Hierarchical and Heterogeneous Bioinspired Composites—Merging Molecular Self-Assembly with Additive Manufacturing

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Biological composites display exceptional mechanical properties owing to a highly organized, heterogeneous architecture spanning several length scales. It is challenging to translate this ordered and multiscale structural organization in synthetic, bulk composites. Herein, a combination of top-down and bottom-up approach is demonstrated, to form a polymer-ceramic composite by macroscopically aligning the self-assembled nanostructure of polymerizable lyotropic liquid crystals via 3D printing. The polymer matrix is then uniformly reinforced with bone-like apatite via in situ biomimetic mineralization. The combinatorial method enables the formation of macrosized, heterogeneous composites where the nanostructure and chemical composition is locally tuned over microscopic distances. This enables precise control over the mechanics in specific directions and regions, with a unique intrinsic–extrinsic toughening mechanism. As a proof-of-concept, the method is used to form large-scale composites mimicking the local nanostructure, compositional gradients and directional mechanical properties of heterogeneous tissues like the bone-cartilage interface, for mechanically stable osteochondral plugs. This work demonstrates the possibility to create hierarchical and complex structured composites using weak starting components, thus opening new routes for efficient synthesis of high-performance materials ranging from biomaterials to structural nanocomposites.

1. Introduction

Inspired by the elegant design principles and outstanding mechanical properties of biological composites, a much sought after goal in fabricating high performance synthetic materials is the ability to control structural and compositional heterogeneity at multiple length scales.\cite{1-4} Solving this challenging task opens the potential toward developing new composites for load-bearing engineering applications and biomedical implants with mechanical functionalities in tailored orientations and fail-safe toughening mechanisms. This includes specialized systems such as mechanically graded materials with improved lifetimes and low propensity for interfacial failure.\cite{3-8}

Heterogeneity in biological materials are often encountered from the nanoscale where structure and composition are locally tuned, which endows the materials with the ability to combine high strength and stiffness together with a sophisticated intrinsic–extrinsic fracture toughening mechanism.\cite{3,6-15} Notable examples of such biological materials are graded tissues like the bone-cartilage or ligament-bone composite that utilize multiscale architectural heterogeneity to efficiently integrate soft and hard constituents at the macro-scale. Such tissues exhibit a local variation in collagen fiber...
alignment and mineral concentration in order to firmly attach the soft tissue to the underlying bone. The gradient in structure and composition translates into a significant stiffness gradient of over four orders of magnitude thus facilitating uniform load transfer and preventing delamination of the cartilage.[16–18]

Control at the nanostructural level and using it to construct a hierarchical and heterogeneous composite has been a grand challenge in the fabrication of synthetic 3D constructs. Recent focus on the formation of bioinspired composites has been dedicated to controlling structural features at the microstructural level (5–100 µm) via top-down methods such as additive manufacturing (AM) and freeze casting.[3] AM is especially powerful, which when applied together with magnetic field or shear, can locally control the orientation of micron-sized particles/constituents in a 3D organic matrix.[19–22] Conversely, extending structural control down to the nanoscale has shown to be difficult due to the top-down nature of AM.[3,23] Biological composites are instead formed from the bottom-up via an intricate process of molecular self-assembly (MS) and biomineralization followed by cellular mechanisms, which build up the ordered nanostructure into higher level architectures.[15,24,25] Material formation using MS is very effective because it allows detailed structural control of materials between the molecular and microscopic length-scales (2–500 nm), although it is no easy task to manipulate the MS based nanostructure and form scaled-up, load bearing composite materials.[26–30] In this work, we merge MS and bioinspired mineralization of polymerizable lyotropic liquid crystals with top-down extrusion 3D printing. The combinatorial technique (MS+AM) permits the formation of load-bearing, large-scale composites with well-defined structural features and toughening mechanisms at length scales comparable to biological composites like bone. As a proof to the flexibility of the MS+AM design concept, we show that the formed materials can closely mimic the macroarchitecture, local nanostructure, mineral gradients, and consequent mechanical gradients of complex heterogeneous tissues like the bone-cartilage composite for potential osteochondral grafts. Using finite element analysis, we further provide a model for an osteochondral knee plug using our synthetic composite.

