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Hybrid 3D Printing of Synthetic and Cell-Laden Bioinks for Shape Retaining Soft Tissue Grafts

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Abstract

Despite recent advances in clinical procedures, the repair of soft tissue remains a reconstructive challenge. Current technologies such as synthetic implants and dermal flap autografting result in inefficient shape retention and unpredictable aesthetic outcomes. 3D printing, however, can be leveraged to produce superior soft tissue grafts that allow enhanced host integration and volume retention. Here, a novel dual bioink 3D printing strategy is presented that utilizes synthetic and natural materials to create stable, biomimetic soft tissue constructs. A double network ink composed of covalently crosslinked poly(ethylene) glycol and ionically crosslinked alginate acts as a physical support network that promotes cell growth and enables long-term graft shape retention. This is coupled with a cell-laden, biodegradable gelatin methacrylate bioink in a hybrid printing technique, and the composite scaffolds are evaluated in their mechanical properties, shape retention, and cytotoxicity. Additionally, a new shape analysis technique utilizing CloudCompare software is developed that expands the available toolbox for assessing scaffold aesthetic properties. With this dynamic 3D bioprinting strategy, complex geometries with robust internal structures can be easily modulated by varying the print ratio of non-degradable to sacrificial strands. The versatility of this hybrid printing fabrication platform can inspire the design of future multi-material regenerative implants.

Graphical Abstract

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Supporting Information

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A hybrid 3D printing strategy that capitalizes on the strengths of non-degradable and biodegradable hydrogels is developed to create stable, biomimetic soft tissue constructs. By tuning the print ratio of nondegradable to sacrificial strands, various shape-retaining and mechanical properties can be obtained. Additionally, a new shape analysis technique is developed and expands the available toolbox for assessing scaffold aesthetic properties.

Keywords

3D printing; poly(ethylene) glycol; composite scaffold; shape retention; double network hydrogels

1. Introduction

Soft tissue defects remain as irremediable obstacles in current reconstructive cases. These defects may occur due to several causes, ranging from simple contour incongruencies due to congenital deformities to large volume tissue loss following deep burns, trauma, or tumor resection. The term ‘soft tissue’ can refer to both connective tissues (such as tendons, ligaments, skin, adipose fat, and synovial membranes) and non-connective tissues (such as muscles, nerves, and blood vessels).^[1] Because soft tissue defects range in size, shape, and tissue composition, there is no single solution capable of reconstructing replacement tissue with identical volume and mechanical properties. Current clinical techniques fill these voids with natural materials, such as free adipose tissue grafts and autologous dermal fat flaps, and synthetic materials, such as commercially available silicone implants.^[2,3] Unfortunately all these strategies present significant setbacks. Though free adipose tissue can be resected safely with a minimally invasive procedure, the autologous fat transfer experiences significant volume augmentation due to the limited survival of mature adipocytes post

liposuction trauma. Contour irregularity, lumpiness, and graft resorption make this grafting technique inefficient and unsuccessful.^[4] Dermal flat flap harvest, a technique that completely resects large sections of skin and fat tissue in attempt to retain its vasculature and structure, is a technique limited by the donor site and can lead to site morbidity, pain, scarring, and often experience shrinkage and fibrosis post implantation.^[5–7] Synthetic materials have the advantage of permanently retaining mechanical properties; however, they are commonly associated with implant migration, rupture and/or breakdown, and fibrotic encapsulation from the immune system's foreign body response.^[8,9] Although for different reasons, these clinical treatments result in low patient satisfaction due to the absence of long-term graft retention and aesthetic restoration, necessitating a more robust strategy to repair soft tissue defects.

Extrusion-based 3D printing can be leveraged in the fabrication of custom-shaped soft tissue equivalents via the precise placement of both cells and matrix materials in an organized layer-by-layer manner that is characteristic of native tissue.^[10] Though this fabrication process is highly beneficial, there are similar material concerns when this technology is applied to soft tissue engineering. For instance, various naturally-derived polymers such as collagen/gelatin, alginate, fibrin, and hyaluronic acid are highly printable with minimal donor-site morbidity and immune response, but undergo rapid remodeling and collapse *in vivo*.^[11–18] Conversely, synthetic polymers such as polycaprolactone and poly(D,L)-lactide have a mechanical mismatch with the native soft tissue, an absence of surface ligands for cell attachment, and degradation products that negatively impact cellular metabolism and proliferation.^[19–22] Despite advances in bioink formulations, no ink is singularly capable of mimicking soft tissue with long-term shape and size retention, mechanical stability, and host integration.

