ADVANCES IN BIOMIMETICS

Edited by **Anne George**

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Preface

Biomimetics is the science of emulating nature's design. In nature, living organisms synthesize mineralized tissues and this process of biomineralization is under strict biological control. It involves the interactions of several biological macromolecules among themselves and with the mineral components. Generally, natures design principles are based on a "Bottom-Up" strategy. Such processes lead to the formation of hierarchically structured organic-inorganic composites with mechanical properties optimized for a given function. A common theme in mineralized tissues is the intimate interaction between the organic and inorganic phases and this leads to the unique properties seen in biological materials. Therefore, understanding natures design principles and ultimately mimicking the process may provide new approaches to synthesize biomaterials with unique properties for various applications. Biomimetics as a scientific discipline has experienced an exceptional development. Its potential in several applications such as medical, veterinary, dental science, material science and nanotechnology bears witness to the importance of understanding the processes by which living organisms exert an exquisite control on the fabrication of various materials. Despite several breakthroughs, there exist only a limited number of methods for the preparation of advanced materials. Consequently, precisely controlling the architecture and composition of inorganic materials still remain enigmatic. Biological organisms have the extraordinary ability to fabricate a wide variety of inorganic materials into complex morphologies that are hierarchically structured on the nano, micro and macroscales with high fidelity. The next generation of biologically inspired materials fabrication methods must draw inspiration from complex biological systems.

The interaction between cells, tissues and biomaterial surfaces are the highlights of the book "Advances in Biomimetics". In this regard the effect of nanostructures and nanotopographies and their effect on the development of a new generation of biomaterials including advanced multifunctional scaffolds for tissue engineering are discussed. The 2 volumes contain articles that cover a wide spectrum of subject matter such as different aspects of the development of scaffolds and coatings with enhanced performance and bioactivity, including investigations of material surface-cell interactions.

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Novel Biomaterials with Parallel Aligned Pore Channels by Directed Ionotropic Gelation of Alginate: Mimicking the Anisotropic Structure of Bone Tissue

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1. Introduction

Regenerative medicine intends to restore lost functionality by healing tissues defects. For this novel types of biodegradable implants have to be used that first foster healing and later take part in the natural remodelling cycle of the body. In this way, patient's cells can reconstruct and adapt the tissue according to the local situation and needs. Ideally, the implant should mimic the desired tissue. That means that the biomaterial should resemble the extracellular matrix (ECM) which is expressed by specific cells and acts as the biological scaffold of living tissues. The closer an artificial scaffold material mimics the pattern the easier it can be involved in the natural healing and remodelling processes, which is why more and more researchers try to establish biomimetic approaches for the development of tissue engineering scaffolds. Biological materials are seldom isotropic and for many tissue engineering applications distinct anisotropic materials are needed. E. g. compact bone exhibits a honeycomb-like structure with overlapping, cylindrical units (osteons) with the so-called Haversian canal in the centre. Scaffolds with parallel aligned pores, mimicking the osteon structure of compact bone can be synthesised by directed ionotropic gelation of the naturally occurring polysaccharide alginate. The parallel channels are formed *via* a sol-gel-process when di- or multivalent cations diffuse into the sol in broad front, forming an alginate hydrogel. The pore size and pore alignment of such gels is influenced by the starting materials (e.g. concentrations, additives like powders or polymers) and the preparation process (e.g. temperature, drying process). The phenomenon was discovered already in the 50th of the last century but the biomedical potential of alginate scaffolds with parallel aligned pores structured by ionotropic gelation has been explored for osteoblasts, stem cell based tissue engineering, axon guiding or co-culture of vascular and muscle cells only in the past few years.

2. Biomimetic approaches for biomaterials and Tissue Engineering (TE)

In natural tissues, cells are embedded in three dimensional, fibrous environments – the so called extracellular matrix (ECM). General task of the ECM is to act as a scaffold for cell

adhesion, to provide certain mechanical stability and elasticity, to protect the cells and to facilitate the development of the proper cell morphology. In addition, ECM is the space of nutrient and oxygen supply, of intercellular communication and it is relevant for storage of water and soluble substances. Each ECM is perfectly adapted to the special needs of a distinct tissue and its dedicated cells.
When developing artificial tissues in terms of tissue engineering a biomaterial called scaffold has to take over the basic functions of the natural ECM, at least until the construct has been fully integrated and remodelled by the host tissue after implantation. It is obvious that it is difficult to design artificial materials which meet all the requirements described above. Therefore many researchers started to mimic the natural ECM with their scaffold material, either concerning chemical composition, micro- or nanostructure or special properties like anisotropy which is also an important feature of most tissues (Ma, 2008). Biomimetic strategies can include the utilisation of ECM components like natural biopolymers (e. g. collagen), material synthesis under physiological conditions (37°C, pH of 7.4, buffered aqueous solutions etc.) or the creation of structural features similar to those of extracellular matrices.
The better an artificial scaffold material mimics its biological model, the faster it will be integrated by the host tissue after implantation and the easier it will be included in the remodelling cycle, leading finally to a complete degradation and healing of the defect.

3. Bone tissue: a natural, highly anisotropic nanocomposite material

In humans (general in mammals), different types of bone exist or are formed intermediately during development or healing, mainly cortical (compact), spongy (trabecular) and woven bone (Weiner & Wagner, 1998). Their organisation is highly hierarchical, but at the lowest level all consist of the same nanocomposite, made of fibrillar collagen type I and the calcium phosphate phase hydroxyapatite (HAP). Collagen is produced by bone cells called osteoblasts, which also express the enzyme alkaline phosphatise (ALP), necessary for calcium phosphate mineral formation. A variety of non-collagenous proteins, also synthesised by osteoblasts, are responsible for control of the matrix formation and mineralisation processes, but the molecular mechanisms are not completely understood yet. With the exception of woven bone, collagen fibrils are deposited in an alternating, sheet-like manner and with a parallel fibre alignment (called "lamellae") into the free space, created by resorbing osteoclasts during bone remodelling. Lamellae form osteons in compact bone – always aligned parallel to the bone axis – and trabecules in spongy bone (Rho et al., 1998). These structure elements are responsible for the outstanding mechanical properties of bone tissue and its perfect adaptation to the local force distribution.
Compact bone has only pores with diameters in the micrometer range, filled either with blood capillaries (Haversian canals, located in the centre of the osteons) or osteocytes (*lacunae* – interconnected by the *canaliculi* pore system). In contrast, the trabecules in spongy bone form a highly open porous structure with pore widths of up to a few millimetres. Fig. 1 shows the hierarchical organisation of (cortical) bone tissue – from the macroscopic organ down to the nanometre scale.

4. Directed ionotropic gelation of alginate – a biomimetic method for generating anisotropic materials

Alginate is the structural saccharid of brown algae. Being a co-polymer, it consists of mannuronic (M) and guluronic acid (G) monosaccharide units, possessing identical

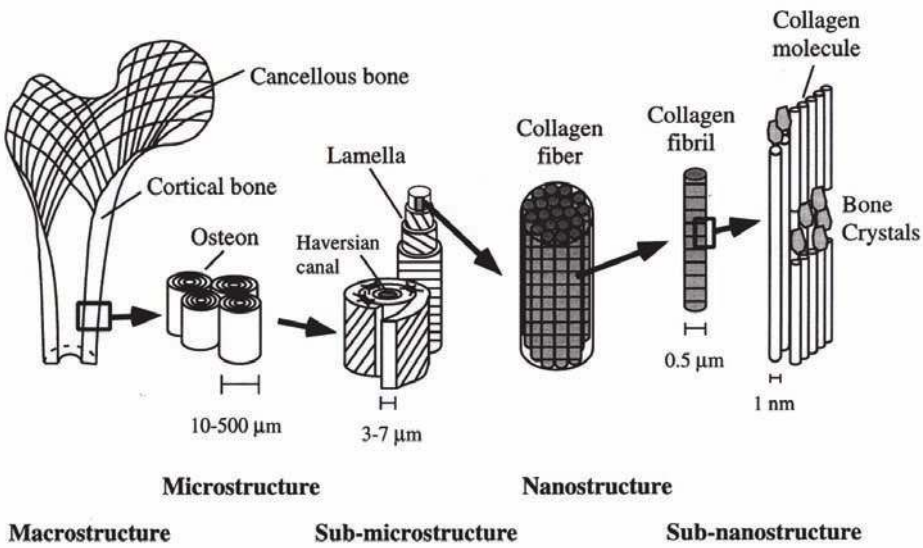


Fig. 1. Hierarchical organisation of cortical bone tissue from the centimetre to the nanometre scale (taken from Roh et al. (1998) with permission)

carboxylic and hydroxyl functional groups but differing in their configuration. These functional groups coordinate multivalent cations and build intermolecular complexes which results in the formation of a stable hydrogel. Straight MM-sequences do not exhibit sites for specific binding of cations (Braccini et al., 1999); the interaction takes place between GG-sequences leading to so-called egg-box motifs (Grant et al., 1973; Braccini & Perez, 2001). Alternating MG-sequences may also contribute but to a much lower extent (Donati et al., 2005). The composition of the alginates derived from different algae varies; the flexible stipes of algae, growing next to the sea surface, contain M-rich alginate whereas those exposed to strong flow exhibit high G-content (Zimmermann et al., 2007).

If an alginate sol gets into contact with gelling ions (electrolyte), the molecules gel immediately by covering the sol with a dense skin or membrane. Microbeads are produced by dropping small volumes into electrolyte solutions whereas the skin is trapping the sol which gets radially transformed into a gel by the diffusing ions. Anisotropic gels with channel-like pores develop when cations diffuse in broad front from one direction into an alginate sol whereas the saccharide molecules get arranged and complexed. Together with the gelation parallel aligned, channel-like pores are formed which can run through the whole length of the gel (Fig. 2).