2. Results and Discussion

2.1. Design of the Polymer Matrix with a Macroscopically Aligned Nanostructure

The core material of the nanocomposite is a hexagonally ordered and polymerizable lyotropic liquid crystal (H1 PLLC) gel. The PLLC gel is formed by mixing di-acrylate modified, amphiphilic triblock copolymers (BCP), Pluronic F127 or P123 (MF127, M wt. 12600 g mol⁻¹; MP123, M wt. 5500 g mol⁻¹) with Milli-q water and butanol according to specific ratios (Table S1, Supporting Information).[30] On equilibration, the amphiphilic BCPs self-assemble into cylindrical and hexagonally ordered nano micellar fibrils (NMF) having a high aspect ratio (d = 10–100 nm and l = 100–10 000 nm) and randomly oriented as micrometer sized grains in arbitrary directions.[31,32] By applying extrusion shear, NMFs are forced to align with the flow direction (Figure 1A).[33,34] The fibrils are subsequently crosslinked by the acrylate groups using UV irradiation, which keeps the aligned nanostructure intact forming a solid, macrocrystalline, tough, and elastic polymerized lyotropic liquid crystal (PLL C).[30,32] This is clearly observed using atomic force microscopy (AFM) and scanning electron microscopy (SEM) where a mold-cast PLLC of a MF127-W (Table S1, Supporting Information and the Experimental Section) sample shows random NMF orientation while an extruded PLLC shows strict and uniform NMF alignment (Figure 1B). Additional SEM images of cross-sections (100–200 µm into the filament) and across the length of the filament (2–3 mm) showed that the degree of alignment is the same throughout the whole extruded piece (Figure S1, Supporting Information). The directional long-range order is further demonstrated as the extruded PLLC exhibits birefringence under cross-polarized light only in the transverse orientation, as seen in the inset of Figure 1B. Thus, the extrusion and UV crosslinking of the PLLC gel forms a structurally hierarchical material, where a single PLLC filament is a microscopic fiber (200–800 µm) composed of uniformly spaced NMFs (10–11 nm), aligned over macroscopic length scales (cm). The NMFs show strong visual similarity to aligned collagen fibrils in cortical bone as seen in Figure 1C. Due to the ease of extrusion through fine nozzles (800–1000 µm), PLLC gels could effortlessly be 3D printed into discrete solid structures using a custom-designed 3D printer (Figure S3, Supporting Information). Figure 1D shows a computer generated and the actual 3D printed rectangular mesh that demonstrates how MS and 3D printing offer mesoscale dimensional control at the nano, micro and macroscale. 2D small angle X-ray scattering (SAXS) data at specific points of the printed mesh (Figure 1D) showed strong anisotropic X-ray scattering. The orientation of the scattering patterns is in convincing agreement with the printing direction. This demonstrates that the NMF align parallel to the printing or extrusion shear direction over macroscopic distances in 3D. For example, when the sample is placed in the SAXS chamber with the print orientation horizontal to the detector, the scattering patterns showed a vertical orientation in the 2D space. This shows that the (100) and (200) planes of the H1 PLLC is preferentially aligned to the X-ray beam in 3D and over macroscopic distances. Adjacent SEM images confirm the alignment of the NMF fibrils. The scattering pattern from the sample rotate by 90° when the sample is rotated by the same angle in the same plane, with respect to the detector, which further confirms the NMF alignment (Figure 1D). On integration of the 2D SAXS patterns to 1D plots, the adjacent Bragg peaks showed q-ratios corresponding to: 1:3:2:7:3, which agree with the hexagonal geometry of the fibrils (Figure S2, Supporting Information).[35] The nanostructural dimensions (d_{NMF} = 9.73 nm and d_{n} = 8.40 nm) displayed in the schematic representation of the mesh (Figure 1D) were accurately calculated for a MP123-W PLLC from the 1D SAXS plots (Figure S2 and Table S2, Supporting Information).
Figure 1. A) Schematic of shear induced alignment of nano micellar fibrils (NMF) during LLC extrusion. B) Nanoscale structure of NMF in a MF127-W (left) mold-cast polymerized LLC (PLLC) with randomly oriented NMF domains and (right) extruded PLLC with macroscopically aligned NMF domains, insets show polarized light images of extruded filaments. C) Aligned and mineralized collagen fibers obtained from a section of a mandible bone of a moose. D) Extrusion 3D printing of the LLC to control structural features at the macro, micro and nanoscale with corresponding SEM and 2D SAXS data. The 2D SAXS data shows anisotropic scattering of the X-ray from an aligned MP123-W PLLC nanostructure, with respect to the printing direction. White arrows in the SAXS panels indicate the extrusion-printing direction.
2.2. Bioinspired Mineralization of Calcium Phosphates within the Aligned PLLC Matrix