In order to address these limitations, we have developed a novel hybrid printing approach that capitalizes on the strengths of both nondegradable and biodegradable hydrogels to create biomimetic constructs with long-term shape and volume retention (Figure 1). Our design incorporates two inks: a double network (DN) of poly(ethylene) glycol (PEG) covalently linked with naturally derived and physically crosslinked alginate, and a biodegradable cell-laden bioink of gelatin methacrylate (GelMA). The DN ink provides robust physical support necessary to retain long-term graft volume and architecture, while the degradable bioink provides the biological environment necessary to stimulate tissue regeneration (Figure 1A). This printing method results in the precise placement of hydrogel strands that functionally complement each other and enables for the construction of complex geometries such as noses, ears, and thyroid cartilage (Figure 1B).

2. Results and Discussion

2.1 Double Network Support Structure

To begin, 4-arm thiol-ene PEG hydrogels were chosen due to this system's predictable swelling characteristics, low toxicity, inertness, and resistance to common enzymes.^[23] Because this solution exhibits low viscosity at low monomer concentrations, it cannot be loaded into a cartridge and printed alone; this solution is not self-supporting for layer-by-layer fabrication and must either be made very viscous by increasing PEG content or

including a thickener. Therefore, alginate was chosen as a thickener for our PEG ink solution since it has been shown to be an effective agent in creating extrudable hydrogels and can provide the opportunity for a second physically crosslinked interpenetrating network when subjected to calcium ions.^[26,27] We found that an alginate concentration of 4% (w/v) produced the most ideal filament (Supplemental Figure 1) when mixed with a 10% 4-arm PEG solution, and this composition was further investigated for its swelling, mechanical, cell viability, and shape retention properties.

The thiol-ene chemistry provides the added advantages of high crosslinking speed when exposed to UV light, a key factor used in our printing process to intermittently crosslink layers, and its cytocompatibility, as the crosslinking of step-growth thiol-norbornene hydrogels is not oxygen-inhibited.^[24,25] The thiol-norbornene reaction not only can be initiated several orders of magnitude faster than the classic method of random chain-growth photopolymerization, but also results in a more uniformly crosslinked gel since each monomer with a defined functionality serves as a single crosslinking point.^[28] The degree of network heterogeneity is also at a minimum when compared to random chain-growth polymerization, since chain-polymerized gels contain an unfixed number of arms per crosslinking point due to the reaction's random nature of radical propagation and termination.^[29] Thus, the thiol-ene chemistry allows for a greater control when designing the swelling and mechanical properties of our ink. To test our material's swelling properties, PEG hydrogels composed of either 5k, 10k, or 20k Da monomers were first casted into discs to characterize the amount of swelling in excess water (Figure 2). Hydrogels composed of higher molecular weight monomer experience more swelling when mass was held constant as observed in previous studies. For example, previous literature has shown poly(ethylene) glycol hydrogels swell greatly under physiological conditions and often become undesirably weak or brittle after swelling, which limits their applications.^[30] Mass swelling ratios are reported in 20–100 range, nearly tripling the hydrogel's originally casted size.^[28,31] In our experiments, the smallest molecular weight monomer (5k) pure polymer network originally had the smallest swelling ratio of 14.8 ± 2.4 . However, the addition of alginate to any ink formulation for printing purposes inhibited perfect PEG crosslink formation, which ultimately caused the once favorable 5k swelling ratio to dramatically increase (Figure 2E). While the higher molecular weight inks can be printed, significant hydrogel swelling and scaffold disfigurement make these ink formulations suboptimal as an engineered soft tissue replacement. When the alginate thickener was ionically crosslinked with calcium ions (after photopolymerization of the thiol-ene network), the swelling was significantly reduced due to the restriction of the interpenetrating secondary polymeric network. Though double network chemistry is known to create extremely strong hydrogels able to withstand significant stress with minimal distortion, they have only been created with cytotoxic materials previously in literature (polyacrylamide).^[32,33] Our choice in alginate as both the ink thickener and the secondary polymeric network is cell-friendly and has been dependably used for 3D printing practices by others.^[14,26,27] Overall, alginate physical crosslinking prevented swelling by $32 \pm 0.2\%$ in the 5k ink, $53 \pm 1\%$ in the 10k ink, and $208 \pm 0.1\%$ in the 20k ink (Figure 2D). The 5k ink swelled the least when alginate ionic crosslinking was introduced (mass swelling ratio of 2.4 ± 0.1), with casted disc samples experiencing $5 \pm 0.2\%$ increase in diameter, $6 \pm 3\%$ in thickness, and $23 \pm 1\%$ in mass. The limited swelling of the 5k ink made it an ideal

