Biomaterials by freeze casting

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The functional requirements for synthetic tissue substitutes appear deceptively simple: they should provide a porous matrix with interconnecting porosity and surface properties that promote rapid tissue ingrowth; at the same time, they should possess sufficient stiffness, strength and toughness to prevent crushing under physiological loads until full integration and healing are reached. Despite extensive efforts and first encouraging results, current biomaterials for tissue regeneration tend to suffer common limitations: insufficient tissue–material interaction and an inherent lack of strength and toughness associated with porosity. The challenge persists to synthesize materials that mimic both structure and mechanical performance of the natural tissue and permit strong tissue–implant interfaces to be formed. In the case of bone substitute materials, for example, the goal is to engineer high-performance composites with effective properties that, similar to natural mineralized tissue, exceed by orders of magnitude the properties of its constituents. It is still difficult with current technology to emulate in synthetic biomaterials multi-level hierarchical composite structures that are thought to be the origin of the observed mechanical property amplification in biological materials. Freeze casting permits to manufacture such complex, hybrid materials through excellent control of structural and mechanical properties. As a processing technique for the manufacture of biomaterials, freeze casting therefore has great promise.

Keywords: tissue scaffolds; hierarchical structures; processing; porosity; mechanical properties; biomimetics

1. Introduction

Biomaterials are designed to replace injured or diseased tissue. Ideally, they are scaffolds for tissue regeneration with properties similar to those of the healthy natural tissue that they replace. Designed to cover a two-dimensional surface or to fill a three-dimensional void, they should, in parallel to healing, gradually be resorbed so that, ultimately, the site of injury becomes almost indistinguishable from the surrounding tissue (Muschler et al. 2004). To achieve these goals, the biomaterial must fulfil several design requirements: it has to possess a sufficiently large porosity (more than 40%) (Hutmacher 2000; Karageorgiou & Kaplan 2005); its surface chemistry and topography must be suited for cell adhesion, proliferation and differentiation; it needs to possess an appropriate

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hierarchical architecture to guide tissue regeneration; and it should allow for controlled resorption when the scaffold is no longer required. Additionally, the scaffold must, despite its high overall porosity that considerably weakens all mechanical properties, possess sufficient stiffness, strength and toughness to perform the natural tissue’s function while the wound is healing. The great diversity in body tissues also means that there is no one ideal tissue scaffold, but that each application requires a specific structure with a well-defined mechanical response. The design problem is thus to find, for each application, the optimal compromise between the biomaterial’s architecture and the material’s mechanical performance.

2. Structure and properties of biological materials versus biomaterials

Natural tissues are biological materials that differ considerably from biomaterials, their synthetic counterparts. They are frequently composites with a cellular structure, created by cells that initially produce and later adapt and remodel the material’s architecture (Wegst & Ashby 2004). The pores of biological materials can vary greatly in size and form and are usually highly interconnected; they form networks that permit the flow of liquids and gases and aid the migration of cells. The porosity provides the living tissue with an infrastructure for the transport of nutrients, oxygen and waste, enabling individual cells to survive, and thus a tissue to grow, adapt and regenerate during its entire lifetime and after injury. Mechanically, the resulting complex architecture and often high overall porosity are important prerequisites for the function, efficiency and multi-functionality of biological materials. Thus, if we wish to create biomaterials that promote healing and serve functions similar to those of the natural tissue, and if we intend the biomaterial to be fully integrated or even resorbed, we need to create substances that emulate the properties of the natural tissue’s overall porosity and pore structure as closely as possible, as this is expected to help sustain cells beyond a scaffold depth of approximately 250 μm, which is the current state of the art (Muschler et al. 2004).

Current tissue scaffolds not only differ structurally from biological materials, but they are also mechanically and chemically very different from the natural tissue for which they substitute. Figure 1, a material property chart plotting fracture toughness against Young’s modulus for natural mineralized tissues and biomaterials, allows us to compare mechanical properties and performance. Metals such as steel and titanium (red) both have a Young’s modulus and a toughness significantly higher than those of natural bone. Fully dense ceramics such as zirconia and bioglass (blue) have a toughness similar to that of natural bone (yellow), but too high a Young’s modulus. The components (green) of mineralized biological materials, such as hydroxyapatite (e.g. in bone, dentin, enamel and antler) and calcium carbonate in the form of calcite or aragonite (e.g. in nacre), have, as bulk materials, a higher Young’s modulus, but a significantly lower toughness than both compact bone and engineering ceramics.

Additionally, synthetic bone substitute materials are frequently monolithic. In contrast, biological ceramics, such as bone, dentin, enamel, antler and nacre (yellow and light blue), achieve their unique property combination and an amplification of their components’ mechanical properties owing to their composite
structure and architecture. The stiffness of the composite derives from the mineral nano- and microparticles, the fracture resistance from a brick-and-mortar-like microstructure in which the polymeric mortar phase transfers loads and absorbs energy (Gao et al. 2003; Fratzl & Weinkamer 2007).

Bone, in which collagen fibrils are mineralized with hydroxyapatite (45 wt% in mature bone (Taylor 2003)), is also an excellent example of a porous biological material. It is the introduction of porosity that further allows tailoring of otherwise single-value physical and mechanical properties. Figure 1 illustrates that the properties of dense cortical or compact bone are almost two magnitudes higher than those of highly porous cancellous (trabecular or spongy) bone that fills the medullary cavities of the femur and skull. It is thus the microstructure of a porous composite material that allows bone to fulfil two particularly important, but contradictory, design requirements: to provide a large overall porosity and sufficiently high mechanical properties such as stiffness, strength and toughness.

