Robust fluidic connections to freestanding microfluidic hydrogels

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Biomimetic scaffolds approaching physiological scale, whose size and large cellular load far exceed the limits of diffusion, require incorporation of a fluidic means to achieve adequate nutrient/metabolite exchange. This need has driven the extension of microfluidic technologies into the area of biomaterials. While construction of perfusable scaffolds is essentially a problem of microfluidic device fabrication, functional implementation of free-standing, thick-tissue constructs depends upon successful integration of external pumping mechanisms through optimized connective assemblies. However, a critical analysis to identify optimal materials/assembly components for hydrogel substrates has received little focus to date. This investigation addresses this issue directly by evaluating the efficacy of a range of adhesive and mechanical fluidic connection methods to gelatin hydrogel constructs based upon both mechanical property analysis and cell compatibility. Results identify a novel bioadhesive, comprised of two enzymatically modified gelatin compounds, for connecting tubing to hydrogel constructs that is both structurally robust and non-cytotoxic. Furthermore, outcomes from this study provide clear evidence that fluidic interconnect success varies with substrate composition (specifically hydrogel versus polydimethylsiloxane), highlighting not only the importance of selecting the appropriately tailored components for fluidic hydrogel systems but also that of encouraging ongoing, targeted exploration of this issue. The optimization of such interconnect systems will ultimately promote exciting scientific and therapeutic developments provided by microfluidic, cell-laden scaffolds. © 2015 AIP Publishing LLC.

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INTRODUCTION

The fabrication of 3D cell-laden tissue constructs is among the forefront of emerging bioengineering technologies, with considerable potential to facilitate scientific discovery and revolutionize regenerative medicine. The development of three-dimensional tissue scaffolds approaching physiological scale, however, requires incorporating means to accommodate increasing cellular metabolic demands no longer adequately supported by diffusion. Perfusable, prevascularized 3D tissue scaffolds, equipped with microfluidic lumen networks, allow for enhanced cell viability and function by providing a means for active nutrient/metabolite exchange, thus enabling convective transport of necessary soluble compounds throughout the scaffold volume and overcoming the roughly $\sim 200\ \mu\text{m}$ thickness limitation imposed by diffusion.¹ Although fabrication of such devices remains a substantial engineering challenge, tremendous effort has been devoted to methodological innovation and discovery as evidenced by an ongoing, steady efflux of scientific literature in this field. One critical aspect of fabrication and functional implementation is the integration of external perfusion machinery through optimized fluidic connective assemblies. However, to the best of our knowledge, there are no reports

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specifically focusing on identifying fluidic interfacing approaches best suited for long-term perfusion of free-standing hydrogel scaffolds.

The field of microfluidics has expanded to include a wide range of device materials, fabrication techniques, and applications. Initial development of microfluidic devices utilized rigid materials such as silicon²⁻⁴ and glass,⁵⁻⁷ but current efforts rely heavily on the use of moldable elastomers, with polydimethylsiloxane (PDMS) being the dominant material.⁸⁻¹⁰ Microfluidic devices fabricated in PDMS have been used for applications varying from point-of-care biomedical diagnostic assays,¹¹⁻¹³ small volume cellular isolation,¹⁴ single cell assays,^{15,16} and most recently, organ-on-a-chip efforts.¹⁷⁻²² The need to produce prevascularized tissue constructs has led to the extension of microfluidic technologies into the area of biomaterials. Microfluidic hydrogels are an attractive construct system because they mimic the mechanical properties of natural tissue, allow for diffusion and interstitial flow of soluble compounds through the gel, and support convective transport of soluble compounds through the microfluidic channel network (thereby mimicking a vascular system). Because gases and soluble biochemicals can readily diffuse and flow through hydrogels, these materials allow the formation of truly 3D cell culture systems with encapsulated cells that better mimic the natural extracellular matrix.