candidate for soft tissue printing, and therefore was further evaluated for cytotoxicity and mechanical properties when co-printed in various patterns with GelMA.

2.2 Hybrid Printing

GelMA is chosen for its resemblance to native extracellular matrix, and offers significant advantages such as its ease in printability, UV photopolymerization, and natural cell binding motifs.^[34–37] It is widely known as a tunable biomaterial whose composition (weight percentage) and crosslinking degree (UV exposure, photoinitiator concentration, and degree of methacrylation) can be customized to match its rate in degradation to the regenerated tissue it is replacing.^[38,39] By strategically co-printing GelMA and the DN inks, our hybrid constructs can be tailored in both stiffness and degradation rate by varying the ratio of each ink in the print. Soft tissue engineering requires matching of mechanical properties to the native tissue, otherwise detrimental effects to the tissue aesthetics can occur. The compressive moduli, toughness, and fracture strength were characterized for various print patterns of DN ink and GelMA bioink (Figure 3). Patterns of DN-GelMA varied from 1–0 (no tissue regenerative capacity, control) down to 0–1 (completely degradable GelMA, control) (Figure 3A). Since the GelMA network can degrade by collagenase, a naturally occurring enzyme in the body, the long-term mechanical properties of the composite scaffold are mainly dictated by the presence of the DN ink's two tightly crosslinked, interpenetrating polymeric networks. Therefore, the aforementioned mechanical properties of the hydrogel were characterized before and after exposure to collagenase IV (500 Units/mL) overnight. Under uniaxial compression, the composite hydrogels are elastic within 15% strain with moduli scaling a 4-fold variation in stiffness, dependent on DN ink presence— a range congruent with the stiffness values of soft tissue (e.g. skeletal muscle (500kPa) to adipose (10kPa) compressive moduli values, Figure 3B).^[40–42] Decreasing the double network fraction from the highest 1–0 pattern to the lowest 1–3 DN-GelMA pattern manifested a 74.5% decline in modulus. Printing GelMA alone resulted in a significantly less stiff scaffold (36.8 ± 15.6 kPa), revealing that the coprint benefits from the incorporation of the DN ink for scaffold reinforcement. Scaffold toughness and fracture strength are critical mechanical properties that display the scaffold's ability to maintain shape while under compressive forces during tissue remodeling and implant healing. Both toughness and fracture strength remained high in all patterns before exposure to collagenase IV (1517.8–1836.3 kPa, 26.7–28.9 kPa respectively). Post-digestion analysis similarly revealed that toughness and fracture strength most closely resembled the dominant matrix of the hybrid's material makeup. Together, these results show that the hybrid printing strategy can be used to produce novel tissue constructs with controllable degradation rates, while simultaneously retaining the desired mechanical properties upon further *in vivo* incorporation.

2.3 Scaffold Aesthetic Properties Mapped with CloudCompare

In addition to maintaining similar mechanical properties to native soft tissue, shape retention is of upmost importance to ensure long-term success of the soft tissue reconstruction. To this end, a model of an adult human nose was designed and scaled down to 1/3 of the average physiological size and printed with the hybrid inks (Figure 4). Again, the alternating patterns of DN ink to sacrificial GelMA bioink were used to print the complex nose shapes, and the scaffolds were 3D scanned with a Hexagon ROMER Absolute Arm both before and after

digestion to determine variations in scaffold surface and shape. Iconic characteristics of the scaffold (i.e. width, length, height, and curvature, Figure 4B) were measured from each surface scan. Across all patterns, a high degree of shape retention was observed with the highest deviation of $14.9 \pm 3.0\%$ change in width, $13.0 \pm 2.7\%$ change in length, and $4.9 \pm 3.3\%$ change in height for the 1–3 pattern. Significant changes in scaffold curvature was only displayed in the 1–2 and 1–3 print patterns by $15.0 \pm 4.8\%$ and $23.3 \pm 8.3\%$ respectively when compared to pre-digestive values. As the GelMA content increased, the peak height of the scaffold decreased post digestion, which resulted in subsequent decreases in scaffold curvature and increases in scaffold width and length.