A promising route for the synthesis of bone substitute materials thus emerges: to mimic the hierarchical composite structure and porosity of the natural tissue because this approach will allow to custom design material properties at several structural length scales so that the biomaterial can simultaneously and optimally fulfil biological and mechanical requirements.

3. Porous materials

In a first-order approximation, two types of porous materials can be distinguished: foams and honeycombs. Foams consist of spherical or equiaxed pores and have isotropic properties, whereas honeycombs consist of highly aligned, prismatic pores that result in anisotropic properties. The main difference between these two porous materials is that the deformation of foams is bending-dominated, which means that they exhibit a post-yield stress–strain curve with a long, flat plateau, whereas that of honeycombs is stretch-dominated, showing initial yield followed by brittle collapse or plastic buckling (Gibson & Ashby 1999; Gibson 2005; Ashby 2006). For a given relative density of the material \( \rho/\rho_s \), where \( \rho \) is the density of the cellular material divided by the density \( \rho_s \) of the solid material from which it is made, both the elastic modulus and the initial collapse strength are significantly greater in a stretch-dominated honeycomb structure than those in a bending-dominated foam. This is why honeycombs are particularly efficient for structural applications in which a high overall porosity is desirable and anisotropy is acceptable, as is the case in biomaterials.

The three most important factors that influence the properties of a cellular scaffold material are: (i) the properties of the solid from which the scaffold is made, (ii) the cell geometry, and (iii) the relative density \( \rho/\rho_s \) of the scaffold (Gibson & Ashby 1999; Gibson 2005; Ashby 2006). For foam-like structures in which the pores can be considered equiaxed, Young’s modulus \( E \) typically scales with Young’s modulus \( E_s \) of the solid from which it is made as \( E \propto E_s(\rho/\rho_s)^2 \), and its compressive strength \( \sigma \) typically scales as \( \sigma \propto \sigma_s(\rho/\rho_s)^{3/2} \), with \( \sigma_s \) being the yield strength (if the cell-wall material is ductile) or the modulus of rupture (if the cell-wall material is brittle) of the solid from which it is made. The fracture toughness \( K_{IC} \) of a cellular scaffold is more difficult to model, but typically scales as \( K_{IC} \propto \sigma_s(\rho/\rho_s)^{3/2} \) with the failure strength of the cell-wall material.
Figure 1. A material property chart showing fracture toughness plotted against Young's modulus for mineralized biological materials and biomaterials. Biomaterials currently used in bone repair (e.g. steel, titanium, zirconia and bioglass) have properties that greatly differ from those of natural bone (Wegst 2009).
realizing that even a seemingly simple property such as porosity cannot currently be described in a way that fully captures what a cell senses. Research attempting to correlate pore diameter, pore curvature, pore aspect ratio, pore surface area-to-volume ratio, connectivity, surface roughness, surface chemistry, which is interdependent with geometry, and cell responses to these are ongoing. Once established, such property correlations are expected to greatly aid tissue-scaffold development.

Biomaterials made by freeze casting, a process that takes advantage of the principle of directional solidification of a liquid vehicle such as water and is therefore also known as ‘ice-templating’, are ideally suited to contribute to a systematic study linking structural features to cell responses. They offer particular potential for such research because all classes of materials (polymers, ceramics, metals and composites) can be prepared by this process, and because many of the properties of the hierarchical structures produced can be varied in a carefully controlled fashion for one given material composition.

4. The process of freeze casting

Interest in the effect of freezing on solutions and suspensions dates back at least to the beginning of the twentieth century, when first qualitative observations were reported by, for example, Bobertag et al. (1908) and Lottermoser (1908). Lottermoser was probably among the first who described the formation of materials with honeycomb structures upon freezing. Bobertag et al. (1908) noted structural changes in the first frozen, then remelted material, which, they suggested, are due to the expanding ice crystals exerting forces on the matter, which, upon freezing, is expelled from the liquid carrier and trapped between the crystals. About 50 years later, Maxwell et al. (1954) described freeze casting as we understand it today. They reported the successful preparation of intricately shaped objects such as turbocharger blades from an extremely thick ceramic slip by casting, freezing and liquid sublimation before sintering.

Initially, freeze casting was identified by Maxwell et al. (1954), as a means to manufacture exceptionally dense ceramics with minimal shrinkage for technical applications. Three decades later, Tong et al. (1984) and Tong & Gryte (1985) applied the technique to a water-soluble polymer; they created, from agar gels, highly porous materials with a well-controlled cellular architecture and studied the effect of freezing velocity and diffusion conditions on pore geometry. More recently, the potential of freeze casting as a means to create ceramics with a controlled and complex cellular architecture has started to attract research attention (Fukasawa et al. 2001, 2002), and the first reports on freeze-cast biomedical materials prepared from collagen solutions have appeared (Schoof et al. 1998, 2000, 2001; von Heimburg et al. 2001; Kuberka et al. 2002). Freeze casting of ceramic-based biomaterials followed (Chow et al. 2001; Araki & Halloran 2005; Zhang et al. 2005; Deville et al. 2006a; Deville 2008; Fu et al. 2008a,b; Macchetta et al. 2009). Most recently, freeze casting of the first metal, titanium, has been reported (Chino & Dunand 2008; Yook et al. 2008, 2009; Fife et al. 2009). Current research focuses on both the fundamentals of the freeze-casting process, drawing on the well-established field of directional solidification of alloys, and the properties of the materials created (Deville 2008).
One reason for freeze casting having quickly gained popularity as a manufacturing route for biomaterials is that it is a comparatively straightforward, physical process, based on benign, biocompatible liquid carriers such as water. Another reason is that the structure of freeze-cast materials, such as their overall porosity, pore size and pore geometry, can easily be controlled across several length scales. Of equal importance is the ability to custom design both during and after freeze casting the scaffold’s cell-wall properties, such as surface roughness and chemistry, and with it the interface properties that are of critical importance to tissue–material interactions and a scaffold’s successful tissue integration. These structural features, combined with the remarkable mechanical properties that directionally solidified materials offer despite their high overall porosity, are the basis of the great promise of freeze-cast biomaterials.