4.1 Theoretical models for the phenomenon

The discoverer of the phenomenon, the German colloid scientist Heinrich Thiele, proposed the phase separation mechanism of droplet segregation. The gelation process

$$\text{Sol} + \text{Electrolyte (A)} \leftrightarrow \text{Gel} + \text{Electrolyte (B)} + \text{Water} \quad (1)$$

is accompanied by dehydration. The finely distributed drops of water are trapped within the zone of sol-gel-transition. Further delivered water molecules will accumulate and are

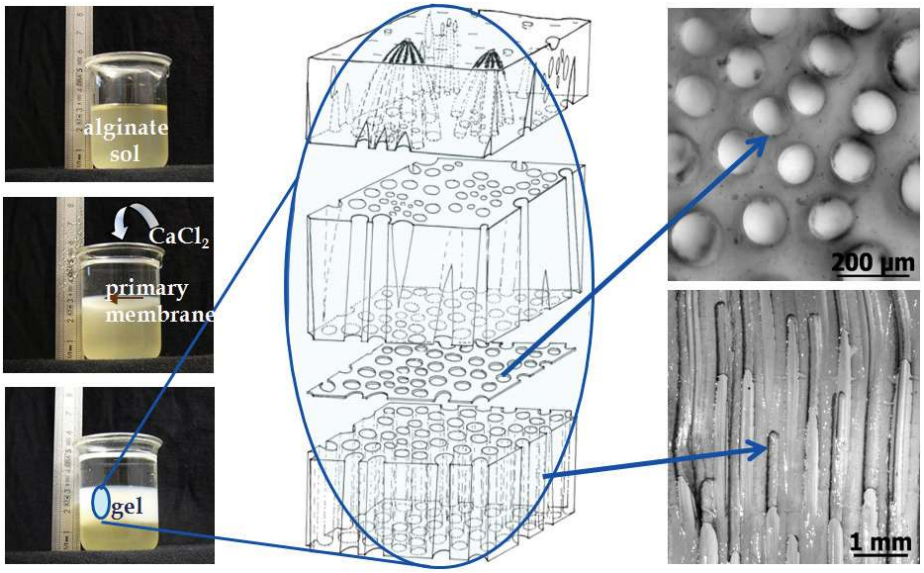


Fig. 2. Sketch of the process of ionotropic gelation of alginate. The scheme in the middle was adapted from Wenger (1998)

pushed by the gelation front towards the sol creating electrolyte containing and alginate free pore channels (Thiele & Hallich, 1957; Thiele, 1967b). Khairou and co-workers described the sol-gel-formation as diffusion controlled process which one step of primary membran formation and further growth of the anisotropic gel (Khairou et al., 2002).

In a series of 5 articles, Kohler and his group developed the theory of chemically fixed dissipative structure formation from the first idea (Kohler & Thumbs, 1995) until the summary of the work (Treml et al., 2003). Based on the observation, that there was a movement in the sol next to already gelled alginate visualized by tiny glass beads, they assumed a coupled mechanism of convection and diffusion. The alginate chains are subject to a conformational change during the complexation by the cations. If the sol exhibits an adequate viscosity, this contraction will induce a movement of the sol which resembles to pattern of the Rayleigh-Benard-Konvection. This pattern gets fixed by the sol-gel-transition. For a stable reaction, a sufficient mass transport is needed to ensure a certain contraction velocity of the alginate molecules. The mathematical description consists of the Navier-Stokes equation for the hydro-dynamical model (Kohler & Thumbs, 1995; Thumbs & Kohler, 1996), Fick's law for the diffusional macroscopic part (Treml & Kohler, 2000) and the results from random walk simulations of a phantom chain (Woelki & Kohler, 2003). The phenomenon of capillary creation due to the ionotropic gelation was postulated as chemically fixed dissipative formation, which is based on the concentration of the alginate sol and gel as well as the electrolyte, the diffusion coefficients of the reactants, the degree of polymerization, length and number of rigid segments of the alginate chain and the gelation rate constant (a fitting parameter obeying to boundary conditions) (Treml et al., 2003).

So far about growth but what about the initiation of the pores? Thiele and Hallich postulated periodic water droplets which segregate by the dehydration during gelation (Thiele & Hallich, 1957). The contraction of the alginate causes accumulations and lower

concentrated areas as nucleation seeds (Purz, 1972). Lateral variations in chain mass fraction and composition were also considered which would laterally vary the contraction capacity (Thumbs & Kohler, 1996). The origin of first segregation and pore creation was tried to identify by Purz and coworkers by electron microscopy – interestingly not with alginate but cellulose xanthate (Purz, 1972; Purz et. al., 1985). The ionotropic gelation is not specific for alginate but can occur also with other polymers (e.g. pectin, cellulose) and even inorganic anisometric colloids (e. g. V_2O_5) get oriented by the flux of counter ions.

4.2 History of ionotropic gelation

The phenomenon of ionotropic gelation was discovered by Heinrich Thiele, professor at the chemical department of Kiel University, Germany. Initially he studied in- and organic anisometric colloids which were oriented by diffusing ions. He created the term ionotropy (*ionos* = ion, *trepein* = turn) (Thiele, 1964) as a special case of gelation (Higdon, 1958). The properties of the gels were birefringence, anisotropic swelling and reversible ion exchange. He was fascinated by the similarity between structures of biological origin and the artificially created anisotropic gels (Thiele & Andersen, 1953). In his pioneering work, Thiele intensively studied parameters which influence the structure formation and different methods to characterise the oriented colloids (Thiele, 1967b). He restlessly compared the structure of ionotropic gels with those of tissues or other biological specimens and found a variety of similarities (Thiele, 1954b; Thiele, 1967a). Based on this comparison, he predicted a model for the principle of biological structure formation – especially supported by studies on dissolution and re-constitution of an eye lens (Thiele et al., 1964). His last publication on ionotropic gelation was dealing with mineralisation of the gels especially with calcium phosphates (Thiele & Awad, 1969).

More than 25 years later, the phenomenon was theoretically investigated with a new vision on the mechanism (Kohler & Thumbs, 1995) as well as towards the kinetics of ionotropic gelation (Khairou et al., 2002) – and finally, the capillary formation could be described by a mathematical model (Treml et al., 2003). At the same time, the idea re-emerged to use the membranes, produced by ionotropic gelation, as filters with adjustable pore diameter. Not only the hydrogels could be utilised for this application (Thiele & Hallich, 1959; Moll, 1963), but also sintered ceramics, derived by structuring slurries of alginate mixed with ceramic powders like e.g. Al_2O_3 (Weber et al., 1997) or even with the mineral phase of bone, hydroxyapatite (HAP) (Dittrich et al., 2002). The pore distribution and run was characterized by µCT in ceramic (Goebbels et al., 2002) or composite (Despang et al., 2005b) state. Since 2005/6, the anisotropic structures have been subject of research in the area of tissue engineering with human cells for hard tissue (Despang et al., 2005a, Dittrich et al., 2006**)** and vascularisation (Yamamoto et al., 2010), in *in vitro* and *in vivo* studies in rats for nerve regeneration (Prang et al., 2006) and with murine embryonic stem cells opening opportunities for the formation of many types of tissue (Willenberg et al., 2006). A more detailed and chronological list of scientific contributions to the field with short summaries of their content follows (Table 1).

4.3 Anisotropic hydrogels

The phenomenon of ionotropic gelation was discovered for alginate leading to a hydrogel with parallel aligned, channel-like pores. At the early beginning, the gelation was carried out solely with Cu^{2+} which needs to be replaced in case of medical applications by acidic exchange or ion substitution for a biocompatible one such as Ca^{2+}. Since 2005, hydrogels

Author(s) Year	Content
Thiele, 1947 [in German]	Alignment and gelation of anisometric particles in colloidal solutions (thin layer), resulting in birefringence pattern in polarized light
Thiele & Micke, 1948 [German]	First full article on alignment and gelation of anisometric particles in colloidal solutions, but not yet about capillary formation
Thiele & Kienast, 1952 [German]	Dependence of alignment of anisometric particles on type and concentration of ions of electrolyte including electron microscopy images of sol and thixotropic gel
Thiele & Andersen, 1953 [Ger.]	Identical structure and pattern of decalcified femur (collagen) and ionotropic gel (Cu^{2+} gelled pectin) observed in polarised light
Thiele, 1954a [German]	Change in experimental set-up: diffusion of electrolyte from outside into the sol, from thin layer of sol to beads and cylinders, direction of ion diffusion from radial to broad front
Thiele, 1954b	English summary of previous work; differentiation of ionotropic gels from other structures, claim on model for some biological patterns: bone (collagen), see weed (alginate) and ripe fruits (pectin)
Thiele & Andersen, 1955a [German]	Transition from inorganic to organic colloids for ionotropic gelation (alginate, pectin); first thoughts on theory of droplet demixing; swelling and birefringence antipodal
Thiele & Andersen, 1955b [Ger.]	Effect of chain length of alginate and pectin on ionotropic gelation (viscosity); first images of radial pore channels in multiphasic gels
Schuur, 1955 [Ge.]	Structure formation of ionotropic gels through material flux
Thiele & Kroenke, 1955 [German]	Reversible Pb-based mineralisation of ionotropic gels (cellulose glyconat) within the cavities or pore walls of gel
Thiele & Hallich, 1957 [German]	**Channel-like pores in 3D gels of alginate through ionotr. gelation including images and theory of droplet demixing; influence of type and concentration of cations and sol on pore channel diameter**
Thiele & Hallich, 1959 [German]	Application of capillary structure of ionotropic alginate gels as filters: void volume, permeability (water, gas), pore size distribution
Thiele et al., 1962 [German]	Distinction between 5 zones of ionotropic gels with parallel aligned pores; focus on primary membrane and diffusion induced membrane potential; ion exchange after cross-linking with DIC
Moll, 1963 [German]	Application of Al-alginate gels with channel-like pores as reversible filter for bacteria and viruses, filtering of a 5 nm gold sol
Thiele, 1964 [German]	Diverting overview about ionotropic gelation (theory, helices, mineralisation) as model of biological pattern formation
Thiele et al., 1964 [German]	Ionotropic gelation as principle of biological pattern formation based on similarities to natural tissues in appearance (osteons in bone, layers of pearl) and reversible gelation of eye lens and cornea etc.
Thiele & Cordes, 1967 [German]	Influence of counter ions on gel formation; ligand field theory
Thiele, 1967 [German]	Short summary of principles of structure formation: bone, eye lens, cornea