The development of this new printing technique also demands the development of a new approach to qualitatively assess scaffold shape maintenance. The application of finite element mesh analysis via a 3D evaluation software named CloudCompare allows both quantitative and qualitative characterization of shape deviations with such high detail that has not yet been visualized in the Tissue Engineering field. Thus, heat map analysis was performed where the surface mesh data sets were superimposed on each other for pairwise comparisons (MeshLab), and deviations between the pre and post-digested scans were determined using CloudCompare (Figure 5). Detailed methods regarding analysis of the scaffold design using the CloudCompare software can be found in the Supplemental Methods section. This data holistically shows the DN ink's ability to dictate scaffold shape during degradative remodeling processes. Color maps illustrated a spectrum of yellow (indicating the standards of a perfect match), red and blue color (both indicating the lack of coincidence) depending on the DN-GelMA pattern used. The distribution of the points in relation to the distance from the referenced pre-digested surface can be visualized on the right side of these maps. Together, the color maps and graphic data displayed in Figure 5 show a gradient in shape maintenance depending on the pattern used during scaffold fabrication. In agreement with the above-mentioned results, the control pattern 1–0 DN-GelMA displayed the best ability to maintain the complex shape of the nose post exposure to collagenase IV, as this scaffold did not experience any degradation. This mirrors the mechanical results previously shown in Figure 3, as an increase in modulus is predicted to be from this pattern's slight swelling. Patterns 2–1 and 1–1 similarly swelled; this is hypothesized to have occurred once the constricting GelMA strands dissolved post collagenase IV digestion and PEG strands were able to relax and swell. Pattern 1–2 displayed adequate ability in preserving the nose shape when compared to its original printed structure, with <1 mm deviation. Lastly, pattern 1–3 showed the least in ability to maintain shape, equivalent to 2mm deviation from its original shape.

Taken together, these results indicate that the double network hydrogel can have a gradient effect on dictating scaffold shape during degradative remodeling processes, allowing the ability to tailor each fabricated scaffold to its intended application. However, overall scaffold shape is maintained for all patterns examined. Consequently, an effective degree of the non-degradable DN ink can be chosen to tailor the desired mechanical properties of the scaffold, such as delicate fat adipose tissues, or stiffer muscular tissues, with promising long-term results.

2.4 Cytotoxicity Testing

To test the cytotoxicity of the DN ink, GelMA bioink was seeded with primary adult human dermal fibroblasts (2 million cells mL⁻¹ concentration) and extruded alongside DN ink in a two-layered disc print (1–1 DN-GelMA pattern) to study the effect of PEG presence on cell viability (Supplemental Figure 2). Discs consisting merely of GelMA and cells were also fabricated as a control to compare the effect of PEG material presence. Samples were stained with Calcein AM and Ethidium Homodimer on days 0, 7, and 14 of culture, imaged, and later digested for DNA quantification. Across all days, cell viability ranged from 87±5% (day 0) down to 72±8% (day 14), a high and acceptable range for bioprinted constructs.^[43] Our quantitative DNA concentration assay mirrored these results, with day 7 containing 0.33 ± 0.1 ratio and day 14 exhibiting 0.55 ± 0.2 ratio in normalized DNA concentration when compared to day 0 values, demonstrating cell proliferation over time. In addition, the fibroblasts displayed a healthy morphology by spreading throughout culture within the prints. Day 0 images portray rounded cell morphology, due to cells' recent experience to encapsulation into a new environment and pressure during the printing process; but by day 7 and day 14, the average cell spreading area gradually increased (Supplementary Figure 3). These results indicate that the current co-printing method of the DN ink and GelMA bioink supports viable cell culture over extended periods of time. Because the DN ink's presence is shown to not have detrimental effects on cell viability, we predict similar positive cell responses in other print patterns. Furthermore, the bioink allows for local remodeling by the cells, an essential requirement for scaffold-host integration and tissue ingrowth upon implantation *in vivo*. Overall, the high biocompatibility and low cytotoxicity of the complementary DN-GelMA hybrid bioinks have promising potential as an effective strategy for the repair and replacement of native soft tissue with long-term stability and integration. Additionally, the readily-modified chemistries of GelMA and PEG can be utilized to sequester desired growth factors (e.g. pro-angiogenic, anti-inflammatory, etc.) to further improve the functionality of the 3D printed grafts.^[44,45]