Because all classes of materials can be processed, a great range of potential applications for freeze-cast biomaterials exists: collagen scaffolds, for example, are being explored for use in the peripheral nervous system and spinal-cord repair (Bozkurt et al. 2007; Moellers et al. 2009), in which excellent pore alignment, pores diameters of 10–50 μm and Young’s modulus of 1–10 kPa are required (Bilston & Thibault 1996; Ozawa et al. 2001); calcium phosphate (hydroxyapatite, tricalcium phosphate and composites of the two) scaffolds with pore diameters of 100–500 μm, Young’s modulus of 0.05–1 GPa and a strength of more than 50 MPa are investigated for bone substitution in low to medium load-bearing applications (Saiz et al. 2008).

Great potential for freeze-cast biomaterials also exists in applications in which porosity is not required. By filling the pores with a second phase, ceramic–polymer or ceramic–metal composites can be created that, similar to their biological counterparts (compact bone and nacre), have a toughness several magnitudes higher than that of their constituents (Munch et al. 2008). Freeze casting is further ideally suited for the manufacture of materials with property gradients. It is thus a process with great promise for the development of biomimetic biomaterials that emulate several design principles of the natural tissue that they are designed to replace.

5. Processing biomaterials by freeze casting

Freeze casting is a physical process. This is why all classes of materials and composites made from them can, in principle, be prepared by freezing polymer solutions, metal or ceramic particle suspensions or novel micro- and nanocomposites consisting of polymer, ceramic or metal fibres and particles in a polymer solution. While the majority of freeze-cast materials is still prepared using aqueous solutions or slurries, other liquid vehicles, such as camphene (Sofie & Dogan 2001; Araki & Halloran 2005; Shanti et al. 2006; Lee et al. 2007a,b; Yoon et al. 2007; Yook et al. 2008, 2009), camphene–naphthalene (Araki & Halloran 2004a) and tert-butyl alcohol (Chen et al. 2007), are also increasingly being used. Important in the choice of the liquid carrier are properties such as its crystal structure and type of growth during solidification and its interaction with the solids dissolved or suspended in it. This is to ensure that a homogeneous dispersion can be achieved from which a material with the desired homogeneous microstructure can be formed by precisely controlling the processing...
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parameters. Because the freeze casting of ceramic particle suspensions, slurries or slips by directional solidification is, to date, the most studied, we will concentrate on these in the following sections. The principles described are, however, equally applicable to metal particle suspensions and polymer solutions.

(a) Slurry properties

Ceramic slurries for freeze casting are typically composed of micro- or submicrometre-sized particles suspended in water to which dispersants (e.g. Darvan C, R.T. Vanderbilt Co, Norwalk, CT, USA) and binders (e.g. polyvinyl alcohol) are commonly added (Deville et al. 2006b). Dispersants, which can be anionic, cationic or neutral and which depend on the liquid carrier and particles suspended in it, reduce particle aggregation and flocculation (Fu et al. 2008b,c). Both are undesirable because they lead to heterogeneities in the final product and they change the slurry’s physical properties such as its sedimentation behaviour and viscosity.

For a given particle size, the viscosity depends on the type of interaction between particles and liquid carrier, whether by van der Waals forces, hydrogen bonds or polar interactions. For a given type of interaction, the viscosity depends on the particle size. In the case of van der Waals forces, for example, the forces grow with molecular weight and particle size; the higher the molecular weight or the larger the agglomerate, the higher the forces and the higher the viscosity. For a given particle size, an increase in solid-volume fraction has the same effect, because the particles are more closely packed, resulting in more and stronger particle–particle interactions increasing the resistance to flow. The viscosity of the slurry is a particularly important processing parameter in freeze-casting. It determines, for a given particle size, both the velocity of sedimentation and the critical freezing-front velocity at which particle trapping occurs during solidification. Viscosity is thus a structural parameter that determines the material’s structural and mechanical properties.

Binders are added to the slurry to increase the strength of the green ceramic bodies before sintering or to form composite materials for which further processing is not necessary.

(b) Sedimentation

Through the careful control of particle sedimentation, which depends on the particle’s density and size distribution and the liquid vehicle’s density and viscosity, it is possible either to avoid or to deliberately generate before and during freezing structural gradients within a sample. When a spherical particle of radius $r$ and mass $m$ is immersed in a fluid of density $\rho_L$, it will experience two forces, one due to the gravitational acceleration $g = 9.807 \, \text{m} \, \text{s}^{-2}$ and a force opposing it, which, according to Archimedes’ principle, is equal to the weight of the volume $V$ of the fluid that it displaces. The resulting force on the sphere is

$$F = (m - \rho_L V) g = (\rho_P - \rho_L) g \frac{4}{3} \pi r^3.$$

If the sphere’s density is higher or lower than that of the fluid in which it is immersed, it will, according to Stokes’s law, experience a frictional force resisting its motion. This resisting force depends on the dynamic viscosity $\eta$ of the liquid
vehicle and both the radius and the velocity \( v_P \) of the particle,

\[ F_I = 6\pi r\eta v_P. \]  

(5.2)

Equating expressions (5.1) and (5.2) and solving for the velocity \( v_P \), the particle’s terminal velocity of the sphere can be calculated,

\[ v_P = \frac{2(\rho_P - \rho_L)g r^2}{9\eta}. \]  

(5.3)

Thus, to ensure minimal sedimentation, the density difference between the sphere and liquid needs to be minimized, and, for a given material pairing and terminal velocity, the viscosity needs to increase quadratically with an increase in the particle diameter.