Several techniques have been developed to form microfluidic channels within hydrogels. Perhaps, the simplest technique is to simply pour the uncrosslinked solution over a thin needle or similar relief structure, which is subsequently removed after the gel has set, yielding straight, reproducible microchannels.^{24,25} Initial work on patterning more complex systems utilized soft lithographic techniques to form 2D fluidic networks in alginate²⁶ and collagen/fibrin substrates.²⁷ This technique, along with emerging 3D printing²⁸⁻³² and stereolithographic³³ approaches, produces 2D patterns which must be stacked to achieve 3D complexity. Recently, there has been an increase in the use of rapid prototyping techniques to pattern sacrificial elements used to make microchannels³⁴⁻³⁶ that yield 3D channel architecture in a single fabrication step.

Regardless of the fabrication technique, material, or application, microfluidic devices must be capable of interfacing to external fluids. For some devices, such as paper-based microfluidic devices, this is simply a matter of placing a drop of solution in a given area on the device surface.^{3,37} Other systems, however, require more complex means of fluidic control through integrated valving³⁸⁻⁴⁰ and connections to external pump/suction mechanisms.⁸ This necessity has driven the development of various techniques to form fluidic interfaces to microfluidic systems, including implementation of external scaffolding/compression rigs,^{41,42} silicon compatible adhesives,^{43,44} friction/pressure fit tubing,^{23,45,46} mechanical interlock,⁴⁷ among others.⁴⁸ While it is critical that these interfaces be able to sustain high pressures over long times without leaking, other important features may include reusability, multiplexing, or compatibility with harsh chemicals or biological systems.²³

However, such biomimetic hydrogel scaffolds present unique challenges that complicate fluidic assembly, largely due to structural fragility and biocompatibility requirements. Unlike the majority of traditional elastomeric microfluidic devices for medical diagnostic applications, microfluidic biomaterials are often impregnated with cells, therapeutic compounds, soluble signalling factors, or other environmental conditioning reagents. Thus, biological reactivity becomes an even more critical consideration in the platform design as the separation between cellular compartments and interconnect components diminishes. These are just a few of the more prominent reasons that prevent translation of PDMS-based fluidic interfacing solutions to hydrogel systems.

In this report, we characterize the efficacy of a variety of adhesive-based and mechanical connection modalities for optimal perfusion of microchannel networks in free-standing, gelatin scaffolds. Specifically, we focus upon identifying fluidic assemblies that are both cell-compatible and possess the material properties conducive to long-term perfusion of fragile, free-standing hydrogels; properties most critical to the functional success of our goals of generating biomimetic, multicellular tissue construct complete with endothelialized microvascular lumen. While this investigation is based upon the needs of one particular hydrogel system (gelatin),

outcomes ultimately highlight the importance and need for greater attention focused upon the fluidic connects and implementation of vascularized tissue constructs.

METHODS

Device substrate composition

Gelatin: Under sterile conditions, within a BSL II rated biosafety hood, porcine gelatin (Sigma, G1890) was suspended in sterile 1X PBS (Sigma) at concentrations of 7%, 10%, or 12% (w/v), then transferred to 37 °C water bath for one hour to allow full dissolution. The gels were enzymatically crosslinked using microbial transglutaminase (mTG) (Modernist Pantry) in a 1:10 (mTG:gelatin) mass ratio, regardless of initial concentration.⁴⁹ Immediately prior to casting, the appropriate volume of 10% (w/v) mTG stock solution in 1X PBS, filter sterilized (Corning) and pre-warmed to 37 °C, was added to aqueous gelatin. If needed, gelatin/mTG solutions were degassed for 30 s in 37 °C sonicating bath. Gelatin/mTG hydrogels were incubated at 37 °C overnight before demolding. Hydrogels not designated for immediate use were autoclaved at 121 °C for 30 min in 1X PBS and stored at room temperature.