Author(s) Year	Content
Thiele, 1967b [German book]	Exhaustive summary and overview on ionotropic gelation (book)
Thiele, 1967c [German]	Ionotropic gels as template for oriented intra- or intercapillary mineralisation in native and cross-linked gels by ion waves
Thiele & Awad, 1969	Mineralisation of alginate hydrogels with parallel aligned pores with calcium phosphate phase brushit by ion waves followed by conversion to hydroxyapatite
Purz, 1972	Anisotropic hydrogels based on cellulose-xanthate structured via ionotropic gelation by thallium or zinc ions; SEM investigations
El-Cheik & Awad, 1976	Conductance of ions-free-washed metal alginate inversely proportional to polarisability of gelling cations
Awad et al., 1980	Kinetic of ionotropic gel formation in two steps (quick membrane formation, slow gel growth) evaluated by change in concentration of electrolyte and description as diffusion controlled process
Purz et al., 1985 [German]	Morphology of anisotropic cellulose-derivate gels structured by ions of Tl, Pb, Zn, La and combinations studied by electron microscopy
Hassan et al., 1989	Latest of 3 similar articles on kinetics of sol-gel-transformation of alginate with different ions (nickel, copper and cobalt)
Heinze et al., 1990 [German]	Structure and application of carboxy-containig polysaccharides, especially anisotropic alginate hydrogels for cell immobilisation, drug release; rheological investigations
Hassan et al., 1991	Structure formation of alginate by interaction of cations with two carboxylic and two hydroxy groups
Hassan, 1991	Kinetics of acidic ion exchange of cations (Ni^{2+}, Co^{2+}, Cu^{2+}) in anisotropic alginate hydrogels by conductimetry
Hassan, 1993	Kinetics of anisotropic Ni-alginate gels: idea for application on separation of ion mixtures and capture of isotopes based on selective alginate binding
Kohler & Thumbs, 1995 [German]	New idea on theory of capillary development by ionotropic gelation of alginate as chemically fixed dissipative structure: contraction of alginate during gelling yields a movement of sol next to gelation front which was visualised by adding 0.3 μm glass beads
Thumbs & Kohler, 1996	Mathematical description of ionotropic gelation similar to Rayleigh-Benard convection by Navier-Stokes equation and introduction of critical convection velocity
Weber et al., 1997	**Al_2O_3 membranes with capillaries produced by Cu^{2+}-gelled alginate-Al_2O_3-slurries and change in volume by drying procedures**
Treml & Kohler, 2000	Mathematical description of diffusive mass transport of alginate and gelling ions: correlation of convective transport to bulk concentrations
Dittrich et al., 2002	Synthesis of ceramic membrans (Al_2O_3, TiO_2, HAP) by ionotropic gelation of alginate/ceramic powder-slurries (drying process,

Author(s) Year	Content
	influence of sintering temperature on density, macro-structure)
Goebbels et al., 2002	Non-destructive analysis (µCT) of pore structure of ceramic membranes (Al_2O_3, TiO_2, HAP), synthesised by ionotropic gelation
Khairou et al., 2002	Kinetic study of ionotropic gelation induced by heavy metal ions and interpretation of change of electrolyte concentration: influence of ionic radius and electrolyte density; model of intra- and intermolecular binding of cations to alginate chains
Woelki & Kohler, 2003	Modelling of the integration of alginate chains to the growing gel by conformational changes/degree of contraction (length of chain, velocity of gelation front, velocity of cross-linking reaction)
Treml et al., 2003	**Summary of new theory on capillary formation as chemically fixed dissipative structure depending on bulk concentrations, diffusion constants, properties of alginate chain (number, length of Kuhn segments), rate constant of gelation reaction**
Despang et al., 2005a	Ca-alginate hydrogels and composites of alginate/HAP for bone TE: addition of HAP powder or synchronous mineralisation *in situ*
Despang et al., 2005b	µCT-evaluation of composites of alginate-gelatine, reinforced with HAP (powder and synchronous mineralisation)
Renzo et al., 2005	Pore channels in Cu-alginate microbeads and mineralisation
Dittrich et al., 2006	**Alginate-gelatine-composites reinforced with HAP or ß-TCP mimicking composition of bone (70:30 in- : organic) and biocompatibility test by cultivation of osteogenically induced hMSC**
Willenberg et al., 2006	**Cu-gelled alginate scaffold as polyelectrolyte with chitosan as matrix for TE with murine embryonic stem cells: structure and *in vitro* experiment for 4 days**
Prang et al., 2006	**Oriented axonal regrowth on isocyanate cross-linked, Cu-gelled alginate hydrogels with *in vitro* (entorhinal-hippocampal slice culture) & *in vivo* (spinal cord) experiments in rats**
Mueller et al., 2006	Axonal regrowth on Cu^{2+}-, Ni^{2+}- or Ba^{2+}-alginate hydrogels (after ion exchange) with *in vitro* & *in vivo* experiments in rats
Eljaouhari et al., 2006	Al_2O_3 membrans based on Cu^{2+}- or Ca^{2+}-alginate-slurries including optimized drying procedure, consolidation and permeability data
Dittrich et al., 2007	Influence of processing parameters on pore structure of Ca^{2+}-alginate-HAP-slurries (drying process, pore run (µCT), influence of media on softening, hMSC *in vitro* culture)
Gelinsky et al., 2007	Biphasic but monolithic scaffolds for therapy of osteochondral defect with 2 layers (alginate/hyaluronate and alginate/HAP)
Despang et al., 2008	Scaffolds for bone TE produced by ceramic processing chain; composite, brown-body & ceramic: change of microstructure and biocompatibility of hMSC
Bernhardt et al., 2009	Biocompatibility of alginate-gelatine-HAP-scaffolds evaluated with osteogenically induced human mesenchymal stem cells (hMSC) over 4

Author(s) Year	Content
	weeks (incl. mechanical testing)
Mueller et al., 2009a	Axonal regrowth on Ba- or Ni-gelled alginate with more and longer linear axon ingrowth in dorsal ganglion *in vitro* culture with 10 µm than 120 µm pore diameters
Mueller et al., 2009b	Summary on axonal regrowth guided by anisotropic alginate hydrogels
Khan et al., 2009	Alginate or polyelectrolyte dextran/alginate w/o particle reinforcement of Au, TiO_2 and Fe_3O_4
Yamamoto et al., 2010	Co-culture of HUVEC w/o smooth muscle cells seeded onto Ca-alginate hydrogel for revascularization – static and perfusion cultures

Table 1. Chronology of scientific publications on ionotropic gelation leading to structures with parallel aligned pores (excluding PhD theses and patents); milestones highlighted bold. Abbreviations: DIC - diisocyanate, hMSC - human mesenchymal stem cells, HUVEC - human umbilical vein endothelial cells, HAP – hydroxyapatite.

with channel-like pores created by ionotropic gelation of alginate were in focus for tissue engineering. The idea of creating a tube-like template for capillary tissue structures e. g. for blood vessels (Yamamoto et al., 2010) is fascinating. Depending on the needs, the pore diameter can be adjusted between 30-460 µm by the processing conditions, meanly type and concentration of alginate and electrolyte (Table 2). The swollen hydrogels exhibit a macro-porosity of approx. 30% due to the pore channel diameter but the walls consist of an alginate network with a high nano-porosity. The pore density was found to be 530/mm² and the mean pore diameter around 30 µm for Cu^{2+} as cation (Willenberg et al., 2006; Prang et al., 2006). Interestingly, using a different type of alginate gelled with Cu^{2+}, we found a pore density of 124/mm² with an mean pore diameter of only 20 µm. Anisotropic hydrogels based on this type of alginate (ISP Manugel DMB) gelled by diffusion of Ca^{2+} ions exhibited a pore density of 77/mm² whereas ISP Manucol DM yields 5/mm². The mean pore diameter is inversely related to the pore density. Using Ba^{2+} or Ni^{2+} ions instead of Cu^{2+} the pore density was 960/mm² and 30/mm², respectively, and the mean pore diameter 10 and 120 µm, respectively (Müller et al., 2008).

Target tissue	Dimension (ØH or LWH)	Pore-Ø	Alginate concentr.	Mol. weight	Electrolyte	Reference
	[mm]	[µm]	[Ma.%]	[kDa]	[M]	
Bone	10x5	40-230	2	40-60	1 M $CaCl_2$	Despang et al., 2005a
Embryonic stem cells	7x5x3	30	2	12-80	0.5 M $CuSO_4$	Willenberg et al., 2006
Neuronal tissue	0.5x0.5x3	27	2	100	1 M $Cu(NO_3)_2$	Prang et al., 2006
Vascularisation	5x2	220-460	0.5-4	64-110	0.5-1.5 M $CaCl_2$	Yamamoto et al., 2010

Table 2. Alginate hydrogel scaffolds designed for different tissue engineering applications

The pore diameter is also influenced by the pH value (Fig. 3), mainly, because the alginate conformation (coiled or stretched) can be changed with the pH. Adjusting the pH value, mostly HCl or NaOH is used which also changes the ion strength and therefore the electrostatic conditions within the sol. To achieve a homogenous alginate sol, the aqueous solution should be buffered because at low pH isotropic gelation can occur due to the ability of H^+ ions to interact with the alginate molecules (Thiele & Hallich, 1957).