Stokes’s law neglects particle–particle interactions and only applies to laminar flow, a type of fluid flow in which adjacent layers do not mix, except on the molecular scale. The transition from laminar to turbulent flow occurs at a critical velocity

\[ v_{\text{trans}} = \frac{Re\eta}{2\rho_L r}. \]  

(5.4)

and Reynolds numbers \( Re < 0.2 \). Inserting \( Re = 0.2 \) into equation (5.4) and equating it with equation (5.3), the maximum particle radius for laminar flow can be calculated as

\[ r_{\text{max}} = \left(\frac{0.45\eta^2}{(\rho_P - \rho_L)\rho_L g}\right)^{1/3}. \]  

(5.5)

For a suspension of hydroxyapatite (\( \rho_P = 3150 \text{ kg m}^{-3} \)) spheres in 0°C water (\( \rho_L = 999 \text{ kg m}^{-3} \) and \( \eta = 1.793 \text{ mPa s} \)), the maximum radius for laminar flow is approximately 41μm. The terminal sedimentation velocity of such a particle is 4.4 mm s\(^{-1}\). Diameters of hydroxyapatite particles used in freeze casting are typically much smaller than this, ranging from 100 nm to 2 μm (Lee & Shin 2007; Moritz & Richter 2007; Suetsugu et al. 2007; Yoon et al. 2007; Deville 2008). They are thus in the laminar-flow regime, and their sedimentation velocities range from 26 nm s\(^{-1}\) to 11 μm s\(^{-1}\), respectively.

6. The freezing process

Once a slurry with appropriate sedimentation properties has been prepared, it is degassed and filled into a mould, which is typically made from polytetrafluoroethylene (PTFE) and sealed by a copper bottom plate (figure 2). The slurry may be degassed once again before the mould is placed on the copper cold finger for directional freezing. Most experiments described in the literature use a one-sided freezing system in which the top of the mould remains open (e.g. Deville et al. 2006a; Fu et al. 2008a; Pekor et al. 2008). The advantage of two-sided freeze-casting systems, in which the bottom and top of the mould are closed by copper plates whose temperature can be varied independently, is that the temperature gradient and freezing-front velocity within the sample can be better controlled (Deville et al. 2006a,c; Waschkies et al. 2009). In both systems,
Figure 2. Schematic of the freeze-casting system. The sample solution or slurry is poured into a PTFE mould and placed with its copper bottom plate onto the copper cold finger. The copper cold finger is cooled by a liquid nitrogen bath; temperature and cooling rate at the mould bottom plate are controlled using a band heater.

a resting period before freezing is frequently used either to adjust the overall sample temperature to a given value close to the solidification temperature or to create a well-defined temperature gradient within it.

To freeze the sample, the heating applied to the cold finger is reduced in a controlled fashion with typical cooling rates ranging from 0.1°C min\(^{-1}\) to 10°C min\(^{-1}\). In most freeze-casting systems, the applied cooling rate is measured at the cold-finger–sample interface, but not the velocity of the freezing front during processing itself. However, in systems that are equipped with a thermocouple array embedded in the mould, the freezing-front velocity can be measured directly and the cold-finger cooling rate adjusted to achieve a desired value (Waschkies et al. 2009). Once the temperature of the cold finger is lower than the solidification temperature of the slurry, a freezing front, defined as the interface between the solid and the liquid phases, is formed and starts to travel through the sample. How the applied cooling rate and the freezing-front velocity within the sample are correlated will be discussed below.

Both the freezing-front velocity and the form in which the liquid vehicle crystallizes determine sample structure and mechanical properties. An important criterion for the selection of the liquid vehicle is, among others, whether it will solidify in a dendritic or a faceted manner. This can be predicted by the liquid vehicle’s α-value (Jackson & Hunt 1965; Araki & Halloran 2004b, 2005)

\[
\alpha = \frac{h_E \xi}{RT_E},
\]  

(6.1)
where \( h_E \) is the latent heat, \( R \) is the gas constant, \( T_E \) is the equilibrium temperature between the two phases and \( \xi \) is a factor that depends on the crystallography of the interface. The value of \( \xi \) typically ranges from 0.5 to 1 for the closest packed face of a crystal and decreases for higher index planes. The factor \( h_E/RT_E \) is the entropy of melting. Materials that have a value of \( \alpha < 1 \) grow in a dendritic fashion. Water and camphene, the most commonly used liquid vehicles used in freeze casting, have \( h_E/RT_E \) values of approximately 2.6 and 1.1, respectively, and show dendritic growth (Araki & Halloran 2005).

While water and camphene are similar in that they both grow dendrites, they greatly differ in their crystal structure. Under ambient temperature and pressure conditions, camphene forms cubic crystals with an isotropic structure, whereas water solidifies into anisotropic hexagonal ice crystals with a crystal growth velocity that is approximately 100 times faster in the \( a \)-direction of the hexagonal base than perpendicular to it in the \( c \)-direction (Petrenko & Whitworth 2002). As a result, ice crystals grow with a lamellar microstructure parallel to the \( a \)-direction during freeze casting. When the crystal growth conditions result in very small lamellar spacing, owing to a high freezing-front velocity, for example, the growth rate in the \( c \)-direction can be sufficiently large for bridges to be formed between the lamellae. Such bridges are often desirable because they significantly stiffen and strengthen the freeze-cast material.