The advantage of using PLLCs as a polymeric matrix is its unprecedented precision both as a template and ordered organic matrix for the preparation of inorganic nanostructures like silica and calcium phosphates (CaPs).\[^{36,37}\] Our earlier works introduced a bioinspired mineralization technique where CaP nanoparticles could be precipitated in situ to form ordered composites.\[^{30,38,39}\] However, due to random orientation of the NMF fibrils, there was no observable particle preferential orientation on the micro and macroscopic length scales. Here, we combine the in situ mineralization method with extrusion induced alignment, where the NMF and inorganic particles are intimately connected and oriented in user-defined directions to form bulk 3D nanocomposites. In the interest of biomedical applications, we formed CaP within the relevant LLC phases which were then printed into bone-mimetic tissue models (Table S1, Supporting Information).

After printing, the PLLC was treated with ammonia gas to raise the pH within the nanometric aqueous domains and precipitate CaP nanoparticles (see the Experimental Section). The cylindrical PLLC-CaP (MF127-20Ca) construct in Figure 2A shows a hierarchical structure with a defined macroscopic geometry \((r = h = 1 \text{ cm})\), microscopic pore sizes (200 µm after drying) and nanoscale alignment of the ordered NMF (variation by at most ± 5°) in each printed filament. More importantly, the images show an interpenetrating interaction between spherical CaPs of homogeneous size \((d \approx 30 \text{ nm})\) and tightly packed bundles of NMFs (Figure 2A). The fibrils wrap tightly around the particles, now organized in a pearl-necklace fashion, through elastic deformation which creates strong contact points between the NMF and CaP. The particles were uniformly distributed and identified as amorphous calcium phosphates (ACP) using X-ray diffraction (XRD) and energy-dispersive X-ray spectroscopy (EDS) (Figures S4, S5A and Table S3, Supporting Information). ACP is a metastable CaP phase that transforms to poorly crystalline,
needle-like hydroxyapatite (HAp) platelets on aging (Figure S5B and Table S3, Supporting Information). In a printed and aged prototype of a human jaw-condyle bone, aligned needle-like HAp nanoparticles formed along the crystallographic c-axis and parallel to the length of the NMF (Figure 2B). This proves that the PLLC can be effectively used to (i) mineralize CaP in situ, (ii) template, and (iii) align nanoparticles of homogeneous size distribution over macroscopic lengths to form ordered, anisotropic bulk composites. Furthermore, by controlling precipitation over macroscopic lengths to form ordered, anisotropic and (iii) align nanoparticles of homogeneous size distribution over macroscopic lengths to form ordered, anisotropic and (iii) align nanoparticles of homogeneous size distribution over macroscopic lengths to form ordered, anisotropic