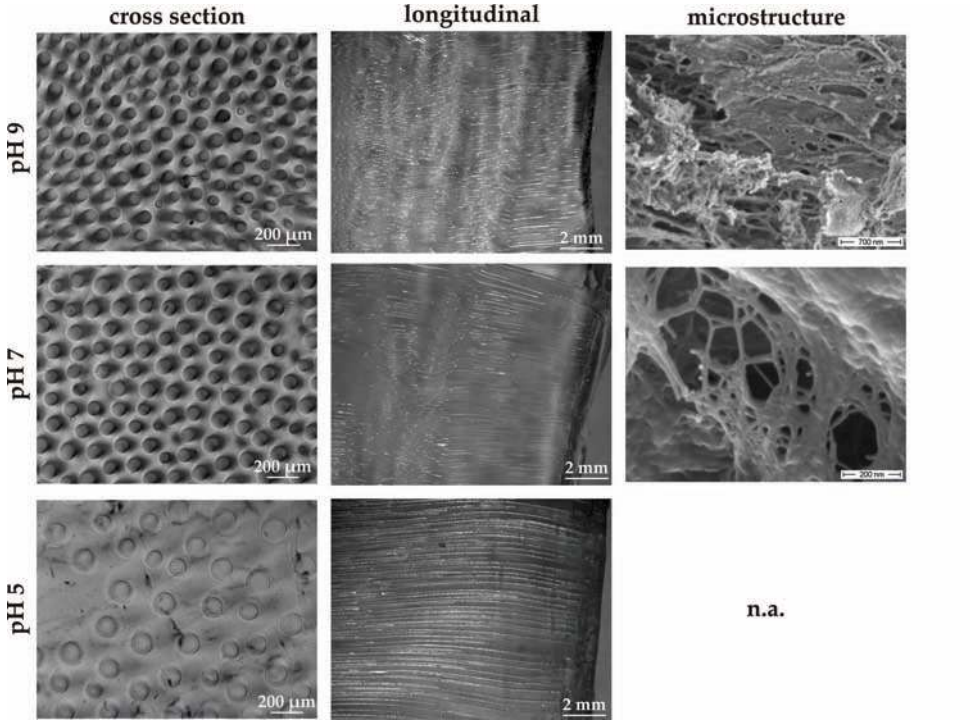


Fig. 3. Variation of pore diameter and pore distribution in the cross section of hydrogels, prepared at different pH values (1% Alginate Manugel, ISP) – microstructure after freeze drying

Up to distinct ranges, other biopolymers can be added to the alginate sol without preventing the process of ionotropic gelation. This also allows to further stabilise the hydrogels by means of covalent cross-linking, e. g. applying carbodiimide chemistry. If a cationic polymer like chitosan is chosen, polyelectrolytic hydrogels which means symplexes of two differently charged polymers are formed (Fig. 4). For a biomimetic approach, we incorporated successfully fibrillar collagen type I as the main component of most mammalian ECMs, but only minor amounts could be used without disturbing the ionotropic gelation process. Also addition of gelatine (thermally denaturised collagen) is possible but the mixture has than to be kept above 30°C to prevent untimely gelation of gelatine.

The most stable (concerning degradation under cell culture conditions) polyelectrolytic hydrogel was found while adding chitosan which additionally facilitated mineralisation by immersion in simulated body fluids (SBF).

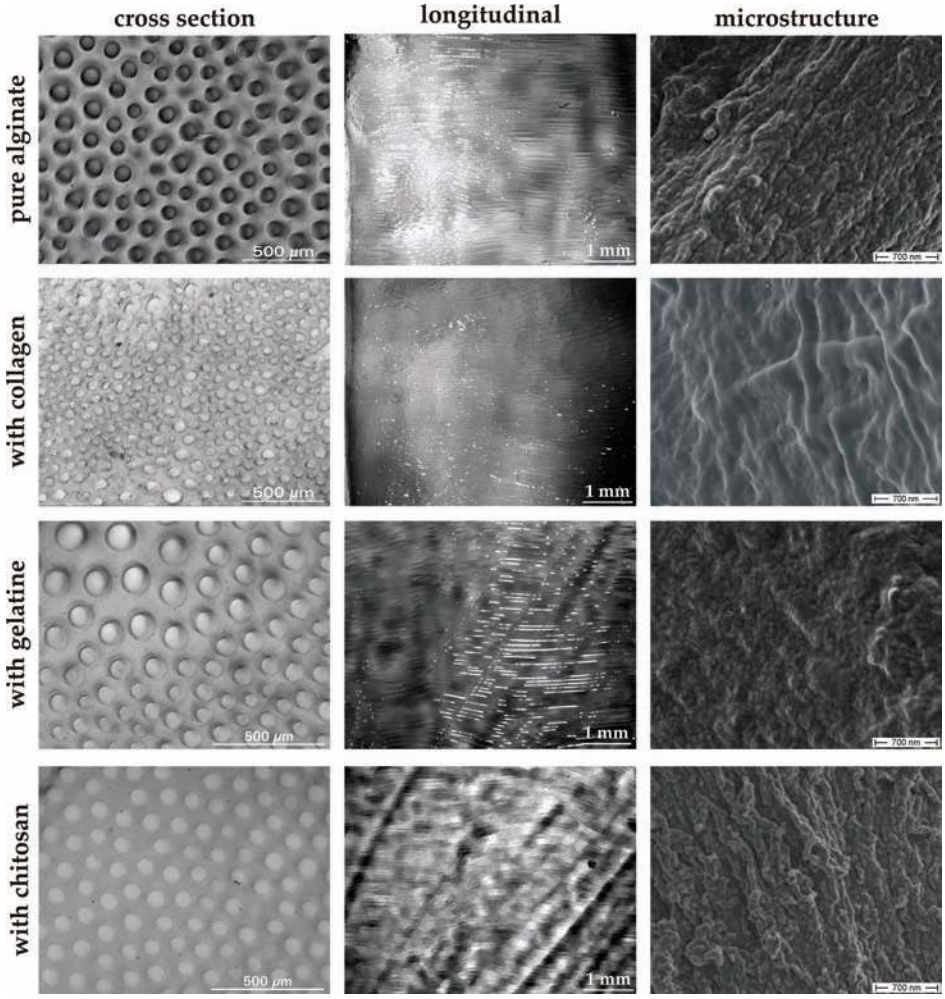


Fig. 4. Polyelectrolytic hydrogels of the negatively charged alginate and positively charged biopolymers (microstructure after air drying)

4.4 Anisotropic composites

Heading for regeneration of hard tissue, the mineral phase of bone, the calcium phosphate hydroxyapatite (HAP), should be incorporated. Alginate-HAP-composites with parallel aligned pores can be achieved following different strategies (Fig. 5), either by mineralisation of the hydrogels after gelation or directly during the sol-gel-process. Possible routes are:

- Immersion of the structured gel in simulated body fluid (SBF) and heterogeneous precipitation of HAP,
- Ion waves, i.e. diffusion of ions (alternating calcium and phosphate ions) in broad front into the hydrogel in some runs creating initially brushit which can be transformed into HAP (Thiele & Awad, 1969),

- Synchronous mineralisation, i.e. precipitation of calcium phosphate during the sol-gel-process (Despang et al., 2005),
- HAP powder, i.e. addition of HAP powder to the alginate sol and structuring of this slurry *via* ionotropic gelation (Despang et al., 2005; Dittrich et al., 2006; Dittrich et al., 2007; Bernhardt et al., 2009),
- Biphasic but monolithic scaffolds for the therapy of osteochondral defects can be produced through deposition of sol layers differing in composition prior to the gelation (Gelinsky et al., 2007).

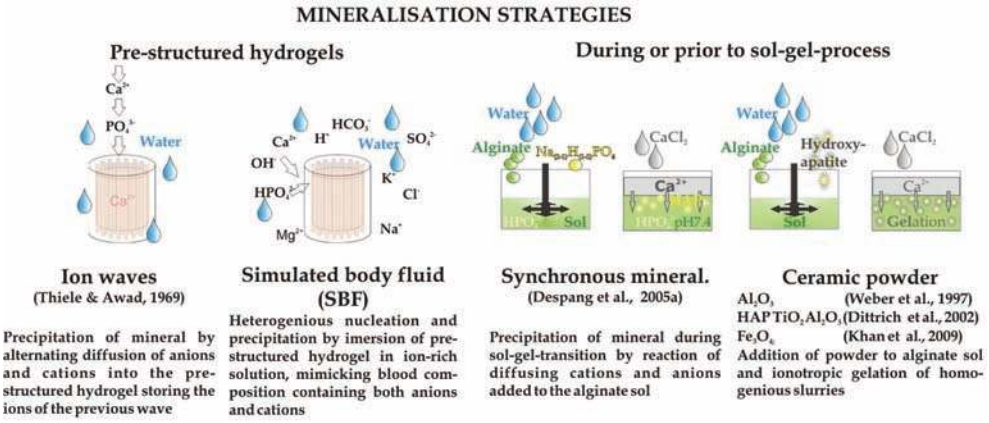


Fig. 5. Strategies of mineralisation – which also can be used in combination

The mineral content of the composites, which was determined by ignition loss, varied between the methods. A dried hydrogel, obtained from a 2% alginate sol without any calcium phosphate phase exhibits approx. 11% of ash due to the gelling ions (Ca^{2+}) and reaction productes ($CaCO_3$ or CaO) during combustion (Despang et al., 2005). 5-9% more mineral content was found for composites which were mineralised simultaneously during the ionotropic gelation. In this case, the Ca^{2+} ions not only orientated the alginate chains but also reacted with the phosphate ions which had been added to the alginate sol before the sol-gel-transition was initiated. Immersion in SBF increased the mineral content up to 11%. Higher contents, mimicking the inorganic-to-organic-ratio of bone (aprox. 70:30), and even more could only be realised by mixing HAP powder to the alginate sol. Thiele reached 50% i.e. a little less than the ratio of bone ECM and each wave led to shrinkage of the structure and therefore the pore diameter decreased (Thiele & Awad, 1969). Interestingly, the place of mineralisation, either intracapillar or in the pore walls, could be adjusted by the processing conditions as well as the shape of the precipitate was changed from round to needle-like by addition of citrate. The different approaches of mineralisation are expressed in varying microstructures (Fig. 6) and change the mechanical properties of the composite materials. The high amount of HAP introduced by addition of ceramic powder results in improved strength compared to the synchronously mineralised composites which was evaluated in wet state (Despang et al., 2005; Bernhardt et al., 2009).