(a) Particle trapping and rejection

The velocity of crystal growth during solidification defines the freezing-front velocity, which determines whether a particle in the slurry will be rejected and pushed ahead, or engulfed and trapped by the approaching liquid–solid interface. The other important factor is the balance between two opposing forces that act on each particle: a repulsive force, which keeps the particle in the liquid phase owing to the system’s resistance against a change in the surface energy, and an attractive force that, due to viscous drag, pushes the particle towards the solid–liquid interface (figure 3).

Thermodynamically, a particle can only be trapped if the free energy of the system \( \Delta \sigma_0 \) is negative

\[
\Delta \sigma_0 = \sigma_{ps} - (\sigma_{pl} + \sigma_{sl}) < 0,
\]

where \( \sigma_{ps} \), \( \sigma_{pl} \) and \( \sigma_{sl} \) are the surface energies between the particle and the solid, the particle and the liquid and the solid and the liquid, respectively. Thus, the particle will be engulfed and trapped, if the energy of the newly formed particle–solid surface is smaller than the sum of the energies of the particle–liquid and the solid–liquid surfaces that disappear from the system; otherwise, the particle remains in the liquid (Körber et al. 1985; Asthana & Tewari 1993).

In the liquid phase, a particle with radius \( r \) experiences a repulsive force owing to molecular van der Waals interactions at the solid–liquid interface

\[
F_R = 2\pi r \Delta \sigma,
\]

where

\[
\Delta \sigma = \Delta \sigma_0 \left( \frac{a_0}{d} \right)^n,
\]

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Figure 3. Schematic of particle–freezing-front interactions. A particle of radius $r$ separated from the solid–liquid interface by a liquid film of thickness $d$ experiences an attractive force $F_h$ and a repulsive force $F_R$. Balancing the forces, a critical freezing-front velocity $v$ can be calculated, above which the particle will be engulfed and trapped, and below which the particles will be rejected and pushed ahead by the solid–liquid interface.

in which $\Delta \sigma_0$ is the free energy of the system, $a_0$ is the mean distance between the molecules in the liquid layer and $d$ is the thickness of the liquid layer between the particle and the solid–liquid interface. The term in brackets with exponent $n$ is a correction to the repulsive forces that act on the particle; values for $n$ typically range from 1 to 4 (Uhlmann et al. 1964; Körber et al. 1985; Zhang et al. 2005). Neglecting this correction by assuming $n = 1$, the repulsive force acting on the particle becomes

$$F_R = 2\pi r \Delta \sigma_0 \left( \frac{a_0}{d} \right).$$  \hspace{1cm} (6.5)

In addition to this repulsive force, the particle also experiences an attractive force owing to viscous drag. The expression for the viscous-drag force is derived from Stokes’s law (equation (5.2)) and adapted to the specific case of a thin film of the liquid that fills the gap between the solid–liquid interface of the freezing front and the particle (Bolling & Cisse 1971; Stefanescu et al. 1988),

$$F_n = \frac{6\pi \eta \nu r^2}{d}. \hspace{1cm} (6.6)$$

From an equilibrium of repulsive and attractive forces, a critical velocity $v_{cr}$ of the freezing front can be calculated at which particle entrapment takes place (Lipp & Körber 1993; Zhang et al. 2005),

$$v_{cr} = \frac{\Delta \sigma_0 a_0}{3 \eta r}. \hspace{1cm} (6.7)$$

At freezing-front velocities below this value, the particles are pushed ahead by the solid–liquid interface.
Experimentally, significant differences were observed by Azouni et al. (1990) and Yemmou et al. (1993) in the encapsulation process in the dependence of the thermal conductivity ratio
\[ \mu = \frac{\lambda_P}{\lambda_L}, \]
where \( \lambda_P \) and \( \lambda_L \) are the thermal conductivities of the particles and the liquid vehicle, respectively. Particles that have a lower thermal conductivity than the liquid result in a convex bending of the interface towards the particle; particles with a higher thermal conductivity than the liquid cause a concave deformation of the interface away from it. Theoretically, this phenomenon was described by Shangguan et al. (1992) and Sen et al. (1997). To account for it, Lipp & Körber (1993) suggest a modified expression for the critical velocity,
\[ v_{cr} = \frac{\Delta \sigma a_0}{12 \eta \mu r}. \]
Critical freezing-front velocities for hydroxyapatite particles in water can be estimated using this expression and typical values for the variables are \( \Delta \sigma \approx \sigma_{sl} \approx 0.1 \text{ J m}^{-2}, \ a_0 = 10 \text{ nm}, \ \eta = 1.793 \text{ mPa s and } \mu = 1.8; \) for particles with radii ranging from \( r = 0.10 \mu \text{m} \) to \( r = 2 \mu \text{m}, \) they range from \( v_{cr} = 0.26 \text{ m s}^{-1} \) to \( v_{cr} = 0.013 \text{ m s}^{-1}, \) respectively.

In addition to differences in thermal conductivity between particles and liquid, which cause undulations of the solid–liquid interface, local changes in the slurry’s concentration ahead of the solid–liquid line lead to instabilities in the freezing front. Assuming that the freezing front that emerges from the mould–liquid interface is plane and that its velocity is below the critical velocity, particles will be rejected. As a consequence, a concentration gradient forms in the slurry immediately ahead of the freezing front, which results in a zone with an increased particle concentration and decreased solidification point, the constitutional undercooling zone. When this finally breaks down, Mullins–Sekerka instabilities (Mullins & Sekerka 1964) are formed with a wavelength that determines the lamellar spacing between the ice crystals (Butler 2001, 2002a,b; Zhang et al. 2005). The architecture observed in freeze-cast samples provides experimental support of the structural differences predicted by freezing-front velocity considerations.