Alginate: As an alternative to gelatin-based hydrogels, 3% (w/v) alginate test devices were produced to further characterize adhesive/interconnect properties. To facilitate device uniformity, a combination of CaCO₃ (Sigma) and gluconolactone (GDL, Sigma) was used to achieve slow (~19 h) calcium-dependent crosslinking of alginate gels. Specifically, 3 g sodium alginate (Protanal LF10/60, FMC Biopolymer) was dissolved in 76 ml of deionized water using a Thinky ARE-250 planetary centrifugal mixer for 6 min at 2000 rpm. This mix cycle was repeated following the addition of 12 ml of 37.5 mM CaCO₃ suspended in DIW. Finally, 12 ml of 75 mM GDL in DI water was mixed into the alginate solution for 30 s at 2000 rpm, then poured into test-device molds. Alginate hydrogels were allowed to fully crosslink for 24 h at room temperature before immediate use in adhesive pressure and tensile strength assays.

Polydimethylsiloxane: Sylgard 184 elastomer base and curing agent (Dow Corning) were combined in a 10:1 (w/w), respectively, and degassed for 20 min under vacuum. PDMS filled molds were allowed to cure at room temperature for 24 h prior to use.

Device fabrication

Burst pressure: To approximate the pressures generated at the intersection of inlet channels (~1–3 mm diameter) designed to facilitate tubing connection using readily available fluidic fitting components with microchannels (5–200 μm diameter) that perfuse cellularized scaffolds, we designed easily reproducible, standardized hydrogel test-device molds consisting of a single macrochannel/microchannel junction. Large (40 × 22 × 20 mm) Peel-A-Way tissue embedding molds (Fisher Scientific) were perforated on opposing ends with stainless steel, blunt tipped, syringe needles (McMaster-Carr), gauges 12 (dia.: 2.8 mm) and 30 (dia.: 300 μm) positioned such that they intersected at the center of the mold (Figure 1). The molds were then filled with 25 ml of the indicated hydrogel or polymer in accordance with the appropriate protocols.

Tensile strength: In order to measure the tensile strength of microfluidic port attachment, Peel-A-Way molds were prepared as for those intended for burst pressure testing, but using only a 12 G syringe needle that yielded a single open channel approximately 2.8 mm in diameter (Figure 1). Each device was cut into three equal sections (~12.5 mm thick) to mitigate the influence of structural instability of the hydrogel upon measurements intended to characterize port attachment efficacy.

Microchannel devices: Using Peel-A-Way molds, “prevascularized” hydrogel devices were prepared using methods reported previously.³⁶ Briefly, Joncryl 678 (BASF) is melt-spun using a cotton-candy machine (Nostalgia Electrics) to generate a delicate fiber mesh that is placed into the Peel-A-Way embedding molds, bonded with 100% ethanol vapour for 10 min under vacuum, plasma treated for 10 min to enhance wetting properties, embedded in the preferred hydrogel solution, and allowed to polymerize for 24 h at 37 °C and 95% humidity. The sacrificial fibers were removed from crosslinked gelatin via dissolution in a series of 30 mM

FIG. 1. Test device fabrication process. This illustration outlines the fabrication procedure for generating reproducible test device for characterizing integrity of fluidic assembly components (not to scale).

ammonium-hydroxide (Sigma) in 1X PBS baths over 24 h to yield hydrogel scaffolds consisting of a dense interconnected network of hollow microchannels.

Port attachment

In order to qualitatively assess relative efficacy of gelatin-based adhesive, we evaluated six means of microfluidic port attachment: cyanoacrylate adhesive (Loctite[®] 4641), fibrin adhesive, high molecular weight (HMWG) fish gelatin (fish glue) adhesive, L-DOPA modified gelatin adhesive (DOPA glue), a combination of HMWG fish gelatin and L-DOPA modified gelatin adhesive (“DOPA-Fish glue”), as well as barb and tubing “friction fit” adhesive alternatives.

Superglue: A thin layer of Loctite[®] 4641 instant adhesive (McMaster Carr) was applied to interfacing surface of the microfluidic Nano-Port (Upchurch Scientific), which was then gently pressed onto the hydrogel/polymer surface whilst taking care to prevent adhesive leakage into macrochannel. Up to two additional layers of adhesive were applied to the outer surface of the port interface, allowing for adequate setting time between applications prior to use.