All changes in composition of the sol or slurry prior to ionotropic gelation will influence the pore formation (diameter, length, density) during the sol-gel-process (Dittrich et al., 2007). However, the gel or composite with parallel aligned pores can be influenced after gelation

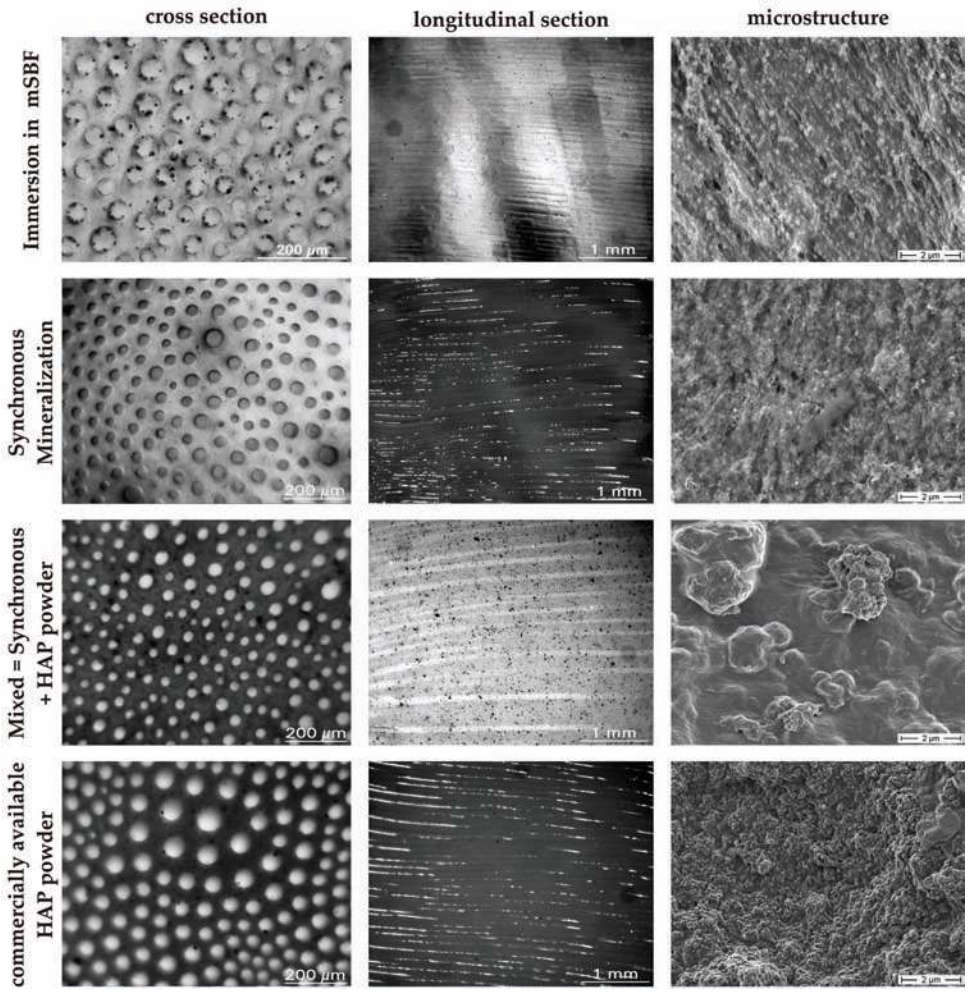


Fig. 6. Composite materials of biopolymer and mineral

by exposure to organic solvents benefiting of the different swelling behaviour. Other strategies are exchange of the gelling ions or different drying procedures. Freeze, air and supercritical drying were studied when the interest on ceramic membranes aroused (Weber et al., 1997) and was further optimised (Dittrich et al., 2002; Eljaouhari et al., 2006). Investigations by micro computer tomography (µCT) revealed that the pore structure was destroyed by ice crystals during freeze drying whereas the structure remained intact when water was exchanged against tert. butanol. Following the run of pore channels, this non destructive method also unveiled that pore channels can merge with distance from the primary membrane (Dittrich et al., 2007).

A mineral gradient in the direction of the long axis of the pore channels can be obtained by carefully covering layers of alginate sol on top of each other which differ in composition (Gelinsky et al., 2007). Bi-phasic but monolithic scaffolds consisting of a hydrogel-part and a

mineralised part were under current investigations for regeneration of osteochondral defect. Both parts contain additional components of the respective ECM, i.e. hyaluronic acid for articular cartilage and hydroxyapatite for bone. Furthermore, living chondrocytes were successfully embedded into the cartilage portion and stayed alive within 2 weeks of *in vitro* culture. Incorporating living cells into the process of ionotropic gelation demands of course work under sterile conditions and with sterile components during all process steps.
For medical applications, scaffolds need to be sterile but alginate (like other biopolymers) is affected by all common sterilisation methods (Despang et al., 2008b). Since tissue engineering comprises the degradation of the scaffold after implantation, the sterilisation method enables to adapt this kinetic to the tissue or application of interest. For use in hard tissue regeneration, the type of calcium phosphate powder incorporated in the composites de- or accelerates the degradation because HAP and tricalcium phosphate (TCP) possess different solubility (Dittrich et al., 2006). *In vitro* studies of the degradation kinetics should be carried out under conditions as close as possible to those *in vivo*, i.e. in the incubator at 37°C/5%CO_2 and in cell culture medium (Bernhardt et al., 2009). Also the mechanical stability over time is differently affected by cell culture medium compared to water or PBS (Dittrich et al., 2007).
The biocompatibility of alginate-gelatine-HAP composites with a pore diameter of approx. 90 µm was evaluated by human mesenchymal stem cells (hMSC) which were osteogenically induced. The seeding efficiency was 10-34% and cell number increased by a factor of 4-7 within 4 weeks (Dittrich et al., 2006; Bernhardt et al., 2009). Osteogenic differentiation was confirmed by reverse transcriptase-PCR by gene expression of ALP and BSPII which were not present at day 1 but were found clearly at day 21 (Bernhardt et al., 2009). A clear difference between osteogenically induced and non-induced cells was observed, too. Cells adhere at the face surface but were also found inside the channel-like pores visualised by confocal laser scanning microscopy (Bernhardt et al., 2009).

4.5 Anisotropic ceramics

Two observations paved the road for the synthesis of anisotropic inorganic materials. First of all, the channel-like structure was conserved in the ash after burning the organic part of mineralised alginate which was intended to determine the mineral content (Thiele, 1967c). Additionally, impurities or additives are not segregated like in the case of crystallisation but incorporated into the hydrogel (Thiele, 1964). Only Weber et al. (1997) described the synthesis of ceramic membranes based on structuring via sol-gel-process of ionotropic gelation of alginate/powder-slurries followed by calcination.
Ceramic processing for membrane manufacturing was studied with Al_2O_3 or TiO_2 including development of adapted drying regimes for the wet composites applying method inherent shrinkage, followed by heat treatment to obtain a sintered ceramic without cracks (Weber et al., 1997; Dittrich et al., 2002; Eljaouhari et al., 2006). Dittrich et al. (2002) for the first time synthesised such ceramics consisting of the mineral phase of bone, hydroxyapatite, with parallel aligned pores and investigated their structure by µCT in cooperation with Goebbels et al. (2002). The pore size and wall thickness was adjusted by the ratio of alginate-to-HAP powder (Dittrich et al., 2002).
Anisotropic Al_2O_3 ceramics with a pore diameter of 19 µm (Dittrich et al., 2002), approximately 70 µm (Eljaouhari et al., 2006) or even 250-320 µm (Weber et al. 1997) were manufactured. For TiO_2 ceramics a range of 10-30 µm was reported (Goebbels et al. 2002).

Lower sintering temperatures result in less shrinkage and larger pore diameters (Dittrich et al., 2002). For tissue engineering of bone, pores in the range of 100-300 µm are demanded. Additionally, HAP which was exposed to temperatures of more than ca. 1000°C is no longer resorbable *in vivo* by osteoclasts (the bone degrading cells). Therefore heat treatment at a lower temperature is required to achieve biodegradable implant materials. Sintering is performed for consolidating ceramic materials. A first HAP ceramic without organic components can be derived as intermediate state after bisquit firing, leading to a material called brown body which normally is consolidated in a further sintering step (Fig. 7).

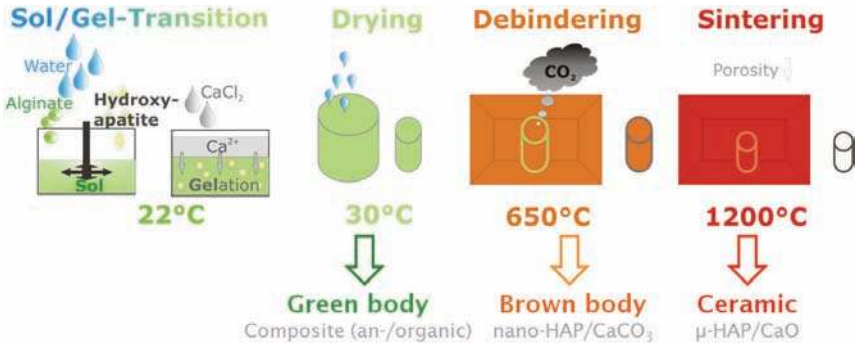


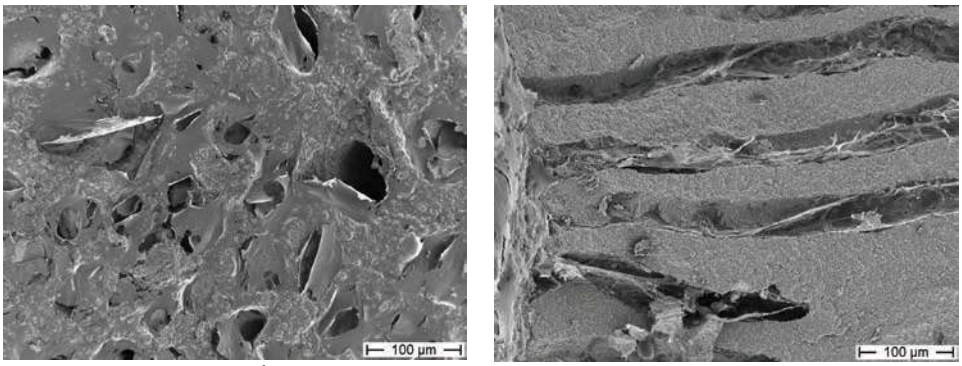
Fig. 7. Sketch of ceramic processing using sol-gel-technique of ionotropic gelation of alginate slurries with ceramic powders

Mechanical tests of HAP brown bodies with parallel aligned pores revealed a compressive strength of 4.5 MPa. This value is quite comparable to that of cancellous bone of human origin (5.9 MPa) tested at the same instrument under similar conditions (Rauh et al., 2009). The brown body exhibited a crystallite size of 41 nm compared to 238 nm of the sintered ceramic after treatment at 1200°C (Despang et al., 2008). Biocompatibility was evaluated by proliferation and differentiation of human mesenchymal stem cells (hMSC). The typical maximum of the specific alkaline phosphatase (ALP) activity was observed at day 14 after seeding. Cell number increase by a factor of 2.5 within 3 weeks for osteogenically induced hMSC cultivated on brown body samples as well as on sintered ceramics (Despang et al., 2008). Osteogenically induced hMSC adhered at the face surface as well as inside the channel-like pores and grew to a confluent layer (Fig. 8).