Nanoindentation and macroscopic compression tests were performed to study the effect of NMF alignment on elasticity and strength of the 3D printed PLLC polymers and PLLC-CaP composites. Specimens were printed as cubic blocks (5 × 5 × 5 mm) with the printed filaments oriented in transverse and longitudinal directions to the load (Figure S6A, Supporting Information). Nanoindentation was performed on individual filaments (~800 µm) of the MF127-W PLLC polymer (Figure S6B, Supporting Information). An estimated 4000 NMFs interact with the indenter surface at any moment, based on effective contact of the indenter (D = 40 µm) and individual fibril diameter (d_{NMF} = 9.33 nm). Under oscillating stress, the NMF bundles showed greater resistance to deformation by a factor of 1.7 in the longitudinal direction, E_{eff} = 131.4 kPa, compared to transverse orientation, E_{eff} = 74.5 kPa (Figure 3A). The load-indentation curves offer insight into how the nanofibrils react to applied force, where the loading-unloading behavior for a longitudinal sample showed significantly larger hysteresis. This corresponds to deformation of the fibrils at higher loads, where they entangle, slide over each other or fibrillate, thereby inhibiting recuperation of the initial structure. In contrast, the transverse NMF showed lower hysteresis due to the elastic behavior (recovery of 60% strain) of sheet-like lamellar arrangement of the fibril bundles. Similar values for stiffness and strength were obtained when the PLLC cubes were macroscopically compressed, which confirms that NMF alignment is also evident at the macroscale (Figure S6C and Table S4, Supporting Information). In addition, the compressive properties of PLLCs were strongly dependent on the amphiphile molecular weight (Figure S7A and the supporting text, Supporting Information). The MP123-W PLLC showed 4–6 times higher stiffness and strength (E = 0.79 ± 0.02 MPa, σ_{eff} = 0.47 ± 0.07 MPa) than MF127 (E = 0.16 ± 0.01 MPa, σ_{eff} = 0.11 ± 0.00 MPa). The smaller MP123 molecule (5500 g mol⁻¹) is less flexible compared to the much larger MF127 molecule (12600 g mol⁻¹) (Figure S7A and Tables S4 and S5, Supporting Information). Therefore, at the nanostructural level, the elastic modulus, and strength of the PLLCs primarily depend on the molecular chain length and NMF alignment. A simplified model can be derived where compressive stress (σₐ) can be approximately correlated to the amphiphile size (m_{amp}) and a dimensionless alignment factor (n) at constant water and amphiphile concentrations (Equation (1)):

\[ \sigma_a = \frac{F_i}{A_i} = \frac{M_i \cdot a \cdot n}{A_i} \]

where \( a = f(m_{amp}), M_i = \text{mass of indenter}, \) and \( A_i = \text{initial area of PLLC sample and assuming } n = 2 \) for longitudinal alignment and \( n = 1 \) for transverse.

Controlling water and mineral content significantly changed the compressive behavior of the PLLC and PLLC-CaP composites. In general, decreasing water and increasing mineral content resulted in enhanced compressive strength and stiffness of the PLLCs. A mineralized MF127-20Ca composite with 12.80 wt% water and 11.76 wt% CaP displayed 600 times higher compressive stiffness (\( E = 74.96 ± 23.24 \) MPa) and 100 times higher strength (10.18 ± 2.61 MPa) than the parent PLLC (Figure 3B, Figures S7B and S8, Supporting Information). The MP123-50Ca samples still showed greater compressive strength than MF127-20Ca owing to a denser polymer network and higher mineral loading (17.39 wt%). Moreover, the CaP reinforced PLLCs showed a clear directionality in the compressive stiffness where the E-modulus for longitudinally oriented composites is approximately twice that of the transverse samples (Figure 3B). Tables S4 and S5 in the Supporting Information summarize the compressive properties of the PLLC and PLLC-CaP composites with respect to NMF orientation, filament orientation, composition, and amphiphile molecular weight.