Fish glue: Stock solutions of 30% (w/v) HMWG, cold-water fish gelatin (HMWG, Norland) and 60% (w/v) mTG were dissolved in 1X PBS at 37 °C for at least 1 h, then filter-sterilized (0.2 μ m cellulose membrane, Corning) and stored at 4 °C. Prior to use, mTG solutions were warmed to 45 °C for 1 h to maximize enzyme activity and efficiency.⁴⁹ Although aqueous at room temperature, HMWG fish gelatin was also warmed to 37 °C to accelerate cure time. To form adhesive, mTG and fish gelatin solutions were combined and by pipet in a 1:2 ratio, respectively, in a microcentrifuge tube (Fisher Scientific) until viscosity began to noticeably decrease (~1–5 min). Fish glue was then applied drop-wise to the outer circumference of device macrochannel opening immediately in advance of attaching the NanoPort. This initial junction was allowed set at least 5 min to increase stability before applying a second layer of fish glue to the outer port/device surface. The final layer of adhesive was given a minimum of 15 min at room temperature to cure before use.

DOPA-Fish glue: This two-stage adhesive system consists of a combination of the aforementioned fish glue adhesive with porcine gelatin enzymatically modified with mushroom tyrosinase (Sigma, >1000 U/ μ g)) to generate sticky DOPA residues as described by Choi *et al.*⁵⁰ Briefly,

1 μg of trypsinase was dissolved in 1 ml of aqueous 10% gelatin and allowed to react for 60 min at 37 °C. Enzyme activity was then heat-inactivated for 30 min at 60 °C. For use as initial sealant, DOPA-gelatin and 100 mM FeCl_3 were warmed to 37 °C. Due to the rapid effect of FeCl_3 , the solutions were not mixed prior to application. Instead, DOPA-gelatin was applied to the device surface surrounding macrochannel. Subsequently, 100 mM FeCl_3 was stirred in the DOPA-gelatin to enhance crosslinking and covalent bonding capacity. FeCl_3 -crosslinked DOPA-gelatin (DOPA glue) formed a fluidic seal in approximately 60 s. Fish glue was then applied to the outer port surface to further stabilize port assembly.

Fibrin glue: Bovine fibrinogen (5 mg/ml, Fisher Scientific) and 5 KU/ml thrombin (Fisher Scientific) in 1 mM CaCl_2 were combined 1:1 by pipet to form the fibrin glue adhesive. Because fibrin glue cures rapidly (<10 s), the two components were mixed directly on test device surface when attaching the NanoPort fitting.

Direct connect fittings: Polypropylene barb fittings (1/8 in., Upchurch Scientific) with a luer lock connection were pressure fitted directly into test device macrochannel for perfusion. Similarly, 1/8 in. OD silicone tubing (VWR) was carefully inserted into test device macrochannels to achieve “friction fit” attachments.

Burst pressure measurements

An illustration of the experimental setup for obtaining burst pressure measurements is shown in Figure 2(a). Specifically, a fluidic circuit consisting of syringe pump (NE-300, New Era Pumps) equipped with a 60 ml syringe (Becton Dickinson) containing blue food coloring (Enoglaucine Disodium Salt, Sigma) dissolved in 1X PBS was connected in series to an inline pressure sensor (30 PSI, Honeywell), then to the test device. Readings from the pressure sensor were collected and recorded using customized LabVIEW (National Instruments) code. Perfusion rates were set initially at 100 $\mu\text{l}/\text{min}$, then increased gradually to a maximum of 20 ml/min, or until failure. Mode of failure was documented based upon location; either at seal (fluidic interface), within the device structure itself or system (i.e. burst pressure exceeded system capacity). All tests were performed in triplicate ($N \geq 3$) and averaged to determine relative burst pressure.