The pore diameter (40-165 µm) was larger for the brown body than for the sintered ceramics with approximately 30-115 µm (Fig. 9), depending on the type of alginate used for structure formation. The pore density varied between 20-90 pores/mm² for the brown bodies and 50-100 pores/mm² for the sintered ceramics. Largest samples prepared were 11x8 mm (ØxH).

Since alginate is gelled by many cations, anisotropic ceramics could also be synthesised by different complexing metal ions. Within the non-toxic elements, Zn^{2+} generates hydrogels with pores larger than those derived from Ca^{2+} (Thiele, 1967b). The more the cations orient the alginates molecules, the smaller the pore diameter of the gelled structure which also leads to an increase of the mechanical strength (Thiele & Hallich, 1957). But this does not primarily apply for the ceramics because the fibrous organic part was burnt. Even so using Zn^{2+} for gelling alginate-HAP-slurries led to larger pore channels than Ca^{2+} (Fig. 10), but the biocompatibility was poor i.e. no cell proliferation was observed within 4 weeks on composite material – *in vitro* studies on HAP bioceramics based on Zn-alginate-HAP-slurry still need to be accomplished.

Beside the actual pore size, the specific surface is an important parameter for scaffolds in tissue engineering by regulating protein and growth factor adsorption. The specific surface (BET) was measured for all states of the ceramic processing including the starting HAP powder, green body (composite of alginate and HAP after drying), brown body (thermal removal of organic phase) and consolidated ceramic (Fig. 11). Highest value is reached for the initial powder whereas in the composite material, the alginate is occupying some space and therefore the specific surface decreased. During heat treatment, the organic phase was removed and the consolidation started by sintering. Nano-sized pores of the walls were filled during sintering but the macro-porosity as relevant parameter for cell ingrowth remained unaffected.



Face surface Longitudinal section

Fig. 8. Osteogenically induced hMSC after 14 days of *in vitro* cultivation on nano-crystalline HAP scaffolds in the state as brown body (Ca^{2+} gelled slurry) – SEM (200x) after supercritical drying

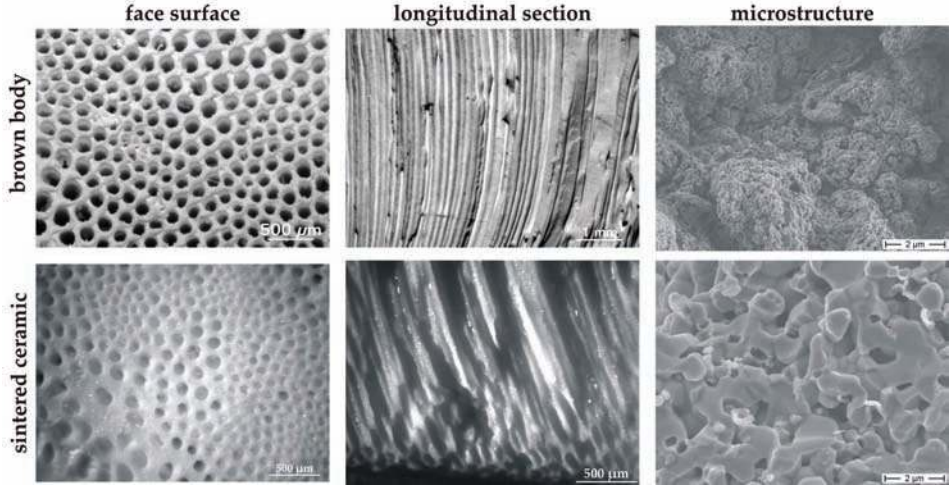


Fig. 9. Hydroxyapatite bioceramic based on Ca-alginate-HAP-slurry (top: state after thermal treatment at 650°C) with channel-like pores (bottom: sintered ceramic)

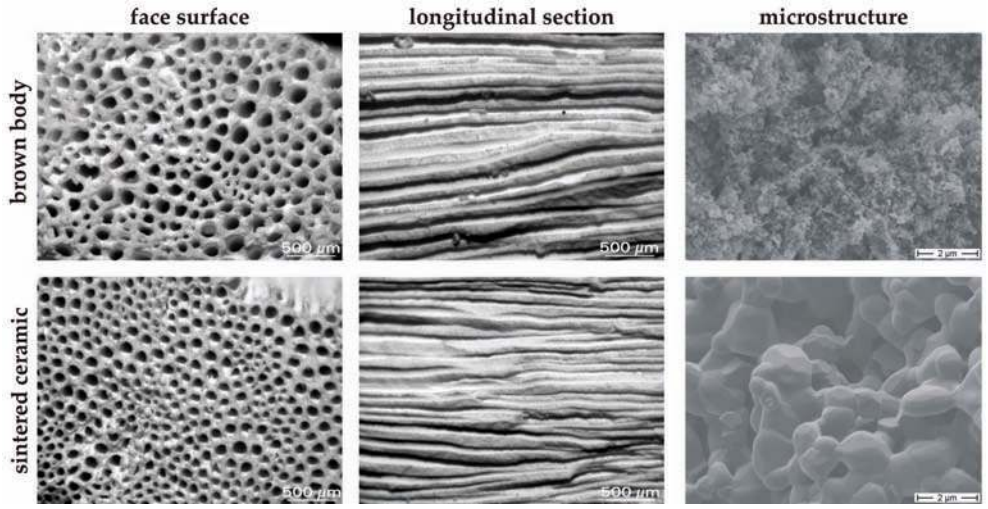


Fig. 10. Ceramics based on Zn-alginate-HAP-slurries (top: brown body, bottom: sintered)

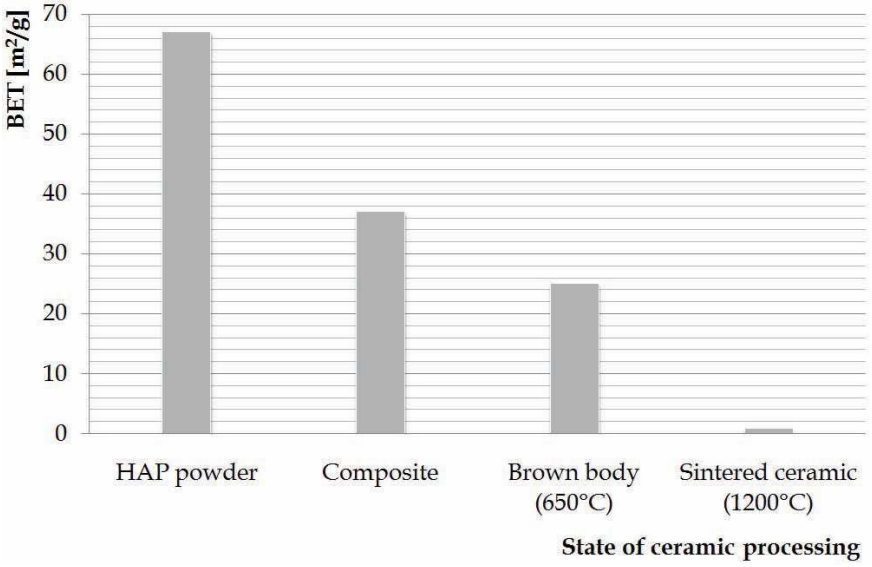


Fig. 11. Change of the specific surface (BET) during ceramic processing through thermal loss of alginate and due to sintering effects

5. Similarities between natural tissues and materials, generated by ionotropic gelation of alginate

In section 3, the highly hierarchical organisation of bone tissue has been described. Many attempts has been undertaken up to now to develop biomimetic materials which resemble

the nanocomposite, consisting of collagen type I and nanoscopical HAP crystals and therefore bone ECM at the smallest length scale (Gelinsky & Heinemann, 2010). In contrast, only a few methods are known which opens the possibility to mimic organisation and structure of (compact) bone at the micrometre scale. By applying directed ionotropic gelation of alginate, osteon-like patterns can be achieved. Addition of collagen (or gelatine) and nanoscopical HAP adapts the chemical composition to that of bone ECM and leads also to mechanical strengthening. Finally, also vital cells can be incorporated during the gelation process, leading to a model for living bone tissue which can be utilised for regenerative therapies or research purposes. Figure 12 demonstrates the several levels of hierarchy which can be realised and controlled during ionotropic gelation.

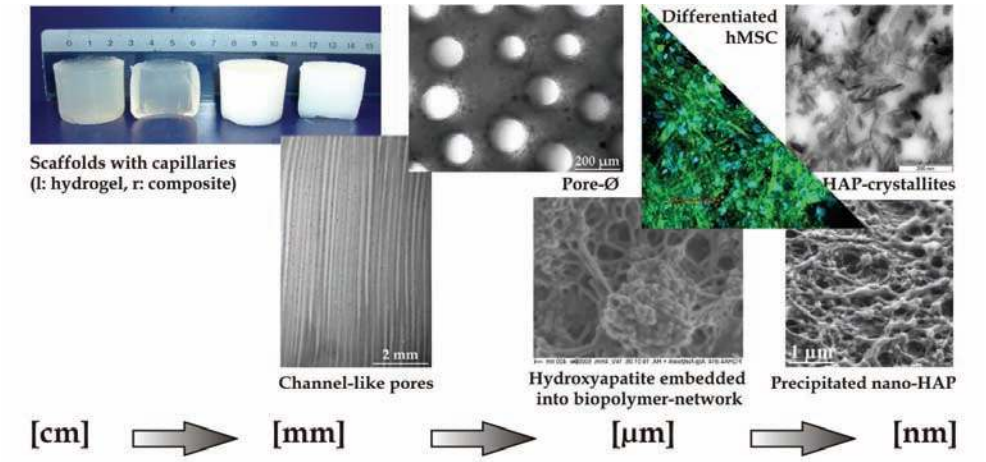


Fig. 12. Example for hierarchically structured composite material synthesised by ionotropic gelation of alginate-gelatine-HAP-slurry; compare with Fig. 2 (hierarchical organisation of cortical bone)

6. Conclusion

With the method of directed ionotropic gelation of alginate-based sols, a variety of materials can be synthesised, mimicking the highly anisotropic properties of natural tissues like bone. By controlling the composition of the starting materials and the reaction conditions, the properties of the product can be adjusted in a wide range. Pure alginate or mixtures of alginate with other biopolymers lead after gelation to elastic hydrogels. By addition of ceramic phases prior to the gelation or mineralisation of the hydrogels afterwards, composites can be achieved with which the composition of bone ECM and the patterns of cortical bone in the micrometre scale nicely can be mimicked.