2.4. Crack Propagation and Hierarchical Toughening of the PLLC-CaP Composites

We correlated the stress-strain curves of the MF127-20Ca composite in Figure 3B to the changes in microstructure using SEM (Figure 3C and Figure S9, Supporting Information). Longitudinal samples showed a distinct yield point (\( \varepsilon = 0.15–0.20 \)) due to buckling of the filaments (Figure S9,1–2a, Supporting Information) while the transverse composites showed a linear elastic behavior even at higher strains (Figure S9,3–4, Supporting Information). At \( \varepsilon > 0.35, \) multiple micrometer-sized cracks formed within the individual printed filaments (200 µm) of the transverse samples (Figure 3C and Figure S9, Supporting Information). The cracks propagate in the direction perpendicular to the filament length. Width of the cracks was in the range of 1–5 µm and run to lengths of 10–50 µm (Figure 3C). On some of the growing cracks, we observed fibrous threads of diameter 500 nm stretching between the cracks (Figure 3C). This could be bundles of the mineralized NMF, trying to bridge the cracks in the printed filament. To understand if and how macroscopic forces affected the nanoscale architecture of the composite, we imaged sections of a composite that was subjected to rapid impact of the microtome knife. The knife tip measures 500 µm and cuts the sample in one swift motion thereby creating large...
amounts of local compressive forces which could rupture the nanostructure of the composite (cuts perpendicular to alignment). SEM images in Figure 3D,E shows regions of rupture along the transverse section of NMF-ACP arrangement of a MF127-20Ca composite. Sharp delamination of the NMF fibrils from the adjacent ACP nanoparticles was observed where the fibrils slide past each other and disintegrate, resulting in an increased fibril-fibril and fibril-ACP spacing. We correlated the SEM images of the ruptured nanostructure and microcracks on the larger filament to hypothesize on the following steps that take place ahead and behind the microcrack tip, (i) molecular uncoiling and disintegration of the ordered arrangement of the cross-linked amphiphiles, within each NMF, (ii) stretching of mineralized and aligned NMF leading to delamination and disintegration, to a point where they rupture at multiple regions, (iii) nonuniform rupture and bridging of microscopic NMF bundles leads to formation of several cracks throughout the larger printed filament, (iv) at

Figure 3. A) Nanoindentation curves of individual MF127-W PLLC filaments. B) Macroscopic compression curves of MF127-20Ca and MP123-50Ca composites. Numbers marked on the plot correspond to SEM images presented in Figure S9 in the Supporting Information. C) Microcrack propagation in compressed filaments of transverse PLLC-CaP composite show extrinsic cracking and fibrous stretching. D) Mineralized NMF-ACP region show mildly disturbed NMF-ACP on microtome impact. E) Low and high magnifications of severely ruptured, NMF-ACP nanostructure on microtome impact show fibril delamination and breakage from mineral particles. F) Schematic of hierarchical crack arrest mechanism in the PLLC-CaP composite.
the macroscale, the transverse lamellar arrangement of the printed filaments further resists compression forces in an almost elastic manner (Figure 3F and Figure S10, Supporting Information). Thus, the data points toward a combination of molecular + nanoscale structure (intrinsc) and micro + macroscopic architecture (extrinsic) contributing to the crack resist character of the PLLC-CaP composites. We believe that our aligned composites exhibit damage tolerance similar to, albeit at a simpler level, how bone utilizes both intrinsic (molecular uncoiling of collagen, sacrificial bonds, and fibrillar sliding) and extrinsic toughening mechanisms (fibrillar bridging and other microfeatures) to achieve damage tolerance. [3,10,41,42] This is a remarkable way of realizing multiscale toughness in polymer composites and our work shows that it might be possible to mimic such mechanisms also in synthetic materials. Two reasons; however, hinder the composites from achieving compressive strength and stiffness values as high as cortical bone, (i) the PLLC-CaP composites lack a chemical interface between the NMF and CaP particles and (ii) low mineral content (~12%-18% by wt) within the PLLC. Nevertheless, it is interesting to note that the compressive strength of the composites is equivalent to or higher than mineralized biological tissues that possess similar structure, water and a much higher CaP content, like calcified cartilage and lumbar cancellous bone (Figure 4A). [18,43,44]