3. Conclusion

We have developed a 3D printing strategy for soft tissue engineering which involves a hybrid scaffold composed of sacrificial layers that allows for tissue integration and a skeletal network that maintains mechanical and shape integrity. The novelty of this paper lies in the versatile and cell-friendly 3D printing platform that we have devised, and the revolutionary application of mesh analysis for assessing scaffold aesthetic properties for soft tissue engineering. We have successfully applied these strategies to commonly-used hydrogels such as GelMA, PEG-based, and alginate materials utilizing a process of multi-material printing that can be readily accessed by the 3D printing and biomedical community. DN-GelMA printing patterns used to fabricate complex scaffolds highly dictate both the composite's mechanical properties and ability to maintain shape post degradation processes. Cytotoxicity characterization of the hybrid print revealed high proliferation and cell spreading after multiple weeks of *in vitro* culture. Our key innovation is producing a biomimetic hydrogel graft consisting of both degradable and non-degradable parts that harmoniously coexist to promote tissue integration while maintaining its original printed structure.

To our best knowledge, there are no previous reports on the creation of volume-stable, mechanically relevant, biocompatible soft tissue grafts fabricated with neither single nor multi-material printing. Overall, a modular and biocompatible ink formulation was developed that can be used to fill soft tissue defects and integrate with the surrounding environment while providing long term mechanical integrity and shape retention.

Experimental Section

Double Network Ink: The double network ink was synthesized by first dissolving 5% (w/v) 4-arm PEG Norbornene and 5% (wv⁻¹) 4-arm PEG Thiol in deionized water. Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP, Sigma Aldrich) with a 0.2% w/v ratio and alginate powder (Sigma) with a w/v ratio varying from 1–5% (derived from brown algae, Sigma) was added and thoroughly mixed. The ideal amount of alginate used for thickening the PEG solution was then tested by systematically extruding the various formulations through the printer cartridge.

Swelling of DN Ink: For each ink formulation (5K, 10K, and 20K), solid discs with a 5 mm diameter and 2 mm thickness were casted using previously printed molds. Disc dimensions, wet/dry weight, and bright field images of each sample were taken before and after a 24 h incubation at 37°C. Percent increase measurements (in mass, thickness, and diameter) were calculated by dividing the difference of swollen vs. unswollen measurements by the original unswollen value. Mass swelling ratio was similarly calculated by dividing the difference of swollen vs. the lyophilized dry weight by the lyophilized dry weight value.

GelMA Bioink: GelMA was synthesized as previously described with further details listed in Supplemental Methods.^[34] For printing, lyophilized GelMA was dissolved at 7% (wv⁻¹) in fibroblast media at 50°C for 20 mins. Irgacure 2959 (0.2% wv⁻¹, BASF) was added at 50°C for 15 min. Fibroblasts (Passage 3) were then added at a concentration of 2×10⁶ cells mL⁻¹ and homogenously mixed throughout the solution. The prepolymer solution was then loaded into a syringe barrel and allowed to equilibrate for 30 min at 23°C.

Hybrid Scaffold Fabrication: Complex architectures consisting of human nose, ear, and thyroid cartilage (adopted from 3D scans, GrabCAD) were printed in a 1–1 DN-GelMA pattern. All model scaffolds were sliced into layers with a slicing thickness equal to 80% (0.32 mm) of the needle size (0.4mm) before printing. Each printed layer was exposed to UV light (5 mW cm⁻² intensity) for 5 s, and the final print was placed in a UV box (5 mW cm⁻²) and exposed further for 6 min. For the remaining assays, 3D cylinder models with 5 mm diameter and 2 mm thickness were designed in SolidWorks (Waltham, MA). For the cytotoxicity assay (1–1 pattern), prints were immediately transferred to 6-well tissue culture plates and submerged in media, with media changes every 2 days for 2 weeks. Cylindrical scaffolds were printed with varying ratios of bioinks (DN-GelMA: 1–0, 2–1, 1–1, 1–2, and 1–3) for mechanical testing. Half of the produced scaffolds were exposed to collagenase IV (500 Units mL⁻¹, Worthington Biochemical Corporation) to evaluate the DN's impact on the composite mechanical properties.