(b) Structural effects of the freezing-front velocity

Azouni et al. (1997) monitored the unidirectional freezing process in doubly distilled water with a shadow graph, in which thermal disturbances refract light and the resulting shadows image the thermal flow. They observed that varying the freezing rate resulted in three distinctly different ice structures; first, a dense ice layer followed by an intermediate columnar zone and finally a lamellar structure with highly aligned porosity. Deville et al. (2007) observed the same three distinct structural regions in bulk materials freeze cast from ceramic slurries.

Initially, when the cooling rate is fast, a plane ice front of high velocity emerges, which traps the particles, thereby forming a relatively dense and homogeneous layer. Gradually, the freezing-front velocity slows owing to an insulating layer of solid being formed. The plane solid–liquid interface starts to undulate owing to interactions with the particles close to the solid–liquid interface.
and particle rejection, which causes constitutional undercooling. As a result, the slurry solidifies with a columnar structure. On further slowing of the freezing-front velocity, local ‘pinning’ of the solid–liquid interface with a well-defined wavelength causes the transition from the columnar to the final, highly ordered lamellar structure. Gradually, the ice lamellae, which may initially be slightly inclined, align with the preferred growth direction parallel to the temperature gradient. They extend fast along their \( a \)-axis and very slowly along their \( c \)-axis. The lamellar structure that they create dominates the rest of the length of the sample. It is the desired structure for biomedical applications. Empirically, the lamellar spacing \( w \) has been found to vary with the freezing-front velocity \( v \) as

\[
w \propto \frac{1}{v^n},
\]

where \( n \) ranges from 1 to 4 (Deville et al. 2007). Because the freezing-front velocity is such an important processing parameter, it is critical to be able to carefully control it.

(c) Control of freezing-front velocity

The freezing-front velocity, and with it the time it takes to solidify an entire sample, primarily depend on two quantities: the temperature gradient between the solidifying layer in the sample and the copper cooling plate and the thermal properties of the solid phase that has already formed. When a layer of thickness \( ds \) of a liquid solidifies at its thermal transition temperature \( T_E \), the solidification enthalpy \( h_E \) is set free as heat, and has to be conducted away through the solid phase to the cooled surface. How quickly this heat can be conducted away determines the speed of solidification and thus the velocity at which the freezing front travels.

Assuming that, at \( x = 0 \), the temperature is kept constant at a temperature \( T_0 < T_E \), and neglecting heat storage in the solidified material, which is permissible because, with a specific heat \( c = 2.11 \text{Jg}^{-1}\text{C}^{-1} \) and latent heat \( h_E = 333.6 \text{Jg}^{-1} \), ice has a phase-transition number (ratio between latent heat and difference in internal energies of the solid at \( T_E \) and \( T_0 \)) of

\[
Ph = \frac{h_E}{c(T_E - T_0)} > 7,
\]

and a quasi-static approximation of the solidification time, which always underestimates the actual solidification time, can be calculated (Stefan problem) (Baehr & Stephan 2003) as

\[
t = \frac{\rho_L h_E}{2\lambda_L(T_E - T_0)^2},
\]

for a liquid vehicle with density \( \rho_L \) and thermal conductivity \( \lambda_L \).

This approximation permits to assume different boundary conditions at the cooled end of the solid and to describe the solidification of a liquid at the surface of a cooling plate of thickness \( \delta_C \) and thermal conductivity \( \lambda_C \), which is cooled by a liquid of temperature \( T_0 \) and which has the heat-transfer coefficient \( \alpha \). Under these conditions, at a given time \( t \), a solid layer of thickness \( s \), density \( \rho_S \) and thermal conductivity \( \lambda_S \) is formed. The heat freed during solidification is,
as before, conducted away through both the solid layer and the plate. Assuming quasi-static conditions and equating the heat generated during solidification with that transferred, the progression of the freezing front can be described as (Baehr & Stephan 2003)

$$\frac{dt}{ds} = \frac{\rho h_E}{k \lambda_S (T_E - T_0)} \left( s + \frac{\lambda_S}{k} \right)$$

(6.13)

where the heat-transfer resistance

$$1 \frac{1}{k} = \frac{\delta_C}{\lambda_C} + \frac{1}{\alpha}$$

(6.14)

is, in our case, that of the copper bottom plate of the freeze-casting mould.

The velocity at which the solidification front progresses is

$$v(s) = \frac{d s}{d t} = \frac{\lambda_S (T_E - T_0)}{\rho h_E} \left( s + \frac{\lambda_S}{k} \right)^{-1}.$$  

(6.15)

Integrating equation (6.13) yields an expression for the solidification time \( t \) of a layer of thickness \( s \)

$$t(s) = \frac{\rho h_E}{\lambda_S (T_E - T_0)} \left( \frac{s^2}{2} + \frac{\lambda_S}{k} s \right).$$

(6.16)

For very thin plates \( \delta_C \to 0, 1/k \to 0 \) and the result equals that of the above-described Stefan problem. For plates with a finite thickness and a heat-transfer resistance \( 1/k > 0 \), solidification is slower.

To achieve a constant freezing-front velocity

$$v = \frac{\lambda_S (T_E - T_0)}{\rho h_E} \left( s + \frac{\lambda_S}{k} \right)^{-1} = \text{const}. \quad (6.17)$$

throughout the sample, the temperature \( T_0 \) of the cooling bottom plate has to be varied as

$$T_0(t) = T_E - \frac{v^2 \rho_l h_E}{\lambda_S \nu k} \left( t + \frac{\lambda_S}{v k} \right).$$

(6.18)

The last unknown in this equation is the thermal conductivity of the solidified slurry.