### 2.5. Designing Heterogeneous Gradient Composites Using the MS+AM Technique

To demonstrate the potential applicability of our MS+AM method, we designed and fabricated a model of the bone-cartilage interface also known as the osteochondral tissue (OT) of the knee joint (Figure 4B). The OT is important for articular joint function such as smooth load transfer from the synovial joints to the bone. Damage to OT leads to a degenerative condition called osteoarthritis. [45] Recent inventions of synthetic osteochondral grafts have a combination of a bi- or tri-phasic materials formed via layer-by-layer methods. [46,47] A problem with such grafts is the propensity for layer delamination under high stresses. Natural OT possesses carefully organized interfaces at the micro and nanoscale, where an intermediate calcified cartilage acts as a load-transition layer from soft cartilage to stiff bone. Each layer in an OT have varying levels of nanostructural alignment of collagen fibers, starting with transverse alignment in the superficial cartilage which changes to longitudinal orientation in the deep and calcified layers. [48,49] Moreover, there exists a mineral gradient and a consequent stiffness gradient along the depth of the tissue (3–5 mm) from the cartilage to subchondral bone. [17,50] Here, we show that the PLLC polymers and PLLC-CaP composite systems can be fabricated into a heterogeneous material with a compositional, structural and mechanical gradient similar to natural OT. To test the formation of a mineral gradient in the PLLC matrix, we immersed a mold-casted MF127-W PLLC in a reservoir of CaP precursor for 10 h such that only half the PLLC was in contact with the precursor solution (Figures S11 and S12, Supporting Information), which allows CaP ions to diffuse into the nanometric aqueous domains of the PLLC. The samples were then mineralized and the composites showed a visual gradient from a transparent region to dense white color indicating the formation of a gradient of CaP nanoparticles along the length of the PLLC (Figure S11A, Supporting Information). SEM and EDS data confirmed the CaP particles and SAXS on different points of the gradient showed that the hexagonal order of the NMF also remained intact (Figure S11B,C, Supporting Information). Next, the concepts of shear induced alignment of the NMF fibrils and diffusion based mineral-gradient were combined to design and 3D print an OT mimetic plug. A 3D model representing the natural tissue was designed with print paths generated in such a way to mimic the alignment of superficial, deep, and calcified cartilage layers and an underlying porous subchondral bone with layer-thickness values closely matching the natural tissue (Figure S12, Supporting Information). [50] After printing, the construct was UV-crosslinked followed by soaking it in CaP precursor solutions of different concentrations, time and at different depths to match the natural mineral gradient of an OT (Figure S13A–F, Supporting Information). The resulting material showed a clear mineral gradient, visually similar to a biological OT (Figure 4C, Figure S13, Supporting Information). SEM images of different sections showed that NMF were aligned in transverse directions in the superficial layer (region I, 0–500 µm) and changed to longitudinal orientations in the deep (region II, 500–2000 µm) and calcified layer (region III, 2000–3000 µm). More importantly, we see an increase in HAP mineral content along the aligned NMF fibrils in the longitudinally printed section of the construct. In region IV, dense mineralization was observed showing aligned HAP nanoparticles in the bone-mimetic region (Figure 4C). A compositional gradient in water was also observed, where the richest section was the superficial and deep layer of the nonmineralized regions (50–60 wt% water) followed by a gradual decrease down to 10–25 wt% water in the calcified and subchondral sections (Figure 4D and Figure S13G,H, Supporting Information). Since the Young’s modulus of the PLLC was measured earlier with respect to the NMF alignment and chemical composition (Tables S4–5, Supporting Information), the stiffness along the length of the gradient construct was mapped. The E-modulus of the plug spanned over four orders of magnitude (0.16–75 MPa), with respect to each region marked in Figure 4C (Figure 4E). Note that the substantial stiffness gradient in a single structure was obtained by merely controlling the CaP diffusion within the matrix and NMF alignment. Based on literature values of layer thicknesses and stiffness values of each layer in an OT, the PLLC construct closely matched the stiffness vs. distance regime of a human articular OT (Figure 4E). [18,50–53]

