pivotal role. In this introductory part, we have presented some selected biomaterial concepts which integrate a hierarchical component in varying degrees into the engineering design of biomaterials.

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