\( (d) \) Thermal conductivity of the freeze-cast solid

To calculate the thermal conductivity of the solidified slurry, which consists of pure-ice lamellae that alternate with lamellae that consist of a particle–ice composite, we assume the latter to be packed beds, in which the particles are randomly close packed. The thermal conductivity of the packed bed \( \lambda_B \) can be
described according to the Krischer–Kröll model (Krischer & Kröll 1956) as an arrangement of serial and parallel components,

\[
\lambda_B = \frac{\lambda_I}{(1 - a)(\psi + (1 - \psi)(\lambda_P/\lambda_I))^{-1} + a(\psi + (1 - \psi)(\lambda_I/\lambda_P))},
\]

where the parameter \( a \) describes the amount of the serial component, \( \psi \) is the volume fraction of the continuous pure-ice phase and \( \lambda_P \) and \( \lambda_I \) are the thermal conductivities of the particles and the pure ice, respectively, as shown in figure 4 (Krischer & Kröll 1956; Tsotsas & Martin 1987). Assuming the packed bed to consist of 64 vol% particles and 36 vol% ice, Tsotsas & Martin (1987) suggest to use \( a = 0.2 \) for an appropriate description of its thermal conductivity. Using these values and the material properties listed in table 1, the thermal conductivity of a single packed-bed lamella of hydroxyapatite is calculated to be \( \lambda_B = 1.16 \text{ W m}^{-1}\text{K}^{-1} \).

At the next higher level of the structural hierarchy, the freeze-cast material consists of highly aligned, alternating lamellae of packed-bed material and pure ice that have the thermal conductivities \( \lambda_B \) and \( \lambda_I \), respectively. Assuming the lamellae to be perfectly parallel to the direction of heat flow, the thermal

\[\]

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Table 1. Properties of water, ice and hydroxyapatite.

<table>
<thead>
<tr>
<th>Material</th>
<th>Density $\rho$ (kg m$^{-3}$)</th>
<th>Solidification temperature $T_E$ ($^\circ$C, K)</th>
<th>Latent heat of fusion $h_E$ (J g$^{-1}$)</th>
<th>Thermal conductivity $\lambda$ (W m$^{-1}$ K$^{-1}$)</th>
<th>Specific heat at constant pressure $c_p$ (J g$^{-1}$ K$^{-1}$)</th>
<th>Viscosity $\eta$ (mPa s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water at 0°C</td>
<td>0.99984</td>
<td>0, 273.16</td>
<td>333.6</td>
<td>0.561</td>
<td>4.2176</td>
<td>1.793</td>
</tr>
<tr>
<td>Ice at 0°C</td>
<td>0.9167</td>
<td>0, 273.16</td>
<td>333.6</td>
<td>2.14</td>
<td>2.11</td>
<td>—</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>3.150</td>
<td>—</td>
<td>—</td>
<td>0.77</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 2. Volume fraction of pure-ice lamellae $\psi$ and thermal conductivity $\lambda_S$ of the solidified composite consisting of a parallel arrangement of particle-bed and pure-ice lamellae.

<table>
<thead>
<tr>
<th>Hydroxyapatite (vol%) slurry properties</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\psi$</td>
<td>0.92</td>
<td>0.84</td>
<td>0.69</td>
<td>0.53</td>
<td>0.37</td>
<td>0.22</td>
</tr>
<tr>
<td>$\lambda_S$ (W m$^{-1}$ K$^{-1}$)</td>
<td>2.06</td>
<td>1.99</td>
<td>1.83</td>
<td>1.68</td>
<td>1.53</td>
<td>1.38</td>
</tr>
</tbody>
</table>

The conductivity of the freeze-cast solid may be described by $a = 0$. With this assumption, the expression for the thermal conductivity reduces to the rule of mixture

$$\lambda_S = \psi \lambda_I + (1 - \psi) \lambda_B,$$

where $\psi$, as before, is the volume fraction of the pure-ice lamellae. It should be noted that this value of $\psi$ differs from the overall volume fraction of ice in the solid when calculating the thermal conductivities of the freeze-cast material. This is because 36 vol% of the particle bed is formed by the ice matrix in which the particles are embedded. As a result, a slurry that contains a 10 vol% fraction of hydroxyapatite will form a structure that consists of 15.6 vol% of particle-bed lamellae and 84.4 vol% of pure-ice lamellae. To illustrate this, the thermal conductivities $\lambda_S$ of solidified slurries with hydroxyapatite particle loadings ranging from 5 to 50 vol% are calculated using the material properties of Table 1 and listed in Table 2.

(e) Lamellar surface roughness control through additives

At the next lower level of the hierarchy, the lamellar surface roughness plays an important role in biomaterials design, as it is known to greatly affect tissue-scaffold interface properties. Munch et al. (2009) explored this through the use of chemical additives that are known to affect the interfacial energies, growth kinetics and microstructure of ice, as well as the topology of the ice–water interface. Most of the additives used by Munch et al. (2009) form a eutectic phase diagram with water, resulting in the modification of the solvent that broadens the number of possible pore-shape morphologies. Their results indicate...
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polymer solution or ceramic slurry is poured into mould at room temperature as ice lamellae grow, they force polymer molecules and suspended particles into voids frozen structure is lyophilized, sublimating ice and generating porosity

Figure 5. Phase diagram illustrating the phase changes of the liquid vehicle (water here) during the freeze-casting process.