When applying thermal treatments, the alginate phase is burnt out and pure inorganic materials remain which preserve the fascinating channel-like and aligned pores. Combinations of the calcium phosphate phase HAP and alginate resulted in astonishingly stable brown bodies after alginate burn-out at 650°C. These materials show similar compressive strength than trabecular human bone but are still degradable after implantation

due to the small particle size. Further heating to sintering temperatures of 1200°C leads to stable ceramics with higher mechanical strength. But during the sintering step, the HAP grains grow, making the product hardly degradable for osteoclasts (and therefore *in vivo*).
Fig. 13 gives an overview about the four main types of materials which can be derived from the process of ionotropic gelation. An increasing number of publications on this topic demonstrates that the phenomenon is still intensively under investigation. Furthermore, more and new fields of application, especially in the biomedical field, have been opened up in the last ten years, including embedding of living human cells during the gelation process. More and astonishing discoveries will follow.

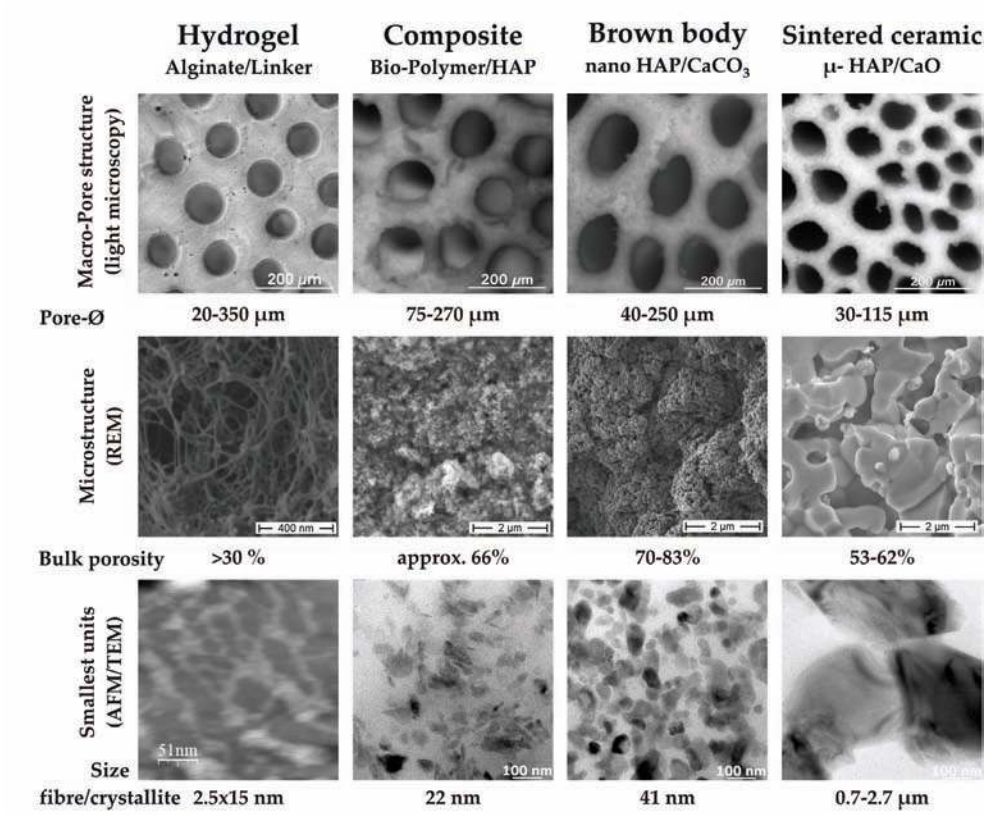


Fig. 13. Overview of anisotropic scaffolds, prepared using the process of ionotropic gelation, consisting of different materials

7. Acknowlegdement

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8. References

Awad, A.; El-Cheikh, F. & Shaker, A. (1980). Kinetic studies of cobalt alginate gels. *Colloid and Polymer Science,* 258, 1244-1249

Bernhardt, A.; Despang, F.; Lode, A.; Demmler, A.; Hanke, Th. & M. Gelinsky (2009). Proliferation and osteogenic differentiation of human bone marrow stromal cells on alginate-gelatine-hydroxyapatite scaffolds with anisotropic pore structure. *Journal of Tissue Engineering and Regenerative Medicine,* 3, 54-62

Braccini, I.; Grasso, R. P. & Perez, S. (1999). Conformational and configurational features of acidic polysaccharides and their interactions with calcium ions: a molecular modeling investigation. *Carbohydrate Research,* 317, 119-130

Braccini, I. & Perez, S. (2001). Molecular basis of Ca^{2+}-induced gelation in alginates and pectins: the egg-box model revisited. *Biomacromolecules,* 2, 1089-1099.

Despang, F.; Börner, A.; Dittrich, R.; Tomandl, G.; Pompe, W. & Gelinsky, M. (2005a). Alginate/calcium phosphate scaffolds with oriented, tube-like pores. *Materialwissenschaft und Werkstofftechnik,* 36, 761-767

Despang, F.; Bernhardt, R.; Mund, E.; Dittrich, R.; Beckmann, F.; Tomandl, G.; Pompe, W. & Gelinsky, M. (2005b). Investigations of alginate/calcium phosphate scaffolds with oriented tube-like pores by Microcomputed tomography. *Annual Report 2005 of HASYLAB DESY,* 1, 409-410

Despang, F.; Dittrich, R.; Bernhardt, A.; Hanke, Th.; Tomandl, G. & Gelinsky, M. (2008a). Channel-Like Pores in Hap-Containing Scaffolds for Bone Engineering: Hydrogels vs. Ceramics. *Tissue Engineering Part A,* 14, 819

Despang, F; Demmler, A.; Bernhardt, A.; Hanke, Th.; Dittrich, R. & Gelinsky, M. (2008b). Evaluation of sterilisation methods for alginate/hydroxyapatite scaffolds for hard tissue engineering. Proceedings of the 8th World Biomaterials Congress, Amsterdam (The Netherlands), 28.5-1.6.2008

Dittrich, R.; Tomandl, G. & Mangler, M. (2002). Preparation of Al_2O_3, TiO_2 and Hydroxyapatite Ceramics with Pores Similar to a Honeycomb Structure. *Advanced Engineering Materials,* 4, 487-490

Dittrich, R.; Despang, F.; Bernhardt, A.; Mannschatz, A.; Hanke, Th.; Tomandl, G.; Pompe, W. & Gelinsky, M. (2006). Mineralized Scaffolds for hard tissue engineering by ionotropic gelation of alginate, In: Vincenzini, P. *(ed.) Advances in Science and Technology,* Vol. 49, 159-164, trans tech publications inc.

Dittrich, R.; Despang, F.; Bernhardt, A.; Hanke, Th.; Tomandl, G.; Pompe, W. & Gelinsky, M. (2007). Scaffolds for hard tissue engineering by ionotropic gelation of alginate - influence of selected preparation parameters. *Journal of the American Ceramic Society,* 90, 1703-1708

Donati, I.; Holtan, S.; Morch, Y. A.; Borgogna, M.; Dentini, M. & Skjaek-Break, G. (2005). New Hypothesis on the Role of Alternating Sequences in Calcium-Alginate Gels. *Biomacromolecules,* 6, 1031–1040

El-Cheikh, F. & Awad, A. (1976). The electric conductance of some metal alginates. *Colloid and Polymer Science,* 254, 120-120

Eljaouhari, A.; Müller, R.; Kellermeier, M.; Heckmann, K. & Kunz W. (2006). New Anisotropic Ceramic Membranes from Chemically Fixed Dissipative Structures. *Langmuir,* 22, 11353-11359

Gelinsky, M.; Eckert, M. & Despang F. (2007). Biphasic, but monolithic scaffolds for the therapy of osteochondral defects. *International Journal of Materials Research,* 98, 749-755

Gelinsky, M. & Heinemann, S. (2010). Nanocomposites for tissue engineering, In: *Nanocomposites for Life Sciences*, Kumar, Ch. (ed.), 405-434. Wiley-VCH, ISBN 978-3-527-32168-1, Weinheim (Germany)

Goebbels, J.; Weidemann, G.; Dittrich, R.; Mangler, M. & Tomandl, G. (2002). Functionally graded porosity in ceramics – Analysis with high resolution computed tomography, In: *Innovative processing and synthesis of ceramics, glasses and composites V*, Singh, J. & Bansal, N. *(ed.),* Ceramic Transactions Series 129, 113-124, Wiley-VCH

Grant, G. T.; Morris, E. R.; Rees, D.A.; Smith, P. J. C. & Thom, D. (1973). Biological interactions between polysaccharides and divalent cations: The egg-box model. *FEBS Letters,* 32, 195-198

Hassan, R.; Summan, A.; Hassan, M. & El-Shatoury, S. (1989). Kinetics and mechanism of sol-gel transformation on polyelectrolytes of some transition metal ions, especially cobalt alginate ionotropic membranes. *European Polymer Journal,* 25, 25, 1209-1212