### 2.6. Finite Element Analysis of the Synthetic Gradient Plug in a Human Knee-Model

We conducted finite element analysis (FEA) to test the mechanical stability of the plug in a human knee model.
of a 70-year-old female (Figure S14, Supporting Information). The location of the OT-plug was chosen such that the highest von Mises stress on the interface between the cartilage and meniscus will occur on the plugs surface. Due to the complexity in simulating dynamic loads around the knee joint, the current model considers the person to be standing on two feet where the knee joint experiences a static compressive load of 45 kg. As expected, the highest stress regions are at the plug-meniscus contact point while minimum stress fields are observed along the depth of the plug-tissue interface. Simulations show that the von Mises stress fields on and around the plug do not exceed 1.36 Pa which is well below the failure stresses of the plug ($\sigma_f = 0.11 \pm 0.00$ MPa) and the surrounding tissue. Importantly, the von Mises stresses for a graded plug gradually decrease from the soft plug surface to the stiffer side (Figure 5A). However, homogeneous plugs show rather uniform stress fields. A fully soft plug ($\sigma_f = 0.11 \pm 0.00$ MPa) takes very little load and the rest...
is transferred to the surrounding tissue which can lead to poor regeneration and damage to the native OT. A stiff plug ($\sigma_f = 14.62 \pm 0.41$ MPa) experience higher stress throughout the plug, which will lead to failure of the plug and hinder the regeneration processes (Figure 5B and Figures S15 and 16, Supporting Information). The FEA analysis showed that a stiffness gradient is important for the plug to transfer loads effectively to the surrounding tissue without resulting in high stresses in the plug and surrounding tissue. This renders the material presented here particularly optimal, as the control of the gradient stiffness is intrinsic to our composite design.

3. Conclusion

In summary, we have demonstrated a new method to fabricate bioinspired composites based on a combination of bottom-up molecular self-assembly and top-down additive manufacturing. The combination enables us to tune material properties from the bottom-up, by controlling amphiphile chain length, choice of nanoscale inorganic phase, nanocomposite structure and alignment, microscale porosity and macroscale geometry. The mechanical behavior of the composites showed directional elastic modulus and an exceptional intrinsic–extrinsic crack behavior similar to only observed in natural mineralized composites. Finally, the concept was adopted to form a composite material carefully mimicking the structural hierarchy, compositional and mechanical character of an osteochondral tissue. We believe the technique and materials thereof will provide new and inexpensive pathways to form scaled-up bioinspired composites that truly mimic the structure and properties of biological composite materials.

4. Experimental Section

Preparation of Polymerizable LLC Gels for Casting and 3D Printing: All chemicals were purchased from Sigma-Aldrich. LLC gels with hexagonal nanostructure were prepared by mechanically mixing diacrylate modified triblock copolymer (BCP), water (or calcium phosphate precursor), and an oil phase, in specific weight ratios as detailed in Tables S1 in the Supporting Information. 2-Hydroxy 2-methylpropiophenone (1 wt% of the MF127 or MP123) was added as photoinitiator. The LLC gels were equilibrated for 1 h at room temperature (RT) before use in vials or syringes. The gel for extrusion-3D printing was stored in 10 mL syringes with blunt needles ($d = 800$ or $1000 \mu$m).