FIG. 2. Experimental setup for mechanical property analysis of fluidic interfaces. (a) Schematic of experimental setup for measuring burst pressure. Inset shows perfusion of PDMS test device using barb fluidic connection. (b) Time-lapse images of 12% gelatin test device fitted with NanoPort using Loctite[®] 4641 (superglue) adhesive during progression of burst pressure analysis. (c) Instron 5944 instrumentation setup and implementation for characterizing the tensile properties of tubing connections. Inset shows 12% gelatin tensile test device fitted with NanoPort assembly using Loctite[®] 4641 (superglue) adhesive just prior to tension test. (d) Time lapse image of tensile strength test.

Tensile strength measurements

As shown in Figure 2(c), tensile strength of fluidic interconnects were measured using an Instron 5944 and accompanying mechanical analysis software (Bluehill). The bases of tensile strength test device sections were affixed to test platform using Loctite® 4641 adhesive before commencing vertical displacement of port attachment (50 N capacity grip) at a rate of 2 mm/min. Each interconnect modality was tested in cohorts of 3 or more ($N \geq 3$), with results averaged to determine overall tensile strength.

Cell viability

Primary human umbilical vein endothelial cells expressing red fluorescent protein (RFP-HUVECs, Angioprotemie) were maintained in BSL II rated cell culture facilities using endothelial growth medium (EGM-2, Lonza). Prior to cell seeding, 10% gelatin pressure test devices were conditioned in EGM-2 for 24 h within a cell culture incubator (HeraCell 150i, Thermo Scientific) at 37 °C and 5% CO₂, using re-closable T-150 flasks (TPP) containing 100–150 ml EGM-2 media supplemented with 1% penicillin/streptomycin (Sigma) and 0.1% amphotericin (Gibco). Approximately 200 μ l of RFP-HUVECs suspended in EGM-2 at a concentration of 500 000 cells/ml ($\sim$ 10 000 cells) were injected by pipet into the macrochannel before attaching tubing with either adhesive or direct-connect modalities as previously described. Cells were allowed to attach to the gelatin surface under static conditions for 1 h before inverting device to promote seeding along entire circumference of channel. After 24 h, cells were stained with 1 μ M Calcein Green (Life Technologies) and 2 μ M Sytox Blue (Life Technologies) in 1X PBS for 30 min to identify live and dead cell populations, respectively, using confocal fluorescence microscopy (LSM 710, Zeiss). Peristaltic pumps (VWR, 61161–354) were then engaged to actively perfuse devices at 100 μ l/min for the remainder of the assay. Cell viability was reassessed at 24 h intervals. Imaging and post-processing analysis was conducted using Zen Black (Zeiss) and Fiji/ImageJ.⁵¹

RESULTS AND DISCUSSION

In the process of conducting mechanical property analysis, surgical haemostatic adhesives,^{52–54} DOPA-gelatin and fibrin glue, were found to lack the structural robustness to withstand testing and were thus omitted from further analysis. Although considered outside the scope of this report, it is important to note that modification to the specified formulations would undoubtedly affect the material properties of both adhesives. Structural integrity of fibrin glue is influenced by concentrations of primary constituents, fibrinogen and thrombin, as well as calcium, collagen, Factor XIII, pH, and temperature.⁵⁴ Alternatively, the number of sticky DOPA residues giving rise to adhesive properties of DOPA-gelatin correlates directly to the availability of Tyrosine; the relative abundance of which is greater in human collagen. Given the evidence of intra-operative biocompatibility, optimization of both fibrin and DOPA-based adhesives for applications supporting perfusion of thick-tissue constructs warrants further investigation.

Outcomes of material property analysis of remaining connection modalities (superglue, barb fitting, tubing friction fit, fish-glue, and DOPA-Fish glue) are shown in Figure 3. As expected, interconnect efficacy is highly dependent upon substrate composition. While robust connections mediated by superglue and mechanical fittings were achieved in PDMS and alginate substrates, structural fragility, swelling propensity, deformation, and degradation over time threatened long-term integrity in gelatin hydrogel constructs.