Figure 6. Visualization of a cylindrical freeze-cast ceramic composite sample with a diameter of 0.6 mm imaged by X-ray micro-tomography at a pixel resolution of 0.3 μm. Freezing rate: (a) 20°C min⁻¹ and (b) 5°C min⁻¹.

that the microstructure can be altered from lamellar (e.g. no additives, trehalose or sucrose) to cellular (e.g. gelatin, glycerol, sucrose with citric acid), or to a lamellar structure with a bimodal pore-width distribution (ethanol). Additives were further shown to strongly modify the roughness of the ceramic walls. They may also aid the formation of bridges between the lamellae that result in improved mechanical properties of the scaffolds.

Surprisingly, Munch et al. (2009) found, despite the obvious structural differences, that the relationship between the structural wavelength and the average freezing-front velocity is almost independent of the additive used. More work is needed, however, to assess the precise influence of additives on final properties and architectures of the scaffolds.

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7. Freeze drying and further processing

After the freezing process is completed, the sample is carefully removed from the mould and lyophilized (freeze dried) for 24–48 h, depending on size, in order to sublimate the ice from the structure (figure 5). Lyophilization parameters have not been found to significantly impact the final microstructure of freeze-cast samples. Because ceramic green bodies are generally very brittle after lyophilization, they are usually sintered. Densification conditions, such as temperature and time, depend on the ceramic used and can be optimized to achieve a desired grain size and density.

8. Structure property correlations in freeze-cast materials

Surprisingly and despite significant research efforts, no exact design requirements exist as to which combination of overall porosity, average pore size, pore connectivity, material surface roughness, surface chemistry, mechanical properties, etc. in a freeze-cast scaffold is best suited for each respective tissue or organ. The results of our ongoing systematic studies of such correlations are expected to provide future guidance.

However, first structure–property linkages relating material architecture to mechanical performance for freeze-cast materials have been established. These correlations can already be influenced during slurry preparation because slurry composition and formulation have a significant effect on the scaffold’s microstructure and subsequently its mechanical properties. In polar solvents, such as water, surface charges on particles are desirable because they aid particle dispersion and thereby improve the homogeneity of the sample. Indirectly, they also affect other important processing parameters, such as the viscosity of the slurry and particle sedimentation, as these depend on the particle size. It is possible to adjust surface charges through, for example, variations of the liquid carrier’s pH and surface oxidation.

Additives in the slurry further allow to control the surface structure and roughness of the lamellae created during the freezing process. The shape of the pores templated by the liquid carrier depends on the crystal type and growth characteristics with which it solidifies. This is why materials prepared with water as the liquid vehicle have structures that greatly differ from those using camphene.

The amount of solid loading determines the overall porosity of the sample; the higher the loading, the lower the overall porosity. Achievable overall porosities depend on the material used and range from approximately 40 to 97 vol% (Deville et al. 2006a; U. G. K. Wegst 2009, unpublished data). The honeycomb-like structure achieved by directional solidification results in mechanical properties that are significantly higher than those achieved by non-directional freezing and lyophilization or other processing techniques that result in a foam-like material. Both Young’s modulus and the strength of honeycombs loaded parallel to the long pore axis are predicted to scale linearly with the relative density of the material. The properties of freeze-cast ceramics approach this value and are significantly higher than those predicted for foams (Deville et al. 2006c). For a given overall porosity, the lamellar thickness and spacing in a freeze-cast material can be controlled. An increase in the cooling rate to achieve an increase
in the freezing-front velocity results in smaller lamellar spacing (figure 6a,b). As a common trend, a decrease in the lamellar spacing results in an increase in compressive strength (Hunger 2008). This is also thought to be due to an increasing number of bridges between lamellae. However, different trends have also been observed, particularly in freeze-cast polymers (U. G. K. Wegst 2009, unpublished data).

Because the porosity generated by freeze-casting is highly connected, it is possible to create hierarchical microstructures by sequential freeze casting, thereby exploring the potential to introduce another level and another direction of porosity into the material. The high connectivity also makes it possible to coat the sample once or several times and to infiltrate it with another phase. Both offer great potential to integrate into the biomaterial growth factors and other biochemical cues to stimulate tissue ingrowth.

9. Conclusion

Initially developed for the manufacture of highly dense ceramics, freeze casting, a process that uses the solidification of a liquid carrier such as water or camphene for templating, has, in recent years, been discovered as a route to create highly porous biomaterials with complex, hierarchical architectures. Several reasons make freeze casting highly attractive for the manufacture of materials for biomedical applications: all classes of materials, polymers, ceramics, metals and their composites can be shaped with it; the processing can be carried out with benign, biocompatible liquid carriers such as water; the hierarchical microstructure created can be carefully controlled by the physical and chemical properties of the components used and processing parameters such as freezing velocity.

The amount, type, size and geometry of the particles and the type of liquid carrier determine the slurry’s viscosity and amount of sedimentation, as well as the slurry’s thermal properties and freezing behaviour. In combination with the freezing velocity and additives, these also determine pore connectivity and morphometry, such as the thickness and spacing of the lamellae and the size and the number of the material bridges between them, the cell-wall’s bulk and surface properties, and thus both tissue–material interaction and mechanical properties of the freeze-cast honeycomb scaffolds. Material composition and processing parameters further determine the anisotropy ratio between the strong and stiff freezing direction and the relatively weaker direction perpendicular to it. As a result, the freeze-casting process is ideally suited for the custom-designed manufacture of complex, hybrid biomaterials with hierarchical structures. By freeze casting, it is possible to emulate in synthetic materials multi-level hierarchical composite structures that are thought to be the origin of the mechanical-property amplification that is frequently observed in biological materials. Systematic studies are under way to correlate structure, mechanical and chemical properties of freeze-cast scaffolds with tissue–interface formation and integration.

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