Hassan, R. M. (1991). Alginate polyelectrolyte ionotropic gels. Part III: Kinetics of exchange of chelated divalent transition metal ions especially cobalt(II) and copper(II) by hydrogen ions in capillary ionotropic metal alginate polymembrane gels. *Journal of Materials Science,* 26, 5806-5810

Hassan, R. M.; Awad, A. & Hassan, A. (1991). Seperation of metal alginate ionotropic gels to polymembranes with special evidence on the position of chelation in copper alginate complex. *Journal of Polymer Science Part A: Polymer Chemistry,* 29, 1645-1648

Hassan, R. M. (1993). Alginate polyelectrolyte ionotropic gels. *Journal of Materials Science,* 28, 384-388

Heinze, T.; Klemm, D.; Loth, F. & Philipp, B. (1990). Herstellung, Struktur und Anwendung von ionotropen Gelen aus carboxygruppenhaltigen Polysacchariden. Fortschrittsbericht Acta Polymerica, 41, 259-269

Higdon, W. T. (1958). Studies of Ionotropy: A Special Case of Gelation. *Journal of Physical Chemistry,* 62, 10, 1277-1281

Khan, F.; Walsh, D.; Patil, A. J.; Perriman, A. W. & Mann, S. (2009). Self-organized structural hierarchy in mixed polysaccharide sponges. *Soft Matter, 5*, 3081-3085

Khairou, K.; Al-Gethami, W. M. & Hassan, R. M. (2002). Kinetics and mechanism of sol-gel transformation between sodium alginate polyelectrolyte and some heavy divalent metal ions with formation of capillary structure polymembranes ionotropic gels. *Journal of Membrane Science,* 209, 445-456

Kohler, H. H. & Thumbs, J. (1995). Wo kommen die kapillaren im Alginatgel her? *Chemie Ingenieur Technik,* 67, 489-492

Ma, P. X. (2008). Biomimetic materials for tissue engineering. *Advanced Drug Delivery Reviews* 60, 2, 184-198

Moll, G. (1963). Über die löslichen Ultrafilter aus Aluminium- und Lanthan-Alginat und die elektronenmikroskopische Demonstration ihrer Filtrationseigenschaften. *Zeitschrift für Hygiene,* 149, 297-314

Müller, R.; Eljaouhari, A.; Prang, P.; Weidner, N. (2006). Preparation of alginate-based anisotropic capillary hydrogels for the promotion of oriented axonal re-growth in the injured spinal cord. *Cytotherapy,* 8, 55

Müller, R.; Pawar, K.; Weidner, N. (2009a). Alginate-based Anisotropic Capillary Hydrogels as Scaffolds for Guided Axon Regeneration. *Tissue Engineering Part A,* 15, 717-718

Müller, R.; Ferreira, I.; Pawar, K. & Weidner, N. (2009b) Application of Hydrogels to Rewire the Injured Spinal Cord, In *Handbook of Hydrogels: Properties, Preparation & Applications,* Stein, D. B. (Ed.), 397-426, NOVA Publishers, Hauppauge, NY

Prang, P.; Müller, R.; Eljaouhari, A.; Heckmann, K.; Kunz, W.; Weber, T.; Faber, C.; Vroemen, M.; Bogdahn, U. & Weidner, N. (2006). The promotion of oriented axonal regrowth in the injured spinal cord by alginate-based anisotropic capillary hydrogels. *Biomaterials,* 27, 3560-3569

Purz, H. J. (1972). Morphological investigations of ordered gel formation in polyelectrolytes. *Journal of Polymer Science Part C: Polymer Symposia,* 38, 405-417

Purz, H. J.; Tiersch, B. & Philipp, B. (1985). Zur Morphologie ionotroper Gele aus anionischen Cellulosederivaten. *Acta Polymerica,* 36, 569-574

Rauh, J.; Despang, F.; Gelinsky, M.; Pruss, A.; Günther, K.-P. & Stiehler, M. (2009) Einfluss von Peressigsäure-Ethanol Sterilisation auf die biomechanischen Eigenschaften humaner spongiöser Knochentransplantate" Proceedings of the *Deutscher Kongress für Orthopädie und Unfallchirurgie.* Berlin, 21.-24. 10. 2009, *DocPO17-23,* German Medical Science GMS Publishing House

Renzo, F. D.; Valentin, R.; Boissiére, M.; Tourrette, A.; Sparapano, G.; Molvinger, K.; Devoisselle, J.-M.; Gérardin, C. & Quignard, F. (2005). Hierarchical Macroporosity Induced by Constrained Syneresis in Core-Shell Polysaccharide Composites. *Chemistry of Materials,* 17, 4693-4699

Rho, J.Y.; Kuhn-Spearing, L. & Zioupos, P. (1998). Mechanical properties and the hierarchical structure of bone. *Medical Engineering and Physics* 20, 92-102

Schuur, G. (1955). Bemerkungen zu den Untersuchungen Ionotropen Gelen von Polyuronsäuren von H. Thiele und G. Andersen. *Colloid and Polymer Science,* 143, 31-32

Thiele, H. (1947). Richtwirkung von Ionen auf anisotrope Kolloide – Ionotropie. *Naturwissenschaften,* 34, 123

Thiele, H. & Micke, H. (1948). Über Strukturen in ionotropen Gelen. *Colloid and Polymer Science,* 111, 73-79

Thiele, H. & Kienast, G. (1952). Einfluß von Ionen auf die Struktur bei der Gelbildung. *Colloid and Polymer Science,* 127, 134-144

Thiele, H. & Andersen, G. (1953). Modellversuch zum Wachstum der Knochensubstanz. *Naturwissenschaften,* 40, 366-366

Thiele, H. (1954a). Modellversuche zur Synthese mizellarer Strukturen. *Colloid and Polymer Science,* 136, 80-84

Thiele, H. (1954b). Ordered coagulation and gel formation. *Discussions of the Faraday Society,* 18, 294-301

Thiele, H. & Andersen, G. (1955a). Ionotrope Gele von Polyuronsäuren: I. Bildung und Verhalten. *Colloid and Polymer Science,* 140, 76-102

Thiele, H. & Andersen, G. (1955b). Ionotrope Gele von Polyuronsäuren: II Ordnungsgrad. *Colloid and Polymer Science,* 142, 5-24

Thiele, H. & Krönke, H. (1955). Geordnete Kristallisation in ionotropen Gelen. *Naturwissenschaften,* 42, 389-389

Thiele, H. & Hallich, K. (1957). Kapillarstrukturen in ionotropen Gelen. *Colloid and Polymer Science,* 151, 1-12

Thiele, H. & Hallich, K. (1959). Ionotrope Gele mit Kapillarstruktur als Filter. *Colloid and Polymer Science,* 163, 115-122

Thiele, H.; Plohnke, K.; Brandt, E. & Moll, G. (1962). Ordnen von Polyelektrolyten durch Ionendiffusion. *Colloid and Polymer Science,* 182, 24-35

Thiele, H. & Cordes, J. (1967). Zur Theorie der Bildung von Gelen. *Colloid and Polymer Science,* 216/217, 361-370

Thiele, H. (1964). Ordnen von Fadenmolekülen durch Ionendiffusion – ein Prinzip der Strukturbildung. *Protoplasma,* 58, 318-341

Thiele, H.; Joraschky, W.; Plohnke, K.; Wiechen, A.; Wolf, R. & Wollmer, A. (1964). Prinzip einer Strukturbildung Ionen ordnen Fadenmoleküle. *Colloid and Polymer Science,* 197, 26-35

Thiele, H. (1967a). Prinzip einer Strukturbildung. *Naturwissenschaften,* 54, 136-139

Thiele, H. (1967b). *Histolyse und Histogenese: Gewebe und ionotrope Gele – Prinzip einer Strukturbildung*. Akademische Verlagsgesellschaft

Thiele, H. (1967c). Geordnete Kristallisation. Nucleation und Mineralisation. *Journal of Biomedical Materials Research,* 1, 213-238

Thiele, H. & Awad, A. (1969). Nucleation and oriented crystallization apatite in ionotropic gels. *Journal of Biomedical Materials Research,* 3, 431-442

Thumbs, H. & Kohler, H. H. (1996). Capillaries in alginate gels as an example of dissipative structure formation. *Chemical Physics,* 208, 9-24

Treml, H. & Kohler, H. H. (2000). Coupling of diffusion and reaction in the process of capillary formation in alginate gel. *Chemical Physics,* 252, 199-208

Treml, H.; Woelki, S. & Kohler, H. H. (2003). Theory of capillary formation in alginate gels. *Chemical Physics,* 293, 341-353

Weber, K.; Tomandl, G.; Wenger, T. & Heckmann, K. (1997). Preparation of Structured Ceramics for Membrane. *Key Engineering Materials,* 132-136, 1754-1757

Weiner, S. & Wagner, H. D. (1998). The material bone: Structure-mechanical function relations. *Annual Review of Materials Science* 28, 271-298

Wenger, Th. (1998). Herstellung gerichtet-strukturierter Keramiken. Dissertation, University of Regensburg (Germany)

Willenberg, B. J.; Hamazaki, T.; Meng, F. W.; Terada, N. & Batich, C. (2006). Self-assembled copper-capillary alginate gel scaffolds with oligochitosan support embryonic stem cell growth. *Journal of Biomedical Materials Research Part A,* 79A, 440-450

Woelki, S. & Kohler, H. H. (2003). Orientation of chain molecules in ionotropic gels: a Brownian dynamics model. *Chemical Physics,* 293, 323-340

Yamamoto, M.; James, D.; Li, H.; Butler, J.; Rafii, S. & Rabbany, S. Y. (2010). Generation of Stable Co-cultures of Vascular Cells in a Honeycomb Alginate Scaffold. *Tissue Engineering Part A*, 16, 299-308

Zimmermann, H.; Ehrhart, F.; Zimmermann, D.; Müller, K.; Katsen-Globa, A.; Behringer, M.; Feilen, P.; Gessner, P.; Zimmermann, G.; Shirley, S.; Weber, M.; Metze, J. & Zimmermann, U. (2007). Hydrogel-based encapsulation of biological, functional tissue: fundamentals, technologies and applications. *Applied Physics A: Materials Science & Processing,* 89, 909-922