Gelatin concentration also influenced outcomes. Low seal failure rates for microchannel and 7% gelatin hydrogels during burst pressure testing suggest that adhesive integrity exceeded the structural capacity of the substrate to withstand the high pressures reported. Increased rigidity of higher gelatin concentrations yielded hydrogels capable of accommodating greater pressures that challenged the interface between adhesive and substrate. With the exception of PDMS, Fish glue and DOPA-Fish glue out-performed tubing friction fit and barb connections for all substrates, as well as superglue for microchannel, 7%, and 10% gelatin hydrogels.

FIG. 3. Robustness of fluidic connection modalities vary with device composition. Outcomes of (a) burst pressure analysis and (b) tensile strength analysis with respect to device composition. In (a), the percentage of respective failures that occurred due to compromised seal (adhesive) is indicated by ($\diamond$) symbols according to the right-most axis. All burst pressure failures were classified by location; either seal or device, with the exceptions of points marked with (*), indicating the robustness of interface exceeding the loading capacity of the testing system (i.e. non-failure). Error bars indicate $\pm$ one standard deviation of the mean from cohorts of $N \geq 3$.

Fish glue was both structurally robust and compliant to environmental changes during testing. However, complete assembly required up to 30 min, including up to 10 min before the adhesive established secure support of the NanoPort. DOPA glue, as characterized by Choi *et al.*,²⁶ is reported to form covalent bonds between ECM components and DOPA residues that are enhanced by the presence of FeCl_3 . Adhesion occurs rapidly and has been shown to act as an effective fluidic seal. Incorporation of DOPA glue to initially stabilize port attachment prior to application of Fish glue enabled rapid (<30 s) port stabilization and establishment of a fluidic seal, and a reduction in overall assembly time by up to 30% compared to Fish glue alone, while maintaining the overall robustness provided by Fish glue. This novel combination adhesive yielded a more consistent and reliable method of port attachment that greatly enhanced the overall success of the microfluidic system.

For gelatin substrates, especially those of lower concentration, Fish glue and DOPA-Fish glue produced the strongest adhesive bond. Tensile strength analysis indicated maximal values for Fish glue in microchannel, 7%, 10%, and 12% gelatin hydrogels, while DOPA-Fish glue performed optimally according to outcomes of burst pressure analysis. Considering that DOPA modified gelatin alone lacked the structural robustness to support port attachment, it is likely that inclusion of DOPA glue as an initial sealant weakened the overall tensile strength of the DOPA-Fish glue adhesive. However, in exchange for the reduction in tensile strength, rapid stabilization of the NanoPort in the vertical position increased integrity of initial fluidic seal and reduced leakage of Fish glue into the macrochannel, thus facilitating a more reliable fluidic interface as demonstrated by capacity to withstand greater fluidic back pressure. Long-term functionality of microfluidic hydrogels depends most heavily upon the ability of the adhesive to withstand back pressure generated by the high fluidic resistance of microchannels. For this reason, along with the significant reduction in assembly time, DOPA-Fish glue is a more attractive adhesive for attaching external tubing to gelatin hydrogels.

Mechanical testing also revealed promising functionality of DOPA-Fish glue for non-gelatin hydrogels. Specifically, for 3% alginate hydrogels, performance of Fish glue and DOPA-Fish glue followed a trend similar to that of gelatin cohorts, although with more severe

FIG. 4. Cell seeding of gelatin hydrogels. (a) Macro-channels of 10% gelatin test devices were seeded with RFP-HUVECs under static conditions. Images depict Z-projection of cell-laden channel surface 48 h after seeding stained with (b) 1 μ M Calcein Green and (c) 2 μ M Sytox Blue to identify live and dead cell, respectively. (d) Orthogonal projections show confluent cell growth spanning entire circumference of channel walls. Particle analysis (ImageJ) of corresponding Z-stacks confirm greater than 85% viable cells prior to port attachment. Scale bars indicate 500 μ m.

disparity in tensile strength outcomes. Alginate gels were structurally more rigid than 12% gelatin substrates. This, combined with the influence of different hydrogel chemistries upon adhesive properties, likely reduced the overall strength of DOPA glue adhesion, thus decreasing the tensile force required for port detachment. However, DOPA-Fish glue outperformed all other fluidic interface modalities except for superglue in burst pressure testing. Enhanced success of superglue, for alginate as well as 12% gelatin and PDMS, is also likely related greater substrate rigidity.