3D Printing of LLC Gels: The gels were extruded at 0.3 mL min$^{-1}$ to analyze NMF alignment using SAXS and PLM. For printing, the loaded syringe was mounted onto a custom-made syringe pump. The syringe pump was fabricated in house using the open-source design developed by Wijnen et al. (Figure S3, Supporting Information). It was electronically coupled to a desktop 3D printer (Printbot Simple). The NEMA 17 stepper of the syringe pump was driven by the printer control board, allowing feeding rate as a means of extrusion control. All 3D models reported in this work were designed and printed using manually written G-code or using the modeling software, OpenSCAD. (G-codes available on request). The printed and cast LLC gels were UV-crosslinked ($\lambda_{\text{max}} = 252$ nm, 90 W) to form a solid PLLC. UV exposure varied from 10–30 min as per sample thickness (mold-cast LLC gels and extruded LLC filaments, 10 min; all 3D printed bulk samples, 30 min).

Mineralization and Drying Procedure: The cast and 3D printed PLLCs containing CaP precursor were mineralized with CaP using NH$_3$ gas (99.999%, Hi-Q 5.0) in a sealed chamber for 3 h (2 bar, RT, pH inside chamber = 12.9, measured using Milli-q water). To form CaP gradients within the PLLC, the sample was soaked to a desired depth in the CaP precursor solutions for 4 h before NH$_3$
treatment (Figure S12, Supporting Information). The PLLC-monometal composites were mineralized in a vacuum oven at 40 °C.

Characterization of NMM and CaP Alignment in Pure and Mineralized, 3D Printed Matrices: The AFM of mold-cast PLLC was obtained on a Bruker Dimension ICON SPM using tapping mode with golden silicon probes (Force constant = 1.45–15.1 N m−2). Polarized light microscopy (PLM) was performed using a Carl Zeiss Axio Imager, polarization contrast mode on the extruded PLLC. Polarized light microscopy (PLM) was performed using a Carl Zeiss, Axio Imager, polarization contrast mode on the extruded PLLC filaments (thickness ≈800 μm). SEM samples were microtomed as thin sections of 200 μm (American Optical, model 820). The samples were analyzed using a LEO Ultra 55 FEG (5 kV) with Oxford Inca EDS detector (10 kV). XRD was used to analyze the crystallinity of the CaP particles (Bruker D8 Advance, λ = 1.54 Å). SAXS was performed at the I-911 beamline, MAX-Lab, Lund, Sweden (2D MarCCD 165 mm detector and λ = 0.91 Å). SAXS samples were placed in a small slit enclosed between Kapton tapes. Thermogravimetric analysis (TGA) was conducted (Pyris 1, PerkinElmer and Mettler Toledo) at a heating rate of 10 °C min−1 from 30 to 600 °C (N2) in alumina crucibles. Nanoindentation measurements (Pluama Nanoindenter, Optics 11, probe diameter = 248 μm) were performed on individual filaments of printed PLLC cubes. 16 points, at a distance of 4 μm was indented during one measurement cycle (three cycles in total) on both the transverse and longitudinal face of a printed PLLC filament. Macroscopic compression on the samples (5 mm × 5 mm × 5 mm, n = 3 for each sample type) was performed by compressing the PLLC and PLLC-CaP composites between two platens, using an Instron 5600 UTM (strain rate = 0.02 mm s−1). Certain 3D printed samples were dried at 35 °C in vacuum for 24 and 96 h to achieve semi-dry and dry state. FEA was performed using an existing knee model from the Open Knee project.[55] The computer aided design tool called ANSA was used to prepare the geometry and the mesh. Abaqus Standard was used to perform the numerical simulations.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements
The authors thank Riccardo Borgani (Royal Institute of Technology, Stockholm) for assistance with AFM images, Milene Gomes, Simon Isaksson, and Jonatan Bergek (Chalmers University of Technology) for help with SAXS data and for assistance with preparing media files respectively, Ernst Breel (Optics 11, Amsterdam) for assistance with nanoindentation measurements. The authors thank MAX-Lab, Sweden for support with SAXS measurements. The authors acknowledge funding from the Knut and Alice Wallenberg foundation and the Area of Advance Materials Sciences (Chalmers University of Technology).

Conflict of Interest
The authors declare no conflict of interest.


Received: February 18, 2017
Revised: April 15, 2017
Published online: June 1, 2017