Mechanical Testing of Hybrid Scaffolds: Compression testing was performed on a Dynamic Mechanical Analyzer (DMA Q800, TA Instruments) with a strain sweep (0–15%) and load 0.01N at 1Hz frequency. Elastic modulus for each sample was calculated by determining the slope of the linear region of the stress-strain curve (strain region of 3–5%). Fracture strength was recorded (force at which the hydrogel plastically yields) as well as toughness (area under stress-strain curve).

Point Cloud Generation: The ROMER Absolute Arm (Hexagon) was used to generate point clouds containing high geometric detail of the scanned surface. Scaffolds were sprayed with water soluble paint prior to scanning for greater ease in detecting surfaces. The noses were sequentially scanned at multiple angles and the surfaces were selected using the software's editing brush tool. After the total region of interest was created, a volume was generated and exported as a stereolithography (STL) file.

Surface Alignment: STL files of the same sample (both pre and post digestion scans) were imported into the open-source software MeshLab (Visual Computing Lab- ISTI CNR). With this software, the alignment tool's ('point-based alignment', 'process tool alignment') were used by selecting four possible points along the base face of each model.^[46] Further details on STL alignment can be found in Supplementary Methods.

Cloud Comparison: The two repositioned STL meshes for each pattern were uploaded into the CloudCompare software and point cloud comparison was performed by selecting the pre-digestion mesh as the reference and the postdigestion mesh as the compared object. A color map was generated, which calculated the distance of points in the compared cloud to the nearest point in the reference pre-digestion cloud. Further CloudCompare methods is described in the Supplemental section.

Statistical Analysis.—Data was analyzed using single factor analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test assuming normal data distribution with a confidence of 95% ($p < 0.05$).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Description of printed inks and hybrid scaffold.

A) 4-arm PEG monomers functionalized with norbornene and thiol end groups react when photoinitiator LAP is present within the solution, as alginate ionically crosslinks with itself with the presence of calcium ions; GelMA monomers mixed with cells and Irgacure 2959 results in a crosslinked cell-encapsulated bioink strand; B) visual representation of alternating PEG and GelMA inks in 1–1 pattern to create a hybrid scaffold. Complex architectures can be created using this printing technique, such as C) human nose, D) ear, and E) thyroid cartilage (commonly known as Adam's Apple).

Figure 2. Swelling characteristics.

A) Description of ink formulations with varying PEG molecular weight monomers where either i) a pure PEG crosslinked network, ii) added alginate for thickening purposes, and iii) a double network consisting of covalently crosslinked PEG and physically crosslinked alginate was formed. Brightfield images revealed that the interpenetrating networks resulted in the least visual swelling (red box). B) diameter percent increase, C) thickness percent increase, and D) mass percent increase E) mass swelling ratio were recorded. $n=6$ and mean $\pm$ standard deviation is depicted, $p<0.05$.

Figure 3. Mechanical testing of various print patterns.

A) Visual description of the various print patterns used to fabricate hybrid scaffolds. Uniaxial compression testing was performed on hybrid scaffolds both before and after experiencing GelMA digestion via collagenase IV. Mechanical properties, such as B) compressive modulus, C) fracture strength, and D) toughness were characterized to ensure hybrid scaffolds exhibited similar properties to soft tissue. $n=5$ and mean $\pm$ standard deviation is depicted, $p<0.05$.

Figure 4. Complex scaffold shape retention.

A) Noses were printed in various patterns of double network ink and GelMA and further subjected to collagenase IV digestion. B) Scaffold dimensions were recorded and compared to its original fabricated form, specifically C) width, length, and peak height. D) Magnitude of curvature was also calculated and recorded for each hybrid pattern. $n=4$ and mean $\pm$ standard deviation is depicted, $p<0.05$.

Figure 5. Color maps displaying point cloud comparisons.

Hybrid nose prints were 3D scanned with a Hexagon Romer Absolute Arm both before and after GelMA digestion, such that finite element meshes could be produced and compared. Color maps display deviations of the digested scaffold to its original form, where red/orange designate positive deviations (swelling) and green/blue designate negative deviations (shrinkage). Histograms displayed on the right graphically show the percentage of mesh points that deviated from their initial positions. Scale bar displays 8 mm.