Softer physiological hydrogels, such as collagen or fibrin, are frequently used as substrates for cell scaffolds, but are less suited to tissue-scale, free-standing constructs as a result of low structural integrity in the absence of external support. In anticipation of advances in biofabrication techniques involving these materials, we conducted a similar subset of experiments using 5% collagen hydrogels. Despite hydrogel fragility, DOPA-Fish glue again demonstrated promising adhesive efficacy in collagen based substrates (see supplementary material).⁵⁵ Considering gelatin is a derivative of collagen, this outcome aligns well with findings associated with the gelatin cohorts.

In addition demonstrating adequate structural and adhesive robustness to support long-term perfusion of free-standing hydrogels, it is equally critical to evaluate fluidic assembly components for any signs of cytotoxicity. Viability of RFP-HUVECs seeded along channel walls of 10% gelatin burst pressure test devices exceeds 85% for up to 48 h under static conditions (Figure 4). Subsequent perfusion of cellularized macro-channels reveals that the use of superglue results in rapid and profound cell death within 24 h (Figure 5(A)). Cyanoacrylate based adhesives used clinically in lieu of traditional sutures are known to be associated with cellular

FIG. 5. Evaluating cellular toxicity of fluidic adhesives. RFP-HUVECs were seeded along gelatin channels under static conditions for 48 h before subjected to continuous perfusion facilitated by (A) superglue or (B) DOPA-Fish glue adhesives. Cellular viability following port attachments was assessed by observing (a, g) RFP expression, (b, h) Calcein Green (live), and (c, i) Sytox Blue (dead). After 24 h of perfusion, fluorescence imaging of cell-laden channels was repeated, revealing marked cellular loss and death in channels perfused with superglue adhered ports (d-f). DOPA-Fish glue adhered ports was associated with unchanged populations (j-l), providing positive indication of cellular compatibility. Scale bars represent 500 μ m.

	Mechanical Strength	Cell Compatibility	Ease of Use	Long-Term Stability	Overall Rating
Superglue	••	◦	•••	•••	POOR
Barb	•	•••	•••	◦	OK
Tubing Friction Fit	•	•••	•••	◦	OK
Fish Glue	•••	•••	•	•••	GOOD
DOPA-Fish Glue	•••	•••	••	•••	EXCELLENT

FIG. 6. Overall rating of adhesive efficacy for perfusion of freestanding gelatin scaffolds. Based upon material property analysis and cell compatibility assays, direct connect and adhesive based fluidic interface methods are rated from poor (◦) to excellent (•••) to simplify overall performance and utility in perfusion of gelatin hydrogels.

necrosis in immediate vicinity of wound.⁵⁶ In contrast, fluidic assemblies facilitated using DOPA-Fish glue were associated with no marked decrease in cell viability after 48 h (Figure 5(B)), providing positive evidence regarding biocompatibility with endothelial cell culture. Furthermore, cell growth in perfused microchannel devices facilitated by DOPA-Fish glue adhesive has subsequently been maintained for nearly two weeks (see supplementary material).⁵⁵

CONCLUSIONS

Overall, substrate composition is the primary determinant influencing success of fluidic interconnect modality. Material property analysis clearly demonstrates that the highest performing interfacing mechanism with respect to PDMS and alginate, specifically mechanical direct-connect and cyanoacrylate-based adhesives, functioned poorly against gelatin hydrogel substrates, and vice-versa. In terms of gelatin-based hydrogel scaffolds, overall functionality of fluidic interconnects are summarized in Figure 6. From this investigation, we identified a novel combination of L-DOPA modified porcine gelatin and fish gelatin as a promising adhesive that is both mechanically robust and non-cytotoxic. This report highlights the importance of selecting the appropriately tailored components and adhesives for fluidic hydrogel systems.

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